# Evaluation of *de novo* Transcriptome Assemblies from RNA-Seq Data

with Bo Li and Colin Dewey

CIBM Seminar, February 12, 2012

# Background - The Transcriptome

Definition: The collection of RNA molecules in a cell or sample.

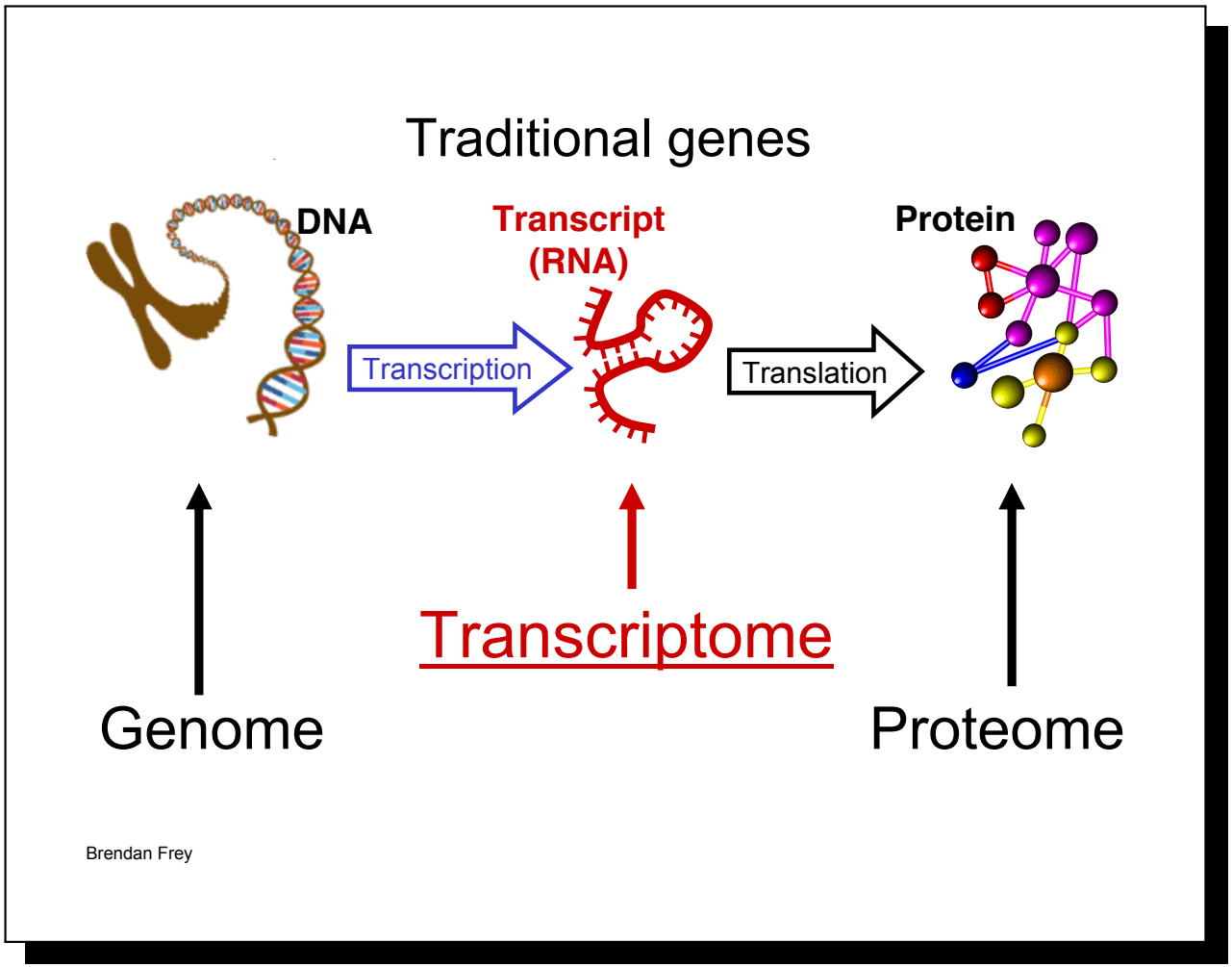


Figure from Brendan Frey, http://www.psi.toronto.edu/isit2006/.

# Background - RNA-Seq

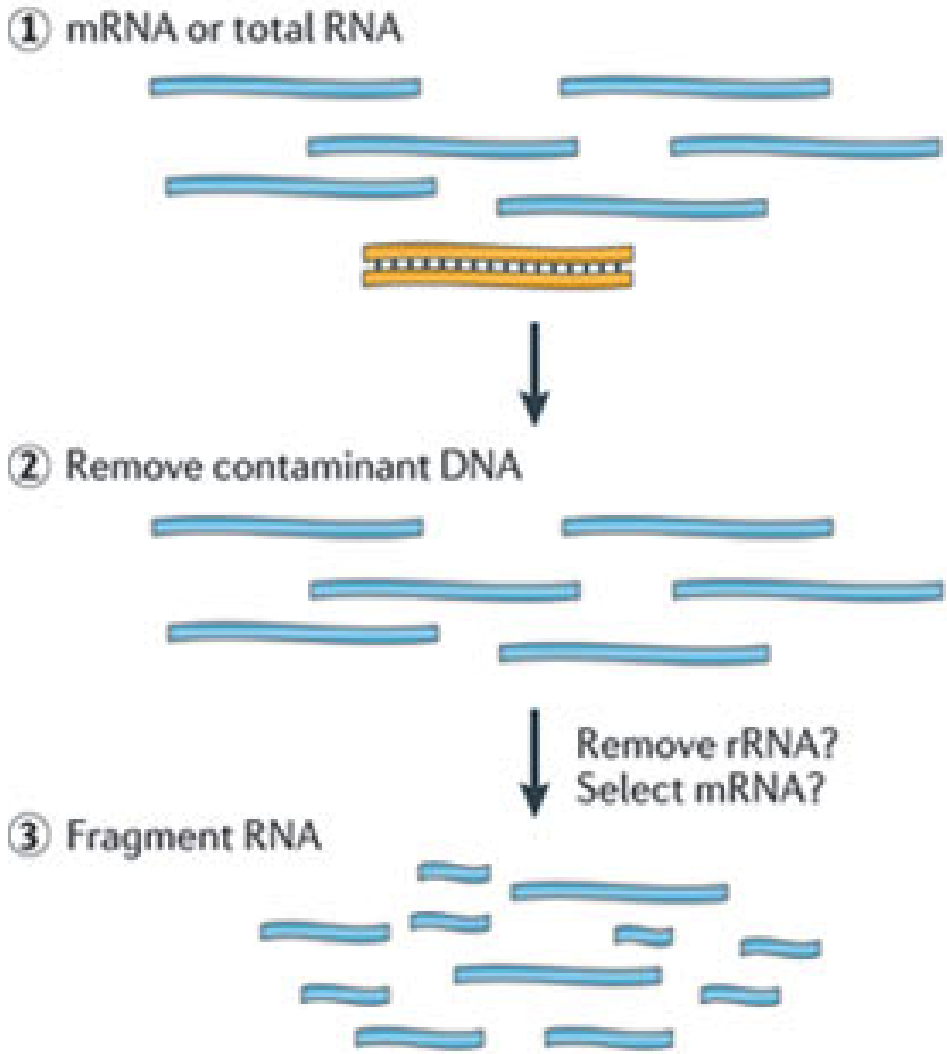


Figure from Martin and Wang, “Next-generation transcriptome assembly”, Nature Reviews Genetics, 2011.

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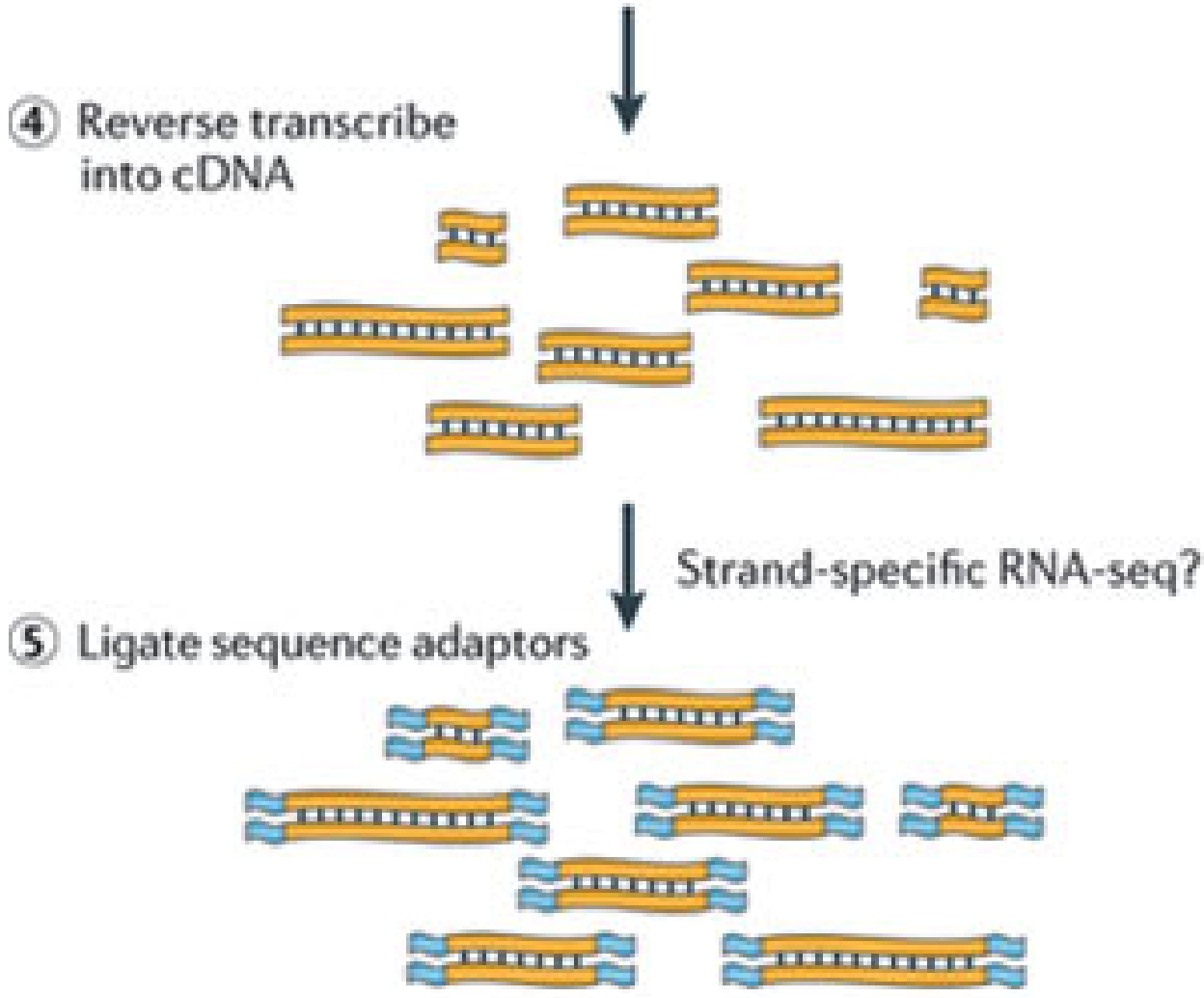


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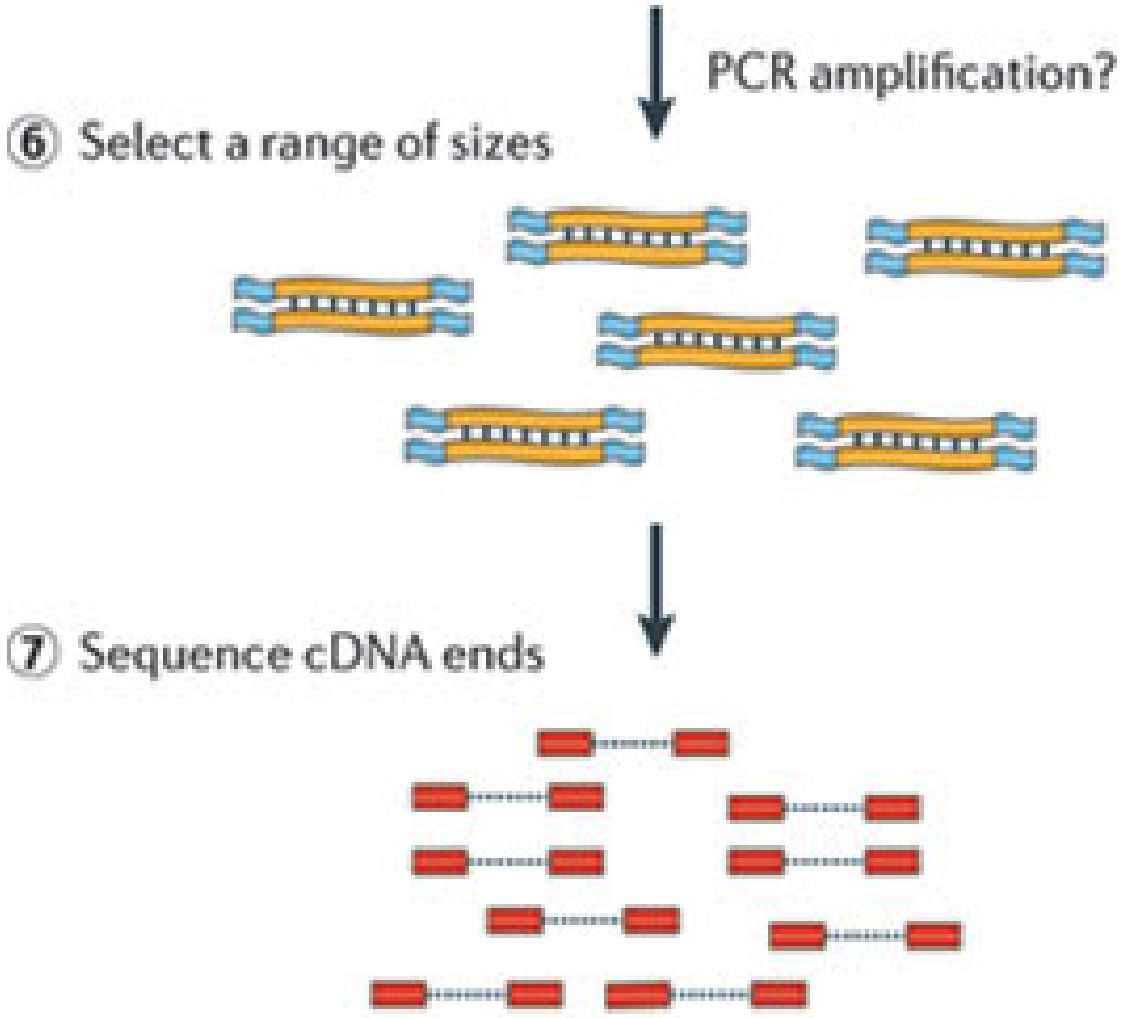
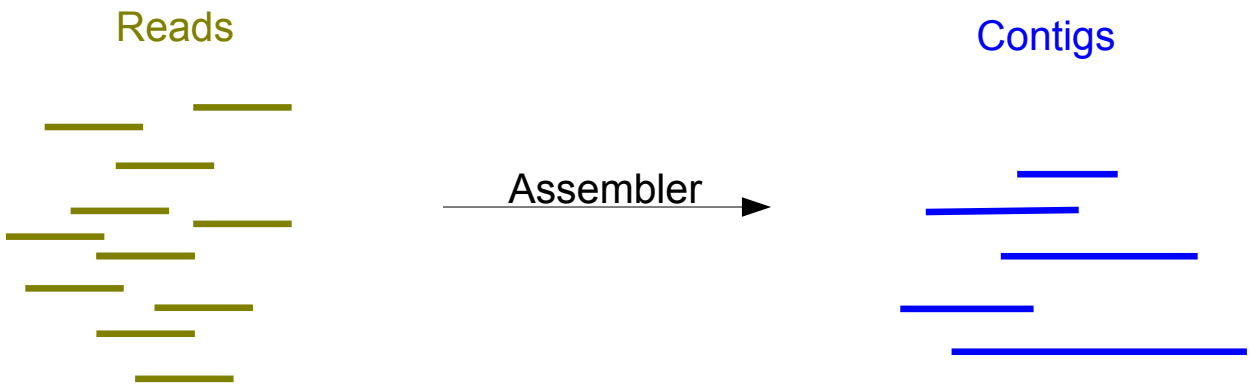


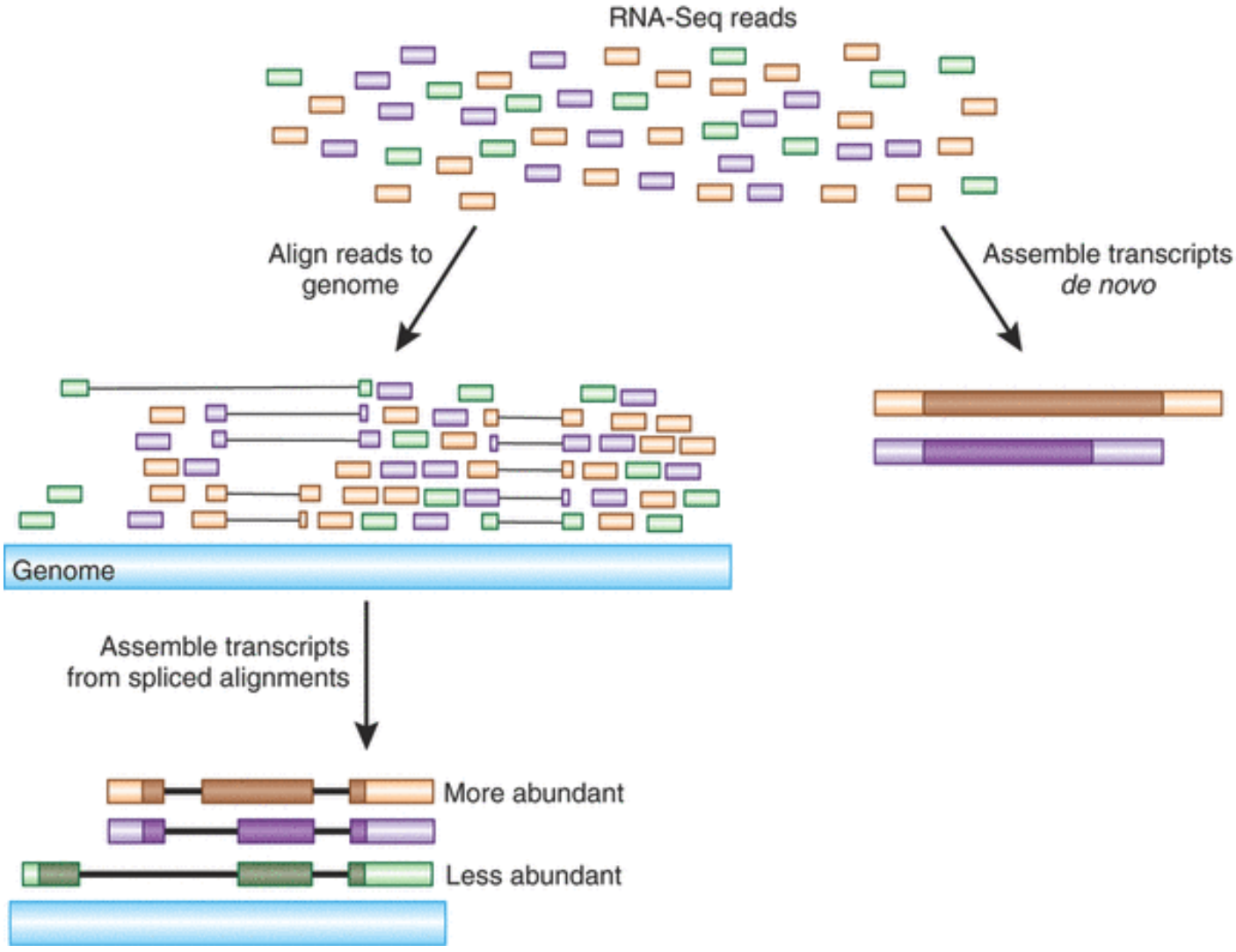

Figure from Martin and Wang, “Next-generation transcriptome assembly”, Nature Reviews Genetics, 2011.

# Background - Assembly




A contig is a continguous subsequence of a transcript sequence.

# Background - Assembly




Edited figure from Haas and Zody, “Advancing RNA-Seq analysis”, Nature Biotechnology, 2010.

# Background - Complications

- Non-uniform expression.
- Alternative splicing.

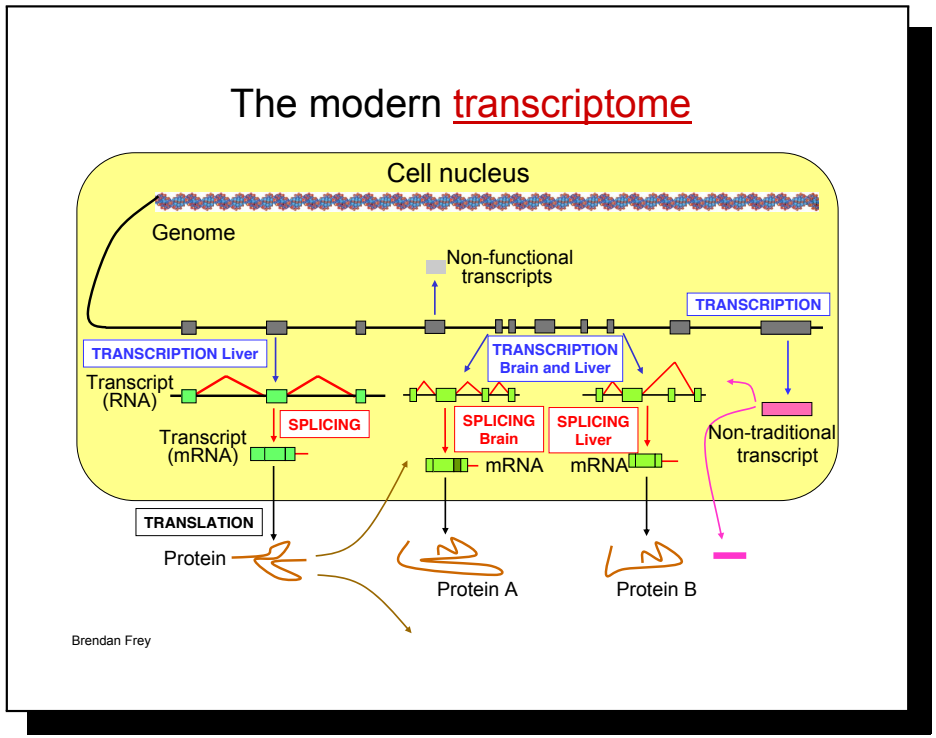



Figure from Brendan Frey, http://www.psi.toronto.edu/isit2006/.

# Background - *de novo* Assembly

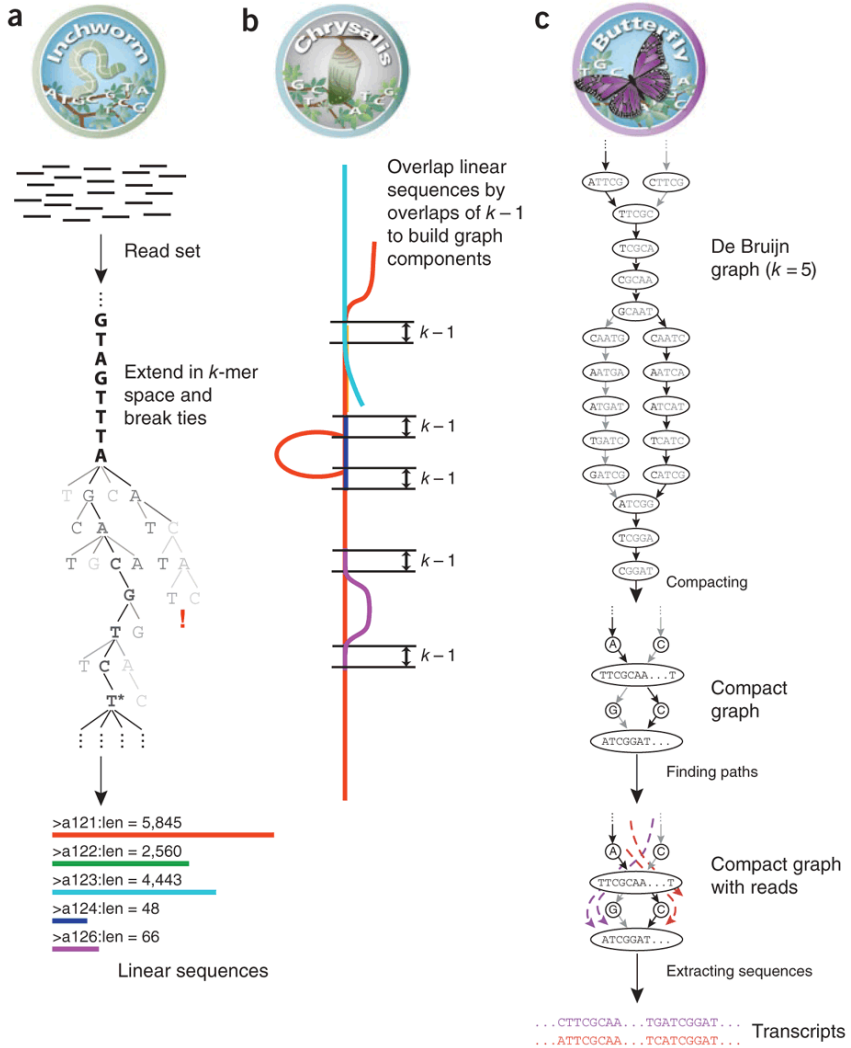



Figure from Grabherr et al., “Full-length transcriptome assembly from RNA-Seq data without a reference genome”, Nature Biotechnology,

# Evaluation Problem

Without the ground truth reference transcript set,

determine which assembly is best

based only on the RNA-Seq data

from which the assemblies were constructed.

# Evaluation Problem - Desiderata

- Start from first principles.
- Avoid trivialalities.
- Achieve the same ordering as a simple reference-based score.

Non-solution: N50, the largest $n$ such that the contigs with length $\geq n$ compose at least 50% of the total bases of the contigs set.

# Our Contributions

- A score that satisfies the given desiderata.
- A reference-based precision/recall framework for transcriptome assembly.
- A software package, DETONATE, that implements the above.
- A comprehensive meta-evaluation of the score.

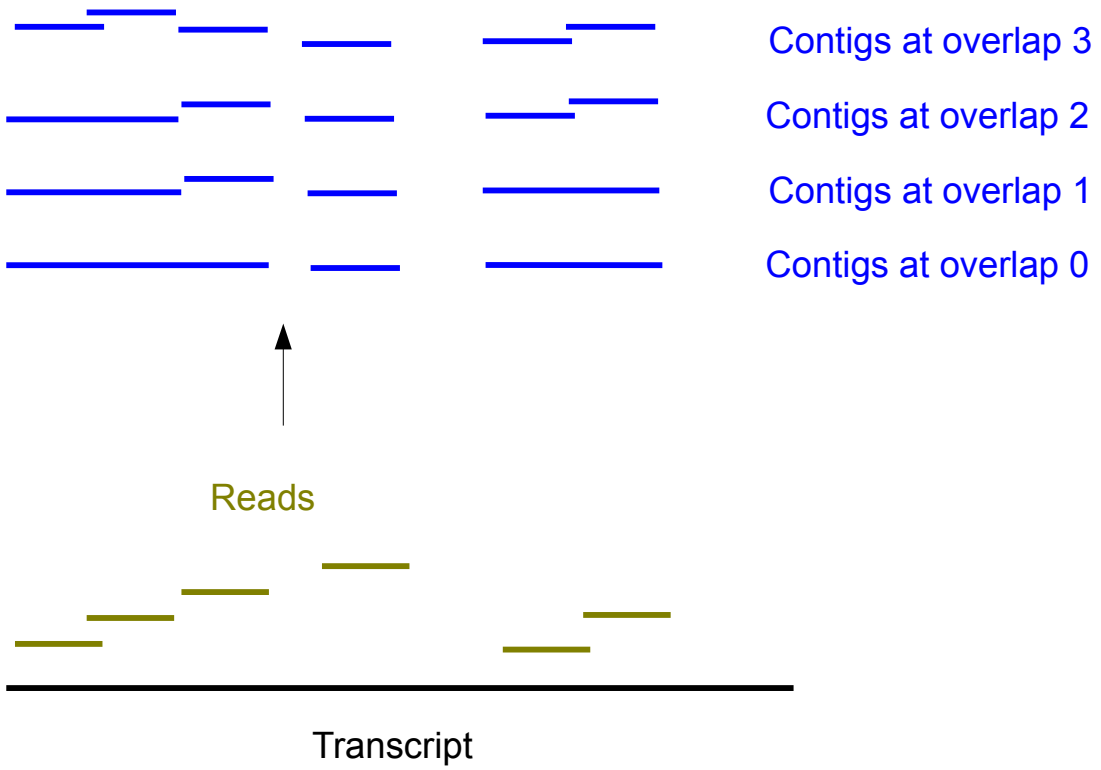
# The Score Is Based on a Probability Model

Our score: $P(\text{assembly}, \text{reads}) \propto P(\text{assembly}|\text{reads})$.

$$
\begin{aligned}
&P(\text{assembly}, \text{reads}) \\
&= \int P(\text{assembly}, \text{coverage}, \text{reads})\, d\text{coverage} \\
&= \int \underbrace{P(\text{assembly}, \text{coverage})}_{\text{prior}} \underbrace{P(\text{reads}|\text{assembly}, \text{coverage})}_{\text{likelihood}}\, d\text{coverage}
\end{aligned}
$$

A contig's "coverage" is the expected number of reads generated from each position of the contig's original transcript.

# The Probability Model Is Based on Ideal Assembly



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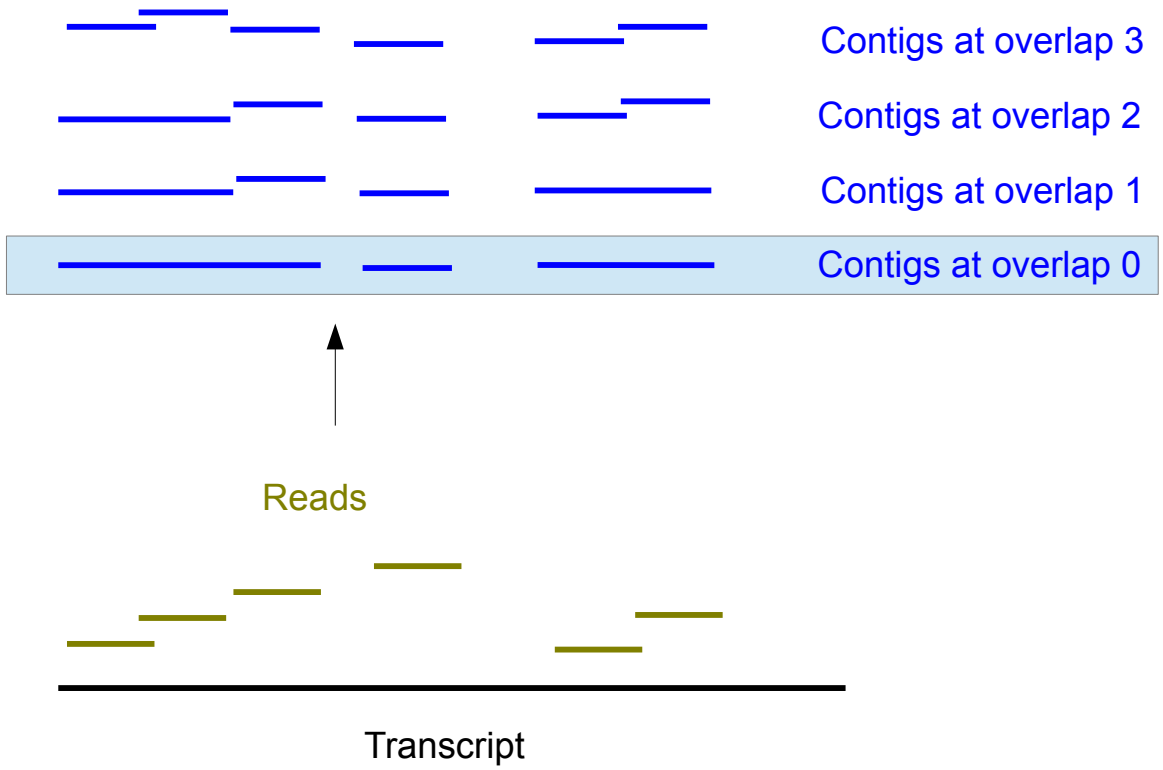


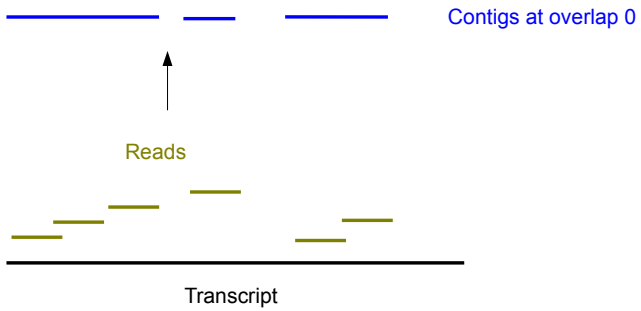
# The Probability Model - Prior

$$
\begin{aligned}
& P(\text{assembly}, \text{reads}) \\
&= \int P(\text{assembly}, \text{coverage}, \text{reads})\, d\text{coverage} \\
&= \int \underbrace{P(\text{assembly}, \text{coverage})}_{\text{prior}} \underbrace{P(\text{reads} | \text{assembly}, \text{coverage})}_{\text{likelihood}}\, d\text{coverage}
\end{aligned}
$$

# The Probability Model - Prior

Generative story:

- Transcript lengths $\sim_{iid}$ negative binomial.
- Given the transcript lengths:
  - Transcript sequences $\sim_{iid}$ uniform.
  - Number of reads starting at each position of a transcript $\sim_{iid}$ Poisson (mean = coverage).
- The assembly is formed from the reads at overlap 0.



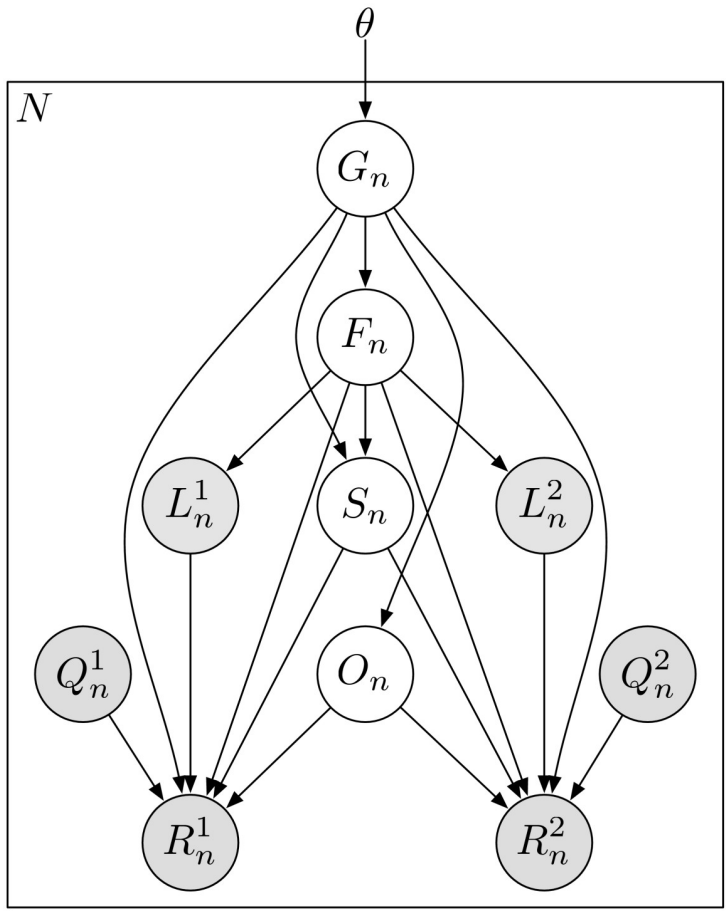

One can work out a recurrence for the prior probability of the assembly and coverage.

# The Probability Model - Likelihood

$$
\begin{aligned}
& P(\text{assembly}, \text{reads}) \\
&= \int P(\text{assembly}, \text{coverage}, \text{reads})\, d\text{coverage} \\
&= \int \underbrace{P(\text{assembly}, \text{coverage})}_{\text{prior}} \boxed{\underbrace{P(\text{reads}|\text{assembly}, \text{coverage})}_{\text{likelihood}}}\, d\text{coverage}
\end{aligned}
$$

# The Probability Model - Likelihood

Previous work, RSEM, introduced a generative model of reads, given transcripts and their expression.



# The Probability Model - Likelihood

Key observation:

- Generating from contigs $\equiv$ generating from transcripts, except that contigs are guaranteed to be covered by reads.

Therefore, we stipulate:

$$P(\text{reads}|\text{assembly}, \text{coverage}) = \frac{P_{RSEM}(\text{reads}|\text{transcripts} = \text{assembly}, \text{expression} = f(\text{coverage}))}{P_{RSEM}(\text{reads cover assembly}|\text{transcripts} = \text{assembly}, \text{expression} = f(\text{coverage}))}$$

# The Probability Model - Marginalization

$$P(\text{assembly}, \text{reads})$$

$$= \int P(\text{assembly}, \text{coverage}, \text{reads}) \, d\text{coverage}$$

$$= \int \underbrace{P(\text{assembly}, \text{coverage})}_{\text{prior}} \underbrace{P(\text{reads}|\text{assembly}, \text{coverage})}_{\text{likelihood}} \, d\text{coverage}$$

# The Probability Model - Marginalization

Approximate the integral by BIC:

$$\begin{aligned}
&\log P(\text{assembly}, \text{reads}) \\
&= \log \int P(\text{assembly}, \text{coverage}, \text{reads})\, d\text{coverage} \\
&= \log P(\text{assembly}, \text{reads} | \text{coverage}^*) - \frac{1}{2} M \log N
\end{aligned}$$

where $M =$ number of contigs, $N =$ number of reads,
coverage$^*$ = maximum likelihood estimate.

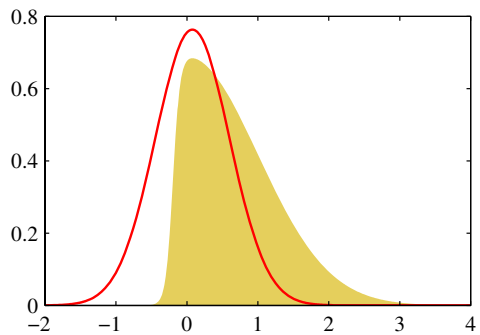
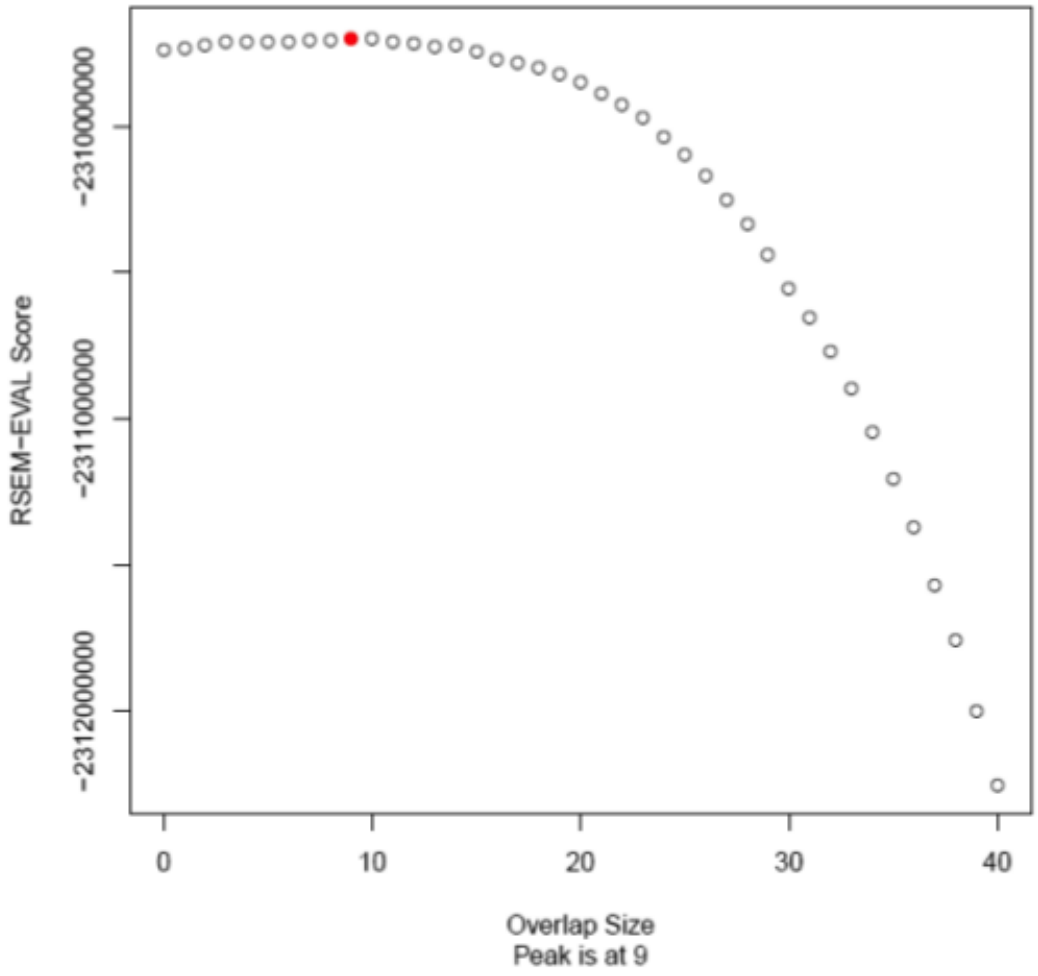



Figure from Bishop, *Pattern Recognition and Machine Learning*, Springer, 2009.

# Experiment 0 - Setup

- Goal: Make sure we have avoided trivialities.
- Procedure:
  - Construct ideal assembly at every possible overlap.
  - Compute score.
- Desired result: Best overlap is fairly close to 0.

# Experiment 0 - Results



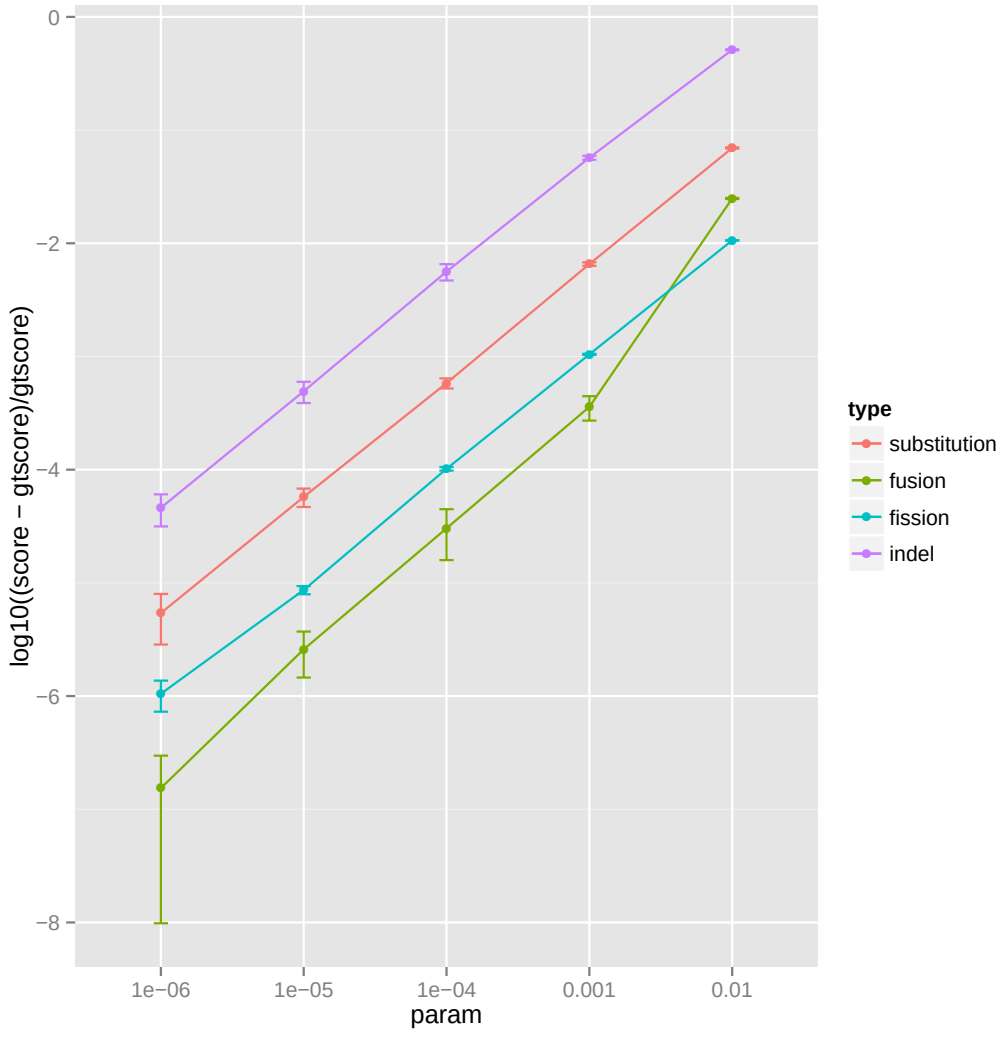
# Experiment 1 - Setup

- Goal: Make sure the true best assembly has the best score, on average.

- Procedure:
  - Construct ideal assembly at overlap 0.
  - Perturb this assembly:
    - Substitution - substitute a base.
    - Fusion - join two contigs into one contig.
    - Fission - split one contig into two contigs.
    - Indel - insert or delete a fragment from a contig.
  - Compute score for ideal and perturbed assemblies.

- Desired result: The ideal assembly has the best score.

# Experiment 1 - Results



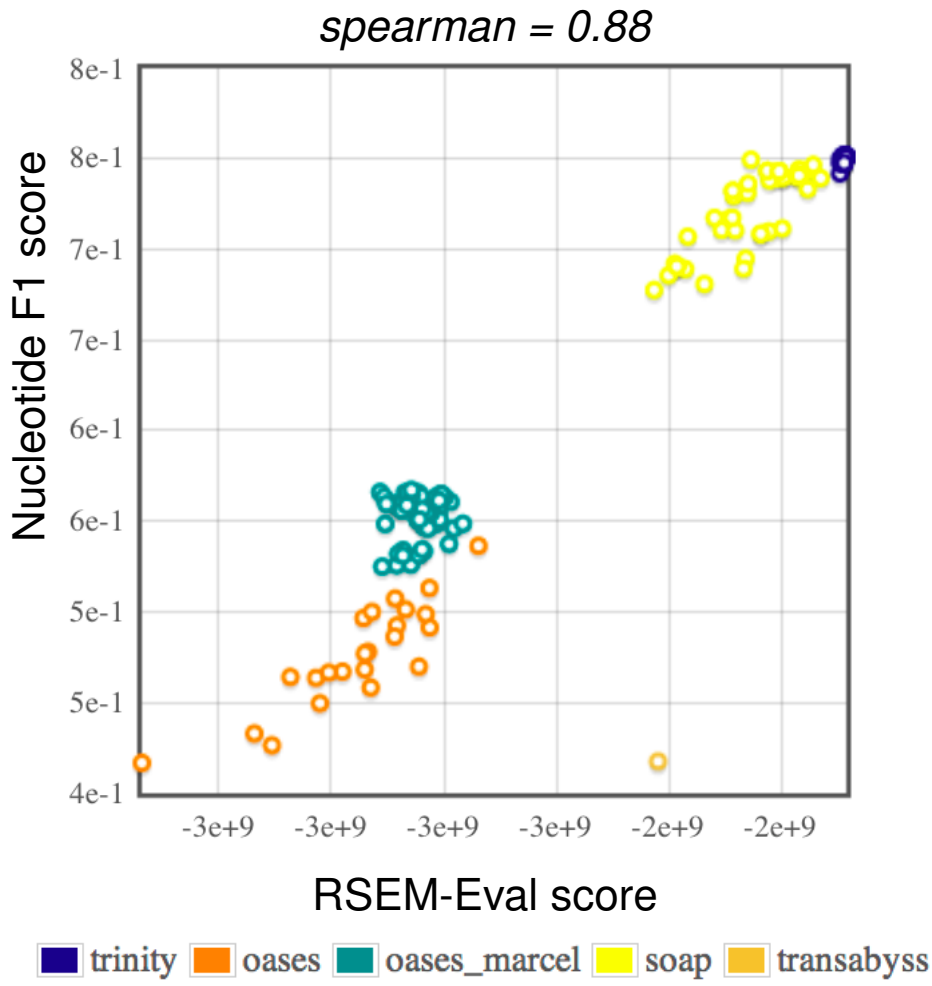
# Experiment 2 - Setup

- Goal: Study the correlation between our score and simple reference-based scores.
- Five datasets:
  - Mouse from Trinity paper.
  - Mouse from Oases paper.
  - Yeast from Trinity paper.
  - Axolotl from Thompson lab.
  - Simulated mouse.
- $\sim$100 assemblies per dataset, using:
  - Trinity.
  - Oases.
  - SOAPdenovo-trans.
  - Trans-ABySS.

# Experiment 2 - Setup

- 3 reference-based F1 scores (harmonic mean of precision and recall):
  - Nucleotide F1.
  - Transcript F1.
  - Pair F1.
- 1 reference-based “k-mer” score:
  - Jensen-Shannon divergence between k-mer distributions.
- Procedure:
  - For each assembly: compute our *de novo* score and each reference-based score.
- Expected result:
  - Monotone relationship between the scores.

# Experiment 2 - Results



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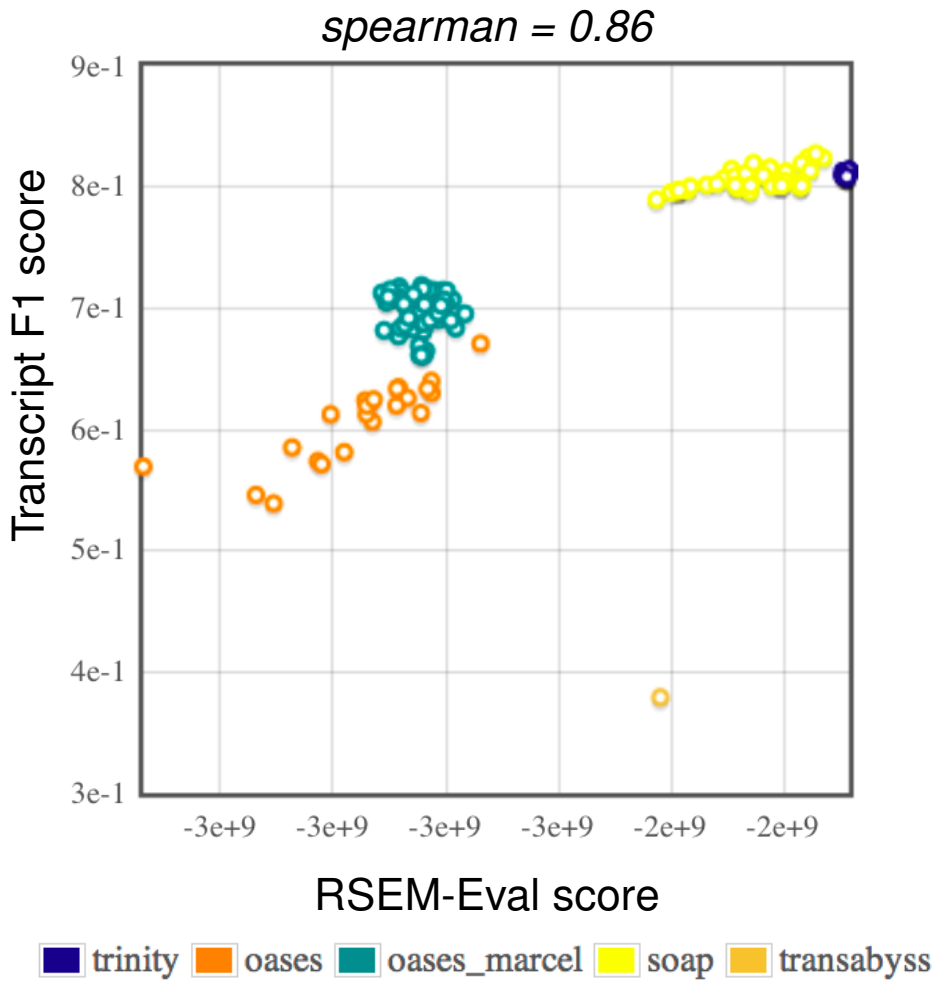

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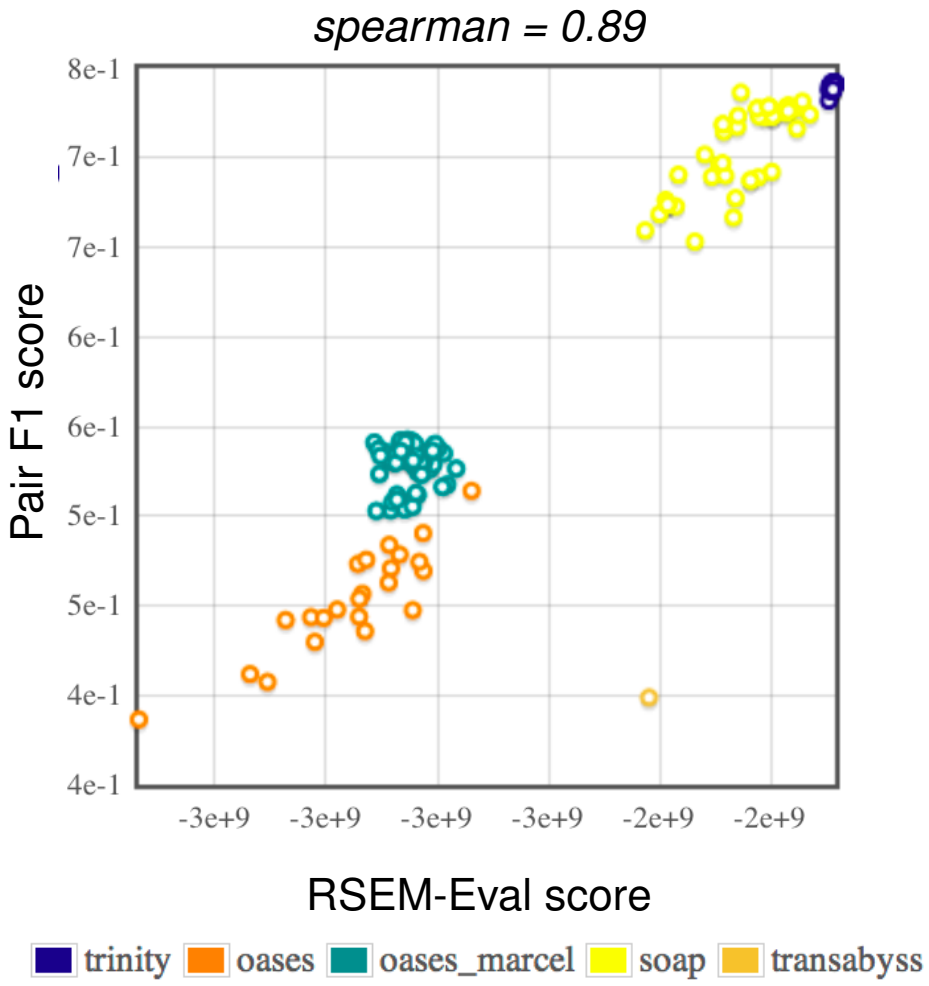

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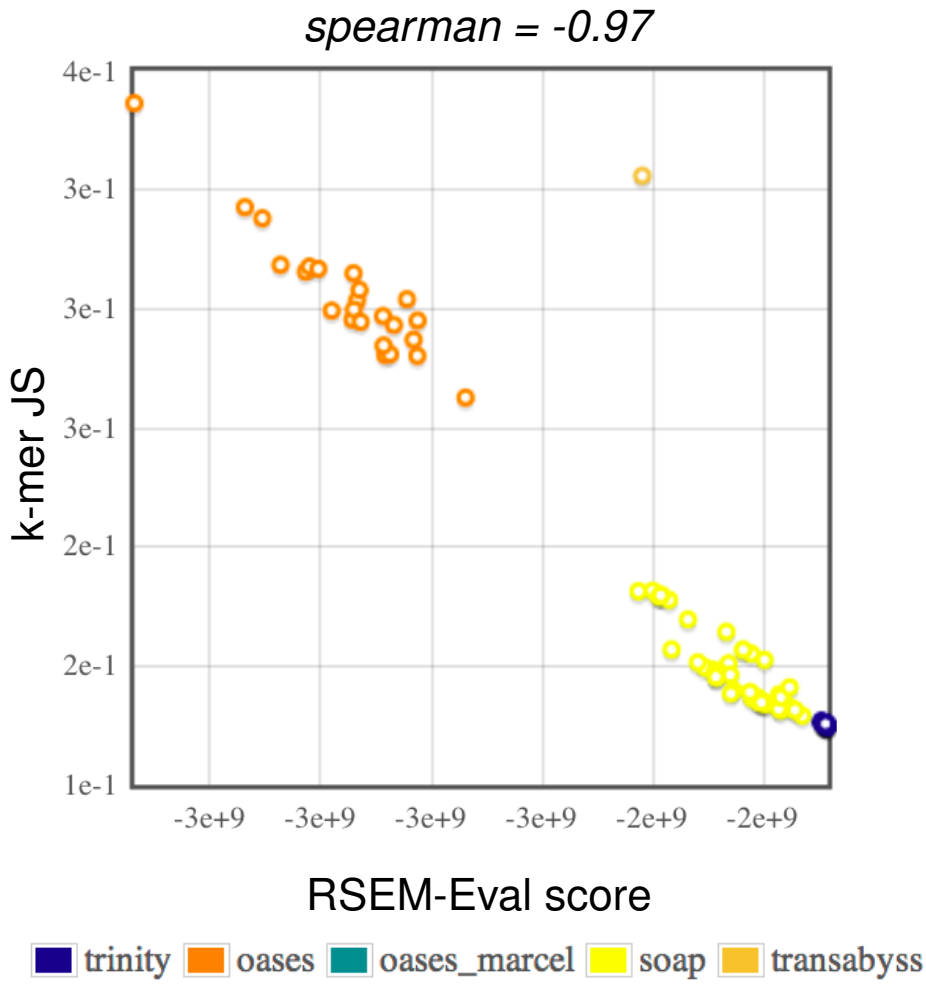

Thanks.

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