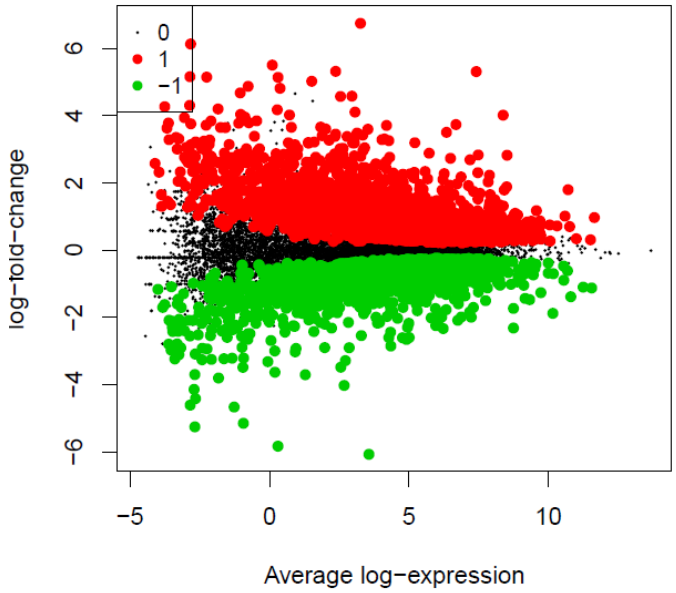
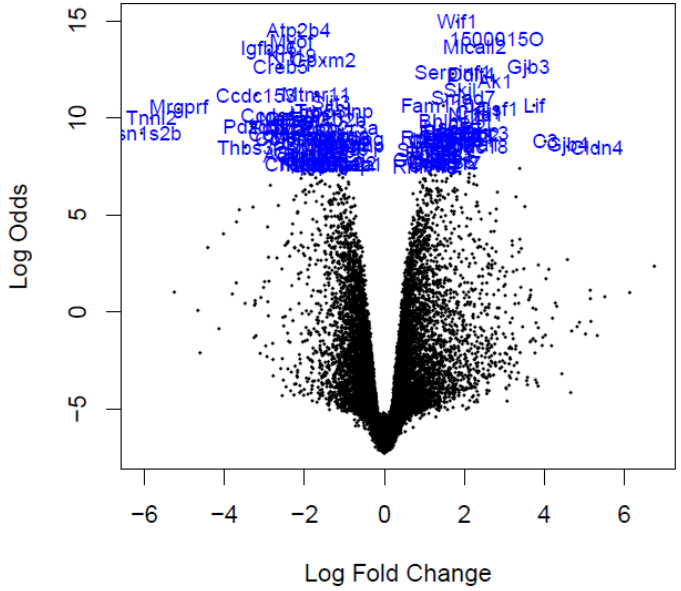
Gene set testing in *limma*

COMBINE RNA-seq Workshop

Why?

- Sometimes after differential expression testing, we have a long list of 1000's of genes
- Too difficult to go through one by one
- Or there may be very few / no genes that make statistical significance (small effect sizes + experimental noise)
- Want to understand pathways involved in the biological system being studied



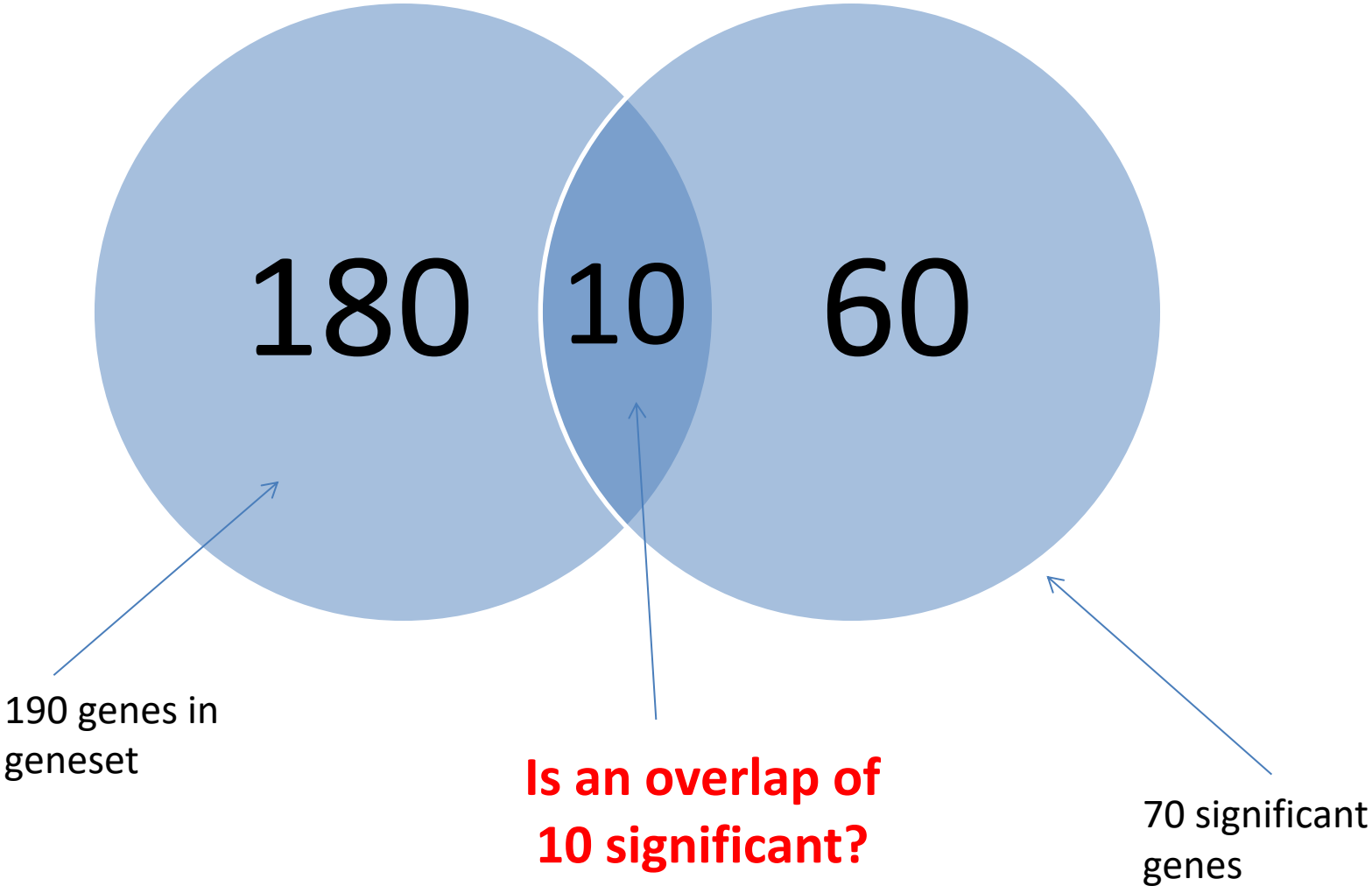


Gene set tests available in *limma*

- Want to test LOTS of gene sets?
  - `goana()` function
    - Test Gene Ontology (GO) categories
  - `kegga()` function
    - Test KEGG pathways
  - `camera()` function
    - User specified gene sets
- Want to test just a few gene sets?
  - `mroast()` / `fry()` functions

Basic principles behind gene set testing

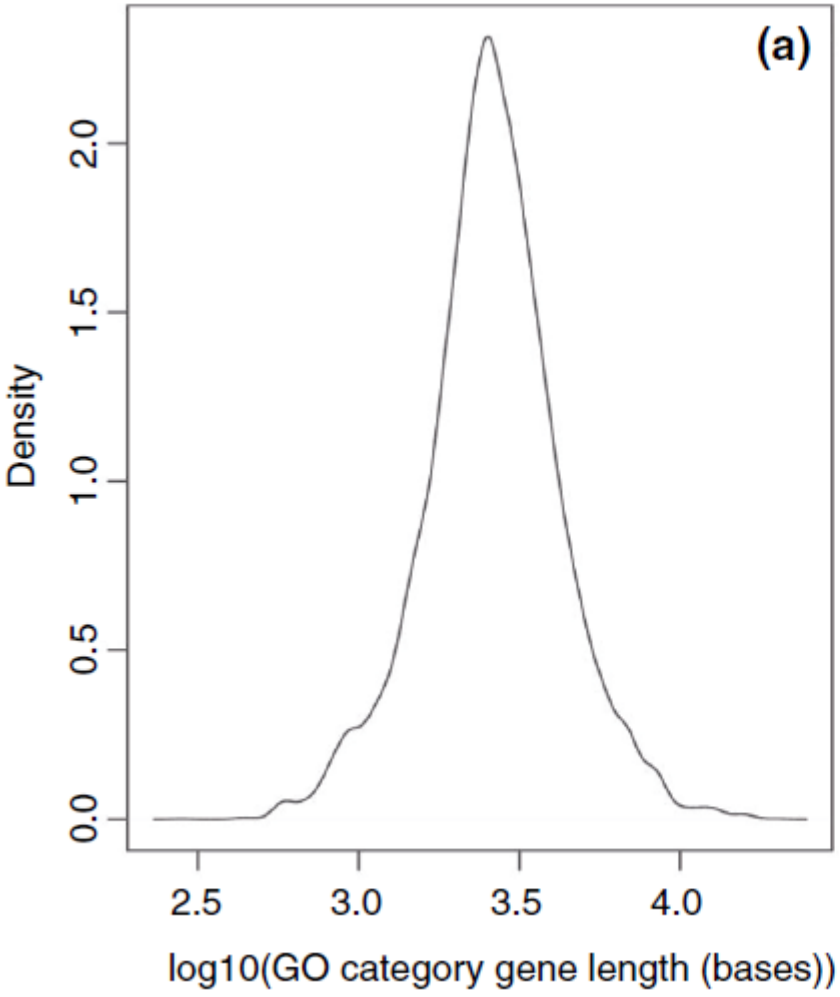
“Overlap” analysis: goana, DAVID, ToppFun, GOstats (& most web-based tools)

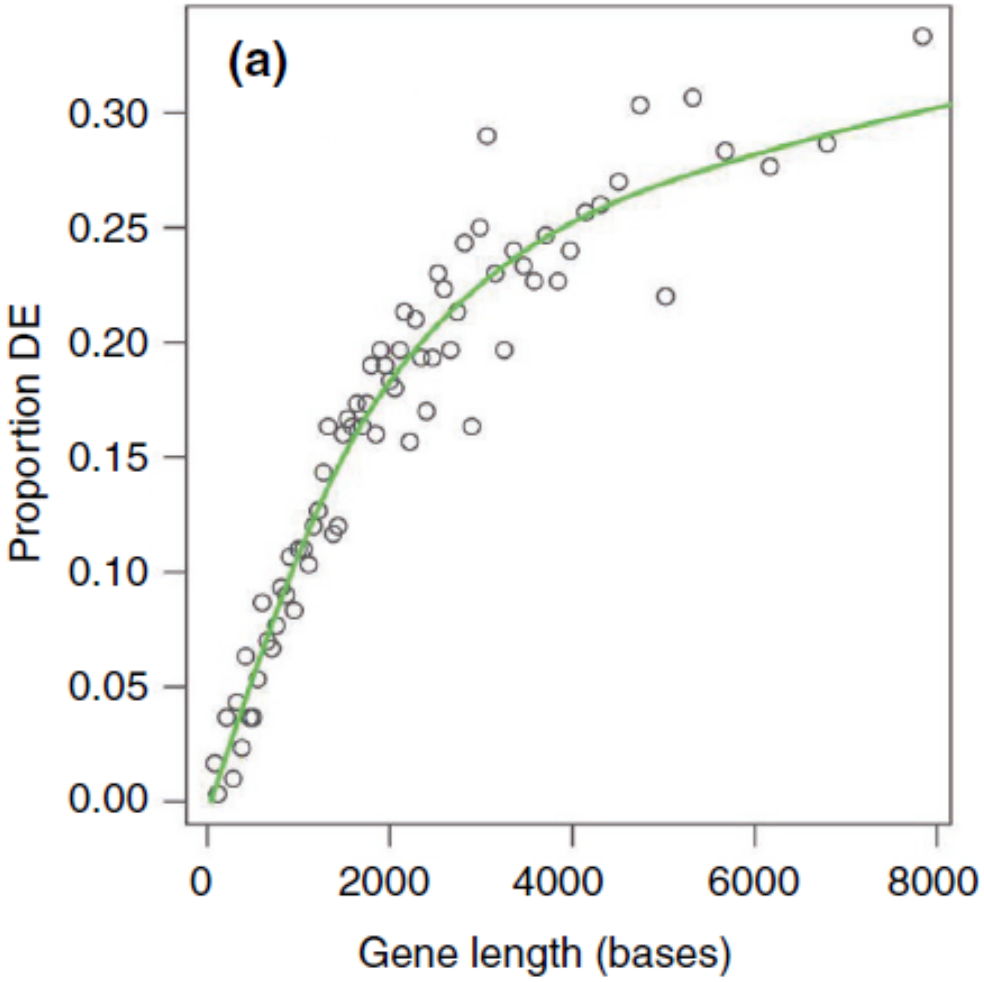


Problem: this test is biased due to the fact that longer genes tend to have more reads assigned to them

Oshlack and Wakefield (2009) Transcript length bias in RNA-seq data confounds systems biology, *Biology Direct*, 4:14.

GO categories have different avg gene lengths





GOseq, Young et al, 2010

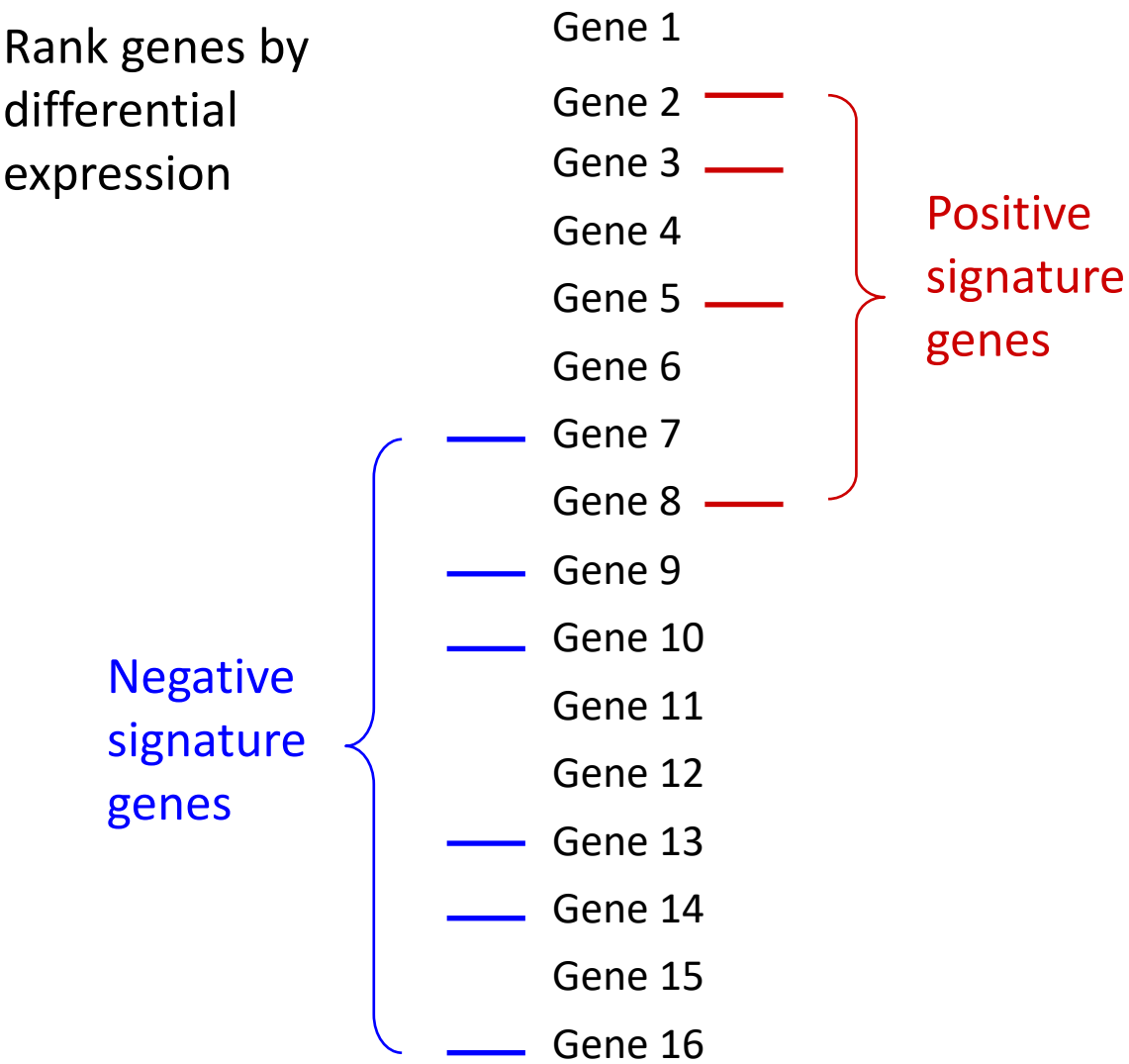
Solution: take into account gene length in your GO analysis

- `goana()` has the ability to take into account gene length using the "covariate" argument
- The GOseq bioconductor package contains the original method

CAMERA

- An "overlap" analysis assumes the genes are independent
- CAMERA tests the ranking of the gene set relative to the other genes in the experiment, while taking into account inter-gene correlations
- It also takes into account strength of evidence of DE by using the moderated *t*-statistics

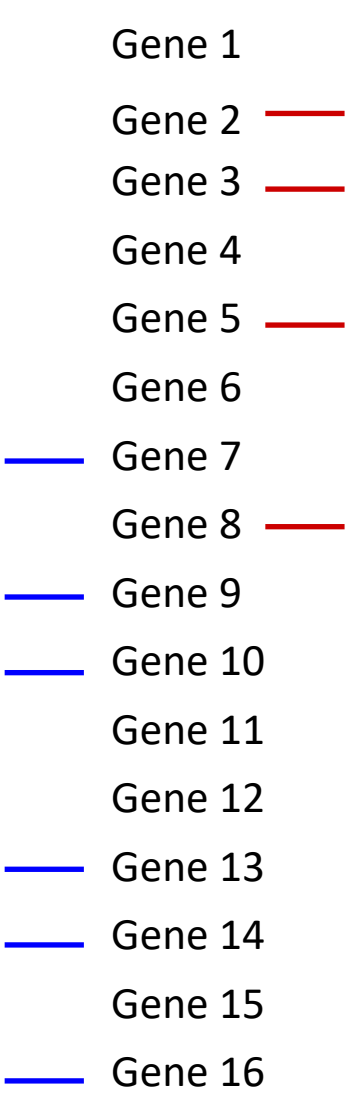
Rank genes and mark signature

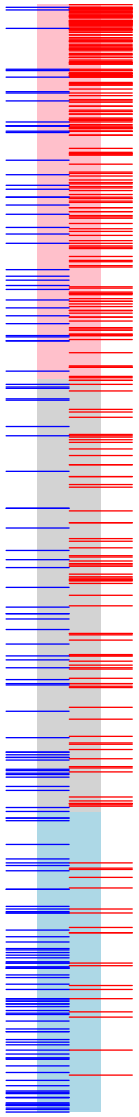


Slide courtesy of Gordon Smyth

Rank genes and mark signature

Rank genes by differential expression

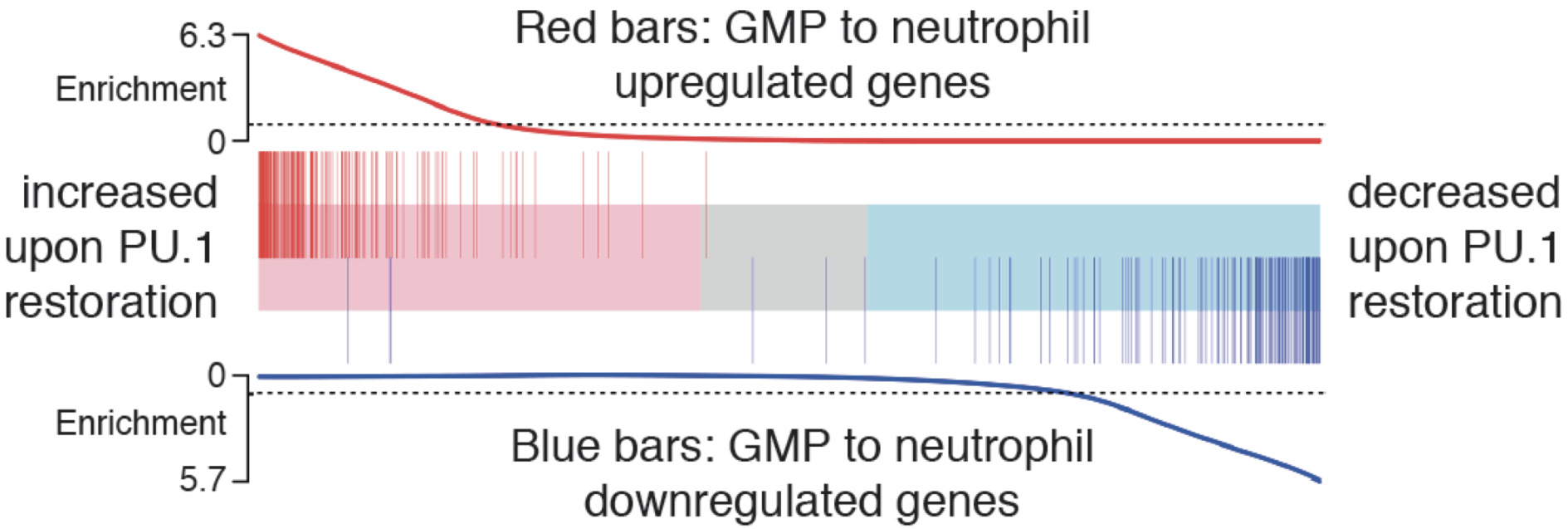




Genome-wide barcode plot

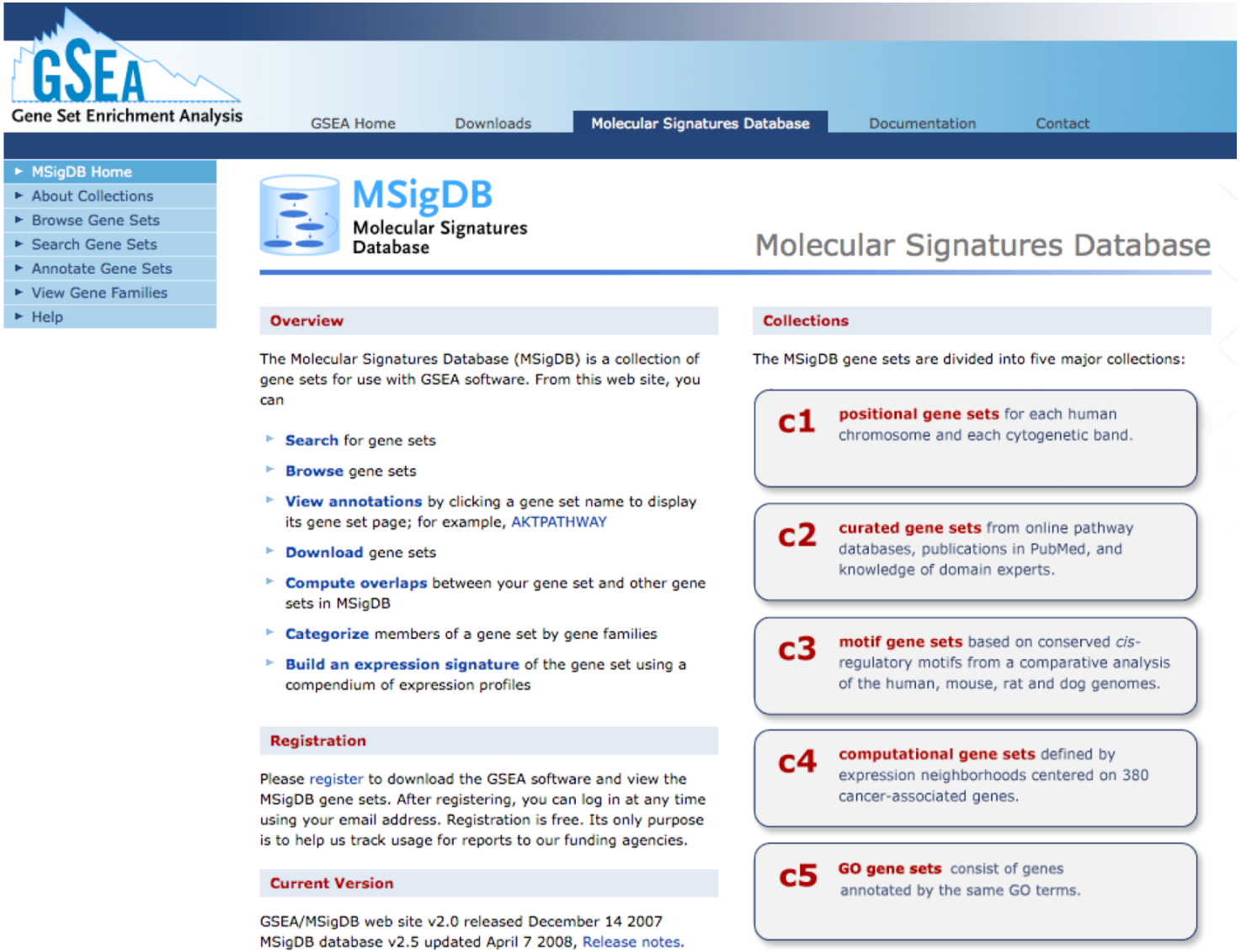
Slide courtesy of Gordon Smyth

Visualisation:
Barcodeplot + enrichment worm



Data courtesy of Mark McKenzie

Gene signature collections



ROAST gene set test

- The question asked is "Do the genes in this gene set tend to be differentially expressed?"
- It is NOT compared relative to other genes
- It is designed such that if > 25-50% of genes in the gene set are differentially expressed it will be significant
- It uses sophisticated techniques (rotation) to preserve gene-gene dependence in the data.
- fry is a fast implementation of roast that assumes constant gene-wise variance

Summary

- Gene set testing techniques range from simple (overlap analysis) to quite complex (CAMERA and ROAST)
- Which test you choose depends on what your hypothesis is
- Sometimes we just do them all…

Acknowledgements

- Gordon Smyth
- Belinda Phipson