

Run GENESPACE vignettes - Rho duplication of grasses

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1. Global Parameters

```
baseDir <- "/Users/jlovell/Desktop/GENESPACE_data/results/"
rawAnnotationDir <- "/Users/jlovell/Desktop/GENESPACE_data"
mcscanDir <- "/Users/jlovell/Documents/comparative_genomics/programs/MCScanX"
nThreads <- 6
path2of <- "orthofinder" # since orthofinder is in the path via conda
```

2. Set parameters

NOTE: Wheat, Maize and Switchgrass WGD occurred after MRCA of all genomes below. Therefore, treat them as polyploid.

Since some ploidy > 1, run orthofinder again in syntenic blocks.

```
rho <- list(
  wd = file.path(baseDir, "rho"),
  speciesIDs = "grasses",
  genomes = data.table(do.call("rbind", list(
    c(genome = "Phallii", version = "Phallii_HAL2_v2.1", ploidy = 1),
    c("switchgrass", "switchgrass_v5.1", 2),
    c("Sviridis", "Sviridis_v2.1", 1),
    c("Sorghum", "Sbicolor_BTx623_v3.1", 1),
    c("maize", "maize_refgen_v5", 2),
    c("rice", "rice_kitaake_v3.1", 1),
    c("brachy", "Bdistachyon_v3.1", 1),
    c("wheat", "wheat_v4.0", 3))))))

rhoParams <- list(
  orthofinderInBlk = T,
  pepString = "fa",
  nSecondHits = 2, # look for one additional hit after masking primary synteny
  nGapsSecond = 10, # relax mcscan -m parameter
  onlyOgAnchorsSecond = F) # initial secondary syntenic regions defined w/o requiring hits to be in the
```

3 Initialize the rho run with the above specified parameters

```
gparRho <- with(rho, init_genespace(  
  genomeIDs = genomes$genome,  
  versionIDs = genomes$version,  
  ploidy = genomes$ploidy,  
  speciesIDs = rep(speciesIDs, length(genomes$genome)),  
  orthofinderInBlk = rhoParams$orthofinderInBlk,  
  pepString = rhoParams$pepString,  
  wd = wd,  
  nCores = nThreads,  
  path2orthofinder = path2of,  
  path2mcscanx = mcscanDir,  
  rawGenomeDir = rawAnnotationDir))  
  
## set working directory to /Users/jlovell/Desktop/GENESPACE_data/results/rho  
##  
## found raw gff files:  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/Phallii_HAL2_v2.1/annotation/PhalliiHAL_496_v2.1.gene.gff3  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/switchgrass_v5.1/annotation/Pvirgatum_516_v5.1.gene.gff3  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/Sviridis_v2.1/annotation/Sviridis_500_v2.1.gene.gff3  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/Sbicolor_BTx623_v3.1/annotation/Sbicolor_313_v3.1.gene.gff3  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/maize_refgen_v5/annotation/GCF_902167145.1_Zm-B73-REFGenome.gff3  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/rice_kitaake_v3.1/annotation/OsativaKitaake_499_v3.1.gene.gff3  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/Bdistachyon_v3.1/annotation/Bdistachyon_314_v3.1.gene.gff3  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/wheat_v4.0/annotation/GCA_002220415.3_Triticum_4.0_genome.gff3  
##  
## found raw peptide files:  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/Phallii_HAL2_v2.1/annotation/PhalliiHAL_496_v2.1.protein.faa  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/switchgrass_v5.1/annotation/Pvirgatum_516_v5.1.protein.faa  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/Sviridis_v2.1/annotation/Sviridis_500_v2.1.protein.faa  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/Sbicolor_BTx623_v3.1/annotation/Sbicolor_313_v3.1.protein.faa  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/maize_refgen_v5/annotation/GCF_902167145.1_Zm-B73-REFGenome.protein.faa  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/rice_kitaake_v3.1/annotation/OsativaKitaake_499_v3.1.protein.faa  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/Bdistachyon_v3.1/annotation/Bdistachyon_314_v3.1.protein.faa  
##   /Users/jlovell/Desktop/GENESPACE_data/grasses/wheat_v4.0/annotation/GCA_002220415.3_Triticum_4.0_transcript.faa  
##  
## Can't find all parsed annotation files ... need to run parse_annotations, parse_ncbi or parse_phytozome  
##  
## GENESPACE run initialized:  
##   Initial orthofinder database generation method: default inside R  
##   Orthology graph method: inBlock
```

4 Parse the raw annotations.

This is a good example of the flexibility of GENESPACE annotation parsing. The maize genome is from NCBI, the wheat from an independent server with different gff specifications, and the rest from phytozome.

```
parse_ncbi(gsParam = gparRho, genomeIDs = "maize")
```

```
## Parsing annotations: maize  
## Reading gff ... found 34295 protein coding genes
```

```

## Reading peptide fasta ... found 57578 / 34295 total and unique entries
## Merging fa and gff found 34294 matching entires

parse_phytozome(
  gsParam = gparRho,
  genomeIDs = c("Phallii", "switchgrass", "Sviridis", "Sorghum", "rice", "brachy"))

## Parsing annotations: Phallii
## Reading gff ... found 33263 protein coding genes
## Reading peptide fasta ... found 33263 / 33263 total and unique entries
## Merging fa and gff found 33263 matching entires
## Parsing annotations: switchgrass
## Reading gff ... found 80278 protein coding genes
## Reading peptide fasta ... found 80278 / 80278 total and unique entries
## Merging fa and gff found 80278 matching entires
## Parsing annotations: Sviridis
## Reading gff ... found 38334 protein coding genes
## Reading peptide fasta ... found 38334 / 38334 total and unique entries
## Merging fa and gff found 38334 matching entires
## Parsing annotations: Sorghum
## Reading gff ... found 34211 protein coding genes
## Reading peptide fasta ... found 34211 / 34211 total and unique entries
## Merging fa and gff found 34211 matching entires
## Parsing annotations: rice
## Reading gff ... found 35594 protein coding genes
## Reading peptide fasta ... found 35594 / 35594 total and unique entries
## Merging fa and gff found 35594 matching entires
## Parsing annotations: brachy
## Reading gff ... found 34310 protein coding genes
## Reading peptide fasta ... found 34310 / 34310 total and unique entries
## Merging fa and gff found 34310 matching entires

parse_annotations(
  gsParam = gparRho, genomeIDs = "wheat", gffEntryType = "gene",
  gffIdColumn = "ID", headerEntryIndex = 2, headerSep = "locus_tag=",
  headerStripText = "].*", gffStripText = "gene-")

## Parsing annotation files ...
## wheat ...
## Importing gff ... found 109335 gff entires, and 109335 gene entries
## Importing fasta ... found 135934 fasta entires
##
## **FOUND DUPLICATE PEPTIDE FASTA HEADERS. IS THIS JUST A PRIMARY TRANSCRIPT FILE?***
## subsetting to longest model for each gene
## 107351 gff-peptide matches
## Done!

```

5 Get orthofinder results

Since they already have been created, just use the orthofinder run from the previous grass run.

```

load(file.path(baseDir, "gparGrasses.rda"))
file.copy(from = gparGrasses$paths$orthofinder,
  to = dirname(gparRho$paths$orthofinder),

```

```
recursive = T)
```

```
## [1] TRUE
```

```
rm(list = "gparGrasses")
```

Also set the synteny parameters as default.

```
gparRho <- with(rhoParams, set_syntenyParams(  
  gsParam = gparRho,  
  nSecondHits = nSecondHits, # look for one additional hit after masking primary synteny  
  nGapsSecond = nGapsSecond, # relax mcscan -m parameter  
  onlyOgAnchorsSecond = onlyOgAnchorsSecond))
```

6 Build synteny data

```
gparRho <- find_orthofinderResults(gsParam = gparRho)
```

```
gparRho <- synteny(gsParam = gparRho, overwrite = T)
```

```
## Parsing the gff files ...
```

```
## Reading the gffs and adding orthofinder IDs ... Done!
```

```
## Found 84963 global OGs for 397635 genes
```

```
## QC-ing genome to ensure chromosomes/scaffolds are big enough...
```

```
##           Genome: n. chrs PASS/FAIL, n. genes PASS/FAIL, n. OGs PASS/FAIL
```

```
##       Phallii: 10/5, 33253/10, 29545/9
```

```
##       Sorghum: 14/80, 34115/96, 29721/95
```

```
##       Sviridis: 9/1, 38330/4, 32779/1
```

```
##       brachy: 5/5, 34303/7, 28764/7
```

```
##       maize: 28/124, 34057/237, 30031/219
```

```
##       rice: 15/11, 35566/28, 30812/26
```

```
##       switchgrass: 45/68, 80108/170, 69114/138
```

```
##       wheat: 46/292, 106863/488, 82182/435
```

```
## All look good!
```

```
## Defining collinear orthogroup arrays ...
```

```
## Found the following counts of arrays / genome:
```

```
##       Phallii: 4593 genes in 1849 collinear arrays
```

```
##       Sorghum: 5569 genes in 2064 collinear arrays
```

```
##       Sviridis: 6514 genes in 2459 collinear arrays
```

```
##       brachy: 4927 genes in 1904 collinear arrays
```

```
##       maize: 3532 genes in 1412 collinear arrays
```

```
##       rice: 6341 genes in 2226 collinear arrays
```

```
##       switchgrass: 11848 genes in 4699 collinear arrays
```

```
##       wheat: 31292 genes in 11472 collinear arrays
```

```
## Pulling synteny for 36 unique pairwise combinations of genomes
```

```
## Running 6 chunks of 6 combinations each:
```

```
## Chunk 1 / 6 (09:02:55) ... Done!
```

```
## wheat  -wheat    : 2626051 (tot), 584250/562 (reg), 217255/2143 (blk)
```

```
## wheat  -switchgr: 1902112 (tot), 199399/470 (reg), 91095/1892 (blk)
```

```
## switchgr-switchgr: 1437845 (tot), 259625/265 (reg), 144007/543 (blk)
```

```
## wheat  -Sviridis: 1446490 (tot), 121842/257 (reg), 48078/1114 (blk)
```

```
## wheat  -rice     : 1427173 (tot), 128213/210 (reg), 48897/1004 (blk)
```

```
## wheat  -brachy   : 1413797 (tot), 126507/231 (reg), 47218/1045 (blk)
```

```
## Chunk 2 / 6 (09:13:27) ... Done!
```

```

## wheat -maize : 1359956 (tot), 113894/447 (reg), 50105/1684 (blk)
## wheat -Sorghum : 1410558 (tot), 117013/220 (reg), 45943/961 (blk)
## wheat -Phallii : 1367531 (tot), 113728/229 (reg), 47567/1022 (blk)
## switchgr-Sviridis: 883204 (tot), 126401/166 (reg), 55448/563 (blk)
## switchgr-rice : 835715 (tot), 111503/152 (reg), 49694/521 (blk)
## switchgr-brachy : 829708 (tot), 94492/175 (reg), 44424/741 (blk)
## Chunk 3 / 6 (09:18:44) ... Done!
## switchgr-maize : 862408 (tot), 105394/303 (reg), 54404/1115 (blk)
## switchgr-Sorghum : 830520 (tot), 114644/148 (reg), 51211/391 (blk)
## switchgr-Phallii : 852578 (tot), 119777/133 (reg), 57339/505 (blk)
## Sviridis-Sviridis: 538615 (tot), 85119/48 (reg), 43952/118 (blk)
## Sviridis-rice : 489227 (tot), 64016/112 (reg), 26470/380 (blk)
## Sviridis-brachy : 477019 (tot), 53306/124 (reg), 23393/479 (blk)
## Chunk 4 / 6 (09:21:56) ... Done!
## Sviridis-maize : 485865 (tot), 57738/180 (reg), 28050/682 (blk)
## Sviridis-Sorghum : 479821 (tot), 63641/109 (reg), 26801/300 (blk)
## Sviridis-Phallii : 461905 (tot), 65393/107 (reg), 29128/388 (blk)
## rice -rice : 491825 (tot), 87242/58 (reg), 42277/112 (blk)
## rice -brachy : 457159 (tot), 54301/96 (reg), 23906/412 (blk)
## rice -maize : 471177 (tot), 54083/172 (reg), 27063/622 (blk)
## Chunk 5 / 6 (09:23:46) ... Done!
## rice -Sorghum : 461197 (tot), 57895/102 (reg), 24989/273 (blk)
## rice -Phallii : 444201 (tot), 58003/104 (reg), 25960/351 (blk)
## brachy -brachy : 468654 (tot), 67401/49 (reg), 39180/162 (blk)
## brachy -maize : 448307 (tot), 48736/182 (reg), 24996/736 (blk)
## maize -maize : 557804 (tot), 77556/305 (reg), 49633/628 (blk)
## brachy -Sorghum : 442224 (tot), 50025/104 (reg), 22598/413 (blk)
## Chunk 6 / 6 (09:25:40) ... Done!
## maize -Sorghum : 459164 (tot), 58649/162 (reg), 29318/574 (blk)
## Sorghum -Sorghum : 473892 (tot), 74808/131 (reg), 39899/183 (blk)
## brachy -Phallii : 425856 (tot), 48870/111 (reg), 23178/440 (blk)
## maize -Phallii : 451803 (tot), 55019/168 (reg), 28051/648 (blk)
## Sorghum -Phallii : 431866 (tot), 58572/100 (reg), 26792/271 (blk)
## Phallii -Phallii : 445814 (tot), 67967/55 (reg), 39414/134 (blk)
## Defining synteny-constrained orthogroups ...
## Found 141907 synteny-split OGs for 397635 genes
## Running orthofinder by region ...
## genome combinat. : n. non-self genes, nOGs global/syntenic/inblk
## Phallii -Phallii : 7000 genes, 5645 / 5708 / 4003
## switchgr-Phallii : 72705 genes, 26387 / 28955 / 25356
## switchgr-switchgr: 47768 genes, 24623 / 26267 / 22466
## switchgr-Sviridis: 70778 genes, 25948 / 28350 / 24840
## switchgr-Sorghum : 64924 genes, 24244 / 26052 / 22747
## switchgr-maize : 64326 genes, 22947 / 25141 / 21548
## switchgr-rice : 63001 genes, 23955 / 25545 / 22318
## switchgr-brachy : 59719 genes, 22885 / 24821 / 21672
## Sviridis-Phallii : 46464 genes, 21777 / 23234 / 20990
## Sviridis-Sviridis: 6423 genes, 5240 / 5303 / 3668
## Sviridis-Sorghum : 43375 genes, 20768 / 22057 / 19861
## Sviridis-maize : 43494 genes, 19389 / 20691 / 17890
## Sviridis-rice : 42574 genes, 20762 / 21868 / 19387
## Sviridis-brachy : 39828 genes, 19653 / 20878 / 18499
## Sorghum -Phallii : 42358 genes, 20336 / 21427 / 19138
## Sorghum -Sorghum : 6483 genes, 5283 / 5339 / 3681

```

```

## maize -Phallii : 43324 genes, 19369 / 20536 / 17643
## maize -Sorghum : 45361 genes, 19919 / 21200 / 18384
## maize -maize : 14825 genes, 8888 / 9347 / 7087
## rice -Phallii : 41762 genes, 20423 / 21427 / 18945
## rice -Sorghum : 40331 genes, 19813 / 20834 / 18468
## rice -maize : 41434 genes, 18686 / 19780 / 16794
## rice -rice : 7682 genes, 6034 / 6109 / 4310
## rice -brachy : 39851 genes, 19518 / 20564 / 18256
## brachy -Phallii : 38848 genes, 19276 / 20270 / 17922
## brachy -Sorghum : 38194 genes, 19024 / 20124 / 17703
## brachy -maize : 39492 genes, 17936 / 19147 / 16228
## brachy -brachy : 6041 genes, 4842 / 4931 / 3397
## wheat -Phallii : 62490 genes, 23355 / 24627 / 21524
## wheat -switchgr: 79936 genes, 26109 / 27540 / 22904
## wheat -Sviridis: 63466 genes, 23712 / 25091 / 21986
## wheat -Sorghum : 62996 genes, 23579 / 25093 / 22123
## wheat -maize : 61941 genes, 22361 / 23934 / 21139
## wheat -rice : 64677 genes, 23787 / 25176 / 22047
## wheat -brachy : 66486 genes, 24158 / 26020 / 23483
## wheat -wheat : 70147 genes, 29701 / 33214 / 27785
## Combining synteny-constrained and inblock orthogroups ...
## syn OGs: 141907, inblk OGs: 146806, combined OGs: 119181
## Found the following counts of arrays / genome:
## Phallii: 6820 genes in 2557 collinear arrays
## Sorghum: 7477 genes in 2636 collinear arrays
## Sviridis: 8748 genes in 3078 collinear arrays
## brachy: 6537 genes in 2464 collinear arrays
## maize: 5146 genes in 2082 collinear arrays
## rice: 8229 genes in 2788 collinear arrays
## switchgrass: 16620 genes in 6175 collinear arrays
## wheat: 36892 genes in 12963 collinear arrays
## Pulling synteny for 36 unique pairwise combinations of genomes
## Running 6 chunks of 6 combinations each:
## Chunk 1 / 6 (10:57:42) ... Done!
## wheat -wheat : 2630289 (tot), 636662/770 (reg), 223301/2868 (blk)
## wheat -switchgr: 1902518 (tot), 218271/518 (reg), 85333/2105 (blk)
## switchgr-switchgr: 1438091 (tot), 274086/339 (reg), 141527/716 (blk)
## wheat -Sviridis: 1446527 (tot), 132060/280 (reg), 44266/1222 (blk)
## wheat -rice : 1427342 (tot), 138718/225 (reg), 45295/1110 (blk)
## wheat -brachy : 1414558 (tot), 140270/262 (reg), 45129/1231 (blk)
## Chunk 2 / 6 (11:10:00) ... Done!
## wheat -maize : 1360491 (tot), 126445/534 (reg), 49132/1882 (blk)
## wheat -Sorghum : 1411037 (tot), 129923/258 (reg), 43164/1103 (blk)
## wheat -Phallii : 1367813 (tot), 125562/272 (reg), 43766/1173 (blk)
## switchgr-Sviridis: 883440 (tot), 131597/184 (reg), 49523/609 (blk)
## switchgr-rice : 835799 (tot), 115711/157 (reg), 44632/543 (blk)
## switchgr-brachy : 829849 (tot), 98566/188 (reg), 40683/775 (blk)
## Chunk 3 / 6 (11:17:14) ... Done!
## switchgr-maize : 862777 (tot), 109476/329 (reg), 50840/1153 (blk)
## switchgr-Sorghum : 830480 (tot), 118460/157 (reg), 45782/416 (blk)
## switchgr-Phallii : 852672 (tot), 123961/132 (reg), 50866/523 (blk)
## Sviridis-Sviridis: 538615 (tot), 89461/80 (reg), 44120/202 (blk)
## Sviridis-rice : 489310 (tot), 66512/109 (reg), 23626/404 (blk)
## Sviridis-brachy : 476830 (tot), 55934/125 (reg), 21404/513 (blk)

```

```
## Chunk 4 / 6 (11:20:35) ... Done!
## Sviridis-maize : 485899 (tot), 59862/194 (reg), 26252/712 (blk)
## Sviridis-Sorghum : 479959 (tot), 66352/111 (reg), 23950/335 (blk)
## Sviridis-Phallii : 462027 (tot), 67944/104 (reg), 25717/407 (blk)
## rice -rice : 491825 (tot), 90691/83 (reg), 42118/185 (blk)
## rice -brachy : 457189 (tot), 56882/96 (reg), 21922/428 (blk)
## rice -maize : 471036 (tot), 55846/180 (reg), 25325/654 (blk)
## Chunk 5 / 6 (11:22:39) ... Done!
## rice -Sorghum : 461202 (tot), 59921/86 (reg), 22353/279 (blk)
## rice -Phallii : 444238 (tot), 60302/93 (reg), 23178/363 (blk)
## brachy -brachy : 468654 (tot), 70937/69 (reg), 39492/261 (blk)
## brachy -maize : 448398 (tot), 50832/202 (reg), 23669/766 (blk)
## maize -maize : 557950 (tot), 83192/382 (reg), 50485/852 (blk)
## brachy -Sorghum : 442314 (tot), 52836/105 (reg), 20746/422 (blk)
## Chunk 6 / 6 (11:24:50) ... Done!
## maize -Sorghum : 459191 (tot), 60872/181 (reg), 27577/618 (blk)
## Sorghum -Sorghum : 473892 (tot), 78887/162 (reg), 39974/288 (blk)
## brachy -Phallii : 425938 (tot), 51496/115 (reg), 21178/452 (blk)
## maize -Phallii : 451796 (tot), 56988/185 (reg), 26253/687 (blk)
## Sorghum -Phallii : 432030 (tot), 60529/86 (reg), 23595/285 (blk)
## Phallii -Phallii : 445814 (tot), 71680/81 (reg), 39328/219 (blk)
## Found 119181 OGs across 397635 genes. gff3-like text file written to:
## /Users/jlovell/Desktop/GENESPACE_data/results//rho/results/gffWithOgs.txt.gz
## Calculating syntenic block breakpoints ...
## Found 52483 blocks. Text file written to:
## /Users/jlovell/Desktop/GENESPACE_data/results//rho/results/syntenicBlocks.txt.gz:
```

7 Print session info

```
save(gparRho, file = file.path(baseDir, "gparRho.rda"))
sessionInfo()

## R version 4.1.2 (2021-11-01)
## Platform: x86_64-apple-darwin17.0 (64-bit)
## Running under: macOS Big Sur 10.16
##
## Matrix products: default
## BLAS: /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/4.1/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats graphics grDevices utils datasets methods base
##
## other attached packages:
## [1] GENESPACE_0.9.3 data.table_1.14.2
##
## loaded via a namespace (and not attached):
## [1] Rcpp_1.0.8 compiler_4.1.2
## [3] restfulr_0.0.13 GenomeInfoDb_1.30.1
```

## [5] XVector_0.34.0	MatrixGenerics_1.6.0
## [7] R.methodsS3_1.8.1	bitops_1.0-7
## [9] R.utils_2.11.0	tools_4.1.2
## [11] zlibbioc_1.40.0	digest_0.6.29
## [13] lattice_0.20-45	evaluate_0.14
## [15] pkgconfig_2.0.3	rlang_1.0.0
## [17] Matrix_1.4-0	igraph_1.2.11
## [19] DelayedArray_0.20.0	cli_3.1.1
## [21] rstudioapi_0.13	yaml_2.2.2
## [23] parallel_4.1.2	xfun_0.29
## [25] fastmap_1.1.0	GenomeInfoDbData_1.2.7
## [27] rtracklayer_1.54.0	stringr_1.4.0
## [29] knitr_1.37	Biostrings_2.62.0
## [31] S4Vectors_0.32.3	IRanges_2.28.0
## [33] grid_4.1.2	stats4_4.1.2
## [35] Biobase_2.54.0	BiocParallel_1.28.3
## [37] XML_3.99-0.8	rmarkdown_2.11
## [39] magrittr_2.0.2	matrixStats_0.61.0
## [41] GenomicAlignments_1.30.0	Rsamtools_2.10.0
## [43] GenomicRanges_1.46.1	htmltools_0.5.2
## [45] BiocGenerics_0.40.0	SummarizedExperiment_1.24.0
## [47] stringi_1.7.6	RCurl_1.98-1.5
## [49] rjson_0.2.21	crayon_1.4.2
## [51] dbscan_1.1-10	BiocIO_1.4.0
## [53] R.oo_1.24.0	