Machine Learning Methods for RNA-seq-based Transcriptome Reconstruction

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Max Planck Society, Tübingen, Germany

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Friedrich Miescher Laboratory
of the Max Planck Society

MAX-PLANCK-GESELLSCHAFT

Discovery of the Nuclein
(Friedrich Miescher, 1869)

Discovery of Nuclein:

- from lymphocyte & salmon
- "multi-basic acid" (≥ 4)

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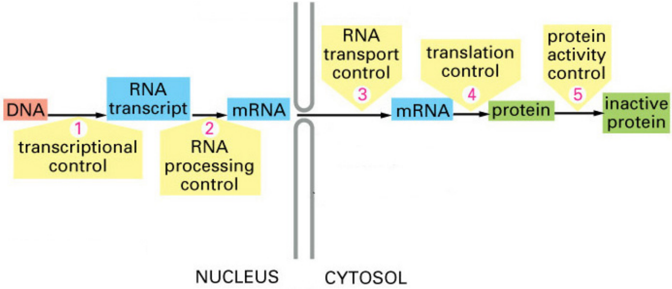
Learning about the Transcriptome

⇝ **What is encoded on the genome and how is it processed?**



Computational Point of View

- How to learn to predict what these processes accomplish?
- How well can we predict it from the available information?

Biological View

- What can we not predict yet? What is missing?
- Can we derive a deeper understanding of these processes?

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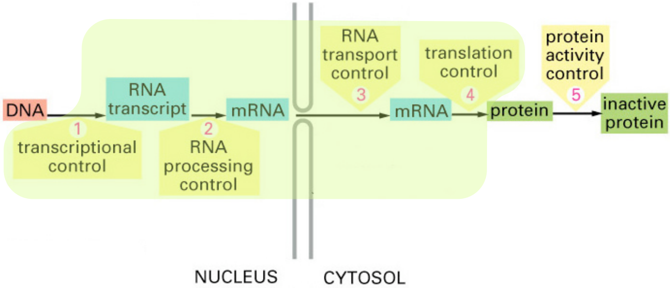

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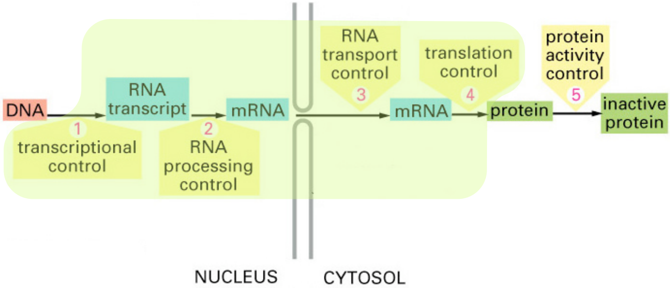

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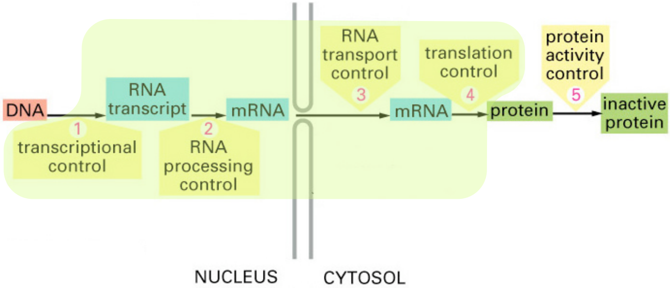

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Given: Observations of some complex phenomenon

Goal: Learn from data & build predictive models

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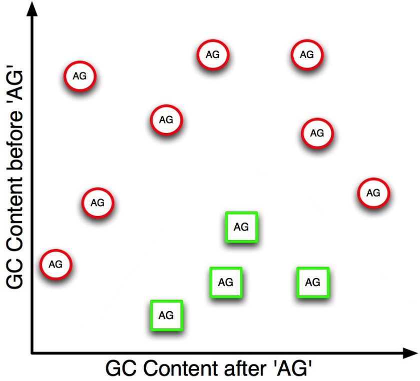

Two different classes of observations

Machine Learning
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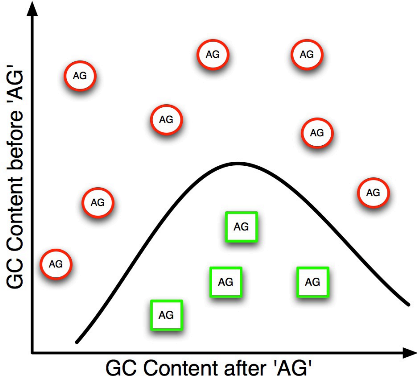


Inferred classification rule

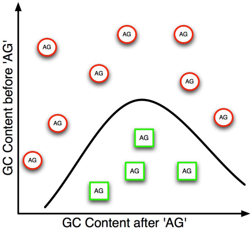
Machine Learning

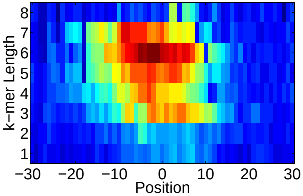
Learning from empirical observations

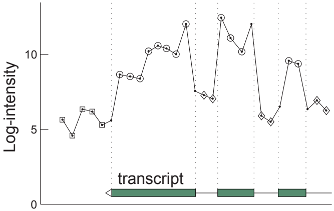
Given: Observations of some complex phenomenon

Goal: Learn from data & build predictive models

1. Large scale sequence classification
2. Analysis and explanation of learning results
3. Sequence segmentation & structure prediction







Deep RNA Sequencing (RNA-Seq)

RNA-Seq allows . . .

- High-throughput transcriptome measurements
- Qualitative studies
  - New transcripts
  - Improved gene models
- Quantitative studies at high resolution
  - Differential expression in tissues, conditions, genotypes, etc.

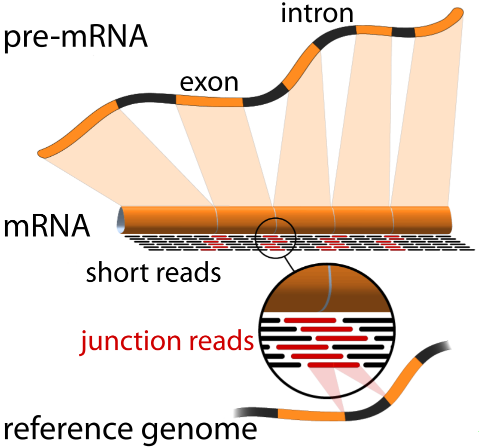


Figure adapted from Wikipedia

Goal: Obtain complete transcriptome for further analyses

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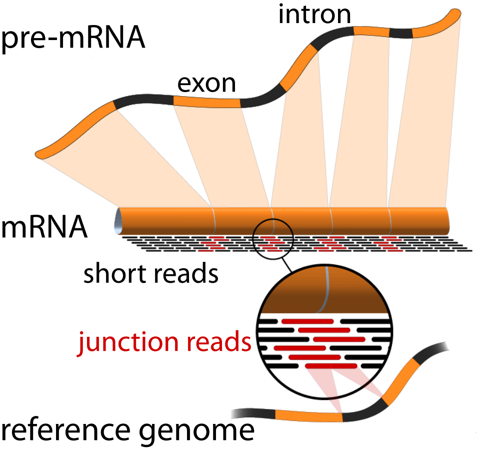

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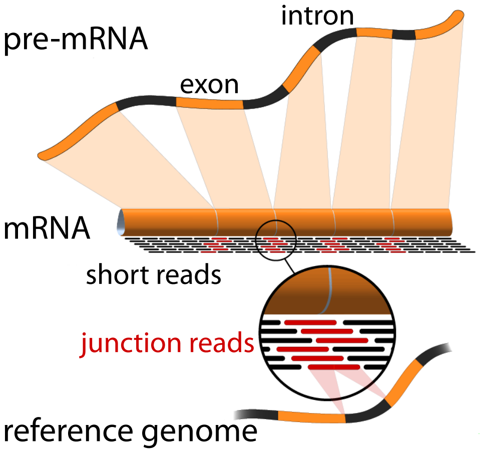

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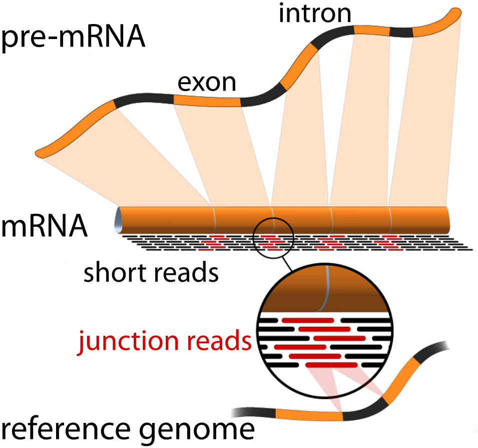

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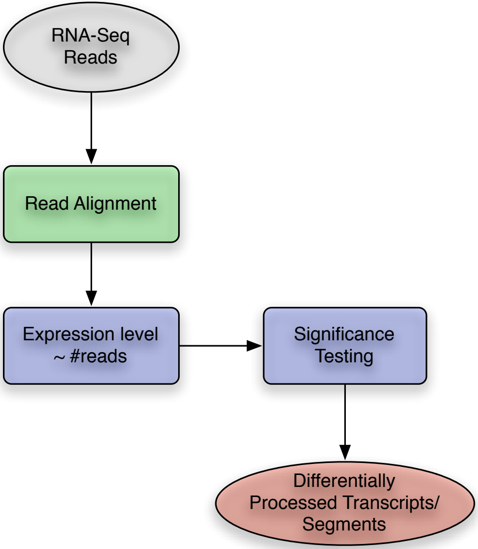
Common RNA-Seq Analysis Steps


RNA-Seq Reads
Read Alignment
Expression level ~ #reads
Significance Testing
Differentially Processed Transcripts/ Segments

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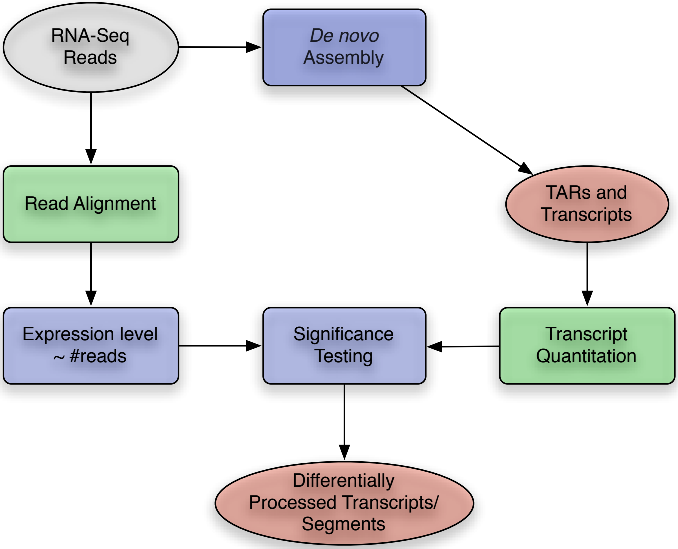

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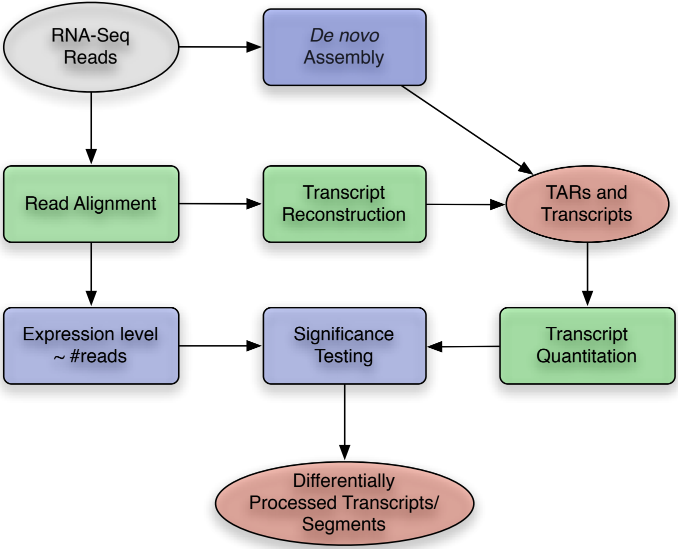
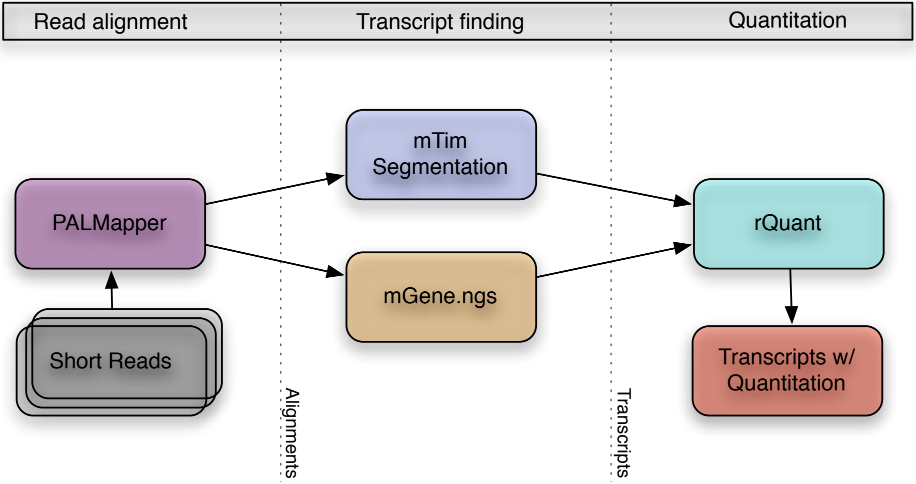


RNA-Seq Pipeline Overview



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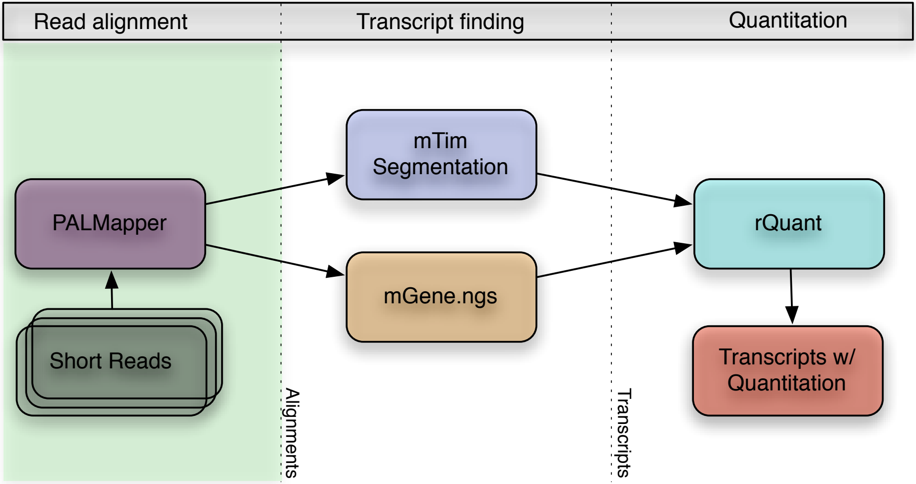

Step 1: PALMapper Read Alignment

(PALMapper = QPALMA + GenomeMapper)

GenomeMapper for (unspliced) read mapping:

- Alignments based on GenomeMapper developed in Tübingen for the 1001 plant genome project [Schneeberger et al., 2009]
- *k*-mer based index, well suited for smaller genomes

More info: `http://fml.mpg.de/raetsch/suppl/palmapper`

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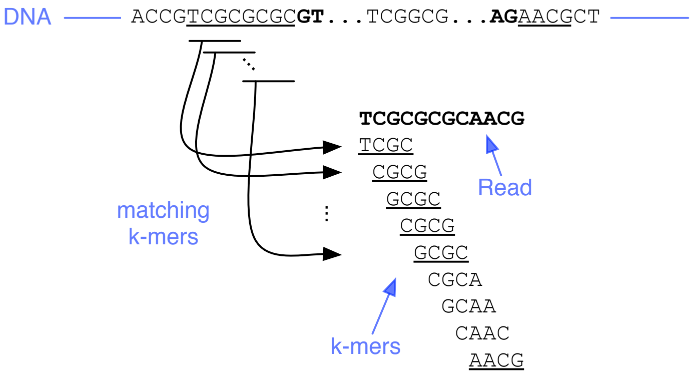

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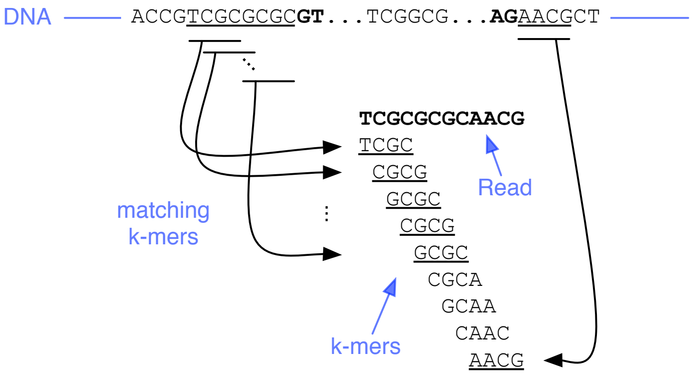

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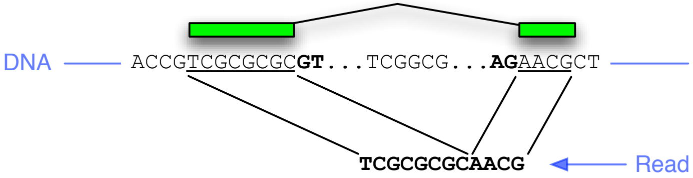
QPALMA for spliced read alignments:

- GenomeMapper identifies *seed regions*
- *Spliced alignments* by QPALMA [De Bona et al., 2008]



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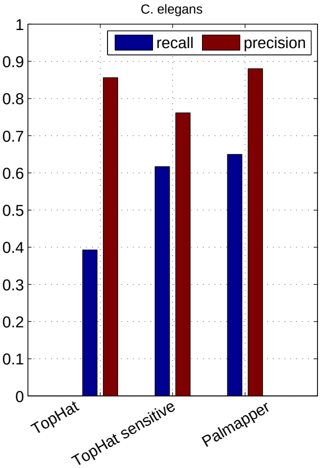
PALMapper Accuracy Evaluation

How accurately can PALMapper identify introns?



PALMapper (3.5h) and TopHat (3.5h/10h) aligning 24M reads

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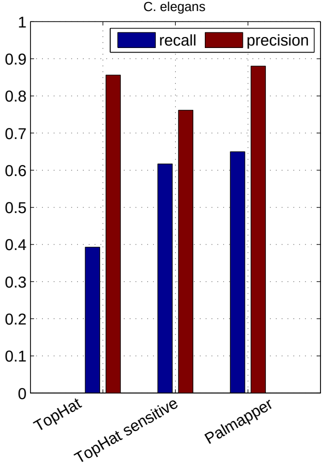
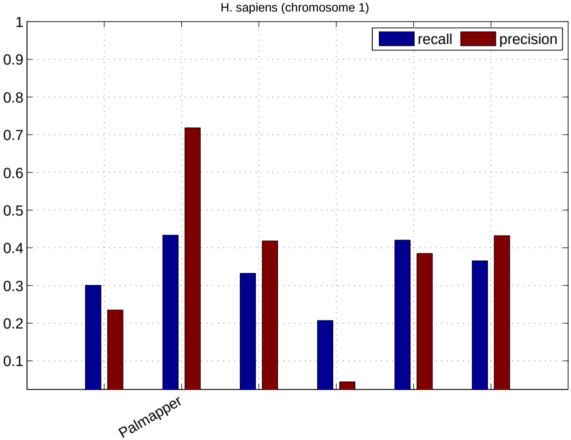
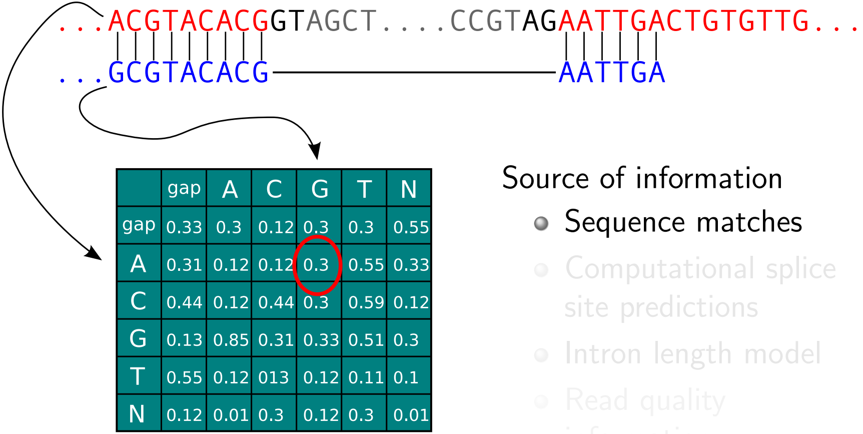




PALMapper (3.5h) and TopHat (3.5h/10h) aligning 24M reads

Comparison of PALMapper with other alignment programs within the RGASP project (preliminary)

QPALMA: Extended Smith-Waterman Scoring



	gap	A	C	G	T	N
gap	0.33	0.3	0.12	0.3	0.3	0.55
A	0.31	0.12	0.12	0.3	0.55	0.33
C	0.44	0.12	0.44	0.3	0.59	0.12
G	0.13	0.85	0.31	0.33	0.51	0.3
T	0.55	0.12	013	0.12	0.11	0.1
N	0.12	0.01	0.3	0.12	0.3	0.01

Source of information

- Sequence matches
- Computational splice site predictions
- Intron length model
- Read quality information

Classical scoring $f : \Sigma \times \Sigma \to \mathbb{R}$

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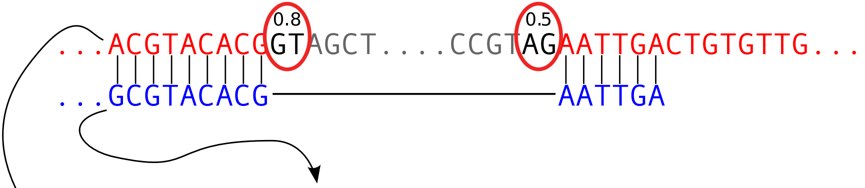

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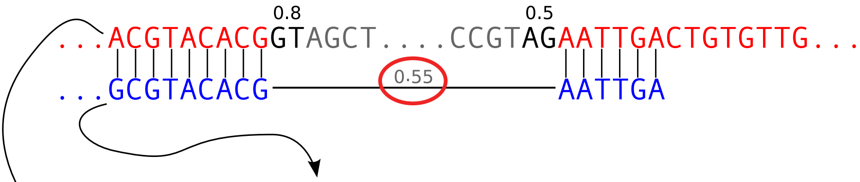

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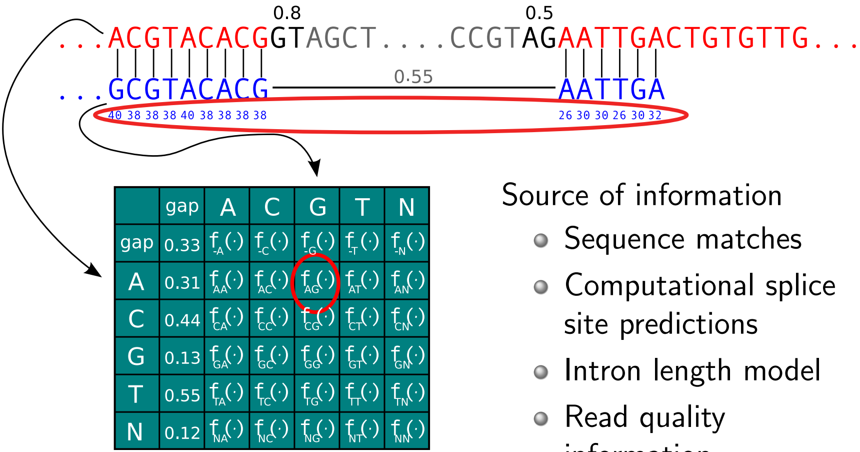

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Quality scoring $f : (\Sigma \times \mathbb{R}) \times \Sigma \rightarrow \mathbb{R}$

[De Bona et al., 2008]

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- What are optimal parameters?
- How do we jointly optimize the 336 parameters?

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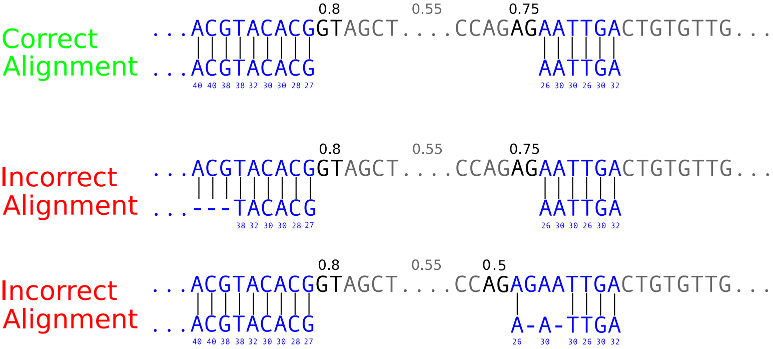

Correct Alignment

```
                0.8       0.55     0.75
...ACGTACACGGTAGCT....CCAGAGAATTGACTGTGTTG...
   |||||||||                  ||||||
...ACGTACACG                  AATTGA
   40 40 38 38 32 30 30 28 27   26 30 30 26 30 32
```

Incorrect Alignment

```
                0.8       0.55     0.75
...ACGTACACGGTAGCT....CCAGAGAATTGACTGTGTTG...
   |||||||||                  ||||||
...---TACACG                  AATTGA
   38 32 30 30 28 27           26 30 30 26 30 32
```

Incorrect Alignment

```
                0.8       0.55   0.5
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   |||||||||                |   ||||
...ACGTACACG                A-A-TTGA
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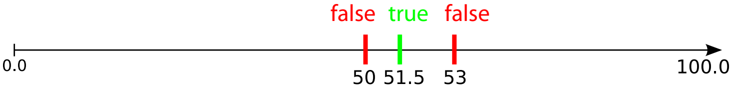
Cartoon: Maximize the Margin



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- Correct alignment is highest scoring one
- Can we do better?

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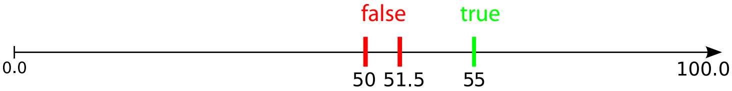

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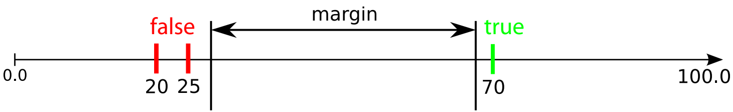

- Technique motivated by SVMs ("large-margin")
- Enforce a margin between correct and incorrect examples
- One has to solve a big quadratic problem

How Can We Generate Data for Training?

- How do we obtain true alignments for training QPalma?
- Simulate *realistic* transcriptome reads with known origin

Strategy:

1. Estimate relationship between quality score and error probability from given reads
2. Use annotation of a few genes to simulate spliced reads
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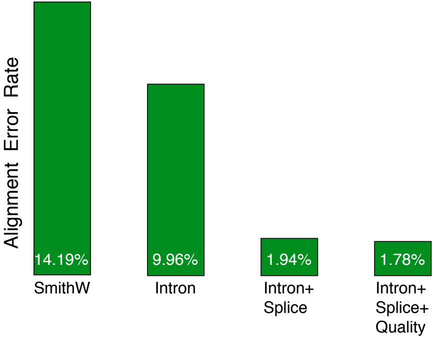
QPALMA RNA-Seq Read Alignment

Generate set of artificially spliced reads

- Genomic reads with quality information
- Genome annotation for artificially splicing the reads
- Use $10,000$ reads for training and $30,000$ for testing



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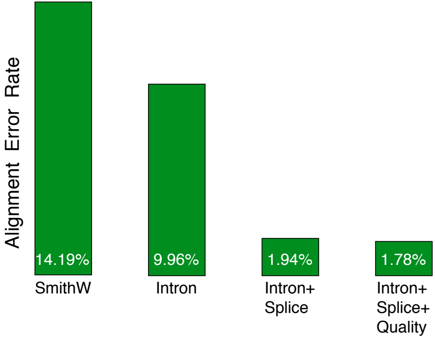
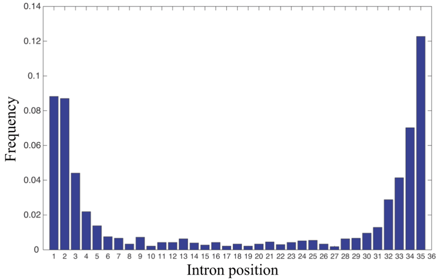


Error vs. intron position



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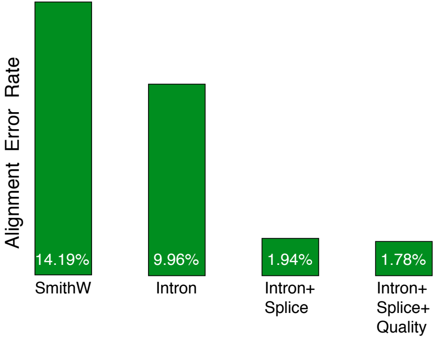

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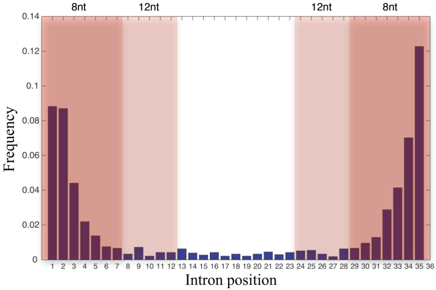

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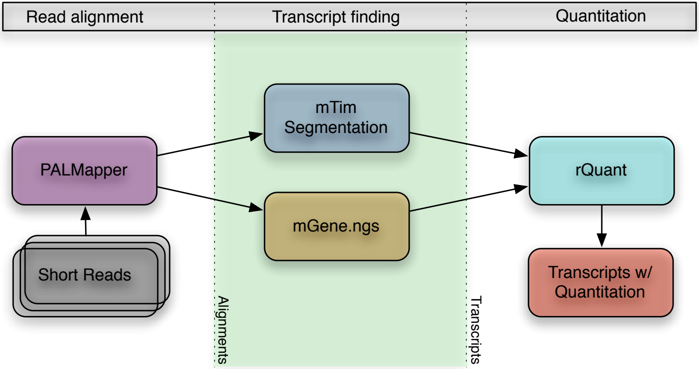
Step 2: Transcript Prediction

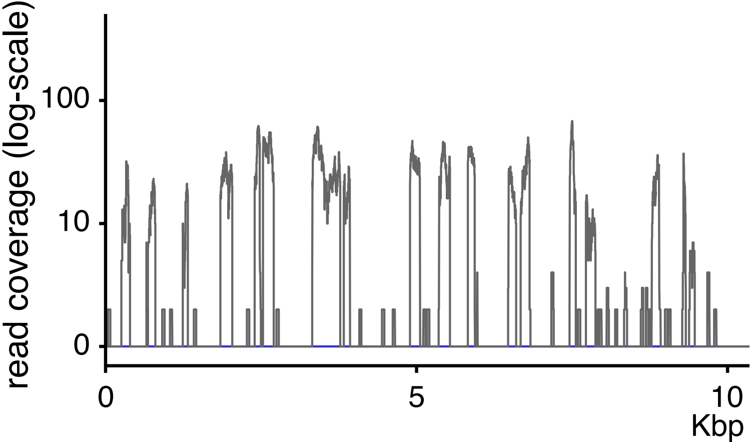


A. Coverage segmentation algorithm **mTiM** for general transcripts (no coding bias/assumption)

B. Extension of the mGene gene finding system to use NGS data for protein coding transcript prediction (**mGene.ngs**)

mTiM: Read Coverage Segmentation

Goal: Characterize each base as *intergenic*, *exonic*, or *intronic*



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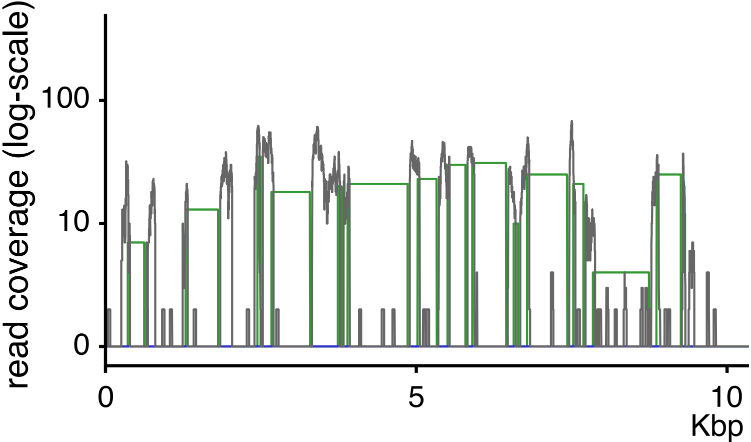
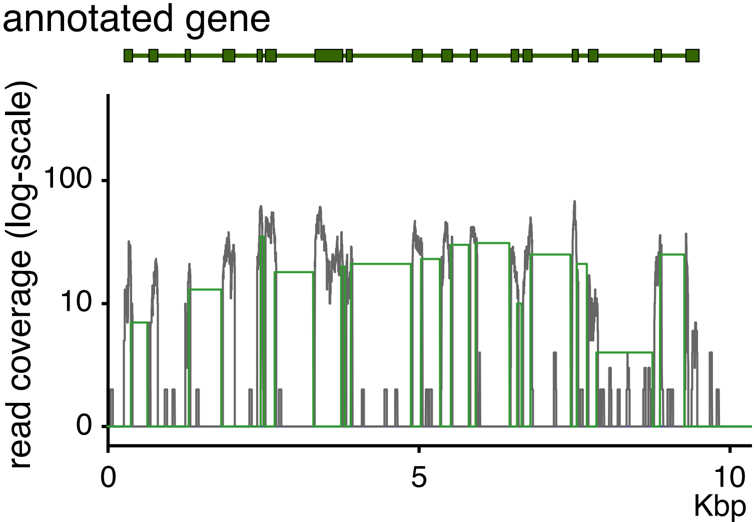
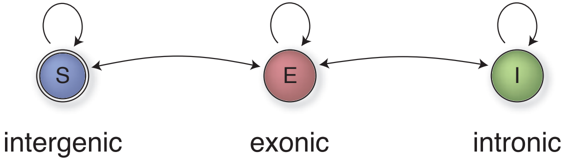


mTiM: Read Coverage Segmentation

Goal: Characterize each base as *intergenic*, *exonic*, or *intronic*



The mTiM Segmentation Approach



- Learn to associate a state with each position given its read coverage and local context
- HM-SVM training: Optimize transformations: signal → score
- Extension: Score spliced reads and splice sites

(G. Zeller et al., 2008; G. Zeller et al., in prep., 2009)

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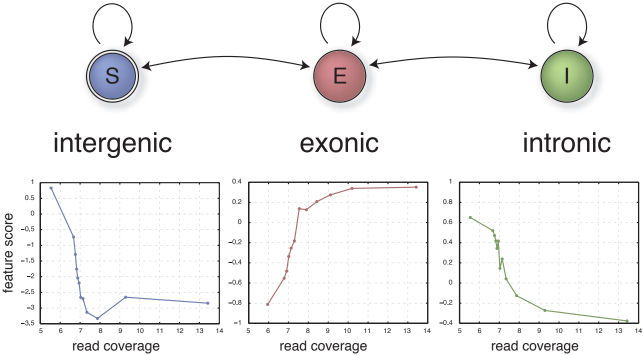

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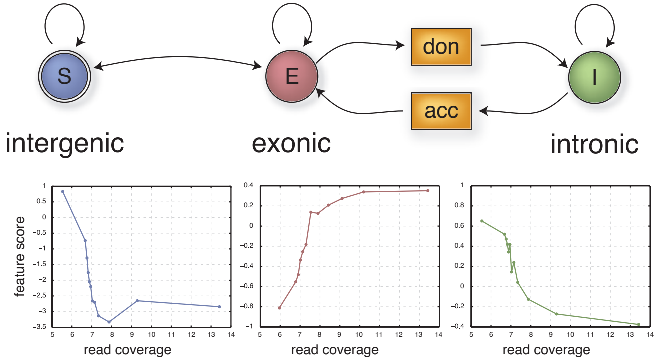

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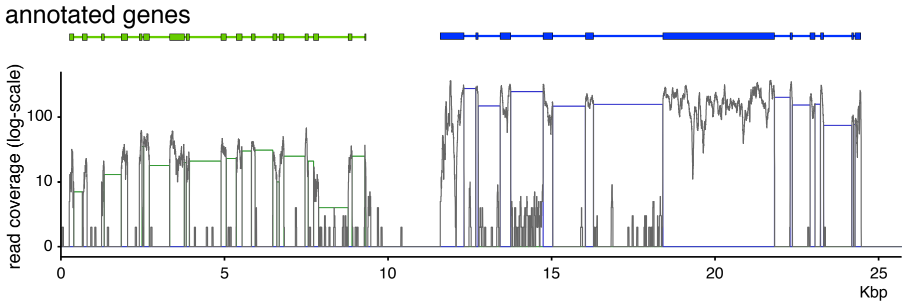
The mTiM Segmentation Approach

Idea: Assume uniform read coverage within exons of same transcript



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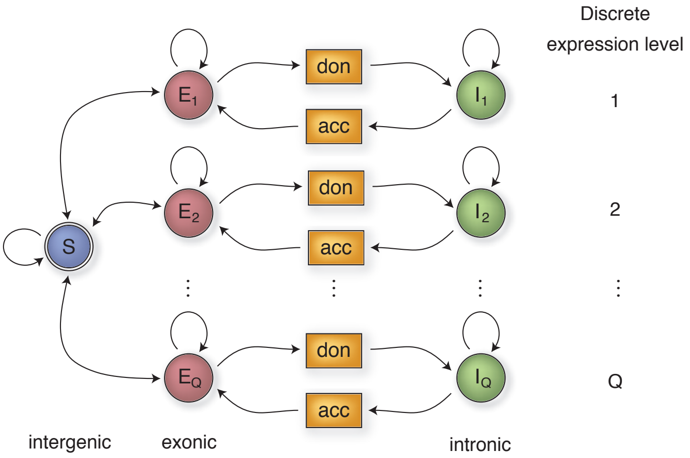

Carry “expression level” information between exons of same transcript

(G. Zeller et al., 2008; G. Zeller et al., in prep., 2010)

Discriminative training of HM-SVMs

$f : \mathbb{R}^\star \rightarrow \Sigma^\star$

given a sequence of hybridization measurements $\boldsymbol{\chi} \in \mathbb{R}^\star$
predicts a state sequence (path) $\boldsymbol{\sigma} \in \Sigma^\star$

Discriminant function $F_{\boldsymbol{\theta}} : \mathbb{R}^\star \times \Sigma^\star \rightarrow \mathbb{R}$ such that for decoding: $f(\chi) = \underset{\boldsymbol{\sigma} \in \mathcal{S}^\star}{\operatorname{argmax}} F_{\boldsymbol{\theta}}(\chi, \boldsymbol{\sigma})$.

Training:

For each training example $(\chi^{(i)}, \boldsymbol{\sigma}^{(i)})$, enforce a large margin of separation

$$F_\theta(\chi^{(i)}, \boldsymbol{\sigma}^{(i)}) - F_\theta(\chi^{(i)}, \overline{\boldsymbol{\sigma}}) \geq \rho$$

between the correct path $\boldsymbol{\sigma}^{(i)}$ and *any* other wrong path $\overline{\boldsymbol{\sigma}} \neq \boldsymbol{\sigma}^{(i)}$.

A quadratic programming problem (QP) is solved to optimize $\boldsymbol{\theta}$.

[Altun et al., 2003, Rätsch et al., 2007, Zeller et al., 2008b]

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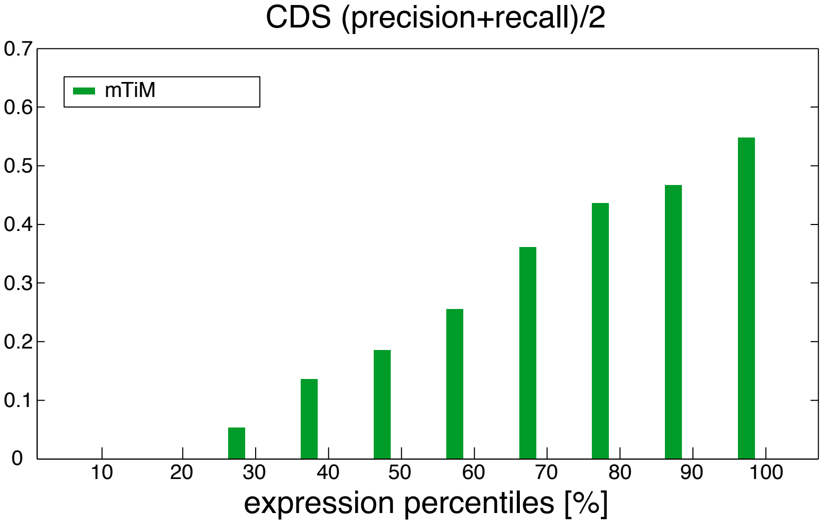
Preliminary Evaluation (*C. elegans*)



Sensitivity heavily depends on read density

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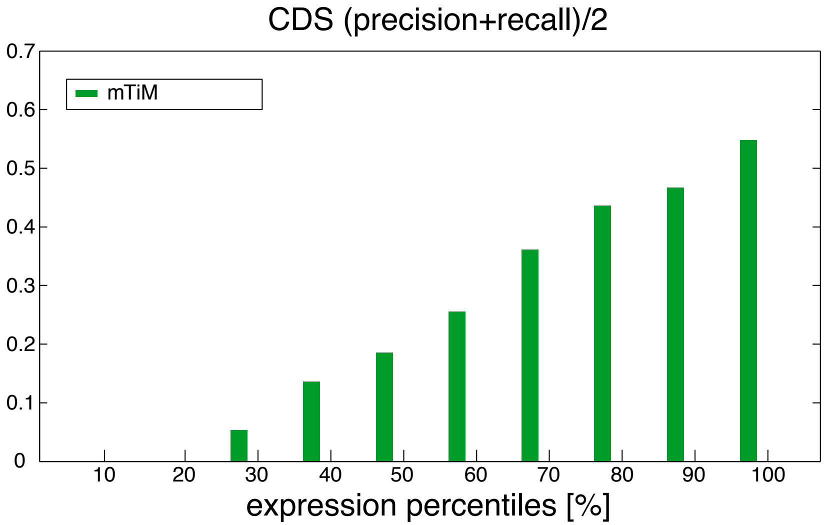

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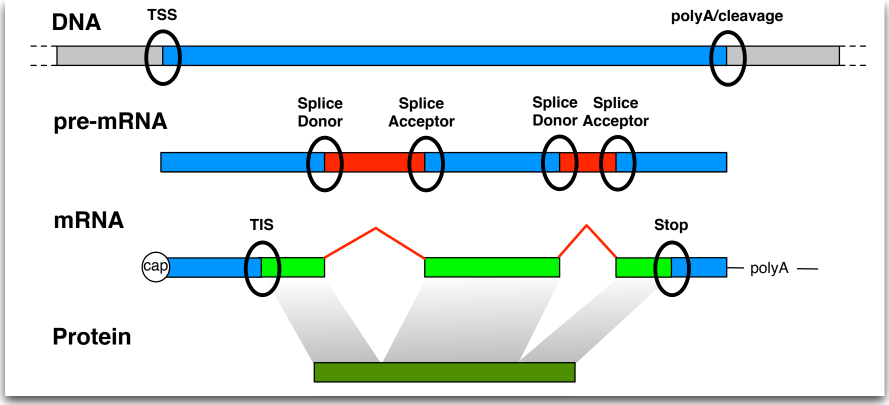
Computational Gene Finding

⇝ Labeling the Genome



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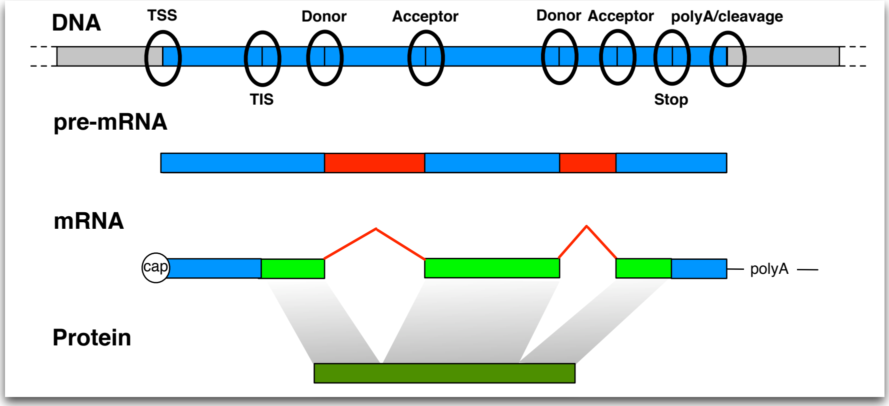

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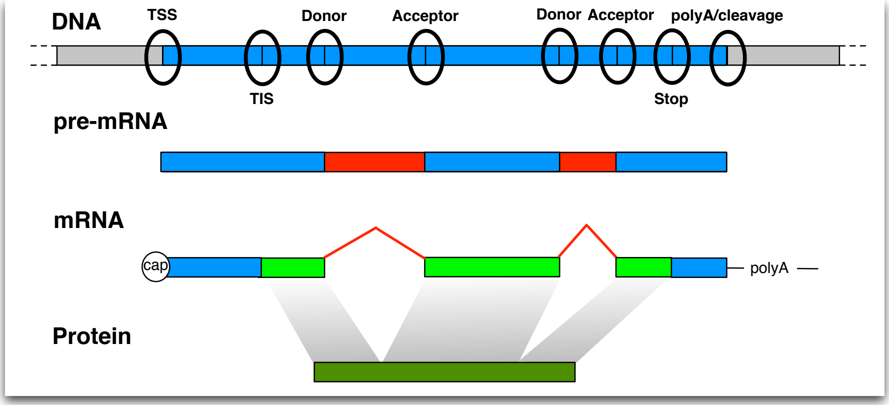

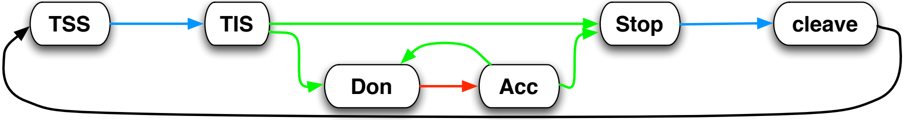

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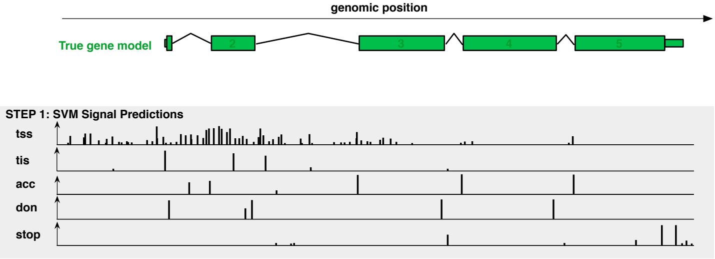

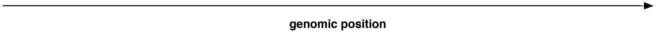

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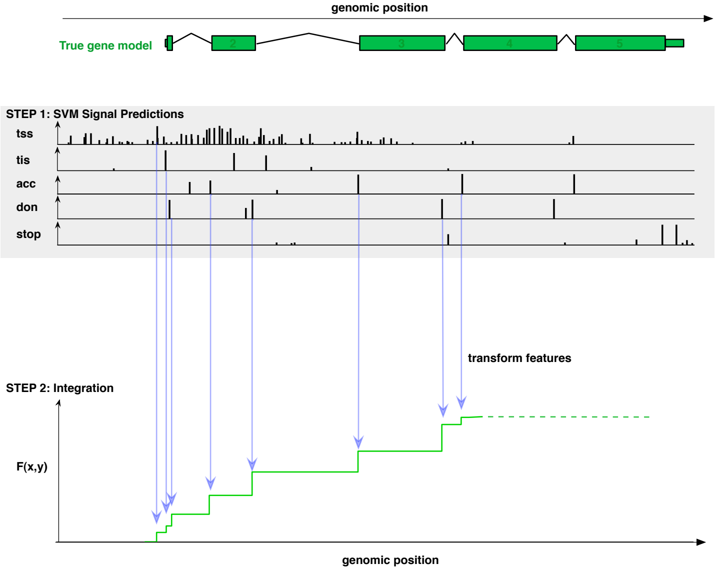

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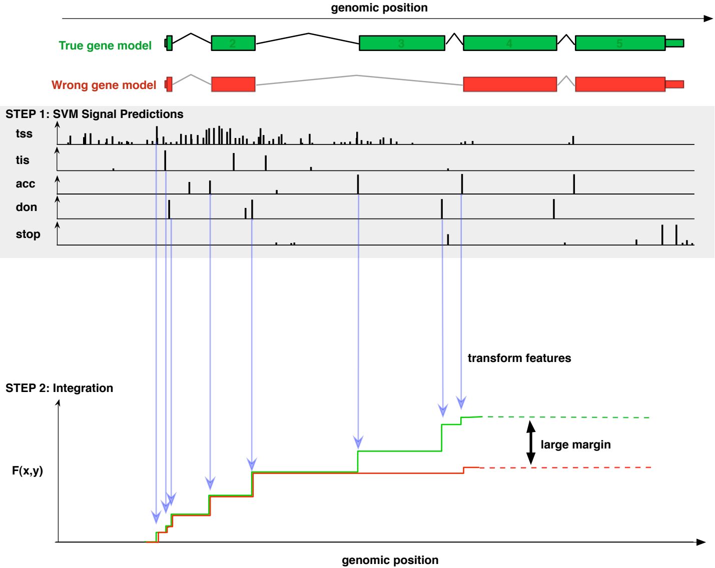

Learning to use Expression Measurements

Two approaches:

- Heuristic to incorporate ESTs/reads/tiling array measurements to *refine predictions*
- Directly *use evidence during learning* to learn to appropriately weight its importance

	Exon Level			Transcript Level		
	SN	**SP**	**F**	**SN**	**SP**	**F**
ab initio	82.3	82.6	82.5	43.1	49.5	46.1
ESTs heuristic	85.3	84.7	85.0	49.5	56.4	52.7
ESTs trained	84.8	85.8	**85.3**	50.5	57.8	**53.9**

Gene prediction in *C. elegans* (CDS evaluation)

Behr et al., in pre., 2010

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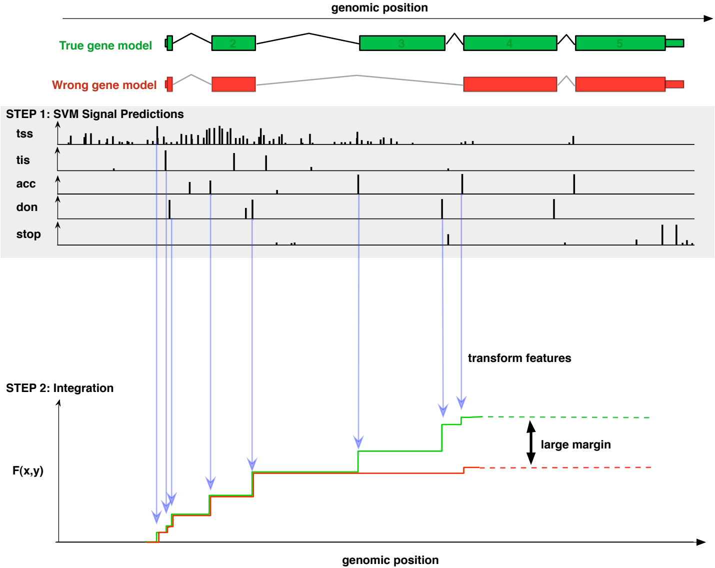

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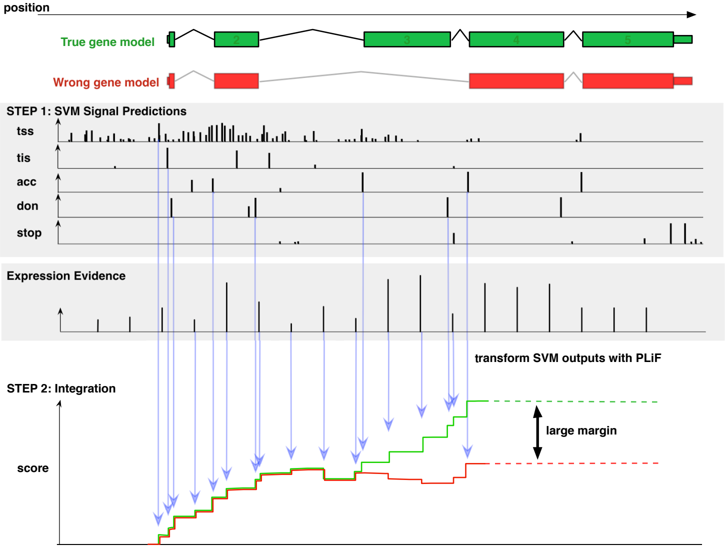

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RNA-Seq trained	84.6	84.9	84.8	49.1	55.2	52.0
RNA-Seq/ESTs trained	84.7	**86.9**	85.8	50.3	60.5	**54.9**

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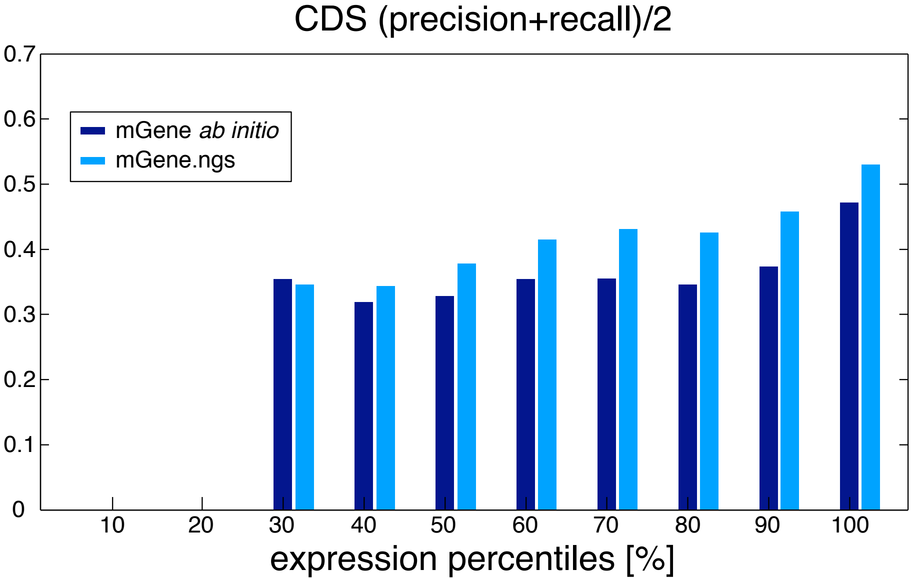

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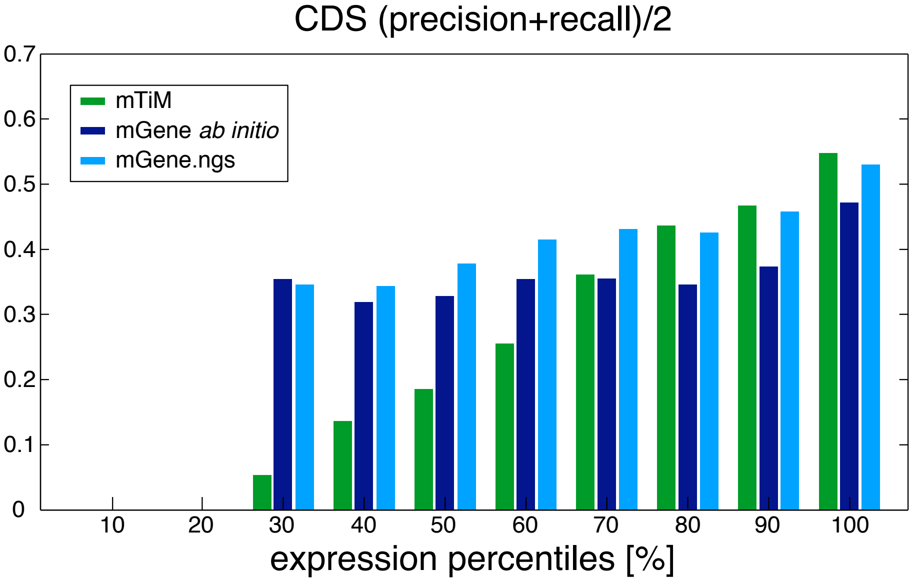

Digestion

- **mTiM** and **mGene.ngs** predict single transcripts
- **mTiM** exploits "uniformity" of read coverage among exons of same transcript
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- **Alt. Transcripts:** Spliced reads for splicing graph completion:
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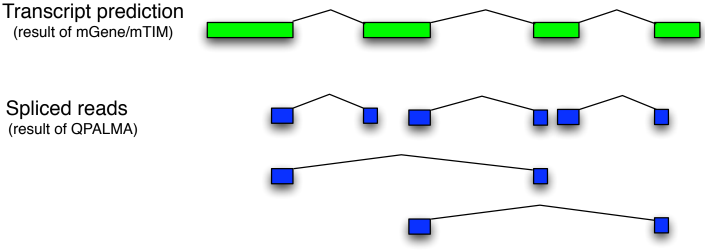

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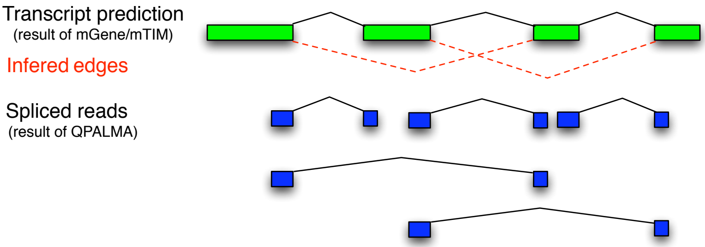

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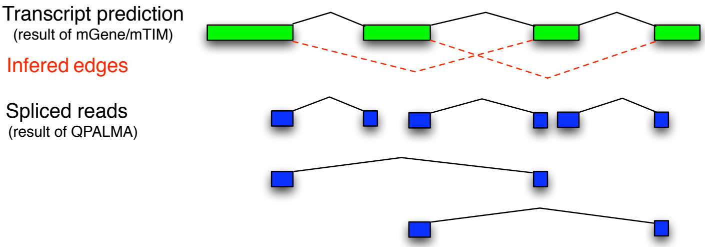

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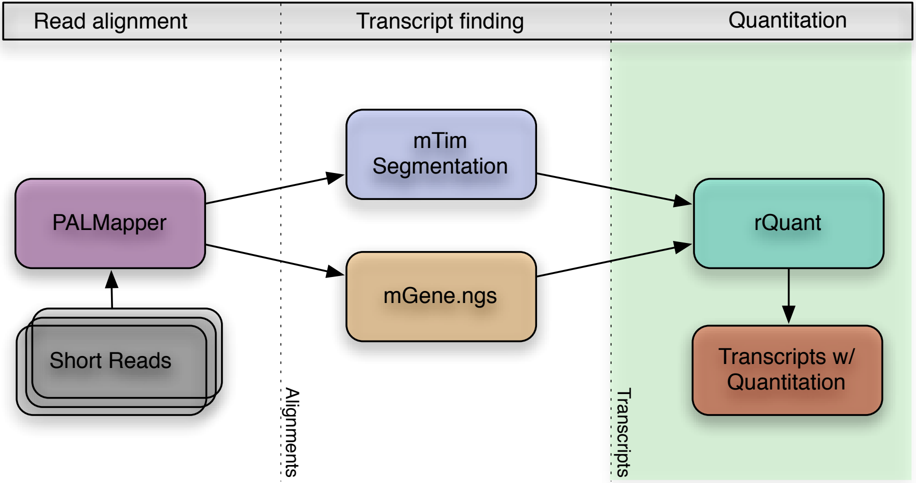
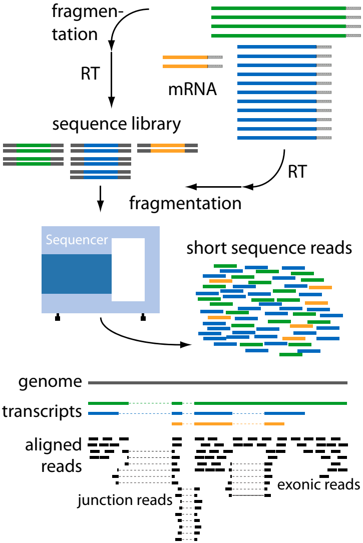
RNA-Seq Pipeline Overview

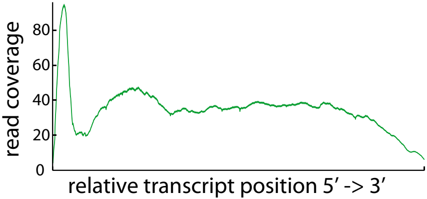


RNA-Seq Biases and Quantitation



Biases due to . . .

- cDNA library construction
- Sequencing
- Read mapping



(average over annotated transcripts of length ≈1kb for the *C. elegans* SRX001872 dataset)

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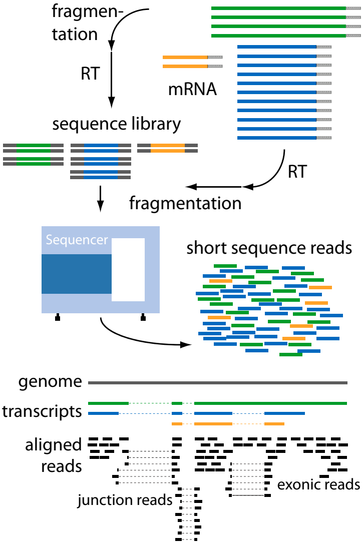

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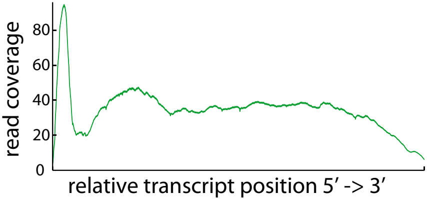

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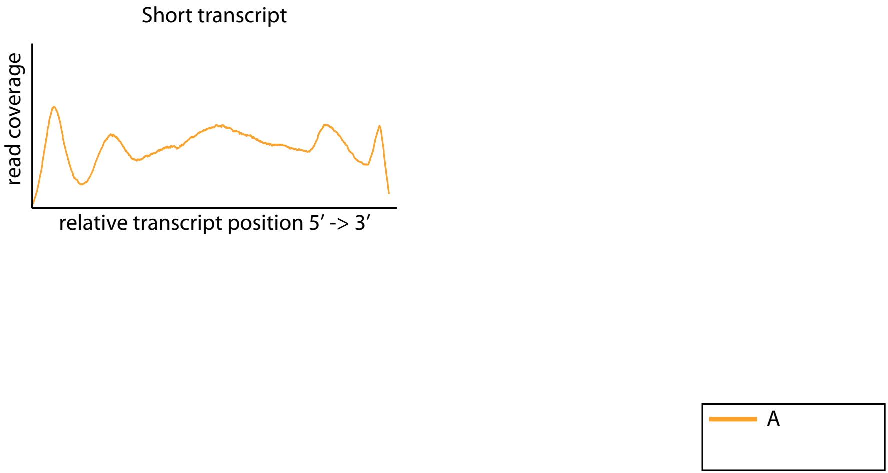
rQuant – Basic Idea



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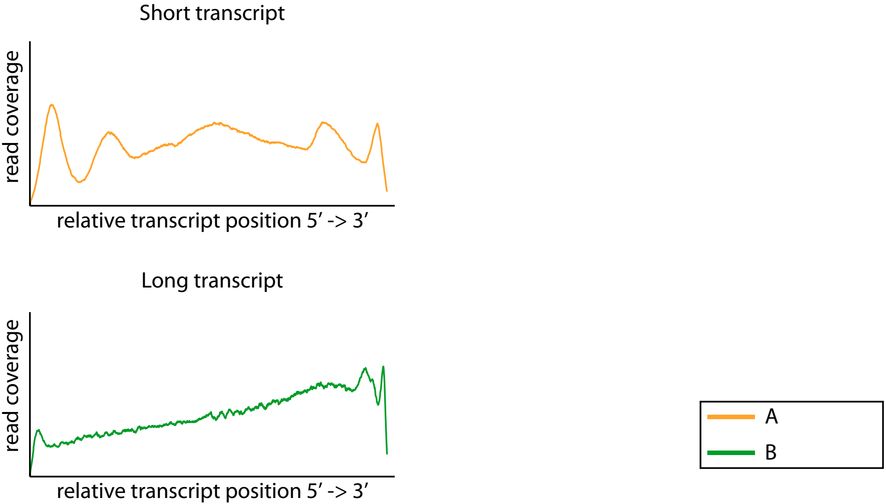

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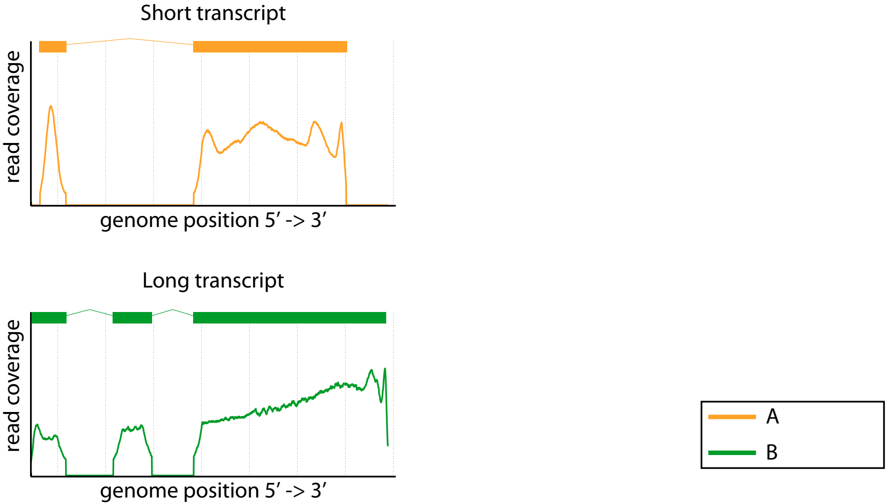

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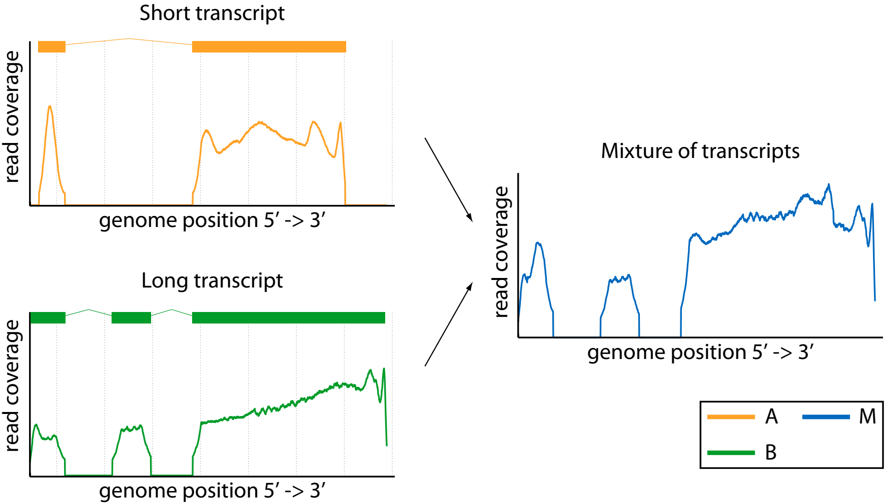

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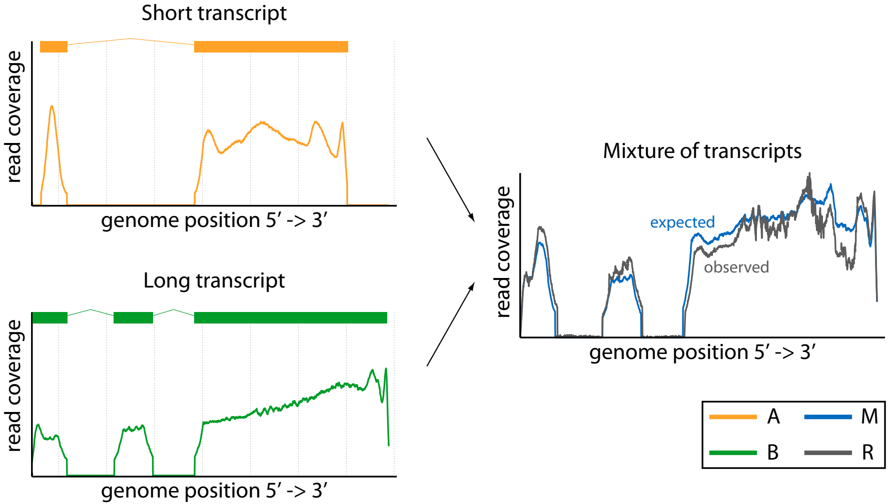

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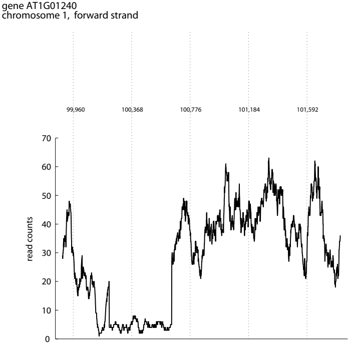

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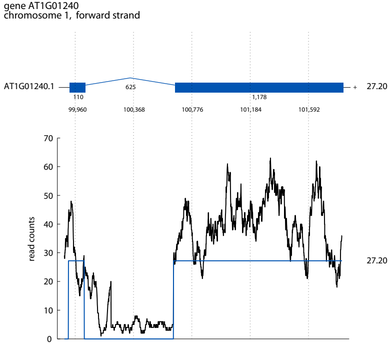

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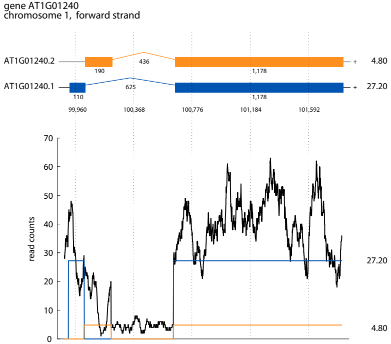

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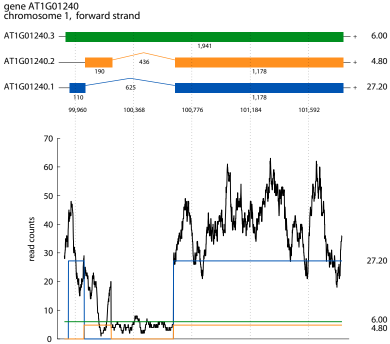

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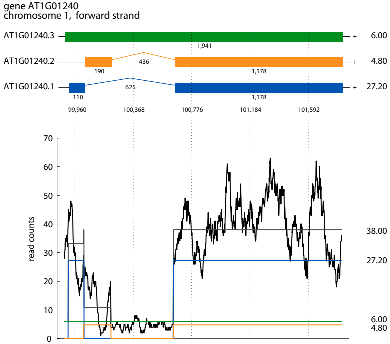

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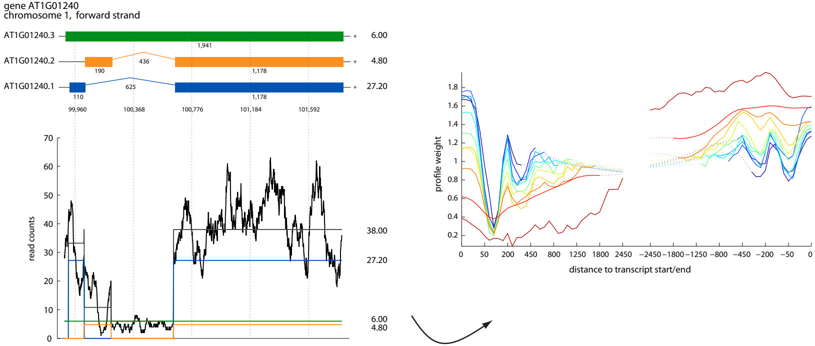

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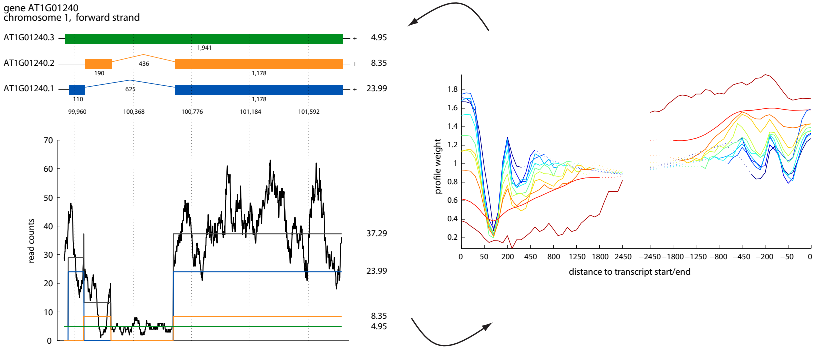

rQuant Evaluation I

rQuant: Position-wise with profiles (estimating library and mapping bias)

compared to

- Position-wise, without profiles
- Segment-wise, without profiles (e.g., Jiang and Wong [2009])
- Segment-wise, with profiles (e.g. Flux Capacitor [Sammeth, 2009a])

Estimate transcript abundances

- Using simulated data for *A. thaliana* (Flux Simulator [Sammeth, 2009b])
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rQuant: Position-wise with profiles (estimating library and mapping bias)

compared to

- Position-wise, without profiles
- Segment-wise, without profiles (e.g., Jiang and Wong [2009])
- Segment-wise, with profiles (e.g. Flux Capacitor [Sammeth, 2009a])

Estimate transcript abundances

- Using simulated data for *A. thaliana* (Flux Simulator [Sammeth, 2009b])
- Subset of alternatively spliced genes

Evaluation: Spearman correlation between

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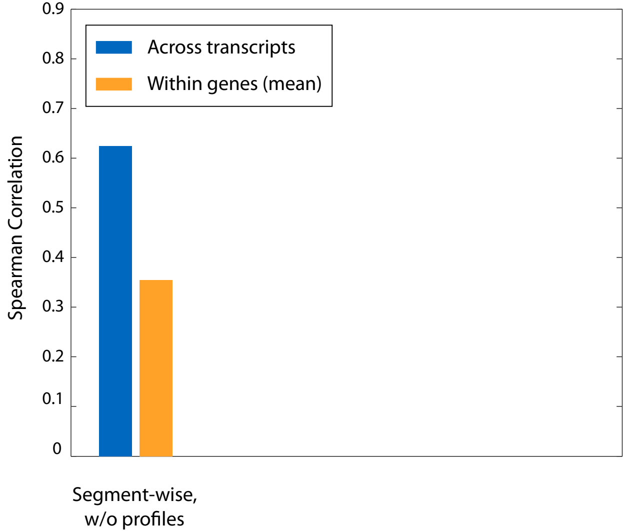
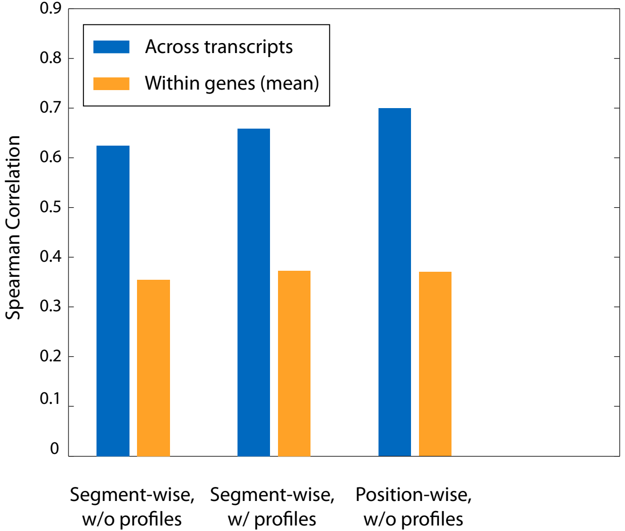
rQuant Evaluation II



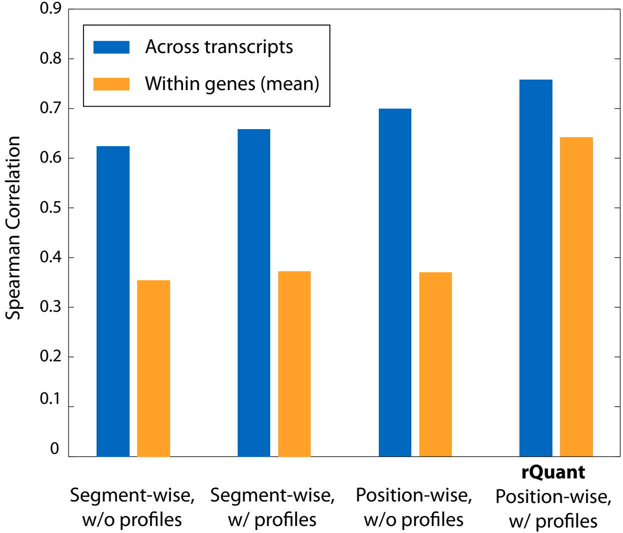
(Bohnert et al., submitted, 2010)

rQuant Evaluation II



(Bohnert et al., submitted, 2010)

rQuant Evaluation II

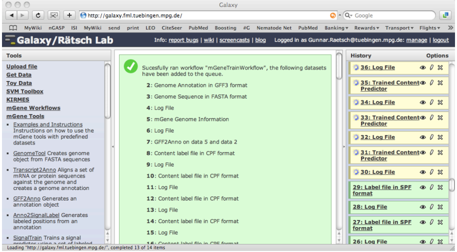


(Bohnert et al., submitted, 2010)

Galaxy-based Web Services for NGS Analyses

Galaxy-based web service http://galaxy.fml.mpg.de

- PALMapper http://fml.mpg.de/raetsch/suppl/palmapper
- mGene http://mgene.org/web
- mTIM http://fml.mpg.de/raetsch/suppl/mtim (in prep.)
- rQuant http://fml.mpg.de/raetsch/suppl/rquant/web


Galaxy
http://galaxy.fml.tuebingen.mpg.de/
Google
MyWiki nGASP ISI MyWiki send print LEO CiteSeer PubMed Boosting #G Nematode Net PubMed Banking Rewards Transport Flights
Galaxy/Rätsch Lab
Info: report bugs | wiki | screencasts | blog
Logged in as Gunnar.Raetsch@tuebingen.mpg.de: manage | logout
Tools
History
Options

Upload file
Get Data
Toy Data
SVM Toolbox
KIRMES
mGene Workflows
mGene Tools

- Examples and Instructions Instructions on how to use the mGene tools with predefined datasets
- GenomeTool Creates genome object from FASTA sequences
- Transcript2Anno Aligns a set of mRNA or protein sequences against the genome and creates a genome annotation
- GFF2Anno Generates an annotation object
- Anno2SignalLabel Generates labeled positions from an annotation
- SignalTrain Trains a signal predictor using a set of labeled

✓ Sucesfully ran workflow "mGeneTrainWorkflow", the following datasets have been added to the queue.

2: Genome Annotation in GFF3 format
3: Genome Sequence in FASTA format
4: Log File
5: mGene Genome Information
6: Log File
7: GFF2Anno on data 5 and data 2
8: Content label file in CPF format
9: Log File
10: Content label file in CPF format
11: Log File
12: Content label file in CPF format
13: Log File
14: Content label file in CPF format
15: Log File
16: Content label file in CPF format

36: Log File
35: Trained Content Predictor
34: Log File
33: Trained Content Predictor
32: Log File
31: Trained Content Predictor
30: Log File
29: Label file in SPF format
28: Log File
27: Label file in SPF format
26: Log File

Loading "http://galaxy.fml.tuebingen.mpg.de/", completed 13 of 14 items

(Rätsch et al., in preparation, 2010)

Summary

- PALMapper
  - Splice site predictions improve alignment performance
  - Outperforms many other read mappers in intron accuracy
- mTiM
  - High specificity, sensitivity depends on read coverage
  - Better for identifying transcripts specific to experimental data
- mGene
  - High sensitivity (also for lowly expressed genes)
  - Identifies also non-expressed genes ⇒ good for annotation
- rQuant
  - Models library prep., sequencing, alignment biases
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Quantitation

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