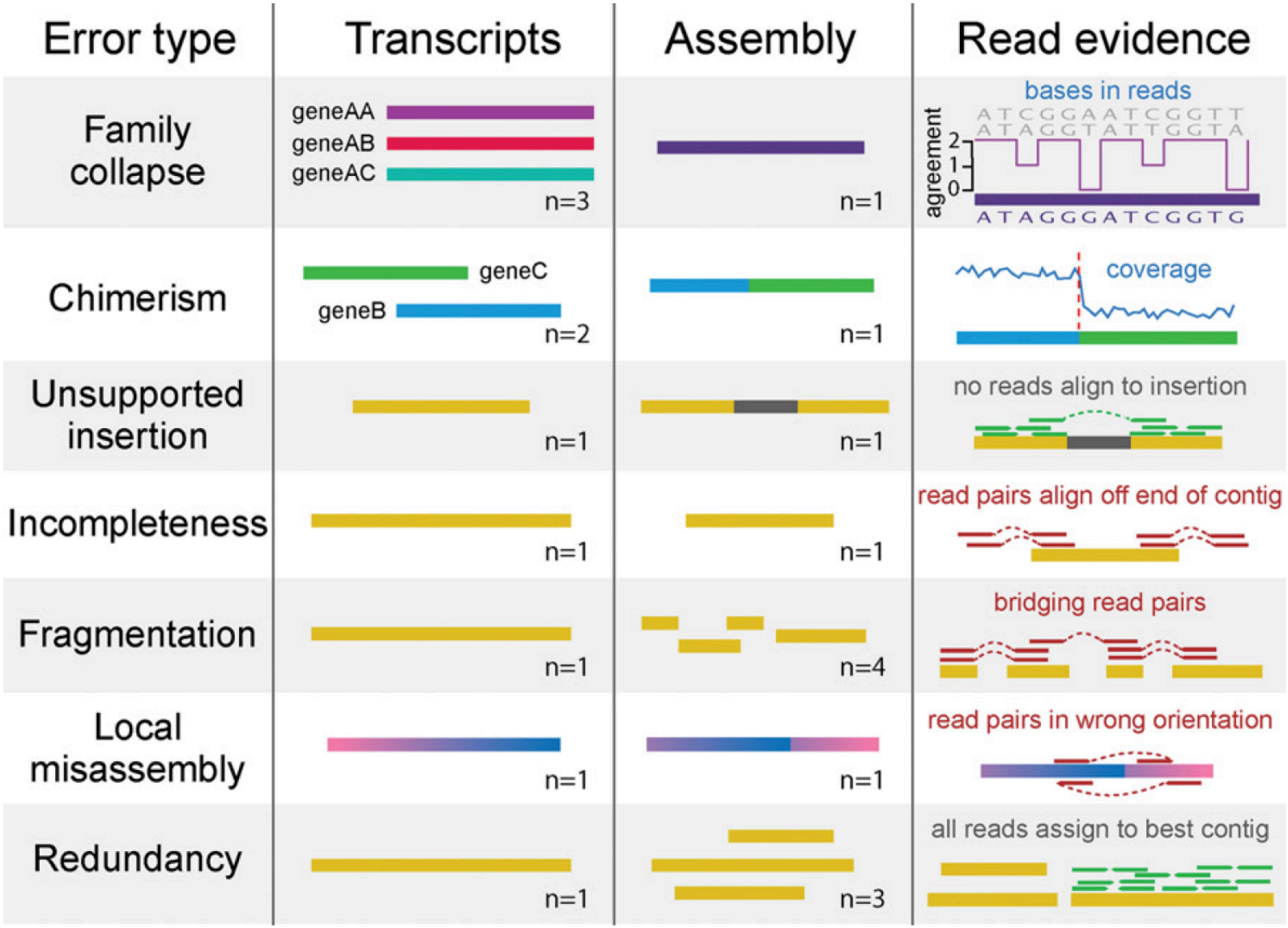
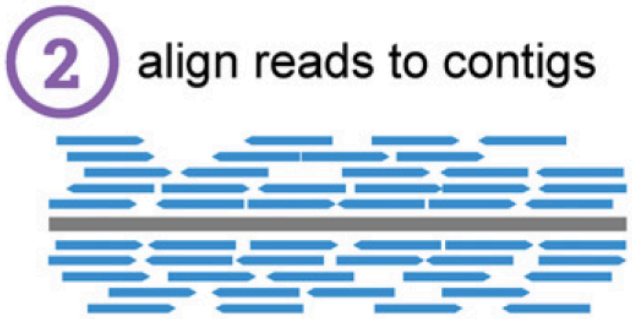
Evaluating the quality of your transcriptome assembly

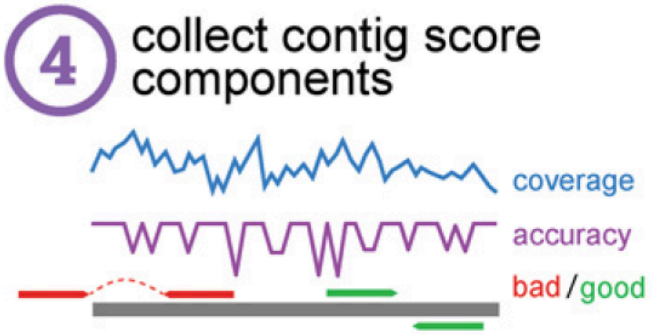
De novo Transcriptome Assembly is Prone to Certain Types of Errors

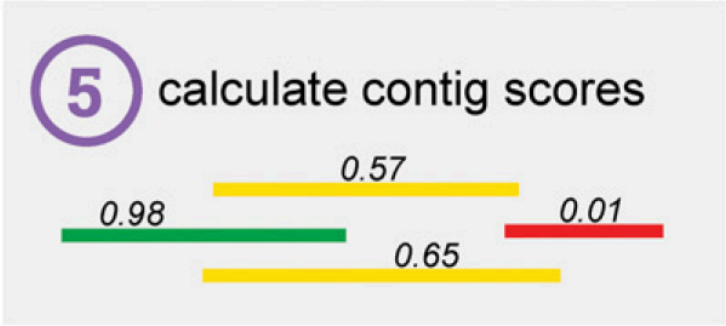


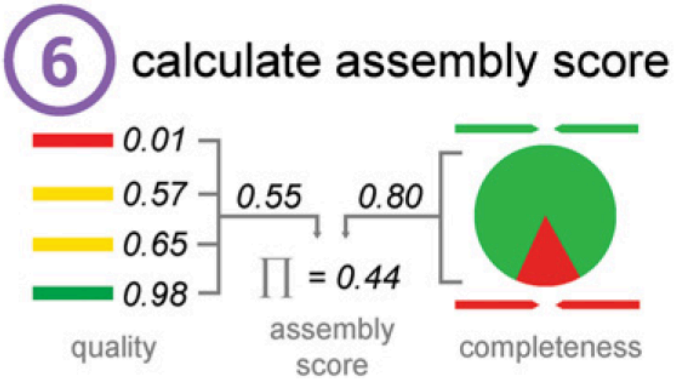
Smith-Unna et al. Genome Research, 2016

TransRate









Smith-Unna et al. Genome Research, 2016

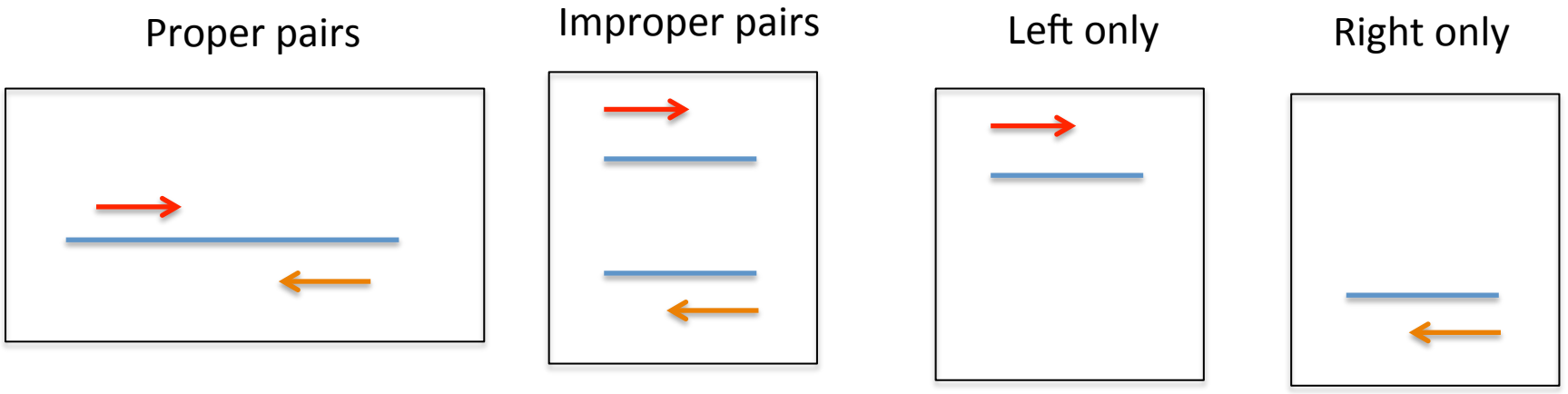
Simple Quantitative and Qualitative Assembly Metrics

Read representation by assembly

Align reads to the assembled transcripts using Bowtie.
A typical 'good' assembly has ~80 % reads mapping to the assembly and ~80% are properly paired.

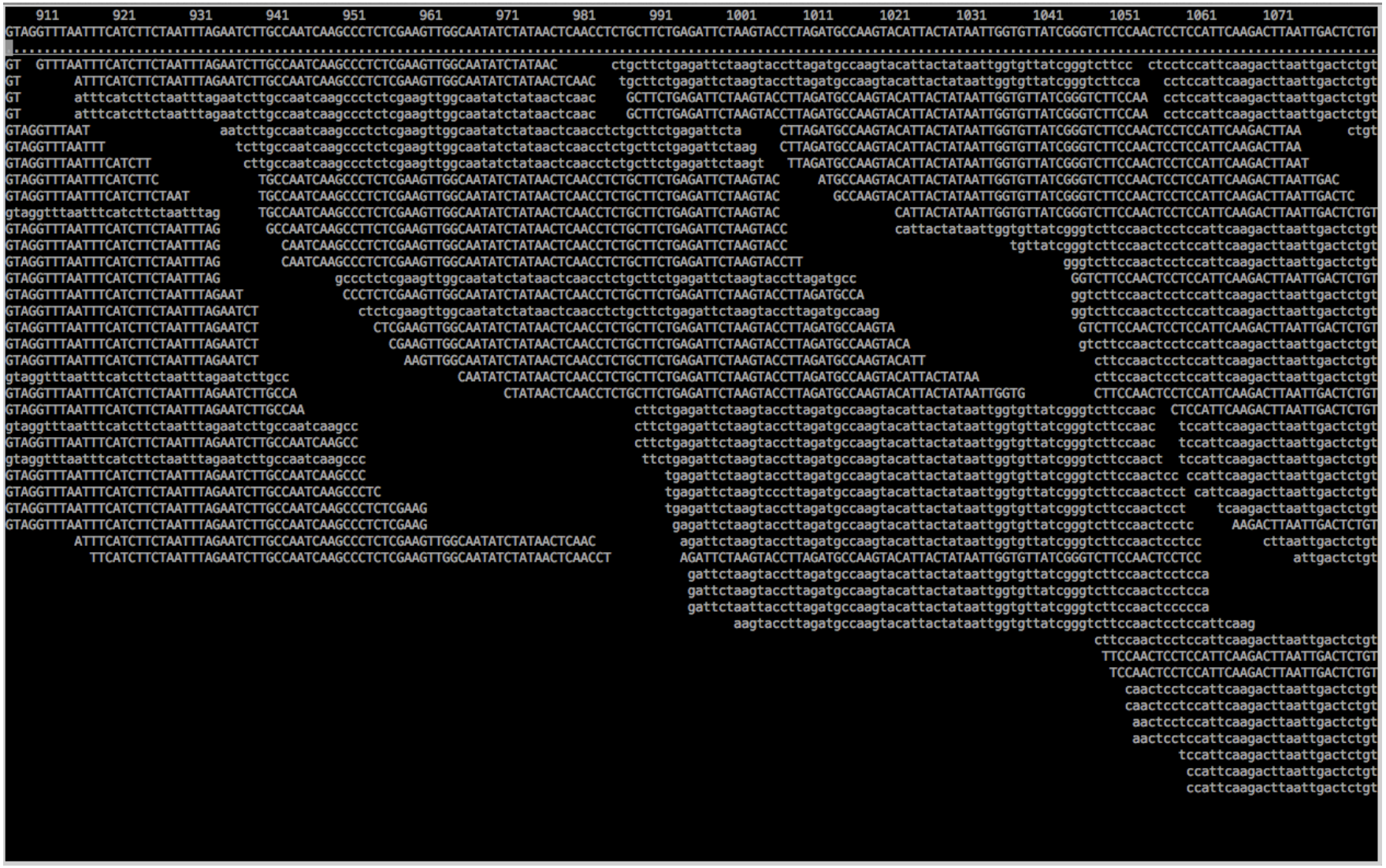
Given read pair: 

Possible mapping contexts in the Trinity assembly are reported:



Assembled transcript contig is only as good as its read support.

% samtools tview alignments.bam target.fasta



IGV

www.broadinstitute.org/igv/

Integrative Genomics Viewer

- Home
- Downloads
- Documents
  - Hosted Genomes
  - FAQ
  - IGV User Guide
  - File Formats
  - Release Notes
  - Credits
- Contact

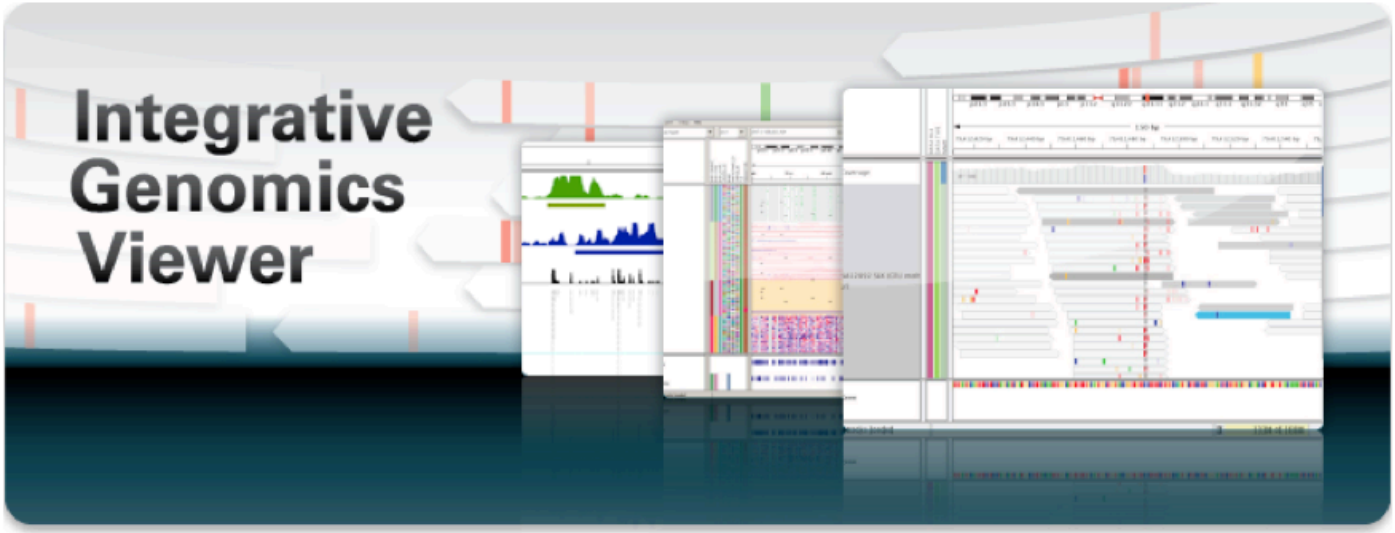
Search website

search

Broad Home
Cancer Program

BROAD INSTITUTE

Home



What's New

July 3, 2012. Soybean (Glycine max) and Rat (rn5) genomes have been updated.

April 20, 2012. IGV 2.1 has been released. See the release notes for more details.

April 19, 2012. See our new IGV paper in Briefings in Bioinformatics.

Overview

Citing IGV

To cite your use of IGV in your publication:

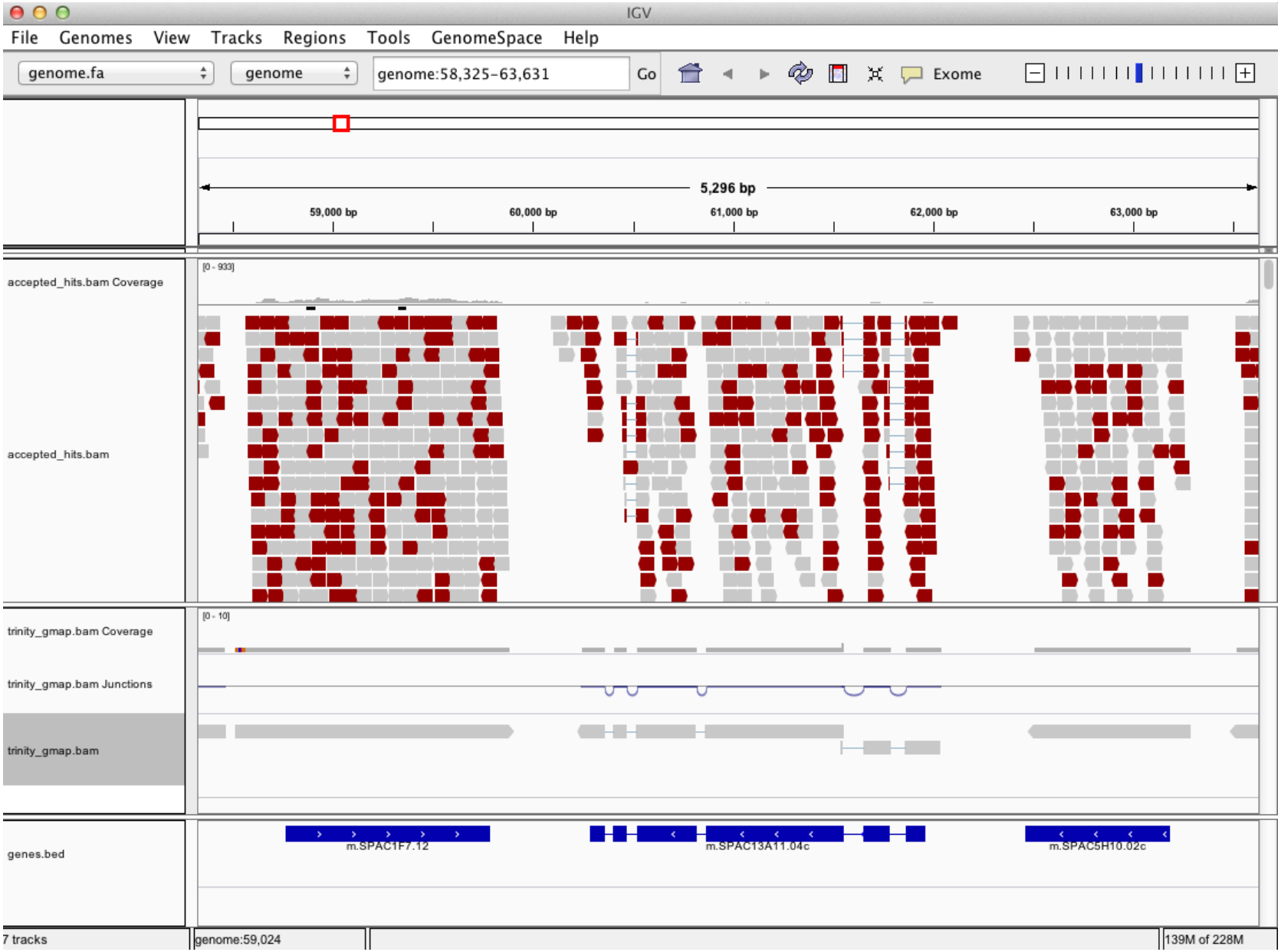
James T. Robinson, Helga Thorvaldsdóttir, Wendy Winckler, Mitchell Guttman, Eric S. Lander, Gad Getz, Jill P. Mesirov. **Integrative Genomics Viewer**. Nature Biotechnology 29, 24–26 (2011), or

Helga Thorvaldsdottir, James T. Robinson, Jill P. Mesirov. **Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration.**

Can Examine Transcript Read Support Using IGV

Can align Trinity transcripts to genome scaffolds to examine intron/exon structures

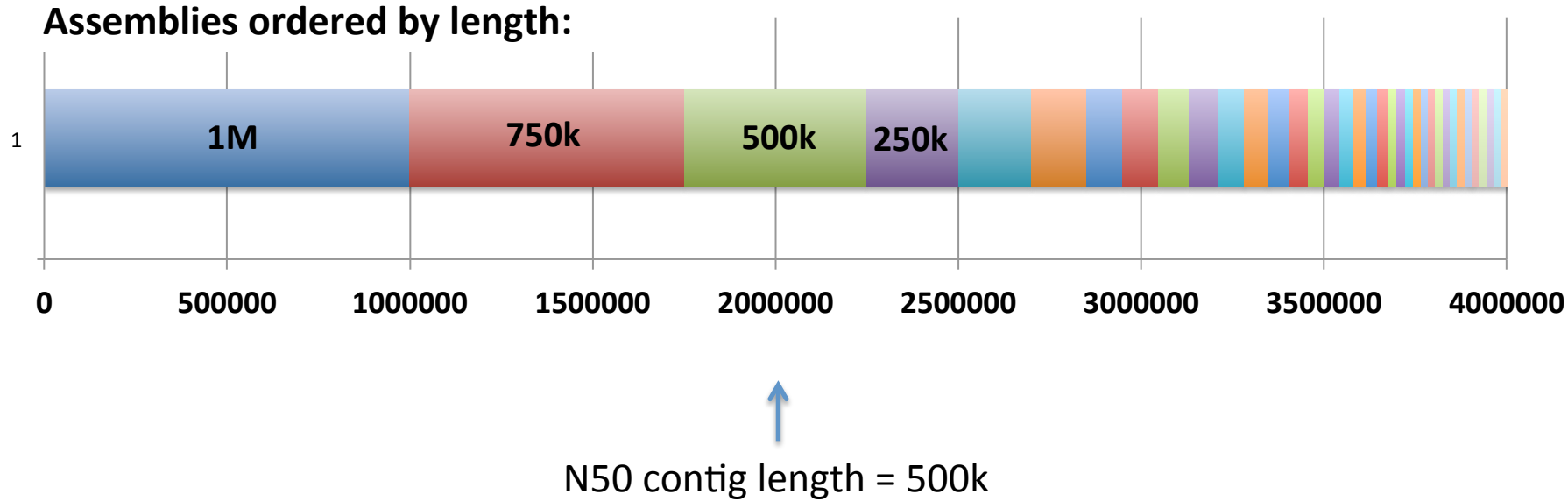
(Trinity transcripts aligned to the genome using GMAP)



The Contig N50 statistic

"At least half of assembled bases are in contigs that are at least ***N50*** bases in length"

In genome assemblies – used often to judge 'which assembly is better'



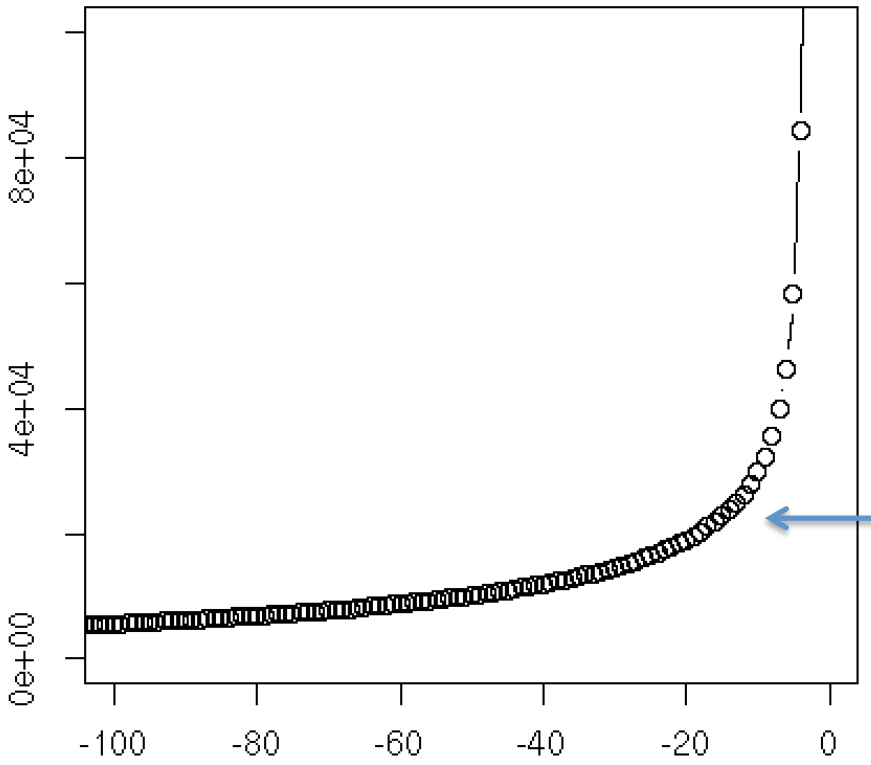
Often, most assembled transcripts are *very* lowly expressed

(How many 'transcripts & genes' are there really?)

1.4 million Trinity transcript contigs

N50 ~ 500 bases

Cumulative # of Transcripts

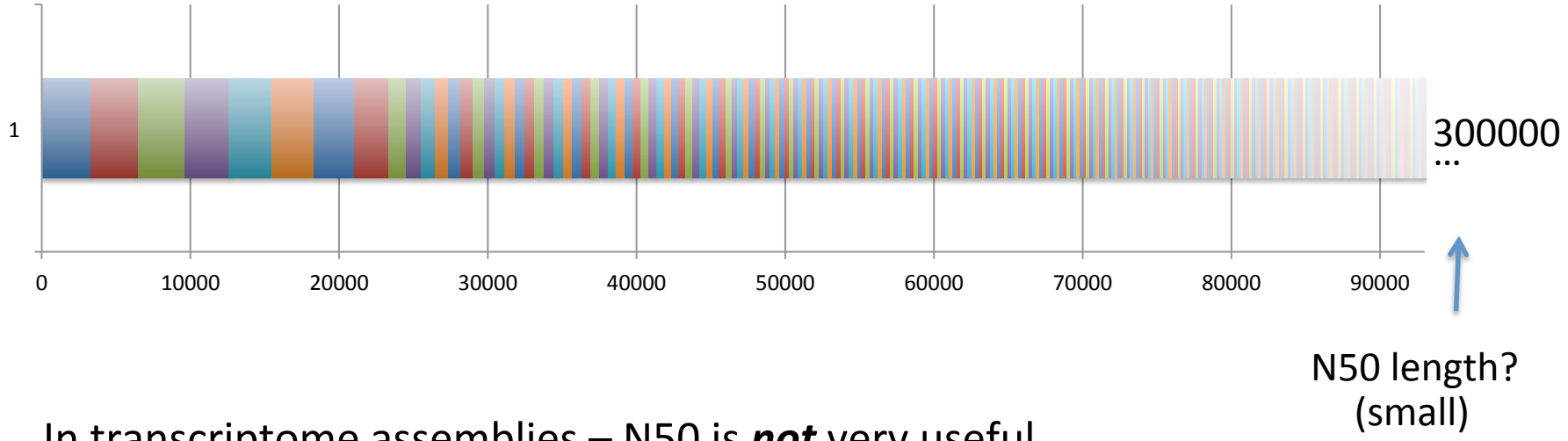


20k transcripts

Expression

* Salamander transcriptome

N50 Calculation for *Transcriptome* Assemblies??

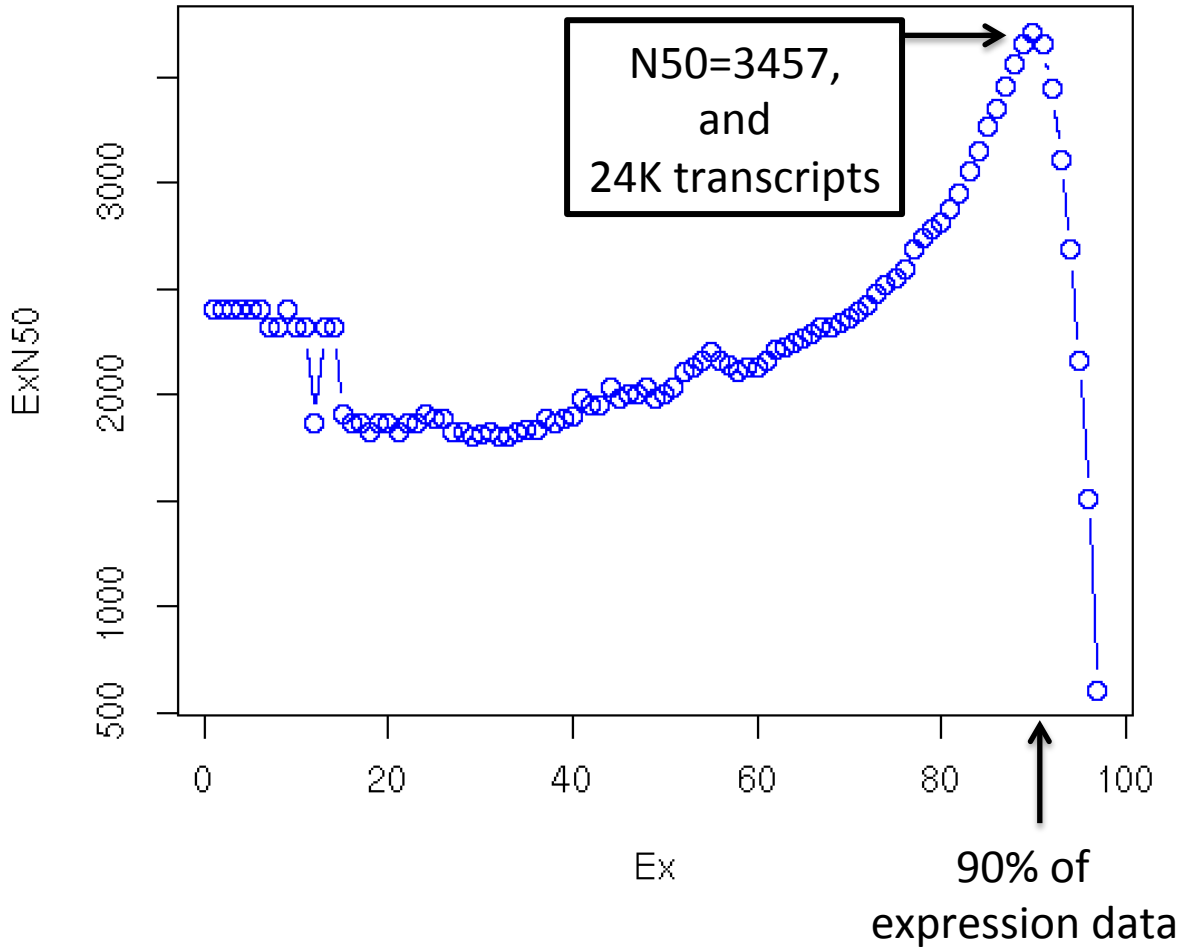


In transcriptome assemblies – N50 is ***not*** very useful.

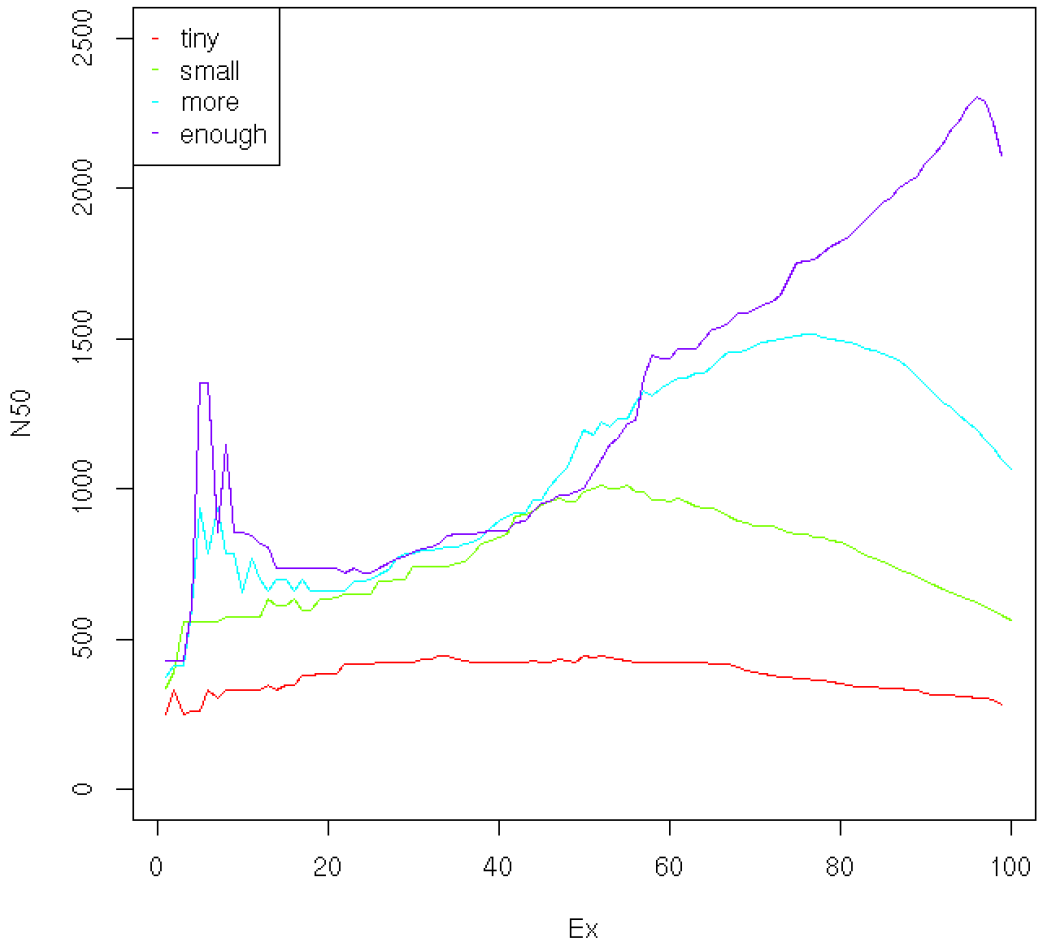
- Overzealous isoform annotation for long transcripts drives higher N50
- Very sensitive reconstruction for short lowly expressed transcripts drives lower N50

Compute N50 Based on the Top-most Highly Expressed Transcripts (ExN50)

- Sort contigs by expression value, descendingly.
- Compute N50 given minimum % total expression data thresholds => ExN50



ExN50 Profiles for Different Trinity Assemblies Using Different Read Depths



Millions of Reads

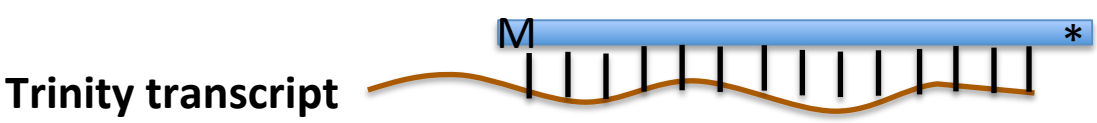
Thousands of Reads

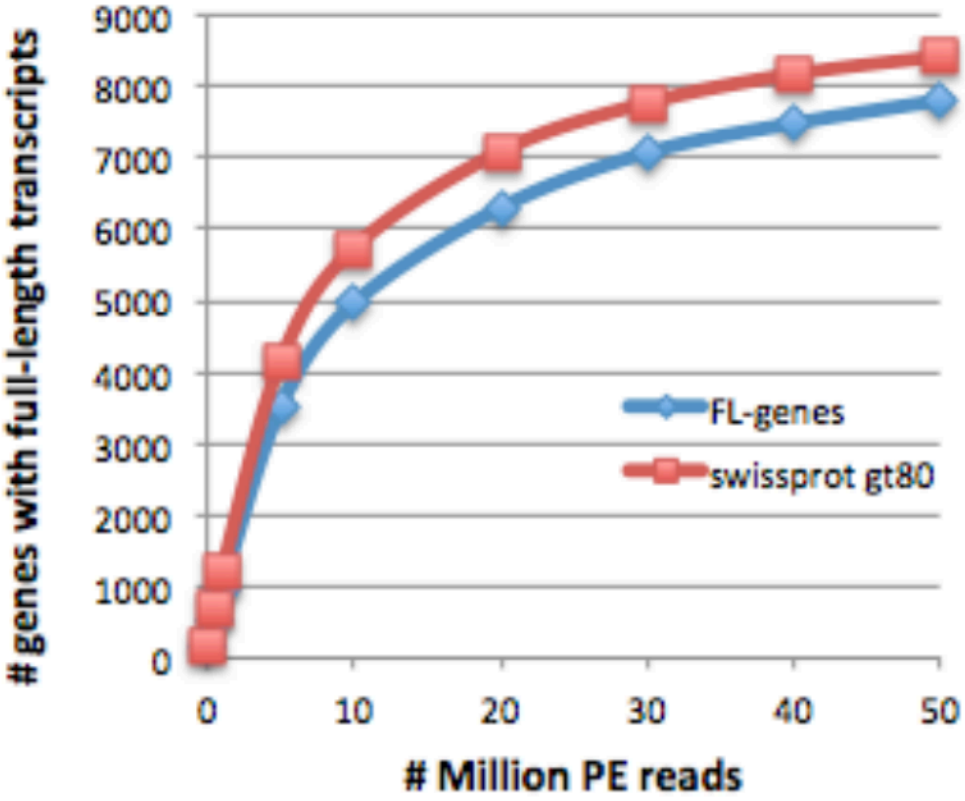
Note shift in ExN50 profiles as you assemble more and more reads.

* Candida transcriptome

Evaluating the quality of your transcriptome assembly

Full-length Transcript Detection via BLASTX





Have you sequenced deeply enough?

* Mouse transcriptome

Haas et al. Nat. Protoc. 2013

busco.ezlab.org

UNIVERSITÉ DE GENÈVE
FACULTÉ DE MÉDECINE

Zdobnov's Computational Evolutionary Genomics group

CEGG Home | OrthoDB *v9* | BUSCO *v2*

BUSCO v2

Assessing genome assembly and annotation completeness with **B**enchmarking **U**niversal **S**ingle-**C**opy **O**rthologs

About BUSCO

BUSCO *v2* provides quantitative measures for the assessment of genome assembly, gene set, and transcriptome completeness, based on evolutionarily-informed expectations of gene content from near-universal single-copy orthologs selected from OrthoDB *v9*.

BUSCO assessments are implemented in open-source software, with a large selection of lineage-specific sets of Benchmarking Universal Single-Copy Orthologs. These conserved orthologs are ideal candidates for large-scale phylogenomics studies, and the annotated BUSCO gene models built during genome assessments provide a comprehensive gene predictor training set for use as part of genome annotation pipelines.

busco.ezlab.org

UNIVERSITÉ DE GENÈVE
FACULTÉ DE MÉDECINE

Zdobnov's Computational Evolutionary Genomics group

CEGG Home | OrthoDB *v9* | BUSCO *v2*

BUSCO v2

Assessing genome assembly and annotation completeness with Benchmarking Universal Single-Copy Orthologs

#Summarized BUSCO benchmarking for file: Trinity.fasta
#BUSCO was run in mode: trans

Summarized benchmarks in BUSCO notation:

C:88%[D:53%],F:4.5%,M:7.3%,n:3023

Representing:

1045 Complete Single-copy BUSCOs
1617 Complete Duplicated BUSCOs
139 Fragmented BUSCOs
222 Missing BUSCOs
3023 Total BUSCO groups searched

Detonate: Which assembly is better?

"RSEM-EVAL [sic] uses a novel probabilistic model-based method to compute the joint probability of both an assembly and the RNA-Seq data as an evaluation score."

$$\text{score}_{\text{RSEM-EVAL}}(A) = \log P(A, D)$$

"the RSEM-EVAL score of an assembly is defined as the log joint probability of the assembly A and the reads D used to construct it"

$$\begin{aligned}\log P(A, D) &= \log \int_{\Lambda} P(D|A, \Lambda) P(A|\Lambda) P(\Lambda) d\Lambda \\ &\approx \underbrace{\log P(D|A, \Lambda_{\text{MLE}})}_{\text{likelihood}} + \underbrace{\log P(A|\Lambda_{\text{MLE}})}_{\text{assembly prior}} \\ &\quad - \underbrace{\frac{1}{2}(M+1)\log N}_{\text{BIC penalty}},\end{aligned}$$

Li et al. **Evaluation of de novo transcriptome assemblies from RNA-Seq data**, Genome Biology 2014

Detonate: Which assembly is better?

"RSEM-EVAL [sic] uses a novel probabilistic model-based method to compute the joint probability of both an assembly and the RNA-Seq data as an evaluation score."

$$\text{score}_{\text{RSEM-EVAL}}(A) = \log P(A, D)$$

"the RSEM-EVAL score of an assembly is defined as the log joint probability of the assembly A and the reads D used to construct it"

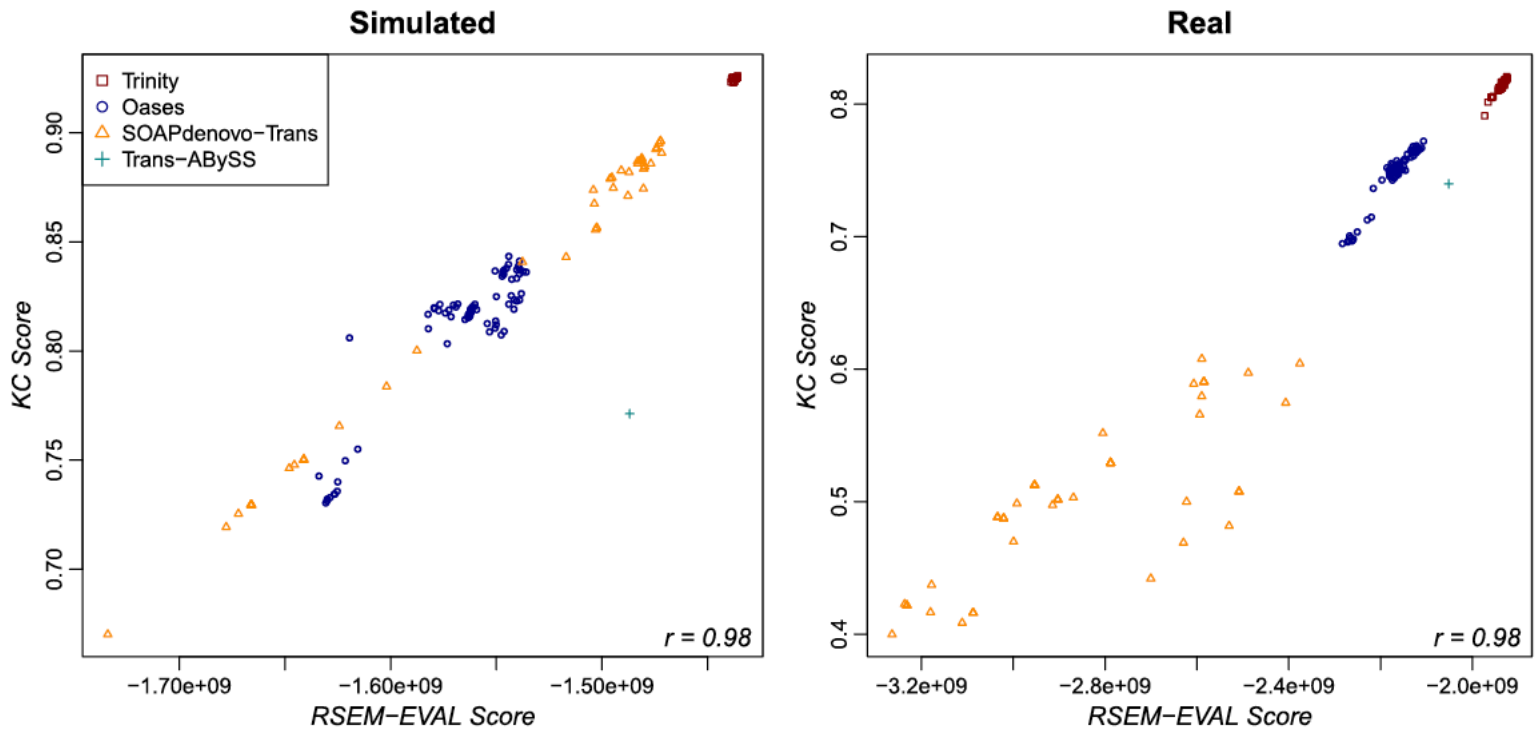
$$\log P(A,D) = \log \int_{\Lambda} P(D|A,\Lambda)P(A|\Lambda)P(\Lambda)d\Lambda$$
$$\approx \underbrace{\log P(D|A,\Lambda_{\text{MLE}})}_{\text{likelihood}} + \underbrace{\log P(A|\Lambda_{\text{MLE}})}_{\text{assembly prior}}$$
$$\underbrace{-\frac{1}{2}(M+1)\log N,}_{\text{BIC penalty}}$$

Bigger Score = Better Assembly

Li et al. **Evaluation of de novo transcriptome assemblies from RNA-Seq data**, Genome Biology 2014

Detonate: Which assembly is better?

"RSEM-EVAL [sic] uses a novel probabilistic model-based method to compute the joint probability of both an assembly and the RNA-Seq data as an evaluation score."



Yay Trinity!!

Li et al. **Evaluation of de novo transcriptome assemblies from RNA-Seq data**, Genome Biology 2014