A very short, sketchy, introduction to Bioconductor

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BIOF 339

Bioconductor

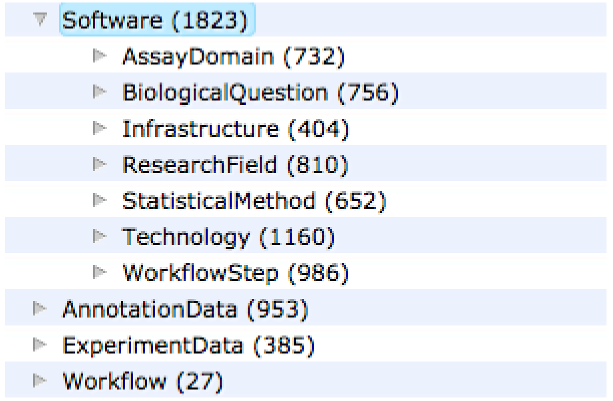
Bioconductor provides tools for the analysis and comprehension of high-throughput genomic and biological data, using R.

- 1823 packages
- Covers the bioinformatic pipeline

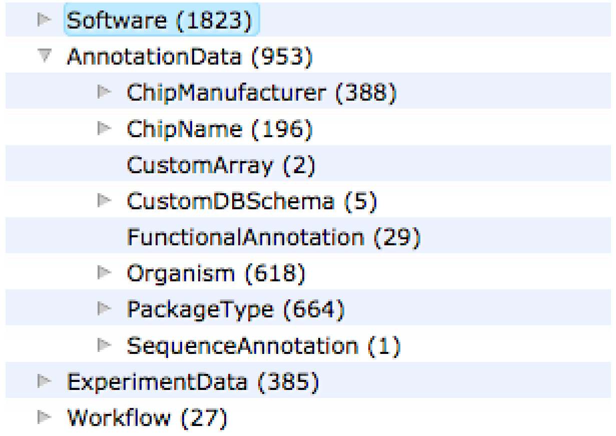
- Analysis [`GenomicRanges`, `Biostrings`, `GenomicAlignments`, `SummarizedExperiment`]
- Annotation (species/platform specific, system) [`biomaRt`, `org.Hs.eg.db`, `GO.db`, `KEGG.db`]
- Experiments [`TENxPBMCData`, `airway`, `ALL`]
- Workflows [`rnaseqGene`, `TCGAWorkflow`]

Bioconductor

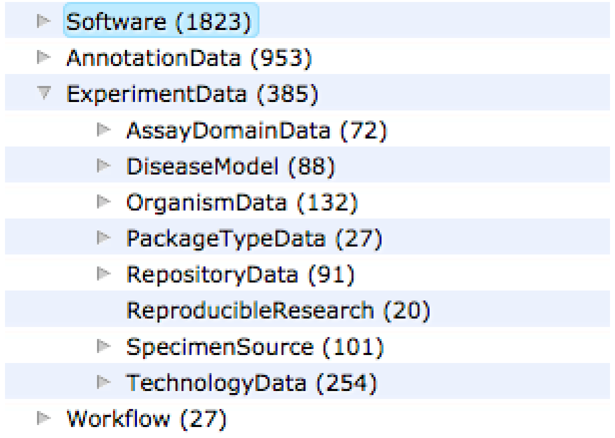
Bioconductor v. 3.10 packages



- Software (1823)
  - AssayDomain (732)
  - BiologicalQuestion (756)
  - Infrastructure (404)
  - ResearchField (810)
  - StatisticalMethod (652)
  - Technology (1160)
  - WorkflowStep (986)
- AnnotationData (953)
- ExperimentData (385)
- Workflow (27)



- Software (1823)
- AnnotationData (953)
  - ChipManufacturer (388)
  - ChipName (196)
  - CustomArray (2)
  - CustomDBSchema (5)
  - FunctionalAnnotation (29)
  - Organism (618)
  - PackageType (664)
  - SequenceAnnotation (1)
- ExperimentData (385)
- Workflow (27)



- Software (1823)
- AnnotationData (953)
- ExperimentData (385)
  - AssayDomainData (72)
  - DiseaseModel (88)
  - OrganismData (132)
  - PackageTypeData (27)
  - RepositoryData (91)
  - ReproducibleResearch (20)
  - SpecimenSource (101)
  - TechnologyData (254)
- Workflow (27)

Installing Bioconductor packages

Bioconductor is a separate repository and system which uses R. So the process is a bit different than `install.packages`. The following works for R version 3.5 and higher.

```r
install.packages("BiocManager")
BiocManager::install(c('Biobase','limma','hgu95av2.db','Biostrings'))
```

There are several packages that are often installed for each Bioconductor package, and some have functions that have the same name as one's you've used. So

- Use `package::function` format for calling functions from non-Bioconductor packages

Bioconductor basics

```
library(Biostrings)
dna <- DNAStringSet(c("AACAT", "GGCGCCT"))
reverseComplement(dna)
```

```
DNAStringSet object of length 2:
    width seq
[1]     5 ATGTT
[2]     7 AGGCGCC
```

```
library(Biostrings)
data("phiX174Phage")
phiX174Phage
```

```
DNAStringSet object of length 6:
    width seq                                               names
[1]  5386 GAGTTTTATCGCTTCCATGACGC...ATGATTGGCGTATCCAACCTGCA Genbank
[2]  5386 GAGTTTTATCGCTTCCATGACGC...ATGATTGGCGTATCCAACCTGCA RF70s
[3]  5386 GAGTTTTATCGCTTCCATGACGC...ATGATTGGCGTATCCAACCTGCA SS78
[4]  5386 GAGTTTTATCGCTTCCATGACGC...ATGATTGGCGTATCCAACCTGCA Bull
[5]  5386 GAGTTTTATCGCTTCCATGACGC...ATGATTGGCGTATCCAACCTGCA G97
[6]  5386 GAGTTTTATCGCTTCCATGACGC...ATGATTGGCGTATCCAACCTGCA NEB03
```

Bioconductor basics

```
letterFrequency(phiX174Phage, 'GC', as.prob=TRUE)
```

```
           G|C
[1,] 0.4476420
[2,] 0.4472707
[3,] 0.4472707
[4,] 0.4470850
[5,] 0.4472707
[6,] 0.4470850
```

Bioconductor data structures

- So far we've seen the `data.frame` or `tibble` be the unit of data storage
- In Bioconductor, data are stored in **containers** which can contain many elements of data for an experiment
    - Actual quantitative results of experiments
    - Phenotype data
    - Genotype meta-data
    - Results of analysis
- In Bioconductor workflows, the same container is updated with new elements, which can then be accessed using accessor functions

An ExpressionSet

```r
library(Biobase)
data('sample.ExpressionSet')
str(sample.ExpressionSet)
```

```
Formal class 'ExpressionSet' [package "Biobase"] with 7 slots
  ..@ experimentData   :Formal class 'MIAME' [package "Biobase"] with 13 slots
  .. .. ..@ name             : chr "Pierre Fermat"
  .. .. ..@ lab              : chr "Francis Galton Lab"
  .. .. ..@ contact          : chr "pfermat@lab.not.exist"
  .. .. ..@ title            : chr "Smoking-Cancer Experiment"
  .. .. ..@ abstract         : chr "An example object of expression set (ExpressionSet) class"
  .. .. ..@ url              : chr "www.lab.not.exist"
  .. .. ..@ pubMedIds        : chr ""
  .. .. ..@ samples          : list()
  .. .. ..@ hybridizations   : list()
  .. .. ..@ normControls     : list()
  .. .. ..@ preprocessing    : list()
  .. .. ..@ other            :List of 1
  .. .. .. ..$ notes: chr "An example object of expression set (exprSet) class"
  .. .. ..@ .__classVersion__:Formal class 'Versions' [package "Biobase"] with 1 slot
  .. .. .. .. ..@ .Data:List of 2
  .. .. .. .. .. ..$ : int [1:3] 1 0 0
  .. .. .. .. .. ..$ : int [1:3] 1 1 0
  ..@ assayData        :<environment: 0x7fee0594eb58>
  ..@ phenoData        :Formal class 'AnnotatedDataFrame' [package "Biobase"] with 4 slots
  .. .. ..@ varMetadata      :'data.frame':    3 obs. of  1 variable:
  .. .. .. ..$ labelDescription: chr [1:3] "Female/Male" "Case/Control" "Testing Score"
  .. .. ..@ data             :'data.frame':    26 obs. of  3 variables:
```

An ExpressionSet

These objects are based on a different R structure. Instead of extracting elements using `$`, this structure uses **slots** which are accessed using `@`

```
slotNames(sample.ExpressionSet)
```

```
[1] "experimentData"    "assayData"          "phenoData"
[4] "featureData"       "annotation"         "protocolData"
[7] ".__classVersion__"
```

```
sample.ExpressionSet@phenoData
```

```
An object of class 'AnnotatedDataFrame'
  sampleNames: A B ... Z (26 total)
  varLabels: sex type score
  varMetadata: labelDescription
```

An ExpressionSet

We almost never use `@`. Instead we use *accessor functions* to extract data

```
pData(sample.ExpressionSet) # Phenotype data
```

```
     sex    type score
A Female Control  0.75
B   Male    Case  0.40
C   Male Control  0.73
D   Male    Case  0.42
E Female    Case  0.93
F   Male Control  0.22
G   Male    Case  0.96
H   Male    Case  0.79
I Female    Case  0.37
J   Male Control  0.63
K   Male    Case  0.26
L Female Control  0.36
M   Male    Case  0.41
N   Male    Case  0.80
O Female    Case  0.10
P Female Control  0.41
Q Female    Case  0.16
R   Male Control  0.72
S   Male    Case  0.17
T Female    Case  0.74
U   Male Control  0.35
V Female Control  0.77
```

An ExpressionSet

We almost never use `@`. Instead we use *accessor functions* to extract data

```
head(exprs(sample.ExpressionSet)) # Expression data
```

```
                       A         B        C        D        E       F        G
AFFX-MurIL2_at  192.7420  85.75330 176.7570 135.5750 64.49390 76.3569 160.5050
AFFX-MurIL10_at  97.1370 126.19600  77.9216  93.3713 24.39860 85.5088  98.9086
AFFX-MurIL4_at   45.8192   8.83135  33.0632  28.7072  5.94492 28.2925  30.9694
AFFX-MurFAS_at   22.5445   3.60093  14.6883  12.3397 36.86630 11.2568  23.0034
AFFX-BioB-5_at   96.7875  30.43800  46.1271  70.9319 56.17440 42.6756  86.5156
AFFX-BioB-M_at   89.0730  25.84610  57.2033  69.9766 49.58220 26.1262  75.0083
                      H       I         J        K       L       M       N
AFFX-MurIL2_at  65.9631 56.9039 135.60800 63.44320 78.2126 83.0943 89.3372
AFFX-MurIL10_at 81.6932 97.8015  90.48380 70.57330 94.5418 75.3455 68.5827
AFFX-MurIL4_at  14.7923 14.2399  34.48740 20.35210 14.1554 20.6251 15.9231
AFFX-MurFAS_at  16.2134 12.0375   4.54978  8.51782 27.2852 10.1616 20.2488
AFFX-BioB-5_at  30.7927 19.7183  46.35200 39.13260 41.7698 80.2197 36.4903
AFFX-BioB-M_at  42.3352 41.1207  91.53070 39.91360 49.8397 63.4794 24.7007
                      O       P        Q        R         S       T         U
AFFX-MurIL2_at  91.0615 95.9377 179.8450 152.4670 180.83400 85.4146 157.98900
AFFX-MurIL10_at 87.4050 84.4581  87.6806 108.0320 134.26300 91.4031  -8.68811
AFFX-MurIL4_at  20.1579 27.8139  32.7911  33.5292  19.81720 20.4190  26.87200
AFFX-MurFAS_at  15.7849 14.3276  15.9488  14.6753  -7.91911 12.8875  11.91860
AFFX-BioB-5_at  36.4021 35.3054  58.6239 114.0620  93.44020 22.5168  48.64620
AFFX-BioB-M_at  47.4641 47.3578  58.1331 104.1220 115.83100 58.1224  73.42210
                       V       W         X       Y         Z
AFFX-MurIL2_at  146.8000 93.8829 103.85500 64.4340 175.61500
```

SummarizedExperiment

This is a more common structure related to modern experiments with different technologies

An Example

```
# BiocManager::install('SummarizedExperiment')
library(SummarizedExperiment)
data(airway, package="airway")
se <- airway
se
```

```
class: RangedSummarizedExperiment
dim: 64102 8
metadata(1): ''
assays(1): counts
rownames(64102): ENSG00000000003 ENSG00000000005 ... LRG_98 LRG_99
rowData names(0):
colnames(8): SRR1039508 SRR1039509 ... SRR1039520 SRR1039521
colData names(9): SampleName cell ... Sample BioSample
```

An Example

Count data from the scRNA-seq experiment

```
assay(se)
```

```
                SRR1039508 SRR1039509 SRR1039512 SRR1039513 SRR1039516
ENSG00000000003        679        448        873        408       1138
ENSG00000000005          0          0          0          0          0
ENSG00000000419        467        515        621        365        587
ENSG00000000457        260        211        263        164        245
ENSG00000000460         60         55         40         35         78
ENSG00000000938          0          0          2          0          1
ENSG00000000971       3251       3679       6177       4252       6721
ENSG00000001036       1433       1062       1733        881       1424
ENSG00000001084        519        380        595        493        820
ENSG00000001167        394        236        464        175        658
ENSG00000001460        172        168        264        118        241
ENSG00000001461       2112       1867       5137       2657       2735
ENSG00000001497        524        488        638        357        676
ENSG00000001561         71         51        211        156         23
ENSG00000001617        555        394        905        415        727
ENSG00000001626         10          2          9          2         10
ENSG00000001629       1660       1251       2259       1079       2462
ENSG00000001630         59         54         66         23         84
ENSG00000001631        729        692        943        475       1034
ENSG00000002016        201        161        256         99        268
ENSG00000002079          3          0          3          1          4
ENSG00000002330        206        174        184        111        194
```

An Example

Genomic ranges for each transcript

```
rowRanges(se)
```

```
GRangesList object of length 64102:
$ENSG00000000003
GRanges object with 17 ranges and 2 metadata columns:
       seqnames            ranges strand |   exon_id       exon_name
          <Rle>         <IRanges>  <Rle> | <integer>     <character>
   [1]        X 99883667-99884983      - |    667145 ENSE00001459322
   [2]        X 99885756-99885863      - |    667146 ENSE00000868868
   [3]        X 99887482-99887565      - |    667147 ENSE00000401072
   [4]        X 99887538-99887565      - |    667148 ENSE00001849132
   [5]        X 99888402-99888536      - |    667149 ENSE00003554016
   ...      ...               ...    ... .       ...             ...
  [13]        X 99890555-99890743      - |    667156 ENSE00003512331
  [14]        X 99891188-99891686      - |    667158 ENSE00001886883
  [15]        X 99891605-99891803      - |    667159 ENSE00001855382
  [16]        X 99891790-99892101      - |    667160 ENSE00001863395
  [17]        X 99894942-99894988      - |    667161 ENSE00001828996
  -------
  seqinfo: 722 sequences (1 circular) from an unspecified genome

...
<64101 more elements>
```

An Example

Phenotype data

```
colData(se)
```

```
DataFrame with 8 rows and 9 columns
           SampleName     cell      dex    albut        Run avgLength
             <factor> <factor> <factor> <factor>   <factor> <integer>
SRR1039508 GSM1275862  N61311     untrt    untrt SRR1039508       126
SRR1039509 GSM1275863  N61311     trt      untrt SRR1039509       126
SRR1039512 GSM1275866  N052611    untrt    untrt SRR1039512       126
SRR1039513 GSM1275867  N052611    trt      untrt SRR1039513        87
SRR1039516 GSM1275870  N080611    untrt    untrt SRR1039516       120
SRR1039517 GSM1275871  N080611    trt      untrt SRR1039517       126
SRR1039520 GSM1275874  N061011    untrt    untrt SRR1039520       101
SRR1039521 GSM1275875  N061011    trt      untrt SRR1039521        98
           Experiment    Sample    BioSample
             <factor>  <factor>     <factor>
SRR1039508  SRX384345 SRS508568 SAMN02422669
SRR1039509  SRX384346 SRS508567 SAMN02422675
SRR1039512  SRX384349 SRS508571 SAMN02422678
SRR1039513  SRX384350 SRS508572 SAMN02422670
SRR1039516  SRX384353 SRS508575 SAMN02422682
SRR1039517  SRX384354 SRS508576 SAMN02422673
SRR1039520  SRX384357 SRS508579 SAMN02422683
SRR1039521  SRX384358 SRS508580 SAMN02422677
```

An Example

Experimental meta-data

```
metadata(se)
```

```
[[1]]
Experiment data
  Experimenter name: Himes BE
  Laboratory: NA
  Contact information:
  Title: RNA-Seq transcriptome profiling identifies CRISPLD2 as a glucocorticoid responsive gene that modulates cy
  URL: http://www.ncbi.nlm.nih.gov/pubmed/24926665
  PMIDs: 24926665

  Abstract: A 226 word abstract is available. Use 'abstract' method.
```

An Example

Data subsetting

```
se[1:5, 1:3]
```

```
class: RangedSummarizedExperiment
dim: 5 3
metadata(1): ''
assays(1): counts
rownames(5): ENSG00000000003 ENSG00000000005 ENSG0000
  ENSG00000000457 ENSG00000000460
rowData names(0):
colnames(3): SRR1039508 SRR1039509 SRR1039512
colData names(9): SampleName cell ... Sample BioSampl
```

```
se[,se$cell=='N61311']
```

```
class: RangedSummarizedExperiment
dim: 64102 2
metadata(1): ''
assays(1): counts
rownames(64102): ENSG00000000003 ENSG00000000005 ...
rowData names(0):
colnames(2): SRR1039508 SRR1039509
colData names(9): SampleName cell ... Sample BioSampl
```

Annotation

biomaRt

The `biomaRt` package allows access to many public annotation databases

```
# BiocManager::install('biomaRt')
library(biomaRt)
ensemblDB <- useMart('ensembl')
searchDatasets(mart=ensemblDB, pattern='Human')
```

```
                 dataset              description    version
80 hsapiens_gene_ensembl Human genes (GRCh38.p13) GRCh38.p13
```

```
ensemblHuman <- useMart('ensembl', dataset='hsapiens_gene_ensembl', host='useast.ensembl.org')
```

Identifying attributes

```
searchAttributes(mart=ensemblHuman, pattern='affy')
```

```
                     name                  description         page
104           affy_hc_g110         AFFY HC G110 probe feature_page
105          affy_hg_focus        AFFY HG Focus probe feature_page
106          affy_hg_u133a        AFFY HG U133A probe feature_page
107        affy_hg_u133a_2      AFFY HG U133A 2 probe feature_page
108          affy_hg_u133b        AFFY HG U133B probe feature_page
109    affy_hg_u133_plus_2    AFFY HG U133 Plus 2 probe feature_page
110           affy_hg_u95a         AFFY HG U95A probe feature_page
111         affy_hg_u95av2       AFFY HG U95Av2 probe feature_page
112           affy_hg_u95b         AFFY HG U95B probe feature_page
113           affy_hg_u95c         AFFY HG U95C probe feature_page
114           affy_hg_u95d         AFFY HG U95D probe feature_page
115           affy_hg_u95e         AFFY HG U95E probe feature_page
116           affy_hta_2_0          AFFY HTA 2 0 probe feature_page
117    affy_huex_1_0_st_v2    AFFY HuEx 1 0 st v2 probe feature_page
118          affy_hugenefl       AFFY HuGeneFL probe feature_page
119  affy_hugene_1_0_st_v1  AFFY HuGene 1 0 st v1 probe feature_page
120  affy_hugene_2_0_st_v1  AFFY HuGene 2 0 st v1 probe feature_page
121         affy_primeview      AFFY PrimeView probe feature_page
122          affy_u133_x3p        AFFY U133 X3P probe feature_page
```

Identifying attributes

```
searchAttributes(mart=ensemblHuman, pattern='hgnc')
```

```
              name         description         page
61         hgnc_id             HGNC ID feature_page
62     hgnc_symbol         HGNC symbol feature_page
93 hgnc_trans_name Transcript name ID feature_page
```

Annotating probsets

We first grab some probesets from the `sample.ExpressionSet` Affy experiment

```
affyIDs <- rownames(featureData(sample.ExpressionSet))
```

Now let's find annotation

```
getBM(attributes = c('affy_hg_u95av2','entrezgene_id'),
      filters = 'affy_hg_u95av2',
      values = affyIDs[200:203],
      mart = ensemblHuman)
```

```
  affy_hg_u95av2 entrezgene_id
1       31440_at          6932
2     31439_f_at          6007
3     31439_f_at          6006
```

A RNA-seq pipeline

The workflow

- Exploratory data analysis
- Differential expression analysis with DESeq2
- Visualization

- We will start after reads have been aligned to a reference genome and reads overlapping known genes have been counted

The experiment

- In the experiment, four primary human airway smooth muscle cell lines were treated with 1 micromolar dexamethasone for 18 hours.
- For each of the four cell lines, we have a treated and an untreated sample.

Get data

```
# BiocManager::install('airway')
library(airway)
data(airway)
se <- airway
head(assay(airway))
```

```
                SRR1039508 SRR1039509 SRR1039512 SRR1039513 SRR1039516
ENSG00000000003        679        448        873        408       1138
ENSG00000000005          0          0          0          0          0
ENSG00000000419        467        515        621        365        587
ENSG00000000457        260        211        263        164        245
ENSG00000000460         60         55         40         35         78
ENSG00000000938          0          0          2          0          1
                SRR1039517 SRR1039520 SRR1039521
ENSG00000000003       1047        770        572
ENSG00000000005          0          0          0
ENSG00000000419        799        417        508
ENSG00000000457        331        233        229
ENSG00000000460         63         76         60
ENSG00000000938          0          0          0
```

Create a DESeqDataSet

```r
# BiocManager::install('DESeq2')
library("DESeq2")
dds <- DESeqDataSet(se, design = ~ cell + dex)
dds
```

```
class: DESeqDataSet
dim: 64102 8
metadata(2): '' version
assays(1): counts
rownames(64102): ENSG00000000003 ENSG00000000005 ... LRG_98 LRG_99
rowData names(0):
colnames(8): SRR1039508 SRR1039509 ... SRR1039520 SRR1039521
colData names(9): SampleName cell ... Sample BioSample
```

Run differential expression pipeline

```
dds <- DESeq(dds)
```

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

Run differential expression pipeline

```
(res <- results(dds))
```

```
log2 fold change (MLE): dex untrt vs trt
Wald test p-value: dex untrt vs trt
DataFrame with 64102 rows and 6 columns
                 baseMean log2FoldChange     lfcSE      stat      pvalue
                <numeric>      <numeric> <numeric> <numeric>   <numeric>
ENSG00000000003  708.6022      0.3812540  0.100654  3.787752 0.000152016
ENSG00000000005    0.0000             NA        NA        NA          NA
ENSG00000000419  520.2979     -0.2068126  0.112219 -1.842943 0.065337292
ENSG00000000457  237.1630     -0.0379204  0.143445 -0.264356 0.791505742
ENSG00000000460   57.9326      0.0881682  0.287142  0.307054 0.758801924
...                   ...            ...       ...       ...         ...
LRG_94                  0             NA        NA        NA          NA
LRG_96                  0             NA        NA        NA          NA
LRG_97                  0             NA        NA        NA          NA
LRG_98                  0             NA        NA        NA          NA
LRG_99                  0             NA        NA        NA          NA
                      padj
                 <numeric>
ENSG00000000003 0.00128363
ENSG00000000005         NA
ENSG00000000419 0.19654609
ENSG00000000457 0.91145948
ENSG00000000460 0.89503278
...                    ...
LRG_94                  NA
LRG_96                  NA
```

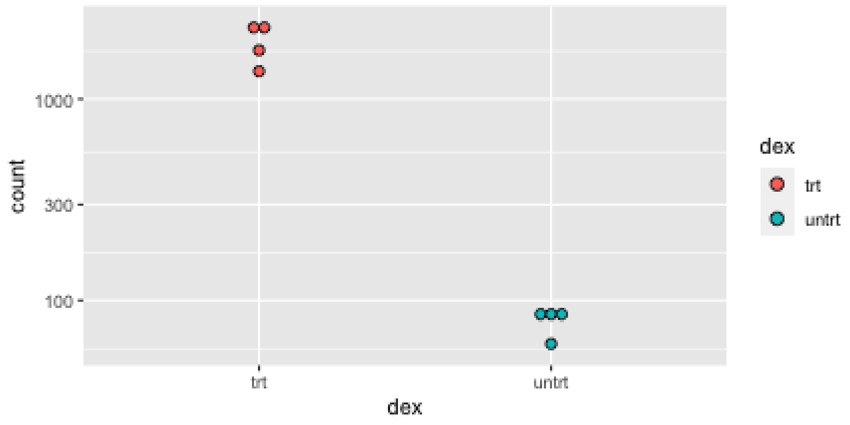
Summarizing results

```
library(tidyverse)
as.data.frame(res) %>%
  rownames_to_column(var = 'ID') %>%
  filter(padj < 0.1) %>%
  arrange(desc(abs(log2FoldChange))) %>% head()
```

```
                ID   baseMean log2FoldChange     lfcSE       stat       pvalue
1 ENSG00000179593  67.243048      -9.505975 1.0545032  -9.014647 1.975049e-19
2 ENSG00000109906 385.071029      -7.352626 0.5363887 -13.707645 9.137621e-43
3 ENSG00000250978  56.318194      -6.327383 0.6777973  -9.335214 1.007876e-20
4 ENSG00000132518   5.654654      -5.885114 1.3240439  -4.444803 8.797262e-06
5 ENSG00000128285   6.624741       5.325904 1.2578147   4.234251 2.293144e-05
6 ENSG00000127954 286.384119      -5.207158 0.4930818 -10.560435 4.545484e-26
          padj
1 1.253739e-17
2 2.256617e-40
3 7.210311e-19
4 1.000612e-04
5 2.380012e-04
6 5.058395e-24
```

A visualization

```
topGene <- rownames(res)[which.min(res$padj)]
dat <- plotCounts(dds, gene=topGene, intgroup=c("dex"), returnData=TRUE)
ggplot(dat, aes(x = dex, y = count, fill=dex))+
  geom_dotplot(binaxis='y', stackdir='center')+
  scale_y_log10()
```



A Seurat pipeline

Grab the data and convert for Seurat

```
# BiocManager::install('TENxPBMCData')
library(TENxPBMCData)
library(Seurat)

pbmc_3k <- TENxPBMCData(dataset='pbmc3k')
colnames(pbmc_3k) <- paste0('Cell', seq_len(ncol(pbmc_3k)))
pbmc <- CreateSeuratObject( counts=assay(pbmc_3k, 'counts'),
                            project='pbmc3k',
                            min.cells=3,
                            min.features = 200)

pbmc
```

```
An object of class Seurat
13714 features across 2700 samples within 1 assay
Active assay: RNA (13714 features, 0 variable features)
```

A bit of QC

```
# The [[ operator can add columns to object metadata. This is a great place to stash QC stats
rownames(pbmc@assays$RNA@counts) <- r2
rownames(pbmc[['RNA']]@meta.features) <- r2
rownames(pbmc@assays$RNA@data) <- r2 # Change to gene names from Ensembl
pbmc[["percent.mt"]] <- PercentageFeatureSet(pbmc, pattern = "^MT-") # percentage in mitochondria genome
head(pbmc@meta.data)
```

```
      orig.ident nCount_RNA nFeature_RNA percent.mt
Cell1     pbmc3k       2419          779  3.0177759
Cell2     pbmc3k       4903         1352  3.7935958
Cell3     pbmc3k       3147         1129  0.8897363
Cell4     pbmc3k       2639          960  1.7430845
Cell5     pbmc3k        980          521  1.2244898
Cell6     pbmc3k       2163          781  1.6643551
```

> See how analyses results are added to the same container. The idea is to keep all the experimental information together. This was a philosophic choice maide by the Bioconductor developers, inspired by the MIAME requirements and how data are stored on genomics databases like GEO

Visualization

```
# Visualize QC metrics as a violin plot
(plt <- VlnPlot(pbmc, features = c("nFeature_RNA", "nCount_RNA", "percent.mt"), ncol = 3))
```

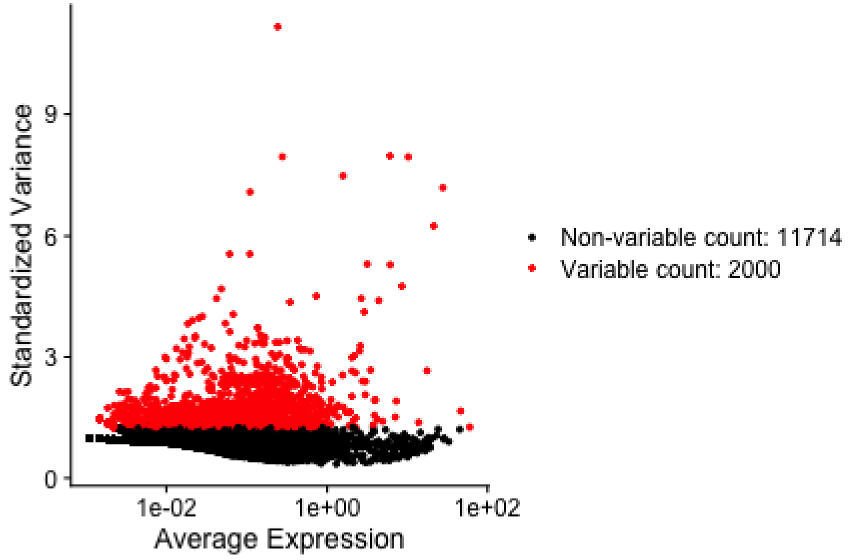
Normalization

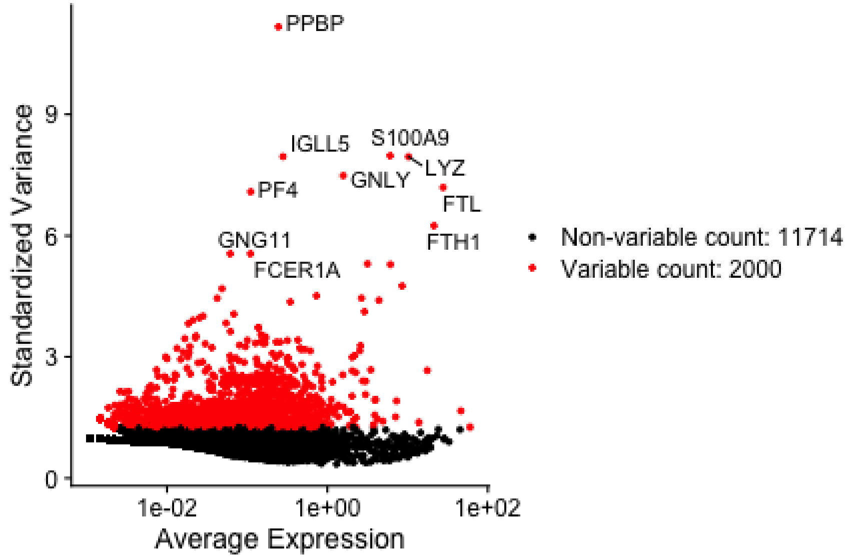
```
pbmc <- NormalizeData(pbmc)
```

> Note, we're saving in the same object

Feature selection

```
rownames(pbmc[['RNA']]@meta.features) <- r2
pbmc <- FindVariableFeatures(pbmc, selection.method = "vst", nfeatures = 2000)

# Identify the 10 most highly variable genes
top10 <- head(VariableFeatures(pbmc), 10)

# plot variable features with and without labels
plot1 <- VariableFeaturePlot(pbmc)
plot2 <- LabelPoints(plot = plot1, points = top10, repel = TRUE)
CombinePlots(plots = list(plot1, plot2))
```





PCA

```
pbmc <- ScaleData(pbmc)
pbmc <- RunPCA(pbmc, features = VariableFeatures(object = pbmc))
print(pbmc[["pca"]], dims = 1:5, nfeatures = 5)
```

```
PC_ 1
Positive:  MALAT1, LTB, IL32, CD2, ACAP1
Negative:  CST3, TYROBP, LST1, AIF1, FTL
PC_ 2
Positive:  CD79A, MS4A1, TCL1A, HLA-DQA1, HLA-DRA
Negative:  NKG7, PRF1, CST7, GZMA, GZMB
PC_ 3
Positive:  HLA-DQA1, CD79A, CD79B, HLA-DQB1, HLA-DPB1
Negative:  PPBP, PF4, SDPR, SPARC, GNG11
PC_ 4
Positive:  HLA-DQA1, CD79A, CD79B, HIST1H2AC, HLA-DQB1
Negative:  VIM, S100A8, S100A6, S100A4, S100A9
PC_ 5
Positive:  GZMB, FGFBP2, NKG7, GNLY, PRF1
Negative:  LTB, VIM, AQP3, PPA1, MAL
```

> Important to note that each step just adds elements to the Seurat object

PCA

```r
DimPlot(pbmc, reduction = "pca")
```

t-SNE

```
pbmc <- RunTSNE(pbmc, dims=1:10)
DimPlot(pbmc, reduction='tsne')
```

Heatmaps

Heatmaps

There are several ways of doing heatmaps in R:

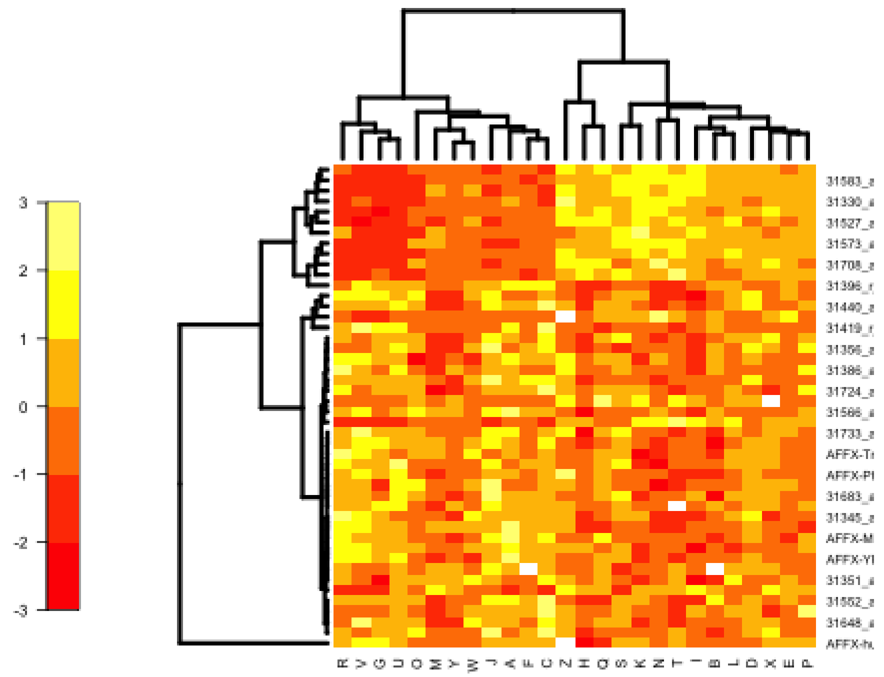
- http://sebastianraschka.com/Articles/heatmaps_in_r.html
- https://plot.ly/r/heatmaps/
- http://moderndata.plot.ly/interactive-heat-maps-for-r/
- http://www.siliconcreek.net/r/simple-heatmap-in-r-with-ggplot2
- https://rud.is/b/2016/02/14/making-faceted-heatmaps-with-ggplot2/

Some example data

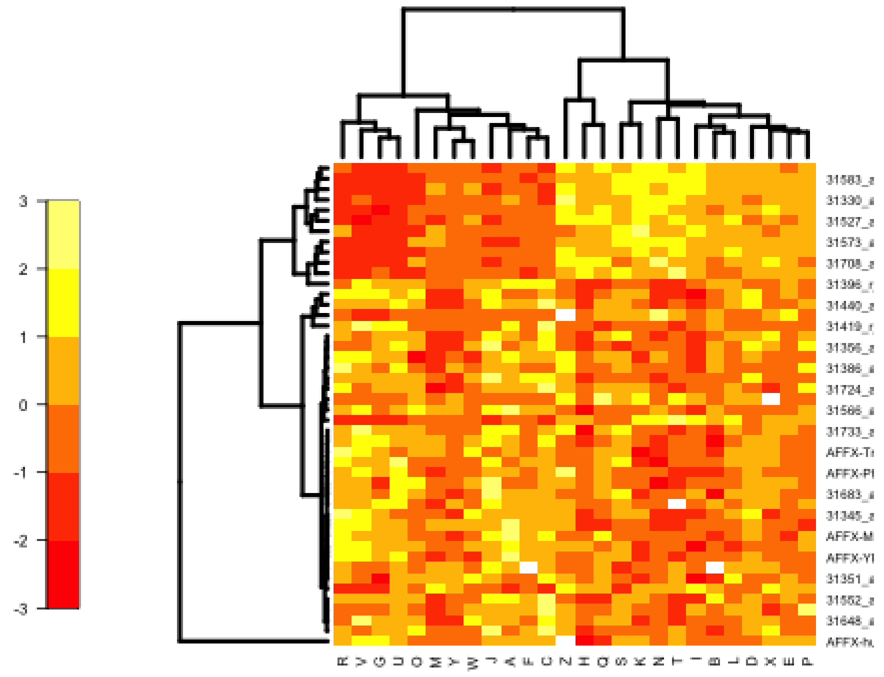
```
library(Biobase)
data(sample.ExpressionSet)
exdat <-  sample.ExpressionSet
library(limma)
design1 <- model.matrix(~type, data=pData(exdat))
lm1 <- lmFit(exprs(exdat), design1)
lm1 <- eBayes(lm1) # compute linear model for each probeset
geneID <- rownames(topTable(lm1, coef=2, num=100, adjust='none',p.value=0.05))
exdat2 <- exdat[geneID,] # Keep features with p-values < 0.05
exdat2
```

```
ExpressionSet (storageMode: lockedEnvironment)
assayData: 46 features, 26 samples
  element names: exprs, se.exprs
protocolData: none
phenoData
  sampleNames: A B ... Z (26 total)
  varLabels: sex type score
  varMetadata: labelDescription
featureData: none
experimentData: use 'experimentData(object)'
Annotation: hgu95av2
```

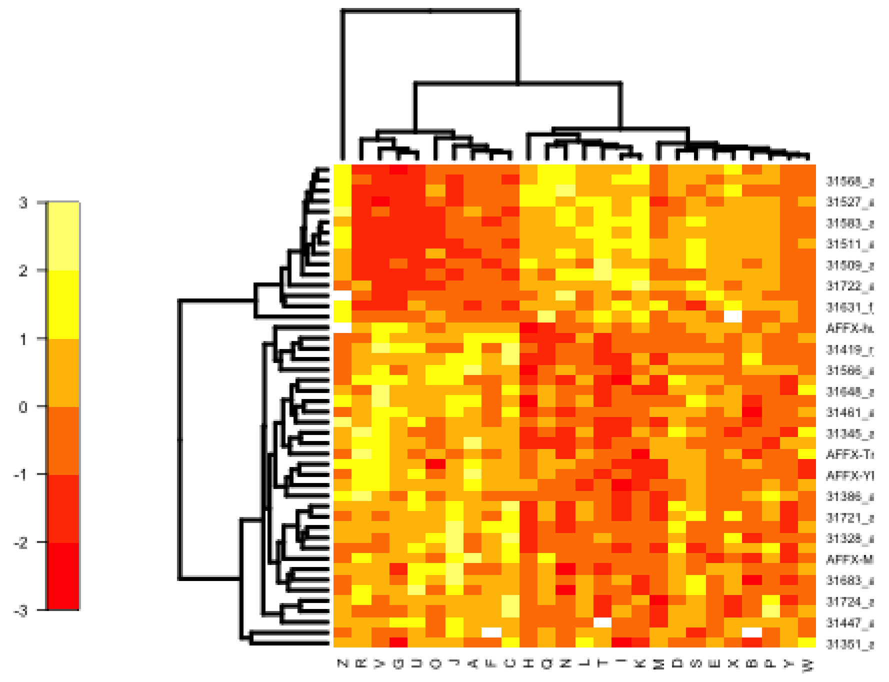
```r
# BiocManager::install('Heatplus')

library(Heatplus)
reg1 <- regHeatmap(exprs(exdat2), legend=2, col=heat.colors,
                   breaks=-3:3)
plot(reg1)
```



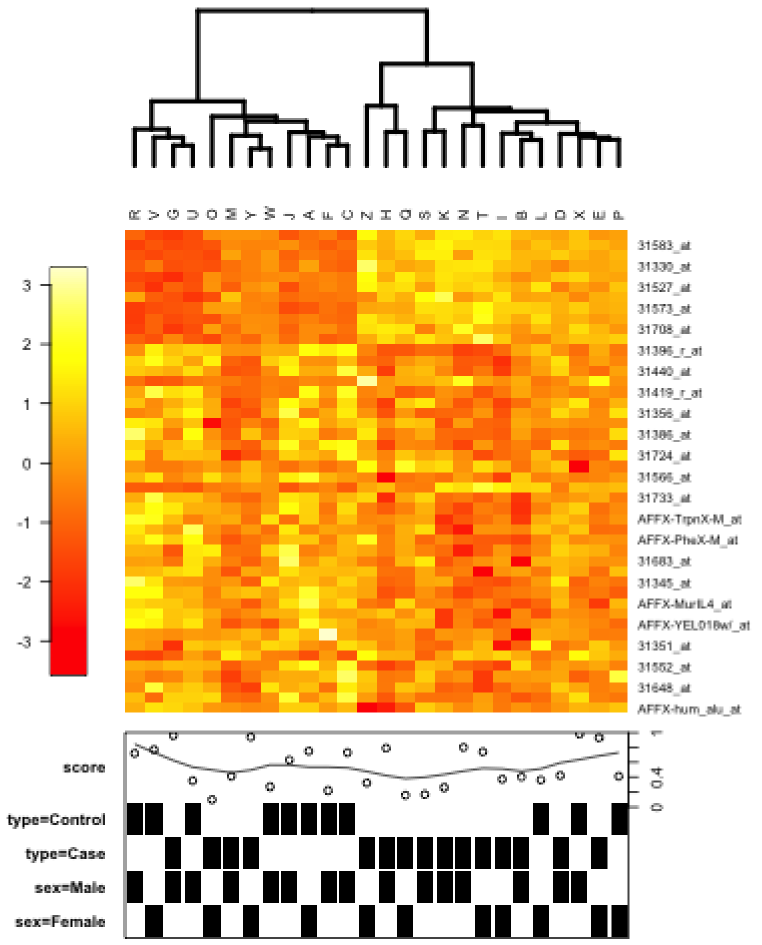
```r
library(Heatplus)
reg1 <- regHeatmap(exprs(exdat2), legend=2, col=heat.colors,
                   breaks=-3:3)
plot(reg1)
```



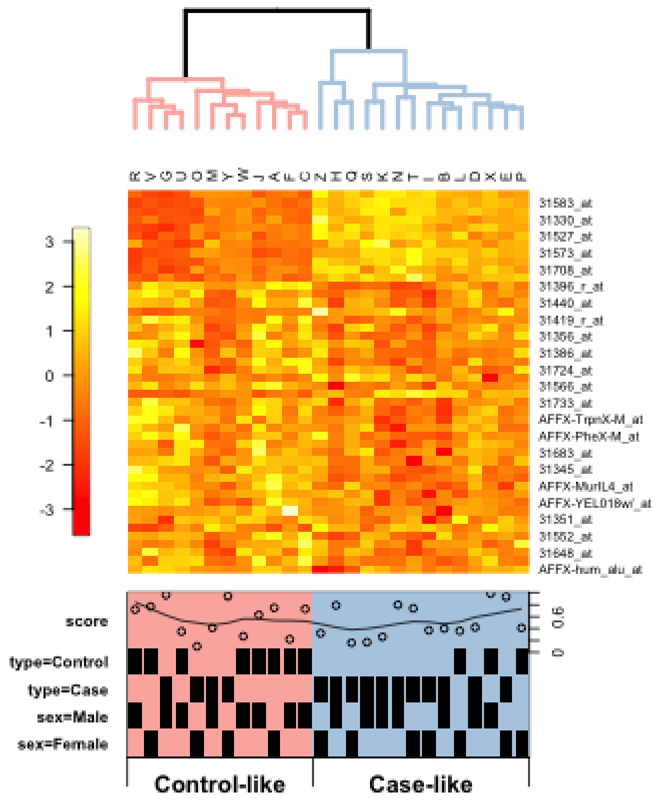
```r
corrdist <- function(x) as.dist(1-cor(t(x)))
hclust.avl <-  function(x) hclust(x, method='average')
reg2 <- regHeatmap(exprs(exdat2), legend=2, col=heat.colors,
                   breaks=-3:3,
                   dendrogram = list(clustfun=hclust.avl, distfun=corrdist))
plot(reg2)
```



```
ann1 <- annHeatmap(exprs(exdat2), ann=pData(exdat2), col = heat.colors)
plot(ann1)
```



```r
ann2 <- annHeatmap(exprs(exdat2), ann=pData(exdat2), col = heat.colors,
                   cluster = list(cuth=7500,
                                  label=c('Control-like','Case-like')))
plot(ann2)
```



```
# devtools::install_github("rstudio/d3heatmap")
d3heatmap(exprs(exdat2), distfun = corrdist,
          hclustfun = hclust.avl, scale='row')
```

Link

Put your mouse over each point :)

Resources

- Organizing Single Cell Analysis with Bioconductor
- Bioconductor Courses
- 2019 Bioconductor workshops