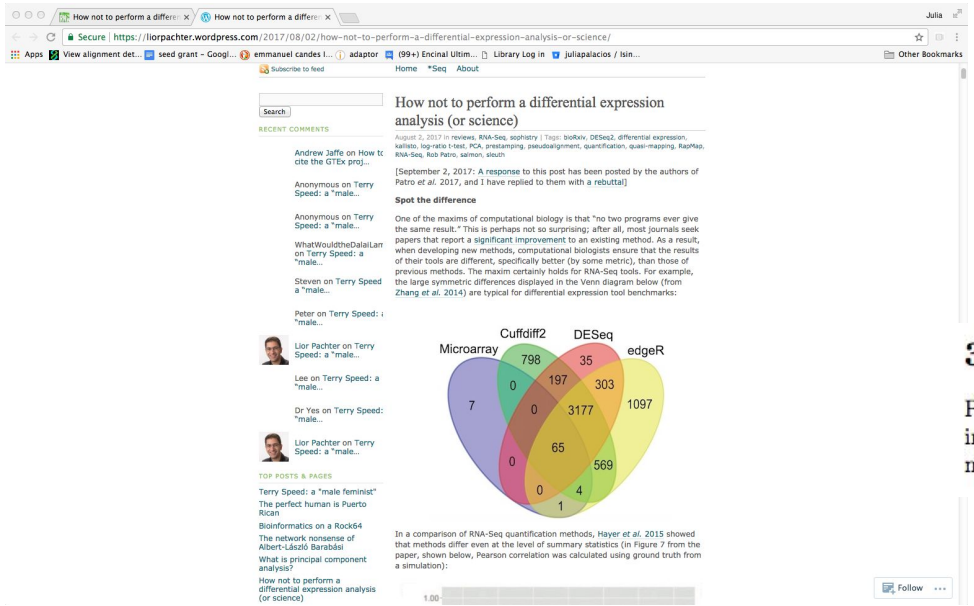
Lecture 5, Permutation Bootstrap tests

1. Permutation/ randomization tests in genomics
2. Failures of Randomization
3. Fixes
4. Bootstrap for RNAseq (JS lecture 6)

- Recall permutation tests: assume F=G and simulate the null distribution of the test statistic
  - How? X~F (1,..n); Y~G (1,..,m), permute X and Y by unif sampling from S_{m+n}

Why is resampling important in genomics?

(Unexplained/unknown/extreme) bias means exact null distributions for tests cannot be found in closed form



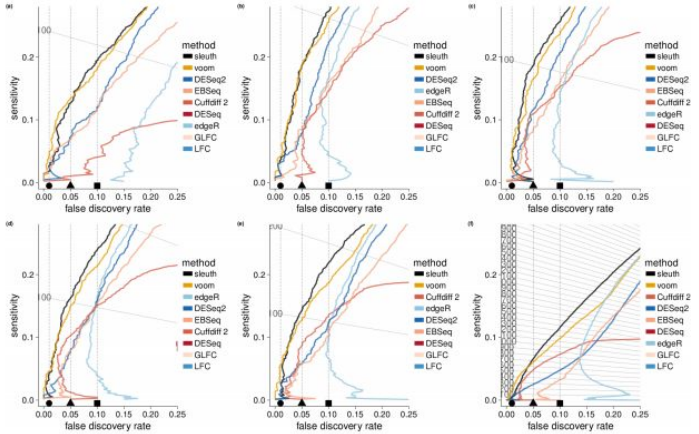
3 Filtering prior to parameter estimation

Prior to estimating parameters of the model we filter low abundance transcripts. This helps in fitting the model. We ignore transcripts where there are less than 5 estimated counts in more than 47% of the samples, i.e. when $|\{i : c_{ti} < 5\}| \geq 0.47 \cdot n$.

Why is resampling important in genomics?

Empirical performance raises ‘red flags’

https://media.nature.com/original/nature-assets/nmeth/journal/v14/n7/extref/nmeth.4324-S1.pdf



Supplementary Figure SN23: Zoomed in version of performance on independent isoform simulation at the isoform level stratified by number of isoforms per gene. (a), (b), ..., (f) contain isoforms with 1, 2, ..., 6+ isoforms per gene.

Quantification with RNA-seq in a world of bias

https://www.nature.com/articles/nmeth.4324

Differential analysis of RNA-seq incorporating quantification uncertainty

Harold Pimentel[1], Nicolas L Bray[2], Suzette Puente[3], Páll Melsted[4] & Lior Pachter[5]

We describe sleuth (http://pachterlab.github.io/sleuth), a method for the differential analysis of gene expression data that utilizes bootstrapping in conjunction with response error linear modeling to decouple biological variance from inferential variance. sleuth is implemented in an interactive shiny app that utilizes kallisto quantifications and bootstraps for fast and accurate analysis of data from RNA-seq experiments.

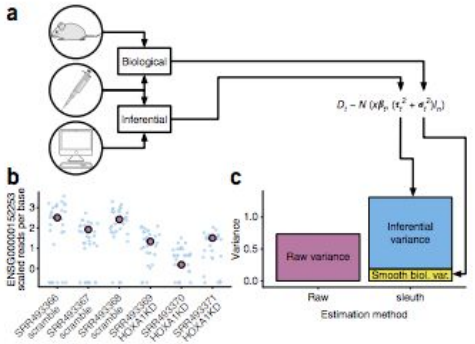
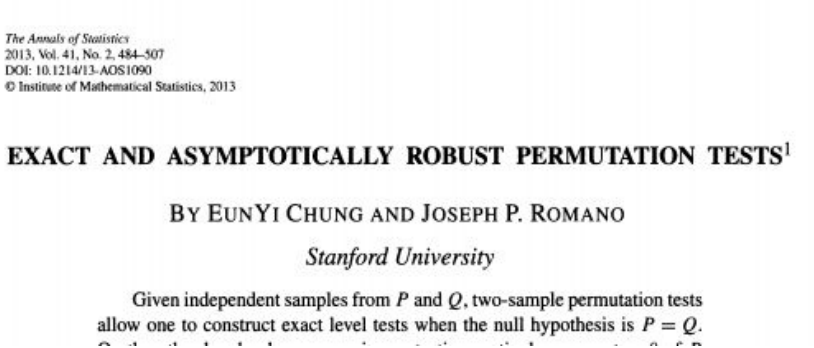


Figure 1 | Overview of sleuth. (**a**) sleuth models different sources of variance to predict differentially expressed transcripts and genes. Biological variance (biol. var.) results from differences in RNA content between replicates and from stochastic biochemistry during library preparation, while inferential variance arises from random sequencing and computational analysis of reads. See Online Methods for description of terms. (**b**) Results for an example gene after running kallisto on RNA-seq data from Trapnell *et al.*[12] generated from human lung fibroblasts transfected with scrambled siRNA (scramble condition) and HOXA1 siRNA (HOXA1KD condition). DESeq2 and voom identify the gene as differentially expressed, but high inferential variance causes sleuth to find no difference. Red dots, point estimates. Blue dots, results for bootstrap samples to assess inferential variance. (**c**) The between-sample raw variance leads to a small estimated biological variance that fails to account for uncertainty introduced when quantifying the samples.

Failures of Permutation/randomization tests



The Annals of Statistics
2013, Vol. 41, No. 2, 484–507
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EXACT AND ASYMPTOTICALLY ROBUST PERMUTATION TESTS[1]

BY EUNYI CHUNG AND JOSEPH P. ROMANO

Stanford University

Given independent samples from P and Q, two-sample permutation tests allow one to construct exact level tests when the null hypothesis is $P = Q$.

On the other hand, when comparing or testing particular parameters θ of P and Q, such as their means or medians, permutation tests need not be level α, or even approximately level α in large samples. Under very weak assump-

Romano, Annals Stats 2013

Failures of Permutation/randomization tests

TABLE 1

Monte Carlo simulation results for studentized permutation median test (one-sided, $\alpha = 0.05$)

Distributions		m: / n:	5 / 5	13 / 21	51 / 101	101 / 101	101 / 201	201 / 201	401 / 401
$N(0, 1)$	Not studentized		0.1079	0.1524	0.1324	0.2309	0.2266	0.2266	0.2249
$N(0, 1)$	Not studentized		0.0646	0.1871	0.2411	0.1769	0.1849	0.1849	0.1853
Logistic(0, 1)	Not studentized		0.0991	0.1413	0.1237	0.2258	0.2233	0.2233	0.2261
Laplace(ln 2, 1)	Not studentized		0.0420	0.0462	0.0477	0.048	0.0493	0.0461	0.0501

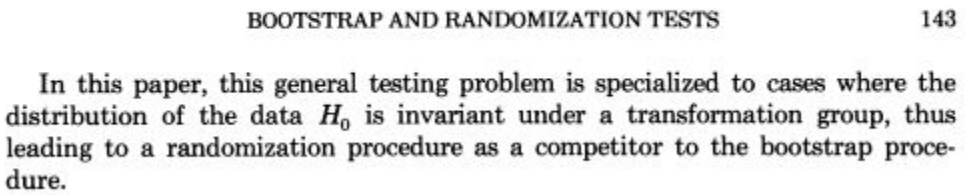
? why and fixes

Data from Romano et al, 2013

Permutation Bootstrap tests

Intuition:

- Bootstrap tests: resample X and Y from their empirical distribution
- Permutation tests: assume F=G and simulate the null distribution of the test statistic
  - How? X~F (1,..n); Y~G (1,...,m), permute X and Y by unif sampling from S_{m+n}



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In this paper, this general testing problem is specialized to cases where the distribution of the data H_0 is invariant under a transformation group, thus leading to a randomization procedure as a competitor to the bootstrap procedure.

Romano, Annals Stats 1989

Permutation vs Bootstrap tests

- Permutation tests and Bootstrap tests: asymptotically equivalent but permutation tests can be exact
- Bootstrap- cannot give exact tests in finite samples
  - May be better to use when sample sizes are unequal and “fix” isn’t known
- Pitfalls- but correctable in some cases

the power of both tests tends to the same value. The result is that the power functions of both tests may be said to be asymptotically equivalent. Hence, the randomization test may be preferable since it has exact level α for finite samples.

Romano, Annals Stats 1989