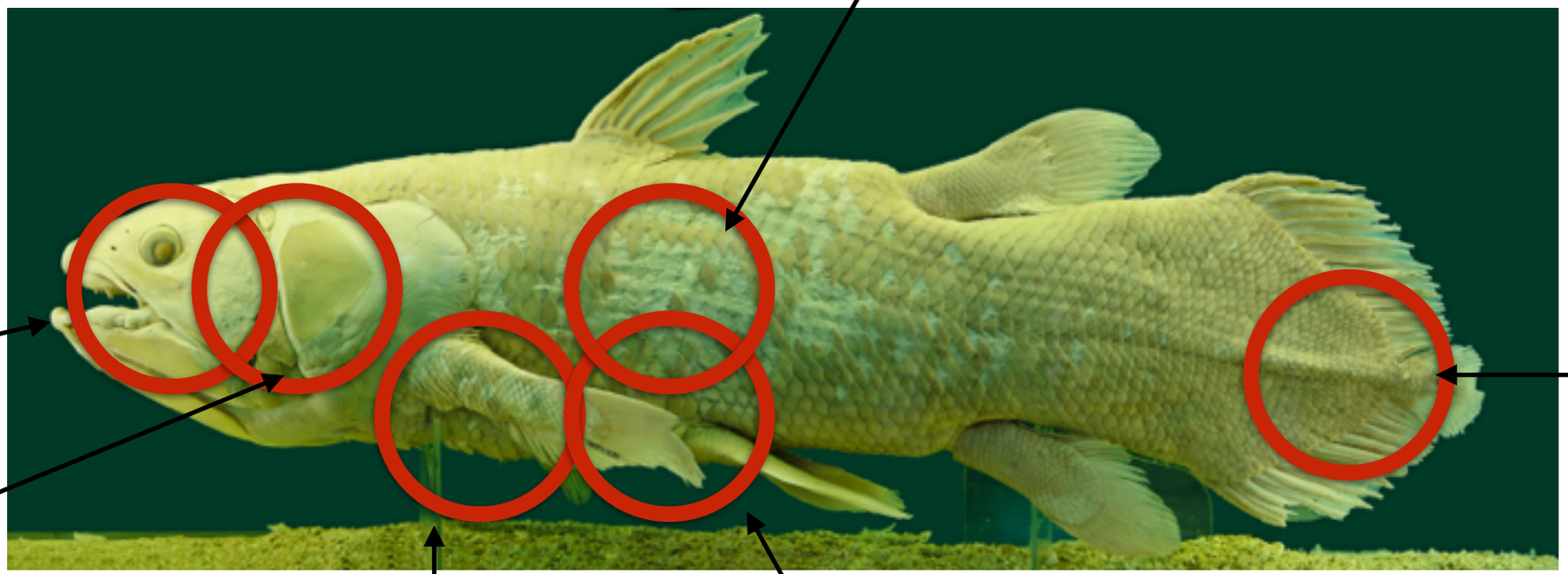
# Live sleuth demo

Harold Pimentel

# Sample to condition table

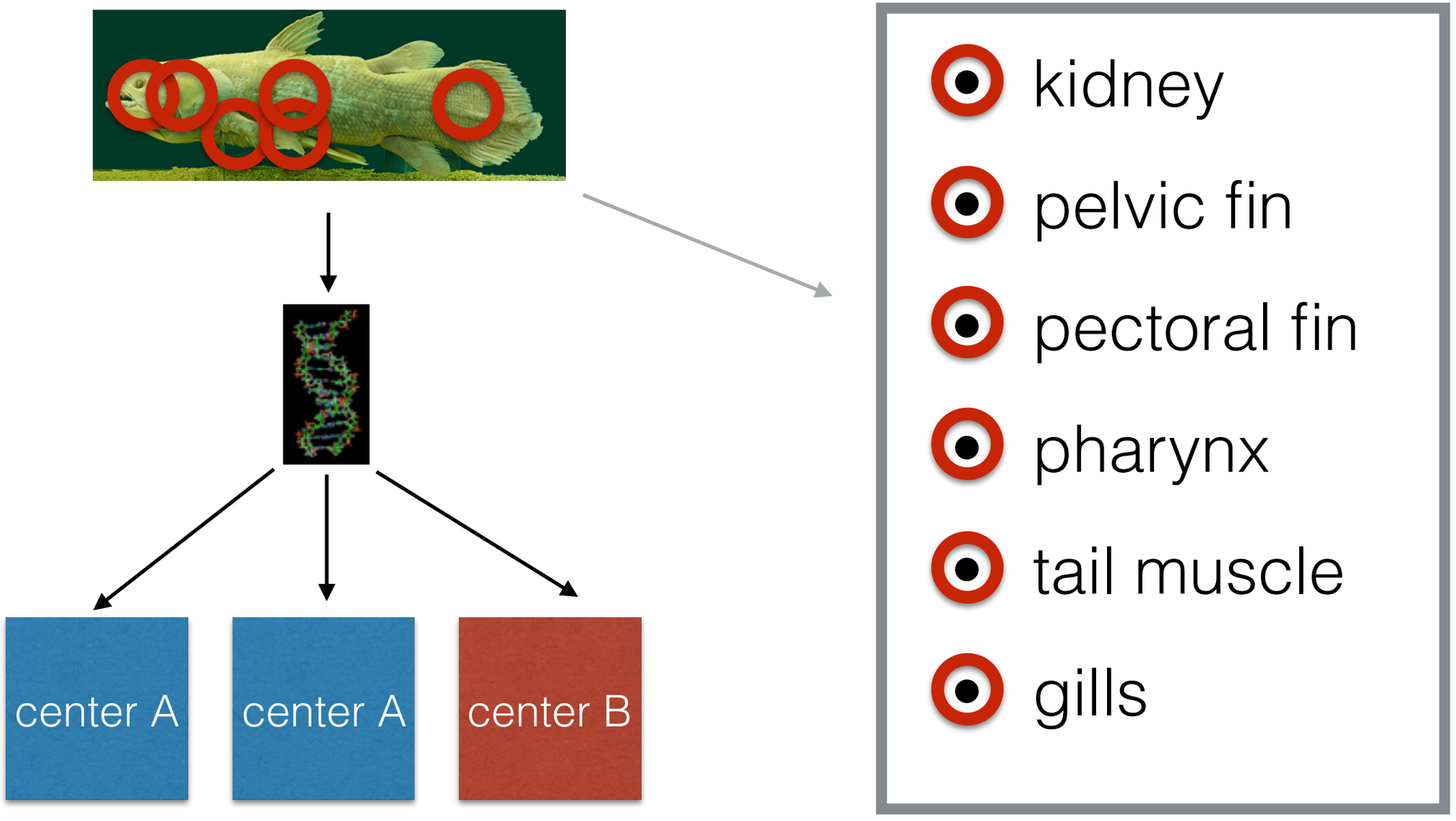
| | sample | center | tissue | path |
|---|---|---|---|---|
| 1 | DRR002302 | NIG | kidney | results/DRR002302/kallisto |
| 2 | DRR002303 | NIG | pelvic fin | results/DRR002303/kallisto |
| 3 | DRR002304 | NIG | pectoral fin | results/DRR002304/kallisto |
| 4 | DRR002305 | NIG | pharynx | results/DRR002305/kallisto |
| 5 | DRR002306 | NIG | gill | results/DRR002306/kallisto |
| 6 | DRR002307 | NIG | tail muscle | results/DRR002307/kallisto |
| 7 | DRR002308 | NIG | kidney | results/DRR002308/kallisto |
| 8 | DRR002309 | NIG | pelvic fin | results/DRR002309/kallisto |
| 9 | DRR002310 | NIG | pectoral fin | results/DRR002310/kallisto |
| 10 | DRR002311 | NIG | pharynx | results/DRR002311/kallisto |
| 11 | DRR002312 | NIG | gill | results/DRR002312/kallisto |
| 12 | DRR002313 | NIG | tail muscle | results/DRR002313/kallisto |
| 13 | DRR002314 | NIG | tail muscle | results/DRR002314/kallisto |
| 14 | DRR002319 | UT-MGS | gill | results/DRR002319/kallisto |
| 15 | DRR002320 | UT-MGS | tail muscle | results/DRR002320/kallisto |
| 16 | DRR002317 | UT-MGS | pectoral fin | results/DRR002317/kallisto |
| 17 | DRR002316 | UT-MGS | pelvic fin | results/DRR002316/kallisto |
| 18 | DRR002315 | UT-MGS | kidney | results/DRR002315/kallisto |
| 19 | DRR002318 | UT-MGS | pharynx | results/DRR002318/kallisto |

# Coelacanth analysis




*all figures from wikipedia (or me)

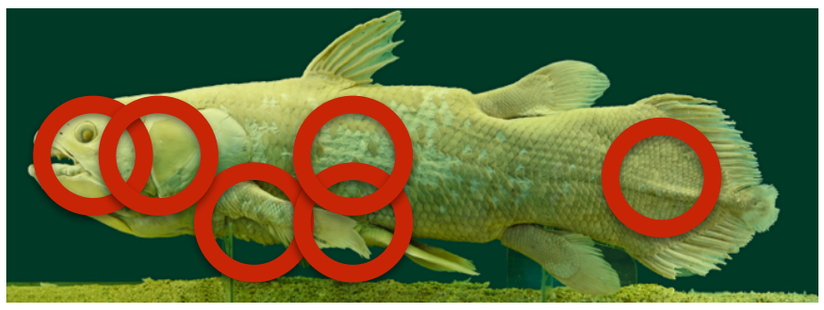
# Experimental design



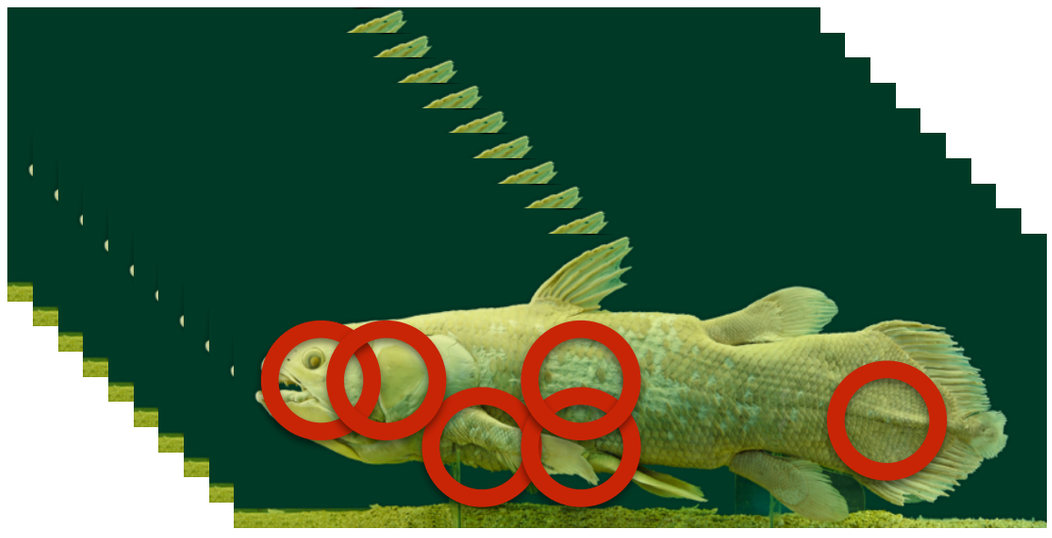
# Testing for tissue specific expression

- Ideally, we sample from **multiple** individuals

**what we have**
N = 1



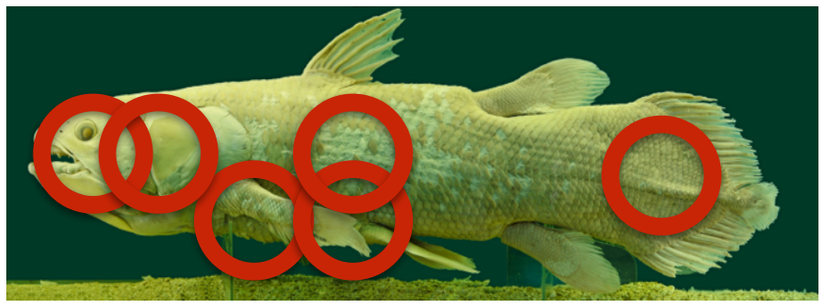
**what we want**
N = many



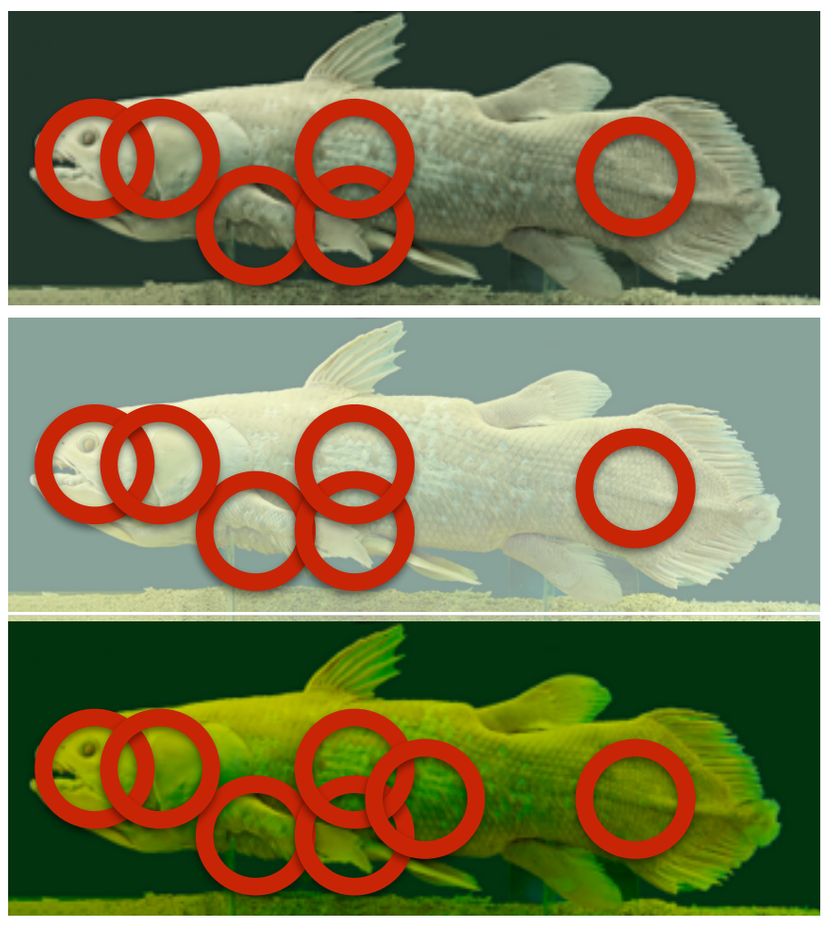
# Testing for tissue specific

- Ideally, we sample from **multiple** individuals

**what we have**
N = 1



**what we settle for**
N = 3

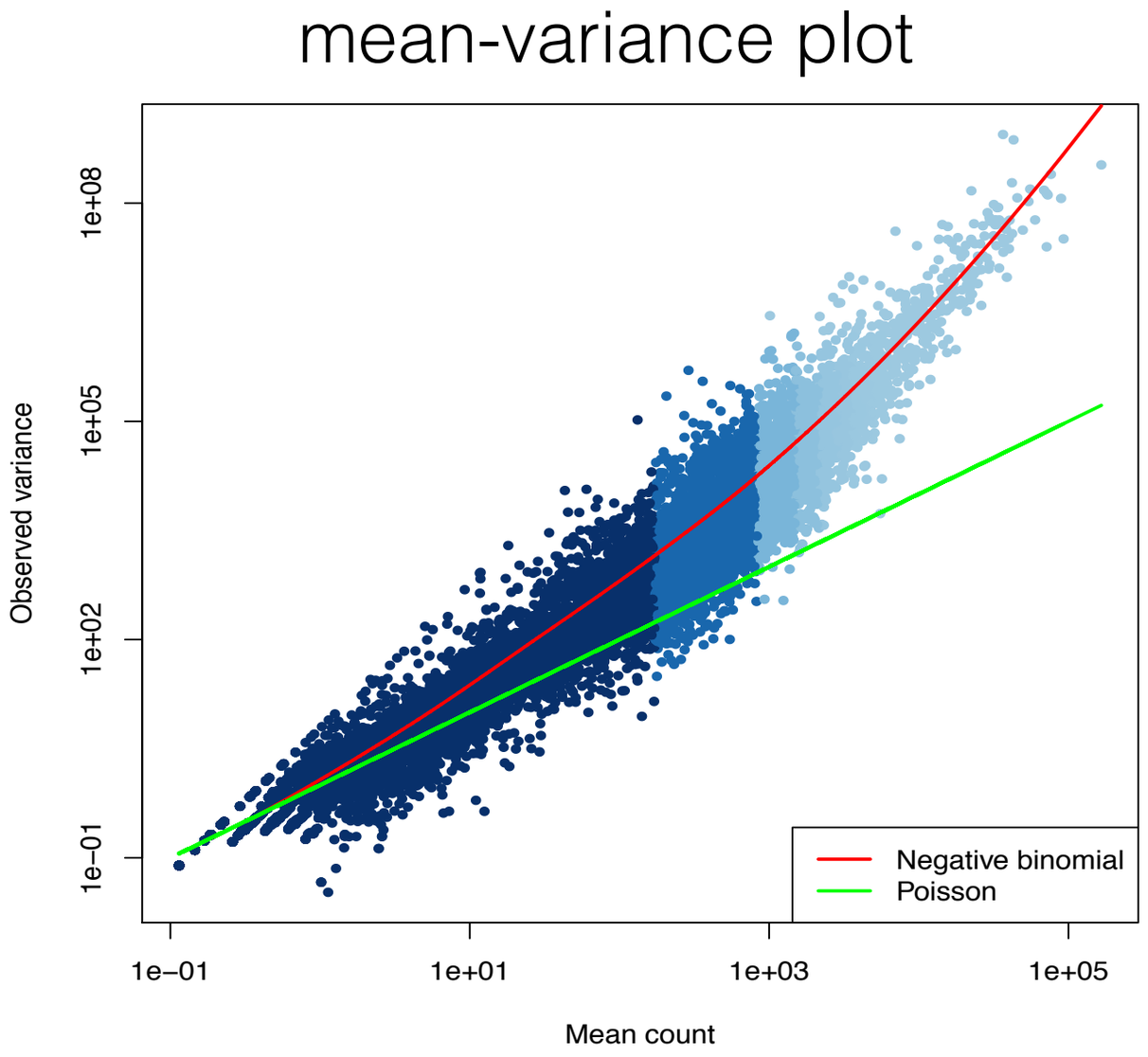


# How to estimate variance with few replicates

- (Simple case) samples from two experimental conditions with few replicates
  - Which features likely have a different population mean?
- Essentially the classic *two-sample* problem with some complications:
- **Transcript abundances must be *estimated* from transcript compatibility counts**
- **Few replicates make variance estimation challenging**
- **Multiple tests reduce power**

# Shrinkage

- Make parametric assumption for distribution of counts
- Assume a smooth mean-variance count relationship to share information between transcripts (genes)
- Most methods do not incorporate "inferential variance" estimates



# Sources of variation

- Technical variance
  - Library preparation

assumed to be fixed

- Biological variance
  - Variation between individuals

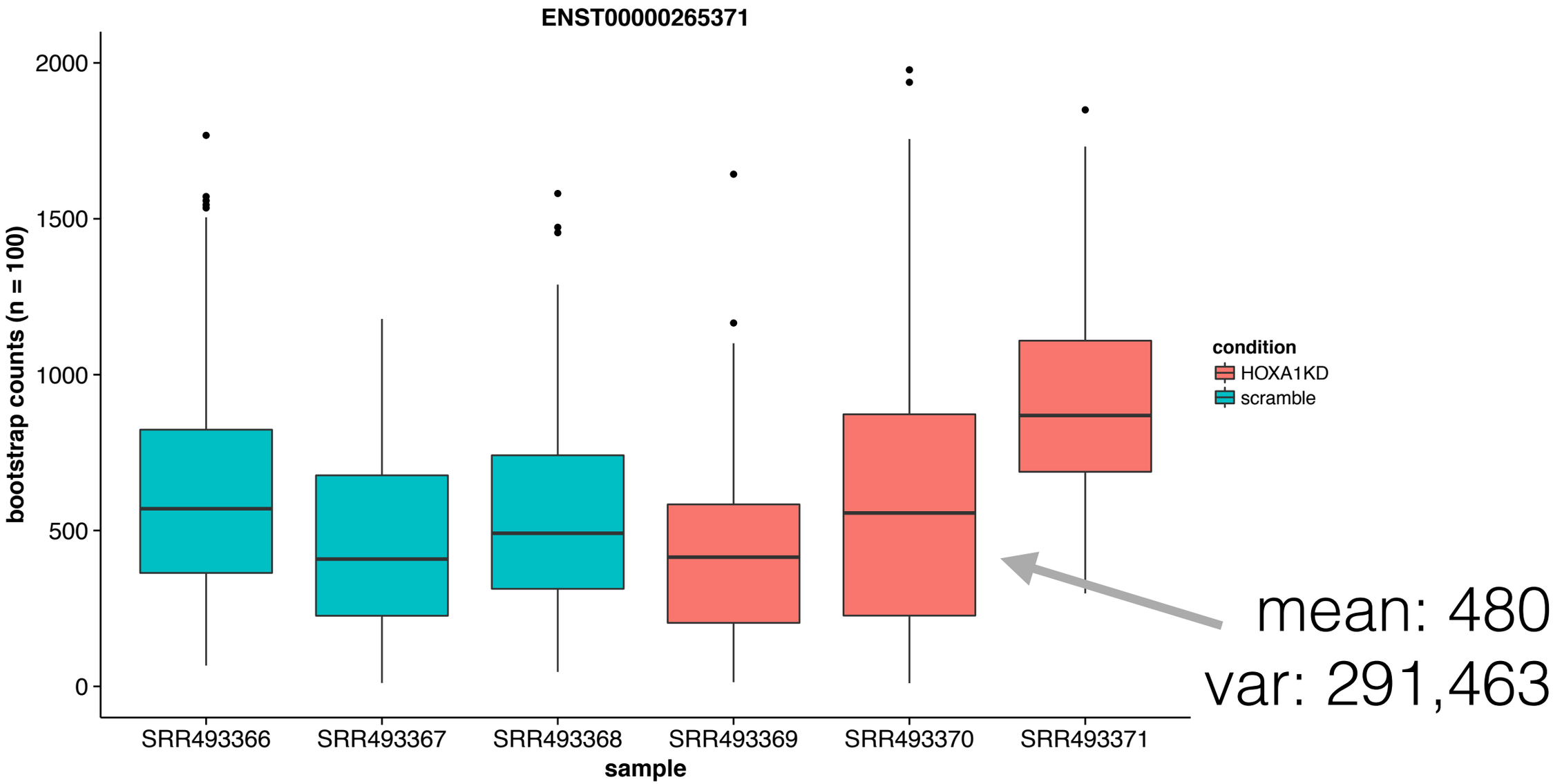
shrinkage estimation

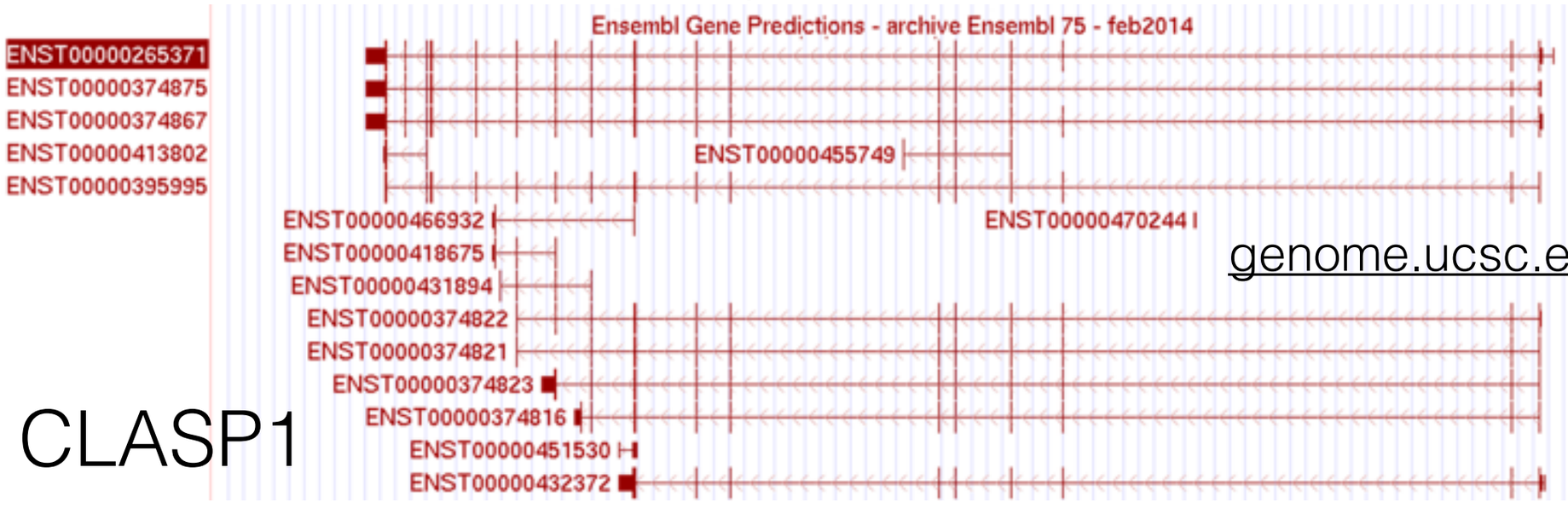
- Inferential variance
  - Variation due to algorithms

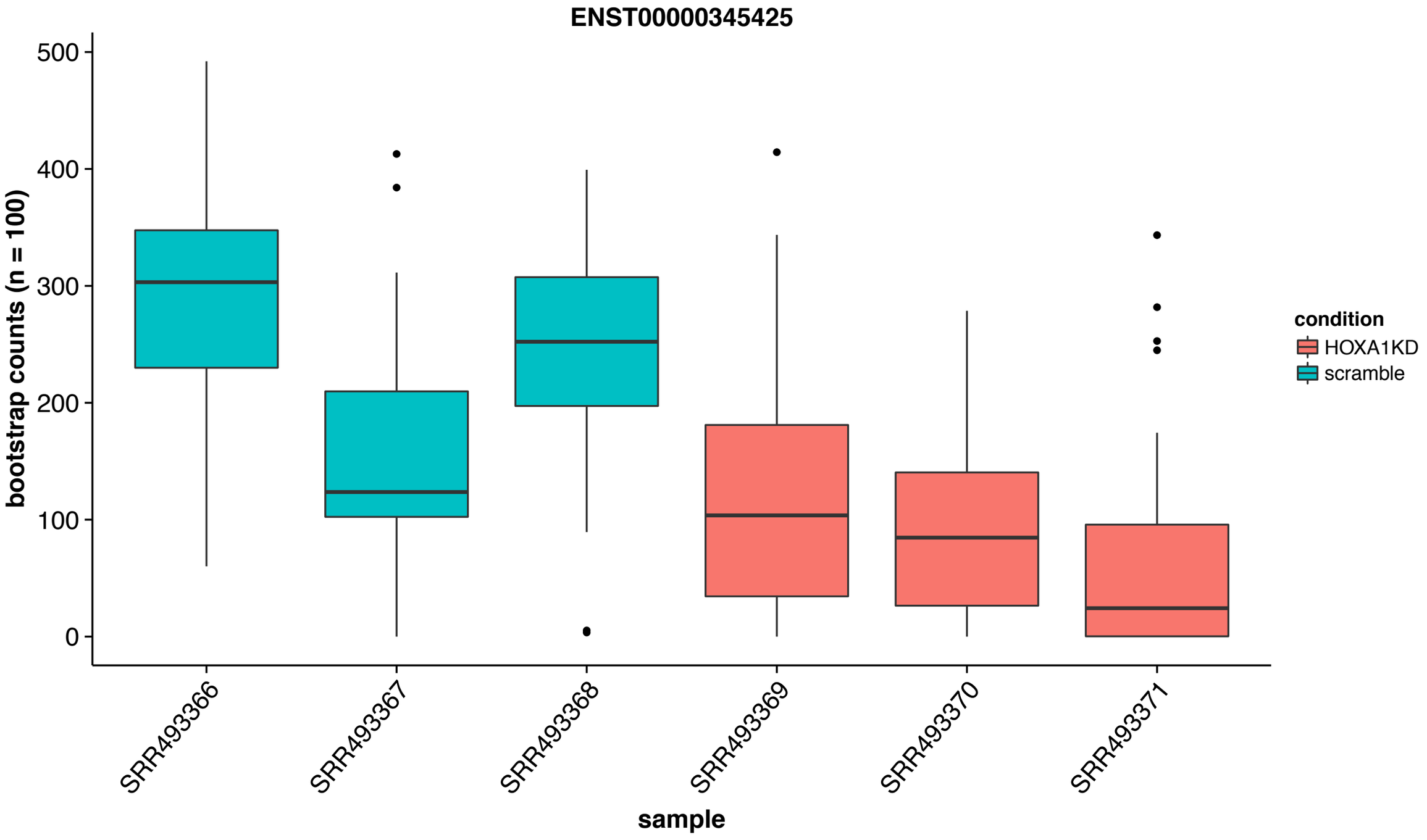
normally ignored,
but modeled in sleuth

# How much inferential variance?

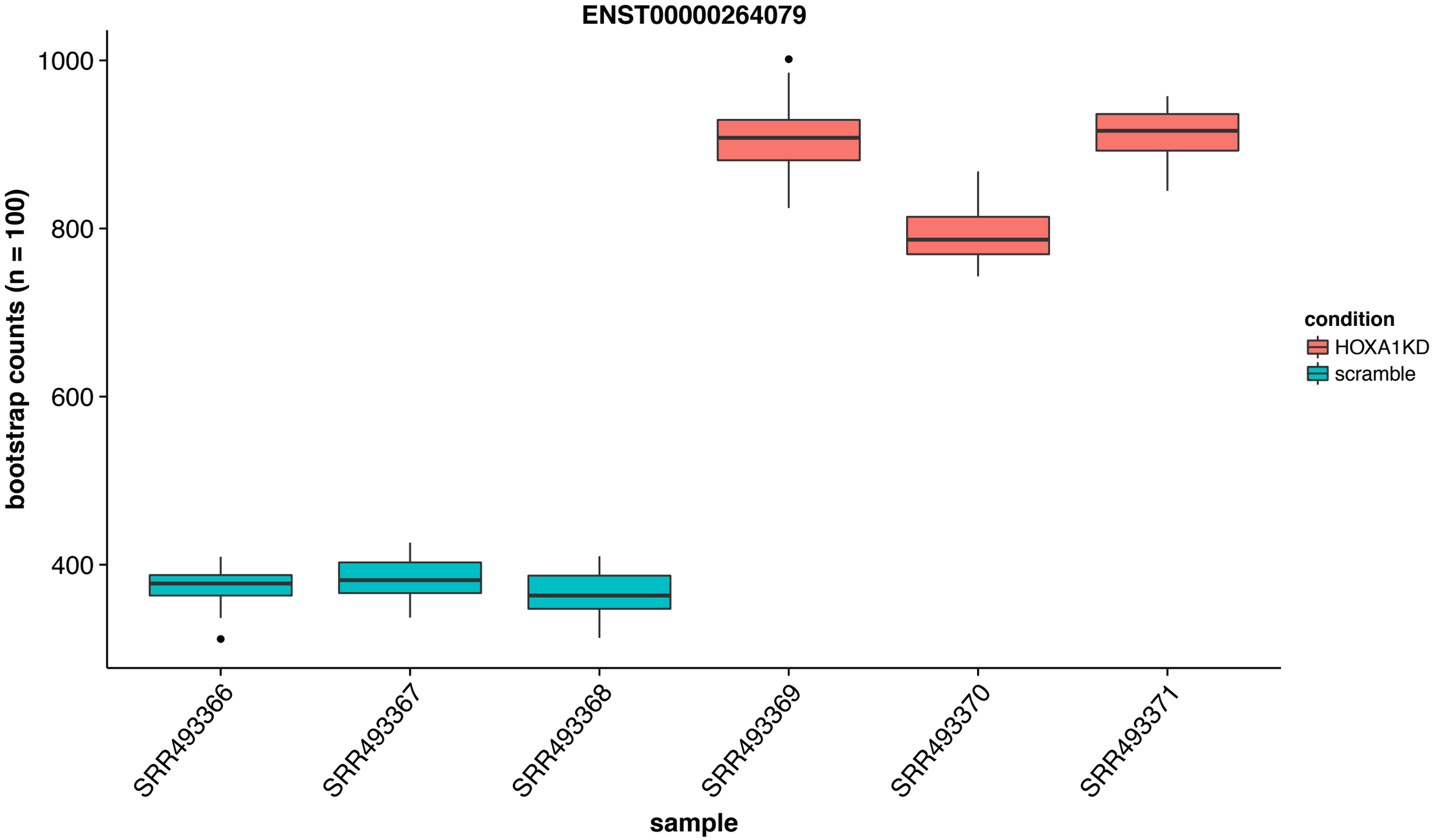
- Often, it is not so bad, but some genes are really complicated and short reads aren't that informative
- Case study:
  - Cuffdiff2 HiSeq data set ~15-20M fragments/sample
    - 3 HOXA1 knockdown in lung fibroblasts
    - 3 with scrambled siRNA
    - Ran *kallisto* with 100 bootstraps


ENST00000265371
2000
1500
1000
500
0
bootstrap counts (n = 100)
SRR493366
SRR493367
SRR493368
SRR493369
SRR493370
SRR493371
sample
condition
HOXA1KD
scramble
mean: 480
var: 291,463

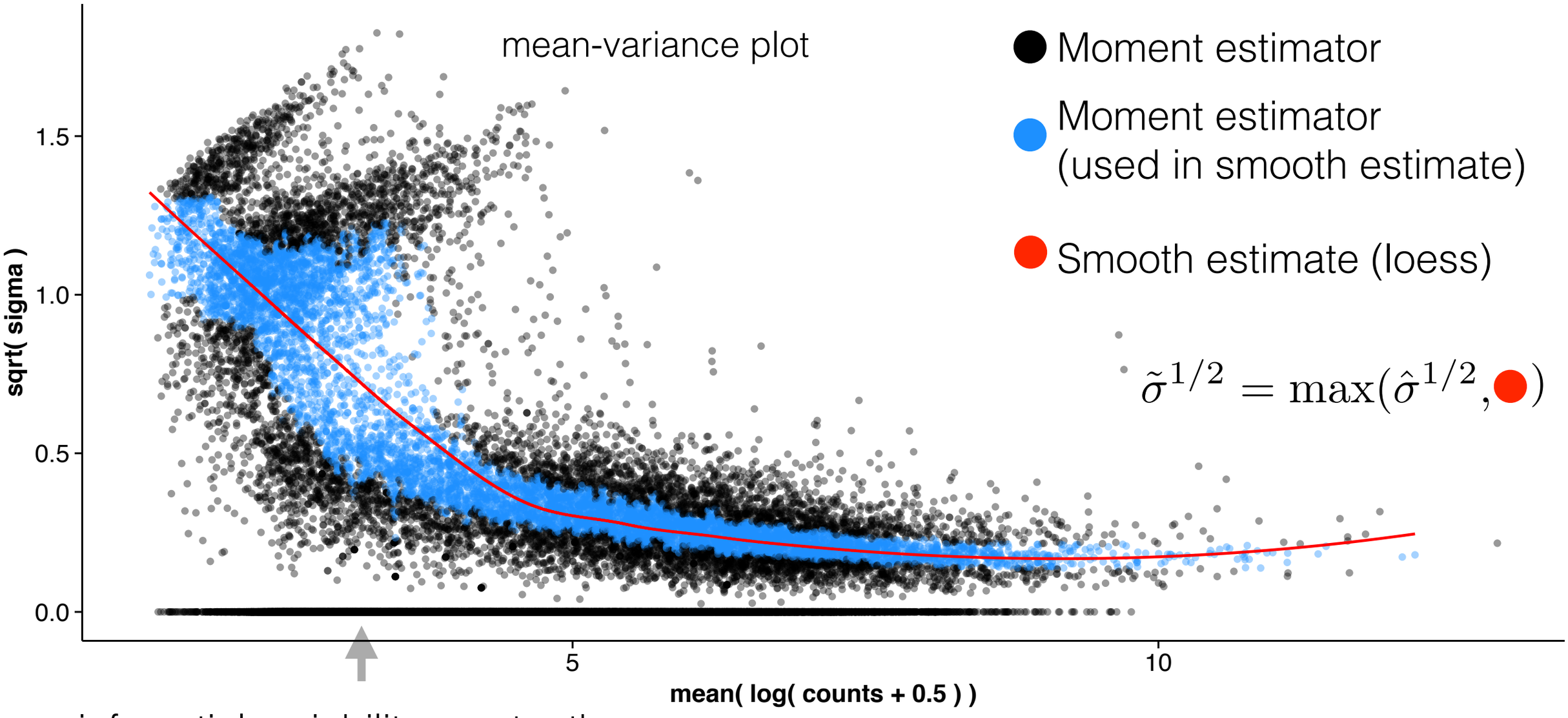

Ensembl Gene Predictions - archive Ensembl 75 - feb2014
ENST00000265371
ENST00000374875
ENST00000374867
ENST00000413802
ENST00000395995
ENST00000455749
ENST00000466932
ENST00000418675
ENST00000431894
ENST00000374822
ENST00000374821
ENST00000374823
ENST00000374816
ENST00000451530
ENST00000432372
genome.ucsc.edu
CLASP1




not significant by sleuth: FDR = 0.634
significant by DESeq2: FDR = 0.040

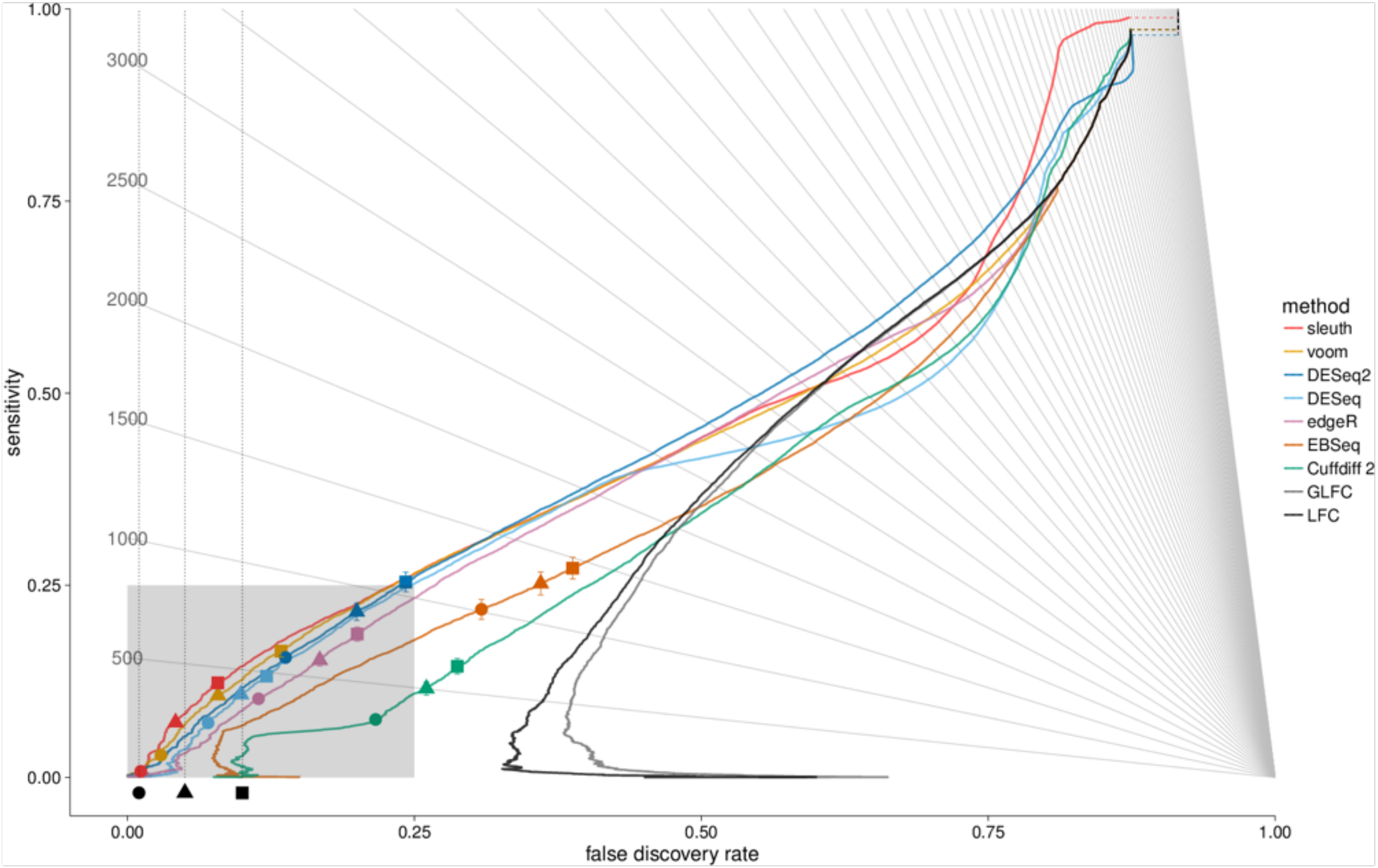

ENST00000264079
bootstrap counts (n = 100)
1000
800
600
400
SRR493366
SRR493367
SRR493368
SRR493369
SRR493370
SRR493371
sample
condition
HOXA1KD
scramble

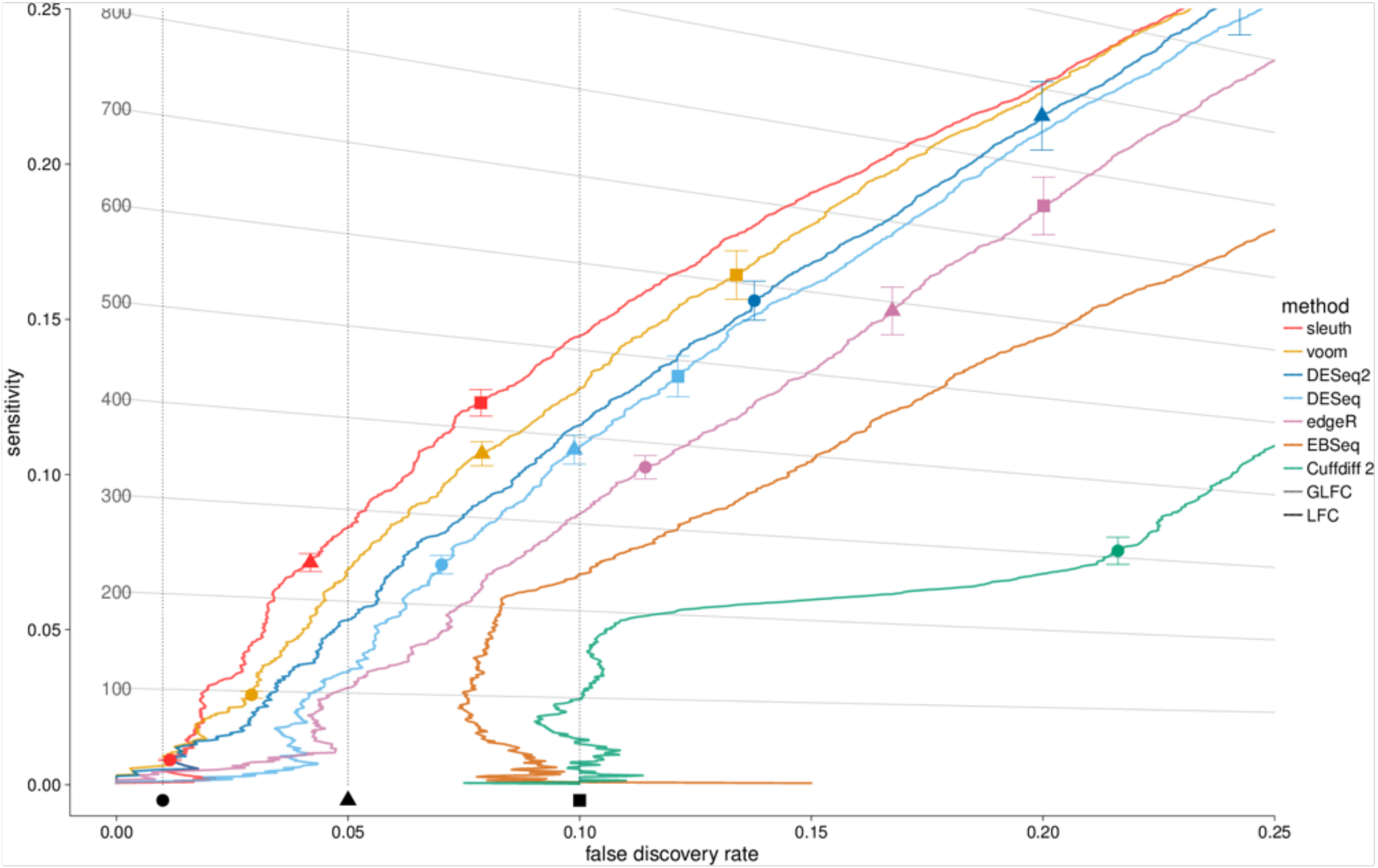
# Biological variance estimation



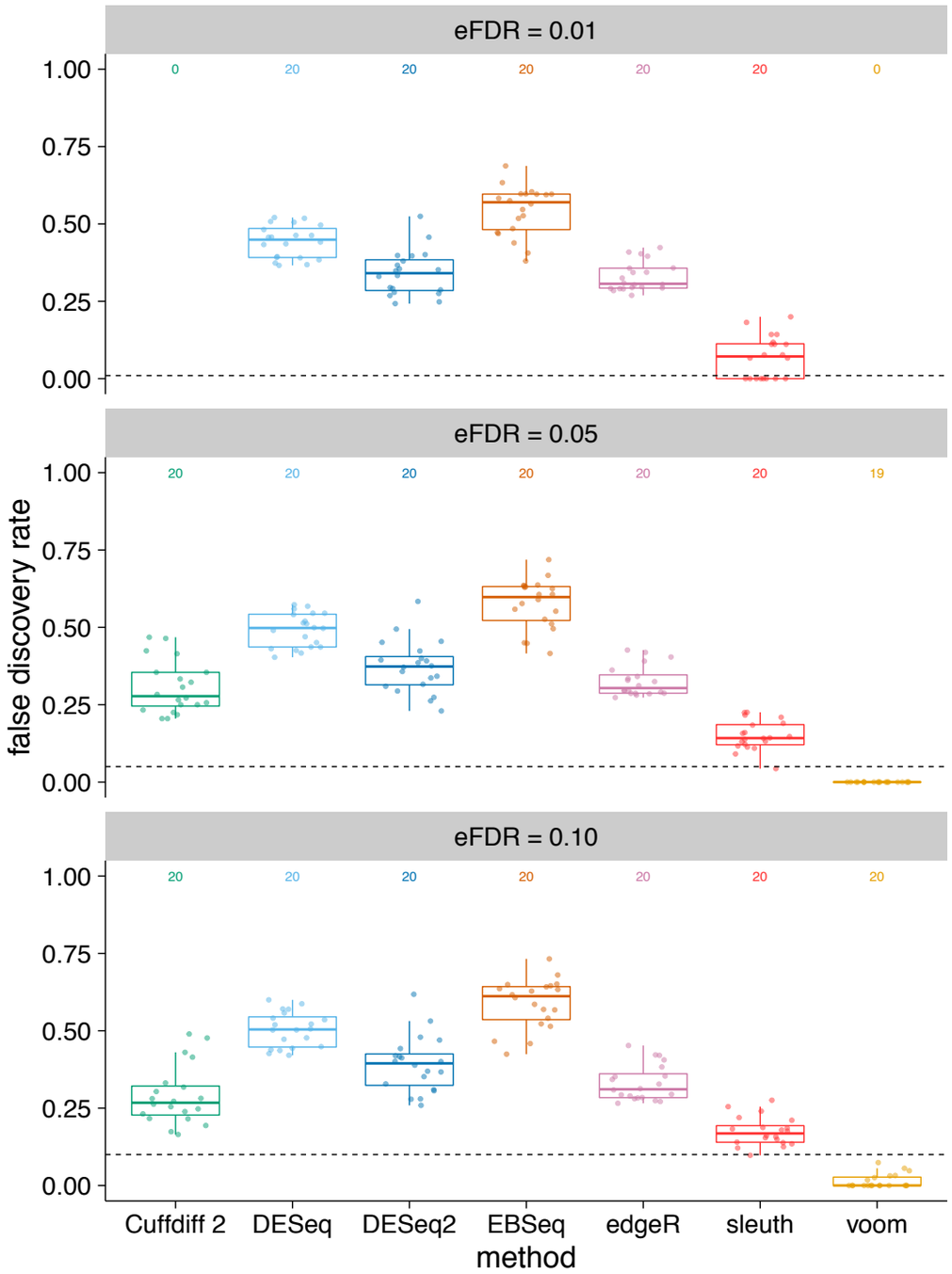

inferential variability greater than
(estimated) biological variability

$$\hat{\sigma}^2 = \frac{1}{n-2}\sum_i r_i^2 - \hat{\tau}^2$$

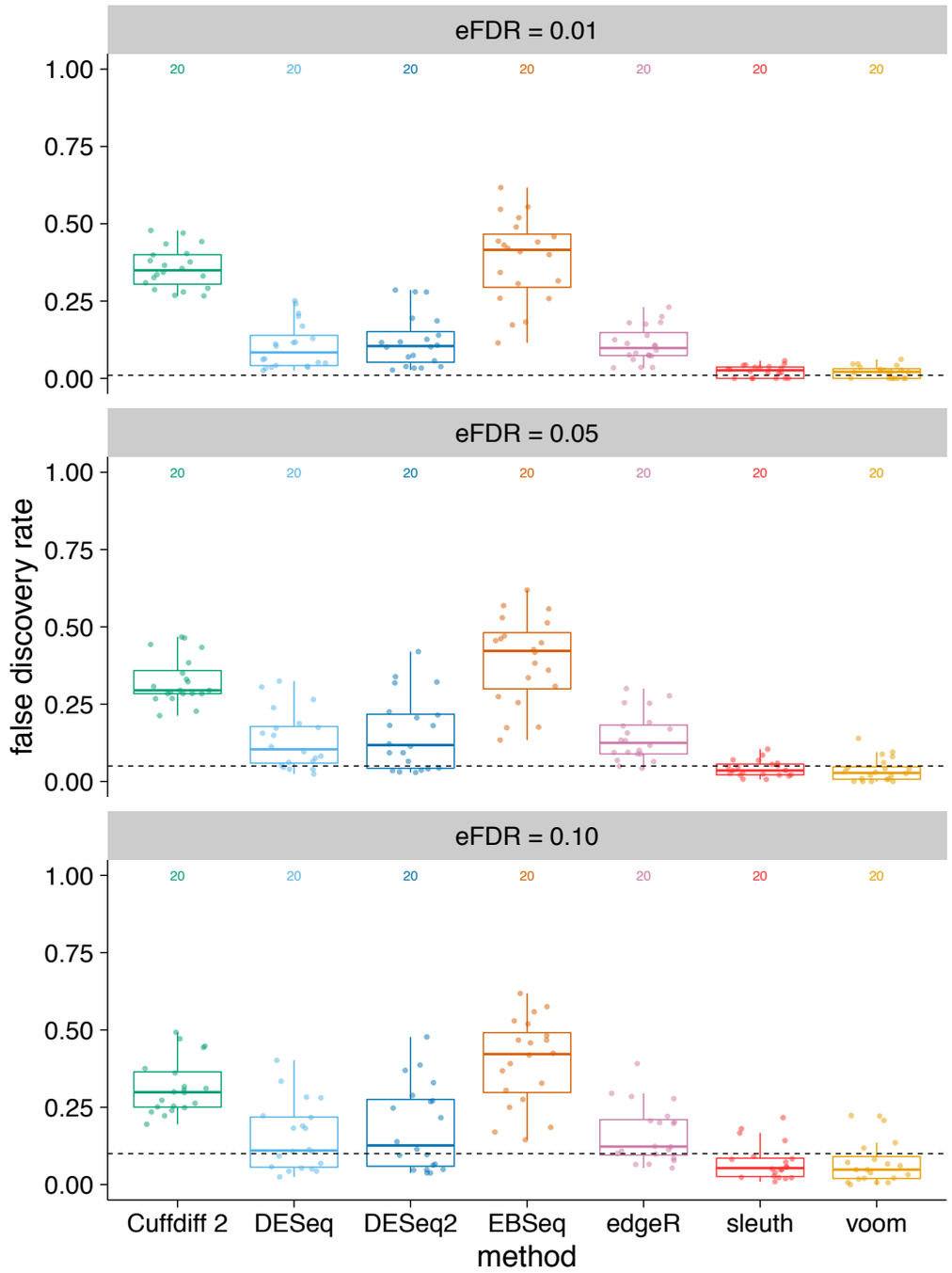

1.00
0.75
0.50
0.25
0.00
sensitivity
3000
2500
2000
1500
1000
500
0.00
0.25
0.50
0.75
1.00
false discovery rate
method
sleuth
voom
DESeq2
DESeq
edgeR
EBSeq
Cuffdiff 2
GLFC
LFC


sensitivity
0.25
0.20
0.15
0.10
0.05
0.00
800
700
600
500
400
300
200
100
0.00
0.05
0.10
0.15
0.20
0.25
false discovery rate
method
sleuth
voom
DESeq2
DESeq
edgeR
EBSeq
Cuffdiff 2
GLFC
LFC

# Sleuth benchmark on Bottomly *et al.*




isoform




gene

# Tissue specific expression