CellTagR CellTag Object Testing

Compiled: June 11, 2019

Single-Cell RNA-Seq CellTag Extraction

Loading the package (Will be changed later)

```
library(devtools)
devtools::install_github("morris-lab/CellTagR")
```

```
## broom      (NA    -> 0.5.2) [CRAN]
## cellranger (NA    -> 1.1.0) [CRAN]
## dbplyr     (NA    -> 1.4.1) [CRAN]
## ellipsis   (NA    -> 0.1.0) [CRAN]
## forcats    (NA    -> 0.4.0) [CRAN]
## generics   (NA    -> 0.0.2) [CRAN]
## haven      (NA    -> 2.1.0) [CRAN]
## lubridate  (NA    -> 1.7.4) [CRAN]
## markdown   (0.9   -> 1.0  ) [CRAN]
## modelr     (NA    -> 0.1.4) [CRAN]
## networkD3  (NA    -> 0.4  ) [CRAN]
## readr      (NA    -> 1.3.1) [CRAN]
## readxl     (NA    -> 1.3.1) [CRAN]
## rematch    (NA    -> 1.0.1) [CRAN]
## reprex     (NA    -> 0.3.0) [CRAN]
## rvest      (NA    -> 0.3.4) [CRAN]
## selectr    (NA    -> 0.4-1) [CRAN]
## tibble     (2.1.2 -> 2.1.3) [CRAN]
## tidyverse  (NA    -> 1.2.1) [CRAN]
##


✔  checking for file '/tmp/RtmpP8xeYK/remotes4c95ab4a261/morris-lab-CellTagR-f53de19/DES
CRIPTION'
##

─  preparing 'CellTagR':
##    checking DESCRIPTION meta-information ...

   checking DESCRIPTION meta-information ...

   checking DESCRIPTION meta-information ... OK

✔  checking DESCRIPTION meta-information
##







   excluding invalid files

─  excluding invalid files
##

   Subdirectory 'R' contains invalid file names:
##      'scripts.zip'
##
```

```
   checking for LF line-endings in source and make files and shell scripts

−  checking for LF line-endings in source and make files and shell scripts
##




   checking for empty or unneeded directories

−  checking for empty or unneeded directories
##




   building

   building ‘CellTagR_0.0.0.9000.tar.gz’

−  building ‘CellTagR_0.0.0.9000.tar.gz’
##


##
```

```
library(CellTagR)
```

Download the bam file from the URL

Note: This download might take a large space and a long time

```
download.file("https://sra-download.ncbi.nlm.nih.gov/traces/sra65/SRZ/007347/SRR7347033/hf1.d15.possorted_genome_bam.bam", params$fastq.bam.data.path)
```

Bam File read

```
bam.test.obj <- CellTagObject(params$object.name, fastq.bam.directory=params$fastq.bam.data.path)
```

V1

Extract the CellTag Information From the bam file

```
bam.test.obj <- CellTagExtraction(bam.test.obj, "v1")
```

```
## Loading required package: GenomeInfoDb
```

```
## Loading required package: BiocGenerics
```

```
## Loading required package: parallel
```

```
##
## Attaching package: 'BiocGenerics'
```

```
## The following objects are masked from 'package:parallel':
##
##     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##     clusterExport, clusterMap, parApply, parCapply, parLapply,
##     parLapplyLB, parRapply, parSapply, parSapplyLB
```

```
## The following objects are masked from 'package:dplyr':
##
##     combine, intersect, setdiff, union
```

```
## The following objects are masked from 'package:Matrix':
##
##     colMeans, colSums, rowMeans, rowSums, which
```

```
## The following objects are masked from 'package:igraph':
##
##     normalize, path, union
```

```
## The following object is masked from 'package:gridExtra':
##
##     combine
```

```
## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs
```

```
## The following objects are masked from 'package:base':
##
##     anyDuplicated, append, as.data.frame, basename, cbind,
##     colMeans, colnames, colSums, dirname, do.call, duplicated,
##     eval, evalq, Filter, Find, get, grep, grepl, intersect,
##     is.unsorted, lapply, lengths, Map, mapply, match, mget, order,
##     paste, pmax, pmax.int, pmin, pmin.int, Position, rank, rbind,
##     Reduce, rowMeans, rownames, rowSums, sapply, setdiff, sort,
##     table, tapply, union, unique, unsplit, which, which.max,
##     which.min
```

```
## Loading required package: S4Vectors
```

```
## Loading required package: stats4
```

```
##
## Attaching package: 'S4Vectors'
```

```
## The following objects are masked from 'package:dplyr':
##
##     first, rename
```

```
## The following object is masked from 'package:tidyr':
##
##     expand
```

```
## The following object is masked from 'package:Matrix':
##
##     expand
```

```
## The following objects are masked from 'package:reshape':
##
##     expand, rename
```

```
## The following object is masked from 'package:plyr':
##
##     rename
```

```
## The following objects are masked from 'package:data.table':
##
##     first, second
```

```
## The following object is masked from 'package:base':
##
##     expand.grid
```

```
## Loading required package: IRanges
```

```
##
## Attaching package: 'IRanges'
```

```
## The following objects are masked from 'package:dplyr':
##
##     collapse, desc, slice
```

```
## The following object is masked from 'package:purrr':
##
##     reduce
```

```
## The following object is masked from 'package:plyr':
##
##     desc
```

```
## The following object is masked from 'package:data.table':
##
##     shift
```

```
## Loading required package: GenomicRanges
```

```
## Loading required package: Biostrings
```

```
## Loading required package: XVector
```

```
##
## Attaching package: 'XVector'
```

```
## The following object is masked from 'package:purrr':
##
##     compact
```

```
## The following object is masked from 'package:plyr':
##
##     compact
```

```
##
## Attaching package: 'Biostrings'
```

```
## The following object is masked from 'package:base':
##
##     strsplit
```

```
head(bam.test.obj@bam.parse.rslt)
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## $v1
##                     Cell.BC        UMI Cell.Tag
##      1: GCAGTTAAGGAGTTGC-1 CCGATAATAT GCAATTGG
##      2: GCAGTTAAGGAGTTGC-1 CCGATAATAT GCAATTGG
##      3: ACTATCTCAGTATCTG-1 CGCCGAAGTG GCAATTGG
##      4: TCTTCGGTCTTAGAGC-1 GTTCCCGGCA GCAATTGG
##      5: CTCTAATGTACTTGAC-1 CGGTGTTACG ATGACCTT
##     ---
## 479939: TTTGTCATCGTTTGCC-1 GAAACGCGCG GACATACG
## 479940: TTTGTCATCGTTTGCC-1 CCTCCCGGTC GACATACG
## 479941: TTTGTCATCGTTTGCC-1 GTAGTCCTTT CCTGAGAA
## 479942: TTTGTCATCTACTTAC-1 AGGCCTGTCA CAGCGTAG
## 479943: TTTGTCATCTACTTAC-1 AGGCCTGTCA CAGCGTAG
```

Generate the Count Matrix for CellTag

```
bam.test.obj <- CellTagMatrixCount(bam.test.obj, params$barcode.file)
```

```
## Warning in `[.data.table`(alltagCounts, , `:=`((tagsRemove), NULL)):
## length(LHS)==0; no columns to delete or assign RHS to.
```

```
bam.test.obj@celltag.stats
```

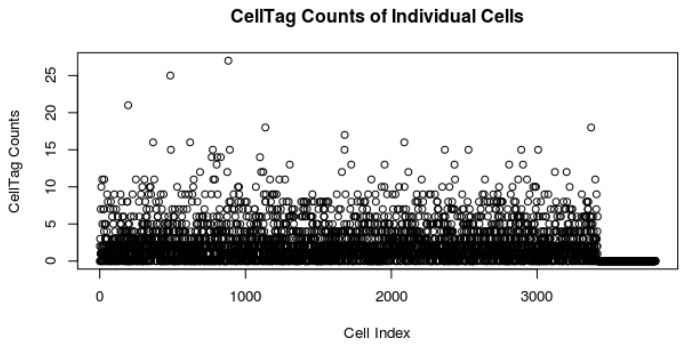
```
## NULL
```

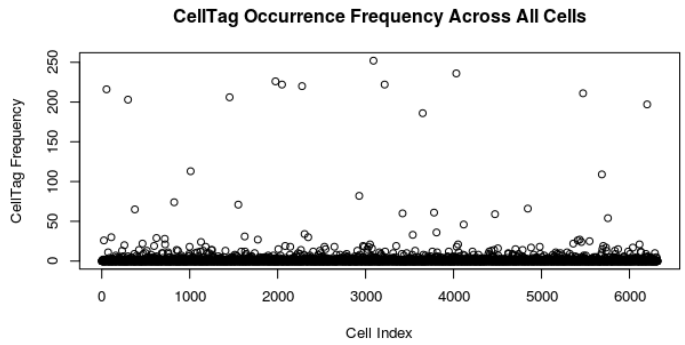
Generate the Binary Matrix from the Count Matrix

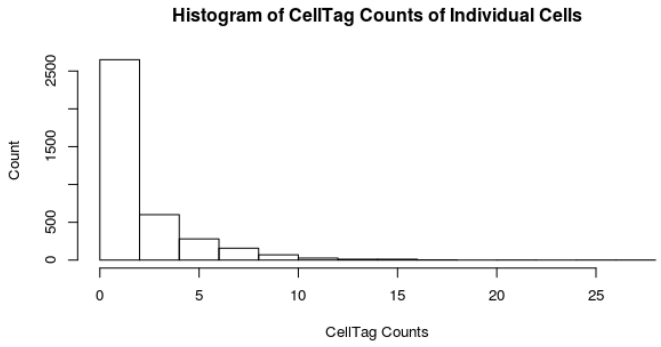
```
bam.test.obj <- SingleCellDataBinatization(bam.test.obj, 2)
```

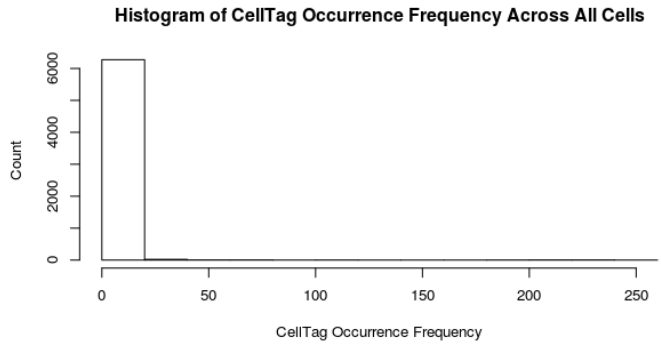
Metric Plots to Facilitate for Additional Filtering

```
MetricPlots(bam.test.obj)
```










```
## Average:  2.221668
## Frequency:  1.340244
```

Apply the V1 whitelisted CellTags

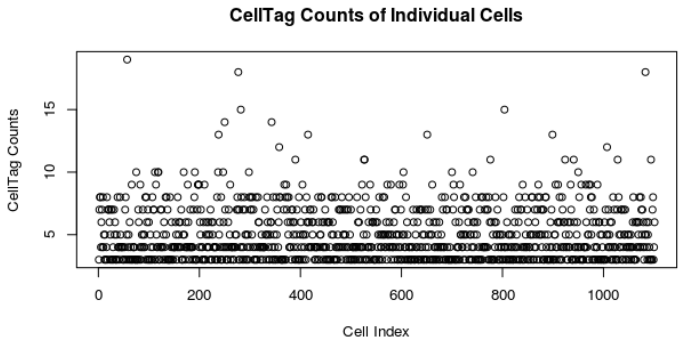
```
bam.test.obj <- SingleCellDataWhitelist(bam.test.obj, params$v1.whitelist.file)
```

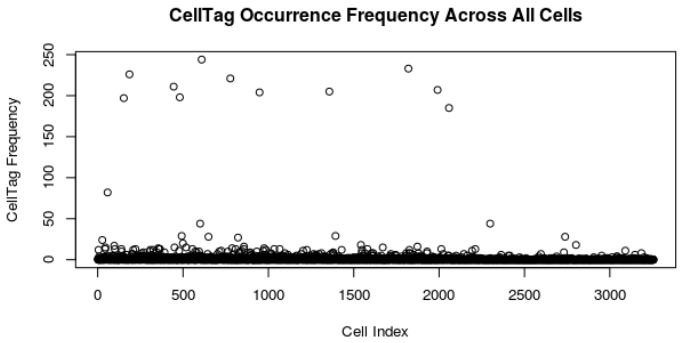
Metric Based Filtering

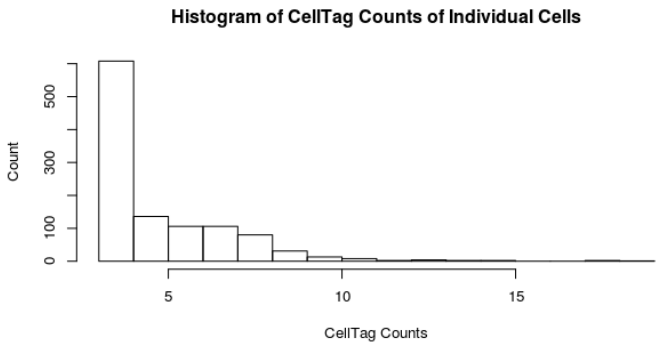
```
bam.test.obj <- MetricBasedFiltering(bam.test.obj, 20, comparison = "less")
bam.test.obj <- MetricBasedFiltering(bam.test.obj, 2, comparison = "greater")
```

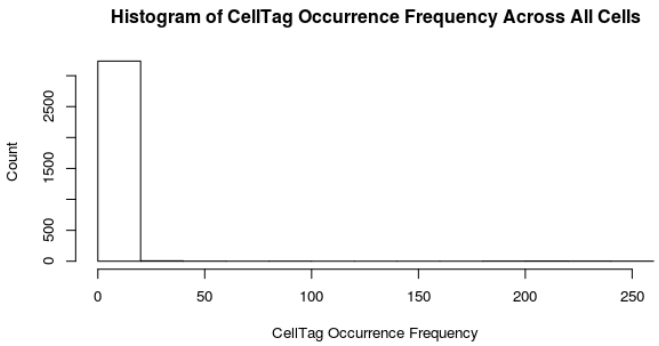
Metric Plots Again to Check for Additional Filtering

```
MetricPlots(bam.test.obj)
```





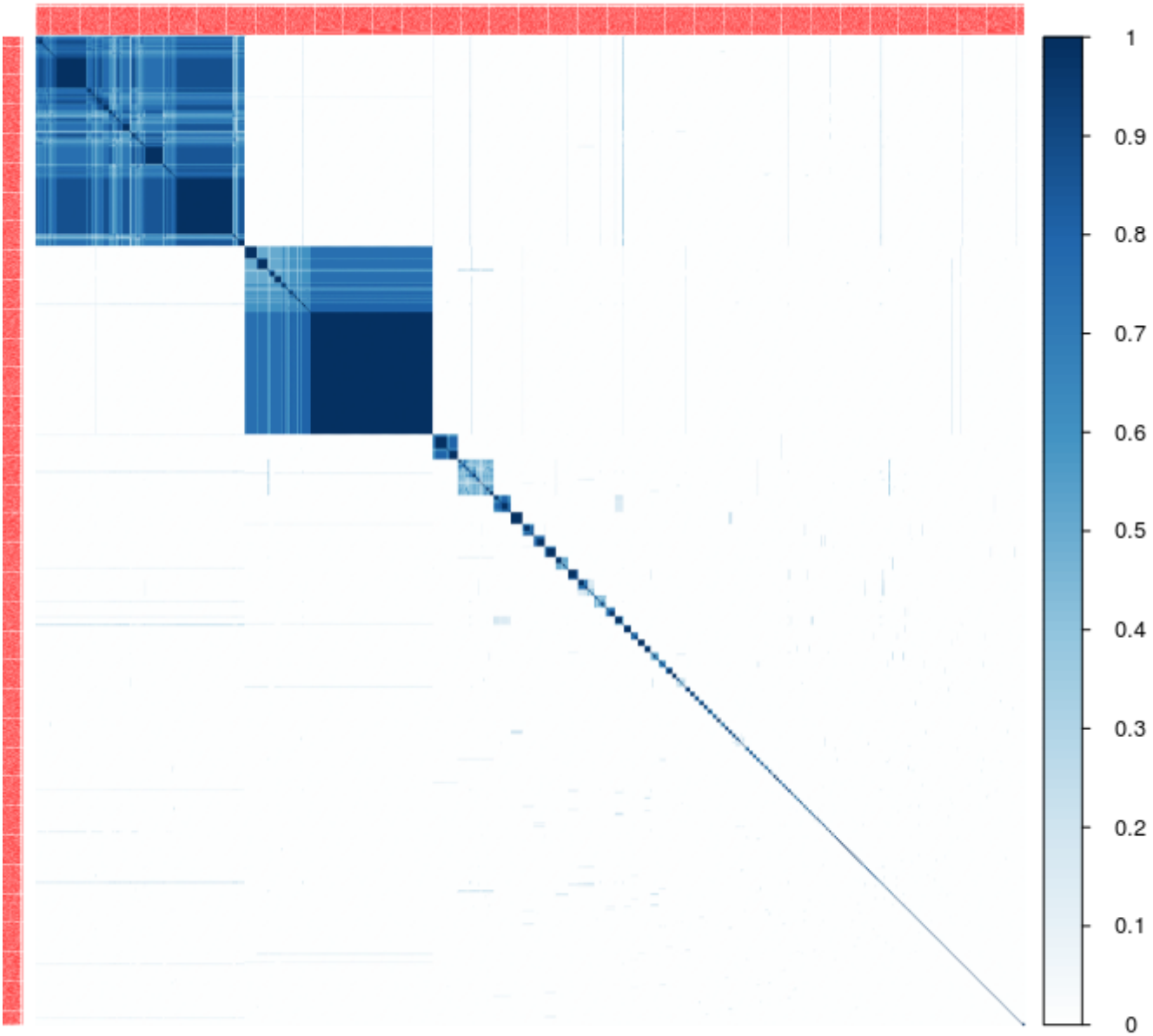





```
## Average:  4.974569
## Frequency:  1.682125
```

Jaccard Analysis

```
bam.test.obj <- JaccardAnalysis(bam.test.obj)
```



Clone Calling

```
bam.test.obj <- CloneCalling(celltag.obj = bam.test.obj, correlation.cutoff=0.7)
```

Checking the stats and Saving the object

```
show(bam.test.obj)
saveRDS(bam.test.obj, paste0(params$object.saving.dir, "/bam_v1_obj.Rds"))
```

```
## Object name:  hf1.d15.test
## Raw CellTag Counts =  6319
## Raw Number of Cells with CellTag =  3812
## Collapsed CellTag Counts =  0
## Whitelisted CellTag Counts =  3256
## Whitelisted Number of Cells with CellTag =  3812
```

V2

Extract the CellTag Information From the bam file

```
bam.test.obj <- CellTagExtraction(bam.test.obj, "v2")
head(bam.test.obj@bam.parse.rslt)
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## $v1
##                     Cell.BC        UMI Cell.Tag
##      1: GCAGTTAAGGAGTTGC-1 CCGATAATAT GCAATTGG
##      2: GCAGTTAAGGAGTTGC-1 CCGATAATAT GCAATTGG
##      3: ACTATCTCAGTATCTG-1 CGCCGAAGTG GCAATTGG
##      4: TCTTCGGTCTTAGAGC-1 GTTCCCGGCA GCAATTGG
##      5: CTCTAATGTACTTGAC-1 CGGTGTTACG ATGACCTT
##     ---
## 479939: TTTGTCATCGTTTGCC-1 GAAACGCGCG GACATACG
## 479940: TTTGTCATCGTTTGCC-1 CCTCCCGGTC GACATACG
## 479941: TTTGTCATCGTTTGCC-1 GTAGTCCTTT CCTGAGAA
## 479942: TTTGTCATCTACTTAC-1 AGGCCTGTCA CAGCGTAG
## 479943: TTTGTCATCTACTTAC-1 AGGCCTGTCA CAGCGTAG
##
## $v2
##                     Cell.BC        UMI Cell.Tag
```

```
##       1: CTACACCTCTTTACAC-1 CTGTGTGTGT TGTGCTTG
##       2: CTACACCTCTTTACAC-1 CTGTGTGTGT TGTGCTTG
##       3: CTTAACTCAAGTCTGT-1 CTCCCTGTGT TGTGCTTG
##       4:                <NA> GTGTGTGTGT TGTGCTTG
##       5: GTCTCGTGTGCAGACA-1 CTCTTCTCGC TGTGCTTG
##      ---
## 479470: TTTGTCATCGAGAGCA-1 AGATAGTTGA CCACTTAT
## 479471: TTTGTCATCGAGAGCA-1 AGATAGTTGA CCACTTAT
## 479472: TTTGTCATCGAGAGCA-1 TACATCTCTC CAAATTTT
## 479473: TTTGTCATCGAGAGCA-1 TACATCTCTC CAAATTTT
## 479474: TTTGTCATCGCCAGCA-1 CTCACGGAGC TCCTGCAA
```

Generate the Count Matrix for CellTag

```
bam.test.obj <- CellTagMatrixCount(bam.test.obj, params$barcode.file)
```

```
## Warning in `[.data.table`(alltagCounts, , `:=`((tagsRemove), NULL)):
## length(LHS)==0; no columns to delete or assign RHS to.
```

```
bam.test.obj@celltag.stats
```

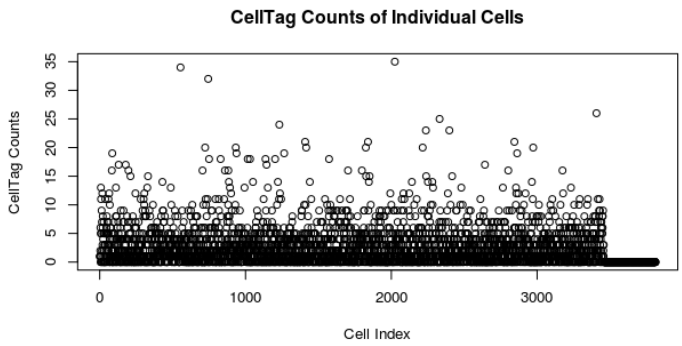
```
## NULL
```

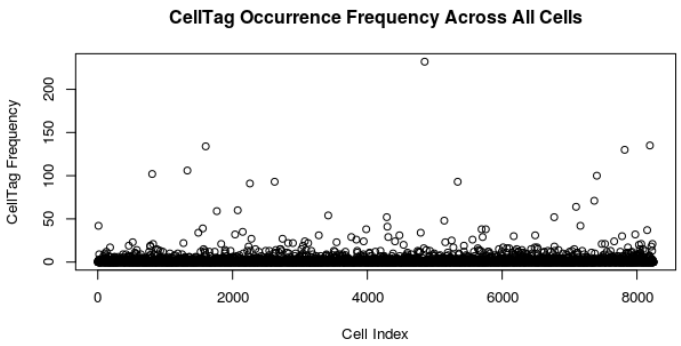
Generate the Binary Matrix from the Count Matrix

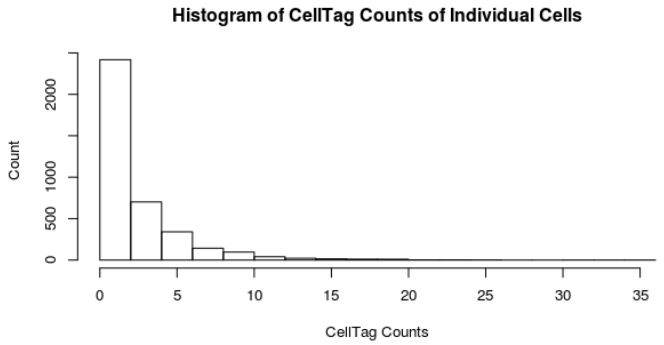
```
bam.test.obj <- SingleCellDataBinatization(bam.test.obj, 2)
```

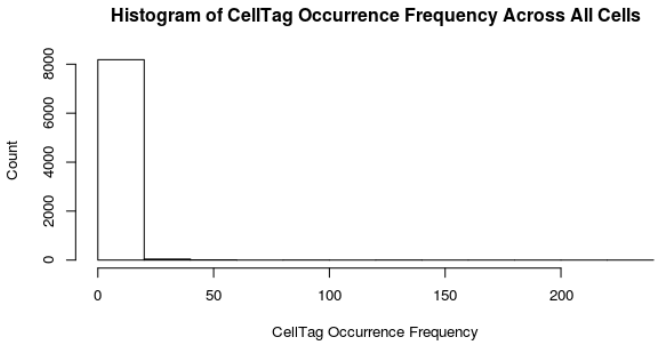
Metric Plots to Facilitate for Additional Filtering

```
MetricPlots(bam.test.obj)
```










```
## Average:  2.5383
## Frequency:  1.173417
```

Apply the V2 whitelisted CellTags

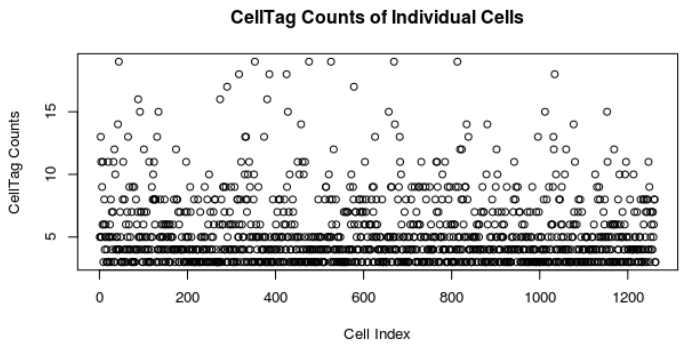
```
bam.test.obj <- SingleCellDataWhitelist(bam.test.obj, params$v2.whitelist.file)
```

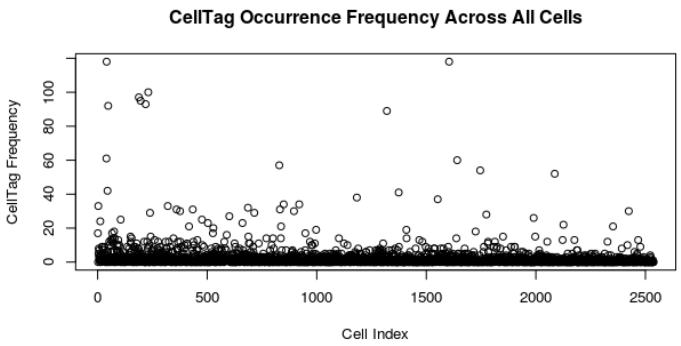
Metric Based Filtering

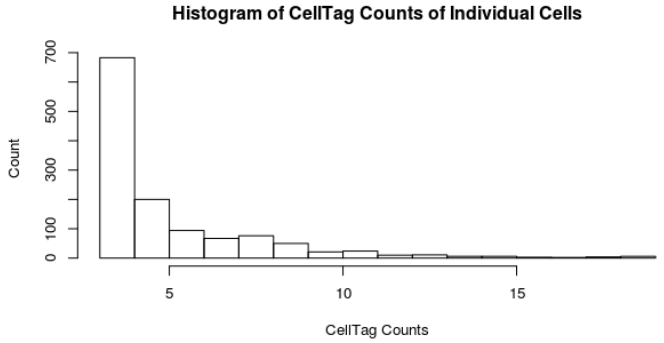
```
bam.test.obj <- MetricBasedFiltering(bam.test.obj, 20, comparison = "less")
bam.test.obj <- MetricBasedFiltering(bam.test.obj, 2, comparison = "greater")
```

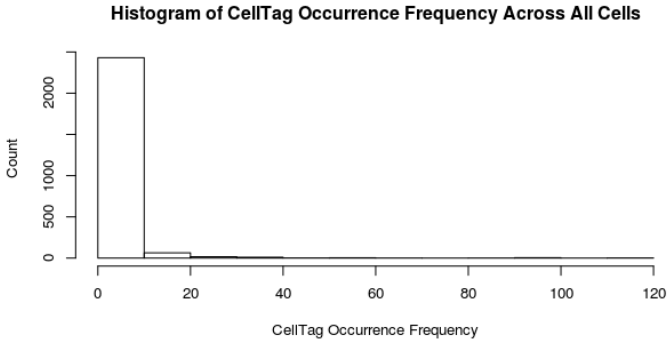
Metric Plots Again to Check for Additional Filtering

```
MetricPlots(bam.test.obj)
```





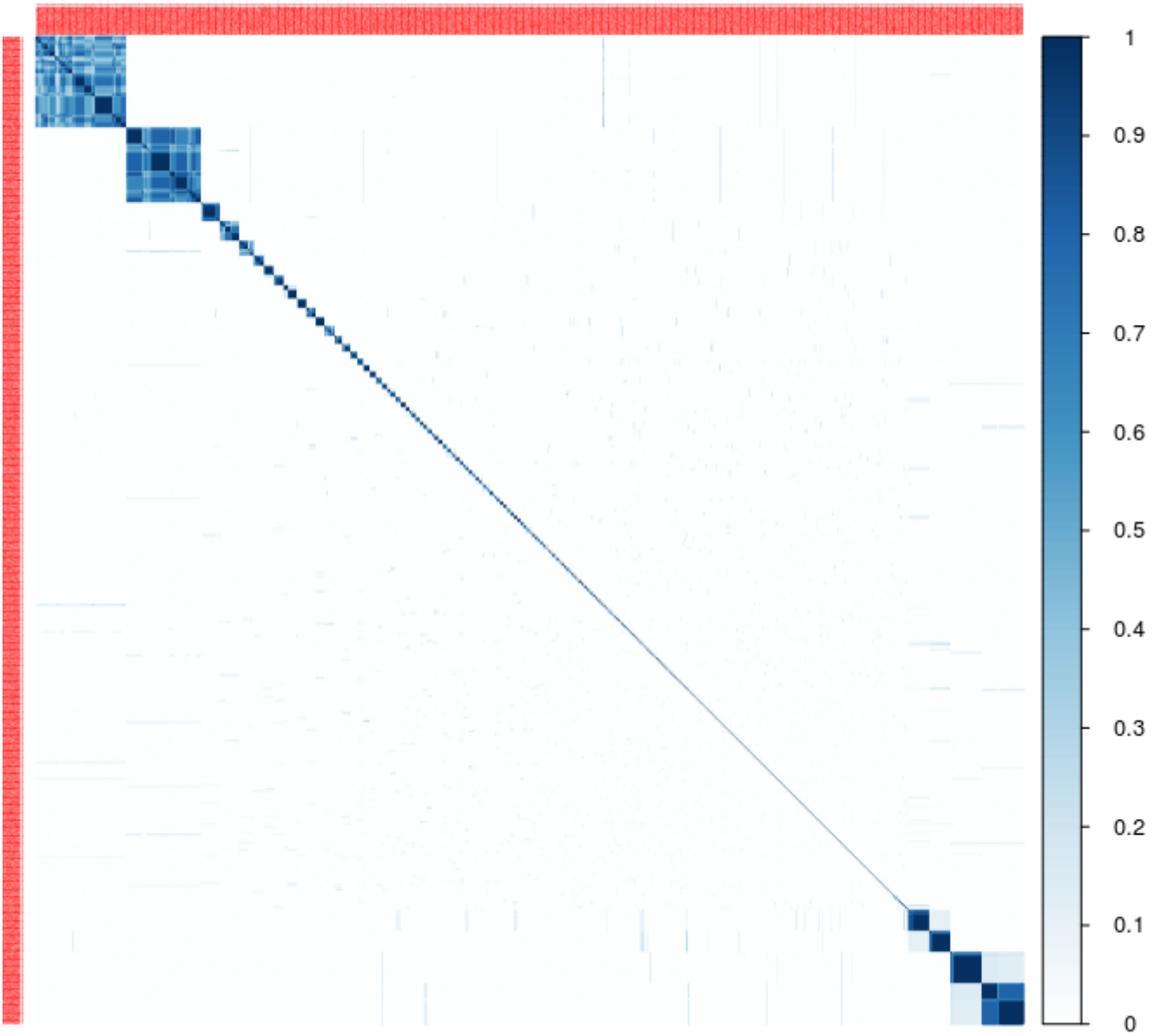





```
## Average:  5.251781
## Frequency:  2.614505
```

Jaccard Analysis

```
bam.test.obj <- JaccardAnalysis(bam.test.obj)
```



Clone Calling

```
bam.test.obj <- CloneCalling(celltag.obj = bam.test.obj, correlation.cutoff=0.7)
```

Checking the stats and Saving the object

```
show(bam.test.obj)
saveRDS(bam.test.obj, paste0(params$object.saving.dir, "/bam_v12_obj.Rds"))
```

```
## Object name:  hf1.d15.test
## Raw CellTag Counts =  14565
## Raw Number of Cells with CellTag =  3812
## Collapsed CellTag Counts =  0
## Whitelisted CellTag Counts =  5793
## Whitelisted Number of Cells with CellTag =  3812
```

V3

Extract the CellTag Information From the bam file

```
bam.test.obj <- CellTagExtraction(bam.test.obj, "v3")
head(bam.test.obj@bam.parse.rslt)
```

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## $v1
##                     Cell.BC        UMI Cell.Tag
##      1: GCAGTTAAGGAGTTGC-1 CCGATAATAT GCAATTGG
##      2: GCAGTTAAGGAGTTGC-1 CCGATAATAT GCAATTGG
##      3: ACTATCTCAGTATCTG-1 CGCCGAAGTG GCAATTGG
##      4: TCTTCGGTCTTAGAGC-1 GTTCCCGGCA GCAATTGG
##      5: CTCTAATGTACTTGAC-1 CGGTGTTACG ATGACCTT
##     ---
## 479939: TTTGTCATCGTTTGCC-1 GAAACGCGCG GACATACG
## 479940: TTTGTCATCGTTTGCC-1 CCTCCCGGTC GACATACG
## 479941: TTTGTCATCGTTTGCC-1 GTAGTCCTTT CCTGAGAA
## 479942: TTTGTCATCTACTTAC-1 AGGCCTGTCA CAGCGTAG
## 479943: TTTGTCATCTACTTAC-1 AGGCCTGTCA CAGCGTAG
##
## $v2
##                     Cell.BC        UMI Cell.Tag
```

```
##       1: CTACACCTCTTTACAC-1 CTGTGTGTGT TGTGCTTG
##       2: CTACACCTCTTTACAC-1 CTGTGTGTGT TGTGCTTG
##       3: CTTAACTCAAGTCTGT-1 CTCCCTGTGT TGTGCTTG
##       4:                <NA> GTGTGTGTGT TGTGCTTG
##       5: GTCTCGTGTGCAGACA-1 CTCTTCTCGC TGTGCTTG
##      ---
## 479470: TTTGTCATCGAGAGCA-1 AGATAGTTGA CCACTTAT
## 479471: TTTGTCATCGAGAGCA-1 AGATAGTTGA CCACTTAT
## 479472: TTTGTCATCGAGAGCA-1 TACATCTCTC CAAATTTT
## 479473: TTTGTCATCGAGAGCA-1 TACATCTCTC CAAATTTT
## 479474: TTTGTCATCGCCAGCA-1 CTCACGGAGC TCCTGCAA
##
## $v3
##                     Cell.BC        UMI Cell.Tag
##       1: TCAGCTCCATCCGTGG-1 GTGTAGTCCG GCAGCCAT
##       2: AAATGCCAGTTATCGC-1 CAACGAGAGA GCAGCTAT
##       3: ATCATGGTCTTCGGTC-1 TATTCAGATA CTCACGAT
##       4: CGACCTTAGGACCACA-1 ACAACTTCCG AGGAGCAT
##       5: TTCTACACATGCTAGT-1 GGTGGCCGGG TCGCTTAT
##      ---
## 130013: TTTGTCATCCGAATGT-1 GAGAGGTCAA TACCGTTC
## 130014: TTTGTCATCCGAATGT-1 GAACAAGTAC TACCGTTC
## 130015: TTTGTCATCCGAATGT-1 CCCCAGAAAT GTAGTTCT
## 130016: TTTGTCATCCGAATGT-1 GAGAGGTCAA TACCGTTC
## 130017: TTTGTCATCTTACCTA-1 TCGACATTGA ACTAACAA
```

Generate the Count Matrix for CellTag

```
bam.test.obj <- CellTagMatrixCount(bam.test.obj, params$barcode.file)
```

```
## Warning in `[.data.table`(alltagCounts, , `:=`((tagsRemove), NULL)):
## length(LHS)==0; no columns to delete or assign RHS to.
```

```
bam.test.obj@celltag.stats
```

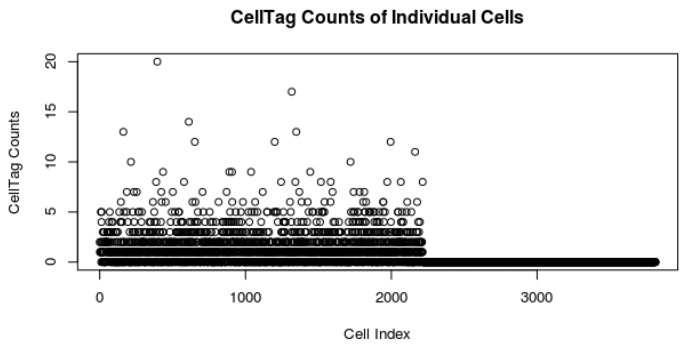
```
## NULL
```

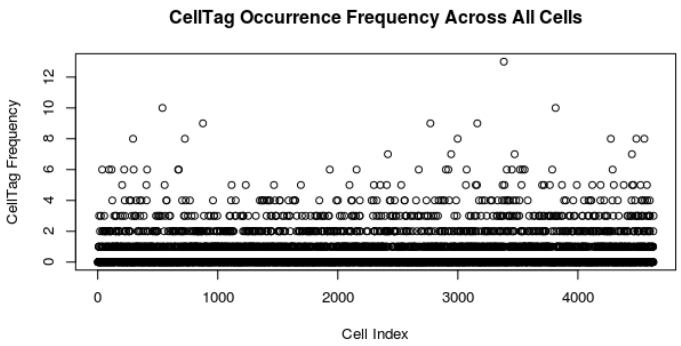
Generate the Binary Matrix from the Count Matrix

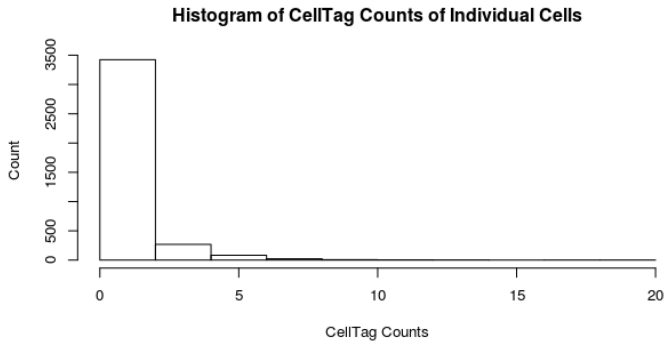
```
bam.test.obj <- SingleCellDataBinatization(bam.test.obj, 2)
```

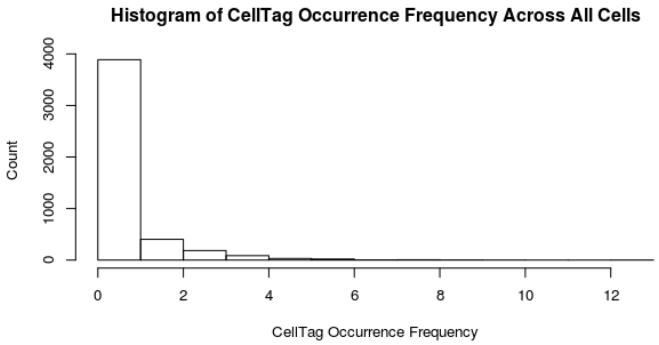
Metric Plots to Facilitate for Additional Filtering

```
MetricPlots(bam.test.obj)
```










```
## Average:  0.8355194
## Frequency:  0.687905
```

Apply the V3 whitelisted CellTags

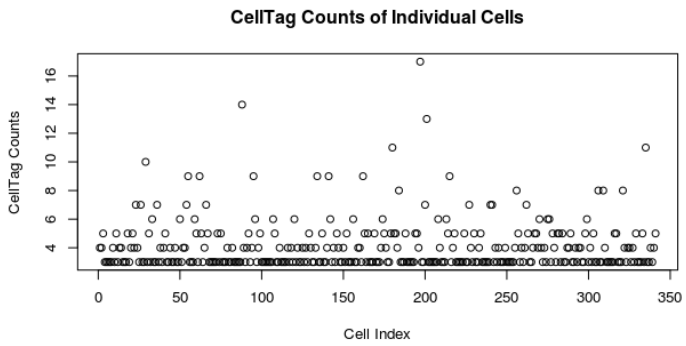
```
bam.test.obj <- SingleCellDataWhitelist(bam.test.obj, params$v3.whitelist.file)
```

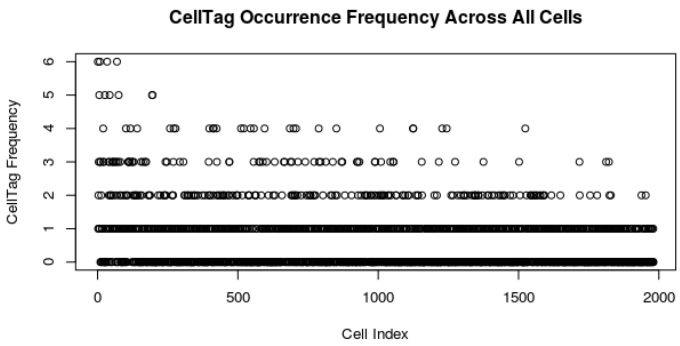
Metric Based Filtering

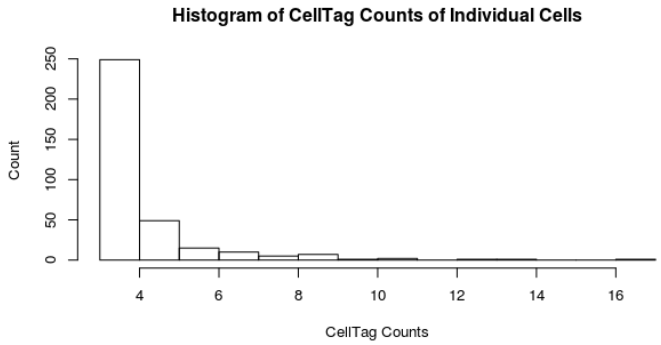
```
bam.test.obj <- MetricBasedFiltering(bam.test.obj, 20, comparison = "less")
bam.test.obj <- MetricBasedFiltering(bam.test.obj, 2, comparison = "greater")
```

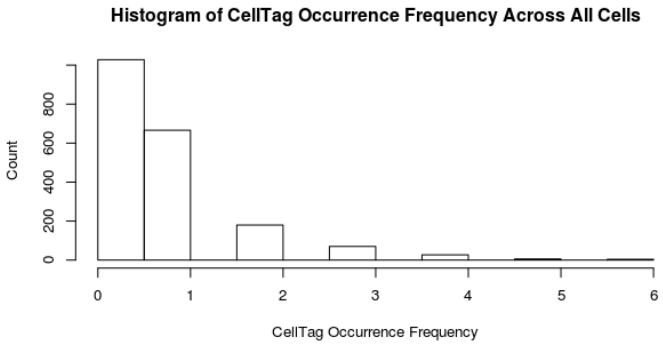
Metric Plots Again to Check for Additional Filtering

```
MetricPlots(bam.test.obj)
```





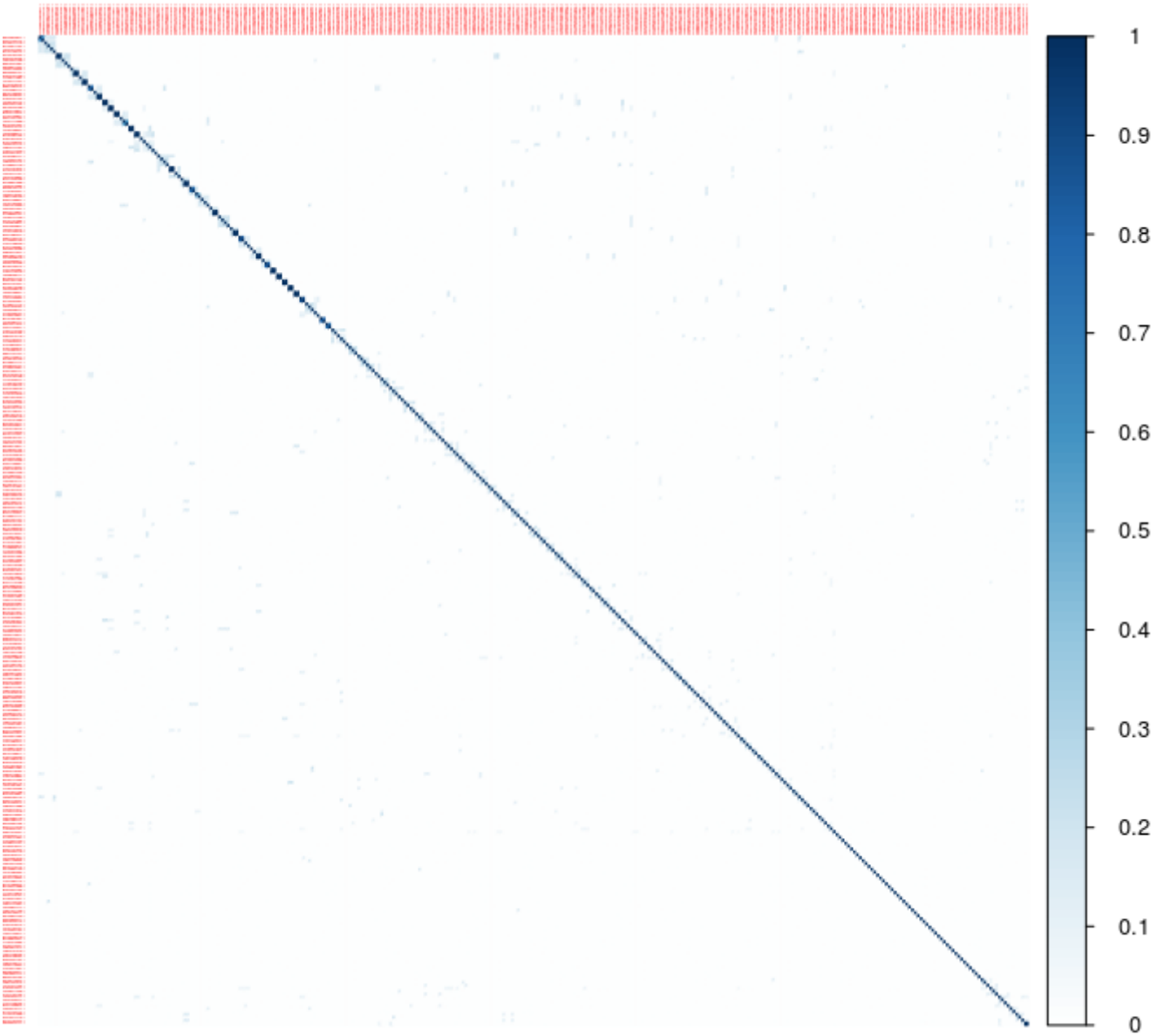





```
## Average:  4.099707
## Frequency:  0.7057042
```

Jaccard Analysis

```
bam.test.obj <- JaccardAnalysis(bam.test.obj)
```



Clone Calling

```
bam.test.obj <- CloneCalling(celltag.obj = bam.test.obj, correlation.cutoff=0.7)
```

Checking the stats and Saving the object

```
show(bam.test.obj)
saveRDS(bam.test.obj, paste0(params$object.saving.dir, "/bam_v123_obj.Rds"))
```

```
## Object name:  hf1.d15.test
## Raw CellTag Counts =  19195
## Raw Number of Cells with CellTag =  3812
## Collapsed CellTag Counts =  0
## Whitelisted CellTag Counts =  7774
## Whitelisted Number of Cells with CellTag =  3812
```