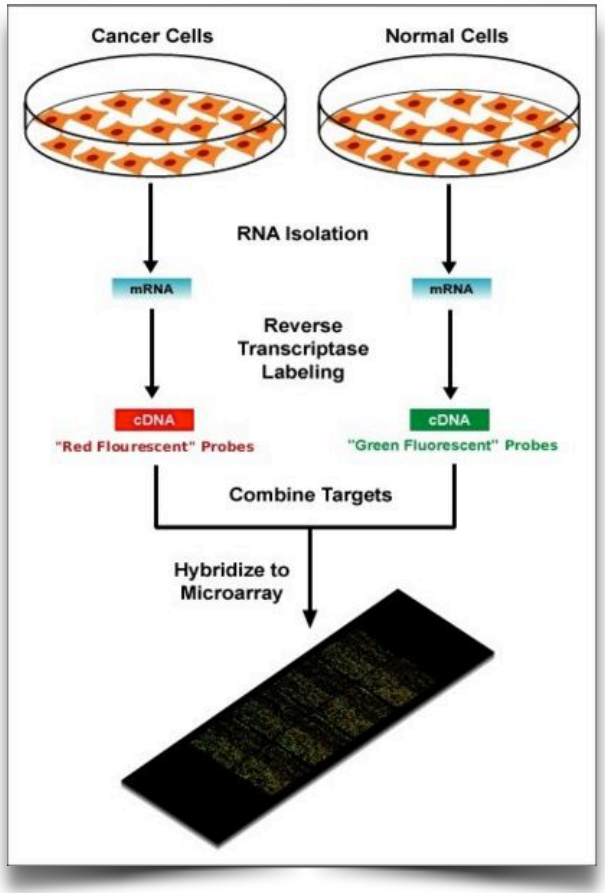
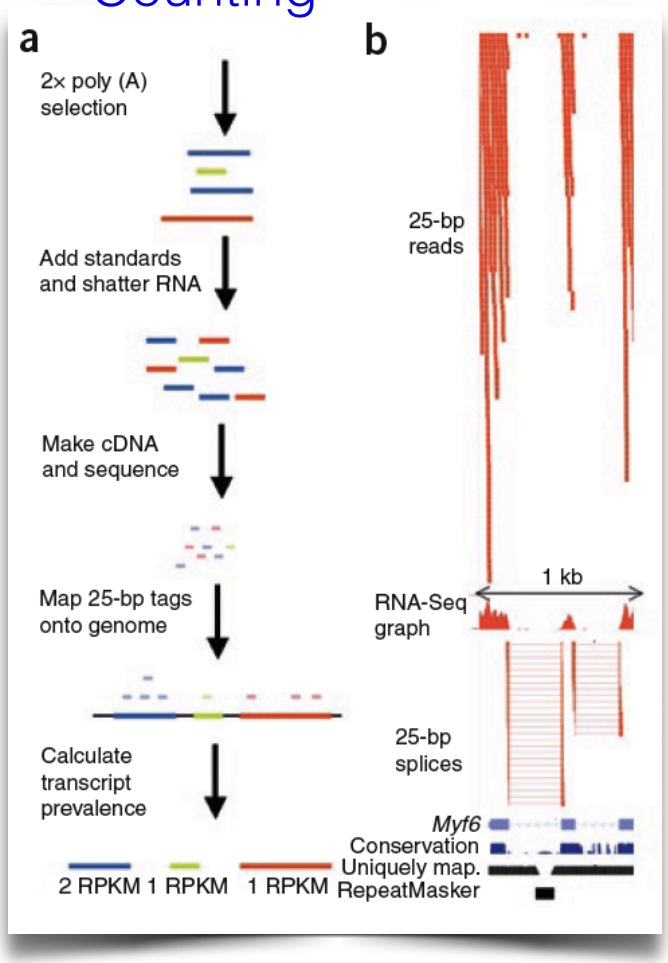
Count data analysis

Abundance by Fluorescence Intensity



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

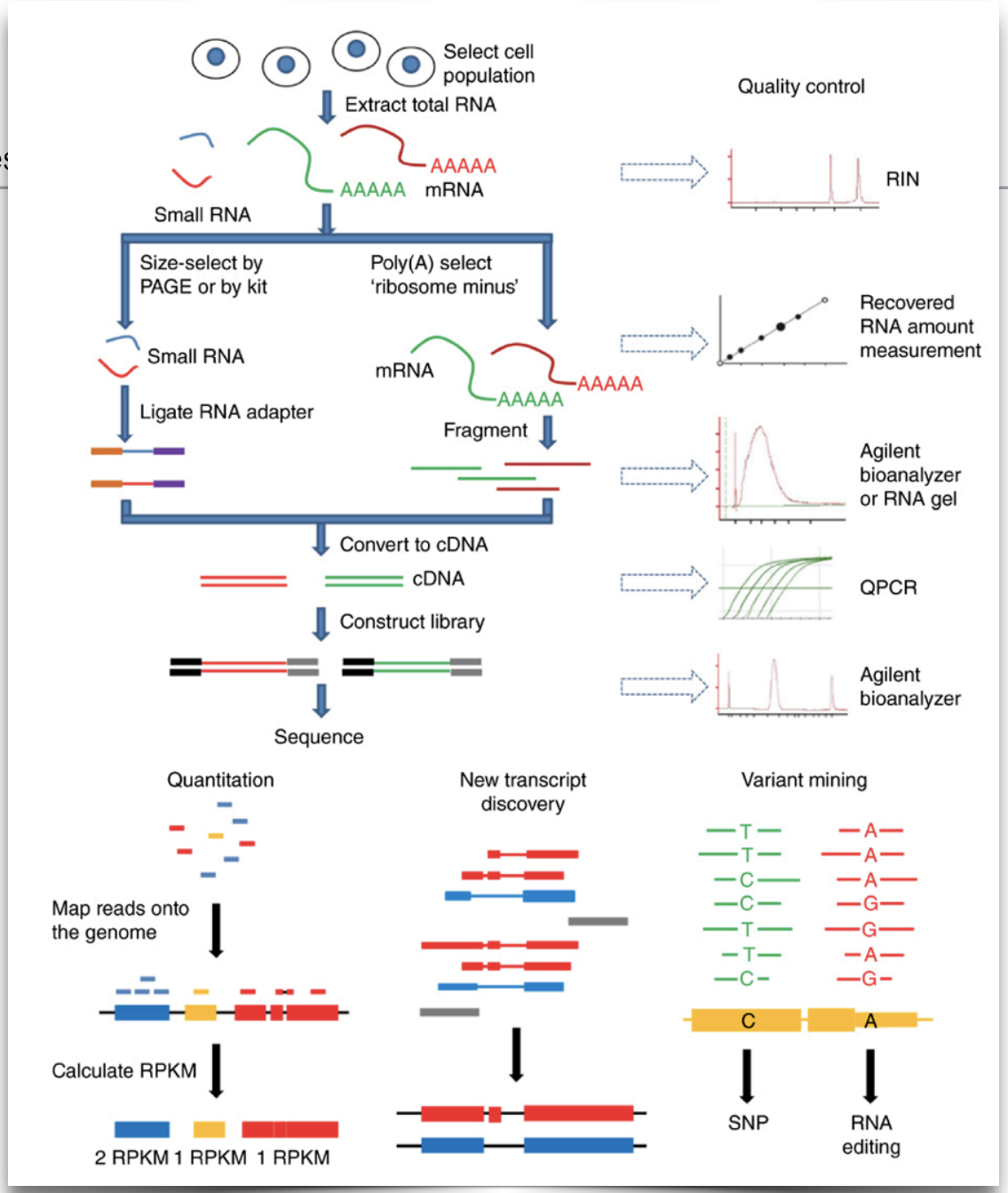
Abundance by Counting



Mortazavi et al., Nature Methods, 2008

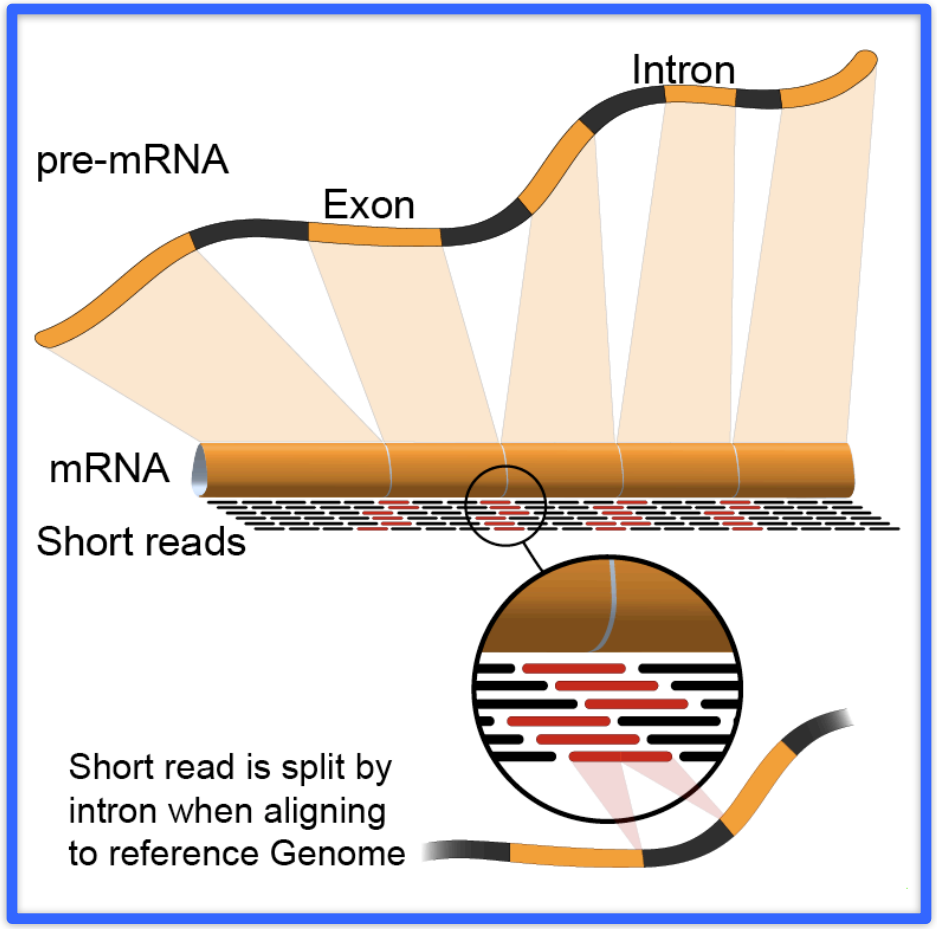
RNA-seq differential expression analyses

1. **Map** the reads to reference sequences
2. **"Count"** reads that map to genes (quantify)
3. Compute DE **Statistics**

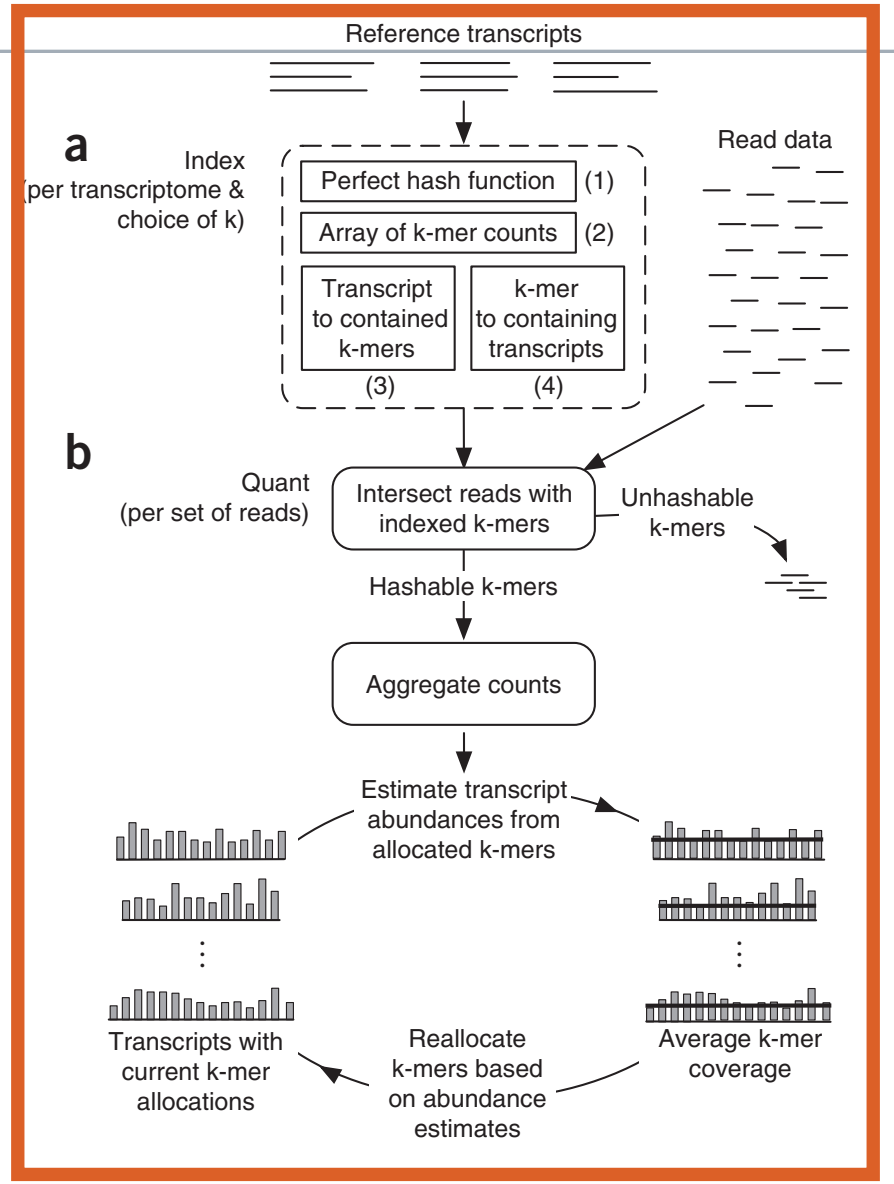


Zeng & Mortazavi, Nature Immunology, 2012

Alignment versus quasi-alignment



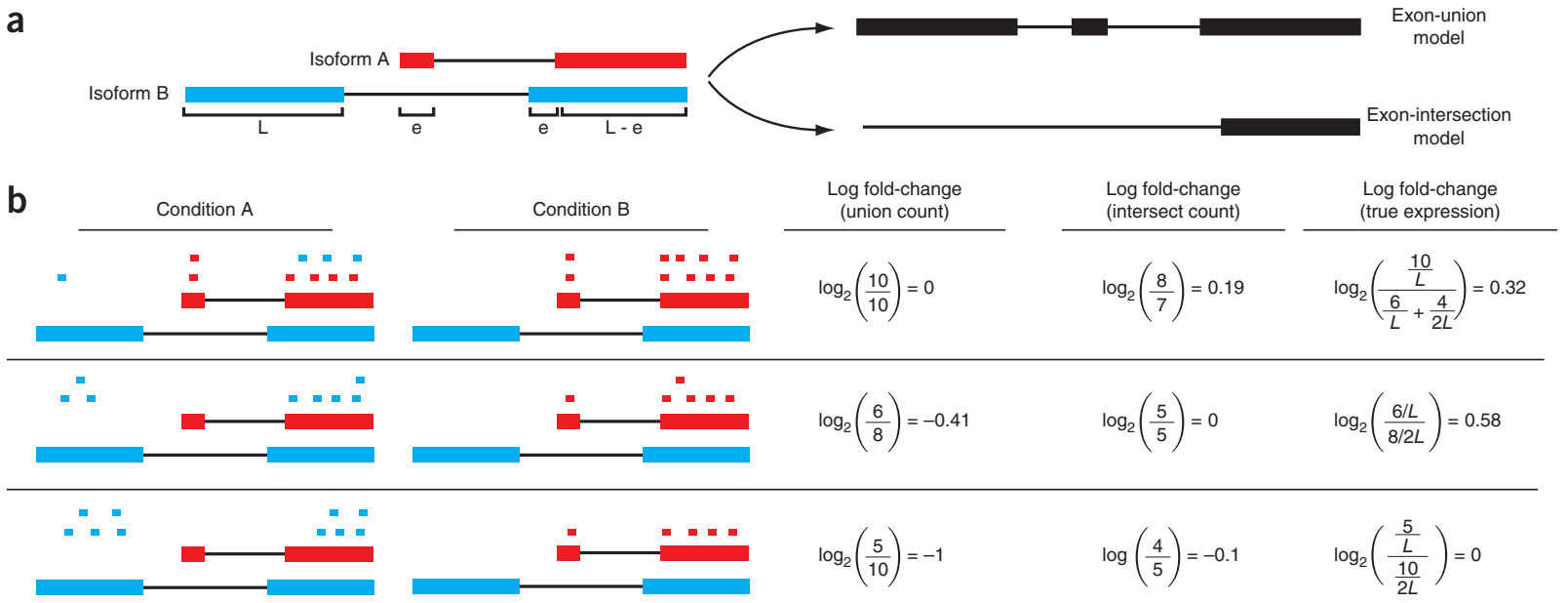
https://en.wikipedia.org/wiki/RNA-Seq



sailfish (Patro et al. 2014)

Caveat: simple gene-level counting not perfect, but good first approximation

Trapnell et al. 2013 Nat Biotech

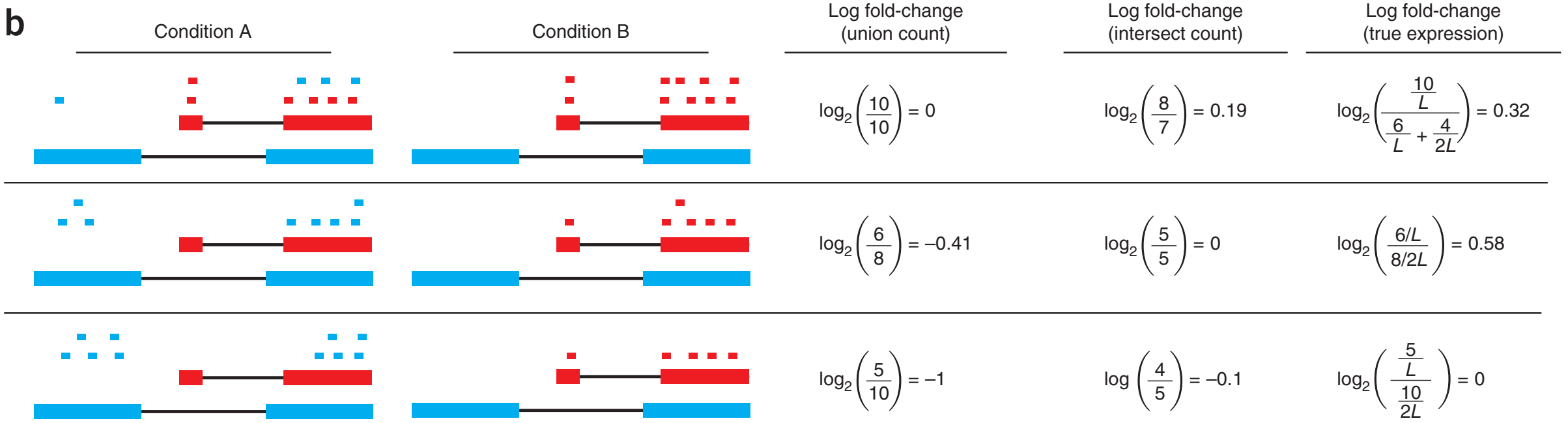


Transcriptome analysis of human tissues and cell lines reveals one dominant transcript per gene

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

Counting/Quantification

union counters —> simple sum of all reads
transcript counters —> sum of length-normalized reads
(often unknown which reads
map to which transcript —> portioning)



adapted from Trapnell et al. 2013 Nat Biotech

How do all these methods of counting affect DE analyses?

You've been doing your RNA-Seq all wrong

Posted by: RNA-Seq Blog in Expression and Quantification November 12, 2015 13,162 Views

In recent years, RNA-seq is emerging as a powerful technology in estimation of gene and/or transcript expression, and RPKM (Reads Per Kilobase per Million reads) is widely used to represent the relative abundance of mRNAs for a gene. In general, the methods for gene quantification can be largely divided into two categories: transcript-based approach and 'union exon'-based approach. Transcript-based approach is intrinsically more difficult because different isoforms of the gene typically have a high proportion of genomic overlap. On the other hand, 'union exon'-based approach method is much simpler and thus widely used in RNA-seq gene quantification. Biologically, a gene is expressed in one or more transcript isoforms. Therefore, transcript-based approach is logistically more meaningful than 'union exon'-based approach. Despite the fact that gene quantification is a fundamental task in most RNA-seq studies, however, it remains unclear whether 'union exon'-based approach for RNA-seq gene quantification is a good practice or not.

Researchers at Pfizer Worldwide Research & Development carried out a side-by-side comparison of 'union exon'-based approach and transcript-based method in RNA-seq gene quantification. It was found that the

F1000Research F1000Research 2016, 4:1521 Last updated: 05 APR 2016

CrossMark click for updates

METHOD ARTICLE

REVISED **Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences [version 2; referees: 2 approved]**

Charlotte Soneson[1,2], Michael I. Love[3,4], Mark D. Robinson[1,2]

[1]Institute for Molecular Life Sciences, University of Zurich, Zurich, 8057, Switzerland
[2]SIB Swiss Institute of Bioinformatics, University of Zurich, Zurich, 8057, Switzerland
[3]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA, 02210, USA
[4]Department of Biostatistics, Harvard TH Chan School of Public Health, Boston, MA, 02115, USA

v2 **First published:** 30 Dec 2015, **4**:1521 (doi: 10.12688/f1000research.7563.1)
Latest published: 29 Feb 2016, **4**:1521 (doi: 10.12688/f1000research.7563.2)

Open Peer Review

Differential expression: why not use methods developed for microarrays?

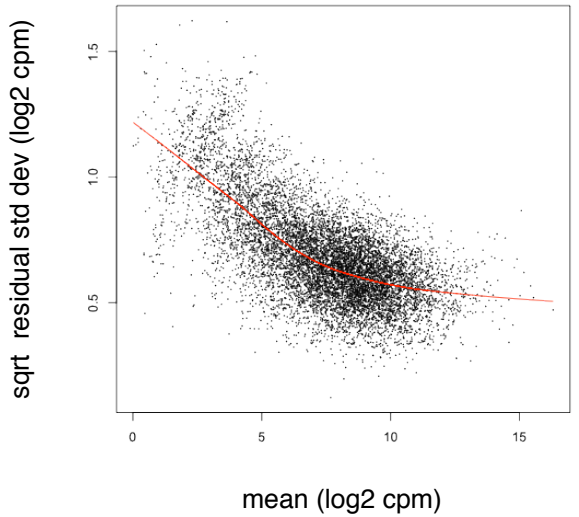
Count data is discrete, not continuous.

Methods designed for microarrays are not directly appli ble and suboptimal **(more on this later)**

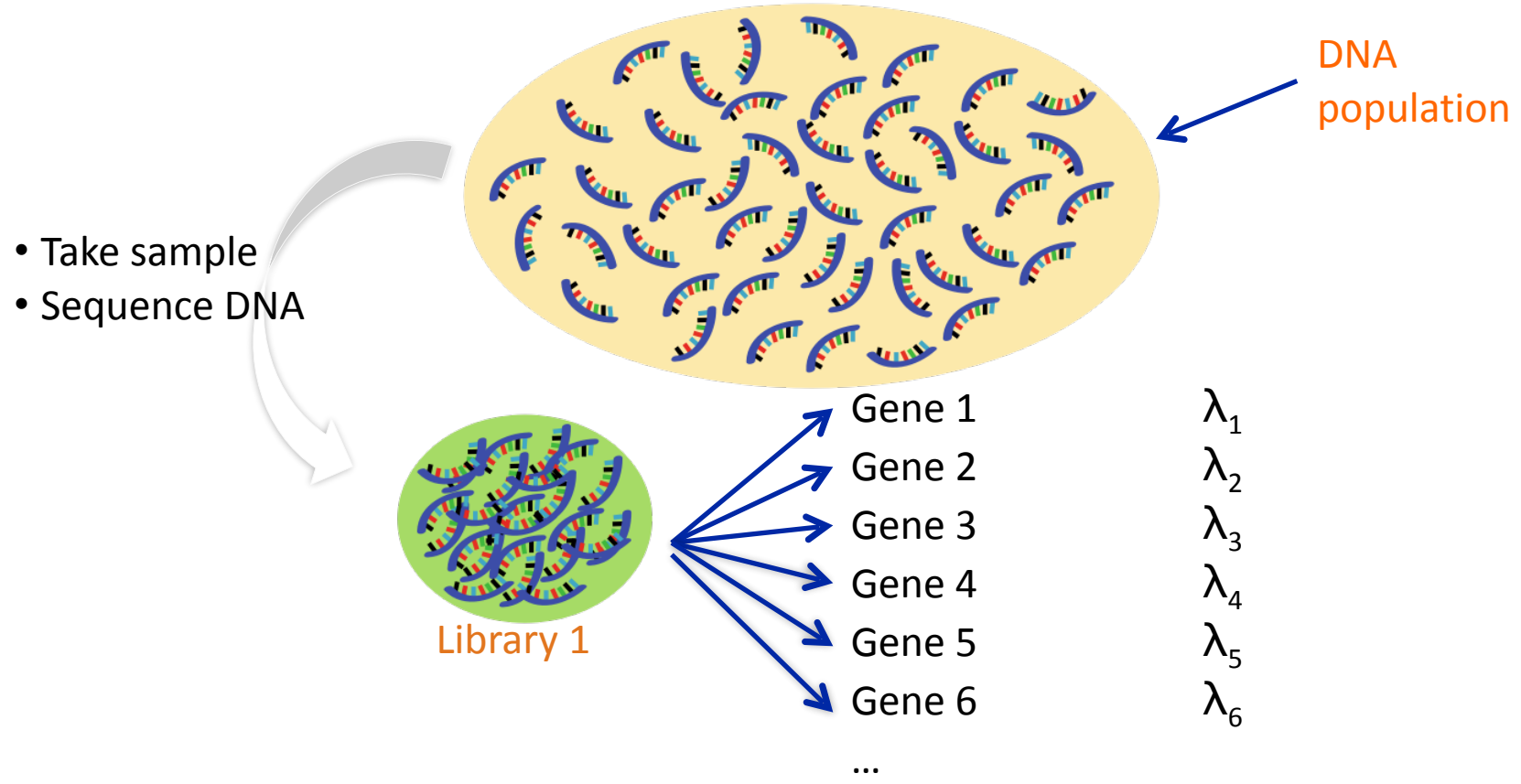
Two options:

Transform count data and apply standard methodology

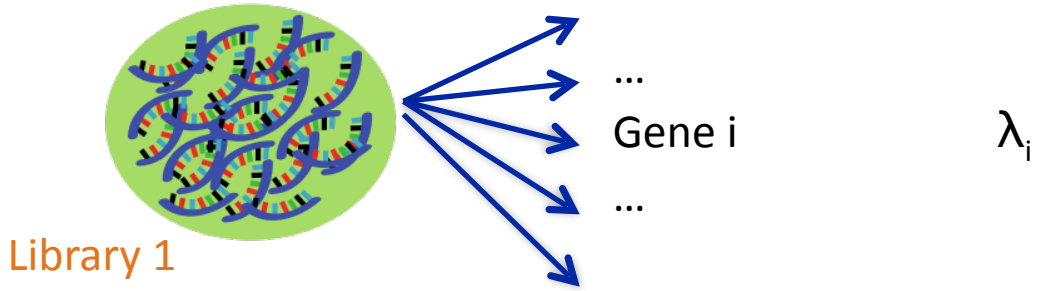
Analyze using models for count data



Sampling reads from population of DNA fragments is multinomial



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



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

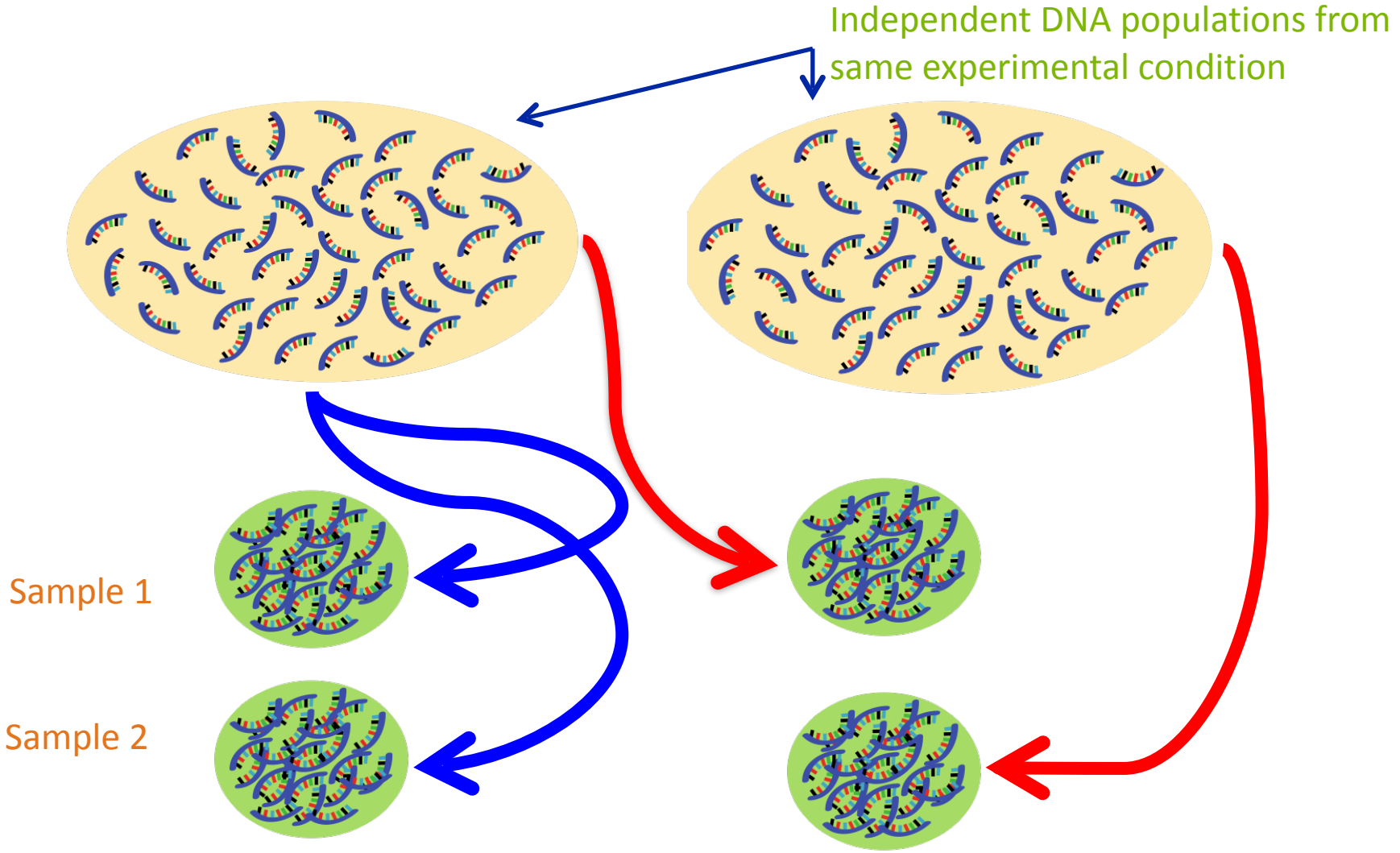
Y_i - observed number of reads for gene i

M - total number of sequences

λ_i - proportion

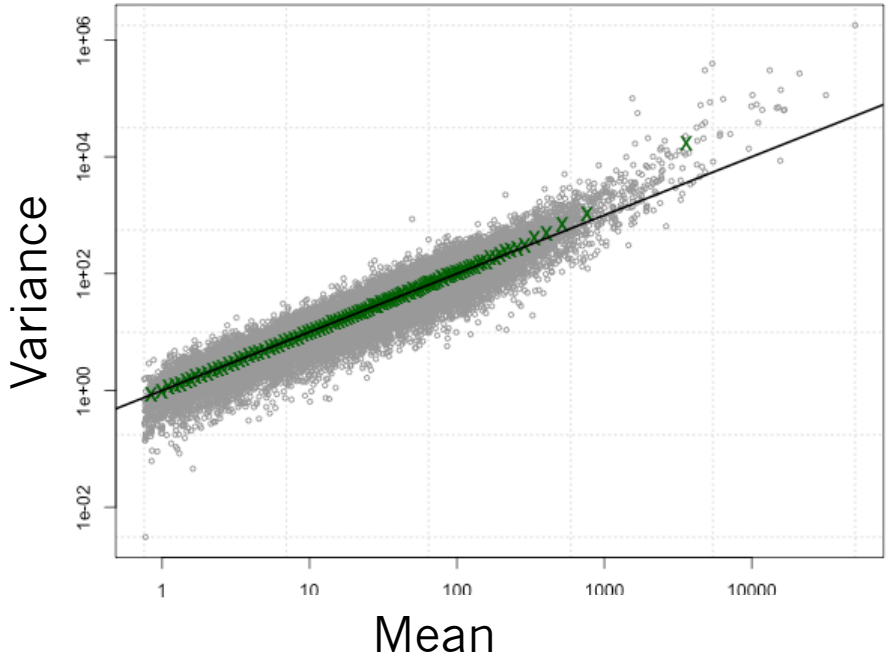
Large M, small λ_i → approximated well by Poisson($\mu_i = M \bullet \lambda_i$)

Technical replication versus biological replication



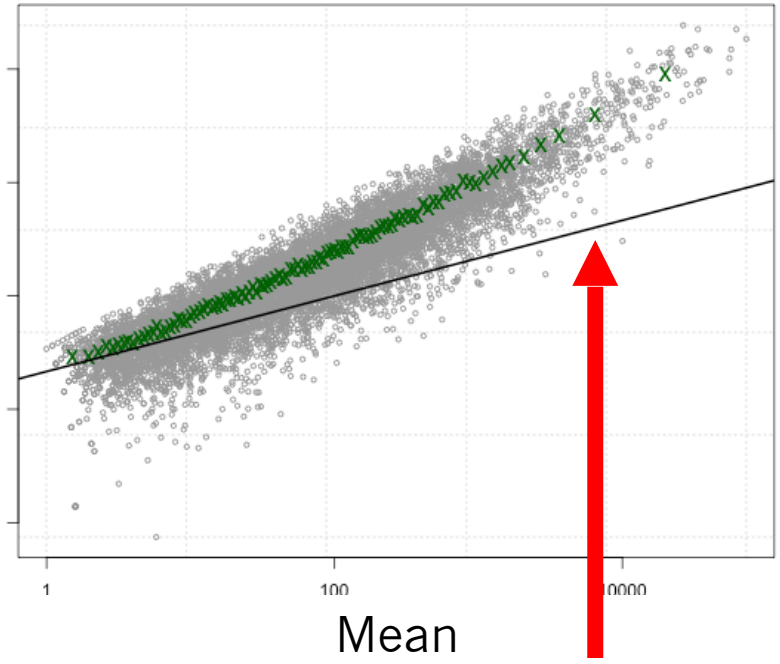
Mean-Variance plots: What we see in real data

Technical replicates



Data from Marioni et al. Genome Research 2008

Biological replicates



Data from Parikh et al. *Genome Biology* 2010

Count data modeling assumptions

Poisson adequately describes technical variation

$Y_i \sim Pois(M * \lambda_i)$

$mean(Y_i) = variance(Y_i) = M * \lambda_i$

Negative binomial (gamma-Poisson) model is a natural extension that allows **biological** variability:

$Y_i \sim NB(\mu_i = M * \lambda_i , \phi_i)$

Same mean, variance is quadratic in the mean:

$variance(Y_i) = \mu_i (1 + \mu_i \phi_i)$

M = library size

λ_i = relative contribution of gene i

Similar interpretation

$Y_i \sim NB(\mu_i = Ni * \lambda_i, \phi_i)$

$$E(y_{gi}) = \mu_{gi} = N_i \pi_{gi}.$$

(Coefficient of variation = standard deviation/mean)

$$\text{var}(y_{gi}) = E_\pi[\text{var}(y|\pi)] + \text{var}_\pi[E(y|\pi)] = \mu_{gi} + \phi_g \mu_{gi}^2.$$

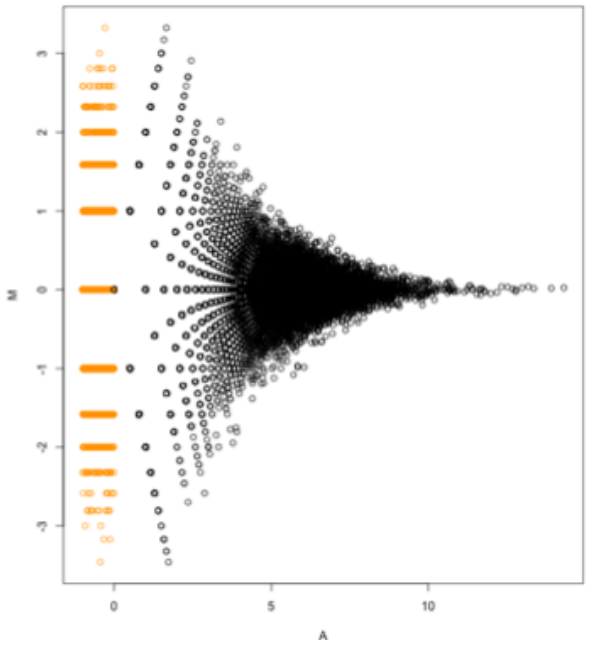
Dividing both sides by μ_{gi}^2 gives

$$\text{CV}^2(y_{gi}) = 1/\mu_{gi} + \phi_g.$$

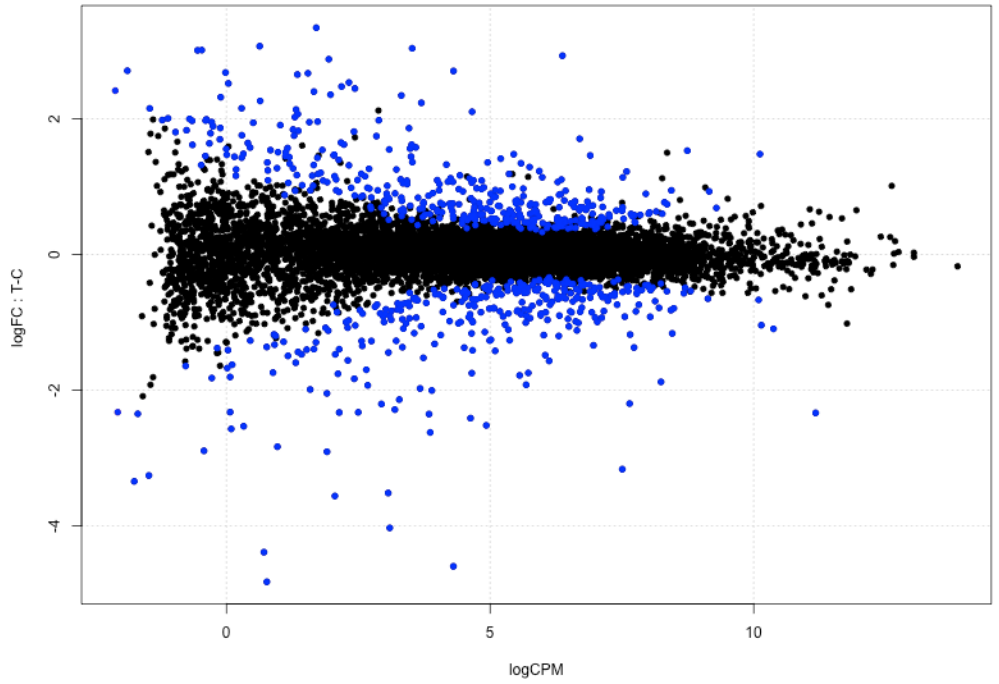
$$\mathrm{CV}^2(y_{gi}) = 1/\mu_{gi} + \phi_g.$$

A confirmation of what the theory states

Technical replicates (~Poisson)



Biological replicates



Differential expression, small sample inference —> **with counts**

- Table of data (e.g., microarray gene expression data with replicates of each of condition A, condition B)
  - rows = features (e.g., genes), columns = experimental units (samples)
- Most common problem in statistical bioinformatics: want to infer whether there is a change in the response —> a statistical test for each row of the table.

What test might you use? Why is this hard? What issues arise? How much statistical power is there [1] ?

```
> head(y)
       group0     group0     group0     group1     group1     group1
gene1 -0.1874854  0.2584037 -0.05550717 -0.4617966 -0.3563024 -0.03271432
gene2 -3.5418798 -2.4540999  0.11750996 -4.3270442 -5.3462622 -5.54049106
gene3 -0.1226303  0.9354707 -1.10537767 -0.1037990  0.5221678 -1.72360854
gene4 -2.3394536 -0.3495697 -3.47742610 -3.2287093  6.1376670 -2.23871974
gene5 -3.7978820  1.4545702 -7.14796503 -4.0500796  4.7235714 10.00033769
gene6  1.4627078 -0.3096070 -0.26230124 -0.7903434  0.8398769 -0.96822312
```

What was successful with microarray data: classical/moderated/ shrunken t-tests

$$t_g = \frac{\overline{y}_{\text{mu}} - \overline{y}_{\text{wt}}}{s_g\, c}$$

Feature-specific

$$\tilde{t}_g = \frac{\overline{y}_{\text{mu}} - \overline{y}_{\text{wt}}}{\tilde{s}_g\, u}$$

Moderated

$$t_{g,\text{pooled}} = \frac{\overline{y}_{\text{mu}} - \overline{y}_{\text{wt}}}{s_0\, c}$$

Common

Let's try the same strategy with counts

At one extreme, assume all genes have same dispersion (too strong)

At other extreme, estimate dispersion separately/independently for each gene (poor estimates)

Shrink individual estimates toward common/trend (how?)

No hierarchical model (e.g. limma) to do this —> **approximations, weighted likelihood**

No t-distribution theory to formulate statistical tests.

Count data modeling assumptions

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$Y_i \sim Pois(M * \lambda_i)$

$mean(Y_i) = variance(Y_i) = M * \lambda_i$

Negative binomial (gamma-Poisson) model is a natural extension that allows **biological** variability:

$Y_i \sim NB(\mu_i = M * \lambda_i , \phi_i)$

Same mean, variance is quadratic in the mean:

$variance(Y_i) = \mu_i (1 + \mu_i \phi_i)$

M = library size

λ_i = relative contribution of gene i

Second challenge: Moderate dispersion estimate

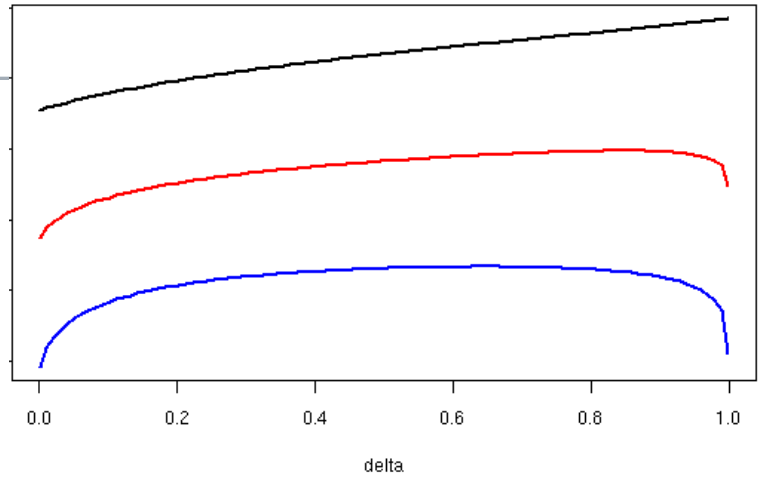
Weighted likelihood -- individual log-likelihood plus a weighted version of the common log-likelihood:

$$\mathrm{WL}(\phi_g) = l_g(\phi_g) + \alpha\, l_C(\phi_g)$$

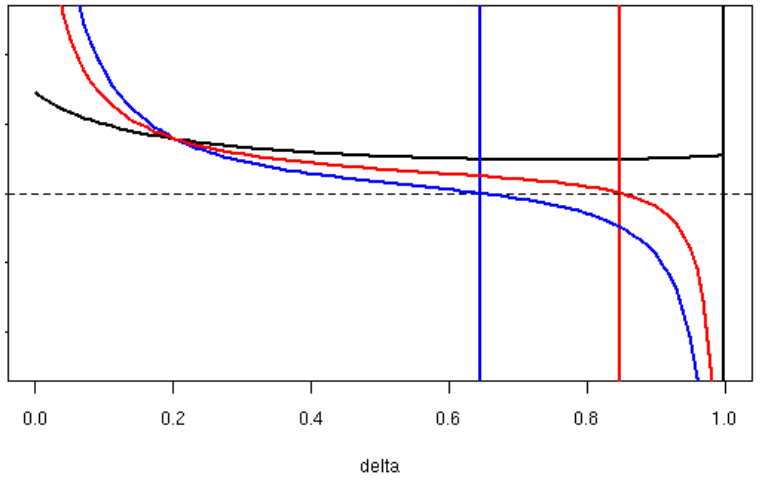
l_g - quantile-adjusted conditional likelihood

Black: single tag
Blue: common dispersion
Red: Linear combination of the two

Log-Likelihood



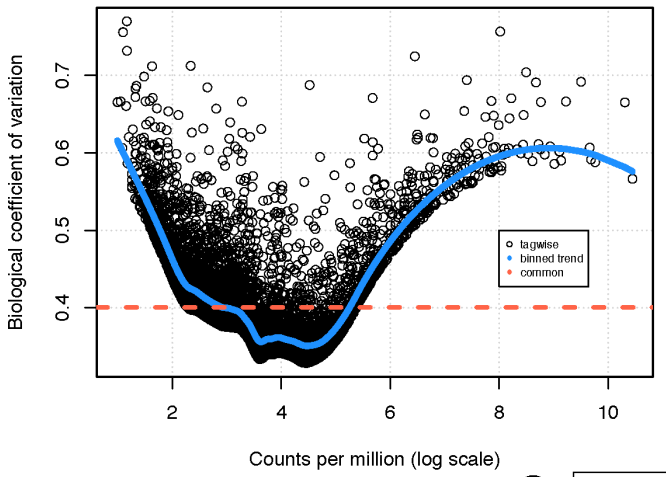
Score (1st derivative of LL)

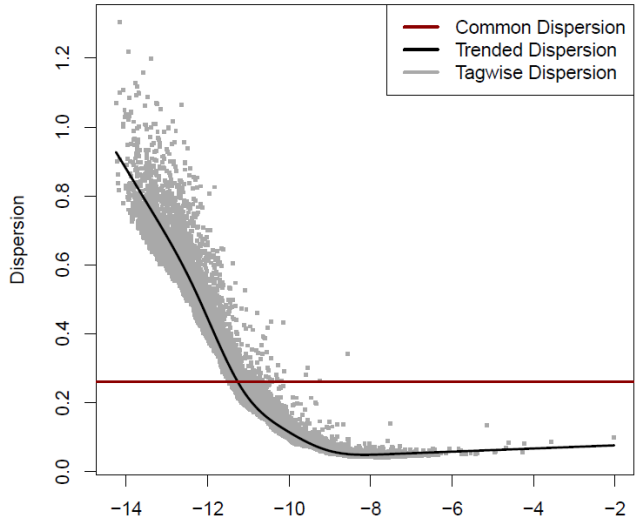


$$\delta = \frac{\phi}{\phi+1}$$

Dispersion varies with mean: moderate dispersion towards **trend**

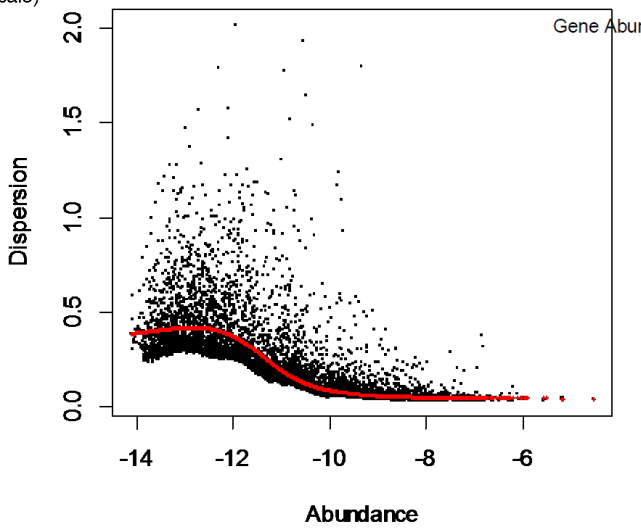
Data:
Tuch et al., 2008





Mouse hemapoeitic stem cells

Advantage: genes are allowed to have their own variance.



Mouse lymphomas

Nature Reviews Genetics | AOP, published online 18 November 2008; doi:10.1038/nrg2484

INNOVATION

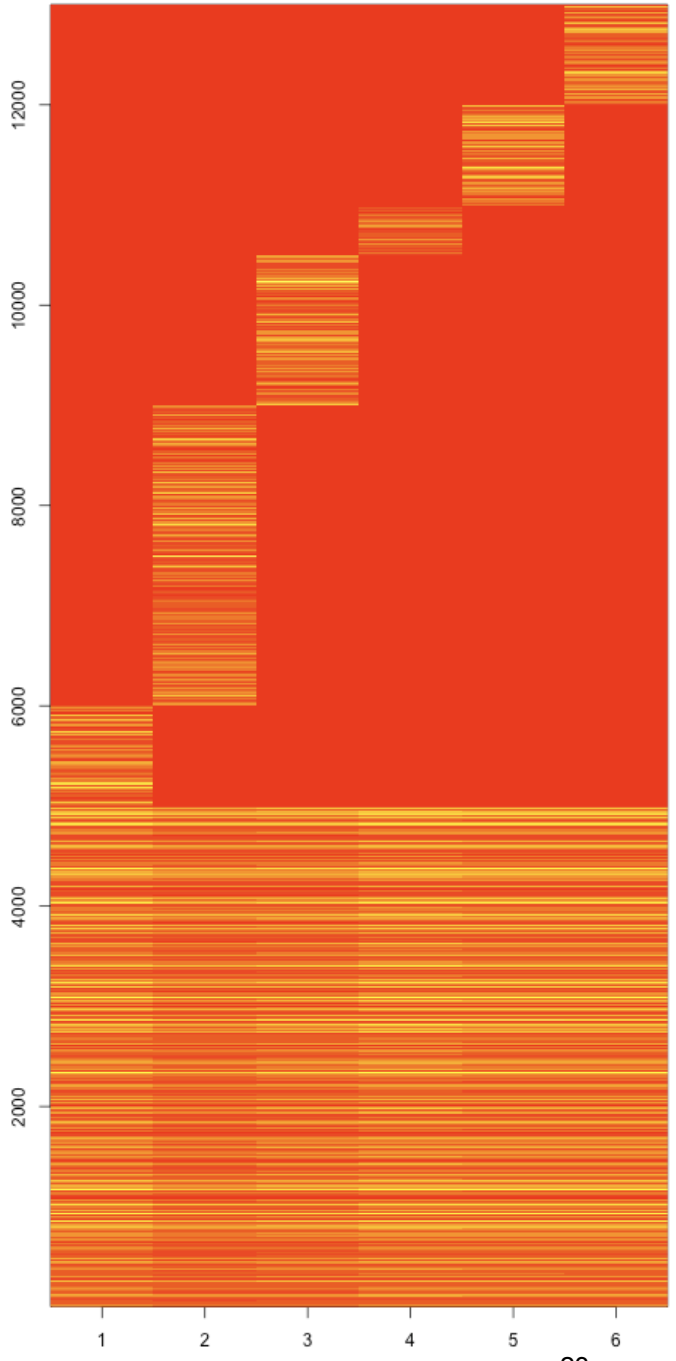
RNA-Seq: a revolutionary tool for transcriptomics

Zhong Wang, Mark Gerstein and Michael Snyder

One particularly powerful advantage of RNA-Seq is that it can capture transcriptome dynamics across different tissues or conditions without sophisticated normalization of data sets[19,20,22].

“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

Kidney and Liver RNA have very different composition

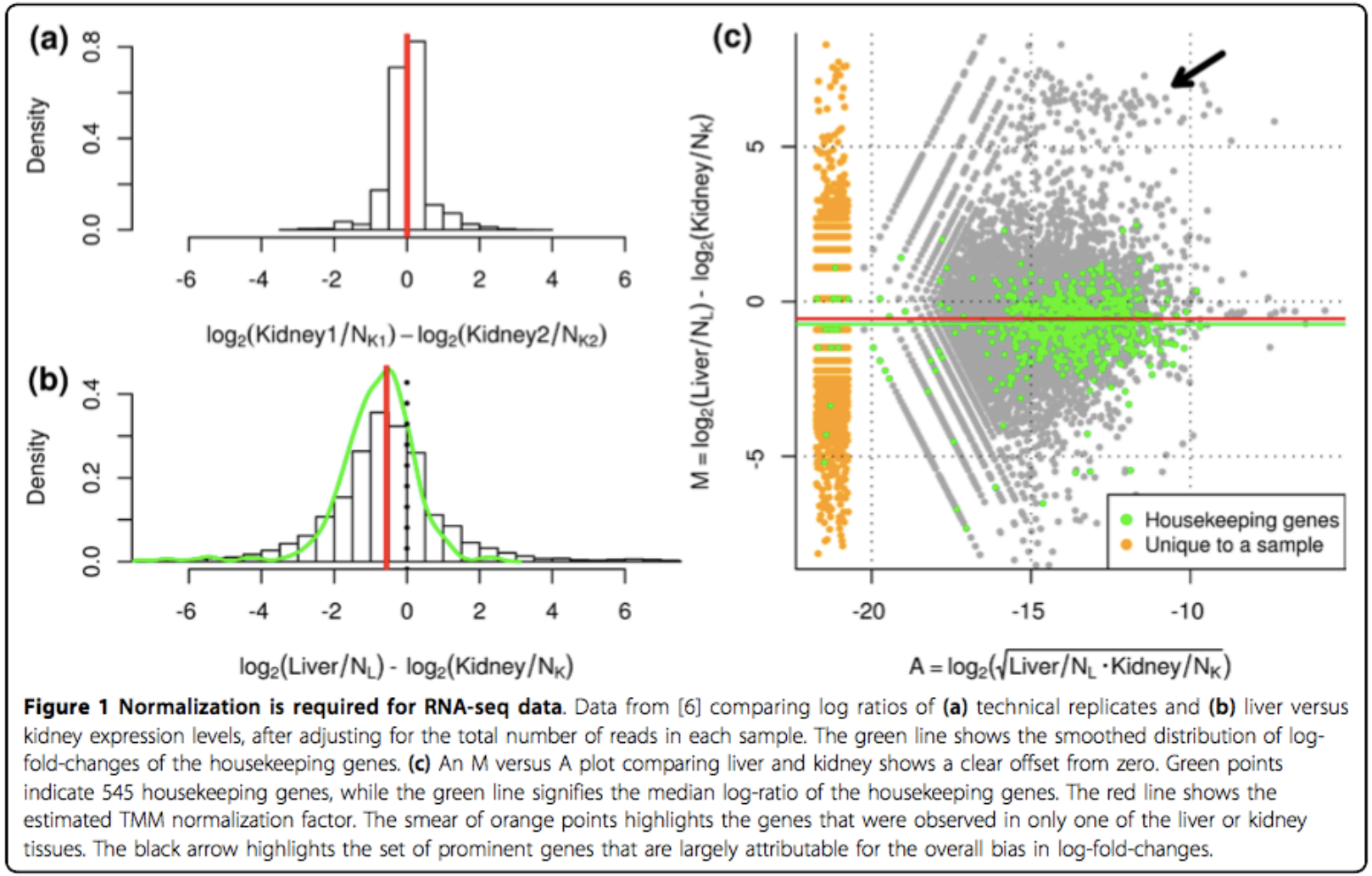


Figure 1 Normalization is required for RNA-seq data. Data from [6] comparing log ratios of **(a)** technical replicates and **(b)** liver versus kidney expression levels, after adjusting for the total number of reads in each sample. The green line shows the smoothed distribution of log-fold-changes of the housekeeping genes. **(c)** An M versus A plot comparing liver and kidney shows a clear offset from zero. Green points indicate 545 housekeeping genes, while the green line signifies the median log-ratio of the housekeeping genes. The red line shows the estimated TMM normalization factor. The smear of orange points highlights the genes that were observed in only one of the liver or kidney tissues. The black arrow highlights the set of prominent genes that are largely attributable for the overall bias in log-fold-changes.

Robinson and Oshlack (2010) Genome Biology

Use scaling factor ("offset") in statistical model

Assumption: core set of genes/loci that do not change in expression.

Our Pick a reference sample, compute a weighted trimmed mean of M-values (TMM) to reference

Adjustment to statistical analysis:

- Use "effective" library size (edgeR)
- Use additional offset (GLM)

Note: count data is not modified

Robinson and Oshlack (2010) Genome Biology

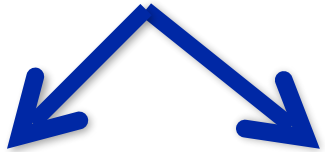
Differential expression: why not use methods developed for microarrays?

Count data is discrete, not continuous.

Methods designed for microarrays are not directly applicable and suboptimal

Transforming count data with logs, with some special treatment, can give very good results

Two options:



Transform count data and apply standard methodology

Analyze using models for count data

What does transformation do to M-V relationship?

For Poisson data, square-root should stabilize

Logarithm is too strong – variance decreases to asymptote (Neg Bin) or 0 (Poisson)

How to pick? Doesn't matter —> voom

voom: mean-variance modeling at the observational level

```
voom                        package:limma                        R Documentation

Transform RNA-Seq Data Ready for Linear Modelling

Description:

     Transform count data to log2-counts per million, estimate the
     mean-variance relationship and use this to compute appropriate
     observational-level weights. The data are then ready for linear
     modeling.
```

Model log counts per million

log counts per million:

$$z_{gi} = \log_2\left(1e6\frac{\text{count}_{gi}+0.5}{\text{libsize}_{gi}+1.0}\right) = \log_2\left(1e6\frac{y_{gi}+0.5}{M_{gi}+1.0}\right)$$

normalize libsize in advance or normalize z_{gi} as for microarrays.

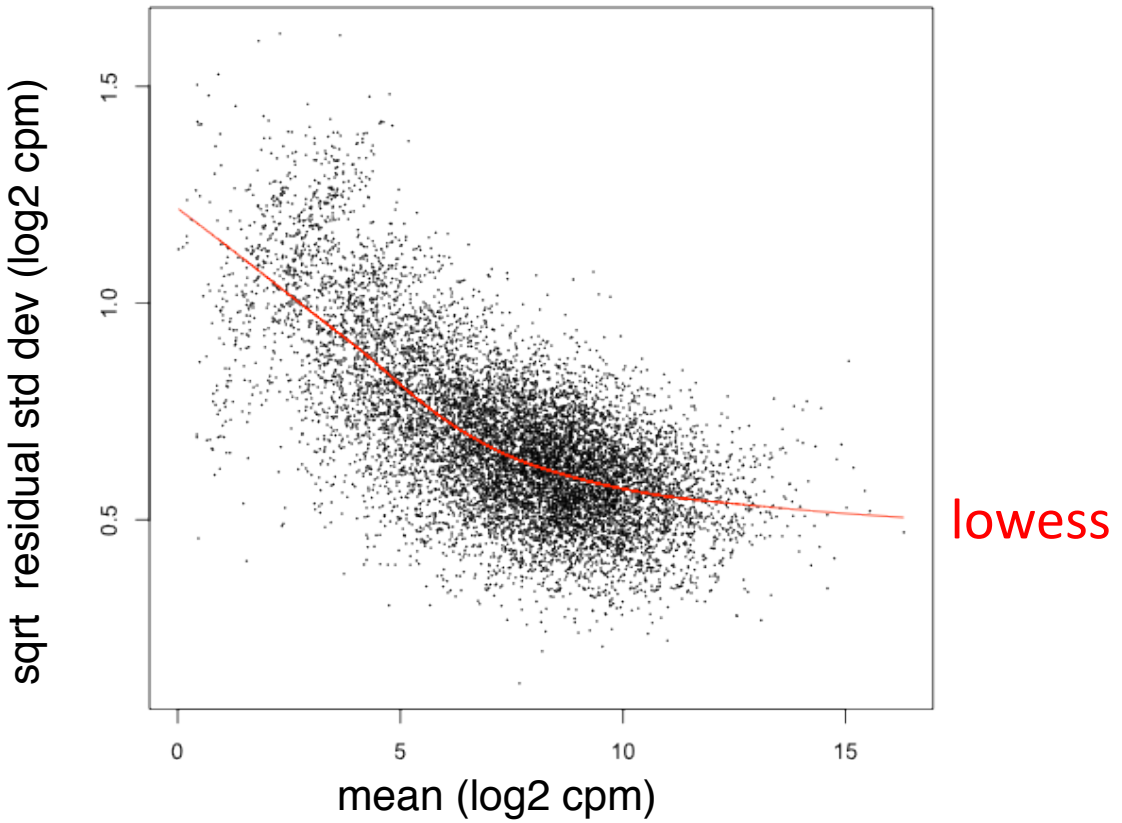
Linear modelling:

$$E\left(z_{gi}\right) = \mu_{gi} = x_i^T\beta_g$$

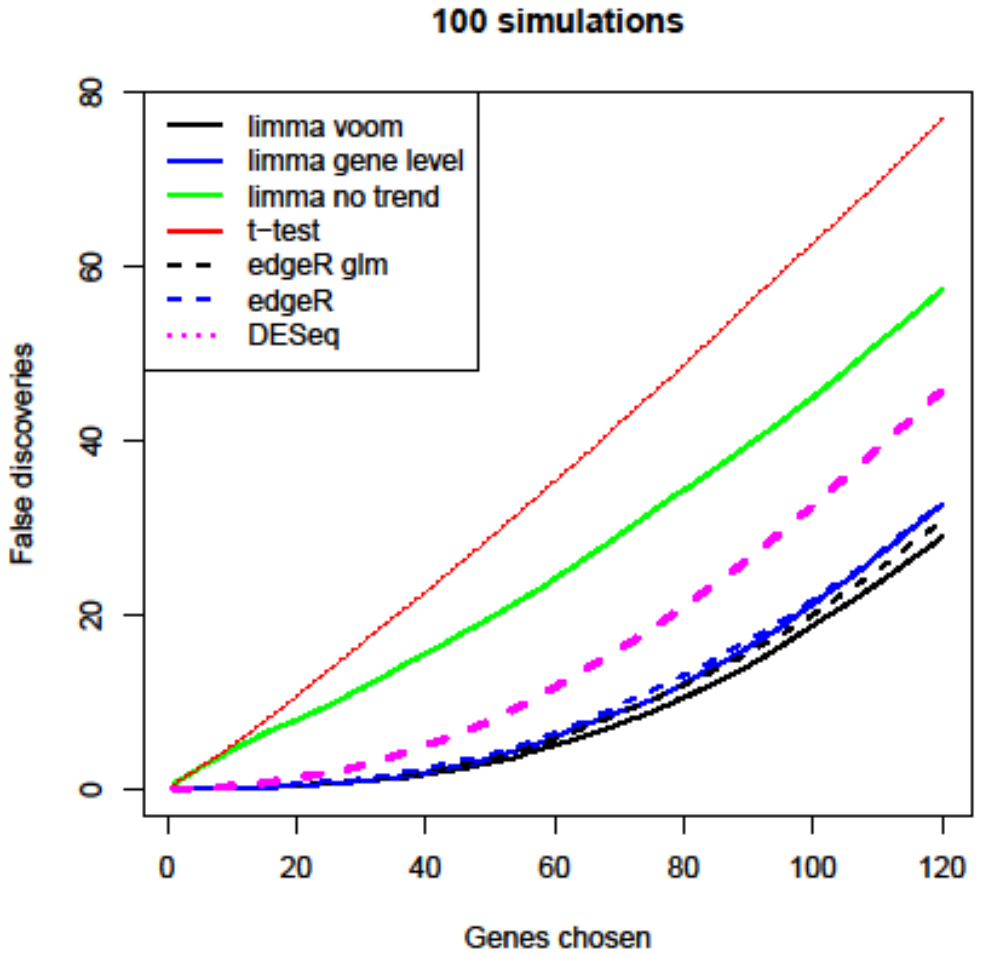
$$\text{var}\left(z_{gi}\right) = s(\mu_{gi})\sigma_g^2$$

Smooth function of mean

voom fits a lowess trend to the mean-variance relationship …



—> Use weights (1/var) in limma analysis .. i.e., **heteroscedastic regression**



Model mean-var relationship

Law et al. 2014 Genome Biology

Linear Models (microarray setting)

In general, need to specify:

- Dependent variable
- Explanatory variables (experimental design, covariates, etc.)

More generally:

$$y = X\beta + \epsilon$$

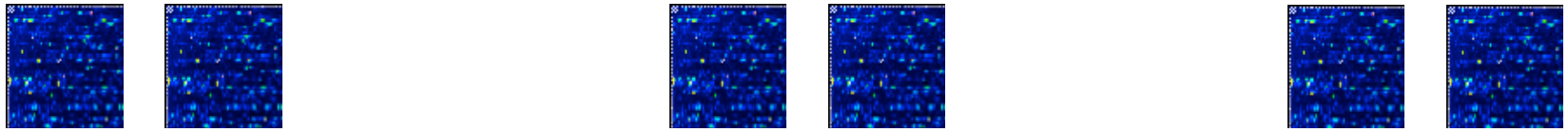
vector of observed data

design matrix

Vector of parameters to estimate

Analysis of Variance → Linear model

WT x 2 | Cond A x 2 | Cond B x 2



$$\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \\ y_6 \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 1 & 1 & 0 \\ 1 & 1 & 0 \\ 1 & 0 & 1 \\ 1 & 0 & 1 \end{bmatrix} \begin{bmatrix} \alpha_1 \\ \alpha_2 \\ \alpha_3 \end{bmatrix} + \begin{bmatrix} \epsilon_1 \\ \epsilon_2 \\ \epsilon_3 \\ \epsilon_4 \\ \epsilon_5 \\ \epsilon_6 \end{bmatrix}$$

α_1 = wt log-expression

α_2 = Cond A - wt

α_3 = Cond B - wt

$E[y_1]=E[y_2]=\alpha_1$ $E[y_3]=E[y_4]=\alpha_1+\alpha_2$ $E[y_5]=E[y_6]=\alpha_1+\alpha_3$

Applications: paired designs, multi-factor designs, interactions

—> This particular model only valid for continuous response

Generalized linear models: a more general framework

Gaussian (normal) distributed response —> various other (common) types.

Three components:

1. Probability distribution of response (in exponential family)
2. Linear predictor (covariates; design matrix)
3. Link function (link mean to linear predictor)

Link function and linear predictor

Link function

$$E(Y_i) = \mu_i \qquad g(\mu_i) = \eta_i$$

$$\eta_i = \beta_0 + \beta_1 x_{1i} + \ldots + \beta_p x_{pi}$$

Linear predictor (covariates)

$$\text{var}(Y_i) = \phi V(\mu)$$

Provides a way to link the mean of response to a linear predictor.

Data is not transformed.

Variance is a function of mean.

Common distributions, "Canonical" link functions

Common distributions with typical uses and canonical link functions

Distribution	Support of distribution	Typical uses	Link name	Link function	Mean function
Normal	real: $(-\infty, +\infty)$	Linear-response data	Identity	$\mathbf{X}\boldsymbol{\beta} = \mu$	$\mu = \mathbf{X}\boldsymbol{\beta}$
Exponential Gamma	real: $(0, +\infty)$	Exponential-response data, scale parameters	Inverse	$\mathbf{X}\boldsymbol{\beta} = \mu^{-1}$	$\mu = (\mathbf{X}\boldsymbol{\beta})^{-1}$
Inverse Gaussian	real: $(0, +\infty)$		Inverse squared	$\mathbf{X}\boldsymbol{\beta} = \mu^{-2}$	$\mu = (\mathbf{X}\boldsymbol{\beta})^{-1/2}$
Poisson	integer: $[0, +\infty)$	count of occurrences in fixed amount of time/space	Log	$\mathbf{X}\boldsymbol{\beta} = \ln(\mu)$	$\mu = \exp(\mathbf{X}\boldsymbol{\beta})$
Bernoulli	integer: $[0, 1]$	outcome of single yes/no occurrence	Logit	$\mathbf{X}\boldsymbol{\beta} = \ln\left(\frac{\mu}{1-\mu}\right)$	$\mu = \frac{\exp(\mathbf{X}\boldsymbol{\beta})}{1+\exp(\mathbf{X}\boldsymbol{\beta})} = \frac{1}{1+\exp(-\mathbf{X}\boldsymbol{\beta})}$
Binomial	integer: $[0, N]$	count of # of "yes" occurrences out of N yes/no occurrences			
Categorical	integer: $[0, K)$ K-vector of integer: $[0, 1]$, where exactly one element in the vector has the value 1	outcome of single K-way occurrence			
Multinomial	K-vector of integer: $[0, N]$	count of occurrences of different types (1 .. K) out of N total K-way occurrences			

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

http://en.wikipedia.org/wiki/Score_(statistics)

$$\mathcal{I}(\theta) = \mathbb{E}\left\{ \left[\frac{\partial}{\partial\theta} \log L(\theta; X) \right]^2 \middle| \theta \right\}.$$

Large sample theory – Result 2 (score is asymptotically normal)

$$\dot{\ell}_1 = \frac{\partial \ell}{\partial \boldsymbol{\theta}_1}$$

$$\dot{\ell}_2 = \frac{\partial \ell}{\partial \boldsymbol{\theta}_2}$$

The "score" function is the first derivative (gradient) of the log-likelihood function, is (asymptotically) normally distributed with mean 0 and variance(-covariance) Fisher information.

Say, we to test H_0: $\theta_2=0$, θ_1 is/are "nuisance" parameter(s)

$$\mathcal{I}_{2.1} = \mathcal{I}_{22} - \mathcal{I}_{21}\mathcal{I}_{11}^{-1}\mathcal{I}_{12}.$$

$$\mathcal{I} = \begin{pmatrix} \mathcal{I}_{11} & \mathcal{I}_{12} \\ \mathcal{I}_{21} & \mathcal{I}_{22} \end{pmatrix}$$

$$S = \dot{\ell}_2^T \mathcal{I}_{2.1}^{-1} \dot{\ell}_2$$

Large sample theory – Result 3 (likelihood ratio test)

$$D = -2 \ln \left(\frac{\text{likelihood for null model}}{\text{likelihood for alternative model}} \right)$$
$$= -2 \ln(\text{likelihood for null model}) + 2 \ln(\text{likelihood for alternative model})$$

http://en.wikipedia.org/wiki/Likelihood-ratio_test

General form (exponential family)

$$-2 \log \lambda = 2 \sum_{i=1}^{n} \frac{y_i(\tilde{\theta}_i - \hat{\theta}_i) - b(\tilde{\theta}_i) + b(\hat{\theta}_i)}{a_i(\phi)}$$

`edgeR::glmLRT()`

Again, large sample theory says this is approx. χ^2 with degrees of freedom according to the difference in the number of parameters between null and alternative (assuming they are nested).

Some interesting generalizations of NB modeling for RNA-seq (3)

$$LRT_k = 2\left(\ell_k(\hat{\mu}_k|y_k) - \ell_k(\tilde{\mu}_k|y_k)\right) \longrightarrow LRT_k \sim \Phi_k \chi^2_q + O_p(n^{-1/2})$$

$$\hat{\Phi}_k = \frac{2\left(\ell_k(y_k|y_k) - \ell_k(\hat{\mu}_k|y_k)\right)}{n-p}$$

$$F_{QL} = \frac{LRT_k/q}{\hat{\Phi}_k}$$

Accounting for the uncertainty in estimating dispersion

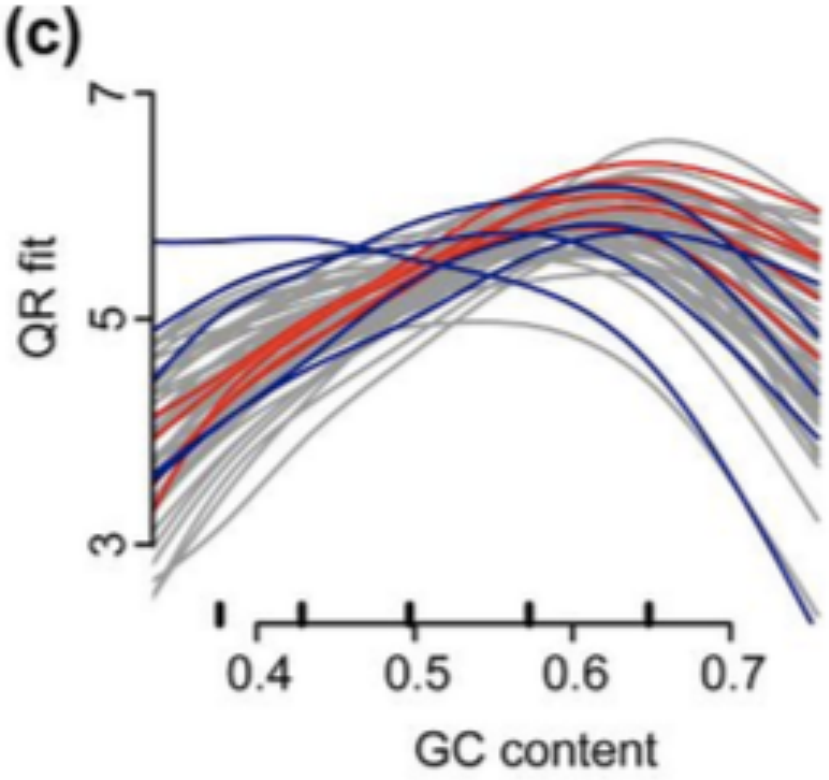
`edgeR::glmQLF`

Lund et al., SAGMB 2012; 11(5):8

Offset ξ explicitly in GLM:

$$E[Y] = \mu = g^{-1}(\eta) = g^{-1}(X\beta + \xi)$$

Some interesting generalizations of NB modeling for RNA-seq (4)



Integrate sample-specific normalization via offset

Profiles vary from sample to sample:
GC content
Gene length

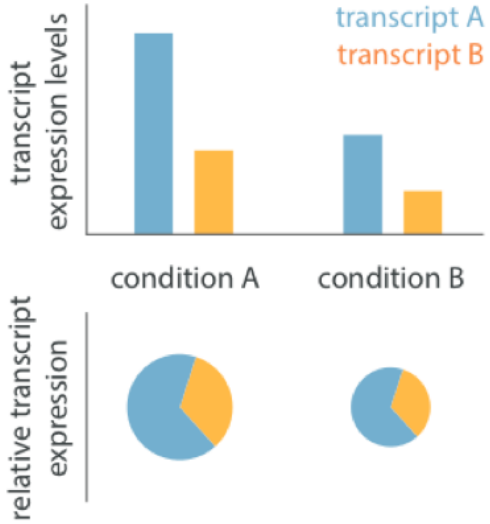
DOES NOT change data, use offsets to modify expected mean

Give a sample (or gene)-specific offset to edgeR/DESeq2

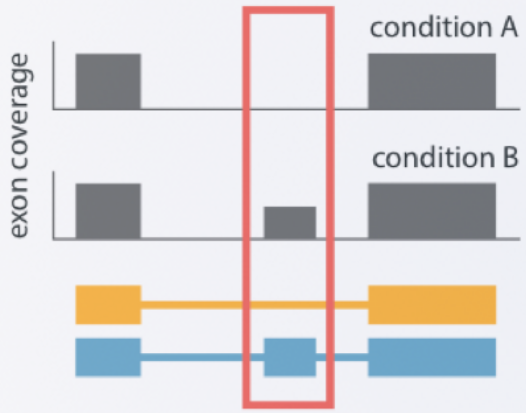
Hansen, Irizarry, Wu Biostatistics 2012

Some terms: DTE, DEU, DTU

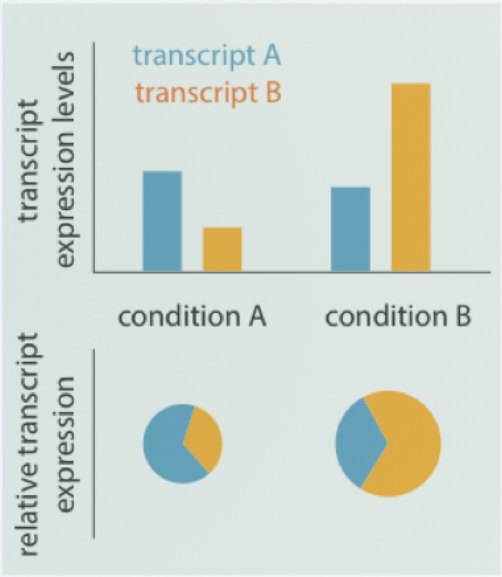
Differential transcript expression (DTE)



Differential exon usage (DEU)



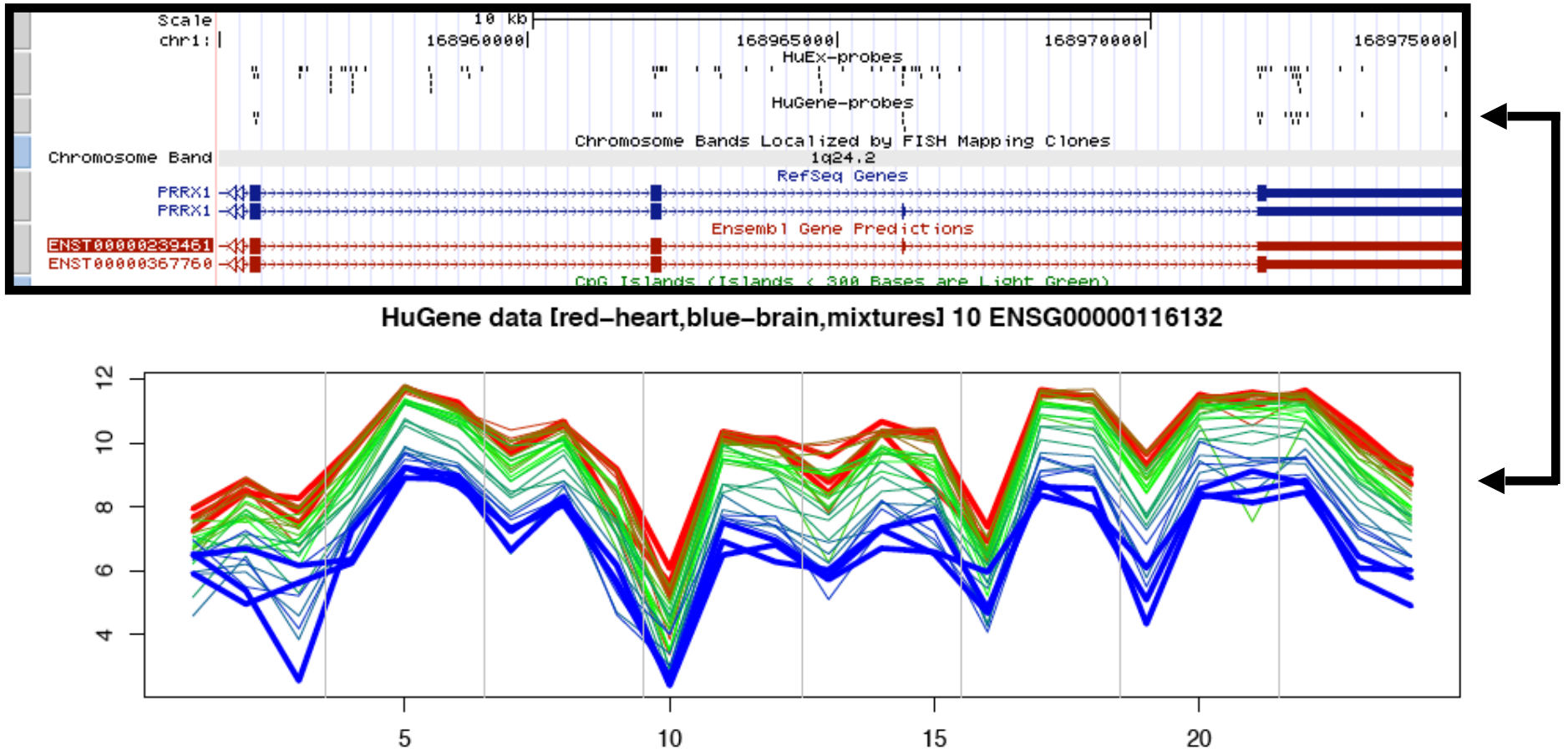
Differential transcript usage (DTU)



differential splicing

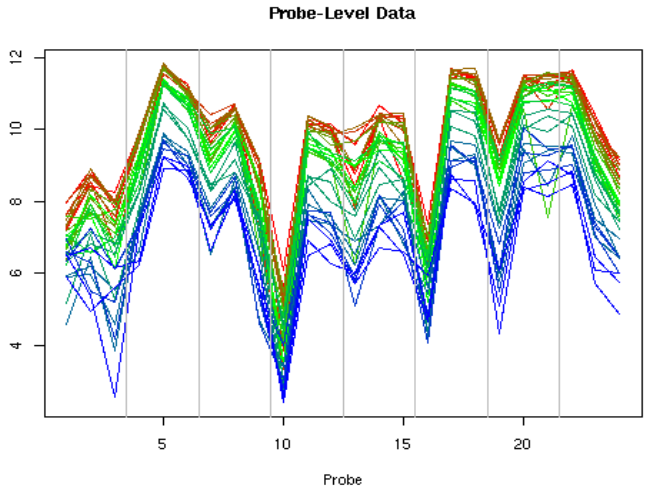
Slide adapted from Mar Gonzàlez-Porta's talk at ECCB 2014
http://radiant-project.eu/ECCB/gonzalez-porta-140907065638-phpapp01.pdf

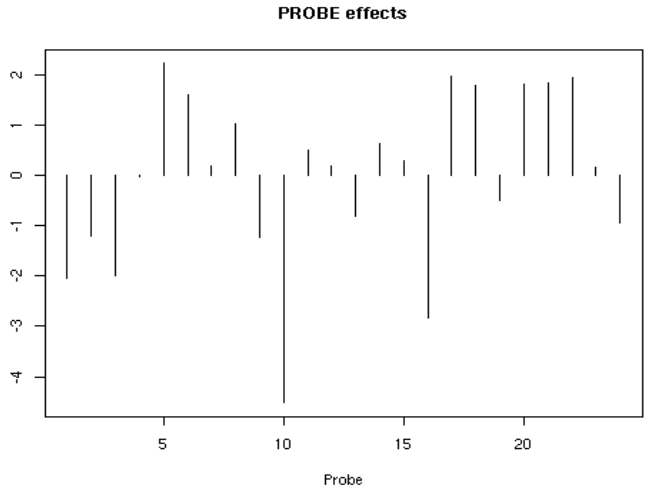
Digression 1/3: The nature of Affymetrix Probe Level Data



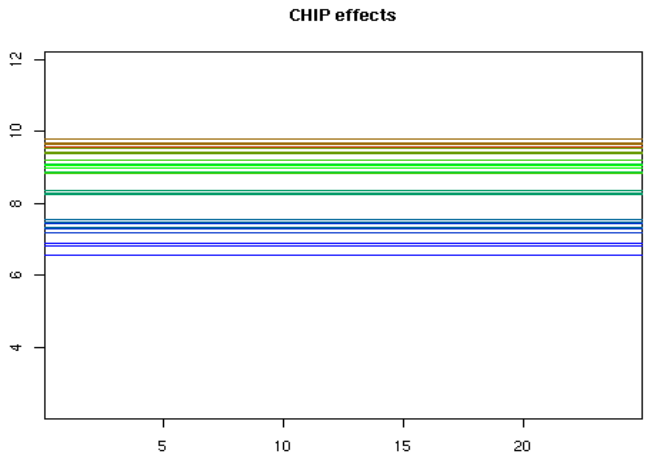
- Data for gene that is DE between heart (red=100% heart) and brain (blue=100% brain).
- 11 mixtures x 3 replicates = 33 samples (33 lines)
- Note the parallelism: probes have different affinities

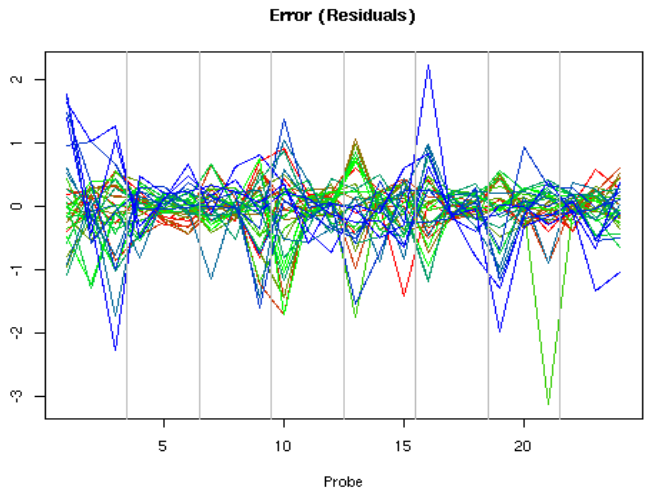
(Digression 2/3) Differential expression: Affy microarrays



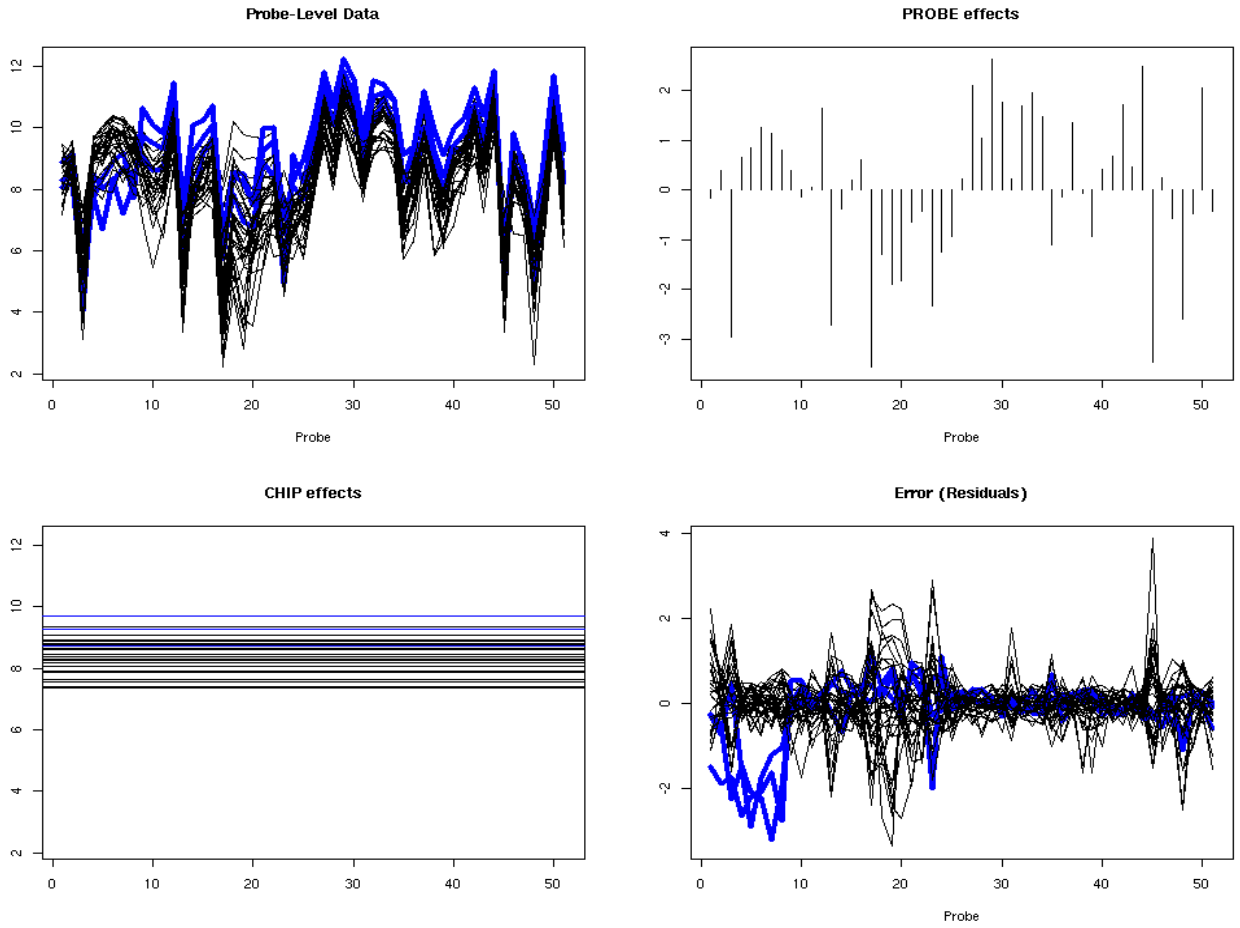


$$y_{ik} = g_i + p_k + e_{ik}$$





Digression 3/3: “Differential splicing” or “Differential isoform usage”: Affy microarrays



$$y_{ik} = g_i + p_k + e_{ik}$$

(back to RNA-seq) Beyond differential expression: differential splicing

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

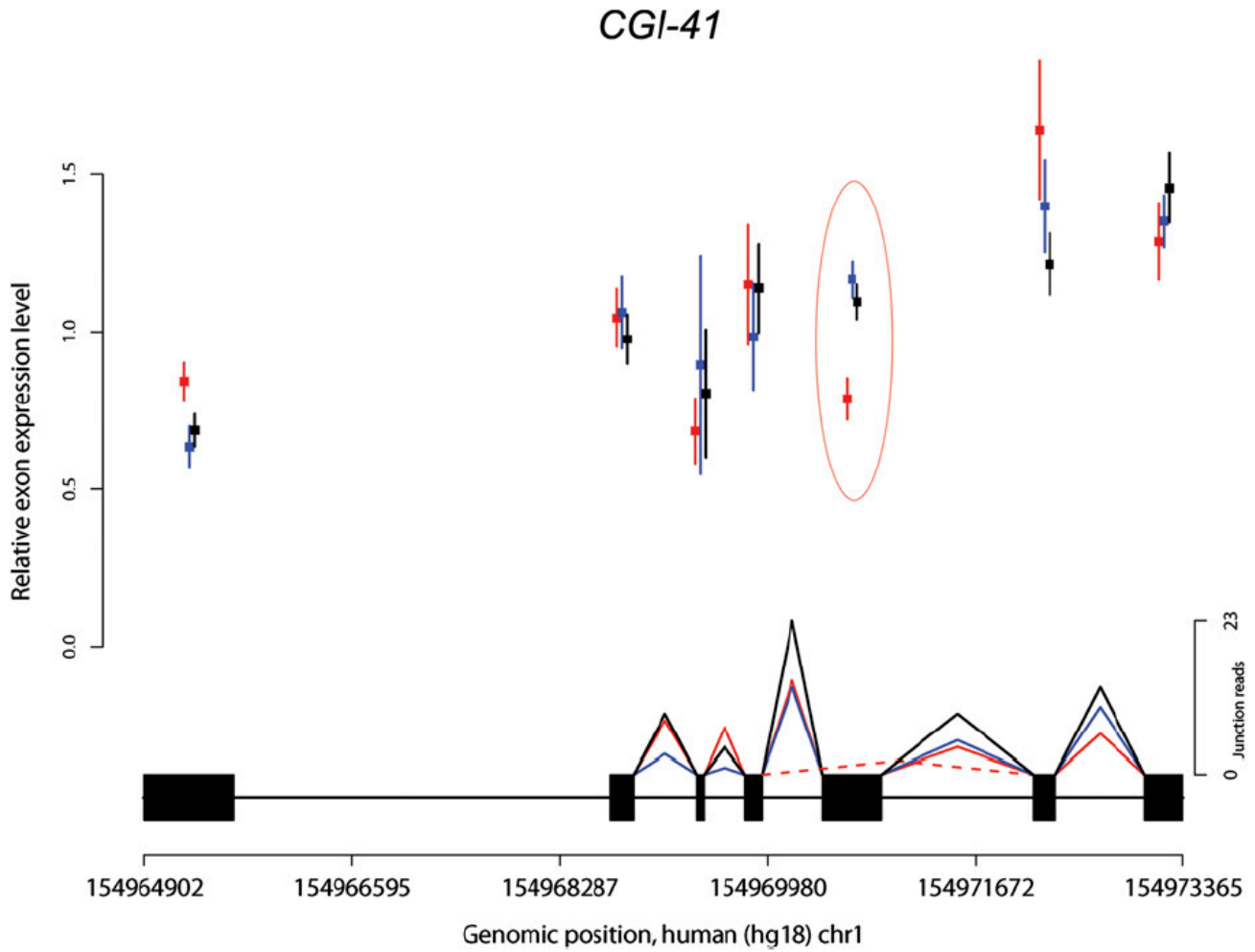
Hugues Richard[1,*], Marcel H. Schulz[1,2], Marc Sultan[3], Asja Nürnberger[3], Sabine Schrinner[3], Daniela Balzereit[3], Emilie Dagand[3], Axel Rasche[3], Hans Lehrach[3], Martin Vingron[1], Stefan A. Haas[1] and Marie-Laure Yaspo[3]

[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)

All the downstream statistical methods start with a count table.

How to get one?

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

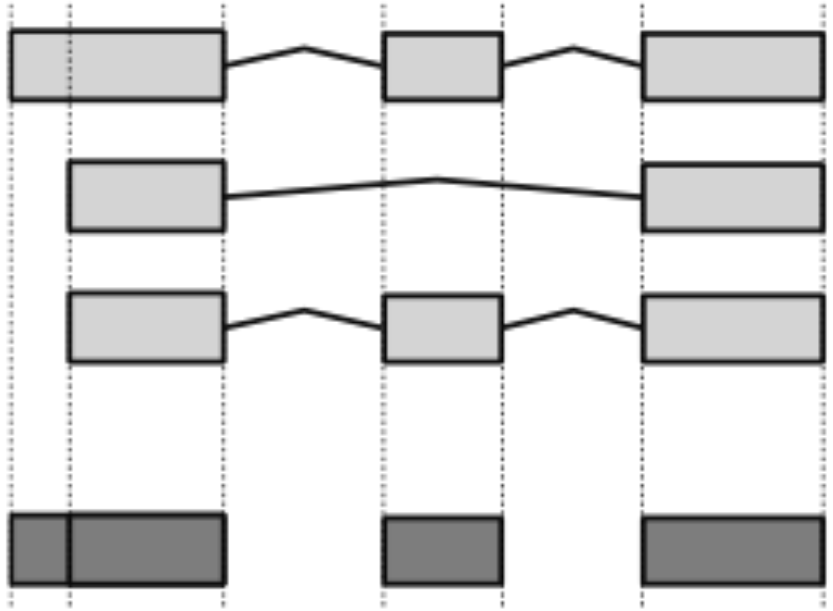


Figure 1. Flattening of gene models: This (fictional) gene has three annotated transcripts involving three exons (light shading), one of which has alternative boundaries. We form counting bins (dark shaded boxes) from the exons as depicted; the exon of variable length gets split into two bins.

Anders et al. 2012 Genome Research

Method

Detecting differential usage of exons from RNA-seq data

Simon Anders,[1,2] Alejandro Reyes,[1] and Wolfgang Huber
European Molecular Biology Laboratory, 69111 Heidelberg, Germany

DEXSeq

Transcript inventory versus differential expression

Shotgun RNA-seq data can be used both for identification of transcripts and for differential expression analysis. In the former, one annotates the regions of the genome that can be expressed, i.e., the exons, and how the pre-mRNAs are spliced into transcripts. In differential expression analysis, one aims to study the regulation of these processes across different conditions. For the method described here, we assume that a transcript inventory has already been defined, and focus on differential expression.

DEXSeq – general structure: exon-level models

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:

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

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

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

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

DEXSeq: sig. interaction terms = differential exon usage

(DEXSeq vignette)

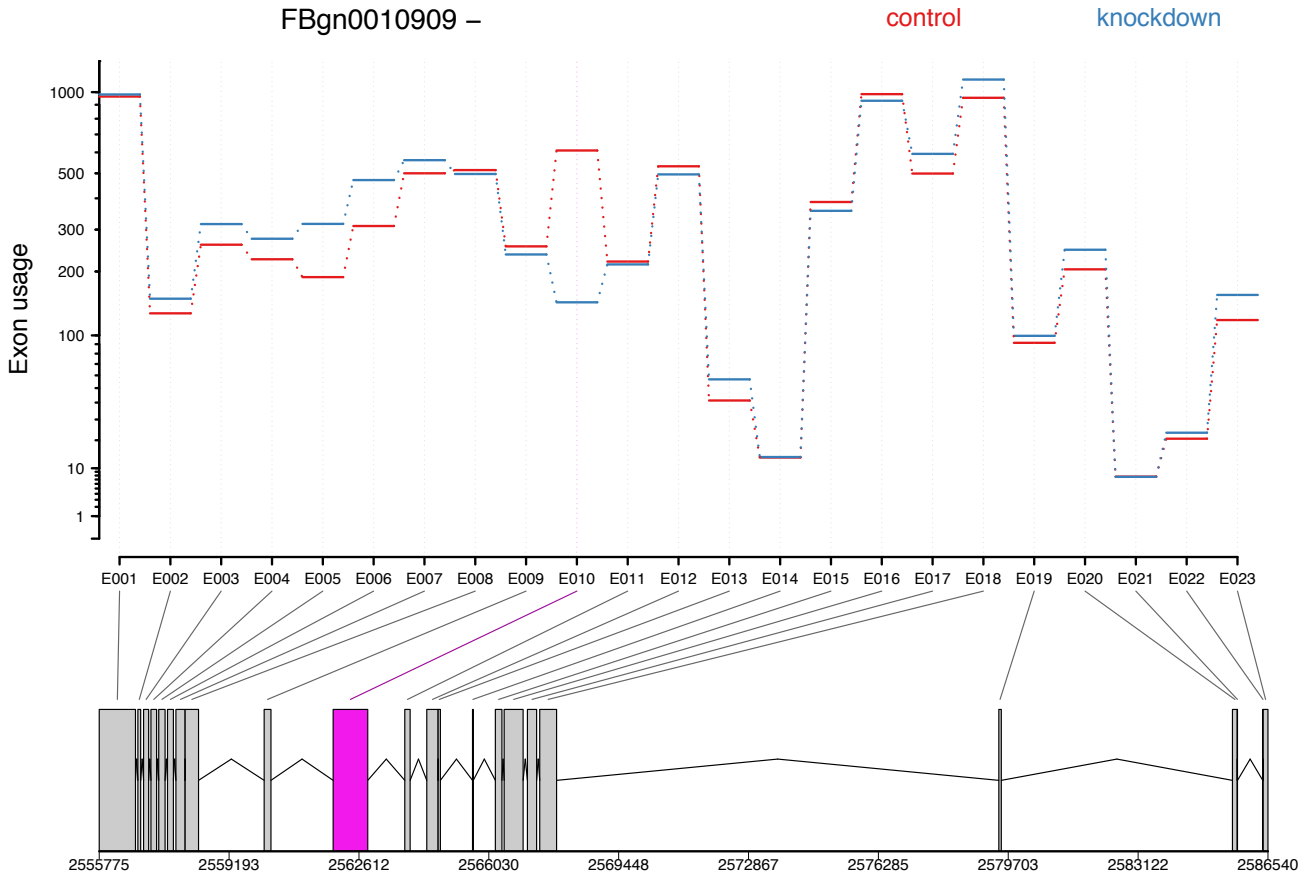
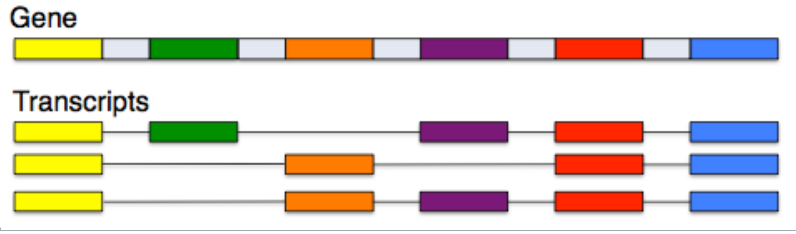


Figure 6: Fitted splicing
The plot represents the estimated effects, as in Figure 3, but after subtraction of overall changes in gene expression.



DTU —> dirichlet-multinomial distribution

Estimated:

- transcript ratios $\Pi = (\pi_1, \pi_2, \pi_3)$

Observed:

- transcript counts $Y = (y_1, y_2, y_3)$
- gene expression $n = \sum_{j=1}^{k} y_j$

Multinomial: $$P(\mathbf{Y} = \mathbf{y} | \mathbf{\Pi} = \pi) = \binom{n}{\mathbf{y}} \prod_{j=1}^{k} \pi_j^{y_j}$$

Dirichlet: $$P(\mathbf{\Pi} = \pi) = \frac{\Gamma(\gamma_+)}{\prod_{j=1}^{k} \Gamma(\gamma_j)} \prod_{j=1}^{k} \pi_j^{\gamma_j - 1}, \gamma_+ = \sum_{j=1}^{k} \gamma_j$$

Dirichlet-multinomial: $$P(\mathbf{Y} = \mathbf{y}) = \binom{n}{\mathbf{y}} \frac{\Gamma(\gamma_+)}{\Gamma(n + \gamma_+)} \prod_{j=1}^{k} \frac{\Gamma(y_j + \gamma_j)}{\Gamma(\gamma_j)}, \gamma_j = \pi_j \gamma_+$$

Nowicka and Robinson 2016, F1000 Research

https://peerj.com/preprints/27283/

Many more details here (228 cited papers!)

RNA sequencing data: hitchhiker's guide to expression analysis

Literature review | Bioinformatics | Computational Biology | Genomics | Data Science

Koen Van Den Berge *[1], Katharina Hembach *[2], Charlotte Soneson *[2,3], Simone Tiberi *[2], Lieven Clement [1], Michael I Love [4], Rob Patro [5], Mark Robinson ✉[2]

November 24, 2018