Novel Methods for Computational Transcriptome Analysis

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Friedrich Miescher Laboratory

Max Planck Society

Tübingen, Germany

Berlin Late Summer Meeting

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Friedrich Miescher Laboratory
of the Max Planck Society



Machine Learning in Biology

Research Topics



Machine Learning

1. Inference methods for structured data
   ⇒ *Develop fast and accurate learning methods*

Molecular Biology

2. Genome annotation
   ⇒ *Predict features encoded on DNA*
3. Biological networks
   ⇒ *Understand interactions between gene products*
4. Analysis of polymorphisms
   ⇒ *Discover polymorphisms and associate with phenotypes*

Machine Learning

$\rightsquigarrow$ Develop fast, accurate and interpretable learning methods



Given: Observations of some complex phenomenon
Goal: Learn from data & build predictive models

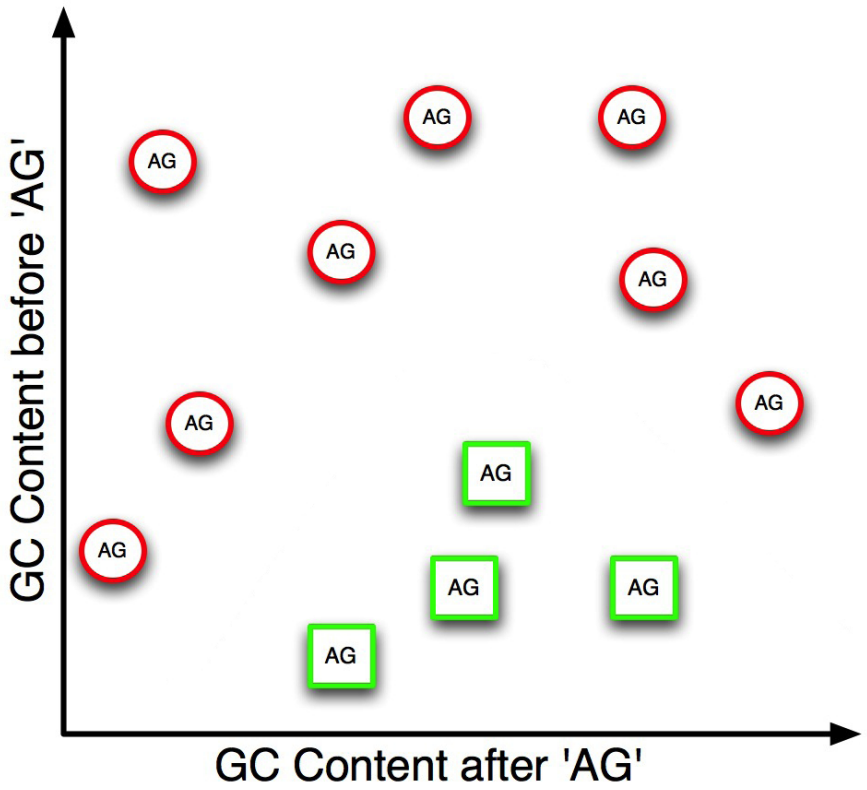
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Example:



Two different classes of observations

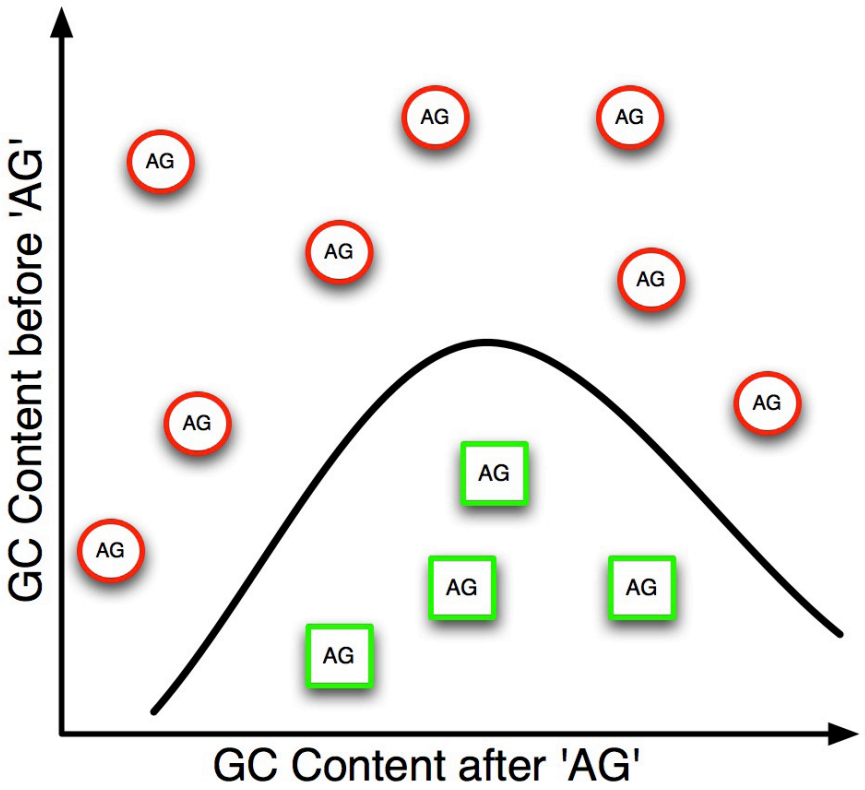
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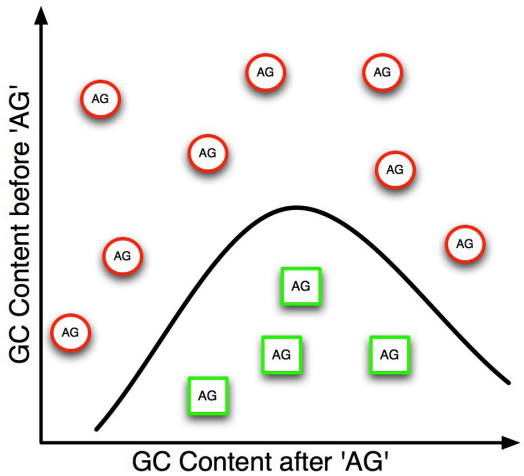
Inferred classification rule

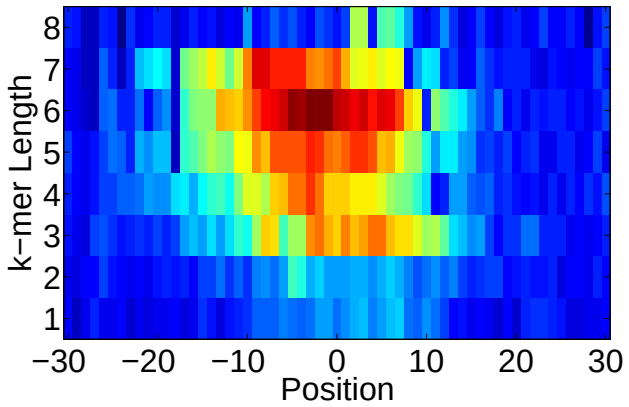
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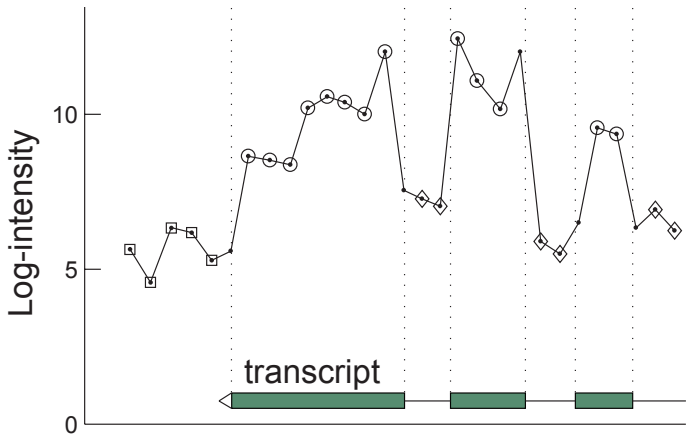
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1. Large scale sequence classification
2. Analysis and explanation of learning results
3. Sequence segmentation & structure prediction

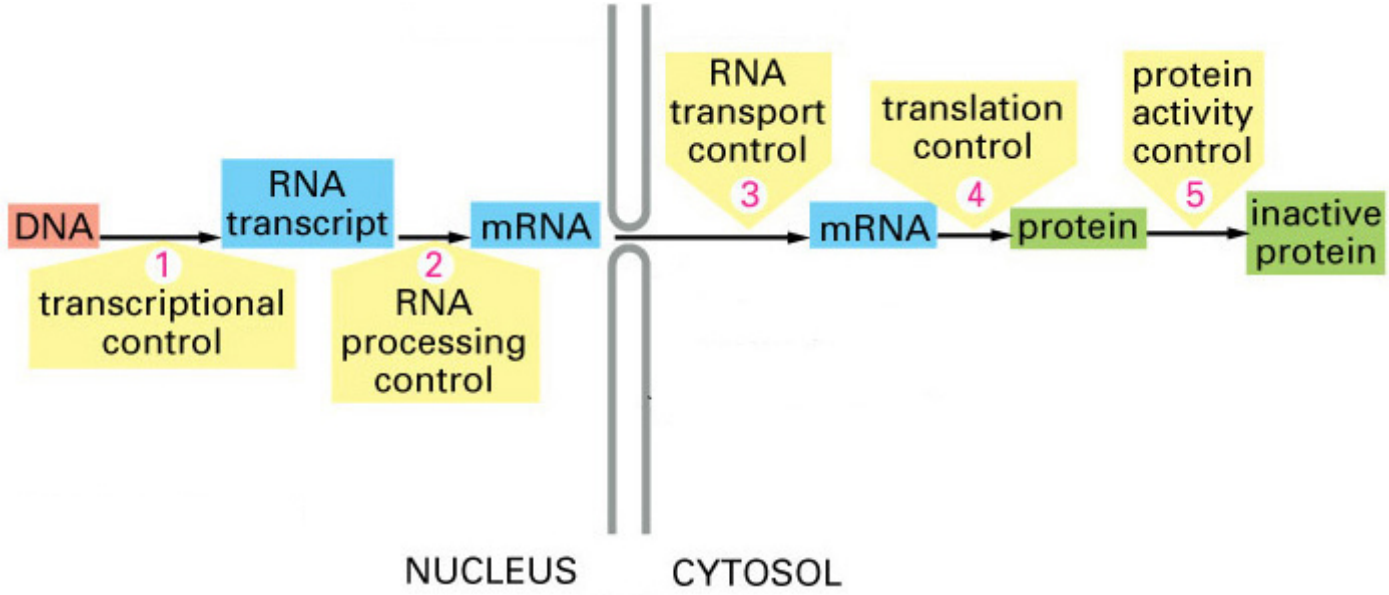






Learning about the Transcriptome

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



Machine Learning View

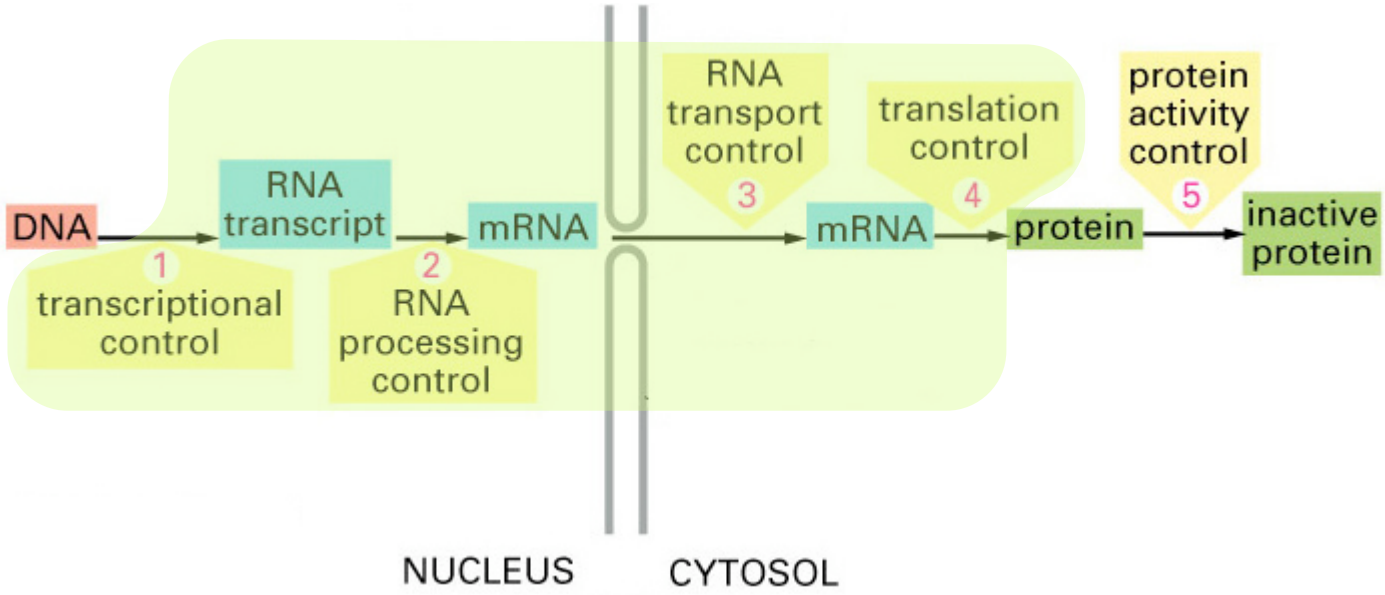
- How to learn to predict what these processes accomplish?
- How well can we predict it from the available information?

Biological View

1. What can we not predict yet? What is missing?
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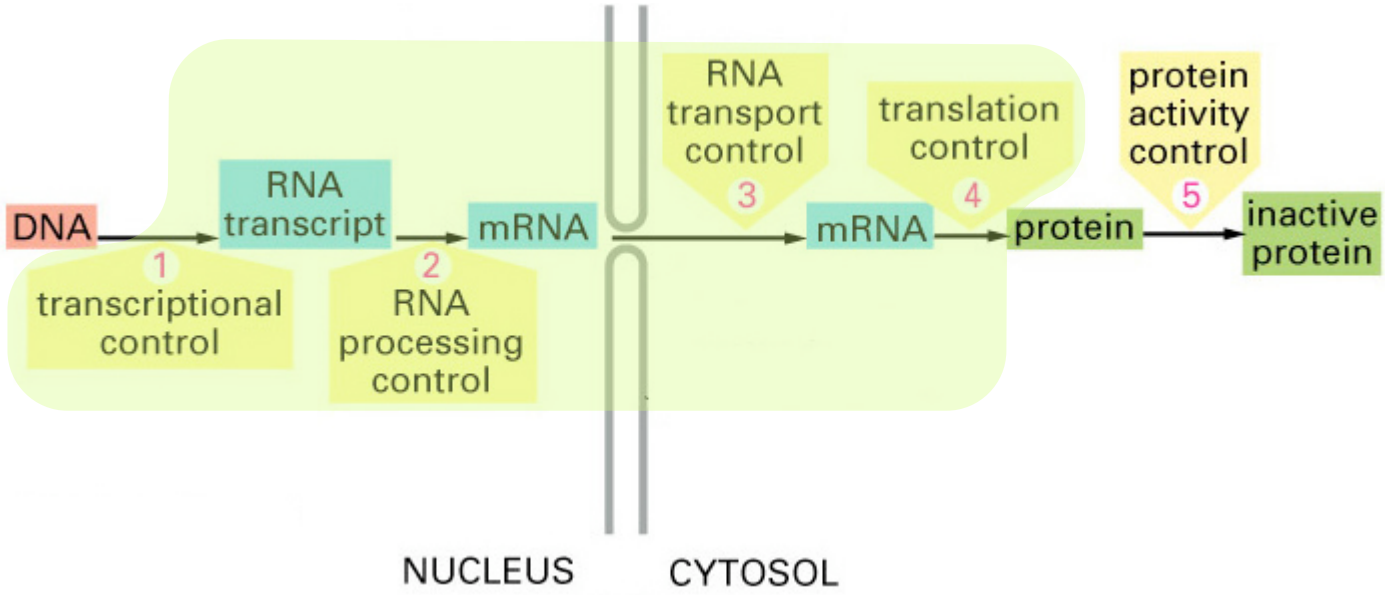
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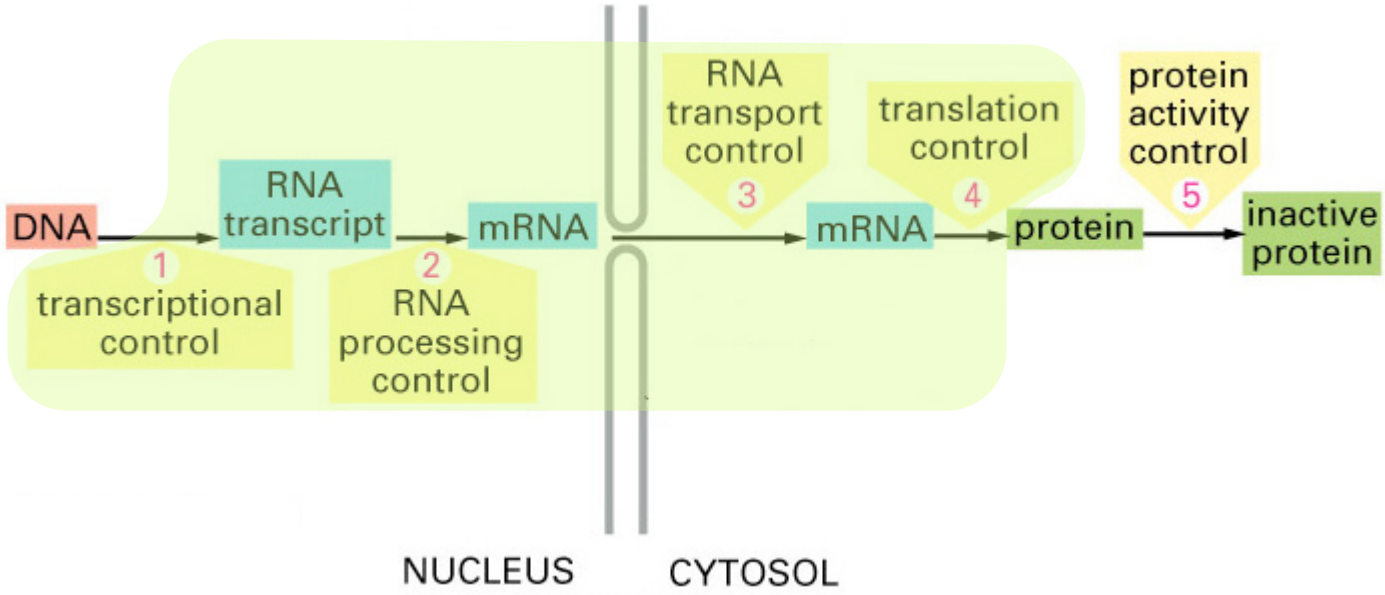
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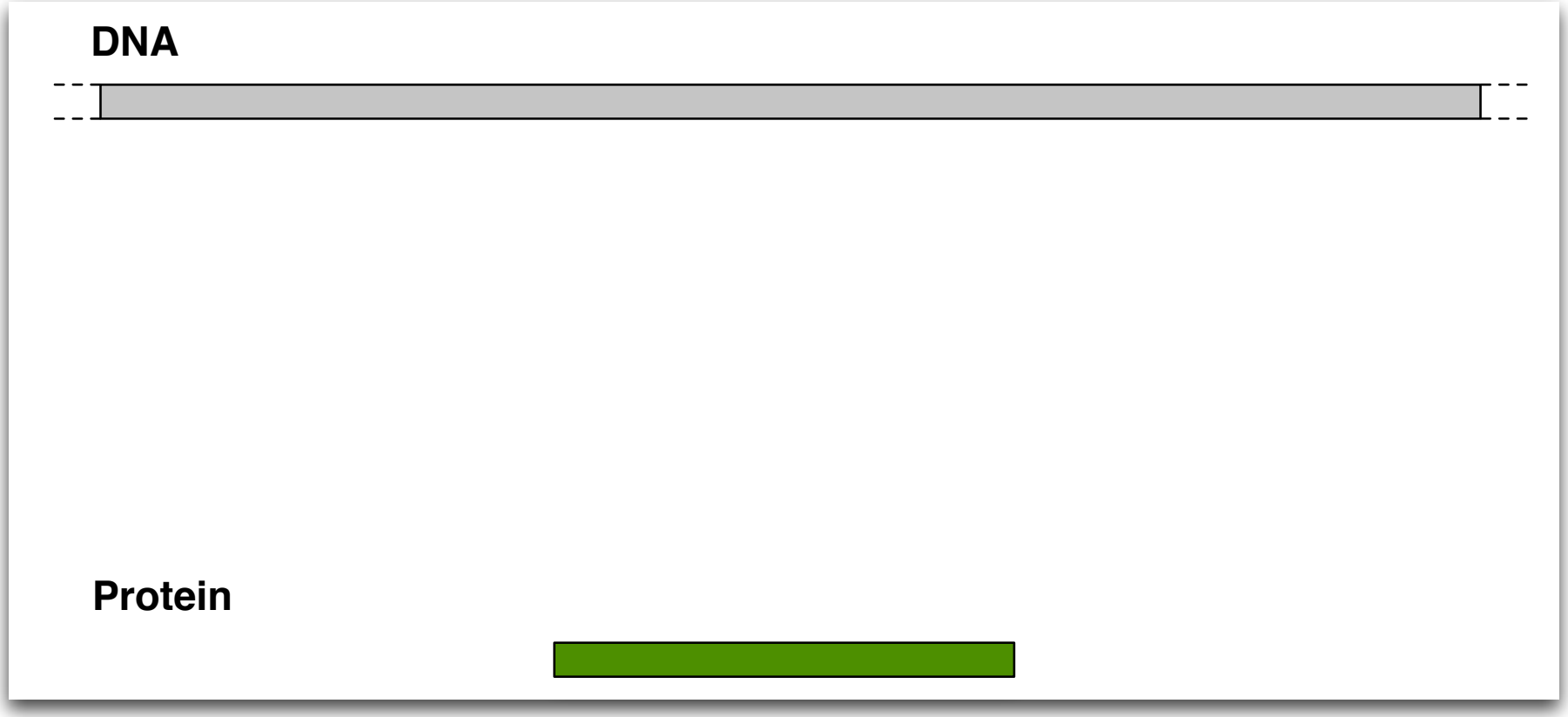
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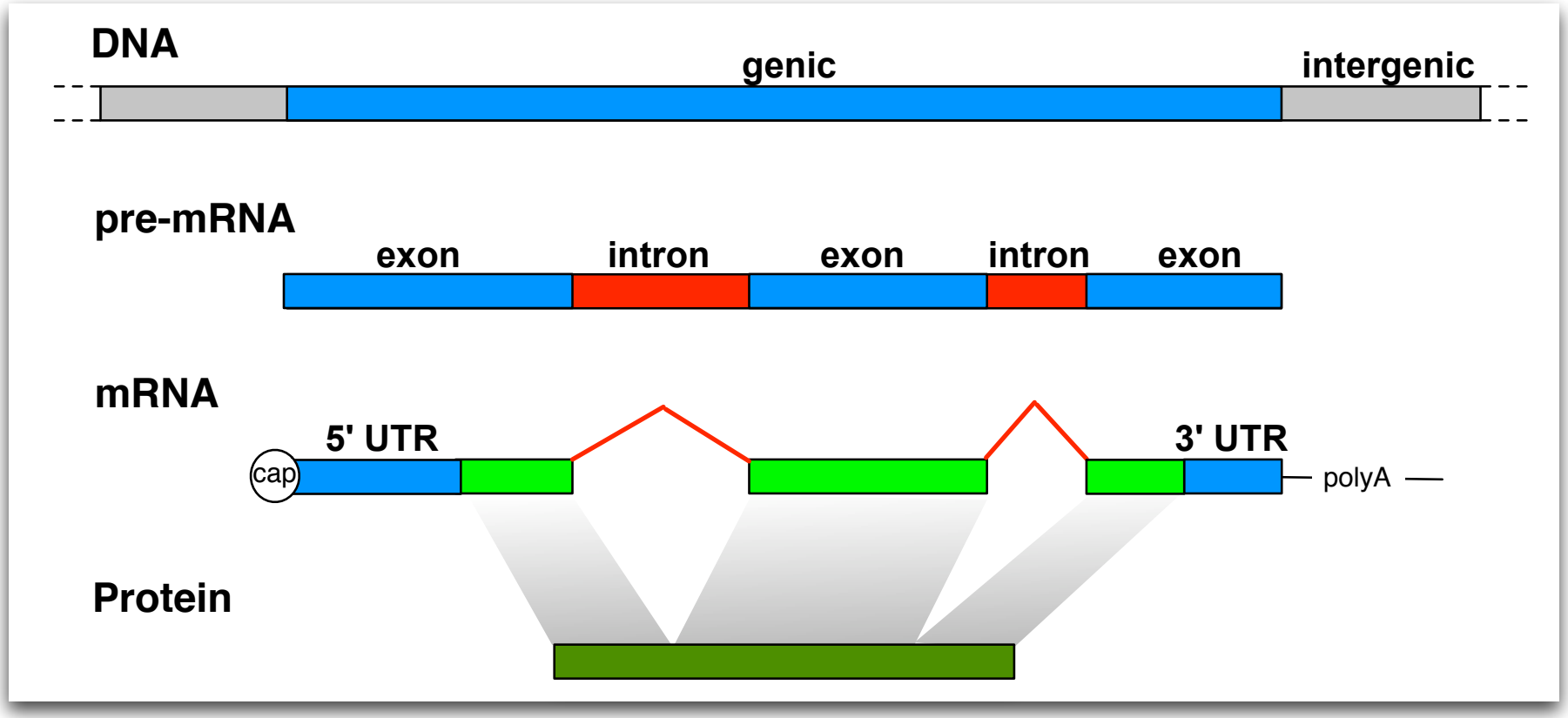
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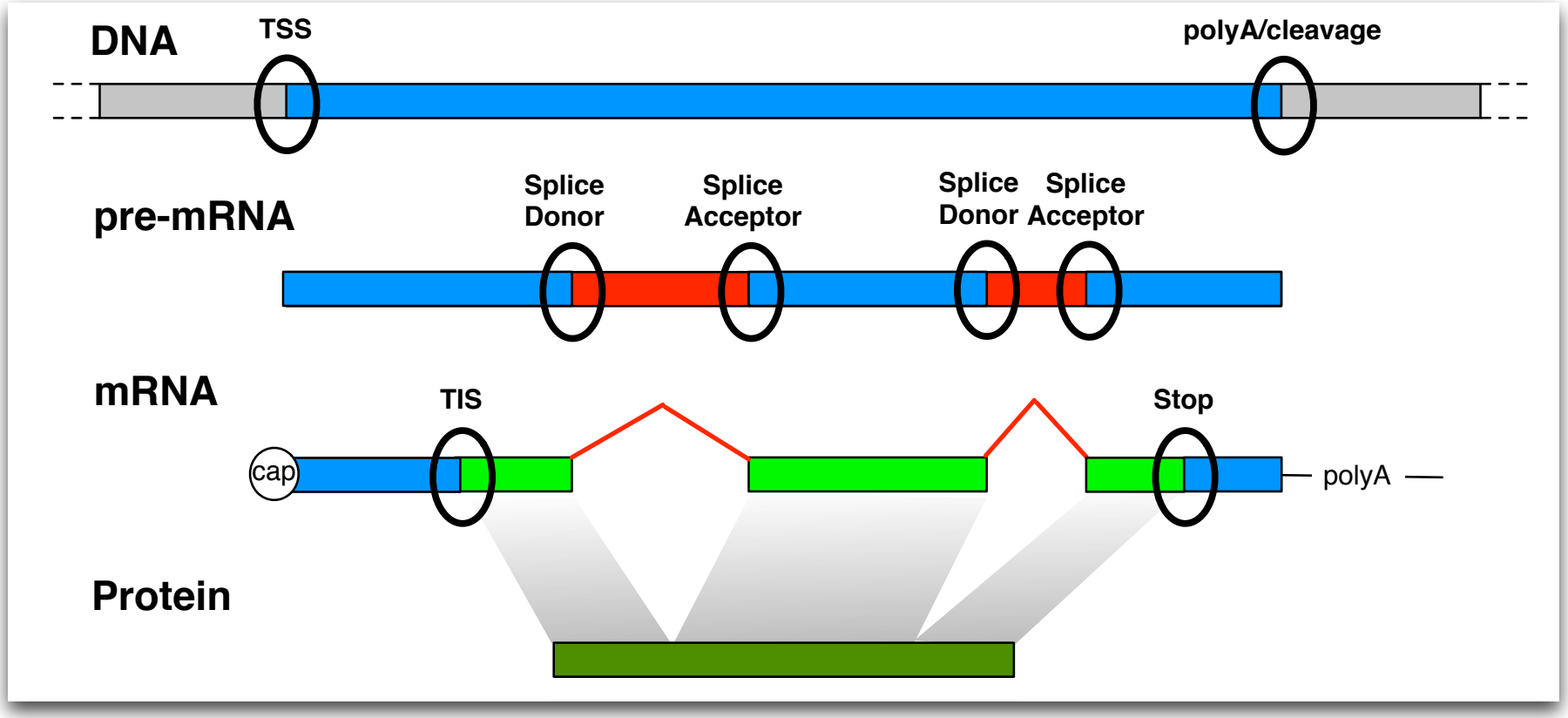
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- Predict gene products

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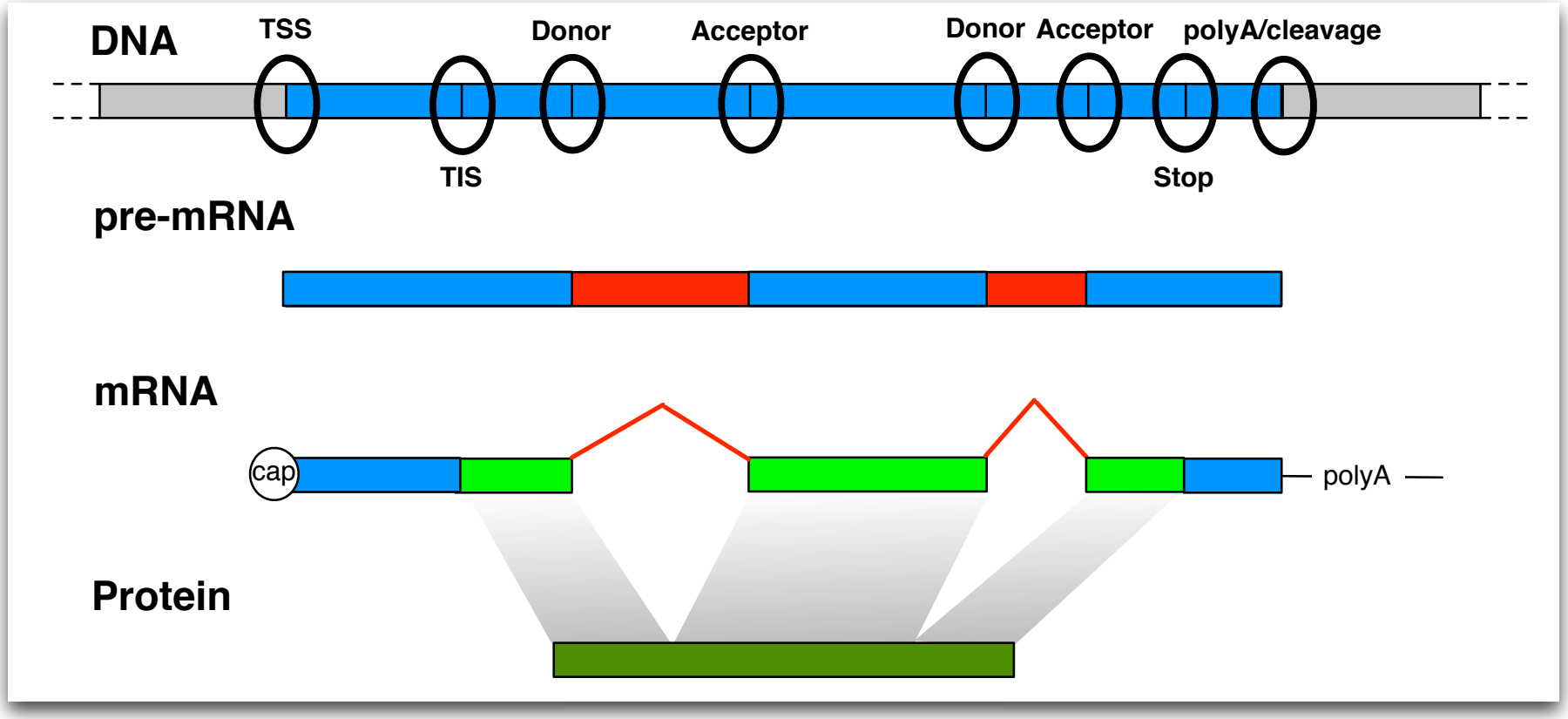
- Given a piece of DNA sequence
- Predict gene products inluding the intermediate processing steps

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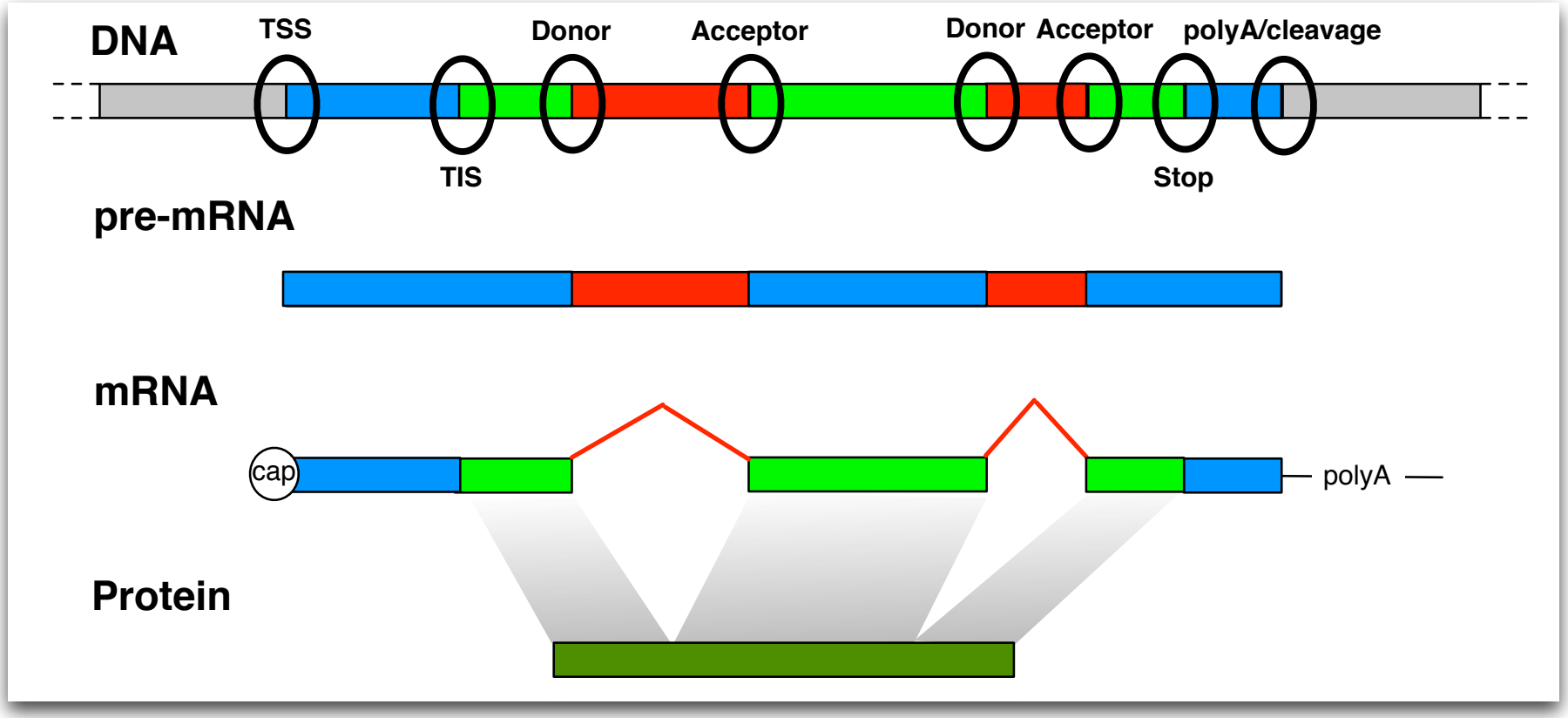
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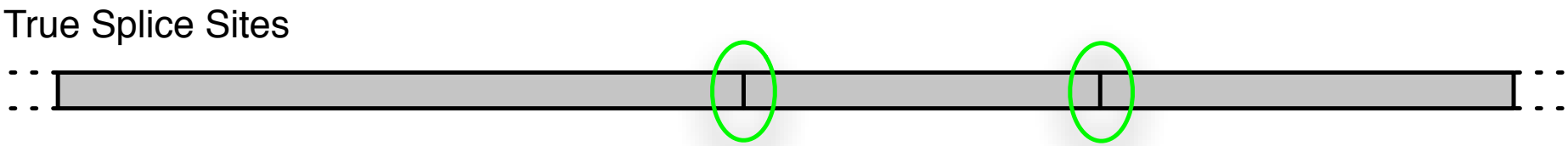
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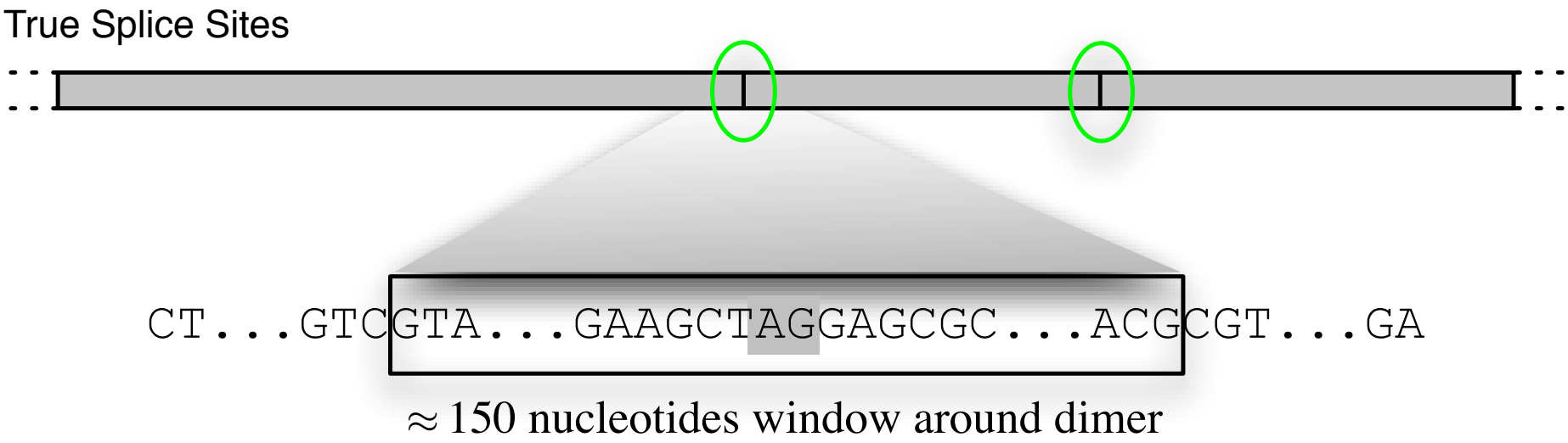


- Predict signals used during processing
- Predict the correct corresponding **label sequence** with labels "intergenic", "exon", "intron", "5' UTR", etc.

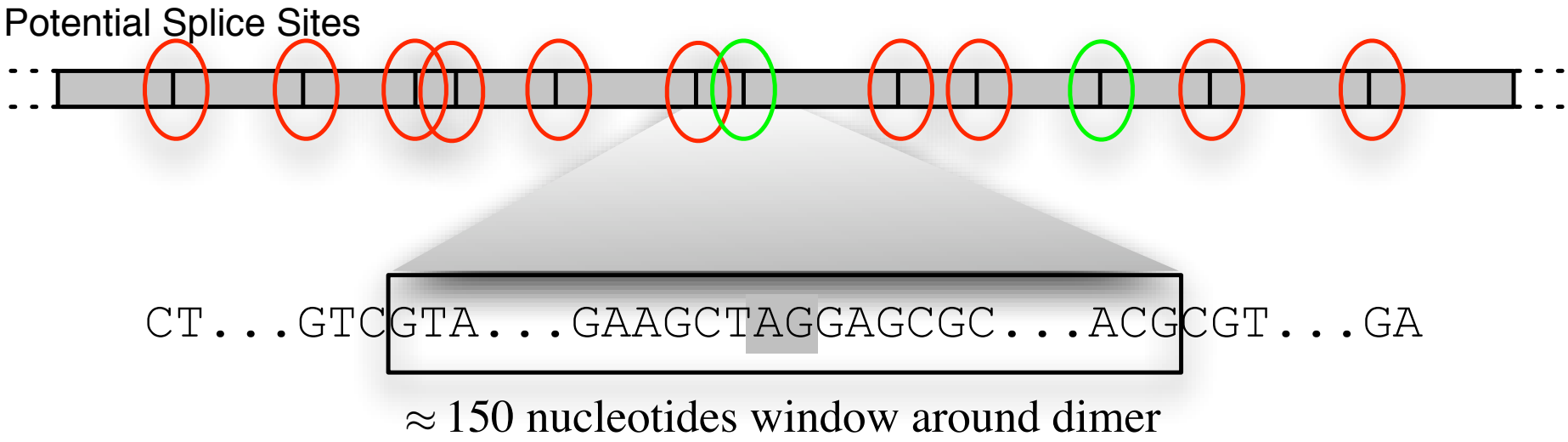
Example: Splice Site Recognition



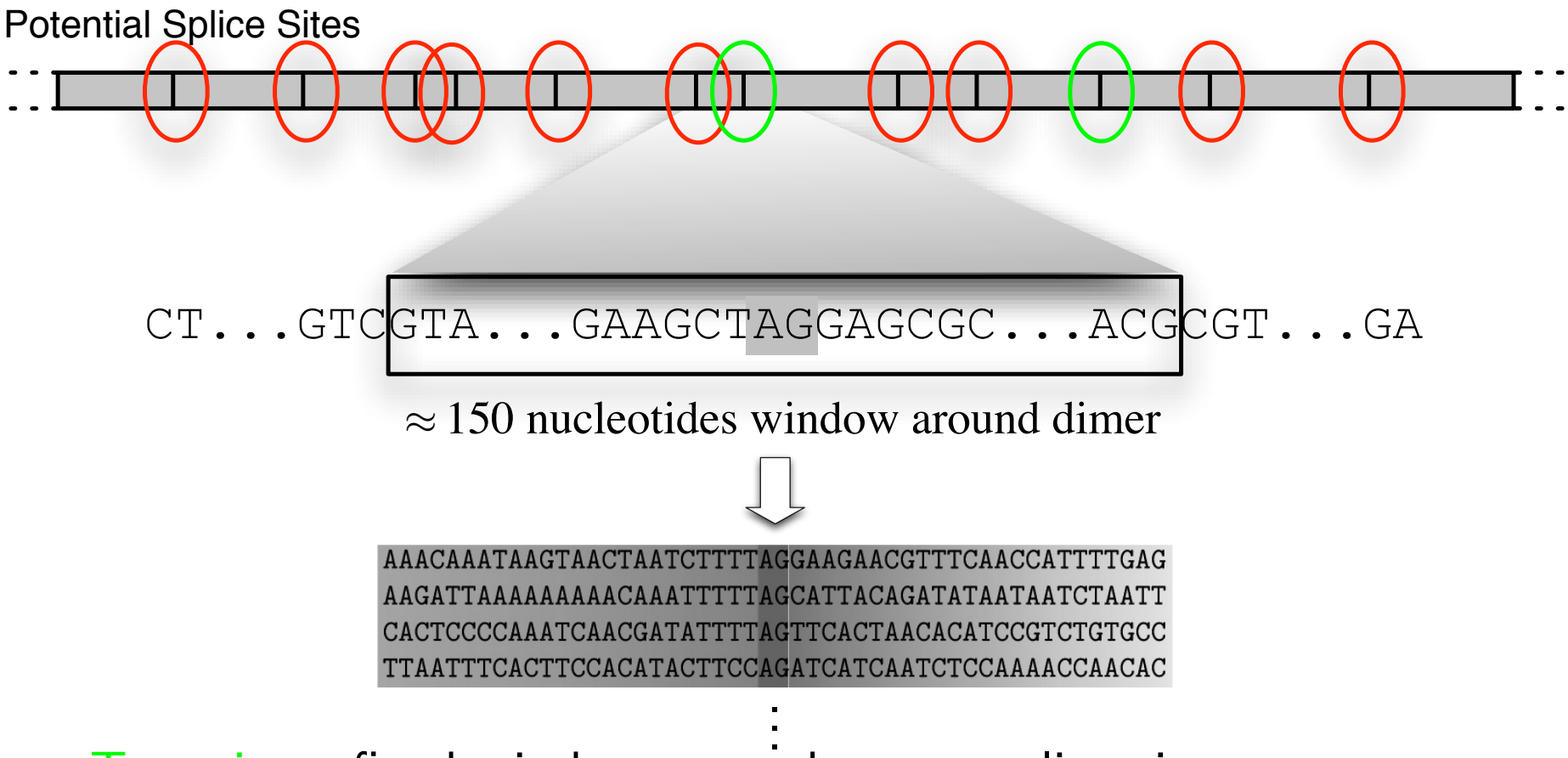
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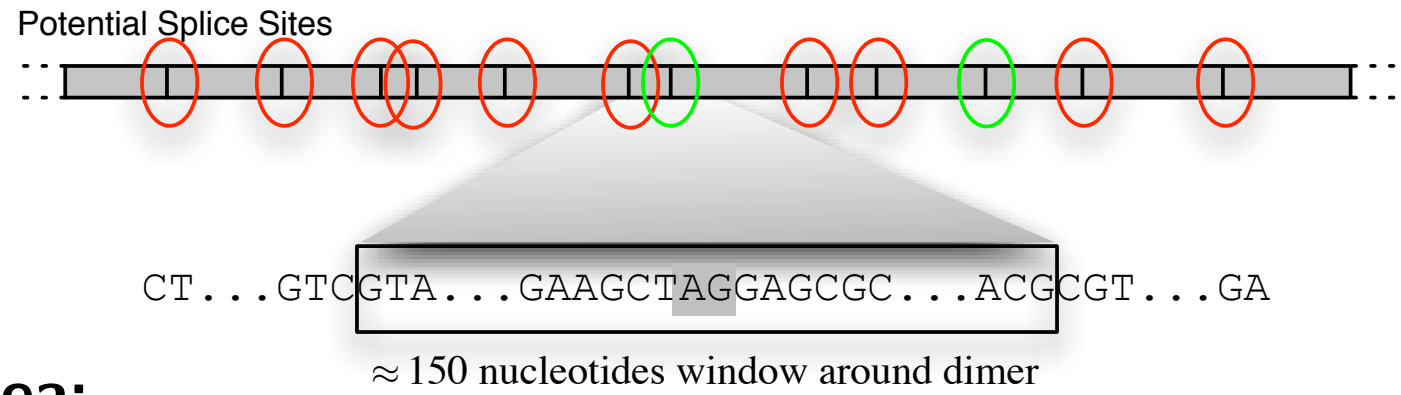
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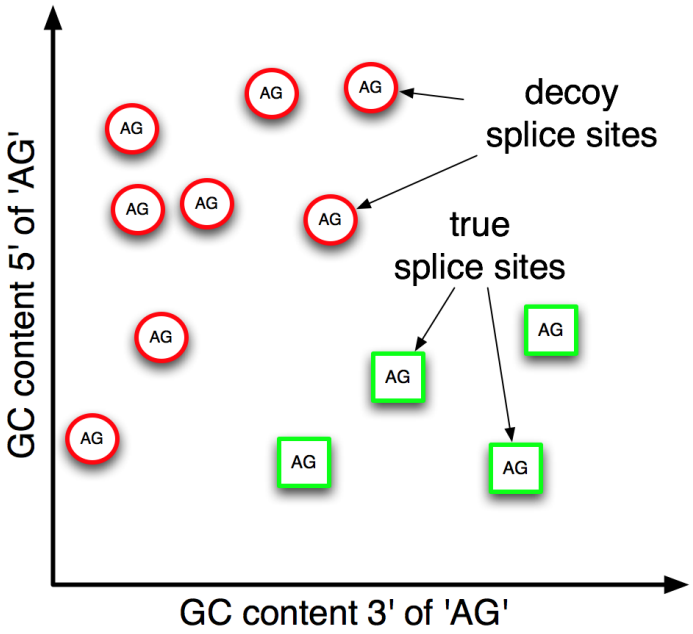
- True sites: fixed window around a true splice site
- Decoy sites: all other consensus sites

$\Rightarrow$ **Millions of examples from EST databases**

Example: Splice Site Recognition



Basic idea:



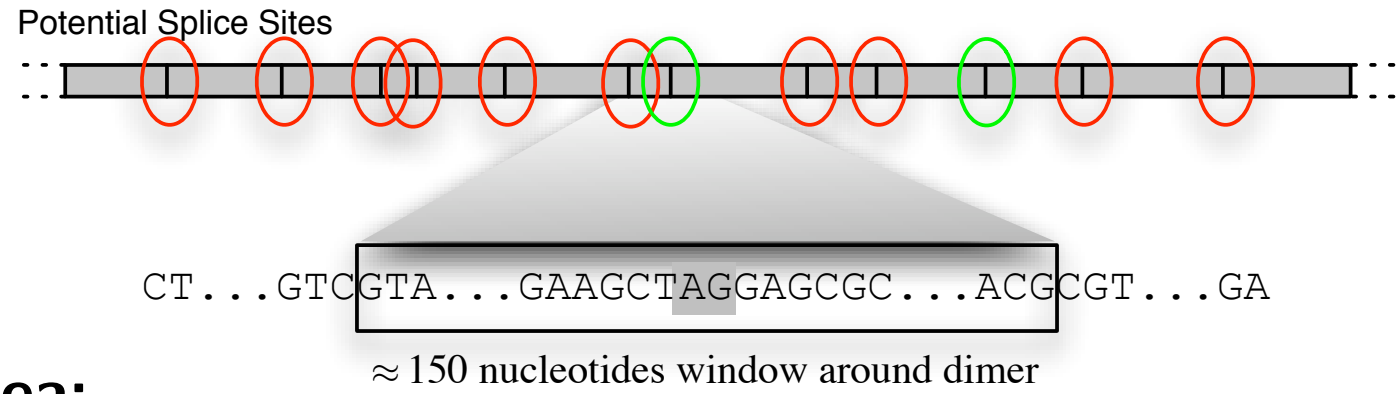
For instance, exploit that exons have higher GC content

or

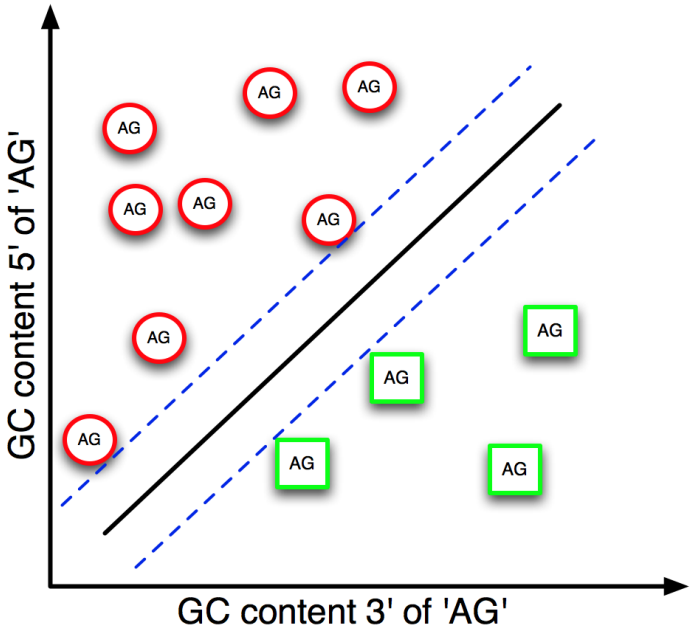
that specific motifs appear near splice sites.

[Sonnenburg et al., 2007]

Example: Splice Site Recognition



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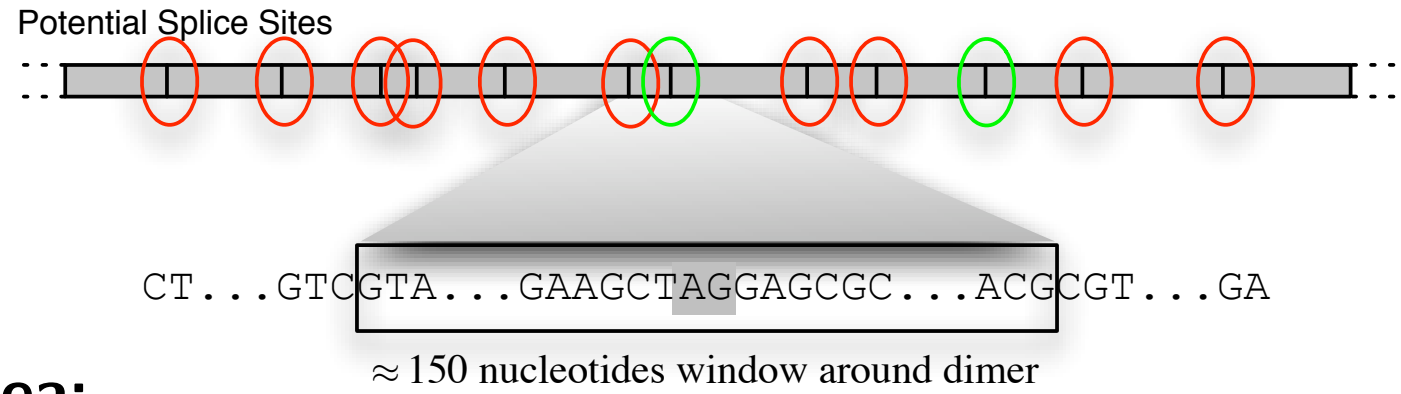
In practice: One feature per substring (e.g. $\leq$20) at all positions

$150 \cdot (4^1 + \ldots + 4^{20}) \approx 2 \cdot 10^{14}$ features

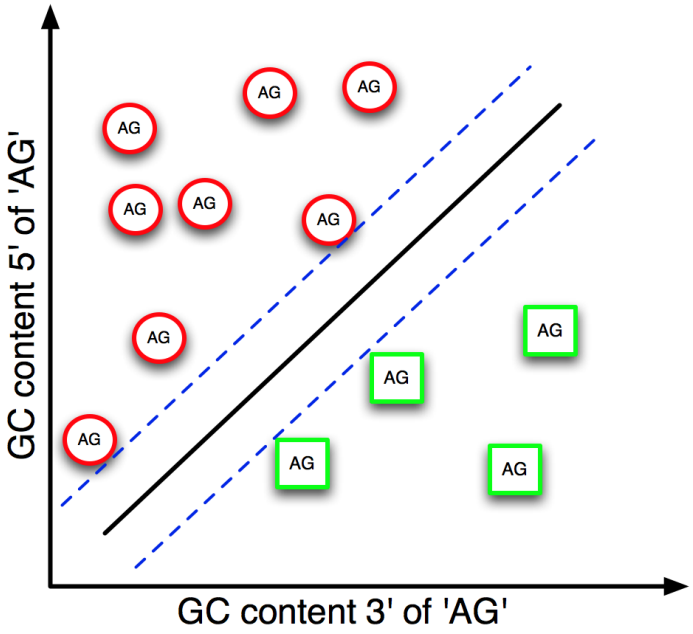
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- Leads to most accurate predictors that currently exist

$\Rightarrow$ www.shogun-toolbox.org

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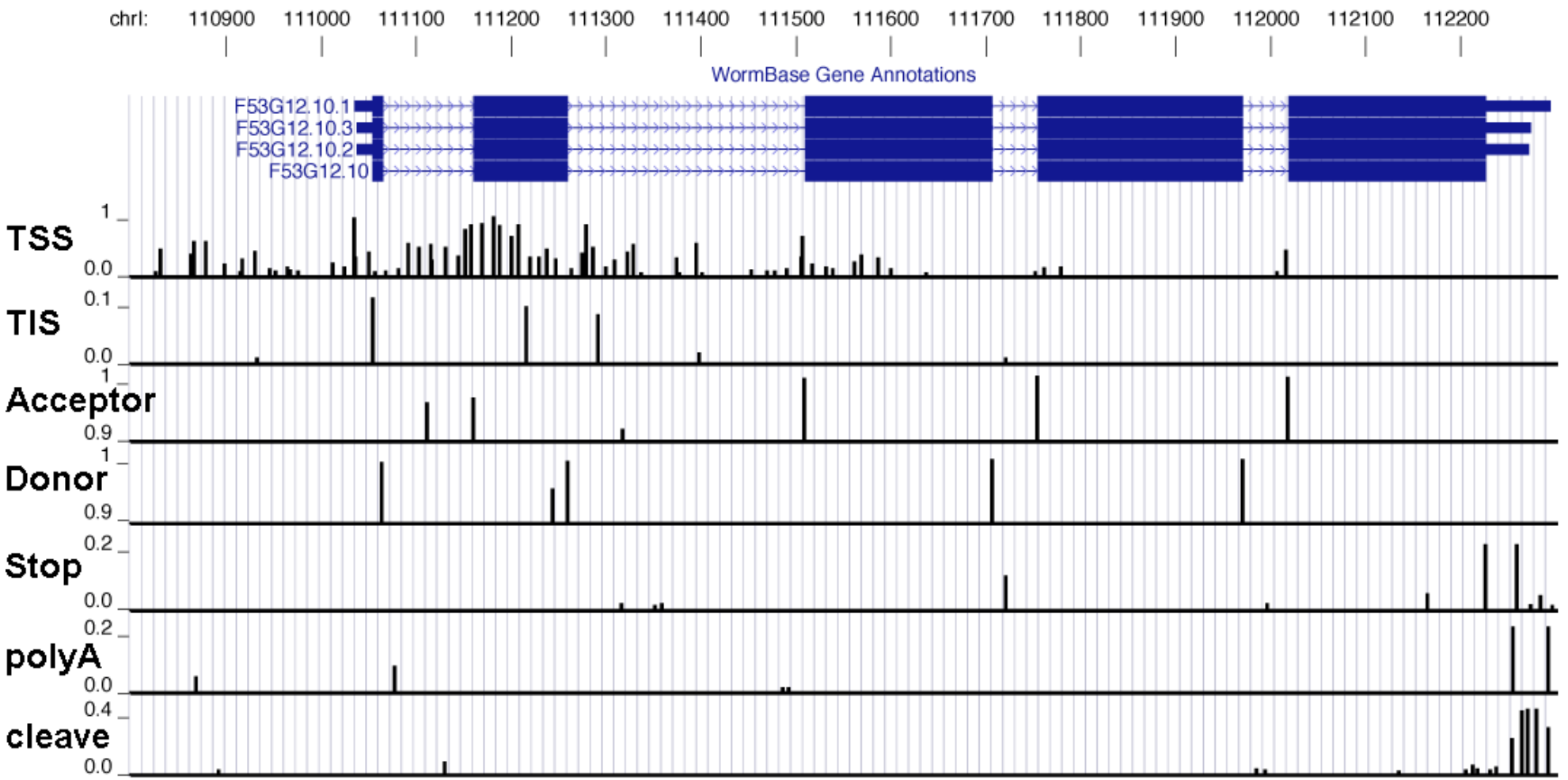
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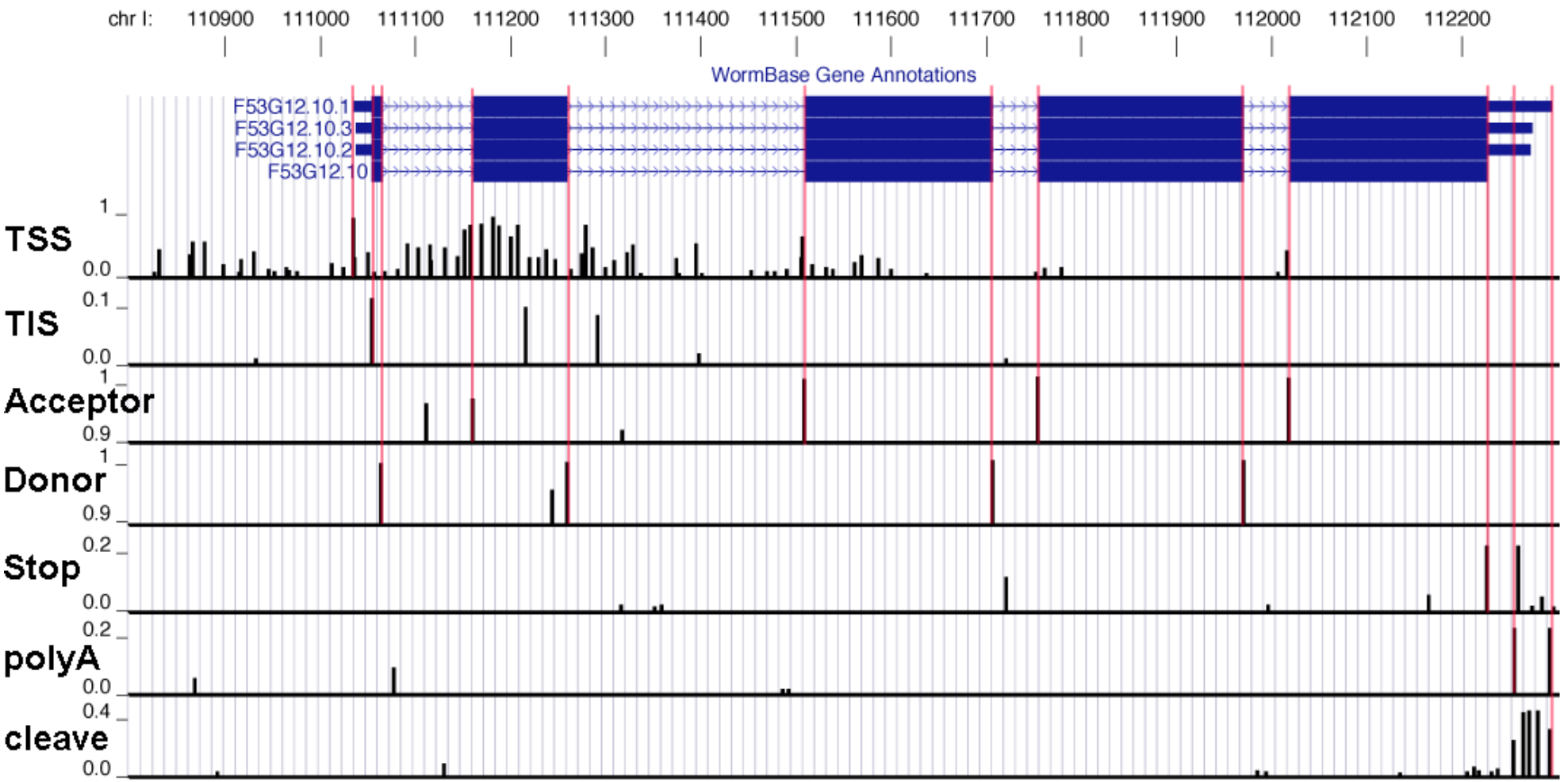
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Example: Predictions in UCSC Browser



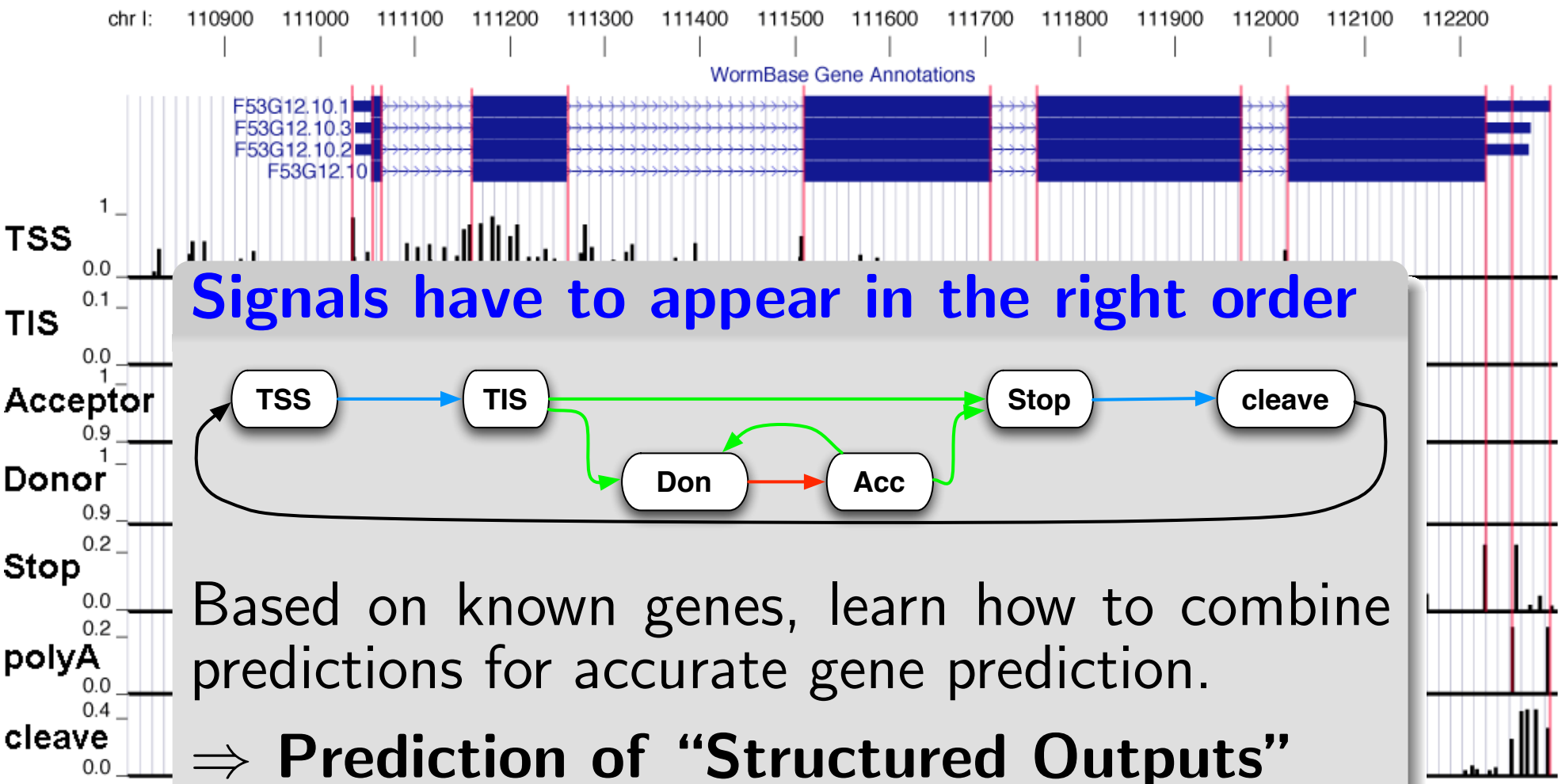
[Rätsch et al., 2007, Schweikert et al., 2009b]

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Example: Predictions in UCSC Browser



Signals have to appear in the right order

Based on known genes, learn how to combine predictions for accurate gene prediction.

⇒ **Prediction of "Structured Outputs"**

[Rätsch et al., 2007, Schweikert et al., 2009b]

Results using mGene

- Most accurate *ab initio* method in the nGASP genome annotation challenge for *C. elegans* [Coghlan et al., 2008]
- Validation of gene predictions for *C. elegans*: [Schweikert et al., 2009b]

	No. of genes	No. of genes analyzed	Frac. of genes w/ expression
New genes	2,197	57	$\approx 42\%$
Missing unconf. genes	205	24	$\approx 8\%$

- Annotation of other nematode genomes: [Schweikert et al., 2009b]

Genome	Genome size [Mbp]	No. of genes	No. exons/gene (mean)	mGene accuracy	best other accuracy
C. remanei	235.94	31503	5.7	**96.6%**	93.8%
C. japonica	266.90	20121	5.3	**93.3%**	88.7%
C. brenneri	453.09	41129	5.4	**93.1%**	87.8%
C. briggsae	108.48	22542	6.0	**87.0%**	82.0%

- Web service for *ab initio* gene predictions [Schweikert et al., 2009a]

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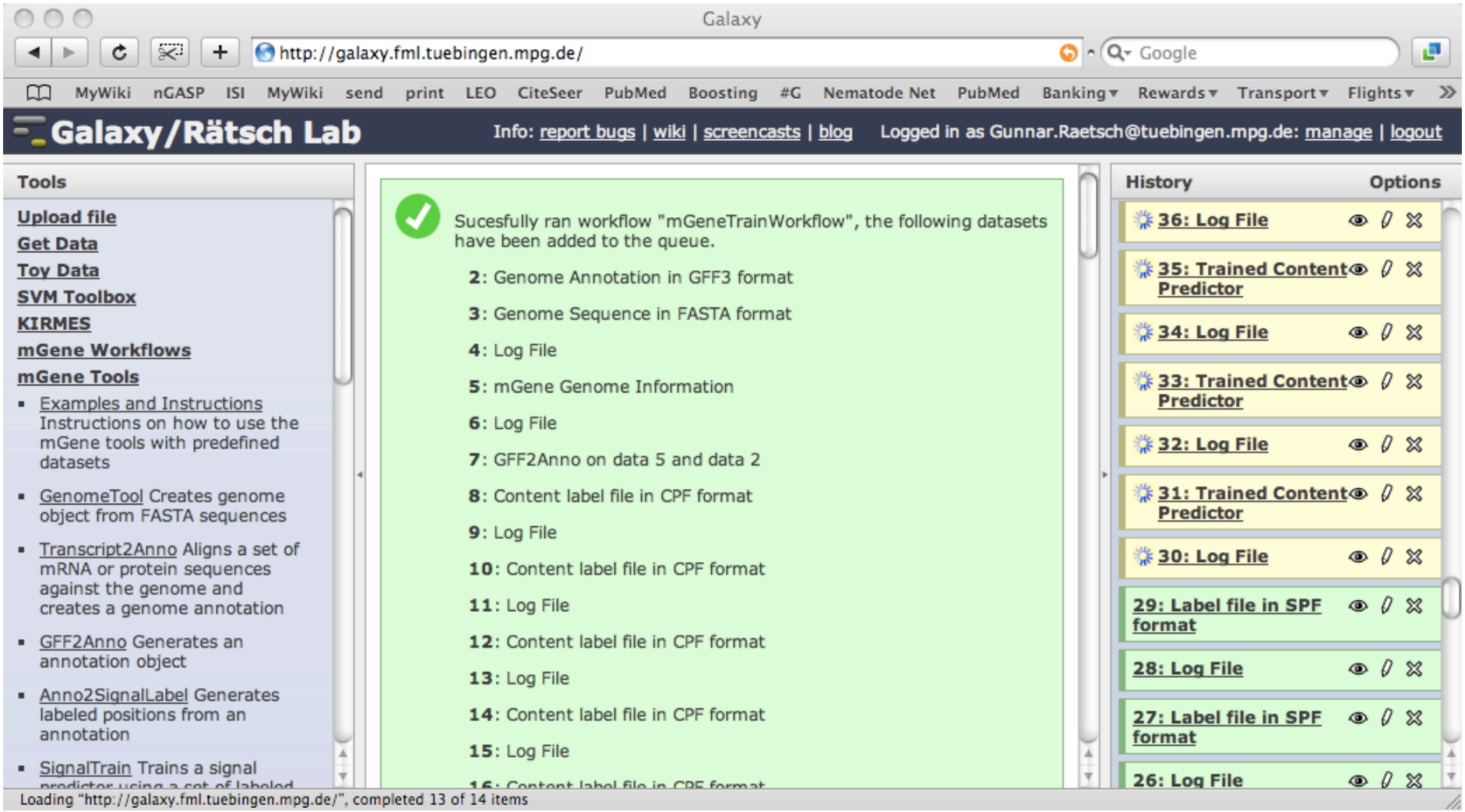
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mGene.web: Gene Finding for Everybody ;-)

(Schweikert et al., NAR Webserver Issue, 2009)



mgene.org/web

Limitations/Extensions

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Gene Finding + Auxiliary "Measurements"

[Behr et al., 2009, in prep.]



Is there other information that may be used by cellular processes that can improve prediction results?

Preliminary study: (*A. thaliana*) transcript level $(SN + SP)/2$

1. mGene (*ab initio*) ... **73.3%**
2. ...with DNA methylation (1 tissue) **76.1%**
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In progress:

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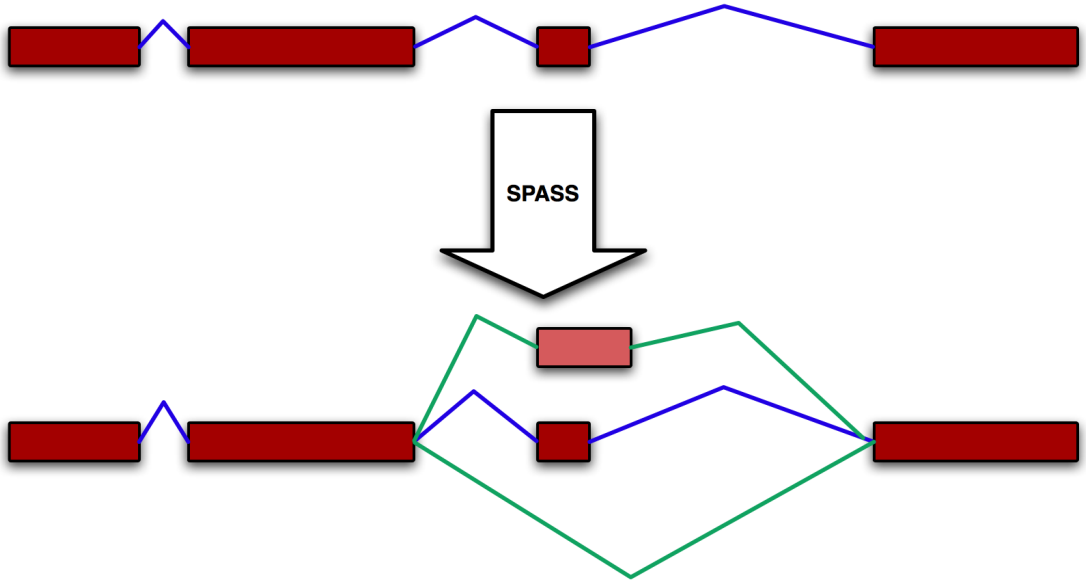
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Alternative Transcripts: First Steps

Predictions of alternative splicing



- Predict novel alternative splicing as independent events
- Only use sequence information [Rätsch et al., 2005]
- Requires known gene structures: Use *ab initio* predictions

Alternative Transcripts: More Steps



- Combine gene finding with prediction of alternative splicing
  - Machine learning challenge:

    Input: DNA sequence

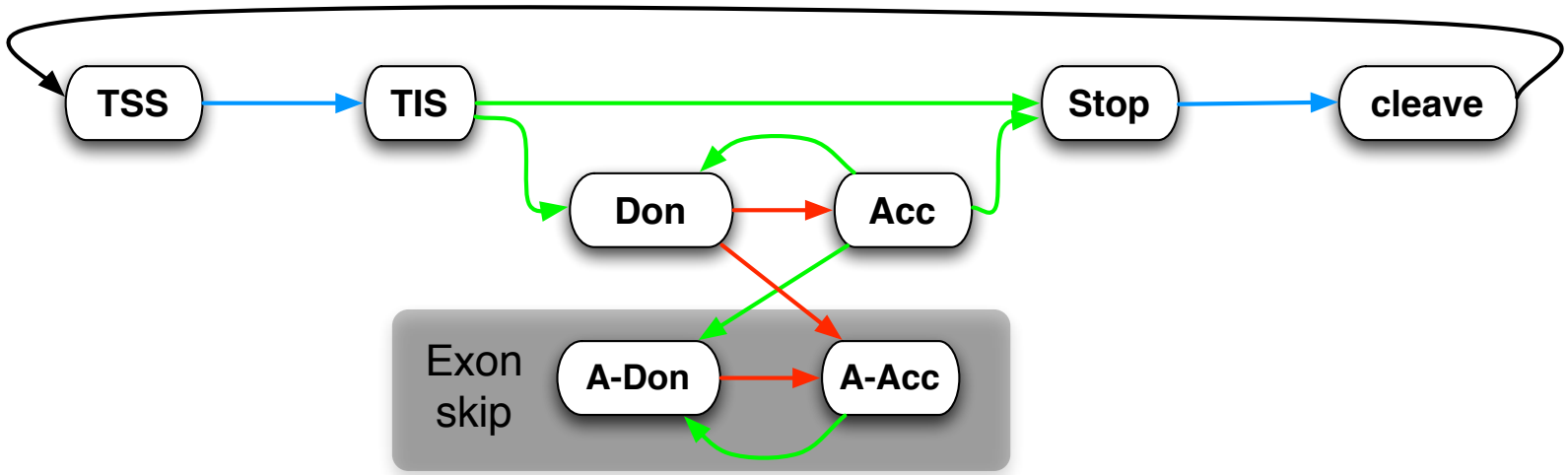
    Output: Splicing graph

- mGene approach can be extended to
  - Predict **"simple splicing graphs"**

- But, without **clean data** we cannot train the model ...

Alternative Transcripts: More Steps

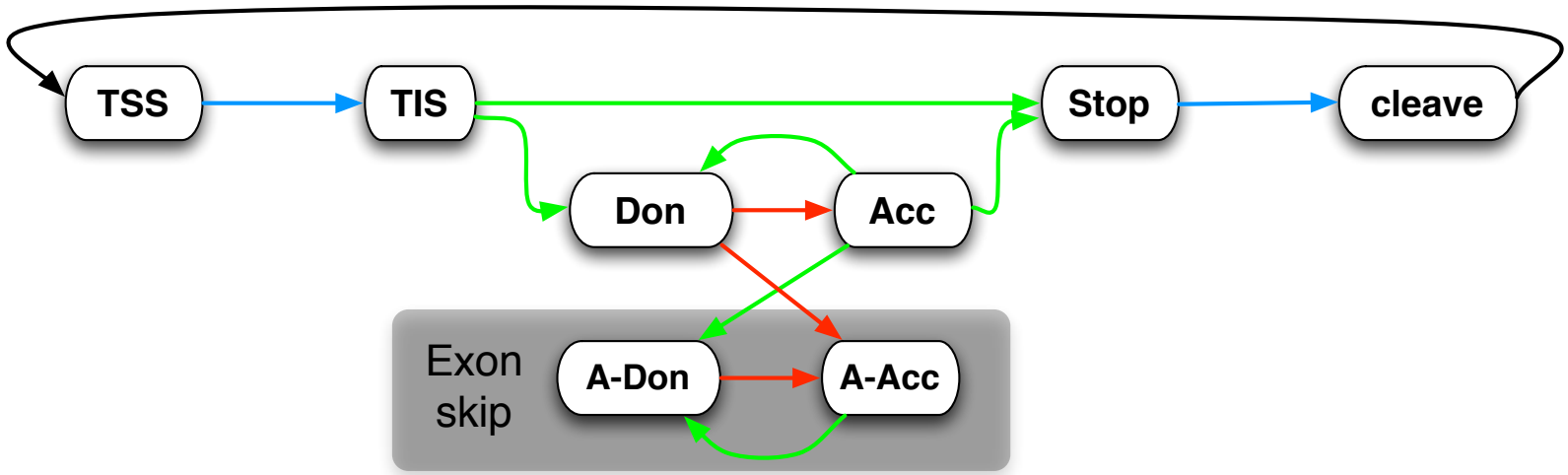
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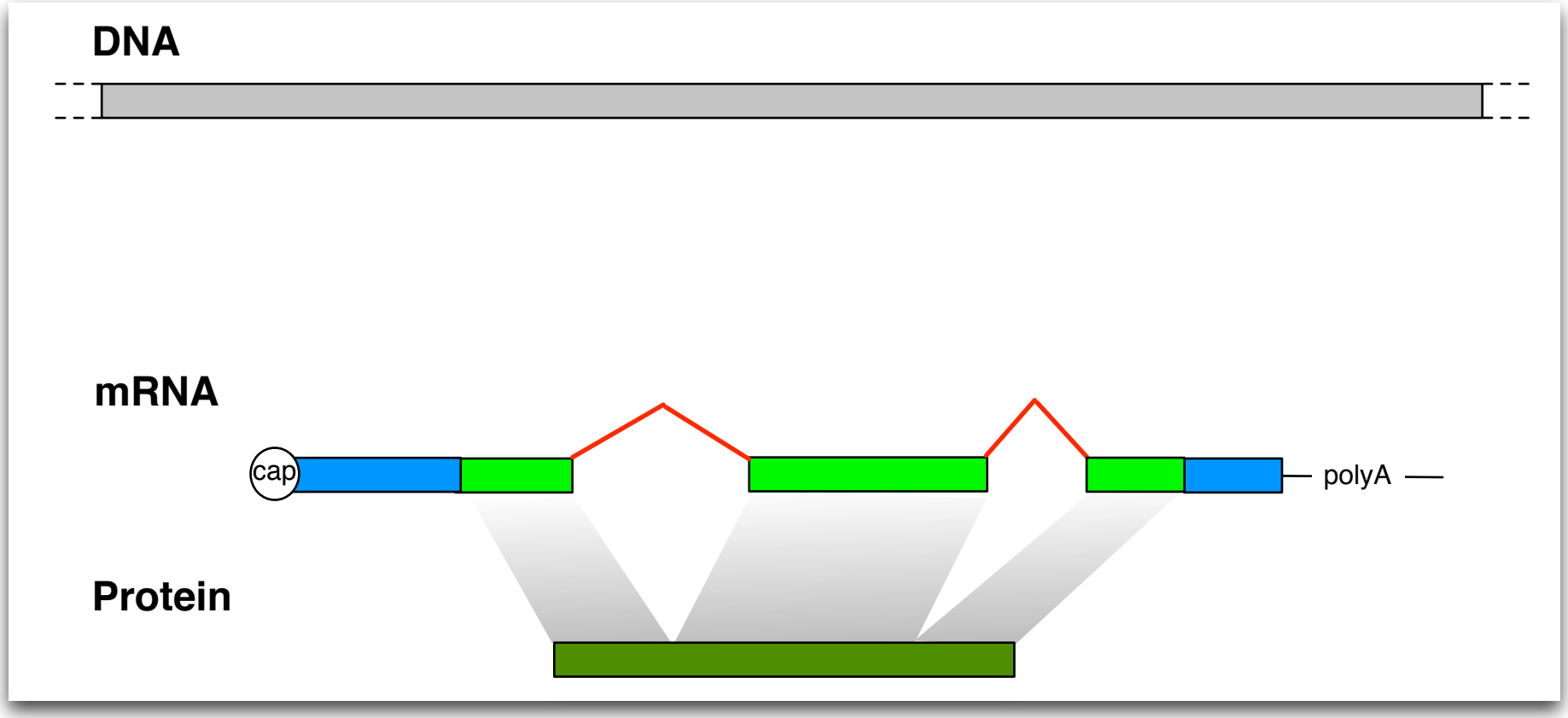
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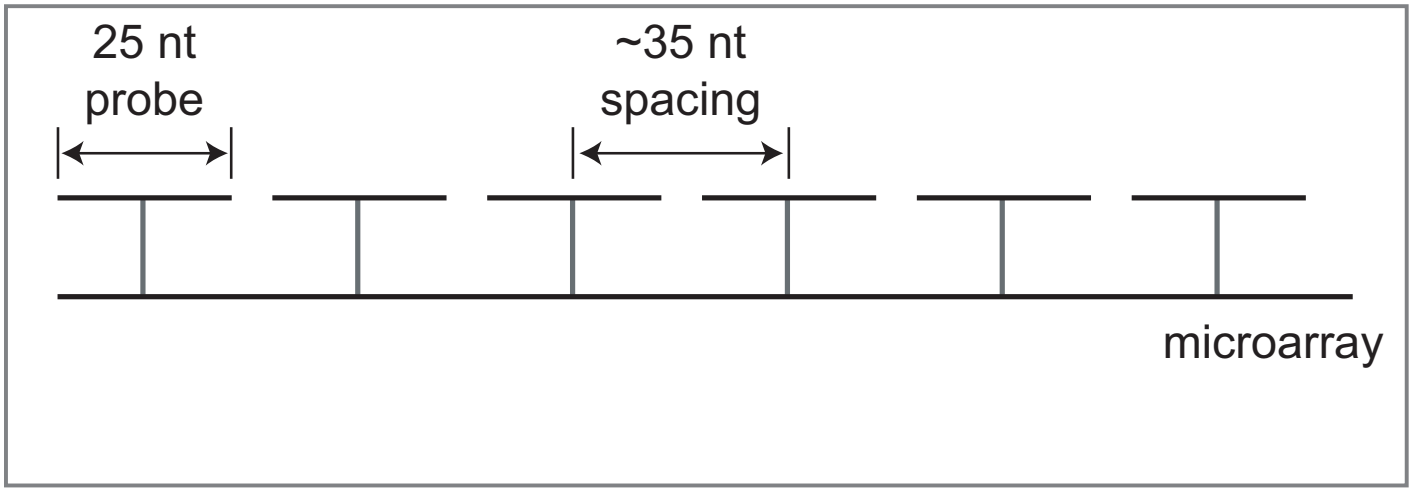
Reconstructing the Transcriptome



Directly measure the transcriptome

- Whole genome tiling arrays
- Transcriptome sequencing (Sanger or Next Generation Sequencing)

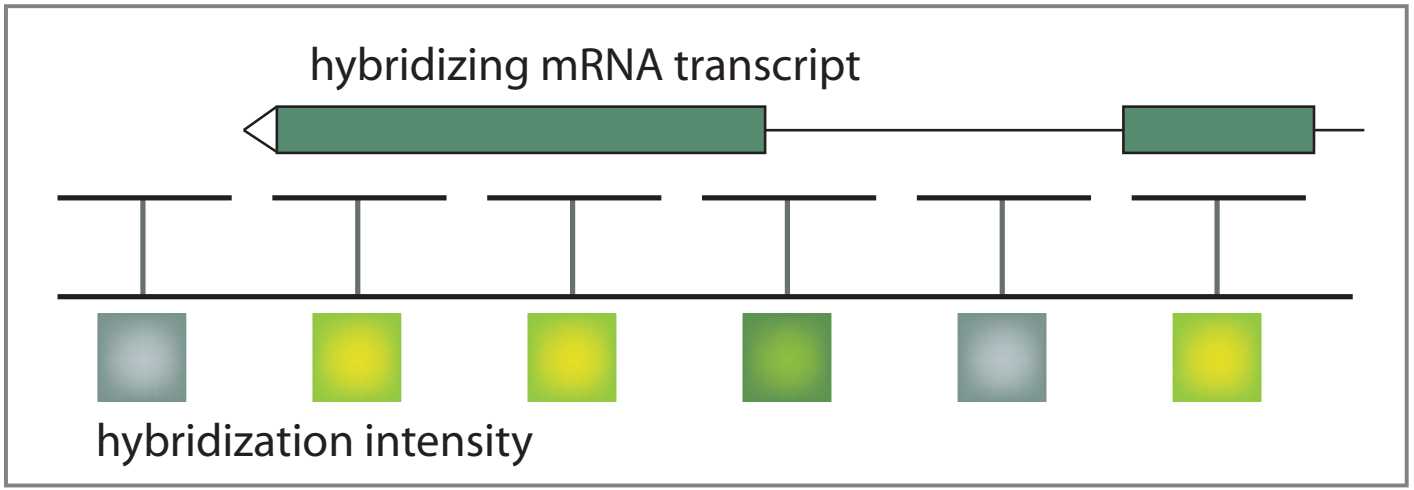
Tiling Arrays for Transcriptome Analysis



- Whole-genome quantitative measurements
- Cost-effective
  - ⇒ Replicates affordable, many tissues / mutants / conditions
- Unbiased
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(Work in collaboration with Detlef Weigel's lab, Max Planck Institute for Developmental Biology)

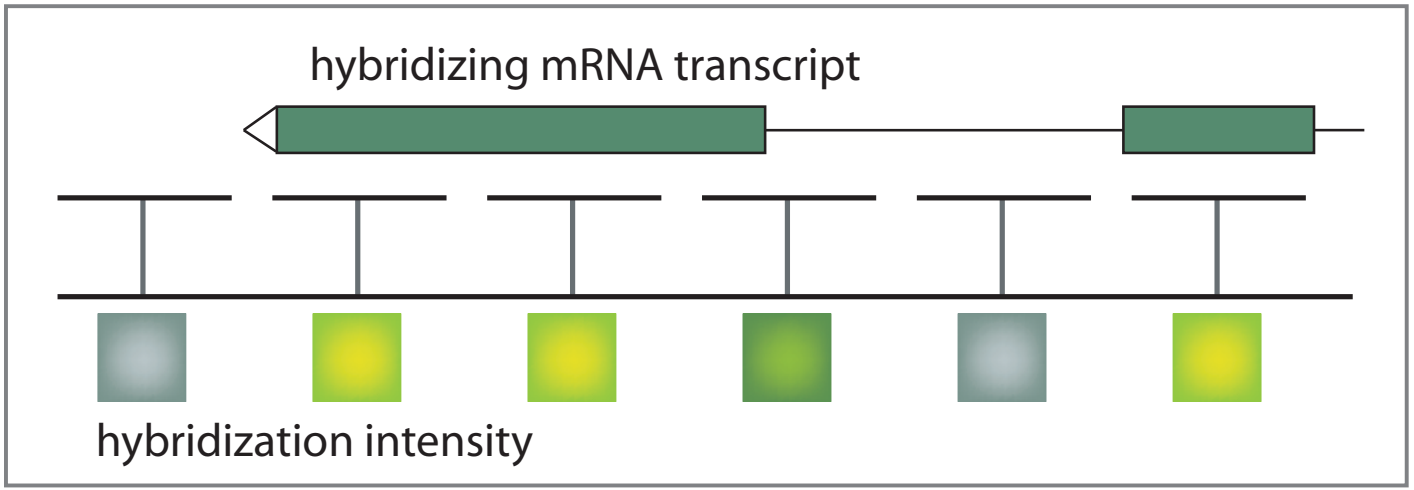
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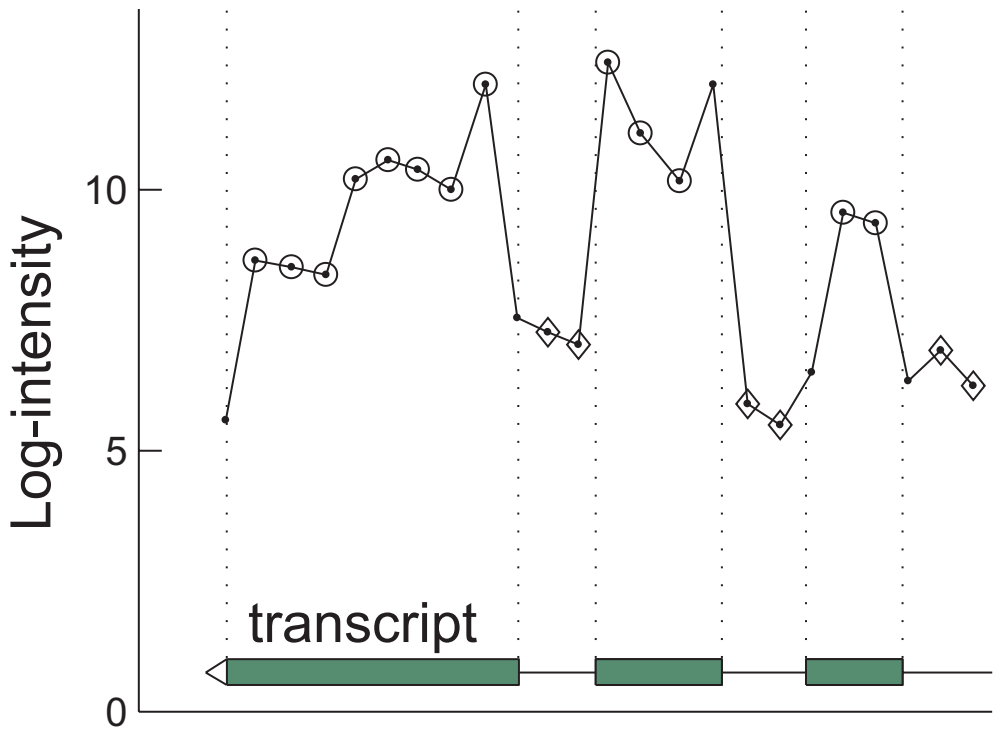
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[Laubinger et al., 2008b]

Intensities are Noisy Measurements

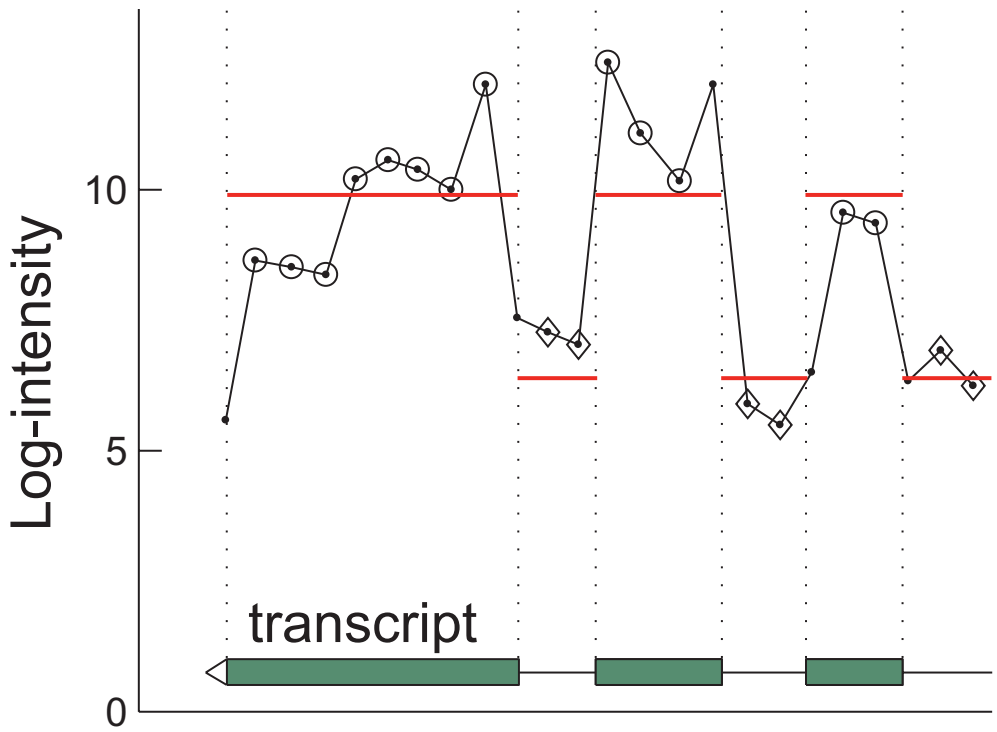


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Systematic bias induced by probe sequence effects

$\Rightarrow$ Model effect for normalization [Zeller et al., 2008b]

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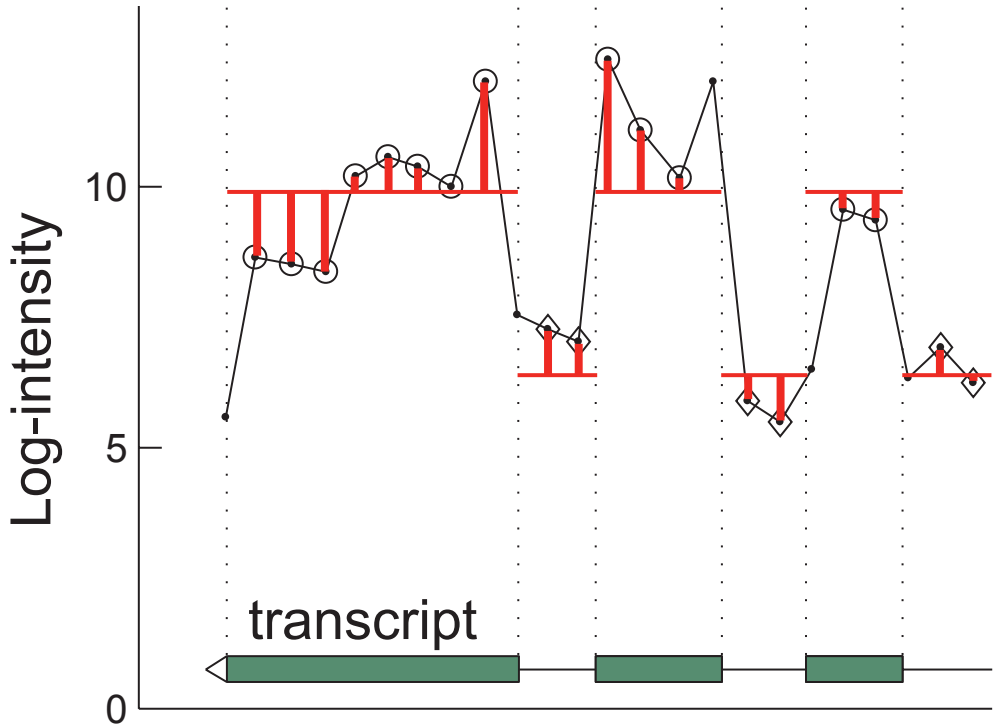


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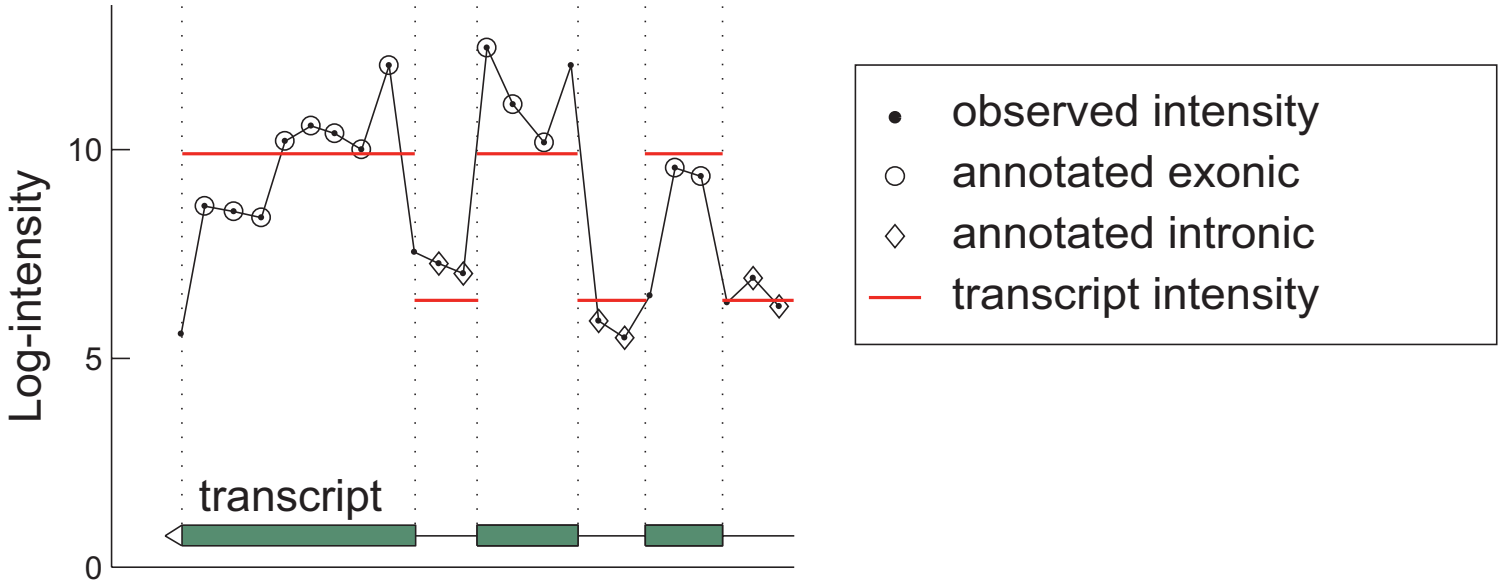
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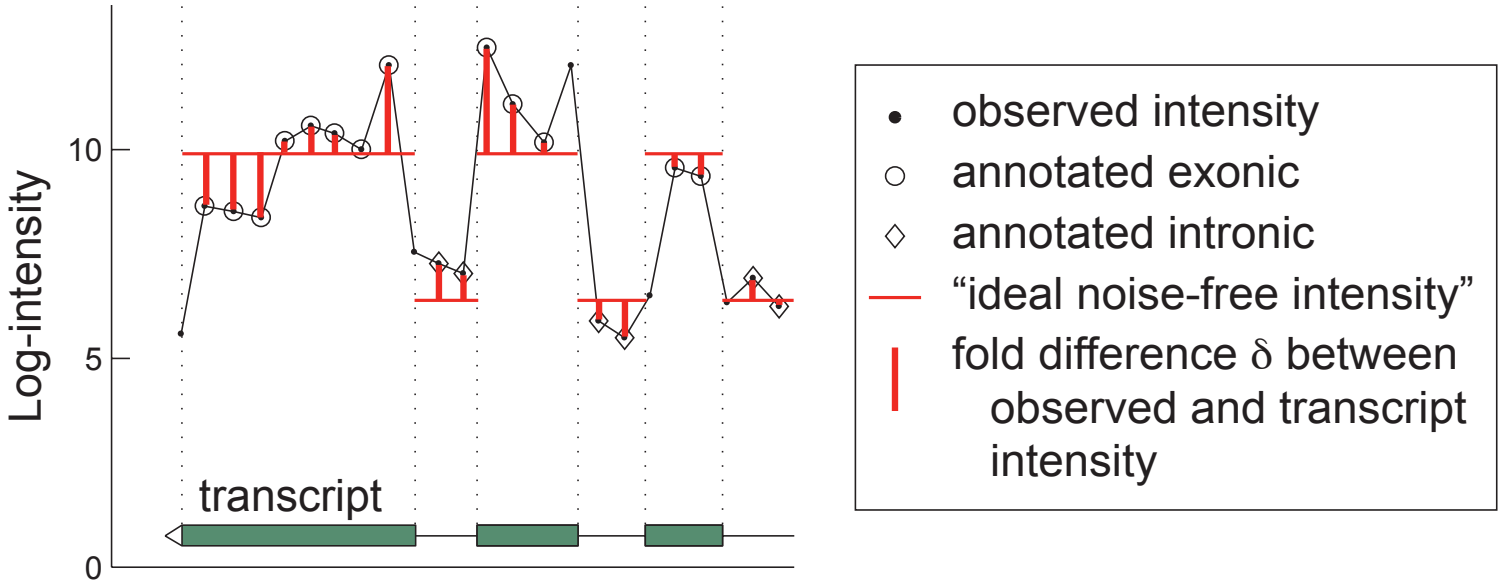
Tiling Array Normalization

- Transcript normalization assumes constant transcript intensities $\overline{y}_i$ (median estimate) [Zeller et al., 2008b]
- Learns intensity deviation from transcript intensity $\delta_i := y_i - \overline{y}_i$
- Takes probe sequence $\mathbf{x}_i$ (positional information on mono-, di- and tri-mer occurrence) as input for regression.
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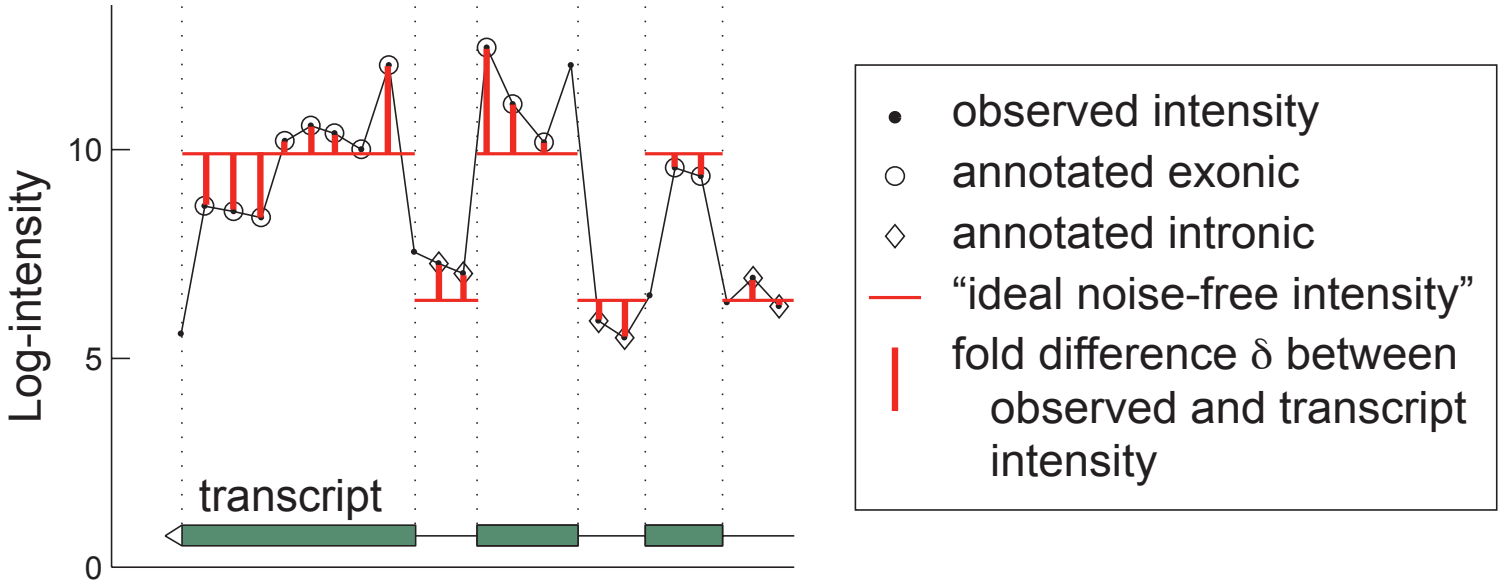
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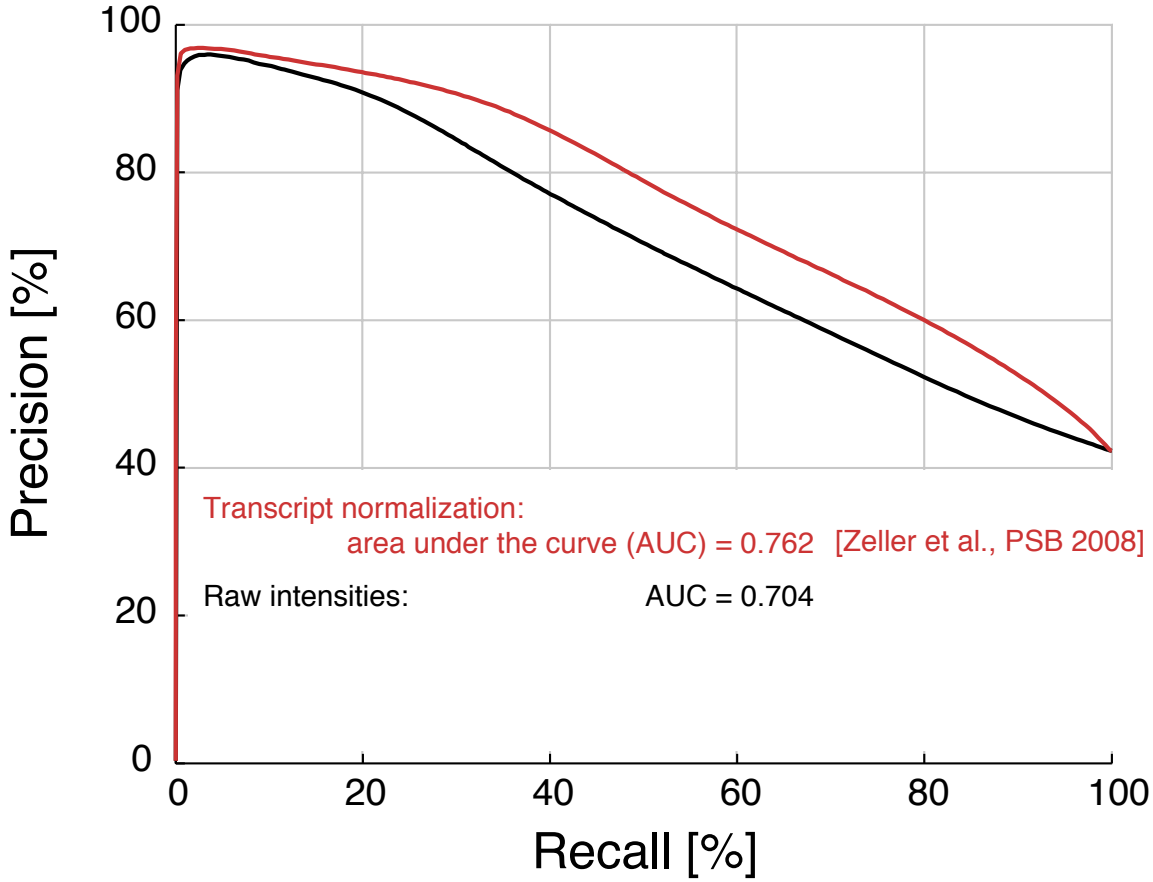


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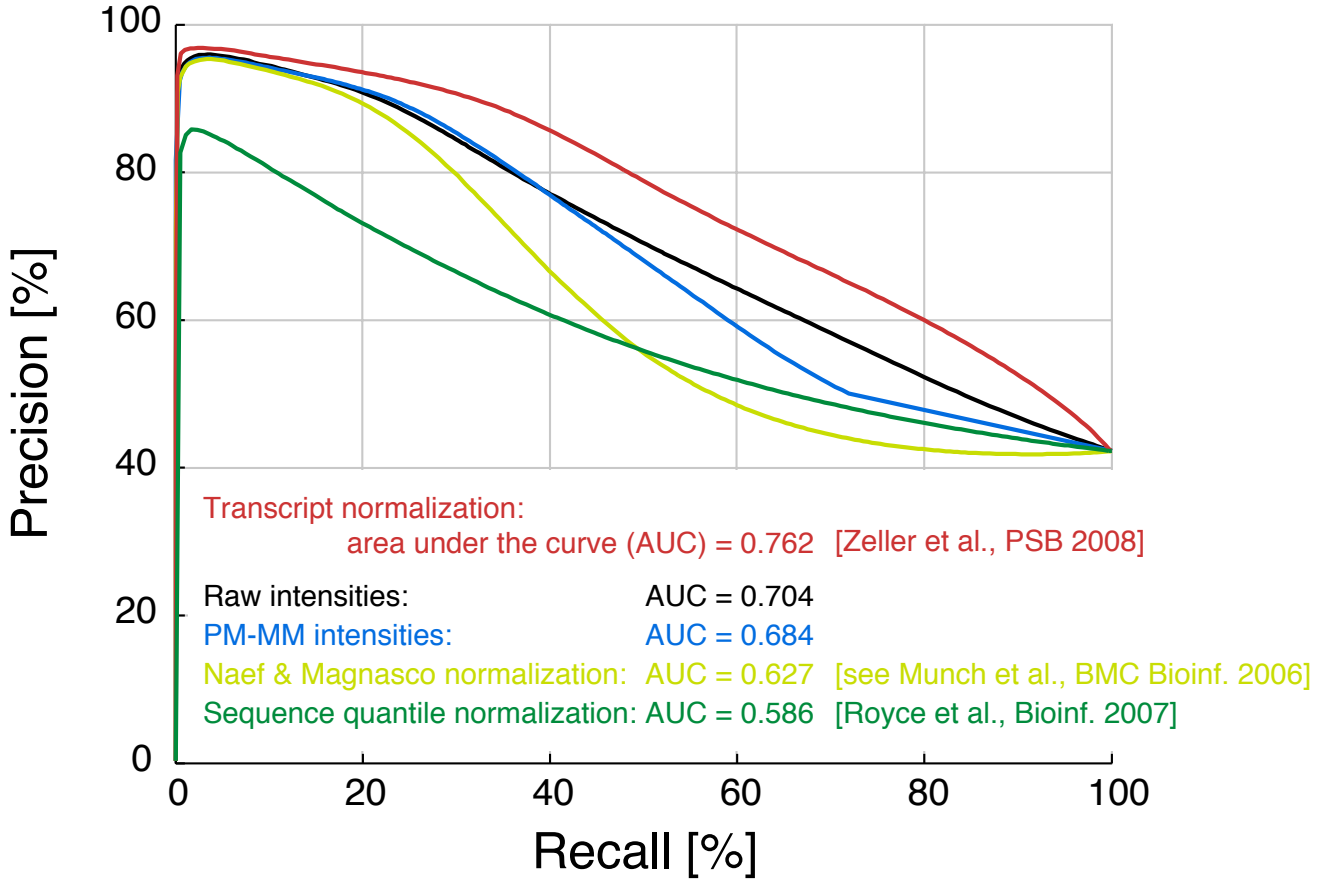
Transcript Normalization Results



Transcript normalization (TN) results in better separation between background and exon signals $\longrightarrow$ improves transcript identification

`www.fml.mpg.de/raetsch/research/tiling`

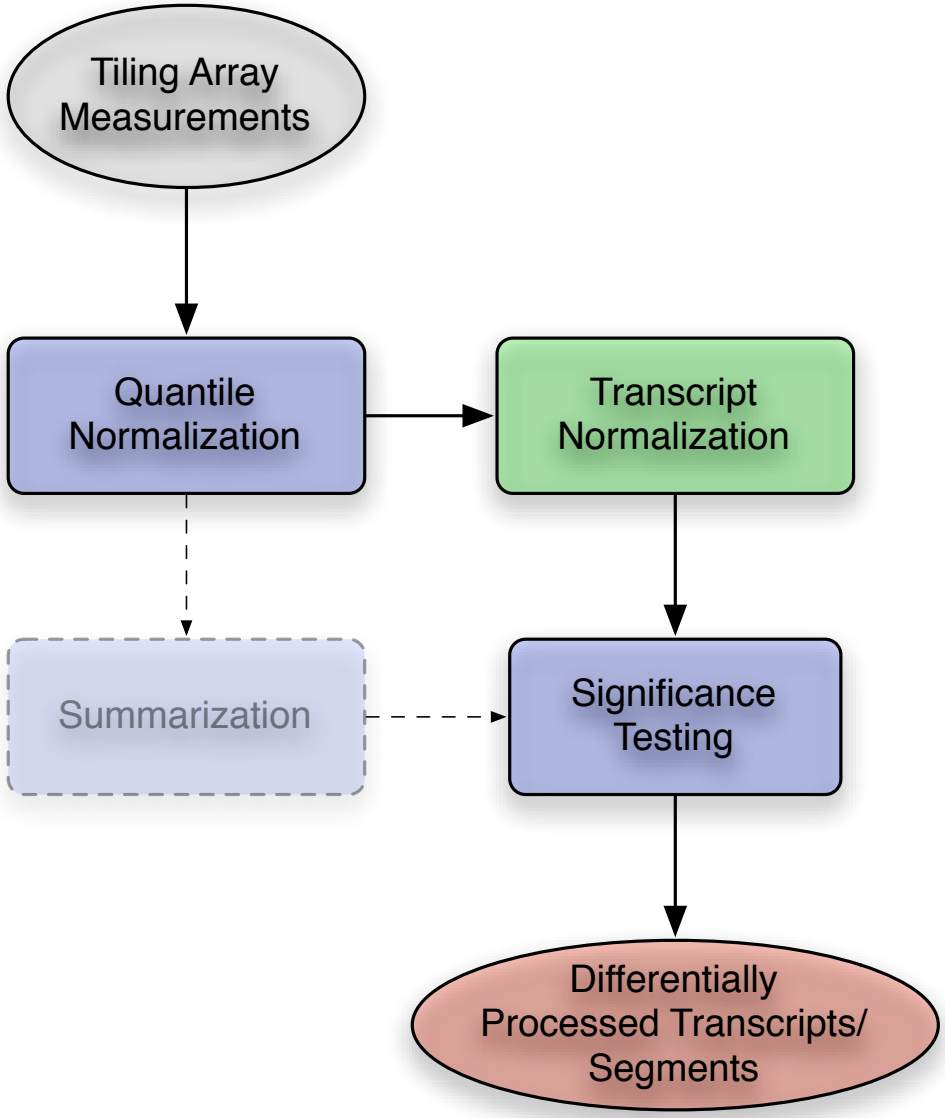
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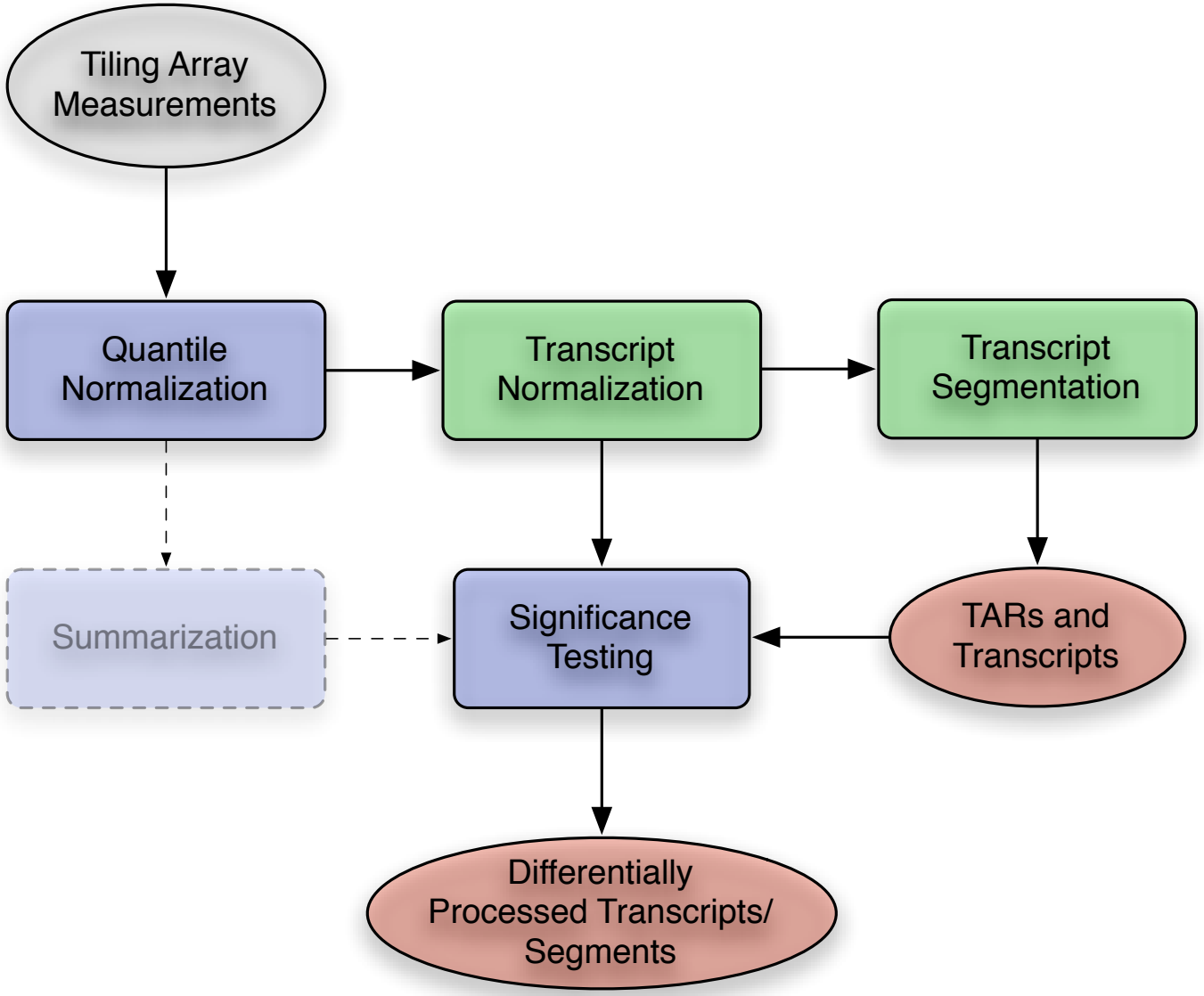
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Analysis Pipelines

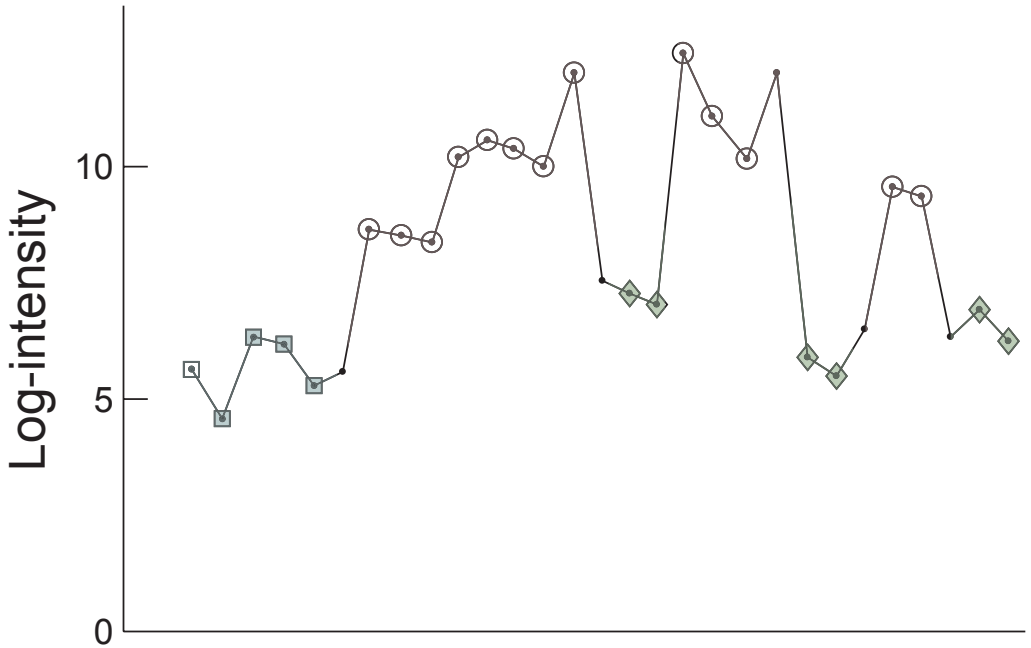


Analysis Pipelines



Tiling Array Segmentation

Goal: Characterize each probe as either intergenic, exonic or intronic



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- annotated intergenic

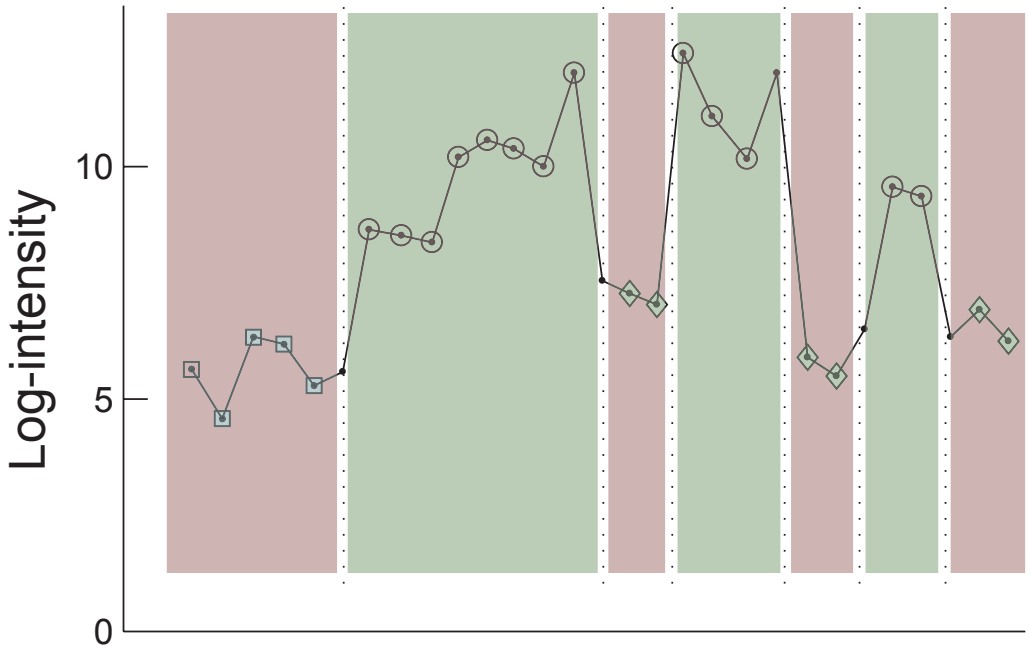
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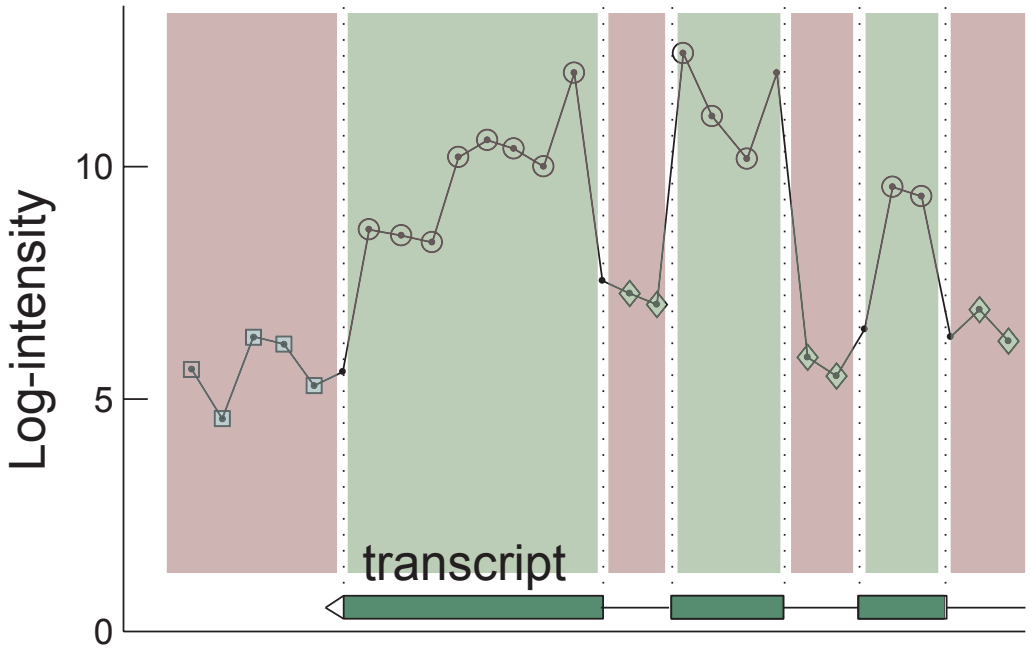
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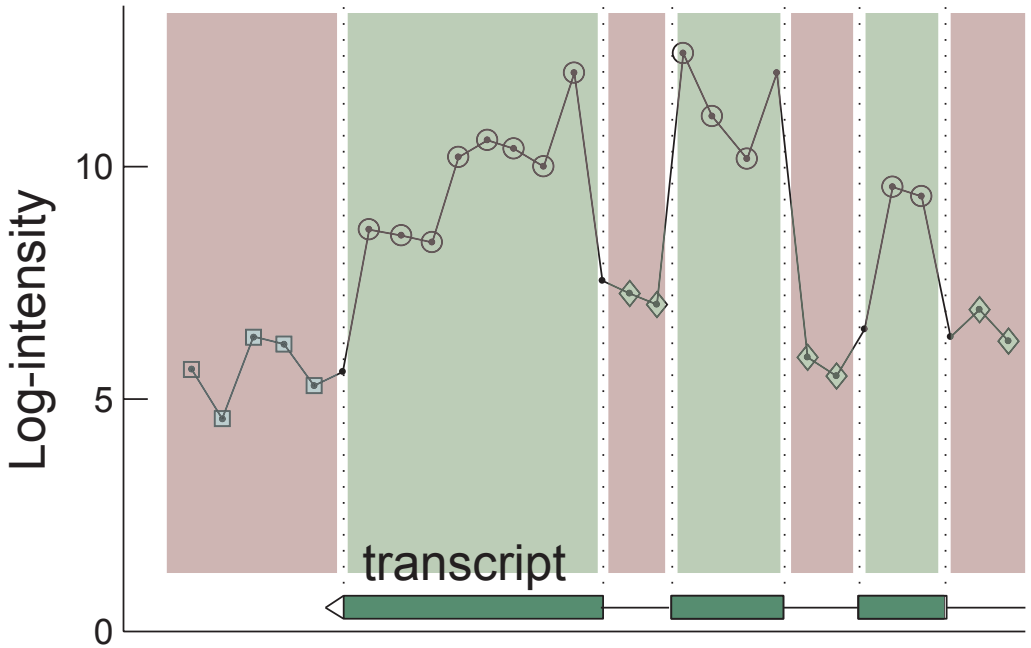
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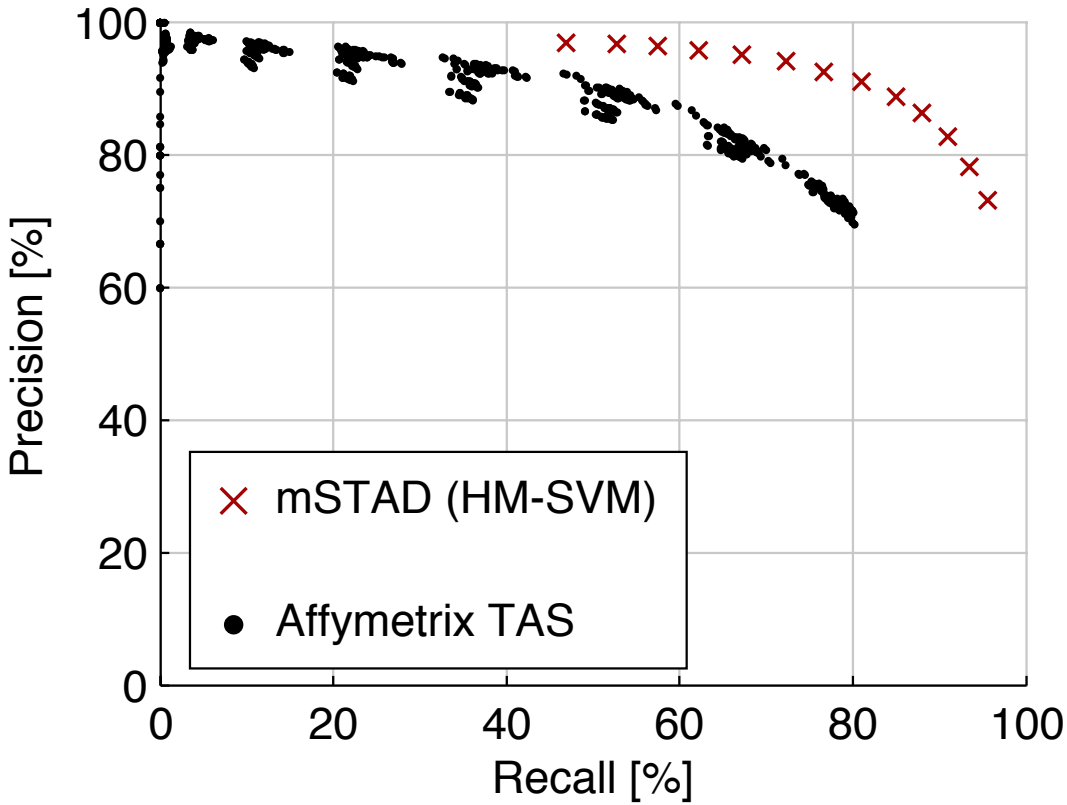
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TAR Identification: Method Comparison
(Combining Normalization and Segmentation Method)



Substantially improved exon probe recognition over the most widely used "transfrag" method

`www.fml.mpg.de/raetsch/research/tiling`

Genome-wide Analyses of Alternative Transcripts



- *A. thaliana* transcriptomes in 11 different tissues, 5 different abiotic stresses, RNA processing mutants (3 replicates)
- 1^{st} exon/intron-resolution analysis in plants [Laubinger et al., 2008b]
- Identified ≈7,000 cases of intron retentions (est. FDR=14%)
- Many of them show differential splicing [Eichner et al., 2009, in prep.]
- Profiling cap-binding/miRNA processing mutants (CBP80/CBP20)

⇒ Influence splicing efficiency

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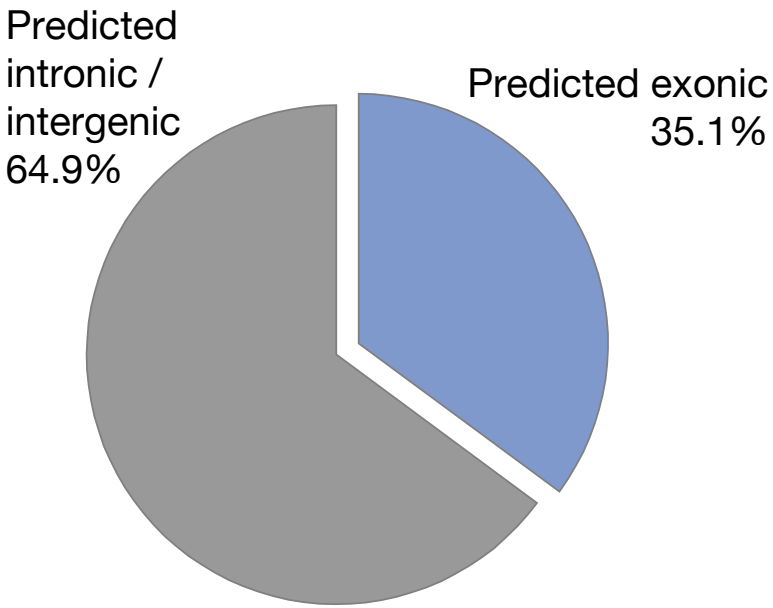
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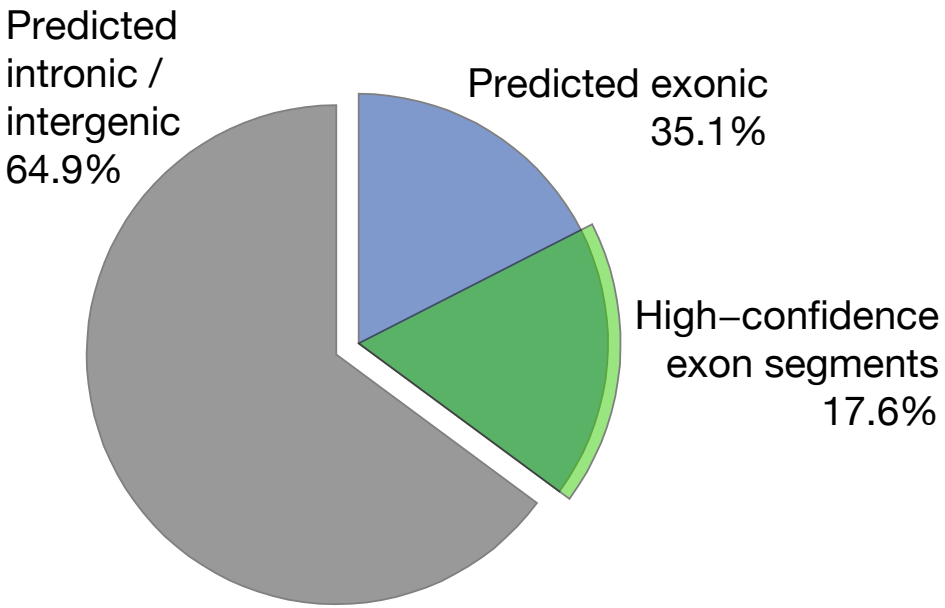
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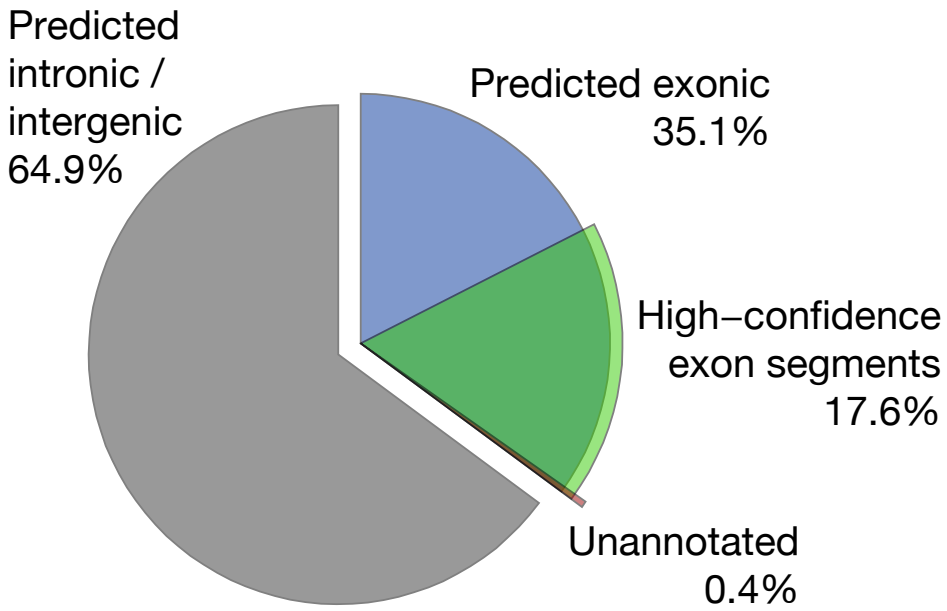
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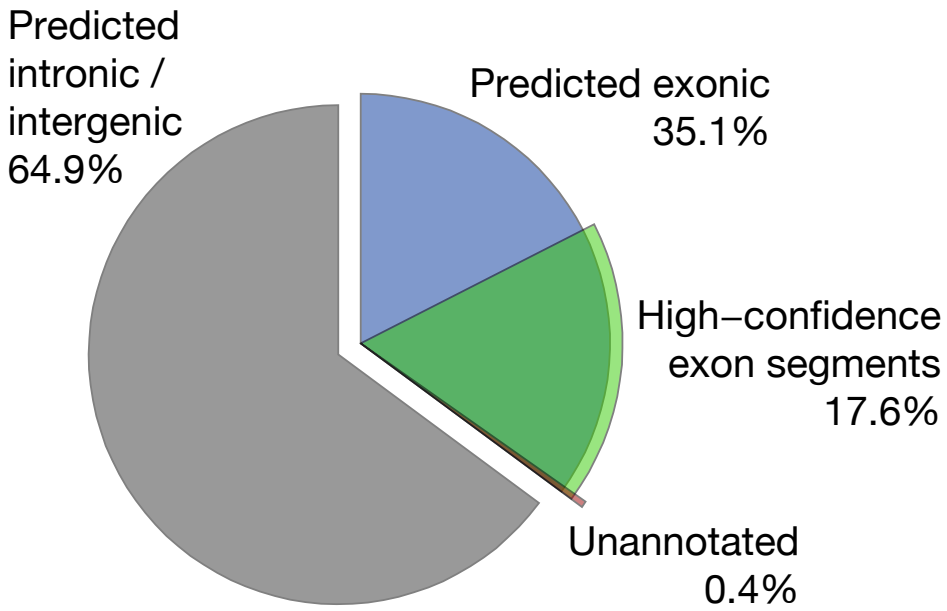
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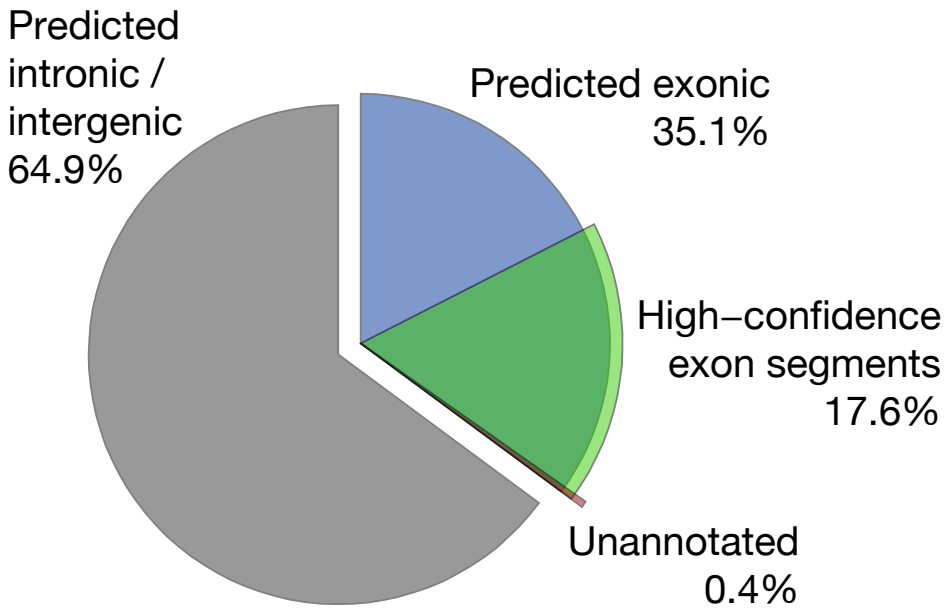
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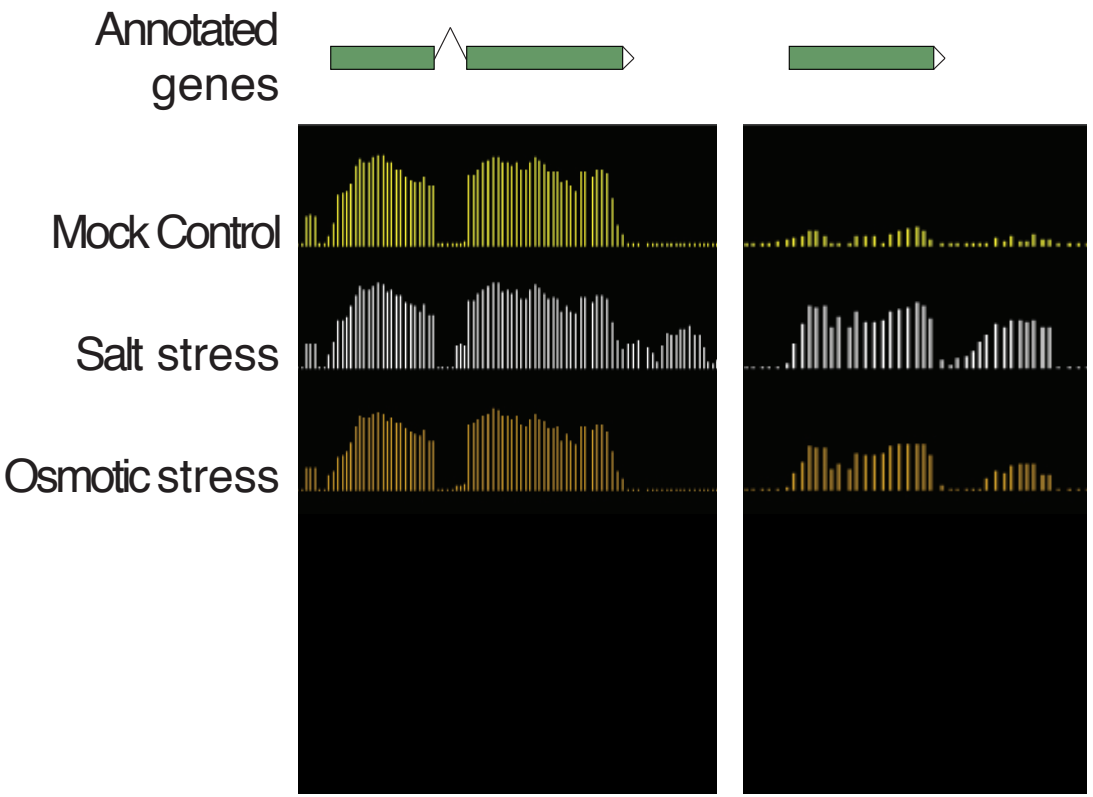


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New Stress-Induced TARs

[Zeller et al., 2009]

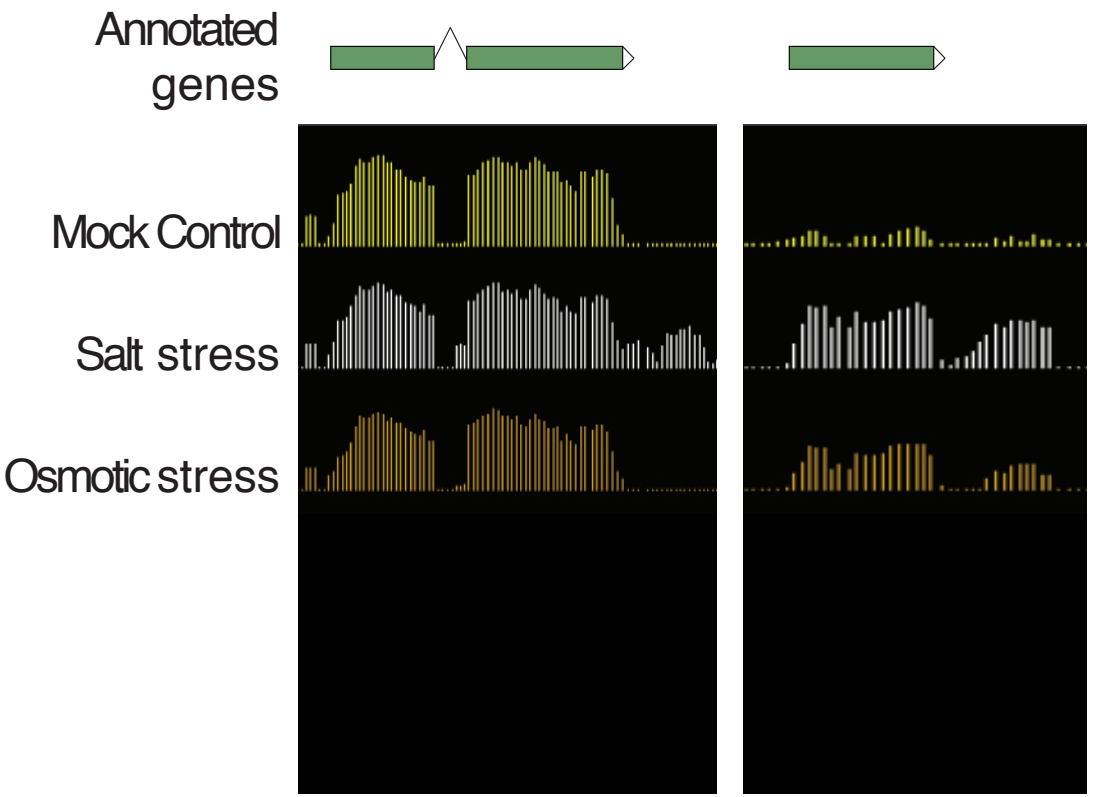


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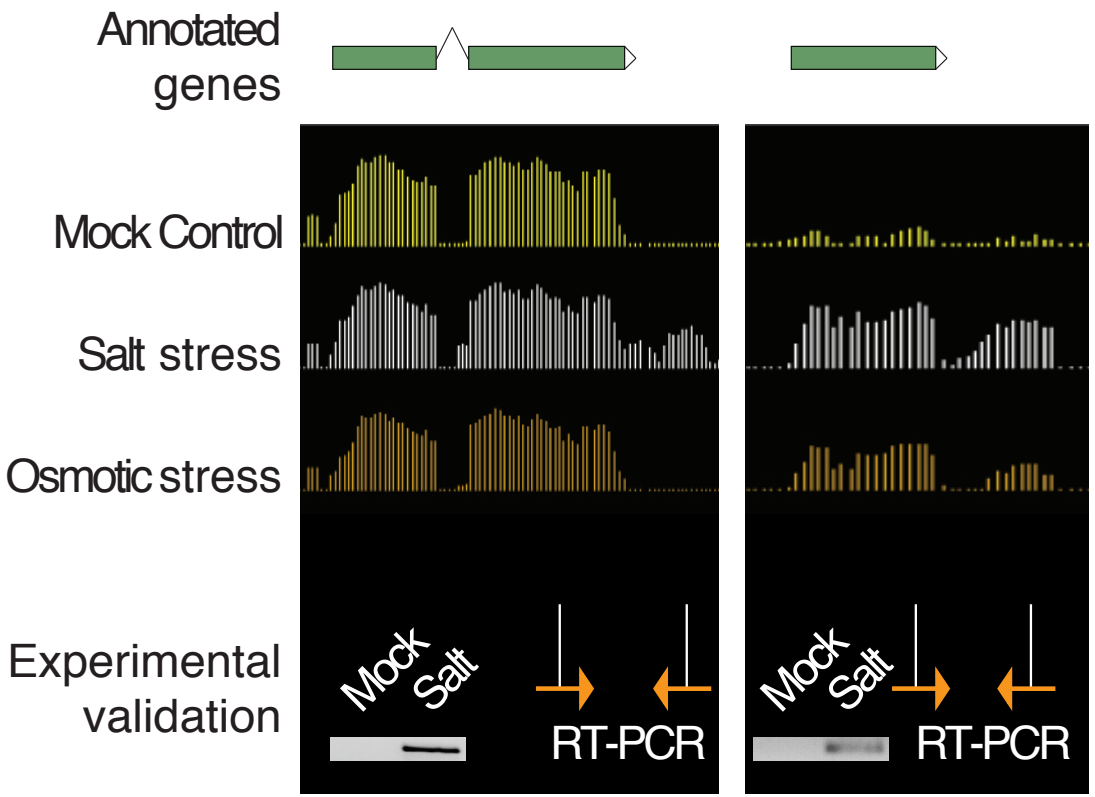


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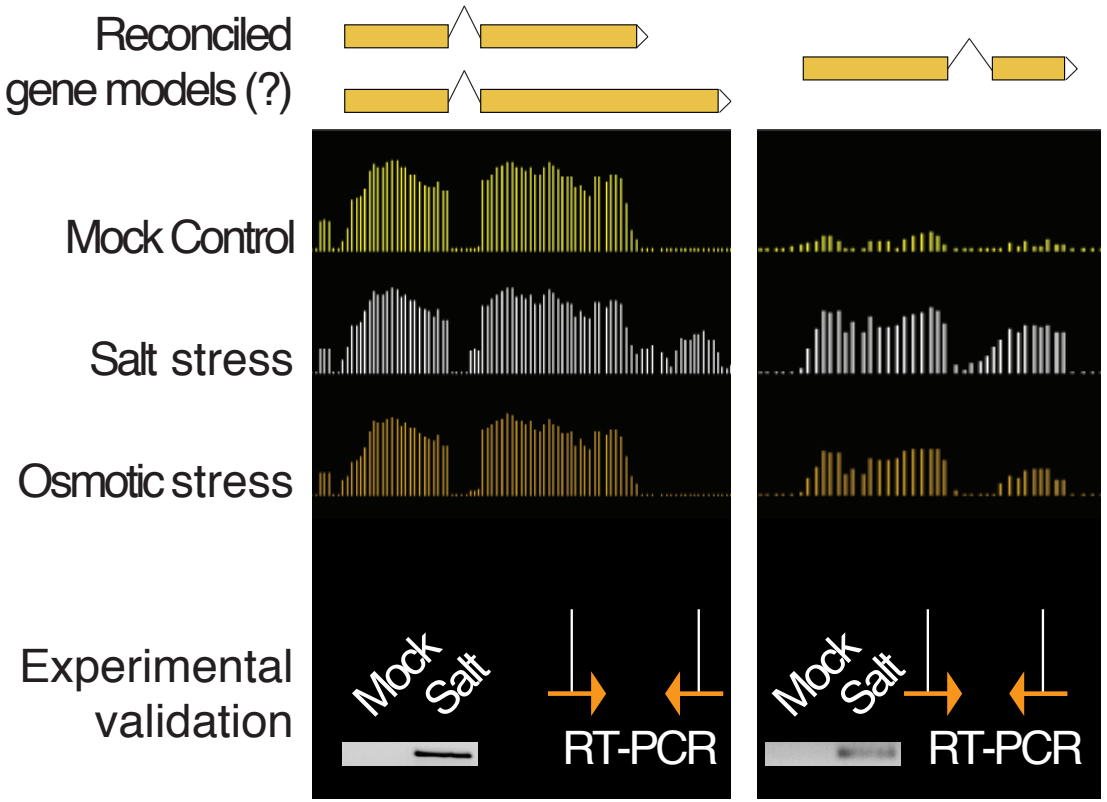


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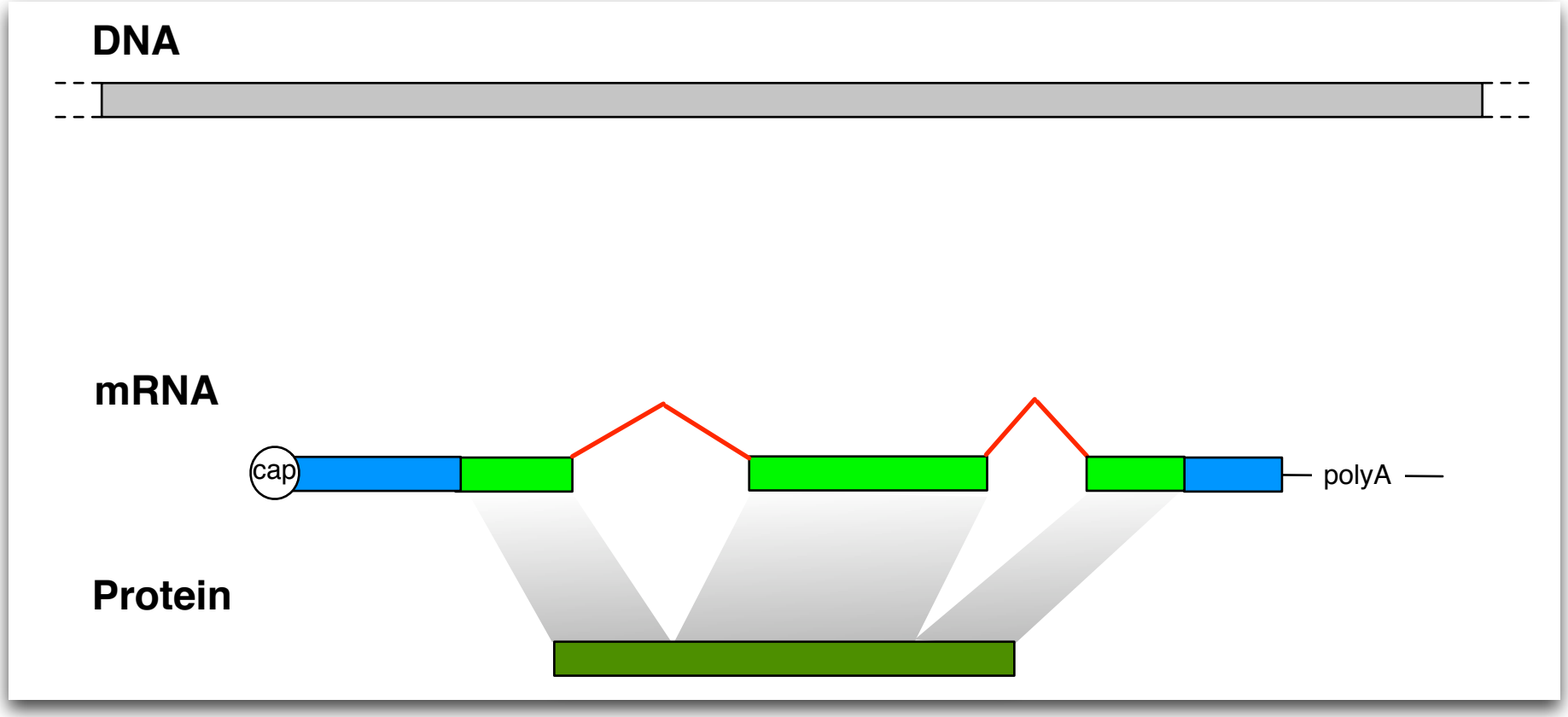
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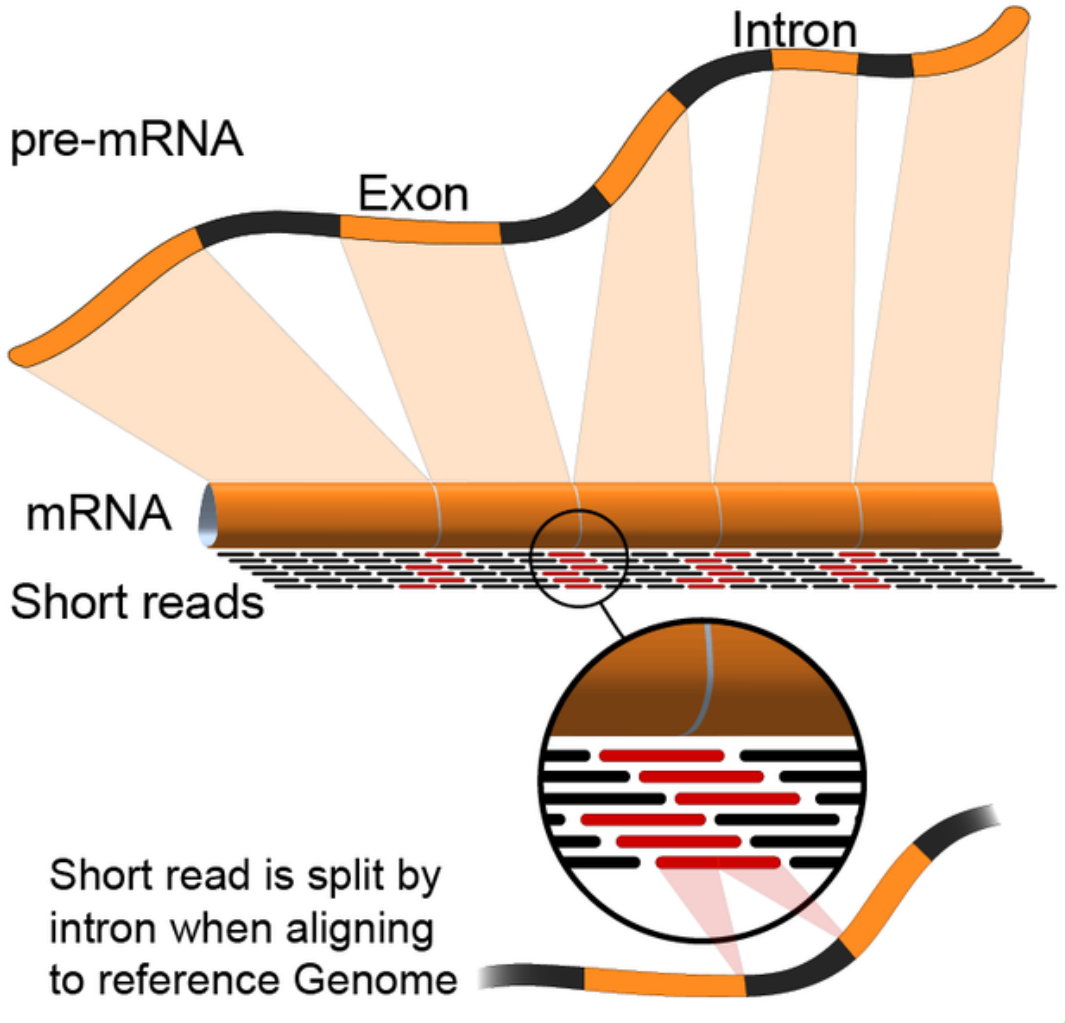
Reconstructing the Transcriptome



Directly measure the transcriptome

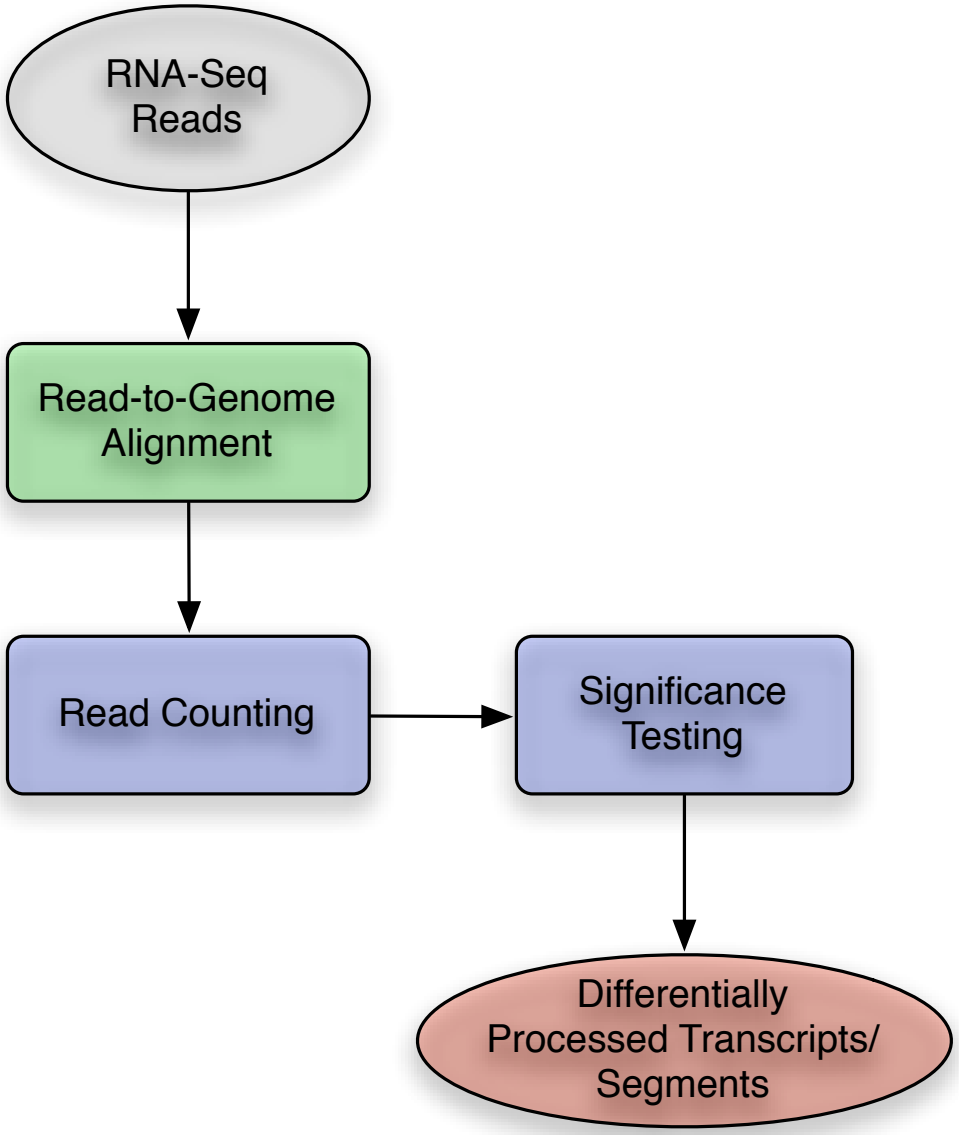
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mRNA Deep Sequencing

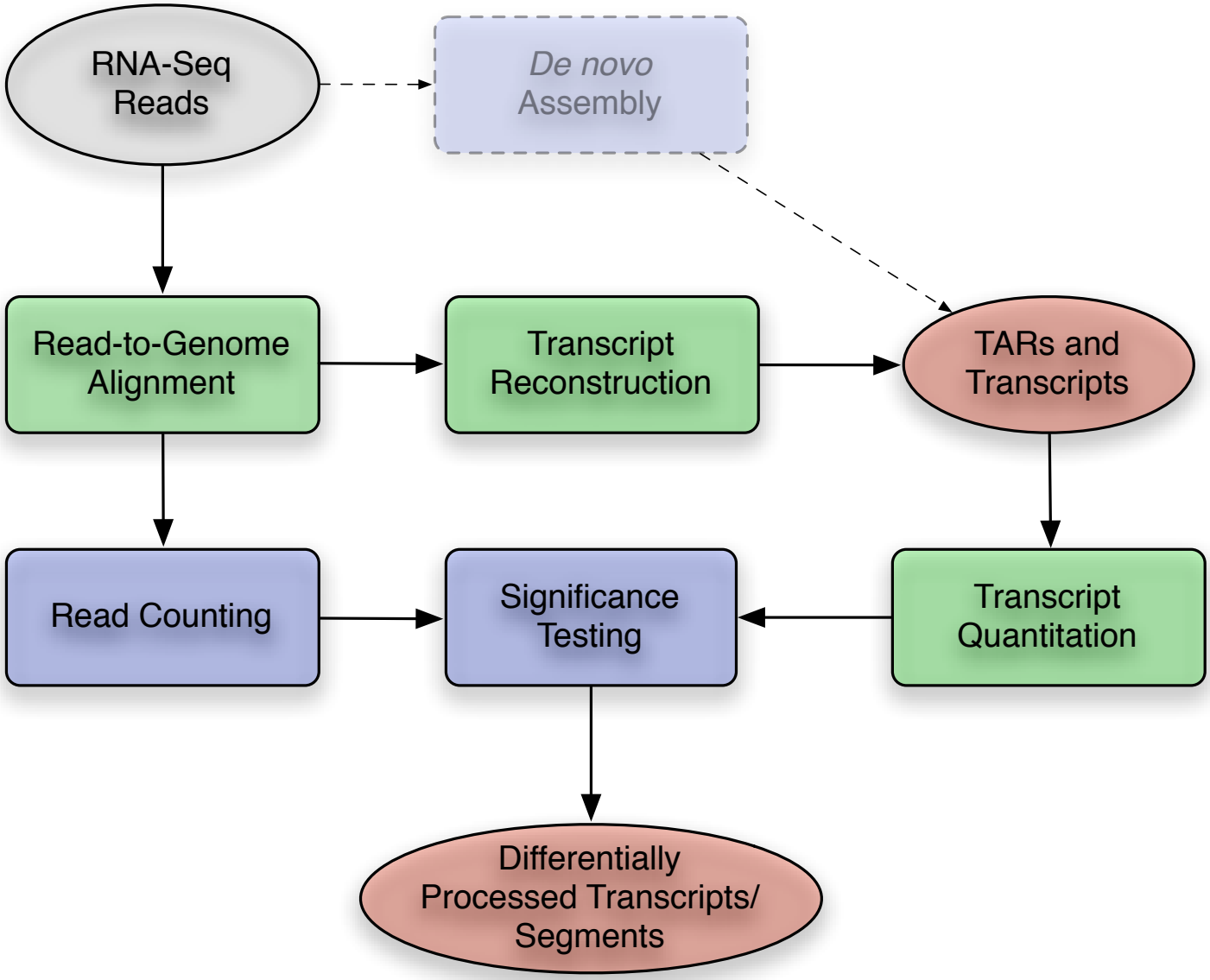


[Wikipedia]

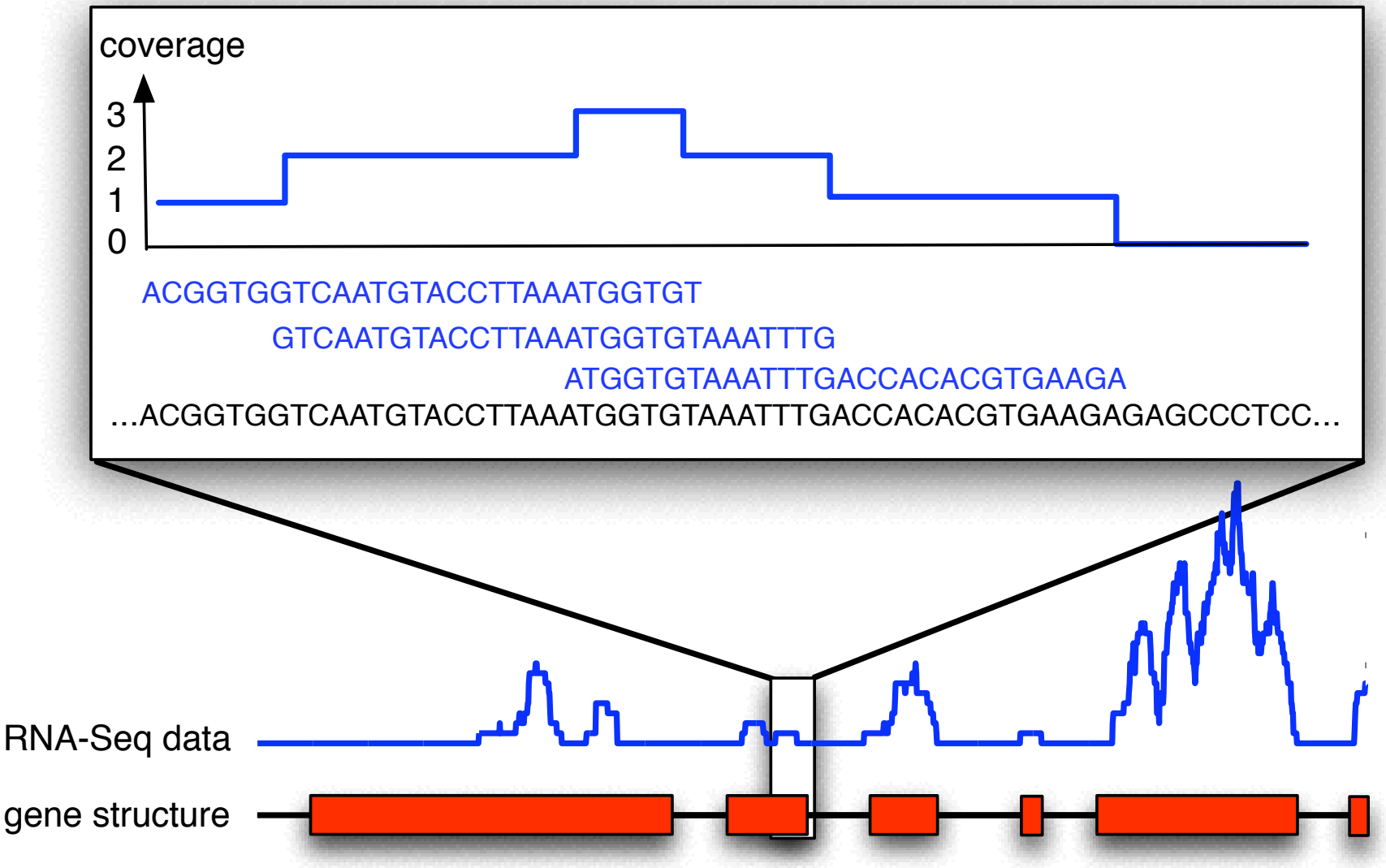
RNA-Seq Analysis Steps



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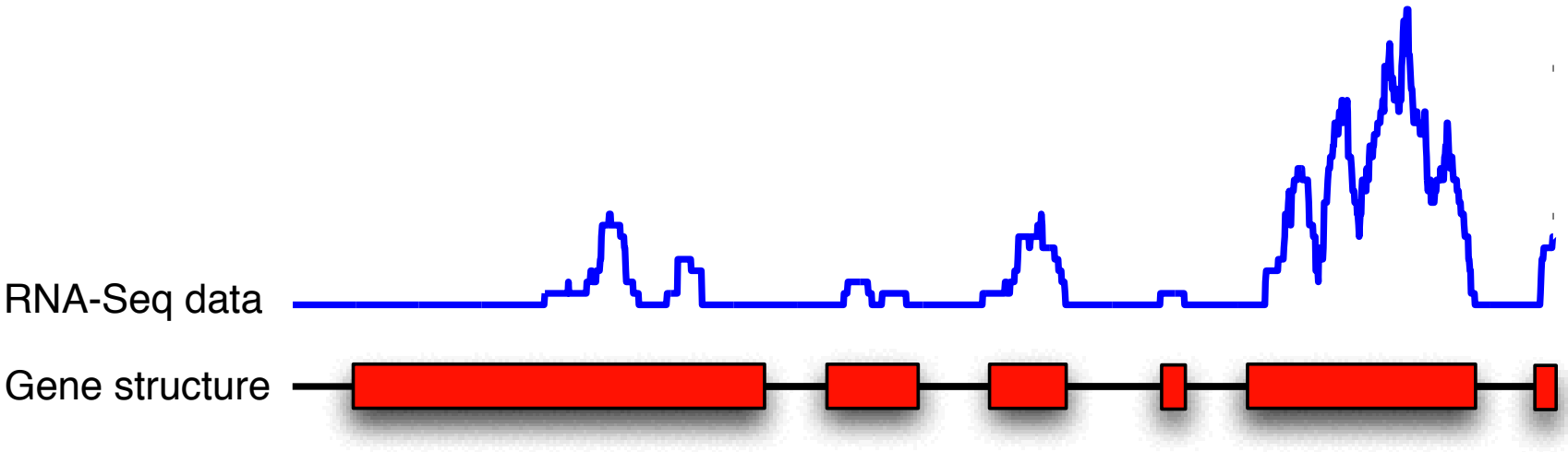


RNA-Seq Read Alignment



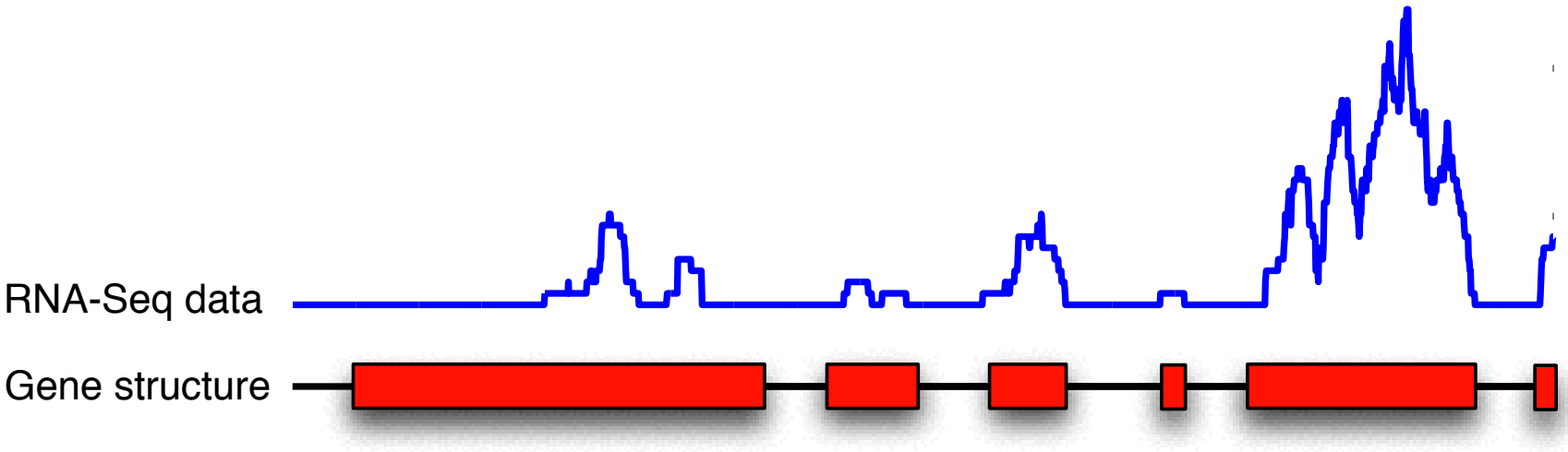
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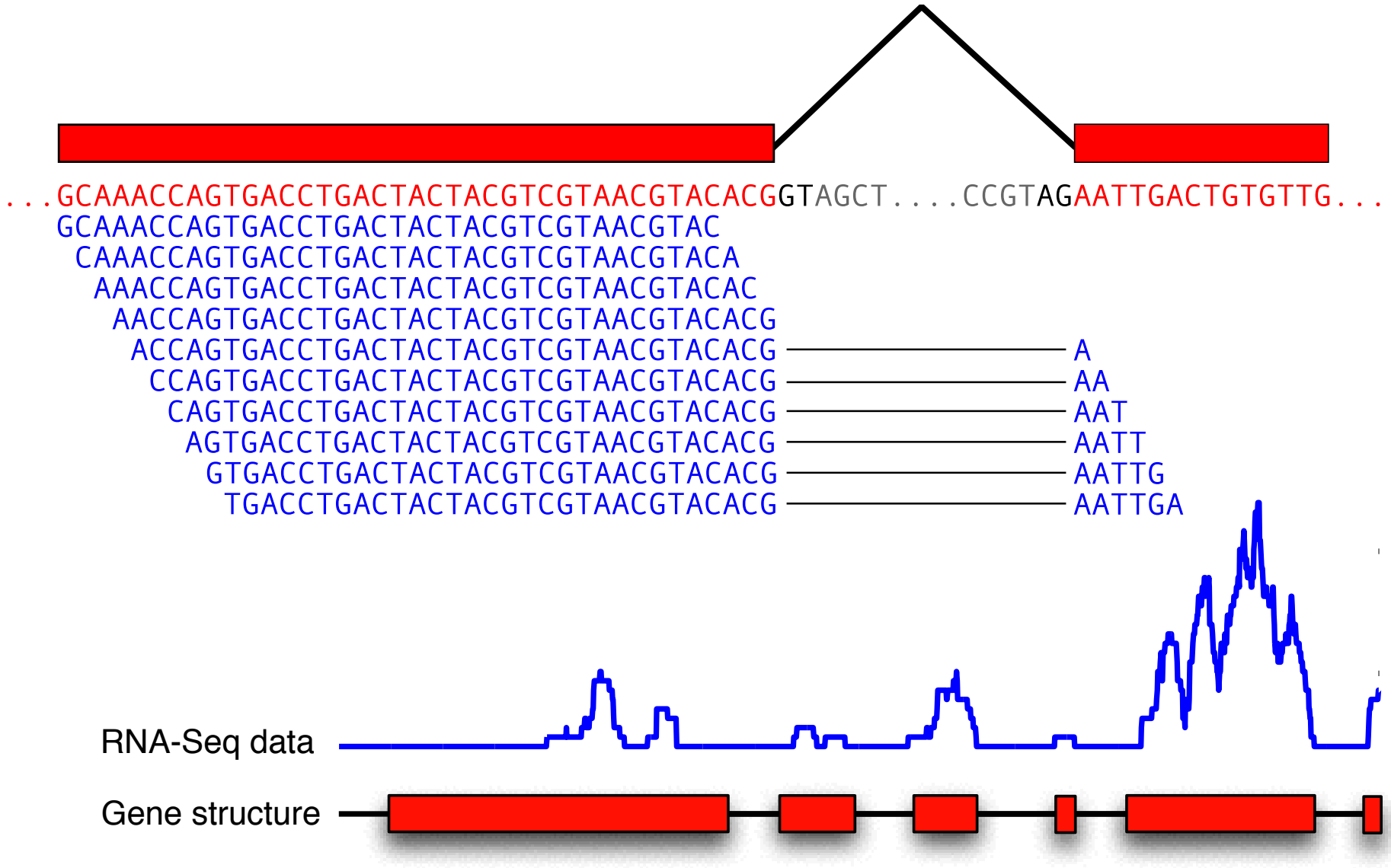


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```
...GCAAACCAGTGACCTGACTACTACGTCGTAACGTACACGGTAGCT....CCGTAGAATTGACTGTGTTG...
   GCAAACCAGTGACCTGACTACTACGTCGTAACGTAC
    CAAACCAGTGACCTGACTACTACGTCGTAACGTACA
     AAACCAGTGACCTGACTACTACGTCGTAACGTACAC
      AACCAGTGACCTGACTACTACGTCGTAACGTACACG
```



RNA-Seq Read Alignment



Extended Smith-Waterman Algorithm

⇒ Combine heterogeneous evidence for accurate alignment



	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

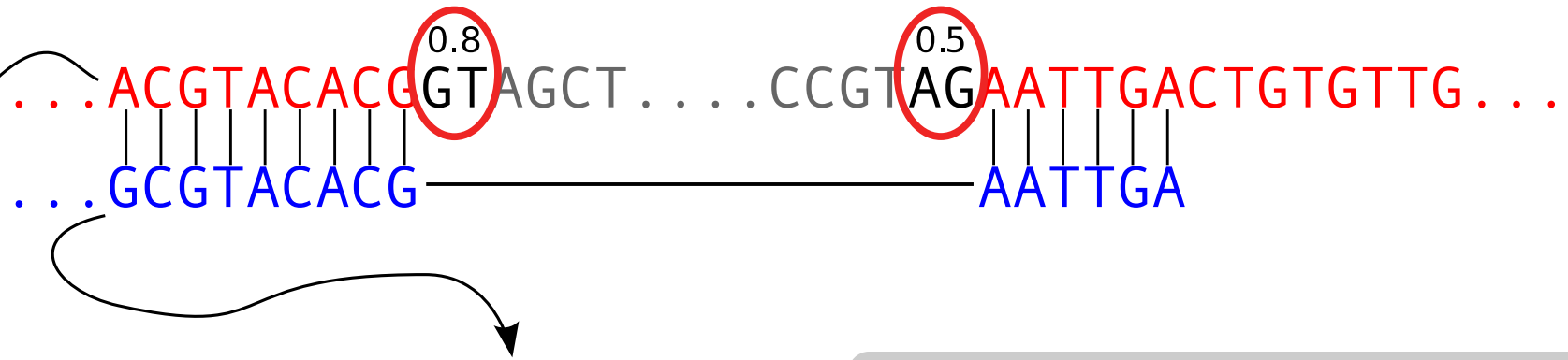
Source of Information

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

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

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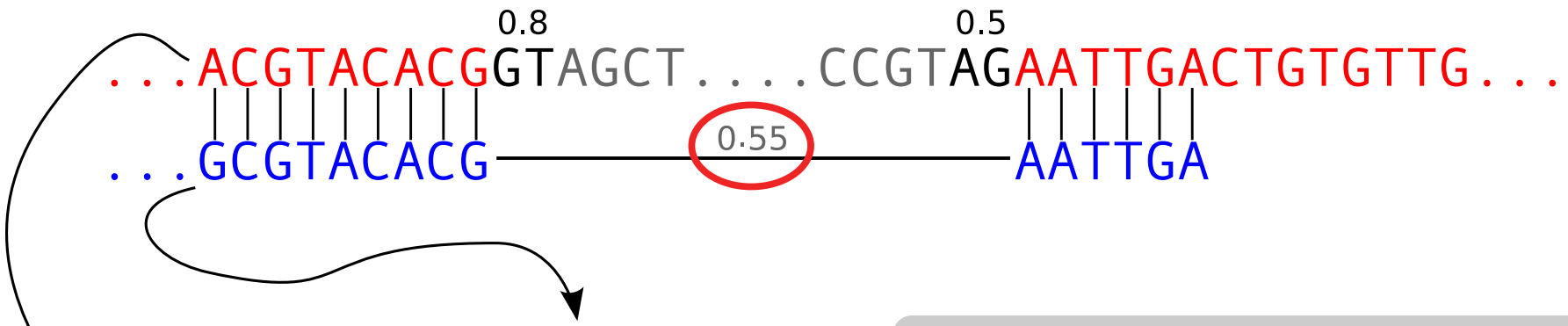
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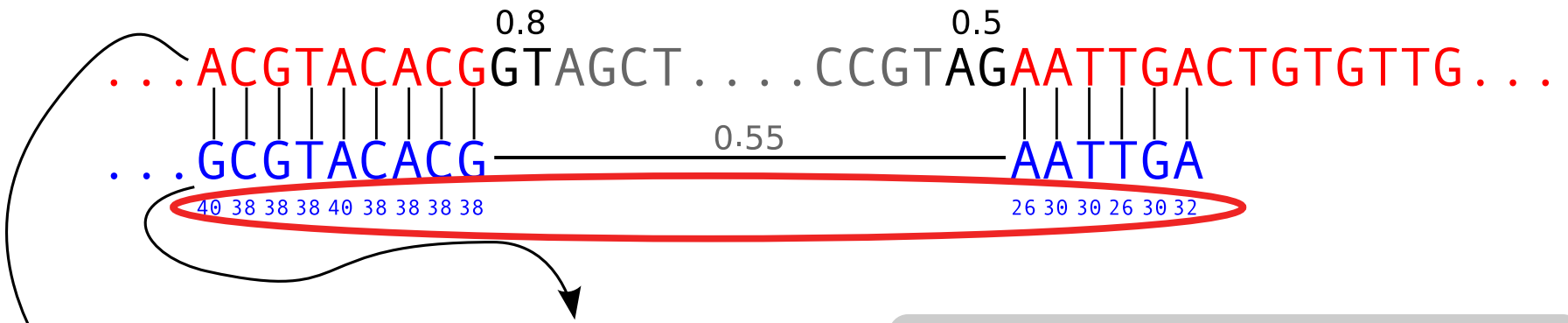
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gap	0.33	$f_{-A}(\cdot)$	$f_{-C}(\cdot)$	$f_{-G}(\cdot)$	$f_{-T}(\cdot)$	$f_{-N}(\cdot)$
A	0.31	$f_{AA}(\cdot)$	$f_{AC}(\cdot)$	$f_{AG}(\cdot)$	$f_{AT}(\cdot)$	$f_{AN}(\cdot)$
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T	0.55	$f_{TA}(\cdot)$	$f_{TC}(\cdot)$	$f_{TG}(\cdot)$	$f_{TT}(\cdot)$	$f_{TN}(\cdot)$
N	0.12	$f_{NA}(\cdot)$	$f_{NC}(\cdot)$	$f_{NG}(\cdot)$	$f_{NT}(\cdot)$	$f_{NN}(\cdot)$

Source of Information

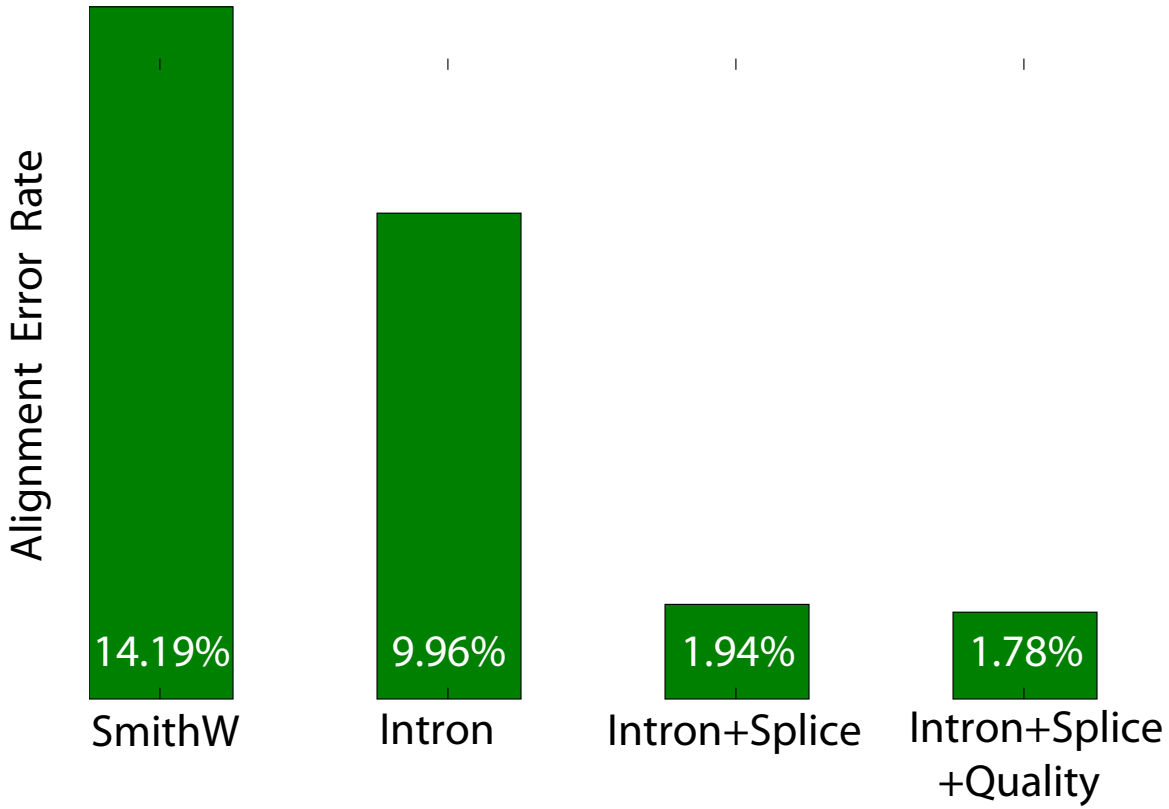
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Quality scoring $f : (\Sigma \times \mathbb{R}) \times \Sigma \rightarrow \mathbb{R}$ [De Bona et al., 2008]

QPalma's Accurate Alignments

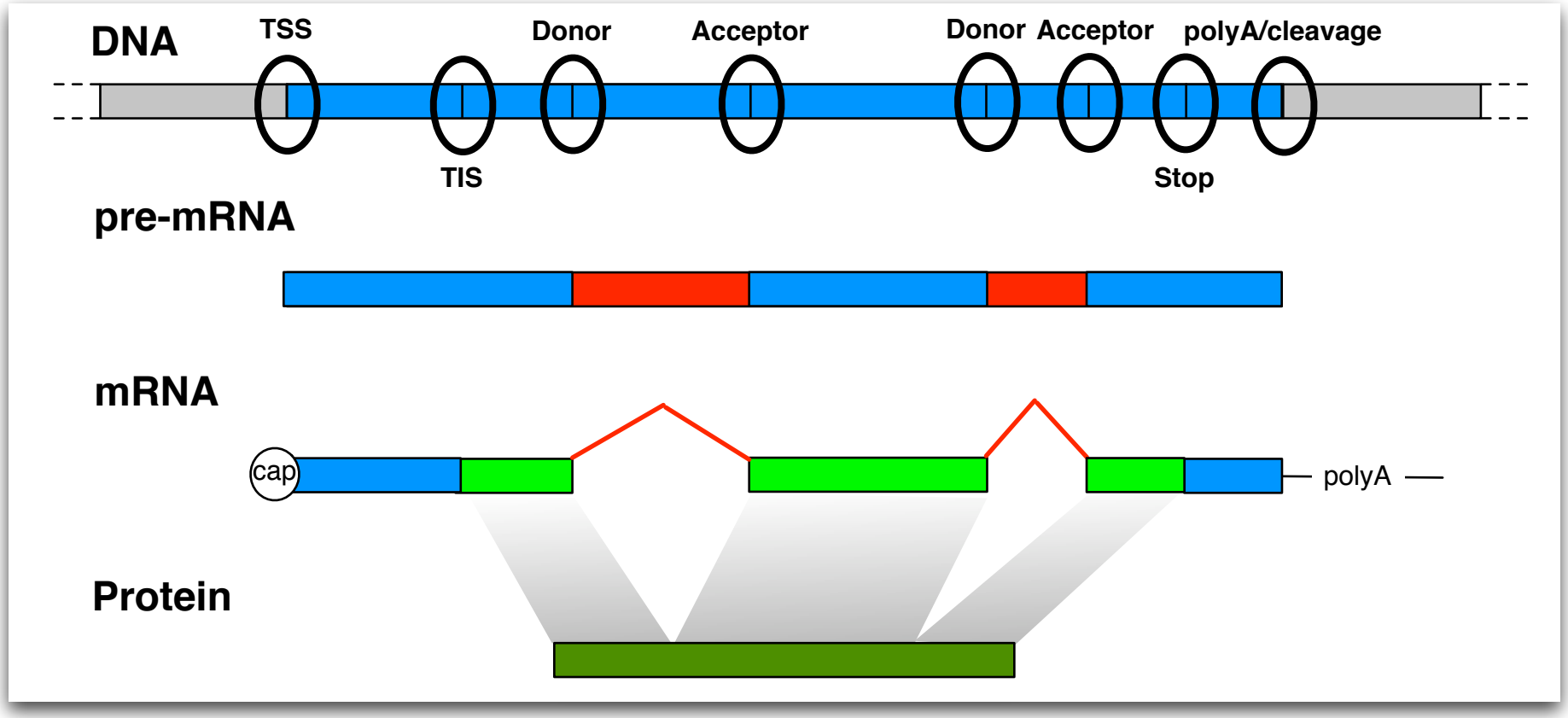
Generate set of artificially spliced reads

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



[De Bona et al., 2008]

Gene Finding + Transcriptome Measurements



Combination

- Higher accuracy
- Condition/tissue specific predictions

Gene Finding + Transcriptome Measurements

[Behr et al., 2009, in prep.]



Idea: Use transcriptome measurements to enhance recognition of exonic regions.

Results for A. thaliana: (Comparison with known gene models)

	transcript level $(SN + SP)/2$
1. mGene (*ab initio*) ...	**73.3%**
2. ...with tiling arrays (11 tissues)	**82.1%**
3. ...with mRNA-seq (1 tissue)	**81.1%**

Transcriptome measurements certainly help, but predictions are still quite far from perfect.

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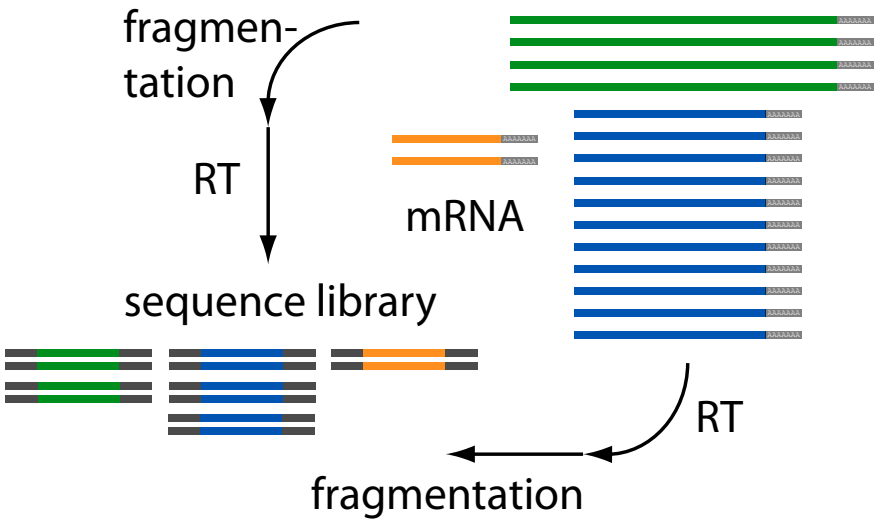
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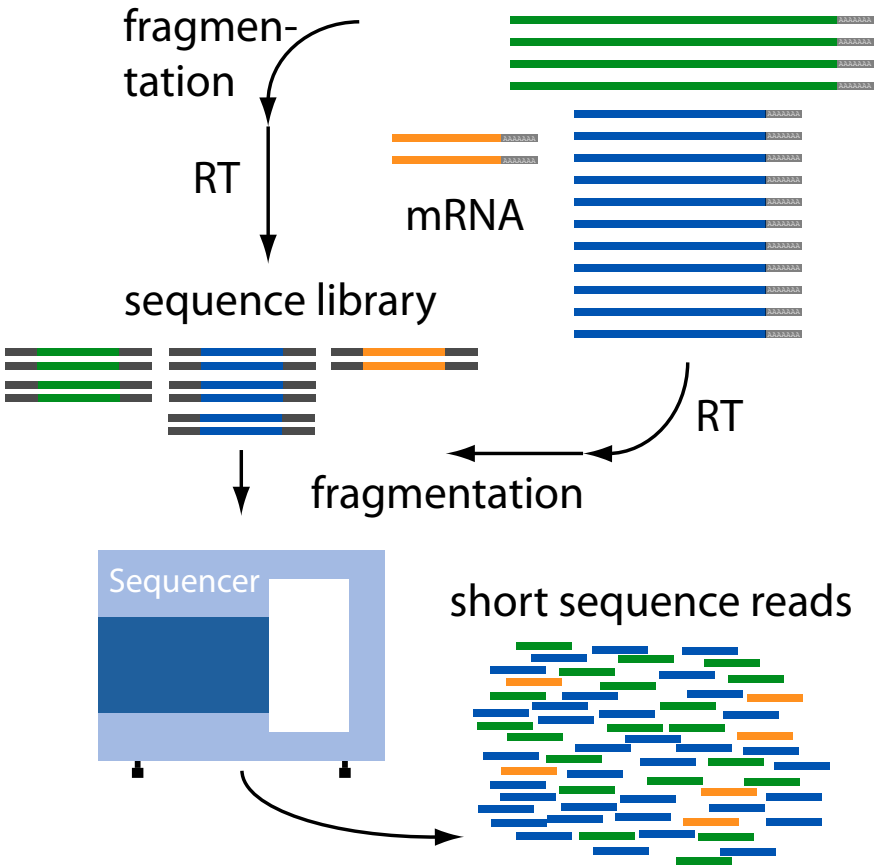
RNA Quantification and Biases



Biases due to . . .

- cDNA library construction
- Sequencing
- Read mapping

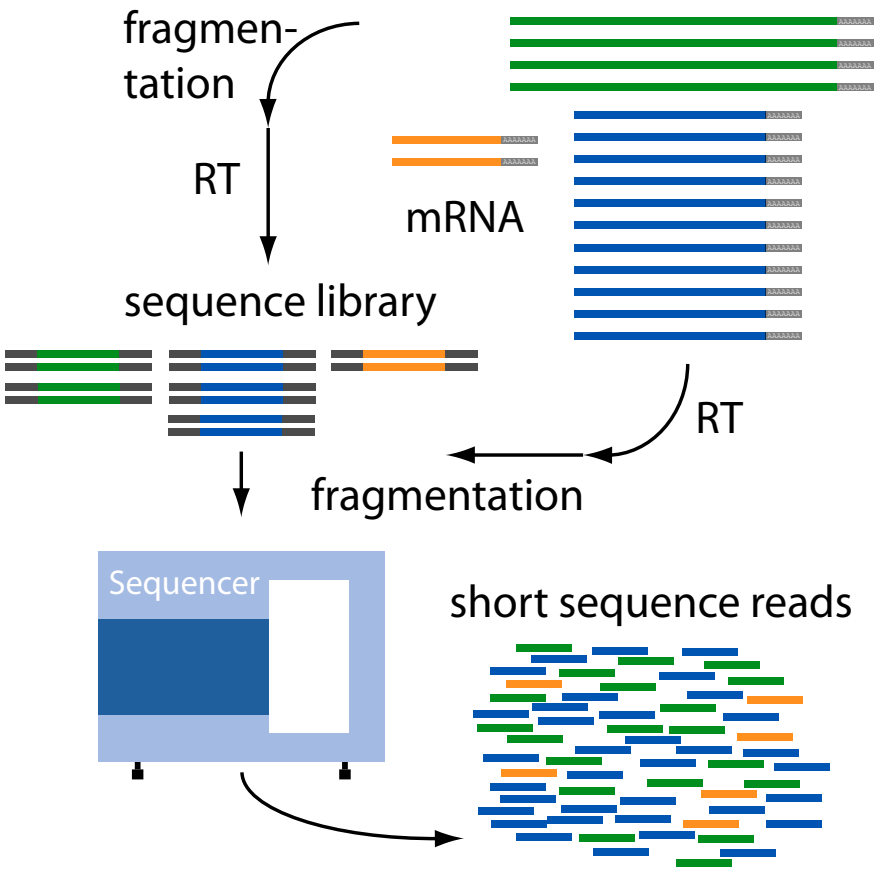
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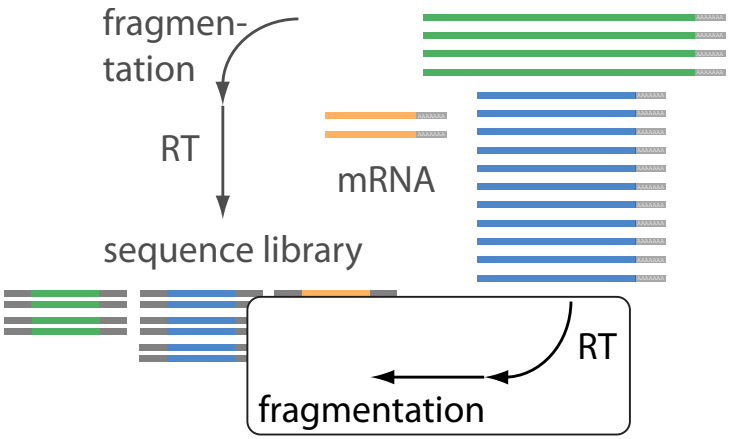
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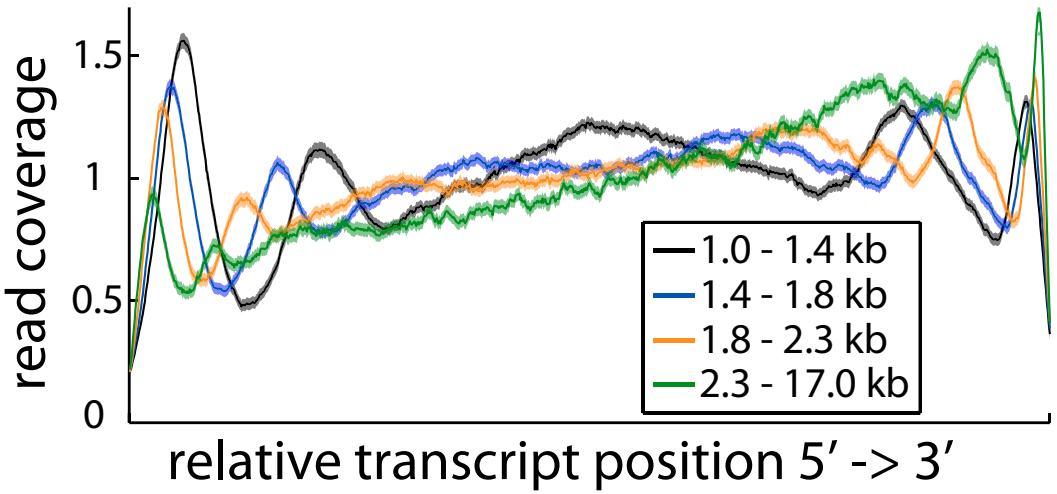


Priming and Fragmentation Biases

Profile: normalised positional read coverage along the transcript



Transcript profiles for different lengths

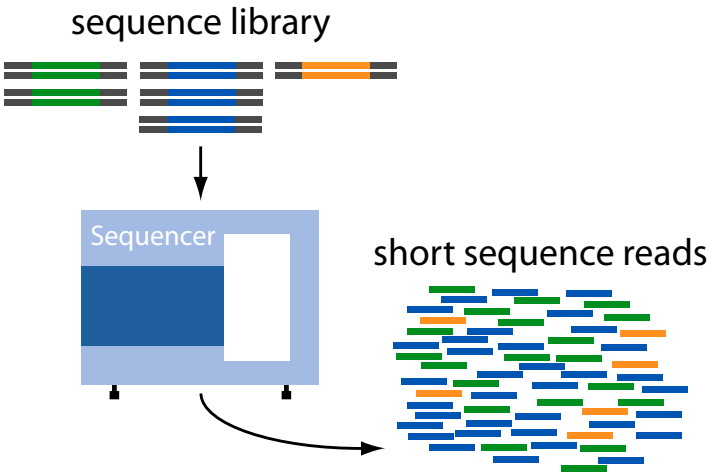


Data generated for *A. thaliana* with Flux Simulator v1.0 [Sammeth, 2009b]

- Oligo-dT priming
- Physical cDNA fragmentation

Sequencing Bias

PCR Bias



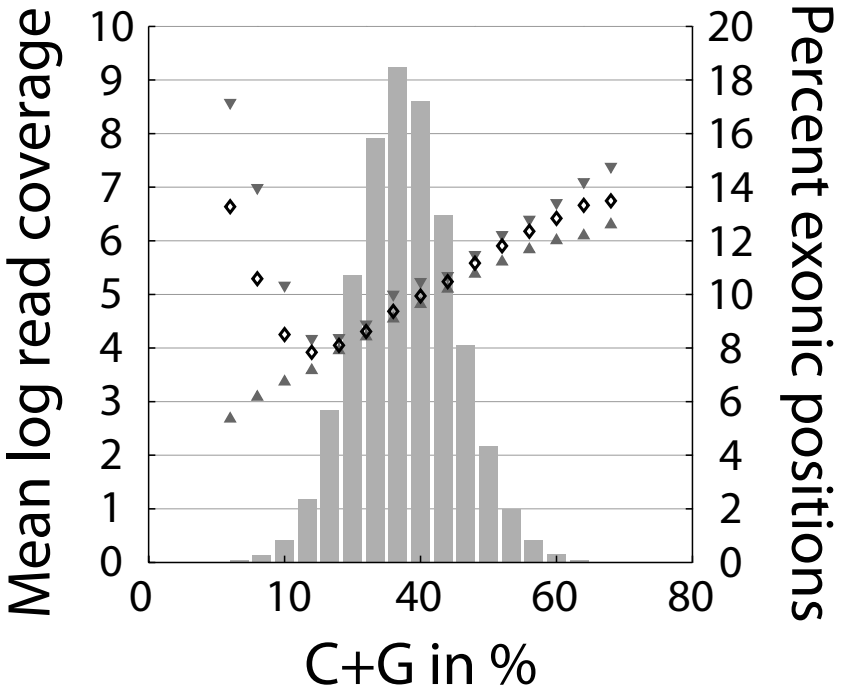


Figure provided by Georg Zeller

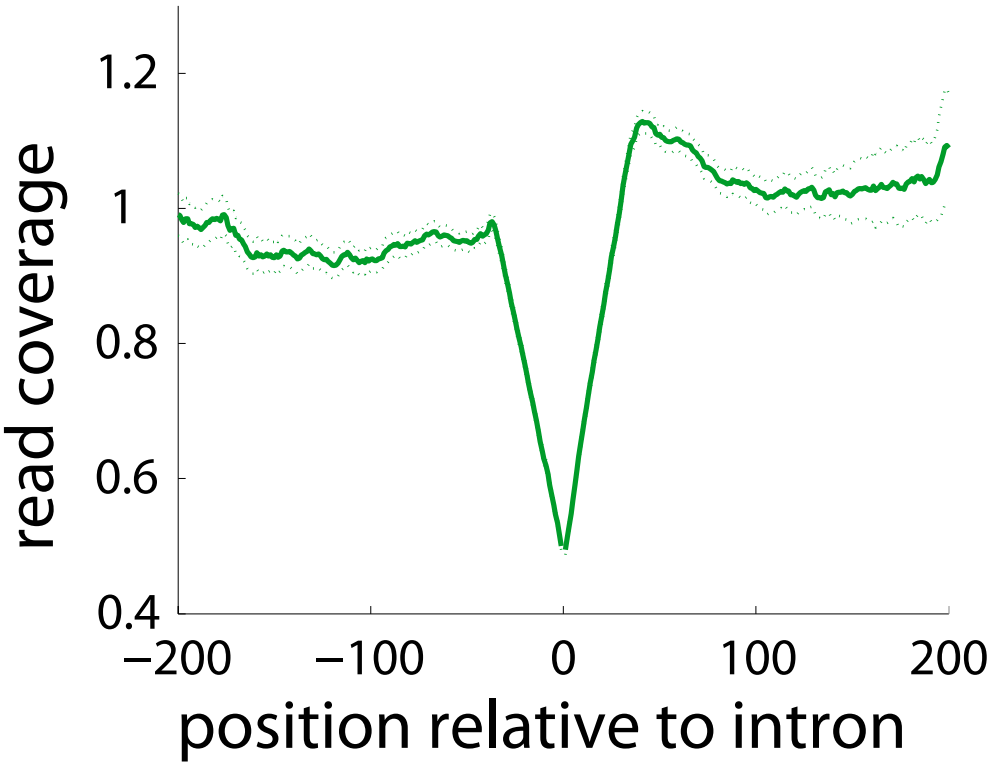
RNA-Seq data (*A. thaliana*)

- Exonic GC content

Mapping Bias

Read Mapping Bias

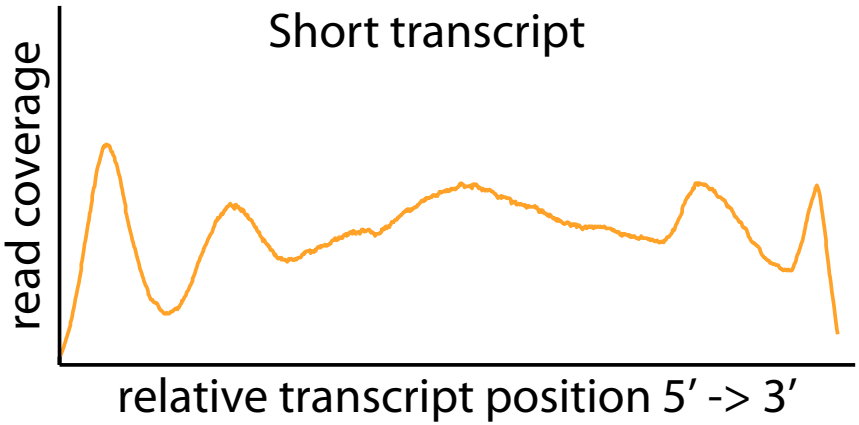




RNA-Seq data (*A. thaliana*)

- Exon boundaries

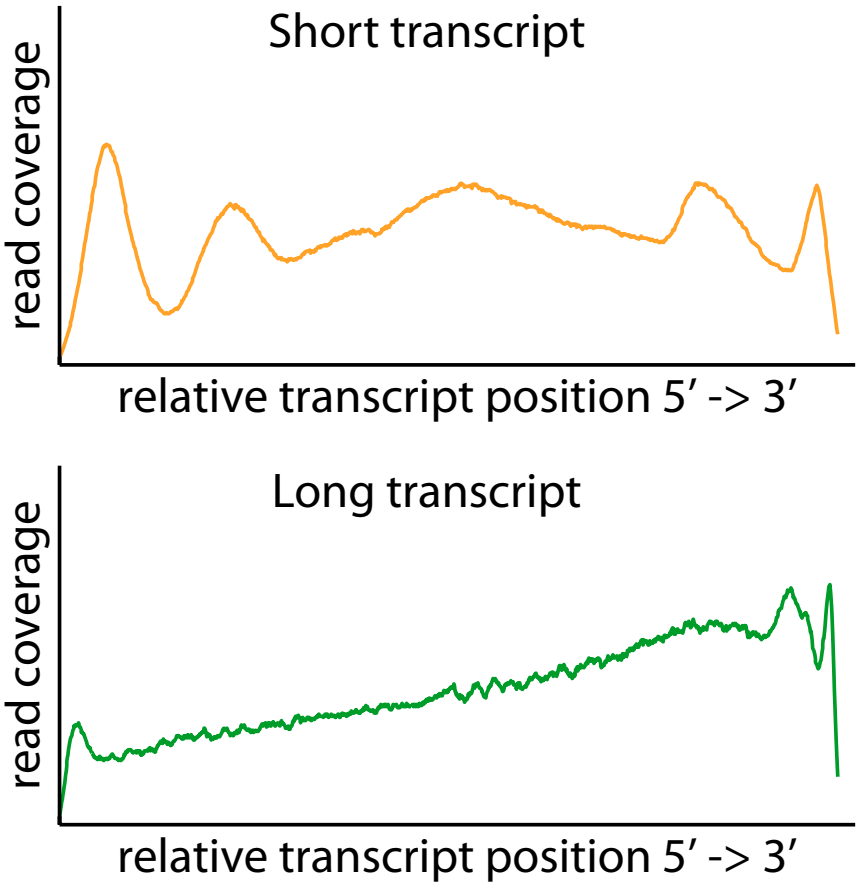
rQuant – Basic Idea





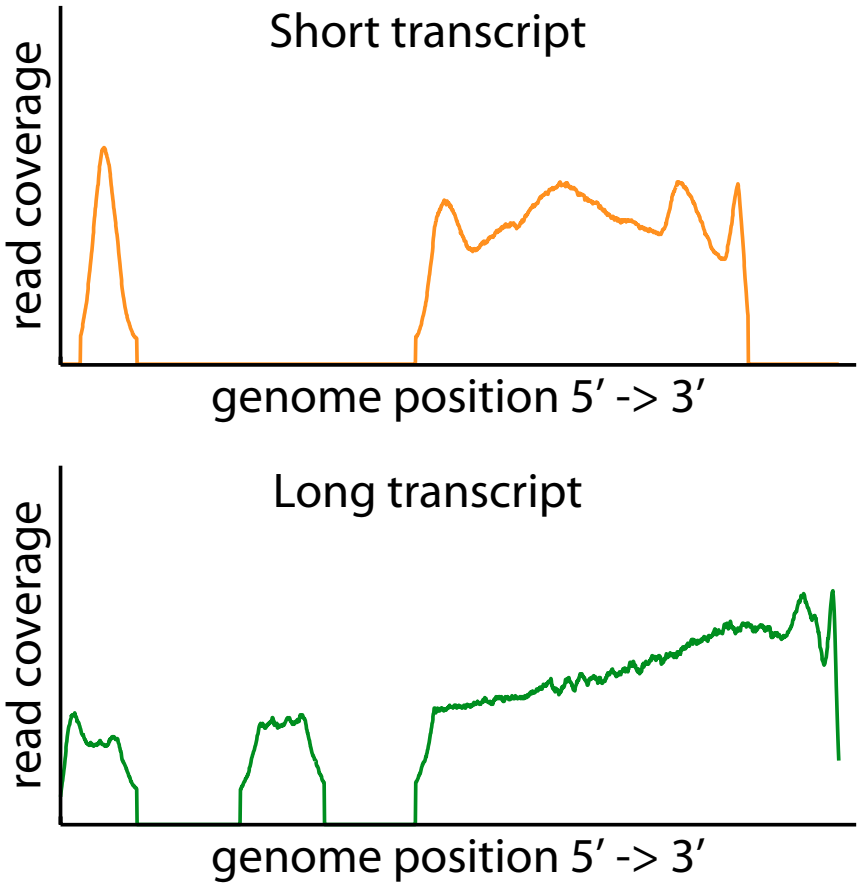
$M_i = w_A A_i + w_B B_i \quad \Rightarrow \quad \min_{w_A, w_B} \sum_i \ell(M_i, R_i)$

rQuant – Basic Idea



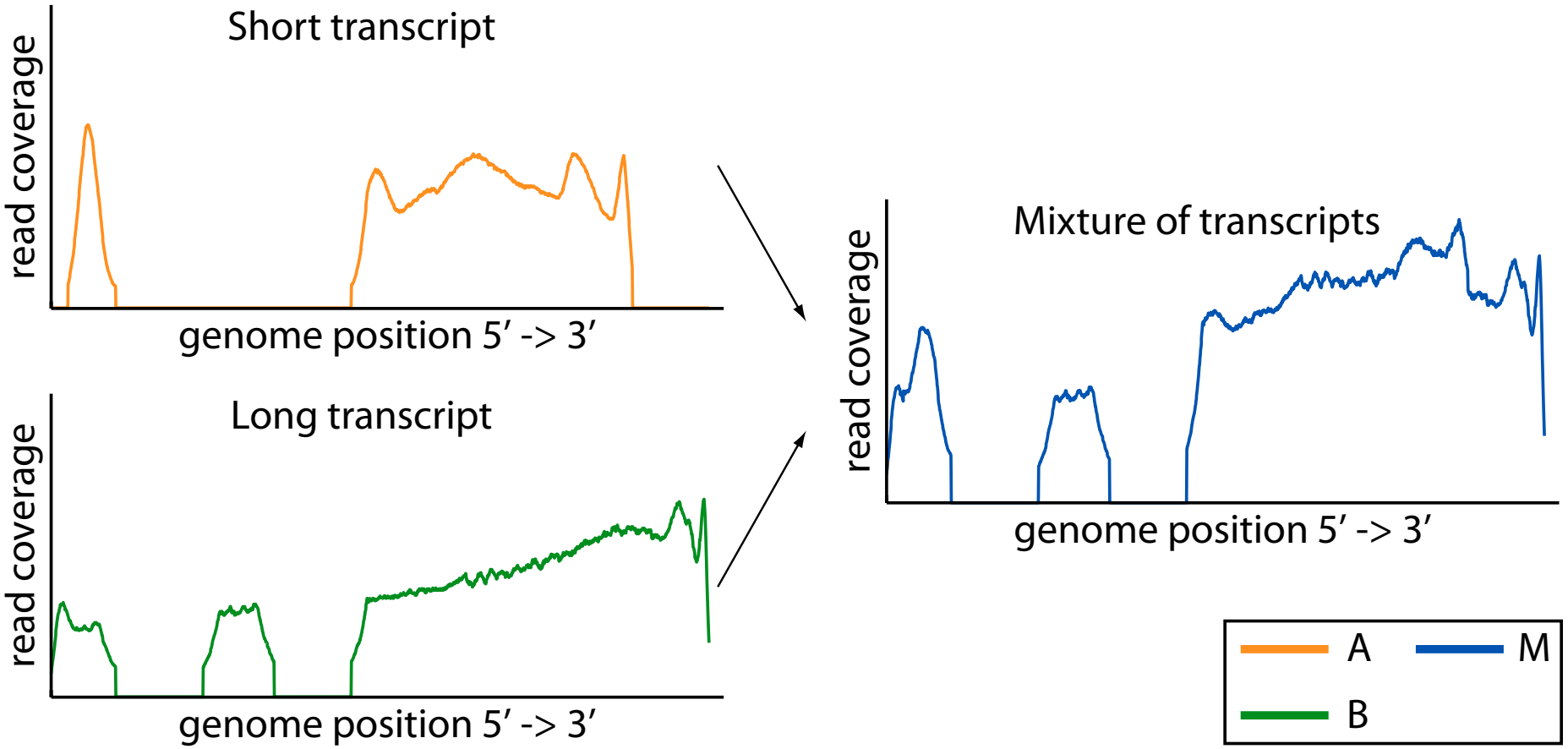
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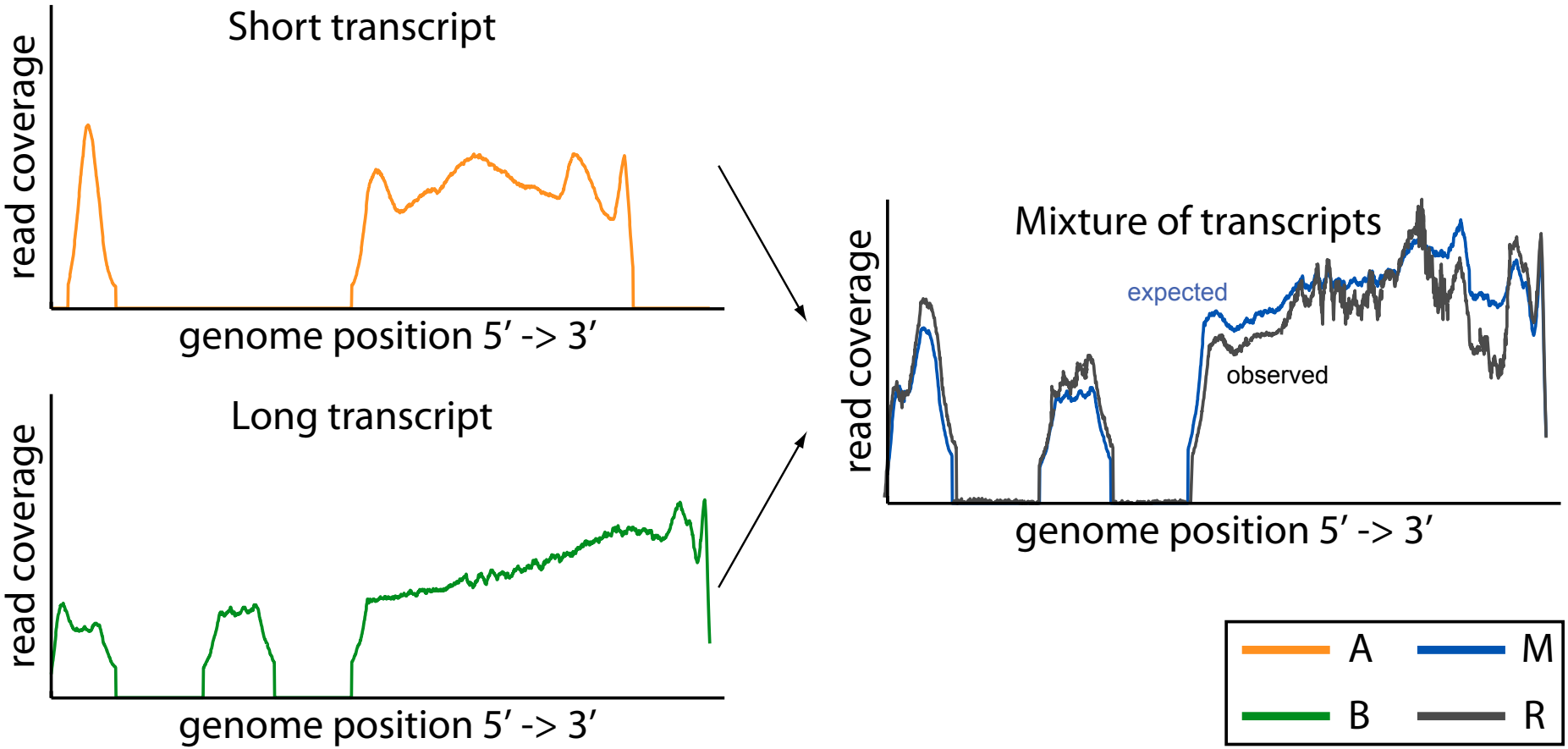
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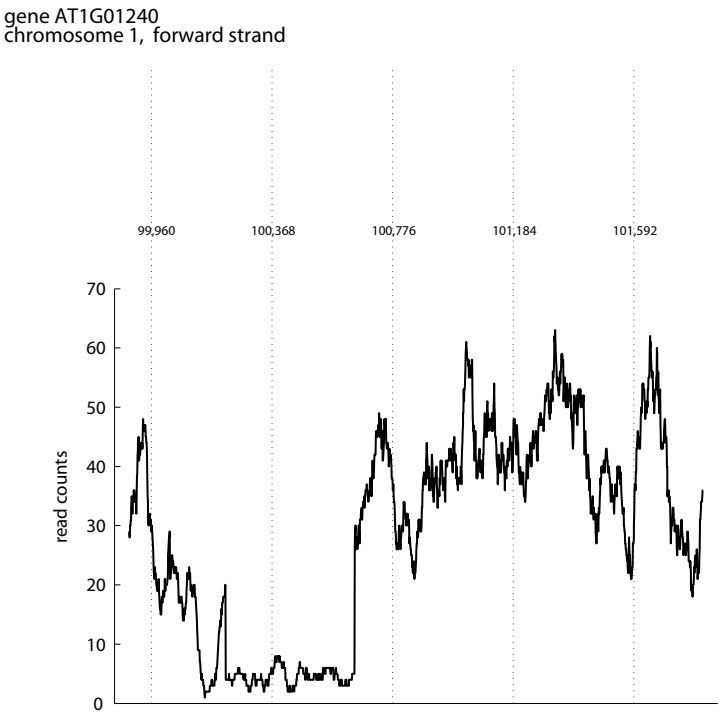
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rQuant – Iterative Algorithm

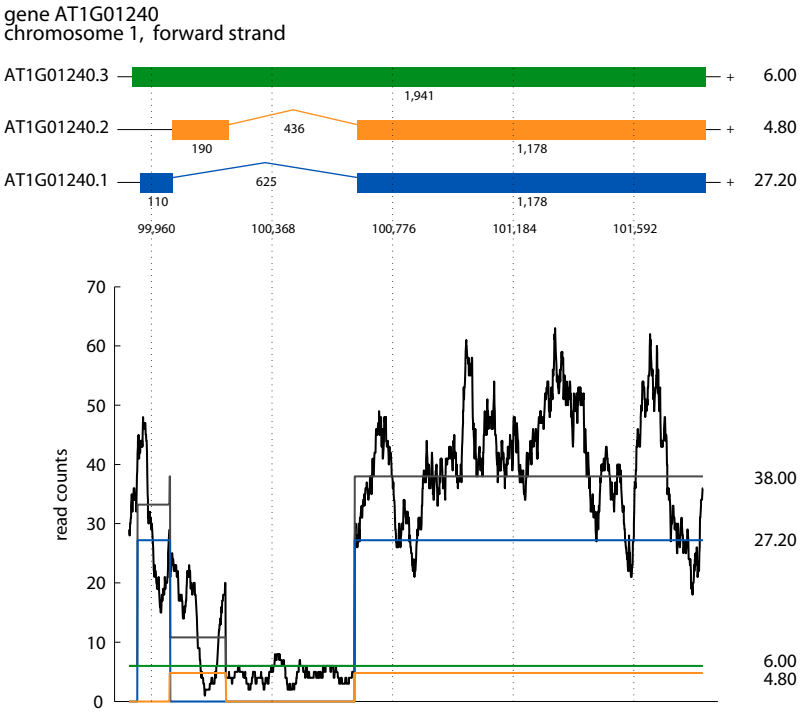
1. Optimise transcript weights: $\min_{\mathbf{w}} \sum_i \ell\left(\sum_t w^{(t)} p_i^{(t)}, R_i\right)$
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[Bohnert et al., 2009]

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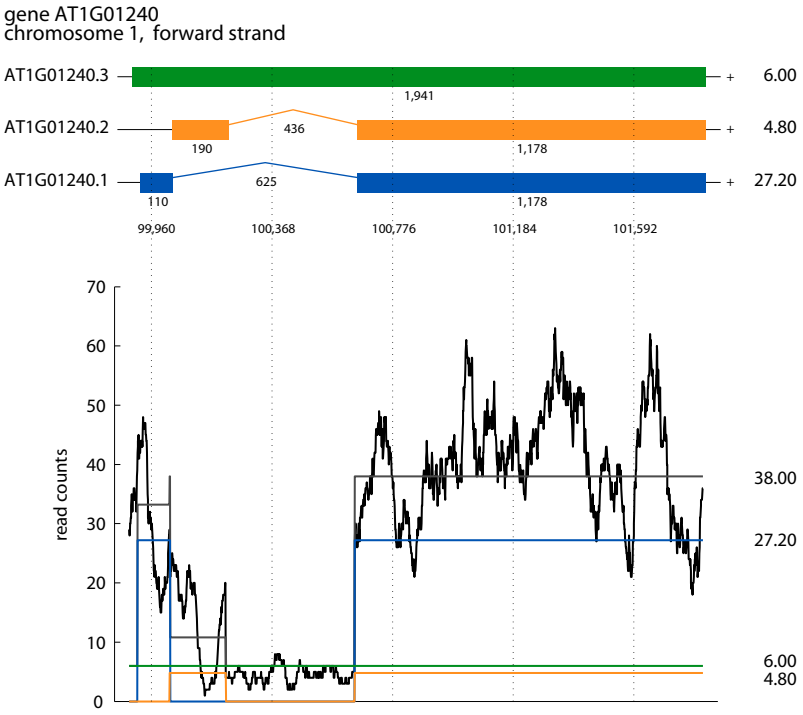
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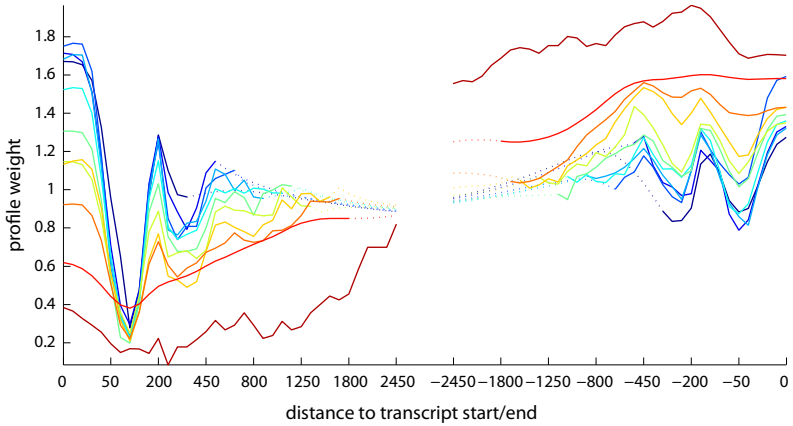


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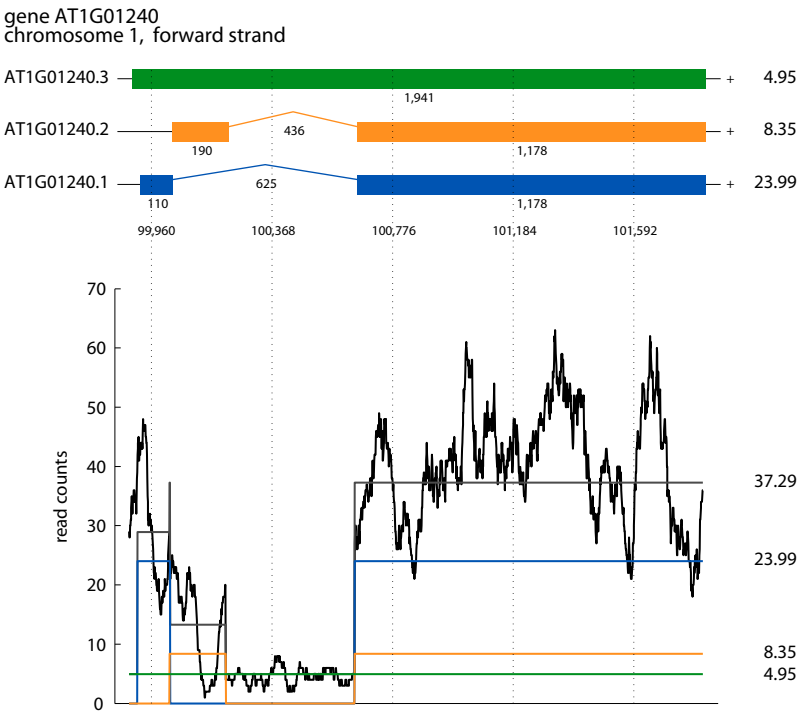


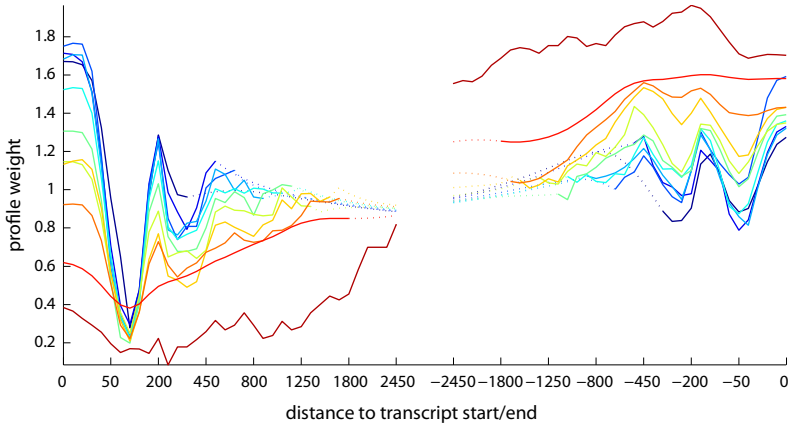


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Evaluation I



Our method rQuant: Position-wise, with profiles

compared to

- Position-wise, without profiles
- Segment-wise, without profiles [Jiang and Wong, 2009]
- Segment-wise, with profiles [Sammeth, 2009a]

Estimate transcript abundances

- Using simulated data for *A. thaliana* (Flux Simulator)
- Subset of alternatively spliced genes

Evaluation: Spearman correlation between

- Simulated RNA expression level and
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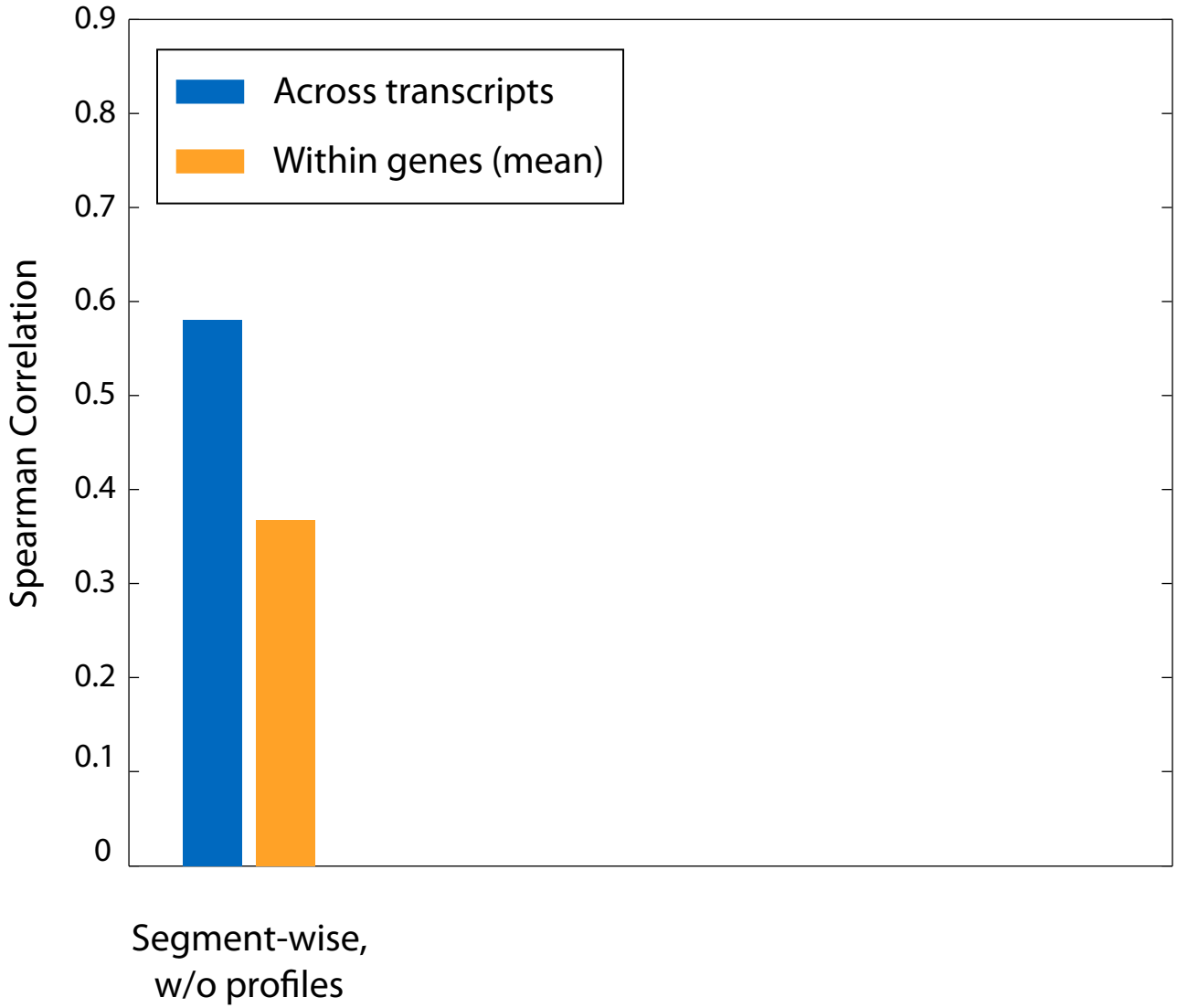
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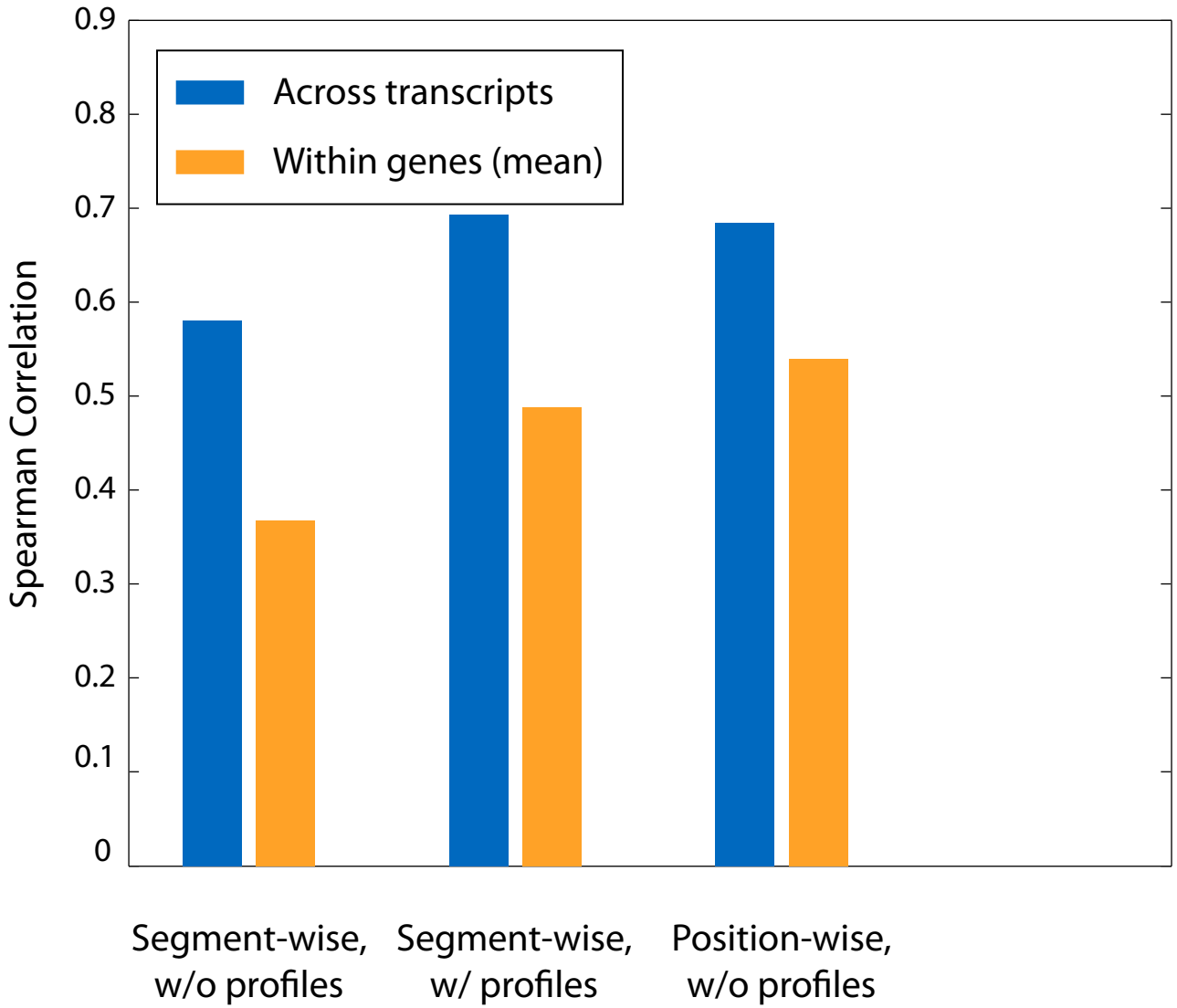
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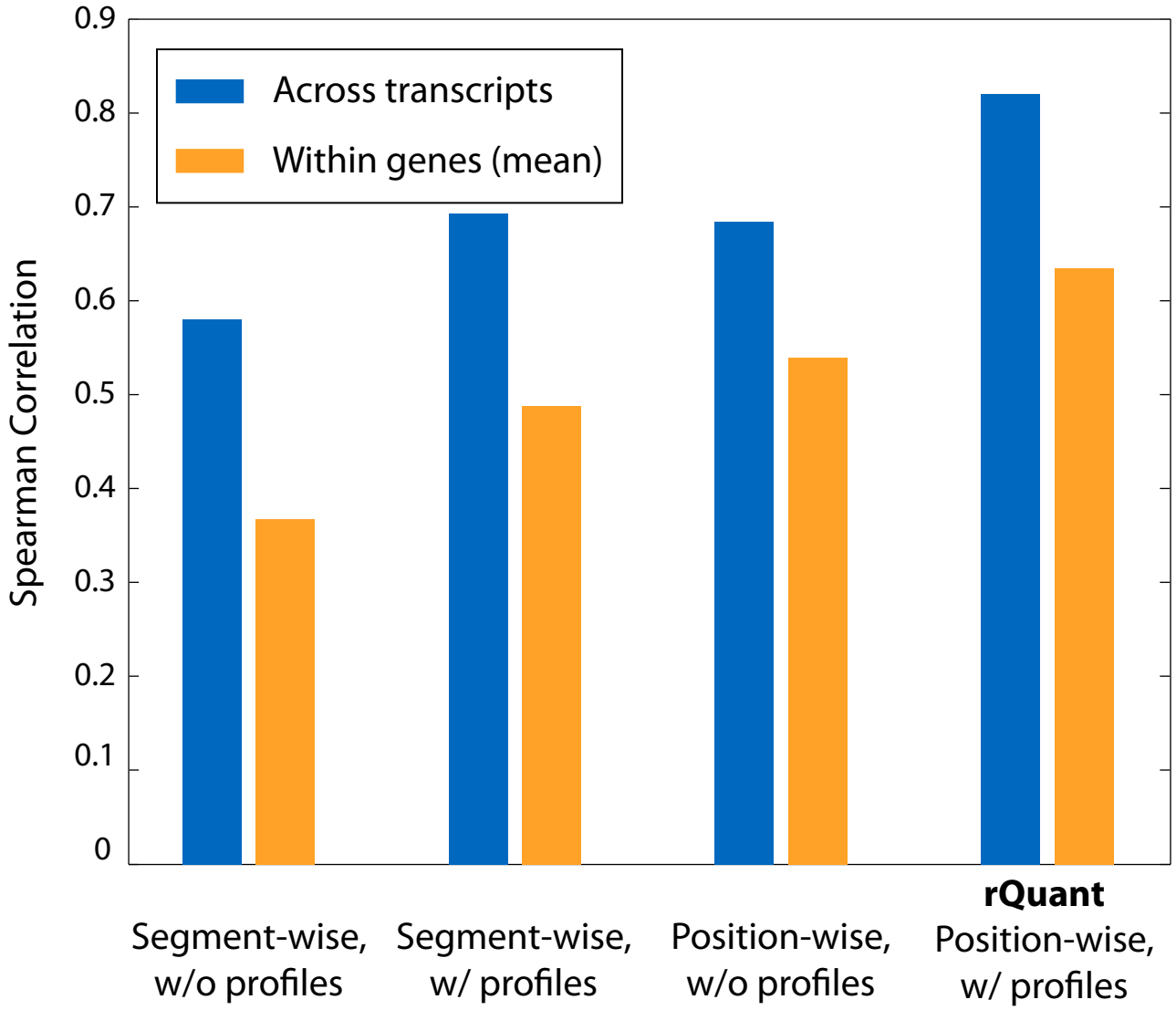
Evaluation II



Evaluation II



Evaluation II



Conclusions



- Methods for characterizing transcriptomes
  - in different organisms
  - under different conditions (development/environment)
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  - Improved accuracy due to novel inference methods
  - No alternative transcripts; ignores other available information
- Analysis of transcriptome measurements
  - Identification of alternative transcripts
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- Bernhard Schölkopf (MPI)

Nematode Material/Methods

- Adrian Streit (MPI)

Acknowledgments

Gene Finding

- Gabi Schweikert (FML, MPIs)
- Jonas Behr (FML)
- Alex Zien (FML & FIRST)
- Georg Zeller (FML & MPI)

Short Read Analysis

- Fabio De Bona (FML)
- Regina Bohnert (FML)
- Stephan Ossowski (MPI)
- Korbinian Schneeberger (MPI)

Tiling Arrays

- Georg Zeller (FML & MPI)
- Johannes Eichner (FML & UniTü)
- Sascha Laubinger (MPI)
- Detlef Weigel (MPI)

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Slides are available online: www.fml.mpg.de/raetsch/talks

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