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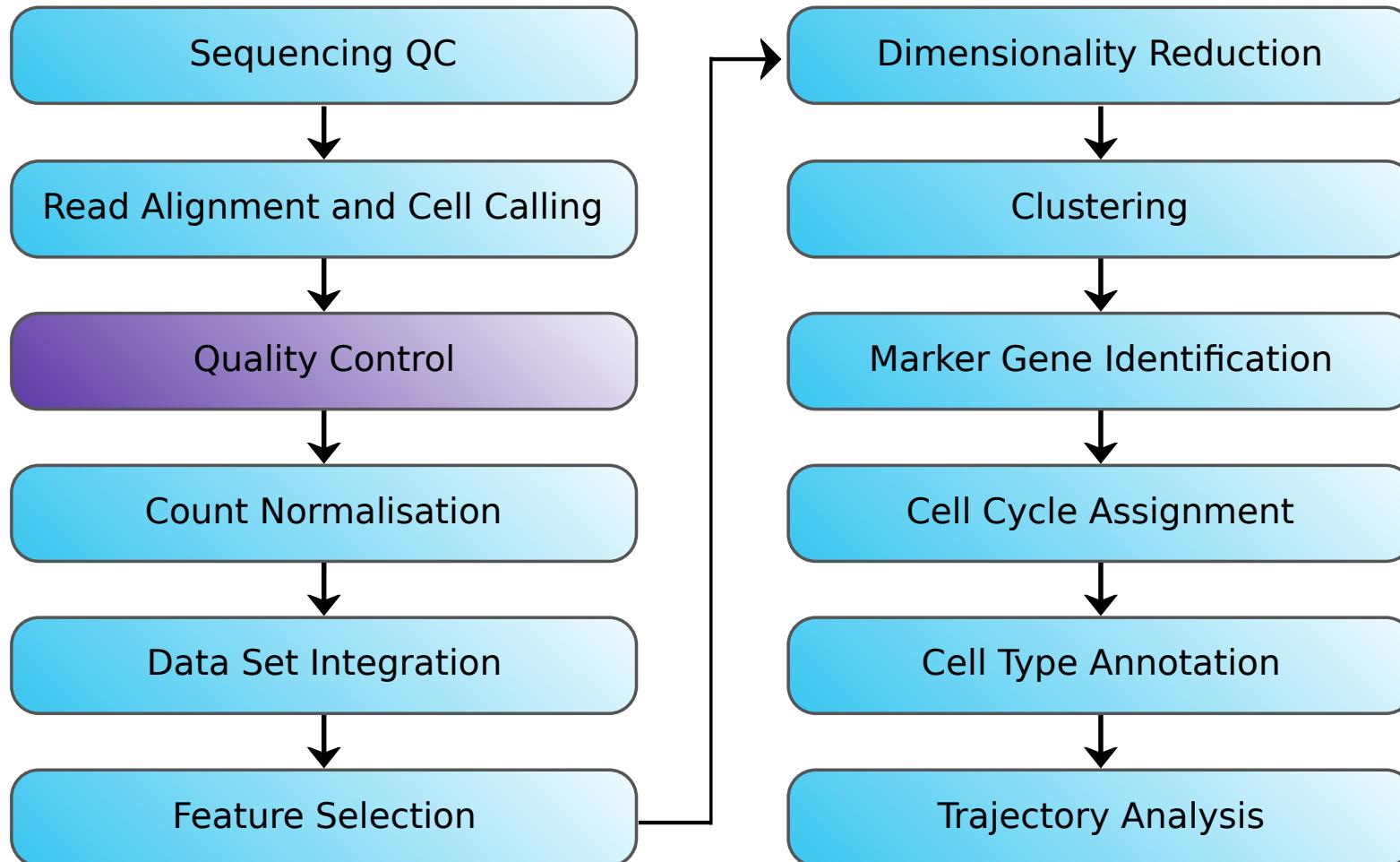
Introduction to single-cell RNA-seq analysis

Quality Control

Ashley Sawle

29th October 2021

Single Cell RNAseq Analysis Workflow



Quality Control

We will now check the quality of the data further:

- Mapping quality
- Cell counts
- Distribution of keys quality metrics

We will then:

- Filter genes with very low expression
- Identify low-quality cells
- Filter and/or mark low quality cells

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Quality Control

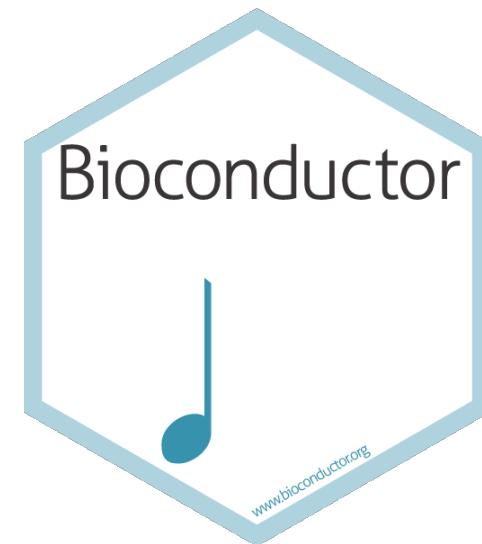
Bioconductor R packages:

- *scater*
- *DropletUtils*

Orchestrating Single-Cell Analysis with Bioconductor

Robert Amezquita, Aaron Lun, Stephanie Hicks, Raphael Gottardo

<http://bioconductor.org/books/release/OSCA/>



Read CellRanger outputs into R

```
%h%-$ ls SRR9264343/outs/raw_feature_bc_matrix  
barcodes.tsv.gz  
features.tsv.gz  
matrix.mtx.gz
```

Loading a single sample

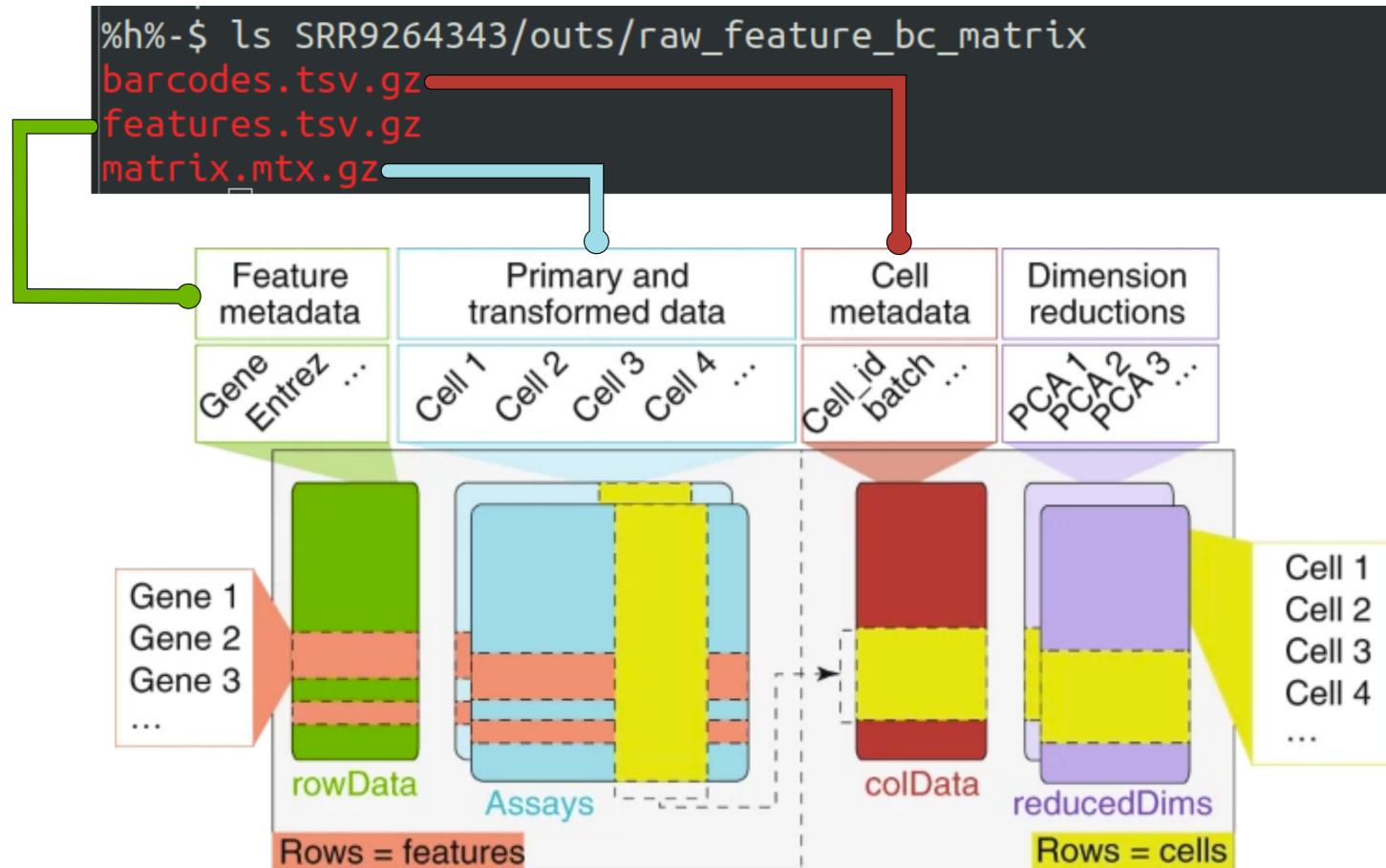
```
sample.path <- c(SRR9264343 = "CellRanger_Outputs/SRR9264343/outs/filtered_feature_bc_matrix/")  
sce <- read10xCounts(sample.path, col.names=TRUE)  
sce <- read10xCounts(sample.path, col.names=FALSE)
```

Loading multiple samples

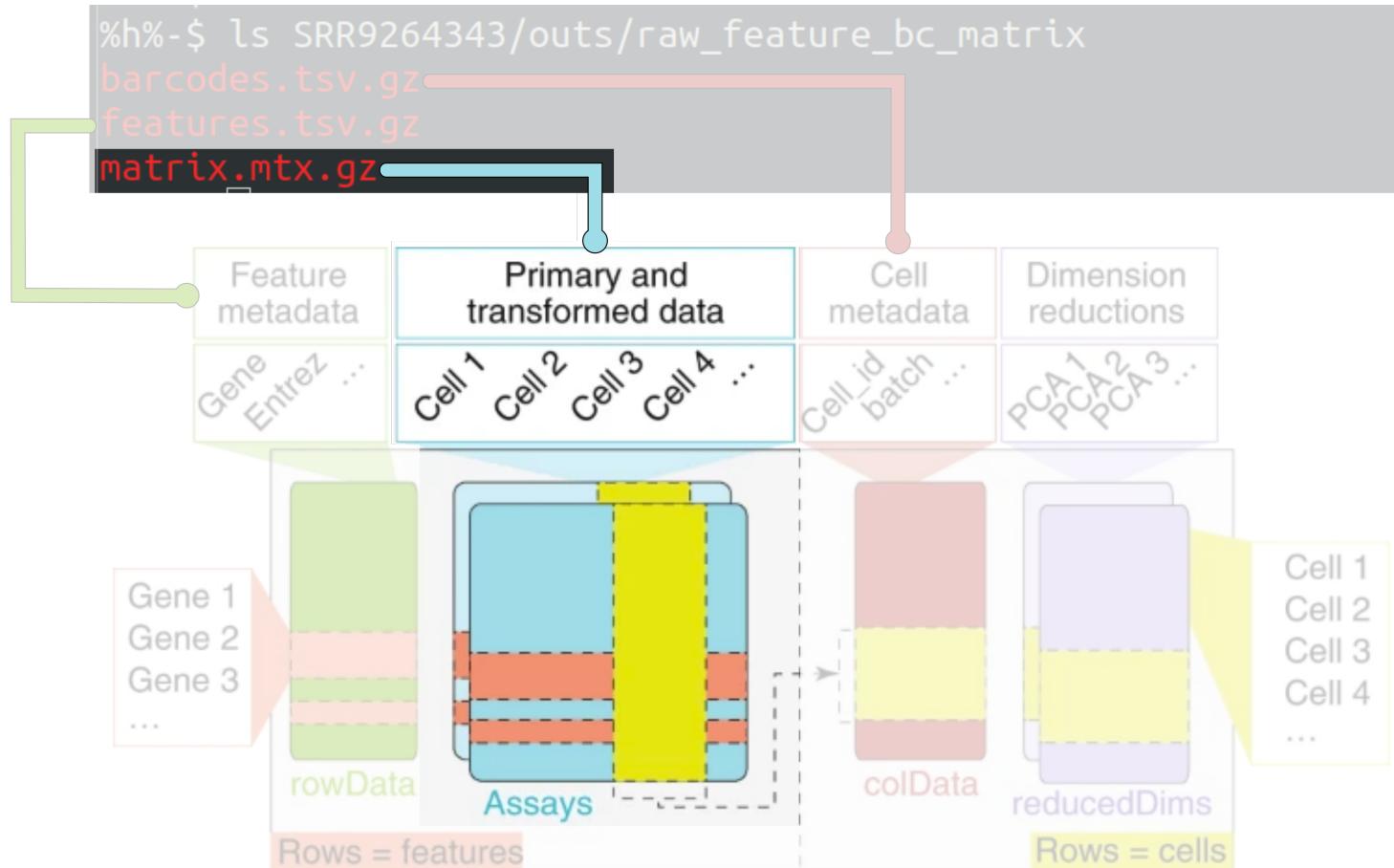
```
list_of_files <- c(SRR9264343 = "CellRanger_Outputs/SRR9264343/outs/filtered_feature_bc_matrix",  
                   SRR9264344 = "CellRanger_Outputs/SRR9264344/outs/filtered_feature_bc_matrix",  
                   SRR9264347 = "CellRanger_Outputs/SRR9264347/outs/filtered_feature_bc_matrix")  
sce <- read10xCounts(sample.path, col.names=TRUE)
```

→ *SingleCellExperiment* object

The *SingleCellExperiment* object



The Counts Matrix

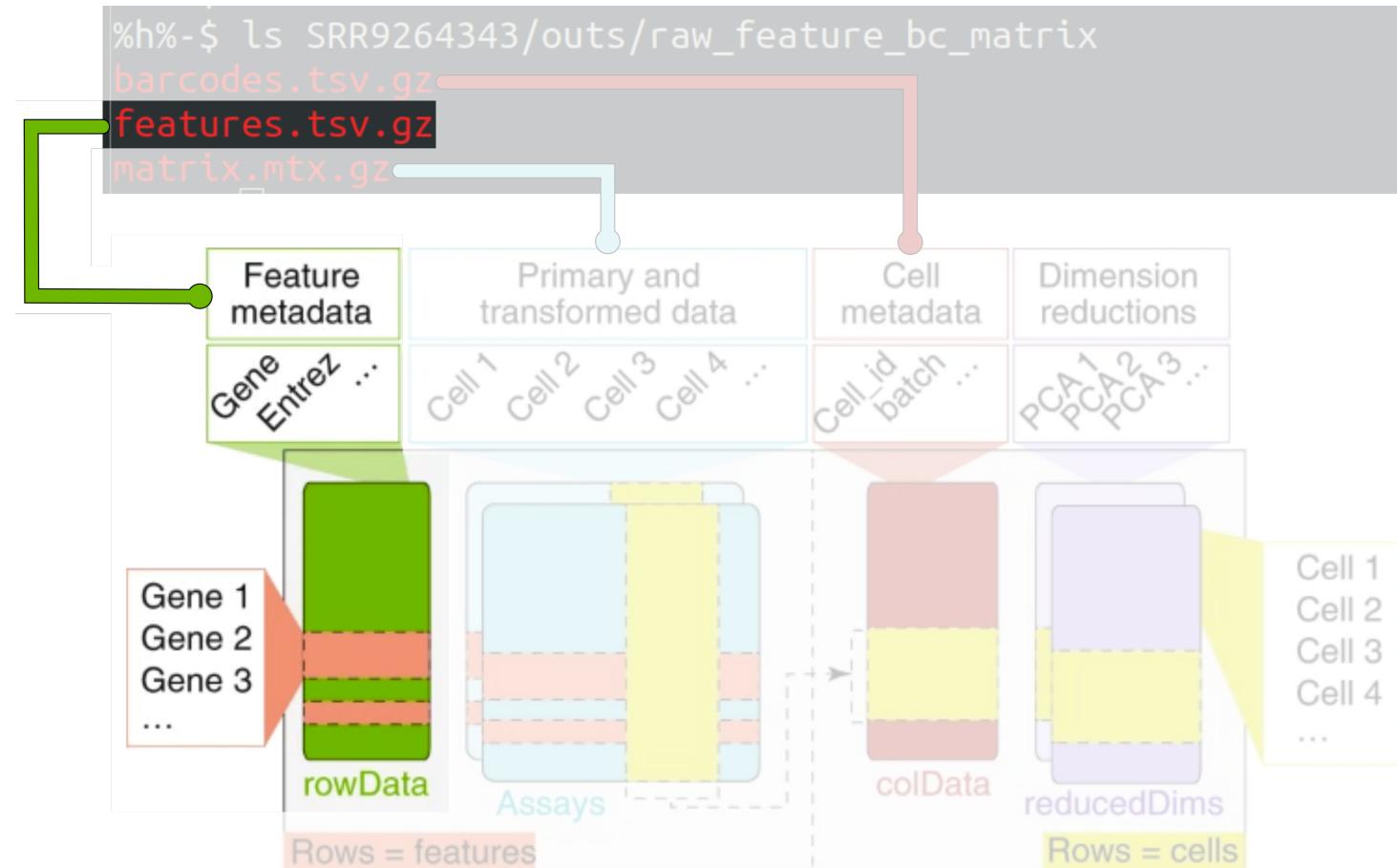


The Counts Matrix

```
counts(sce)
```

```
## 36601 x 3094 sparse Matrix of class "dgCMatrix"
##
## ENSG00000175756 1 2 . 1 1 . . 1 . . 2 1 . 1 2 2 1 . . 1 . 1 3 1 1 2 1 2 2 . 6 . . 1 1 1 . . 1 3 1 . .
## ENSG00000221978 . 2 . . . . . 3 . . . 1 . . . . . 1 . . . 1 . . . 1 . . . 1 . . . 1 . . .
## ENSG00000224870 . . . . . . . . 1 1 . . . . . . . . . . . . . . . . 1 . . .
## ENSG00000242485 1 2 . 1 2 . . 1 . 1 1 4 . . 1 . 1 2 . 2 . 1 2 . . . 1 . 2 2 . . 1 1 1 1 . 1 . 1 .
## ENSG00000272455 . . . . . . . . . . . . . . . . . . . . . . . . . . . .
## ENSG00000235098 . . . . . . . . . . . . . . . . . . . . . . . . . . .
## ENSG00000225905 . . . . . . . . . . . . . . . . . . . . . . . . . . .
## ENSG00000205116 . . . . . . . . . . . . . . . . . . . . . . . . . . .
## ENSG00000225285 . . . . . . . . . . . . . . . . . . . . . . . . . . .
## ENSG00000179403 . . . . . . . . . . . . . . . . . . . . . . . . . . .
## ENSG00000215915 . . . . . . . . . . . . . . . . . . . . . . . . . . .
## ENSG00000160072 . . . . . . . . . . . . . . . . . . . . . . . . . 1 .
## ENSG00000197785 1 . . . . . . . . . . . . . . . . . . . . . . . . 1 .
## ENSG00000205090 . . . . . . . . . . . . . . . . . . . . . . . . . .
## ENSG00000160075 2 1 . . . 1 3 1 4 2 3 1 . . . 1 1 . 2 2 1 . 4 2 1 1 1 . . 1 2 . . 1 . . 1 . 1 2 . .
## ENSG00000215014 . . . . . 1 . . . . . . . . . . . . . . . . . . 1 .
## ENSG00000228594 . . . . . . . . . . . . . . . . . . . . . . . . . .
## ENSG00000286989 . . . . . . . . . . . . . . . . . . . . . . . . . .
## ENSG00000272106 . . . . . . . . . . . . . . . . . . . . . . . . . .
## ENSG00000197530 . . . . . . . . . . 1 . . . 1 . . . . . . . . 1 . . 1 .
## ENSG00000189409 . . . . . . . . . . . . . . . . . . . . . . . . . .
## ENSG00000248333 . 1 . . . . 1 2 . . . . . 1 1 . . . . . . . . . 1 . 1 .
## ENSG00000272004 . . . . . . . . . . . . . . . . . . . . . . . . . .
##
## suppressing 3050 columns and 36556 rows in show(); maybe adjust 'options(max.print= *, width = *)'
##
```

Feature metadata

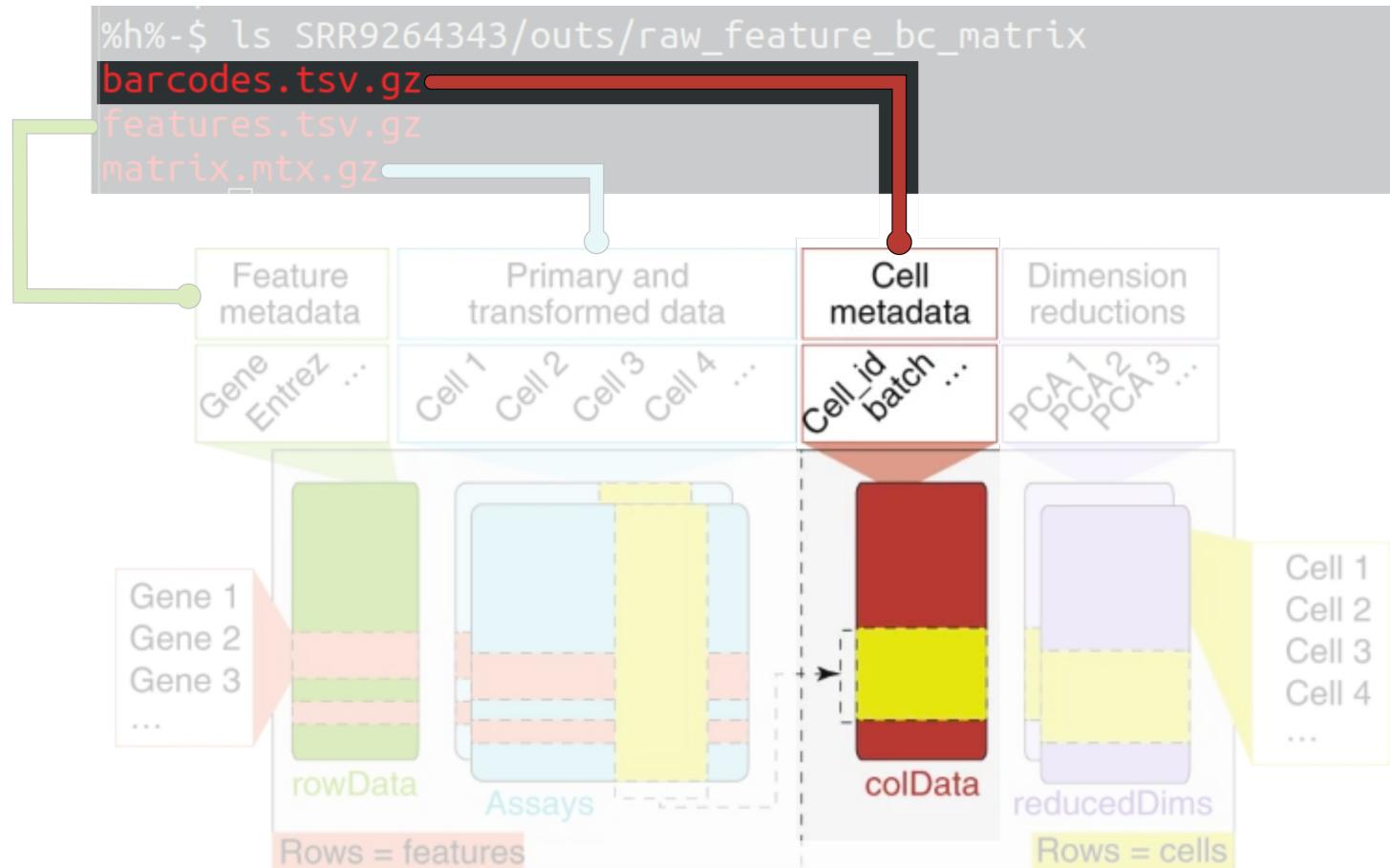


Feature metadata

```
rowData(sce)
```

```
## DataFrame with 36601 rows and 3 columns
##           ID      Symbol        Type
##           <character> <character> <character>
## ENSG00000175756 ENSG00000175756 AURKAIP1 Gene Expression
## ENSG00000221978 ENSG00000221978 CCNL2 Gene Expression
## ENSG00000224870 ENSG00000224870 MRPL20-AS1 Gene Expression
## ENSG00000242485 ENSG00000242485 MRPL20 Gene Expression
## ENSG00000272455 ENSG00000272455 AL391244.2 Gene Expression
## ...
##           ...      ...        ...
## ENSG00000240731 ENSG00000240731 AL139287.1 Gene Expression
## ENSG00000224051 ENSG00000224051 CPTP Gene Expression
## ENSG00000169962 ENSG00000169962 TAS1R3 Gene Expression
## ENSG00000107404 ENSG00000107404 DVL1 Gene Expression
## ENSG00000162576 ENSG00000162576 MXRA8 Gene Expression
```

Droplet annotation (Cell metadata)



Droplet annotation (Cell metadata)

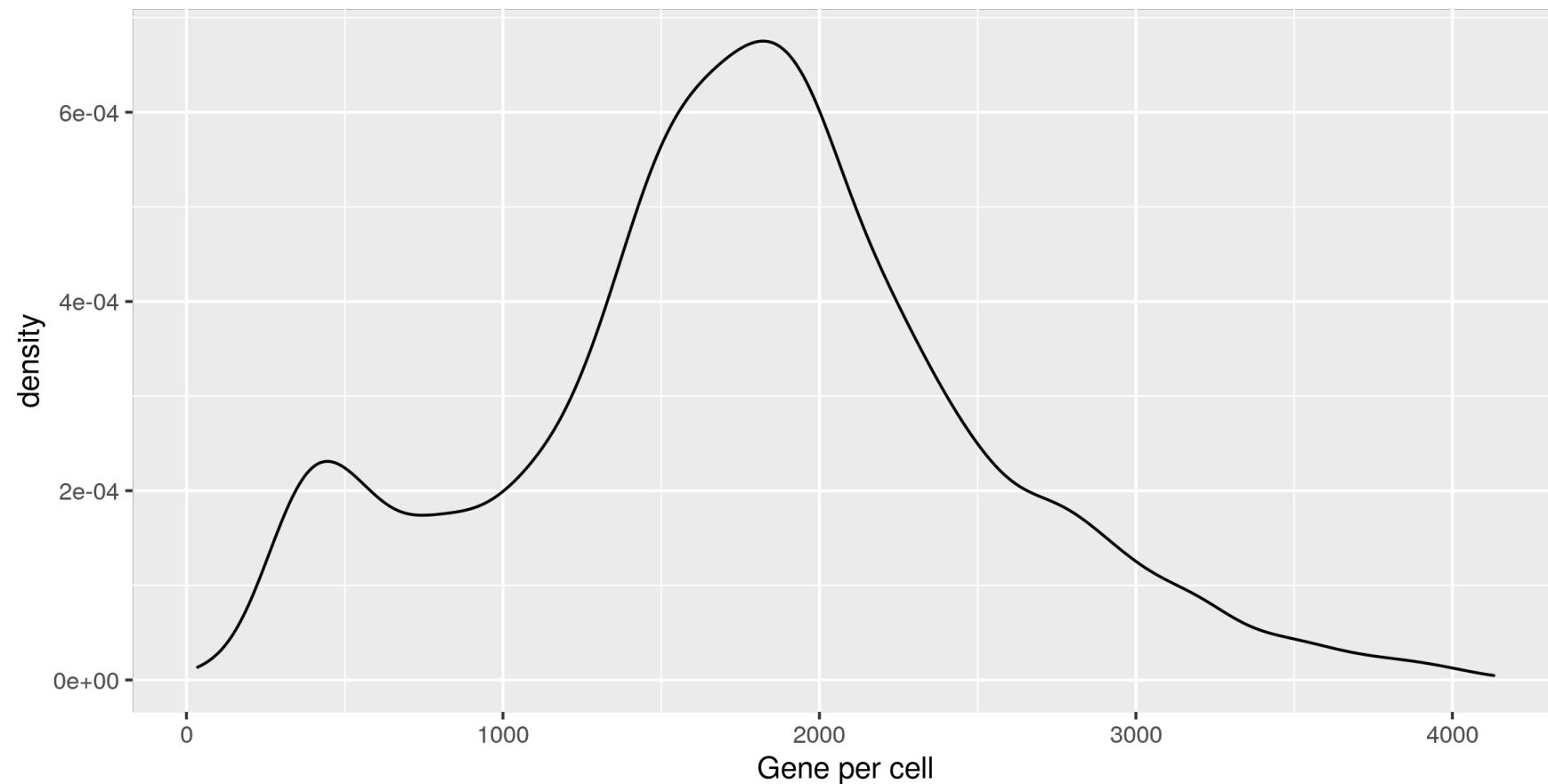
```
colData(sce)
```

```
## DataFrame with 3094 rows and 2 columns
##      Sample          Barcode
##      <character>    <character>
## 1   SRR9264343  AAACCTGAGACTTCG-1
## 2   SRR9264343  AAACCTGGTCTTCAAG-1
## 3   SRR9264343  AAACCTGGTGCAACTT-1
## 4   SRR9264343  AAACCTGGTGTGAGG-1
## 5   SRR9264343  AAACCTGTCCCAAGTA-1
## ...
## 3090 SRR9264343  TTTGGTTCTTAGGG-1
## 3091 SRR9264343  TTTGTCAAGAACGAG-1
## 3092 SRR9264343  TTTGTCAAGGACGAAA-1
## 3093 SRR9264343  TTTGTCACAGGCTCAC-1
## 3094 SRR9264343  TTTGTCAGTTCGGCAC-1
```

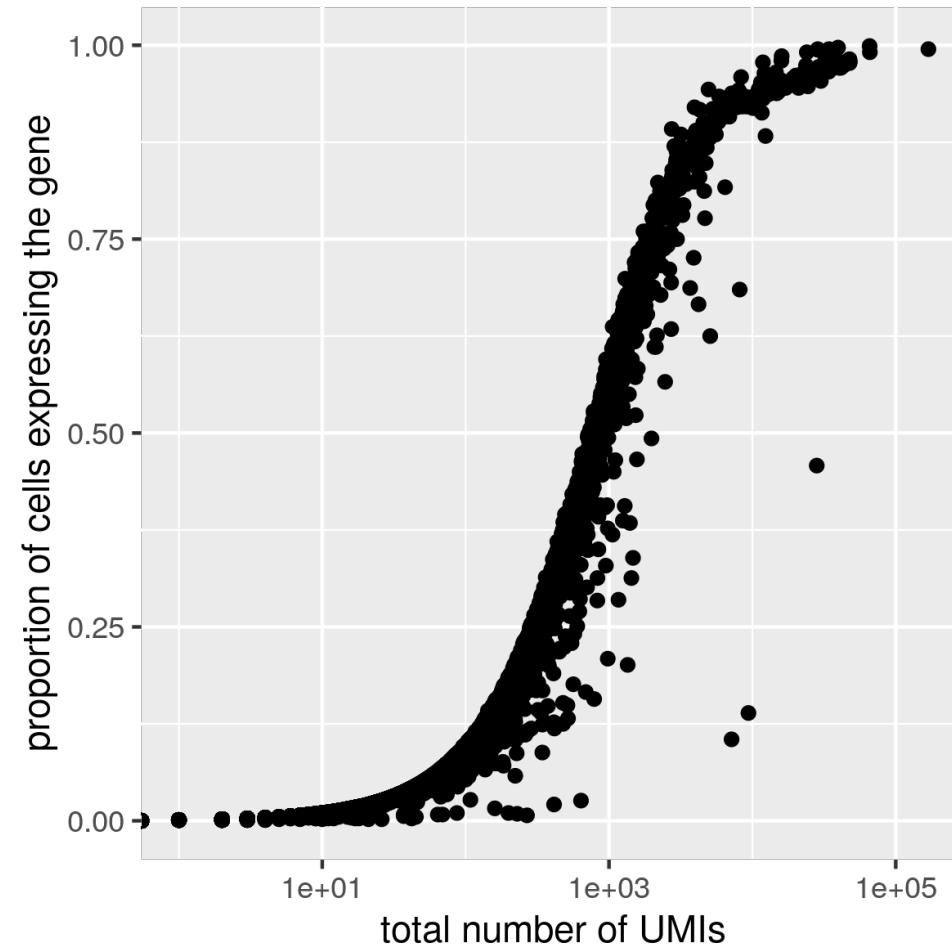
```
colnames(counts(sce))
```

```
## NULL
```

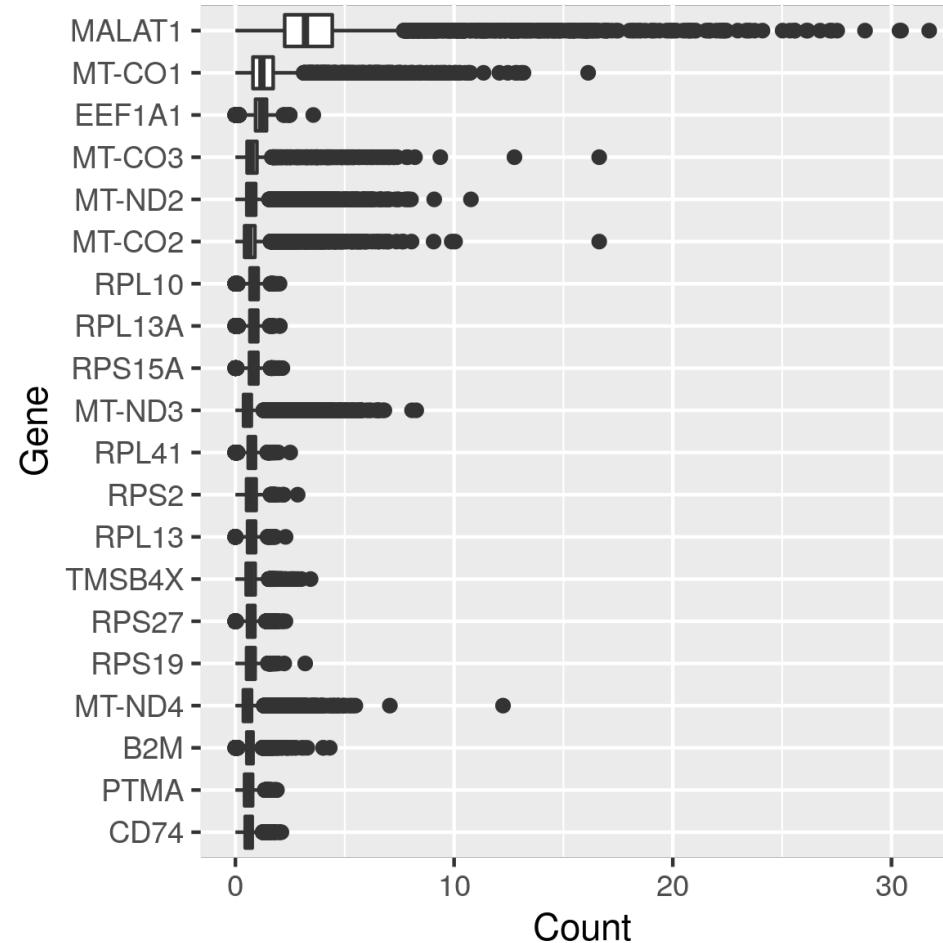
Properties of RNAseq data - Number of genes detected per cell



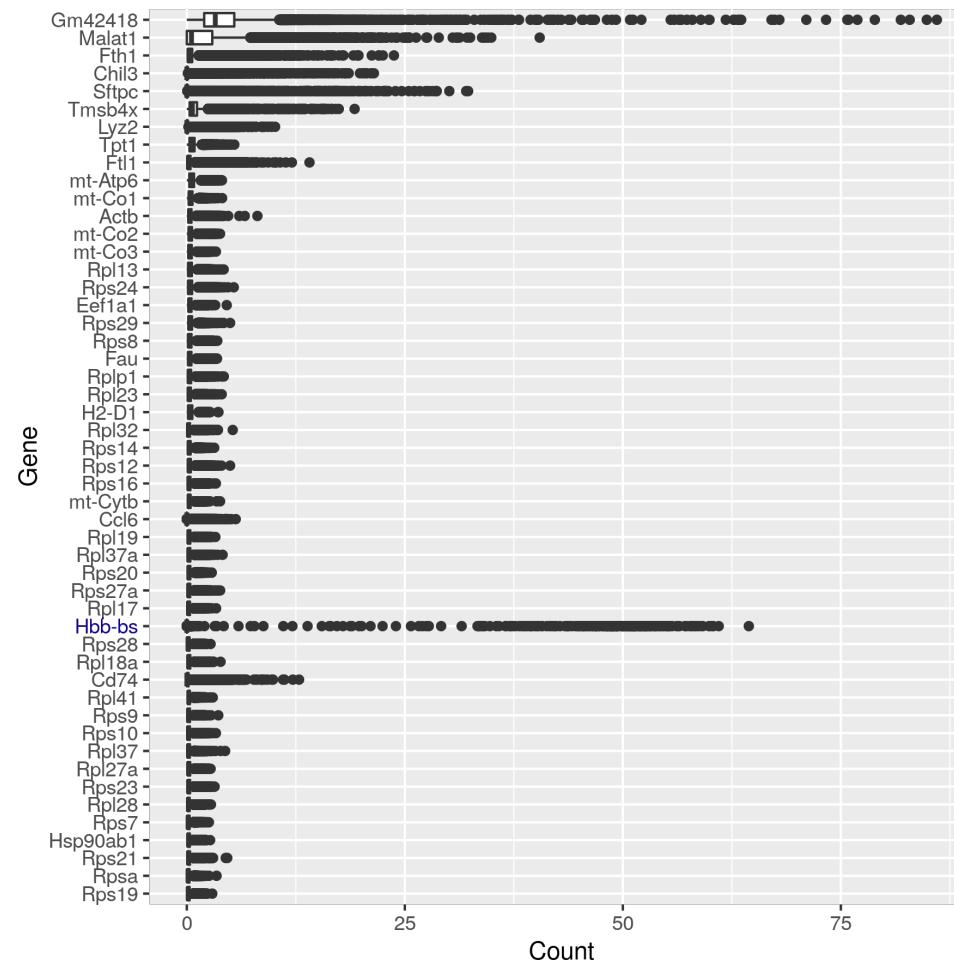
Properties of RNAseq data - Total UMIs



Properties of RNAseq data - Distribution of counts for a gene across cells



Properties of RNAseq data - Distribution of counts for a gene across cells



Remove undetected genes

Although the count matrix has 36601 genes, many of these will not have been detected in any droplet. We can remove these to reduce the size of the count matrix.

```
undetected_genes <- rowSums(counts(sce)) == 0  
sce <- sce[!undetected_genes,]  
sce
```

```
## class: SingleCellExperiment  
## dim: 19938 3094  
## metadata(1): Samples  
## assays(1): counts  
## rownames(19938): ENSG00000175756 ENSG00000221978 ... ENSG00000169962 ENSG00000107404  
## rowData names(3): ID Symbol Type  
## colnames: NULL  
## colData names(2): Sample Barcode  
## reducedDimNames(0):  
## mainExpName: NULL  
## altExpNames(0):
```

Quality Control

- Not all of the droplets called as cells by CellRanger will contain good quality cells
- Poor quality droplets will adversely affect downstream analysis
- We can use QC metrics to filter out poor quality droplets:
 - Total UMIs (Library size)
 - Number of gene detected
 - Proportion of UMIs mapping to mitochondrial genes

Quality Control

- Add gene annotation to identify Mt genes - AnnotationHub

```
rowData(sce)
```

```
## DataFrame with 36601 rows and 4 columns
##           ID      Symbol       Type Chromosome
##           <character> <character> <character> <character>
## ENSG00000175756 ENSG00000175756 AURKAIP1 Gene Expression     1
## ENSG00000221978 ENSG00000221978 CCNL2 Gene Expression     1
## ENSG00000224870 ENSG00000224870 MRPL20-AS1 Gene Expression     1
## ENSG00000242485 ENSG00000242485 MRPL20 Gene Expression     1
## ENSG00000272455 ENSG00000272455 AL391244.2 Gene Expression     1
## ...
##           ...      ...       ...      ...
## ENSG00000212907 ENSG00000212907 MT-ND4L Gene Expression    MT
## ENSG00000198886 ENSG00000198886 MT-ND4 Gene Expression    MT
## ENSG00000198786 ENSG00000198786 MT-ND5 Gene Expression    MT
## ENSG00000198695 ENSG00000198695 MT-ND6 Gene Expression    MT
## ENSG00000198727 ENSG00000198727 MT-CYB Gene Expression    MT
```

Quality Control

```
is.mito <- which(rowData(sce)$Chromosome=="MT")
sce <- addPerCellQC(sce, subsets = list(Mito = is.mito))
```

Adds six columns to the droplet annotation:

- **sum**: total UMI count
- **detected**: number of features (genes) detected
- **subsets_Mito_sum**: number of UMIs mapped to mitochondrial transcripts
- **subsets_Mito_detected**: number of mitochondrial genes detected
- **subsets_Mito_percent**: percentage of UMIs mapped to mitochondrial transcripts
- **total**: also the total UMI count

Quality Control

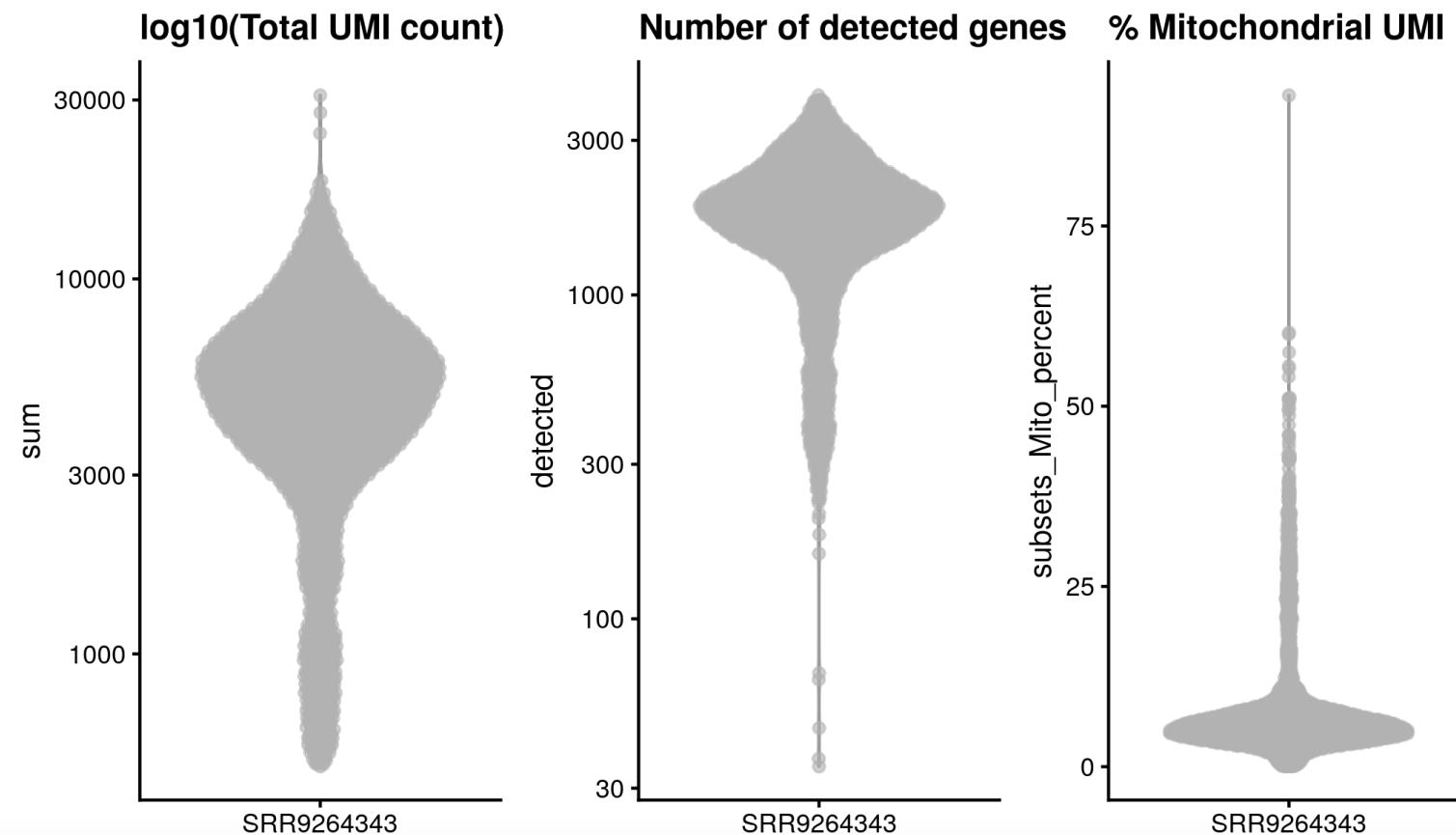
```
is.mito <- which(rowData(sce)$Chromosome=="MT")
sce <- addPerCellQC(sce, subsets = list(Mito = is.mito))
```

```
colData(sce)
```

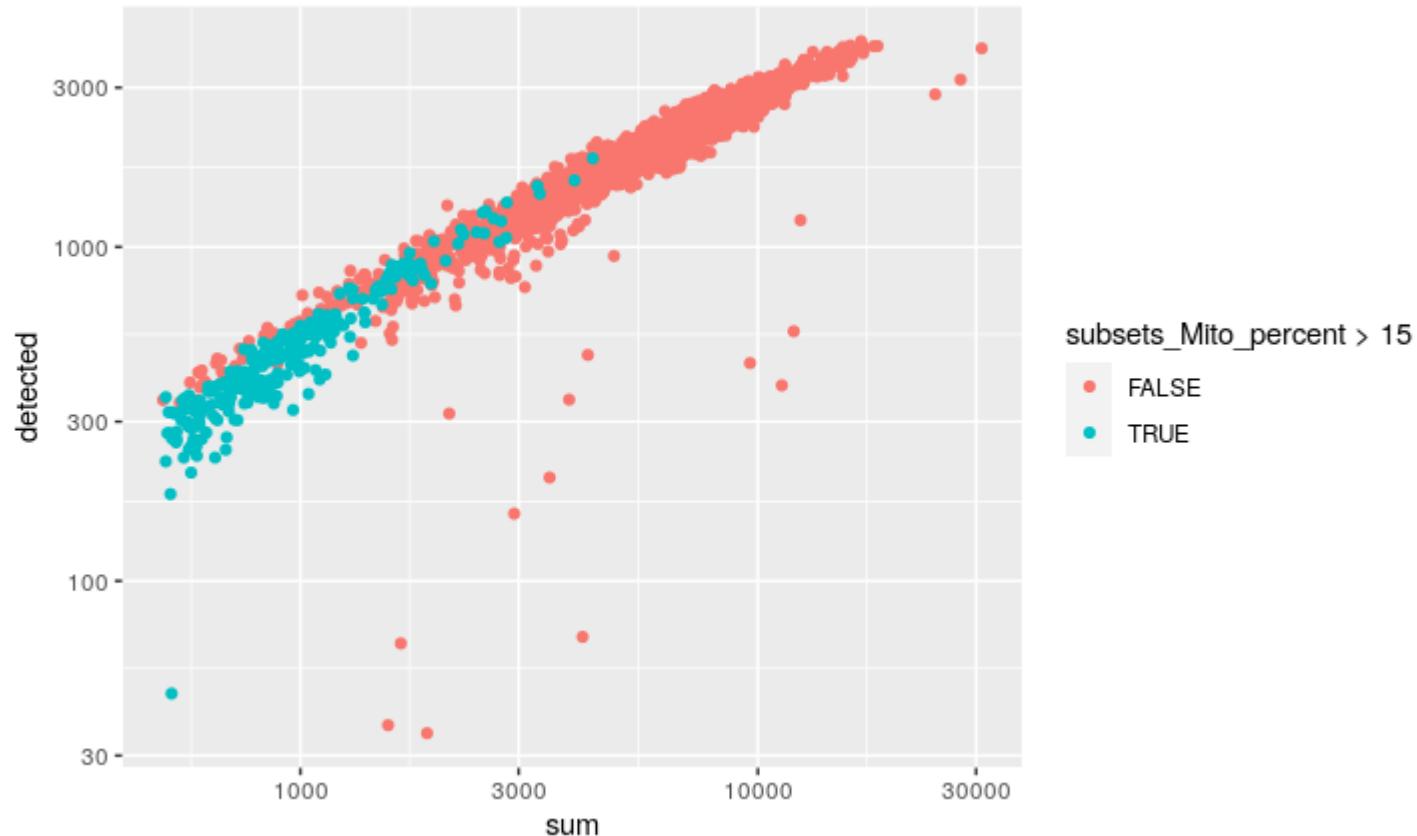
```
## DataFrame with 3094 rows and 8 columns
##           Sample      Barcode    sum detected subsets_Mito_sum
## AACCTGAGACTTCG-1 SRR9264343 AACCTGAGACTTCG-1   6677    2056        292
## AACCTGGTCTTCAAG-1 SRR9264343 AACCTGGTCTTCAAG-1 12064    3177        575
## AACCTGGTCAACTT-1  SRR9264343 AACCTGGTCAACTT-1    843     363        428
## AACCTGGTGTGAGG-1  SRR9264343 AACCTGGTGTGAGG-1   8175    2570        429
## AACCTGTCCCAAGTA-1 SRR9264343 AACCTGTCCCAAGTA-1  8638    2389        526
## ...
## TTTGGTTTCTTAGGG-1 SRR9264343 TTTGGTTTCTTAGGG-1  3489    1600        239
## TTTGTCAAGAACGAG-1 SRR9264343 TTTGTCAAGAACGAG-1  7809    2415        548
## TTTGTCAAGGACGAAA-1 SRR9264343 TTTGTCAAGGACGAAA-1  9486    2589        503
## TTTGTACAGGCTCAC-1 SRR9264343 TTTGTACAGGCTCAC-1  1182     591        224
## TTTGTCAGTCGGCAC-1 SRR9264343 TTTGTCAGTCGGCAC-1 10514    2831        484
##           subsets_Mito_detected subsets_Mito_percent total
## AACCTGAGACTTCG-1                12       4.37322   6677
## AACCTGGTCTTCAAG-1               12       4.76625  12064
## AACCTGGTCAACTT-1                11      50.77106   843
## AACCTGGTGTGAGG-1                12       5.24771   8175
## AACCTGTCCCAAGTA-1               13       6.08937  8638
## ...
## TTTGGTTTCTTAGGG-1                11       6.85010   3489
## TTTGTCAAGAACGAG-1                12       7.01754  7809
## TTTGTCAAGGACGAAA-1               12       5.30255  9486
```

QC metrics - distribution

```
plotColData(sce, x="Sample", y="sum") + scale_y_log10()
plotColData(sce, x="Sample", y="detected") + scale_y_log10()
plotColData(sce, x="Sample", y="subsets_Mito_percent")
```

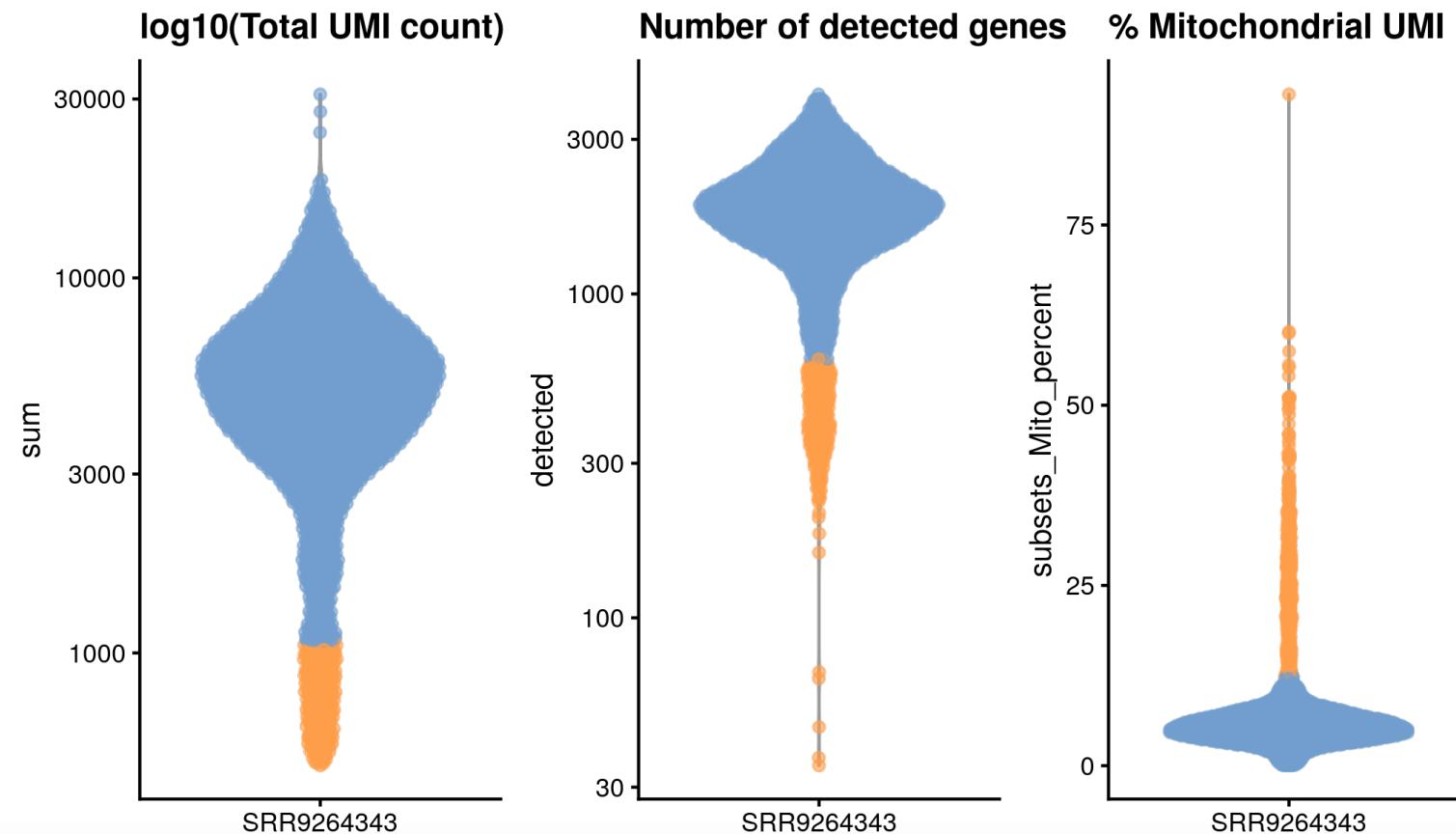


QC metrics - relationship



Identification of low-quality cells with adaptive thresholds

```
sce$low_lib_size <- isOutlier(sce$sum, log=TRUE, type="lower")
sce$low_n_features <- isOutlier(sce$detected, log=TRUE, type="lower")
sce$high_Mito_percent <- isOutlier(sce$subsets_Mito_percent, type="higher")
```



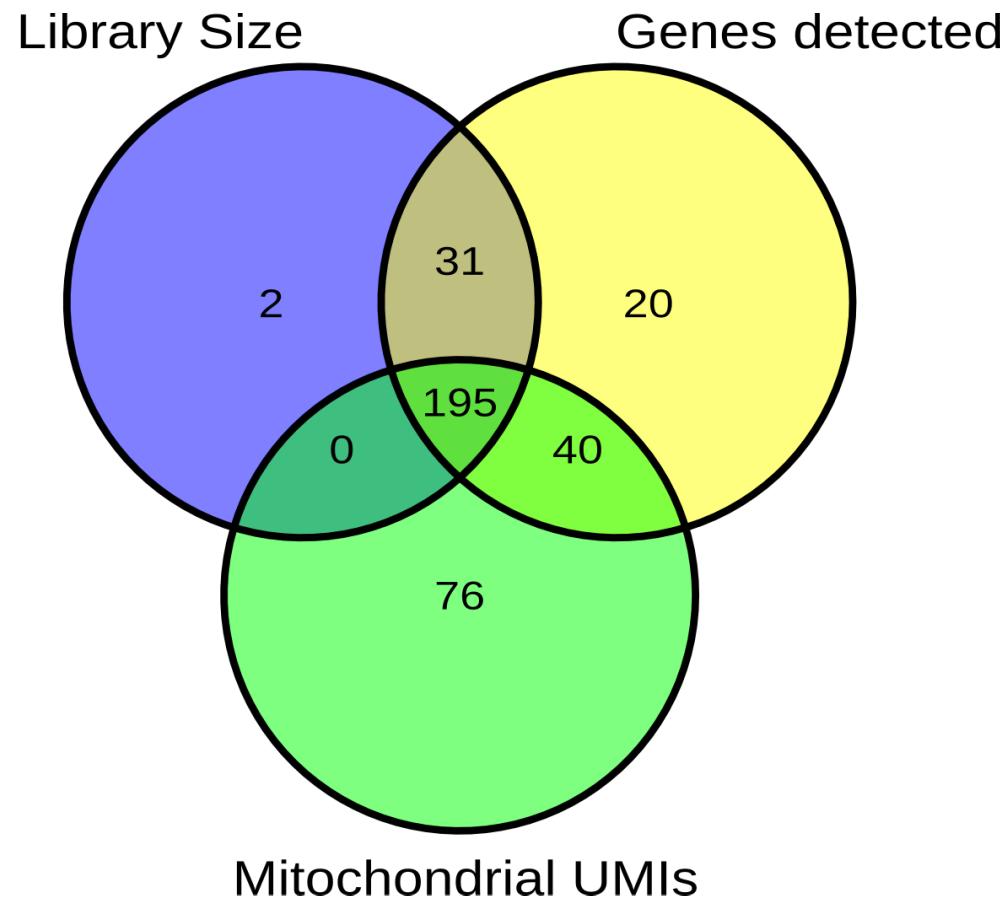
All three filter steps at once

```
cell_qc_results <- quickPerCellQC(colData(sce), percent_subsets=c("subsets_Mito_percent"))
```

```
## DataFrame with 3094 rows and 4 columns
##   low_lib_size low_n_features high_subsets_Mito_percent discard
##   <outlier.filter> <outlier.filter> <outlier.filter> <logical>
## 1 FALSE          FALSE          FALSE          FALSE
## 2 FALSE          FALSE          FALSE          FALSE
## 3 TRUE           TRUE           TRUE           TRUE
## 4 FALSE          FALSE          FALSE          FALSE
## 5 FALSE          FALSE          FALSE          FALSE
## ...
## 3090 FALSE         FALSE          FALSE          FALSE
## 3091 FALSE         FALSE          FALSE          FALSE
## 3092 FALSE         FALSE          FALSE          FALSE
## 3093 FALSE         TRUE           TRUE           TRUE
## 3094 FALSE         FALSE          FALSE          FALSE
```

All three filter steps at once

```
cell_qc_results <- quickPerCellQC(colData(sce), percent_subsets=c("subsets_Mito_percent"))
```



Filter the Single Cell Object

- Filter cells according to QC metrics.

```
sce.Filtered <- sce[, !cell_qc_results$discard]  
sce.Filtered  
  
## class: SingleCellExperiment  
## dim: 36601 2730  
## metadata(1): Samples  
## assays(1): counts  
## rownames(36601): ENSG00000175756 ENSG00000221978 ... ENSG00000107404 ENSG00000162576  
## rowData names(4): ID Symbol Type Chromosome  
## colnames(2730): AACCTGAGACTTCG-1 AACCTGGTCTTCAAG-1 ... TTTGTCAAGGACGAAA-1  
## TTTGTCAGTTCGGCAC-1  
## colData names(8): Sample Barcode ... subsets_Mito_percent total  
## reducedDimNames(0):  
## mainExpName: NULL  
## altExpNames(0):
```