main

July 12, 2021

1 Building consensus network with WGCNA

```
[1]: PARAM_NETWORK_TYPE = 'signed'
```

1.1 Functions

```
[2]: filter_outliers = function(expression, z_threshold = 2.5)
          # Input: an expression matrix
          # Output: an expression matrix with outliers removed
          # Remove samples with z normalized total distance from other samples >\sqcup
      \rightarrow z threshold
          sample_distance = dist(expression)
          dist_z = scale(colSums(as.matrix(sample_distance)))
          stopifnot(all(rownames(dist_z) == rownames(expression)))
          keepSamples = dist_z < z_threshold
          new_expression = expression[keepSamples,]
          new_expression
     }
     prepare_data=function(setLabels)
         suppressMessages(library(dplyr))
         # Load sample data
         load("../../../differential_analysis/caudate/_m/genes/voomSVA.RData")
         phenotypes = v$targets %>% as.data.frame %>% select(RNum, Race)
         sample_table0 = v$design %>% as.data.frame %>% select(-Intercept) %>%
             rename("Ancestry"="EA", "Sex"="Male")
         sample_table = phenotypes %>%
             inner_join(tibble::rownames_to_column(sample_table0, "RNum"),__
      \rightarrowby=c("RNum")) %>%
             mutate("V1"=RNum) %>% tibble::column_to_rownames("V1")
         ## Filter by ancestry
         aa_samples = phenotypes %>% filter(Race == "AA")
         ea_samples = phenotypes %>% filter(Race == "CAUC")
```

```
print(dim(aa_samples))
    print(dim(ea_samples))
    # Load residualized expression
    vsd <- data.table::fread(paste0("../../../differential_analysis/caudate/</pre>
 \hookrightarrow ",
                                     " m/genes/residualized expression.tsv")) %>%
        replace(is.na(.), "") %>% tibble::column_to_rownames("V1")
    print(dim(vsd))
    # Keep only the columns and rows that are present in
    # both the sample table and vsd file
    samples_aa = intersect(colnames(vsd), rownames(aa_samples))
    samples_ea = intersect(colnames(vsd), rownames(ea_samples))
    vsd_aa = vsd[,samples_aa]
    vsd_ea = vsd[,samples_ea]
    # WGCNA data import
    suppressMessages(library(WGCNA))
    nSets = 2; shortLabels = c("AA", "EA")
    multiExpr0 = vector(mode="list", length=nSets)
    multiExpr0[[1]] = list(data=as.data.frame(t(vsd aa)))
    names(multiExpr0[[1]]$data) = rownames(vsd_aa)
    rownames(multiExpr0[[1]]$data) = colnames(vsd aa)
    multiExpr0[[2]] = list(data=as.data.frame(t(vsd ea)))
    names(multiExpr0[[2]]$data) = rownames(vsd_ea)
    rownames(multiExpr0[[2]]$data) = colnames(vsd_ea)
    exprSize = checkSets(multiExpr0)
    print(exprSize)
    # Remove offending genes and samples from the data
    gsg = goodSamplesGenesMS(multiExpr0, verbose = 3);
    if (!gsg$allOK)
        for(set in 1:exprSize$nSets){
            multiExpr0[[set]]$data = multiExpr0[[set]]$data[gsg$goodSamples,__
 →gsg$goodGenes]
        }
    # Secondary sample filtering
    for(set in 1:exprSize$nSets){
        multiExpr0[[set]]$data = filter_outliers(multiExpr0[[set]]$data, 2.5)
    }
    multiExpr <- multiExpr0</pre>
    exprSize = checkSets(multiExpr)
    samples_aa = intersect(rownames(multiExpr[[1]]$data), rownames(aa_samples))
    samples_ea = intersect(rownames(multiExpr[[2]]$data), rownames(ea_samples))
    samples = c(samples_aa, samples_ea)
    sample_table = sample_table[samples,]
    save(multiExpr, exprSize, sample_table, shortLabels, file = '00.RData')
}
```

```
plot_sample_clustering <- function(setLabels){</pre>
    lnames = load('00.RData')
    sampleTrees = list()
    for(set in 1:exprSize$nSets){
        sampleTrees[[set]] = hclust(dist(multiExpr[[set]]$data),__
 →method="average")
    pdf(file='sample_clustering.pdf', height=12, width=12)
    par(mfrow=c(2,1))
    par(mar=c(0,4,2,0))
    for(set in 1:exprSize$nSets){
        plot(sampleTrees[[set]],
             main=paste("Sample clustering on all genes in ", setLabels[set]),
             xlab="", sub="", cex=0.7)
    }
    dev.off()
}
```

```
[3]: prepare_traits = function()
         lnames = load('00.RData')
         Traits <- vector(mode="list", length=exprSize$nSets)</pre>
         # Associate traits with samples
         for(set in 1:exprSize$nSets){
             setSamples = rownames(multiExpr[[set]]$data)
             traitRows = match(setSamples, sample_table$RNum)
             Traits[[set]] = list(data=sample_table[traitRows, c(-1, -2)])
             rownames(Traits[[set]]$data) = sample_table[traitRows, 1]
         }
         nGenes = exprSize$nGenes
         nSamples = exprSize$nSamples
         save(multiExpr, exprSize, sample_table, shortLabels,
              Traits, nGenes, nSamples, file = "01.RData")
     }
     plot_power_parameter <- function(nSets, multiExpr, RsquaredCut = 0.85){</pre>
         # Choose a set of soft-thresholding powers
         powers = seq(from = 4, to=20, by=1)
         # Initialize a list to hold the results of scale-free analysis
         powerTables = vector(mode = "list", length = nSets)
         softPowerTables = vector(mode = "list", length = nSets)
         # Call the network topology analysis function for each set in turn
         for (set in 1:nSets){
```

```
powerTables[[set]] = list(data =
→pickSoftThreshold(multiExpr[[set]]$data,
                                                           powerVector=powers,__
\rightarrowverbose = 2,
                                                          Ш
→networkType=PARAM_NETWORK_TYPE) [[2]])
       # Calculated softpower from fitted values
       cond = powerTables[[set]]$data$`SFT.R.sq` > RsquaredCut
       softPowerTables[[set]] = min(powerTables[[set]]$data[cond, "Power"])
   softpower = max(unlist(softPowerTables))
   print(softpower)
   # Plot the results:
   colors = c("black", "red")
   # Will plot these columns of the returned scale free analysis tables
   plotCols = c(2,5,6,7)
   colNames = c("Scale Free Topology Model Fit", "Mean connectivity",
                "Median connectivity", "Max connectivity")
   # Get the minima and maxima of the plotted points
   ylim = matrix(NA, nrow = 2, ncol = 4)
   for (set in 1:nSets){
       for (col in 1:length(plotCols)){
           ylim[1, col] = min(ylim[1, col],
                              powerTables[[set]]$data[, plotCols[col]],
                              na.rm = TRUE)
           ylim[2, col] = max(ylim[2, col],
                              powerTables[[set]]$data[, plotCols[col]],
                              na.rm = TRUE)
       }
   }
   # Plot the quantities in the chosen columns vs. the soft thresholding power
   sizeGrWindow(8, 6)
   pdf(file = "power_parameter_selection.pdf", wi = 8, he = 6)
   par(mfcol = c(2,2))
   par(mar = c(4.2, 4.2, 2.2, 0.5))
   cex1 = 0.7
   for (col in 1:length(plotCols)) for (set in 1:nSets){
       if (set==1){
           plot(powerTables[[set]]$data[,1],__
→-sign(powerTables[[set]]$data[,3])*powerTables[[set]]$data[,2],
                xlab="Soft Threshold (power)", ylab=colNames[col], type="n", u
→ylim = ylim[, col],
                main = colNames[col])
           addGrid()
       }
       if (col==1){
```

```
text(powerTables[[set]]$data[,1],__
 →-sign(powerTables[[set]]$data[,3])*powerTables[[set]]$data[,2],
                 labels=powers,cex=cex1,col=colors[set])
        } else {
            text(powerTables[[set]]$data[,1],__
 →powerTables[[set]]$data[,plotCols[col]],
                 labels=powers,cex=cex1,col=colors[set])
        }
        if (col==1){
            legend("bottomright", legend = setLabels, col = colors, pch = 20)
        } else {
            legend("topright", legend = setLabels, col = colors, pch = 20)
        }
    }
    dev.off()
}
figure_out_power_parameter <- function()</pre>
{
    suppressMessages(library(WGCNA))
    #enableWGCNAThreads()
    lnames = load('01.RData')
    nSets = exprSize$nSets
    plot_power_parameter(nSets, multiExpr, 0.85)
}
```

```
[4]: construct network <- function(softPower){
         suppressMessages(library(WGCNA))
         enableWGCNAThreads()
         lnames = load("01.RData")
         # softPower value from previous plot power_parameter_selection.pdf
         cor <- WGCNA::cor</pre>
         net = blockwiseConsensusModules(multiExpr, maxBlockSize=30000,
                                          power=softPower, minModuleSize=30,
                                          deepSplit=2, pamRespectsDendro=FALSE,
                                          mergeCutHeight=0.25, numericLabels=TRUE,
                                          minKMEtoStay=0, corType="bicor",
                                          saveTOMFileBase="TOM", saveTOMs=TRUE,
                                          networkType=PARAM_NETWORK_TYPE,
                                          TOMType=PARAM_NETWORK_TYPE, verbose=3)
         consMEs = net$multiMEs
         moduleLabels = net$colors
         moduleColors = labels2colors(moduleLabels)
         consTree = net$dendrograms[[1]]
         save(net, consMEs, moduleLabels, moduleColors, consTree, file="02.RData")
     }
```

```
[5]: consensus_eigengene_network <- function(){
         suppressMessages(library(WGCNA))
         lnames = load(file = "01.RData")
         lnames = load(file = "02.RData")
         nSets = exprSize$nSets
         # Create a variable weight that will hold just the body weight of mice in
      \rightarrowboth sets
         ancestry = vector(mode = "list", length = nSets);
         for (set in 1:nSets){
             ancestry[[set]] = list(data = as.data.
      →frame(Traits[[set]]$data$Ancestry))
             names(ancestry[[set]]$data) = "ancestry"
         }
         # Recalculate consMEs to give them color names
         consMEsC = multiSetMEs(multiExpr, universalColors = moduleColors)
         # Plot eigengene network
         sizeGrWindow(8,10)
         pdf(file = "eigengene_networks.pdf", width=8, height=10)
         par(cex = 0.9)
         plotEigengeneNetworks(consMEsC, setLabels, marDendro=c(0,2,2,1),
                                marHeatmap=c(3,3,2,1), xLabelsAngle=0,
                                zlimPreservation=c(0.5, 1))
         dev.off()
         # We add the weight trait to the eigengenes and order them by consesus_{\sqcup}
      \rightarrow hierarchical clustering:
         MET = consensusOrderMEs(addTraitToMEs(consMEsC, ancestry))
         # Plot eigengene network
         sizeGrWindow(8,10)
         pdf(file = "eigengene_networks_ancestry.pdf", width=8, height=10)
         par(cex = 0.9)
         plotEigengeneNetworks(MET, setLabels, marDendro=c(0,2,2,1),
                                marHeatmap=c(3,3,2,1), xLabelsAngle=0,
                                zlimPreservation=c(0.5, 1)
```

```
dev.off()
    save(MET, consMEsC, ancestry, file="03.RData")
}
export_eigengene_tables = function(){
    suppressMessages(library(WGCNA))
    lnames = load(file = "01.RData")
    lnames = load(file = "02.RData")
    lnames = load(file = "03.RData")
    nSets = exprSize$nSets
    ## Export eigengene tables
    for(set in 1:nSets){
        write.csv(consMEsC[[set]]$data,
                  paste0('eigengenes_',shortLabels[[set]],'.csv'))
    }
    # Write modules
    modules = data.frame(row.names=colnames(multiExpr[[1]]$data),
                         module=moduleColors)
    write.csv(modules, 'modules.csv')
}
```

1.2 Main

[1] 22374

[6]: setLabels = c("AA Caudate", "EA Caudate")

```
prepare_data(setLabels)
plot_sample_clustering(setLabels)
prepare_traits()
figure_out_power_parameter()
Loading required package: limma
[1] 122
          2
[1] 117
          2
Warning message in
data.table::fread(paste0("../../../differential_analysis/caudate/", :
"Detected 239 column names but the data has 240 columns (i.e. invalid file).
Added 1 extra default column name for the first column which is guessed to be
row names or an index. Use setnames() afterwards if this guess is not correct,
or fix the file write command that created the file to create a valid file."
[1] 22374
            239
$nSets
[1] 2
$nGenes
```

\$nSamples [1] 122 117 \$structureOK [1] TRUE Flagging genes and samples with too many missing values... ..step 1 ..bad gene count: 0, bad sample counts: 0, 0 **png:** 2 pickSoftThreshold: will use block size 1999. pickSoftThreshold: calculating connectivity for given powers... ..working on genes 1 through 1999 of 22374 Warning message: "executing %dopar% sequentially: no parallel backend registered" ..working on genes 2000 through 3998 of 22374 ..working on genes 3999 through 5997 of 22374 ..working on genes 5998 through 7996 of 22374 ..working on genes 7997 through 9995 of 22374 ..working on genes 9996 through 11994 of 22374 ..working on genes 11995 through 13993 of 22374 ..working on genes 13994 through 15992 of 22374 ..working on genes 15993 through 17991 of 22374 ..working on genes 17992 through 19990 of 22374 ..working on genes 19991 through 21989 of 22374 ..working on genes 21990 through 22374 of 22374 Power SFT.R.sq slope truncated.R.sq mean.k. median.k. max.k. 1 4 0.452 - 12.400.900 1570.00 1550.000 1990.0 2 5 0.685 - 10.500.919 841.00 825.000 1240.0 3 6 0.836 -8.32 0.950 458.00 444.000 811.0 4 7 0.917 -6.770.973 255.00 242.000 556.0 5 8 0.955 -5.570.986 144.00 134.000 398.0 -4.64 83.00 6 9 0.974 0.994 74.600 295.0 7 10 0.981 -3.950.995 48.90 42.100 225.0 8 11 0.981 -3.460.993 29.50 24.100 179.0 9 0.977 - 3.100.991 13.900 147.0 12 18.20 10 13 0.967 -2.840.986 11.50 8.150 123.0 11 0.955 - 2.620.980 7.50 4.810 105.0 14 12 -2.440.973 5.02 90.6 15 0.941 2.880 13 0.937 -2.280.973 3.45 1.730 79.0 16 14 2.44 69.5 17 0.926 -2.150.970 1.060 15 18 0.922 - 2.040.971 1.77 0.651 61.6 16 19 0.912 - 1.950.966 1.32 0.405 54.9

1.00

0.255

49.2

0.970

17

20

0.918 - 1.85

pickSoftThreshold: will use block size 1999.

```
pickSoftThreshold: calculating connectivity for given powers...
       ..working on genes 1 through 1999 of 22374
       ..working on genes 2000 through 3998 of 22374
       ..working on genes 3999 through 5997 of 22374
       ..working on genes 5998 through 7996 of 22374
       ..working on genes 7997 through 9995 of 22374
       ..working on genes 9996 through 11994 of 22374
       ..working on genes 11995 through 13993 of 22374
       ..working on genes 13994 through 15992 of 22374
       ..working on genes 15993 through 17991 of 22374
       ..working on genes 17992 through 19990 of 22374
       ..working on genes 19991 through 21989 of 22374
       ..working on genes 21990 through 22374 of 22374
       Power SFT.R.sq slope truncated.R.sq mean.k. median.k. max.k.
    1
                0.334 - 9.21
                                      0.874 1570.000
                                                       1550.000 1960.0
    2
           5
                0.539 - 8.48
                                      0.890 838.000
                                                        824.000 1170.0
    3
           6
                0.723 - 7.27
                                      0.921 456.000
                                                        443.000
                                                                727.0
    4
           7
                                      0.948 252.000
                                                        241.000 474.0
                0.851 - 6.16
    5
           8
                                      0.973 142.000
                                                        133.000 326.0
                0.935 - 5.06
    6
           9
                0.963 - 4.40
                                      0.980
                                              81.800
                                                        73.800 242.0
    7
          10
                0.963 - 3.93
                                      0.978
                                              48.000
                                                         41.500
                                                                191.0
    8
          11
                0.968 - 3.45
                                      0.980
                                               28.800
                                                         23.600
                                                                156.0
    9
          12
                0.976 - 3.04
                                      0.985
                                              17.700
                                                        13.600
                                                                130.0
    10
          13
                0.982 - 2.71
                                      0.990
                                               11.200
                                                          7.910
                                                                111.0
    11
          14
                0.985 - 2.46
                                      0.993
                                               7.270
                                                          4.650
                                                                 96.3
    12
          15
                0.985 - 2.27
                                      0.994
                                               4.860
                                                          2.760
                                                                  85.3
    13
                                                                  76.3
          16
                0.983 - 2.11
                                      0.994
                                               3.350
                                                          1.660
    14
          17
                0.977 - 1.98
                                      0.989
                                               2.380
                                                          1.000
                                                                  68.7
    15
                                                                  62.3
          18
                0.977 - 1.87
                                      0.995
                                               1.730
                                                          0.614
    16
          19
                0.977 - 1.78
                                      0.996
                                               1.300
                                                          0.379
                                                                  56.7
    17
          20
                0.971 - 1.71
                                      0.994
                                               0.998
                                                          0.236
                                                                  51.8
    [1] 7
    png: 2
[7]: softpower = 11 ## Based on Dentate Gyrus and Hippocampus
     construct_network(softpower)
     plot_cluster_dendrogram()
    Allowing parallel execution with up to 63 working processes.
     Calculating consensus modules and module eigengenes block-wise from all genes
     Calculating topological overlaps block-wise from all genes
       Flagging genes and samples with too many missing values...
        ..step 1
     ...Working on set 1
        TOM calculation: adjacency...
        ..will use 63 parallel threads.
         Fraction of slow calculations: 0.000000
        ..connectivity..
```

```
..matrix multiplication (system BLAS)..
         ..normalization..
         ..done.
     ...Working on set 2
        TOM calculation: adjacency..
         ..will use 63 parallel threads.
         Fraction of slow calculations: 0.000000
         ..connectivity..
         ..matrix multiplication (system BLAS)..
        ..normalization..
         ..done.
     ..Working on block 1 .
     ...Working on set 1
     ...Working on set 2
     ...Calculating consensus network
     ..Working on block 1 .
     ...clustering and detecting modules...
     ...calculating eigengenes...
     ...checking consensus modules for statistical meaningfulness..
     ...checking for genes that should be reassigned...
     ..merging consensus modules that are too close..
         mergeCloseModules: Merging modules whose distance is less than 0.25
           Calculating new MEs...
    png: 2
[8]: consensus_eigengene_network()
     export_eigengene_tables()
     multiSetMEs: Calculating module MEs.
       Working on set 1 ...
       Working on set 2 ...
    1.3 Repreducibility Information
[9]: Sys.time()
     proc.time()
     options(width = 120)
     sessioninfo::session_info()
    [1] "2021-07-12 11:27:25 EDT"
                system elapsed
    5060.883 1628.467 1264.950
      Session info
     setting value
     version R version 4.0.3 (2020-10-10)
     os
              Arch Linux
     system
              x86_64, linux-gnu
```

ui X11 language (EN)

collate en_US.UTF-8
ctype en_US.UTF-8
tz America/New_York

date 2021-07-12

Packages

package	*	version	date	lib	source
AnnotationDbi		1.52.0	2020-10-27	[1]	Bioconductor
assertthat		0.2.1	2019-03-21	[1]	CRAN (R 4.0.2)
backports		1.2.1	2020-12-09	[1]	CRAN (R 4.0.2)
base64enc		0.1-3	2015-07-28	[1]	CRAN (R 4.0.2)
Biobase		2.50.0	2020-10-27	[1]	Bioconductor
BiocGenerics		0.36.1	2021-04-16	[1]	Bioconductor
bit		4.0.4	2020-08-04	[1]	CRAN (R 4.0.2)
bit64		4.0.5	2020-08-30	[1]	CRAN (R 4.0.2)
blob		1.2.1	2020-01-20	[1]	CRAN (R 4.0.2)
cachem		1.0.5	2021-05-15	[1]	CRAN (R 4.0.3)
checkmate		2.0.0	2020-02-06	[1]	CRAN (R 4.0.2)
cli		3.0.0	2021-06-30	[1]	CRAN (R 4.0.3)
cluster		2.1.0	2019-06-19	[2]	CRAN (R 4.0.3)
codetools		0.2-16	2018-12-24	[2]	CRAN (R 4.0.3)
colorspace		2.0-2	2021-06-24	[1]	CRAN (R 4.0.3)
crayon		1.4.1	2021-02-08	[1]	CRAN (R 4.0.3)
data.table		1.14.0	2021-02-21	[1]	CRAN (R 4.0.3)
DBI		1.1.1	2021-01-15	[1]	CRAN (R 4.0.2)
digest		0.6.27	2020-10-24	[1]	CRAN (R 4.0.2)
doParallel		1.0.16	2020-10-16	[1]	CRAN (R 4.0.3)
dplyr	*	1.0.7	2021-06-18	[1]	CRAN (R 4.0.3)
${\tt dynamicTreeCut}$	*	1.63-1	2016-03-11	[1]	CRAN (R 4.0.3)
ellipsis		0.3.2	2021-04-29	[1]	CRAN (R 4.0.3)
evaluate		0.14	2019-05-28	[1]	CRAN (R 4.0.2)
fansi		0.5.0	2021-05-25	[1]	CRAN (R 4.0.3)
fastcluster	*	1.2.3	2021-05-24	[1]	CRAN (R 4.0.3)
fastmap		1.1.0	2021-01-25	[1]	CRAN (R 4.0.2)
foreach		1.5.1	2020-10-15	[1]	CRAN (R 4.0.2)
foreign		0.8-80	2020-05-24	[2]	CRAN (R 4.0.3)
Formula		1.2-4	2020-10-16	[1]	CRAN (R 4.0.2)
generics		0.1.0	2020-10-31	[1]	CRAN (R 4.0.2)
ggplot2		3.3.5	2021-06-25	[1]	CRAN (R 4.0.3)
glue		1.4.2	2020-08-27	[1]	CRAN (R 4.0.2)
GO.db		3.12.1	2021-04-08	[1]	Bioconductor
gridExtra		2.3	2017-09-09	[1]	CRAN (R 4.0.2)
gtable		0.3.0	2019-03-25	[1]	CRAN (R 4.0.2)
Hmisc		4.5-0	2021-02-28	[1]	CRAN (R 4.0.3)
htmlTable		2.2.1	2021-05-18	[1]	CRAN (R 4.0.3)
htmltools		0.5.1.1	2021-01-22	[1]	CRAN (R 4.0.2)

```
htmlwidgets
                  1.5.3
                          2020-12-10 [1] CRAN (R 4.0.2)
impute
                  1.64.0
                          2020-10-27 [1] Bioconductor
IRanges
                  2.24.1
                          2020-12-12 [1] Bioconductor
                  1.0
                          2021-01-20 [1] CRAN (R 4.0.2)
IRdisplay
                          2021-05-11 [1] CRAN (R 4.0.3)
IRkernel
                  1.2
                          2020-10-15 [1] CRAN (R 4.0.2)
iterators
                  1.0.13
                  0.1-8.1 2019-10-24 [1] CRAN (R 4.0.2)
jpeg
jsonlite
                  1.7.2
                          2020-12-09 [1] CRAN (R 4.0.2)
                          2021-04-24 [1] CRAN (R 4.0.3)
knitr
                  1.33
lattice
                  0.20-41 2020-04-02 [2] CRAN (R 4.0.3)
                          2019-12-19 [1] CRAN (R 4.0.2)
                  0.6 - 29
latticeExtra
                  1.0.0
                          2021-02-15 [1] CRAN (R 4.0.3)
lifecycle
               * 3.46.0
                          2020-10-27 [1] Bioconductor
limma
                          2020-11-17 [1] CRAN (R 4.0.2)
magrittr
                  2.0.1
Matrix
                  1.3 - 4
                          2021-06-01 [1] CRAN (R 4.0.3)
                  0.59.0
                          2021-06-01 [1] CRAN (R 4.0.3)
matrixStats
memoise
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preprocessCore
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R.6
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Rcpp
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WGCNA
               * 1.70-3
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                          2021-06-15 [1] CRAN (R 4.0.3)
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- [1] /home/jbenja13/R/x86_64-pc-linux-gnu-library/4.0
 [2] /usr/lib/R/library