UPSC Umeå Plant Science Center

easyRNAseq
Where from, where to?

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Outline

- From where it comes
  - – history and rationale
- To where it stands
  - – current workflow and caveats
- And where it should be
  - – current and planned changes

History

- Started at the EMBL (European Molecular Biology Laboratory), HD, in 2008
  - The data: a large transcriptome dataset (>30 RNA-Seq samples, fruitfly)
  - Not much (pre-)processing available.
  - R/Bioc capabilities of handling large dataset and performing statistical analyses.
  - At the same time, Bioconductor started to develop packages specifically for NGS data.

4 years later

- Bioc 2.11
  - more than 1,150 packages (550+ soft., 600+ annot.)
  - among which the Bioconductor "core" packages for NGS: 8 of them that leveraged R "base" to work conveniently with "biological" objects
  - but as well, 50+ contributed packages just for NGS analyses, and 18 RNA-Seq specific

Rationale

- As for 3 years ago, the rationale remains:
  - to simplify (to ease) my pre-processing because analyses is what's the most gratifying!
  - so I wanted a function to which I would give
    - aligned reads
    - chromosomic
    - genic annotation
  - and from which I would get a count table.

Challenges

- Computationally relevant

Data volume	disk space and computing capacity
R	complex architecture

- Biologically relevant

Reference selection	Genome, transcriptome?
Reference validity	e.g. different strains
Alignment policies	quality trimming, gapped alignments, multimapping
Replicates	etc...

easyRNASeq (for me)

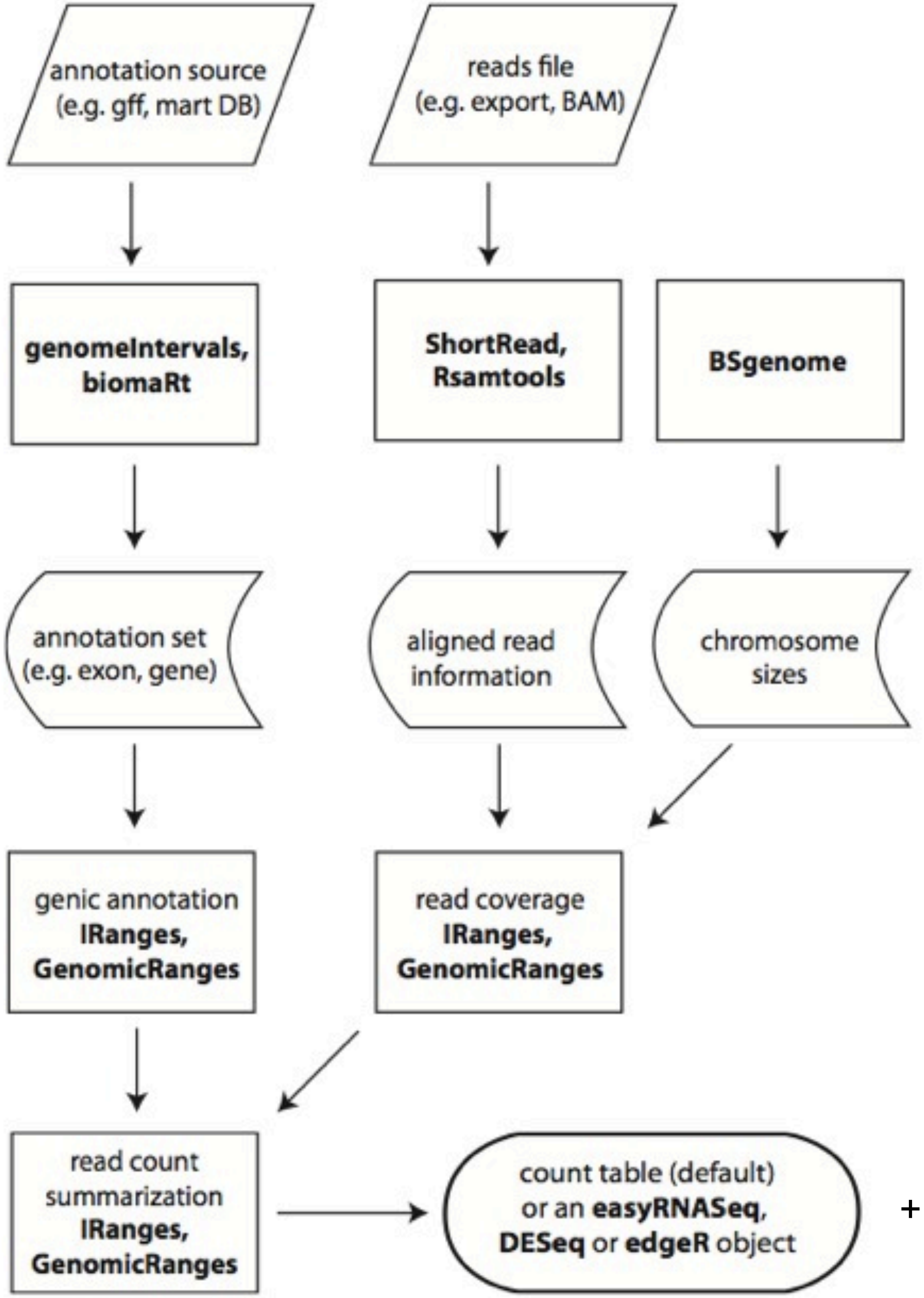
- An R package
  - to ease various RNA-Seq analyses (flexibility)
  - that wraps and combines the functionalities of many R packages
  - all encapsulated into a single function call: easyRNAseq
  - additional functions
    - e.g. de-multiplex data

easyRNASeq (for the user)

- The same, but as less a black box as possible!

- a non neglectable part of the computation time is spent on assessing the validity of the user input

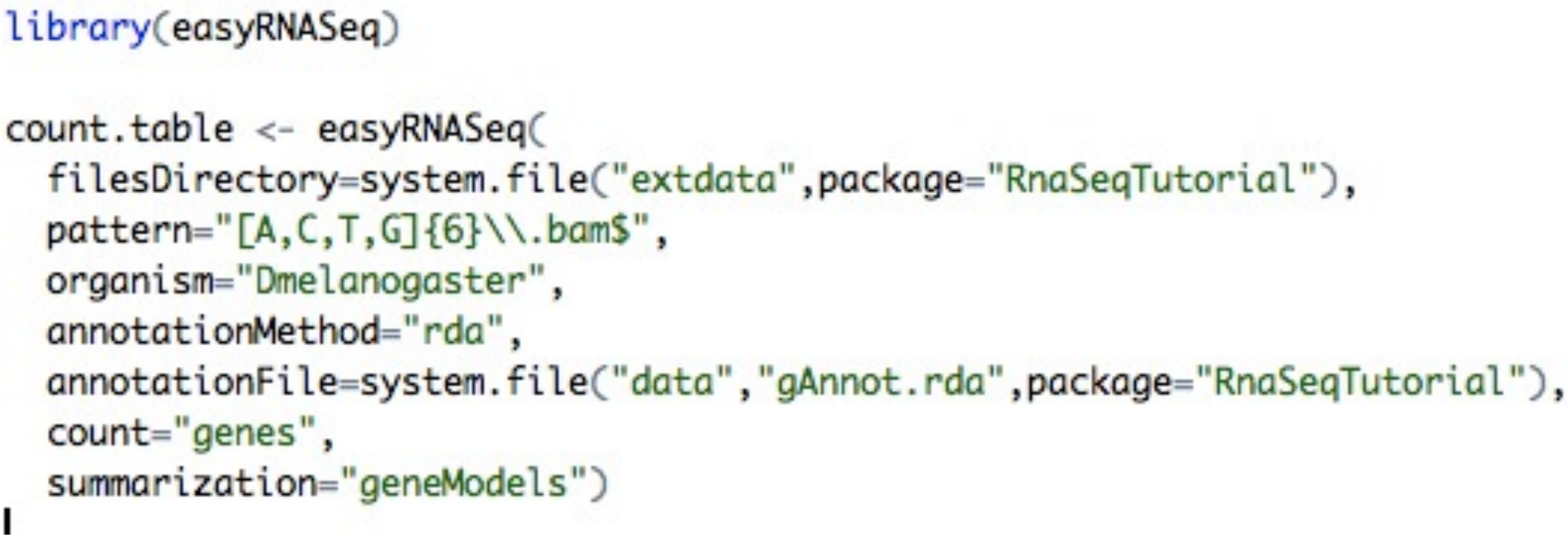
Where it stands



annotation source (e.g. gff, mart DB)
reads file (e.g. export, BAM)
genomeIntervals, biomaRt
ShortRead, Rsamtools
BSgenome
annotation set (e.g. exon, gene)
aligned read information
chromosome sizes
genic annotation **IRanges, GenomicRanges**
read coverage **IRanges, GenomicRanges**
read count summarization **IRanges, GenomicRanges**
count table (default) or an **easyRNASeq, DESeq** or **edgeR** object

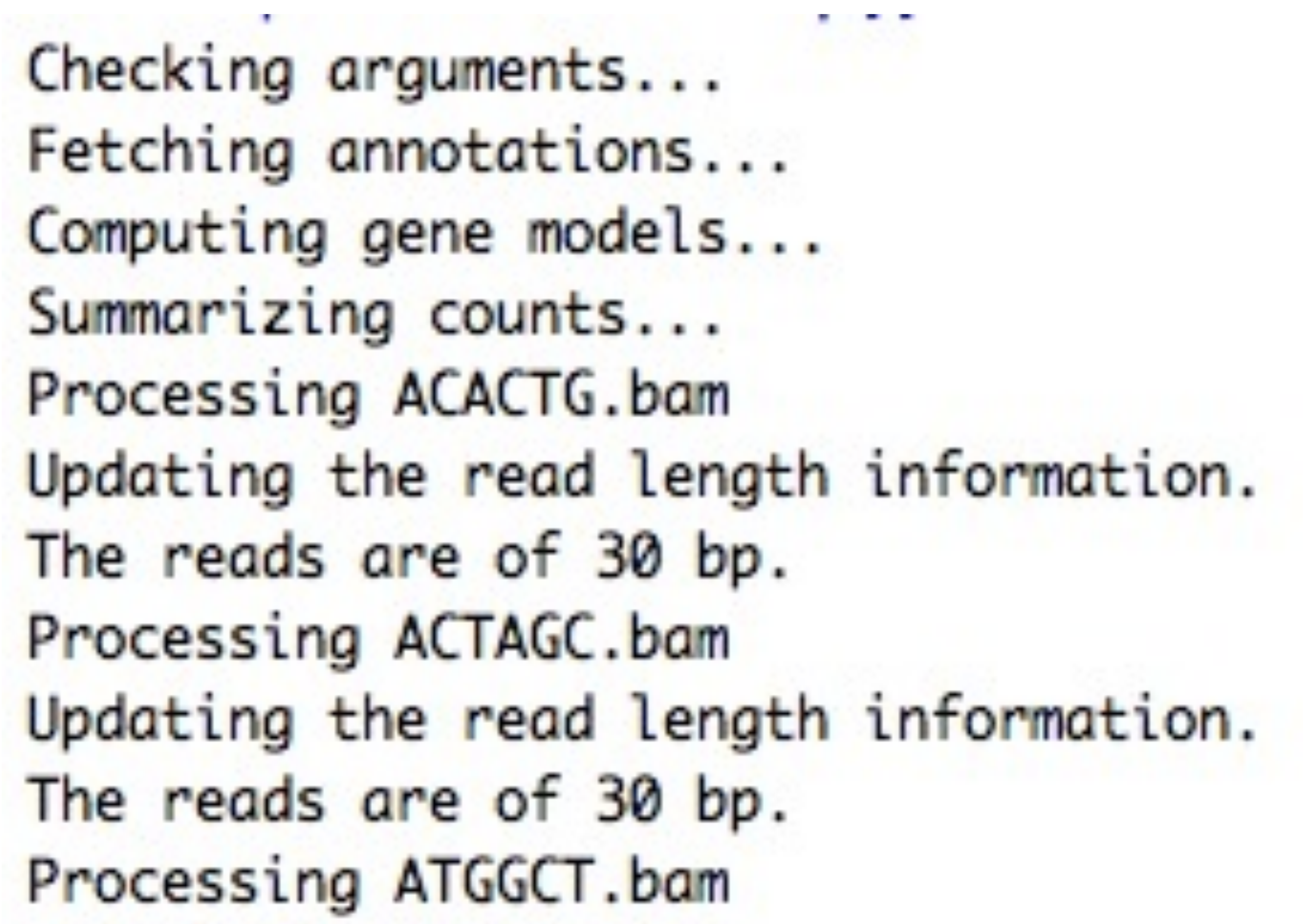
+ SummarizedExperiment

An example call



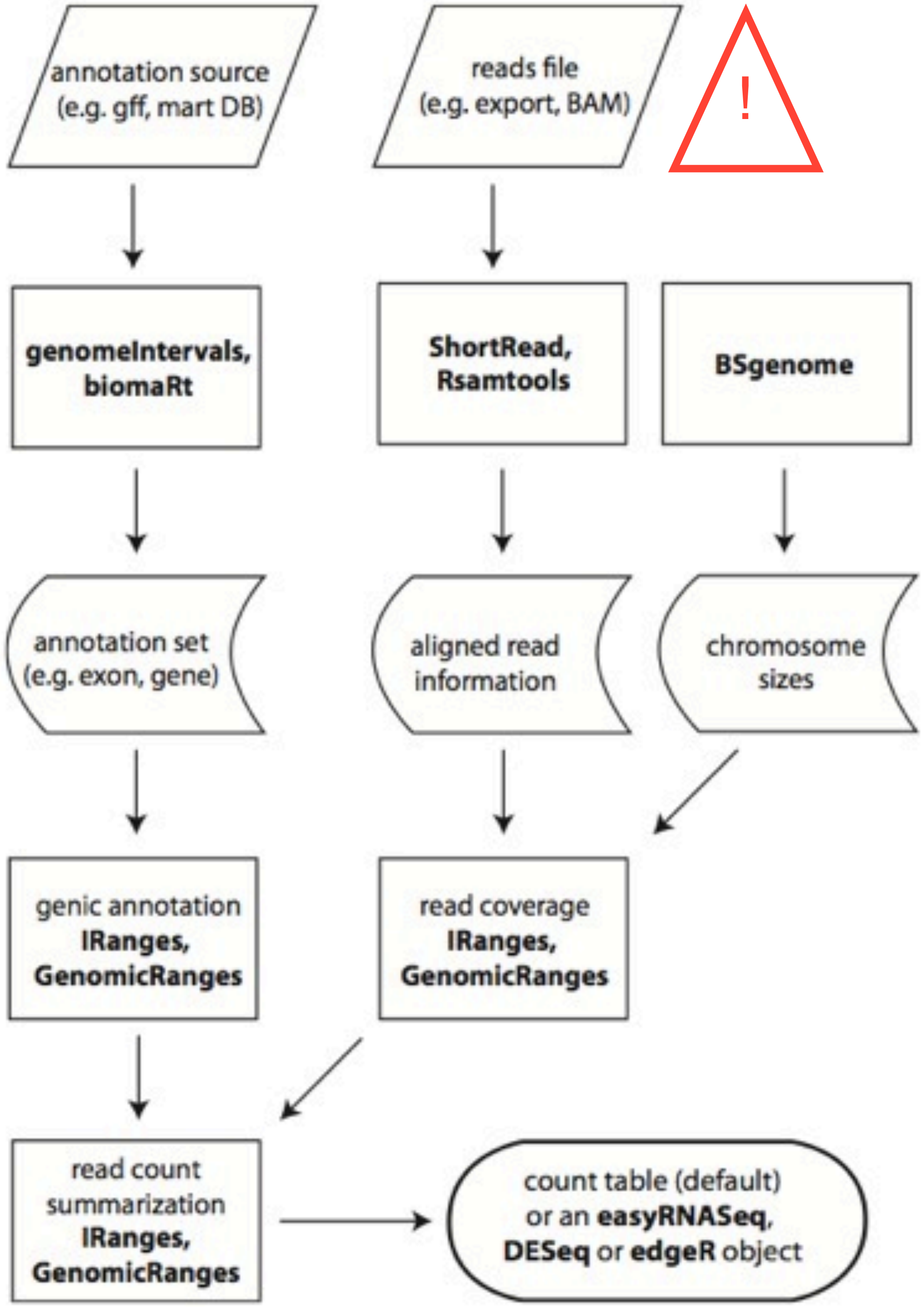
```
library(easyRNASeq)

count.table <- easyRNASeq(
  filesDirectory=system.file("extdata",package="RnaSeqTutorial"),
  pattern="[A,C,T,G]{6}\\.bam$",
  organism="Dmelanogaster",
  annotationMethod="rda",
  annotationFile=system.file("data","gAnnot.rda",package="RnaSeqTutorial"),
  count="genes",
  summarization="geneModels")
```

An example call II



```
Checking arguments...
Fetching annotations...
Computing gene models...
Summarizing counts...
Processing ACACTG.bam
Updating the read length information.
The reads are of 30 bp.
Processing ACTAGC.bam
Updating the read length information.
The reads are of 30 bp.
Processing ATGGCT.bam
```

Estimating expression

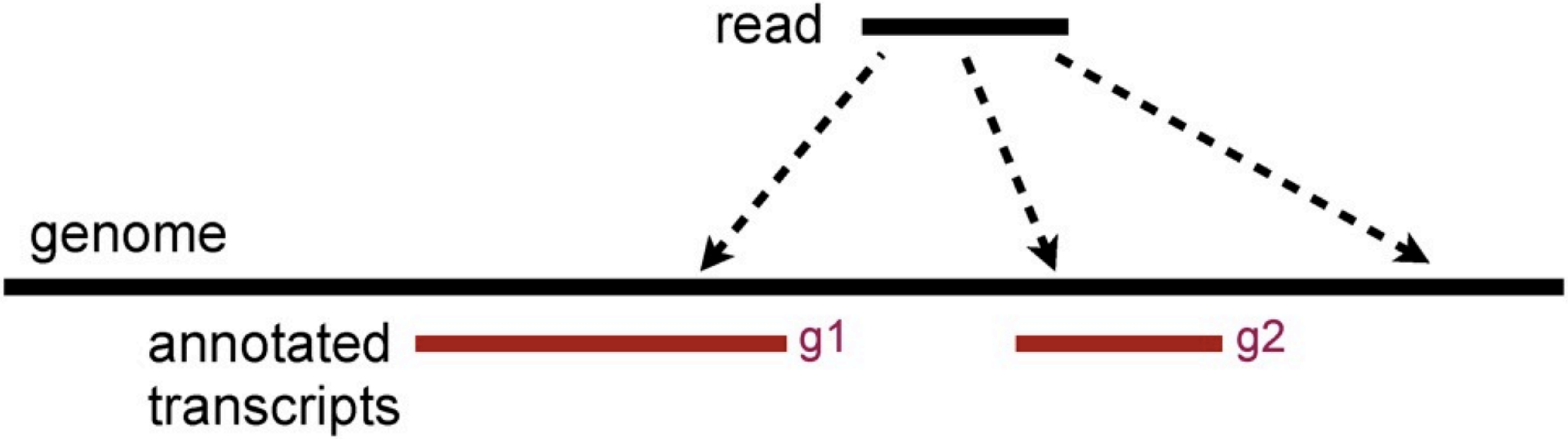


Delhomme et al. easyRNASeq: a bioconductor package for processing RNA-Seq data. Bioinformatics (2012) pp.

Quantifying expression

"Easy": count the number of reads that aligned to a gene, exon, splice-junction...

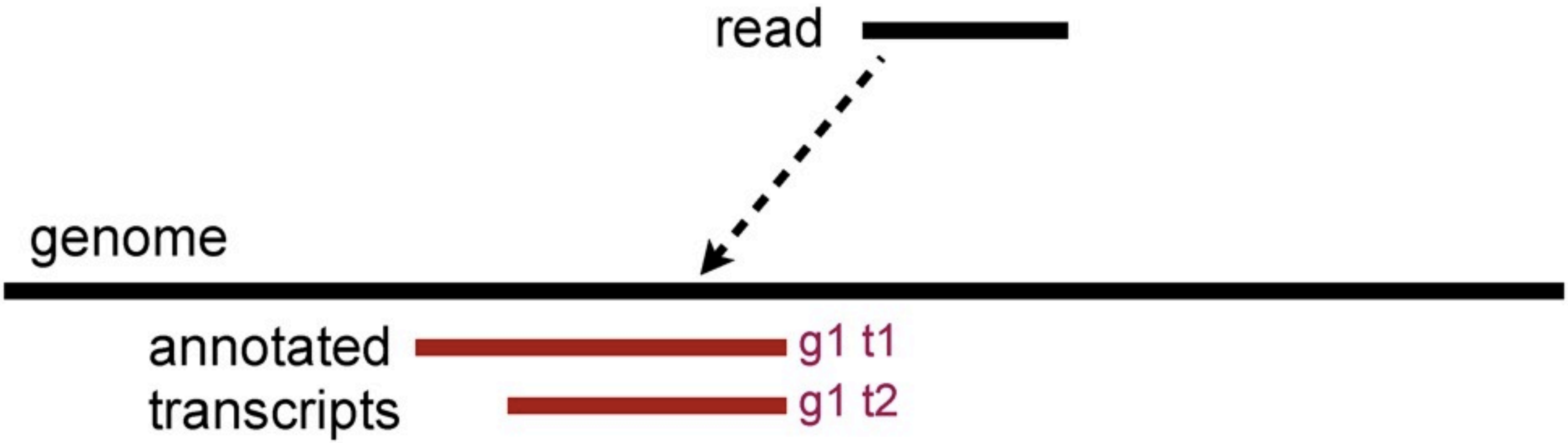
What about multi-mapping reads?



Courtesy of Angela Gonçalves, EBI, Cambridge, UK

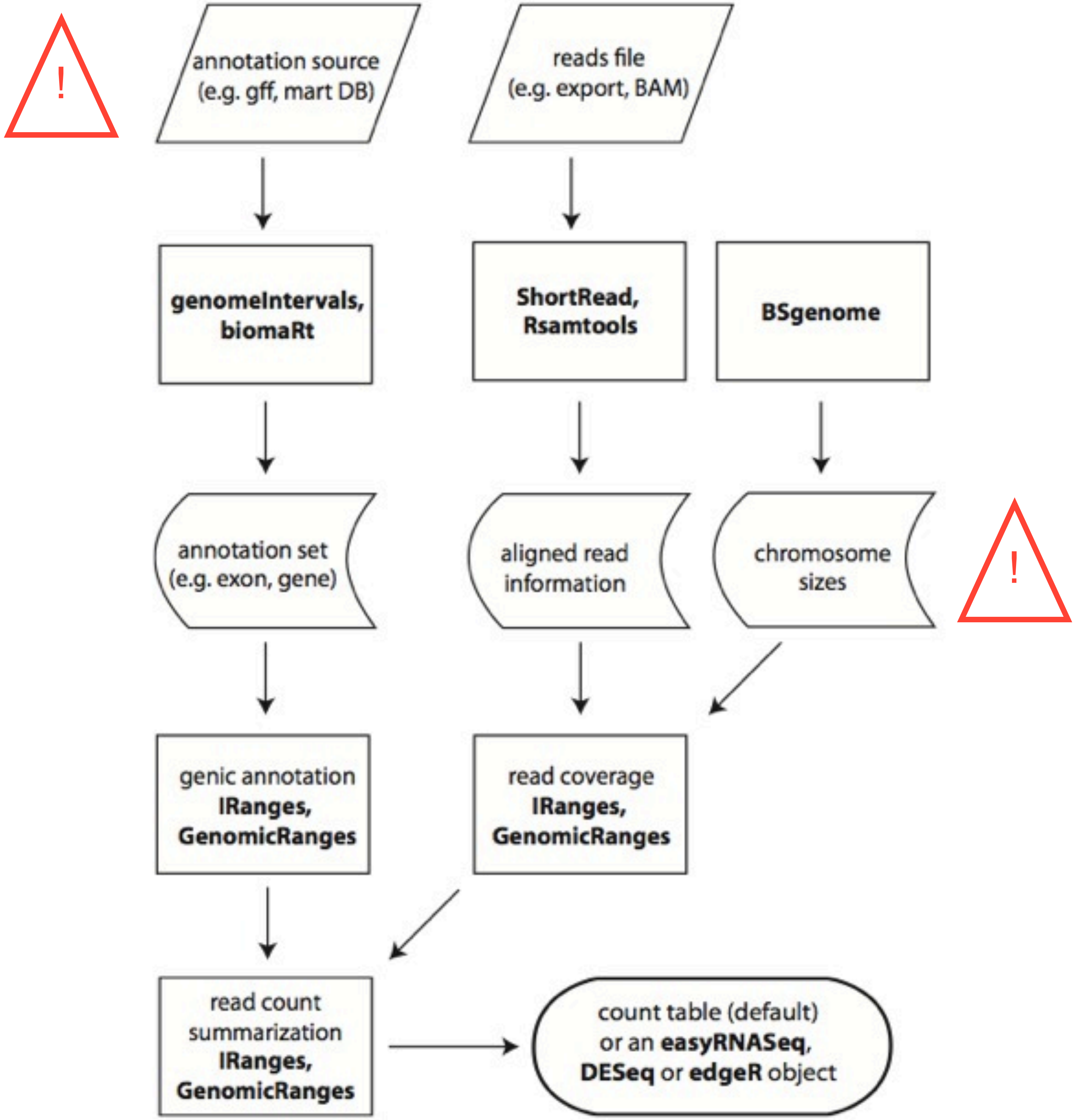
Quantifying expression II

And what about isoform levels?

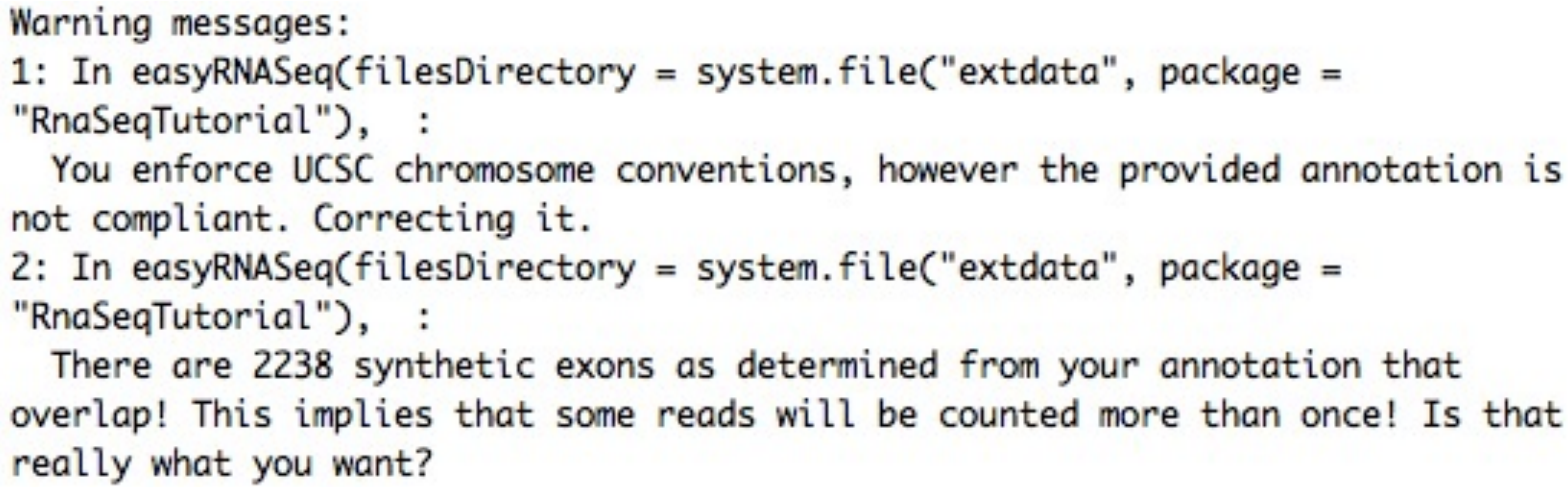


Courtesy of Angela Gonçalves, EBI, Cambridge, UK

Pitfalls


!
annotation source
(e.g. gff, mart DB)
reads file
(e.g. export, BAM)
genomeIntervals,
biomaRt
ShortRead,
Rsamtools
BSgenome
annotation set
(e.g. exon, gene)
aligned read
information
chromosome
sizes
!
genic annotation
IRanges,
GenomicRanges
read coverage
IRanges,
GenomicRanges
read count
summarization
IRanges,
GenomicRanges
count table (default)
or an easyRNASeq,
DESeq or edgeR object

An example call III



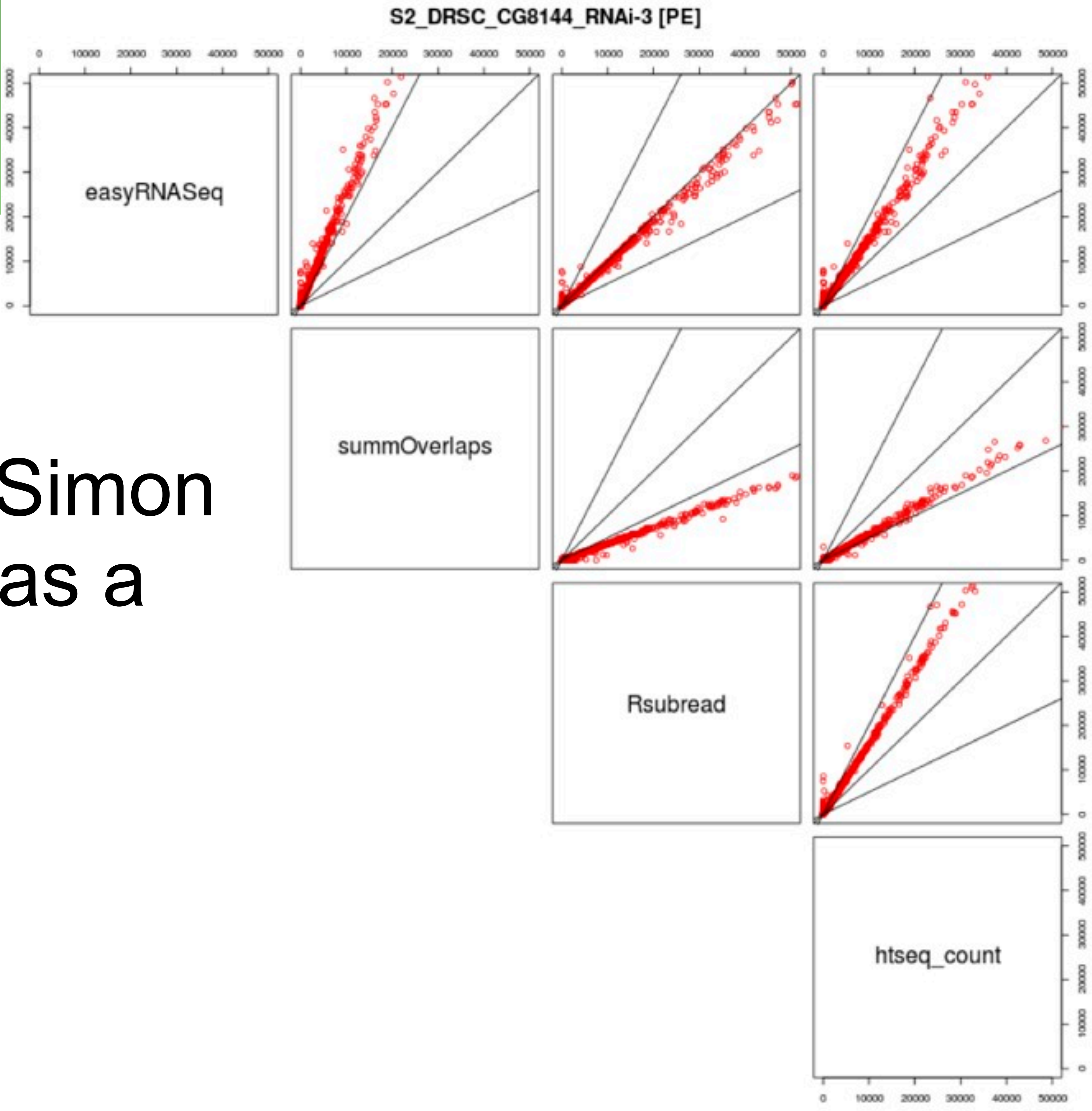
```
Warning messages:
1: In easyRNASeq(filesDirectory = system.file("extdata", package =
"RnaSeqTutorial"),  :
  You enforce UCSC chromosome conventions, however the provided annotation is
not compliant. Correcting it.
2: In easyRNASeq(filesDirectory = system.file("extdata", package =
"RnaSeqTutorial"),  :
  There are 2238 synthetic exons as determined from your annotation that
overlap! This implies that some reads will be counted more than once! Is that
really what you want?
```

Pitfalls

- Mind the alignment specificities
- Mind the “reference” used
- Mind the correspondence of the names between
  - the annotation
  - the read alignments
  - Most frequent subject of users’ email.
- Many of these are controlled for (which is why easyRNAseq emits so many warnings)

BioC package for summarizing expression

- GenomicRanges (summarizeOverlaps())
- easyRNASeq (easyRNASeq())
- Rsubread (featureCounts())
- ArrayExpressHTS
- ...



htseq-count[1] (Simon Anders) used as a reference

The figure is a courtesy of Mark Robinson

[1] (http://www-huber.embl.de/users/anders/HTSeq/doc/overview.html

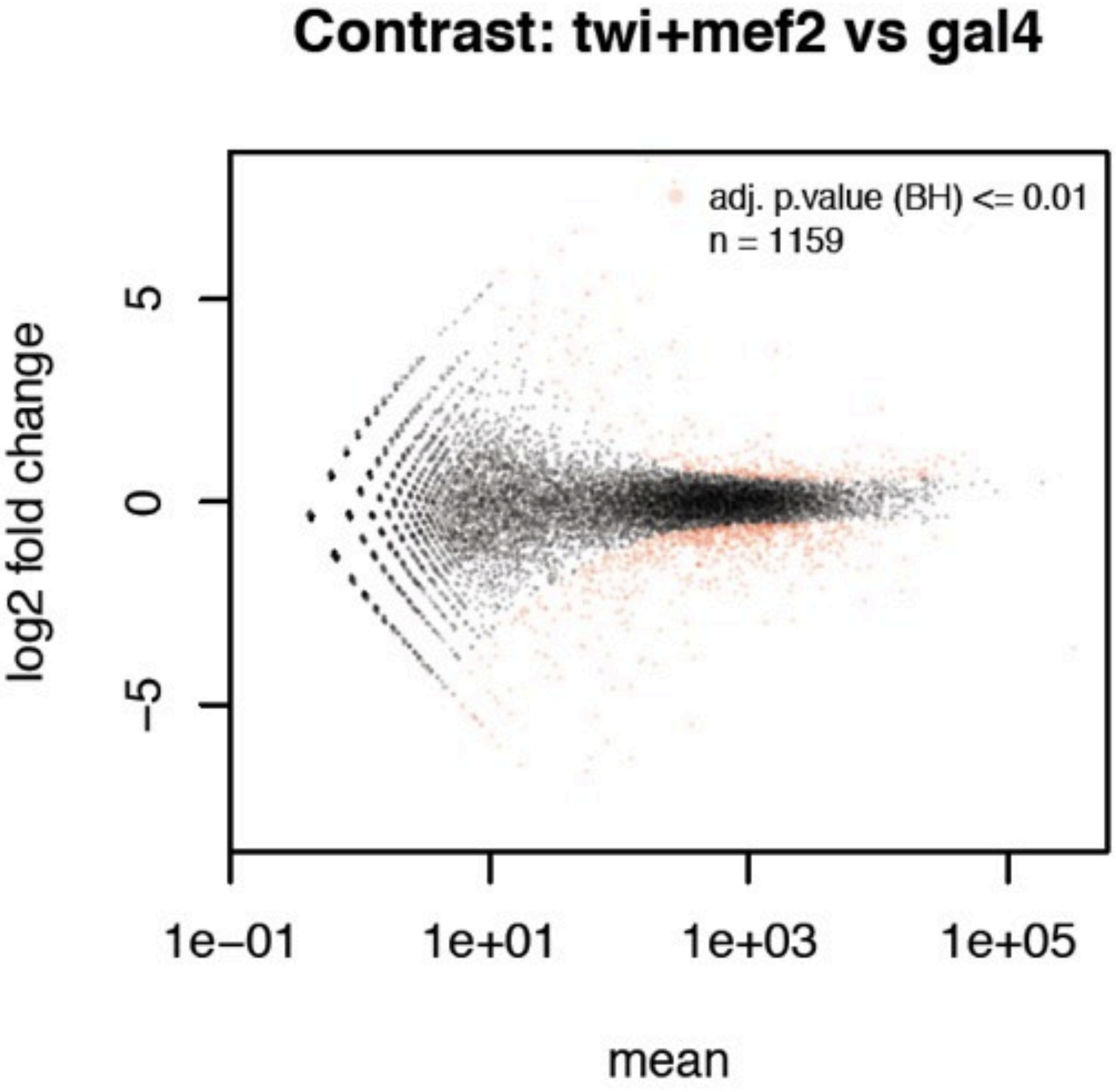
Use cases

- Evident use case:
  - –RNA-Seq differential expression

- useful as well
  - –for further QA
  - –for other experimental analyses
    - short RNA
    - Tag-Seq
    - CaGE-Seq

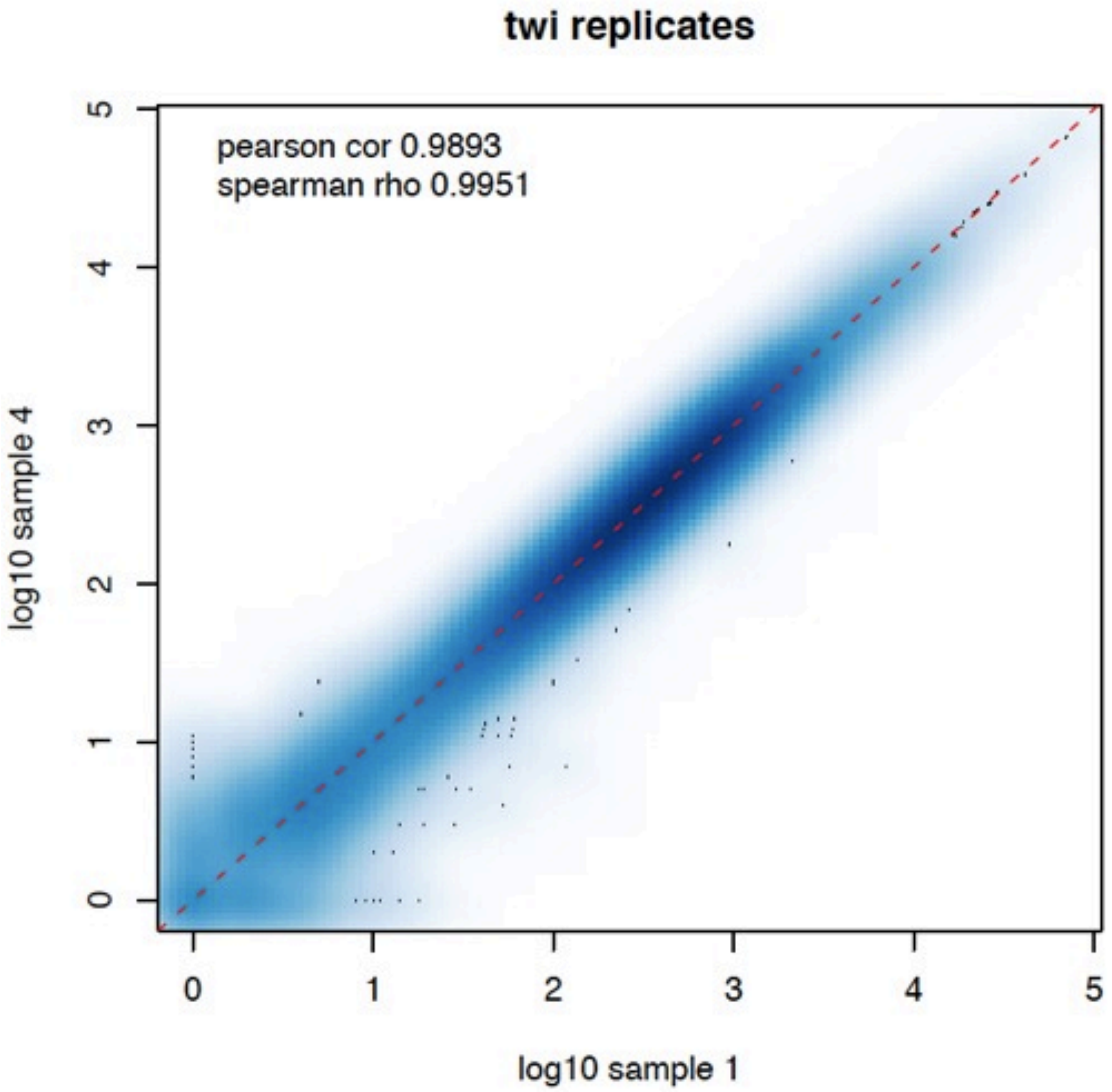
Correction/Normalization

- RPKM
  - Reads Per feature Kb per Million reads in the library
- DESeq
  - based on a Negative Binomial
  - fit a model to correct for the library sizes
- edgeR
  - based on a Negative Binomial
  - use a trimmed mean of M-values to correct for the library sizes



QA example: Replicate comparison

- Automatic if the user requires a DESeq or edgeR ready to use object.



Summary

- Bioc core packages have been consolidated
- but easyRNAseq still needs to mature
  - integrate most recent technologies (multi-mapping)
  - annotation processing
  - vignette pruning and adding an FAQ section

Where to go

- Rsamtools: stream BAM file to minimize mem. footprint.

- SummarizedExperiment as a standard
  - add coercion from/to edgeR - DESeq

- Plugin additional methods (cqn,DEXSeq,...)

- Use BiocParallel instead of parallel

Acknowledgments

- For the content of that talk
  - Angela Gonçalves, Mark Robinson
- For the invitation
  - Mark Robinson
- For precious comments and help
  - The whole Bioconductor “core” group (present and past), Wolgang Huber, Simon Anders, Charles Girardot, Stefan Bonn
- For feedback
  - Numerous Bioc users: Tim Triche Jr., Wade Davis, Richard Friedman
- You, for your attention