DEXSeq paper discussion

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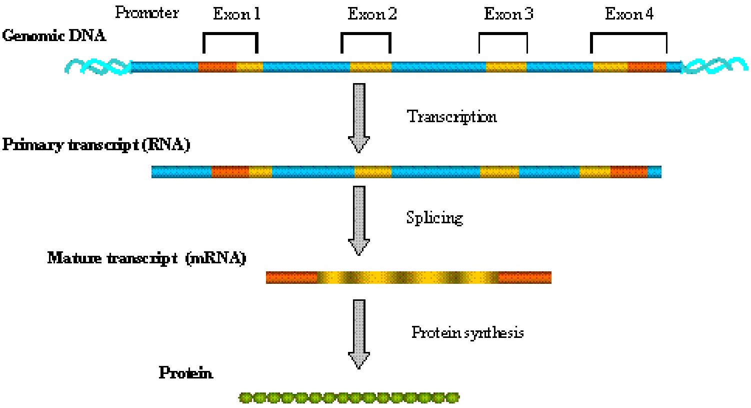
1. Background

2. DEXSeq paper

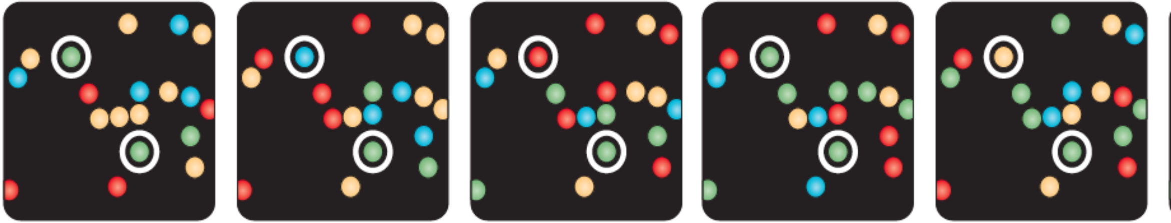
3. Results

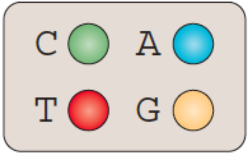
Gene Expression [1]



[1] Source: `http://www.ncbi.nlm.nih.gov/projects/genome/probe/doc/ApplExpression.shtml`

High-Throughput Sequencing [2]

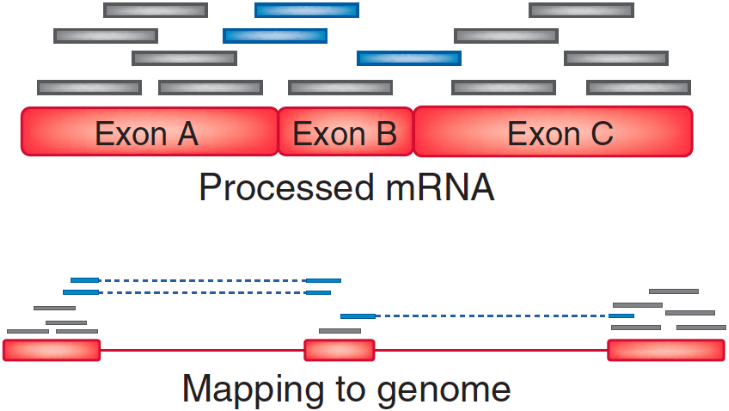




Top: CATCGT
Bottom: CCCCCC

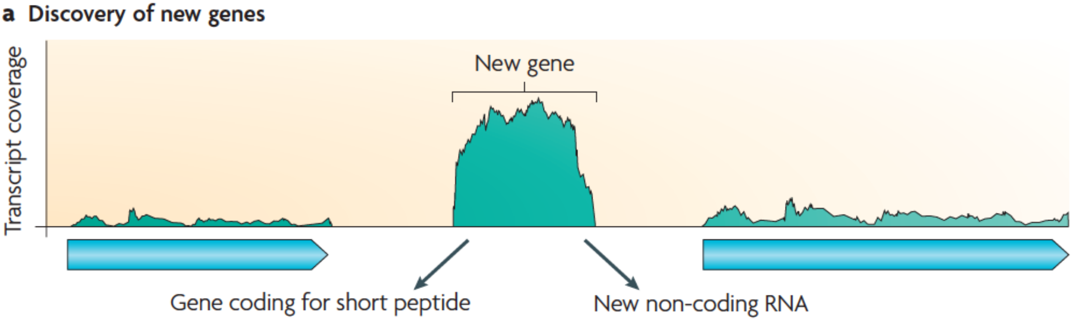
[2] Source: Metzker, Sequencing technologies — the next generation, 2010, Nat Rev Genet

Alignment (Mapping) [3]



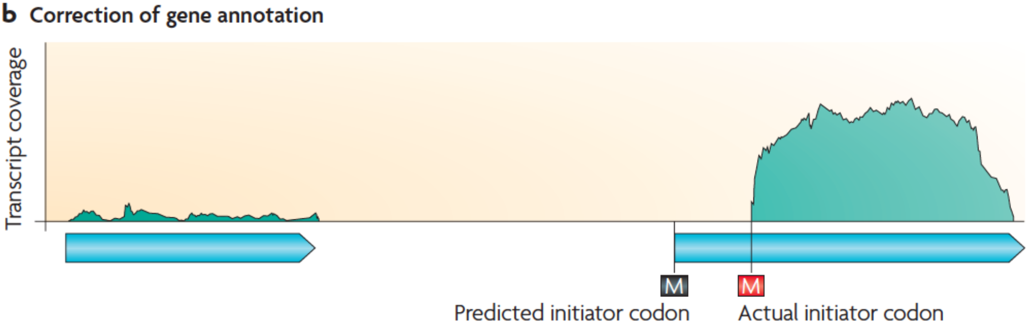
[3] Source: Trapnell *et al*, How to map billions of short reads onto genomes, 2009, Nat Biotech

What can we find? [4]



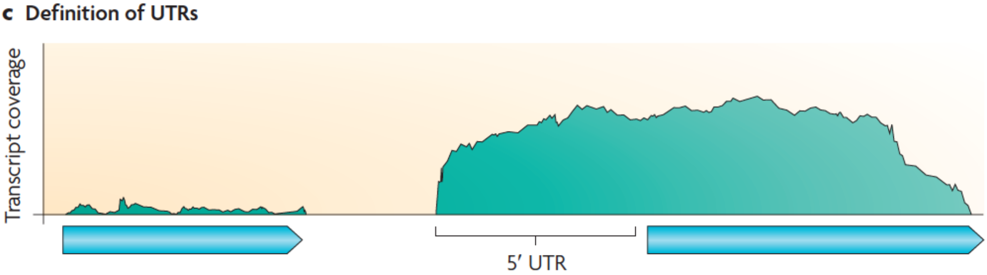
[4] Source: Sorek and Cossart, Prokaryotic transcriptomics a new view on regulation, physiology and pathogenicity, 2010, Nat Rev Genet

What can we find? [5]



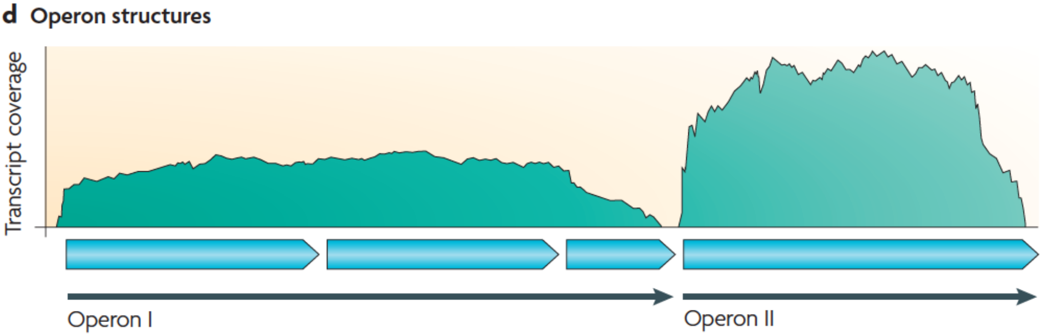
[5] Source: Sorek and Cossart, Prokaryotic transcriptomics a new view on regulation, physiology and pathogenicity, 2010, Nat Rev Genet

What can we find? [6]



[6] Source: Sorek and Cossart, Prokaryotic transcriptomics a new view on regulation, physiology and pathogenicity, 2010, Nat Rev Genet

What can we find? [7]



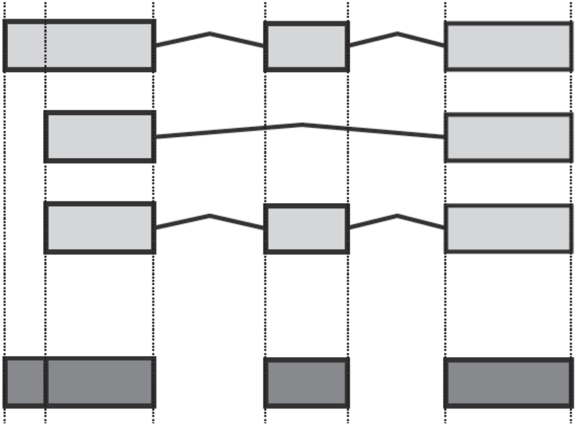
[7] Source: Sorek and Cossart, Prokaryotic transcriptomics a new view on regulation, physiology and pathogenicity, 2010, Nat Rev Genet

Main ideas

Compare two or more conditions of interest to find the DE exons (DEX).

- Focus on DE: assume a transcript inventory
- Account for biological variation
- Use GLMs
- Fine tuning to make it fast, control for false positives, and when possible increase power

Simplifying the exome: *counting bins* [8]



[8] Source: Anders, Reyes, Huber; Detecting differential usage of exons from RNA-seq data, 2012, Genome Research

Model

Using count data and assume it follows a negative binomial distribution

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

- counting bin l
- gene i
- sample $j = 1, \ldots, m$
- size factor s_j: needed because each sample is sequenced at a different *depth*
- α_{il} is the dispersion parameter

Poisson vs NB [10]

Poisson GLM

- Outcome $Y \sim \mathit{Poisson}(\mu)$
- Link function: $\log \mu = x'\beta$
- Variance function $\mathit{Var}(Y) = \mathit{Var}(\mu) = \alpha\mu$ where $\alpha = 1$. $\alpha \neq 1$ is the quasi-likelihood approach.

Negative Binomial Model: Gamma-Poisson mixture construction

- Assume unobserved r.v. E where $E \sim \mathit{Gamma}(\theta, 1/\theta)$.
  - Mean: $\theta \cdot 1/\theta = 1$, Variance: $\theta \cdot 1/\theta^2 = 1/\theta$.
- Assume that $Y|E \sim \mathit{Poisson}(\mu E)$
- Then Y has a negative binomial distribution with mean μ and variance $\mu + \mu^2/\theta = \mu(1 + \mu/\theta)$ [9]
- Variance of Y increases quadratically with the mean rather than linearly.

[9] $\alpha = 1/\theta$ in the DEXSeq paper

[10] Source: 140.654 2012 slides by Roger Peng

Main log-linear model

$$\log \mu_{ijl} = \beta_i^G + \beta_{il}^E + \beta_{i\rho_j}^C + \beta_{i\rho_j l}^{EC} \tag{2}$$

- β_i^G: baseline expression strength of gene i
- β_{il}^E: log of the expected fraction of the reads mapped to gene i that overlap counting bin l
- $\beta_{i\rho_j}^C$: log of the fold change in overall expression of gene i under condition ρ_j
- ρ_j experimental condition of sample j
- $\beta_{i\rho_j l}^{EC}$: effect condition ρ_j has on the fraction of reads falling into bin l

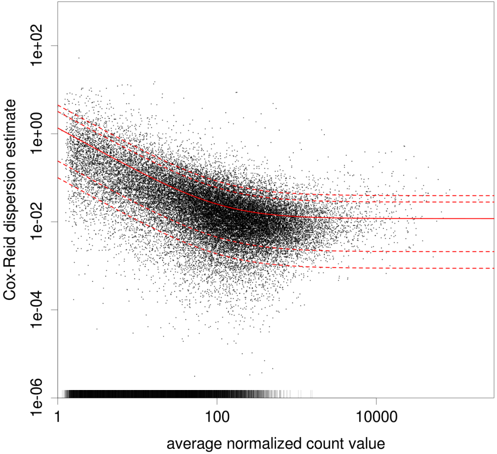
Variability: gene expression + exon usage

- Var. in gene expression: when the total number of transcripts for a gene i differs from the expected value under ρ_j
- Var. in exon usage: using different exons or counting bins

$$\log \mu_{ijl} = \beta_i^G + \beta_{il}^E + \beta_{ij}^S + \beta_{i\rho_j l}^{EC} \tag{3}$$

Change $\beta_{i\rho_j}^C$ by β_{ij}^S. Absorbs var. in gene expression.

Dispersion estimates [11]



[11] Source: Anders, Reyes, Huber; Detecting differential usage of exons from RNA-seq data, 2012, Genome Research

Analysis of Deviance [12]

- Deviance $D(\hat{\beta}) = 2\ell^* - 2\ell(\hat{\beta}; y)$ where ℓ^* is the saturated likelihood
- Two spaces for β: small S (nested) and large L with $H_0 : \beta \in S$ and $H_a : \beta \in L - S$.
- Likelihood ratio

$$LR = \frac{\mathscr{L}(\hat{\beta}_S; y)}{\mathscr{L}(\hat{\beta}_L; y)}$$

- Under H_0, $-2 \log LR \sim \chi^2_{|L|-|S|}$
- Note $D(\hat{\beta}_S) - D(\hat{\beta}_L) = -2[\ell(\hat{\beta}_S; y) - \ell(\hat{\beta}_L; y)] = -2 \log LR$

[12] Source: 140.654 2012 slides by Roger Peng

Testing for DEX: ANODEV

Fit two models

$$\log \mu_{ijl} = \beta_i^G + \beta_{il}^E + \beta_{ij}^S \tag{4}$$

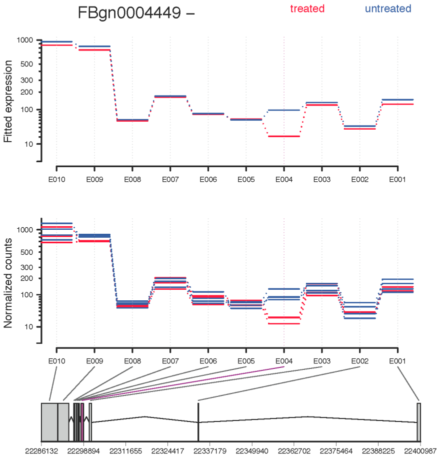
$$\log \mu_{ijl} = \beta_i^G + \beta_{il}^E + \beta_{ij}^S + \beta_{i\rho_j l}^{EC}\delta_{ll'} \tag{5}$$

where

$$\delta_{ll'} = \begin{cases} 1 & \text{if } l = l' \\ 0 & \text{otherwise} \end{cases}$$

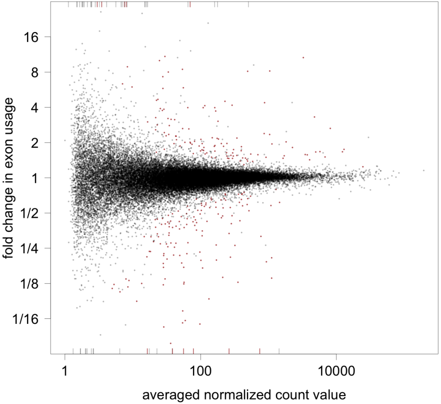
Then test using analysis of deviance (ANODEV)
Control FDR by adjusting p-values using Benjamini-Hochberg's method.

Finding DEX: knockdown of *pasilla* on *Drosophila melanogaster* example [13]



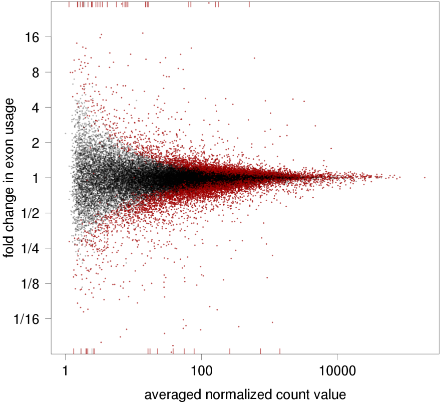
[13] Source `http://www-huber.embl.de/pub/DEXSeq/analysis/brooksetal/`

Detection power depends on mean [14]



[14] Source: reproduced with code from `http://genome.cshlp.org/content/suppl/2012/08/20/gr.133744.111.DC1/Supp_II.html`

Without considering biological variation [15]



[15] Source `http://www-huber.embl.de/pub/DEXSeq/analysis/brooksetal/`

Interesting comparison

- Mock comparison: check for DEX between replicates from a control condition
- Used an FDR of 10%
- DEXSeq: 8 genes (159 in the real control vs treatment comparison)
- Cuffdiff v 1.3.0: 639 genes (37 in real comp.)

This trend continues with other data sets.

Thanks!

- Main source: Anders, Reyes, Huber; Detecting differential usage of exons from RNA-seq data, 2012, Genome Research
- PMID: 22722343.