Ballgown

flexible RNA-seq differential expression analysis

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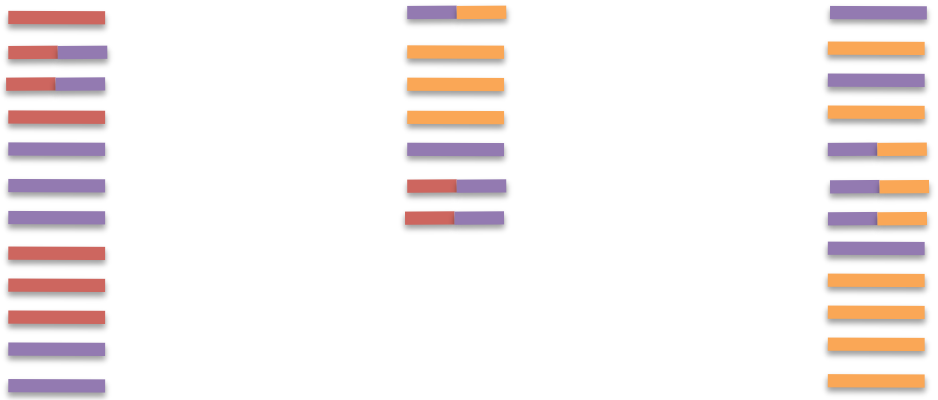
RNA-seq data

Reads
(50-100 bases)

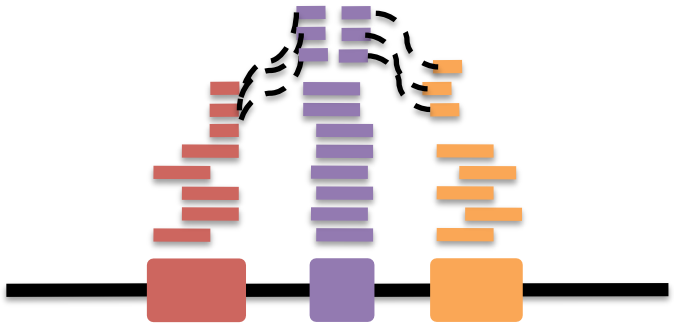


Transcripts
(RNA)

Genome
(DNA)

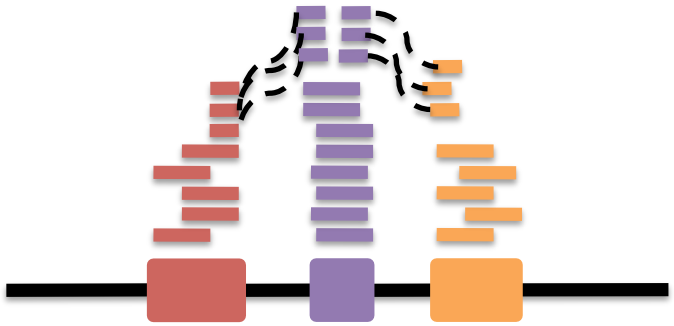
RNA-seq data analysis

align



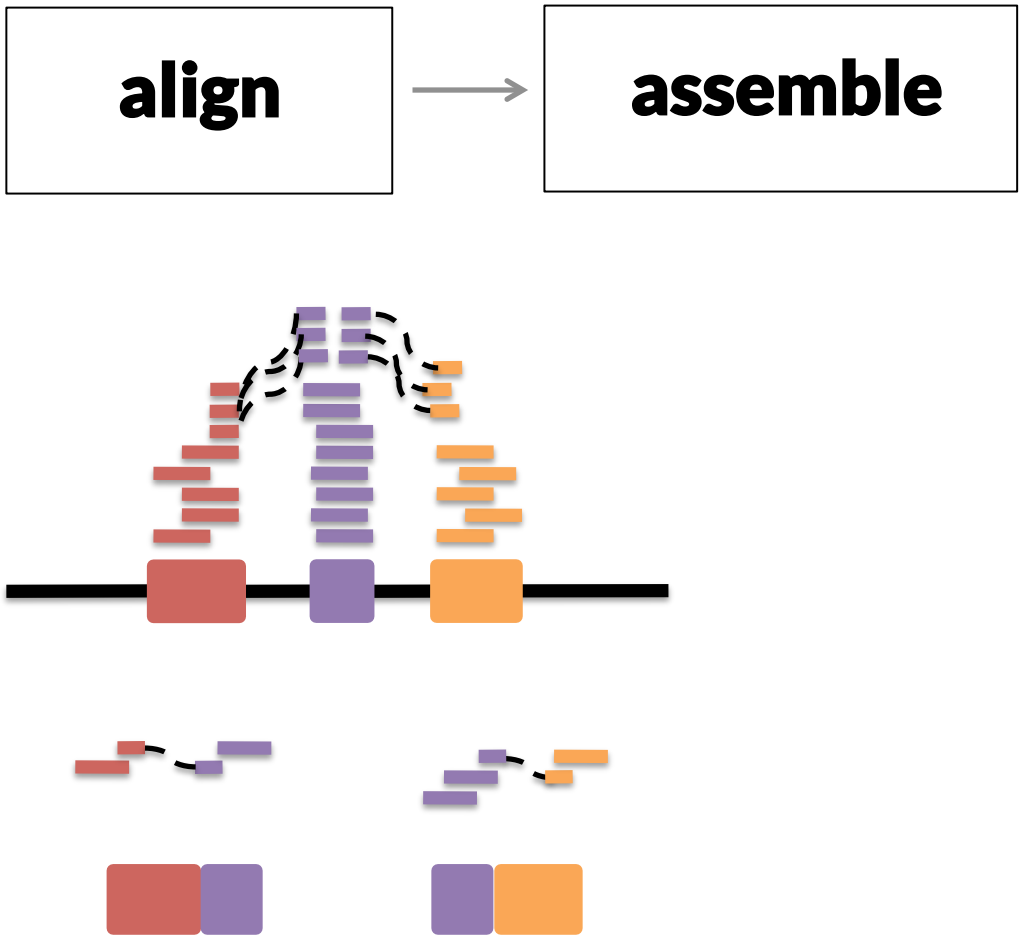
RNA-seq data analysis

align

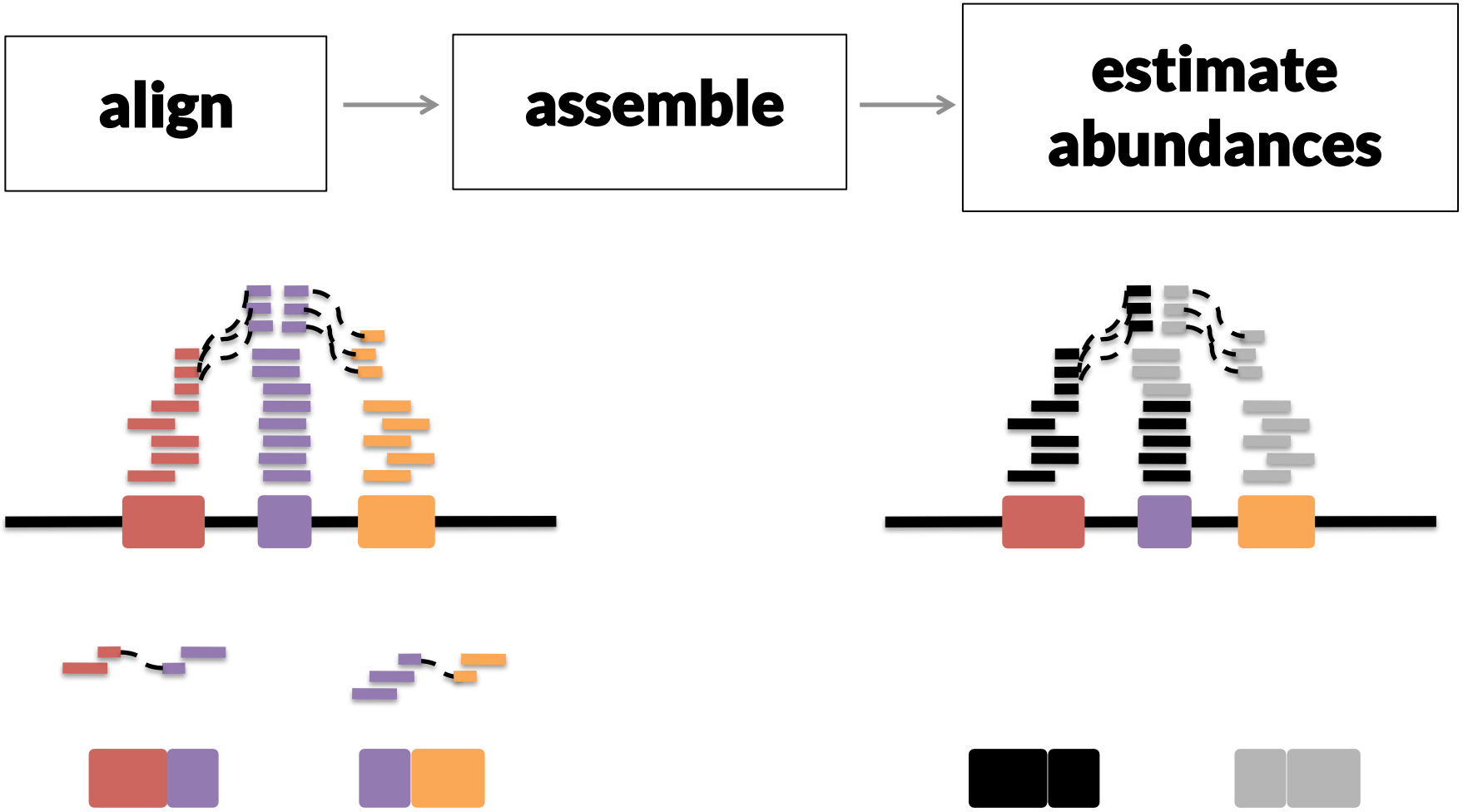


(or not: *de novo* assembly)

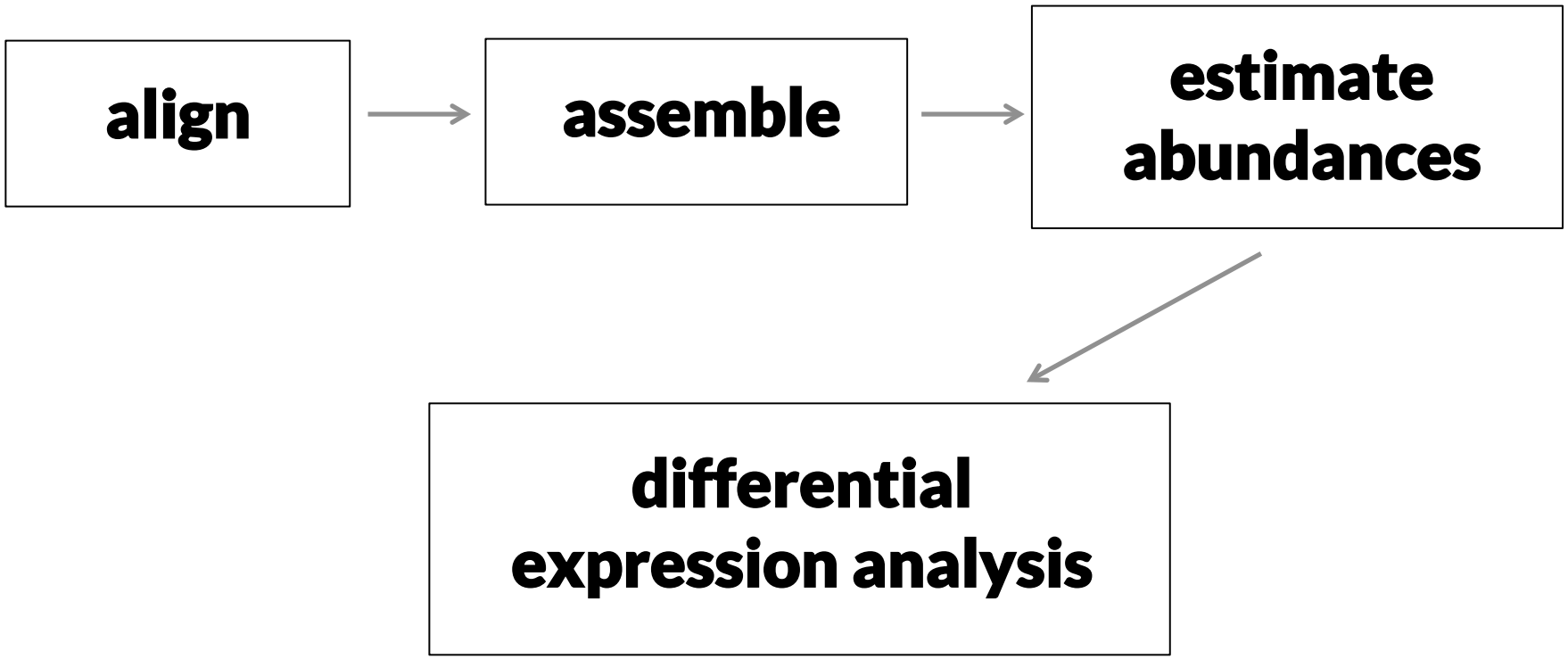
RNA-seq data analysis



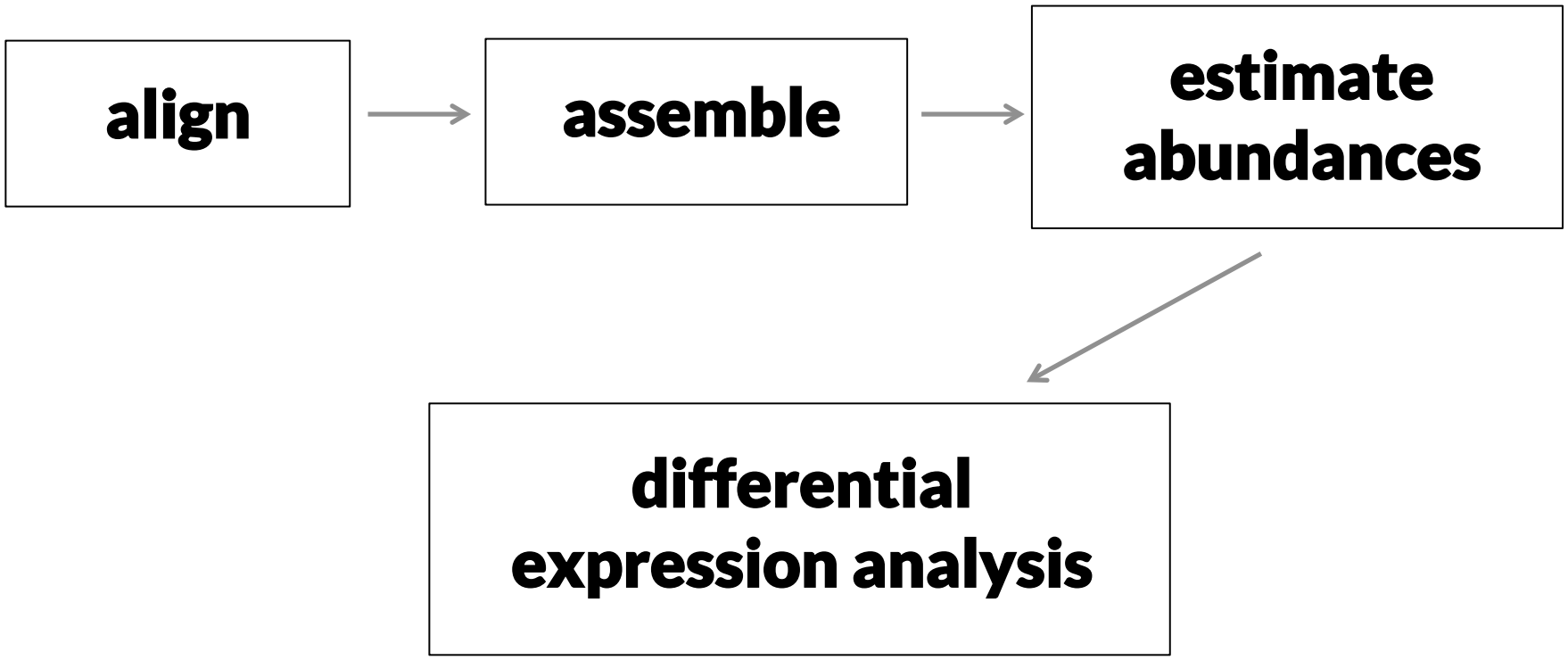
RNA-seq data analysis



RNA-seq data analysis

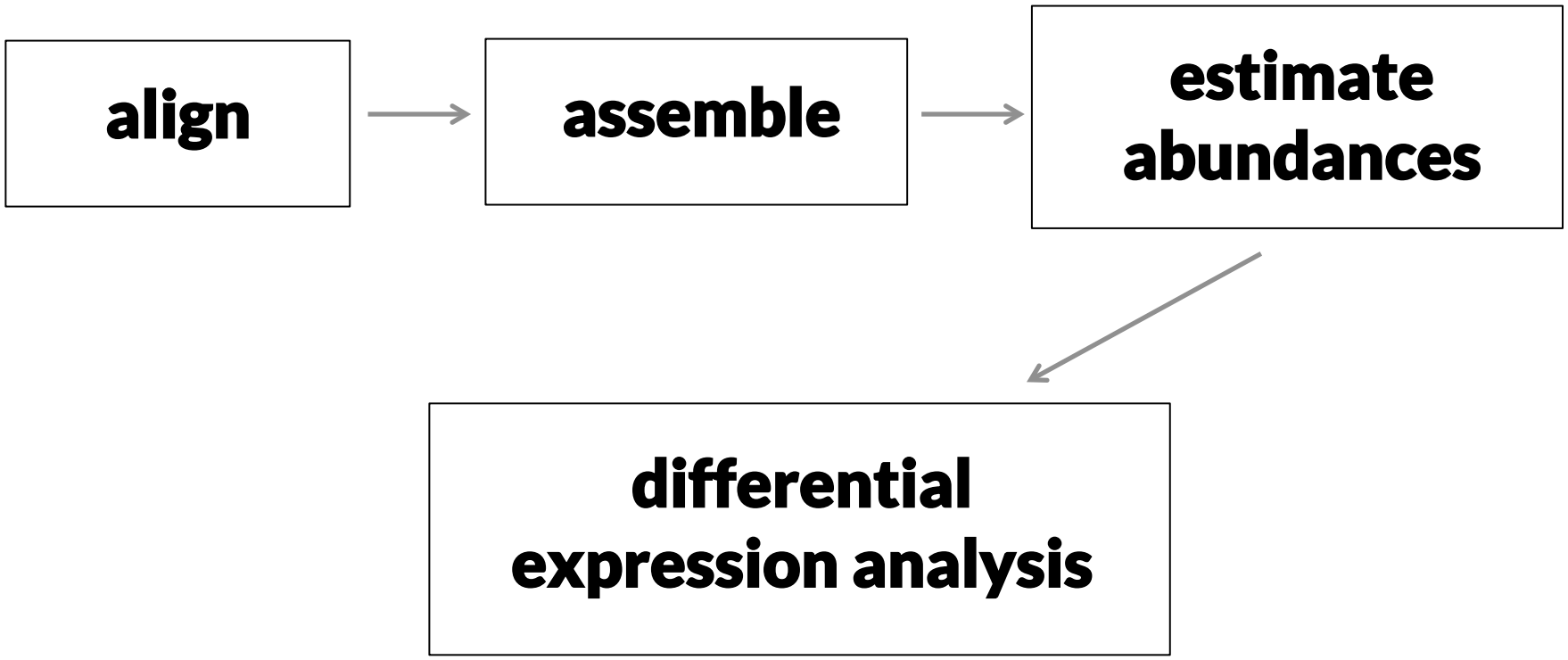


RNA-seq data analysis



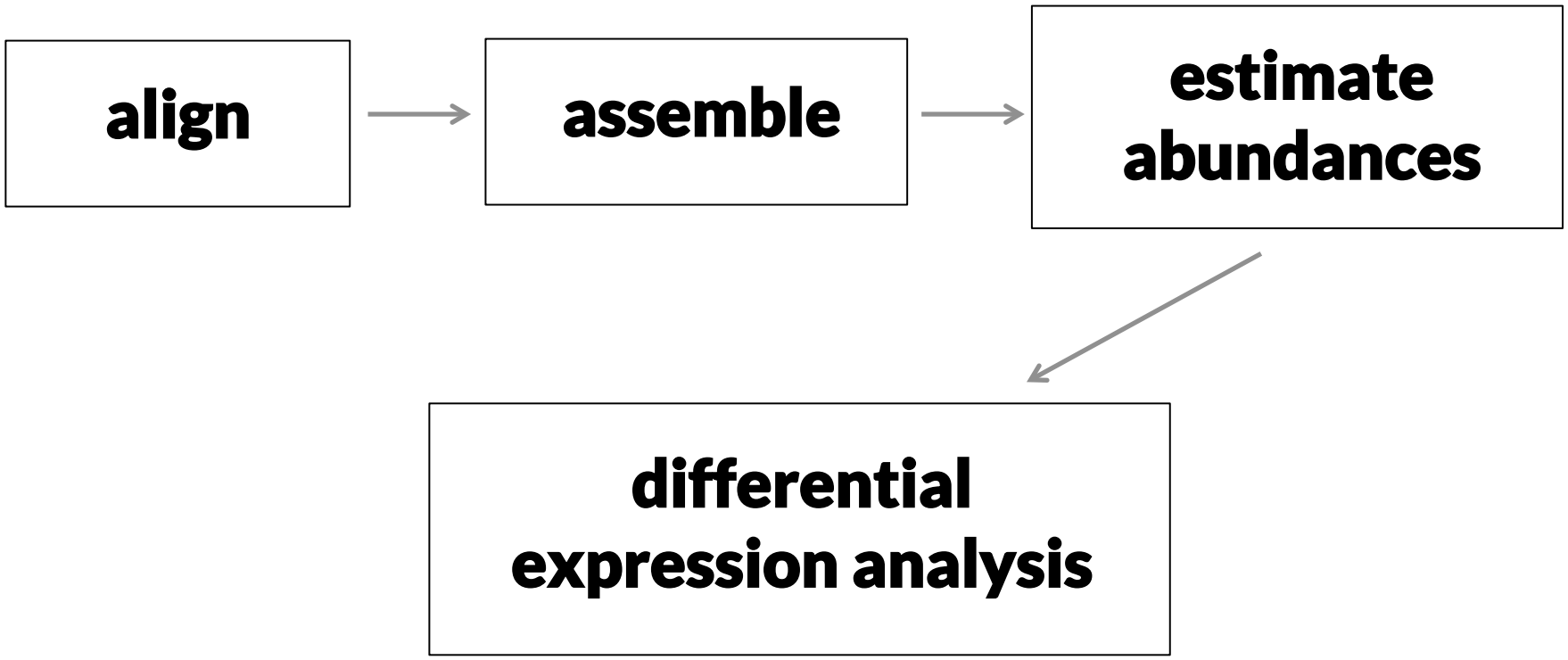
Is transcript X expressed differently in population A vs. population B?

RNA-seq data analysis



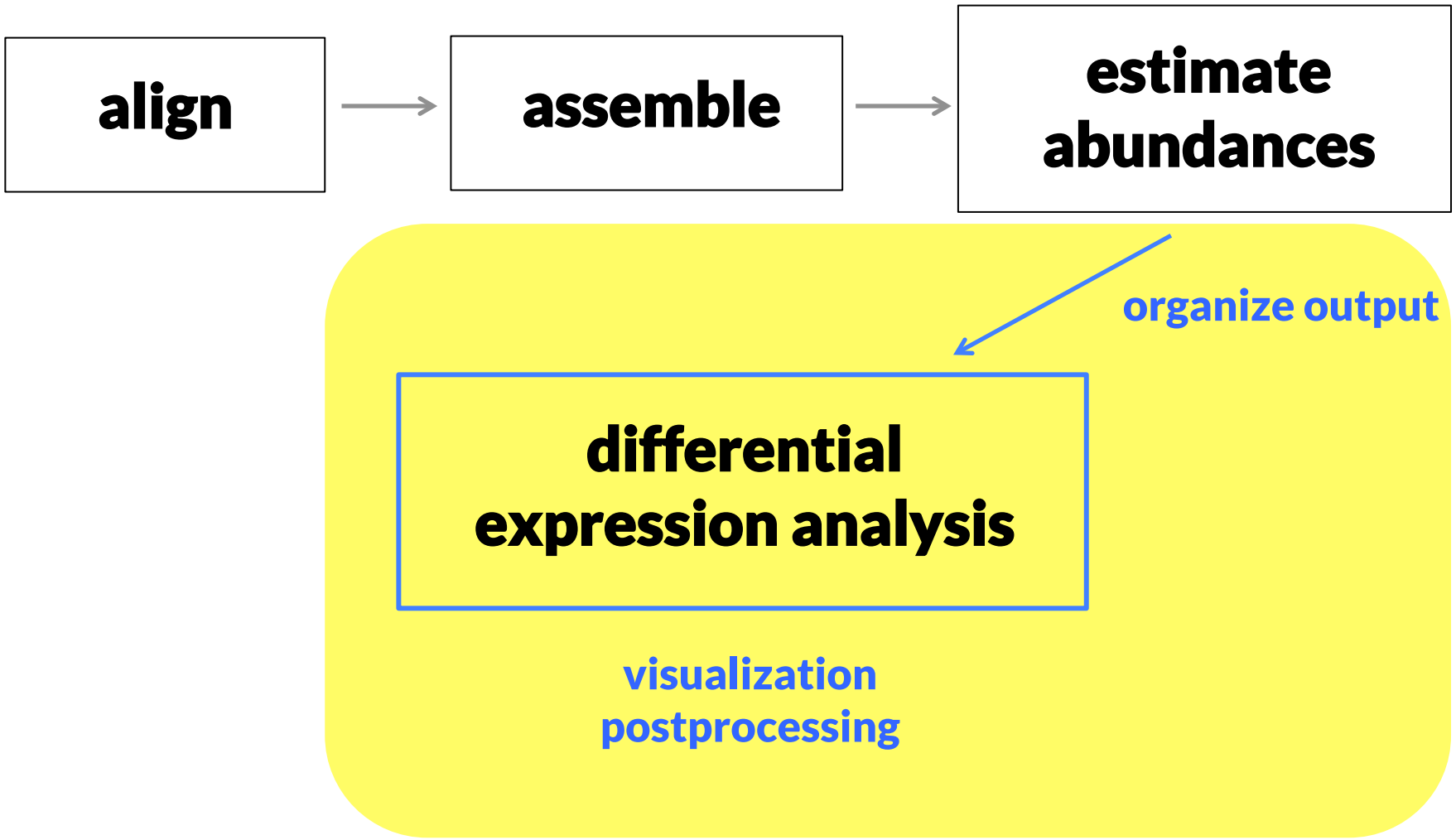
Is transcript X expressed differently in population A vs. population B vs. population C?

RNA-seq data analysis



Is transcript X expressed constantly over the course of time in the study?

Ballgown


align
assemble
estimate abundances
organize output
differential expression analysis
visualization
postprocessing

organize output: Tablemaker

```
top_dir/
    sample01/
        e2t.ctab
        e_data.ctab
        i2t.ctab
        i_data.ctab
        t_data.ctab
    sample02/
        e2t.ctab
        e_data.ctab
        i2t.ctab
        i_data.ctab
        t_data.ctab
    sample03/
        e2t.ctab
        e_data.ctab
        i2t.ctab
        i_data.ctab
        t_data.ctab
```

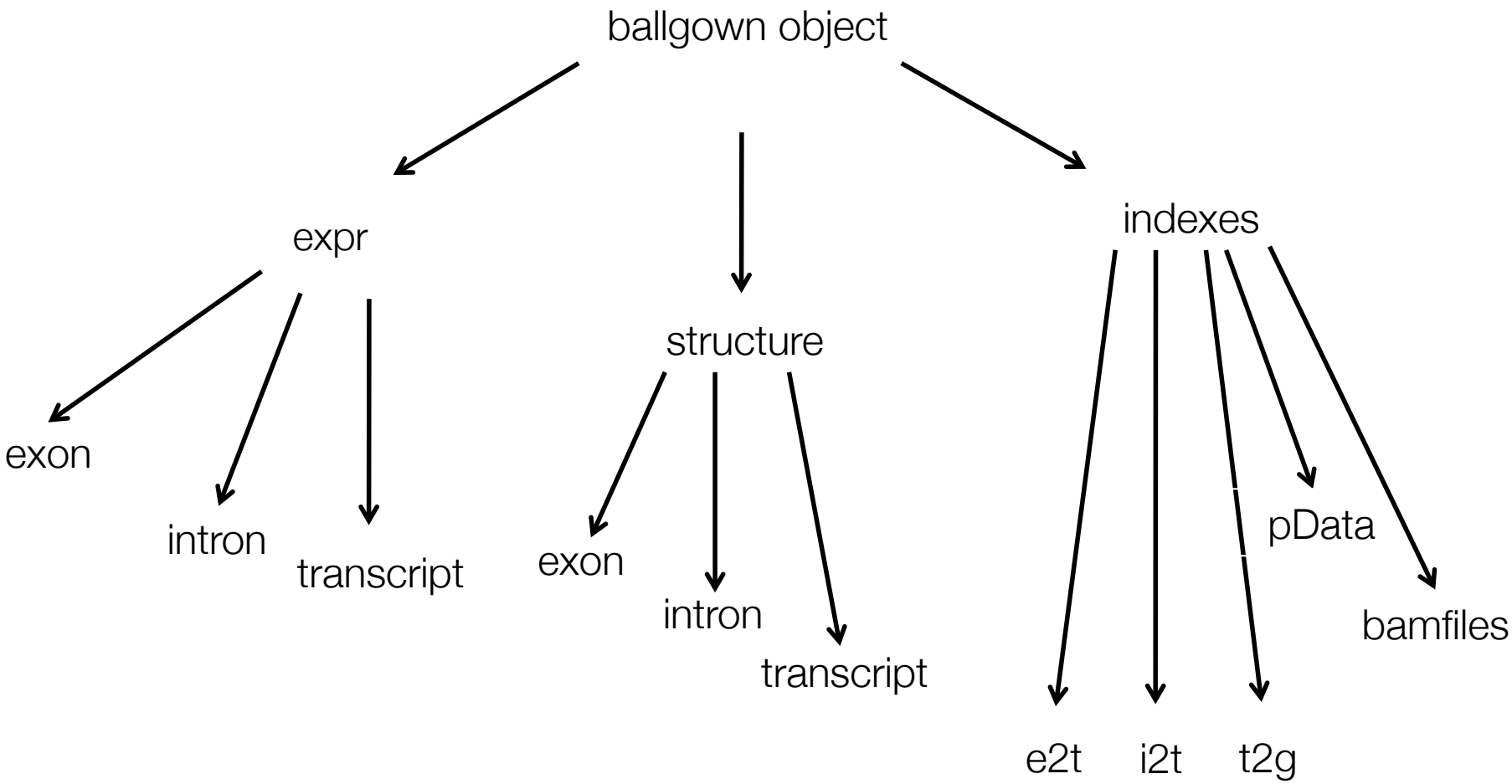
S4 class for transcript assemblies

```
library(ballgown)

my_assembly = ballgown(dataDir='top_dir', samplePattern=
    'sample')
my_assembly
## ballgown instance with 124892 transcripts and 3 samples

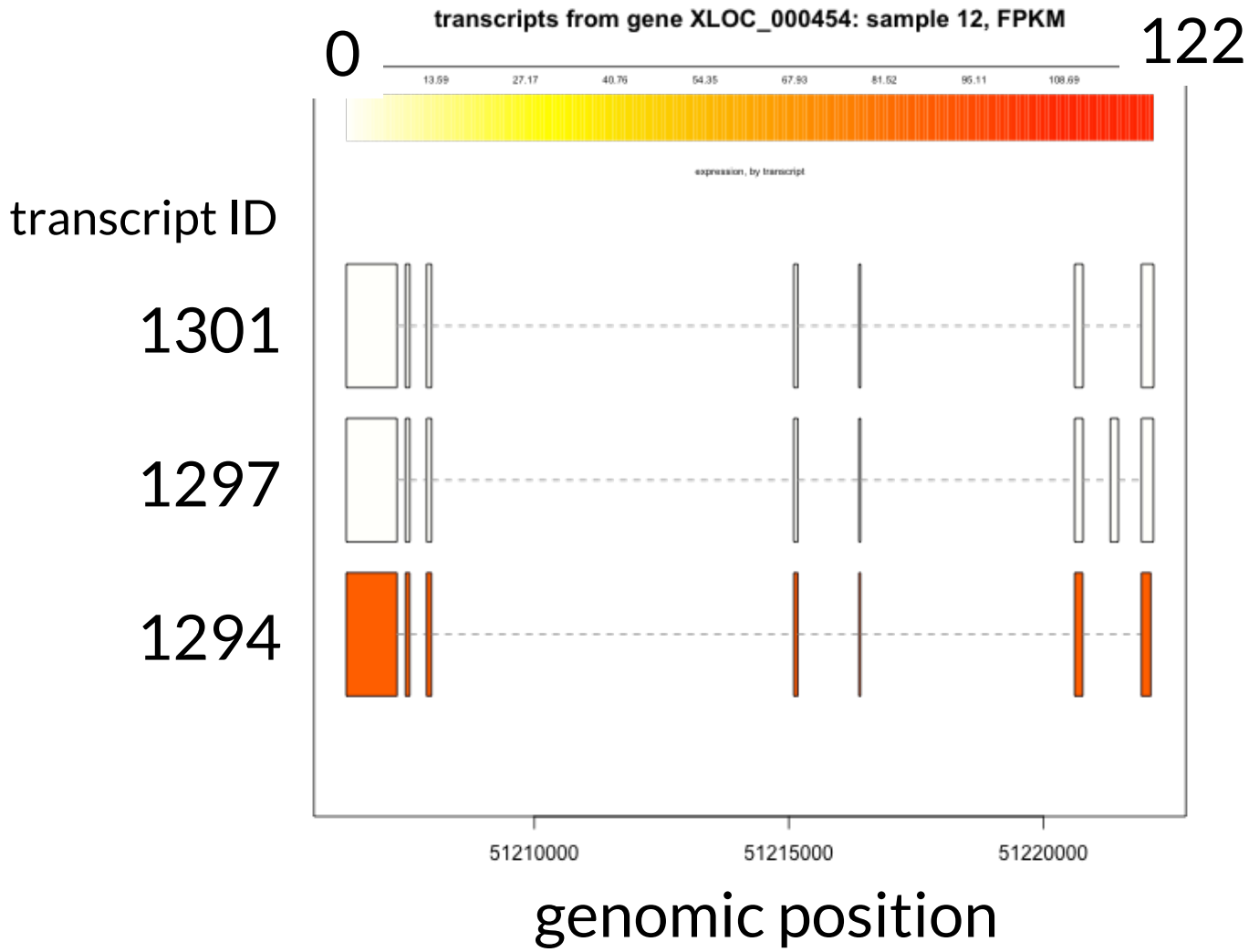
my_rsem_assembly = ballgownrsem(dir='top_dir', samples=
    c('sample01', 'sample02', 'sample03'),
    gtf='hg19_genes.gtf')
my_rsem_assembly
## ballgown instance with 45914 transcripts and 3 samples
```

S4 class for transcript assemblies



convenient visualization

```
plotTranscripts(gene='XLOC_000454', gown=my_assembly,
    samples='sample12', meas='FPKM', colorby='transcript',
    main='transcripts from gene XLOC_000454: sample 12, FPKM')
```



fast, flexible statistical analysis

```
stat_results = stattest(my_assembly, feature='transcript',
    meas='FPKM', covariate='group')

head(stat_results)
## feature      id    pval           qval
## transcript  10    0.01381576     0.105212332
## transcript  25    0.26773622     0.791149753
## transcript  35    0.01085070     0.089518254
## transcript  41    0.47108019     0.902537475
## transcript  45    0.08402948     0.489348136
## transcript  67    0.27317385     0.79114975
```

drop-in replacement for Cuffdiff

fast, flexible statistical analysis

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stat_results = stattest(my_assembly, feature='transcript',
    meas='FPKM', covariate='group')

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## feature      id    pval           qval
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## transcript  45    0.08402948     0.489348136
## transcript  67    0.27317385     0.79114975
```

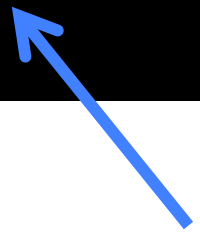
drop-in replacement for Cuffdiff

bonus: handles more experimental designs!

timecourse

```
stat_results = stattest(my_assembly, feature='transcript',
    meas='FPKM', covariate='group')

timecourse_results = stattest(my_assembly,
    feature='transcript', meas='FPKM', covariate='time',
    timecourse=TRUE)
```



models expression over time as smooth curve; compares to model where expression is assumed constant over time

custom models

```
stat_results = stattest(my_assembly, feature='transcript',
    meas='FPKM', covariate='group')

timecourse_results = stattest(my_assembly,
    feature='transcript', meas='FPKM', covariate='time',
    timecourse=TRUE)

mod = model.matrix(~ time + library_size)
mod0 = model.matrix(~ library_size)
my_timecourse_results = stattest(my_assembly,
    feature='transcript', meas='FPKM', mod=mod, mod0=mod0)
```

easy integration with other packages

```
stat_results = stattest(my_assembly, feature='transcript',
    meas='FPKM', covariate='group')

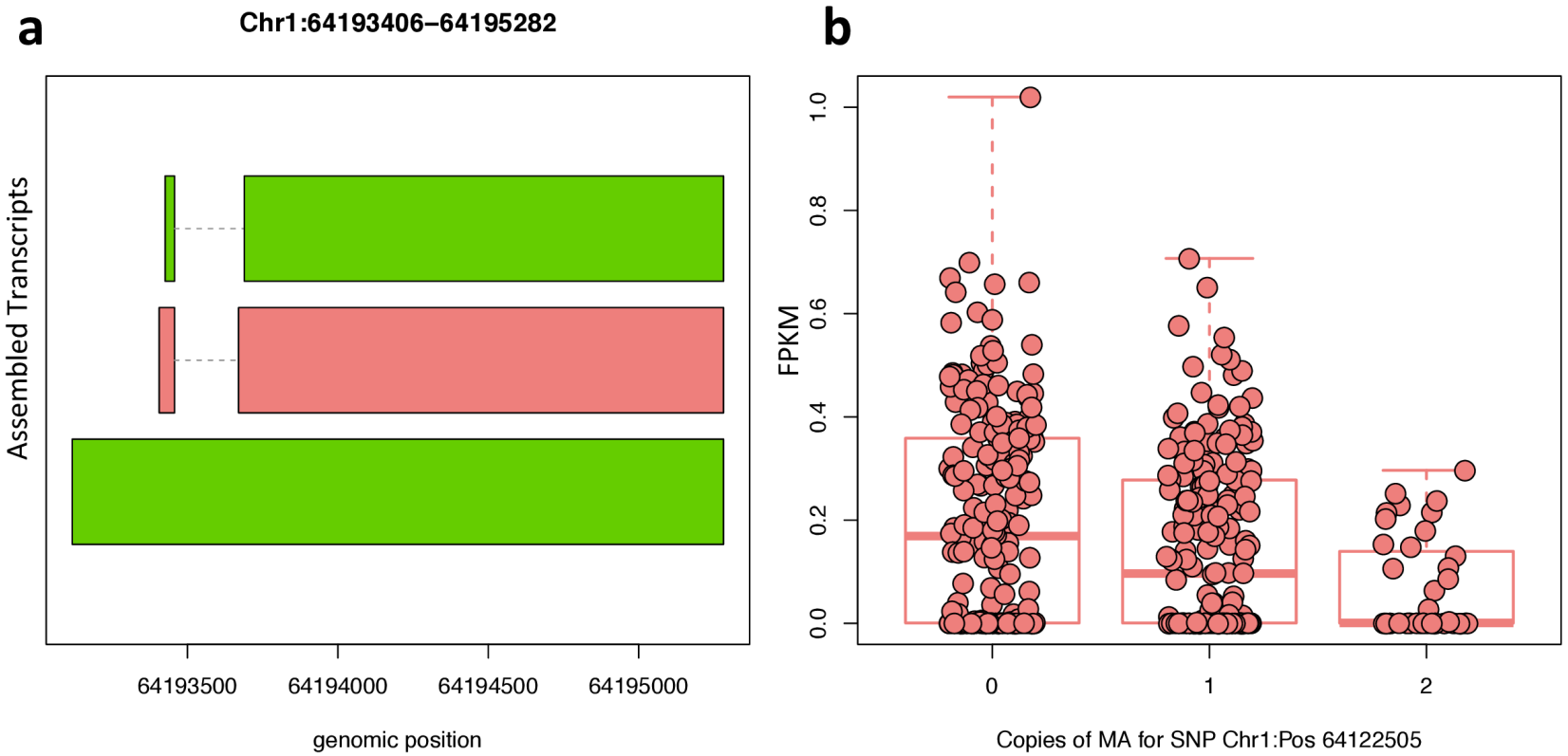
timecourse_results = stattest(my_assembly,
    feature='transcript', meas='FPKM', covariate='time',
    timecourse=TRUE)

mod = model.matrix(~ time + library_size)
mod0 = model.matrix(~ library_size)
my_timecourse_results = stattest(my_assembly,
    feature='transcript', meas='FPKM', mod=mod, mod0=mod0)


library(limma)
expression_mat = log2(texpr(my_assembly, 'FPKM') + 1)
fit = lmFit(y, mod)
fit = eBayes(fit, trend=TRUE)
```

wrangling large datasets

reanalysis of GEUVADIS data: eQTL



wrangling large datasets

reanalysis / processing of GEUVADIS data

Ballgown objects: figshare

TopHat read alignments: ArrayExpress (E-GEUV-6)

analysis code: GitHub,
https://github.com/alyssafrazee/ballgown_code

thanks!

Collaborators: Jeff Leek (advisor), Geo Pertea, Andrew Jaffe, Ben Langmead, Steven Salzberg

References:

manuscript: http://biorxiv.org/content/early/2014/03/30/003665
limma: “Linear models for Microarray Data” by Gordon K Smyth, in Bioinformatics and Computational Biology Solutions using R and Bioconductor, Springer 2005
Cufflinks: PMID 20436464 (Trapnell et al 2010)
RSEM: PMID 21816040 (Li & Dewey 2011)

Software: Ballgown is under Bioconductor review; currently available and fully documented at https://github.com/alyssafrazee/ballgown

differential expression model

for each transcript, compare the fits of the following models using an F-test. Null hypothesis is that the fits of model (a) and model (b) are equally good; alternative is that (a) fits better.

(a) $$\text{expression}_i = \alpha + \beta_0 \text{group}_i + \sum_{p=1}^{P} \gamma_p \text{confounder}_{ip} + \text{noise}_{ip}$$

(b) $$\text{expression}_i = \alpha^* + \sum_{p=1}^{P} \gamma_p^* \text{confounder}_{ip} + \text{noise}_{ip}^*$$