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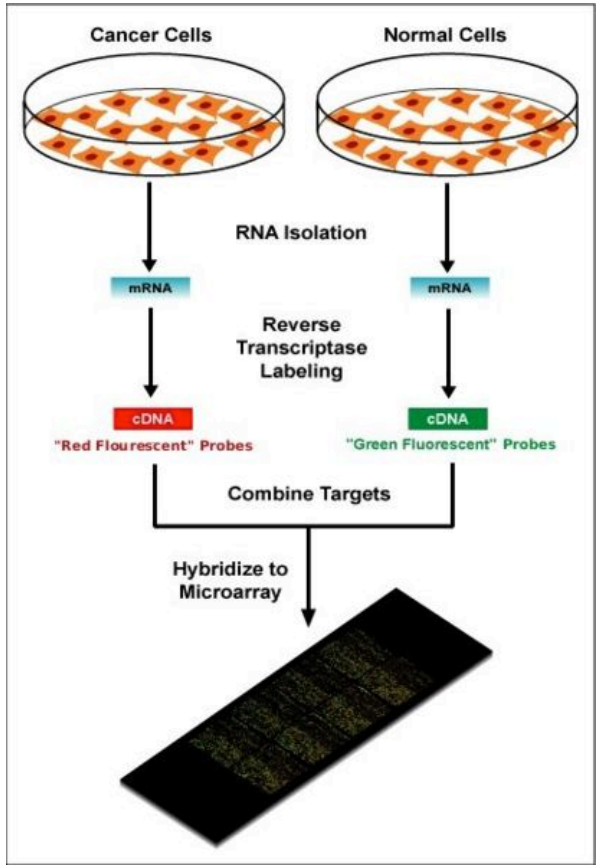
Lecture part: BioC2014
Differential gene- and exon-level expression analyses for RNA-seq data using edgeR, voom and featureCounts

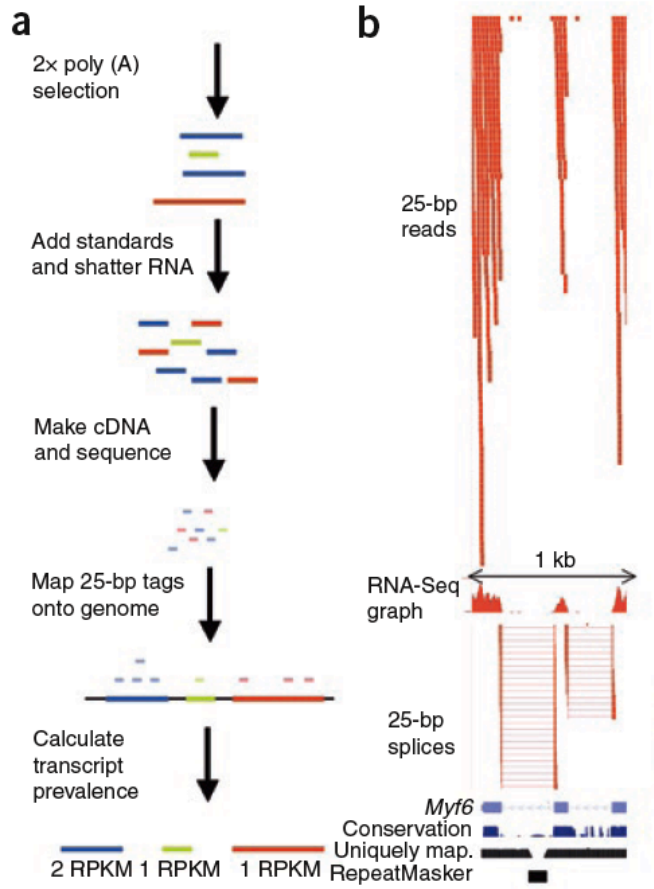
Mark D. Robinson, University of Zurich

Abundance by Fluorescence Intensity



http://en.wikipedia.org/wiki/DNA_microarray

Abundance by Counting



Mortazavi et al., Nature Methods, 2008

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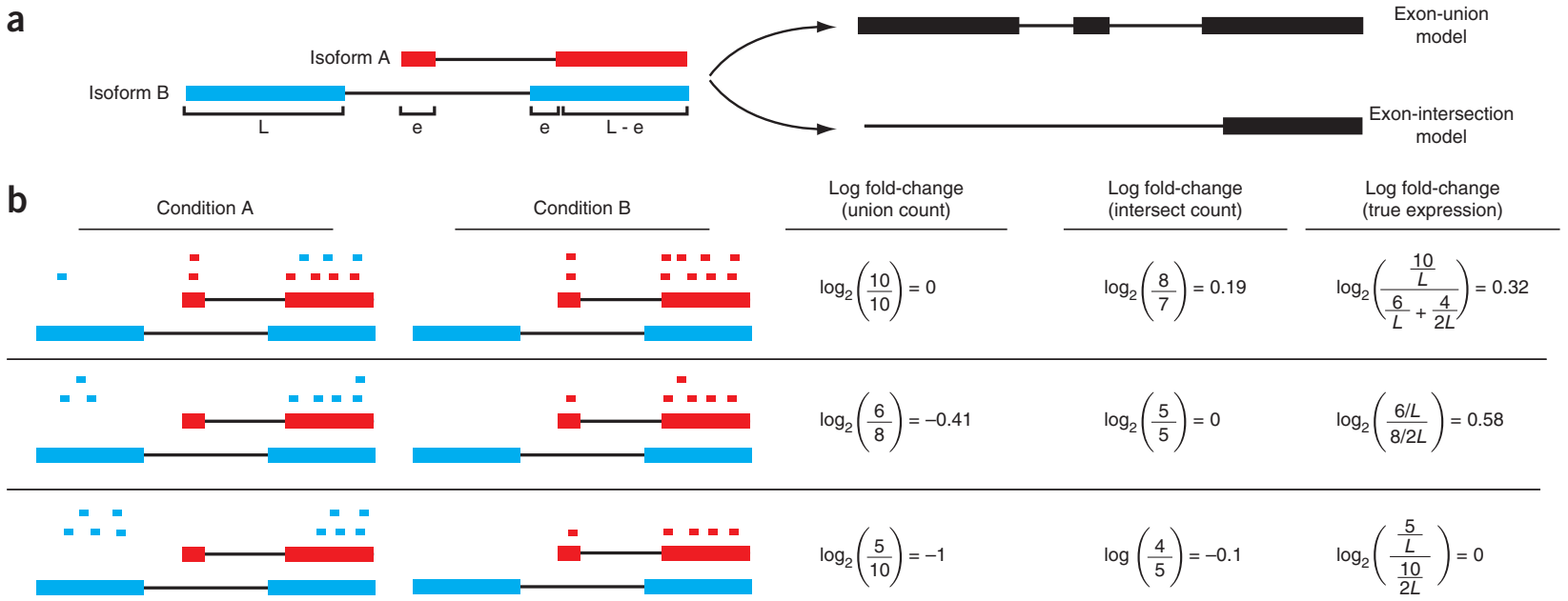
Gene-level counting: issues can be dealt with at second step

Trapnell et al. 2013 Nat Biotech



Transcriptome analysis of human tissues and cell lines reveals one dominant transcript per gene

Mar Gonzàlez-Porta[1], Adam Frankish[2], Johan Rung[1], Jennifer Harrow[2] and Alvis Brazma[1*]

University of Zurich[UZH]

Data analysis pipelines for RNA-seq differential expression

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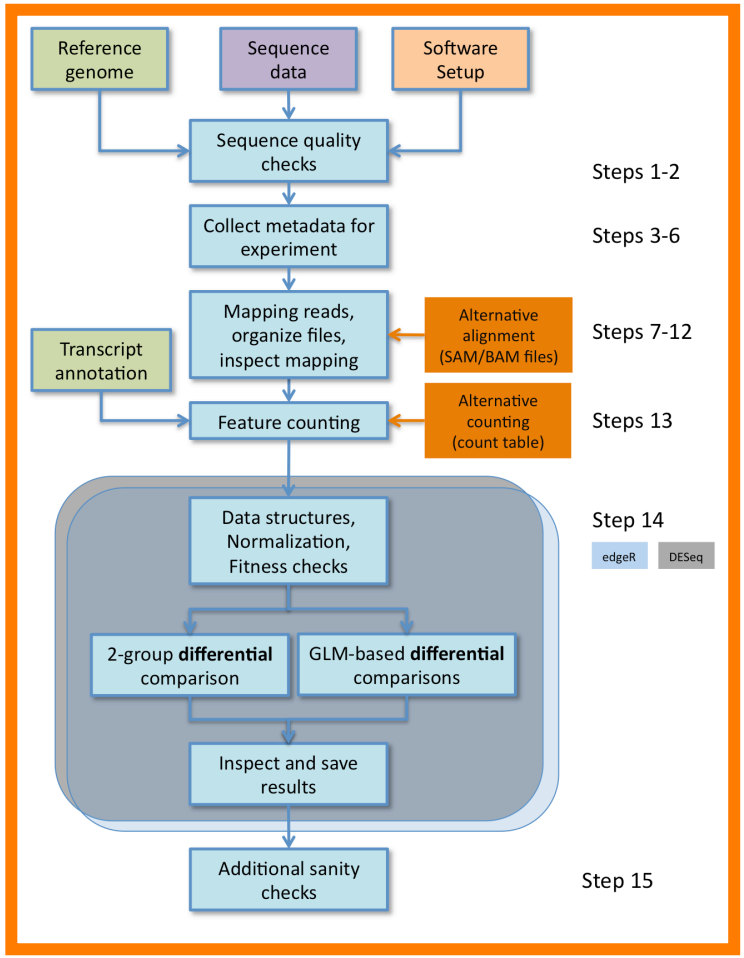
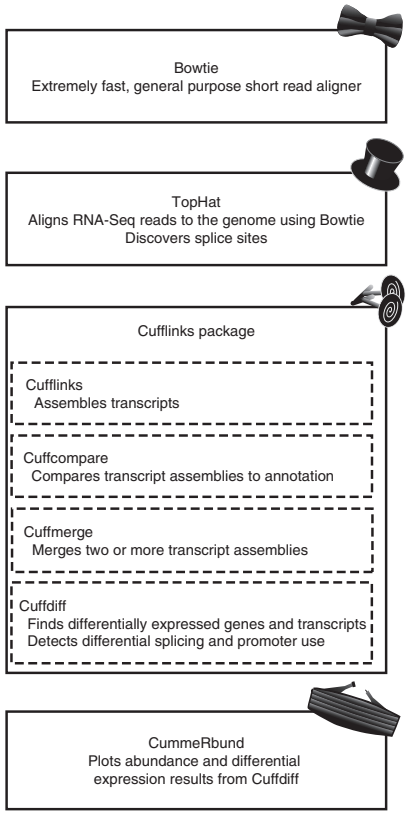
edgeR, DESeq



cufflinks, cuffdiff



Trinity

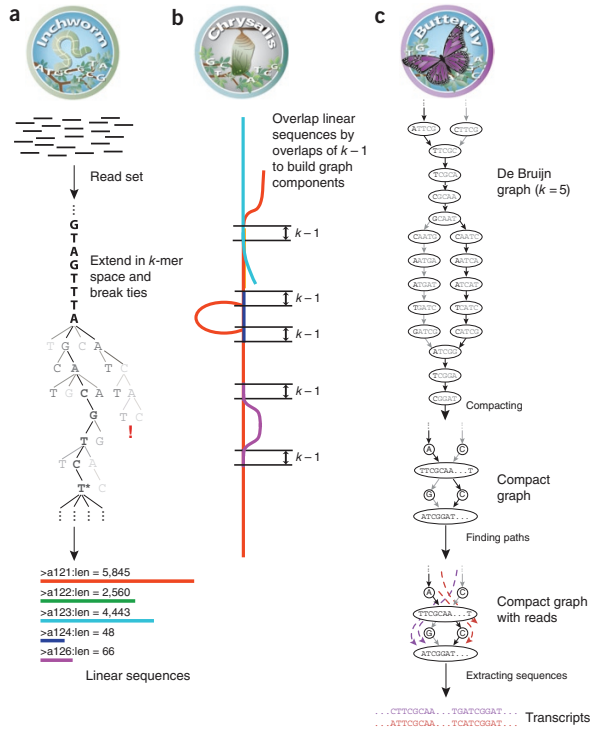


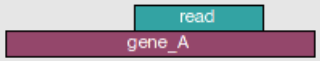
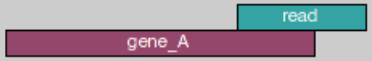
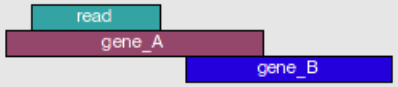
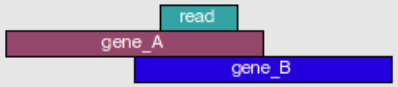
Figure 1 Overview of Trinity. (**a**) Inchworm assembles the read data set (short black lines, top) by greedily searching for paths in a *k*-mer graph (middle), resulting in a collection of linear contigs (color lines, bottom), with each *k*-mer present only once in the contigs. (**b**) Chrysalis pools contigs (colored lines) if they share at least one *k* – 1-mer and if reads span the junction between contigs, and then it builds individual de Bruijn graphs from each pool. (**c**) Butterfly takes each de Bruijn graph from Chrysalis (top), and trims spurious edges and compacts linear paths (middle). It then reconciles the graph with reads (dashed colored arrows, bottom) and pairs (not shown), and outputs one linear sequence for each splice form and/or paralogous transcript represented in the graph (bottom, colored sequences).

Nature Protocols September 2013 (preprint at http://arxiv.org/pdf/1302.3685v3.pdf)

Counting: a few considerations (gene-level)

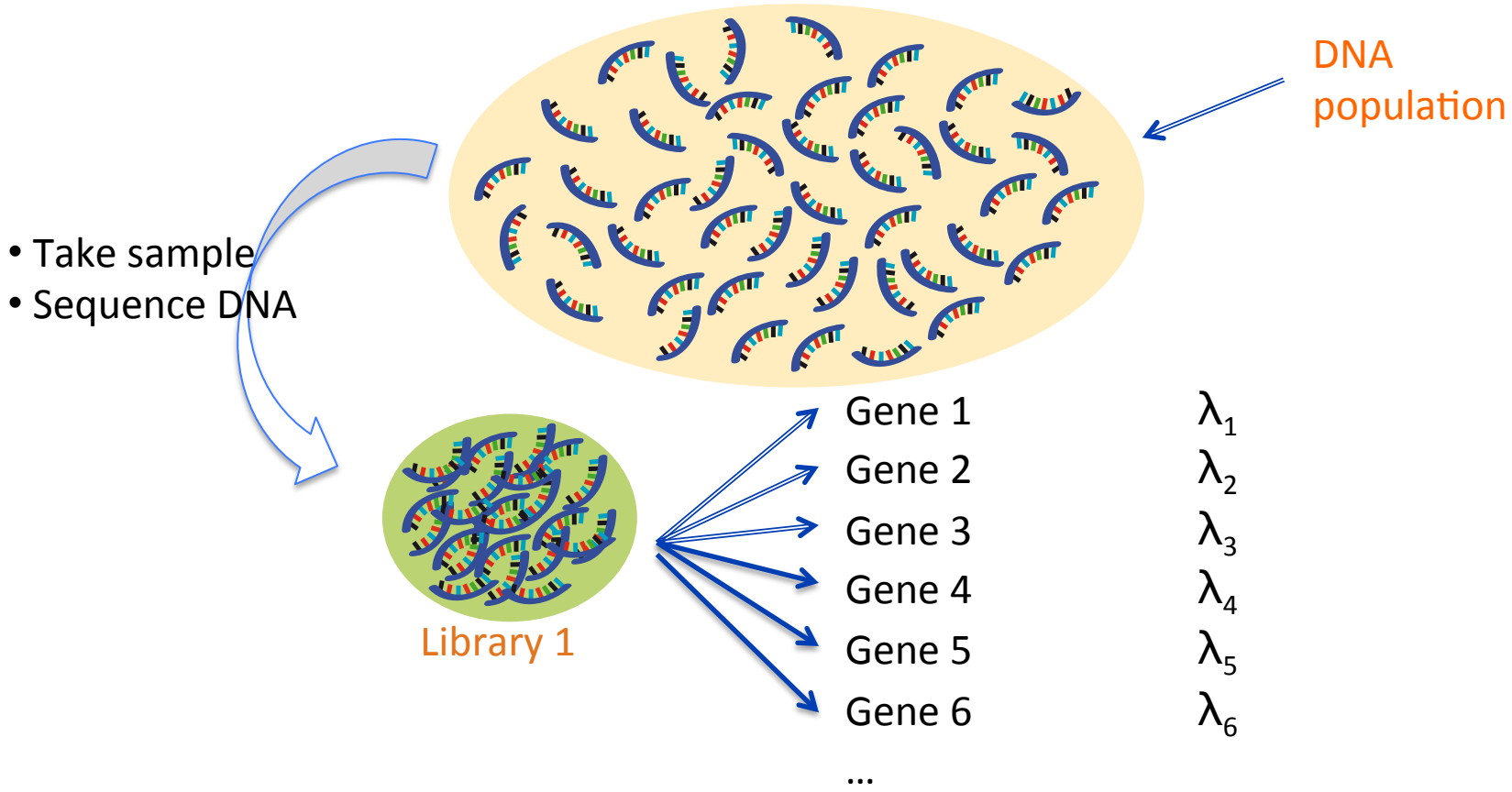
All the downstream statistical methods start with a count table.

- annotation-based? What about novel genes?
- gene-level versus transcript-level? versus exon-level?
- ambiguities

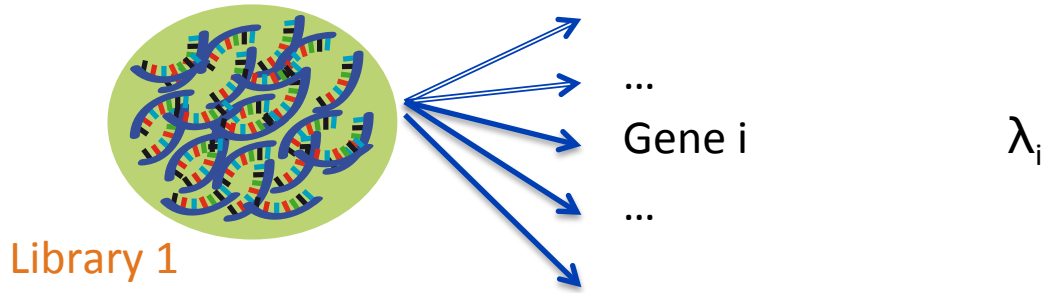
	union	intersection_strict	intersection_nonempty
	gene_A	gene_A	gene_A
	gene_A	no_feature	gene_A
	gene_A	no_feature	gene_A
	gene_A	gene_A	gene_A
	gene_A	gene_A	gene_A
	ambiguous	gene_A	gene_A
	ambiguous	ambiguous	ambiguous

http://www-huber.embl.de/users/anders/HTSeq/doc/overview.html

Sampling reads from population of DNA fragment is multinomial



For a single gene, it's a coin toss, i.e. Binomial

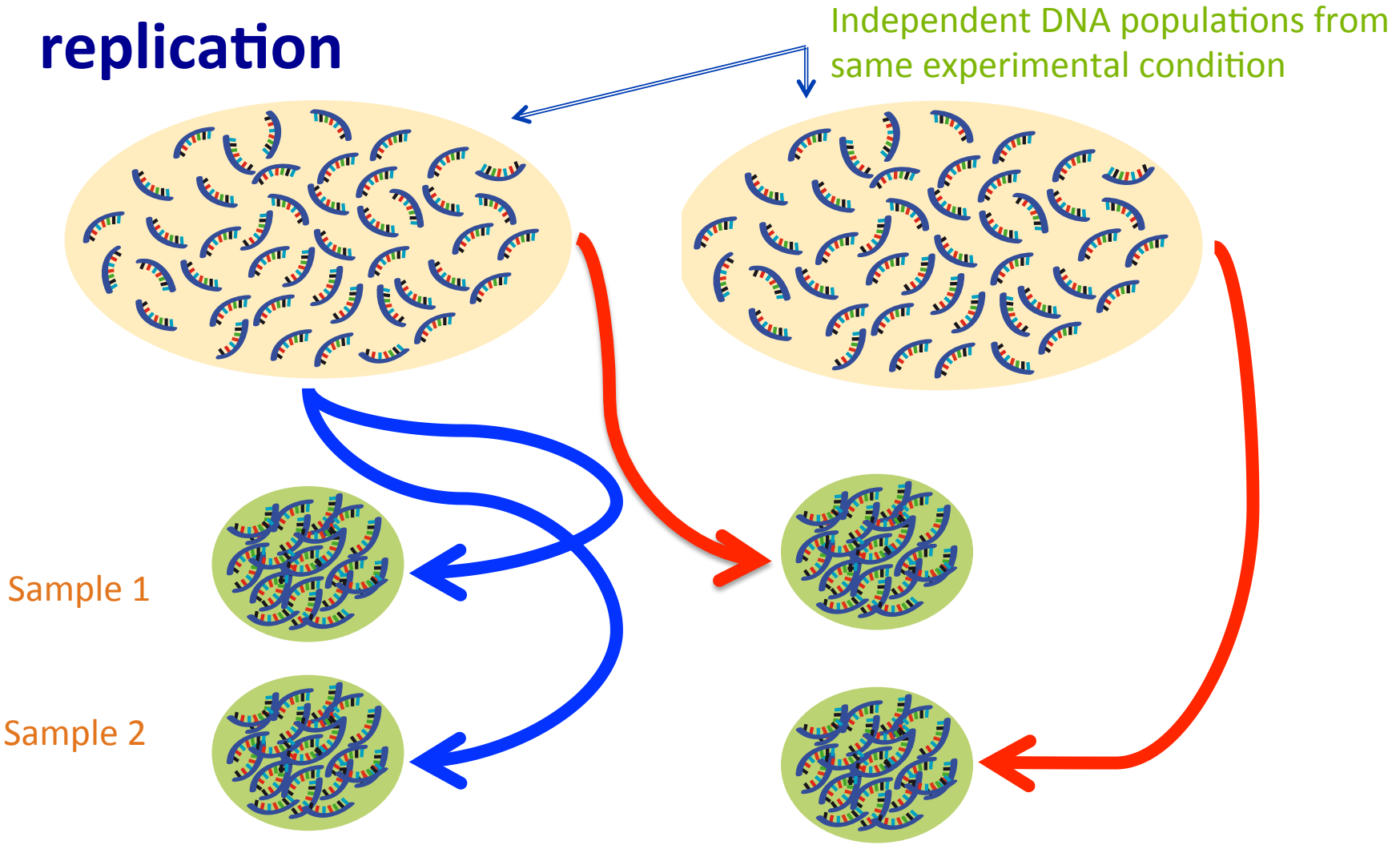


$Y_i \sim \text{Binomial}(M, \lambda_i)$

Y_i - observed number of reads for gene i
M - total number of sequences
λ_i - proportion

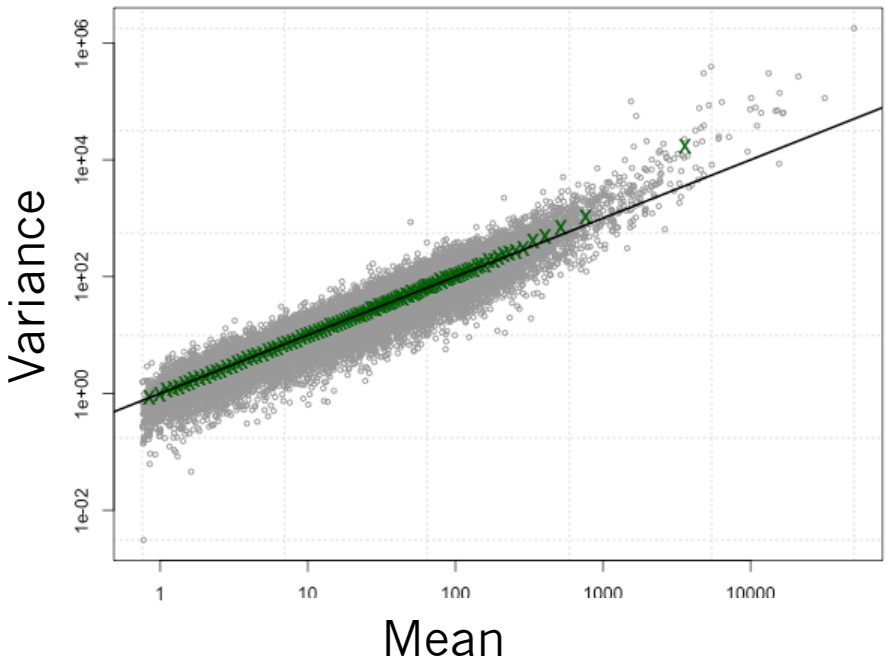
Large M, small λ_i → approximated well by Poisson($\mu_i = M \bullet \lambda_i$)

Technical replication versus biological replication



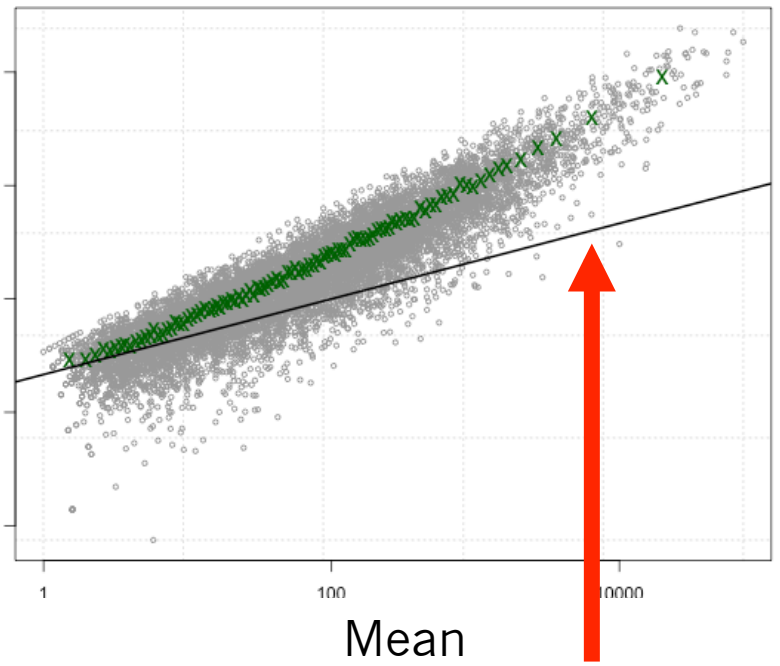
Mean-Variance plots: What we see in real data

Technical replicates



Data from Marioni et al. Genome Research 2008

Biological replicates

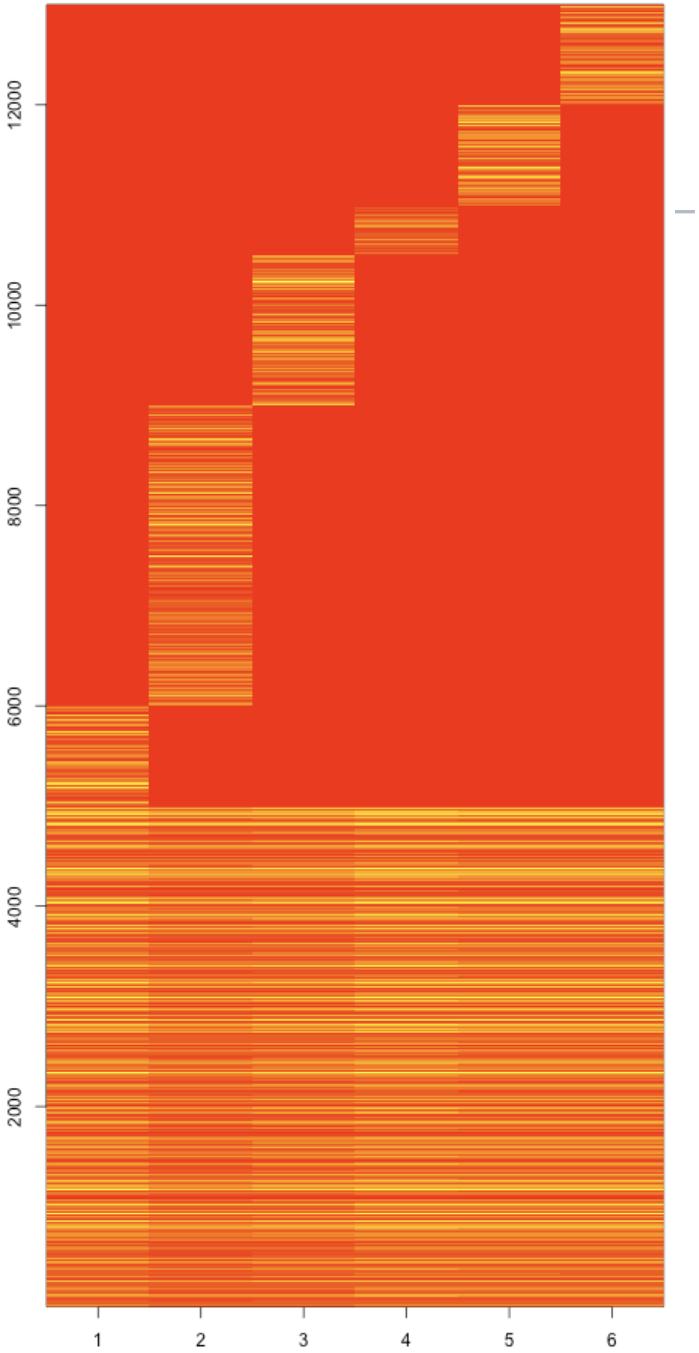


Data from Parikh et al. *Genome Biology* 2010

mean=variance
(Poisson assumption)

Normalization: "Composition" or "Diversity" can affect read depth

- Hypothetical example: Sequence 6 libraries to the **same** depth, with varying levels of *unique-to-sample* counts
- Read depth is affected not only by expression (and length), but also expression levels of other genes
- Composition can induce (sometimes significant) differences in counts



Red=low, goldenyellow=high

Robinson and Oshlack (2010) Genome Biology

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Model assumptions

M_j = library size
λ_{ij} = relative abundance of feature i

Poisson describes technical variation:

$$Y_{ij} \sim Pois(M_j * \lambda_{ij})$$

$$mean(Y_{ij}) = variance(Y_{ij}) = M_j * \lambda_{ij}$$

Negative binomial models **biological** variability using the dispersion parameter φ:

$$Y_{ij} \sim NB(\mu_{ij} = M_j * \lambda_{ij} , \varphi_i)$$

Same mean, variance is quadratic in the mean:

$$variance(Y_{ij}) = \mu_{ij} (1 + \mu_{ij} \varphi_i)$$

Critical parameter to estimate: dispersion

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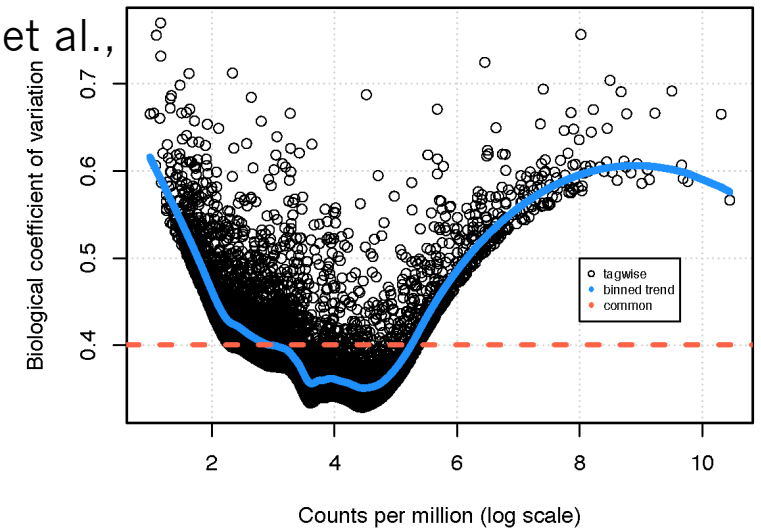
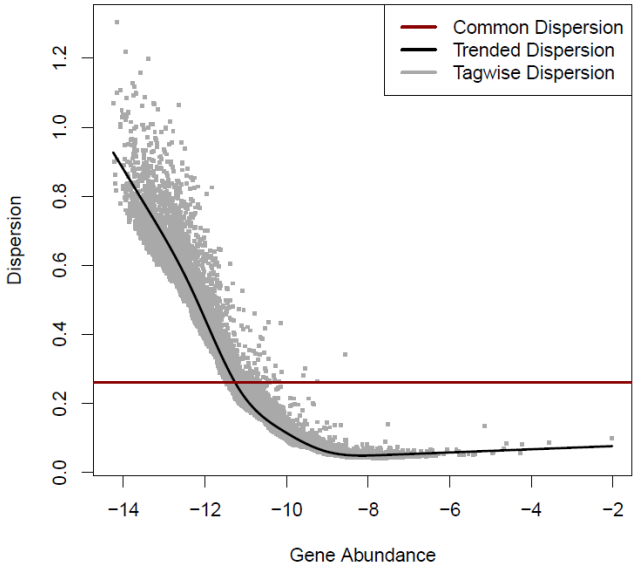
edgeR dispersion estimation: moderate towards trend

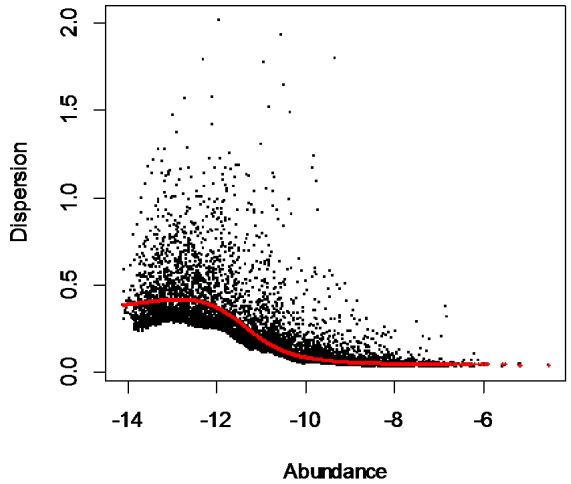
Data:
Tuch et al., 2008





Mouse hemapoeitic stem cells, (Samir Taoudi)

Advantage: share information, but genes are allowed to have their own variance.



Mouse lymphomas (Stan Lee)

Davis McCarthy

Flexibility for various experimental designs: Generalized linear modeling

Response: negative binomial with dispersion fixed (to make it in the exponential family).

Link function (relate mean of response to linear combination of parameters)

For example:

$$Y_i \sim \mathrm{NB}(\mu_i, \phi)$$
$$\mathbf{X}\boldsymbol{\beta} = \ln(\mu)$$

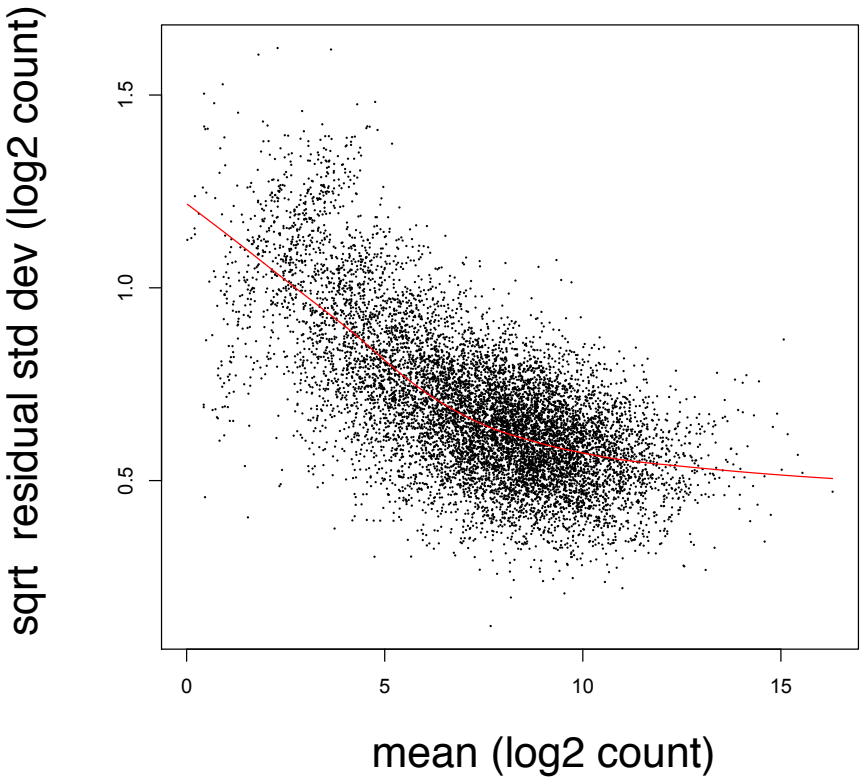
X – design matrix
ln() – link function
β – parameters

Applicability to a wide range of designs

McCarthy et al. 2012, NAR

Voom method

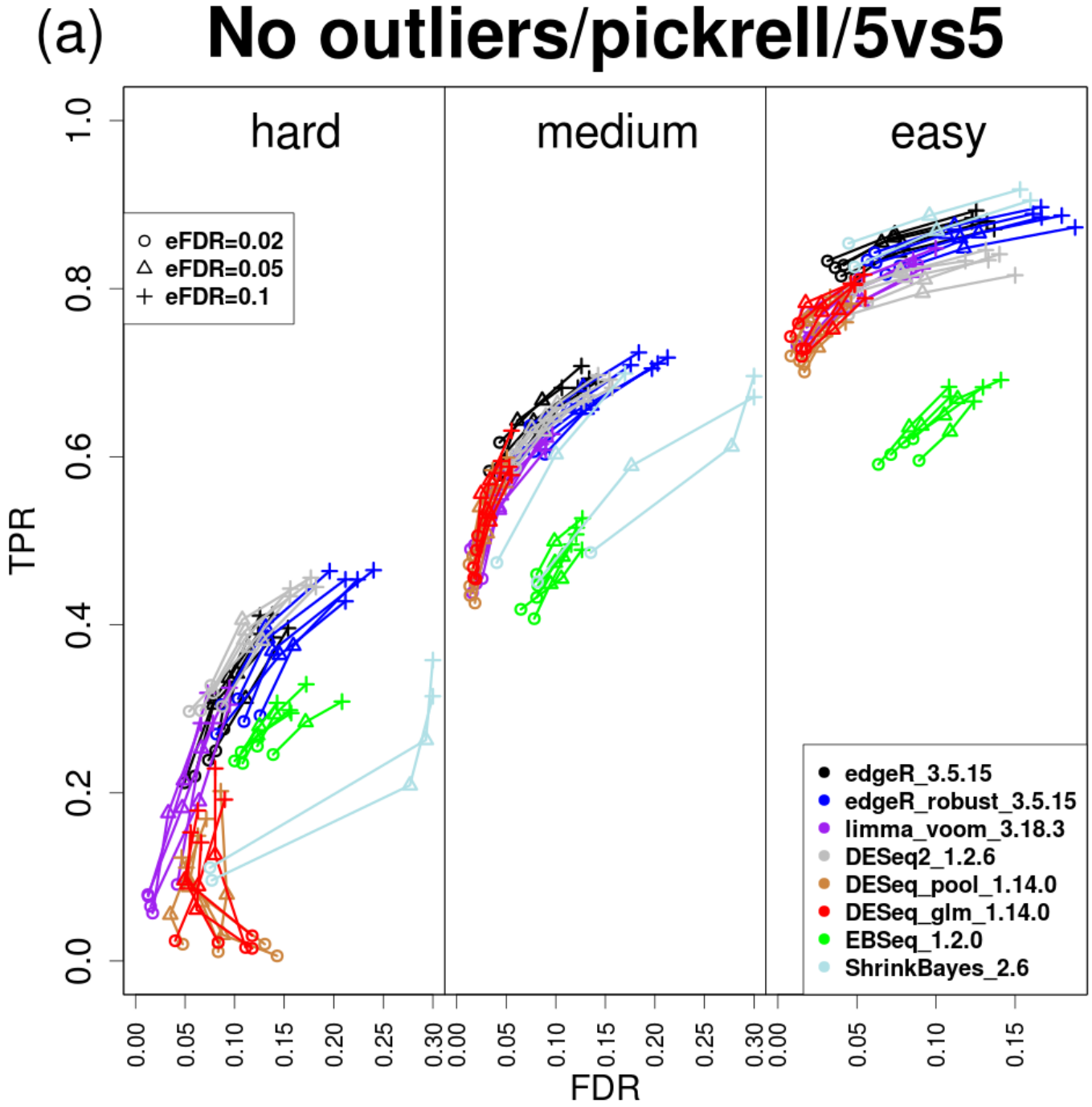
- Converts discrete counts to (log-cpm)
- Removes trend in the variance of counts
- Estimate variances and use inverse as weight



Voom: precision weights unlock linear model analysis tools for RNA-seq read counts. Law et al. 2014. Genome Biology.2014, 15:R29.

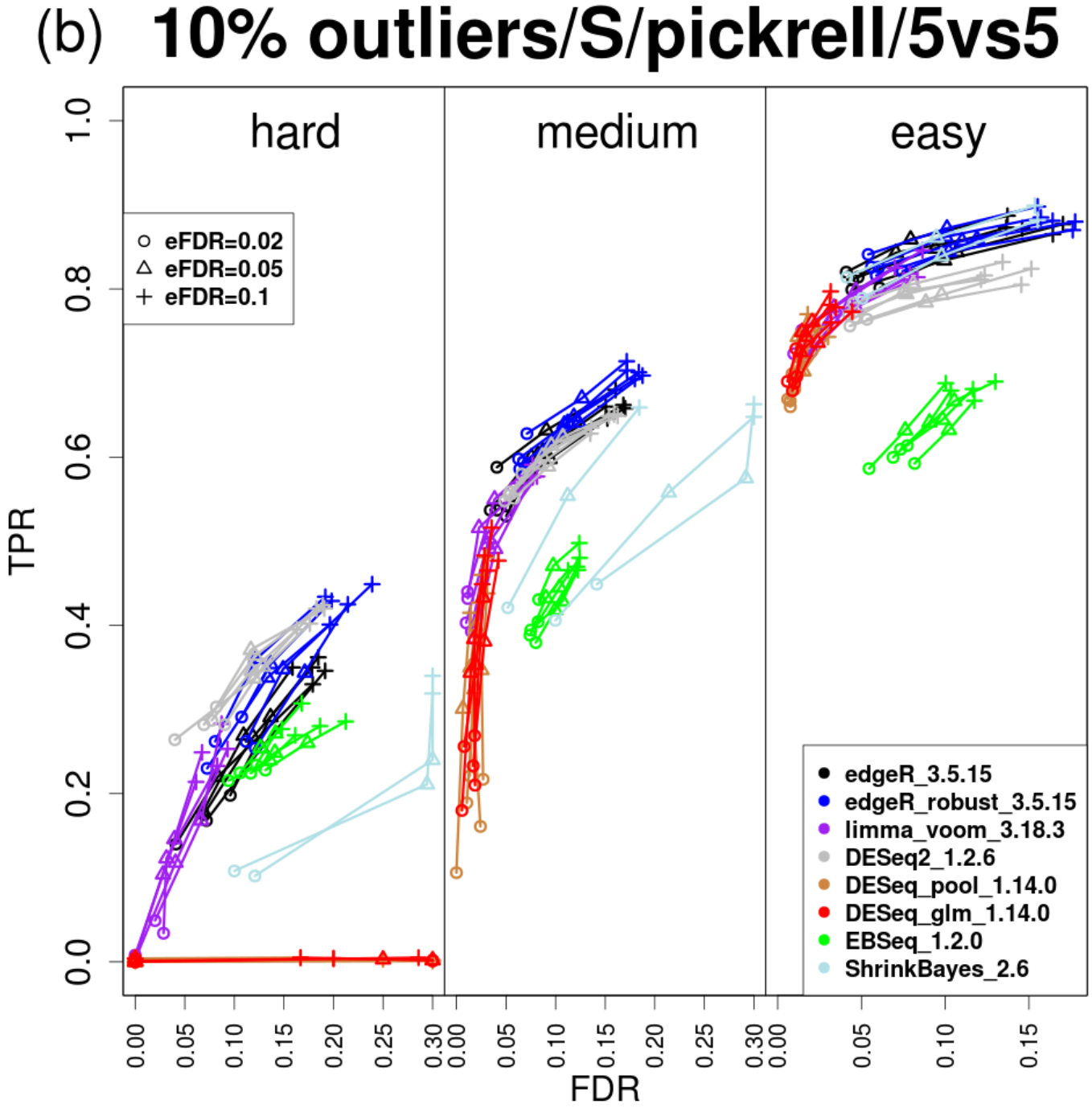
(slide from Charity Law)

Simulation-based comparisons: do methods achieve their FDRs?



Zhou et al., NAR, 2014

Simulation-based comparisons: do methods achieve their FDRs?



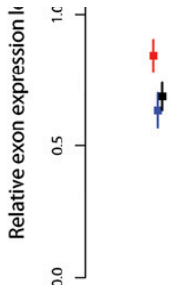
Zhou et al., NAR, 2014

Beyond differential expression: differential splicing

Prediction of alternative isoforms from exon expression levels in RNA-Seq experiments

Hugues Richard[1,*], Marcel H. Schulz[1,2], Marc Sultan[3], Asja Nürnberger[3], Sabine Schrinner[3], Daniela Balzereit[3], Emilie Dagand[3], Axel Rasche[3], Hans Lehrach[3], Martin Vingron[1], Stefan A. Haas[1] and Marie-Laure Yaspo[3]

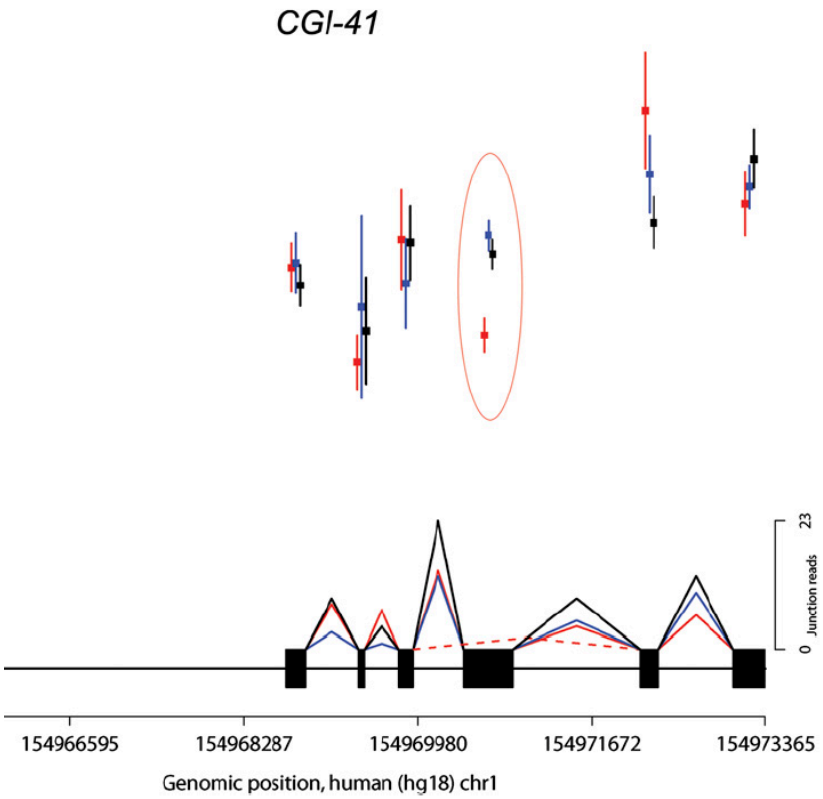
[1]Department of Computational Molecular Biology, Max Planck Institute for Molecular Genetics, Ihnestr. 73, [2]International Max Planck Research School for Computational Biology and Scientific Computing, and [3]Department of Vertebrate Genomics, Max Planck Institute for Molecular Genetics, Ihnestr. 73, 14195 Berlin, Germany



Sex-specific and lineage-specific alternative splicing in primates

Ran Blekhman,[1,4,5] John C. Marioni,[1,4,5] Paul Zumbo,[2] Matthew Stephens,[1,3,5] and Yoav Gilad[1,5]

[1]Department of Human Genetics, University of Chicago, Chicago, Illinois 60637, USA; [2]Keck Biotechnology Laboratory, New Haven, Connecticut 06511, USA; [3]Department of Statistics, University of Chicago, Chicago, Illinois 60637, USA



Counting: a few considerations (exon-level)

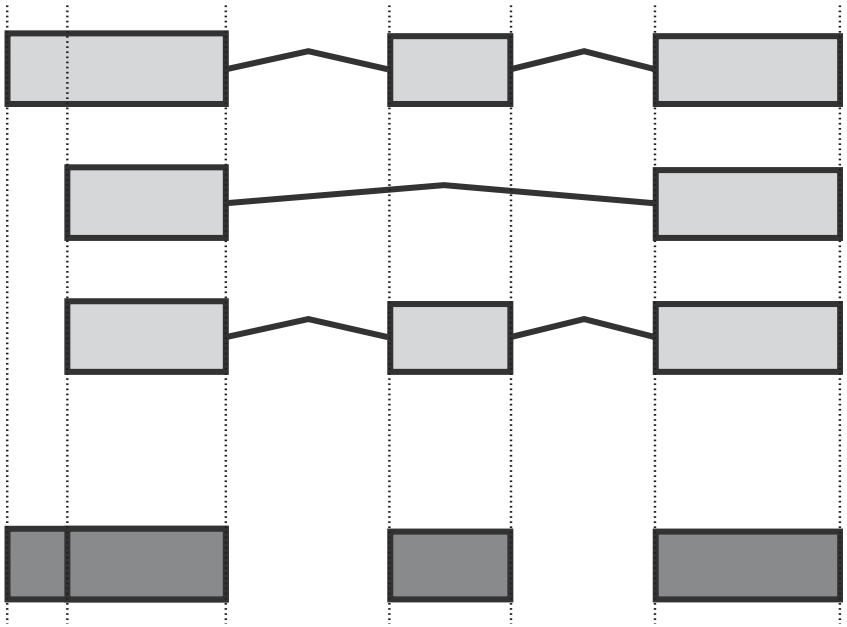


Figure 1. Flattening of gene models: This (fictional) gene has three annotated transcripts involving three exons (light shading), one of which has alternative boundaries. We form counting bins (dark shaded boxes) from the exons as depicted; the exon of variable length gets split into two bins.

Anders et al. 2012 Genome Research

DEXSeq – general structure

We use generalized linear models (GLMs) (McCullagh and Nelder 1989) to model read counts. Specifically, we assume K_{ijl} to follow a negative binomial (NB) distribution:

$$K_{ijl} \sim NB\left(\text{mean} = s_j \mu_{ijl}, \text{dispersion} = \alpha_{il}\right), \qquad (1)$$

where α_{il} is the dispersion parameter (a measure of the distribution's spread; see below) for counting bin (i, l), and the mean is predicted via a log-linear model as

$$\log \mu_{ijl} = \beta_i^{\mathrm{G}} + \beta_{il}^{\mathrm{E}} + \beta_{i\rho_j}^{\mathrm{C}} + \beta_{i\rho_j l}^{\mathrm{EC}}. \qquad (2)$$

i – gene
j – sample … ρ_j is condition (categorical)
l – bin

β^G – baseline "expression strength"
β^E – "exon" (bin) effect
β^C – condition effect
β^{EC} – condition x "exon" interaction

Method

Detecting differential usage of exons from RNA-seq data

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European Molecular Biology Laboratory, 69111 Heidelberg, Germany