Transcript quantification and Analysis of alternative splicing with RNA-Seq

BMI/CS 776
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Overview

- RNA-Seq technology
- The RNA-Seq quantification problem
- Generative probabilistic models and Expectation-Maximization for the quantification task
- Inference of alternative splicing from RNA-Seq data with probabilistic splice graphs
Goals for lecture

• What is RNA-Seq?
• How is RNA-Seq used to measure the abundances of RNAs within cells?
• What probabilistic models and algorithms are used for analyzing RNA-Seq?
Measuring transcription the old way: microarrays

• Each spot has “probes” for a certain gene
• Probe: a DNA sequence complementary to a certain gene
• Relies on complementary hybridization
• Intensity/color of light from each spot is measurement of the number of transcripts for a certain gene in a sample
• Requires knowledge of gene sequences
Advantages of RNA-Seq over microarrays

- No reference sequence needed
  - With microarrays, limited to the probes on the chip
- Low background noise
- Large dynamic range
  - $10^5$ compared to $10^2$ for microarrays
- High technical reproducibility
- Identify novel transcripts and splicing events
RNA-Seq technology

- Leverages rapidly advancing sequencing technology
- Transcriptome analog to whole genome shotgun sequencing
- Two key differences from genome sequencing:
  1. Transcripts sequenced at different levels of coverage - expression levels
  2. Sequences already known (in many cases) - coverage is measurement
A generic RNA-Seq protocol

Sample RNA → RNA fragments → cDNA fragments → reads

- Fragmentation
- Reverse transcription + amplification
- Sequencing machine

DNA Sequencing reads:
- CCTTCNCACTTCGTTCCTCCAC
- TTTTTNCAGAGTTTTTTCTTG
- GAACANTCAACGCTTGGTGA
- GGAAANAAGACCCTGTTGAGC
- CCCGGNGATCCGCTGGGACAA
- GCAGCATATTGATAGATAACT
- CTAGCTACGCCTACGCATCG
- CATCTACGCTACGCATCG
- CCCGCGGATTTAGGCTATCTCG
- TCACACATCTCTAGCTACGACAT
- CATGCTAGCTATGCCTATCTA
RNA-Seq data: FASTQ format

@HWUSI-EAS1789_0001:3:2:1708:1305#0/1
CCTCNCACTTCGTCTCCACTTAGCGATAATTG
+HWUSI-EAS1789_0001:3:2:1708:1305#0/1
VVULVVYVVYZZZZZ\ee[a\^b`[a\a[\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a]\a}\n
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aaaaaBeeeeeffffffehhhhhhhgddhhhhahhhadh

1 Illumina HiSeq 2500 lane
~150 million reads
Tasks with RNA-Seq data

• **Assembly:**
  - Given: RNA-Seq reads (and possibly a genome sequence)
  - Do: Reconstruct full-length transcript sequences from the reads

• **Quantification (our focus):**
  - Given: RNA-Seq reads and transcript sequences
  - Do: Estimate the relative abundances of transcripts ("gene expression")

• **Differential expression or additional downstream analyses:**
  - Given: RNA-Seq reads from two different samples and transcript sequences
  - Do: Predict which transcripts have different abundances between two samples
RNA-Seq is a *relative* abundance measurement technology

- RNA-Seq gives you reads from the ends of a random **sample** of fragments in your library

- Without additional data this only gives information about **relative** abundances

- Additional information, such as levels of “spike-in” transcripts, are needed for absolute measurements
Issues with relative abundance measures

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sample 1 absolute abundance</th>
<th>Sample 1 relative abundance</th>
<th>Sample 2 absolute abundance</th>
<th>Sample 2 relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>10%</td>
<td>20</td>
<td>5%</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>10%</td>
<td>20</td>
<td>5%</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>10%</td>
<td>20</td>
<td>5%</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>10%</td>
<td>20</td>
<td>5%</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>10%</td>
<td>20</td>
<td>5%</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>50%</td>
<td>300</td>
<td>75%</td>
</tr>
</tbody>
</table>

- Changes in absolute expression of high expressors is a major factor
- Normalization is required for comparing samples in these situations
The basics of quantification with RNA-Seq data

• For simplicity, suppose reads are of length one (typically they are > 35 bases)

<table>
<thead>
<tr>
<th>transcripts</th>
<th>reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 200</td>
<td>100 A</td>
</tr>
<tr>
<td>2 60</td>
<td>60 C</td>
</tr>
<tr>
<td>3 80</td>
<td>40 G</td>
</tr>
</tbody>
</table>

• What relative abundances would you estimate for these genes?

• Relative abundance is relative transcript levels in the cell, not proportion of observed reads
Length dependence

• Probability of a read coming from a transcript $\propto$ relative abundance $\times$ length

transcripts

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>100 A</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>60 C</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>40 G</td>
</tr>
</tbody>
</table>

transcript 1 relative abundance

probability of read from transcript 1

\[
\hat{f}_1 \propto \frac{100}{200} = \frac{1}{400}
\]
Length dependence

- Probability of a read coming from a transcript \( \propto \) relative abundance \( \times \) length

\[
\begin{align*}
\hat{f}_1 & \propto \frac{100}{200} = \frac{1}{400} \quad \hat{f}_1 = 0.25 \\
\hat{f}_2 & \propto \frac{60}{60} = \frac{1}{200} \quad \hat{f}_2 = 0.5 \\
\hat{f}_3 & \propto \frac{40}{80} = \frac{1}{400} \quad \hat{f}_3 = 0.25
\end{align*}
\]
The basics of quantification from RNA-Seq data

• Basic assumption:

\[ \theta_i = P(\text{read from transcript } i) = Z^{-1} \tau_i \ell_i' \]

expression level
(relative abundance)

• Normalization factor is the mean length of expressed transcripts

\[ Z = \sum_i \tau_i \ell_i' \]
The basics of quantification from RNA-Seq data

• Estimate the probability of reads being generated from a given transcript by counting the number of reads that align to that transcript

\[ \hat{\theta}_i = \frac{c_i}{N} \]

• Convert to expression levels by normalizing by transcript length

\[ \hat{\tau}_i \propto \frac{\hat{\theta}_i}{\ell'_i} \]
The basics of quantification from RNA-Seq data

• Basic quantification algorithm
  – Align reads against a set of reference transcript sequences
  – Count the number of reads aligning to each transcript
  – Convert read counts into relative expression levels
Counts to expression levels

- RPKM - Reads Per Kilobase per Million mapped reads
  \[ \text{RPKM for gene } i = 10^9 \times \frac{c_i}{\ell_i' N} \]

- FPKM (fragments instead of reads, two reads per fragment, for paired end reads)

- TPM - Transcripts Per Million
  (estimate of) \[ \text{TPM for isoform } i = 10^6 \times Z \times \frac{c_i}{\ell_i' N} \]

- Prefer TPM to RPKM because of normalization factor
  - TPM is a technology-independent measure (simply a fraction)
What if reads do not uniquely map to transcripts?

• The approach described assumes that every read can be uniquely aligned to a single transcript

• This is generally not the case
  – Some genes have similar sequences - gene families, repetitive sequences
  – Alternative splice forms of a gene share a significant fraction of sequence
Central dogma of molecular biology

Griffith et al. PLoS Computational Biology 2015
Alternative splicing

pre-mRNA

splicing

alternatively spliced mRNAs

translation

protein isoforms
# Multi-mapping reads in RNA-Seq

<table>
<thead>
<tr>
<th>Species</th>
<th>Read length</th>
<th>% multi-mapping reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>25</td>
<td>17%</td>
</tr>
<tr>
<td>Mouse</td>
<td>75</td>
<td>10%</td>
</tr>
<tr>
<td>Maize</td>
<td>25</td>
<td>52%</td>
</tr>
<tr>
<td>Axolotl</td>
<td>76</td>
<td>23%</td>
</tr>
<tr>
<td>Human</td>
<td>50</td>
<td>23%</td>
</tr>
</tbody>
</table>

- Throwing away multi-mapping reads leads to
  - Loss of information
  - Potentially biased estimates of abundance
Distributions of alignment counts

Mouse Liver

Mouse Liver

Maize

Maize
What if reads do not uniquely map to transcripts?

- Multiread: a read that could have been derived from multiple transcripts

<table>
<thead>
<tr>
<th>transcripts</th>
<th>reads</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 20 + 180 = 200</td>
<td>90 A</td>
</tr>
<tr>
<td>2 20 + 40 = 60</td>
<td>40 C</td>
</tr>
<tr>
<td>3 80</td>
<td>40 G</td>
</tr>
<tr>
<td></td>
<td>30 T</td>
</tr>
</tbody>
</table>

- How would you estimate the relative abundances for these transcripts?
Some options for handling multireads

• Discard multireads, estimate based on uniquely mapping reads only

• Discard multireads, but use “unique length” of each transcript in calculations

• “Rescue” multireads by allocating (fractions of) them to the transcripts

  – Three step algorithm

    1. Estimate abundances based on uniquely mapping reads only

    2. For each multiread, divide it between the transcripts to which it maps, proportionally to their abundances estimated in the first step

    3. Recompute abundances based on updated counts for each transcript
Rescue method example - Step 1

transcripts

reads

1  60

2  80

3

200

90 A
40 C
40 G
30 T

\[
\hat{f}_{1\text{unique}} = \frac{\frac{90}{200}}{\frac{90}{200} + \frac{40}{60} + \frac{40}{80}} = 0.278
\]

\[
\hat{f}_{2\text{unique}} = 0.412
\]

\[
\hat{f}_{3\text{unique}} = 0.309
\]
Rescue method example - Step 2

**Step 2**

\[ c_1^{\text{rescue}} = 90 + 30 \times \frac{0.278}{0.278 + 0.412} = 102.1 \]

\[ c_2^{\text{rescue}} = 40 + 30 \times \frac{0.412}{0.278 + 0.412} = 57.9 \]

\[ c_3^{\text{rescue}} = 40 + 0 = 40 \]
Rescue method example - Step 3

transcripts reads

1 60 90 A
2 80 40 C
3 200 40 G
30 T

Step 3

\[ \hat{f}_{\text{rescue}} = \frac{\frac{102.1}{200}}{\frac{102.1}{200} + \frac{57.9}{60} + \frac{40}{80}} = 0.258 \]

\[ \hat{f}_{\text{rescue}} = \frac{\frac{57.9}{60}}{\frac{102.1}{200} + \frac{57.9}{60} + \frac{40}{80}} = 0.488 \]

\[ \hat{f}_{\text{rescue}} = \frac{\frac{40}{80}}{\frac{102.1}{200} + \frac{57.9}{60} + \frac{40}{80}} = 0.253 \]
An observation about the rescue method

- Note that at the end of the rescue algorithm, we have an updated set of abundance estimates.
- These new estimates could be used to reallocate the multireads.
- And then we could update our abundance estimates once again.
- And repeat!
- This is the intuition behind the statistical approach to this problem.
RSEM (RNA-Seq by Expectation-Maximization) - a generative probabilistic model

- Simplified view of the model (plate notation)
  - Grey – observed variable
  - White – latent (unobserved) variables
RSEM - a generative probabilistic model

$$P(g, f, s, o, \ell, q, r | \theta) = \prod_{n=1}^{N} P(g_n | \theta)P(f_n | g_n)P(s_n | f_n, g_n)P(o_n | g_n)P(q_n)P(\ell_n | f_n)P(r_n | g_n, f_n, s_n, o_n, \ell_n, q_n)$$
Quantification as maximum likelihood inference

- Observed data likelihood
  \[ P(r, \ell, q|\theta) = \prod_{n=1}^{N} \sum_{i=0}^{M} \theta_i \sum_{j=0}^{L_i} \sum_{k=0}^{L_i} \sum_{o=0}^{1} P(R_n = r_n, L_n = \ell_n, Q_n = q_n, S_n = j, F_n = k, O_n = o|G_n = i) \]

- Likelihood function is concave with respect to \( \theta \)
  - Has a global maximum (or global maxima)

- Expectation-Maximization for optimization

“RNA-Seq gene expression estimation with read mapping uncertainty”
Li, B., Ruotti, V., Stewart, R., Thomson, J., Dewey, C.
Bioinformatics, 2010
Approximate inference with read alignments

\[ P(r, \ell, q|\theta) = \prod_{n=1}^{N} \sum_{i=0}^{M} \theta_i \sum_{j=0}^{L_i} \sum_{k=0}^{L_i} \sum_{o=0}^{1} P(R_n = r_n, L_n = \ell_n, Q_n = q_n, S_n = j, F_n = k, O_n = o|G_n = i) \]

- Full likelihood computation requires \( O(NM^2L^2) \) time
  - \( N \) (number of reads) \( \sim 10^7 \)
  - \( M \) (number of transcripts) \( \sim 10^4 \)
  - \( L \) (average transcript length) \( \sim 10^3 \)

- Approximate by alignment

\[ P(r, \ell, q|\theta) = \prod_{n=1}^{N} \sum_{(i,j,k,o) \in \pi_n^x} \theta_i P(R_n = r_n, L_n = \ell_n, Q_n = q_n, Z_{nijk\ell o} = 1|G_n = i) \]

all local alignments of read \( n \) with at most \( x \) mismatches
EM Algorithm

• Expectation-Maximization for RNA-Seq
  – E-step: Compute expected read counts given current expression levels
  – M-step: Compute expression values maximizing likelihood given expected read counts

• Rescue algorithm $\approx 1$ iteration of EM
Expected read count visualization
Improved accuracy over unique and rescue

Mouse gene-level expression estimation
Improving accuracy on repetitive genomes: maize

Maize gene-level expression estimation
RNA-Seq and RSEM summary

• RNA-Seq is the preferred technology for transcriptome analysis in most settings
• The major challenge in analyzing RNA-Seq data: the reads are much shorter than the transcripts from which they are derived
• Tasks with RNA-Seq data thus require handling hidden information: which gene/isoform gave rise to a given read
• The Expectation-Maximization algorithm is extremely powerful in these situations
Recent developments in RNA-Seq

• Long read sequences: PacBio and Oxford Nanopore

• Single-cell RNA-Seq: review
  – Observe heterogeneity of cell populations
  – Model technical artifacts (e.g. artificial 0 counts)
  – Detect sub-populations
  – Predict pseudotime through dynamic processes
  – Detect gene-gene and cell-cell relationships

• Alignment-free quantification:
  – Kallisto
  – Salmon
Public sources of RNA-Seq data

  – Both microarray and sequencing data
• Sequence Read Archive (SRA): http://www.ncbi.nlm.nih.gov/sra
  – All sequencing data (not necessarily RNA-Seq)
• ArrayExpress: https://www.ebi.ac.uk/arrayexpress/
  – European version of GEO
• Homogenized data: MetaSRA, Toil, recount2, ARCHS⁴
Inference of alternative splicing from RNA-Seq data

• Part I - Alternative splicing and the challenges it poses
• Part II - A solution: Probabilistic Splice Graphs (PSGs)
• Part III - Evaluating PSG methodology
Alternative splicing

pre-mRNA

splicing

alternatively spliced mRNAs

translation

protein isoforms
Classes of alternative splicing events

- Exon skipping
- Mutually exclusive exons
- Alternative 5' donor sites
- Alternative 3' acceptor sites
- Intron retention
Complication 1: De novo transcriptome assembly

- RNA-Seq reads/fragments are relatively short
- Often insufficient to reconstruct full-length isoforms in the presence of alternative splicing
- Transcriptome assemblies perhaps best left in “graph” form
  - De Bruijn graph
  - String graphs

Graph constructed by the “Butterfly” module of Trinity (Grabherr et al. 2011)
Complication 2: Non-identifiability of full-length isoform models

two (of infinitely many) possible isoform abundances

LeGault et al. 2013
Complication 3: Combinatorial explosion of distinct isoforms

- Combinatorial explosion of the number of possible isoforms for each gene
- Insufficient data to accurately estimate abundances of thousands of isoforms

Drosophila Dscam: more than 38,000 possible isoforms (Schmucker et al., 2000)
Inference of alternative splicing from RNA-Seq data

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• Part III - Evaluating PSG methodology
Splice Graphs

• Heber et al. 2002
• Compact **data structure** for representing the possible isoforms of a gene
Splice Graphs with EST and RNA-Seq data

• Xing et al. 2006
  – EM algorithm for estimating abundances of all possible isoforms given splice graph and EST data
  – Expressed Sequence Tag (EST), 74.2 million in 2013

• Montgomery et al. 2010, Singh et al. 2011
  – Graph flow-based methods for quantification/differential splicing given RNA-Seq data

• Rogers et al. 2012
  – SpliceGrapher: construct splice graph structure given RNA-Seq data
Probabilistic Splice Graphs

- Jenkins et al. 2006
- Compact **probabilistic model** representing isoform frequencies in terms of frequencies of individual splice events
- Originally used by Jenkins et al. for EST analysis
Probabilistic Splice Graph Complexity

known isoforms

“line graph”

“exon graph”

“higher-order exon graph”

“unfactorized graph”
Advantages of PSGs

• **Compact** description of the possible isoforms of a gene
  – Models the **frequencies** of potentially exponentially many isoforms with a **polynomial** number of parameters
  – Models **dependence or independence** of splice events

• The parameters of a PSG are more often **identifiable** than a model that has a parameter for every possible isoform

• Splice graphs are **naturally-produced structures** from transcriptome assemblers
PSGs are alternative "parsimonious" models

- Other methods find smallest set of isoform structures that explain the data
  - Cufflinks (Trapnell et al., 2010)
  - IsoLasso (Li et al., 2011)
  - NSMAP (Xia et al., 2011)
  - SLIDE (Li et al., 2011)

- PSG models are another form of parsimonious model
  - Minimize the number of splice event parameters
  - Assumption of independence between splice events
Application of PSGs to RNA-Seq data

  - Combined model of PSG with RNA-Seq generative model
  - Efficient PSG parameter estimation with EM and dynamic programming
  - Identifiability proofs for PSG with RNA-Seq data
  - Differential processing (splicing) tests
The PSG parameter inference task

• Given: RNA-Seq reads and a PSG structure

• Do: Estimate the (ML or MAP) parameters for the model
PSG notations

- A directed acyclic graph (DAC)
- Vertex $v_i$ is a sequence with length $l_i$
- Edge $(v_i, v_j)$ with weight $0 \leq \alpha_{i,j} \leq 1$
- An isoform is a path $s$ with weight $w(s) = \prod_{i=1}^{s-1} \alpha_{s_i, s_{i+1}}$
A model of RNA-Seq from PSGs

• RSEM model extended to probabilistic splice graphs
  – fragment length distribution, quality scores, read mapping ambiguity
• Dynamic programming algorithms → polynomial time inference for genes with an exponential number of isoforms

Probability of including vertex \( j \) given that vertex \( i \) was in transcript

Expected prefix length from \( v_0 \) to \( v_i \)

Expected suffix length from \( v_i \) to \( v_M \)
EM for PSG parameter estimation

- **E-step**: compute the expectation of the number of times edge \((i,j)\) is used
  \[
  E[Z_{ni,j}] = \frac{\sum_{(b,s) \in \pi(r_n)} g(s, i, j)}{\sum_{(b,s) \in \pi(r_n)} g(s)}
  \]
  \[
  g(s) = f(0, s_1)w(s)
  
  g(s, i, j) = \begin{cases}
    f(0, s_1)w(s) & (i, j) \in s \\
    f(0, i)\alpha_{ij}f(j, s_1)w(s) & \text{if } \exists \text{ path from } v_j \text{ to } s_1 \\
    f(0, s_1)w(s)f(s_{|s|}, i)\alpha_{ij} & \text{if } \exists \text{ path from } s_{|s|} \text{ to } v_i \\
    0 & \text{otherwise}
  \end{cases}
  \]

- **M-step**: maximize the completely-observed likelihood given the edge counts
  \[
  \alpha_{ij} = \frac{c_{ij}}{\sum_k \frac{c_{ik}}{(d_p(i)+d_q(k))}}
  
  c_{ij} = E_{\alpha(t)}[Z_{ij}]
  \]
Identifiability of PSGs with RNA-Seq data

- **Identifiability:** \( P(D|M, \theta) = P(D|M, \theta'), \forall D \leftrightarrow \theta = \theta' \)
- **Proposition:** If for all edges \((u, v)\), there exists a read that is uniquely derived from that edge, or \(v\) has indegree 1 and there exists a read that is uniquely derived from \(v\), then the PSG is identifiable.
The differential processing (DP) task

- Given: RNA-Seq reads from two conditions and a PSG structure

<table>
<thead>
<tr>
<th>condition 1</th>
<th>condition 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCTTCNCACTTCGTTCCTCACC</td>
<td>CATATCGTGTAGCTAGTAGC</td>
</tr>
<tr>
<td>TTTTTNCAAGATTTTTTTTTTG</td>
<td>CCACACTAGGCTACGTGCGCA</td>
</tr>
<tr>
<td>GAACANTCCAACGCTTGGTGA</td>
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</tr>
</tbody>
</table>

- Do: Determine if the processing frequencies are different

\[
\alpha_1 = \alpha_1' \quad \text{and} \quad \alpha_2 = \alpha_2' \quad ?
\]

\[
\alpha_1 = \alpha_1' \quad \text{or} \quad \alpha_2 = \alpha_2' \quad ?
\]
Our approach to the differential processing (DP) task

• Simple likelihood ratio tests with PSG model

• Test for null hypothesis that all frequencies are the same

\[ \text{LR} = \frac{P(R^1|\hat{\alpha}^1)P(R^2|\hat{\alpha}^2)}{P(R^1 \cup R^2|\hat{\alpha}^{12})} \]

• Test for null hypothesis that frequencies of edges out of one vertex \((i)\) are the same

\[ \text{LR} = \frac{P(R^1|\hat{\alpha}^1)P(R^2|\hat{\alpha}^2)}{P(R^1, R^2|\hat{\alpha}^1_i, \hat{\alpha}^2_i, \hat{\alpha}^{12}_i)} \]
Inference of alternative splicing from RNA-Seq data

• Part I - The problem
• Part II - A solution: Probabilistic Splice Graphs (PSGs)
• Part III - Evaluating PSG methodology
Efficient inference for highly-spliced genes

• DSCAM running time test
  – 23,976 isoforms

  – 184 read pairs from a modENCODE sample

<table>
<thead>
<tr>
<th>Method</th>
<th>RSEM</th>
<th>Cufflinks</th>
<th>PSG EM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Running time</td>
<td>Not possible</td>
<td>&gt; 6 hours (&gt; 90 GB RAM)</td>
<td>&lt; 3 seconds</td>
</tr>
</tbody>
</table>
A simple method for comparison

• The **Junction-Read (JR)** method

• Keep only reads that align to the splice junctions (edges in the PSG)

• Throws away data, but is very robust to model assumption violations
Convergence with simulated data
Comparisons on real data

- Require notion of “distance” between estimates from different methods
- Our distance measure:
  - per vertex
  - maximum difference between probability estimates on out-edges of vertex (L-$\infty$ norm)

\[
\text{distance}_v(A, B) = \max(|0.6 - 0.2|, |0.5 - 0.3|, |0.3 - 0.1|) = 0.4
\]
How close are the estimates from JR and EM on real data?

Vertices from 88 most abundant (> 5000 reads) alternatively-spliced genes in a modENCODE fly data set
Convergence of estimates on real data
Comparing PSGs of different complexity

- Same set of fly data
- Estimated with three classes of PSG: line, exon, full-length
- Compared estimates to those from JR (gold-standard)
- No statistically-significant difference between exon and full-length graph estimates
Summary of Junction-Read comparison results

• Estimates using PSG models are generally close to those from the simplistic JR-method
  – ⇒ PSG model assumptions appear to be reasonable

• PSG estimates converge more quickly as the data set increases in size
  – ⇒ Our EM estimation procedure uses information from all reads, not just those that span splice junctions

• Exon-graph estimates as good as those using traditional full-length isoform models
  – ⇒ Independence assumptions of exon graphs appear to be reasonable
Differential processing detection

DP Accuracy on real data

<table>
<thead>
<tr>
<th>Sample 1</th>
<th>Sample 2</th>
<th>PSG</th>
<th>FDM</th>
<th>Cuffdiff</th>
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Differential processing detection

## DP accuracy on simulated data

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<tr>
<th>Method</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Predicted DP</th>
<th>Recall</th>
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</tbody>
</table>

Simulations based on two ENCODE cell lines, 10% of genes selected to be DP.
Next steps for modeling RNA-Seq with PSGs

- **Graph construction**
  - Exon discovery
  - Splice junction discovery

- **Model selection**
  - Learning dependencies between splice events
Summary

• Alternative splicing is a significant complication in RNA-Seq analysis

• Probabilistic Splice Graphs enable identifiable models for alternatively spliced genes with efficient inference algorithms

• Differential processing (splicing) tests with PSG models look promising