#Pt4

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt4 iITx/Pt4 07032019.tsv")

source('Z:/Jianing/10x scRNA-seq TCR-seq/Pt15_MJ001, MJ004/alloreactivity_printtofile 09132017.R')

names(data)

```
 [1] "Rearrangement"        "Sum..Templates."      "Present.In"           "Itx.Pt4.POD13.Bx"

 [5] "Itx.Pt4.POD524.Bx"    "Itx.Pt4.POD524.COL_Bx"  "Itx.Pt4.POD674.ILE_Bx"  "Itx.Pt4.POD674.Na_COL_Bx"

 [9] "Itx.Pt4.POD674.Tr_COL_Bx" "Itx.Pt4.PreTx.4U"   "Itx.Pt4.PreTx.8U"     "Itx.Pt4.PreTx.R4H"

[13] "Itx.Pt4.PreTx.R4L"    "Itx.Pt4.PreTx.R8H"    "Itx.Pt4.PreTx.R8L"    "Itx_Pt4_MLN_D4L"

[17] "Itx_Pt4_MLN_D4U"      "Itx_Pt4_MLN_D8L"      "Itx_Pt4_MLN_D8U"      "Itx_Pt4_PBMC_POD324"

[21] "Itx_Pt4_PBMC_POD65"   "Pt4_iITx_PBMC_POD541"
```

Sample name (Pt: patient)	updated rename
Itx_Pt4_MLN_D4L	Pt04_Pre_Donor_CD4_CFSElo
Itx_Pt4_MLN_D4U	Pt04_Pre_Donor_CD4_unstim
Itx_Pt4_MLN_D8L	Pt04_Pre_Donor_CD8_CFSElo
Itx_Pt4_MLN_D8U	Pt04_Pre_Donor_CD8_unstim
Itx-Pt4-POD13-Bx	Pt04_Post_ileum_day0013
Itx-Pt4-POD524-Bx	Pt04_Post_ileum_day0524
Itx-Pt4-POD674-ILE_Bx	Pt04_Post_ileum_day0674
Itx_Pt4_PBMC_POD65	Pt04_Post_PBMC_day0065
Itx_Pt4_PBMC_POD324	Pt04_Post_PBMC_day0324
Pt4_iITx_PBMC_POD541	Pt04_Post_PBMC_day541
Itx-Pt4-PreTx-R4L	Pt04_Pre_Recipient_CD4_CFSElo
Itx-Pt4-PreTx-4U	Pt04_Pre_Recipient_CD4_unstim
Itx-Pt4-PreTx-R8L	Pt04_Pre_Recipient_CD8_CFSElo
Itx-Pt4-PreTx-8U	Pt04_Pre_Recipient_CD8_unstim

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

normalize(data[,c(17,19)])

ratio=5

  c1indices=which(data[,17]>0 & data[,19]>0 & data[,17]>ratio*data[,19])

  c2indices=which(data[,17]>0 & data[,19]>0 & data[,19]>ratio*data[,17])

  ambiindices=which(data[,17]>0 & data[,19]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,19]=0

  data[c2indices,17]=0

  data[ambiindices,c(17,19)]=0

#D4L vs D8L

normalize(data[,c(16,18)])

ratio=5

  c1indices=which(data[,16]>0 & data[,18]>0 & data[,16]>ratio*data[,18])

  c2indices=which(data[,16]>0 & data[,18]>0 & data[,18]>ratio*data[,16])

  ambiindices=which(data[,16]>0 & data[,18]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,18]=0

  data[c2indices,16]=0

  data[ambiindices,c(16,18)]=0

#R4U vs R8U

```
normalize(data[,c(10,11)])

ratio=5

  c1indices=which(data[,10]>0 & data[,11]>0 & data[,10]>ratio*data[,11])

  c2indices=which(data[,10]>0 & data[,11]>0 & data[,11]>ratio*data[,10])

  ambiindices=which(data[,10]>0 & data[,11]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,11]=0

  data[c2indices,10]=0

  data[ambiindices,c(10,11)]=0




#R4L vs R8L

normalize(data[,c(13,15)])

ratio=5

  c1indices=which(data[,13]>0 & data[,15]>0 & data[,13]>ratio*data[,15])

  c2indices=which(data[,13]>0 & data[,15]>0 & data[,15]>ratio*data[,13])

  ambiindices=which(data[,13]>0 & data[,15]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,15]=0

  data[c2indices,13]=0

  data[ambiindices,c(13,15)]=0




rownames(data)=data[,1]

x=data


#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,17]>0 | x[,19]>0)&(x[,10]>0 | x[,11]>0))
```

ambiguous=union(ambiguous,which((x[,17]>0 | x[,19]>0)&(x[,13]>0 | x[,15]>0)))

ambiguous=union(ambiguous,which((x[,10]>0 | x[,11]>0)&(x[,16]>0 | x[,18]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]

#additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L, R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional step of optimization for CD4 and CD8 sorting error.

#R4L vs R8U

normalize(x[,c(13,11)])

ratio=2

  c1indices=which(x[,13]>0 & x[,11]>0 & x[,13]>ratio*x[,11])

  c2indices=which(x[,13]>0 & x[,11]>0 & x[,11]>ratio*x[,13])

  ambiindices=which(x[,13]>0 & x[,11]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,11]=0

  x[c2indices,13]=0

  x[ambiindices,c(13,11)]=0

#R8L vs R4U

normalize(x[,c(15,10)])

ratio=2

  c1indices=which(x[,15]>0 & x[,10]>0 & x[,15]>ratio*x[,10])

  c2indices=which(x[,15]>0 & x[,10]>0 & x[,10]>ratio*x[,15])

  ambiindices=which(x[,15]>0 & x[,10]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

```
x[c1indices,10]=0

x[c2indices,15]=0

x[ambiindices,c(15,10)]=0



#D4L vs D8U

normalize(x[,c(16,19)])

ratio=2

  c1indices=which(x[,16]>0 & x[,19]>0 & x[,16]>ratio*x[,19])

  c2indices=which(x[,16]>0 & x[,19]>0 & x[,19]>ratio*x[,16])

  ambiindices=which(x[,16]>0 & x[,19]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,19]=0

  x[c2indices,16]=0

  x[ambiindices,c(16,19)]=0



#D8L vs D4U

normalize(x[,c(18,17)])

ratio=2

  c1indices=which(x[,18]>0 & x[,17]>0 & x[,18]>ratio*x[,17])

  c2indices=which(x[,18]>0 & x[,17]>0 & x[,17]>ratio*x[,18])

  ambiindices=which(x[,18]>0 & x[,17]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,17]=0

  x[c2indices,18]=0

  x[ambiindices,c(18,17)]=0
```

write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt4 iITx/Pt4 07032019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T, sep="\t")

add "fold=2" below, use 2 fold expansion (default 5 fold)

add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for read counts

#HVG direction

For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]

For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]

cd4.HVG= x[,c(10,13)]

cd8.HVG= x[,c(11,15)]

allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00002)

length(allo.HVG[[1]])

[1] 14446

length(allo.HVG[[2]])

[1] 14361

write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt4 iITx/Pt4 CD4 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt4 iITx/Pt4 CD8 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,10]+x[,13])>0,])

rCD8mappable=rownames(x[(x[,11]+x[,15])>0,])

CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])

6

write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt4 iITx/Pt4 CD4nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt4 iITx/Pt4 CD8nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 168858

length(rCD8mappable)

[1] 181439

length(CD4nonHVG)

[1] 154412

length(CD8nonHVG)

[1] 167078

rmappable=rownames(x[(x[,10]+x[,13]+x[,11]+x[,15])>0,])

length(rmappable)

[1] 350297

write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt4 iITx/Pt4 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)+length(rCD8mappable)

[1] 350297

length(intersect(rCD4mappable,rCD8mappable))

[1] 0

#GVH direction

For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]

For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]

cd4.GVH= x[,c(17,16)]

cd8.GVH= x[,c(19,18)]

allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00002)

length(allo.GVH[[1]])

[1] 9213

length(allo.GVH[[2]])

[1] 1876

write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt4
iITx/Pt4 CD4 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt4
iITx/Pt4 CD8 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

dCD4mappable=rownames(x[(x[,17]+x[,16])>0,])

dCD8mappable=rownames(x[(x[,19]+x[,18])>0,])

CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])

CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])

write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt4
iITx/Pt4 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt4
iITx/Pt4 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)

[1] 128275

length(dCD8mappable)

[1] 59438

length(CD4nonGVH)

[1] 119062

length(CD8nonGVH)

[1] 57562

dmappable=rownames(x[(x[,17]+x[,16]+x[,19]+x[,18])>0,])

length(dmappable)

[1] 187713

write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt4 iITx/Pt4 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)+length(dCD8mappable)

[1] 187713

length(intersect(dCD4mappable,dCD8mappable))

[1] 0

#Pt4 POD13 Bx GVH

cd4= x[,c(17,16,4)]

cd8= x[,c(19,18,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt4 iITx/Pt4 07032019 2x.out')

rownames(x)=x[,1]

cd4= x[,c(17,16)]

cd8= x[,c(19,18)]

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))




#Pt4 POD13 Bx HVG

cd4= x[,c(10,13,4)]

cd8= x[,c(11,15,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt4 iITx/Pt4 07032019 2x.out' )




rownames(x)=x[,1]

cd4= x[,c(10,13)]

cd8= x[,c(11,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))




#Pt4 POD524 ileum Bx GVH

cd4= x[,c(17,16,5)]

cd8= x[,c(19,18,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt4 iITx/Pt4 07032019 2x.out' )




rownames(x)=x[,1]

cd4= x[,c(17,16)]

cd8= x[,c(19,18)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)
```

```
intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))




#Pt4 POD524 ileum Bx HVG

cd4= x[,c(10,13,5)]

cd8= x[,c(11,15,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt4 iITx/Pt4 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(10,13)]

cd8= x[,c(11,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))




#Pt4 POD674 ileum Bx GVH

cd4= x[,c(17,16,7)]

cd8= x[,c(19,18,7)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt4 iITx/Pt4 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,16)]

cd8= x[,c(19,18)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,7]>0,]))
```

```
intersect(allo[[2]], rownames(x[x[,7]>0,]))




#Pt4 POD674 ileum Bx HVG

cd4= x[,c(10,13,7)]

cd8= x[,c(11,15,7)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt4 iITx/Pt4 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(10,13)]

cd8= x[,c(11,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,7]>0,]))

intersect(allo[[2]], rownames(x[x[,7]>0,]))




#Pt4 POD65 PBMC GVH

cd4= x[,c(17,16,21)]

cd8= x[,c(19,18,21)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt4 iITx/Pt4 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,16)]

cd8= x[,c(19,18)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,21]>0,]))

intersect(allo[[2]], rownames(x[x[,21]>0,]))
```

```
#Pt4 POD65 PBMC HVG

cd4= x[,c(10,13,21)]

cd8= x[,c(11,15,21)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt4 iITx/Pt4 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(10,13)]

cd8= x[,c(11,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,21]>0,]))

intersect(allo[[2]], rownames(x[x[,21]>0,]))




#Pt4 POD324 PBMC GVH

cd4= x[,c(17,16,20)]

cd8= x[,c(19,18,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt4 iITx/Pt4 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,16)]

cd8= x[,c(19,18)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))
```

```
#Pt4 POD324 PBMC HVG

cd4= x[,c(10,13,20)]

cd8= x[,c(11,15,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt4 iITx/Pt4 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(10,13)]

cd8= x[,c(11,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))




#Pt4 POD541 PBMC GVH

cd4= x[,c(17,16,22)]

cd8= x[,c(19,18,22)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt4 iITx/Pt4 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,16)]

cd8= x[,c(19,18)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))
```

```
#Pt4 POD541 PBMC HVG

cd4= x[,c(10,13,22)]

cd8= x[,c(11,15,22)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt4 iITx/Pt4 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(10,13)]

cd8= x[,c(11,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))
```

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt7 MVTx/Pt7 07052019.tsv")

source('Z:/Jianing/10x scRNA-seq TCR-seq/Pt15_MJ001, MJ004/alloreactivity_printtofile 09132017.R')

names(data)

[1] "Rearrangement"   "Sum..Templates."   "Present.In"   "Itx.Pt7.PBMC.POD253" "Itx.Pt7.POD101.136.DM"

 [6] "Itx.Pt7.POD101.136.DN" "Itx.Pt7.POD22.50.D"   "Itx.Pt7.POD22.50.R4" "Itx.Pt7.POD22.50.R8"   "Itx.Pt7.POD225.Bx"

[11] "Itx.Pt7.POD24.Bx"   "Itx.Pt7.PreTx.4U"   "Itx.Pt7.PreTx.8U"   "Itx.Pt7.PreTx.D4H" "Itx.Pt7.PreTx.D4L"

[16] "Itx.Pt7.PreTx.D4U"   "Itx.Pt7.PreTx.D8H"   "Itx.Pt7.PreTx.D8L" "Itx.Pt7.PreTx.D8U"   "Itx.Pt7.PreTx.R4H"

[21] "Itx.Pt7.PreTx.R4L"   "Itx.Pt7.PreTx.R8H"   "Itx.Pt7.PreTx.R8L"

Sample name (Pt: patient)	updated rename
Itx-Pt7-PreTx-D4L	Pt07_Pre_Donor_CD4_CFSElo
Itx-Pt7-PreTx-D4U	Pt07_Pre_Donor_CD4_unstim
Itx-Pt7-PreTx-D8L	Pt07_Pre_Donor_CD8_CFSElo
Itx-Pt7-PreTx-D8U	Pt07_Pre_Donor_CD8_unstim
Itx-Pt7-POD24-Bx	Pt07_Post_ileum_day0024
Itx-Pt7-POD225-Bx	Pt07_Post_ileum_day0255
Itx-Pt7-PBMC-POD253	Pt07_Post_PBMC_day0253
Itx-Pt7-PreTx-R4L	Pt07_Pre_Recipient_CD4_CFSElo
Itx-Pt7-PreTx-4U	Pt07_Pre_Recipient_CD4_unstim
Itx-Pt7-PreTx-R8L	Pt07_Pre_Recipient_CD8_CFSElo
Itx-Pt7-PreTx-8U	Pt07_Pre_Recipient_CD8_unstim

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

normalize(data[,c(16,19)])

ratio=5

  c1indices=which(data[,16]>0 & data[,19]>0 & data[,16]>ratio*data[,19])

  c2indices=which(data[,16]>0 & data[,19]>0 & data[,19]>ratio*data[,16])

  ambiindices=which(data[,16]>0 & data[,19]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,19]=0

  data[c2indices,16]=0

  data[ambiindices,c(16,19)]=0

#D4L vs D8L

normalize(data[,c(15,18)])

ratio=5

  c1indices=which(data[,15]>0 & data[,18]>0 & data[,15]>ratio*data[,18])

  c2indices=which(data[,15]>0 & data[,18]>0 & data[,18]>ratio*data[,15])

  ambiindices=which(data[,15]>0 & data[,18]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,18]=0

  data[c2indices,15]=0

  data[ambiindices,c(15,18)]=0

#R4U vs R8U

normalize(data[,c(12,13)])

```
ratio=5

  c1indices=which(data[,12]>0 & data[,13]>0 & data[,12]>ratio*data[,13])

  c2indices=which(data[,12]>0 & data[,13]>0 & data[,13]>ratio*data[,12])

  ambiindices=which(data[,12]>0 & data[,13]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,13]=0

  data[c2indices,12]=0

  data[ambiindices,c(12,13)]=0



#R4L vs R8L

normalize(data[,c(21,23)])

ratio=5

  c1indices=which(data[,21]>0 & data[,23]>0 & data[,21]>ratio*data[,23])

  c2indices=which(data[,21]>0 & data[,23]>0 & data[,23]>ratio*data[,21])

  ambiindices=which(data[,21]>0 & data[,23]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,23]=0

  data[c2indices,21]=0

  data[ambiindices,c(21,23)]=0



rownames(data)=data[,1]

x=data



#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,16]>0 | x[,19]>0)&(x[,12]>0 | x[,13]>0))

ambiguous=union(ambiguous,which((x[,16]>0 | x[,19]>0)&(x[,21]>0 | x[,23]>0)))
```

ambiguous=union(ambiguous,which((x[,12]>0 | x[,13]>0)&(x[,15]>0 | x[,18]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]

#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L, R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional step of optimization for CD4 and CD8 sorting error.

#R4L vs R8U

normalize(x[,c(21,13)])

ratio=2

  c1indices=which(x[,21]>0 & x[,13]>0 & x[,21]>ratio*x[,13])

  c2indices=which(x[,21]>0 & x[,13]>0 & x[,13]>ratio*x[,21])

  ambiindices=which(x[,21]>0 & x[,13]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,13]=0

  x[c2indices,21]=0

  x[ambiindices,c(21,13)]=0

#R8L vs R4U

normalize(x[,c(23,12)])

ratio=2

  c1indices=which(x[,23]>0 & x[,12]>0 & x[,23]>ratio*x[,12])

  c2indices=which(x[,23]>0 & x[,12]>0 & x[,12]>ratio*x[,23])

  ambiindices=which(x[,23]>0 & x[,12]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,12]=0

```
  x[c2indices,23]=0

  x[ambiindices,c(23,12)]=0



#D4L vs D8U

normalize(x[,c(15,19)])

ratio=2

  c1indices=which(x[,15]>0 & x[,19]>0 & x[,15]>ratio*x[,19])

  c2indices=which(x[,15]>0 & x[,19]>0 & x[,19]>ratio*x[,15])

  ambiindices=which(x[,15]>0 & x[,19]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,19]=0

  x[c2indices,15]=0

  x[ambiindices,c(15,19)]=0



#D8L vs D4U

normalize(x[,c(18,16)])

ratio=2

  c1indices=which(x[,18]>0 & x[,16]>0 & x[,18]>ratio*x[,16])

  c2indices=which(x[,18]>0 & x[,16]>0 & x[,16]>ratio*x[,18])

  ambiindices=which(x[,18]>0 & x[,16]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,16]=0

  x[c2indices,18]=0

  x[ambiindices,c(18,16)]=0
```

write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt7 MVTx/Pt7 07052019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T, sep="\t")

add "fold=2" below, use 2 fold expansion (default 5 fold)

add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for read counts

#HVG direction

For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]

For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]

cd4.HVG= x[,c(12,21)]

cd8.HVG= x[,c(13,23)]

allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00002)

length(allo.HVG[[1]])

[1] 13324

length(allo.HVG[[2]])

[1] 2851

write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt7 MVTx/Pt7 CD4 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt7 MVTx/Pt7 CD8 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,12]+x[,21])>0,])

rCD8mappable=rownames(x[(x[,13]+x[,23])>0,])

CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])

write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt7 MVTx/Pt7 CD4nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt7 MVTx/Pt7 CD8nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 101761

length(rCD8mappable)

[1] 41326

length(CD4nonHVG)

[1] 88437

length(CD8nonHVG)

[1] 38475

rmappable=rownames(x[(x[,12]+x[,21]+x[,13]+x[,23])>0,])

length(rmappable)

[1] 143087

write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt7 MVTx/Pt7 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)+length(rCD8mappable)

[1] 143087

length(intersect(rCD4mappable,rCD8mappable))

[1] 0

```
#GVH direction
# For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]
# For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]
cd4.GVH= x[,c(16,15)]
cd8.GVH= x[,c(19,18)]
allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00002)


length(allo.GVH[[1]])
[1] 9145
length(allo.GVH[[2]])
[1] 3534


write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt7
MVTx/Pt7 CD4 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt7
MVTx/Pt7 CD8 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")



dCD4mappable=rownames(x[(x[,16]+x[,15])>0,])
dCD8mappable=rownames(x[(x[,19]+x[,18])>0,])


CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])
CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])


write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt7
MVTx/Pt7 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt7
MVTx/Pt7 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")
```

length(dCD4mappable)

[1] 105621

length(dCD8mappable)

[1] 38058

length(CD4nonGVH)

[1] 96476

length(CD8nonGVH)

[1] 34524

dmappable=rownames(x[(x[,16]+x[,19]+x[,15]+x[,18])>0,])

length(dmappable)

[1] 143679

write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt7 MVTx/Pt7 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)+length(dCD8mappable)

[1] 143679

length(intersect(dCD4mappable,dCD8mappable))

[1] 0

#Pt7 POD24 Bx GVH

cd4= x[,c(16,15,11)]

cd8= x[,c(19,18,11)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt7 MVTx/Pt7 07052019 2x.out')

```
rownames(x)=x[,1]

cd4= x[,c(16,15)]

cd8= x[,c(19,18)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,11]>0,]))

intersect(allo[[2]], rownames(x[x[,11]>0,]))



#Pt7 POD24 Bx HVG

cd4= x[,c(12,21,11)]

cd8= x[,c(13,23,11)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt7 MVTx/Pt7 07052019 2x.out' )



rownames(x)=x[,1]

cd4= x[,c(12,21)]

cd8= x[,c(13,23)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,11]>0,]))

intersect(allo[[2]], rownames(x[x[,11]>0,]))



#Pt7 POD225 Bx GVH

cd4= x[,c(16,15,10)]

cd8= x[,c(19,18,10)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt7 MVTx/Pt7 07052019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(16,15)]

cd8= x[,c(19,18)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))




#Pt7 POD225 Bx HVG

cd4= x[,c(12,21,10)]

cd8= x[,c(13,23,10)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt7 MVTx/Pt7 07052019 2x.out' )




rownames(x)=x[,1]

cd4= x[,c(12,21)]

cd8= x[,c(13,23)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))




#Pt7 POD253 PBMC GVH

cd4= x[,c(16,15,4)]

cd8= x[,c(19,18,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt7 MVTx/Pt7 07052019 2x.out' )




rownames(x)=x[,1]

cd4= x[,c(16,15)]
```

```
cd8= x[,c(19,18)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))



#Pt7 POD253 PBMC HVG

cd4= x[,c(12,21,4)]

cd8= x[,c(13,23,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt7 MVTx/Pt7 07052019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(12,21)]

cd8= x[,c(13,23)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))
```

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt9 iITx/Pt9 07032019.tsv")

source('Z:/Jianing/10x scRNA-seq TCR-seq/Pt15_MJ001, MJ004/alloreactivity_printtofile 09132017.R')

names(data)

 [1] "Rearrangement"   "Sum..Templates."   "Present.In"   "Itx.Pt9.POD20.Bx"
"Itx.Pt9.POD254.Bx"

 [6] "Itx.Pt9.PreTx.4U"   "Itx.Pt9.PreTx.8U"   "Itx.Pt9.PreTx.D4L" "Itx.Pt9.PreTx.D4U"
"Itx.Pt9.PreTx.D8L"

[11] "Itx.Pt9.PreTx.D8U"   "Itx.Pt9.PreTx.R4H"   "Itx.Pt9.PreTx.R4L" "Itx.Pt9.PreTx.R8H"
"Itx.Pt9.PreTx.R8L"

[16] "Itx_Pt9_PBMC_POD156" "Itx_Pt9_PBMC_POD66"

Sample name (Pt: patient)	updated rename
Itx-Pt9-PreTx-D4L	Pt09_Pre_Donor_CD4_CFSElo
Itx-Pt9-PreTx-D4U	Pt09_Pre_Donor_CD4_unstim
Itx-Pt9-PreTx-D8L	Pt09_Pre_Donor_CD8_CFSElo
Itx-Pt9-PreTx-D8U	Pt09_Pre_Donor_CD8_unstim
Itx-Pt9-POD20-Bx	Pt09_Post_ileum_day0020
Itx-Pt9-POD254-Bx	Pt09_Post_ileum_day0254
Itx_Pt9_PBMC_POD66	Pt09_Post_PBMC_day0066
Itx_Pt9_PBMC_POD156	Pt09_Post_PBMC_day0156
Itx-Pt9-PreTx-R4L	Pt09_Pre_Recipient_CD4_CFSElo
Itx-Pt9-PreTx-4U(R)	Pt09_Pre_Recipient_CD4_unstim
Itx-Pt9-PreTx-R8L	Pt09_Pre_Recipient_CD8_CFSElo
Itx-Pt9-PreTx-8U(R)	Pt09_Pre_Recipient_CD8_unstim

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

normalize(data[,c(9,11)])

ratio=5

  c1indices=which(data[,9]>0 & data[,11]>0 & data[,9]>ratio*data[,11])

  c2indices=which(data[,9]>0 & data[,11]>0 & data[,11]>ratio*data[,9])

  ambiindices=which(data[,9]>0 & data[,11]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,11]=0

  data[c2indices,9]=0

  data[ambiindices,c(9,11)]=0

#D4L vs D8L

normalize(data[,c(8,10)])

ratio=5

  c1indices=which(data[,8]>0 & data[,10]>0 & data[,8]>ratio*data[,10])

  c2indices=which(data[,8]>0 & data[,10]>0 & data[,10]>ratio*data[,8])

  ambiindices=which(data[,8]>0 & data[,10]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,10]=0

  data[c2indices,8]=0

  data[ambiindices,c(8,10)]=0

#R4U vs R8U

normalize(data[,c(6,7)])

```
ratio=5

 c1indices=which(data[,6]>0 & data[,7]>0 & data[,6]>ratio*data[,7])

 c2indices=which(data[,6]>0 & data[,7]>0 & data[,7]>ratio*data[,6])

 ambiindices=which(data[,6]>0 & data[,7]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,7]=0

 data[c2indices,6]=0

 data[ambiindices,c(6,7)]=0



#R4L vs R8L

normalize(data[,c(13,15)])

ratio=5

 c1indices=which(data[,13]>0 & data[,15]>0 & data[,13]>ratio*data[,15])

 c2indices=which(data[,13]>0 & data[,15]>0 & data[,15]>ratio*data[,13])

 ambiindices=which(data[,13]>0 & data[,15]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,15]=0

 data[c2indices,13]=0

 data[ambiindices,c(13,15)]=0



rownames(data)=data[,1]

x=data



#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,9]>0 | x[,11]>0)&(x[,6]>0 | x[,7]>0))

ambiguous=union(ambiguous,which((x[,9]>0 | x[,11]>0)&(x[,13]>0 | x[,15]>0)))

ambiguous=union(ambiguous,which((x[,6]>0 | x[,7]>0)&(x[,8]>0 | x[,10]>0)))
```

```
x=x[setdiff(1:nrow(x),ambiguous),]
```

#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L, R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional step of optimization for CD4 and CD8 sorting error.

```
#R4L vs R8U

normalize(x[,c(13,7)])

ratio=2

  c1indices=which(x[,13]>0 & x[,7]>0 & x[,13]>ratio*x[,7])

  c2indices=which(x[,13]>0 & x[,7]>0 & x[,7]>ratio*x[,13])

  ambiindices=which(x[,13]>0 & x[,7]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,7]=0

  x[c2indices,13]=0

  x[ambiindices,c(13,7)]=0
```

```
#R8L vs R4U

normalize(x[,c(15,6)])

ratio=2

  c1indices=which(x[,15]>0 & x[,6]>0 & x[,15]>ratio*x[,6])

  c2indices=which(x[,15]>0 & x[,6]>0 & x[,6]>ratio*x[,15])

  ambiindices=which(x[,15]>0 & x[,6]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,6]=0

  x[c2indices,15]=0

  x[ambiindices,c(15,6)]=0
```

```
#D4L vs D8U
normalize(x[,c(8,11)])
ratio=2
 c1indices=which(x[,8]>0 & x[,11]>0 & x[,8]>ratio*x[,11])
 c2indices=which(x[,8]>0 & x[,11]>0 & x[,11]>ratio*x[,8])
 ambiindices=which(x[,8]>0 & x[,11]>0)
 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
 x[c1indices,11]=0
 x[c2indices,8]=0
 x[ambiindices,c(8,11)]=0


#D8L vs D4U
normalize(x[,c(10,9)])
ratio=2
 c1indices=which(x[,10]>0 & x[,9]>0 & x[,10]>ratio*x[,9])
 c2indices=which(x[,10]>0 & x[,9]>0 & x[,9]>ratio*x[,10])
 ambiindices=which(x[,10]>0 & x[,9]>0)
 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
 x[c1indices,9]=0
 x[c2indices,10]=0
 x[ambiindices,c(10,9)]=0


write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt9 iITx/Pt9
07032019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T, sep="\t")
```

add "fold=2" below, use 2 fold expansion (default 5 fold)

add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for read counts

#HVG direction

For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]

For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]

cd4.HVG= x[,c(6,13)]

cd8.HVG= x[,c(7,15)]

allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00002)

length(allo.HVG[[1]])

[1] 14629

length(allo.HVG[[2]])

[1] 8569

write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt9 iITx/Pt9 CD4 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt9 iITx/Pt9 CD8 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,6]+x[,13])>0,])

rCD8mappable=rownames(x[(x[,7]+x[,15])>0,])

CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])

write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt9 iITx/Pt9 CD4nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt9 iITx/Pt9 CD8nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 197313

length(rCD8mappable)

[1] 159929

length(CD4nonHVG)

[1] 182684

length(CD8nonHVG)

[1] 151360

rmappable=rownames(x[(x[,6]+x[,13]+x[,7]+x[,15])>0,])

length(rmappable)

[1] 357242

write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt9 iITx/Pt9 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)+length(rCD8mappable)

[1] 357242

length(intersect(rCD4mappable,rCD8mappable))

[1] 0

#GVH direction

For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]

For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]

cd4.GVH= x[,c(9,8)]

cd8.GVH= x[,c(11,10)]

allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00002)

length(allo.GVH[[1]])

[1] 12157

length(allo.GVH[[2]])

[1] 3325

write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt9
iITx/Pt9 CD4 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt9
iITx/Pt9 CD8 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

dCD4mappable=rownames(x[(x[,9]+x[,8])>0,])

dCD8mappable=rownames(x[(x[,11]+x[,10])>0,])

CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])

CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])

write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt9
iITx/Pt9 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt9
iITx/Pt9 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)

[1] 182943

length(dCD8mappable)

[1] 142122

length(CD4nonGVH)

[1] 170786

length(CD8nonGVH)

[1] 138797

dmappable=rownames(x[(x[,9]+x[,8]+x[,11]+x[,10])>0,])

length(dmappable)

[1] 325065

write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt9
iITx/Pt9 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)+length(dCD8mappable)

[1] 325065

length(intersect(dCD4mappable,dCD8mappable))

[1] 0

#Pt9 POD20 Bx GVH

cd4= x[,c(9,8,4)]

cd8= x[,c(11,10,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt9 iITx/Pt9 07032019 2x.out')

rownames(x)=x[,1]

cd4= x[,c(9,8)]

cd8= x[,c(11,10)]

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))


#Pt9 POD20 Bx HVG

cd4= x[,c(6,13,4)]

cd8= x[,c(7,15,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt9 iITx/Pt9 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(6,13)]

cd8= x[,c(7,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))


#Pt9 POD254 Bx GVH

cd4= x[,c(9,8,5)]

cd8= x[,c(11,10,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt9 iITx/Pt9 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,8)]

cd8= x[,c(11,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))
```

```
#Pt9 POD254 Bx HVG

cd4= x[,c(6,13,5)]

cd8= x[,c(7,15,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt9 iITx/Pt9 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(6,13)]

cd8= x[,c(7,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt9 POD66 PBMC GVH

cd4= x[,c(9,8,17)]

cd8= x[,c(11,10,17)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt9 iITx/Pt9 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,8)]

cd8= x[,c(11,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))


#Pt9 POD66 PBMC  HVG

cd4= x[,c(6,13,17)]

cd8= x[,c(7,15,17)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt9 iITx/Pt9 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(6,13)]

cd8= x[,c(7,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))



#Pt9 POD156 PBMC GVH

cd4= x[,c(9,8,16)]

cd8= x[,c(11,10,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt9 iITx/Pt9 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,8)]

cd8= x[,c(11,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))



#Pt9 POD156 PBMC  HVG

cd4= x[,c(6,13,16)]

cd8= x[,c(7,15,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt9 iITx/Pt9 07032019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(6,13)]

cd8= x[,c(7,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))
```

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt10 MVTx/Pt10 07032019.tsv")

source('Z:/Jianing/10x scRNA-seq TCR-seq/Pt15_MJ001, MJ004/alloreactivity_printtofile 09132017.R')

names(data)

 [1] "Rearrangement"   "Sum..Templates."   "Present.In"   "Itx.Pt10.Bx.POD338" "Itx.Pt10.Bx_POD17"

 [6] "Itx.Pt10.Bx_POD204" "Itx.Pt10.D4L"   "Itx.Pt10.D4U.MLN"   "Itx.Pt10.D4U.spleen" "Itx.Pt10.D8L"

[11] "Itx.Pt10.D8U.MLN"   "Itx.Pt10.D8U.spleen" "Itx.Pt10.R4L"   "Itx.Pt10.R4U.spleen" "Itx.Pt10.R8L"

[16] "Itx.Pt10.R8U.spleen" "Itx_Pt10_PBMC_POD118" "Pt10MVTx_PBMC_POD8"

Sample name (Pt: patient)	updated rename
Itx-Pt10-D4L	Pt10_Pre_Donor_CD4_CFSElo
Itx-Pt10-D4U-spleen	Pt10_Pre_Donor_CD4_unstim
Itx-Pt10-D8L	Pt10_Pre_Donor_CD8_CFSElo
Itx-Pt10-D8U-spleen	Pt10_Pre_Donor_CD8_unstim
Itx-Pt10-Bx_POD17	Pt10_Post_ileum_day0017
Itx-Pt10-Bx_POD204	Pt10_Post_ileum_day0204
Itx-Pt10-Bx-POD338	Pt10_Post_ileum_day0338
Itx-Pt10-PBMCs-POD8	Pt10_Post_PBMC_day0008
Itx_Pt10_PBMC_POD118	Pt10_Post_PBMC_day0118
Itx-Pt10-R4L	Pt10_Pre_Recipient_CD4_CFSElo
Itx-Pt10-R4U-spleen	Pt10_Pre_Recipient_CD4_unstim
Itx-Pt10-R8U-spleen	Pt10_Pre_Recipient_CD8_unstim
Itx-Pt10-R8L	Pt10_Pre_Recipient_CD8_CFSElo

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

normalize(data[,c(9,12)])

ratio=5

  c1indices=which(data[,9]>0 & data[,12]>0 & data[,9]>ratio*data[,12])

  c2indices=which(data[,9]>0 & data[,12]>0 & data[,12]>ratio*data[,9])

  ambiindices=which(data[,9]>0 & data[,12]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,12]=0

  data[c2indices,9]=0

  data[ambiindices,c(9,12)]=0

#D4L vs D8L

normalize(data[,c(7,10)])

ratio=5

  c1indices=which(data[,7]>0 & data[,10]>0 & data[,7]>ratio*data[,10])

  c2indices=which(data[,7]>0 & data[,10]>0 & data[,10]>ratio*data[,7])

  ambiindices=which(data[,7]>0 & data[,10]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,10]=0

  data[c2indices,7]=0

  data[ambiindices,c(7,10)]=0

#R4U vs R8U

normalize(data[,c(14,16)])

```
ratio=5

  c1indices=which(data[,14]>0 & data[,16]>0 & data[,14]>ratio*data[,16])

  c2indices=which(data[,14]>0 & data[,16]>0 & data[,16]>ratio*data[,14])

  ambiindices=which(data[,14]>0 & data[,16]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,16]=0

  data[c2indices,14]=0

  data[ambiindices,c(14,16)]=0



#R4L vs R8L

normalize(data[,c(13,15)])

ratio=5

  c1indices=which(data[,13]>0 & data[,15]>0 & data[,13]>ratio*data[,15])

  c2indices=which(data[,13]>0 & data[,15]>0 & data[,15]>ratio*data[,13])

  ambiindices=which(data[,13]>0 & data[,15]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,15]=0

  data[c2indices,13]=0

  data[ambiindices,c(13,15)]=0



rownames(data)=data[,1]

x=data



#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by CFSE-MLR sorting

ambiguous=which((x[,9]>0 | x[,12]>0)&(x[,14]>0 | x[,16]>0))

ambiguous=union(ambiguous,which((x[,9]>0 | x[,12]>0)&(x[,13]>0 | x[,15]>0)))

ambiguous=union(ambiguous,which((x[,14]>0 | x[,16]>0)&(x[,7]>0 | x[,10]>0)))
```

```
x=x[setdiff(1:nrow(x),ambiguous),]
```

#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L, R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional step of optimization for CD4 and CD8 sorting error.

```
#R4L vs R8U

normalize(x[,c(13,16)])

ratio=2

  c1indices=which(x[,13]>0 & x[,16]>0 & x[,13]>ratio*x[,16])

  c2indices=which(x[,13]>0 & x[,16]>0 & x[,16]>ratio*x[,13])

  ambiindices=which(x[,13]>0 & x[,16]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,16]=0

  x[c2indices,13]=0

  x[ambiindices,c(13,16)]=0


#R8L vs R4U

normalize(x[,c(15,14)])

ratio=2

  c1indices=which(x[,15]>0 & x[,14]>0 & x[,15]>ratio*x[,14])

  c2indices=which(x[,15]>0 & x[,14]>0 & x[,14]>ratio*x[,15])

  ambiindices=which(x[,15]>0 & x[,14]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,14]=0

  x[c2indices,15]=0

  x[ambiindices,c(15,14)]=0
```

```
#D4L vs D8U

normalize(x[,c(7,12)])

ratio=2

 c1indices=which(x[,7]>0 & x[,12]>0 & x[,7]>ratio*x[,12])

 c2indices=which(x[,7]>0 & x[,12]>0 & x[,12]>ratio*x[,7])

 ambiindices=which(x[,7]>0 & x[,12]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,12]=0

 x[c2indices,7]=0

 x[ambiindices,c(7,12)]=0




#D8L vs D4U

normalize(x[,c(10,9)])

ratio=2

 c1indices=which(x[,10]>0 & x[,9]>0 & x[,10]>ratio*x[,9])

 c2indices=which(x[,10]>0 & x[,9]>0 & x[,9]>ratio*x[,10])

 ambiindices=which(x[,10]>0 & x[,9]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,9]=0

 x[c2indices,10]=0

 x[ambiindices,c(10,9)]=0


write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt10
MVTx/Pt10 07032019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T,
sep="\t")
```

add "fold=2" below, use 2 fold expansion (default 5 fold)

add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for read counts

#HVG direction

For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]

For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]

cd4.HVG= x[,c(14,13)]

cd8.HVG= x[,c(16,15)]

allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00002)

#When you define allo=listAlloreactive(cd4,cd8),then you should get cd4 alloreactives stored in allo[[1]] and cd8 alloreactives stored in allo[[2]]. If you do allo=union(allo[[1]],allo[[2]]), then allo contains all the alloreactives, and you can define nonallo=setdiff(rownames(x),allo)

length(allo.HVG[[1]])

[1] 9350

length(allo.HVG[[2]])

[1] 5134

write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt10 MVTx/Pt10 CD4 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt10 MVTx/Pt10 CD8 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,14]+x[,13])>0,])

rCD8mappable=rownames(x[(x[,16]+x[,15])>0,])

CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])

write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt10 MVTx/Pt10 CD4nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt10 MVTx/Pt10 CD8nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 154536

length(rCD8mappable)

[1] 54173

length(CD4nonHVG)

[1] 145186

length(CD8nonHVG)

[1] 49039

rmappable=rownames(x[(x[,14]+x[,13]+x[,16]+x[,15])>0,])

length(rmappable)

[1] 208709

write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt10 MVTx/Pt10 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)+length(rCD8mappable)

[1] 208709

length(intersect(rCD4mappable,rCD8mappable))

[1] 0

#GVH direction

For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]

For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]

cd4.GVH= x[,c(9,7)]

cd8.GVH= x[,c(12,10)]

allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00002)

length(allo.GVH[[1]])

[1] 4355

length(allo.GVH[[2]])

[1] 5929

write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt10 MVTx/Pt10 CD4 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt10 MVTx/Pt10 CD8 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

dCD4mappable=rownames(x[(x[,9]+x[,7])>0,])

dCD8mappable=rownames(x[(x[,12]+x[,10])>0,])

CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])

CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])

write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt10 MVTx/Pt10 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt10 MVTx/Pt10 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)

[1] 87128

length(dCD8mappable)

[1] 106737

length(CD4nonGVH)

[1] 184042

length(CD8nonGVH)

[1] 4589

dmappable=rownames(x[(x[,9]+x[,7]+x[,12]+x[,10])>0,])

length(dmappable)

[1] 193865

write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt10 MVTx/Pt10 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)+length(dCD8mappable)

[1] 193865

length(intersect(dCD4mappable,dCD8mappable))

[1] 0

#Pt10 POD17 Bx GVH

cd4= x[,c(9,7,5)]

cd8= x[,c(12,10,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt10 MVTx/Pt10 07032019 2x.out')

```
rownames(x)=x[,1]

cd4= x[,c(9,7)]

cd8= x[,c(12,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt10 POD17 Bx HVG

cd4= x[,c(14,13,5)]

cd8= x[,c(16,15,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt10 MVTx/Pt10 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(14,13)]

cd8= x[,c(16,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt10 POD204 Bx GVH

cd4= x[,c(9,7,6)]

cd8= x[,c(12,10,6)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt10 MVTx/Pt10 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,7)]
```

```
cd8= x[,c(12,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,6]>0,]))

intersect(allo[[2]], rownames(x[x[,6]>0,]))


#Pt10 POD204 Bx HVG

cd4= x[,c(14,13,6)]

cd8= x[,c(16,15,6)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt10 MVTx/Pt10 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(14,13)]

cd8= x[,c(16,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,6]>0,]))

intersect(allo[[2]], rownames(x[x[,6]>0,]))



#Pt10 POD338 Bx GVH

cd4= x[,c(9,7,4)]

cd8= x[,c(12,10,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt10 MVTx/Pt10 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,7)]

cd8= x[,c(12,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)
```

```
intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))


#Pt10 POD338 Bx HVG

cd4= x[,c(14,13,4)]

cd8= x[,c(16,15,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt10 MVTx/Pt10 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(14,13)]

cd8= x[,c(16,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))


#Pt10 POD8 PBMC GVH

cd4= x[,c(9,7,18)]

cd8= x[,c(12,10,18)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt10 MVTx/Pt10 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,7)]

cd8= x[,c(12,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,18]>0,]))

intersect(allo[[2]], rownames(x[x[,18]>0,]))
```

```
#Pt10 POD8 PBMC HVG

cd4= x[,c(14,13,18)]

cd8= x[,c(16,15,18)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt10 MVTx/Pt10 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(14,13)]

cd8= x[,c(16,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,18]>0,]))

intersect(allo[[2]], rownames(x[x[,18]>0,]))


#Pt10 POD118 PBMC GVH

cd4= x[,c(9,7,17)]

cd8= x[,c(12,10,17)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt10 MVTx/Pt10 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,7)]

cd8= x[,c(12,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))


#Pt10 POD118 PBMC HVG

cd4= x[,c(14,13,17)]
```

```
cd8= x[,c(16,15,17)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt10 MVTx/Pt10 07032019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(14,13)]

cd8= x[,c(16,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))
```

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt13 iITx/Pt13 06112019.tsv")

source('Z:/Jianing/10x scRNA-seq TCR-seq/Pt15_MJ001, MJ004/alloreactivity_printtofile 09132017.R')

names(data)

 [1] "Rearrangement"   "Sum..Templates."   "Present.In"

 [4] "It.Pt13.MLN.D4U"   "It.Pt13.spleen.D4U"   "Itx.Pt13.D4L"

 [7] "Itx.Pt13.D8L"   "Itx.Pt13.MLN.D8U"   "Itx.Pt13.POD12_Bx"

[10] "Itx.Pt13.POD142_Bx"   "Itx.Pt13.R4L"   "Itx.Pt13.R4U"

[13] "Itx.Pt13.R8L"   "Itx.Pt13.R8U"   "Itx.Pt13.spleen.D8U"

[16] "Itx_Pt13_PBMC_POD453"   "Pt13_iITx_Bx_POD1013_Native_colon" "Pt13_iITx_Bx_POD1013_Tx_colon"

[19] "Pt13_iITx_Bx_POD1013_Tx_ileum"   "Pt13_iITx_Bx_POD1013_Tx_jejunum" "Pt13_iITx_Bx_POD460_Native_colon"

[22] "Pt13_iITx_Bx_POD460_Tx_colon"   "Pt13_iITx_Bx_POD460_Tx_ileum" "Pt13_iITx_POD1032_PBMC.1"

[25] "Pt13_iITx_POD1032_ileum_stoma_IEL.1" "Pt13_iITx_POD1032_ileum_stoma_LPL_T" "Pt13iITx_PBMC_POD41"

Sample name (Pt: patient)	updated rename
Itx-Pt13-D4L	Pt13_Pre_Donor_CD4_CFSElo
It-Pt13-spleen-D4U	Pt13_Pre_Donor_CD4_unstim
Itx-Pt13-D8L	Pt13_Pre_Donor_CD8_CFSElo
Itx-Pt13-spleen-D8U	Pt13_Pre_Donor_CD8_unstim
Itx-Pt13-POD12_Bx	Pt13_Post_ileum_day0012
Itx-Pt13-POD142_Bx	Pt13_Post_ileum_day0142
Pt13_iITx_Bx_POD460_Tx_ileum	Pt13_Post_ileum_day0460
It-Pt13-PBMCs-POD41	Pt13_Post_PBMC_day0041
Itx_Pt13_PBMC_POD453	Pt13_Post_PBMC_day0453
Itx_Pt13_PBMC_POD1032	Pt13_Post_PBMC_day1032
Itx-Pt13-R4L	Pt13_Pre_Recipient_CD4_CFSElo
Itx-Pt13-R4U	Pt13_Pre_Recipient_CD4_unstim
Itx-Pt13-R8L	Pt13_Pre_Recipient_CD8_CFSElo
Itx-Pt13-R8U	Pt13_Pre_Recipient_CD8_unstim

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

normalize(data[,c(5,15)])

ratio=5

  c1indices=which(data[,5]>0 & data[,15]>0 & data[,5]>ratio*data[,15])

  c2indices=which(data[,5]>0 & data[,15]>0 & data[,15]>ratio*data[,5])

  ambiindices=which(data[,5]>0 & data[,15]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,15]=0

  data[c2indices,5]=0

  data[ambiindices,c(5,15)]=0

#D4L vs D8L

normalize(data[,c(6,7)])

ratio=5

  c1indices=which(data[,6]>0 & data[,7]>0 & data[,6]>ratio*data[,7])

  c2indices=which(data[,6]>0 & data[,7]>0 & data[,7]>ratio*data[,6])

  ambiindices=which(data[,6]>0 & data[,7]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,7]=0

  data[c2indices,6]=0

  data[ambiindices,c(6,7)]=0

#R4U vs R8U

normalize(data[,c(12,14)])

```
ratio=5
  c1indices=which(data[,12]>0 & data[,14]>0 & data[,12]>ratio*data[,14])
  c2indices=which(data[,12]>0 & data[,14]>0 & data[,14]>ratio*data[,12])
  ambiindices=which(data[,12]>0 & data[,14]>0)
  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
  data[c1indices,14]=0
  data[c2indices,12]=0
  data[ambiindices,c(12,14)]=0


#R4L vs R8L
normalize(data[,c(11,13)])
ratio=5
  c1indices=which(data[,11]>0 & data[,13]>0 & data[,11]>ratio*data[,13])
  c2indices=which(data[,11]>0 & data[,13]>0 & data[,13]>ratio*data[,11])
  ambiindices=which(data[,11]>0 & data[,13]>0)
  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
  data[c1indices,13]=0
  data[c2indices,11]=0
  data[ambiindices,c(11,13)]=0
```

write.table(data,file ="Z:/Jianing/10x scRNA-seq TCR-seq/Pt13_MJ002, MJ003/Pt13 remove CD4 CD8 ambiguous clones.tsv",quote=F,row.names=F,col.names=T, sep="\t")

write.table(data,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt13 iITx/Pt13 remove CD4 CD8 ambiguous clones.tsv",quote=F,row.names=F,col.names=T, sep="\t")

```
rownames(data)=data[,1]

x=data


#use spleen.D4U and spleen.D8U

#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,5]>0 | x[,15]>0)&(x[,12]>0 | x[,14]>0))

ambiguous=union(ambiguous,which((x[,5]>0 | x[,15]>0)&(x[,11]>0 | x[,13]>0)))

ambiguous=union(ambiguous,which((x[,12]>0 | x[,14]>0)&(x[,6]>0 | x[,7]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]


#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L,
R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional
step of optimization for CD4 and CD8 sorting error.


#R4L vs R8U

normalize(x[,c(11,14)])

ratio=2

  c1indices=which(x[,11]>0 & x[,14]>0 & x[,11]>ratio*x[,14])

  c2indices=which(x[,11]>0 & x[,14]>0 & x[,14]>ratio*x[,11])

  ambiindices=which(x[,11]>0 & x[,14]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,14]=0

  x[c2indices,11]=0

  x[ambiindices,c(11,14)]=0
```

```
#R8L vs R4U

normalize(x[,c(13,12)])

ratio=2

  c1indices=which(x[,13]>0 & x[,12]>0 & x[,13]>ratio*x[,12])

  c2indices=which(x[,13]>0 & x[,12]>0 & x[,12]>ratio*x[,13])

  ambiindices=which(x[,13]>0 & x[,12]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,12]=0

  x[c2indices,13]=0

  x[ambiindices,c(13,12)]=0



#D4L vs D8U

normalize(x[,c(6,15)])

ratio=2

  c1indices=which(x[,6]>0 & x[,15]>0 & x[,6]>ratio*x[,15])

  c2indices=which(x[,6]>0 & x[,15]>0 & x[,15]>ratio*x[,6])

  ambiindices=which(x[,6]>0 & x[,15]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,15]=0

  x[c2indices,6]=0

  x[ambiindices,c(6,15)]=0



#D8L vs D4U

normalize(x[,c(7,5)])

ratio=2

  c1indices=which(x[,7]>0 & x[,5]>0 & x[,7]>ratio*x[,5])
```

```
c2indices=which(x[,7]>0 & x[,5]>0 & x[,5]>ratio*x[,7])

ambiindices=which(x[,7]>0 & x[,5]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

x[c1indices,5]=0

x[c2indices,7]=0

x[ambiindices,c(7,5)]=0
```

write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt13 iITx/Pt13 06112019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T, sep="\t")

add "fold=2" below, use 2 fold expansion (default 5 fold)

add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for read counts

#HVG direction

For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]

For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]

cd4.HVG= x[,c(12,11)]

cd8.HVG= x[,c(14,13)]

allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00002)

length(allo.HVG[[1]])

[1] 18445

length(allo.HVG[[2]])

[1] 8146

write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt13 iITx/Pt13 CD4 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt13 iITx/Pt13 CD8 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,12]+x[,11])>0,])

rCD8mappable=rownames(x[(x[,14]+x[,13])>0,])

CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])

write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt13 iITx/Pt13 CD4nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt13 iITx/Pt13 CD8nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 193548

length(rCD8mappable)

[1] 132361

length(CD4nonHVG)

[1] 175103

length(CD8nonHVG)

[1] 124215

rmappable=rownames(x[(x[,12]+x[,11]+x[,14]+x[,13])>0,])

length(rmappable)

[1] 325909

```
write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt13
iITx/Pt13 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")


length(rCD4mappable)+length(rCD8mappable)

[1] 325909

length(intersect(rCD4mappable,rCD8mappable))

[1] 0


#GVH direction

# For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]

# For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]

cd4.GVH= x[,c(5,6)]

cd8.GVH= x[,c(15,7)]

allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00002)


length(allo.GVH[[1]])

[1] 6735

length(allo.GVH[[2]])

[1] 6891


write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 CD4 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 CD8 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")


dCD4mappable=rownames(x[(x[,5]+x[,6])>0,])

dCD8mappable=rownames(x[(x[,15]+x[,7])>0,])
```

CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])

CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])

write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt13 iITx/Pt13 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt13 iITx/Pt13 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)

[1] 163280

length(dCD8mappable)

[1] 160705

length(CD4nonGVH)

[1] 156545

length(CD8nonGVH)

[1] 153814

dmappable=rownames(x[(x[,5]+x[,6]+x[,15]+x[,7])>0,])

length(dmappable)

[1] 323985

write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt13 iITx/Pt13 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)+length(dCD8mappable)

[1] 323985

```
length(intersect(dCD4mappable,dCD8mappable))

[1] 0


#Pt13 POD41 PBMC GVH

cd4= x[,c(5,6,27)]

cd8= x[,c(15,7,27)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 06112019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(5,6)]

cd8= x[,c(15,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,27]>0,]))

intersect(allo[[2]], rownames(x[x[,27]>0,]))



#Pt13 POD41 PBMC HVG

cd4= x[,c(12,11,27)]

cd8= x[,c(14,13,27)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 06112019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(12,11)]

cd8= x[,c(14,13)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,27]>0,]))
```

```
intersect(allo[[2]], rownames(x[x[,27]>0,]))
```

#Pt13 POD453 PBMC GVH

```
cd4= x[,c(5,6,16)]

cd8= x[,c(15,7,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 06112019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(5,6)]

cd8= x[,c(15,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))
```

#Pt13 POD453 PBMC HVG

```
cd4= x[,c(12,11,16)]

cd8= x[,c(14,13,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 06112019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(12,11)]

cd8= x[,c(14,13)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))
```

```
#Pt13 POD1032 PBMC GVH

cd4= x[,c(5,6,24)]

cd8= x[,c(15,7,24)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 06112019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(5,6)]

cd8= x[,c(15,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,24]>0,]))

intersect(allo[[2]], rownames(x[x[,24]>0,]))




#Pt13 POD1032 PBMC HVG

cd4= x[,c(12,11,24)]

cd8= x[,c(14,13,24)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 06112019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(12,11)]

cd8= x[,c(14,13)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,24]>0,]))

intersect(allo[[2]], rownames(x[x[,24]>0,]))
```

```
#Pt13 POD12 Bx GVH

cd4= x[,c(5,6,9)]

cd8= x[,c(15,7,9)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 06112019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(5,6)]

cd8= x[,c(15,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,9]>0,]))

intersect(allo[[2]], rownames(x[x[,9]>0,]))


#Pt13 POD12 Bx HVG

cd4= x[,c(12,11,9)]

cd8= x[,c(14,13,9)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 06112019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(12,11)]

cd8= x[,c(14,13)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,9]>0,]))

intersect(allo[[2]], rownames(x[x[,9]>0,]))


#Pt13 POD142 Bx GVH

cd4= x[,c(5,6,10)]
```

```
cd8= x[,c(15,7,10)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 06112019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(5,6)]

cd8= x[,c(15,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))


#Pt13 POD142 Bx HVG

cd4= x[,c(12,11,10)]

cd8= x[,c(14,13,10)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 06112019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(12,11)]

cd8= x[,c(14,13)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))


#Pt13 POD460 ileum Bx GVH

cd4= x[,c(5,6,23)]

cd8= x[,c(15,7,23)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 06112019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(5,6)]

cd8= x[,c(15,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,23]>0,]))

intersect(allo[[2]], rownames(x[x[,23]>0,]))


#Pt13 POD460 ileum Bx HVG

cd4= x[,c(12,11,23)]

cd8= x[,c(14,13,23)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt13 iITx/Pt13 06112019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(12,11)]

cd8= x[,c(14,13)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,23]>0,]))

intersect(allo[[2]], rownames(x[x[,23]>0,]))
```

#Pt14

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt14 iITx/Pt14 10242019.tsv")

source('Z:/Jianing/10x scRNA-seq TCR-seq/Pt15_MJ001, MJ004/alloreactivity_printtofile 09132017.R')

names(data)

[1] "Rearrangement"   "Sum..Templates."   "Present.In"

 [4] "Itx.Pt14.Bx_POD09"   "Itx.Pt14.Bx_POD156"   "Itx.Pt14.D4L"

 [7] "Itx.Pt14.D4U.spleen"   "Itx.Pt14.D8L"   "Itx.Pt14.D8U.spleen"

[10] "Itx.Pt14.R4L"   "Itx.Pt14.R4U"   "Itx.Pt14.R8L"

[13] "Itx.Pt14.R8U"   "Itx.Pt14Bx.POD16"   "Itx.Pt14Bx.POD226"

[16] "Itx_Pt14_PBMC_POD30"   "Itx_Pt14_PBMC_POD456"   "Pt14_iITx_Bx_POD527_Native_colon"

[19] "Pt14_iITx_Bx_POD527_Tx_colon"   "Pt14_iITx_Bx_POD527_Tx_ileum"   "Pt14_iITx_Bx_POD717_Native_colon"

[22] "Pt14_iITx_Bx_POD717_Tx_colon"   "Pt14_iITx_Bx_POD717_Tx_ileum"   "Pt14_iITx_POD1764_PBMC_T"

[25] "Pt14_iITx_POD1764_Tx_colon_Bx_T"   "Pt14_iITx_POD1764_ileum_Bx_Chronic_Rej" "Pt14_iITx_POD1764_native_colon_Bx_T"

Sample name (Pt: patient)	updated rename
Itx-Pt14-D4L	Pt14_Pre_Donor_CD4_CFSElo
Itx-Pt14-D4U-spleen	Pt14_Pre_Donor_CD4_unstim
Itx-Pt14-D8L	Pt14_Pre_Donor_CD8_CFSElo
Itx-Pt14-D8U-spleen	Pt14_Pre_Donor_CD8_unstim
Itx-Pt14-Bx_POD09	Pt14_Post_ileum_day0009
Itx-Pt14-Bx_POD16	Pt14_Post_ileum_day0016
Itx-Pt14-Bx_POD156	Pt14_Post_ileum_day0156
Itx-Pt14Bx-POD226	Pt14_Post_ileum_day0226
Pt14_iITx_Bx_POD457_Tx_ileum	Pt14_Post_ileum_day0457
Pt14_iITx_Bx_POD527_Tx_ileum	Pt14_Post_ileum_day0527
Pt14_iITx_Bx_POD717_Tx_ileum	Pt14_Post_ileum_day0717
Itx_Pt14_PBMC_POD30	Pt14_Post_PBMC_day0030

Itx_Pt14_PBMC_POD456	Pt14_Post_PBMC_day0456
Itx-Pt14-R4L	Pt14_Pre_Recipient_CD4_CFSElo
Itx-Pt14-R4U	Pt14_Pre_Recipient_CD4_unstim
Itx-Pt14-R8L	Pt14_Pre_Recipient_CD8_CFSElo
Itx-Pt14-R8U	Pt14_Pre_Recipient_CD8_unstim

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

normalize(data[,c(7,9)])

ratio=5

  c1indices=which(data[,7]>0 & data[,9]>0 & data[,7]>ratio*data[,9])

  c2indices=which(data[,7]>0 & data[,9]>0 & data[,9]>ratio*data[,7])

  ambiindices=which(data[,7]>0 & data[,9]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,9]=0

  data[c2indices,7]=0

  data[ambiindices,c(7,9)]=0

#D4L vs D8L

normalize(data[,c(6,8)])

ratio=5

  c1indices=which(data[,6]>0 & data[,8]>0 & data[,6]>ratio*data[,8])

  c2indices=which(data[,6]>0 & data[,8]>0 & data[,8]>ratio*data[,6])

  ambiindices=which(data[,6]>0 & data[,8]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,8]=0

```
data[c2indices,6]=0

data[ambiindices,c(6,8)]=0




#R4U vs R8U

normalize(data[,c(11,13)])

ratio=5

 c1indices=which(data[,11]>0 & data[,13]>0 & data[,11]>ratio*data[,13])

 c2indices=which(data[,11]>0 & data[,13]>0 & data[,13]>ratio*data[,11])

 ambiindices=which(data[,11]>0 & data[,13]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,13]=0

 data[c2indices,11]=0

 data[ambiindices,c(11,13)]=0




#R4L vs R8L

normalize(data[,c(10,12)])

ratio=5

 c1indices=which(data[,10]>0 & data[,12]>0 & data[,10]>ratio*data[,12])

 c2indices=which(data[,10]>0 & data[,12]>0 & data[,12]>ratio*data[,10])

 ambiindices=which(data[,10]>0 & data[,12]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,12]=0

 data[c2indices,10]=0

 data[ambiindices,c(10,12)]=0
```

```
rownames(data)=data[,1]

x=data


#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,7]>0 | x[,9]>0)&(x[,11]>0 | x[,13]>0))

ambiguous=union(ambiguous,which((x[,7]>0 | x[,9]>0)&(x[,10]>0 | x[,12]>0)))

ambiguous=union(ambiguous,which((x[,11]>0 | x[,13]>0)&(x[,6]>0 | x[,8]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]


#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L,
R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional
step of optimization for CD4 and CD8 sorting error.


#R4L vs R8U

normalize(x[,c(10,13)])

ratio=2

 c1indices=which(x[,10]>0 & x[,13]>0 & x[,10]>ratio*x[,13])

 c2indices=which(x[,10]>0 & x[,13]>0 & x[,13]>ratio*x[,10])

 ambiindices=which(x[,10]>0 & x[,13]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,13]=0

 x[c2indices,10]=0

 x[ambiindices,c(10,13)]=0
```

```
#R8L vs R4U

normalize(x[,c(12,11)])

ratio=2

  c1indices=which(x[,12]>0 & x[,11]>0 & x[,12]>ratio*x[,11])

  c2indices=which(x[,12]>0 & x[,11]>0 & x[,11]>ratio*x[,12])

  ambiindices=which(x[,12]>0 & x[,11]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,11]=0

  x[c2indices,12]=0

  x[ambiindices,c(12,11)]=0



#D4L vs D8U

normalize(x[,c(6,9)])

ratio=2

  c1indices=which(x[,6]>0 & x[,9]>0 & x[,6]>ratio*x[,9])

  c2indices=which(x[,6]>0 & x[,9]>0 & x[,9]>ratio*x[,6])

  ambiindices=which(x[,6]>0 & x[,9]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,9]=0

  x[c2indices,6]=0

  x[ambiindices,c(6,9)]=0



#D8L vs D4U

normalize(x[,c(8,7)])

ratio=2

  c1indices=which(x[,8]>0 & x[,7]>0 & x[,8]>ratio*x[,7])
```

```
c2indices=which(x[,8]>0 & x[,7]>0 & x[,7]>ratio*x[,8])

ambiindices=which(x[,8]>0 & x[,7]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

x[c1indices,7]=0

x[c2indices,8]=0

x[ambiindices,c(8,7)]=0


write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt14 iITx/Pt14
05242019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T, sep="\t")


# add "fold=2" below, use 2 fold expansion (default 5 fold)

# add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for
read counts


#HVG direction

# For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]

# For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]

cd4.HVG= x[,c(11,10)]

cd8.HVG= x[,c(13,12)]

allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00002)


length(allo.HVG[[1]])

[1] 15085

length(allo.HVG[[2]])

[1] 8963

write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 CD4 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 CD8 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")
```

```
rCD4mappable=rownames(x[(x[,11]+x[,10])>0,])

rCD8mappable=rownames(x[(x[,13]+x[,12])>0,])


CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])


write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 CD4nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F,
sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 CD8nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F,
sep="\t")


length(rCD4mappable)

[1] 229182

length(rCD8mappable)

[1] 169186


length(CD4nonHVG)

[1] 214097

length(CD8nonHVG)

[1] 160223


rmappable=rownames(x[(x[,11]+x[,10]+x[,13]+x[,12])>0,])


length(rmappable)

[1] 398368
```

```
write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt14
iITx/Pt14 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")


length(rCD4mappable)+length(rCD8mappable)

[1] 398368

length(intersect(rCD4mappable,rCD8mappable))

[1] 0




#GVH direction

# For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]

# For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]

cd4.GVH= x[,c(7,6)]

cd8.GVH= x[,c(9,8)]

allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00002)


length(allo.GVH[[1]])

[1] 4983

length(allo.GVH[[2]])

[1] 7603


write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 CD4 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 CD8 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")


dCD4mappable=rownames(x[(x[,7]+x[,6])>0,])

dCD8mappable=rownames(x[(x[,9]+x[,8])>0,])
```

CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])

CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])

write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt14 iITx/Pt14 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt14 iITx/Pt14 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)

[1] 159332

length(dCD8mappable)

[1] 171793

length(CD4nonGVH)

[1] 154349

length(CD8nonGVH)

[1] 164190

dmappable=rownames(x[(x[,7]+x[,6]+x[,9]+x[,8])>0,])

length(dmappable)

[1] 331125

write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt14 iITx/Pt14 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)+length(dCD8mappable)

[1] 331125

length(intersect(dCD4mappable,dCD8mappable))

[1] 0

#Pt14 POD9 Bx GVH

cd4= x[,c(7,6,4)]

cd8= x[,c(9,8,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt14 iITx/Pt14 05232019 2x.out')

rownames(x)=x[,1]

cd4= x[,c(7,6)]

cd8= x[,c(9,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))

#Pt14 POD9 Bx HVG

cd4= x[,c(11,10,4)]

cd8= x[,c(13,12,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt14 iITx/Pt14 05232019 2x.out')

rownames(x)=x[,1]

cd4= x[,c(11,10)]

cd8= x[,c(13,12)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))

```
#Pt14 POD16 Bx GVH

cd4= x[,c(7,6,14)]

cd8= x[,c(9,8,14)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 05232019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(7,6)]

cd8= x[,c(9,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,14]>0,]))

intersect(allo[[2]], rownames(x[x[,14]>0,]))


#Pt14 POD16 Bx HVG

cd4= x[,c(11,10,14)]

cd8= x[,c(13,12,14)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 05232019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,10)]

cd8= x[,c(13,12)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,14]>0,]))

intersect(allo[[2]], rownames(x[x[,14]>0,]))


#Pt14 POD156 Bx GVH
```

```
cd4= x[,c(7,6,5)]

cd8= x[,c(9,8,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 05232019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(7,6)]

cd8= x[,c(9,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt14 POD156 Bx HVG

cd4= x[,c(11,10,5)]

cd8= x[,c(13,12,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 05232019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,10)]

cd8= x[,c(13,12)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt14 POD226 Bx GVH

cd4= x[,c(7,6,15)]

cd8= x[,c(9,8,15)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt14 iITx/Pt14 05232019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(7,6)]

cd8= x[,c(9,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))


#Pt14 POD226 Bx HVG

cd4= x[,c(11,10,15)]

cd8= x[,c(13,12,15)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt14 iITx/Pt14 05232019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,10)]

cd8= x[,c(13,12)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))


#Pt14 POD527 ileum Bx GVH

cd4= x[,c(7,6,20)]

cd8= x[,c(9,8,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt14 iITx/Pt14 05232019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(7,6)]

cd8= x[,c(9,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))


#Pt14 POD527 ileum Bx HVG

cd4= x[,c(11,10,20)]

cd8= x[,c(13,12,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 05232019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,10)]

cd8= x[,c(13,12)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))


#Pt14 POD717 ileum Bx GVH

cd4= x[,c(7,6,23)]

cd8= x[,c(9,8,23)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 05232019 2x.out' )


rownames(x)=x[,1]
```

```
cd4= x[,c(7,6)]

cd8= x[,c(9,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,23]>0,]))

intersect(allo[[2]], rownames(x[x[,23]>0,]))




#Pt14 POD717 ileum Bx HVG

cd4= x[,c(11,10,23)]

cd8= x[,c(13,12,23)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 05232019 2x.out' )




rownames(x)=x[,1]

cd4= x[,c(11,10)]

cd8= x[,c(13,12)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,23]>0,]))

intersect(allo[[2]], rownames(x[x[,23]>0,]))




#Pt14 POD30 PBMC GVH

cd4= x[,c(7,6,16)]

cd8= x[,c(9,8,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 05232019 2x.out' )




rownames(x)=x[,1]

cd4= x[,c(7,6)]
```

```
cd8= x[,c(9,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))




#Pt14 POD30 PBMC HVG

cd4= x[,c(11,10,16)]

cd8= x[,c(13,12,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 05232019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,10)]

cd8= x[,c(13,12)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))



#Pt14 POD456 PBMC GVH

cd4= x[,c(7,6,17)]

cd8= x[,c(9,8,17)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 05232019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(7,6)]

cd8= x[,c(9,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)
```

```
intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))


#Pt14 POD456 PBMC HVG

cd4= x[,c(11,10,17)]

cd8= x[,c(13,12,17)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt14 iITx/Pt14 05232019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,10)]

cd8= x[,c(13,12)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))
```

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt15 MVTx/Pt15 06282019.tsv")

source('Z:/Jianing/10x scRNA-seq TCR-seq/Pt15_MJ001, MJ004/alloreactivity_printtofile 09132017.R')

names(data)

[1] "Rearrangement"   "Sum..Templates."   "Present.In"

 [4] "Pt15MVTx.Bx.POD17"   "Pt15MVTx.Bx.POD27"
"Pt15MVTx.Bx.POD55"

 [7] "Pt15MVTx.GVH.D4L"   "Pt15MVTx.GVH.D8L"
"Pt15MVTx.GVH.R4L"

[10] "Pt15MVTx.GVH.R8L"   "Pt15MVTx.PBMCs.POD11"
"Pt15MVTx.PBMCs.POD143.Donor_T"

[13] "Pt15MVTx.PBMCs.POD143.Recipient_T" "Pt15MVTx.PBMCs.POD19"
"Pt15MVTx.PBMCs.POD26"

[16] "Pt15MVTx.pre.Tx.donor_MLN"   "Pt15MVTx.unstim.D4"
"Pt15MVTx.unstim.D8"

[19] "Pt15MVTx.unstim.R4"   "Pt15MVTx.unstim.R8"
"Pt15MVTx_Bx_POD237"

[22] "Pt15MVTx_PBMC_POD255"   "Pt15_MVTx_Bx_POD455"
"Pt15_MVTx_Bx_Tx_colon_POD1018"

[25] "Pt15_MVTx_Bx_duodenum_POD1018"   "Pt15_MVTx_Bx_ileum_POD1018"
"Pt15_MVTx_Bx_native_colon_POD1018"

[28] "Pt15_MVTx_Bx_stomach_POD1018"   "Pt15_MVTx_PBMC_POD1070"
"Pt15_MVTx_PBMC_POD83"

[31] "Pt15_MVTx_PBMC_POD83_MLR_D3L"   "Pt15_MVTx_POD1336_PBMC"
"Pt15_MVTx_POD1336_Tx_colon_Bx"

[34] "Pt15_MVTx_POD1336_duodenum_Bx"   "Pt15_MVTx_POD1336_ileum_Bx_ACR"
"Pt15_MVTx_POD1336_native_colon_Bx"

[37] "Pt15_MVTx_POD1336_stomach_Bx"

Sample name (Pt: patient)	updated rename
Pt15MVTx-GVH-D4L	Pt15_Pre_Donor_CD4_CFSElo
Pt15MVTx-unstim-D4 spleen	Pt15_Pre_Donor_CD4_unstim
Pt15MVTx-GVH-D8L	Pt15_Pre_Donor_CD8_CFSElo
Pt15MVTx-unstim-D8 spleen	Pt15_Pre_Donor_CD8_unstim
Pt15MVTx-Bx-POD17	Pt15_Post_ileum_day0017
Pt15MVTx-Bx-POD27	Pt15_Post_ileum_day0027
Pt15MVTx-Bx-POD55	Pt15_Post_ileum_day0055
Pt15MVTx-Bx-POD237	Pt15_Post_ileum_day0237
Pt15MVTx-Bx-POD319	Pt15_Post_ileum_day0319
Pt15MVTx-Bx-POD455	Pt15_Post_ileum_day0455
Pt15_MVTx_Bx_ileum_POD1018	Pt15_Post_ileum_day1018
Pt15MVTx-PBMCs-POD11	Pt15_Post_PBMC_day0011
Pt15MVTx-PBMCs-POD19	Pt15_Post_PBMC_day0019
Pt15MVTx-PBMCs-POD26	Pt15_Post_PBMC_day0026
Pt15_MVTx_PBMC_POD83	Pt15_Post_PBMC_day0083
Pt15MVTx-PBMCs-POD255	Pt15_Post_PBMC_day0255
Pt15MVTx-PBMCs-POD1070	Pt15_Post_PBMC_day1070
Pt15MVTx-HVG-R4L	Pt15_Pre_Recipient_CD4_CFSElo
Pt15MVTx-unstim-R4	Pt15_Pre_Recipient_CD4_unstim
Pt15MVTx-HVG-R8L	Pt15_Pre_Recipient_CD8_CFSElo
Pt15MVTx-unstim-R8	Pt15_Pre_Recipient_CD8_unstim

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

normalize(data[,c(17,18)])

ratio=5

c1indices=which(data[,17]>0 & data[,18]>0 & data[,17]>ratio*data[,18])

c2indices=which(data[,17]>0 & data[,18]>0 & data[,18]>ratio*data[,17])

ambiindices=which(data[,17]>0 & data[,18]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

data[c1indices,18]=0

data[c2indices,17]=0

```r
  data[ambiindices,c(17,18)]=0


#D4L vs D8L
normalize(data[,c(7,8)])
ratio=5
  c1indices=which(data[,7]>0 & data[,8]>0 & data[,7]>ratio*data[,8])
  c2indices=which(data[,7]>0 & data[,8]>0 & data[,8]>ratio*data[,7])
  ambiindices=which(data[,7]>0 & data[,8]>0)
  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
  data[c1indices,8]=0
  data[c2indices,7]=0
  data[ambiindices,c(7,8)]=0


#R4U vs R8U
normalize(data[,c(19,20)])
ratio=5
  c1indices=which(data[,19]>0 & data[,20]>0 & data[,19]>ratio*data[,20])
  c2indices=which(data[,19]>0 & data[,20]>0 & data[,20]>ratio*data[,19])
  ambiindices=which(data[,19]>0 & data[,20]>0)
  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
  data[c1indices,20]=0
  data[c2indices,19]=0
  data[ambiindices,c(19,20)]=0


#R4L vs R8L
normalize(data[,c(9,10)])
ratio=5
  c1indices=which(data[,9]>0 & data[,10]>0 & data[,9]>ratio*data[,10])
```

```
c2indices=which(data[,9]>0 & data[,10]>0 & data[,10]>ratio*data[,9])

ambiindices=which(data[,9]>0 & data[,10]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

data[c1indices,10]=0

data[c2indices,9]=0

data[ambiindices,c(9,10)]=0


rownames(data)=data[,1]

x=data


#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,17]>0 | x[,18]>0)&(x[,19]>0 | x[,20]>0))

ambiguous=union(ambiguous,which((x[,17]>0 | x[,18]>0)&(x[,9]>0 | x[,10]>0)))

ambiguous=union(ambiguous,which((x[,19]>0 | x[,20]>0)&(x[,7]>0 | x[,8]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]


#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L,
R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional
step of optimization for CD4 and CD8 sorting error.


#R4L vs R8U

normalize(x[,c(9,20)])

ratio=2

 c1indices=which(x[,9]>0 & x[,20]>0 & x[,9]>ratio*x[,20])

 c2indices=which(x[,9]>0 & x[,20]>0 & x[,20]>ratio*x[,9])

 ambiindices=which(x[,9]>0 & x[,20]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,20]=0
```

```
  x[c2indices,9]=0

  x[ambiindices,c(9,20)]=0


#R8L vs R4U

normalize(x[,c(10,19)])

ratio=2

  c1indices=which(x[,10]>0 & x[,19]>0 & x[,10]>ratio*x[,19])

  c2indices=which(x[,10]>0 & x[,19]>0 & x[,19]>ratio*x[,10])

  ambiindices=which(x[,10]>0 & x[,19]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,19]=0

  x[c2indices,10]=0

  x[ambiindices,c(10,19)]=0


#D4L vs D8U

normalize(x[,c(7,18)])

ratio=2

  c1indices=which(x[,7]>0 & x[,18]>0 & x[,7]>ratio*x[,18])

  c2indices=which(x[,7]>0 & x[,18]>0 & x[,18]>ratio*x[,7])

  ambiindices=which(x[,7]>0 & x[,18]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,18]=0

  x[c2indices,7]=0

  x[ambiindices,c(7,18)]=0


#D8L vs D4U

normalize(x[,c(8,17)])

ratio=2
```

```
c1indices=which(x[,8]>0 & x[,17]>0 & x[,8]>ratio*x[,17])

c2indices=which(x[,8]>0 & x[,17]>0 & x[,17]>ratio*x[,8])

ambiindices=which(x[,8]>0 & x[,17]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

x[c1indices,17]=0

x[c2indices,8]=0

x[ambiindices,c(8,17)]=0
```

```
write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt15
MVTx/Pt15 06282019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T,
sep="\t")
```

add "fold=2" below, use 2 fold expansion (default 5 fold)

add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for read counts

#HVG direction

For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]

For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]

```
cd4.HVG= x[,c(19,9)]
```

```
cd8.HVG= x[,c(20,10)]
```

```
allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00002)
```

```
length(allo.HVG[[1]])
```

[1] 9057

```
length(allo.HVG[[2]])
```

[1] 10081

```
write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 CD4 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")
```

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt15 MVTx/Pt15 CD8 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,19]+x[,9])>0,])

rCD8mappable=rownames(x[(x[,20]+x[,10])>0,])

CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])

write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt15 MVTx/Pt15 CD4nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt15 MVTx/Pt15 CD8nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 149037

length(rCD8mappable)

[1] 129754

length(CD4nonHVG)

[1] 139980

length(CD8nonHVG)

[1] 119673

rmappable=rownames(x[(x[,19]+x[,9]+x[,20]+x[,10])>0,])

length(rmappable)

[1] 278791

93

write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt15 MVTx/Pt15 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)+length(rCD8mappable)

[1] 278791

length(intersect(rCD4mappable,rCD8mappable))

[1] 0

#GVH direction

For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]

For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]

cd4.GVH= x[,c(17,7)]

cd8.GVH= x[,c(18,8)]

allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00002)

length(allo.GVH[[1]])

[1] 4499

length(allo.GVH[[2]])

[1] 3999

write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt15 MVTx/Pt15 CD4 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt15 MVTx/Pt15 CD8 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

dCD4mappable=rownames(x[(x[,17]+x[,7])>0,])

dCD8mappable=rownames(x[(x[,18]+x[,8])>0,])

CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])

CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])

write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt15 MVTx/Pt15 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt15 MVTx/Pt15 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)

[1] 127323

length(dCD8mappable)

[1] 128335

length(CD4nonGVH)

[1] 122824

length(CD8nonGVH)

[1] 124336

dmappable=rownames(x[(x[,17]+x[,7]+x[,18]+x[,8])>0,])

length(dmappable)

[1] 255658

write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt15 MVTx/Pt15 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)+length(dCD8mappable)

[1] 255658

```
length(intersect(dCD4mappable,dCD8mappable))
```

[1] 0


```
#Pt15 POD17 Bx GVH

cd4= x[,c(17,7,4)]

cd8= x[,c(18,8,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,7)]

cd8= x[,c(18,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))


#Pt15 POD17 Bx HVG

cd4= x[,c(19,9,4)]

cd8= x[,c(20,10,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(19,9)]

cd8= x[,c(20,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))
```

```r
#Pt15 POD27 Bx GVH

cd4= x[,c(17,7,5)]

cd8= x[,c(18,8,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,7)]

cd8= x[,c(18,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt15 POD27 Bx HVG

cd4= x[,c(19,9,5)]

cd8= x[,c(20,10,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(19,9)]

cd8= x[,c(20,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt15 POD55 Bx GVH

cd4= x[,c(17,7,6)]
```

```
cd8= x[,c(18,8,6)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,7)]

cd8= x[,c(18,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,6]>0,]))

intersect(allo[[2]], rownames(x[x[,6]>0,]))


#Pt15 POD55 Bx HVG

cd4= x[,c(19,9,6)]

cd8= x[,c(20,10,6)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(19,9)]

cd8= x[,c(20,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,6]>0,]))

intersect(allo[[2]], rownames(x[x[,6]>0,]))


#Pt15 POD237 Bx GVH

cd4= x[,c(17,7,21)]

cd8= x[,c(18,8,21)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(17,7)]

cd8= x[,c(18,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,21]>0,]))

intersect(allo[[2]], rownames(x[x[,21]>0,]))


#Pt15 POD237 Bx HVG

cd4= x[,c(19,9,21)]

cd8= x[,c(20,10,21)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(19,9)]

cd8= x[,c(20,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,21]>0,]))

intersect(allo[[2]], rownames(x[x[,21]>0,]))


#Pt15 POD455 Bx GVH

cd4= x[,c(17,7,23)]

cd8= x[,c(18,8,23)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,7)]

cd8= x[,c(18,8)]
```

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,23]>0,]))

intersect(allo[[2]], rownames(x[x[,23]>0,]))


#Pt15 POD455 Bx HVG

cd4= x[,c(19,9,23)]

cd8= x[,c(20,10,23)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(19,9)]

cd8= x[,c(20,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,23]>0,]))

intersect(allo[[2]], rownames(x[x[,23]>0,]))



#Pt15 POD1018 ileum Bx GVH

cd4= x[,c(17,7,26)]

cd8= x[,c(18,8,26)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,7)]

cd8= x[,c(18,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,26]>0,]))
```

```
intersect(allo[[2]], rownames(x[x[,26]>0,]))
```

#Pt15 POD1018 ileum Bx HVG

```
cd4= x[,c(19,9,26)]

cd8= x[,c(20,10,26)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(19,9)]

cd8= x[,c(20,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,26]>0,]))

intersect(allo[[2]], rownames(x[x[,26]>0,]))
```

#Pt15 POD11 PBMC GVH

```
cd4= x[,c(17,7,11)]

cd8= x[,c(18,8,11)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,7)]

cd8= x[,c(18,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,11]>0,]))

intersect(allo[[2]], rownames(x[x[,11]>0,]))
```

```
#Pt15 POD11 PBMC HVG

cd4= x[,c(19,9,11)]

cd8= x[,c(20,10,11)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(19,9)]

cd8= x[,c(20,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,11]>0,]))

intersect(allo[[2]], rownames(x[x[,11]>0,]))




#Pt15 POD19 PBMC GVH

cd4= x[,c(17,7,14)]

cd8= x[,c(18,8,14)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,7)]

cd8= x[,c(18,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,14]>0,]))

intersect(allo[[2]], rownames(x[x[,14]>0,]))
```

```
#Pt15 POD19 PBMC HVG

cd4= x[,c(19,9,14)]

cd8= x[,c(20,10,14)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(19,9)]

cd8= x[,c(20,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,14]>0,]))

intersect(allo[[2]], rownames(x[x[,14]>0,]))




#Pt15 POD26 PBMC GVH

cd4= x[,c(17,7,15)]

cd8= x[,c(18,8,15)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,7)]

cd8= x[,c(18,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))
```

```
#Pt15 POD26 PBMC HVG

cd4= x[,c(19,9,15)]

cd8= x[,c(20,10,15)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(19,9)]

cd8= x[,c(20,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))




#Pt15 POD83 PBMC GVH

cd4= x[,c(17,7,30)]

cd8= x[,c(18,8,30)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,7)]

cd8= x[,c(18,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,30]>0,]))

intersect(allo[[2]], rownames(x[x[,30]>0,]))
```

```
#Pt15 POD83 PBMC HVG

cd4= x[,c(19,9,30)]

cd8= x[,c(20,10,30)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(19,9)]

cd8= x[,c(20,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,30]>0,]))

intersect(allo[[2]], rownames(x[x[,30]>0,]))




#Pt15 POD255 PBMC GVH

cd4= x[,c(17,7,22)]

cd8= x[,c(18,8,22)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,7)]

cd8= x[,c(18,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))
```

```
#Pt15 POD255 PBMC HVG

cd4= x[,c(19,9,22)]

cd8= x[,c(20,10,22)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(19,9)]

cd8= x[,c(20,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))



#Pt15 POD1070 PBMC GVH

cd4= x[,c(17,7,29)]

cd8= x[,c(18,8,29)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,7)]

cd8= x[,c(18,8)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,29]>0,]))

intersect(allo[[2]], rownames(x[x[,29]>0,]))



#Pt15 POD1070 PBMC HVG
```

```
cd4= x[,c(19,9,29)]

cd8= x[,c(20,10,29)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt15 MVTx/Pt15 06282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(19,9)]

cd8= x[,c(20,10)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))
```

#Pt16

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 06182019.tsv")

source('P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/! summary of R codes/alloreactivity_printtofile 09132017.R')

names(data)

 [1] "Rearrangement"   "Sum..Templates."

 [3] "Present.In"   "Pt16LITx.GVH.D4L"

 [5] "Pt16LITx.GVH.D8L"   "Pt16LITx.HVG.R4L"

 [7] "Pt16LITx.HVG.R8L"   "Pt16LITx.PBMCs.POD9"

 [9] "Pt16LITx.unstim.D4"   "Pt16LITx.unstim.D8"

[11] "Pt16LITx.unstim.R4"   "Pt16LITx.unstim.R8"

[13] "Pt16_1st_LITx_POD23_PBMC"   "Pt16_2nd_MVTx_POD494_LN"

[15] "Pt16_2nd_MVTx_POD494_PBMC"
"Pt16_2nd_MVTx_POD494_ileum_stoma_IEL.LPL"

[17] "Pt16_LITx_PBMC_POD463"   "Pt16_reTx_2ndD4U_SP"

[19] "Pt16_reTx_2ndD8U_SP"   "Pt16_reTx_Bx_POD14"

[21] "Pt16_reTx_MLR.16_2ndD4L_SP_vs_R_POD0_MLN"
"Pt16_reTx_MLR.16_2ndD8L_SP_vs_R_POD0_MLN"

[23] "Pt16_reTx_MLR.17_R_POD0_aLN_R4L_vs_2ndD_SP"
"Pt16_reTx_MLR.17_R_POD0_aLN_R8L_vs_2ndD_SP"

[25] "Pt16_reTx_MLR.3_2ndD4L_vs_1stD"   "Pt16_reTx_MLR.3_2ndD8L_vs_1stD"

[27] "Pt16_reTx_MLR.6_1stD4L_vs_2ndD"   "Pt16_reTx_MLR.6_1stD8L_vs_2ndD"

[29] "Pt16_reTx_PBMC_POD285"
"Pt16_reTx_POD0_PBMC_POD786_of_1st_Tx"

[31] "Pt16_reTx_POD126_BM"   "Pt16_reTx_POD126_BM_1st_Donor_CD45"

[33] "Pt16_reTx_POD126_BM_2nd_Donor_CD45"   "Pt16_reTx_POD126_Bx_duodenum"

[35] "Pt16_reTx_POD126_Bx_ileum"   "Pt16_reTx_POD126_Bx_stomach"

[37] "Pt16_reTx_POD126_PBMC"
"Pt16_reTx_POD126_PBMC_1st_Donor_CD45"

[39] "Pt16_reTx_POD126_PBMC_2nd_Donor_CD45"   "Pt16_reTx_POD32_Bx"

[41] "Pt16_reTx_POD33_PBMC"   "Pt16_reTx_POD8_PBMC"

Sample name (Pt: patient)	updated rename
Pt16LITx-GVH-D4L	Pt16_Pre1_Donor1_CD4_CFSElo
Pt16LITx-unstim-D4	Pt16_Pre1_Donor1_CD4_unstim
Pt16LITx-GVH-D8L	Pt16_Pre1_Donor1_CD8_CFSElo
Pt16LITx-unstim-D8	Pt16_Pre1_Donor1_CD8_unstim
Pt16_reTx_MLR-16_2ndD4L_SP_vs_R_POD0_MLN	Pt16_Pre2_Donor2_CD4_CFSElo
Pt16_reTx_2ndD4U_SP	Pt16_Pre2_Donor2_CD4_unstim
Pt16_reTx_MLR-16_2ndD8L_SP_vs_R_POD0_MLN	Pt16_Pre2_Donor2_CD8_CFSElo
Pt16_reTx_2ndD8U_SP	Pt16_Pre2_Donor2_CD8_unstim
Pt16LITx-PBMCs-POD9	Pt16_Post1_PBMC_day0009
Pt16_LITx_PBMC_POD463	Pt16_Post1_PBMC_day0463
Pt16_reTx_POD0_PBMC_POD786_of_1st_Tx	Pt16_Post1_PBMC_day0786_Post2_day0000
Pt16_reTx_POD126_BM	Pt16_Post2_BM_day0126
Pt16_reTx_POD14_Bx	Pt16_Post2_ileum_day0014
Pt16_reTx_POD32_Bx	Pt16_Post2_ileum_day0032
Pt16_reTx_POD126_Bx_ileum	Pt16_Post2_ileum_day0126
Pt16_reTx_POD494_ileum stoma	Pt16_Post2_ileum_day0494
Pt16_reTx_POD8_PBMC	Pt16_Post2_PBMC_day0008
Pt16_reTx_POD33_PBMC	Pt16_Post2_PBMC_day0033
Pt16_reTx_POD126_PBMC	Pt16_Post2_PBMC_day0126
Pt16_reTx_POD285_PBMC	Pt16_Post2_PBMC_day0285
Pt16_reTx_POD494_PBMC	Pt16_Post2_PBMC_day0494
Pt16LITx-unstim-R4	Pt16_Pre1_Recipient_CD4_unstim
Pt16LITx-unstim-R8	Pt16_Pre1_Recipient_CD8_unstim
Pt16LITx-HVG-R8L	Pt16_Pre1_Recipient_CD4_CFSElo_vs_Donor1
Pt16LITx-HVG-R4L	Pt16_Pre1_Recipient_CD8_CFSElo_vs_Donor1

Pt16_reTx_MLR-17_R_POD0_aLN_R4L_vs_2ndD_SP	Pt16_Pre1_Recipient_CD4_CFSElo_vs_Donor2
Pt16_reTx_MLR-17_R_POD0_aLN_R8L_vs_2ndD_SP	Pt16_Pre1_Recipient_CD8_CFSElo_vs_Donor2

#1st Tx, Pt16' LITx

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D14U vs D18U

normalize(data[,c(9,10)])

ratio=5

 c1indices=which(data[,9]>0 & data[,10]>0 & data[,9]>ratio*data[,10])

 c2indices=which(data[,9]>0 & data[,10]>0 & data[,10]>ratio*data[,9])

 ambiindices=which(data[,9]>0 & data[,10]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,10]=0

 data[c2indices,9]=0

 data[ambiindices,c(9,10)]=0

#D14L vs D18L

normalize(data[,c(4,5)])

ratio=5

 c1indices=which(data[,4]>0 & data[,5]>0 & data[,4]>ratio*data[,5])

 c2indices=which(data[,4]>0 & data[,5]>0 & data[,5]>ratio*data[,4])

 ambiindices=which(data[,4]>0 & data[,5]>0)

```
ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

data[c1indices,5]=0

data[c2indices,4]=0

data[ambiindices,c(4,5)]=0




#R4U vs R8U

normalize(data[,c(11,12)])

ratio=5

 c1indices=which(data[,11]>0 & data[,12]>0 & data[,11]>ratio*data[,12])

 c2indices=which(data[,11]>0 & data[,12]>0 & data[,12]>ratio*data[,11])

 ambiindices=which(data[,11]>0 & data[,12]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,12]=0

 data[c2indices,11]=0

 data[ambiindices,c(11,12)]=0




#R4L(D1) vs R8L (D1)

normalize(data[,c(6,7)])

ratio=5

 c1indices=which(data[,6]>0 & data[,7]>0 & data[,6]>ratio*data[,7])

 c2indices=which(data[,6]>0 & data[,7]>0 & data[,7]>ratio*data[,6])

 ambiindices=which(data[,6]>0 & data[,7]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,7]=0

 data[c2indices,6]=0

 data[ambiindices,c(6,7)]=0
```

```
rownames(data)=data[,1]

x=data


#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,9]>0 | x[,10]>0)&(x[,11]>0 | x[12]>0))

ambiguous=union(ambiguous,which((x[,9]>0 | x[,10]>0)&(x[,6]>0 | x[,7]>0)))

ambiguous=union(ambiguous,which((x[,11]>0 | x[,12]>0)&(x[,4]>0 | x[,5]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]


#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L,
R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional
step of optimization for CD4 and CD8 sorting error.


#R4L(D1) vs R8U

normalize(x[,c(6,12)])

ratio=2

 c1indices=which(x[,6]>0 & x[,12]>0 & x[,6]>ratio*x[,12])

 c2indices=which(x[,6]>0 & x[,12]>0 & x[,12]>ratio*x[,6])

 ambiindices=which(x[,6]>0 & x[,12]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,12]=0

 x[c2indices,6]=0

 x[ambiindices,c(6,12)]=0


#R8L(D1) vs R4U

normalize(x[,c(7,11)])

ratio=2

 c1indices=which(x[,7]>0 & x[,11]>0 & x[,7]>ratio*x[,11])
```

```
c2indices=which(x[,7]>0 & x[,11]>0 & x[,11]>ratio*x[,7])
ambiindices=which(x[,7]>0 & x[,11]>0)
ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
x[c1indices,11]=0
x[c2indices,7]=0
x[ambiindices,c(7,11)]=0


#D14L vs D18U
normalize(x[,c(4,10)])
ratio=2
 c1indices=which(x[,4]>0 & x[,10]>0 & x[,4]>ratio*x[,10])
 c2indices=which(x[,4]>0 & x[,10]>0 & x[,10]>ratio*x[,4])
 ambiindices=which(x[,4]>0 & x[,10]>0)
 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
 x[c1indices,10]=0
 x[c2indices,4]=0
 x[ambiindices,c(4,10)]=0


#D18L vs D14U
normalize(x[,c(5,9)])
ratio=2
 c1indices=which(x[,5]>0 & x[,9]>0 & x[,5]>ratio*x[,9])
 c2indices=which(x[,5]>0 & x[,9]>0 & x[,9]>ratio*x[,5])
 ambiindices=which(x[,5]>0 & x[,9]>0)
 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
 x[c1indices,9]=0
 x[c2indices,5]=0
 x[ambiindices,c(5,9)]=0
```

write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 LITx 06192019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T, sep="\t")

add "fold=2" below, use 2 fold expansion (default 5 fold)

add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for read counts

#HVG1 direction

For CD4 HvG1, cd4.HVG1=x[,c(unstim(R4U),stim(R4L))]

For CD8 HvG, cd8.HVG1=x[,c(unstim(R8U),stim(R8L))]

cd4.HVG1= x[,c(11,6)]

cd8.HVG1= x[,c(12,7)]

allo.HVG1=listAlloreactive(cd4.HVG1,cd8.HVG1, fold=2, freq=0.00002)

length(allo.HVG1[[1]])

[1] 9761

length(allo.HVG1[[2]])

[1] 4915

write.table(allo.HVG1[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 CD4 HVG1list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG1[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 CD8 HVG1list.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,11]+x[,6])>0,])

rCD8mappable=rownames(x[(x[,12]+x[,7])>0,])

CD4nonHVG1=setdiff(rCD4mappable,allo.HVG1[[1]])

CD8nonHVG1=setdiff(rCD8mappable,allo.HVG1[[2]])

write.table(CD4nonHVG1,file ="P:/CCTI_USERS/Jiauing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 CD4nonHVG1 rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG1,file ="P:/CCTI_USERS/Jiauing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 CD8nonHVG1 rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 179052

length(rCD8mappable)

[1] 144834

length(CD4nonHVG1)

[1] 169291

length(CD8nonHVG1)

[1] 139919

rmappable=rownames(x[(x[,11]+x[,6]+x[,12]+x[,7])>0,])

length(rmappable)

[1] 323886

write.table(rmappable,file ="P:/CCTI_USERS/Jiauing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)+length(rCD8mappable)

[1] 323886

length(intersect(rCD4mappable,rCD8mappable))

[1] 0

```
#GVH direction
# For CD4 G1VH, cd4.G1VH=x[,c(unstim(D4U),stim(D4L))]
# For CD8 G1VH, cd8.G1VH=x[,c(unstim(D8U),stim(D8L))]
cd4.G1VH= x[,c(9,4)]
cd8.G1VH= x[,c(10,5)]
allo.G1VH=listAlloreactive(cd4.G1VH,cd8.G1VH, fold=2, freq=0.00002)


length(allo.G1VH[[1]])
[1] 6009
length(allo.G1VH[[2]])
[1] 2196


write.table(allo.G1VH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 CD4 G1VHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.G1VH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 CD8 G1VHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")


d1CD4mappable=rownames(x[(x[,9]+x[,4])>0,])
d1CD8mappable=rownames(x[(x[,10]+x[,5])>0,])
CD4nonG1VH=setdiff(d1CD4mappable,allo.G1VH[[1]])
CD8nonG1VH=setdiff(d1CD8mappable,allo.G1VH[[2]])


write.table(CD4nonG1VH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 CD4nonG1VH rmappable list.txt",quote=F,row.names=F,col.names=F,
sep="\t")

write.table(CD8nonG1VH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 CD8nonG1VH rmappable list.txt",quote=F,row.names=F,col.names=F,
sep="\t")
```

length(d1CD4mappable)

[1] 55875

length(d1CD8mappable)

[1] 52065

length(CD4nonG1VH)

[1] 49866

length(CD8nonG1VH)

[1] 49869

d1mappable=rownames(x[(x[,9]+x[,4]+x[,10]+x[,5])>0,])

length(d1mappable)

[1] 107940

write.table(d1mappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 donor1 mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(d1CD4mappable)+length(d1CD8mappable)

[1] 107940

length(intersect(d1CD4mappable,d1CD8mappable))

[1] 0

#Pt16' POD9 PBMC GVH

cd4= x[,c(9,4,8)]

cd8= x[,c(10,5,8)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out')

```
rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,8]>0,]))

intersect(allo[[2]], rownames(x[x[,8]>0,]))
```

#Pt16' POD9 PBMC HVG

```
cd4= x[,c(11,6,8)]

cd8= x[,c(12,7,8)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,8]>0,]))

intersect(allo[[2]], rownames(x[x[,8]>0,]))
```

#Pt16' POD23 PBMC GVH

```
cd4= x[,c(9,4,13)]

cd8= x[,c(10,5,13)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(9,4)]
```

```
cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,13]>0,]))

intersect(allo[[2]], rownames(x[x[,13]>0,]))


#Pt16' POD23 PBMC HVG

cd4= x[,c(11,6,13)]

cd8= x[,c(12,7,13)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,13]>0,]))

intersect(allo[[2]], rownames(x[x[,13]>0,]))


#Pt16' POD463 PBMC GVH

cd4= x[,c(9,4,17)]

cd8= x[,c(10,5,17)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,17]>0,]))
```

```
intersect(allo[[2]], rownames(x[x[,17]>0,]))


#Pt16' POD463 PBMC HVG

cd4= x[,c(11,6,17)]

cd8= x[,c(12,7,17)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))


#Pt16' POD786 PBMC GVH

cd4= x[,c(9,4,30)]

cd8= x[,c(10,5,30)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,30]>0,]))

intersect(allo[[2]], rownames(x[x[,30]>0,]))
```

```
#Pt16' POD786 PBMC HVG

cd4= x[,c(11,6,30)]

cd8= x[,c(12,7,30)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,30]>0,]))

intersect(allo[[2]], rownames(x[x[,30]>0,]))




#Pt16' POD794 (POD8 reTx) PBMC GVH

cd4= x[,c(9,4,42)]

cd8= x[,c(10,5,42)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,42]>0,]))

intersect(allo[[2]], rownames(x[x[,42]>0,]))
```

#Pt16' POD794 (POD8 reTx) PBMC HVG

cd4= x[,c(11,6,42)]

cd8= x[,c(12,7,42)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out')

rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,42]>0,]))

intersect(allo[[2]], rownames(x[x[,42]>0,]))

#Pt16' POD819 (POD33 reTx) PBMC GVH

cd4= x[,c(9,4,41)]

cd8= x[,c(10,5,41)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out')

rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,41]>0,]))

intersect(allo[[2]], rownames(x[x[,41]>0,]))

#Pt16' POD819 (POD33 reTx) PBMC HVG

```
cd4= x[,c(11,6,41)]

cd8= x[,c(12,7,41)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,41]>0,]))

intersect(allo[[2]], rownames(x[x[,41]>0,]))



#Pt16' POD912 (POD126 reTx) PBMC GVH

cd4= x[,c(9,4,37)]

cd8= x[,c(10,5,37)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,37]>0,]))

intersect(allo[[2]], rownames(x[x[,37]>0,]))



#Pt16' POD912 (POD126 reTx) PBMC HVG

cd4= x[,c(11,6,37)]
```

```
cd8= x[,c(12,7,37)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,37]>0,]))

intersect(allo[[2]], rownames(x[x[,37]>0,]))




#Pt16' POD1071 (POD285 reTx) PBMC GVH

cd4= x[,c(9,4,29)]

cd8= x[,c(10,5,29)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,29]>0,]))

intersect(allo[[2]], rownames(x[x[,29]>0,]))




#Pt16' POD1071 (POD285 reTx) PBMC HVG

cd4= x[,c(11,6,29)]
```

```
cd8= x[,c(12,7,29)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,29]>0,]))

intersect(allo[[2]], rownames(x[x[,29]>0,]))


#Pt16' POD800 (POD14 reTx) Bx GVH

cd4= x[,c(9,4,20)]

cd8= x[,c(10,5,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))


#Pt16' POD800 (POD14 reTx) Bx HvG

cd4= x[,c(11,6,20)]

cd8= x[,c(12,7,20)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))



#Pt16' POD818 (POD32 reTx) Bx GVH

cd4= x[,c(9,4,40)]

cd8= x[,c(10,5,40)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,40]>0,]))

intersect(allo[[2]], rownames(x[x[,40]>0,]))



#Pt16' POD818 (POD32 reTx) Bx HvG

cd4= x[,c(11,6,40)]

cd8= x[,c(12,7,40)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,40]>0,]))

intersect(allo[[2]], rownames(x[x[,40]>0,]))



#Pt16' POD912 (POD126 reTx) Bx GVH

cd4= x[,c(9,4,35)]

cd8= x[,c(10,5,35)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,35]>0,]))

intersect(allo[[2]], rownames(x[x[,35]>0,]))



#Pt16' POD912 (POD126 reTx) Bx HvG

cd4= x[,c(11,6,35)]

cd8= x[,c(12,7,35)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,35]>0,]))

intersect(allo[[2]], rownames(x[x[,35]>0,]))



#Pt16' POD1280 (POD494 reTx) ileum stoma GVH

cd4= x[,c(9,4,16)]

cd8= x[,c(10,5,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))



#Pt16' POD1280 (POD494 reTx)  ileum stoma HvG

cd4= x[,c(11,6,16)]

cd8= x[,c(12,7,16)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))



#Pt16' POD912 (POD126 reTx) BM GVH

cd4= x[,c(9,4,31)]

cd8= x[,c(10,5,31)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,31]>0,]))

intersect(allo[[2]], rownames(x[x[,31]>0,]))



#Pt16' POD912 (POD126 reTx) BM HvG

cd4= x[,c(11,6,31)]

cd8= x[,c(12,7,31)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,31]>0,]))

intersect(allo[[2]], rownames(x[x[,31]>0,]))



#Pt16' POD912 (POD126 reTx) BM 1st donor CD45 GVH

cd4= x[,c(9,4,32)]

cd8= x[,c(10,5,32)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,32]>0,]))

intersect(allo[[2]], rownames(x[x[,32]>0,]))



#Pt16' POD912 (POD126 reTx) BM 1st donor CD45 HvG

cd4= x[,c(11,6,32)]

cd8= x[,c(12,7,32)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,32]>0,]))

intersect(allo[[2]], rownames(x[x[,32]>0,]))



#Pt16' POD912 (POD126 reTx) BM 2nd donor CD45 GVH

cd4= x[,c(9,4,33)]

cd8= x[,c(10,5,33)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,33]>0,]))

intersect(allo[[2]], rownames(x[x[,33]>0,]))



#Pt16' POD912 (POD126 reTx) BM 2nd donor CD45 HvG

cd4= x[,c(11,6,33)]

cd8= x[,c(12,7,33)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,33]>0,]))

intersect(allo[[2]], rownames(x[x[,33]>0,]))



#Pt16' POD912 (POD126 reTx) PBMC 2nd donor CD45 GVH

cd4= x[,c(9,4,39)]

cd8= x[,c(10,5,39)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,39]>0,]))

intersect(allo[[2]], rownames(x[x[,39]>0,]))



#Pt16' POD912 (POD126 reTx) PBMC 2nd donor CD45 HvG

cd4= x[,c(11,6,39)]

cd8= x[,c(12,7,39)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,39]>0,]))

intersect(allo[[2]], rownames(x[x[,39]>0,]))




#Pt16' POD1280 (POD494 reTx) PBMC GVH

cd4= x[,c(9,4,15)]

cd8= x[,c(10,5,15)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(9,4)]

cd8= x[,c(10,5)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))




#Pt16' POD1280 (POD494 reTx)  PBMC HVG

cd4= x[,c(11,6,15)]

cd8= x[,c(12,7,15)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 LITx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,6)]

cd8= x[,c(12,7)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))
```

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 06182019.tsv")

source('P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/! summary of R codes/alloreactivity_printtofile 09132017.R')

names(data)

```
 [1] "Rearrangement"                    "Sum..Templates."
 [3] "Present.In"                       "Pt16LITx.GVH.D4L"
 [5] "Pt16LITx.GVH.D8L"                 "Pt16LITx.HVG.R4L"
 [7] "Pt16LITx.HVG.R8L"                 "Pt16LITx.PBMCs.POD9"
 [9] "Pt16LITx.unstim.D4"               "Pt16LITx.unstim.D8"
[11] "Pt16LITx.unstim.R4"               "Pt16LITx.unstim.R8"
[13] "Pt16_1st_LITx_POD23_PBMC"         "Pt16_2nd_MVTx_POD494_LN"
[15] "Pt16_2nd_MVTx_POD494_PBMC"        "Pt16_2nd_MVTx_POD494_ileum_stoma_IEL.LPL"
[17] "Pt16_LITx_PBMC_POD463"            "Pt16_reTx_2ndD4U_SP"
[19] "Pt16_reTx_2ndD8U_SP"              "Pt16_reTx_Bx_POD14"
[21] "Pt16_reTx_MLR.16_2ndD4L_SP_vs_R_POD0_MLN"   "Pt16_reTx_MLR.16_2ndD8L_SP_vs_R_POD0_MLN"
[23] "Pt16_reTx_MLR.17_R_POD0_aLN_R4L_vs_2ndD_SP"   "Pt16_reTx_MLR.17_R_POD0_aLN_R8L_vs_2ndD_SP"
[25] "Pt16_reTx_MLR.3_2ndD4L_vs_1stD"   "Pt16_reTx_MLR.3_2ndD8L_vs_1stD"
[27] "Pt16_reTx_MLR.6_1stD4L_vs_2ndD"   "Pt16_reTx_MLR.6_1stD8L_vs_2ndD"
[29] "Pt16_reTx_PBMC_POD285"            "Pt16_reTx_POD0_PBMC_POD786_of_1st_Tx"
[31] "Pt16_reTx_POD126_BM"              "Pt16_reTx_POD126_BM_1st_Donor_CD45"
[33] "Pt16_reTx_POD126_BM_2nd_Donor_CD45"   "Pt16_reTx_POD126_Bx_duodenum"
[35] "Pt16_reTx_POD126_Bx_ileum"        "Pt16_reTx_POD126_Bx_stomach"
[37] "Pt16_reTx_POD126_PBMC"            "Pt16_reTx_POD126_PBMC_1st_Donor_CD45"
```

[39] "Pt16_reTx_POD126_PBMC_2nd_Donor_CD45"   "Pt16_reTx_POD32_Bx"

[41] "Pt16_reTx_POD33_PBMC"   "Pt16_reTx_POD8_PBMC"

Sample name (Pt: patient)	updated rename
Pt16LITx-GVH-D4L	Pt16_Pre1_Donor1_CD4_CFSElo
Pt16LITx-unstim-D4	Pt16_Pre1_Donor1_CD4_unstim
Pt16LITx-GVH-D8L	Pt16_Pre1_Donor1_CD8_CFSElo
Pt16LITx-unstim-D8	Pt16_Pre1_Donor1_CD8_unstim
Pt16_reTx_MLR-16_2ndD4L_SP_vs_R_POD0_MLN	Pt16_Pre2_Donor2_CD4_CFSElo
Pt16_reTx_2ndD4U_SP	Pt16_Pre2_Donor2_CD4_unstim
Pt16_reTx_MLR-16_2ndD8L_SP_vs_R_POD0_MLN	Pt16_Pre2_Donor2_CD8_CFSElo
Pt16_reTx_2ndD8U_SP	Pt16_Pre2_Donor2_CD8_unstim
Pt16LITx-PBMCs-POD9	Pt16_Post1_PBMC_day0009
Pt16_LITx_PBMC_POD463	Pt16_Post1_PBMC_day0463
Pt16_reTx_POD0_PBMC_POD786_of_1st_Tx	Pt16_Post1_PBMC_day0786_Post2_day0000
Pt16_reTx_POD126_BM	Pt16_Post2_BM_day0126
Pt16_reTx_POD14_Bx	Pt16_Post2_ileum_day0014
Pt16_reTx_POD32_Bx	Pt16_Post2_ileum_day0032
Pt16_reTx_POD126_Bx_ileum	Pt16_Post2_ileum_day0126
Pt16_reTx_POD494_ileum stoma	Pt16_Post2_ileum_day0494
Pt16_reTx_POD8_PBMC	Pt16_Post2_PBMC_day0008
Pt16_reTx_POD33_PBMC	Pt16_Post2_PBMC_day0033
Pt16_reTx_POD126_PBMC	Pt16_Post2_PBMC_day0126
Pt16_reTx_POD285_PBMC	Pt16_Post2_PBMC_day0285
Pt16_reTx_POD494_PBMC	Pt16_Post2_PBMC_day0494
Pt16LITx-unstim-R4	Pt16_Pre1_Recipient_CD4_unstim
Pt16LITx-unstim-R8	Pt16_Pre1_Recipient_CD8_unstim
Pt16LITx-HVG-R8L	Pt16_Pre1_Recipient_CD4_CFSElo_vs_Donor1
Pt16LITx-HVG-R4L	Pt16_Pre1_Recipient_CD8_CFSElo_vs_Donor1
Pt16_reTx_MLR-17_R_POD0_aLN_R4L_vs_2ndD_SP	Pt16_Pre1_Recipient_CD4_CFSElo_vs_Donor2
Pt16_reTx_MLR-17_R_POD0_aLN_R8L_vs_2ndD_SP	Pt16_Pre1_Recipient_CD8_CFSElo_vs_Donor2

#2nd Tx, Pt16" MVTx

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D24U vs D28U

normalize(data[,c(18,19)])

ratio=5

  c1indices=which(data[,18]>0 & data[,19]>0 & data[,18]>ratio*data[,19])

  c2indices=which(data[,18]>0 & data[,19]>0 & data[,19]>ratio*data[,18])

  ambiindices=which(data[,18]>0 & data[,19]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,19]=0

  data[c2indices,18]=0

  data[ambiindices,c(18,19)]=0

#D24L vs D28L

normalize(data[,c(21,22)])

ratio=5

  c1indices=which(data[,21]>0 & data[,22]>0 & data[,21]>ratio*data[,22])

  c2indices=which(data[,21]>0 & data[,22]>0 & data[,22]>ratio*data[,21])

  ambiindices=which(data[,21]>0 & data[,22]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,22]=0

  data[c2indices,21]=0

  data[ambiindices,c(21,22)]=0

```
#R4U vs R8U

normalize(data[,c(11,12)])

ratio=5

 c1indices=which(data[,11]>0 & data[,12]>0 & data[,11]>ratio*data[,12])

 c2indices=which(data[,11]>0 & data[,12]>0 & data[,12]>ratio*data[,11])

 ambiindices=which(data[,11]>0 & data[,12]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,12]=0

 data[c2indices,11]=0

 data[ambiindices,c(11,12)]=0




#R4L(D2) vs R8L (D2)

normalize(data[,c(23,24)])

ratio=5

 c1indices=which(data[,23]>0 & data[,24]>0 & data[,23]>ratio*data[,24])

 c2indices=which(data[,23]>0 & data[,24]>0 & data[,24]>ratio*data[,23])

 ambiindices=which(data[,23]>0 & data[,24]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,24]=0

 data[c2indices,23]=0

 data[ambiindices,c(23,24)]=0


rownames(data)=data[,1]

x=data
```

#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by CFSE-MLR sorting error

ambiguous=which((x[,18]>0 | x[,19]>0)&(x[,11]>0 | x[12]>0))

ambiguous=union(ambiguous,which((x[,18]>0 | x[,19]>0)&(x[,23]>0 | x[,24]>0)))

ambiguous=union(ambiguous,which((x[,11]>0 | x[,12]>0)&(x[,21]>0 | x[,22]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]

#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L, R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional step of optimization for CD4 and CD8 sorting error.

#R4L(D2) vs R8U

normalize(x[,c(23,12)])

ratio=2

  c1indices=which(x[,23]>0 & x[,12]>0 & x[,23]>ratio*x[,12])

  c2indices=which(x[,23]>0 & x[,12]>0 & x[,12]>ratio*x[,23])

  ambiindices=which(x[,23]>0 & x[,12]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,12]=0

  x[c2indices,23]=0

  x[ambiindices,c(23,12)]=0

#R8L(D2) vs R4U

normalize(x[,c(24,11)])

ratio=2

```
c1indices=which(x[,24]>0 & x[,11]>0 & x[,24]>ratio*x[,11])

c2indices=which(x[,24]>0 & x[,11]>0 & x[,11]>ratio*x[,24])

ambiindices=which(x[,24]>0 & x[,11]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

x[c1indices,11]=0

x[c2indices,24]=0

x[ambiindices,c(24,11)]=0




#D24L vs D28U

normalize(x[,c(21,19)])

ratio=2

 c1indices=which(x[,21]>0 & x[,19]>0 & x[,21]>ratio*x[,19])

 c2indices=which(x[,21]>0 & x[,19]>0 & x[,19]>ratio*x[,21])

 ambiindices=which(x[,21]>0 & x[,19]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,19]=0

 x[c2indices,21]=0

 x[ambiindices,c(21,19)]=0




#D28L vs D24U

normalize(x[,c(22,18)])

ratio=2

 c1indices=which(x[,22]>0 & x[,18]>0 & x[,22]>ratio*x[,18])

 c2indices=which(x[,22]>0 & x[,18]>0 & x[,18]>ratio*x[,22])

 ambiindices=which(x[,22]>0 & x[,18]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
```

```
  x[c1indices,18]=0

  x[c2indices,22]=0

  x[ambiindices,c(22,18)]=0
```

write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 MVTx 06192019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T, sep="\t")

add "fold=2" below, use 2 fold expansion (default 5 fold)

add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for read counts

#HVG2 direction

For CD4 HvG2, cd4.HVG2=x[,c(unstim(R4U),stim(R4L))]

For CD8 HvG2, cd8.HVG2=x[,c(unstim(R8U),stim(R8L))]

cd4.HVG2= x[,c(11,23)]

cd8.HVG2= x[,c(12,24)]

allo.HVG2=listAlloreactive(cd4.HVG2,cd8.HVG2, fold=2, freq=0.00002)

length(allo.HVG2[[1]])

[1] 6989

length(allo.HVG2[[2]])

[1] 6442

write.table(allo.HVG2[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 CD4 HVG2list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG2[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 CD8 HVG2list.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,11]+x[,23])>0,])

rCD8mappable=rownames(x[(x[,12]+x[,24])>0,])

CD4nonHVG2=setdiff(rCD4mappable,allo.HVG2[[1]])

CD8nonHVG2=setdiff(rCD8mappable,allo.HVG2[[2]])

write.table(CD4nonHVG2,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 CD4nonHVG2 rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG2,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 CD8nonHVG2 rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 164727

length(rCD8mappable)

[1] 140789

length(CD4nonHVG2)

[1] 157738

length(CD8nonHVG2)

[1] 134347

rmappable=rownames(x[(x[,11]+x[,23]+x[,12]+x[,24])>0,])

length(rmappable)

[1] 305516

write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)+length(rCD8mappable)

[1] 305516

length(intersect(rCD4mappable,rCD8mappable))

[1] 0

#G2VH direction

For CD4 G2VH, cd4.G2VH=x[,c(unstim(D4U),stim(D4L))]

For CD8 G2VH, cd8.G2VH=x[,c(unstim(D8U),stim(D8L))]

cd4.G2VH= x[,c(18,21)]

cd8.G2VH= x[,c(19,22)]

allo.G2VH=listAlloreactive(cd4.G2VH,cd8.G2VH, fold=2, freq=0.00002)

length(allo.G2VH[[1]])

[1] 665

length(allo.G2VH[[2]])

[1] 1262

write.table(allo.G2VH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 CD4 G2VHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.G2VH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 CD8 G2VHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

d2CD4mappable=rownames(x[(x[,18]+x[,21])>0,])

d2CD8mappable=rownames(x[(x[,19]+x[,22])>0,])

CD4nonG2VH=setdiff(d1CD4mappable,allo.G2VH[[1]])

CD8nonG2VH=setdiff(d1CD8mappable,allo.G2VH[[2]])

write.table(CD4nonG2VH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 CD4nonG2VH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonG2VH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 CD8nonG2VH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(d2CD4mappable)

[1] 145522

length(d2CD8mappable)

[1] 106369

length(CD4nonG2VH)

[1] 55875

length(CD8nonG2VH)

[1] 52063

d2mappable=rownames(x[(x[,18]+x[,21]+x[,19]+x[,22])>0,])

length(d2mappable)

[1] 251891

write.table(d2mappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 donor2 mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(d2CD4mappable)+length(d2CD8mappable)

[1] 251891

length(intersect(d2CD4mappable,d2CD8mappable))

[1] 0

```
# venn diagram of Pt16" POD126

#a=Bx=35, b=PBMC=37, C=BM=31


a=rownames(x[x[,35]>0,])

b=rownames(x[x[,37]>0,])

c=rownames(x[x[,31]>0,])

venn_diagram(a,b,c)


[1] "a:"   "9644"

[1] "b:"   "12709"

[1] "c:"   "21207"

[1] "a,b:" "108"

[1] "a,c:" "88"

[1] "b,c:" "2375"

[1] "a,b,c:" "275"



#Pt16" POD14 Bx GVH

cd4= x[,c(18,21,20)]

cd8= x[,c(19,22,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(18,21)]

cd8= x[,c(19,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))
```

```
intersect(allo[[2]], rownames(x[x[,20]>0,]))


#Pt16" POD14 Bx HVG

cd4= x[,c(11,23,20)]

cd8= x[,c(12,24,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,23)]

cd8= x[,c(12,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))


#Pt16" POD32 Bx GVH

cd4= x[,c(18,21,40)]

cd8= x[,c(19,22,40)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(18,21)]

cd8= x[,c(19,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,40]>0,]))

intersect(allo[[2]], rownames(x[x[,40]>0,]))
```

```
#Pt16" POD32 Bx HVG

cd4= x[,c(11,23,40)]

cd8= x[,c(12,24,40)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,23)]

cd8= x[,c(12,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,40]>0,]))

intersect(allo[[2]], rownames(x[x[,40]>0,]))




#Pt16" POD126 Bx GVH

cd4= x[,c(18,21,35)]

cd8= x[,c(19,22,35)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(18,21)]

cd8= x[,c(19,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,35]>0,]))

intersect(allo[[2]], rownames(x[x[,35]>0,]))
```

```
#Pt16" POD126 Bx HVG

cd4= x[,c(11,23,35)]

cd8= x[,c(12,24,35)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,23)]

cd8= x[,c(12,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,35]>0,]))

intersect(allo[[2]], rownames(x[x[,35]>0,]))




#Pt16" POD494 ileum stoma GVH

cd4= x[,c(18,21,16)]

cd8= x[,c(19,22,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(18,21)]

cd8= x[,c(19,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))




#Pt16" POD494 ileum stoma HVG
```

```
cd4= x[,c(11,23,16)]

cd8= x[,c(12,24,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,23)]

cd8= x[,c(12,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))



#Pt16" POD8 PBMC GVH

cd4= x[,c(18,21,42)]

cd8= x[,c(19,22,42)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(18,21)]

cd8= x[,c(19,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,42]>0,]))

intersect(allo[[2]], rownames(x[x[,42]>0,]))



#Pt16" POD8 PBMC HVG

cd4= x[,c(11,23,42)]
```

```
cd8= x[,c(12,24,42)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,23)]

cd8= x[,c(12,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,42]>0,]))

intersect(allo[[2]], rownames(x[x[,42]>0,]))


#Pt16" POD33 PBMC GVH

cd4= x[,c(18,21,41)]

cd8= x[,c(19,22,41)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(18,21)]

cd8= x[,c(19,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,41]>0,]))

intersect(allo[[2]], rownames(x[x[,41]>0,]))


#Pt16" POD33 PBMC HVG

cd4= x[,c(11,23,41)]

cd8= x[,c(12,24,41)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,23)]

cd8= x[,c(12,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,41]>0,]))

intersect(allo[[2]], rownames(x[x[,41]>0,]))




#Pt16" POD126 PBMC GVH

cd4= x[,c(18,21,37)]

cd8= x[,c(19,22,37)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(18,21)]

cd8= x[,c(19,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,37]>0,]))

intersect(allo[[2]], rownames(x[x[,37]>0,]))




#Pt16" POD126 PBMC HVG

cd4= x[,c(11,23,37)]

cd8= x[,c(12,24,37)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,23)]

cd8= x[,c(12,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,37]>0,]))

intersect(allo[[2]], rownames(x[x[,37]>0,]))




#Pt16" POD126 BM GVH

cd4= x[,c(18,21,31)]

cd8= x[,c(19,22,31)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(18,21)]

cd8= x[,c(19,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,31]>0,]))

intersect(allo[[2]], rownames(x[x[,31]>0,]))




#Pt16" POD126 BM HVG

cd4= x[,c(11,23,31)]

cd8= x[,c(12,24,31)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,23)]

cd8= x[,c(12,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,31]>0,]))

intersect(allo[[2]], rownames(x[x[,31]>0,]))



#Pt16" POD126 BM 1st donor CD45 GVH

cd4= x[,c(18,21,32)]

cd8= x[,c(19,22,32)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(18,21)]

cd8= x[,c(19,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,32]>0,]))

intersect(allo[[2]], rownames(x[x[,32]>0,]))



#Pt16" POD126 BM 1st donor CD45 HVG

cd4= x[,c(11,23,32)]

cd8= x[,c(12,24,32)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,23)]

cd8= x[,c(12,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,32]>0,]))

intersect(allo[[2]], rownames(x[x[,32]>0,]))



#Pt16" POD126 BM 2nd donor CD45 GVH

cd4= x[,c(18,21,33)]

cd8= x[,c(19,22,33)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(18,21)]

cd8= x[,c(19,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,33]>0,]))

intersect(allo[[2]], rownames(x[x[,33]>0,]))



#Pt16" POD126 BM 2nd donor CD45 HVG

cd4= x[,c(11,23,33)]

cd8= x[,c(12,24,33)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(11,23)]

cd8= x[,c(12,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,33]>0,]))

intersect(allo[[2]], rownames(x[x[,33]>0,]))


#Pt16" POD285 PBMC GVH

cd4= x[,c(18,21,29)]

cd8= x[,c(19,22,29)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(18,21)]

cd8= x[,c(19,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,29]>0,]))

intersect(allo[[2]], rownames(x[x[,29]>0,]))


#Pt16" POD285 PBMC HVG

cd4= x[,c(11,23,29)]

cd8= x[,c(12,24,29)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(11,23)]

cd8= x[,c(12,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,29]>0,]))

intersect(allo[[2]], rownames(x[x[,29]>0,]))
```

```
#Pt16'' POD494 PBMC GVH

cd4= x[,c(18,21,15)]

cd8= x[,c(19,22,15)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(18,21)]

cd8= x[,c(19,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))
```

```
#Pt16'' POD494 PBMC HVG

cd4= x[,c(11,23,15)]

cd8= x[,c(12,24,15)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt16 reTx/Pt16 MVTx 06192019 2x.out' )
```

```
rownames(x)=x[,1]
cd4= x[,c(11,23)]
cd8= x[,c(12,24)]
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)
intersect(allo[[1]], rownames(x[x[,15]>0,]))
intersect(allo[[2]], rownames(x[x[,15]>0,]))
```

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt17 iITx/Pt17 06172019.tsv")

source('P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/! summary of R codes/alloreactivity_printtofile 09132017.R')

names(data)

```
 [1] "Rearrangement"                    "Sum..Templates."
 [3] "Present.In"                       "Itx_Pt17_PBMC_POD267"
 [5] "Pt17_LITx_POD31_PBMC"             "Pt17_iITx_Bx_POD231"
 [7] "Pt17_iITx_Bx_POD472_Native_colon" "Pt17_iITx_Bx_POD472_Tx_colon"
 [9] "Pt17_iITx_Bx_POD472_Tx_ileum"
"Pt17_iITx_Bx_POD584_Native_colon_sorted_T"
[11] "Pt17_iITx_Bx_POD584_Tx_colon_sorted_T"
"Pt17_iITx_Bx_POD584_Tx_ileum_sorted_T"
[13] "Pt17_iITx_Bx_POD662_Native_colon_sorted_T"
"Pt17_iITx_Bx_POD662_Tx_colon_sorted_T"
[15] "Pt17_iITx_Bx_POD662_Tx_ileum_sorted_T"   "Pt17_iITx_PBMC_POD231"
[17] "Pt17_iITx_PBMC_POD662_sorted_T"          "Pt17iITx.Bx.POD17.21"
[19] "Pt17iITx.Bx.POD57"                "Pt17iITx.Bx.POD7"
[21] "Pt17iITx.GVH.D4L"                 "Pt17iITx.GVH.D8L"
[23] "Pt17iITx.HVG.R4L"                 "Pt17iITx.HVG.R8L"
[25] "Pt17iITx.PBMCs.POD18"             "Pt17iITx.unstim.D4"
[27] "Pt17iITx.unstim.D8"               "Pt17iITx.unstim.R4"
[29] "Pt17iITx.unstim.R8"               "Pt17iITx.Bx.POD98"
```

Sample name (Pt: patient)	updated rename
Pt17iITx-GVH-D4L	Pt17_Pre_Donor_CD4_CFSElo
Pt17iITx-unstim-D4	Pt17_Pre_Donor_CD4_unstim
Pt17iITx-GVH-D8L	Pt17_Pre_Donor_CD8_CFSElo

Pt17iITx-unstim-D8	Pt17_Pre_Donor_CD8_unstim
Pt17iITx-Bx-POD7	Pt17_Post_ileum_day0007
Pt17iITx-Bx-POD17+21	Pt17_Post_ileum_day0017and0021
Pt17iITx-Bx-POD57	Pt17_Post_ileum_day0057
Pt17iITx-Bx-POD98	Pt17_Post_ileum_day0098
Pt17_iITx_Bx_POD231	Pt17_Post_ileum_day0231
Pt17_iITx_Bx_POD472_Tx_ileum	Pt17_Post_ileum_day0472
Pt17_iITx_Bx_POD584_Tx_ileum_sorted_T	Pt17_Post_ileum_day0584
Pt17_iITx_Bx_POD662_Tx_ileum_sorted_T	Pt17_Post_ileum_day0662
Pt17iITx-PBMCs-POD18	Pt17_Post_PBMC_day0018
Pt17iITx-PBMCs-POD31	Pt17_Post_PBMC_day0031
Pt17_iITx_PBMC_POD231	Pt17_Post_PBMC_day0231
Itx_Pt17_PBMC_POD267	Pt17_Post_PBMC_day0267
Pt17_iITx_PBMC_POD662_sorted T	Pt17_Post_PBMC_day0662
Pt17iITx-HVG-R4L	Pt17_Pre_Recipient_CD4_CFSElo
Pt17iITx-unstim-R4	Pt17_Pre_Recipient_CD4_unstim
Pt17iITx-HVG-R8L	Pt17_Pre_Recipient_CD8_CFSElo
Pt17iITx-unstim-R8	Pt17_Pre_Recipient_CD8_unstim

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

normalize(data[,c(26,27)])

ratio=5

 c1indices=which(data[,26]>0 & data[,27]>0 & data[,26]>ratio*data[,27])

 c2indices=which(data[,26]>0 & data[,27]>0 & data[,27]>ratio*data[,26])

 ambiindices=which(data[,26]>0 & data[,27]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,27]=0

 data[c2indices,26]=0

 data[ambiindices,c(26,27)]=0

```
#D4L vs D8L
normalize(data[,c(21,22)])
ratio=5
  c1indices=which(data[,21]>0 & data[,22]>0 & data[,21]>ratio*data[,22])
  c2indices=which(data[,21]>0 & data[,22]>0 & data[,22]>ratio*data[,21])
  ambiindices=which(data[,21]>0 & data[,22]>0)
  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
  data[c1indices,22]=0
  data[c2indices,21]=0
  data[ambiindices,c(21,22)]=0


#R4U vs R8U
normalize(data[,c(28,29)])
ratio=5
  c1indices=which(data[,28]>0 & data[,29]>0 & data[,28]>ratio*data[,29])
  c2indices=which(data[,28]>0 & data[,29]>0 & data[,29]>ratio*data[,28])
  ambiindices=which(data[,28]>0 & data[,29]>0)
  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
  data[c1indices,29]=0
  data[c2indices,28]=0
  data[ambiindices,c(28,29)]=0


#R4L vs R8L
normalize(data[,c(23,24)])
ratio=5
  c1indices=which(data[,23]>0 & data[,24]>0 & data[,23]>ratio*data[,24])
```

```
c2indices=which(data[,23]>0 & data[,24]>0 & data[,24]>ratio*data[,23])

ambiindices=which(data[,23]>0 & data[,24]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

data[c1indices,24]=0

data[c2indices,23]=0

data[ambiindices,c(23,24)]=0


rownames(data)=data[,1]

x=data


#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,26]>0 | x[,27]>0)&(x[,28]>0 | x[,29]>0))

ambiguous=union(ambiguous,which((x[,26]>0 | x[,27]>0)&(x[,23]>0 | x[,24]>0)))

ambiguous=union(ambiguous,which((x[,28]>0 | x[,29]>0)&(x[,21]>0 | x[,22]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]


#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L,
R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional
step of optimization for CD4 and CD8 sorting error.


#R4L vs R8U

normalize(x[,c(23,29)])

ratio=2

 c1indices=which(x[,23]>0 & x[,29]>0 & x[,23]>ratio*x[,29])

 c2indices=which(x[,23]>0 & x[,29]>0 & x[,29]>ratio*x[,23])

 ambiindices=which(x[,23]>0 & x[,29]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,29]=0
```

```
  x[c2indices,23]=0

  x[ambiindices,c(23,29)]=0


#R8L vs R4U

normalize(x[,c(24,28)])

ratio=2

  c1indices=which(x[,24]>0 & x[,28]>0 & x[,24]>ratio*x[,28])

  c2indices=which(x[,24]>0 & x[,28]>0 & x[,28]>ratio*x[,24])

  ambiindices=which(x[,24]>0 & x[,28]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,28]=0

  x[c2indices,24]=0

  x[ambiindices,c(24,28)]=0


#D4L vs D8U

normalize(x[,c(21,27)])

ratio=2

  c1indices=which(x[,21]>0 & x[,27]>0 & x[,21]>ratio*x[,27])

  c2indices=which(x[,21]>0 & x[,27]>0 & x[,27]>ratio*x[,21])

  ambiindices=which(x[,21]>0 & x[,27]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,27]=0

  x[c2indices,21]=0

  x[ambiindices,c(21,27)]=0


#D8L vs D4U

normalize(x[,c(22,26)])

ratio=2
```

```
c1indices=which(x[,22]>0 & x[,26]>0 & x[,22]>ratio*x[,26])

c2indices=which(x[,22]>0 & x[,26]>0 & x[,26]>ratio*x[,22])

ambiindices=which(x[,22]>0 & x[,26]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

x[c1indices,26]=0

x[c2indices,22]=0

x[ambiindices,c(22,26)]=0
```

```
write.table(x,file ="Z:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt17 iITx/Pt17
06182019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T, sep="\t")
```

```
# add "fold=2" below, use 2 fold expansion (default 5 fold)
```

```
# add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for
read counts
```

```
#HVG direction
# For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]
# For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]
cd4.HVG= x[,c(28,23)]
cd8.HVG= x[,c(29,24)]
allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00002)
```

```
length(allo.HVG[[1]])
[1] 14725
length(allo.HVG[[2]])
[1] 10611
```

```
write.table(allo.HVG[[1]],file ="Z:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt17 iITx/Pt17 CD4 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")
```

write.table(allo.HVG[[2]],file ="Z:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt17 iITx/Pt17 CD8 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,28]+x[,23])>0,])

rCD8mappable=rownames(x[(x[,29]+x[,24])>0,])

CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])

write.table(CD4nonHVG,file ="Z:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt17 iITx/Pt17 CD4nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="Z:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt17 iITx/Pt17 CD8nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 196218

length(rCD8mappable)

[1] 141315

length(CD4nonHVG)

[1] 181493

length(CD8nonHVG)

[1] 130704

rmappable=rownames(x[(x[,28]+x[,23]+x[,29]+x[,24])>0,])

length(rmappable)

[1] 337533

```
write.table(rmappable,file ="Z:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt17
iITx/Pt17 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")


length(rCD4mappable)+length(rCD8mappable)

[1] 337533

length(intersect(rCD4mappable,rCD8mappable))

[1] 0


#GVH direction

# For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]

# For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]

cd4.GVH= x[,c(26,21)]

cd8.GVH= x[,c(27,22)]

allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00002)


length(allo.GVH[[1]])

[1] 3852

length(allo.GVH[[2]])

[1] 2570


write.table(allo.GVH[[1]],file ="Z:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt17 iITx/Pt17 CD4 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="Z:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt17 iITx/Pt17 CD8 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")


dCD4mappable=rownames(x[(x[,26]+x[,21])>0,])

dCD8mappable=rownames(x[(x[,27]+x[,22])>0,])
```

CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])

CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])

write.table(CD4nonGVH,file ="Z:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt17 iITx/Pt17 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonGVH,file ="Z:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt17 iITx/Pt17 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)

[1] 139463

length(dCD8mappable)

[1] 97000

length(CD4nonGVH)

[1] 135611

length(CD8nonGVH)

[1] 94430

dmappable=rownames(x[(x[,26]+x[,21]+x[,27]+x[,22])>0,])

length(dmappable)

[1] 236463

write.table(dmappable,file ="Z:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt17 iITx/Pt17 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)+length(dCD8mappable)

[1] 236463

```
length(intersect(dCD4mappable,dCD8mappable))

[1] 0
```

#Pt17 POD7 Bx GVH

```
cd4= x[,c(26,21,20)]

cd8= x[,c(27,22,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(26,21)]

cd8= x[,c(27,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))
```

#Pt17 POD7 Bx HVG

```
cd4= x[,c(28,23,20)]

cd8= x[,c(29,24,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,23)]

cd8= x[,c(29,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))
```

```
#Pt17 POD17-21 Bx GVH

cd4= x[,c(26,21,18)]

cd8= x[,c(27,22,18)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(26,21)]

cd8= x[,c(27,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,18]>0,]))

intersect(allo[[2]], rownames(x[x[,18]>0,]))


#Pt17 POD17-21 Bx HVG

cd4= x[,c(28,23,18)]

cd8= x[,c(29,24,18)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,23)]

cd8= x[,c(29,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,18]>0,]))

intersect(allo[[2]], rownames(x[x[,18]>0,]))
```

```
#Pt17 POD57 Bx GVH

cd4= x[,c(26,21,19)]

cd8= x[,c(27,22,19)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(26,21)]

cd8= x[,c(27,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,19]>0,]))

intersect(allo[[2]], rownames(x[x[,19]>0,]))


#Pt17 POD57 Bx HVG

cd4= x[,c(28,23,19)]

cd8= x[,c(29,24,19)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,23)]

cd8= x[,c(29,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,19]>0,]))

intersect(allo[[2]], rownames(x[x[,19]>0,]))


#Pt17 POD98 Bx GVH

cd4= x[,c(26,21,30)]

cd8= x[,c(27,22,30)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(26,21)]

cd8= x[,c(27,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,30]>0,]))

intersect(allo[[2]], rownames(x[x[,30]>0,]))


#Pt17 POD98 Bx HVG

cd4= x[,c(28,23,30)]

cd8= x[,c(29,24,30)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,23)]

cd8= x[,c(29,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,30]>0,]))

intersect(allo[[2]], rownames(x[x[,30]>0,]))


#Pt17 POD231 Bx GVH

cd4= x[,c(26,21,6)]

cd8= x[,c(27,22,6)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(26,21)]

cd8= x[,c(27,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,6]>0,]))

intersect(allo[[2]], rownames(x[x[,6]>0,]))


#Pt17 POD231 Bx HVG

cd4= x[,c(28,23,6)]

cd8= x[,c(29,24,6)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,23)]

cd8= x[,c(29,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,6]>0,]))

intersect(allo[[2]], rownames(x[x[,6]>0,]))


#Pt17 POD472 ileum Bx GVH

cd4= x[,c(26,21,9)]

cd8= x[,c(27,22,9)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(26,21)]

cd8= x[,c(27,22)]
```

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,9]>0,]))

intersect(allo[[2]], rownames(x[x[,9]>0,]))


#Pt17 POD472 ileum Bx HVG

cd4= x[,c(28,23,9)]

cd8= x[,c(29,24,9)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,23)]

cd8= x[,c(29,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,9]>0,]))

intersect(allo[[2]], rownames(x[x[,9]>0,]))


#Pt17 POD584 ileum Bx GVH

cd4= x[,c(26,21,12)]

cd8= x[,c(27,22,12)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(26,21)]

cd8= x[,c(27,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,12]>0,]))

intersect(allo[[2]], rownames(x[x[,12]>0,]))
```

```
#Pt17 POD584 ileum Bx HVG

cd4= x[,c(28,23,12)]

cd8= x[,c(29,24,12)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,23)]

cd8= x[,c(29,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,12]>0,]))

intersect(allo[[2]], rownames(x[x[,12]>0,]))



#Pt17 POD662 ileum Bx GVH

cd4= x[,c(26,21,15)]

cd8= x[,c(27,22,15)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(26,21)]

cd8= x[,c(27,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))
```

```
#Pt17 POD662 ileum Bx HVG

cd4= x[,c(28,23,15)]

cd8= x[,c(29,24,15)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,23)]

cd8= x[,c(29,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))




#Pt17 POD18 PBMC GVH

cd4= x[,c(26,21,25)]

cd8= x[,c(27,22,25)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(26,21)]

cd8= x[,c(27,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,25]>0,]))

intersect(allo[[2]], rownames(x[x[,25]>0,]))
```

```
#Pt17 POD18 PBMC HVG

cd4= x[,c(28,23,25)]

cd8= x[,c(29,24,25)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,23)]

cd8= x[,c(29,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,25]>0,]))

intersect(allo[[2]], rownames(x[x[,25]>0,]))



#Pt17 POD31 PBMC GVH

cd4= x[,c(26,21,5)]

cd8= x[,c(27,22,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(26,21)]

cd8= x[,c(27,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))



#Pt17 POD31 PBMC HVG
```

```
cd4= x[,c(28,23,5)]

cd8= x[,c(29,24,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,23)]

cd8= x[,c(29,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))




#Pt17 POD231 PBMC GVH

cd4= x[,c(26,21,16)]

cd8= x[,c(27,22,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(26,21)]

cd8= x[,c(27,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))




#Pt17 POD231 PBMC HVG
```

```
cd4= x[,c(28,23,16)]

cd8= x[,c(29,24,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,23)]

cd8= x[,c(29,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))



#Pt17 POD267 PBMC GVH

cd4= x[,c(26,21,4)]

cd8= x[,c(27,22,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(26,21)]

cd8= x[,c(27,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))



#Pt17 POD267 PBMC HVG

cd4= x[,c(28,23,4)]
```

```
cd8= x[,c(29,24,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,23)]

cd8= x[,c(29,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))



#Pt17 POD662 PBMC GVH

cd4= x[,c(26,21,17)]

cd8= x[,c(27,22,17)]

run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(26,21)]

cd8= x[,c(27,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))



#Pt17 POD662 PBMC HVG

cd4= x[,c(28,23,17)]

cd8= x[,c(29,24,17)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='Z:/CCTI_USERS/Jianing Fu/Adaptive samples
and analysis/Pt17 iITx/Pt17 06182019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,23)]

cd8= x[,c(29,24)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))
```

#Pt18

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt18 MVTx/Pt18 05232019.tsv")

source('Z:/Jianing/10x scRNA-seq TCR-seq/Pt15_MJ001, MJ004/alloreactivity_printtofile 09132017.R')

names(data)

[1] "Rearrangement"   "Sum..Templates."   "Present.In"

 [4] "Pt18_MVTx_BM_POD357"   "Pt18_MVTx_BM_POD357_Donor_CD45." "Pt18_MVTx_Bx_POD105"

 [7] "Pt18_MVTx_Bx_POD18"   "Pt18_MVTx_Bx_POD307"   "Pt18_MVTx_Bx_POD35"

[10] "Pt18_MVTx_Bx_POD5.7"   "Pt18_MVTx_Bx_Tx_colon_POD357" "Pt18_MVTx_Bx_duodenum_POD357"

[13] "Pt18_MVTx_Bx_ileum_POD357"   "Pt18_MVTx_Bx_native_colon_POD357" "Pt18_MVTx_Bx_stomach_POD357"

[16] "Pt18_MVTx_PBMC_POD14"   "Pt18_MVTx_PBMC_POD28"   "Pt18_MVTx_PBMC_POD314"

[19] "Pt18_MVTx_PBMC_POD357"   "Pt18_MVTx_PBMC_POD357_Donor_CD45." "Pt18_MVTx_PBMC_POD5"

[22] "Pt18_MVTx_PBMC_POD7"   "Pt18_MVTx_PBMC_POD98"   "Pt18_MVTx_SP_D4U"

[25] "Pt18_MVTx_SP_D8U"   "Pt18_MVTx_SP_GVH_D4L"   "Pt18_MVTx_SP_GVH_D8L"

[28] "Pt18_MVTx_SP_HVG_R4L"   "Pt18_MVTx_SP_HVG_R8L"   "Pt18_MVTx_SP_R4U"

[31] "Pt18_MVTx_SP_R8U"

Sample name (Pt: patient)	updated rename
Pt18_MVTx_SP_GVH_D4L	Pt18_Pre_Donor_CD4_CFSElo
Pt18_MVTx_SP_D4U	Pt18_Pre_Donor_CD4_unstim
Pt18_MVTx_SP_GVH_D8L	Pt18_Pre_Donor_CD8_CFSElo
Pt18_MVTx_SP_D8U	Pt18_Pre_Donor_CD8_unstim
Pt18_MVTx_BM_POD357	Pt18_Post_BM_day0357
Pt18_MVTx_Bx_POD5+7	Pt18_Post_ileum_day0005and0007
Pt18_MVTx_Bx_POD18	Pt18_Post_ileum_day0018
Pt18_MVTx_Bx_POD35	Pt18_Post_ileum_day0035
Pt18_MVTx_Bx_POD105	Pt18_Post_ileum_day0105
Pt18_MVTx_Bx_POD307	Pt18_Post_ileum_day0307
Pt18_MVTx_Bx_ileum_POD357	Pt18_Post_ileum_day0357
Pt18_MVTx_PBMC_POD5	Pt18_Post_PBMC_day0005
Pt18_MVTx_PBMC_POD7	Pt18_Post_PBMC_day0007
Pt18_MVTx_PBMC_POD14	Pt18_Post_PBMC_day0014
Pt18_MVTx_PBMC_POD28	Pt18_Post_PBMC_day0028
Pt18_MVTx_PBMC_POD98	Pt18_Post_PBMC_day0098
Pt18_MVTx_PBMC_POD314	Pt18_Post_PBMC_day0314
Pt18_MVTx_PBMC_POD357	Pt18_Post_PBMC_day0357
Pt18_MVTx_SP_HVG_R4L	Pt18_Pre_Recipient_CD4_CFSElo
Pt18_MVTx_SP_R4U	Pt18_Pre_Recipient_CD4_unstim
Pt18_MVTx_SP_HVG_R8L	Pt18_Pre_Recipient_CD8_CFSElo
Pt18_MVTx_SP_R8U	Pt18_Pre_Recipient_CD8_unstim

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

normalize(data[,c(24,25)])

ratio=5

 c1indices=which(data[,24]>0 & data[,25]>0 & data[,24]>ratio*data[,25])

 c2indices=which(data[,24]>0 & data[,25]>0 & data[,25]>ratio*data[,24])

 ambiindices=which(data[,24]>0 & data[,25]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,25]=0

```
data[c2indices,24]=0

data[ambiindices,c(24,25)]=0



#D4L vs D8L

normalize(data[,c(26,27)])

ratio=5

 c1indices=which(data[,26]>0 & data[,27]>0 & data[,26]>ratio*data[,27])

 c2indices=which(data[,26]>0 & data[,27]>0 & data[,27]>ratio*data[,26])

 ambiindices=which(data[,26]>0 & data[,27]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,27]=0

 data[c2indices,26]=0

 data[ambiindices,c(26,27)]=0



#R4U vs R8U

normalize(data[,c(30,31)])

ratio=5

 c1indices=which(data[,30]>0 & data[,31]>0 & data[,30]>ratio*data[,31])

 c2indices=which(data[,30]>0 & data[,31]>0 & data[,31]>ratio*data[,30])

 ambiindices=which(data[,30]>0 & data[,31]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,31]=0

 data[c2indices,30]=0

 data[ambiindices,c(30,31)]=0
```

```
#R4L vs R8L

normalize(data[,c(28,29)])

ratio=5

  c1indices=which(data[,28]>0 & data[,29]>0 & data[,28]>ratio*data[,29])

  c2indices=which(data[,28]>0 & data[,29]>0 & data[,29]>ratio*data[,28])

  ambiindices=which(data[,28]>0 & data[,29]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,29]=0

  data[c2indices,28]=0

  data[ambiindices,c(28,29)]=0


rownames(data)=data[,1]

x=data


#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,24]>0 | x[,25]>0)&(x[,30]>0 | x[,31]>0))

ambiguous=union(ambiguous,which((x[,24]>0 | x[,25]>0)&(x[,28]>0 | x[,29]>0)))

ambiguous=union(ambiguous,which((x[,30]>0 | x[,31]>0)&(x[,26]>0 | x[,27]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]


#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L,
R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional
step of optimization for CD4 and CD8 sorting error.


#R4L vs R8U

normalize(x[,c(28,31)])

ratio=2

  c1indices=which(x[,28]>0 & x[,31]>0 & x[,28]>ratio*x[,31])
```

```
c2indices=which(x[,28]>0 & x[,31]>0 & x[,31]>ratio*x[,28])
ambiindices=which(x[,28]>0 & x[,31]>0)
ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
x[c1indices,31]=0
x[c2indices,28]=0
x[ambiindices,c(28,31)]=0


#R8L vs R4U
normalize(x[,c(29,30)])
ratio=2
 c1indices=which(x[,29]>0 & x[,30]>0 & x[,29]>ratio*x[,30])
 c2indices=which(x[,29]>0 & x[,30]>0 & x[,30]>ratio*x[,29])
 ambiindices=which(x[,29]>0 & x[,30]>0)
 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
 x[c1indices,30]=0
 x[c2indices,29]=0
 x[ambiindices,c(29,30)]=0


#D4L vs D8U
normalize(x[,c(26,25)])
ratio=2
 c1indices=which(x[,26]>0 & x[,25]>0 & x[,26]>ratio*x[,25])
 c2indices=which(x[,26]>0 & x[,25]>0 & x[,25]>ratio*x[,26])
 ambiindices=which(x[,26]>0 & x[,25]>0)
 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
 x[c1indices,25]=0
 x[c2indices,26]=0
 x[ambiindices,c(26,25)]=0
```

```
#D8L vs D4U

normalize(x[,c(27,24)])

ratio=2

 c1indices=which(x[,27]>0 & x[,24]>0 & x[,27]>ratio*x[,24])

 c2indices=which(x[,27]>0 & x[,24]>0 & x[,24]>ratio*x[,27])

 ambiindices=which(x[,27]>0 & x[,24]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,24]=0

 x[c2indices,27]=0

 x[ambiindices,c(27,24)]=0


write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt18
MVTx/Pt18 05242019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T,
sep="\t")


# add "fold=2" below, use 2 fold expansion (default 5 fold)

# add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for
read counts


#HVG direction

# For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]

# For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]

cd4.HVG= x[,c(30,28)]

cd8.HVG= x[,c(31,29)]

allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00002)


length(allo.HVG[[1]])

[1] 8469
```

length(allo.HVG[[2]])

[1] 12805

write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt18 MVTx/Pt18 CD4 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt18 MVTx/Pt18 CD8 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,30]+x[,28])>0,])

rCD8mappable=rownames(x[(x[,31]+x[,29])>0,])

CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])

write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt18 MVTx/Pt18 CD4nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt18 MVTx/Pt18 CD8nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 46548

length(rCD8mappable)

[1] 145033

length(CD4nonHVG)

[1] 38079

length(CD8nonHVG)

[1] 132228

rmappable=rownames(x[(x[,30]+x[,28]+x[,31]+x[,29])>0,])

length(rmappable)

[1] 191581

write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt18 MVTx/Pt18 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)+length(rCD8mappable)

[1] 191581

length(intersect(rCD4mappable,rCD8mappable))

[1] 0

#GVH direction

For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]

For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]

cd4.GVH= x[,c(24,26)]

cd8.GVH= x[,c(25,27)]

allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00002)

length(allo.GVH[[1]])

[1] 9305

length(allo.GVH[[2]])

[1] 13259

write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt18 MVTx/Pt18 CD4 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt18 MVTx/Pt18 CD8 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

dCD4mappable=rownames(x[(x[,24]+x[,26])>0,])

dCD8mappable=rownames(x[(x[,25]+x[,27])>0,])

CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])

CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])

write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt18 MVTx/Pt18 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt18 MVTx/Pt18 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)

[1] 84428

length(dCD8mappable)

[1] 54370

length(CD4nonGVH)

[1] 75123

length(CD8nonGVH)

[1] 41111

dmappable=rownames(x[(x[,24]+x[,26]+x[,25]+x[,27])>0,])

length(dmappable)

[1] 138798

write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt18 MVTx/Pt18 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)+length(dCD8mappable)

[1] 138798

length(intersect(dCD4mappable,dCD8mappable))

[1] 0

venn diagram of Pt18 POD357

#a=Bx=13, b=PBMC=19, C=BM=4

a=rownames(x[x[,13]>0,])

b=rownames(x[x[,19]>0,])

c=rownames(x[x[,4]>0,])

venn_diagram(a,b,c)

[1] "a:"   "77059"

[1] "b:"   "21400"

[1] "c:"   "3548"

[1] "a,b:" "1679"

[1] "a,c:" "116"

[1] "b,c:" "173"

[1] "a,b,c:" "198"

```
#Pt18 POD5-7 Bx GVH

cd4= x[,c(24,26,10)]

cd8= x[,c(25,27,10)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))


#Pt18 POD5-7 Bx HVG

cd4= x[,c(30,28,10)]

cd8= x[,c(31,29,10)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))


#Pt18 POD18 Bx GVH

cd4= x[,c(24,26,7)]

cd8= x[,c(25,27,7)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,7]>0,]))

intersect(allo[[2]], rownames(x[x[,7]>0,]))


#Pt18 POD18 Bx HVG

cd4= x[,c(30,28,7)]

cd8= x[,c(31,29,7)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,7]>0,]))

intersect(allo[[2]], rownames(x[x[,7]>0,]))


#Pt18 POD35 Bx GVH

cd4= x[,c(24,26,9)]

cd8= x[,c(25,27,9)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,9]>0,]))

intersect(allo[[2]], rownames(x[x[,9]>0,]))


#Pt18 POD35 Bx HVG

cd4= x[,c(30,28,9)]

cd8= x[,c(31,29,9)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,9]>0,]))

intersect(allo[[2]], rownames(x[x[,9]>0,]))


#Pt18 POD105 Bx GVH

cd4= x[,c(24,26,6)]

cd8= x[,c(25,27,6)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]
```

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,6]>0,]))

intersect(allo[[2]], rownames(x[x[,6]>0,]))


#Pt18 POD105 Bx HVG

cd4= x[,c(30,28,6)]

cd8= x[,c(31,29,6)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,6]>0,]))

intersect(allo[[2]], rownames(x[x[,6]>0,]))


#Pt18 POD307 Bx GVH

cd4= x[,c(24,26,8)]

cd8= x[,c(25,27,8)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,8]>0,]))

intersect(allo[[2]], rownames(x[x[,8]>0,]))
```

```
#Pt18 POD307 Bx HVG

cd4= x[,c(30,28,8)]

cd8= x[,c(31,29,8)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,8]>0,]))

intersect(allo[[2]], rownames(x[x[,8]>0,]))


#Pt18 POD357 ileum Bx GVH

cd4= x[,c(24,26,13)]

cd8= x[,c(25,27,13)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,13]>0,]))

intersect(allo[[2]], rownames(x[x[,13]>0,]))


#Pt18 POD357 ileum Bx HVG
```

```
cd4= x[,c(30,28,13)]

cd8= x[,c(31,29,13)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,13]>0,]))

intersect(allo[[2]], rownames(x[x[,13]>0,]))



#Pt18 POD357 BM GVH

cd4= x[,c(24,26,4)]

cd8= x[,c(25,27,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))



#Pt18 POD357 BM HVG

cd4= x[,c(30,28,4)]
```

```
cd8= x[,c(31,29,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))



#Pt18 POD357 BM Donor CD45 GVH

cd4= x[,c(24,26,5)]

cd8= x[,c(25,27,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))



#Pt18 POD357 BM Donor CD45 HVG

cd4= x[,c(30,28,5)]

cd8= x[,c(31,29,5)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt18 POD5 PBMC GVH

cd4= x[,c(24,26,21)]

cd8= x[,c(25,27,21)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,21]>0,]))

intersect(allo[[2]], rownames(x[x[,21]>0,]))


#Pt18 POD5 PBMC HVG

cd4= x[,c(30,28,21)]

cd8= x[,c(31,29,21)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,21]>0,]))

intersect(allo[[2]], rownames(x[x[,21]>0,]))
```

#Pt18 POD7 PBMC GVH

```
cd4= x[,c(24,26,22)]

cd8= x[,c(25,27,22)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))
```

#Pt18 POD7 PBMC HVG

```
cd4= x[,c(30,28,22)]

cd8= x[,c(31,29,22)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]
```

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))
```

```
#Pt18 POD14 PBMC GVH

cd4= x[,c(24,26,16)]

cd8= x[,c(25,27,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))
```

```
#Pt18 POD14 PBMC HVG

cd4= x[,c(30,28,16)]

cd8= x[,c(31,29,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))
```

```
intersect(allo[[2]], rownames(x[x[,16]>0,]))



#Pt18 POD28 PBMC GVH

cd4= x[,c(24,26,17)]

cd8= x[,c(25,27,17)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))



#Pt18 POD28 PBMC HVG

cd4= x[,c(30,28,17)]

cd8= x[,c(31,29,17)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))
```

#Pt18 POD98 PBMC GVH

```
cd4= x[,c(24,26,23)]

cd8= x[,c(25,27,23)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,23]>0,]))

intersect(allo[[2]], rownames(x[x[,23]>0,]))
```


#Pt18 POD98 PBMC HVG

```
cd4= x[,c(30,28,23)]

cd8= x[,c(31,29,23)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,23]>0,]))

intersect(allo[[2]], rownames(x[x[,23]>0,]))
```


#Pt18 POD314 PBMC GVH

```
cd4= x[,c(24,26,18)]
```

```
cd8= x[,c(25,27,18)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,18]>0,]))

intersect(allo[[2]], rownames(x[x[,18]>0,]))


#Pt18 POD314 PBMC HVG

cd4= x[,c(30,28,18)]

cd8= x[,c(31,29,18)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,18]>0,]))

intersect(allo[[2]], rownames(x[x[,18]>0,]))


#Pt18 POD357 PBMC GVH

cd4= x[,c(24,26,19)]

cd8= x[,c(25,27,19)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(24,26)]

cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,19]>0,]))

intersect(allo[[2]], rownames(x[x[,19]>0,]))


#Pt18 POD357 PBMC HVG

cd4= x[,c(30,28,19)]

cd8= x[,c(31,29,19)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,19]>0,]))

intersect(allo[[2]], rownames(x[x[,19]>0,]))


#Pt18 POD357 Donor CD45 PBMC GVH

cd4= x[,c(24,26,20)]

cd8= x[,c(25,27,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(24,26)]
```

```
cd8= x[,c(25,27)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))


#Pt18 POD357 Donor CD45 PBMC HVG

cd4= x[,c(30,28,20)]

cd8= x[,c(31,29,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt18 MVTx/Pt18 05242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(30,28)]

cd8= x[,c(31,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))
```

#Pt19

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt19 MVTx/Pt19 05282019.tsv")

source('Z:/Jianing/10x scRNA-seq TCR-seq/Pt15_MJ001, MJ004/alloreactivity_printtofile 09132017.R')

names(data)

```
 [1] "Rearrangement"              "Sum..Templates."              "Present.In"

 [4] "Pt19_MVTx_BM_POD105"         "Pt19_MVTx_Bx_POD12"
"Pt19_MVTx_Bx_POD22"

 [7] "Pt19_MVTx_Bx_POD43_sorted_T"   "Pt19_MVTx_Bx_Tx_colon_POD356"
"Pt19_MVTx_Bx_duodenum_POD356"

[10] "Pt19_MVTx_Bx_ileum_POD356"      "Pt19_MVTx_Bx_native_colon_POD356"
"Pt19_MVTx_Bx_stomach_POD356"

[13] "Pt19_MVTx_Donor_CD45._BM_POD105" "Pt19_MVTx_PBMC_POD105_sorted_T"
"Pt19_MVTx_PBMC_POD16"

[16] "Pt19_MVTx_PBMC_POD355"          "Pt19_MVTx_PBMC_POD43_sorted_T"
"Pt19_MVTx_PBMC_POD9"

[19] "Pt19_MVTx_POD729_ileum_T"       "Pt19_MVTx_POD734_BM_donor_T"
"Pt19_MVTx_POD734_BM_recipient_T"

[22] "Pt19_MVTx_POD734_PBMC_T"        "Pt19_MVTx_SP_D4U"
"Pt19_MVTx_SP_D8U"

[25] "Pt19_MVTx_SP_GVH_D4L"           "Pt19_MVTx_SP_GVH_D8L"
"Pt19_MVTx_SP_HVG_R4L"

[28] "Pt19_MVTx_SP_HVG_R8L"           "Pt19_MVTx_SP_R4U"
"Pt19_MVTx_SP_R8U"

[31] "Pt19_MVTx_stoma_pieces_POD105"   "Pt19_iITx_Bx_POD127_Tx_ileum"
```

Pt19_MVTx_Donor_CD45+_BM_POD105	Pt19_Post_Donor_BM_sorted_day0105
Pt19_MVTx_SP_GVH_D4L	Pt19_Pre_Donor_CD4_CFSElo
Pt19_MVTx_SP_D4U	Pt19_Pre_Donor_CD4_unstim
Pt19_MVTx_SP_GVH_D8L	Pt19_Pre_Donor_CD8_CFSElo
Pt19_MVTx_SP_D8U	Pt19_Pre_Donor_CD8_unstim
Pt19_MVTx_Recipient_CD45+_BM_POD729	Pt19_Post_Recipient_BM_sorted_day0729
Pt19_MVTx_Bx_POD12	Pt19_Post_ileum_day0012

Pt19_MVTx_Bx_POD22	Pt19_Post_ileum_day0022
Pt19_MVTx_stoma_pieces_POD105	Pt19_Post_ileum_day0105
Pt19_MVTx_Bx_ileum_POD356	Pt19_Post_ileum_day0356
Pt19_MVTx_Bx_ileum_POD729	Pt19_Post_ileum_day0729
Pt19_MVTx_Bx_POD43_sorted_T	Pt19_Post_ileum_day0043
Pt19_MVTx_PBMC_POD9	Pt19_Post_PBMC_day0009
Pt19_MVTx_PBMC_POD16	Pt19_Post_PBMC_day0016
Pt19_MVTx_PBMC_POD355	Pt19_Post_PBMC_day0335
Pt19_MVTx_PBMC_POD734	Pt19_Post_PBMC_day0734
Pt19_MVTx_PBMC_POD43_sorted_T	Pt19_Post_PBMC_day0043
Pt19_MVTx_PBMC_POD105_sorted_T	Pt19_Post_PBMC_day0105
Pt19_MVTx_SP_HVG_R4L	Pt19_Pre_Recipient_CD4_CFSElo
Pt19_MVTx_SP_R4U	Pt19_Pre_Recipient_CD4_unstim
Pt19_MVTx_SP_HVG_R8L	Pt19_Pre_Recipient_CD8_CFSElo
Pt19_MVTx_SP_R8U	Pt19_Pre_Recipient_CD8_unstim

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

normalize(data[,c(23,24)])

ratio=5

 c1indices=which(data[,23]>0 & data[,24]>0 & data[,23]>ratio*data[,24])

 c2indices=which(data[,23]>0 & data[,24]>0 & data[,24]>ratio*data[,23])

 ambiindices=which(data[,23]>0 & data[,24]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,24]=0

 data[c2indices,23]=0

 data[ambiindices,c(23,24)]=0

#D4L vs D8L

```
normalize(data[,c(25,26)])

ratio=5

  c1indices=which(data[,25]>0 & data[,26]>0 & data[,25]>ratio*data[,26])

  c2indices=which(data[,25]>0 & data[,26]>0 & data[,26]>ratio*data[,25])

  ambiindices=which(data[,25]>0 & data[,26]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,26]=0

  data[c2indices,25]=0

  data[ambiindices,c(25,26)]=0




#R4U vs R8U

normalize(data[,c(29,30)])

ratio=5

  c1indices=which(data[,29]>0 & data[,30]>0 & data[,29]>ratio*data[,30])

  c2indices=which(data[,29]>0 & data[,30]>0 & data[,30]>ratio*data[,29])

  ambiindices=which(data[,29]>0 & data[,30]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,30]=0

  data[c2indices,29]=0

  data[ambiindices,c(29,30)]=0




#R4L vs R8L

normalize(data[,c(27,28)])

ratio=5

  c1indices=which(data[,27]>0 & data[,28]>0 & data[,27]>ratio*data[,28])

  c2indices=which(data[,27]>0 & data[,28]>0 & data[,28]>ratio*data[,27])
```

```
ambiindices=which(data[,27]>0 & data[,28]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

data[c1indices,28]=0

data[c2indices,27]=0

data[ambiindices,c(27,28)]=0


rownames(data)=data[,1]

x=data


#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,23]>0 | x[,24]>0)&(x[,29]>0 | x[,30]>0))

ambiguous=union(ambiguous,which((x[,23]>0 | x[,24]>0)&(x[,27]>0 | x[,28]>0)))

ambiguous=union(ambiguous,which((x[,29]>0 | x[,30]>0)&(x[,25]>0 | x[,26]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]


#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L,
R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional
step of optimization for CD4 and CD8 sorting error.


#R4L vs R8U

normalize(x[,c(27,30)])

ratio=2

c1indices=which(x[,27]>0 & x[,30]>0 & x[,27]>ratio*x[,30])

c2indices=which(x[,27]>0 & x[,30]>0 & x[,30]>ratio*x[,27])

ambiindices=which(x[,27]>0 & x[,30]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

x[c1indices,30]=0

x[c2indices,27]=0
```

```
  x[ambiindices,c(27,30)]=0


#R8L vs R4U

normalize(x[,c(28,29)])

ratio=2

  c1indices=which(x[,28]>0 & x[,29]>0 & x[,28]>ratio*x[,29])

  c2indices=which(x[,28]>0 & x[,29]>0 & x[,29]>ratio*x[,28])

  ambiindices=which(x[,28]>0 & x[,29]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,29]=0

  x[c2indices,28]=0

  x[ambiindices,c(28,29)]=0


#D4L vs D8U

normalize(x[,c(25,24)])

ratio=2

  c1indices=which(x[,25]>0 & x[,24]>0 & x[,25]>ratio*x[,24])

  c2indices=which(x[,25]>0 & x[,24]>0 & x[,24]>ratio*x[,25])

  ambiindices=which(x[,25]>0 & x[,24]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,24]=0

  x[c2indices,25]=0

  x[ambiindices,c(25,24)]=0



#D8L vs D4U

normalize(x[,c(26,23)])

ratio=2
```

```
c1indices=which(x[,26]>0 & x[,23]>0 & x[,26]>ratio*x[,23])

c2indices=which(x[,26]>0 & x[,23]>0 & x[,23]>ratio*x[,26])

ambiindices=which(x[,26]>0 & x[,23]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

x[c1indices,23]=0

x[c2indices,26]=0

x[ambiindices,c(26,23)]=0
```

write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt19 MVTx/Pt19 05282019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T, sep="\t")

add "fold=2" below, use 2 fold expansion (default 5 fold)

add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for read counts

#HVG direction

For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]

For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]

cd4.HVG= x[,c(29,27)]

cd8.HVG= x[,c(30,28)]

allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00002)

#When you define allo=listAlloreactive(cd4,cd8),then you should get cd4 alloreactives stored in allo[[1]] and cd8 alloreactives stored in allo[[2]]. If you do allo=union(allo[[1]],allo[[2]]), then allo contains all the alloreactives, and you can define nonallo=setdiff(rownames(x),allo)

length(allo.HVG[[1]])

[1] 13067

length(allo.HVG[[2]])

[1] 10915

write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt19 MVTx/Pt19 CD4 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt19 MVTx/Pt19 CD8 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,29]+x[,27])>0,])

rCD8mappable=rownames(x[(x[,30]+x[,28])>0,])

CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])

write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt19 MVTx/Pt19 CD4nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt19 MVTx/Pt19 CD8nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 156370

length(rCD8mappable)

[1] 142040

length(CD4nonHVG)

[1] 143303

length(CD8nonHVG)

[1] 131125

rmappable=rownames(x[(x[,29]+x[,27]+x[,30]+x[,28])>0,])

length(rmappable)

[1] 298410

write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt19 MVTx/Pt19 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)+length(rCD8mappable)

[1] 298410

length(intersect(rCD4mappable,rCD8mappable))

[1] 0

#GVH direction

For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]

For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]

cd4.GVH= x[,c(23,25)]

cd8.GVH= x[,c(24,26)]

allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00002)

length(allo.GVH[[1]])

[1] 16007

length(allo.GVH[[2]])

[1] 15997

write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt19 MVTx/Pt19 CD4 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt19 MVTx/Pt19 CD8 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

dCD4mappable=rownames(x[(x[,23]+x[,25])>0,])

dCD8mappable=rownames(x[(x[,24]+x[,26])>0,])

CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])

CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])

write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F,
sep="\t")

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F,
sep="\t")

length(dCD4mappable)

[1] 177684

length(dCD8mappable)

[1] 170776

length(CD4nonGVH)

[1] 161677

length(CD8nonGVH)

[1] 154779

dmappable=rownames(x[(x[,23]+x[,25]+x[,24]+x[,26])>0,])

length(dmappable)

[1] 348460

write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt19
MVTx/Pt19 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)+length(dCD8mappable)

[1] 348460

length(intersect(dCD4mappable,dCD8mappable))

[1] 0

[1] "Rearrangement"   "Sum..Templates."   "Present.In"

 [4] "Pt19_MVTx_BM_POD105"   "Pt19_MVTx_Bx_POD12"
"Pt19_MVTx_Bx_POD22"

 [7] "Pt19_MVTx_Bx_POD43_sorted_T"   "Pt19_MVTx_Bx_Tx_colon_POD356"
"Pt19_MVTx_Bx_duodenum_POD356"

[10] "Pt19_MVTx_Bx_ileum_POD356"   "Pt19_MVTx_Bx_native_colon_POD356"
"Pt19_MVTx_Bx_stomach_POD356"

[13] "Pt19_MVTx_Donor_CD45._BM_POD105" "Pt19_MVTx_PBMC_POD105_sorted_T"
"Pt19_MVTx_PBMC_POD16"

[16] "Pt19_MVTx_PBMC_POD355"   "Pt19_MVTx_PBMC_POD43_sorted_T"
"Pt19_MVTx_PBMC_POD9"

[19] "Pt19_MVTx_POD729_ileum_T"   "Pt19_MVTx_POD734_BM_donor_T"
"Pt19_MVTx_POD734_BM_recipient_T"

[22] "Pt19_MVTx_POD734_PBMC_T"   "Pt19_MVTx_SP_D4U"
"Pt19_MVTx_SP_D8U"

[25] "Pt19_MVTx_SP_GVH_D4L"   "Pt19_MVTx_SP_GVH_D8L"
"Pt19_MVTx_SP_HVG_R4L"

[28] "Pt19_MVTx_SP_HVG_R8L"   "Pt19_MVTx_SP_R4U"
"Pt19_MVTx_SP_R8U"

[31] "Pt19_MVTx_stoma_pieces_POD105"   "Pt19_iITx_Bx_POD127_Tx_ileum"

venn diagram of Pt19 POD105

#a=Bx=31, b=PBMC=14, C=BM=4

```
a=rownames(x[x[,31]>0,])
b=rownames(x[x[,14]>0,])
c=rownames(x[x[,4]>0,])
venn_diagram(a,b,c)
```

[1] "a:"   "4575"
[1] "b:"   "14400"
[1] "c:"   "3787"
[1] "a,b:" "176"
[1] "a,c:" "53"
[1] "b,c:" "247"
[1] "a,b,c:" "52"

```
# venn diagram of Pt19 POD734
#a=Bx=19, b=PBMC=22, C=BM=21

a=rownames(x[x[,19]>0,])
b=rownames(x[x[,22]>0,])
c=rownames(x[x[,21]>0,])
venn_diagram(a,b,c)
```

[1] "a:"   "11309"
[1] "b:"   "46562"
[1] "c:"   "84563"
[1] "a,b:" "291"
[1] "a,c:" "485"
[1] "b,c:" "2376"
[1] "a,b,c:" "313"

```
#Pt19 POD12 Bx GVH

cd4= x[,c(23,25,5)]

cd8= x[,c(24,26,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt19 POD12 Bx HVG

cd4= x[,c(29,27,5)]

cd8= x[,c(30,28,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt19 POD22 Bx GVH

cd4= x[,c(23,25,6)]
```

```
cd8= x[,c(24,26,6)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,6]>0,]))

intersect(allo[[2]], rownames(x[x[,6]>0,]))


#Pt19 POD22 Bx HVG

cd4= x[,c(29,27,6)]

cd8= x[,c(30,28,6)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,6]>0,]))

intersect(allo[[2]], rownames(x[x[,6]>0,]))


#Pt19 POD43 Bx GVH

cd4= x[,c(23,25,7)]

cd8= x[,c(24,26,7)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,7]>0,]))

intersect(allo[[2]], rownames(x[x[,7]>0,]))


#Pt19 POD43 Bx HVG

cd4= x[,c(29,27,7)]

cd8= x[,c(30,28,7)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,7]>0,]))

intersect(allo[[2]], rownames(x[x[,7]>0,]))


#Pt19 POD105 Bx GVH

cd4= x[,c(23,25,31)]

cd8= x[,c(24,26,31)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]
```

```
cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,31]>0,]))

intersect(allo[[2]], rownames(x[x[,31]>0,]))


#Pt19 POD105 Bx HVG

cd4= x[,c(29,27,31)]

cd8= x[,c(30,28,31)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,31]>0,]))

intersect(allo[[2]], rownames(x[x[,31]>0,]))


#Pt19 POD105 BM GVH

cd4= x[,c(23,25,4)]

cd8= x[,c(24,26,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))
```

```
intersect(allo[[2]], rownames(x[x[,4]>0,]))
```

#Pt19 POD105 BM HVG

```
cd4= x[,c(29,27,4)]

cd8= x[,c(30,28,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))
```

#Pt19 POD105 BM donor CD45 GVH

```
cd4= x[,c(23,25,13)]

cd8= x[,c(24,26,13)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,13]>0,]))

intersect(allo[[2]], rownames(x[x[,13]>0,]))
```

```
#Pt19 POD105 BM donor CD45 HVG

cd4= x[,c(29,27,13)]

cd8= x[,c(30,28,13)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,13]>0,]))

intersect(allo[[2]], rownames(x[x[,13]>0,]))



#Pt19 POD356 ileum Bx GVH

cd4= x[,c(23,25,10)]

cd8= x[,c(24,26,10)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))
```

```
#Pt19 POD356 ileum Bx HVG

cd4= x[,c(29,27,10)]

cd8= x[,c(30,28,10)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))




#Pt19 POD729 ileum Bx GVH

cd4= x[,c(23,25,19)]

cd8= x[,c(24,26,19)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,19]>0,]))

intersect(allo[[2]], rownames(x[x[,19]>0,]))
```

```
#Pt19 POD729 ileum Bx HVG

cd4= x[,c(29,27,19)]

cd8= x[,c(30,28,19)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,19]>0,]))

intersect(allo[[2]], rownames(x[x[,19]>0,]))


#Pt19 POD734 BM recipient T GVH

cd4= x[,c(23,25,21)]

cd8= x[,c(24,26,21)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,21]>0,]))

intersect(allo[[2]], rownames(x[x[,21]>0,]))


#Pt19 POD734 BM recipient T HVG

cd4= x[,c(29,27,21)]
```

```
cd8= x[,c(30,28,21)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,21]>0,]))

intersect(allo[[2]], rownames(x[x[,21]>0,]))



#Pt19 POD734 BM donor T GVH

cd4= x[,c(23,25,20)]

cd8= x[,c(24,26,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))


#Pt19 POD734 BM donor T HVG

cd4= x[,c(29,27,20)]

cd8= x[,c(30,28,20)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))


#Pt19 POD9 PBMC GVH

cd4= x[,c(23,25,18)]

cd8= x[,c(24,26,18)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,18]>0,]))

intersect(allo[[2]], rownames(x[x[,18]>0,]))


#Pt19 POD9 PBMC HVG

cd4= x[,c(29,27,18)]

cd8= x[,c(30,28,18)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,18]>0,]))

intersect(allo[[2]], rownames(x[x[,18]>0,]))


#Pt19 POD16 PBMC GVH

cd4= x[,c(23,25,15)]

cd8= x[,c(24,26,15)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))


#Pt19 POD16 PBMC HVG

cd4= x[,c(29,27,15)]

cd8= x[,c(30,28,15)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]
```

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))


#Pt19 POD43 PBMC GVH

cd4= x[,c(23,25,17)]

cd8= x[,c(24,26,17)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))


#Pt19 POD43 PBMC HVG

cd4= x[,c(29,27,17)]

cd8= x[,c(30,28,17)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))
```

```
#Pt19 POD105 PBMC GVH

cd4= x[,c(23,25,14)]

cd8= x[,c(24,26,14)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,14]>0,]))

intersect(allo[[2]], rownames(x[x[,14]>0,]))


#Pt19 POD105 PBMC HVG

cd4= x[,c(29,27,14)]

cd8= x[,c(30,28,14)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,14]>0,]))

intersect(allo[[2]], rownames(x[x[,14]>0,]))


#Pt19 POD355 PBMC GVH
```

```
cd4= x[,c(23,25,16)]

cd8= x[,c(24,26,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))


#Pt19 POD355 PBMC HVG

cd4= x[,c(29,27,16)]

cd8= x[,c(30,28,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))


#Pt19 POD734 PBMC GVH

cd4= x[,c(23,25,22)]
```

```
cd8= x[,c(24,26,22)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,25)]

cd8= x[,c(24,26)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))



#Pt19 POD734 PBMC HVG

cd4= x[,c(29,27,22)]

cd8= x[,c(30,28,22)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt19 MVTx/Pt19 05282019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(29,27)]

cd8= x[,c(30,28)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))
```

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20 05292019.tsv")

source('P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/! summary of R codes/alloreactivity_printtofile 09132017.R')

names(data)

[1] "Rearrangement"   "Sum..Templates."

 [3] "Present.In"   "Pt20_iITx_BM_POD125_sorted_T"

 [5] "Pt20_iITx_Bx_POD104"   "Pt20_iITx_Bx_POD16"

 [7] "Pt20_iITx_Bx_POD23"   "Pt20_iITx_Bx_POD306"

 [9] "Pt20_iITx_Bx_POD37"   "Pt20_iITx_Bx_POD68"

[11] "Pt20_iITx_Bx_colon_POD250_sorted_T"   "Pt20_iITx_Bx_ileum_POD250_sorted_T"

[13] "Pt20_iITx_Bx_native_colon_POD250_sorted_T" "Pt20_iITx_MLN_HVG_R4L"

[15] "Pt20_iITx_MLN_HVG_R8L"   "Pt20_iITx_MLN_R4U"

[17] "Pt20_iITx_MLN_R8U"   "Pt20_iITx_PBMC_POD110"

[19] "Pt20_iITx_PBMC_POD16"   "Pt20_iITx_PBMC_POD242"

[21] "Pt20_iITx_PBMC_POD26"   "Pt20_iITx_PBMC_POD306"

[23] "Pt20_iITx_PBMC_POD39"   "Pt20_iITx_PBMC_POD68"

[25] "Pt20_iITx_POD521_BM_T"   "Pt20_iITx_POD523_ileum_Bx_T"

[27] "Pt20_iITx_POD524_PBMC_T"   "Pt20_iITx_SP_D4U"

[29] "Pt20_iITx_SP_D8U"   "Pt20_iITx_SP_GVH_D4L"

[31] "Pt20_iITx_SP_GVH_D8L"

Sample name (Pt: patient)	updated rename
Pt20_iITx_SP_GVH_D4L	Pt20_Pre_Donor_CD4_CFSElo
Pt20_iITx_SP_D4U	Pt20_Pre_Donor_CD4_unstim
Pt20_iITx_SP_GVH_D8L	Pt20_Pre_Donor_CD8_CFSElo
Pt20_iITx_SP_D8U	Pt20_Pre_Donor_CD8_unstim
Pt20_iITx_BM_POD125_sorted T	Pt20_Post_BM_day0125

Pt20_iITx_POD521_BM_T	Pt20_Post_BM_day0521
Pt20_iITx_Bx_POD16	Pt20_Post_ileum_day0016
Pt20_iITx_Bx_POD23	Pt20_Post_ileum_day0023
Pt20_iITx_Bx_POD37	Pt20_Post_ileum_day0037
Pt20_iITx_Bx_POD68	Pt20_Post_ileum_day0068
Pt20_iITx_Bx_POD104	Pt20_Post_ileum_day0104
Pt20_iITx_Bx_POD306	Pt20_Post_ileum_day0306
Pt20_iITx_POD523_ileum_Bx_T	Pt20_Post_ileum_day0523
Pt20_iITx_Bx_POD250_Tx_ileum_sorted_T	Pt20_Post_ileum_day0250
Pt20_iITx_PBMC_POD16	Pt20_Post_PBMC_day0016
Pt20_iITx_PBMC_POD26	Pt20_Post_PBMC_day0026
Pt20_iITx_PBMC_POD39	Pt20_Post_PBMC_day0039
Pt20_iITx_PBMC_POD68	Pt20_Post_PBMC_day0068
Pt20_iITx_PBMC_POD110	Pt20_Post_PBMC_day0110
Pt20_iITx_PBMC_POD242	Pt20_Post_PBMC_day0242
Pt20_iITx_PBMC_POD306	Pt20_Post_PBMC_day0306
Pt20_iITx_POD524_PBMC_T	Pt20_Post_PBMC_day0524
Pt20_iITx_MLN_HVG_R4L	Pt20_Pre_Recipient_CD4_CFSElo
Pt20_iITx_MLN_R4U	Pt20_Pre_Recipient_CD4_unstim
Pt20_iITx_MLN_HVG_R8L	Pt20_Pre_Recipient_CD8_CFSElo
Pt20_iITx_MLN_R8U	Pt20_Pre_Recipient_CD8_unstim

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

normalize(data[,c(28,29)])

ratio=5

 c1indices=which(data[,28]>0 & data[,29]>0 & data[,28]>ratio*data[,29])

 c2indices=which(data[,28]>0 & data[,29]>0 & data[,29]>ratio*data[,28])

 ambiindices=which(data[,28]>0 & data[,29]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,29]=0

```
data[c2indices,28]=0
data[ambiindices,c(28,29)]=0



#D4L vs D8L
normalize(data[,c(30,31)])
ratio=5
 c1indices=which(data[,30]>0 & data[,31]>0 & data[,30]>ratio*data[,31])
 c2indices=which(data[,30]>0 & data[,31]>0 & data[,31]>ratio*data[,30])
 ambiindices=which(data[,30]>0 & data[,31]>0)
 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
 data[c1indices,31]=0
 data[c2indices,30]=0
 data[ambiindices,c(30,31)]=0



#R4U vs R8U
normalize(data[,c(16,17)])
ratio=5
 c1indices=which(data[,16]>0 & data[,17]>0 & data[,16]>ratio*data[,17])
 c2indices=which(data[,16]>0 & data[,17]>0 & data[,17]>ratio*data[,16])
 ambiindices=which(data[,16]>0 & data[,17]>0)
 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
 data[c1indices,17]=0
 data[c2indices,16]=0
 data[ambiindices,c(16,17)]=0
```

```
#R4L vs R8L

normalize(data[,c(14,15)])

ratio=5

  c1indices=which(data[,14]>0 & data[,15]>0 & data[,14]>ratio*data[,15])

  c2indices=which(data[,14]>0 & data[,15]>0 & data[,15]>ratio*data[,14])

  ambiindices=which(data[,14]>0 & data[,15]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,15]=0

  data[c2indices,14]=0

  data[ambiindices,c(14,15)]=0



rownames(data)=data[,1]

x=data



#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,28]>0 | x[,29]>0)&(x[,16]>0 | x[,17]>0))

ambiguous=union(ambiguous,which((x[,28]>0 | x[,29]>0)&(x[,14]>0 | x[,15]>0)))

ambiguous=union(ambiguous,which((x[,16]>0 | x[,17]>0)&(x[,30]>0 | x[,31]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]



#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L,
R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional
step of optimization for CD4 and CD8 sorting error.



#R4L vs R8U

normalize(x[,c(14,17)])

ratio=2
```

```
 c1indices=which(x[,14]>0 & x[,17]>0 & x[,14]>ratio*x[,17])

 c2indices=which(x[,14]>0 & x[,17]>0 & x[,17]>ratio*x[,14])

 ambiindices=which(x[,14]>0 & x[,17]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,17]=0

 x[c2indices,14]=0

 x[ambiindices,c(14,17)]=0


#R8L vs R4U

normalize(x[,c(15,16)])

ratio=2

 c1indices=which(x[,15]>0 & x[,16]>0 & x[,15]>ratio*x[,16])

 c2indices=which(x[,15]>0 & x[,16]>0 & x[,16]>ratio*x[,15])

 ambiindices=which(x[,15]>0 & x[,16]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,16]=0

 x[c2indices,15]=0

 x[ambiindices,c(15,16)]=0


#D4L vs D8U

normalize(x[,c(30,29)])

ratio=2

 c1indices=which(x[,30]>0 & x[,29]>0 & x[,30]>ratio*x[,29])

 c2indices=which(x[,30]>0 & x[,29]>0 & x[,29]>ratio*x[,30])

 ambiindices=which(x[,30]>0 & x[,29]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,29]=0

 x[c2indices,30]=0
```

```
  x[ambiindices,c(30,29)]=0


#D8L vs D4U

normalize(x[,c(31,28)])

ratio=2

  c1indices=which(x[,31]>0 & x[,28]>0 & x[,31]>ratio*x[,28])

  c2indices=which(x[,31]>0 & x[,28]>0 & x[,28]>ratio*x[,31])

  ambiindices=which(x[,31]>0 & x[,28]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,28]=0

  x[c2indices,31]=0

  x[ambiindices,c(31,28)]=0


write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20
05292019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T, sep="\t")


# add "fold=2" below, use 2 fold expansion (default 5 fold)

# add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for
read counts


#HVG direction

# For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]

# For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]

cd4.HVG= x[,c(16,14)]

cd8.HVG= x[,c(17,15)]

allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00002)


length(allo.HVG[[1]])

[1] 12560
```

length(allo.HVG[[2]])

[1] 10069

write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20 CD4 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20 CD8 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,16]+x[,14])>0,])

rCD8mappable=rownames(x[(x[,17]+x[,15])>0,])

CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])

write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20 CD4nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20 CD8nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 190297

length(rCD8mappable)

[1] 162987

length(CD4nonHVG)

[1] 177737

length(CD8nonHVG)

[1] 152918

rmappable=rownames(x[(x[,16]+x[,17]+x[,14]+x[,15])>0,])

length(rmappable)

[1] 353284

write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)+length(rCD8mappable)

[1] 353284

length(intersect(rCD4mappable,rCD8mappable))

[1] 0

#GVH direction

For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]

For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]

cd4.GVH= x[,c(28,30)]

cd8.GVH= x[,c(29,31)]

allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00002)

length(allo.GVH[[1]])

[1] 7089

length(allo.GVH[[2]])

[1] 6654

write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20 CD4 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20 CD8 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

dCD4mappable=rownames(x[(x[,28]+x[,30])>0,])

dCD8mappable=rownames(x[(x[,29]+x[,31])>0,])

CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])

CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])

write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F,
sep="\t")

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F,
sep="\t")

length(dCD4mappable)

[1] 91123

length(dCD8mappable)

[1] 63941

length(CD4nonGVH)

[1] 84034

length(CD8nonGVH)

[1] 57287

dmappable=rownames(x[(x[,28]+x[,30]+x[,29]+x[,31])>0,])

length(dmappable)

[1] 155064

write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20
iITx/Pt20 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

```
length(dCD4mappable)+length(dCD8mappable)
[1] 155064
length(intersect(dCD4mappable,dCD8mappable))
[1] 0


#list individual HvG clones in a certain sample, Pt20 ileum Bx POD23


rmappableearlyACR=rownames(x[x[,7]>0,])


length(rmappableearlyACR)
[1] 14394


CD4HvGearlyACR=intersect(rmappableearlyACR,allo.HVG[[1]])
CD8HvGearlyACR=intersect(rmappableearlyACR,allo.HVG[[2]])


length(allo.HVG[[1]])
[1] 12560
length(CD4HvGearlyACR)
[1] 54
length(allo.HVG[[2]])
[1] 10069
length(CD8HvGearlyACR)
[1] 17


#Pt20 POD16 Bx GVH
cd4= x[,c(28,30,6)]
cd8= x[,c(29,31,6)]
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,6]>0,]))

intersect(allo[[2]], rownames(x[x[,6]>0,]))



#Pt20 POD16 Bx HVG

cd4= x[,c(16,14,6)]

cd8= x[,c(17,15,6)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )



rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,6]>0,]))

intersect(allo[[2]], rownames(x[x[,6]>0,]))



#Pt20 POD23 Bx GVH

cd4= x[,c(28,30,7)]

cd8= x[,c(29,31,7)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,7]>0,]))

intersect(allo[[2]], rownames(x[x[,7]>0,]))
```

```
#Pt20 POD23 Bx HVG

cd4= x[,c(16,14,7)]

cd8= x[,c(17,15,7)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,7]>0,]))

intersect(allo[[2]], rownames(x[x[,7]>0,]))
```

```
#Pt20 POD37 Bx GVH

cd4= x[,c(28,30,9)]

cd8= x[,c(29,31,9)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,9]>0,]))

intersect(allo[[2]], rownames(x[x[,9]>0,]))


#Pt20 POD37 Bx HVG

cd4= x[,c(16,14,9)]

cd8= x[,c(17,15,9)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,9]>0,]))

intersect(allo[[2]], rownames(x[x[,9]>0,]))


#Pt20 POD68 Bx GVH

cd4= x[,c(28,30,10)]

cd8= x[,c(29,31,10)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]
```

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))


#Pt20 POD68 Bx HVG

cd4= x[,c(16,14,10)]

cd8= x[,c(17,15,10)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))



#Pt20 POD104 Bx GVH

cd4= x[,c(28,30,5)]

cd8= x[,c(29,31,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))
```

```
intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt20 POD104 Bx HVG

cd4= x[,c(16,14,5)]

cd8= x[,c(17,15,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt20 POD250 ileum Bx GVH

cd4= x[,c(28,30,12)]

cd8= x[,c(29,31,12)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,12]>0,]))

intersect(allo[[2]], rownames(x[x[,12]>0,]))
```

```
#Pt20 POD250 ileum Bx HVG

cd4= x[,c(16,14,12)]

cd8= x[,c(17,15,12)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,12]>0,]))

intersect(allo[[2]], rownames(x[x[,12]>0,]))



#Pt20 POD306 Bx GVH

cd4= x[,c(28,30,8)]

cd8= x[,c(29,31,8)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,8]>0,]))

intersect(allo[[2]], rownames(x[x[,8]>0,]))
```

```
#Pt20 POD306 Bx HVG

cd4= x[,c(16,14,8)]

cd8= x[,c(17,15,8)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,8]>0,]))

intersect(allo[[2]], rownames(x[x[,8]>0,]))




#Pt20 POD523 Bx GVH

cd4= x[,c(28,30,26)]

cd8= x[,c(29,31,26)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,26]>0,]))

intersect(allo[[2]], rownames(x[x[,26]>0,]))




#Pt20 POD523 Bx HVG
```

```
cd4= x[,c(16,14,26)]

cd8= x[,c(17,15,26)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,26]>0,]))

intersect(allo[[2]], rownames(x[x[,26]>0,]))


#Pt20 POD521 BM_T GVH

cd4= x[,c(28,30,25)]

cd8= x[,c(29,31,25)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,25]>0,]))

intersect(allo[[2]], rownames(x[x[,25]>0,]))


#Pt20 POD521 BM_T HVG

cd4= x[,c(16,14,25)]
```

```
cd8= x[,c(17,15,25)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,25]>0,]))

intersect(allo[[2]], rownames(x[x[,25]>0,]))



#Pt20 POD16 PBMC GVH

cd4= x[,c(28,30,19)]

cd8= x[,c(29,31,19)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,19]>0,]))

intersect(allo[[2]], rownames(x[x[,19]>0,]))



#Pt20 POD16 PBMC HVG

cd4= x[,c(16,14,19)]

cd8= x[,c(17,15,19)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,19]>0,]))

intersect(allo[[2]], rownames(x[x[,19]>0,]))



#Pt20 POD26 PBMC GVH

cd4= x[,c(28,30,21)]

cd8= x[,c(29,31,21)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,21]>0,]))

intersect(allo[[2]], rownames(x[x[,21]>0,]))



#Pt20 POD26 PBMC HVG

cd4= x[,c(16,14,21)]

cd8= x[,c(17,15,21)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20 05292019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,21]>0,]))

intersect(allo[[2]], rownames(x[x[,21]>0,]))


#Pt20 POD39 PBMC GVH

cd4= x[,c(28,30,23)]

cd8= x[,c(29,31,23)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,23]>0,]))

intersect(allo[[2]], rownames(x[x[,23]>0,]))


#Pt20 POD39 PBMC HVG

cd4= x[,c(16,14,23)]

cd8= x[,c(17,15,23)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]
```

```
cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,23]>0,]))

intersect(allo[[2]], rownames(x[x[,23]>0,]))


#Pt20 POD68 PBMC GVH

cd4= x[,c(28,30,24)]

cd8= x[,c(29,31,24)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,24]>0,]))

intersect(allo[[2]], rownames(x[x[,24]>0,]))


#Pt20 POD68 PBMC HVG

cd4= x[,c(16,14,24)]

cd8= x[,c(17,15,24)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,24]>0,]))
```

```
intersect(allo[[2]], rownames(x[x[,24]>0,]))


#Pt20 POD110 PBMC GVH

cd4= x[,c(28,30,18)]

cd8= x[,c(29,31,18)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,18]>0,]))

intersect(allo[[2]], rownames(x[x[,18]>0,]))


#Pt20 POD110 PBMC HVG

cd4= x[,c(16,14,18)]

cd8= x[,c(17,15,18)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,18]>0,]))

intersect(allo[[2]], rownames(x[x[,18]>0,]))
```

```
#Pt20 POD242 PBMC GVH

cd4= x[,c(28,30,20)]

cd8= x[,c(29,31,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))


#Pt20 POD242 PBMC HVG

cd4= x[,c(16,14,20)]

cd8= x[,c(17,15,20)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))


#Pt20 POD306 PBMC GVH

cd4= x[,c(28,30,22)]

cd8= x[,c(29,31,22)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))


#Pt20 POD306 PBMC HVG

cd4= x[,c(16,14,22)]

cd8= x[,c(17,15,22)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))


#Pt20 POD125 BM GVH

cd4= x[,c(28,30,4)]

cd8= x[,c(29,31,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))
```

```
#Pt20 POD125 BM HVG

cd4= x[,c(16,14,4)]

cd8= x[,c(17,15,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))
```

```
#Pt20 POD524 PBMC GVH

cd4= x[,c(28,30,27)]

cd8= x[,c(29,31,27)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(28,30)]

cd8= x[,c(29,31)]
```

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,27]>0,]))

intersect(allo[[2]], rownames(x[x[,27]>0,]))


#Pt20 POD524 PBMC HVG

cd4= x[,c(16,14,27)]

cd8= x[,c(17,15,27)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt20 iITx/Pt20 05292019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(16,14)]

cd8= x[,c(17,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,27]>0,]))

intersect(allo[[2]], rownames(x[x[,27]>0,]))
```

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 10242019.tsv")

source('P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/! summary of R codes/alloreactivity_printtofile 09132017.R')

names(data)

```
 [1] "Rearrangement"                      "Sum..Templates."

 [3] "Present.In"                         "Pt21_MVTx_Bx_POD109"

 [5] "Pt21_MVTx_Bx_POD23"
"Pt21_MVTx_Bx_duodenum_POD243_sorted_T"

 [7] "Pt21_MVTx_Bx_ileum_POD243_sorted_T"
"Pt21_MVTx_Bx_stomach_POD243_sorted_T"

 [9] "Pt21_MVTx_LN_gut_associated_POD262_sorted_T"   "Pt21_MVTx_PBMC_POD109"

[11] "Pt21_MVTx_PBMC_POD11"               "Pt21_MVTx_PBMC_POD23"

[13] "Pt21_MVTx_PBMC_POD262_sorted_T"         "Pt21_MVTx_POD626_LN_T"

[15] "Pt21_MVTx_POD626_PBMC_T"            "Pt21_MVTx_POD626_ileum_Bx"

[17] "Pt21_MVTx_SP_D4U"                   "Pt21_MVTx_SP_D8U"

[19] "Pt21_MVTx_SP_GVH_D4L"               "Pt21_MVTx_SP_GVH_D8L"

[21] "Pt21_MVTx_SP_HVG_R4L"               "Pt21_MVTx_SP_HVG_R8L"

[23] "Pt21_MVTx_SP_R4U"                   "Pt21_MVTx_SP_R8U"

[25] "Pt21_MVTx_ileum_stoma_IEL.LPL_POD262_sorted_T"
```

Sample name (Pt: patient)	updated rename
Pt21_MVTx_SP_GVH_D4L	Pt21_Pre_Donor_CD4_CFSElo
Pt21_MVTx_SP_D4U	Pt21_Pre_Donor_CD4_unstim
Pt21_MVTx_SP_GVH_D8L	Pt21_Pre_Donor_CD8_CFSElo
Pt21_MVTx_SP_D8U	Pt21_Pre_Donor_CD8_unstim
Pt21_MVTx_Bx_POD23	Pt21_Post_ileum_day0023
Pt21_MVTx_Bx_POD109	Pt21_Post_ileum_day0109
Pt21_MVTx_Bx_ileum_POD243_sorted T	Pt21_Post_ileum_day0243

Pt21_MVTx_Bx_ileum stoma_POD262_sorted T	Pt21_Post_ileum_day0262
Pt21_MVTx_PBMC_POD11	Pt21_Post_PBMC_day0011
Pt21_MVTx_PBMC_POD23	Pt21_Post_PBMC_day0023
Pt21_MVTx_PBMC_POD109	Pt21_Post_PBMC_day0109
Pt21_MVTx_PBMC_POD262_sorted T	Pt21_Post_PBMC_day0262
Pt21_MVTx_SP_HVG_R4L	Pt21_Pre_Recipient_CD4_CFSElo
Pt21_MVTx_SP_R4U	Pt21_Pre_Recipient_CD4_unstim
Pt21_MVTx_SP_HVG_R8L	Pt21_Pre_Recipient_CD8_CFSElo
Pt21_MVTx_SP_R8U	Pt21_Pre_Recipient_CD8_unstim

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

normalize(data[,c(17,18)])

ratio=5

 c1indices=which(data[,17]>0 & data[,18]>0 & data[,17]>ratio*data[,18])

 c2indices=which(data[,17]>0 & data[,18]>0 & data[,18]>ratio*data[,17])

 ambiindices=which(data[,17]>0 & data[,18]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,18]=0

 data[c2indices,17]=0

 data[ambiindices,c(17,18)]=0

#D4L vs D8L

normalize(data[,c(19,20)])

ratio=5

 c1indices=which(data[,19]>0 & data[,20]>0 & data[,19]>ratio*data[,20])

 c2indices=which(data[,19]>0 & data[,20]>0 & data[,20]>ratio*data[,19])

```
ambiindices=which(data[,19]>0 & data[,20]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

data[c1indices,20]=0

data[c2indices,19]=0

data[ambiindices,c(19,20)]=0


#R4U vs R8U

normalize(data[,c(23,24)])

ratio=5

 c1indices=which(data[,23]>0 & data[,24]>0 & data[,23]>ratio*data[,24])

 c2indices=which(data[,23]>0 & data[,24]>0 & data[,24]>ratio*data[,23])

 ambiindices=which(data[,23]>0 & data[,24]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,24]=0

 data[c2indices,23]=0

 data[ambiindices,c(23,24)]=0


#R4L vs R8L

normalize(data[,c(21,22)])

ratio=5

 c1indices=which(data[,21]>0 & data[,22]>0 & data[,21]>ratio*data[,22])

 c2indices=which(data[,21]>0 & data[,22]>0 & data[,22]>ratio*data[,21])

 ambiindices=which(data[,21]>0 & data[,22]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,22]=0

 data[c2indices,21]=0

 data[ambiindices,c(21,22)]=0
```

```
rownames(data)=data[,1]

x=data


#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,17]>0 | x[,18]>0)&(x[,23]>0 | x[,24]>0))

ambiguous=union(ambiguous,which((x[,17]>0 | x[,18]>0)&(x[,21]>0 | x[,22]>0)))

ambiguous=union(ambiguous,which((x[,23]>0 | x[,24]>0)&(x[,19]>0 | x[,20]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]


#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L,
R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional
step of optimization for CD4 and CD8 sorting error.


#R4L vs R8U

normalize(x[,c(21,24)])

ratio=2

  c1indices=which(x[,21]>0 & x[,24]>0 & x[,21]>ratio*x[,24])

  c2indices=which(x[,21]>0 & x[,24]>0 & x[,24]>ratio*x[,21])

  ambiindices=which(x[,21]>0 & x[,24]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,24]=0

  x[c2indices,21]=0

  x[ambiindices,c(21,24)]=0


#R8L vs R4U

normalize(x[,c(22,23)])

ratio=2

  c1indices=which(x[,22]>0 & x[,23]>0 & x[,22]>ratio*x[,23])
```

```
c2indices=which(x[,22]>0 & x[,23]>0 & x[,23]>ratio*x[,22])
ambiindices=which(x[,22]>0 & x[,23]>0)
ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
x[c1indices,23]=0
x[c2indices,22]=0
x[ambiindices,c(22,23)]=0


#D4L vs D8U
normalize(x[,c(19,18)])
ratio=2
 c1indices=which(x[,19]>0 & x[,18]>0 & x[,19]>ratio*x[,18])
 c2indices=which(x[,19]>0 & x[,18]>0 & x[,18]>ratio*x[,19])
 ambiindices=which(x[,19]>0 & x[,18]>0)
 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
 x[c1indices,18]=0
 x[c2indices,19]=0
 x[ambiindices,c(19,18)]=0


#D8L vs D4U
normalize(x[,c(20,17)])
ratio=2
 c1indices=which(x[,20]>0 & x[,17]>0 & x[,20]>ratio*x[,17])
 c2indices=which(x[,20]>0 & x[,17]>0 & x[,17]>ratio*x[,20])
 ambiindices=which(x[,20]>0 & x[,17]>0)
 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
 x[c1indices,17]=0
 x[c2indices,20]=0
 x[ambiindices,c(20,17)]=0
```

write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 10242019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T, sep="\t")

add "fold=2" below, use 2 fold expansion (default 5 fold)

add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for read counts

#HVG direction

For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]

For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]

cd4.HVG= x[,c(23,21)]

cd8.HVG= x[,c(24,22)]

allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00002)

length(allo.HVG[[1]])

[1] 12690

length(allo.HVG[[2]])

[1] 13186

write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 CD4 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 CD8 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,23]+x[,21])>0,])

rCD8mappable=rownames(x[(x[,24]+x[,22])>0,])

CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])

write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 CD4nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 CD8nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 137963

length(rCD8mappable)

[1] 109399

length(CD4nonHVG)

[1] 125273

length(CD8nonHVG)

[1] 96213

rmappable=rownames(x[(x[,23]+x[,21]+x[,24]+x[,22])>0,])

length(rmappable)

[1] 247362

write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)+length(rCD8mappable)

[1] 247362

length(intersect(rCD4mappable,rCD8mappable))

[1] 0

#GVH direction

For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]

For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]

cd4.GVH= x[,c(17,19)]

cd8.GVH= x[,c(18,20)]

allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00002)

length(allo.GVH[[1]])

[1] 7837

length(allo.GVH[[2]])

[1] 7277

write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 CD4 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 CD8 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

dCD4mappable=rownames(x[(x[,17]+x[,19])>0,])

dCD8mappable=rownames(x[(x[,18]+x[,20])>0,])

CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])

CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])

write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)

[1] 78613

length(dCD8mappable)

[1] 58958

length(CD4nonGVH)

[1] 70776

length(CD8nonGVH)

[1] 51681

dmappable=rownames(x[(x[17]+x[,19]+x[,18]+x[,20])>0,])

length(dmappable)

[1] 137571

write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)+length(dCD8mappable)

[1] 137571

length(intersect(dCD4mappable,dCD8mappable))

[1] 0

#Pt21 POD626 Bx GVH

cd4= x[,c(17,19,16)]

cd8= x[,c(18,20,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 10242019 2x.out')

```
rownames(x)=x[,1]

cd4= x[,c(17,19)]

cd8= x[,c(18,20)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))


#Pt21 POD626 Bx  HVG

cd4= x[,c(23,21,16)]

cd8= x[,c(24,22,16)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 10242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,21)]

cd8= x[,c(24,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))


#Pt21 POD626 PBMC GVH

cd4= x[,c(17,19,15)]

cd8= x[,c(18,20,15)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 10242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(17,19)]

cd8= x[,c(18,20)]
```

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))


#Pt21 POD626 PBMC  HVG

cd4= x[,c(23,21,15)]

cd8= x[,c(24,22,15)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 10242019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(23,21)]

cd8= x[,c(24,22)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))



#previous .tsv version

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21
MVTx/Pt21 07012019.tsv")

source('P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/! summary of R
codes/alloreactivity_printtofile 09132017.R')


names(data)
 [1] "Rearrangement"                    "Sum..Templates."

 [3] "Present.In"                       "Pt21_MVTx_Bx_POD109"

 [5] "Pt21_MVTx_Bx_POD23"
"Pt21_MVTx_Bx_duodenum_POD243_sorted_T"
```

[7] "Pt21_MVTx_Bx_ileum_POD243_sorted_T"
"Pt21_MVTx_Bx_stomach_POD243_sorted_T"

 [9] "Pt21_MVTx_LN_gut_associated_POD262_sorted_T"   "Pt21_MVTx_PBMC_POD109"

[11] "Pt21_MVTx_PBMC_POD11"   "Pt21_MVTx_PBMC_POD23"

[13] "Pt21_MVTx_PBMC_POD262_sorted_T"   "Pt21_MVTx_SP_D4U"

[15] "Pt21_MVTx_SP_D8U"   "Pt21_MVTx_SP_GVH_D4L"

[17] "Pt21_MVTx_SP_GVH_D8L"   "Pt21_MVTx_SP_HVG_R4L"

[19] "Pt21_MVTx_SP_HVG_R8L"   "Pt21_MVTx_SP_R4U"

[21] "Pt21_MVTx_SP_R8U"
"Pt21_MVTx_ileum_stoma_IEL.LPL_POD262_sorted_T"

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

normalize(data[,c(14,15)])

ratio=5

 c1indices=which(data[,14]>0 & data[,15]>0 & data[,14]>ratio*data[,15])

 c2indices=which(data[,14]>0 & data[,15]>0 & data[,15]>ratio*data[,14])

 ambiindices=which(data[,14]>0 & data[,15]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,15]=0

 data[c2indices,14]=0

 data[ambiindices,c(14,15)]=0

#D4L vs D8L

normalize(data[,c(16,17)])

ratio=5

 c1indices=which(data[,16]>0 & data[,17]>0 & data[,16]>ratio*data[,17])

```
c2indices=which(data[,16]>0 & data[,17]>0 & data[,17]>ratio*data[,16])
ambiindices=which(data[,16]>0 & data[,17]>0)
ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
data[c1indices,17]=0
data[c2indices,16]=0
data[ambiindices,c(16,17)]=0


#R4U vs R8U
normalize(data[,c(20,21)])
ratio=5
 c1indices=which(data[,20]>0 & data[,21]>0 & data[,20]>ratio*data[,21])
 c2indices=which(data[,20]>0 & data[,21]>0 & data[,21]>ratio*data[,20])
 ambiindices=which(data[,20]>0 & data[,21]>0)
 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
 data[c1indices,21]=0
 data[c2indices,20]=0
 data[ambiindices,c(20,21)]=0


#R4L vs R8L
normalize(data[,c(18,19)])
ratio=5
 c1indices=which(data[,18]>0 & data[,19]>0 & data[,18]>ratio*data[,19])
 c2indices=which(data[,18]>0 & data[,19]>0 & data[,19]>ratio*data[,18])
 ambiindices=which(data[,18]>0 & data[,19]>0)
 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
 data[c1indices,19]=0
 data[c2indices,18]=0
 data[ambiindices,c(18,19)]=0
```

```
rownames(data)=data[,1]

x=data


#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,14]>0 | x[,15]>0)&(x[,20]>0 | x[,21]>0))

ambiguous=union(ambiguous,which((x[,14]>0 | x[,15]>0)&(x[,18]>0 | x[,19]>0)))

ambiguous=union(ambiguous,which((x[,20]>0 | x[,21]>0)&(x[,16]>0 | x[,17]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]


#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L,
R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional
step of optimization for CD4 and CD8 sorting error.


#R4L vs R8U

normalize(x[,c(18,21)])

ratio=2

  c1indices=which(x[,18]>0 & x[,21]>0 & x[,18]>ratio*x[,21])

  c2indices=which(x[,18]>0 & x[,21]>0 & x[,21]>ratio*x[,18])

  ambiindices=which(x[,18]>0 & x[,21]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,21]=0

  x[c2indices,18]=0

  x[ambiindices,c(18,21)]=0


#R8L vs R4U

normalize(x[,c(19,20)])

ratio=2
```

```
c1indices=which(x[,19]>0 & x[,20]>0 & x[,19]>ratio*x[,20])

c2indices=which(x[,19]>0 & x[,20]>0 & x[,20]>ratio*x[,19])

ambiindices=which(x[,19]>0 & x[,20]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

x[c1indices,20]=0

x[c2indices,19]=0

x[ambiindices,c(19,20)]=0


#D4L vs D8U

normalize(x[,c(16,15)])

ratio=2

 c1indices=which(x[,16]>0 & x[,15]>0 & x[,16]>ratio*x[,15])

 c2indices=which(x[,16]>0 & x[,15]>0 & x[,15]>ratio*x[,16])

 ambiindices=which(x[,16]>0 & x[,15]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,15]=0

 x[c2indices,16]=0

 x[ambiindices,c(16,15)]=0


#D8L vs D4U

normalize(x[,c(17,14)])

ratio=2

 c1indices=which(x[,17]>0 & x[,14]>0 & x[,17]>ratio*x[,14])

 c2indices=which(x[,17]>0 & x[,14]>0 & x[,14]>ratio*x[,17])

 ambiindices=which(x[,17]>0 & x[,14]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,14]=0

 x[c2indices,17]=0
```

```
x[ambiindices,c(17,14)]=0
```

```
write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21
MVTx/Pt21 07012019 resolve ambiguous updated.tsv",quote=F,row.names=F,col.names=T,
sep="\t")
```

```
# add "fold=2" below, use 2 fold expansion (default 5 fold)
```

```
# add "freq=0.00002" below, use freq=0.00002 for template counts; use default freq=0.00001 for
read counts
```

```
#HVG direction
```

```
# For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]
```

```
# For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]
```

```
cd4.HVG= x[,c(20,18)]
```

```
cd8.HVG= x[,c(21,19)]
```

```
allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00002)
```

```
length(allo.HVG[[1]])
```

```
[1] 12690
```

```
length(allo.HVG[[2]])
```

```
[1] 13186
```

```
write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 CD4 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")
```

```
write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 CD8 HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")
```

```
rCD4mappable=rownames(x[(x[,20]+x[,18])>0,])
```

```
rCD8mappable=rownames(x[(x[,21]+x[,19])>0,])
```

```
CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])
```

```
CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])
```

```
write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 CD4nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F,
sep="\t")
```

```
write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 CD8nonHVG rmappable list.txt",quote=F,row.names=F,col.names=F,
sep="\t")
```

```
length(rCD4mappable)
```

[1] 137963

```
length(rCD8mappable)
```

[1] 109399

```
length(CD4nonHVG)
```

[1] 125273

```
length(CD8nonHVG)
```

[1] 96213

```
rmappable=rownames(x[(x[,20]+x[,18]+x[,21]+x[,19])>0,])
```

```
length(rmappable)
```

[1] 247362

```
write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21
MVTx/Pt21 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")
```

```
length(rCD4mappable)+length(rCD8mappable)
```

[1] 247362

```
length(intersect(rCD4mappable,rCD8mappable))
[1] 0


#GVH direction
# For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]
# For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]
cd4.GVH= x[,c(14,16)]
cd8.GVH= x[,c(15,17)]
allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00002)


length(allo.GVH[[1]])
[1] 7837
length(allo.GVH[[2]])
[1] 7277


write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 CD4 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 CD8 GVHlist.txt",quote=F,row.names=F,col.names=F, sep="\t")


dCD4mappable=rownames(x[(x[,14]+x[,16])>0,])
dCD8mappable=rownames(x[(x[,15]+x[,17])>0,])


CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])
CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])


write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F,
sep="\t")
```

```
write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F,
sep="\t")
```

```
length(dCD4mappable)
```

[1] 78613

```
length(dCD8mappable)
```

[1] 58958

```
length(CD4nonGVH)
```

[1] 70776

```
length(CD8nonGVH)
```

[1] 51681

```
dmappable=rownames(x[(x[14]+x[,16]+x[,15]+x[,17])>0,])
```

```
length(dmappable)
```

[1] 137571

```
write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21
MVTx/Pt21 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")
```

```
length(dCD4mappable)+length(dCD8mappable)
```

[1] 137571

```
length(intersect(dCD4mappable,dCD8mappable))
```

[1] 0

```
#Pt21 POD23 Bx GVH
```

```
cd4= x[,c(14,16,5)]
```

```
cd8= x[,c(15,17,5)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(14,16)]

cd8= x[,c(15,17)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt21 POD23 Bx HVG

cd4= x[,c(20,18,5)]

cd8= x[,c(21,19,5)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(20,18)]

cd8= x[,c(21,19)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,5]>0,]))

intersect(allo[[2]], rownames(x[x[,5]>0,]))


#Pt21 POD109 Bx GVH

cd4= x[,c(14,16,4)]

cd8= x[,c(15,17,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(14,16)]

cd8= x[,c(15,17)]
```

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))


#Pt21 POD109 Bx HVG

cd4= x[,c(20,18,4)]

cd8= x[,c(21,19,4)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(20,18)]

cd8= x[,c(21,19)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,4]>0,]))

intersect(allo[[2]], rownames(x[x[,4]>0,]))


#Pt21 POD243 ileum Bx GVH

cd4= x[,c(14,16,7)]

cd8= x[,c(15,17,7)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(14,16)]

cd8= x[,c(15,17)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,7]>0,]))

intersect(allo[[2]], rownames(x[x[,7]>0,]))
```

#Pt21 POD243 ileum HVG

cd4= x[,c(20,18,7)]

cd8= x[,c(21,19,7)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 07012019 2x.out')

rownames(x)=x[,1]

cd4= x[,c(20,18)]

cd8= x[,c(21,19)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,7]>0,]))

intersect(allo[[2]], rownames(x[x[,7]>0,]))

#Pt21 POD262 ileum stoma Bx GVH

cd4= x[,c(14,16,22)]

cd8= x[,c(15,17,22)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Pt21 MVTx/Pt21 07012019 2x.out')

rownames(x)=x[,1]

cd4= x[,c(14,16)]

cd8= x[,c(15,17)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))

#Pt21 POD262 ileum stoma Bx HVG

```
cd4= x[,c(20,18,22)]

cd8= x[,c(21,19,22)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(20,18)]

cd8= x[,c(21,19)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))


#Pt21 POD11 PBMC GVH

cd4= x[,c(14,16,11)]

cd8= x[,c(15,17,11)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(14,16)]

cd8= x[,c(15,17)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,11]>0,]))

intersect(allo[[2]], rownames(x[x[,11]>0,]))


#Pt21 POD11 PBMC HVG

cd4= x[,c(20,18,11)]

cd8= x[,c(21,19,11)]
```

```
run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(20,18)]

cd8= x[,c(21,19)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,11]>0,]))

intersect(allo[[2]], rownames(x[x[,11]>0,]))


#Pt21 POD23 PBMC GVH

cd4= x[,c(14,16,12)]

cd8= x[,c(15,17,12)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(14,16)]

cd8= x[,c(15,17)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,12]>0,]))

intersect(allo[[2]], rownames(x[x[,12]>0,]))


#Pt21 POD23 PBMC HVG

cd4= x[,c(20,18,12)]

cd8= x[,c(21,19,12)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(20,18)]

cd8= x[,c(21,19)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,12]>0,]))

intersect(allo[[2]], rownames(x[x[,12]>0,]))


#Pt21 POD109 PBMC GVH

cd4= x[,c(14,16,10)]

cd8= x[,c(15,17,10)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(14,16)]

cd8= x[,c(15,17)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))


#Pt21 POD109 PBMC HVG

cd4= x[,c(20,18,10)]

cd8= x[,c(21,19,10)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(20,18)]

cd8= x[,c(21,19)]
```

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))


#Pt21 POD262 PBMC GVH

cd4= x[,c(14,16,13)]

cd8= x[,c(15,17,13)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(14,16)]

cd8= x[,c(15,17)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,13]>0,]))

intersect(allo[[2]], rownames(x[x[,13]>0,]))


#Pt21 POD262 PBMC HVG

cd4= x[,c(20,18,13)]

cd8= x[,c(21,19,13)]

run(cd4,cd8, fold=2,freq=0.00002, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Pt21 MVTx/Pt21 07012019 2x.out' )


rownames(x)=x[,1]

cd4= x[,c(20,18)]

cd8= x[,c(21,19)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00002)

intersect(allo[[1]], rownames(x[x[,13]>0,]))

intersect(allo[[2]], rownames(x[x[,13]>0,]))
```

data1 <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 with cut off.tsv")

names(data1)

[1] "v"   "j"

 [3] "cdr3nt"   "cdr3aa"

 [5] "samples"   "Pt22.MVTx.POD10.PBMC"

 [7] "Pt22.MVTx.POD10.liver.Bx"   "Pt22.MVTx.POD104.IleumBx"

 [9] "Pt22.MVTx.POD20.PBMC"   "Pt22.MVTx.POD24.ileum.Bx"

[11] "Pt22.MVTx.POD83.PBMC"   "Pt22.MVTx.SP.donor.CD4.CFSElow.D4L"

[13] "Pt22.MVTx.SP.donor.CD4.unstim.D4U"   "Pt22.MVTx.SP.donor.CD8.CFSElow.D8L"

[15] "Pt22.MVTx.SP.donor.CD8.unstim.D8U"
"Pt22.MVTx.SP.recipient.CD4.CFSElow.R4L"

[17] "Pt22.MVTx.SP.recipient.CD4.unstim.R4U"
"Pt22.MVTx.SP.recipient.CD8.CFSElow.R8L"

[19] "Pt22.MVTx.SP.recipient.CD8.unstim.R8U" "Total"

#data1

#set the rownames of data

rownames(data1)=paste(data1[,1],data1[,2],data1[,3])

data1[is.na(data1)]=0

write.table(data1,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 MiXCR cdr3ntvj functional.tsv",quote=F,row.names=T,col.names=F, sep="\t")

manullay add in column names

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 MiXCR cdr3ntvj functional.tsv")

```
names(data)

[1] "cdr3ntvj"                    "v"

 [3] "j"                          "cdr3nt"

 [5] "cdr3aa"                      "samples"

 [7] "Pt22.MVTx.POD10.PBMC"           "Pt22.MVTx.POD10.liver.Bx"

 [9] "Pt22.MVTx.POD104.IleumBx"       "Pt22.MVTx.POD20.PBMC"

[11] "Pt22.MVTx.POD24.ileum.Bx"       "Pt22.MVTx.POD83.PBMC"

[13] "Pt22.MVTx.SP.donor.CD4.CFSElow.D4L"     "Pt22.MVTx.SP.donor.CD4.unstim.D4U"

[15] "Pt22.MVTx.SP.donor.CD8.CFSElow.D8L"     "Pt22.MVTx.SP.donor.CD8.unstim.D8U"

[17] "Pt22.MVTx.SP.recipient.CD4.CFSElow.R4L"
"Pt22.MVTx.SP.recipient.CD4.unstim.R4U"

[19] "Pt22.MVTx.SP.recipient.CD8.CFSElow.R8L"
"Pt22.MVTx.SP.recipient.CD8.unstim.R8U"

[21] "Total"


#source('P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/! summary of R
codes/alloreactivity_printtofile 09132017.R')


# resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and
recipient ambiguous clones.


#D4U vs D8U

normalize(data[,c(14,16)])

ratio=5

 c1indices=which(data[,14]>0 & data[,16]>0 & data[,14]>ratio*data[,16])

 c2indices=which(data[,14]>0 & data[,16]>0 & data[,16]>ratio*data[,14])

 ambiindices=which(data[,14]>0 & data[,16]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,16]=0
```

```
data[c2indices,14]=0

data[ambiindices,c(14,16)]=0



#D4L vs D8L

normalize(data[,c(13,15)])

ratio=5

 c1indices=which(data[,13]>0 & data[,15]>0 & data[,13]>ratio*data[,15])

 c2indices=which(data[,13]>0 & data[,15]>0 & data[,15]>ratio*data[,13])

 ambiindices=which(data[,13]>0 & data[,15]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,15]=0

 data[c2indices,13]=0

 data[ambiindices,c(13,15)]=0



#R4U vs R8U

normalize(data[,c(18,20)])

ratio=5

 c1indices=which(data[,18]>0 & data[,20]>0 & data[,18]>ratio*data[,20])

 c2indices=which(data[,18]>0 & data[,20]>0 & data[,20]>ratio*data[,18])

 ambiindices=which(data[,18]>0 & data[,20]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,20]=0

 data[c2indices,18]=0

 data[ambiindices,c(18,20)]=0
```

```
#R4L vs R8L

normalize(data[,c(17,19)])

ratio=5

  c1indices=which(data[,17]>0 & data[,19]>0 & data[,17]>ratio*data[,19])

  c2indices=which(data[,17]>0 & data[,19]>0 & data[,19]>ratio*data[,17])

  ambiindices=which(data[,17]>0 & data[,19]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,19]=0

  data[c2indices,17]=0

  data[ambiindices,c(17,19)]=0


rownames(data)=data[,1]

x=data


#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,14]>0 | x[,16]>0)&(x[,18]>0 | x[,20]>0))

ambiguous=union(ambiguous,which((x[,14]>0 | x[,16]>0)&(x[,17]>0 | x[,19]>0)))

ambiguous=union(ambiguous,which((x[,18]>0 | x[,20]>0)&(x[,13]>0 | x[,15]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]


#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L,
R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional
step of optimization for CD4 and CD8 sorting error.


#R4L vs R8U

normalize(x[,c(17,20)])

ratio=2

  c1indices=which(x[,17]>0 & x[,20]>0 & x[,17]>ratio*x[,20])
```

```
c2indices=which(x[,17]>0 & x[,20]>0 & x[,20]>ratio*x[,17])
ambiindices=which(x[,17]>0 & x[,20]>0)
ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
x[c1indices,20]=0
x[c2indices,17]=0
x[ambiindices,c(17,20)]=0


#R8L vs R4U
normalize(x[,c(19,18)])
ratio=2
c1indices=which(x[,19]>0 & x[,18]>0 & x[,19]>ratio*x[,18])
c2indices=which(x[,19]>0 & x[,18]>0 & x[,18]>ratio*x[,19])
ambiindices=which(x[,19]>0 & x[,18]>0)
ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
x[c1indices,18]=0
x[c2indices,19]=0
x[ambiindices,c(19,18)]=0


#D4L vs D8U
normalize(x[,c(13,16)])
ratio=2
c1indices=which(x[,13]>0 & x[,16]>0 & x[,13]>ratio*x[,16])
c2indices=which(x[,13]>0 & x[,16]>0 & x[,16]>ratio*x[,13])
ambiindices=which(x[,13]>0 & x[,16]>0)
ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
x[c1indices,16]=0
x[c2indices,13]=0
x[ambiindices,c(13,16)]=0
```

```r
 #D8L vs D4U

normalize(x[,c(15,14)])

ratio=2

 c1indices=which(x[,15]>0 & x[,14]>0 & x[,15]>ratio*x[,14])

 c2indices=which(x[,15]>0 & x[,14]>0 & x[,14]>ratio*x[,15])

 ambiindices=which(x[,15]>0 & x[,14]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 x[c1indices,14]=0

 x[c2indices,15]=0

 x[ambiindices,c(15,14)]=0


write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn
data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 MiXCR cdr3ntvj
functional resolve ambiguous.tsv",quote=F,row.names=F,col.names=T, sep="\t")


# add "fold=2" below, use 2 fold expansion (default 5 fold)

# use freq=0.00002 for template counts; use default freq=0.00001 for read counts



#GVH direction

# For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]

# For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]

cd4.GVH= x[,c(14,13)]

cd8.GVH= x[,c(16,15)]

allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00001)


length(allo.GVH[[1]])

[1] 5228

length(allo.GVH[[2]])
```

[1] 3901

write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 CD4GVH list MiXCR.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 CD8GVH list MiXCR.txt",quote=F,row.names=F,col.names=F, sep="\t")

dCD4mappable=rownames(x[(x[,14]+x[,13])>0,])

dCD8mappable=rownames(x[(x[,16]+x[,15])>0,])

CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])

CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])

write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 CD4nonGVH dmappable list MiXCR.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 CD8nonGVH dmappable list MiXCR.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)
[1] 72240
length(dCD8mappable)
[1] 46018

length(CD4nonGVH)
[1] 67012
length(CD8nonGVH)
[1] 42117

```
dmappable=rownames(x[(x[,14]+x[,13]+x[,16]+x[,15])>0,])
```

```
length(dmappable)
```

[1] 118258

```
write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn
data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 donor mappable list
MiXCR.txt",quote=F,row.names=F,col.names=F, sep="\t")
```

```
length(dCD4mappable)+length(dCD8mappable)
```

[1] 118258

```
length(intersect(dCD4mappable,dCD8mappable))
```

[1] 0

```
#HVG direction
# For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]
# For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]
cd4.HVG= x[,c(18,17)]
cd8.HVG= x[,c(20,19)]
allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00001)
```

```
length(allo.HVG[[1]])
```

[1] 3340

```
length(allo.HVG[[2]])
```

[1] 1335

```
write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 CD4 HVGlist
MiXCR.txt",quote=F,row.names=F,col.names=F, sep="\t")
```

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 CD8 HVGlist MiXCR.txt",quote=F,row.names=F,col.names=F, sep="\t")

rCD4mappable=rownames(x[(x[,18]+x[,17])>0,])

rCD8mappable=rownames(x[(x[,20]+x[,19])>0,])

CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])

write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 CD4 nonHVGlist MiXCR.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 CD8 nonHVGlist MiXCR.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)

[1] 48357

length(rCD8mappable)

[1] 16924

length(CD4nonHVG)

[1] 45017

length(CD8nonHVG)

[1] 15589

rmappable=rownames(x[(x[,18]+x[,17]+x[,20]+x[,19])>0,])

length(rmappable)

[1] 65281

```
write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn
data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 recipient mappable list
MiXCR.txt",quote=F,row.names=F,col.names=F, sep="\t")
```

```
length(rCD4mappable)+length(rCD8mappable)
```

[1] 65281

```
length(intersect(rCD4mappable,rCD8mappable))
```

[1] 0

```
#Pt22 POD24 Bx GVH
```

```
cd4= x[,c(14,13,11)]
```

```
cd8= x[,c(16,15,11)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 2x
MiXCR.out' )
```

```
rownames(x)=x[,1]
```

```
cd4= x[,c(14,13)]
```

```
cd8= x[,c(16,15)]
```

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)
```

```
intersect(allo[[1]], rownames(x[x[,11]>0,]))
```

```
intersect(allo[[2]], rownames(x[x[,11]>0,]))
```

```
#Pt22 POD24 Bx HVG
```

```
cd4= x[,c(18,17,11)]
```

```
cd8= x[,c(20,19,11)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(18,17)]

cd8= x[,c(20,19)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,11]>0,]))

intersect(allo[[2]], rownames(x[x[,11]>0,]))



#Pt22 POD104 Bx GVH

cd4= x[,c(14,13,9)]

cd8= x[,c(16,15,9)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(14,13)]

cd8= x[,c(16,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,9]>0,]))

intersect(allo[[2]], rownames(x[x[,9]>0,]))



#Pt22 POD104 Bx HVG


cd4= x[,c(18,17,9)]
```

```
cd8= x[,c(20,19,9)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 2x
MiXCR.out' )
```

```
rownames(x)=x[,1]
```

```
cd4= x[,c(18,17)]
```

```
cd8= x[,c(20,19)]
```

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)
```

```
intersect(allo[[1]], rownames(x[x[,9]>0,]))
```

```
intersect(allo[[2]], rownames(x[x[,9]>0,]))
```

```
#Pt22 POD10 PBMC GVH
```

```
cd4= x[,c(14,13,7)]
```

```
cd8= x[,c(16,15,7)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 2x
MiXCR.out' )
```

```
rownames(x)=x[,1]
```

```
cd4= x[,c(14,13)]
```

```
cd8= x[,c(16,15)]
```

```
allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)
```

```
intersect(allo[[1]], rownames(x[x[,7]>0,]))
```

```
intersect(allo[[2]], rownames(x[x[,7]>0,]))
```

```
#Pt22 POD10 PBMC HVG
```

```
cd4= x[,c(18,17,7)]
```

```
cd8= x[,c(20,19,7)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(18,17)]

cd8= x[,c(20,19)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,7]>0,]))

intersect(allo[[2]], rownames(x[x[,7]>0,]))



#Pt22 POD20 PBMC GVH

cd4= x[,c(14,13,10)]

cd8= x[,c(16,15,10)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(14,13)]

cd8= x[,c(16,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))


#Pt22 POD20 PBMC HVG

cd4= x[,c(18,17,10)]

cd8= x[,c(20,19,10)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(18,17)]

cd8= x[,c(20,19)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))




#Pt22 POD83 PBMC GVH

cd4= x[,c(14,13,12)]

cd8= x[,c(16,15,12)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(14,13)]

cd8= x[,c(16,15)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,12]>0,]))

intersect(allo[[2]], rownames(x[x[,12]>0,]))


#Pt22 POD83 PBMC HVG

cd4= x[,c(18,17,12)]

cd8= x[,c(20,19,12)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-09-2019 updated MiXCR data/Pt22 08092019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(18,17)]

cd8= x[,c(20,19)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,12]>0,]))

intersect(allo[[2]], rownames(x[x[,12]>0,]))
```

data1 <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 with cut off_functional only.tsv")

names(data1)

 [1] "v"   "j"

 [3] "cdr3nt"   "cdr3aa"

 [5] "samples"   "Pt23.MVTx.POD10.PBMC"

 [7] "Pt23.MVTx.POD10.ileum.Bx"   "Pt23.MVTx.POD119.PBMC"

 [9] "Pt23.MVTx.POD149.IleumBx"   "Pt23.MVTx.POD15.17.ileum.Bx"

[11] "Pt23.MVTx.POD18.PBMC"   "Pt23.MVTx.POD346.BM.T"

[13] "Pt23.MVTx.POD346.PBMC.T"   "Pt23.MVTx.POD346.cecum.Bx.T"

[15] "Pt23.MVTx.POD346.duodenum.Bx.T"   "Pt23.MVTx.POD346.ileum.Bx.T"

[17] "Pt23.MVTx.POD346.native.colon.Bx.T"   "Pt23.MVTx.POD346.stomach.Bx.T"

[19] "Pt23.MVTx.POD37.ileum.resection.T"   "Pt23.MVTx.POD44.49.IleumBx"

[21] "Pt23.MVTx.POD46.PBMC"   "Pt23.MVTx.SP.donor.CD4.CFSElow.D4L"

[23] "Pt23.MVTx.SP.donor.CD4.unstim.D4U"   "Pt23.MVTx.SP.donor.CD8.CFSElow.D8L"

[25] "Pt23.MVTx.SP.donor.CD8.unstim.D8U"
"Pt23.MVTx.SP.recipient.CD4.CFSElow.R4L"

[27] "Pt23.MVTx.SP.recipient.CD4.unstim.R4U"
"Pt23.MVTx.SP.recipient.CD8.CFSElow.R8L"

[29] "Pt23.MVTx.SP.recipient.CD8.unstim.R8U" "Sum"

#data1
#set the rownames of data
rownames(data1)=paste(data1[,1],data1[,2],data1[,3])
data1[is.na(data1)]=0

write.table(data1,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 MiXCR cdr3ntvj functional.tsv",quote=F,row.names=T,col.names=F, sep="\t")

manullay add in column names

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 MiXCR cdr3ntvj functional.tsv")

names(data)

 [1] "cdr3ntvj"   "v"

 [3] "j"   "cdr3nt"

 [5] "cdr3aa"   "samples"

 [7] "Pt23.MVTx.POD10.PBMC"   "Pt23.MVTx.POD10.ileum.Bx"

 [9] "Pt23.MVTx.POD119.PBMC"   "Pt23.MVTx.POD149.IleumBx"

[11] "Pt23.MVTx.POD15.17.ileum.Bx"   "Pt23.MVTx.POD18.PBMC"

[13] "Pt23.MVTx.POD346.BM.T"   "Pt23.MVTx.POD346.PBMC.T"

[15] "Pt23.MVTx.POD346.cecum.Bx.T"   "Pt23.MVTx.POD346.duodenum.Bx.T"

[17] "Pt23.MVTx.POD346.ileum.Bx.T"   "Pt23.MVTx.POD346.native.colon.Bx.T"

[19] "Pt23.MVTx.POD346.stomach.Bx.T"   "Pt23.MVTx.POD37.ileum.resection.T"

[21] "Pt23.MVTx.POD44.49.IleumBx"   "Pt23.MVTx.POD46.PBMC"

[23] "Pt23.MVTx.SP.donor.CD4.CFSElow.D4L"   "Pt23.MVTx.SP.donor.CD4.unstim.D4U"

[25] "Pt23.MVTx.SP.donor.CD8.CFSElow.D8L"   "Pt23.MVTx.SP.donor.CD8.unstim.D8U"

[27] "Pt23.MVTx.SP.recipient.CD4.CFSElow.R4L"
"Pt23.MVTx.SP.recipient.CD4.unstim.R4U"

[29] "Pt23.MVTx.SP.recipient.CD8.CFSElow.R8L"
"Pt23.MVTx.SP.recipient.CD8.unstim.R8U"

[31] "Sum"

```
#source('P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/! summary of R
codes/alloreactivity_printtofile 09132017.R')


# resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and
recipient ambiguous clones.


#D4U vs D8U

normalize(data[,c(24,26)])

ratio=5

  c1indices=which(data[,24]>0 & data[,26]>0 & data[,24]>ratio*data[,26])

  c2indices=which(data[,24]>0 & data[,26]>0 & data[,26]>ratio*data[,24])

  ambiindices=which(data[,24]>0 & data[,26]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,26]=0

  data[c2indices,24]=0

  data[ambiindices,c(24,26)]=0


#D4L vs D8L

normalize(data[,c(23,25)])

ratio=5

  c1indices=which(data[,23]>0 & data[,25]>0 & data[,23]>ratio*data[,25])

  c2indices=which(data[,23]>0 & data[,25]>0 & data[,25]>ratio*data[,23])

  ambiindices=which(data[,23]>0 & data[,25]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,25]=0

  data[c2indices,23]=0

  data[ambiindices,c(23,25)]=0
```

```
#R4U vs R8U

normalize(data[,c(28,30)])

ratio=5

 c1indices=which(data[,28]>0 & data[,30]>0 & data[,28]>ratio*data[,30])

 c2indices=which(data[,28]>0 & data[,30]>0 & data[,30]>ratio*data[,28])

 ambiindices=which(data[,28]>0 & data[,30]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,30]=0

 data[c2indices,28]=0

 data[ambiindices,c(28,30)]=0




#R4L vs R8L

normalize(data[,c(27,29)])

ratio=5

 c1indices=which(data[,27]>0 & data[,29]>0 & data[,27]>ratio*data[,29])

 c2indices=which(data[,27]>0 & data[,29]>0 & data[,29]>ratio*data[,27])

 ambiindices=which(data[,27]>0 & data[,29]>0)

 ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

 data[c1indices,29]=0

 data[c2indices,27]=0

 data[ambiindices,c(27,29)]=0


rownames(data)=data[,1]

x=data
```

#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by CFSE-MLR sorting

ambiguous=which((x[,24]>0 | x[,26]>0)&(x[,28]>0 | x[,30]>0))

ambiguous=union(ambiguous,which((x[,24]>0 | x[,26]>0)&(x[,27]>0 | x[,29]>0)))

ambiguous=union(ambiguous,which((x[,28]>0 | x[,30]>0)&(x[,23]>0 | x[,25]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]

#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L, R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional step of optimization for CD4 and CD8 sorting error.

#R4L vs R8U

normalize(x[,c(27,30)])

ratio=2

  c1indices=which(x[,27]>0 & x[,30]>0 & x[,27]>ratio*x[,30])

  c2indices=which(x[,27]>0 & x[,30]>0 & x[,30]>ratio*x[,27])

  ambiindices=which(x[,27]>0 & x[,30]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,30]=0

  x[c2indices,27]=0

  x[ambiindices,c(27,30)]=0

#R8L vs R4U

normalize(x[,c(29,28)])

ratio=2

  c1indices=which(x[,29]>0 & x[,28]>0 & x[,29]>ratio*x[,28])

  c2indices=which(x[,29]>0 & x[,28]>0 & x[,28]>ratio*x[,29])

  ambiindices=which(x[,29]>0 & x[,28]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

```
  x[c1indices,28]=0

  x[c2indices,29]=0

  x[ambiindices,c(29,28)]=0


#D4L vs D8U

normalize(x[,c(23,26)])

ratio=2

  c1indices=which(x[,23]>0 & x[,26]>0 & x[,23]>ratio*x[,26])

  c2indices=which(x[,23]>0 & x[,26]>0 & x[,26]>ratio*x[,23])

  ambiindices=which(x[,23]>0 & x[,26]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,26]=0

  x[c2indices,23]=0

  x[ambiindices,c(23,26)]=0


#D8L vs D4U

normalize(x[,c(25,24)])

ratio=2

  c1indices=which(x[,25]>0 & x[,24]>0 & x[,25]>ratio*x[,24])

  c2indices=which(x[,25]>0 & x[,24]>0 & x[,24]>ratio*x[,25])

  ambiindices=which(x[,25]>0 & x[,24]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,24]=0

  x[c2indices,25]=0

  x[ambiindices,c(25,24)]=0


write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn
data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 MiXCR cdr3ntvj
functional resolve ambiguous.tsv",quote=F,row.names=F,col.names=T, sep="\t")
```

add "fold=2" below, use 2 fold expansion (default 5 fold)

use freq=0.00002 for template counts; use default freq=0.00001 for read counts

#GVH direction

For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]

For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]

cd4.GVH= x[,c(24,23)]

cd8.GVH= x[,c(26,25)]

allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00001)

#When you define allo=listAlloreactive(cd4,cd8),then you should get cd4 alloreactives stored in allo[[1]] and cd8 alloreactives stored in allo[[2]]. If you do allo=union(allo[[1]],allo[[2]]), then allo contains all the alloreactives, and you can define nonallo=setdiff(rownames(x),allo)

length(allo.GVH[[1]])

[1] 1096

length(allo.GVH[[2]])

[1] 1391

write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 CD4GVH list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 CD8GVH list.txt",quote=F,row.names=F,col.names=F, sep="\t")

dCD4mappable=rownames(x[(x[,24]+x[,23])>0,])

dCD8mappable=rownames(x[(x[,26]+x[,25])>0,])

CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])

CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])

write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 CD4nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)

[1] 19842

length(dCD8mappable)

[1] 42160

length(CD4nonGVH)

[1] 18746

length(CD8nonGVH)

[1] 40769

dmappable=rownames(x[(x[,24]+x[,23]+x[,26]+x[,25])>0,])

length(dmappable)

[1] 62002

write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)+length(dCD8mappable)

[1] 62002

length(intersect(dCD4mappable,dCD8mappable))

[1] 0

```
#HVG direction

# For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]

# For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]

cd4.HVG= x[,c(28,27)]

cd8.HVG= x[,c(30,29)]

allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00001)


length(allo.HVG[[1]])

[1] 2399

length(allo.HVG[[2]])

[1] 3043


write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 CD4
HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 CD8
HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")


rCD4mappable=rownames(x[(x[,28]+x[,27])>0,])

rCD8mappable=rownames(x[(x[,30]+x[,29])>0,])


CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])

write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 CD4
nonHVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 CD8
nonHVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")
```

length(rCD4mappable)

[1] 47306

length(rCD8mappable)

[1] 89683

length(CD4nonHVG)

[1] 44907

length(CD8nonHVG)

[1] 86640

rmappable=rownames(x[(x[,28]+x[,27]+x[,30]+x[,29])>0,])

length(rmappable)

[1] 136989

write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 recipient mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(rCD4mappable)+length(rCD8mappable)

[1] 136989

length(intersect(rCD4mappable,rCD8mappable))

[1] 0

#Pt23 POD10 Bx GVH

cd4= x[,c(24,23,8)]

cd8= x[,c(26,25,8)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x MiXCR.out')

rownames(x)=x[,1]

cd4= x[,c(24,23)]

```
cd8= x[,c(26,25)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,8]>0,]))

intersect(allo[[2]], rownames(x[x[,8]>0,]))


#Pt23 POD10 Bx HVG

cd4= x[,c(28,27,8)]

cd8= x[,c(30,29,8)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(28,27)]

cd8= x[,c(30,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,8]>0,]))

intersect(allo[[2]], rownames(x[x[,8]>0,]))


#Pt23 POD15-17 Bx GVH

cd4= x[,c(24,23,11)]

cd8= x[,c(26,25,11)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(24,23)]

cd8= x[,c(26,25)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)
```

```
intersect(allo[[1]], rownames(x[x[,11]>0,]))

intersect(allo[[2]], rownames(x[x[,11]>0,]))


#Pt23 POD15-17 Bx HVG

cd4= x[,c(28,27,11)]

cd8= x[,c(30,29,11)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(28,27)]

cd8= x[,c(30,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,11]>0,]))

intersect(allo[[2]], rownames(x[x[,11]>0,]))


#Pt23 POD37 Bx GVH

cd4= x[,c(24,23,20)]

cd8= x[,c(26,25,20)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(24,23)]

cd8= x[,c(26,25)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))
```

```
#Pt23 POD37 Bx HVG

cd4= x[,c(28,27,20)]

cd8= x[,c(30,29,20)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(28,27)]

cd8= x[,c(30,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,20]>0,]))

intersect(allo[[2]], rownames(x[x[,20]>0,]))


#Pt23 POD44-49 Bx GVH

cd4= x[,c(24,23,21)]

cd8= x[,c(26,25,21)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(24,23)]

cd8= x[,c(26,25)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,21]>0,]))

intersect(allo[[2]], rownames(x[x[,21]>0,]))


#Pt23 POD44-49 Bx HVG

cd4= x[,c(28,27,21)]
```

```
cd8= x[,c(30,29,21)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(28,27)]

cd8= x[,c(30,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,21]>0,]))

intersect(allo[[2]], rownames(x[x[,21]>0,]))


#Pt23 POD149 Bx GVH

cd4= x[,c(24,23,10)]

cd8= x[,c(26,25,10)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(24,23)]

cd8= x[,c(26,25)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))


#Pt23 POD149 Bx HVG

cd4= x[,c(28,27,10)]

cd8= x[,c(30,29,10)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(28,27)]

cd8= x[,c(30,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,10]>0,]))

intersect(allo[[2]], rownames(x[x[,10]>0,]))


#Pt23 POD346 ileum Bx GVH

cd4= x[,c(24,23,17)]

cd8= x[,c(26,25,17)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(24,23)]

cd8= x[,c(26,25)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))


#Pt23 POD346 ileum Bx HVG

cd4= x[,c(28,27,17)]

cd8= x[,c(30,29,17)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(28,27)]

cd8= x[,c(30,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))


#Pt23 POD10 PBMC GVH

cd4= x[,c(24,23,7)]

cd8= x[,c(26,25,7)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(24,23)]

cd8= x[,c(26,25)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,7]>0,]))

intersect(allo[[2]], rownames(x[x[,7]>0,]))


#Pt23 POD10 PBMC HVG

cd4= x[,c(28,27,7)]

cd8= x[,c(30,29,7)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(28,27)]

cd8= x[,c(30,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,7]>0,]))

intersect(allo[[2]], rownames(x[x[,7]>0,]))


#Pt23 POD18 PBMC GVH

cd4= x[,c(24,23,12)]

cd8= x[,c(26,25,12)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(24,23)]

cd8= x[,c(26,25)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,12]>0,]))

intersect(allo[[2]], rownames(x[x[,12]>0,]))


#Pt23 POD18 PBMC HVG

cd4= x[,c(28,27,12)]

cd8= x[,c(30,29,12)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(28,27)]

cd8= x[,c(30,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,12]>0,]))

intersect(allo[[2]], rownames(x[x[,12]>0,]))


#Pt23 POD46 PBMC GVH

cd4= x[,c(24,23,22)]

cd8= x[,c(26,25,22)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(24,23)]

cd8= x[,c(26,25)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))


#Pt23 POD46 PBMC HVG

cd4= x[,c(28,27,22)]

cd8= x[,c(30,29,22)]
```

```r
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(28,27)]

cd8= x[,c(30,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,22]>0,]))

intersect(allo[[2]], rownames(x[x[,22]>0,]))


#Pt23 POD119 PBMC GVH

cd4= x[,c(24,23,9)]

cd8= x[,c(26,25,9)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(24,23)]

cd8= x[,c(26,25)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,9]>0,]))

intersect(allo[[2]], rownames(x[x[,9]>0,]))


#Pt23 POD119 PBMC HVG

cd4= x[,c(28,27,9)]

cd8= x[,c(30,29,9)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(28,27)]

cd8= x[,c(30,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,9]>0,]))

intersect(allo[[2]], rownames(x[x[,9]>0,]))


#Pt23 POD346 PBMC GVH

cd4= x[,c(24,23,14)]

cd8= x[,c(26,25,14)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(24,23)]

cd8= x[,c(26,25)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,14]>0,]))

intersect(allo[[2]], rownames(x[x[,14]>0,]))


#Pt23 POD346 PBMC HVG

cd4= x[,c(28,27,14)]

cd8= x[,c(30,29,14)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(28,27)]

cd8= x[,c(30,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,14]>0,]))

intersect(allo[[2]], rownames(x[x[,14]>0,]))


#Pt23 POD346 BM GVH

cd4= x[,c(24,23,13)]

cd8= x[,c(26,25,13)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(24,23)]

cd8= x[,c(26,25)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,13]>0,]))

intersect(allo[[2]], rownames(x[x[,13]>0,]))


#Pt23 POD346 BM HVG

cd4= x[,c(28,27,13)]

cd8= x[,c(30,29,13)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt23 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(28,27)]

cd8= x[,c(30,29)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,13]>0,]))

intersect(allo[[2]], rownames(x[x[,13]>0,]))
```

data1 <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 with cut off_functional only.tsv")

names(data1)

 [1] "v"   "j"

 [3] "cdr3nt"   "cdr3aa"

 [5] "samples"   "Pt24.iITx.LN.recipient.CD4.CFSElow.HVG.R4L"

 [7] "Pt24.iITx.LN.recipient.CD4.unstim.R4U"
"Pt24.iITx.LN.recipient.CD8.CFSElow.HVG.R8L"

 [9] "Pt24.iITx.LN.recipient.CD8.unstim.R8U"   "Pt24.iITx.POD18.IleumBx.sortedT"

[11] "Pt24.iITx.POD20.PBMC.sortedT"   "Pt24.iITx.POD32.IleumBx"

[13] "Pt24.iITx.POD36.PBMC"   "Pt24.iITx.POD39.IleumBx"

[15] "Pt24.iITx.POD54.BM"   "Pt24.iITx.POD8.PBMC"

[17] "Pt24.iITx.SP.donor.CD4.CFSElow.GVH.D4L"   "Pt24.iITx.SP.donor.CD4.unstim.D4U"

[19] "Pt24.iITx.SP.donor.CD8.CFSElow.GVH.D8L"   "Pt24.iITx.SP.donor.CD8.unstim.D8U"

[21] "Sum"

#data1

#set the rownames of data

rownames(data1)=paste(data1[,1],data1[,2],data1[,3])

data1[is.na(data1)]=0

write.table(data1,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 MiXCR cdr3ntvj functional.tsv",quote=F,row.names=T,col.names=F, sep="\t")

manullay add in column names

data <- read.delim("P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 MiXCR cdr3ntvj functional.tsv")

names(data)

 [1] "cdr3ntvj"   "v"

 [3] "j"   "cdr3nt"

 [5] "cdr3aa"   "samples"

 [7] "Pt24.iITx.LN.recipient.CD4.CFSElow.HVG.R4L" "Pt24.iITx.LN.recipient.CD4.unstim.R4U"

 [9] "Pt24.iITx.LN.recipient.CD8.CFSElow.HVG.R8L" "Pt24.iITx.LN.recipient.CD8.unstim.R8U"

[11] "Pt24.iITx.POD18.IleumBx.sortedT"   "Pt24.iITx.POD20.PBMC.sortedT"

[13] "Pt24.iITx.POD32.IleumBx"   "Pt24.iITx.POD36.PBMC"

[15] "Pt24.iITx.POD39.IleumBx"   "Pt24.iITx.POD54.BM"

[17] "Pt24.iITx.POD8.PBMC"   "Pt24.iITx.SP.donor.CD4.CFSElow.GVH.D4L"

[19] "Pt24.iITx.SP.donor.CD4.unstim.D4U" "Pt24.iITx.SP.donor.CD8.CFSElow.GVH.D8L"

[21] "Pt24.iITx.SP.donor.CD8.unstim.D8U"   "Sum"

#source('P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/! summary of R codes/alloreactivity_printtofile 09132017.R')

abundancePlot(normalize(data[,c(19,21,18,20)]))

abundancePlot(normalize(data[,c(8,10,7,9)]))

resolve CD4 and CD8 ambiguous clones first (ratio=5), then followed by resolve donor and recipient ambiguous clones.

#D4U vs D8U

```
normalize(data[,c(19,21)])

ratio=5

  c1indices=which(data[,19]>0 & data[,21]>0 & data[,19]>ratio*data[,21])

  c2indices=which(data[,19]>0 & data[,21]>0 & data[,21]>ratio*data[,19])

  ambiindices=which(data[,19]>0 & data[,21]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,21]=0

  data[c2indices,19]=0

  data[ambiindices,c(19,21)]=0


#D4L vs D8L

normalize(data[,c(18,20)])

ratio=5

  c1indices=which(data[,18]>0 & data[,20]>0 & data[,18]>ratio*data[,20])

  c2indices=which(data[,18]>0 & data[,20]>0 & data[,20]>ratio*data[,18])

  ambiindices=which(data[,18]>0 & data[,20]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,20]=0

  data[c2indices,18]=0

  data[ambiindices,c(18,20)]=0


#R4U vs R8U

normalize(data[,c(8,10)])

ratio=5

  c1indices=which(data[,8]>0 & data[,10]>0 & data[,8]>ratio*data[,10])

  c2indices=which(data[,8]>0 & data[,10]>0 & data[,10]>ratio*data[,8])

  ambiindices=which(data[,8]>0 & data[,10]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)
```

```
  data[c1indices,10]=0

  data[c2indices,8]=0

  data[ambiindices,c(8,10)]=0


#R4L vs R8L

normalize(data[,c(7,9)])

ratio=5

  c1indices=which(data[,7]>0 & data[,9]>0 & data[,7]>ratio*data[,9])

  c2indices=which(data[,7]>0 & data[,9]>0 & data[,9]>ratio*data[,7])

  ambiindices=which(data[,7]>0 & data[,9]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  data[c1indices,9]=0

  data[c2indices,7]=0

  data[ambiindices,c(7,9)]=0


rownames(data)=data[,1]

x=data


#remove donor/recipient ambiguous clones, either real shared clones, or ambiguous raised by
CFSE-MLR sorting

ambiguous=which((x[,19]>0 | x[,21]>0)&(x[,8]>0 | x[,10]>0))

ambiguous=union(ambiguous,which((x[,19]>0 | x[,21]>0)&(x[,7]>0 | x[,9]>0)))

ambiguous=union(ambiguous,which((x[,8]>0 | x[,10]>0)&(x[,18]>0 | x[,20]>0)))

x=x[setdiff(1:nrow(x),ambiguous),]
```

#Should additionally remove stim vs unstim ambiguous within recipient preTx (R4L, R4U, R8L, R8U) or donor preTx (D4L, D4U, D8L, D8U) samples; which can be considered as additional step of optimization for CD4 and CD8 sorting error.

#R4L vs R8U

normalize(x[,c(7,10)])

ratio=2

  c1indices=which(x[,7]>0 & x[,10]>0 & x[,7]>ratio*x[,10])

  c2indices=which(x[,7]>0 & x[,10]>0 & x[,10]>ratio*x[,7])

  ambiindices=which(x[,7]>0 & x[,10]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,10]=0

  x[c2indices,7]=0

  x[ambiindices,c(7,10)]=0

#R8L vs R4U

normalize(x[,c(9,8)])

ratio=2

  c1indices=which(x[,9]>0 & x[,8]>0 & x[,9]>ratio*x[,8])

  c2indices=which(x[,9]>0 & x[,8]>0 & x[,8]>ratio*x[,9])

  ambiindices=which(x[,9]>0 & x[,8]>0)

  ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

  x[c1indices,8]=0

  x[c2indices,9]=0

  x[ambiindices,c(9,8)]=0

#D4L vs D8U

normalize(x[,c(18,21)])

ratio=2

```
c1indices=which(x[,18]>0 & x[,21]>0 & x[,18]>ratio*x[,21])

c2indices=which(x[,18]>0 & x[,21]>0 & x[,21]>ratio*x[,18])

ambiindices=which(x[,18]>0 & x[,21]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

x[c1indices,21]=0

x[c2indices,18]=0

x[ambiindices,c(18,21)]=0


#D8L vs D4U

normalize(x[,c(20,19)])

ratio=2

c1indices=which(x[,20]>0 & x[,19]>0 & x[,20]>ratio*x[,19])

c2indices=which(x[,20]>0 & x[,19]>0 & x[,19]>ratio*x[,20])

ambiindices=which(x[,20]>0 & x[,19]>0)

ambiindices=setdiff(setdiff(ambiindices,c1indices),c2indices)

x[c1indices,19]=0

x[c2indices,20]=0

x[ambiindices,c(20,19)]=0


write.table(x,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn
data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 MiXCR cdr3ntvj
functional resolve ambiguous.tsv",quote=F,row.names=F,col.names=T, sep="\t")


# add "fold=2" below, use 2 fold expansion (default 5 fold)

# use freq=0.00002 for template counts; use default freq=0.00001 for read counts
```

```
#GVH direction
# For CD4 GVH, cd4.GVH=x[,c(unstim(D4U),stim(D4L))]
# For CD8 GVH, cd8.GVH=x[,c(unstim(D8U),stim(D8L))]
cd4.GVH= x[,c(19,18)]
cd8.GVH= x[,c(21,20)]
allo.GVH=listAlloreactive(cd4.GVH,cd8.GVH, fold=2, freq=0.00001)


length(allo.GVH[[1]])
[1] 514
length(allo.GVH[[2]])
[1] 428


write.table(allo.GVH[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 CD4GVH
list.txt",quote=F,row.names=F,col.names=F, sep="\t")
write.table(allo.GVH[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 CD8GVH
list.txt",quote=F,row.names=F,col.names=F, sep="\t")



dCD4mappable=rownames(x[(x[,19]+x[,18])>0,])
dCD8mappable=rownames(x[(x[,21]+x[,20])>0,])


CD4nonGVH=setdiff(dCD4mappable,allo.GVH[[1]])
CD8nonGVH=setdiff(dCD8mappable,allo.GVH[[2]])


write.table(CD4nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 CD4nonGVH
rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")
```

write.table(CD8nonGVH,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 CD8nonGVH rmappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)

[1] 45418

length(dCD8mappable)

[1] 34023

length(CD4nonGVH)

[1] 44904

length(CD8nonGVH)

[1] 33595

dmappable=rownames(x[(x[,19]+x[,18]+x[,21]+x[,20])>0,])

length(dmappable)

[1] 79441

write.table(dmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 donor mappable list.txt",quote=F,row.names=F,col.names=F, sep="\t")

length(dCD4mappable)+length(dCD8mappable)

[1] 79441

length(intersect(dCD4mappable,dCD8mappable))

[1] 0

#HVG direction
# For CD4 HvG, cd4.HVG=x[,c(unstim(R4U),stim(R4L))]
# For CD8 HvG, cd8.HVG=x[,c(unstim(R8U),stim(R8L))]
cd4.HVG= x[,c(8,7)]

```
cd8.HVG= x[,c(10,9)]

allo.HVG=listAlloreactive(cd4.HVG,cd8.HVG, fold=2, freq=0.00001)


length(allo.HVG[[1]])

[1] 837

length(allo.HVG[[2]])

[1] 650


write.table(allo.HVG[[1]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 CD4
HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(allo.HVG[[2]],file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 CD8
HVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")


rCD4mappable=rownames(x[(x[,8]+x[,7])>0,])

rCD8mappable=rownames(x[(x[,10]+x[,9])>0,])


CD4nonHVG=setdiff(rCD4mappable,allo.HVG[[1]])

CD8nonHVG=setdiff(rCD8mappable,allo.HVG[[2]])


write.table(CD4nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 CD4
nonHVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")

write.table(CD8nonHVG,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 CD8
nonHVGlist.txt",quote=F,row.names=F,col.names=F, sep="\t")


length(rCD4mappable)

[1] 11270

length(rCD8mappable)
```

```
[1] 8553

length(CD4nonHVG)

[1] 10433

length(CD8nonHVG)

[1] 7903


rmappable=rownames(x[(x[,8]+x[,7]+x[,10]+x[,9])>0,])


length(rmappable)

[1] 19823

write.table(rmappable,file ="P:/CCTI_USERS/Jianing Fu/Adaptive samples and analysis/Upenn
data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 recipient mappable
list.txt",quote=F,row.names=F,col.names=F, sep="\t")


length(rCD4mappable)+length(rCD8mappable)

[1] 19823

length(intersect(rCD4mappable,rCD8mappable))

[1] 0


#Pt24 POD18 Bx GVH

cd4= x[,c(19,18,11)]

cd8= x[,c(21,20,11)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(19,18)]

cd8= x[,c(21,20)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)
```

```
intersect(allo[[1]], rownames(x[x[,11]>0,]))

intersect(allo[[2]], rownames(x[x[,11]>0,]))
```

#Pt24 POD18 Bx HVG

```
cd4= x[,c(8,7,11)]

cd8= x[,c(10,9,11)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(8,7)]

cd8= x[,c(10,9)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,11]>0,]))

intersect(allo[[2]], rownames(x[x[,11]>0,]))
```

#Pt24 POD32 Bx GVH

```
cd4= x[,c(19,18,13)]

cd8= x[,c(21,20,13)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(19,18)]

cd8= x[,c(21,20)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)
```

```
intersect(allo[[1]], rownames(x[x[,13]>0,]))

intersect(allo[[2]], rownames(x[x[,13]>0,]))
```

#Pt24 POD32 Bx HVG

```
cd4= x[,c(8,7,13)]

cd8= x[,c(10,9,13)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(8,7)]

cd8= x[,c(10,9)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,13]>0,]))

intersect(allo[[2]], rownames(x[x[,13]>0,]))
```

#Pt24 POD39 Bx GVH

```
cd4= x[,c(19,18,15)]

cd8= x[,c(21,20,15)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )
```

```
rownames(x)=x[,1]

cd4= x[,c(19,18)]

cd8= x[,c(21,20)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,15]>0,]))
```

```
intersect(allo[[2]], rownames(x[x[,15]>0,]))


#Pt24 POD39 Bx HVG

cd4= x[,c(8,7,15)]

cd8= x[,c(10,9,15)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(8,7)]

cd8= x[,c(10,9)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,15]>0,]))

intersect(allo[[2]], rownames(x[x[,15]>0,]))


#Pt24 POD8 PBMC GVH

cd4= x[,c(19,18,17)]

cd8= x[,c(21,20,17)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(19,18)]

cd8= x[,c(21,20)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))
```

```
#Pt24 POD8 PBMC HVG

cd4= x[,c(8,7,17)]

cd8= x[,c(10,9,17)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(8,7)]

cd8= x[,c(10,9)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,17]>0,]))

intersect(allo[[2]], rownames(x[x[,17]>0,]))


#Pt24 POD20 PBMC GVH

cd4= x[,c(19,18,12)]

cd8= x[,c(21,20,12)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(19,18)]

cd8= x[,c(21,20)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,12]>0,]))

intersect(allo[[2]], rownames(x[x[,12]>0,]))
```

```
#Pt24 POD20 PBMC HVG

cd4= x[,c(8,7,12)]

cd8= x[,c(10,9,12)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(8,7)]

cd8= x[,c(10,9)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,12]>0,]))

intersect(allo[[2]], rownames(x[x[,12]>0,]))


#Pt24 POD36 PBMC GVH

cd4= x[,c(19,18,14)]

cd8= x[,c(21,20,14)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(19,18)]

cd8= x[,c(21,20)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,14]>0,]))

intersect(allo[[2]], rownames(x[x[,14]>0,]))


#Pt24 POD36 PBMC HVG

cd4= x[,c(8,7,14)]
```

```
cd8= x[,c(10,9,14)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(8,7)]

cd8= x[,c(10,9)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,14]>0,]))

intersect(allo[[2]], rownames(x[x[,14]>0,]))


#Pt24 POD54 BM GVH

cd4= x[,c(19,18,16)]

cd8= x[,c(21,20,16)]

run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(19,18)]

cd8= x[,c(21,20)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))


#Pt24 POD54 BM HVG

cd4= x[,c(8,7,16)]

cd8= x[,c(10,9,16)]
```

```
run(cd4,cd8, fold=2,freq=0.00001, filename='P:/CCTI_USERS/Jianing Fu/Adaptive samples and
analysis/Upenn data_Immune DB_MiXCR/08-21-2019 updated MiXCR data/Pt24 08212019 2x
MiXCR.out' )


rownames(x)=x[,1]

cd4= x[,c(8,7)]

cd8= x[,c(10,9)]

allo=listAlloreactive(cd4, cd8, fold=2,freq=0.00001)

intersect(allo[[1]], rownames(x[x[,16]>0,]))

intersect(allo[[2]], rownames(x[x[,16]>0,]))
```