Fully Automated Genome Annotation with Deep RNA Sequencing

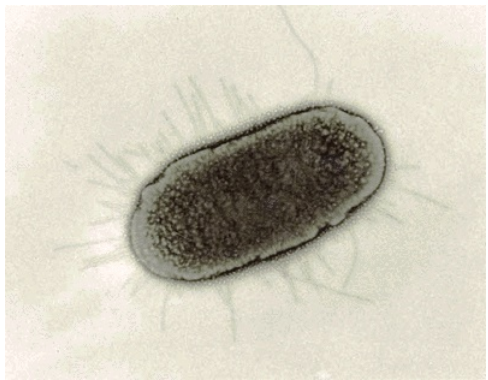
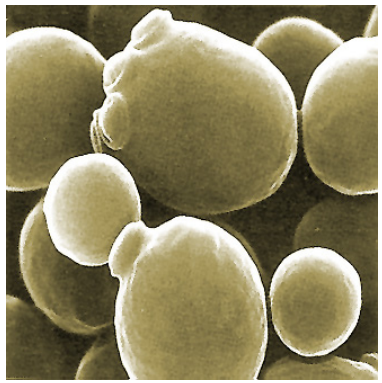
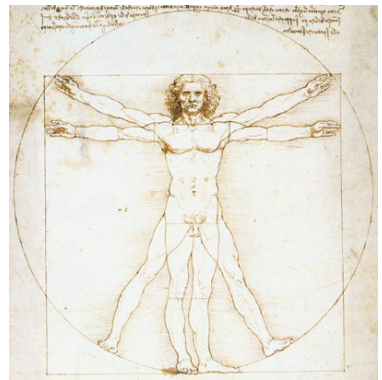
Gunnar Rätsch

Friedrich Miescher Laboratory of the Max Planck Society
Tübingen, Germany

Bio-IT World Europe, October 5, 2010

Sequencing Genomes

- Large, concerted effort to sequence genomes of model organisms

1997	1997	1998	2000
			
E. coli	*S. cerevisiae*	*C. elegans*	*H. sapiens* **$2.7 billion**

- In 2003, NHGRI committed to develop next-generation sequencing technologies to lower the cost of 30x a human genome (∼100 Gbp):
  - $100,000 genome
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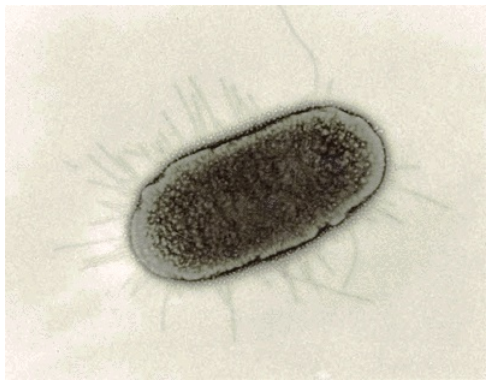	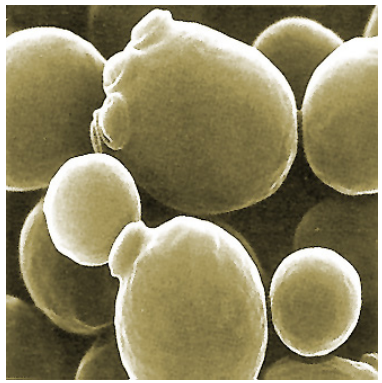		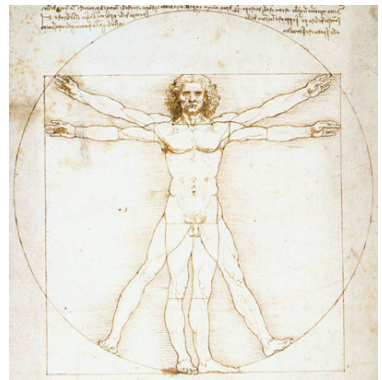
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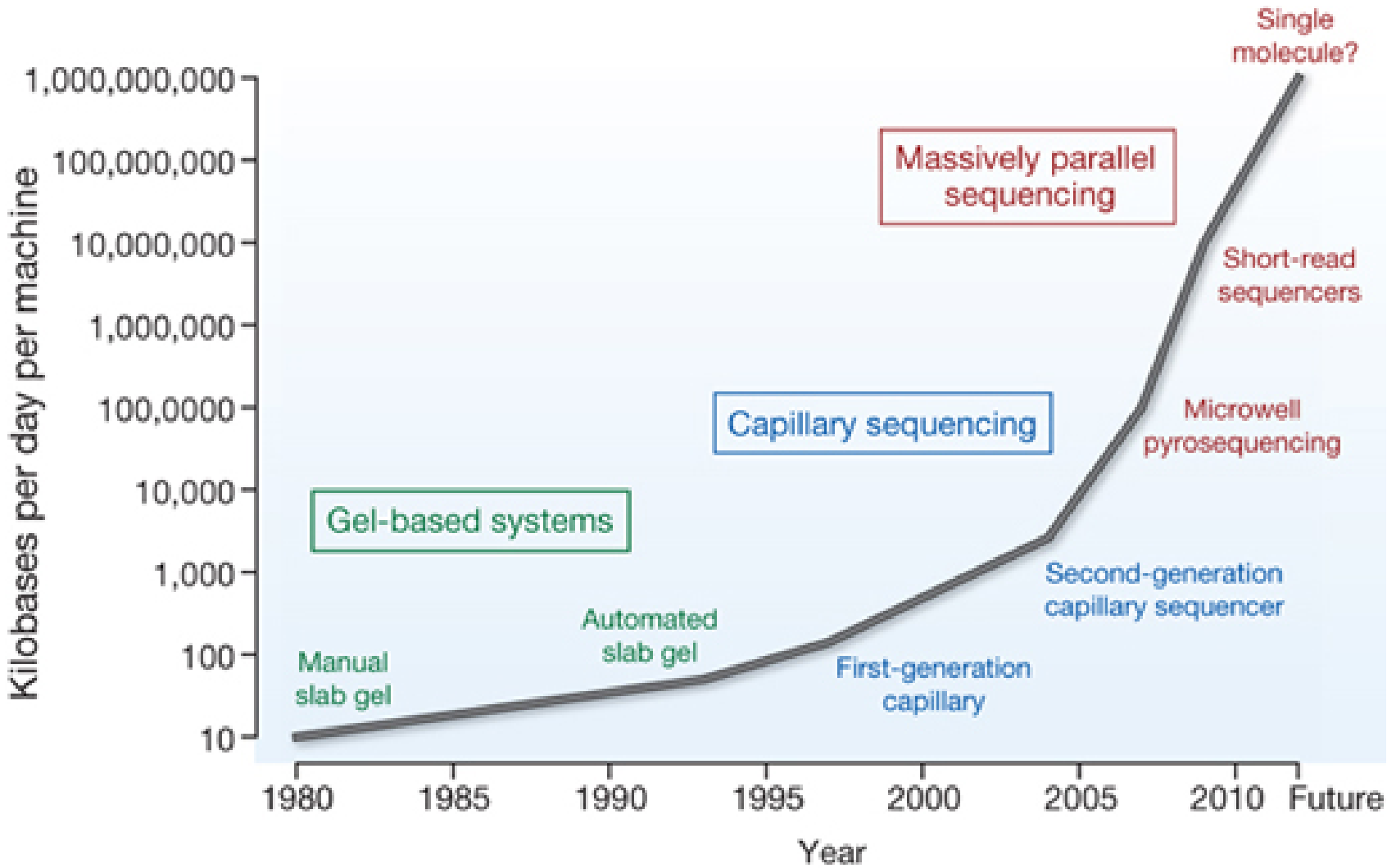

[Nature 458, 719-724 (2009)]

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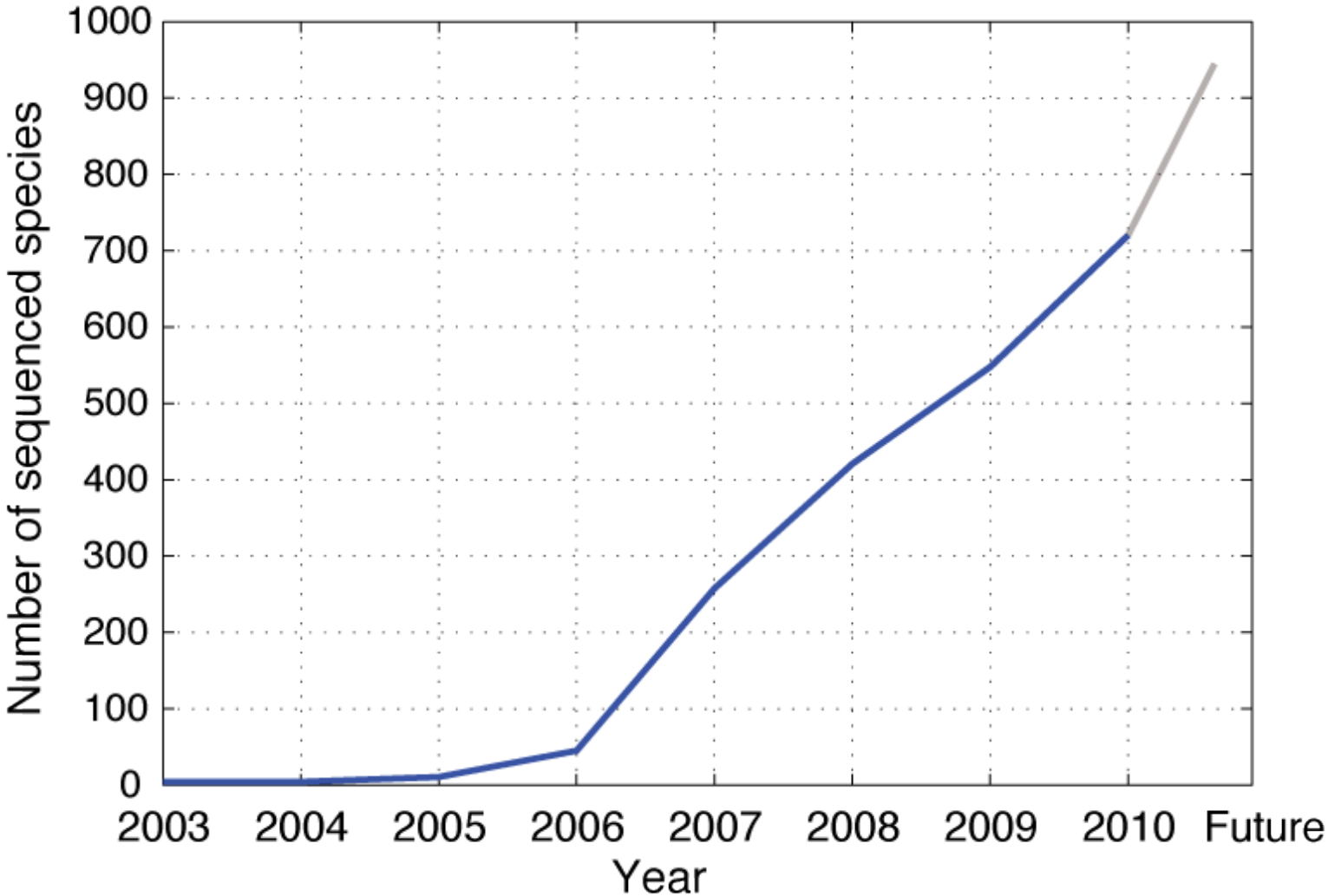
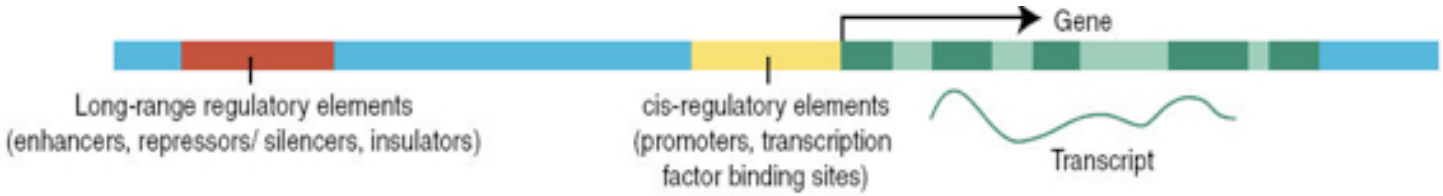


[Generated from NCBI on Sep 30, 2010]

What does it all mean?

- How to make sense of the genome?
  - Which nucleotides are functional?
  - What is their function?



- Complex process of genome annotation
  - Computational gene prediction & manual curation
  - Based on homology
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  - Most accurate annotations only for model organisms
  - Few gene discoveries, inaccurate annotation for new genes
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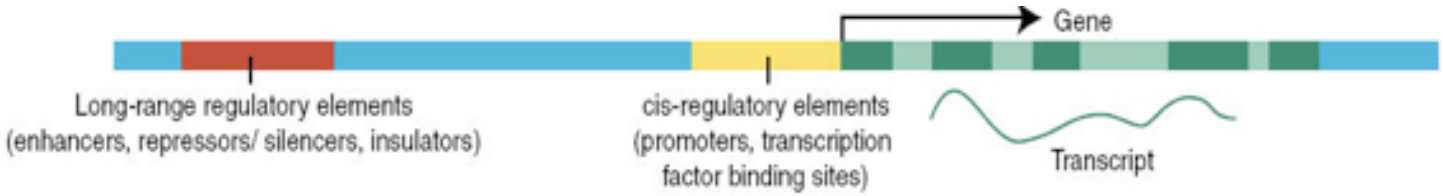

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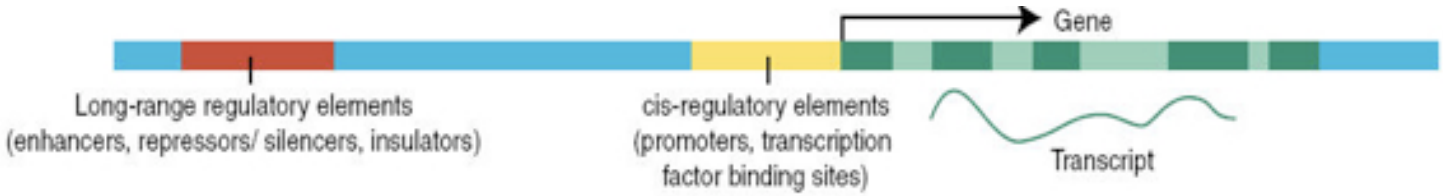

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Automated Genome Annotation

What is needed?

1. Highly accurate annotation of genomes
   - Non-coding RNAs
   - Alternative transcripts
2. With as little as possible prior knowledge
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3. Fully automated such that everybody can do it him/herself

Steps to get there:

- Deep RNA sequencing
- Improved analysis methods
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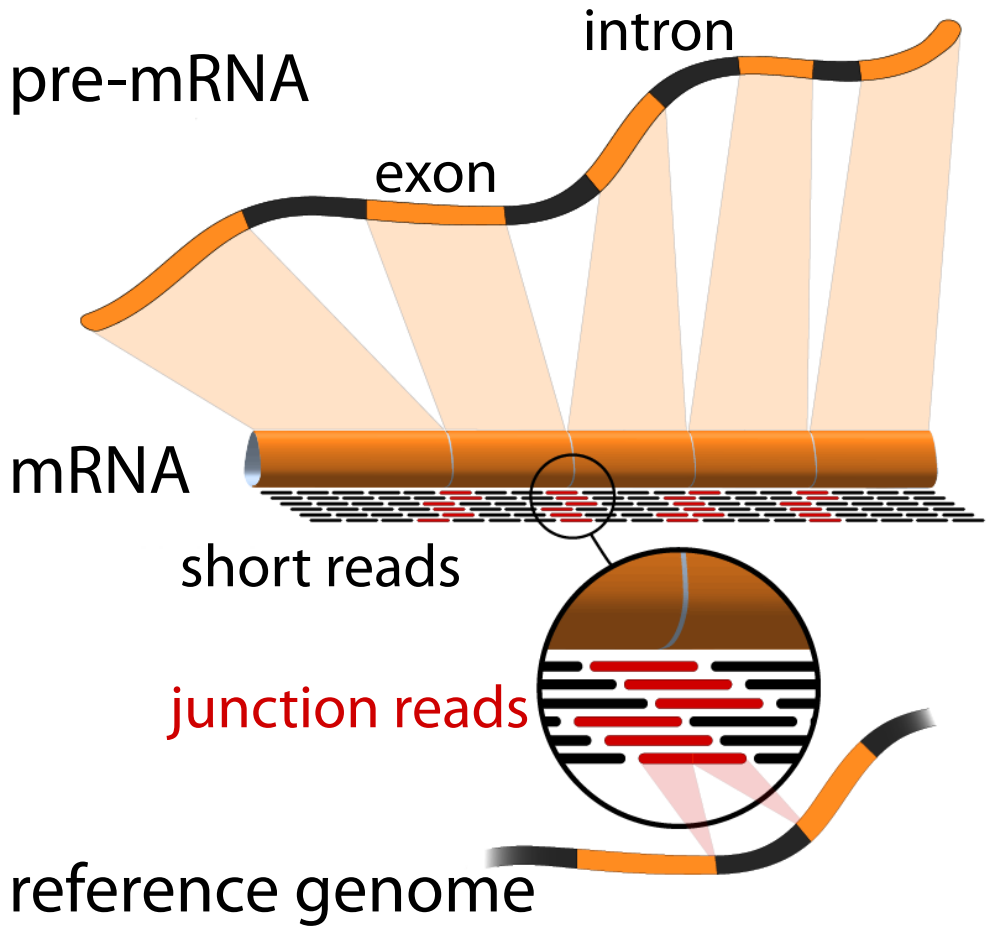
Deep RNA Sequencing (RNA-Seq)

fml

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.



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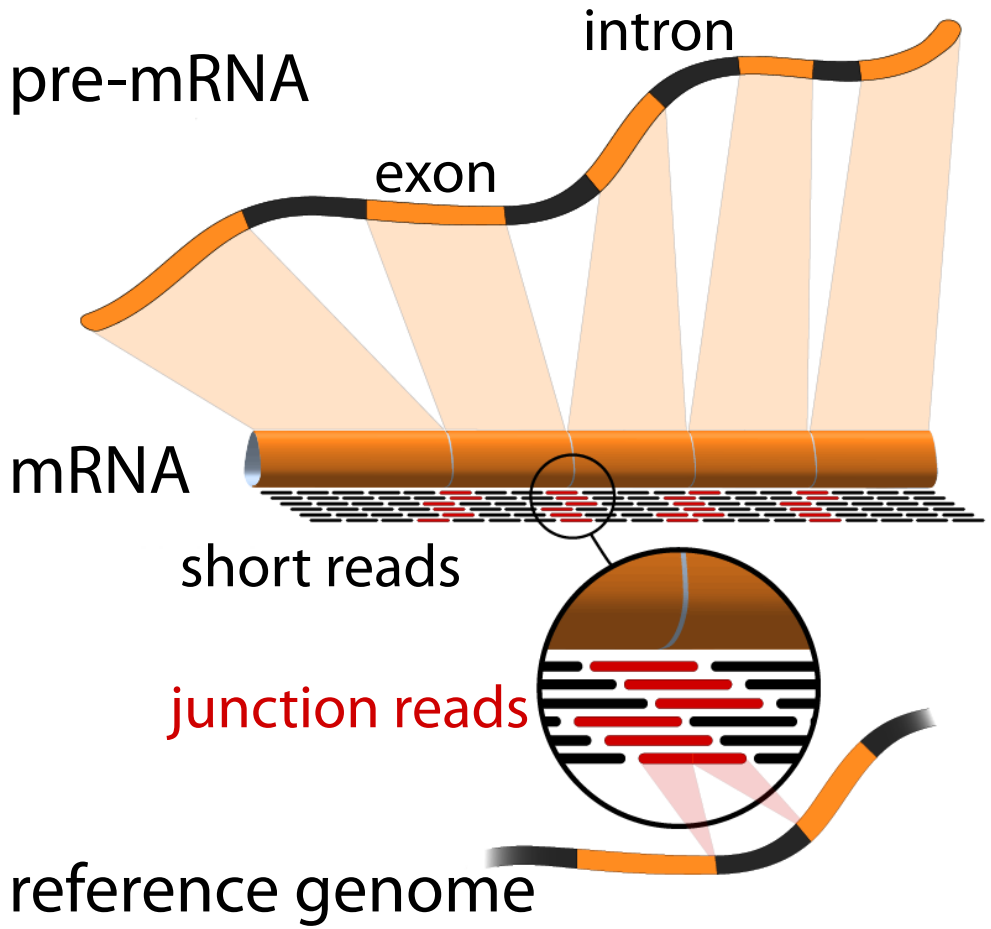

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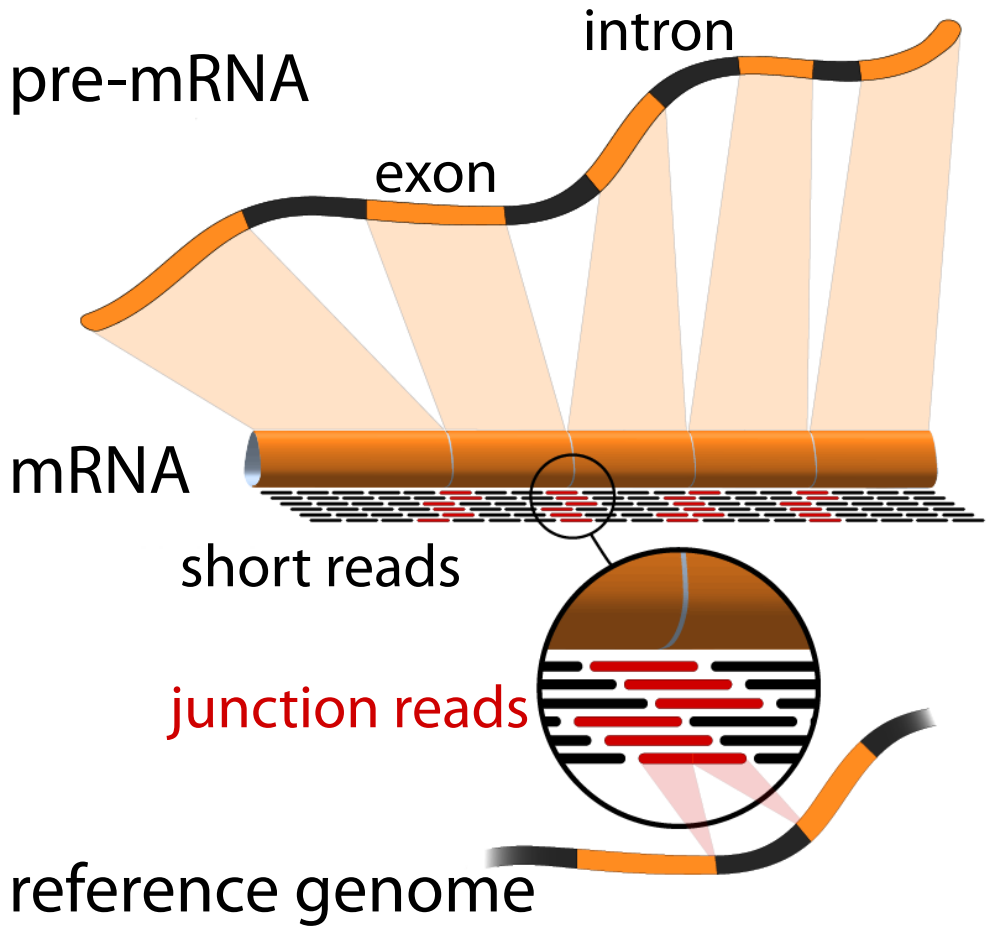

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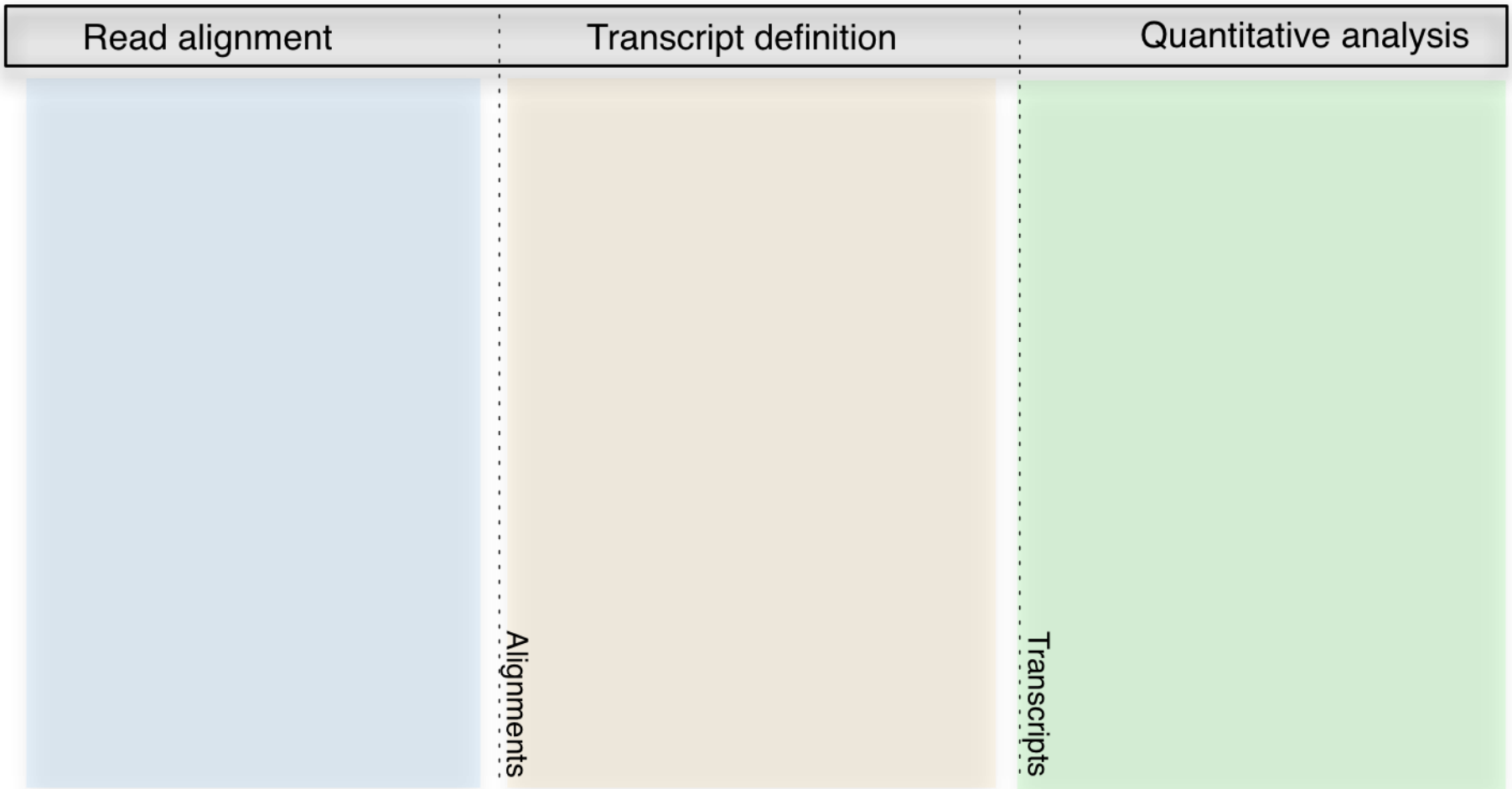

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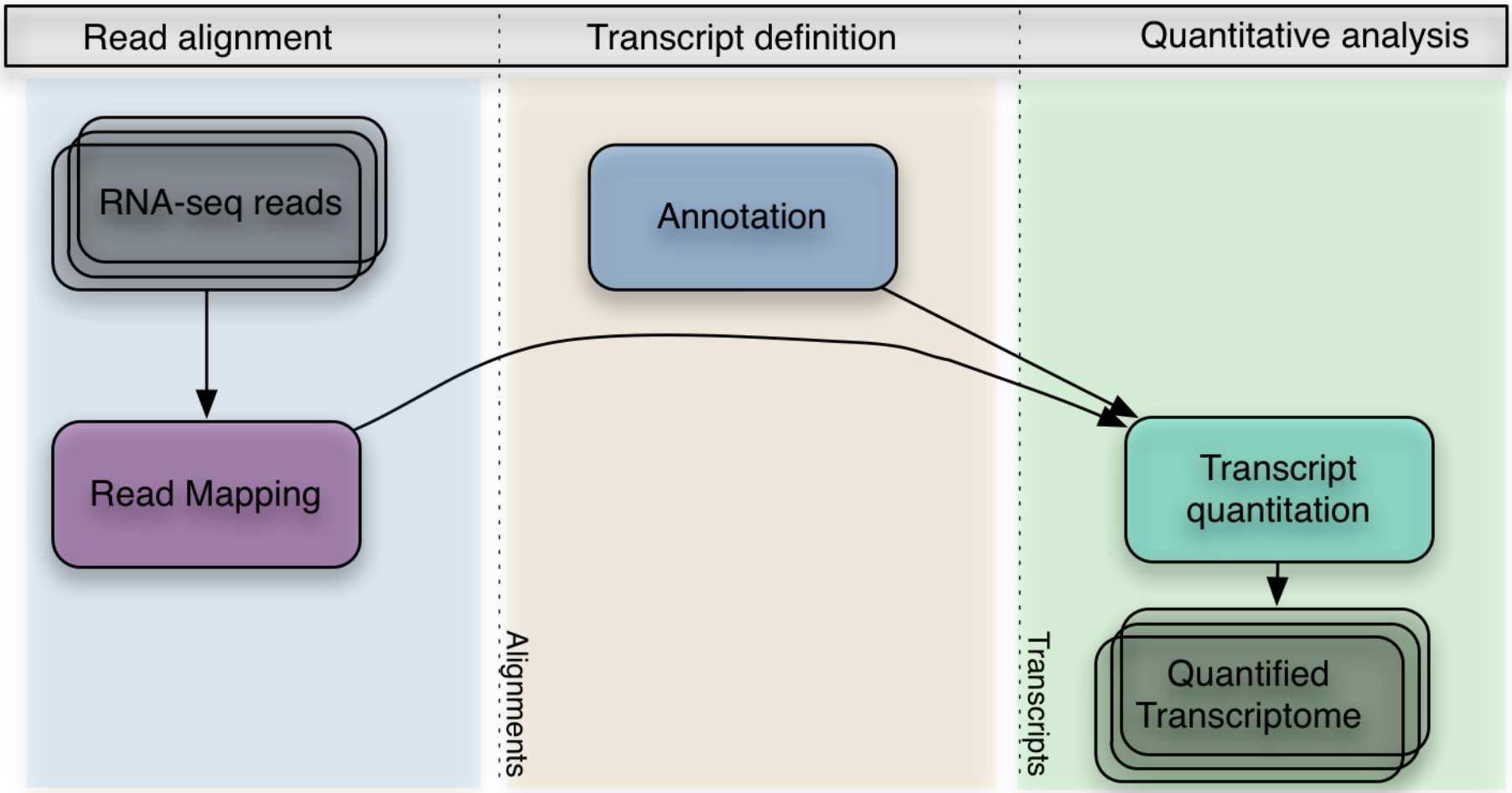

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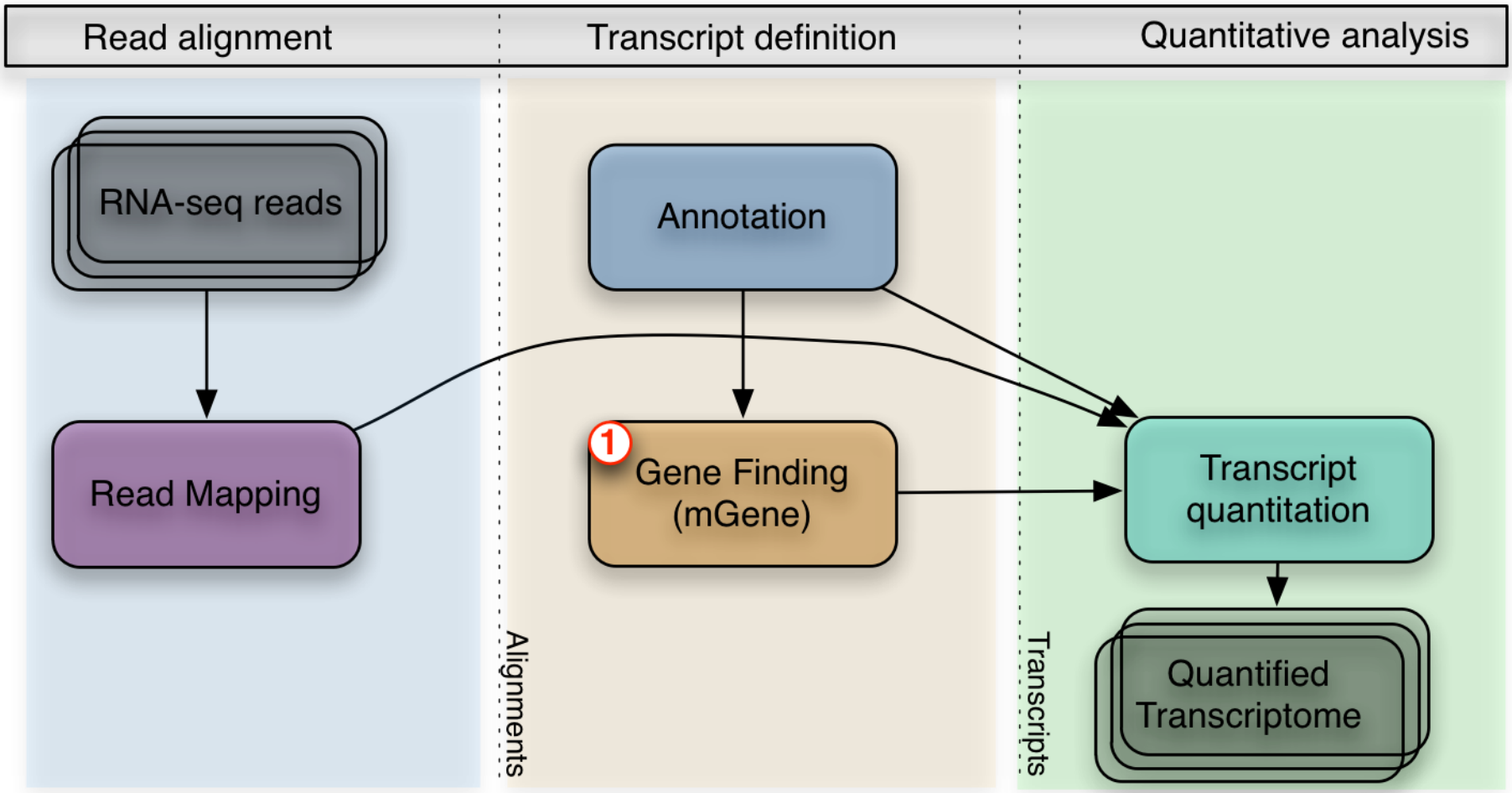

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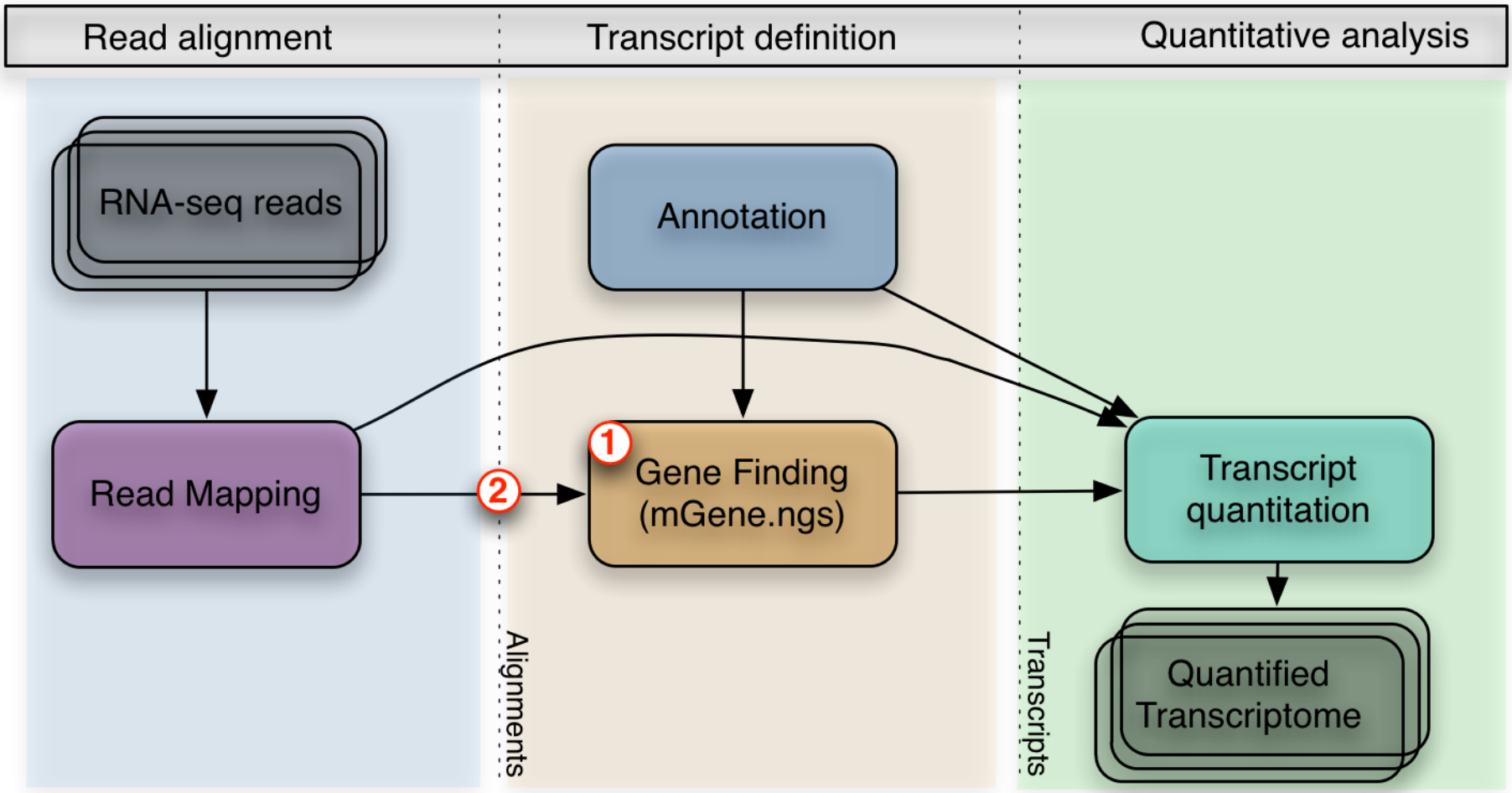

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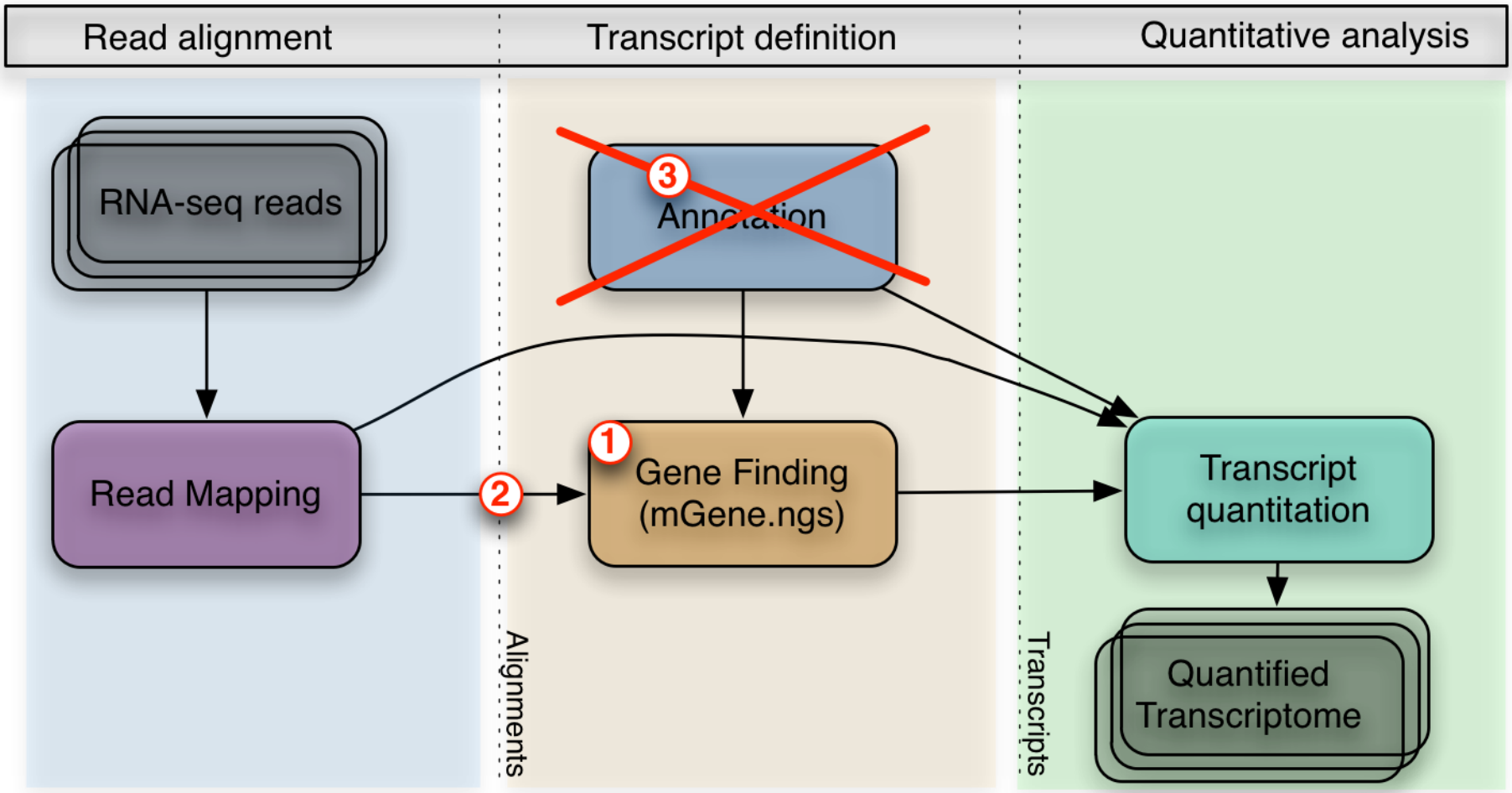
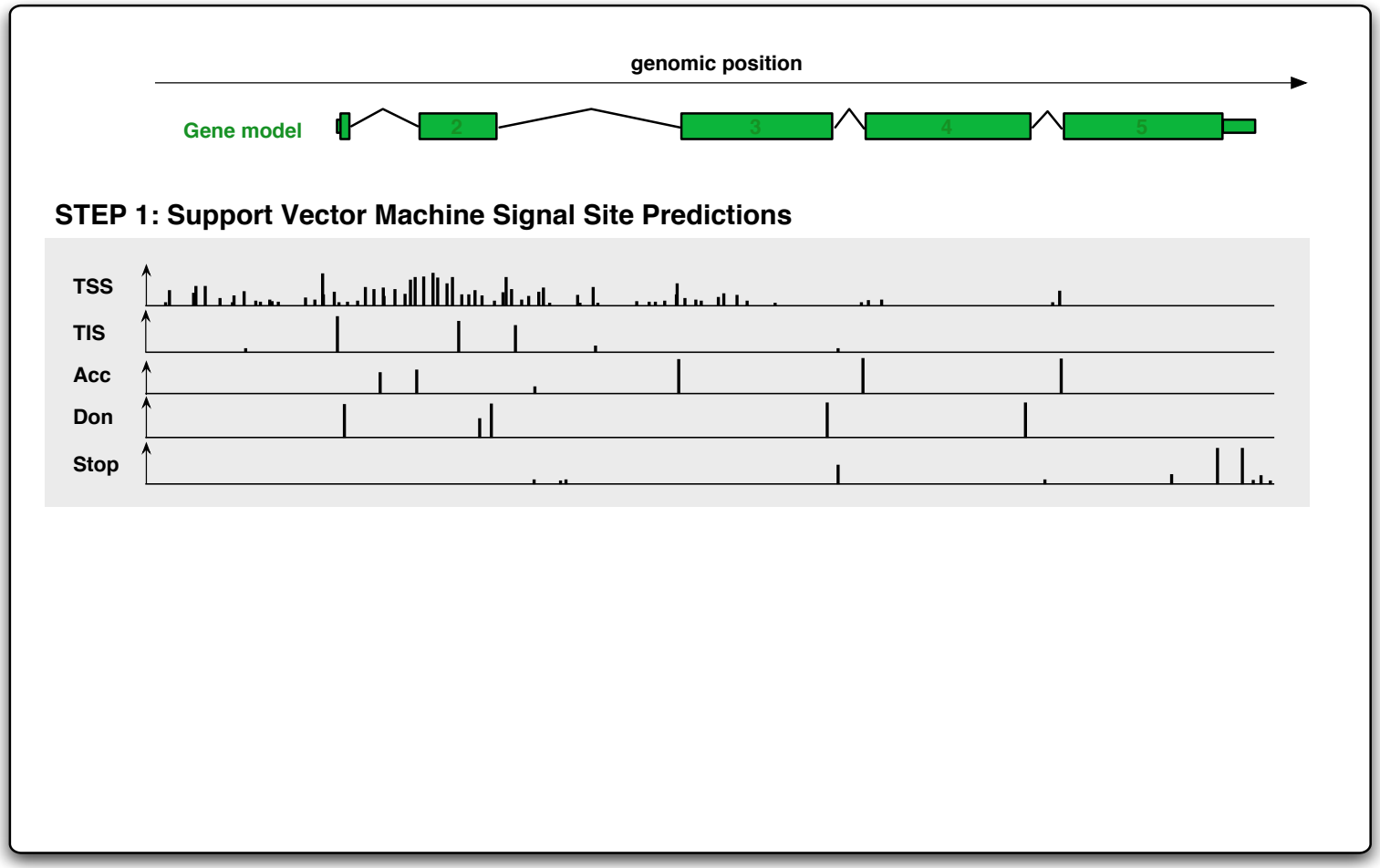


Step 1: Novel Gene Prediction Methods



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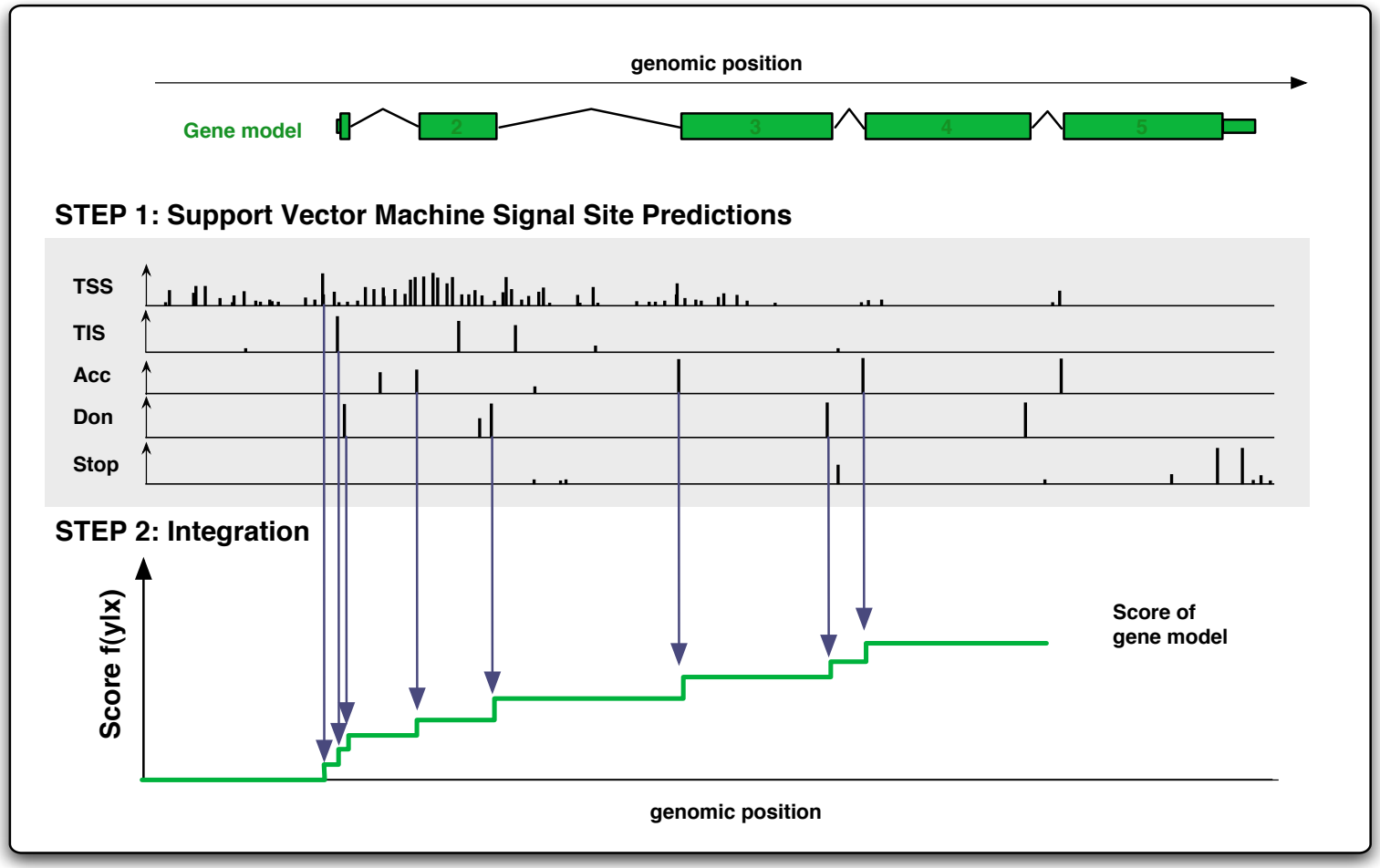

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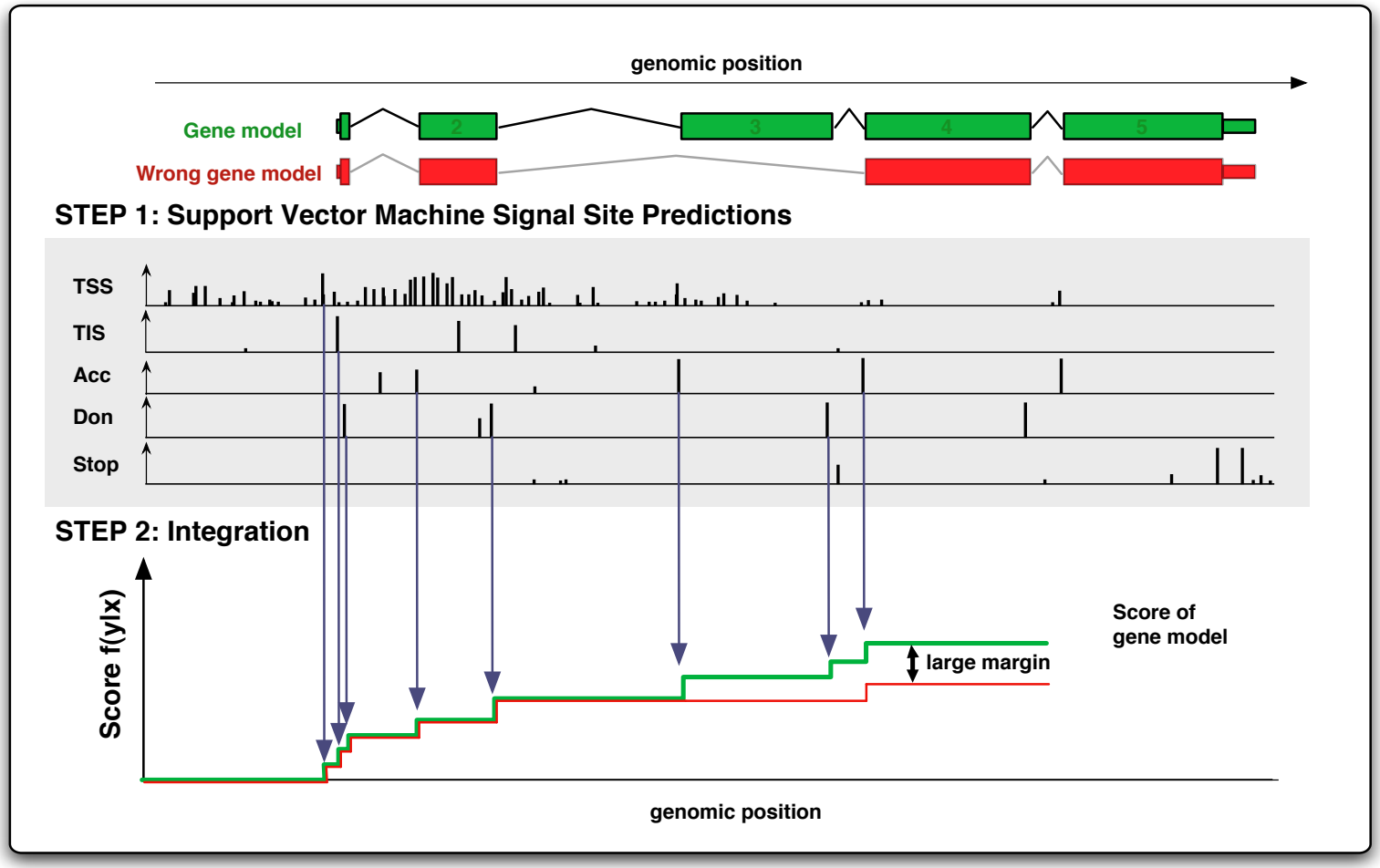

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Results using **mGene**

(Schweikert et al., Genome Research, 2009)

- Most accurate *ab initio* method in the nGASP genome annotation challenge for *C. elegans* [2]
- Validation of novel gene predictions for *C. elegans*: [7]

	No. of genes	No. of genes analyzed	Frac. of genes w/ expression
New genes	2,197	57	$\approx 42\%$
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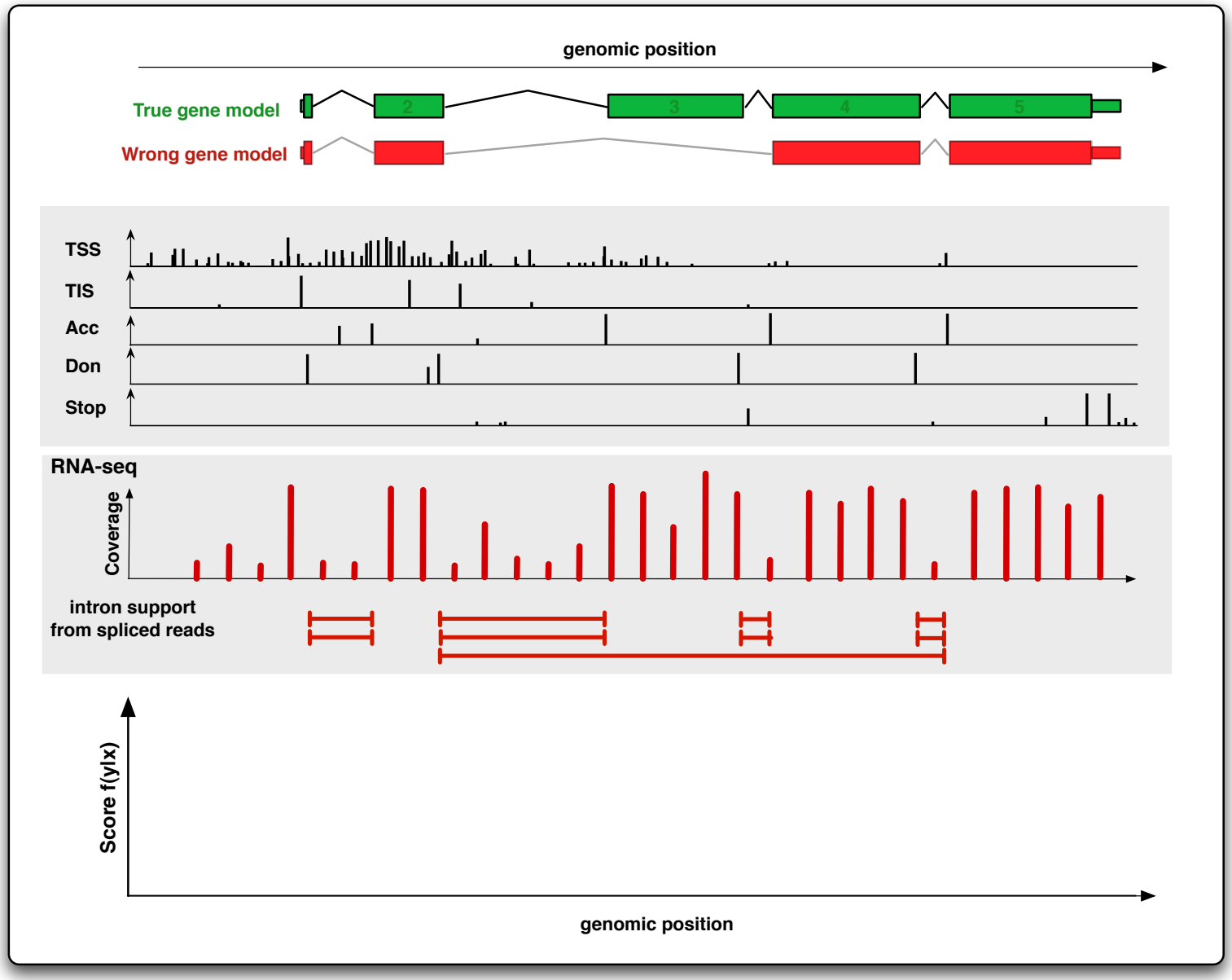

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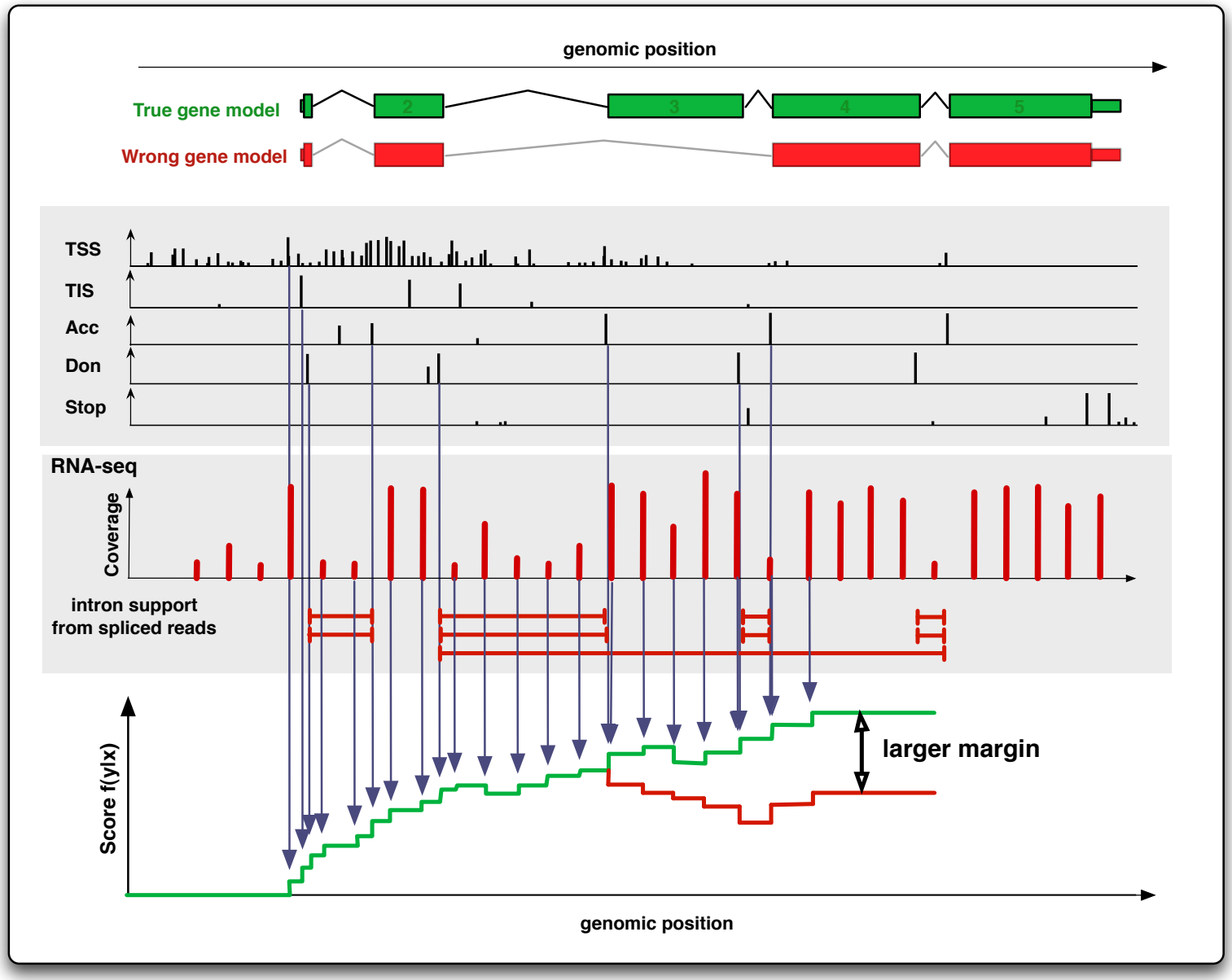

Results for *C. elegans*

RNA-Seq:

- paired-end, strand-specific RNA-Seq (Illumina)
- 76bp reads, 50 million reads (2 lanes, $\approx$ 2.000 Euro)
- Alignment with Palmapper [4]

Evaluation:

- Transcript-level F-score of *coding transcripts*
  ... for different expression levels
- Compare
  - mGene (*ab initio*) [6]
  - mGene.ngs (with RNA-Seq)
  - Cufflinks (only based on RNA-Seq) [10]

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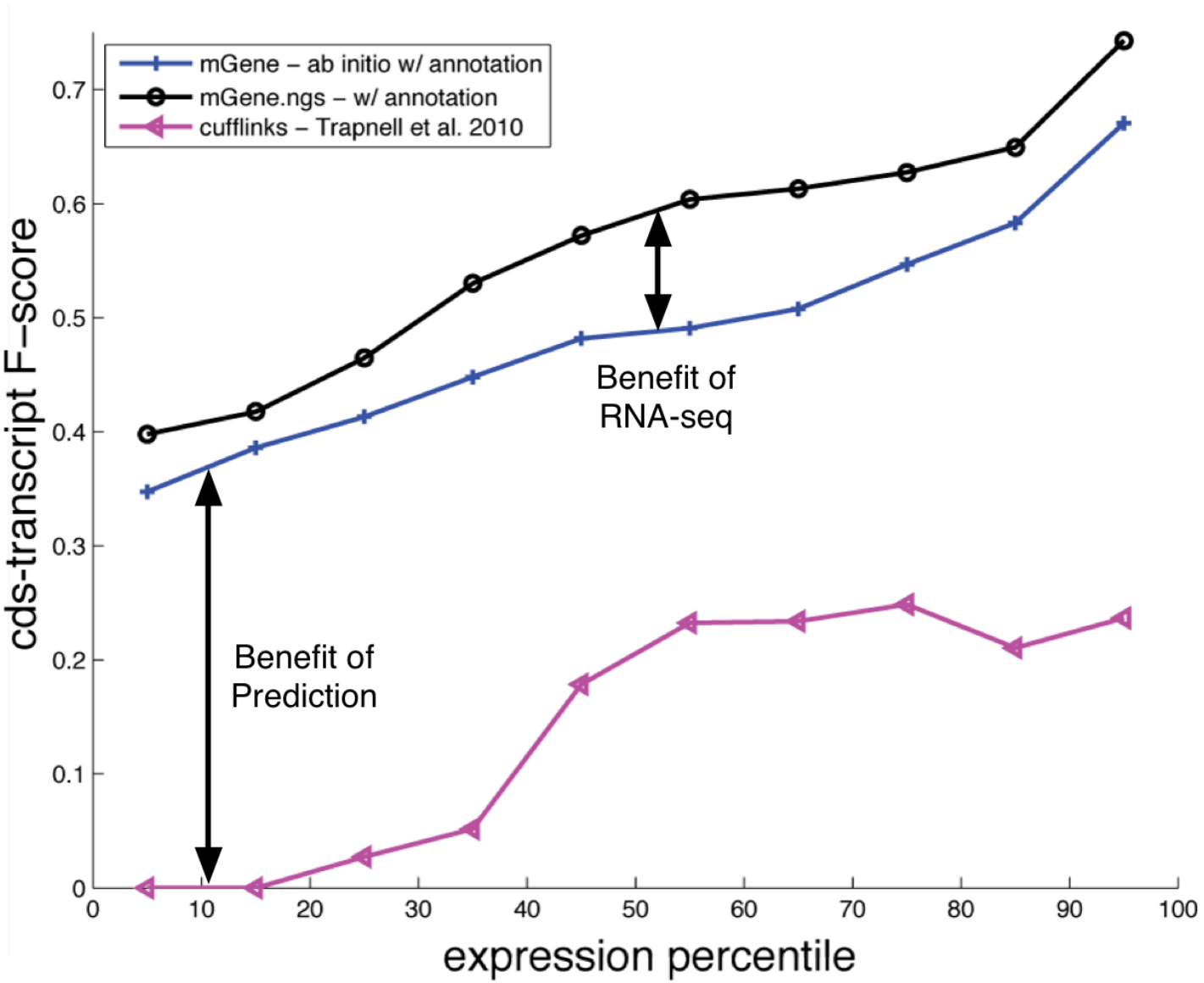

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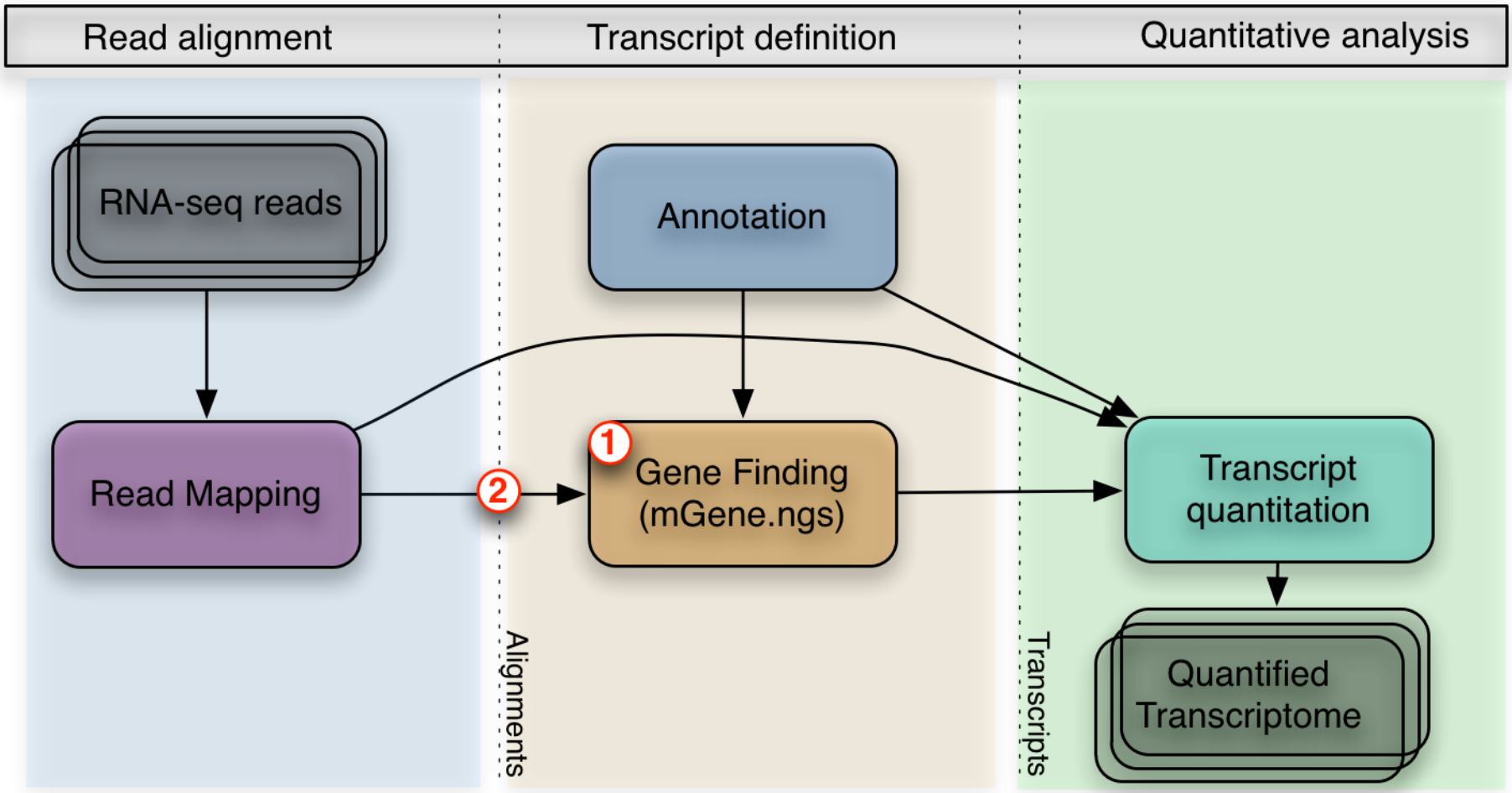

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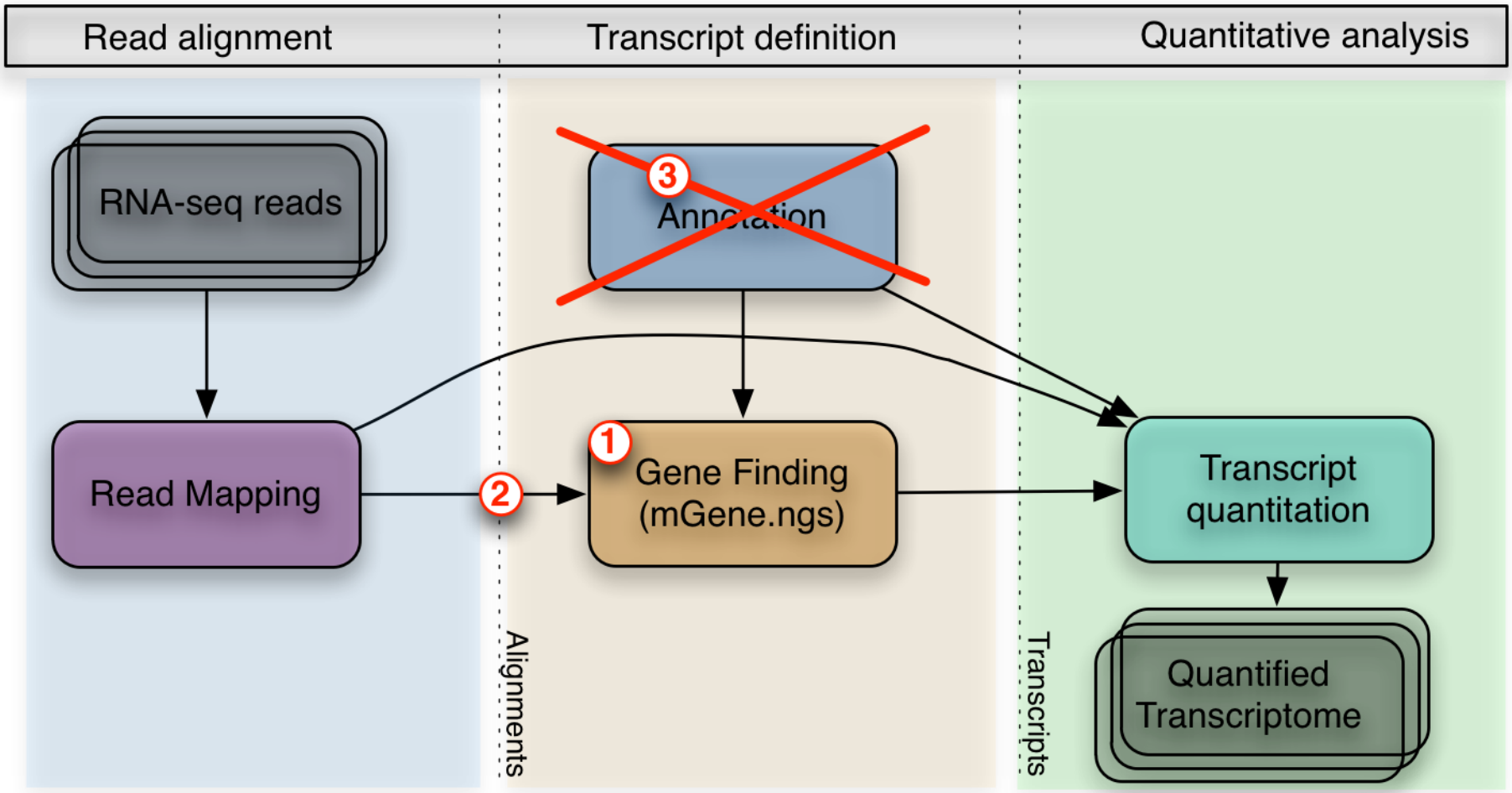
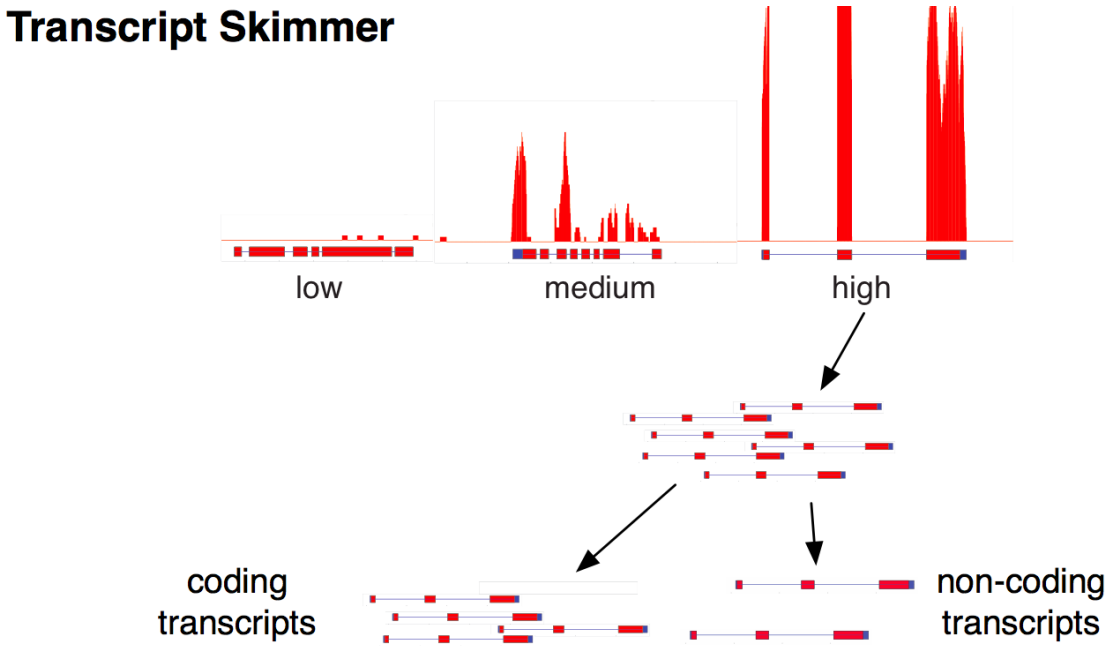


Step 3: Skimming and Non-coding Transcripts



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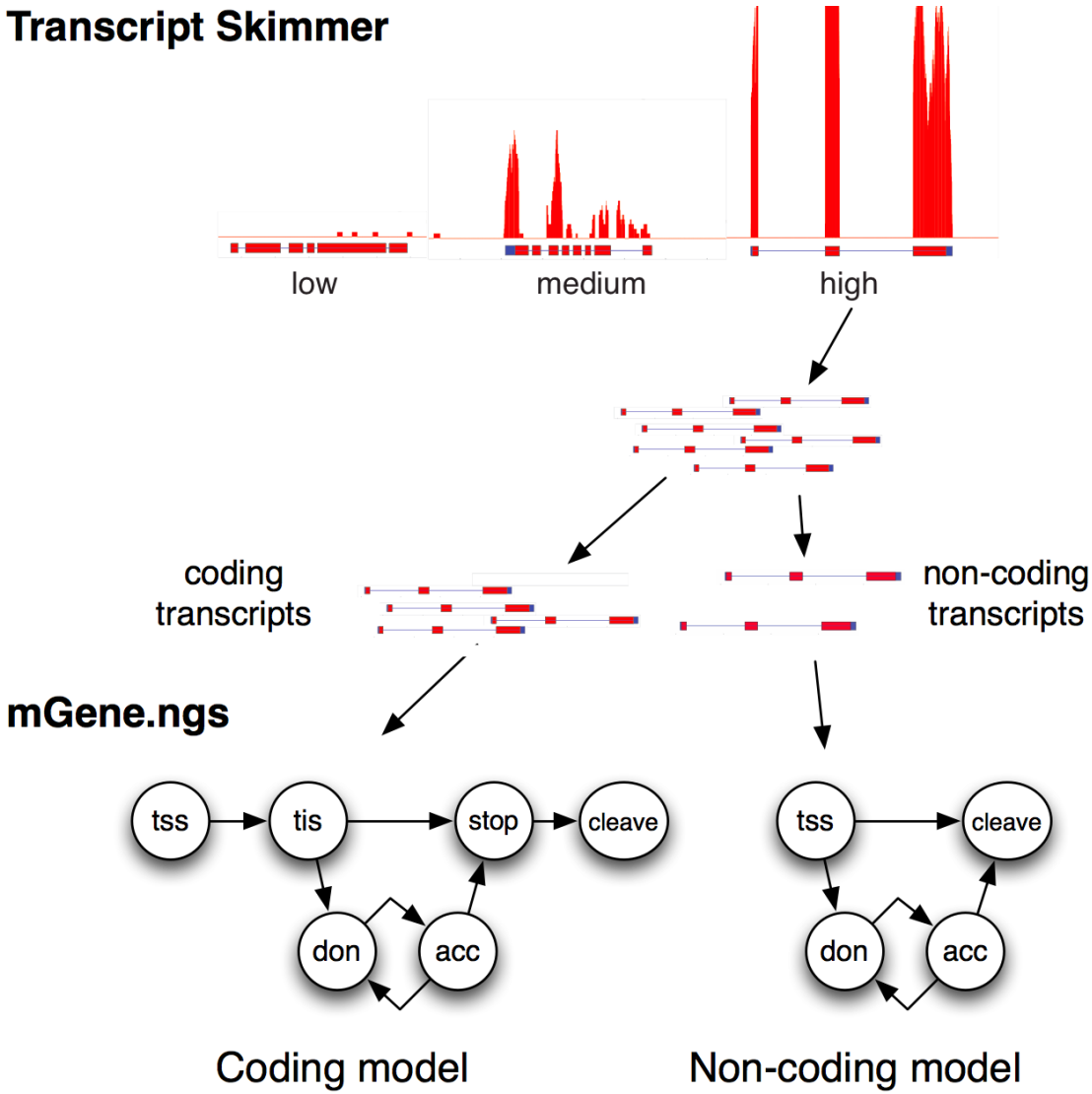
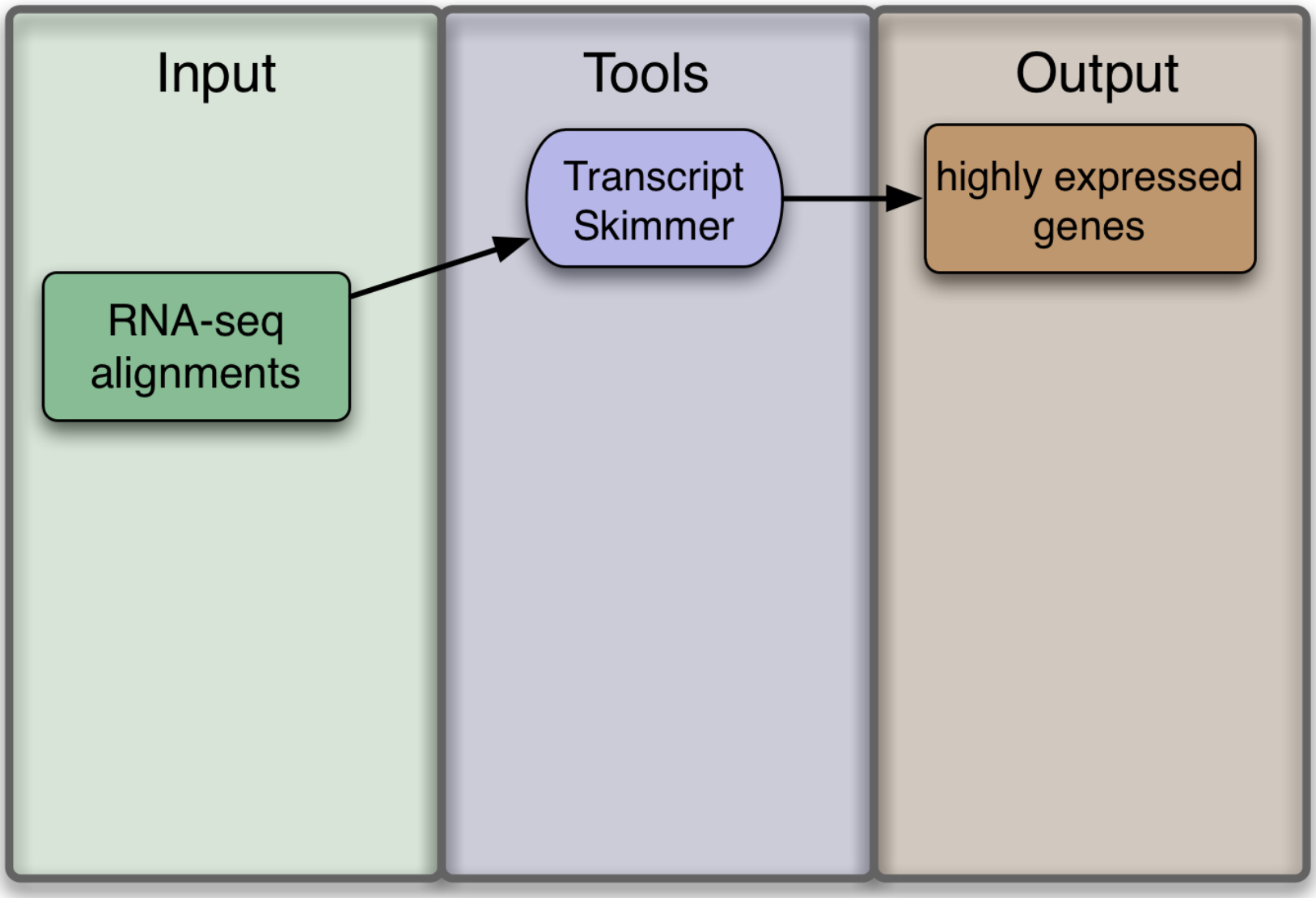


Genome Annotation Workflow



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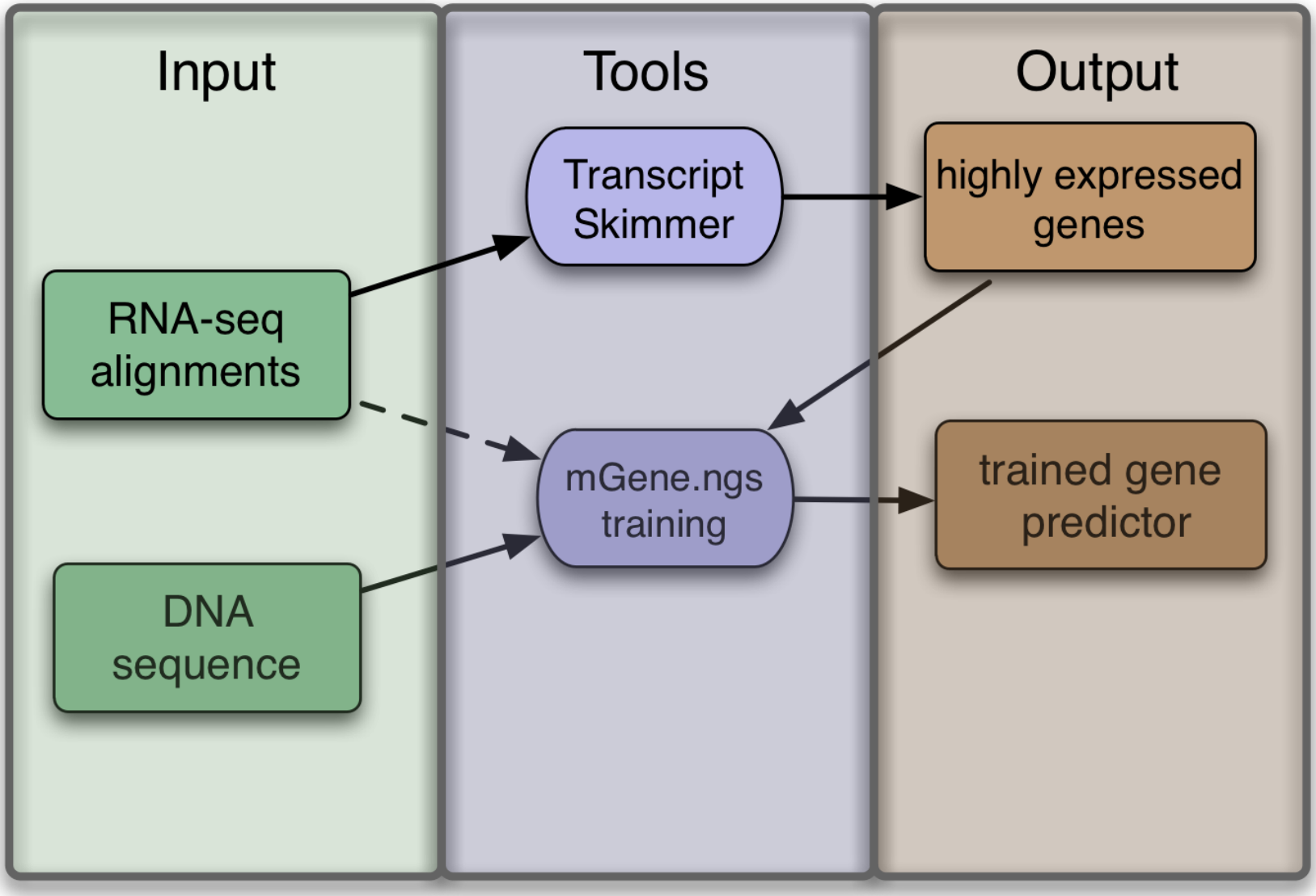

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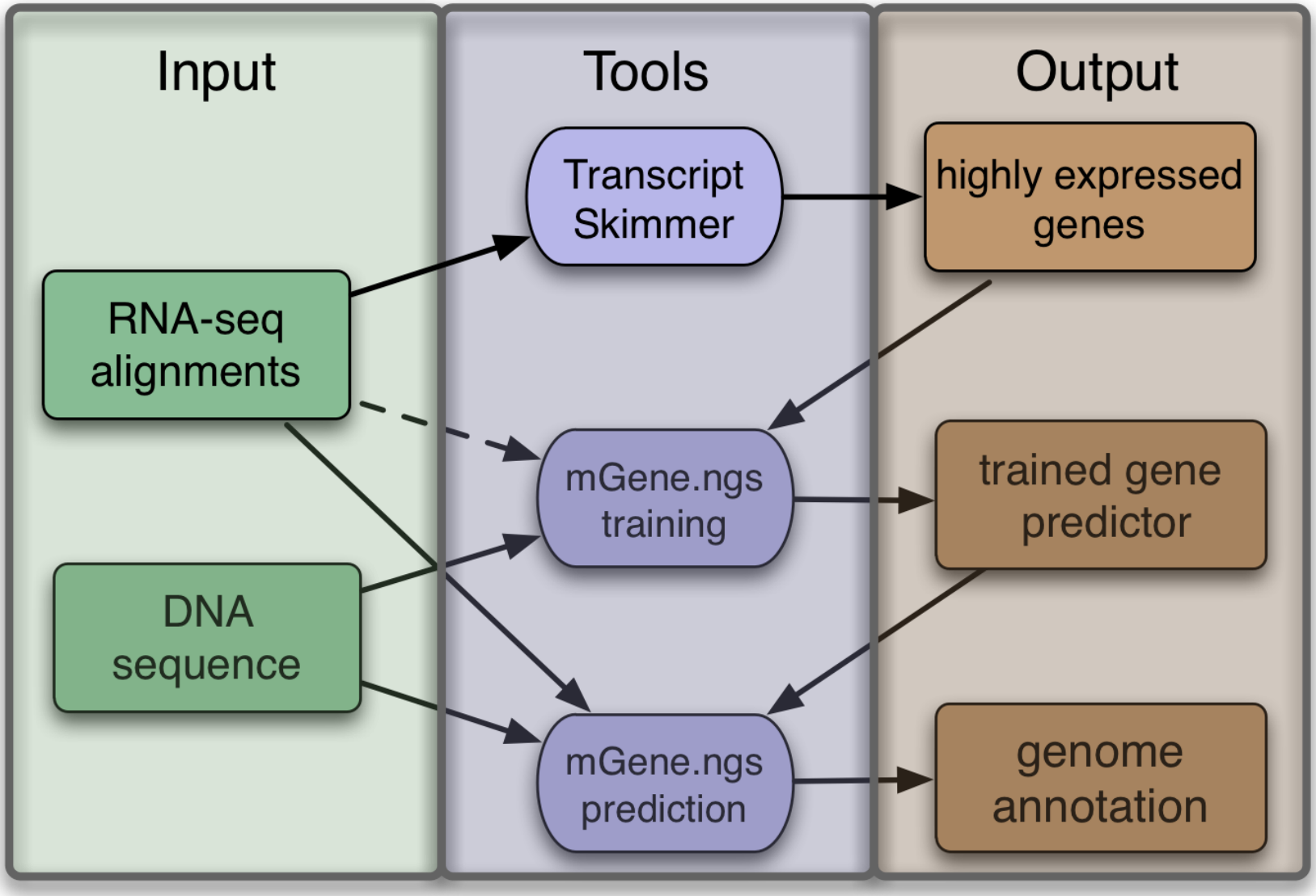

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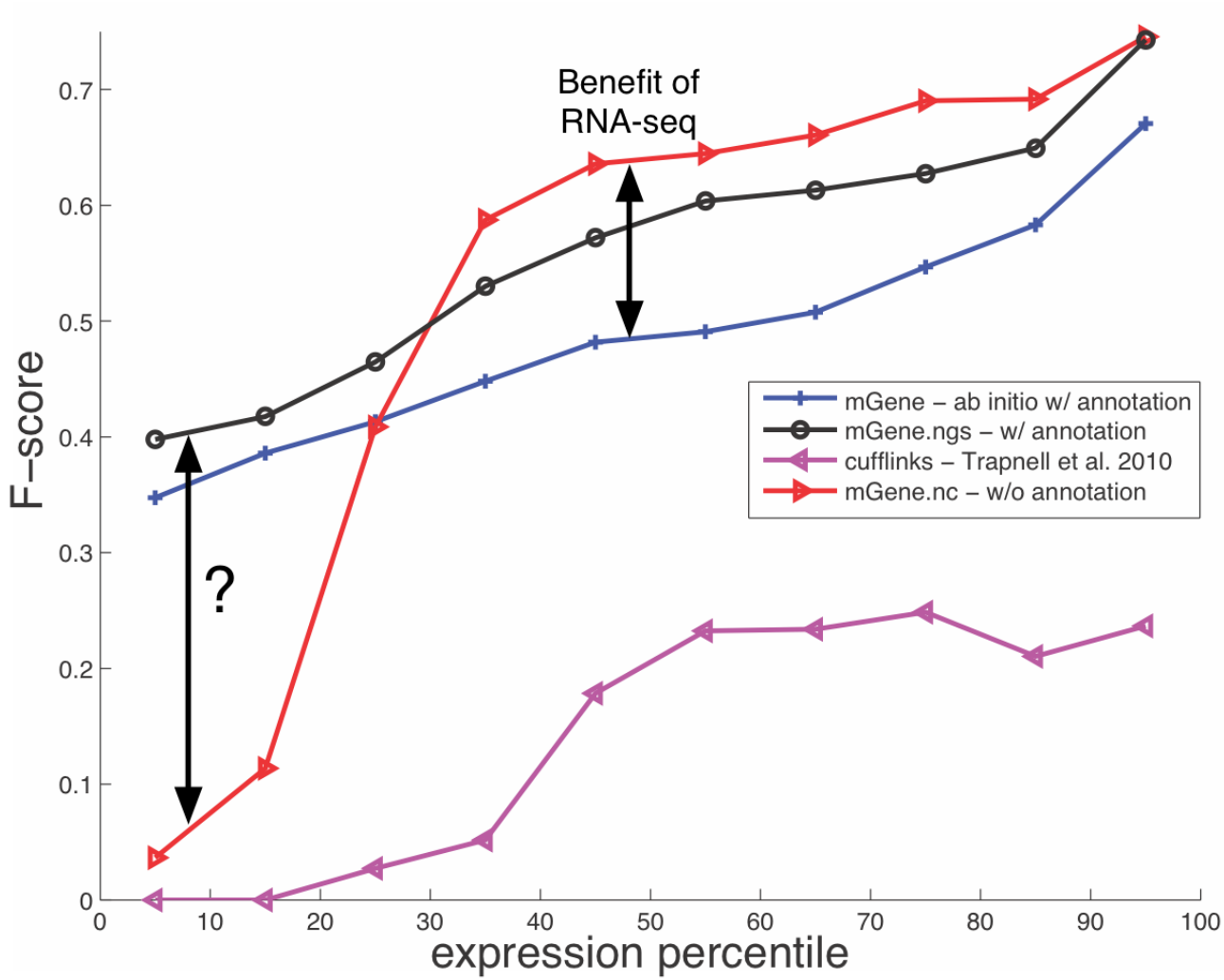
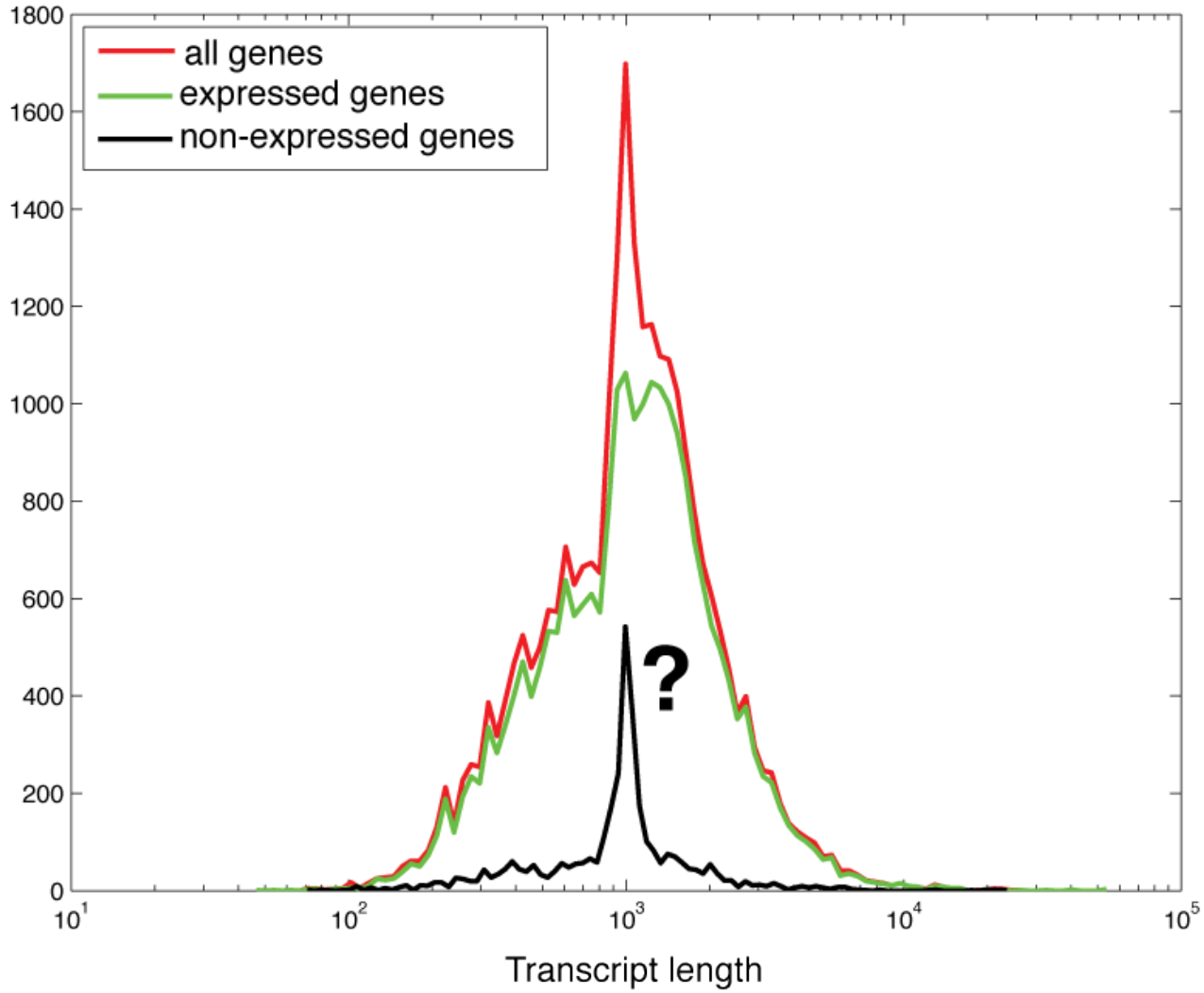
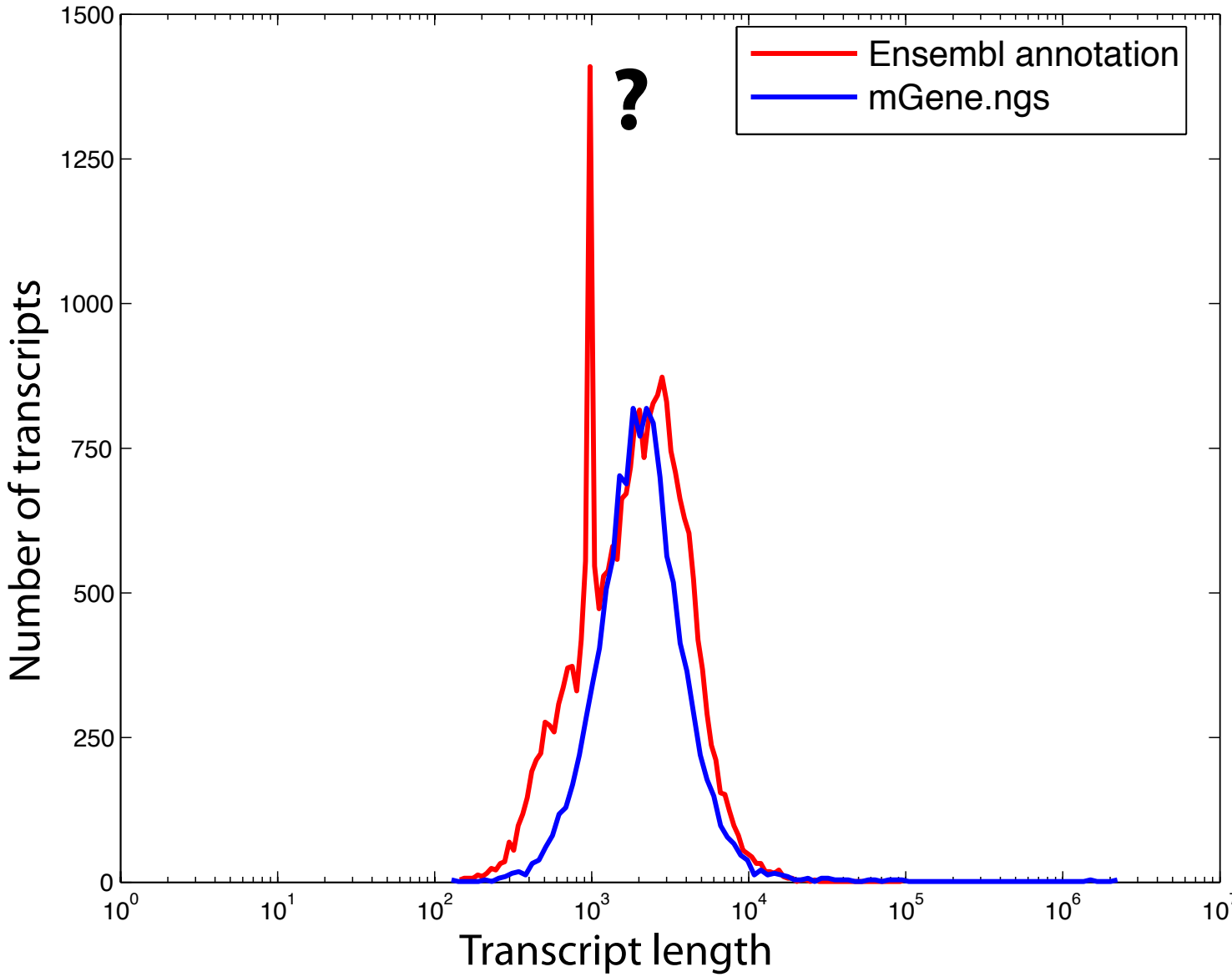


Coding Region Lengths (*C. elegans*)



Artifacts in *C. elegans* annotation?

Coding Region Lengths (mouse)



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  - mGene.ngs integrates RNA-Seq and genomic information
  - Transcript Skimmer identifies highly expressed genes for training
  - Accurate prediction of *coding and non-coding transcripts*
- Fully automated training requiring only
  - Genome sequence
  - RNA-Seq alignments
- Good for annotating new genomes or improving existing ones
- Currently used to (re-)annotate *mouse*, *Drosophila* species, *A. thaliana* strains, *Capsella*, *maize*, ...
- Web service available `galaxy.fml.mpg.de`
- Cloud integration planned
- Source will be free (GPL and other models) `mgene.org`

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Further information

Slides available at:

fml.mpg.de/raetsch/lectures

Jonas Behr

Gabriele Schweikert

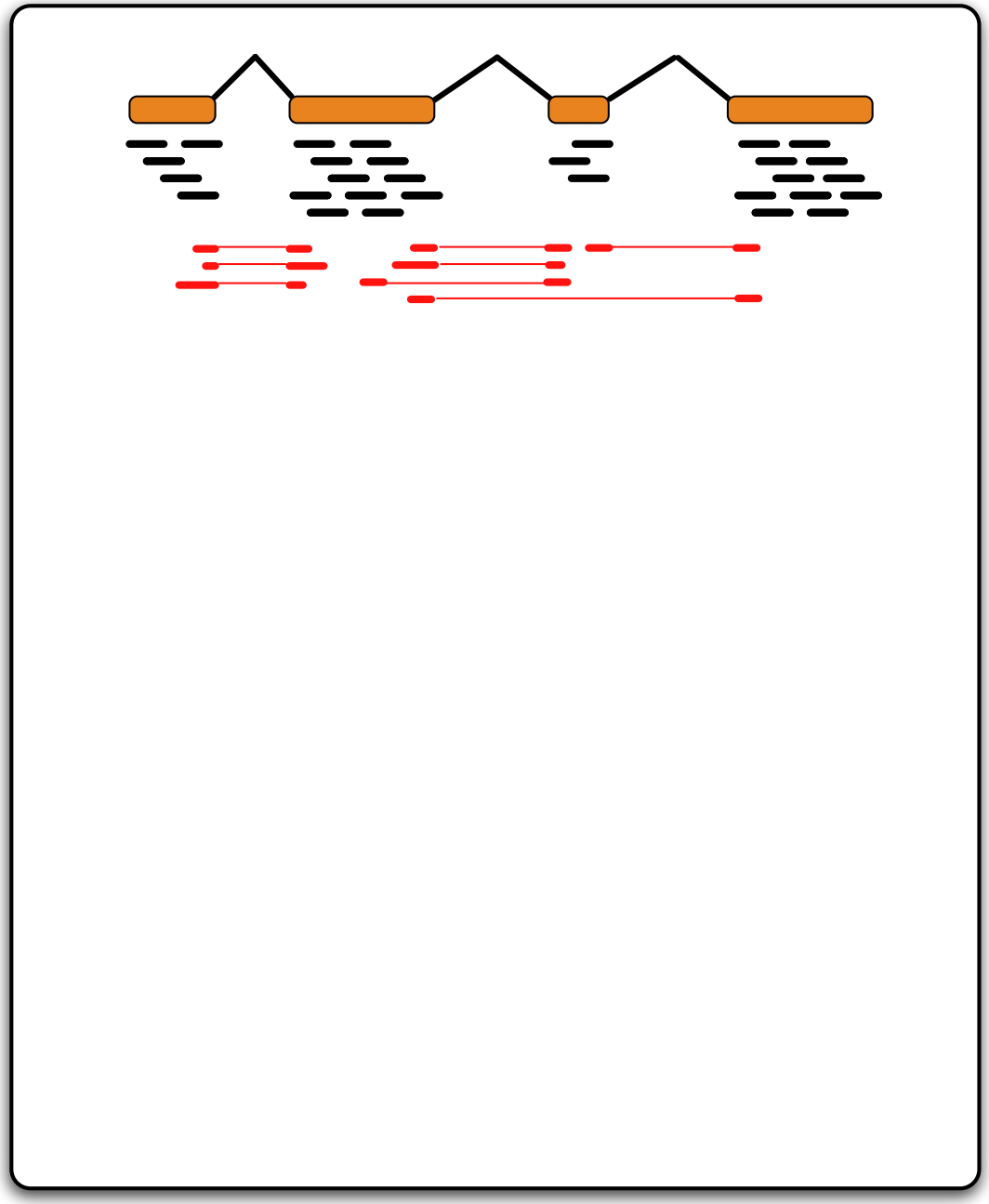
Acknowledgments

Gene finding:	**Jonas Behr, Georg Zeller, Gabriele Schweikert**
Quantification:	**Regina Bohnert**
Library preparation:	**Lisa Hartmann, Lisa M. Smith**
Alignments:	**Andre Kahles, Geraldine Jean, Jonas Behr**
Shogun support:	**Sören Sonnenburg**
Discussions:	**Philipp Drewe, Sebastian Schultheiss, Christian Widmer**

Funding: Max Planck Society and German Research Foundation

Infer alternative isoforms

- mGene.ngs prediction
- Build splicegraph using spliced reads
- Generate transcripts from graph
- rQuant: explain read coverage by combination of transcripts



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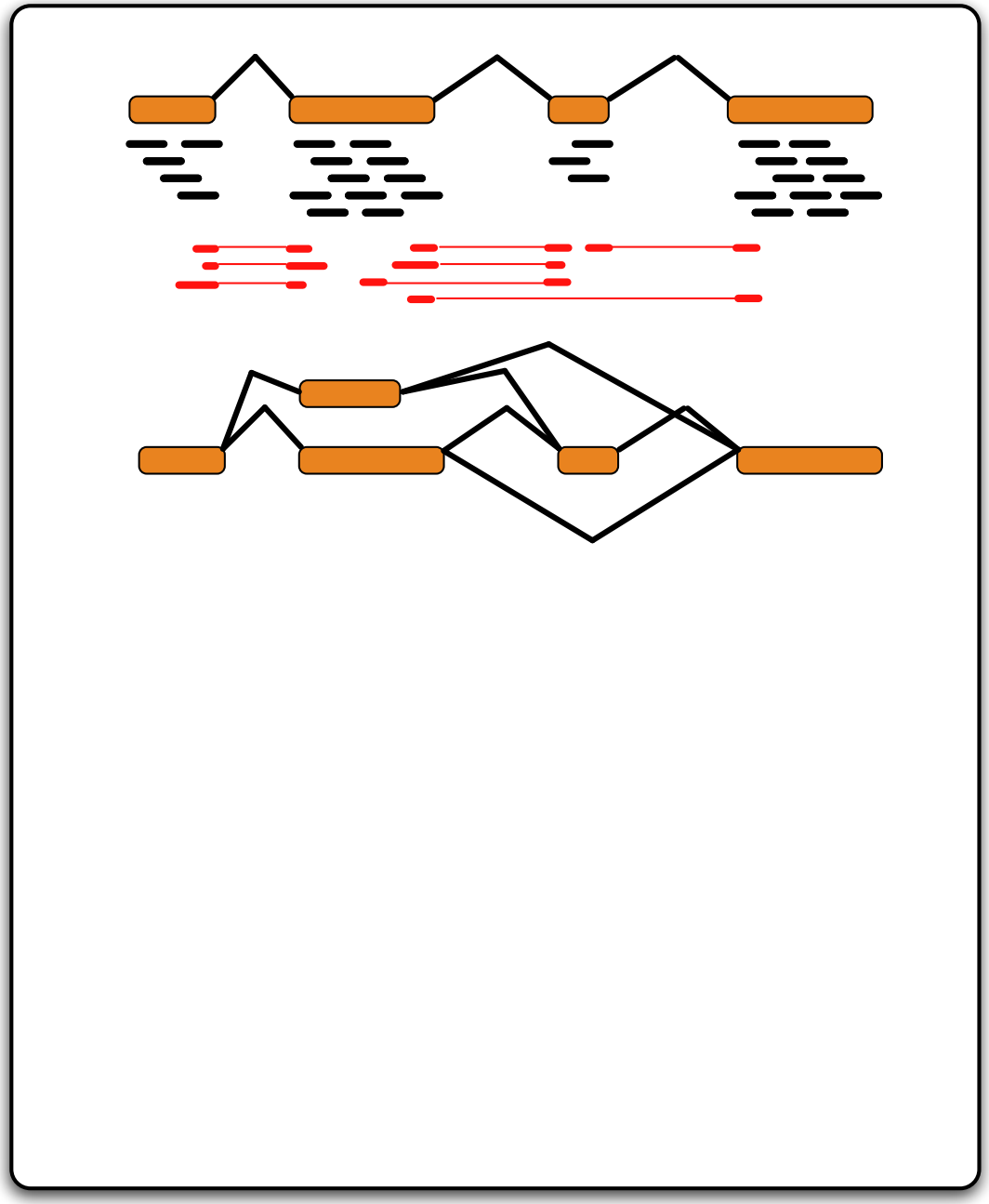

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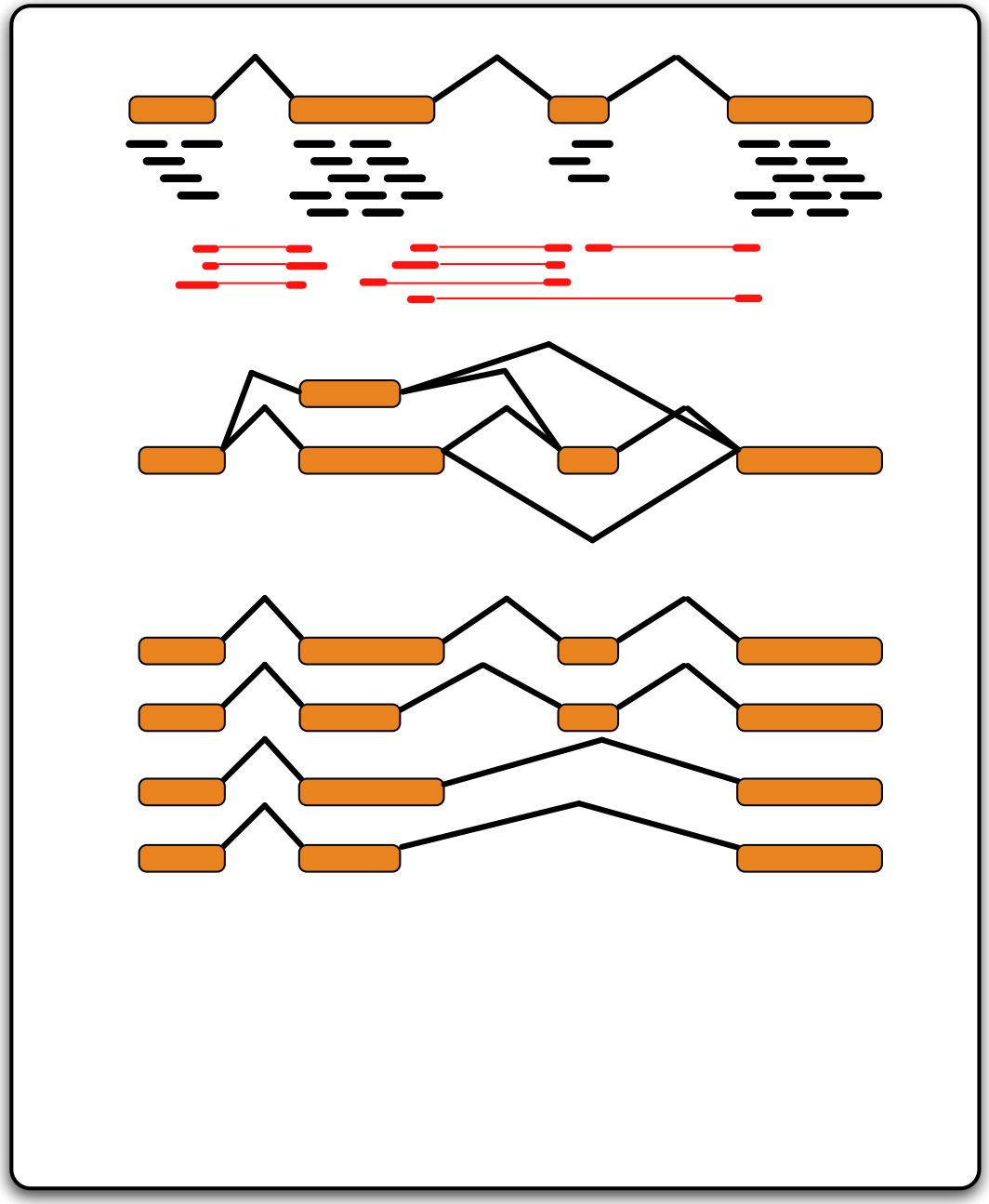

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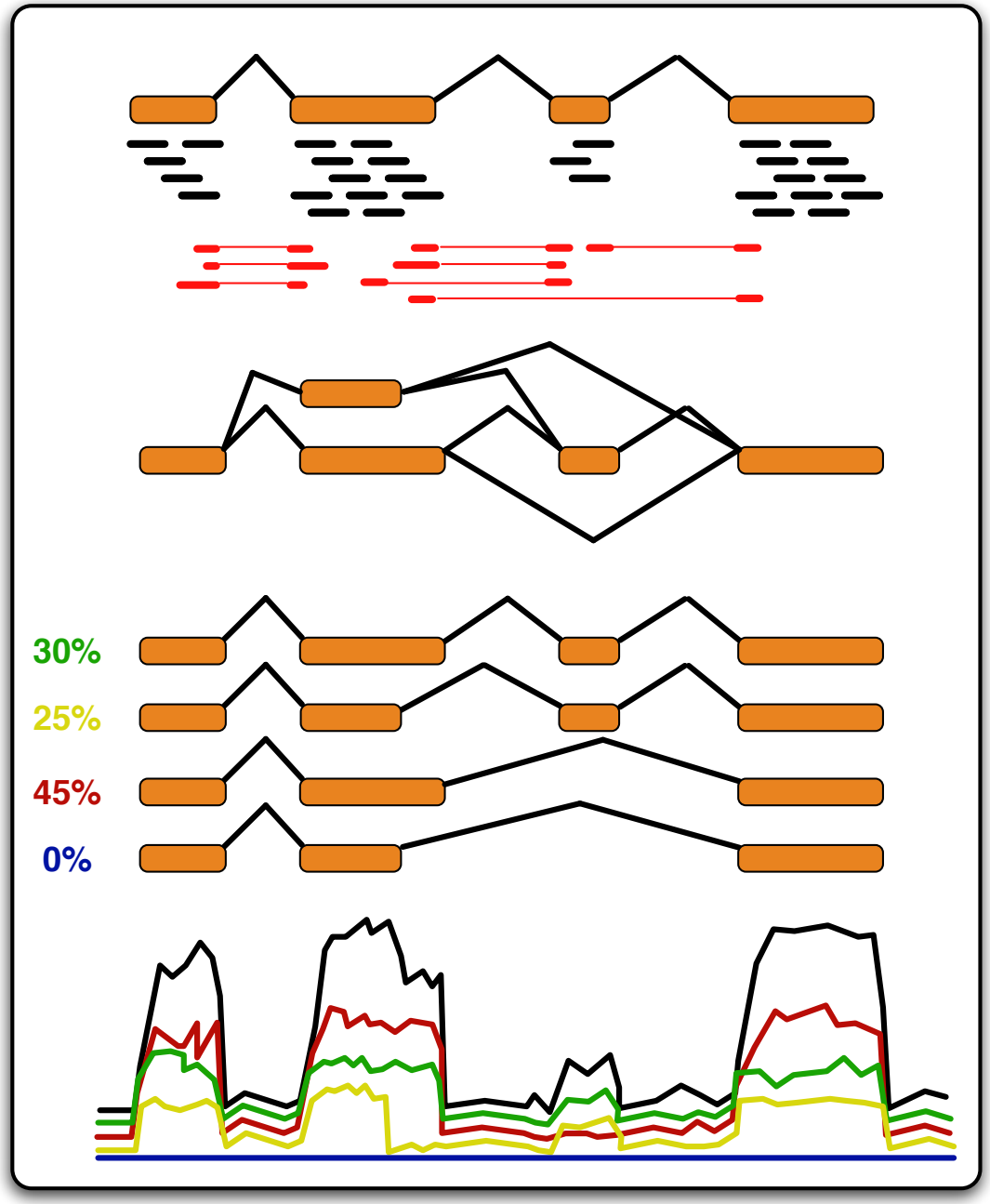

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