The Krumlov Trinity Transcriptomics Experience

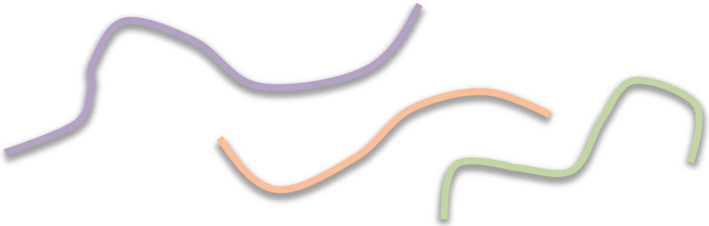
Brian Haas

Broad Institute

Workshop on Genomics, Cesky Krumlov, Jan 2020

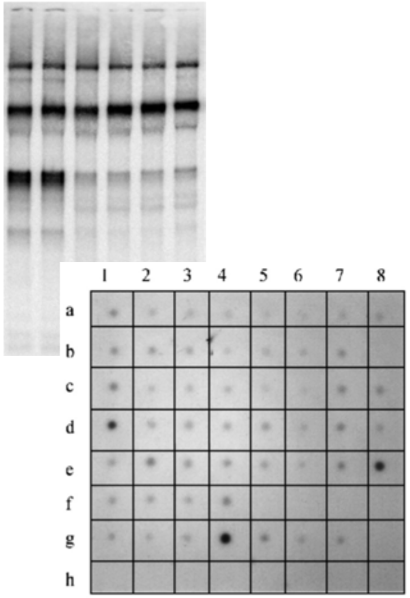
Biological Investigations Empowered by Transcriptomics

Extract RNA,
... some protocol for processing, ...

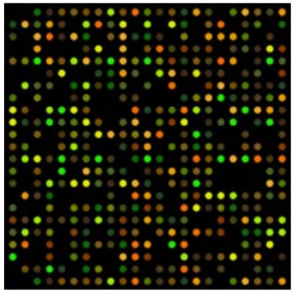


Analysis Method
(pick your favorite)

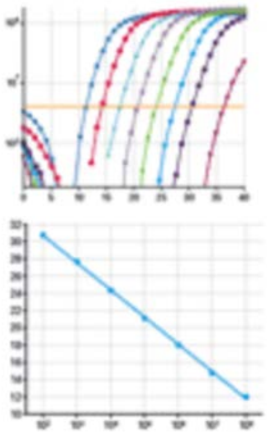
Northern



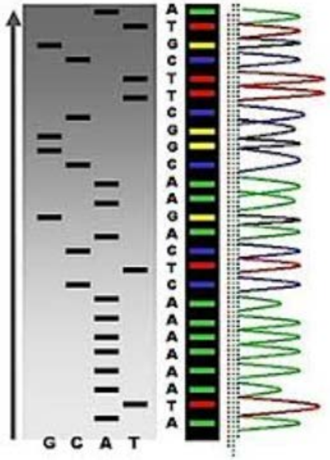
Dot Blot



Microarray



qRT-PCR



Sanger Sequencing

Other...

Minion

MinION MkI: portable, real time biological analyses

MinION

Historical Timeline to Modern Transcriptomics (from 1970)

Reverse Transcription (1970)

Northern Blot
Sanger Sequencing
(1977)

Expressed Sequence Tags (1992)

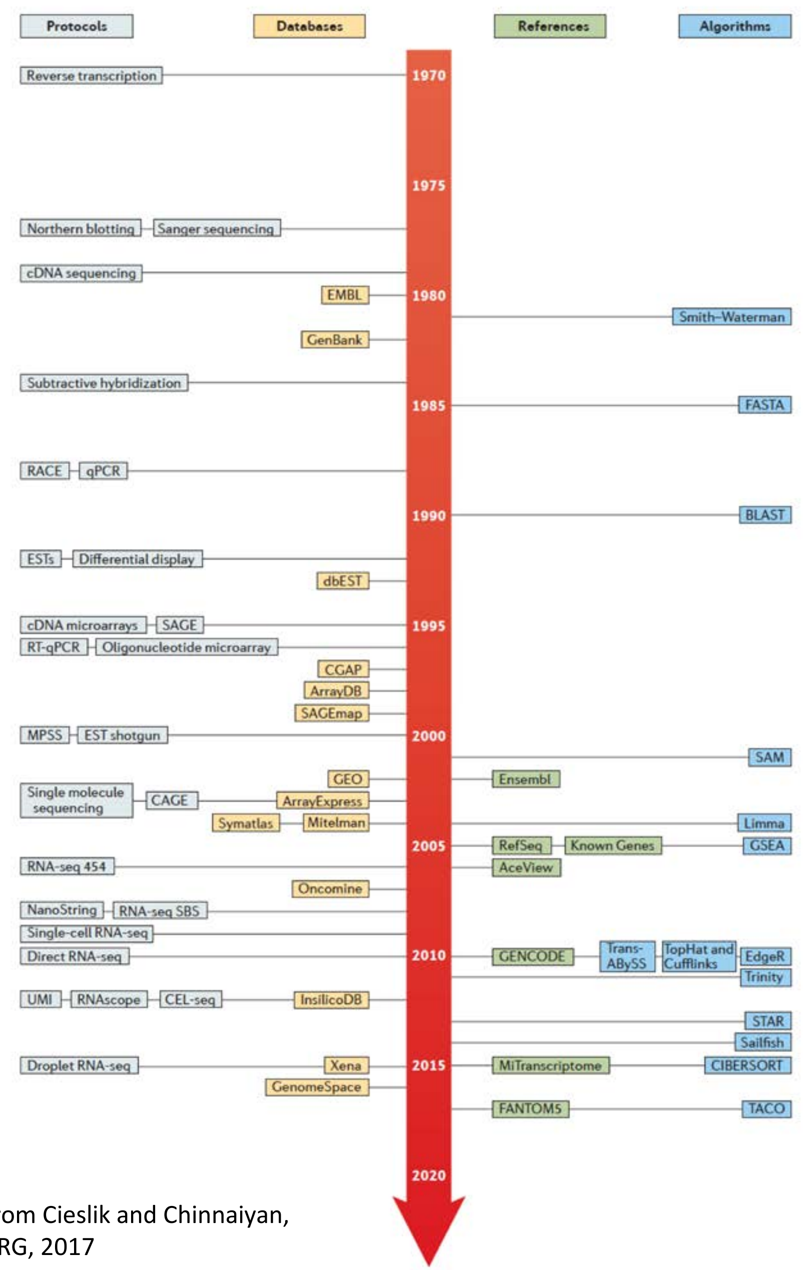
cDNA microarrays (1995)

RNA-Seq (2006-2008)

PacBio IsoSeq (2014)

Droplet single cell RNA-Seq (2015)

Direct RNA Seq Nanopore (2018)



From Cieslik and Chinnaiyan, NRG, 2017

Note: Just a small sampling of what's available.

Smith Waterman (1981)

BLAST (1990)

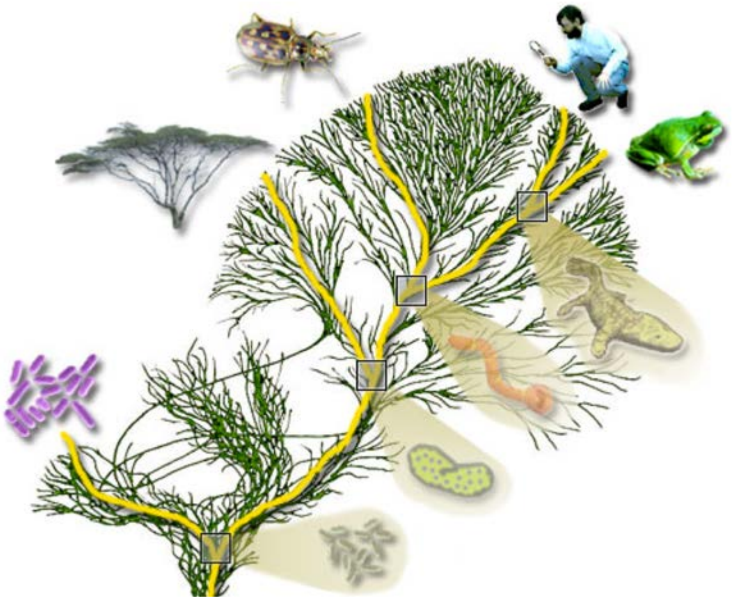
Tophat/Cufflinks (2010)

RSEM

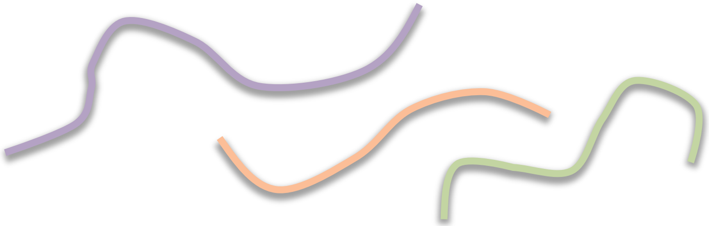
(2011)

Kallisto (2016)
Salmon (2017)

Modern Transcriptome Studies Empowered by RNA-seq



Extract RNA, convert to cDNA

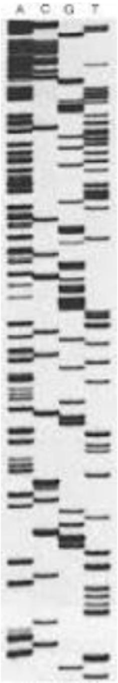


Next-gen Sequencer
(pick your favorite)

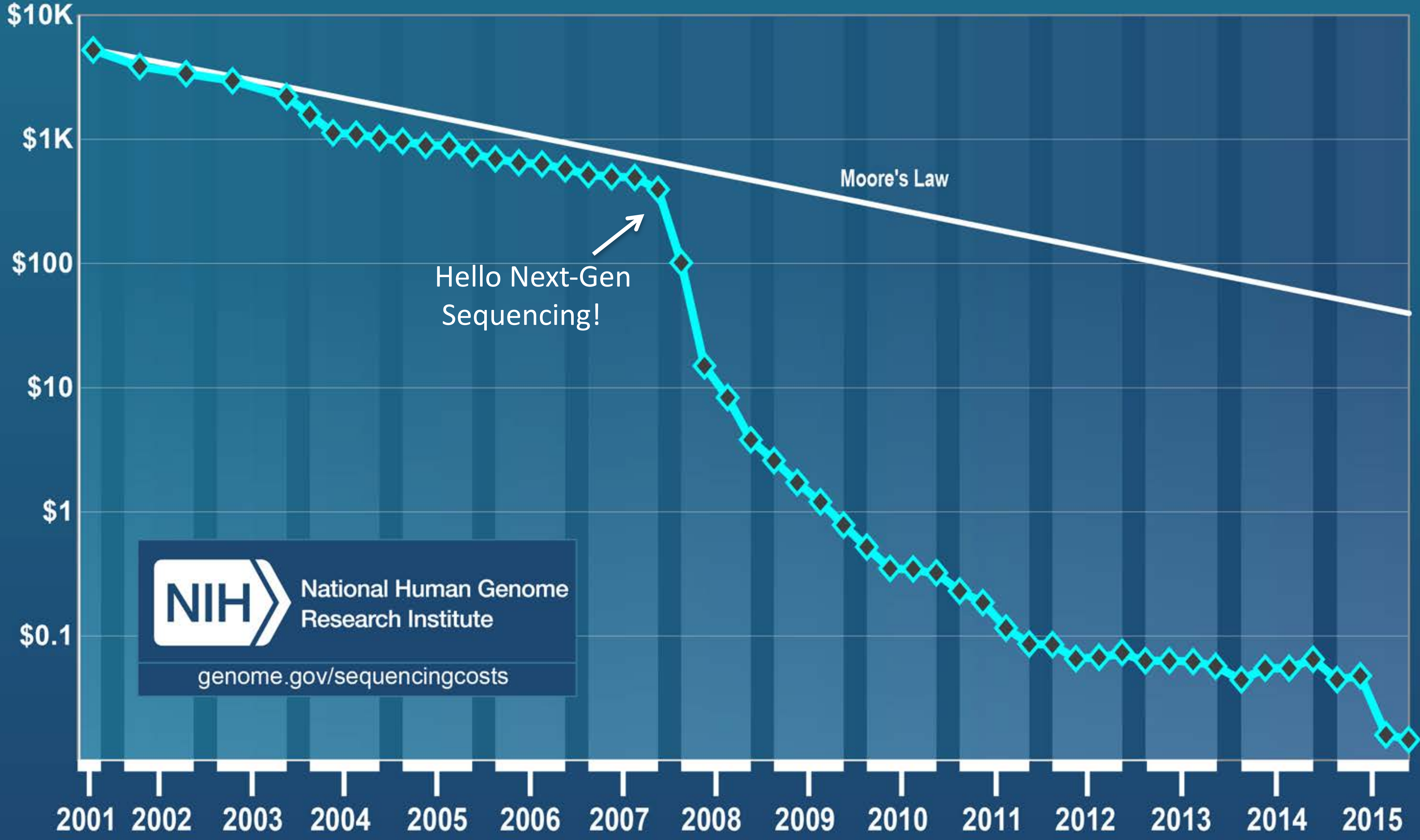
Millions to Billions of Reads

Personal Reflections...

Circa 1995



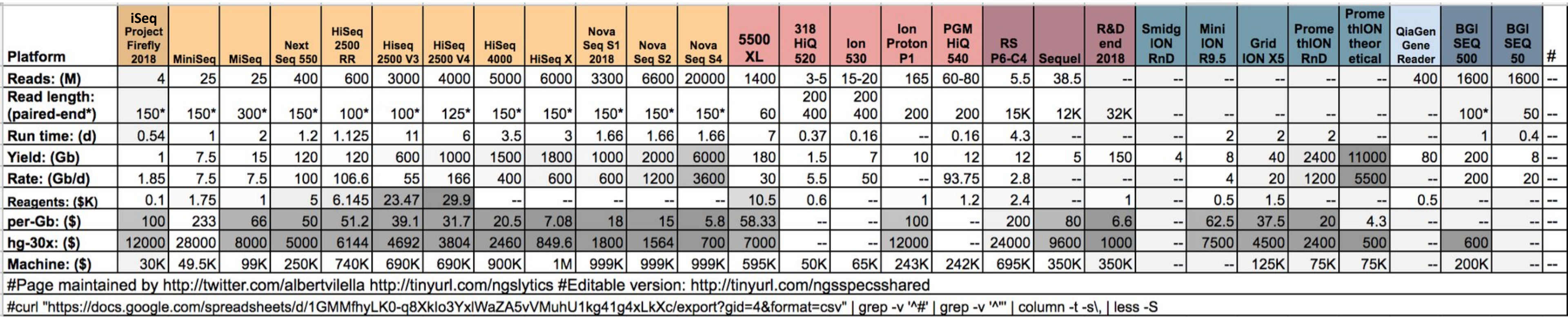
Cost per Raw Megabase of DNA Sequence



Hello Next-Gen Sequencing!

From https://www.genome.gov/sequencingcostsdata/

Generating RNA-Seq: *How to Choose?*



Platform	iSeq Project Firefly 2018	MiniSeq	MiSeq	Next Seq 550	HiSeq 2500 RR	Hiseq 2500 V3	HiSeq 2500 V4	HiSeq 4000	HiSeq X	Nova Seq S1 2018	Nova Seq S2	Nova Seq S4
Reads: (M)	4	25	25	400	600	3000	4000	5000	6000	3300	6600	20000
Read length: (paired-end*)	150*	150*	300*	150*	100*	100*	125*	150*	150*	150*	150*	150*
Run time: (d)	0.54	1	2	1.2	1.125	11	6	3.5	3	1.66	1.66	1.66
Yield: (Gb)	1	7.5	15	120	120	600	1000	1500	1800	1000	2000	6000
Rate: (Gb/d)	1.85	7.5	7.5	100	106.6	55	166	400	600	600	1200	3600
Reagents: ($K)	0.1	1.75	1	5	6.145	23.47	29.9	--	--	--	--	--
per-Gb: ($)	100	233	66	50	51.2	39.1	31.7	20.5	7.08	18	15	5.8
hg-30x: ($)	12000	28000	8000	5000	6144	4692	3804	2460	849.6	1800	1564	700
Machine: ($)	30K	49.5K	99K	250K	740K	690K	690K	900K	1M	999K	999K	999K

Platform	5500 XL	318 HiQ 520	Ion 530	Ion Proton P1	PGM HiQ 540	RS P6-C4	Sequel	R&D end 2018	SmidgION RnD	Mini ION R9.5	Grid ION X5	PromethION RnD	PromethION theoretical	QiaGen Gene Reader	BGI SEQ 500	BGI SEQ 50	#
Reads: (M)	1400	3-5	15-20	165	60-80	5.5	38.5	--	--	--	--	--	--	400	1600	1600	--
Read length: (paired-end*)	60	200 400	200 400	200	200	15K	12K	32K	--	--	--	--	--	--	100*	50	--
Run time: (d)	7	0.37	0.16	--	0.16	4.3	--	--	--	2	2	2	--	--	1	0.4	--
Yield: (Gb)	180	1.5	7	10	12	12	5	150	4	8	40	2400	11000	80	200	8	--
Rate: (Gb/d)	30	5.5	50	--	93.75	2.8	--	--	--	4	20	1200	5500	--	200	20	--
Reagents: ($K)	10.5	0.6	--	1	1.2	2.4	--	1	--	0.5	1.5	--	--	0.5	--	--	--
per-Gb: ($)	58.33	--	--	100	--	200	80	6.6	--	62.5	37.5	20	4.3	--	--	--	--
hg-30x: ($)	7000	--	--	12000	--	24000	9600	1000	--	7500	4500	2400	500	--	600	--	
Machine: ($)	595K	50K	65K	243K	242K	695K	350K	350K	--	--	125K	75K	75K	--	200K	--	--

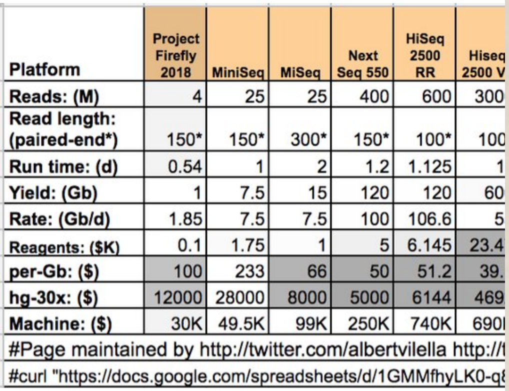
```
#Page maintained by http://twitter.com/albertvilella http://tinyurl.com/ngslytics #Editable version: http://tinyurl.com/ngsspecsshared
#curl "https://docs.google.com/spreadsheets/d/1GMMfhyLK0-q8Xklo3YxlWaZA5vVMuhU1kg41g4xLkXc/export?gid=4&format=csv" | grep -v '^#' | grep -v '^"' | column -t -s\, | less -S
```

Stats circa 2018

For current, see: **https://tinyurl.com/wbgcs65**

*Not all shown at scale

Generating RNA-Seq: *How to Choose?*

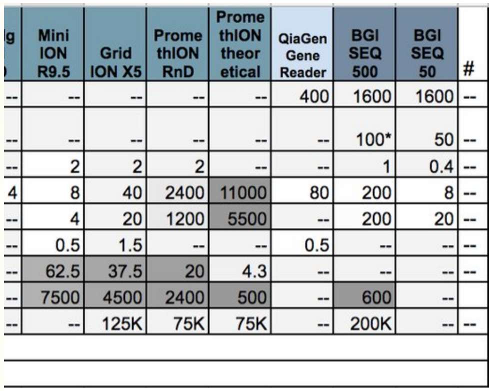


Platform	Project Firefly 2018	MiniSeq	MiSeq	Next Seq 550	HiSeq 2500 RR	Hiseq 2500 V
Reads: (M)	4	25	25	400	600	300
Read length: (paired-end*)	150*	150*	300*	150*	100*	100
Run time: (d)	0.54	1	2	1.2	1.125	1
Yield: (Gb)	1	7.5	15	120	120	60
Rate: (Gb/d)	1.85	7.5	7.5	100	106.6	5
Reagents: ($K)	0.1	1.75	1	5	6.145	23.4
per-Gb: ($)	100	233	66	50	51.2	39.
hg-30x: ($)	12000	28000	8000	5000	6144	469
Machine: ($)	30K	49.5K	99K	250K	740K	690

#Page maintained by http://twitter.com/albertvilella http://t

#curl "https://docs.google.com/spreadsheets/d/1GMMfhyLK0-q8

"What I especially like about this baby is this little drawer where I can keep my lunch."



Platform	g	Mini ION R9.5	Grid ION X5	Prome thION RnD	Prome thION theor etical	QiaGen Gene Reader	BGI SEQ 500	BGI SEQ 50	#
Reads: (M)	--	--	--	--	--	400	1600	1600	--
Read length: (paired-end*)	--	--	--	--	--	--	100*	50	--
Run time: (d)	--	2	2	2	--	--	1	0.4	--
Yield: (Gb)	4	8	40	2400	11000	80	200	8	--
Rate: (Gb/d)	--	4	20	1200	5500	--	200	20	--
Reagents: ($K)	--	0.5	1.5	--	--	0.5	--	--	--
per-Gb: ($)	--	62.5	37.5	20	4.3	--	--	--	--
hg-30x: ($)	--	7500	4500	2400	500	--	600	--	
Machine: ($)	--	--	125K	75K	75K	--	200K	--	--

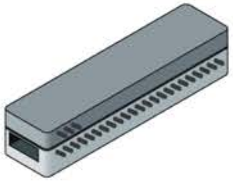
Thx Joshua Levin, for the cartoon. ☺

Each has pros/cons

Today’s Most Popular Sequencing Technologies

Illumina

Pacific Biosciences



Oxford Nanopore

Images from “RNA sequencing: the teenage years”
Rory Stark, Marta Grzelak & James Hadfield
Nature Reviews Genetics volume 20, pages631–656(2019)

Today’s Most Popular Sequencing Technologies

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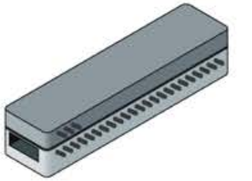
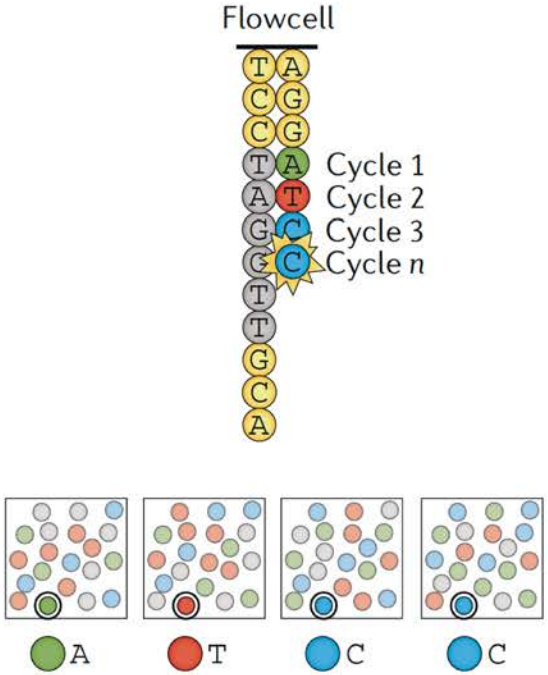


Oxford Nanopore



Hundreds of millions to billions of highly accurate but shorter reads. ($)

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Rory Stark, Marta Grzelak & James Hadfield
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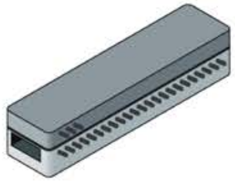
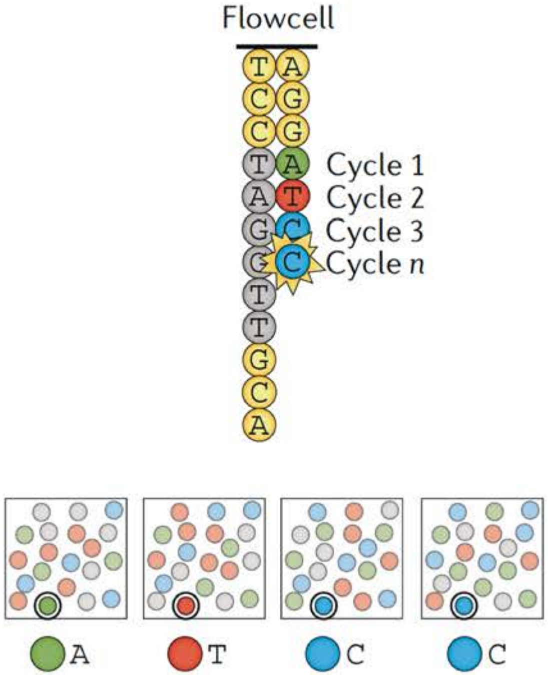
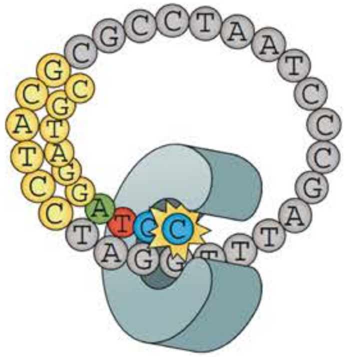
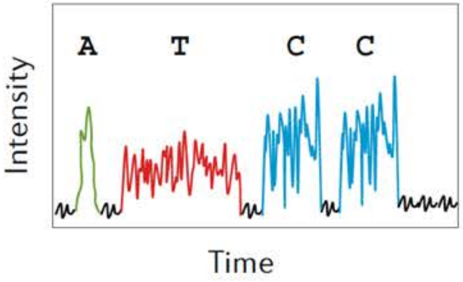


Oxford Nanopore



Hundreds of millions to billions of highly accurate but shorter reads. ($)


C G C C T A A T C C C C G A T T
G C C G A T T A G C A G C T A
A T C C



Limited sequencing depth, but highly accurate full-length single molecule reads. ($$$)

Images from “RNA sequencing: the teenage years”
Rory Stark, Marta Grzelak & James Hadfield
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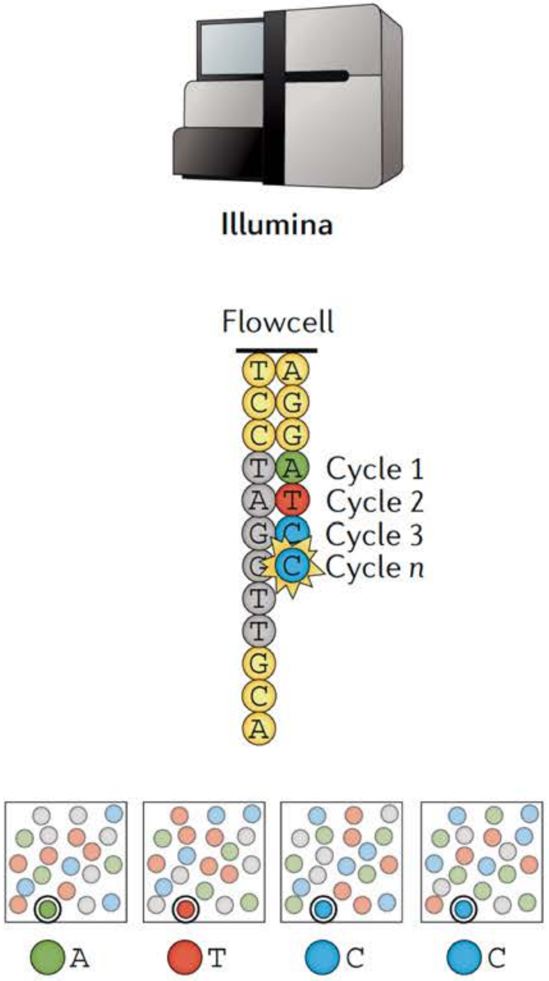
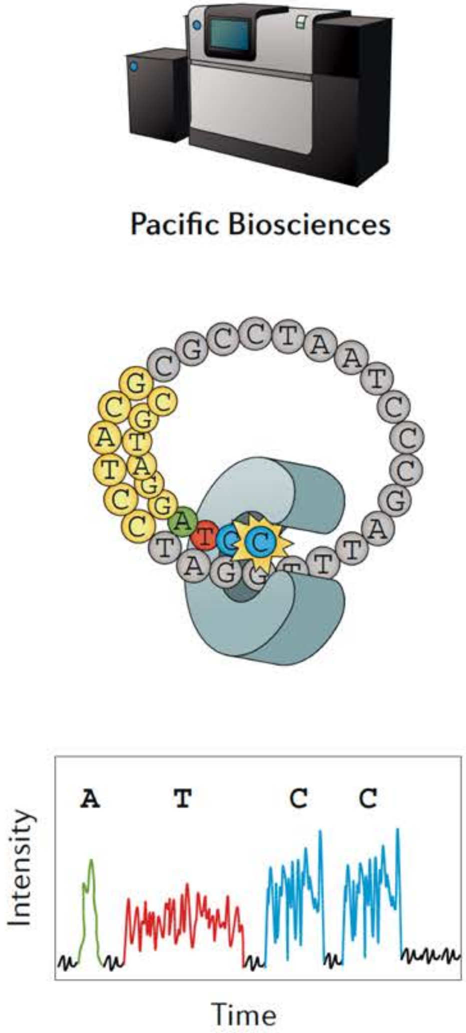
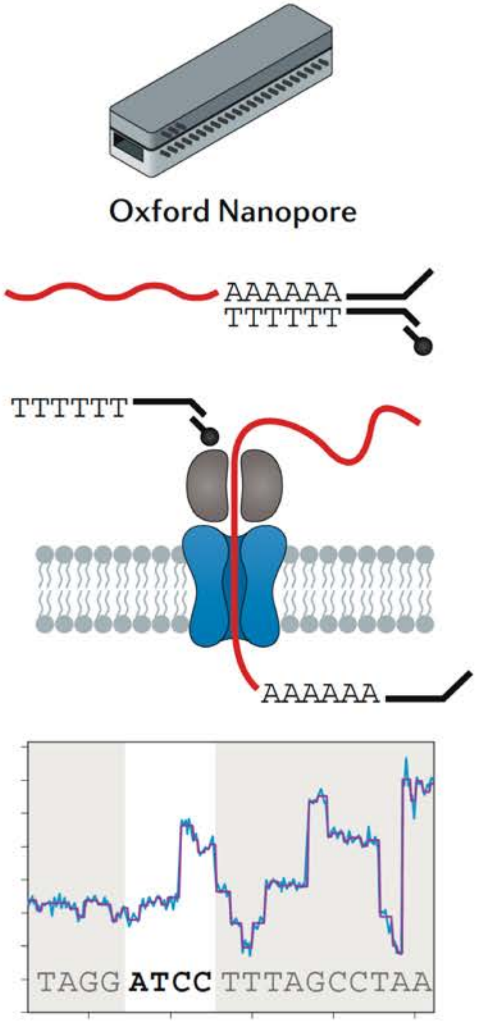
Today's Most Popular Sequencing Technologies



Hundreds of millions to billions of highly accurate but shorter reads. ($)



Limited sequencing depth, but highly accurate full-length single molecule reads. ($$$)



Limited sequencing depth, and moderate-to-highly accurate full-length single molecule reads. ($$)

Can do direct RNA sequencing!
and find evidence for methylation

Images from "RNA sequencing: the teenage years"
Rory Stark, Marta Grzelak & James Hadfield
Nature Reviews Genetics volume 20, pages631–656(2019)

A Plethora of Biological Sequence Analyses Enabled by RNA-Seq

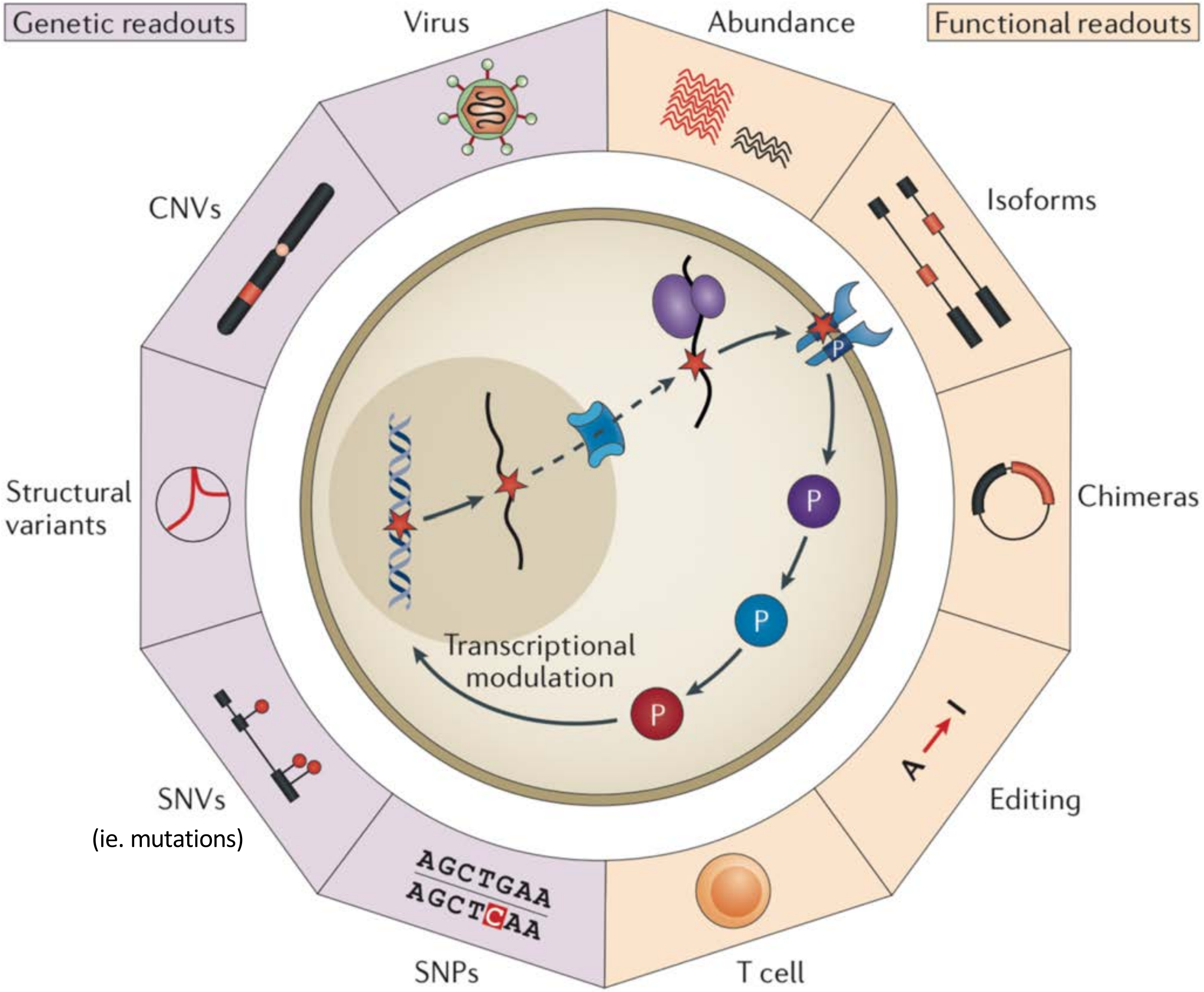
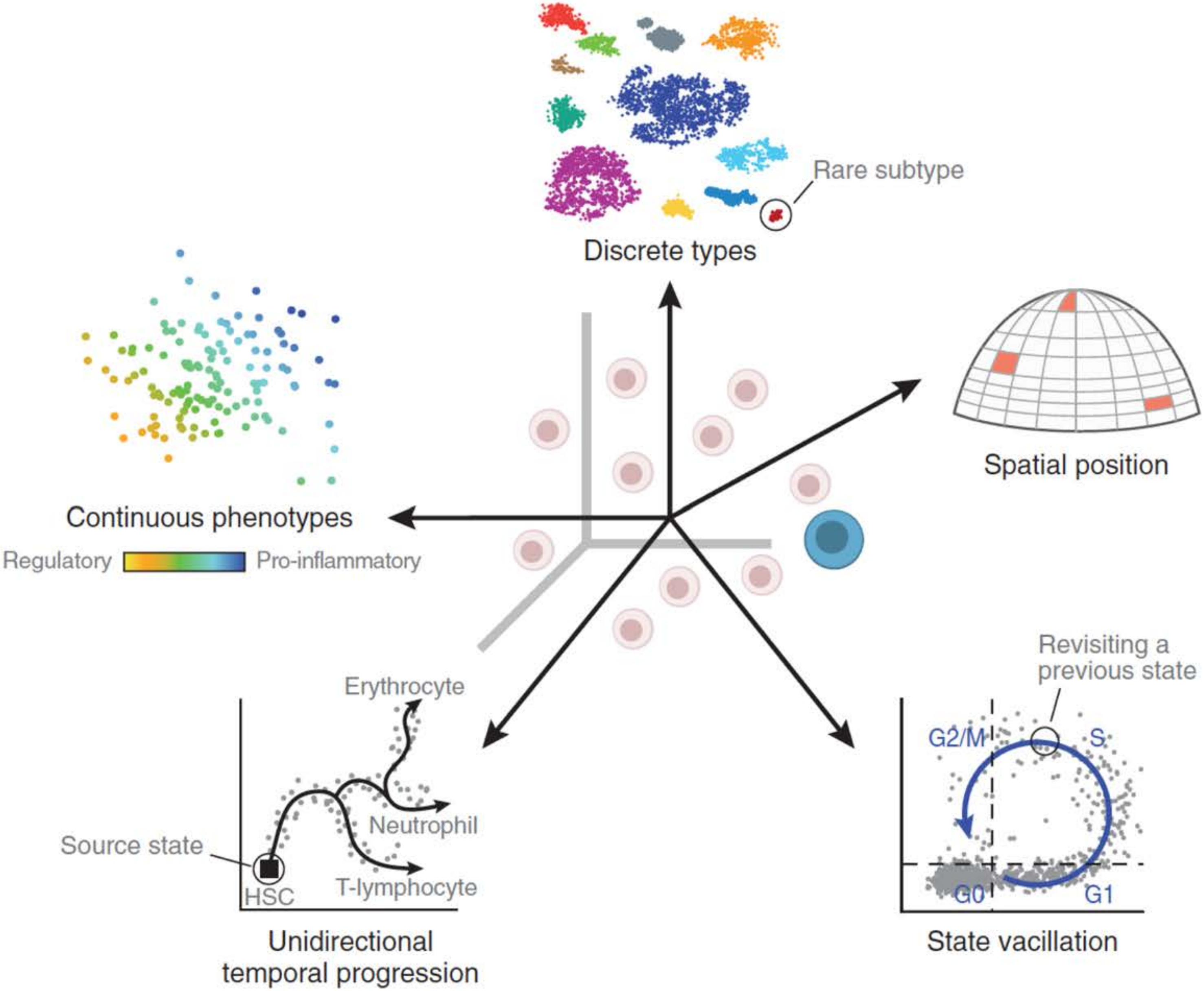


Figure 2 | **Transcriptome profiling for genetic causes and functional phenotypic readouts.**

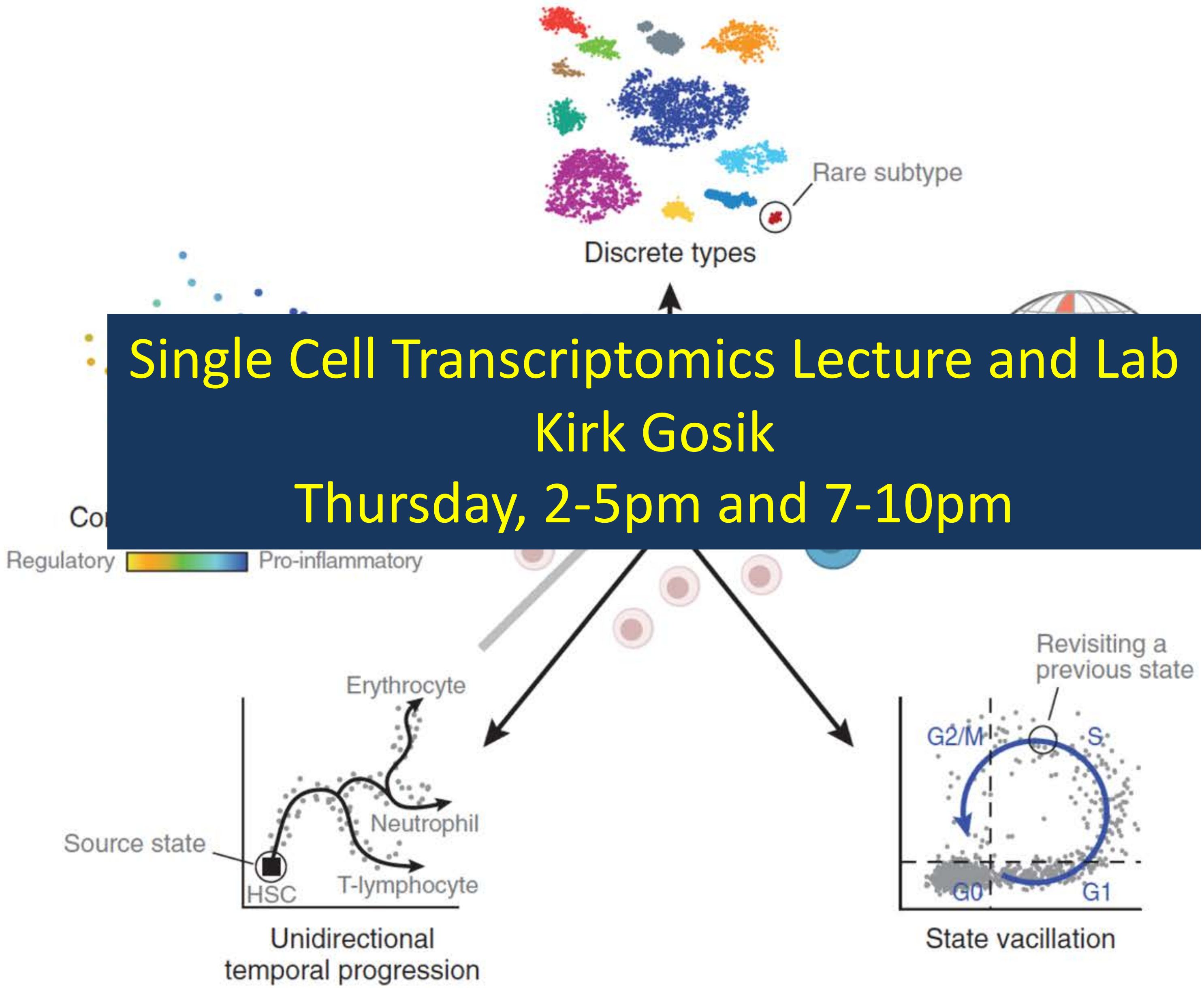
From Cieslik and Chinnaiyan, NRG, 2017

RNA-Seq is Empowering Discovery at Single Cell Resolution



Wagner, Regev, and Yosef. NBT 2016

RNA-Seq is Empowering Discovery at Single Cell Resolution

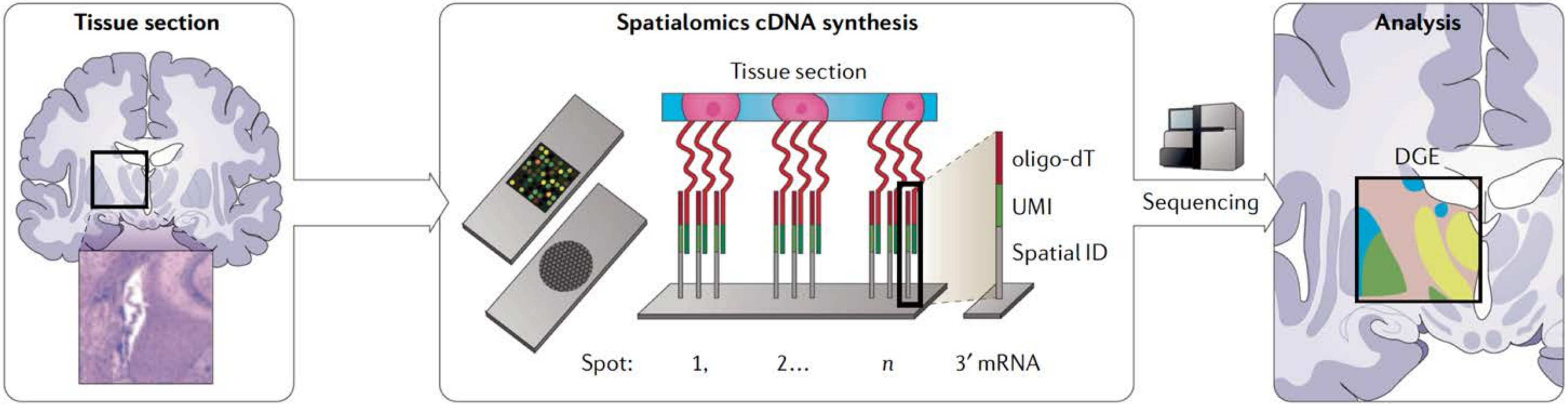


Single Cell Transcriptomics Lecture and Lab
Kirk Gosik
Thursday, 2-5pm and 7-10pm

Wagner, Regev, and Yosef. NBT 2016

Spatial Transcriptomics

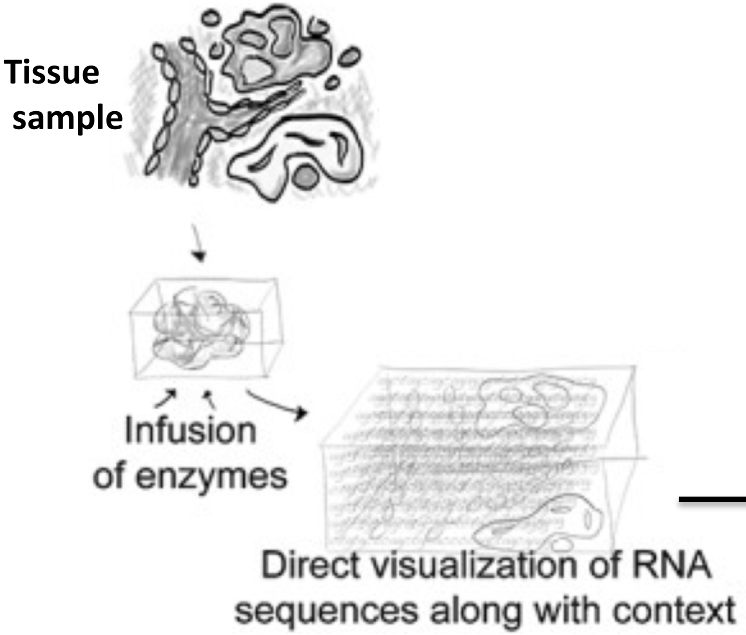
Spatial Encoding

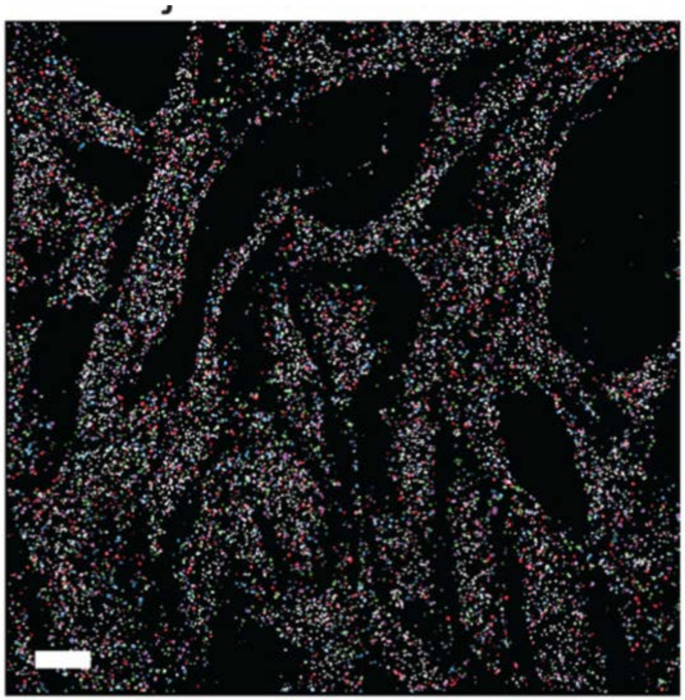


From "RNA sequencing: the teenage years"
Rory Stark, Marta Grzelak & James Hadfield
Nature Reviews Genetics volume 20, pages631–656(2019)

Spatial Transcriptomics

Fluorescent in situ RNA sequencing (FISSEQ)





Fibroblasts, FISSEQ gene pixels

Adapted from:
JH Lee, 2017, PMC5315614
JH Lee, 2014, PMC4140943

A Myriad of Other Specialized RNA-seq -based Applications

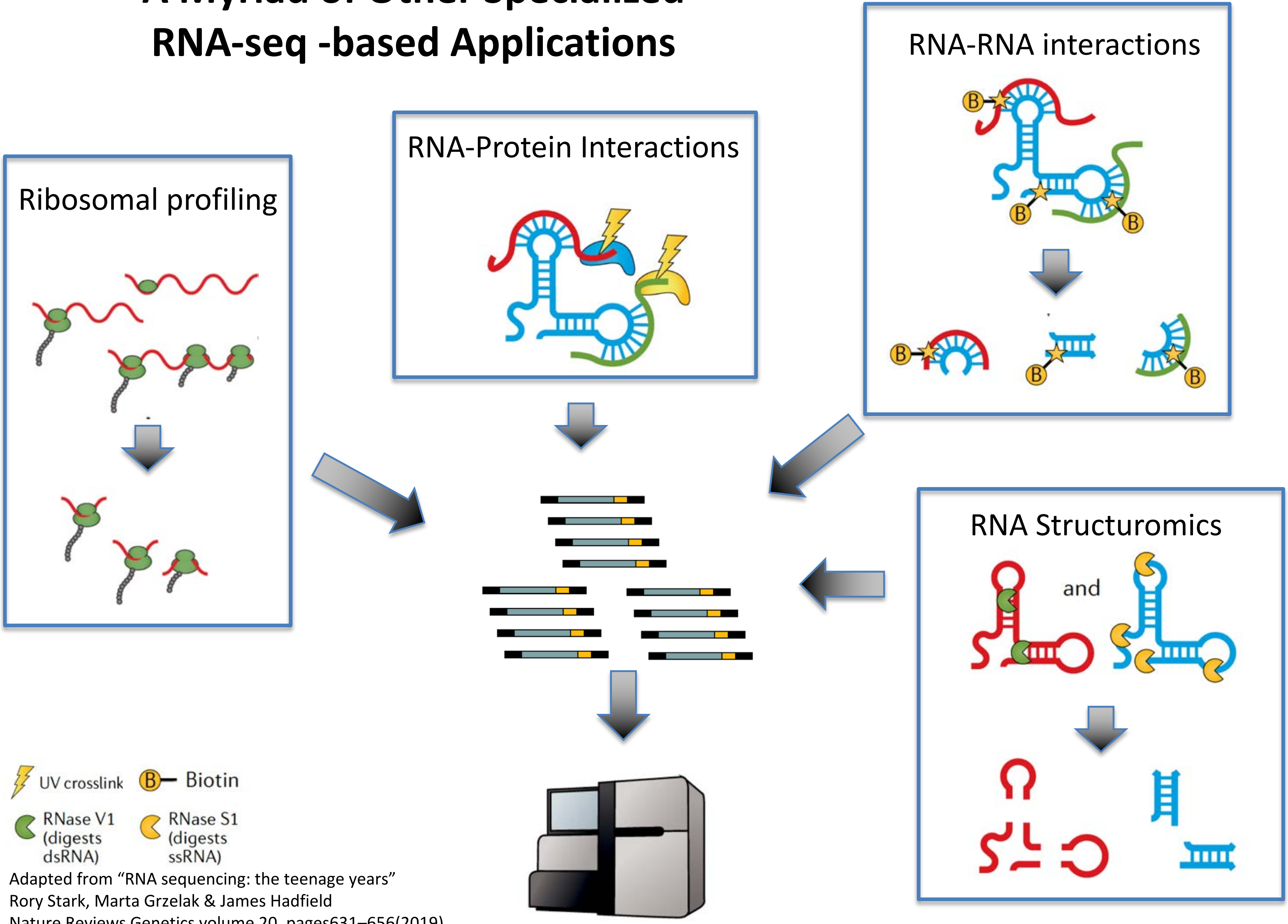
RNA-Sequencing as your lens towards biological discovery

⚡ UV crosslink Ⓑ– Biotin

RNase V1 (digests dsRNA) RNase S1 (digests ssRNA)

Adapted from “RNA sequencing: the teenage years”
Rory Stark, Marta Grzelak & James Hadfield
Nature Reviews Genetics volume 20, pages631–656(2019)

A Myriad of Other Specialized RNA-seq -based Applications



Ribosomal profiling

RNA-Protein Interactions

RNA-RNA interactions

RNA Structuromics

UV crosslink

B— Biotin

RNase V1 (digests dsRNA)

RNase S1 (digests ssRNA)

Adapted from “RNA sequencing: the teenage years”
Rory Stark, Marta Grzelak & James Hadfield
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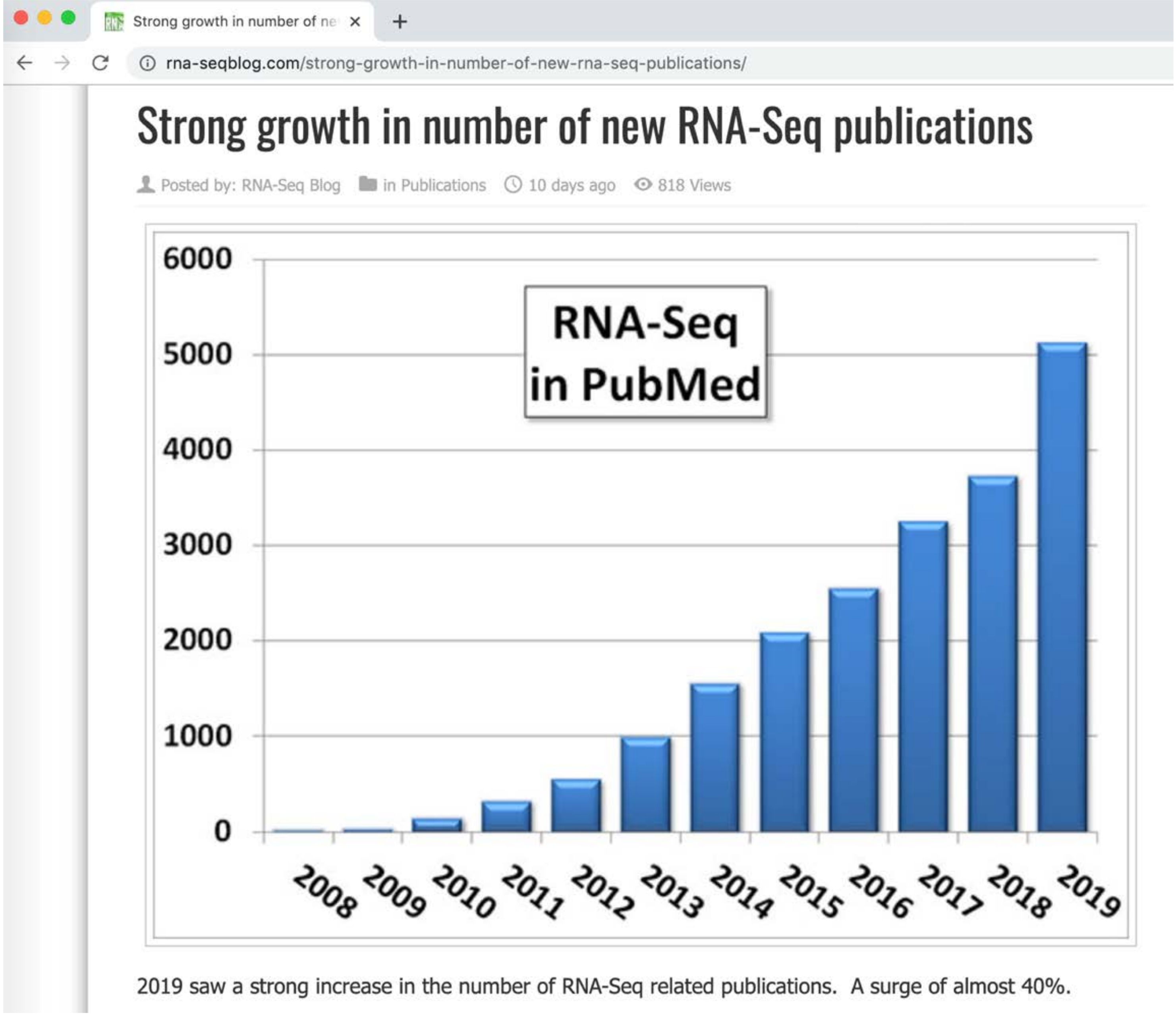
RNA-Seq
Transcriptome Sequencing Research & Industry News



Strong growth in number of ne ×

rna-seqblog.com/strong-growth-in-number-of-new-rna-seq-publications/

Strong growth in number of new RNA-Seq publications

Posted by: RNA-Seq Blog · in Publications · 10 days ago · 818 Views

2019 saw a strong increase in the number of RNA-Seq related publications. A surge of almost 40%.

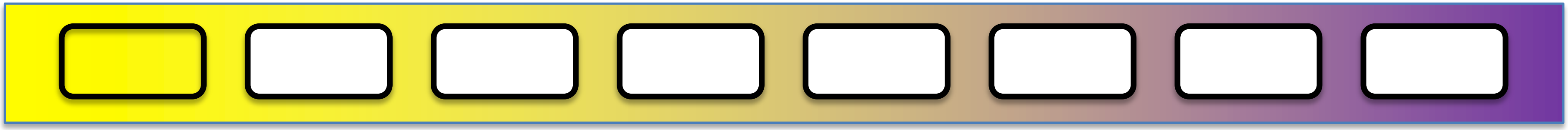
Transcriptomics Lecture Overview

1. Overview of RNA-Seq
2. Transcript reconstruction methods
3. Trinity de novo assembly
4. Transcriptome quality assessment

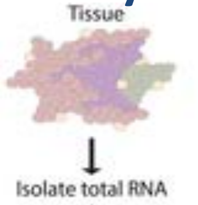
    (coffee break)

5. Expression quantification
6. Differential expression analysis
7. Functional annotation
8. Case study: salamander transcriptome

Part 1. Overview of RNA-Seq



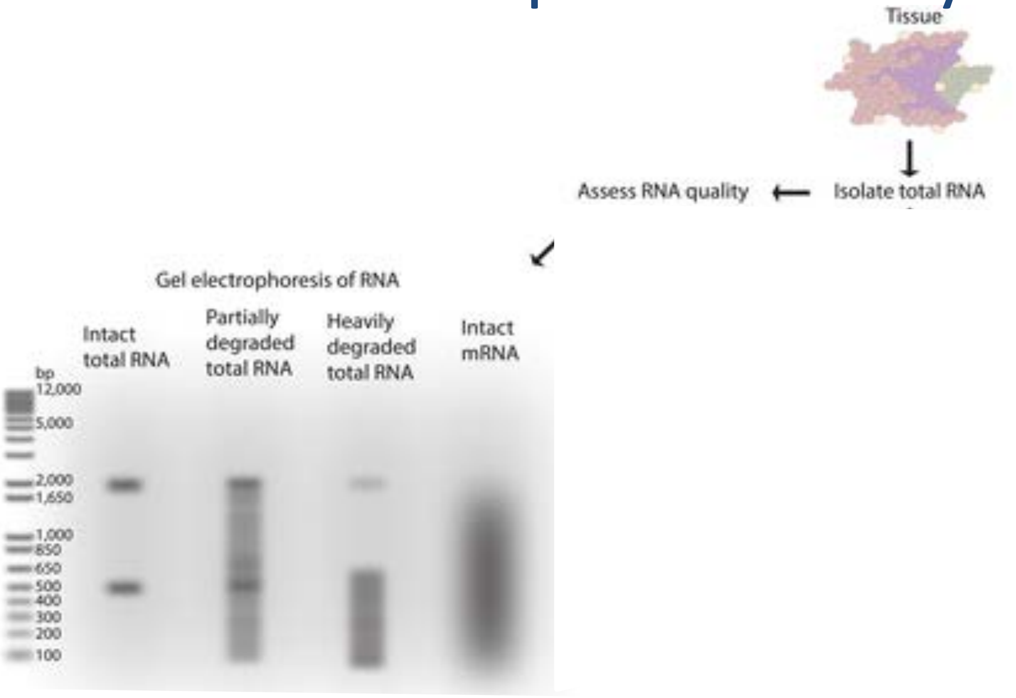
RNA-seq library fragmentation and size selection strategies that influence interpretation and analysis.



http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393 Griffith et al., 2015

PLOS | COMPUTATIONAL BIOLOGY

RNA-seq library fragmentation and size selection strategies that influence interpretation and analysis.



Tissue

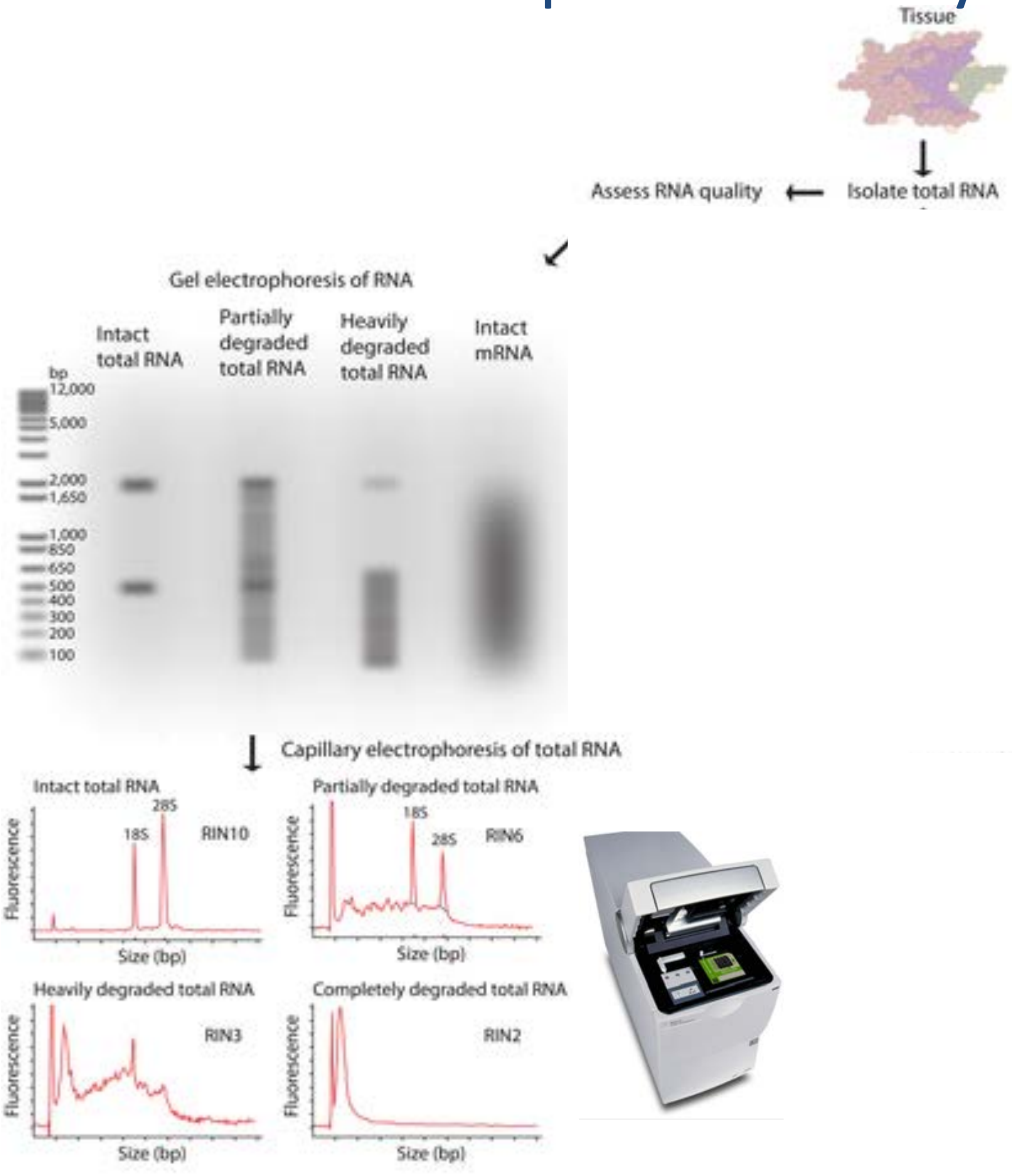
Isolate total RNA

Assess RNA quality

Gel electrophoresis of RNA
bp
12,000
5,000
2,000
1,650
1,000
850
650
500
400
300
200
100
Intact total RNA
Partially degraded total RNA
Heavily degraded total RNA
Intact mRNA

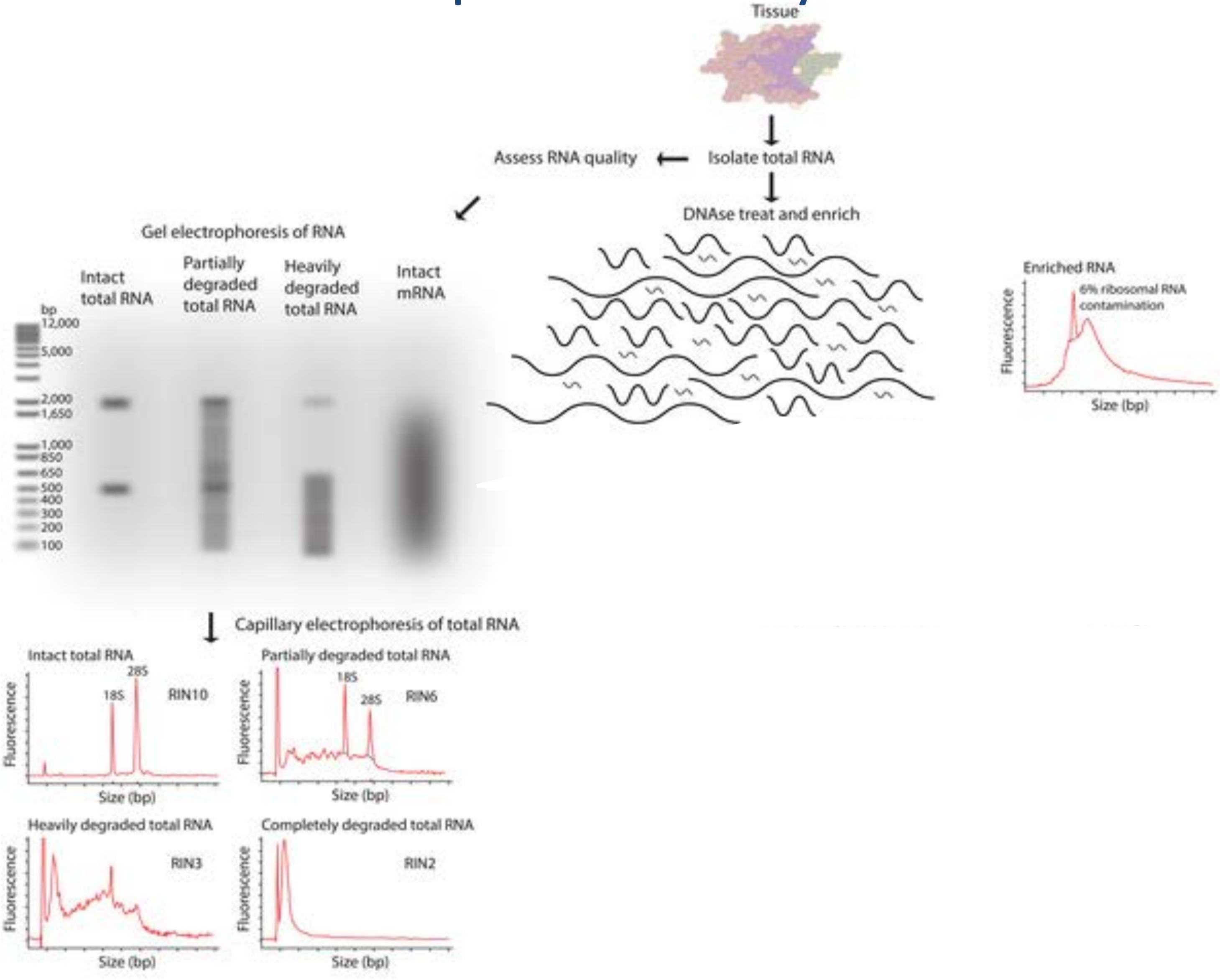
http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393 Griffith et al., 2015

RNA-seq library fragmentation and size selection strategies that influence interpretation and analysis.



http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393 Griffith et al., 2015

RNA-seq library fragmentation and size selection strategies that influence interpretation and analysis.



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PLOS | COMPUTATIONAL BIOLOGY

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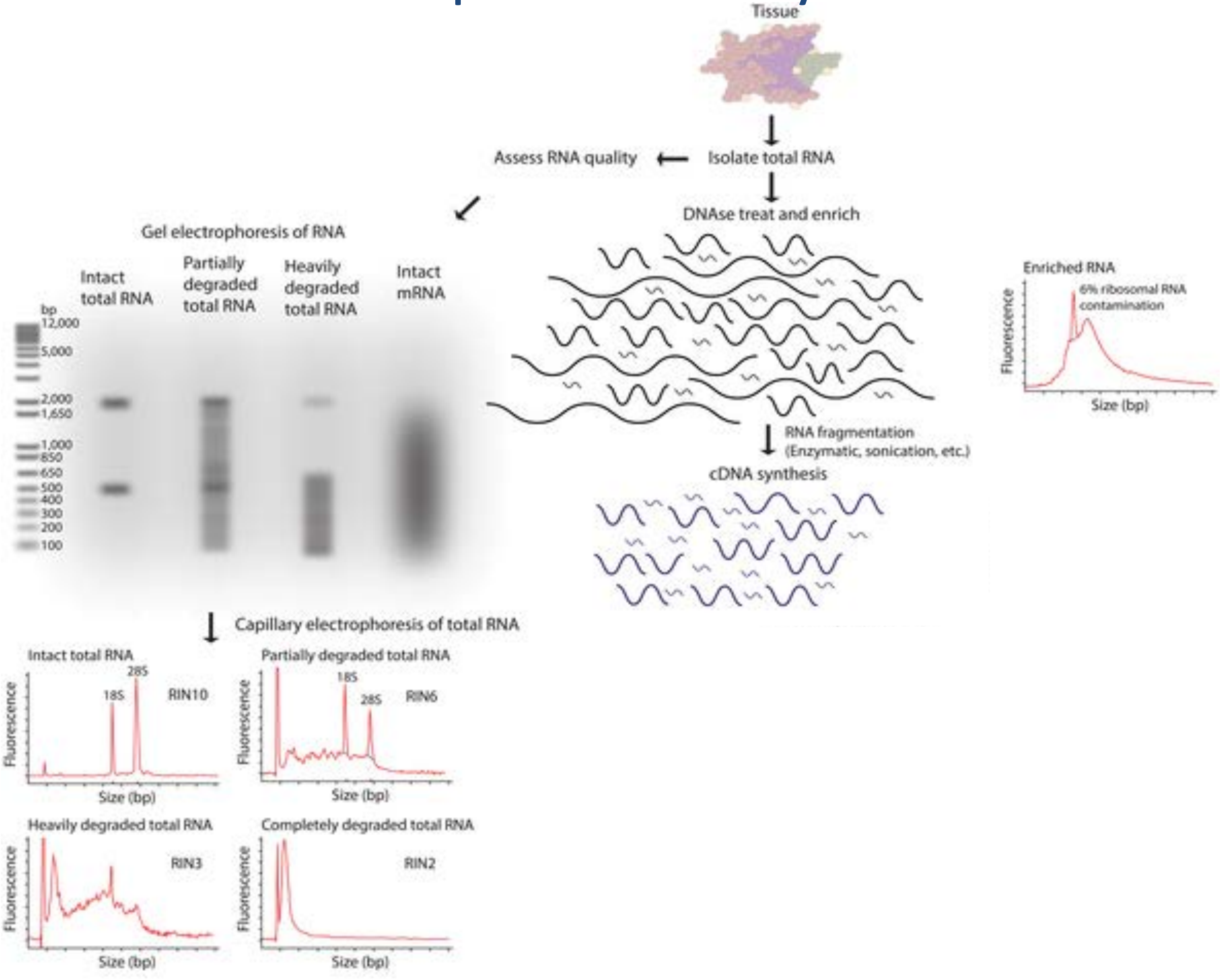
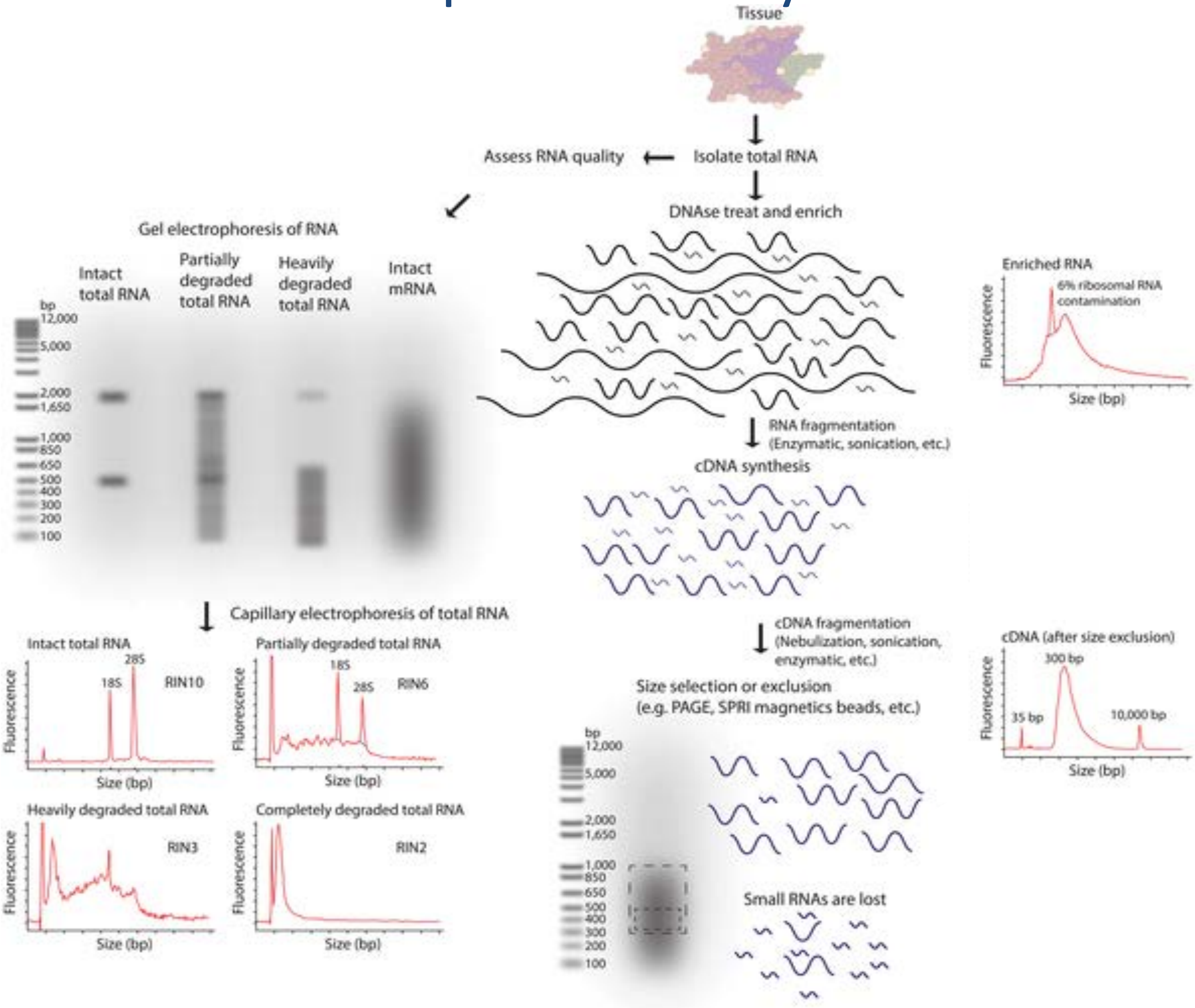


http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393 Griffith et al., 2015

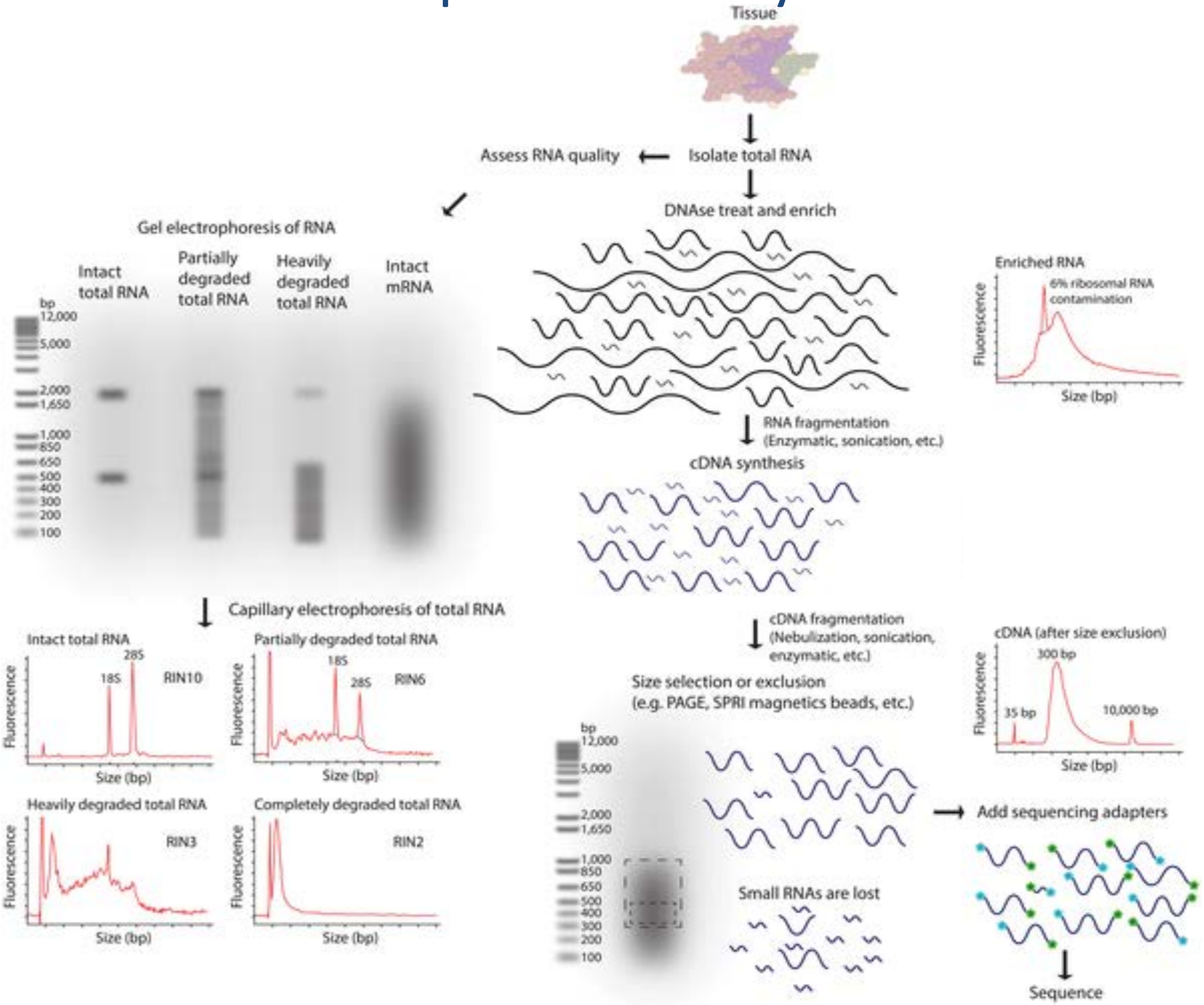
RNA-seq library fragmentation and size selection strategies that influence interpretation and analysis.


Tissue
Isolate total RNA
Assess RNA quality
DNAse treat and enrich
Gel electrophoresis of RNA
Intact total RNA
Partially degraded total RNA
Heavily degraded total RNA
Intact mRNA
bp
12,000
5,000
2,000
1,650
1,000
850
650
500
400
300
200
100
Enriched RNA
6% ribosomal RNA contamination
Fluorescence
Size (bp)
RNA fragmentation (Enzymatic, sonication, etc.)
cDNA synthesis
Capillary electrophoresis of total RNA
Intact total RNA
28S
18S
RIN10
Fluorescence
Size (bp)
Partially degraded total RNA
18S
28S
RIN6
Fluorescence
Size (bp)
Heavily degraded total RNA
RIN3
Fluorescence
Size (bp)
Completely degraded total RNA
RIN2
Fluorescence
Size (bp)
cDNA fragmentation (Nebulization, sonication, enzymatic, etc.)
Size selection or exclusion (e.g. PAGE, SPRI magnetics beads, etc.)
bp
12,000
5,000
2,000
1,650
1,000
850
650
500
400
300
200
100
Small RNAs are lost
cDNA (after size exclusion)
300 bp
35 bp
10,000 bp
Fluorescence
Size (bp)

http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393 Griffith et al., 2015

PLOS | COMPUTATIONAL BIOLOGY

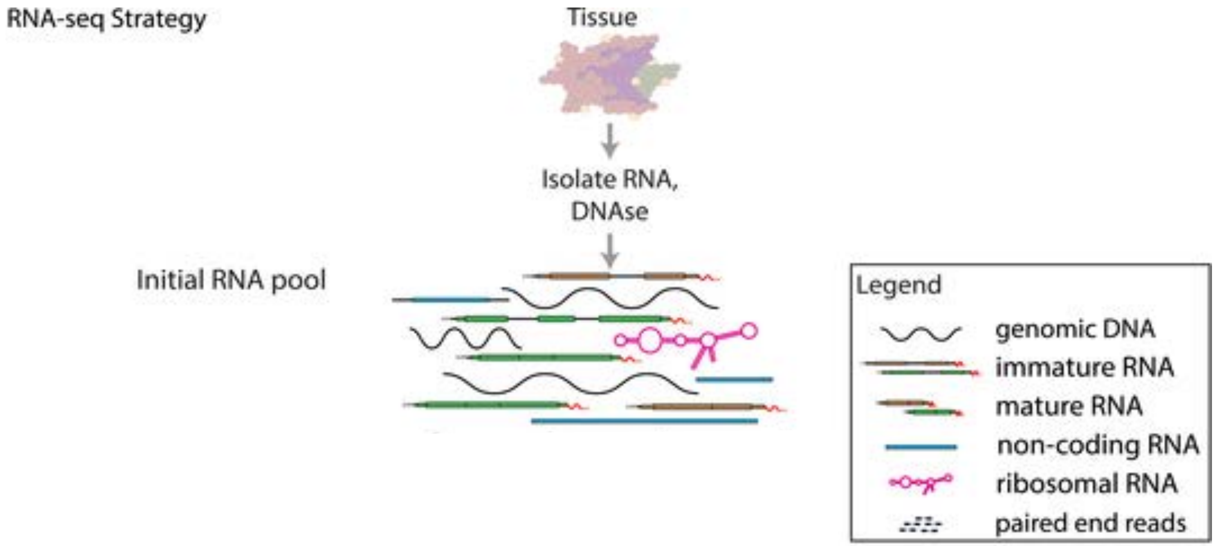
RNA-seq library fragmentation and size selection strategies that influence interpretation and analysis.


Tissue
Isolate total RNA
Assess RNA quality
DNAse treat and enrich
Gel electrophoresis of RNA
Intact total RNA
Partially degraded total RNA
Heavily degraded total RNA
Intact mRNA
bp
12,000
5,000
2,000
1,650
1,000
850
650
500
400
300
200
100
Enriched RNA
6% ribosomal RNA contamination
Fluorescence
Size (bp)
RNA fragmentation (Enzymatic, sonication, etc.)
cDNA synthesis
Capillary electrophoresis of total RNA
Intact total RNA
18S
28S
RIN10
Partially degraded total RNA
18S
28S
RIN6
Heavily degraded total RNA
RIN3
Completely degraded total RNA
RIN2
Fluorescence
Size (bp)
cDNA fragmentation (Nebulization, sonication, enzymatic, etc.)
Size selection or exclusion (e.g. PAGE, SPRI magnetics beads, etc.)
bp
12,000
5,000
2,000
1,650
1,000
850
650
500
400
300
200
100
Small RNAs are lost
cDNA (after size exclusion)
300 bp
35 bp
10,000 bp
Fluorescence
Size (bp)
Add sequencing adapters
Sequence

http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393 Griffith et al., 2015

PLOS COMPUTATIONAL BIOLOGY

RNA-seq library enrichment strategies that influence interpretation and analysis.



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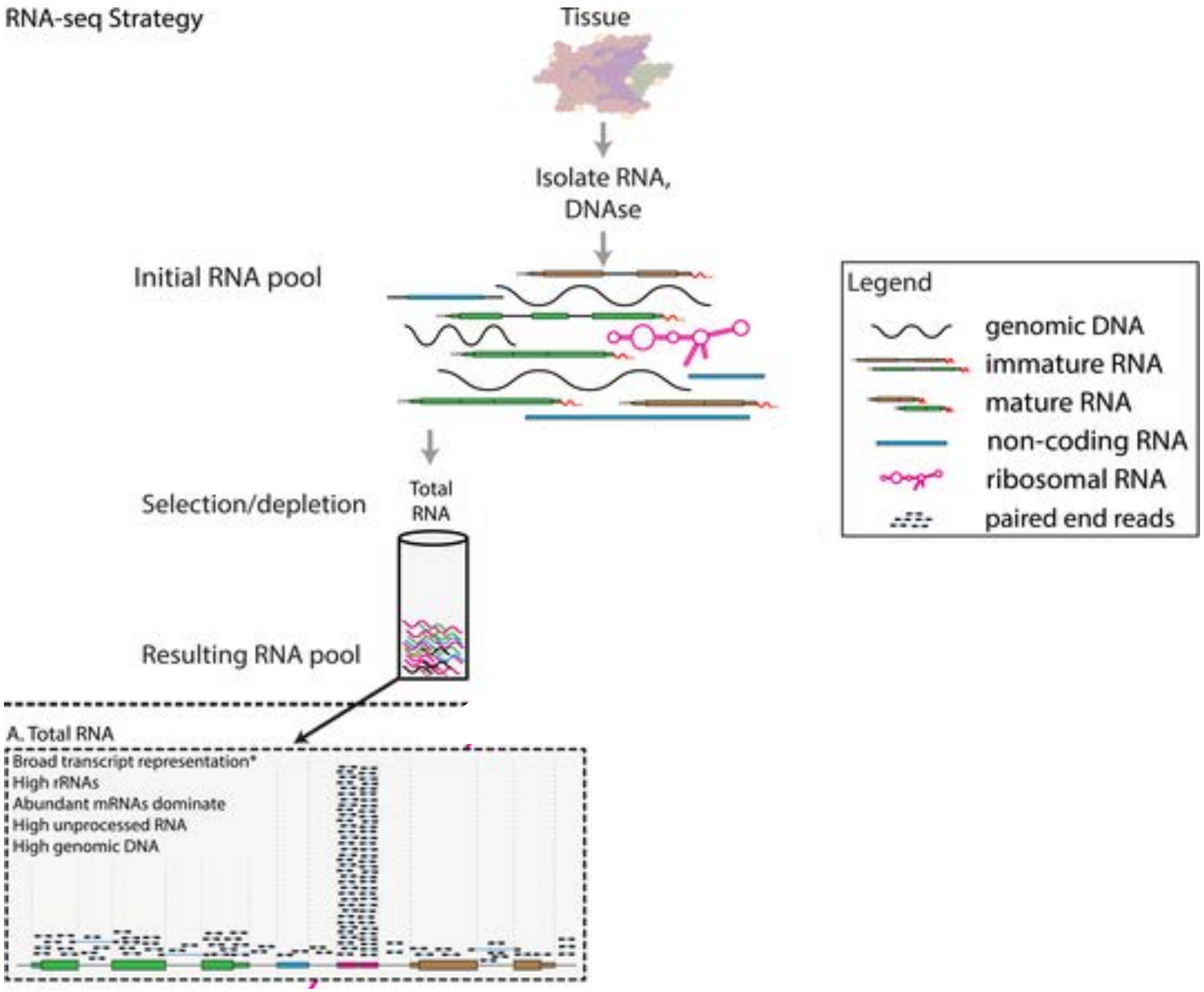
Griffith et al., 2015

RNA-seq library enrichment strategies that influence interpretation and analysis.



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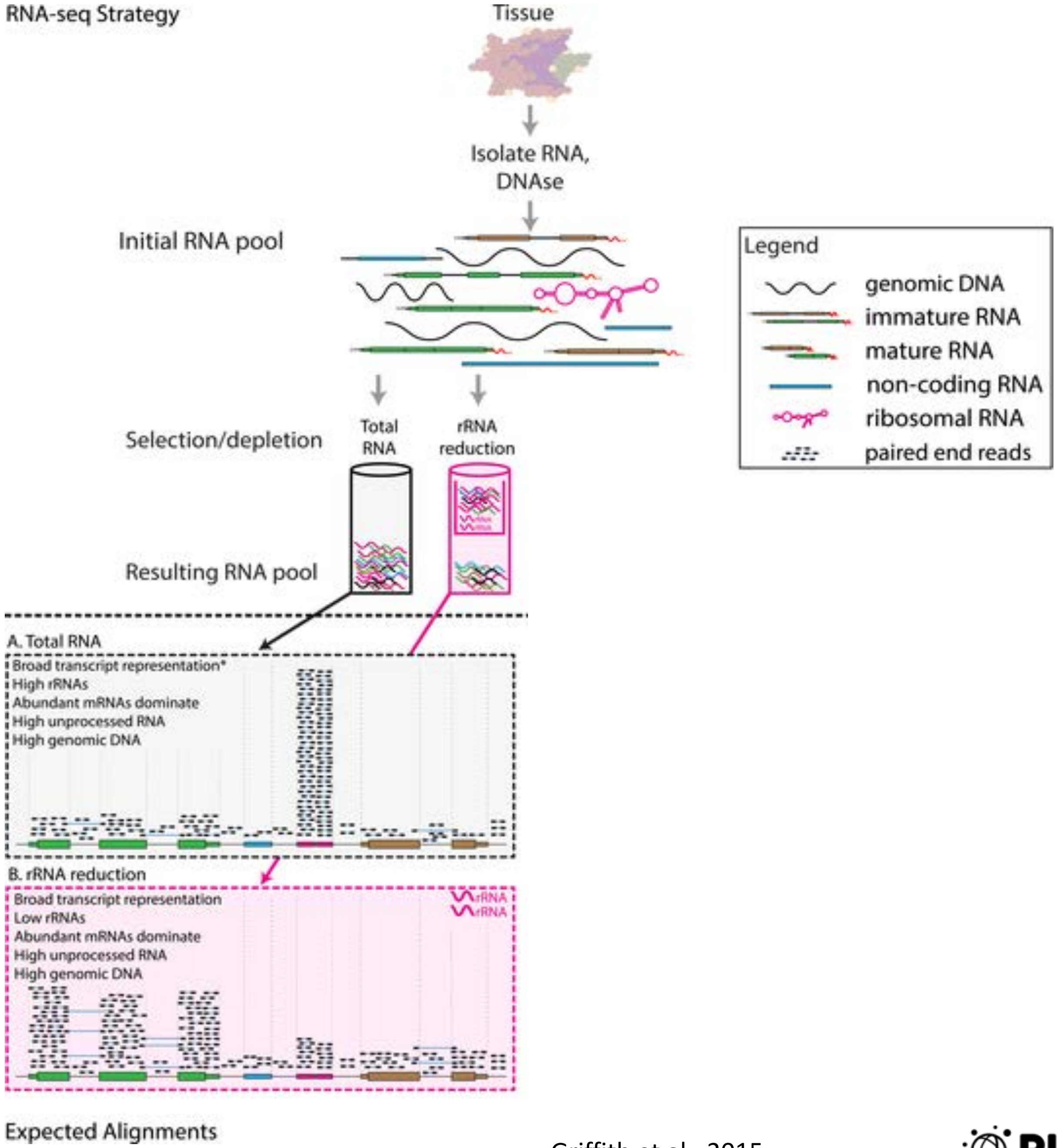

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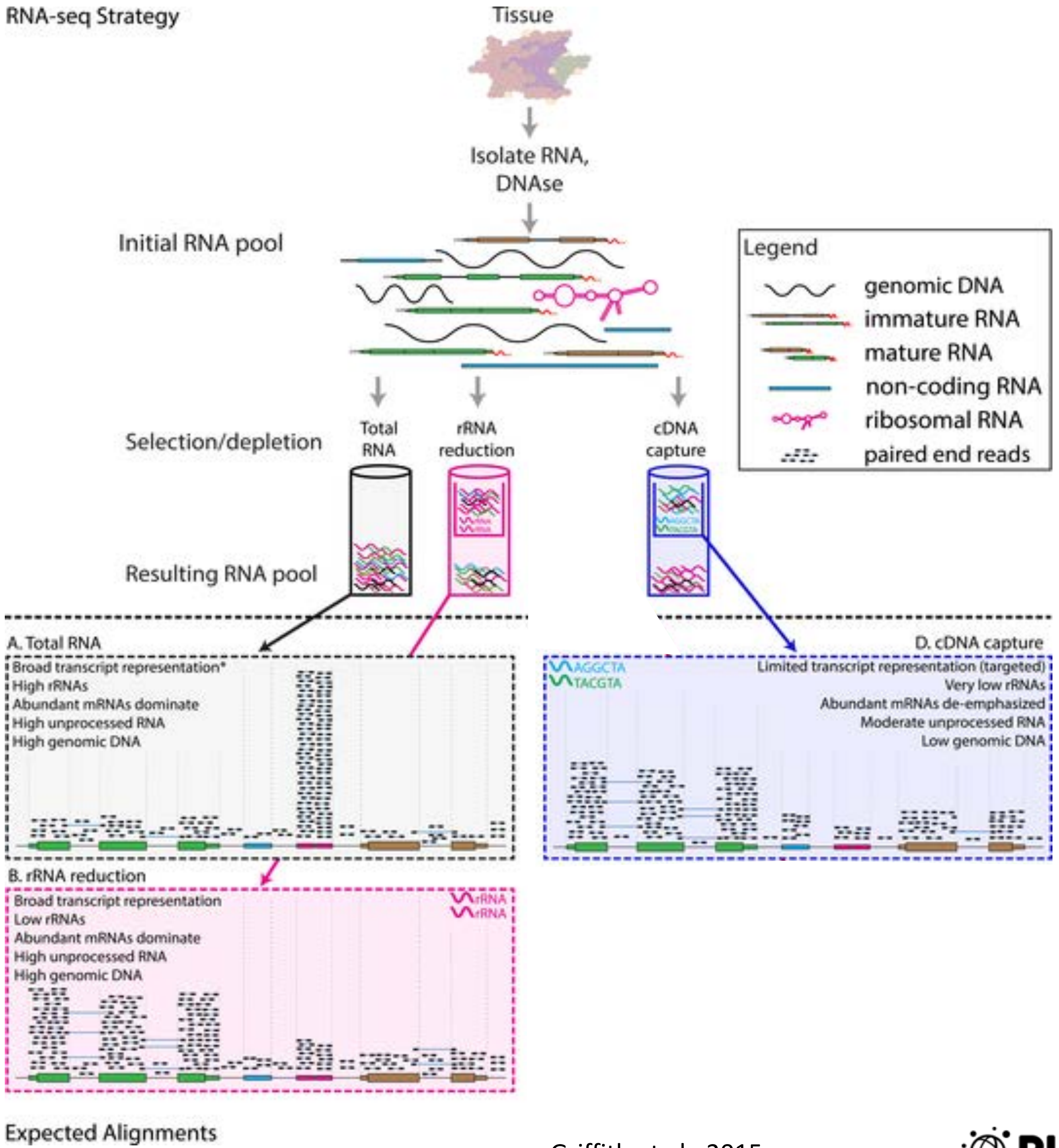

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PLOS | COMPUTATIONAL BIOLOGY

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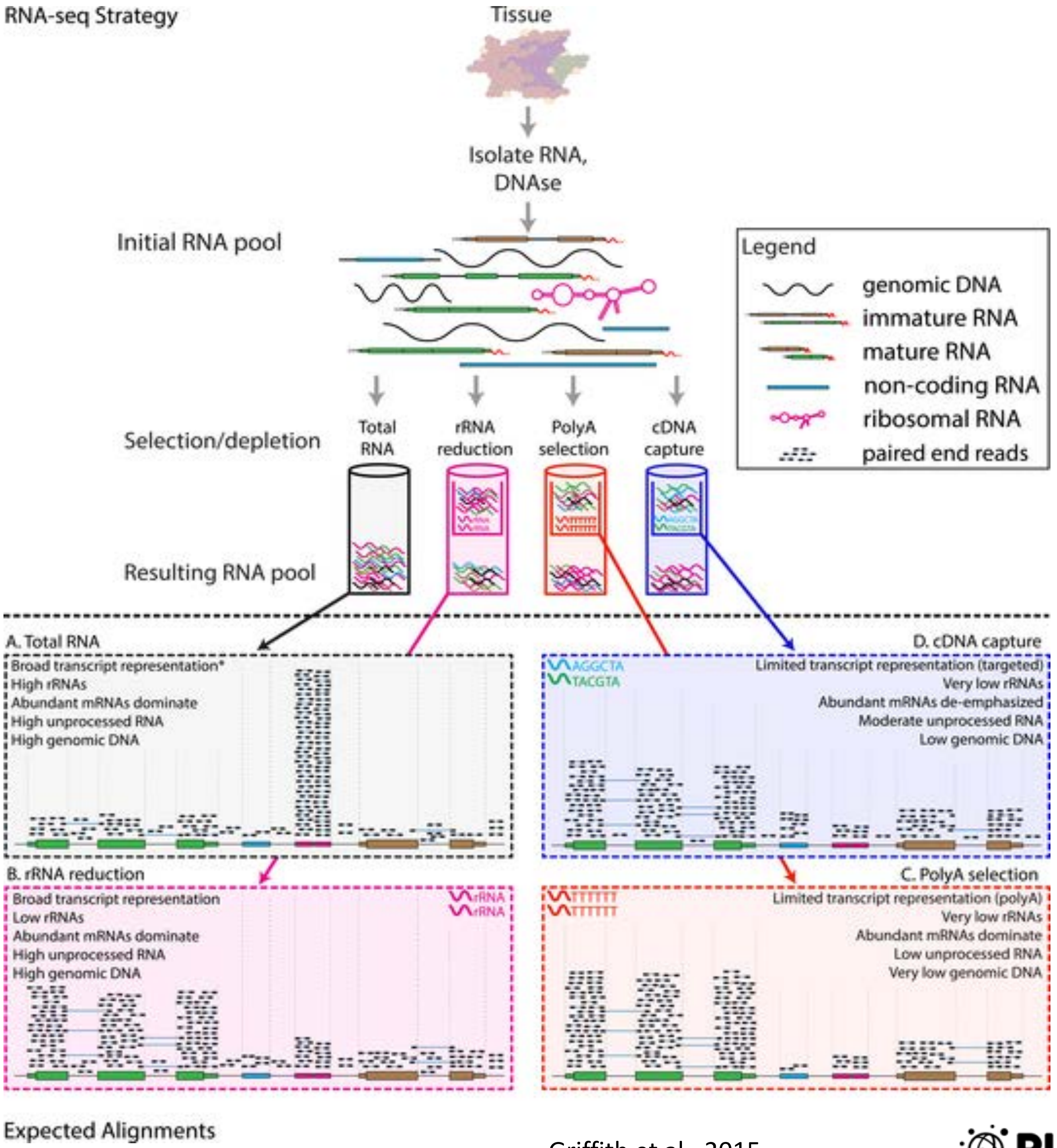
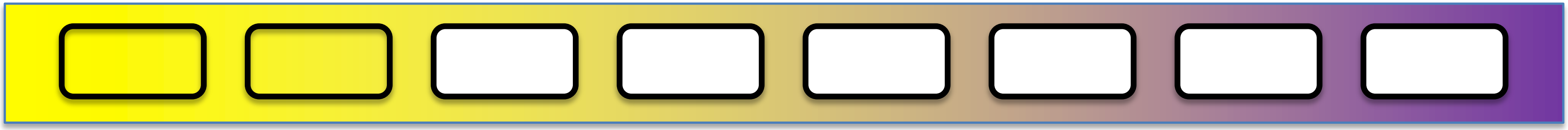


http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1004393

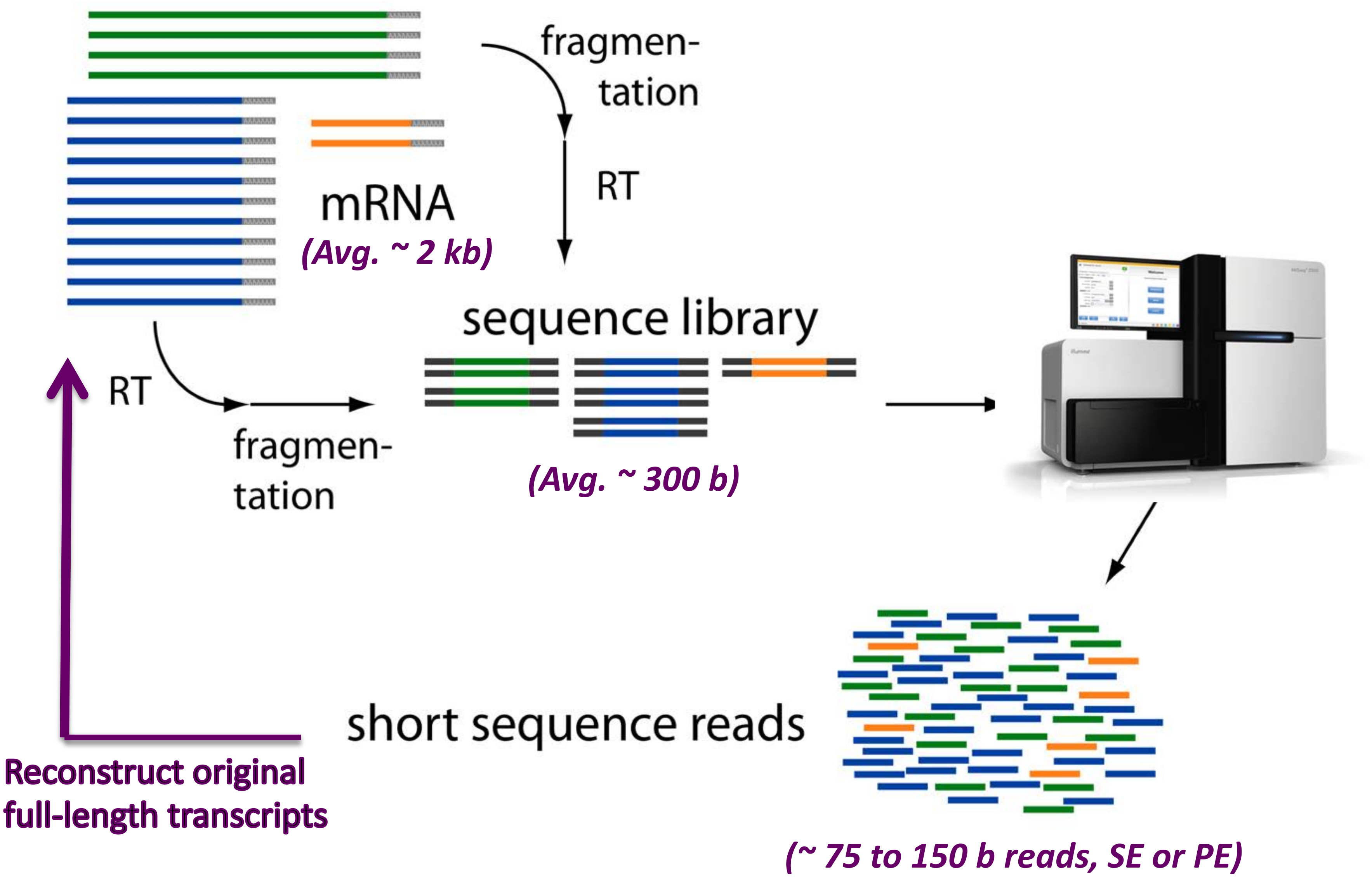
Griffith et al., 2015

PLOS | COMPUTATIONAL BIOLOGY

Part 2. Transcript Reconstruction Methods



RNA-Seq Challenge: Transcript Reconstruction



mRNA

(Avg. ~ 2 kb)

fragmen-
tation

RT

RT

fragmen-
tation

sequence library

(Avg. ~ 300 b)

short sequence reads

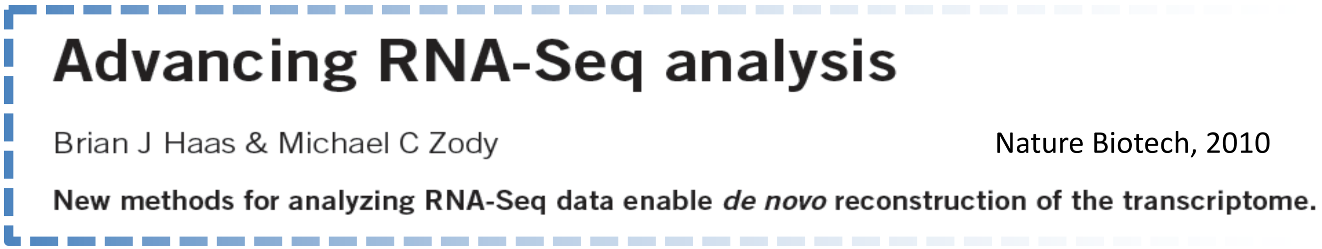
(~ 75 to 150 b reads, SE or PE)

Reconstruct original full-length transcripts

Adapted from: http://www2.fml.tuebingen.mpg.de/raetsch/members/research/transcriptomics.html

Transcript Reconstruction from RNA-Seq Reads

RNA-Seq reads



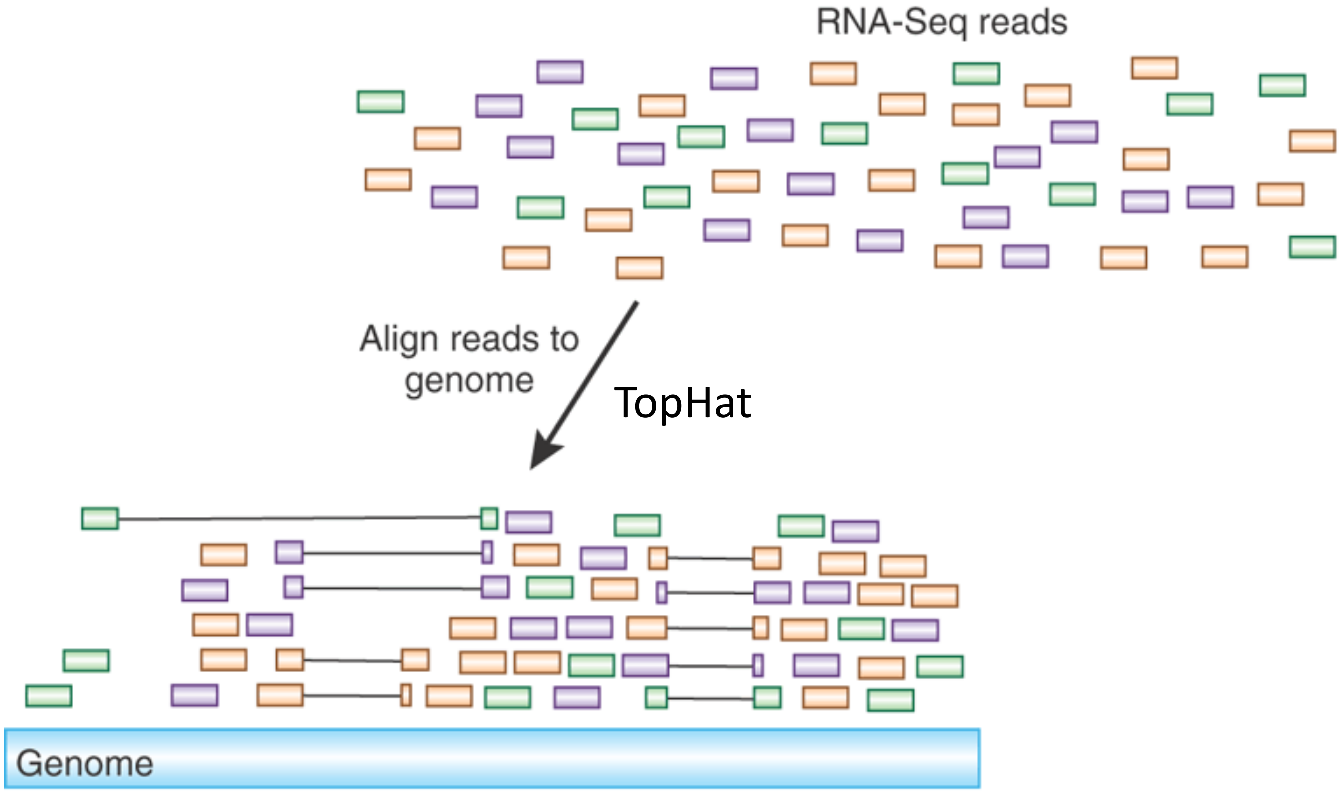
Advancing RNA-Seq analysis

Brian J Haas & Michael C Zody

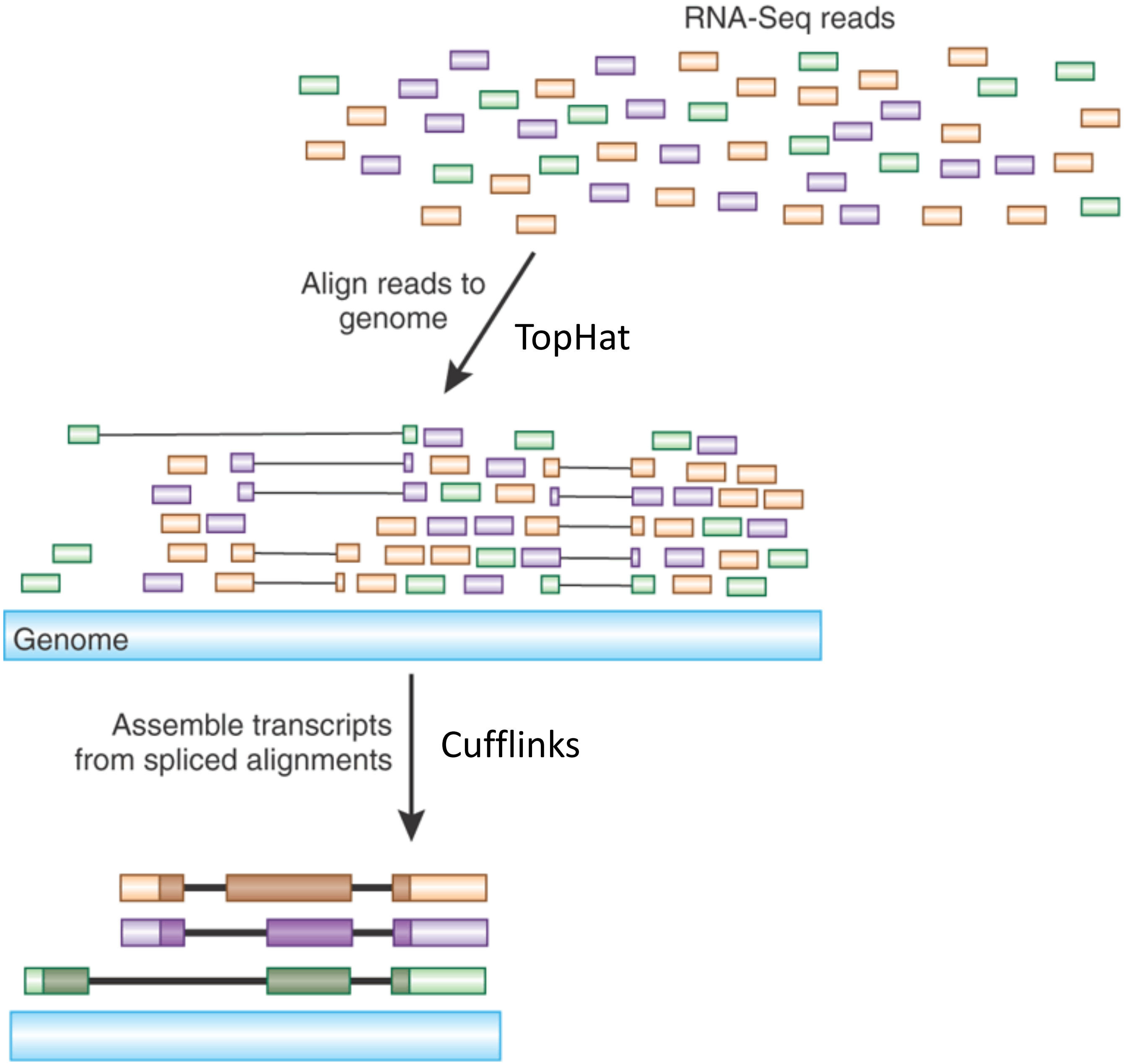
Nature Biotech, 2010

New methods for analyzing RNA-Seq data enable *de novo* reconstruction of the transcriptome.

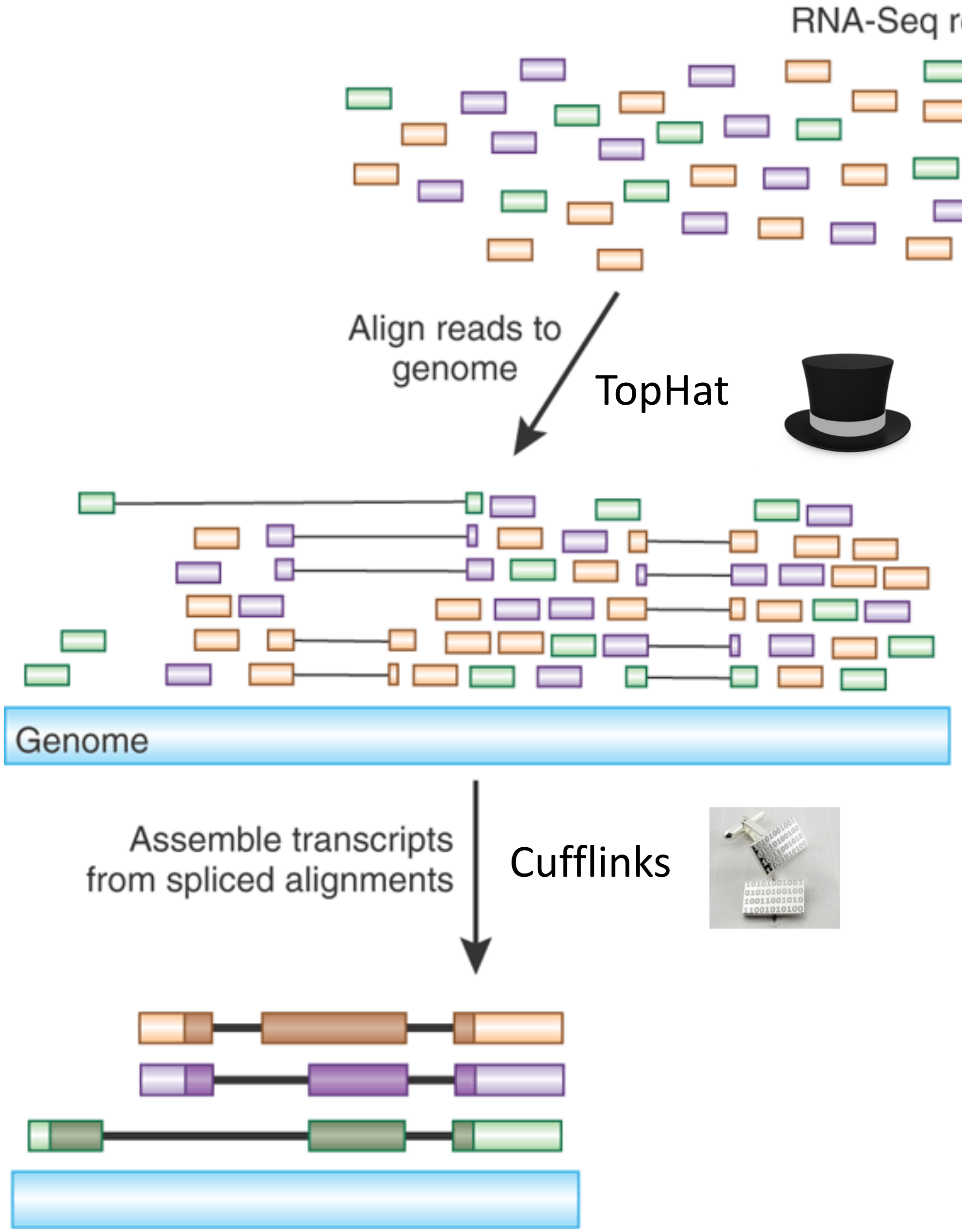
Transcript Reconstruction from RNA-Seq Reads



Transcript Reconstruction from RNA-Seq Reads



Transcript Reconstruction from RNA-Seq Reads



RNA-Seq reads

Align reads to genome

TopHat

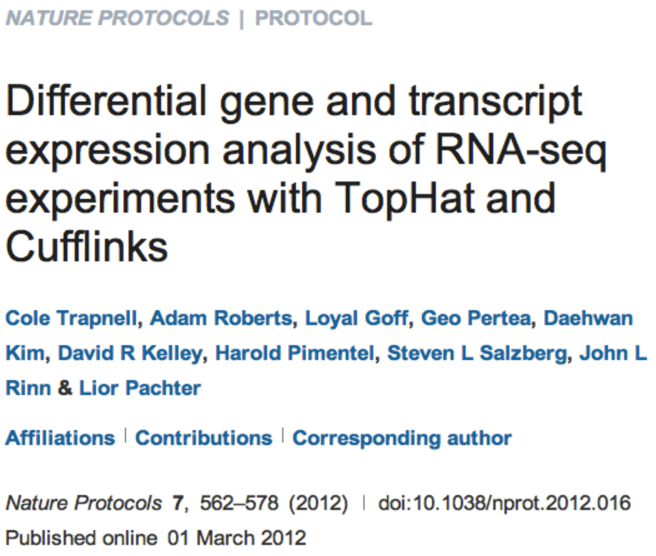
Genome

Assemble transcripts from spliced alignments

Cufflinks

The Tuxedo Suite:

End-to-end **Genome**-based
RNA-Seq Analysis
Software Package



NATURE PROTOCOLS | PROTOCOL

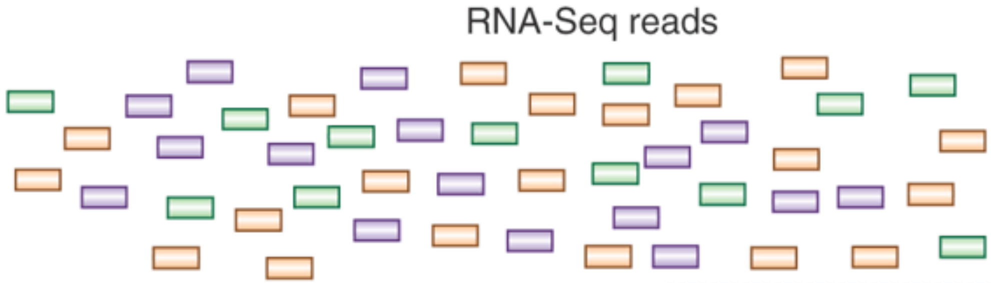
Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks

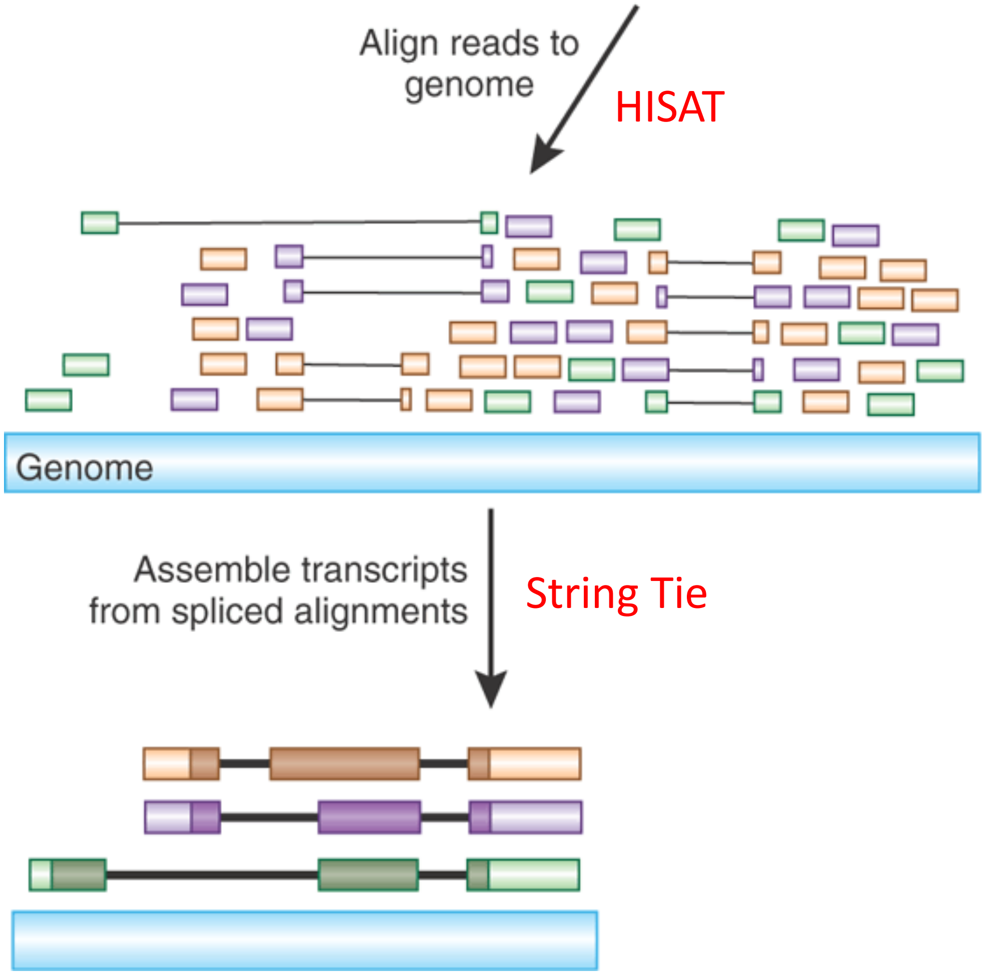
Cole Trapnell, Adam Roberts, Loyal Goff, Geo Pertea, Daehwan Kim, David R Kelley, Harold Pimentel, Steven L Salzberg, John L Rinn & Lior Pachter

Affiliations | Contributions | Corresponding author

Nature Protocols **7**, 562–578 (2012) | doi:10.1038/nprot.2012.016
Published online 01 March 2012

Transcript Reconstruction from RNA-Seq Reads



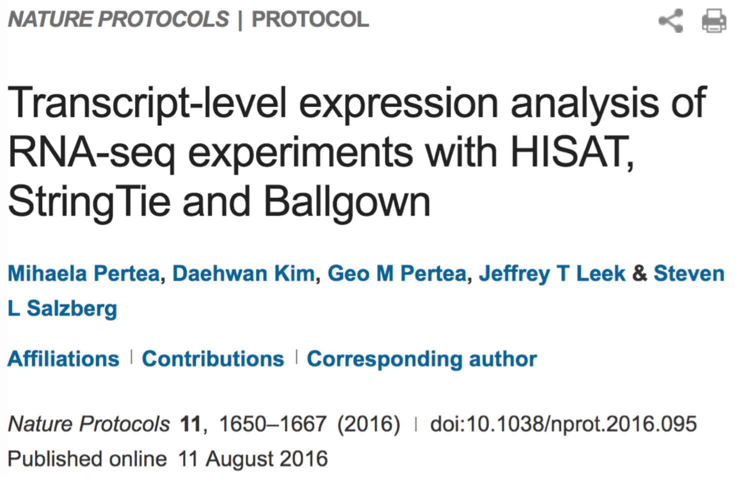


HISAT

String Tie

The “New Tuxedo” Suite:

End-to-end **Genome**-based RNA-Seq Analysis Software Package


NATURE PROTOCOLS | PROTOCOL

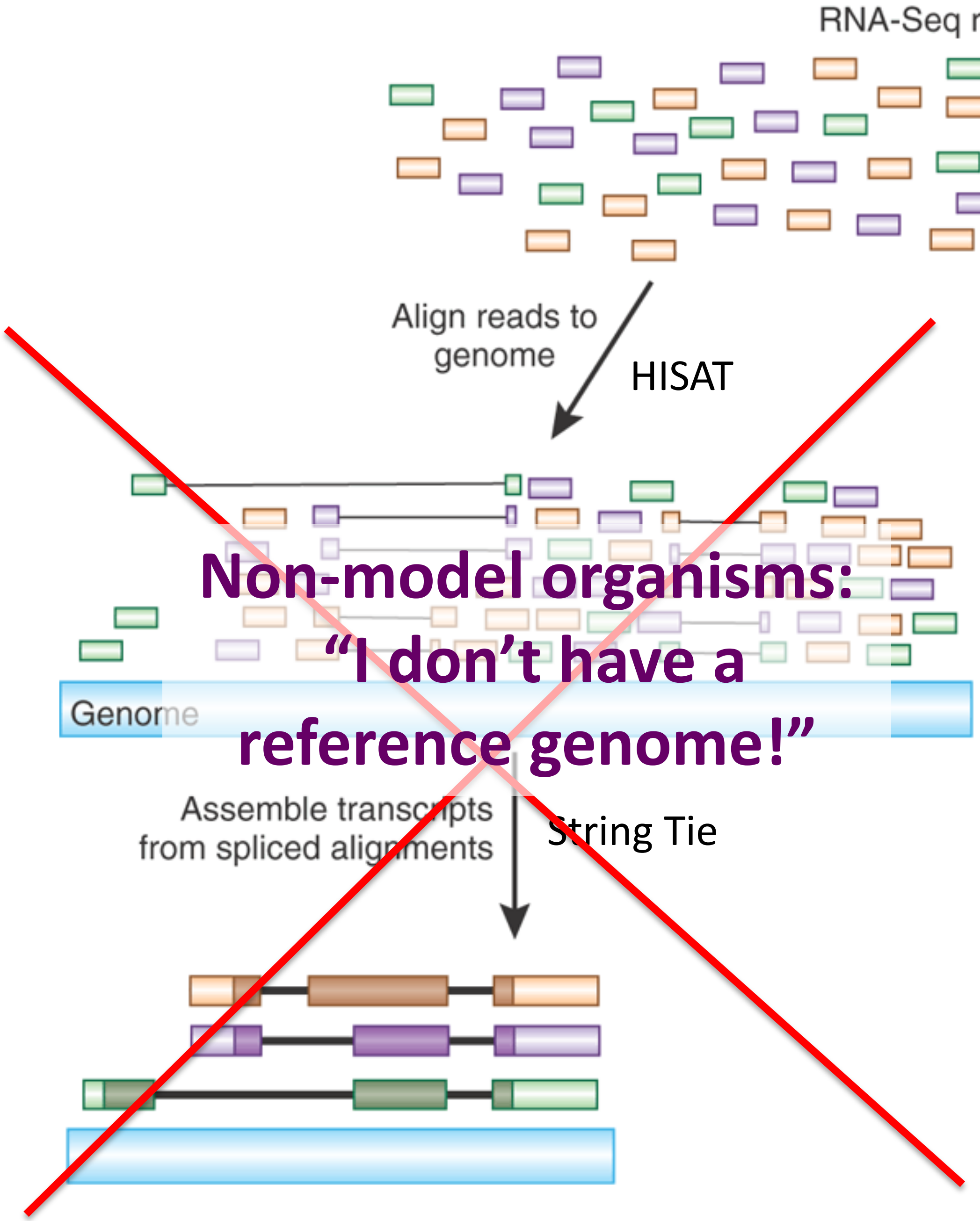
Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown

Mihaela Pertea, Daehwan Kim, Geo M Pertea, Jeffrey T Leek & Steven L Salzberg

Affiliations | Contributions | Corresponding author

Nature Protocols **11**, 1650–1667 (2016) | doi:10.1038/nprot.2016.095
Published online 11 August 2016

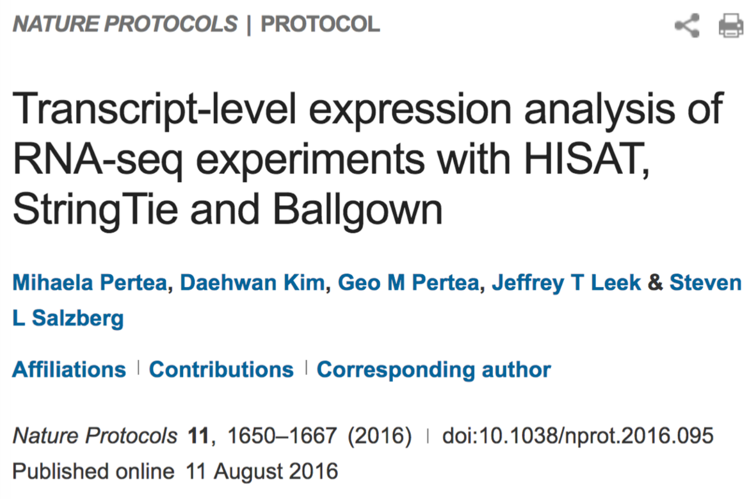
Transcript Reconstruction from RNA-Seq Reads



Non-model organisms: "I don't have a reference genome!"

The "New Tuxedo" Suite:

End-to-end **Genome**-based RNA-Seq Analysis Software Package



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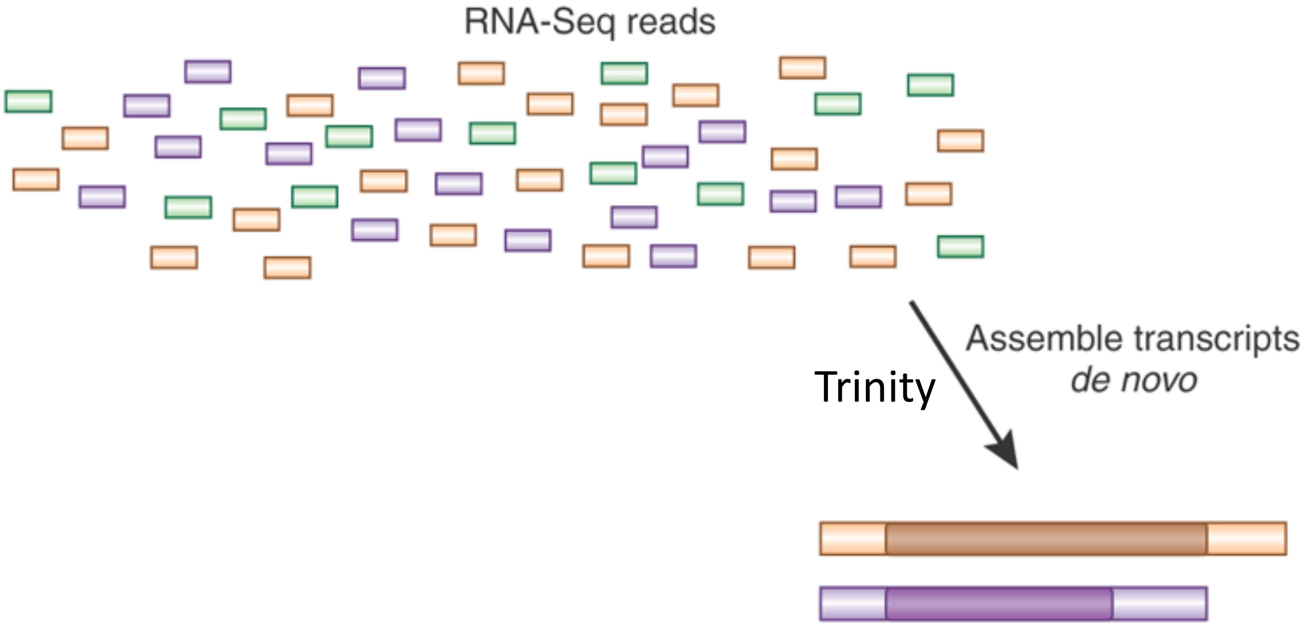
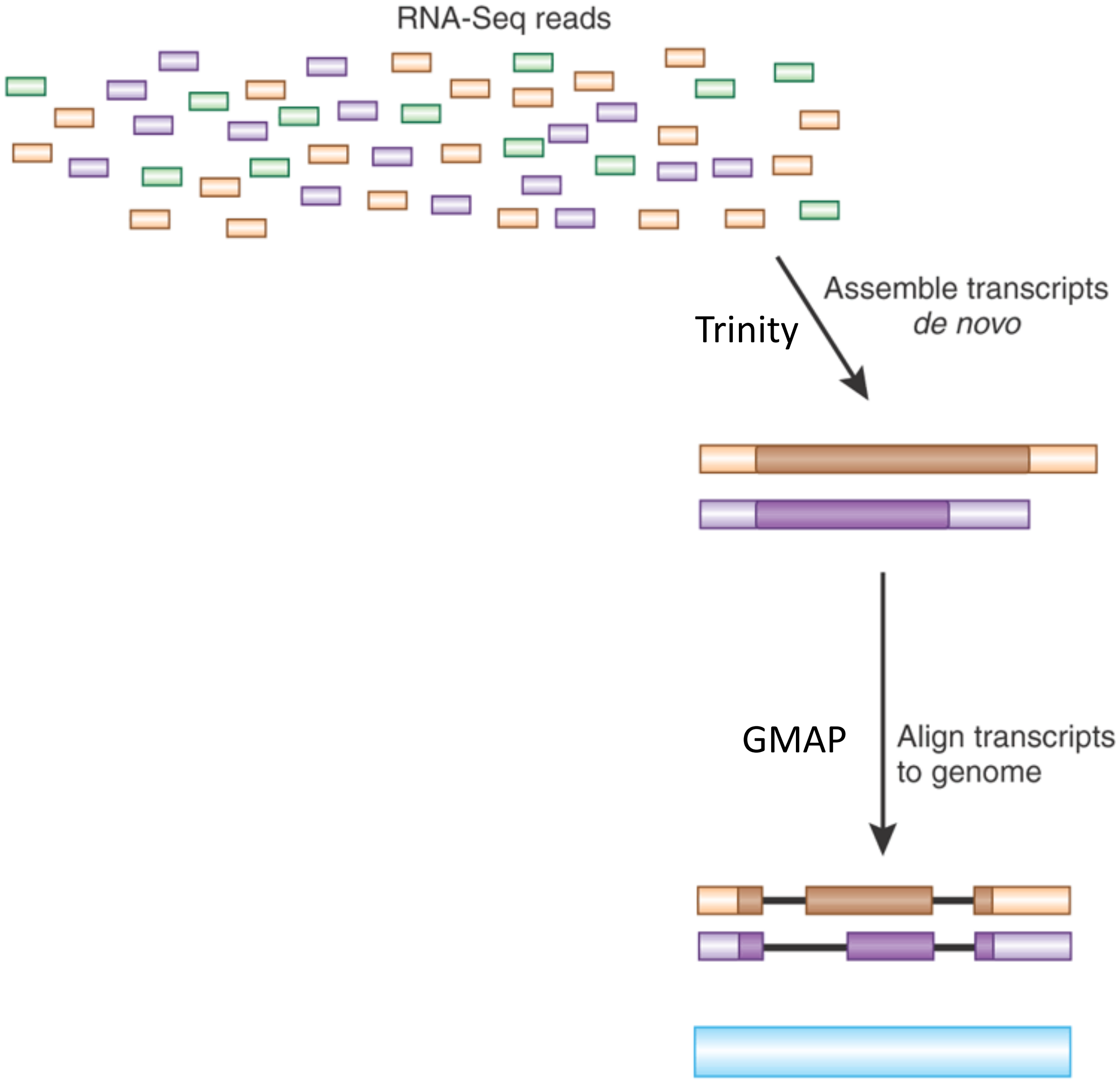


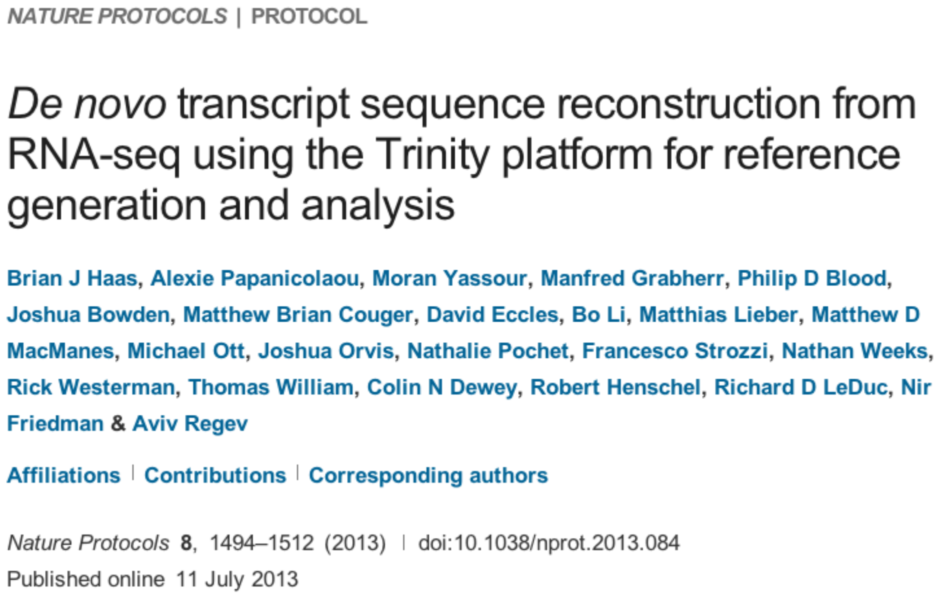
Transcript Reconstruction from RNA-Seq Reads



Transcript Reconstruction from RNA-Seq Reads

RNA-Seq reads

End-to-end **Transcriptome**-based
RNA-Seq Analysis
Software Package



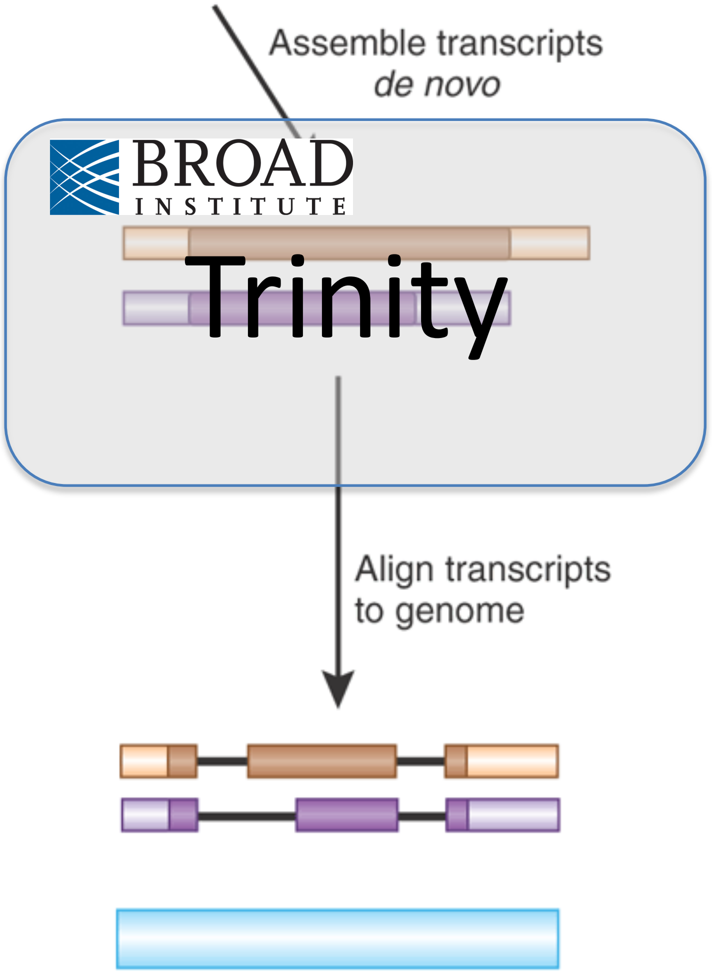
NATURE PROTOCOLS | PROTOCOL

De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis

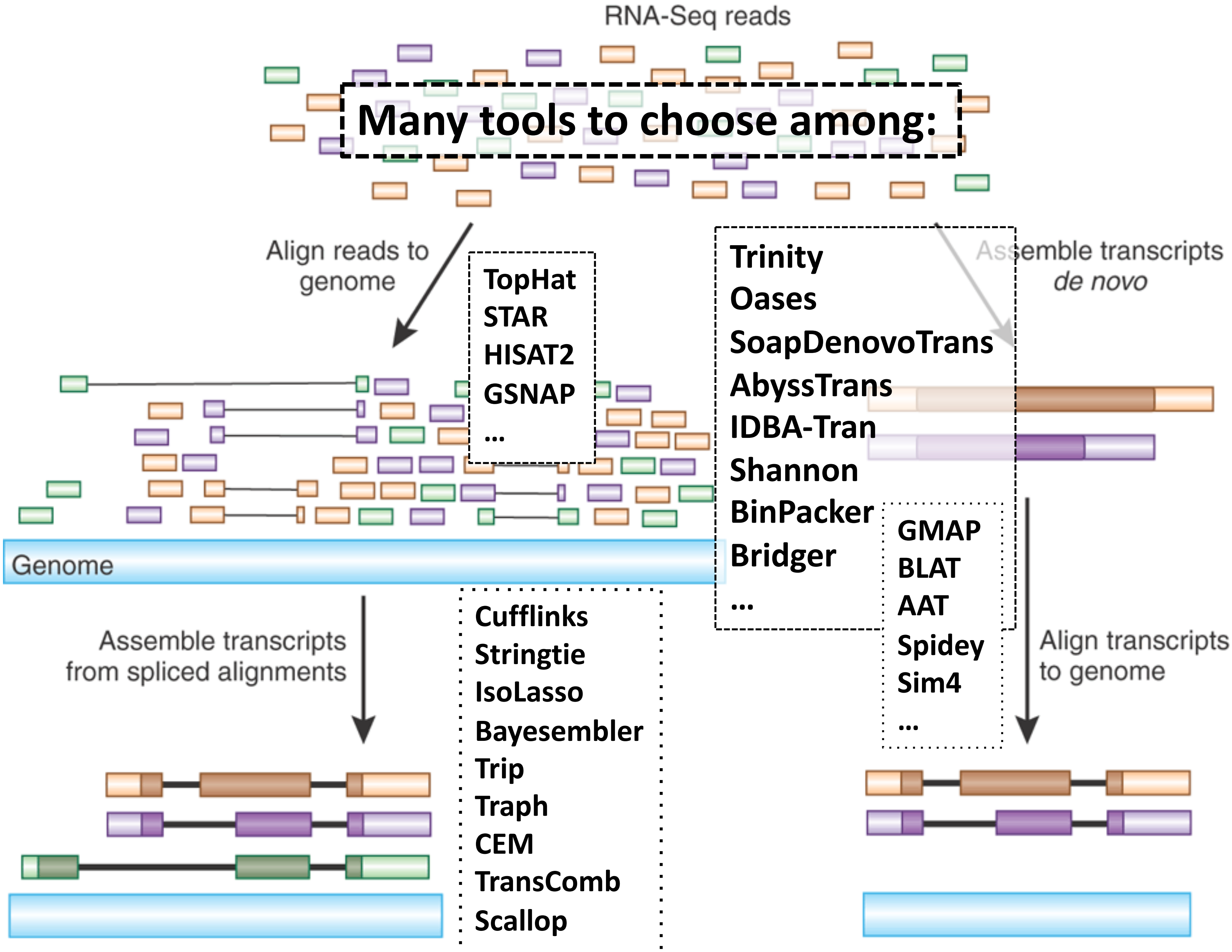
Brian J Haas, Alexie Papanicolaou, Moran Yassour, Manfred Grabherr, Philip D Blood, Joshua Bowden, Matthew Brian Couger, David Eccles, Bo Li, Matthias Lieber, Matthew D MacManes, Michael Ott, Joshua Orvis, Nathalie Pochet, Francesco Strozzi, Nathan Weeks, Rick Westerman, Thomas William, Colin N Dewey, Robert Henschel, Richard D LeDuc, Nir Friedman & Aviv Regev

Affiliations | Contributions | Corresponding authors

Nature Protocols 8, 1494–1512 (2013) | doi:10.1038/nprot.2013.084
Published online 11 July 2013



Transcript Reconstruction from RNA-Seq Reads


RNA-Seq reads
Align reads to genome
Assemble transcripts *de novo*
Genome
Assemble transcripts from spliced alignments
Align transcripts to genome

Many tools to choose among:

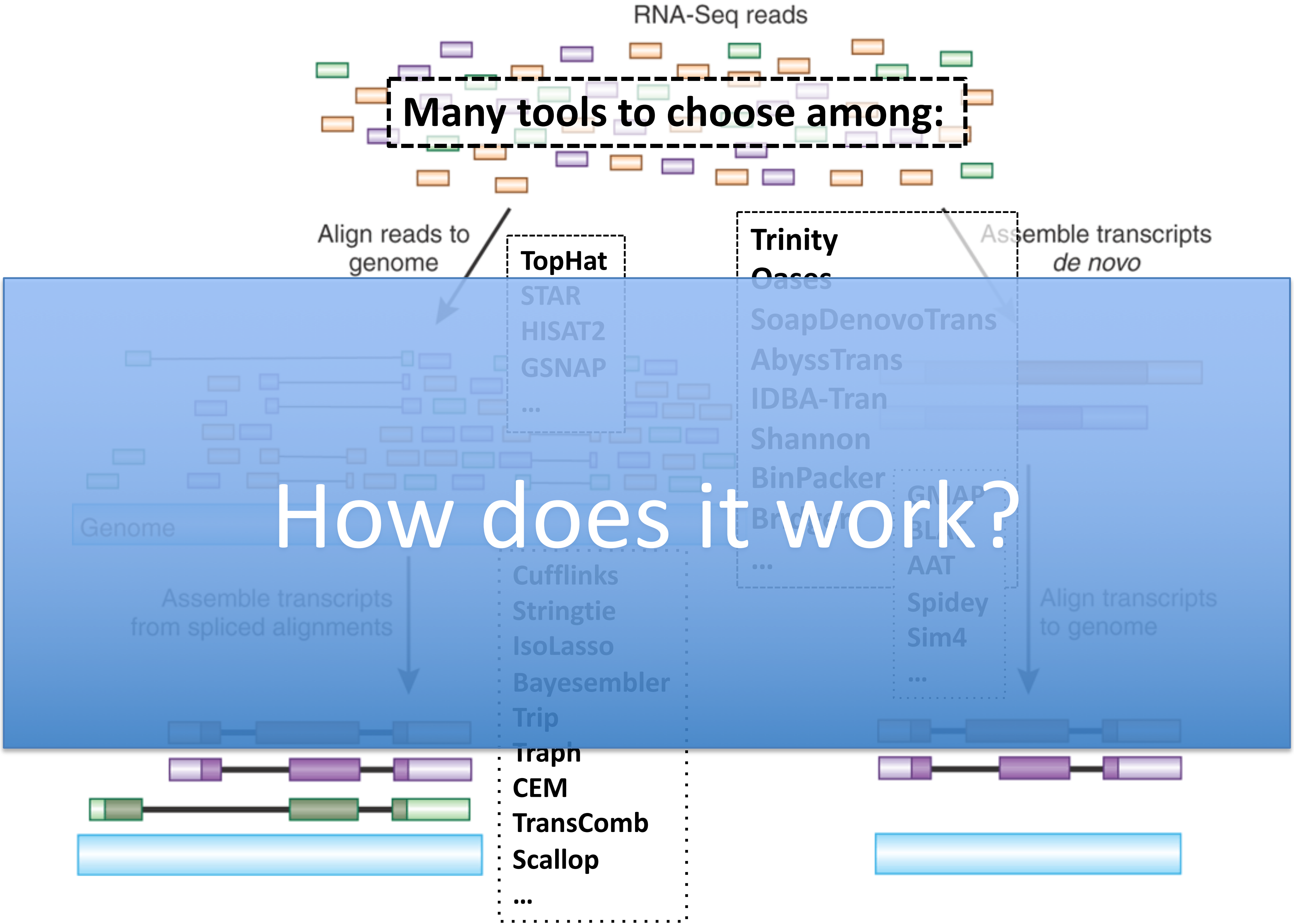
TopHat
STAR
HISAT2
GSNAP
…

Trinity
Oases
SoapDenovoTrans
AbyssTrans
IDBA-Tran
Shannon
BinPacker
Bridger
…

GMAP
BLAT
AAT
Spidey
Sim4
…

Cufflinks
Stringtie
IsoLasso
Bayesembler
Trip
Traph
CEM
TransComb
Scallop
…

Transcript Reconstruction from RNA-Seq Reads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BLAT
AAT
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Sim4
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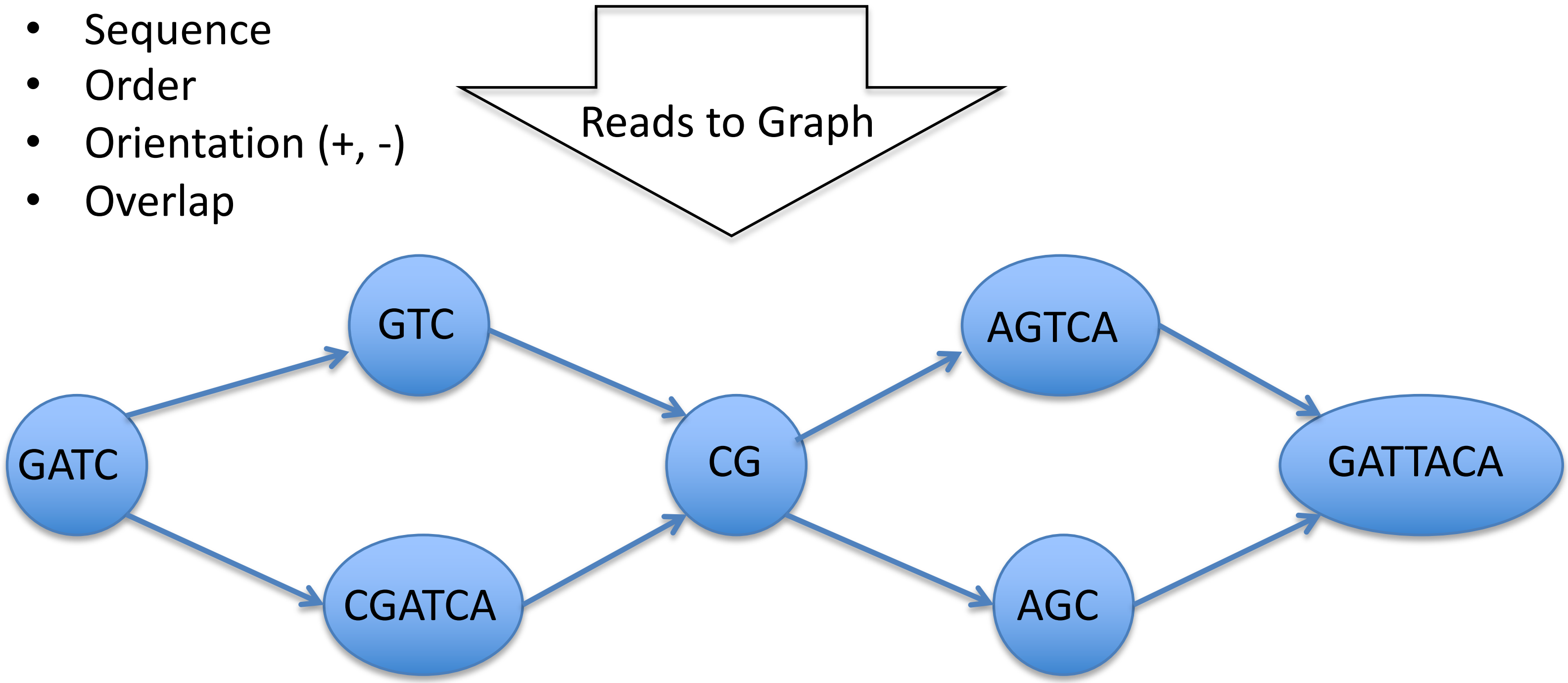
Cufflinks
Stringtie
IsoLasso
Bayesembler
Trip
Traph
CEM
TransComb
Scallop
…

How does it work?

Graph Data Structures Commonly Used For Assembly

- Sequence
- Order
- Orientation (+, -)
- Overlap



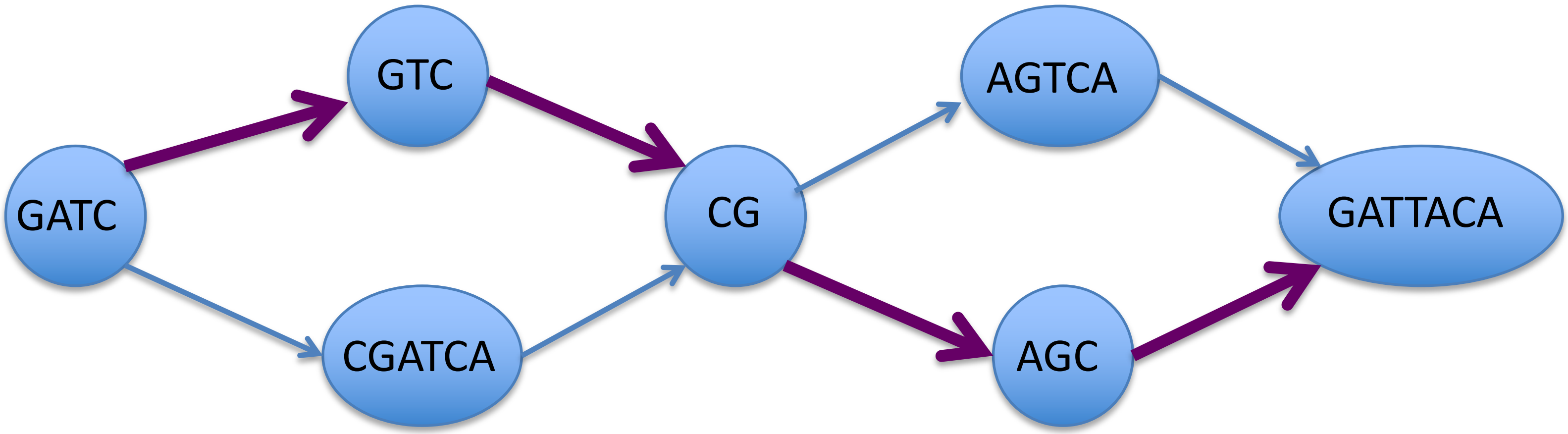
Nodes = sequence (+/-)
Edges = order, overlap

Graph Data Structures Commonly Used For Assembly

RNA-Seq reads

- Sequence
- Order
- Orientation (+, -)
- Overlap

Reads to Graph

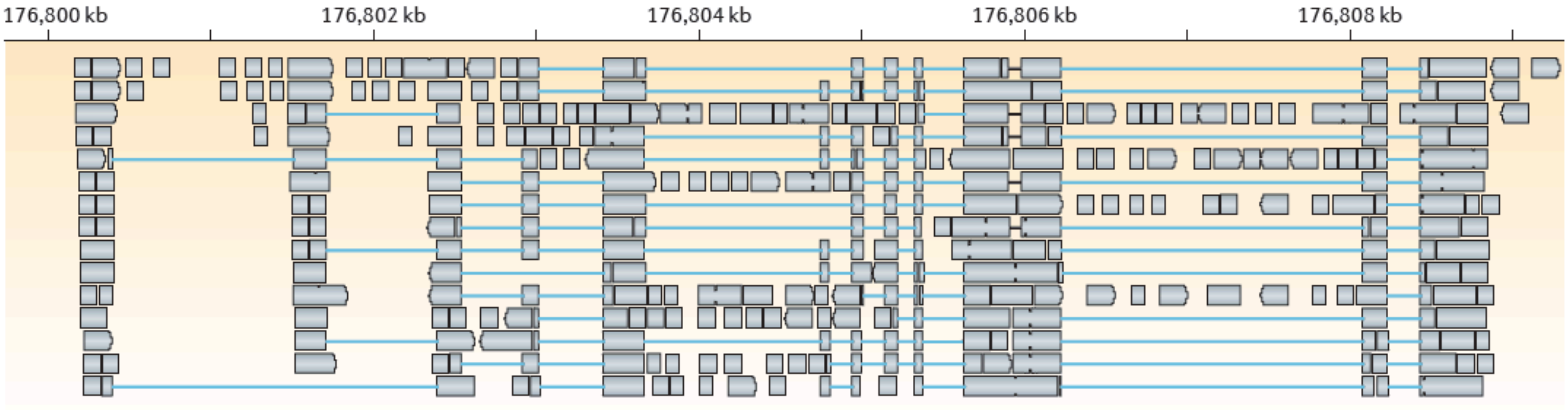


GATCGTCCGAGCGATTACA

Nodes = sequence (+/-)
Edges = order, overlap

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome



From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

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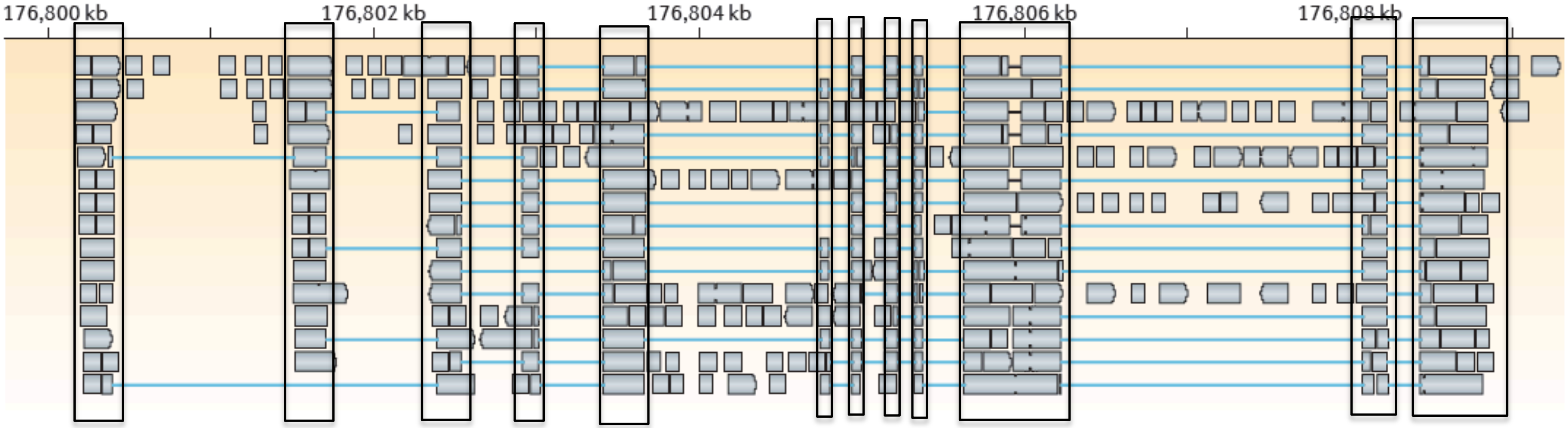
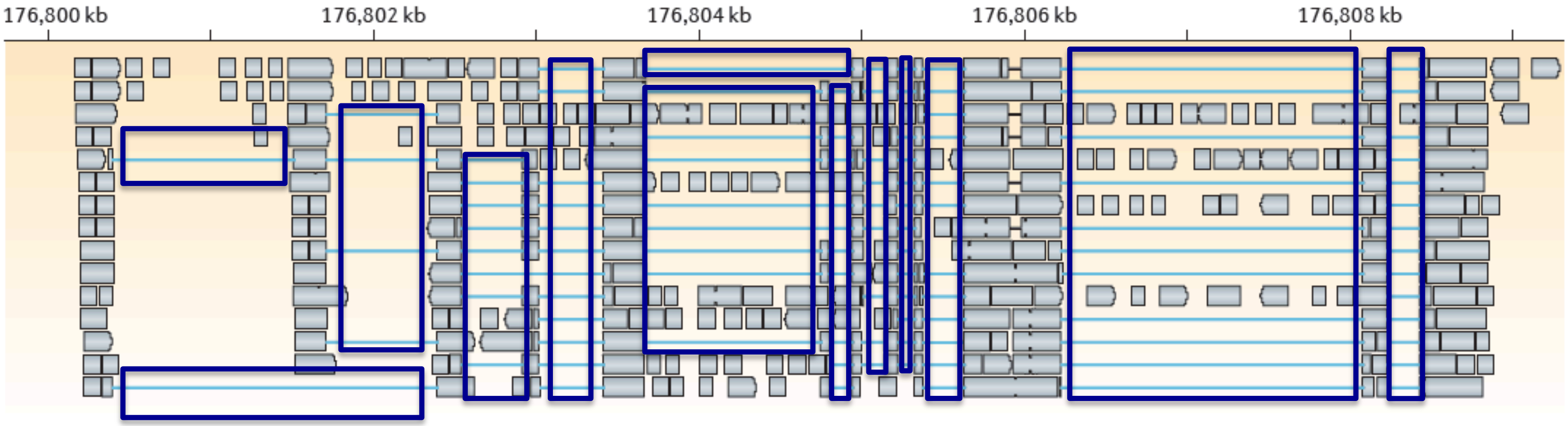


Alignment segment piles => exon regions

From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome

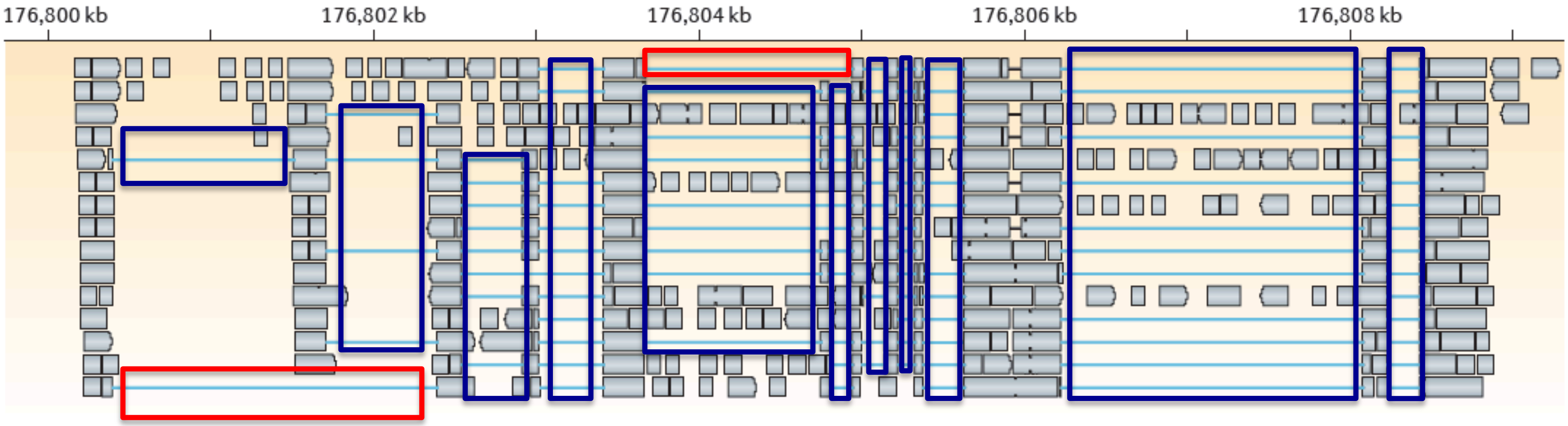


Large alignment gaps => introns

From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome

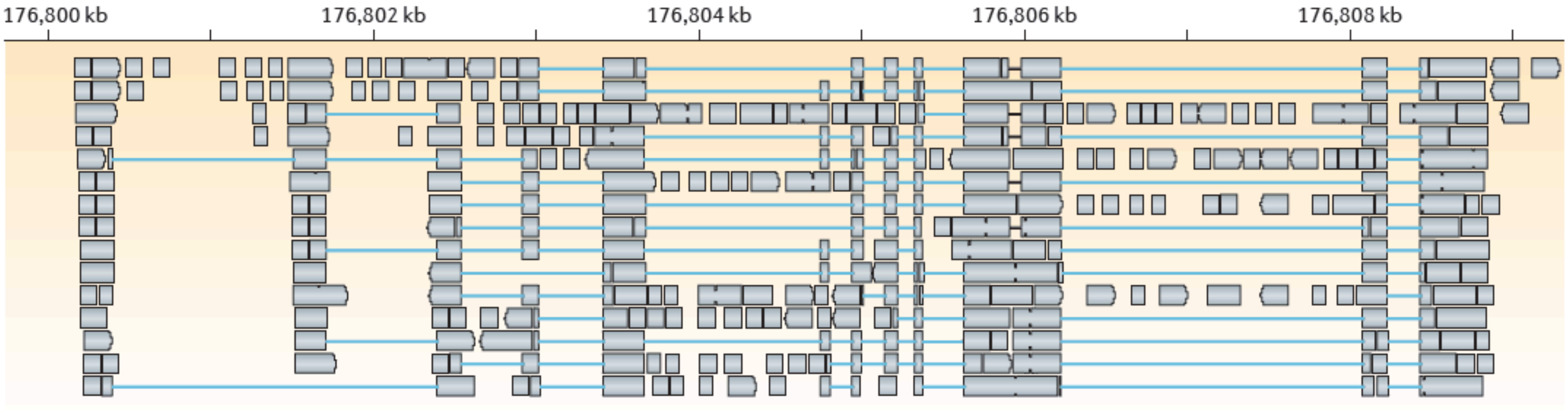


Overlapping but different introns = evidence of alternative splicing

From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome



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Genome-Guided Transcript Reconstruction

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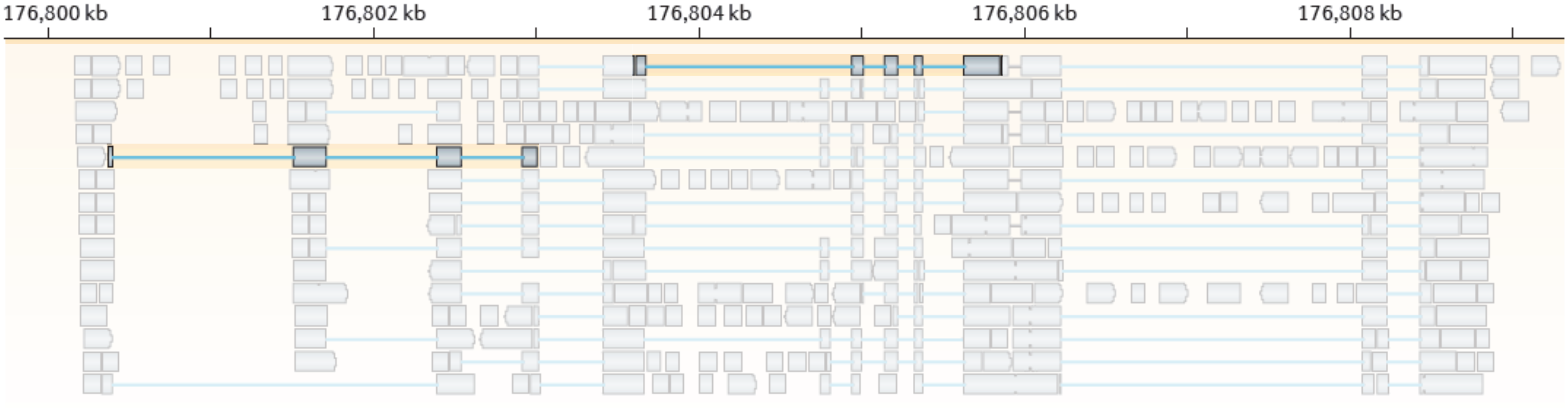
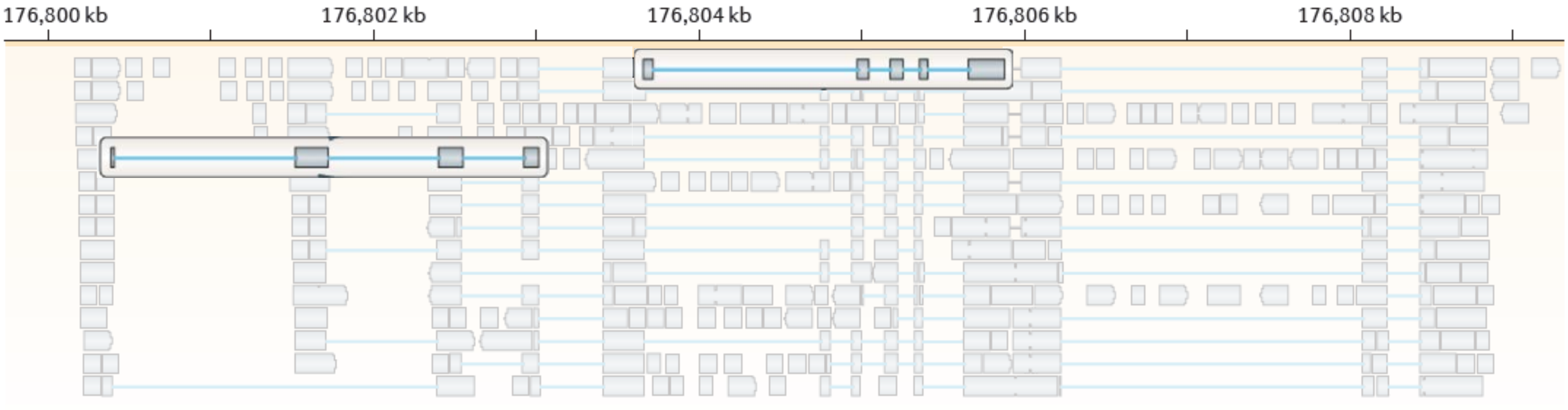


Individual reads can yield multiple exon and intron segments (splice patterns)

From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome

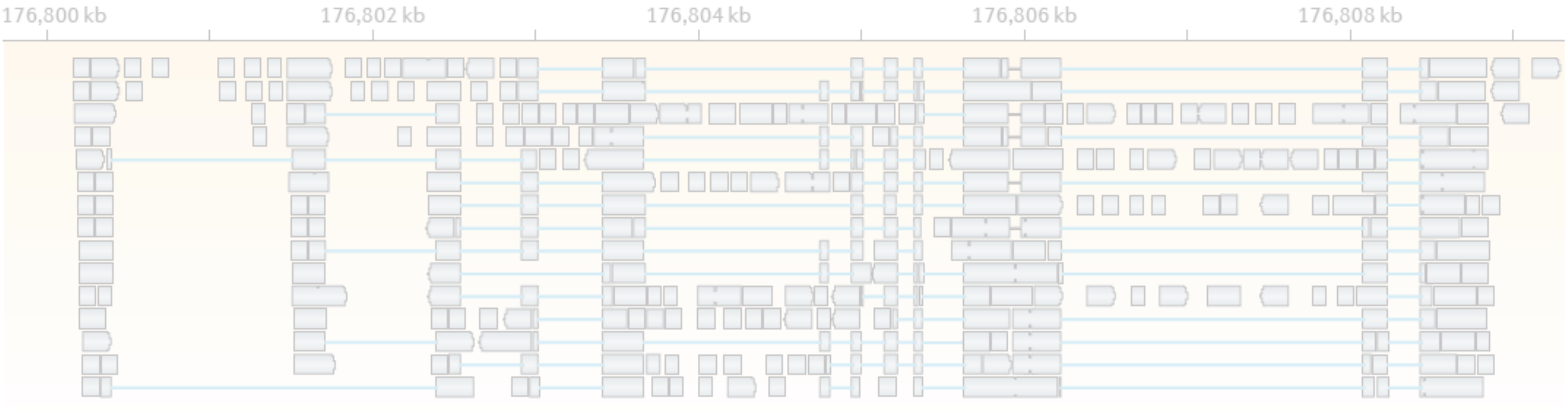


Nodes = unique splice patterns

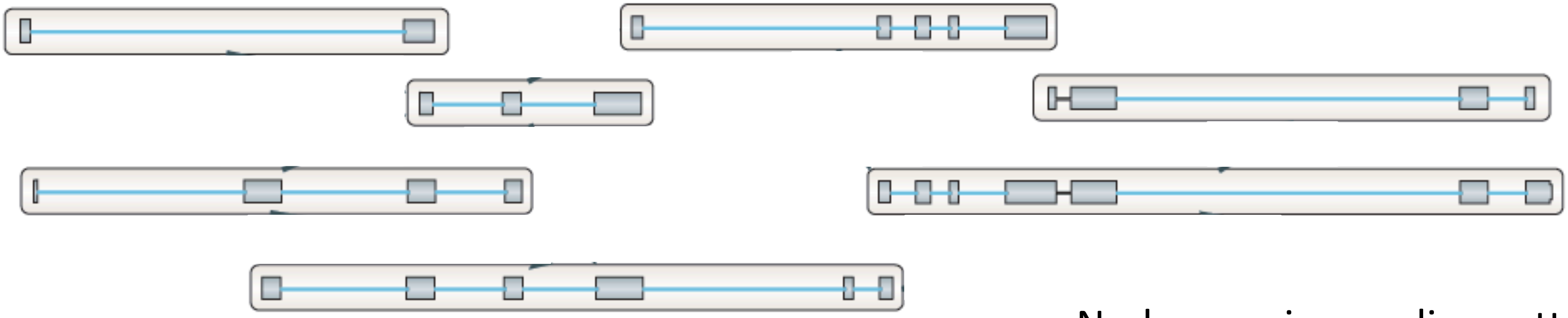
From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome



Construct graph from unique splice patterns of aligned reads.



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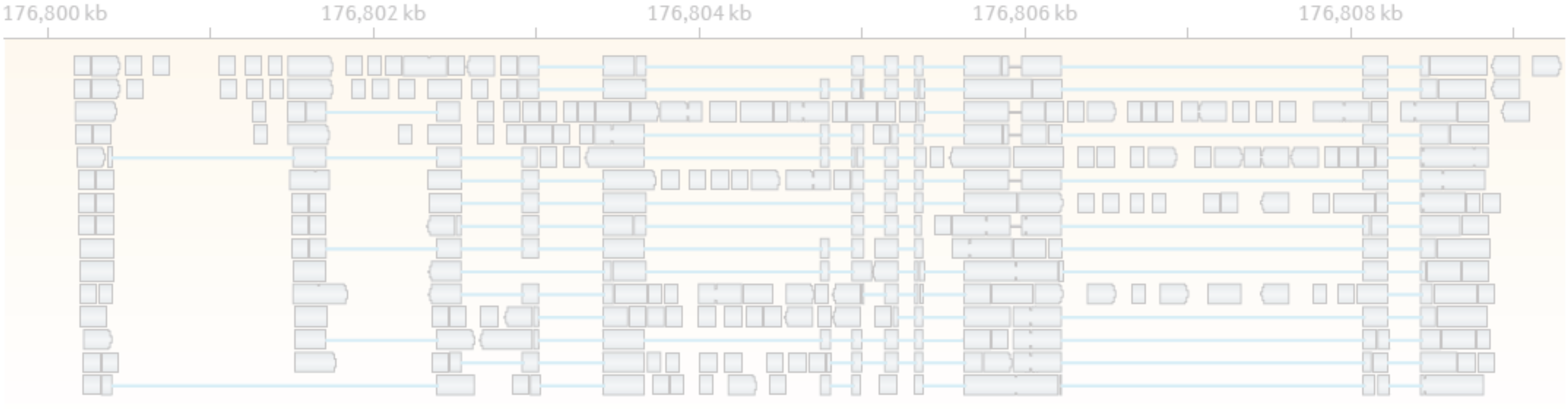

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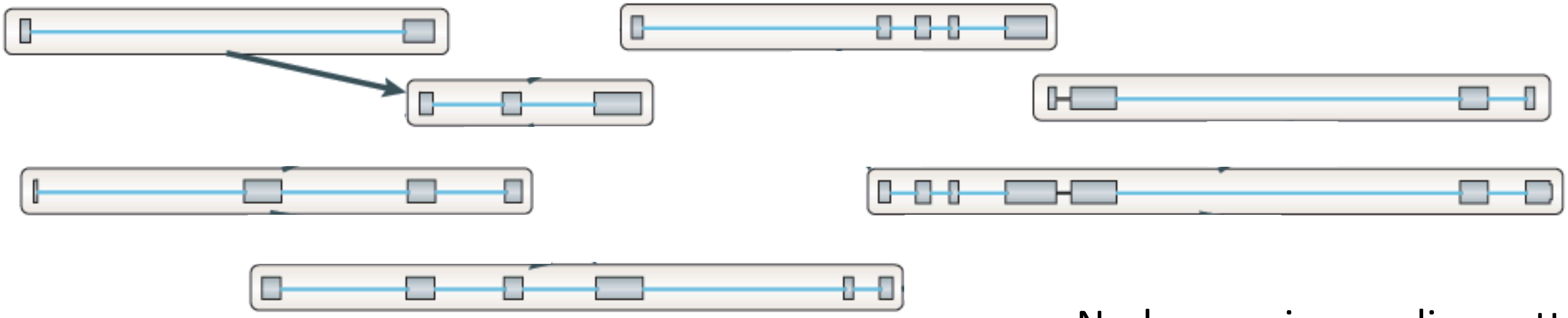
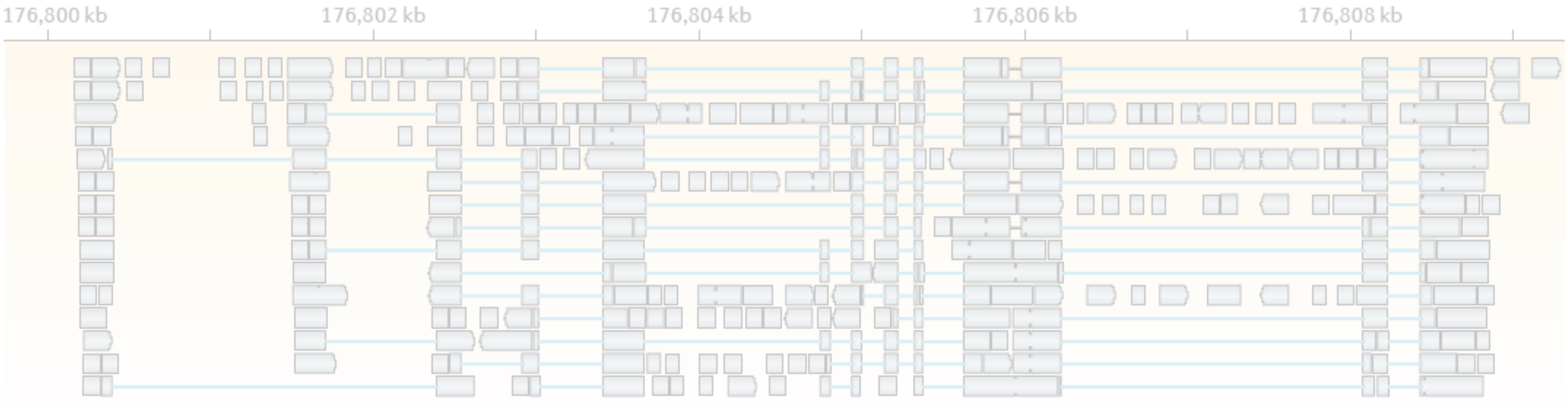


Nodes = unique splice patterns
Edges = compatible patterns

From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome



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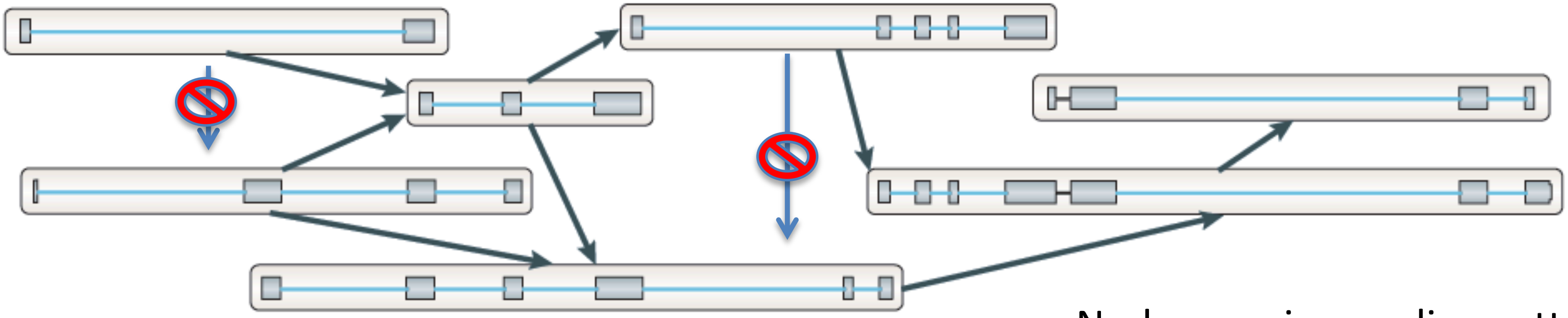

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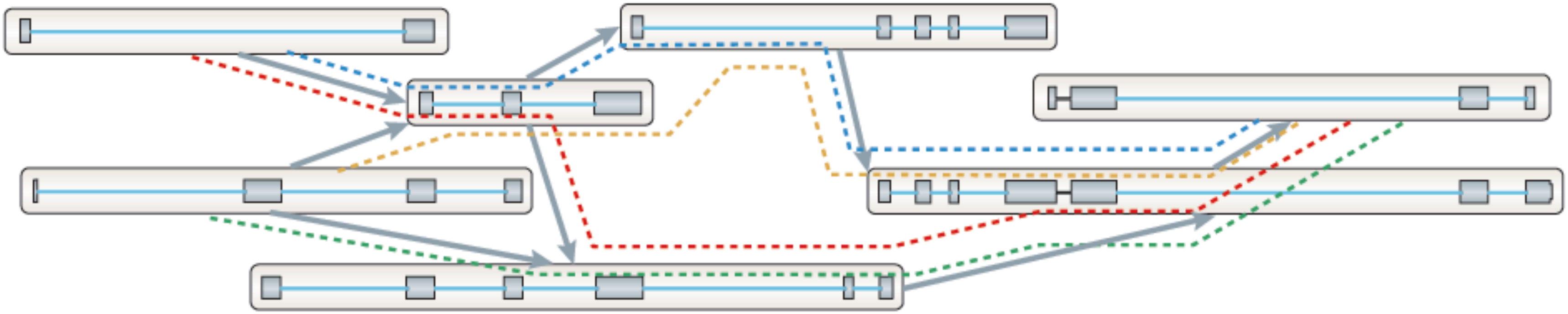
Genome-Guided Transcript Reconstruction

Traverse paths through the graph to assemble transcript isoforms



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Genome-Guided Transcript Reconstruction

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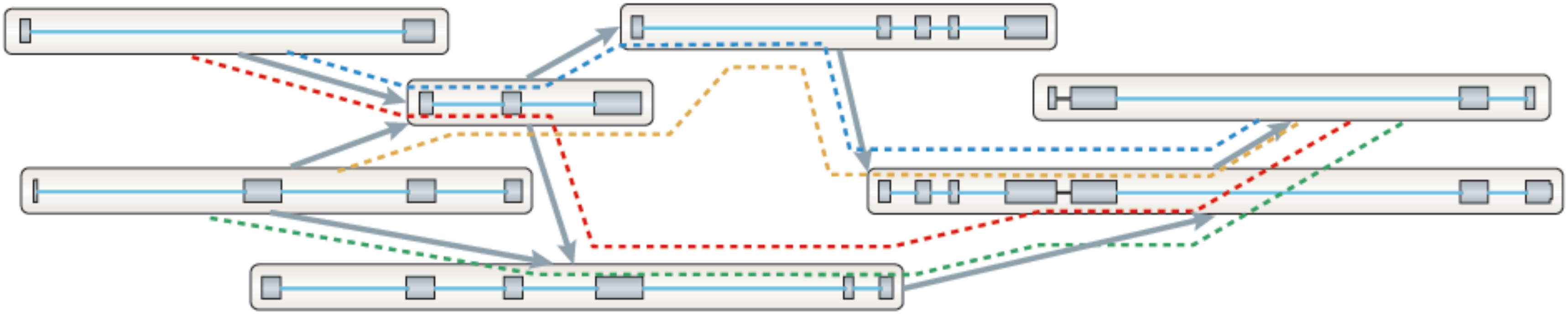
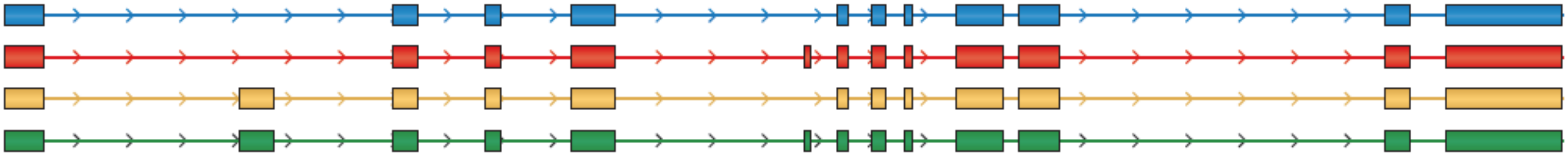


Reconstructed isoforms

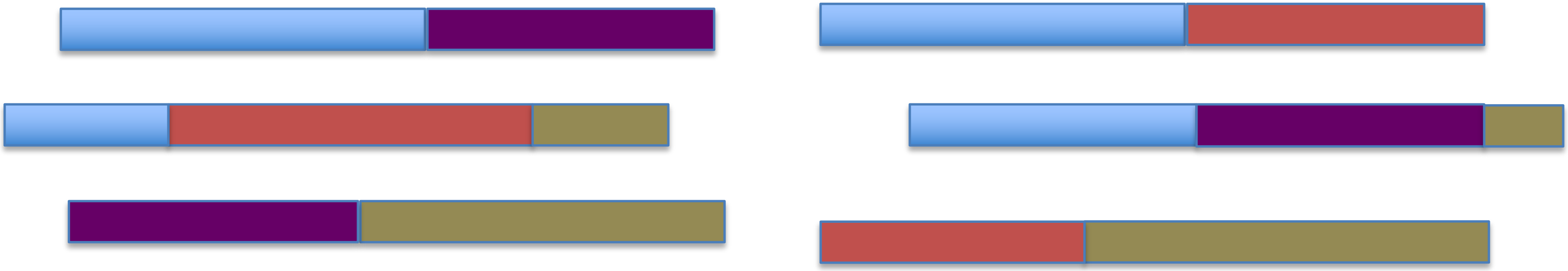


From Martin & Wang. Nature Reviews in Genetics. 2011

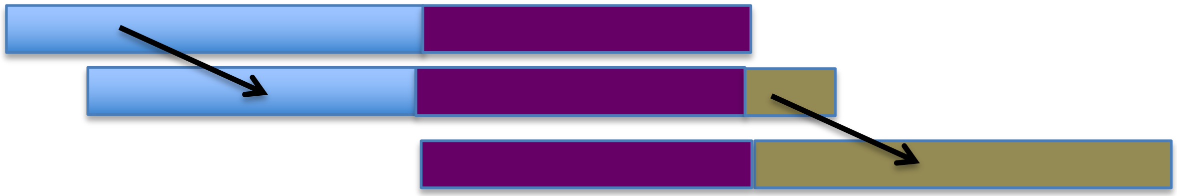
What if you don’t have a high quality reference genome sequence?

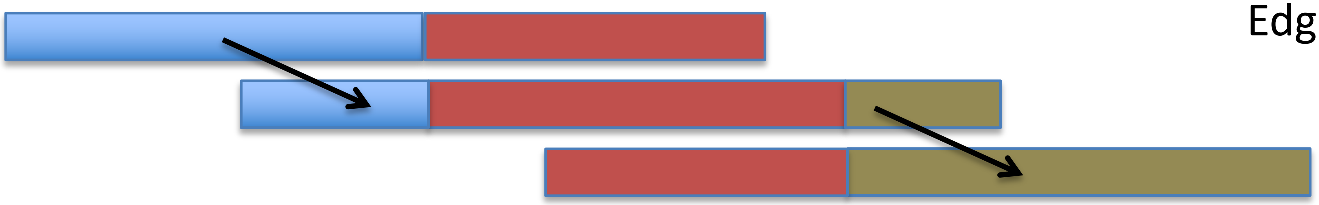
Genome-free de novo transcript reconstruction to the rescue.

Read Overlap Graph: Reads as nodes, overlaps as edges



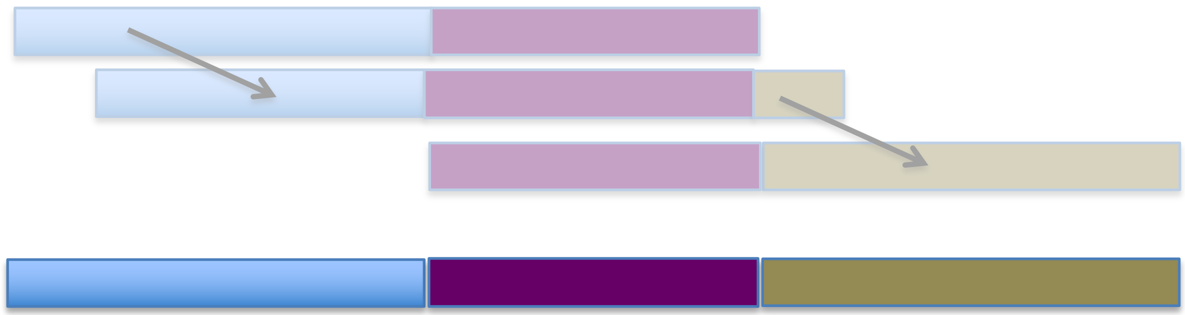
Read Overlap Graph: Reads as nodes, overlaps as edges





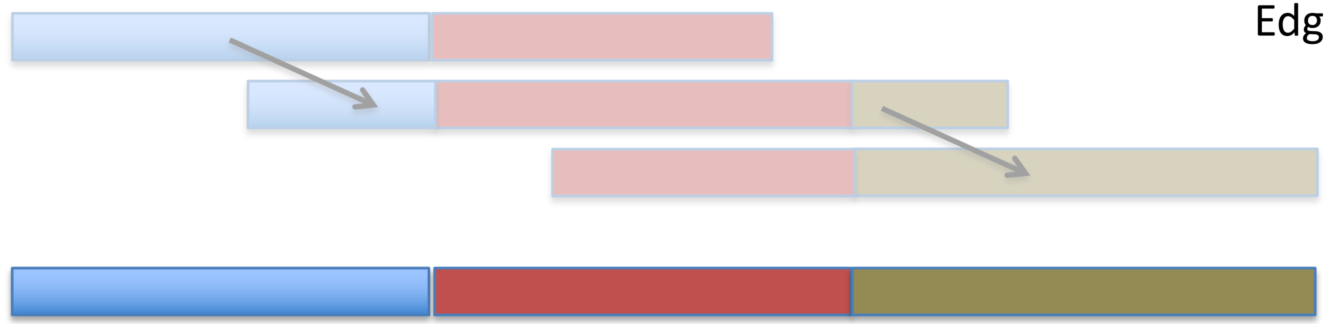
Node = read
Edge = overlap

Read Overlap Graph: Reads as nodes, overlaps as edges

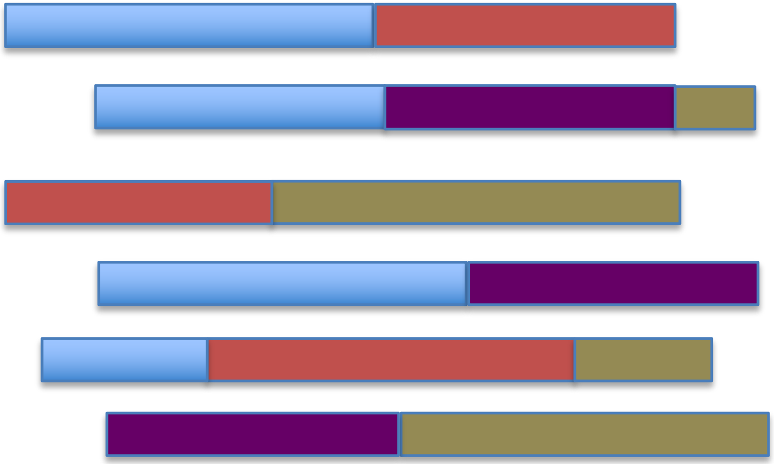


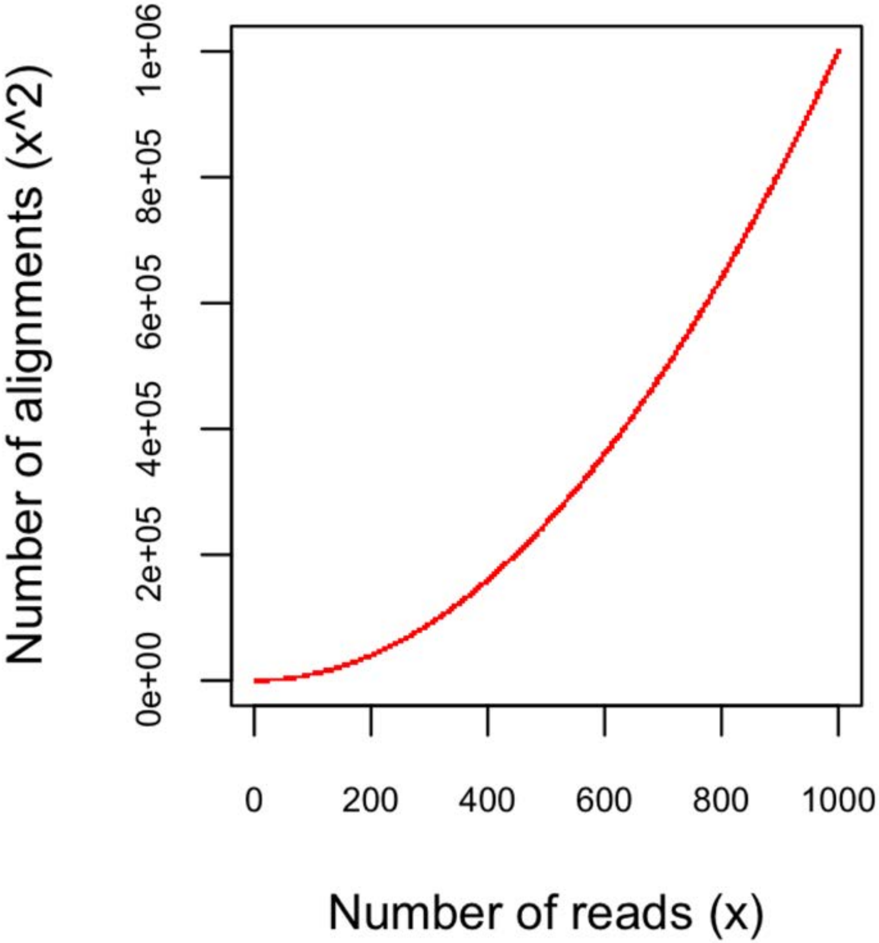
Generate consensus sequence where reads overlap

Node = read
Edge = overlap



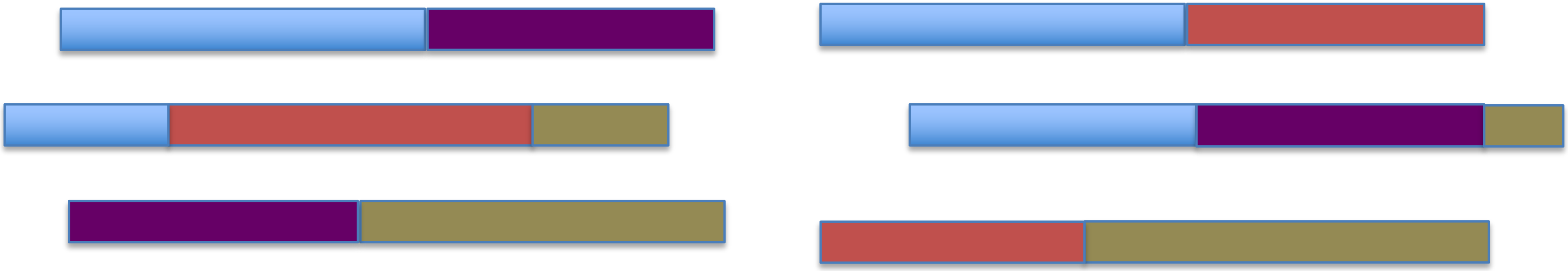
Finding pairwise overlaps between *n* reads involves ~ n^2 comparisons.





Impractical for typical RNA-Seq data (50M reads)

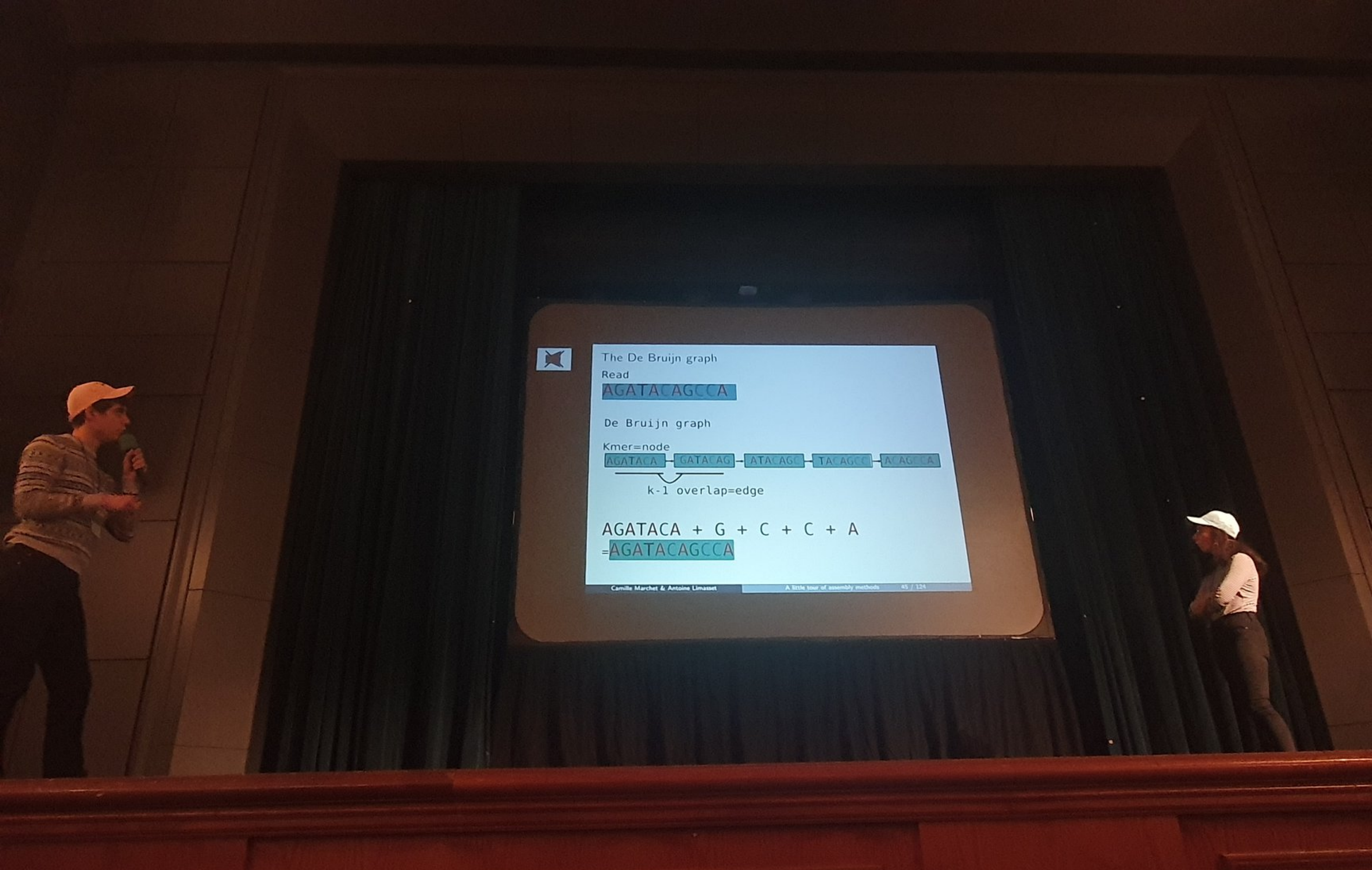
No genome to align to… De novo assembly required



Want to avoid ***n***n^2 read alignments to define overlaps

Use a de Bruijn graph

Have you learned about the de Bruijn graph already?



The De Bruijn graph

Read

AGATACAGCCA

De Bruijn graph

Kmer=node

AGATACA – GATACAG – ATACAGC – TACAGCC – ACAGCCA

k-1 overlap=edge

AGATACA + G + C + C + A

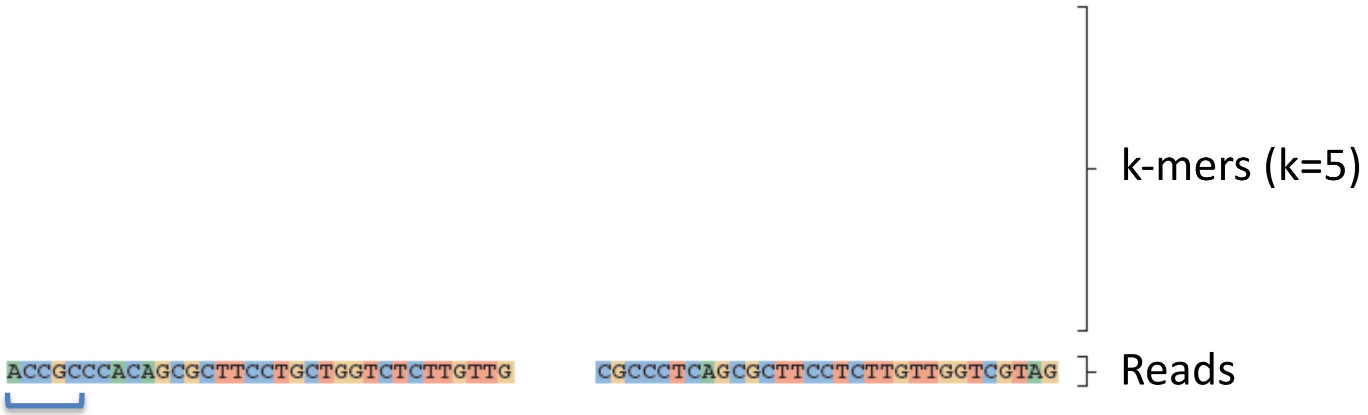
=AGATACAGCCA

Camille Marchet & Antoine Limasset — A little tour of assembly methods — 45 / 124

Yes, you have. ☺

Sequence Assembly via de Bruijn Graphs

Generate all substrings of length k from the reads



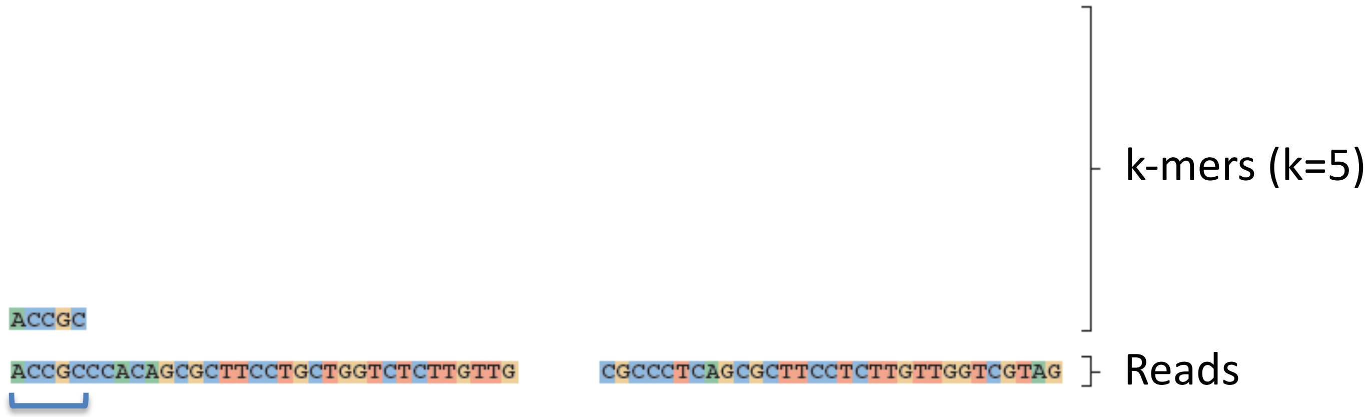
ACCGCCCACAGCGCTTCCTGCTGGTCTCTTGTTG

CGCCCTCAGCGCTTCCTCTTGTTGGTCGTAG

From Martin & Wang, Nat. Rev. Genet. 2011

Sequence Assembly via De Bruijn Graphs

Generate all substrings of length k from the reads



ACCGC

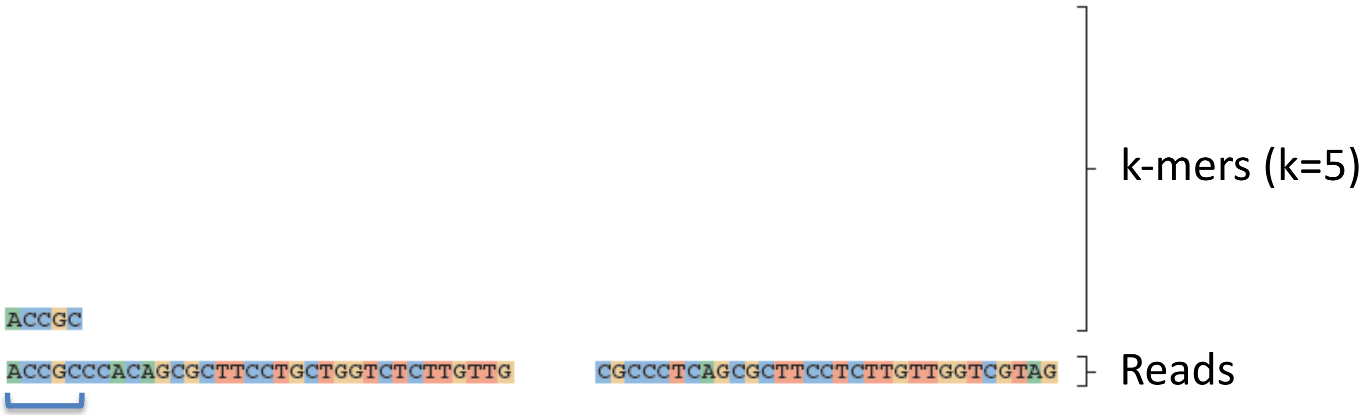
ACCGCCCACAGCGCTTCCTGCTGGTCTCTTGTTG

CGCCCTCAGCGCTTCCTCTTGTTGGTCGTAG

From Martin & Wang, Nat. Rev. Genet. 2011

Sequence Assembly via De Bruijn Graphs

Generate all substrings of length k from the reads



k-mers (k=5)

ACCGC

ACCGCCCACAGCGCTTCCTGCTGGTCTCTTGTTG

CGCCCTCAGCGCTTCCTCTTGTTGGTCGTAG

Reads

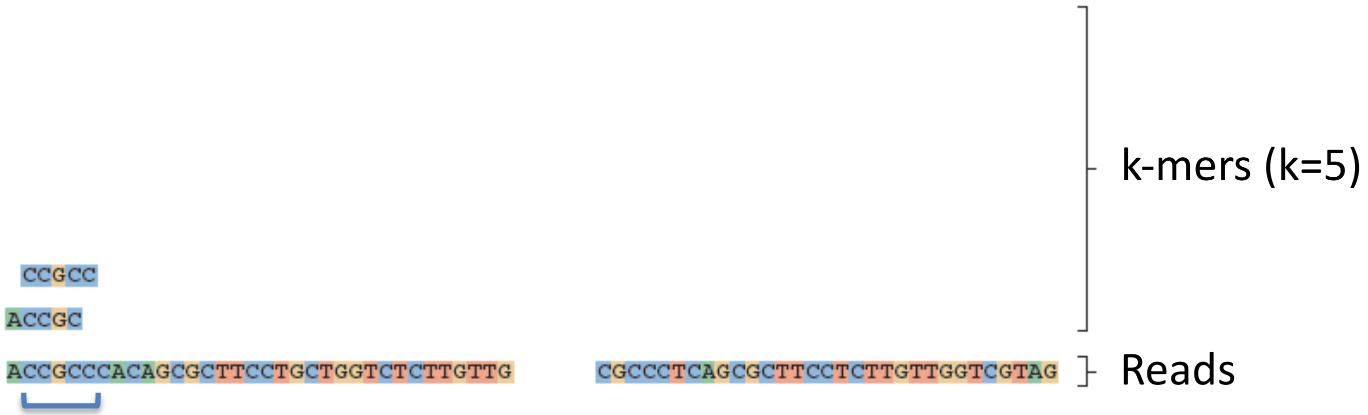
Construct the de Bruijn graph

Nodes = unique k-mers

From Martin & Wang, Nat. Rev. Genet. 2011

Sequence Assembly via De Bruijn Graphs

Generate all substrings of length k from the reads


k-mers (k=5)
CCGCC
ACCGC
ACCGCCCACAGCGCTTCCTGCTGGTCTCTTGTTG
CGCCCTCAGCGCTTCCTCTTGTTGGTCGTAG
Reads

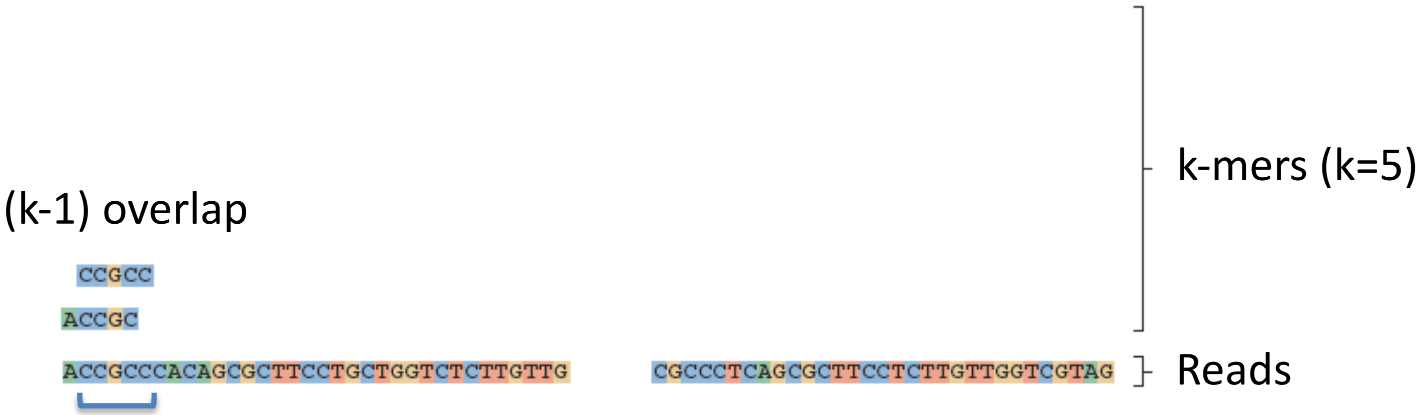
Construct the de Bruijn graph

Nodes = unique k-mers
Edges = overlap by (k-1)

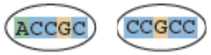
From Martin & Wang, Nat. Rev. Genet. 2011

Sequence Assembly via De Bruijn Graphs

Generate all substrings of length k from the reads


k-mers (k=5)
(k-1) overlap
CCGCC
ACCGC
ACCGCCCACAGCGCTTCCTGCTGGTCTCTTGTTG
CGCCCTCAGCGCTTCCTCTTGTTGGTCGTAG
Reads

Construct the de Bruijn graph



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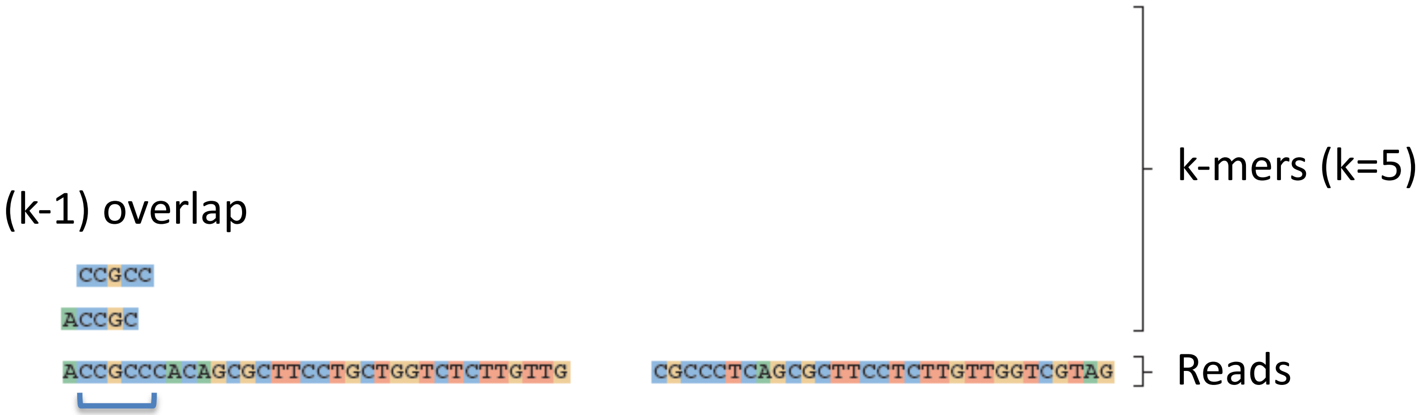
Edges = overlap by (k-1)

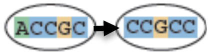
From Martin & Wang, Nat. Rev. Genet. 2011

Sequence Assembly via De Bruijn Graphs

Generate all substrings of length k from the reads


k-mers (k=5)
(k-1) overlap
CCGCC
ACCGC
ACCGCCCACAGCGCTTCCTGCTGGTCTCTTGTTG
CGCCCTCAGCGCTTCCTCTTGTTGGTCGTAG
Reads

Construct the de Bruijn graph



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Edges = overlap by (k-1)

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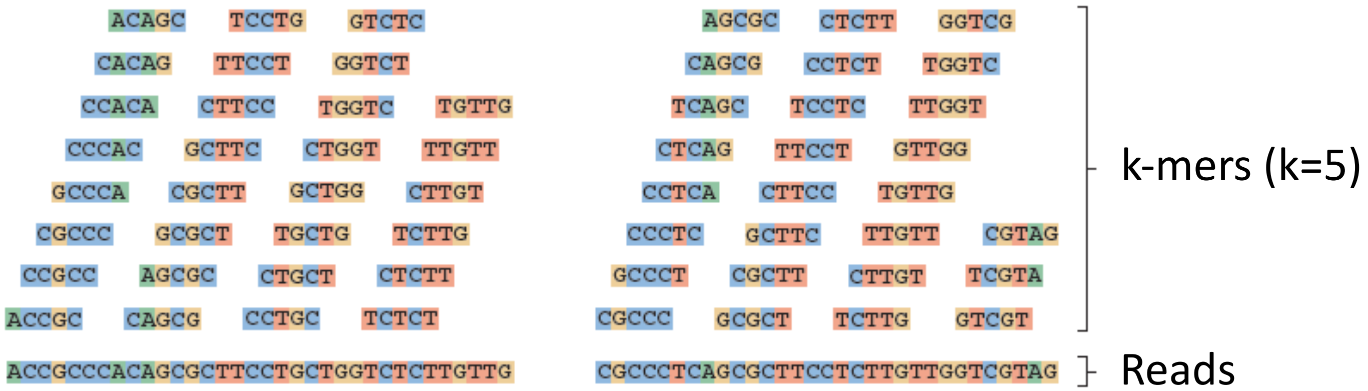
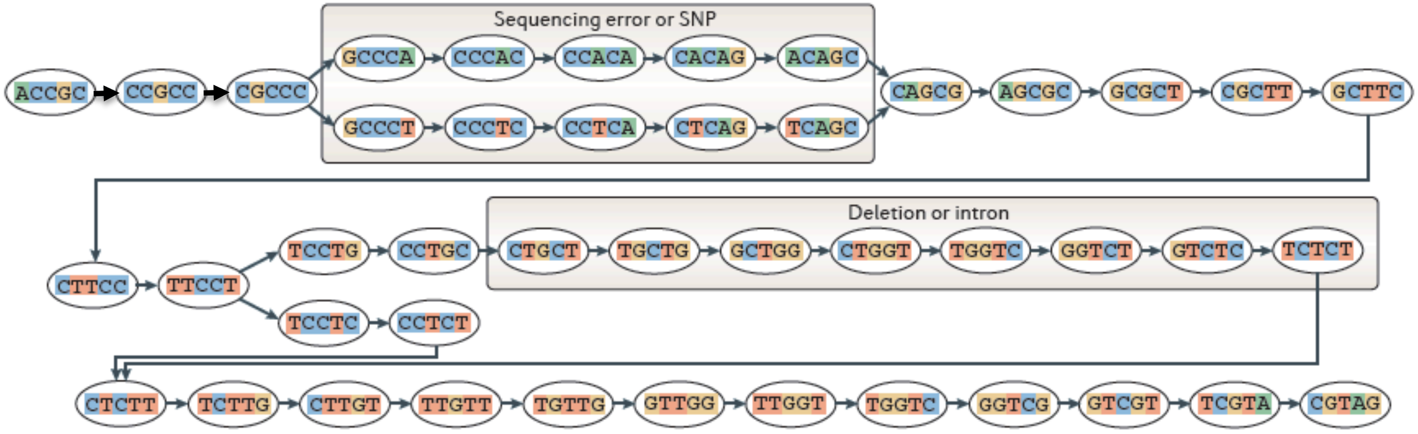
Sequence Assembly via De Bruijn Graphs

Generate all substrings of length k from the reads



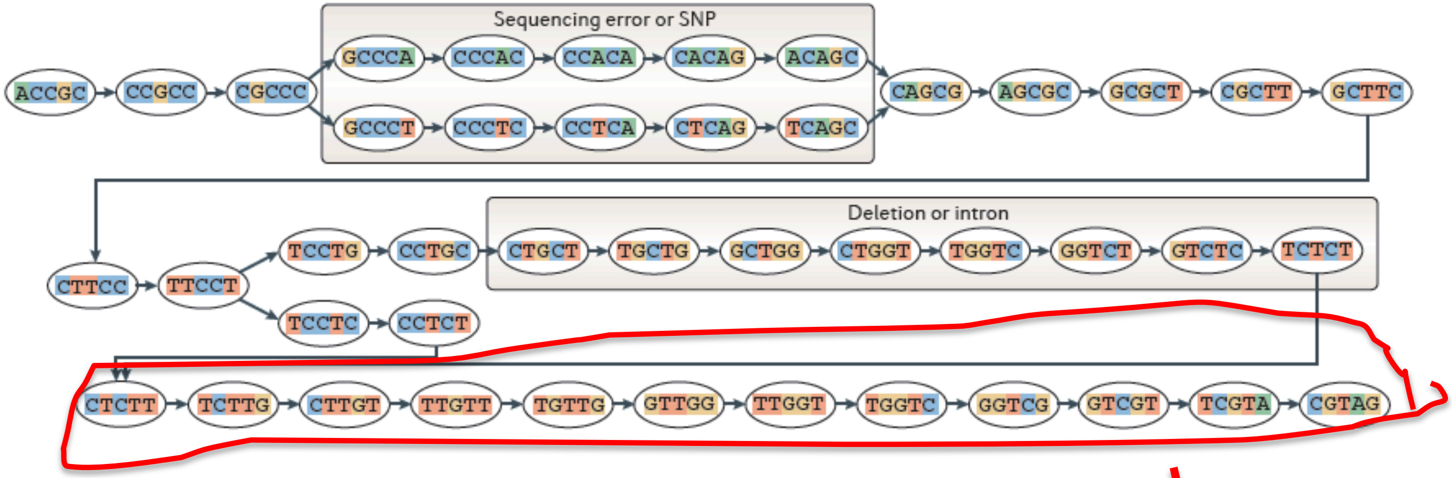
Construct the de Bruijn graph



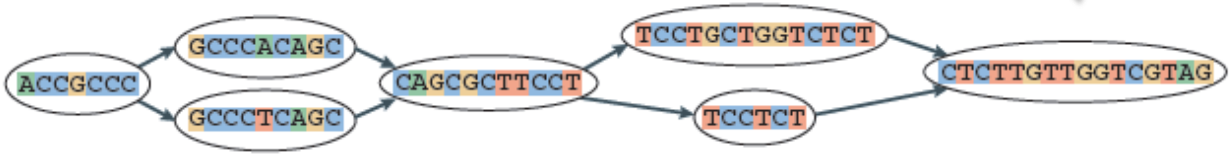
Nodes = unique k-mers
Edges = overlap by (k-1)

From Martin & Wang, Nat. Rev. Genet. 2011

Construct the de Bruijn graph

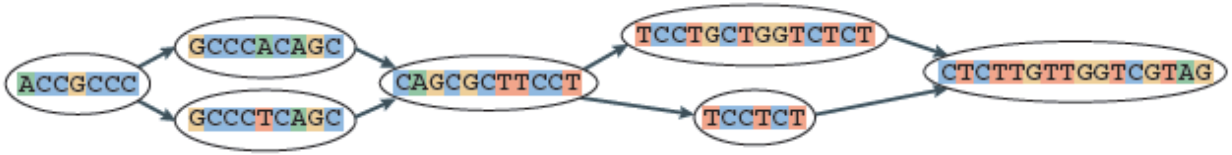


Collapse the de Bruijn graph

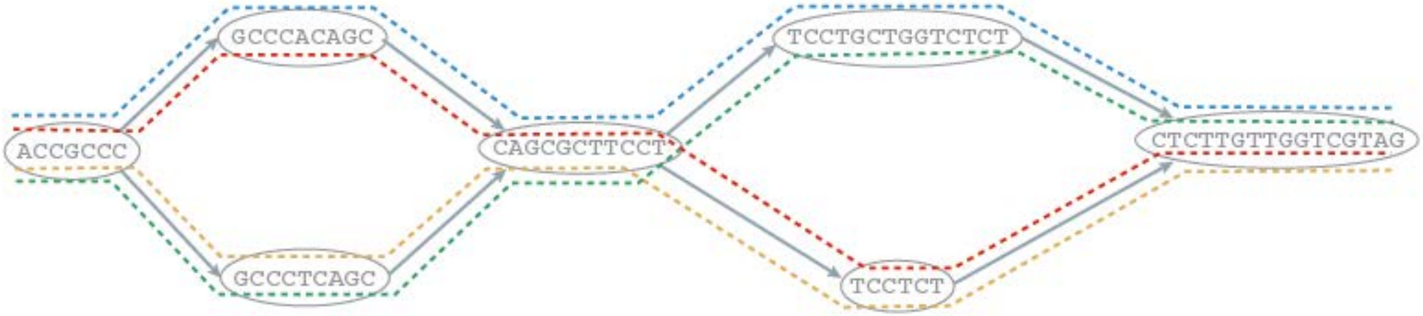


From Martin & Wang, Nat. Rev. Genet. 2011

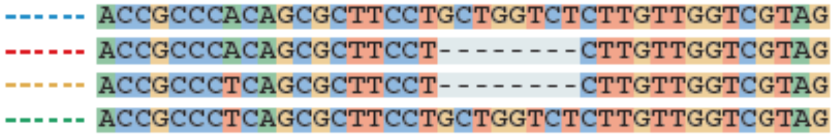
Collapse the de Bruijn graph



Traverse the graph



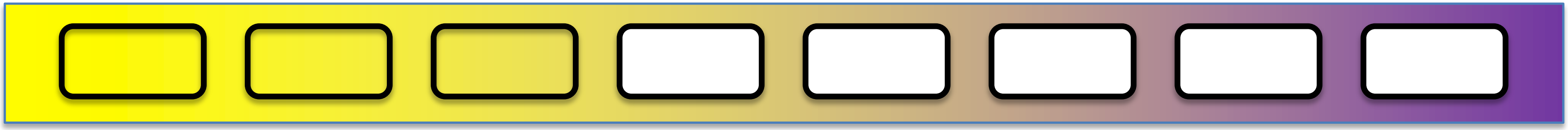
Assemble Transcript Isoforms



```
------ ACCGCCCACAGCGCTTCCTGCTGGTCTCTTGTTGGTCGTAG
------ ACCGCCCACAGCGCTTCCT--------CTTGTTGGTCGTAG
------ ACCGCCCTCAGCGCTTCCT--------CTTGTTGGTCGTAG
------ ACCGCCCTCAGCGCTTCCTGCTGGTCTCTTGTTGGTCGTAG
```

From Martin & Wang, Nat. Rev. Genet. 2011

Part 3. Trinity De novo Assembly



Contrasting Genome and Transcriptome Assembly

Genome Assembly

- Uniform coverage
- Single contig per locus
- Double-stranded

Transcriptome Assembly

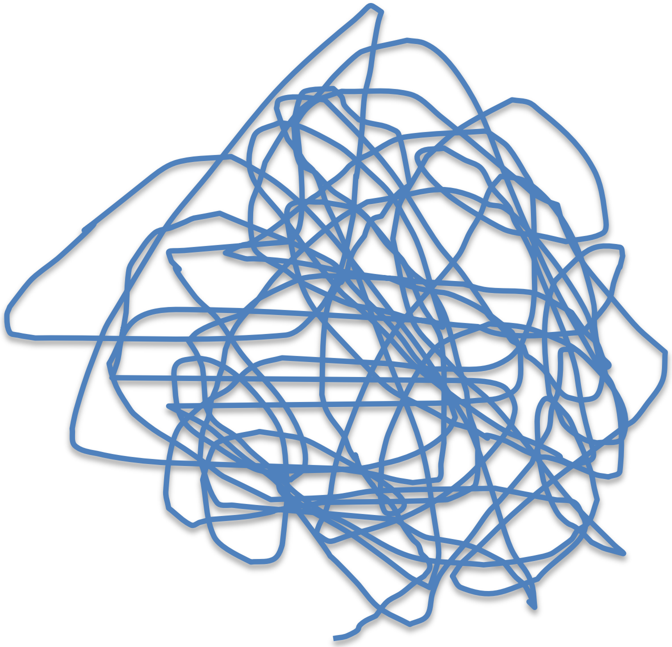
- Exponentially distributed coverage levels
- Multiple contigs per locus (alt splicing)
- Strand-specific

Trinity

Trinity Aggregates Isolated Transcript Graphs

Genome Assembly

Single Massive Graph



Entire chromosomes represented.

Trinity Transcriptome Assembly

Many Thousands of Small Graphs

Ideally, one graph per expressed gene.

Trinity – How it works:

RNA-Seq reads → Linear contigs → de-Bruijn graphs → Transcripts + Isoforms

>a121:len=5845

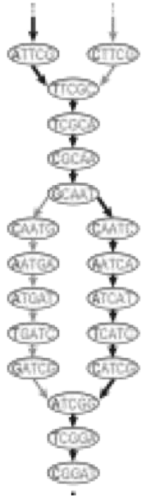
>a122:len=2560

>a123:len=4443

>a124:len=48

>a125:len=8876

>a126:len=66



...CTTCGCAA...TGATCGGAT...

...ATTCGCAA...TCATCGGAT...

Thousands of disjoint graphs

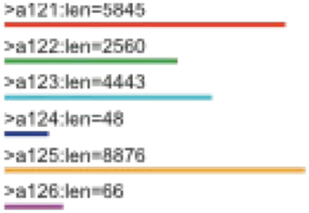
Trinity – How it works:

Younger
me

Manfred
Grabherr

Moran
Yassour

RNA-Seq reads → **Linear contigs** → **de-Bruijn graphs** → **Transcripts + Isoforms**



>a121:len=5845
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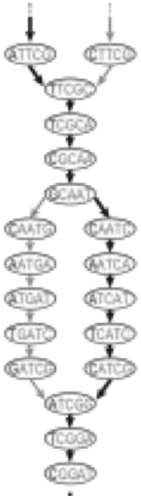
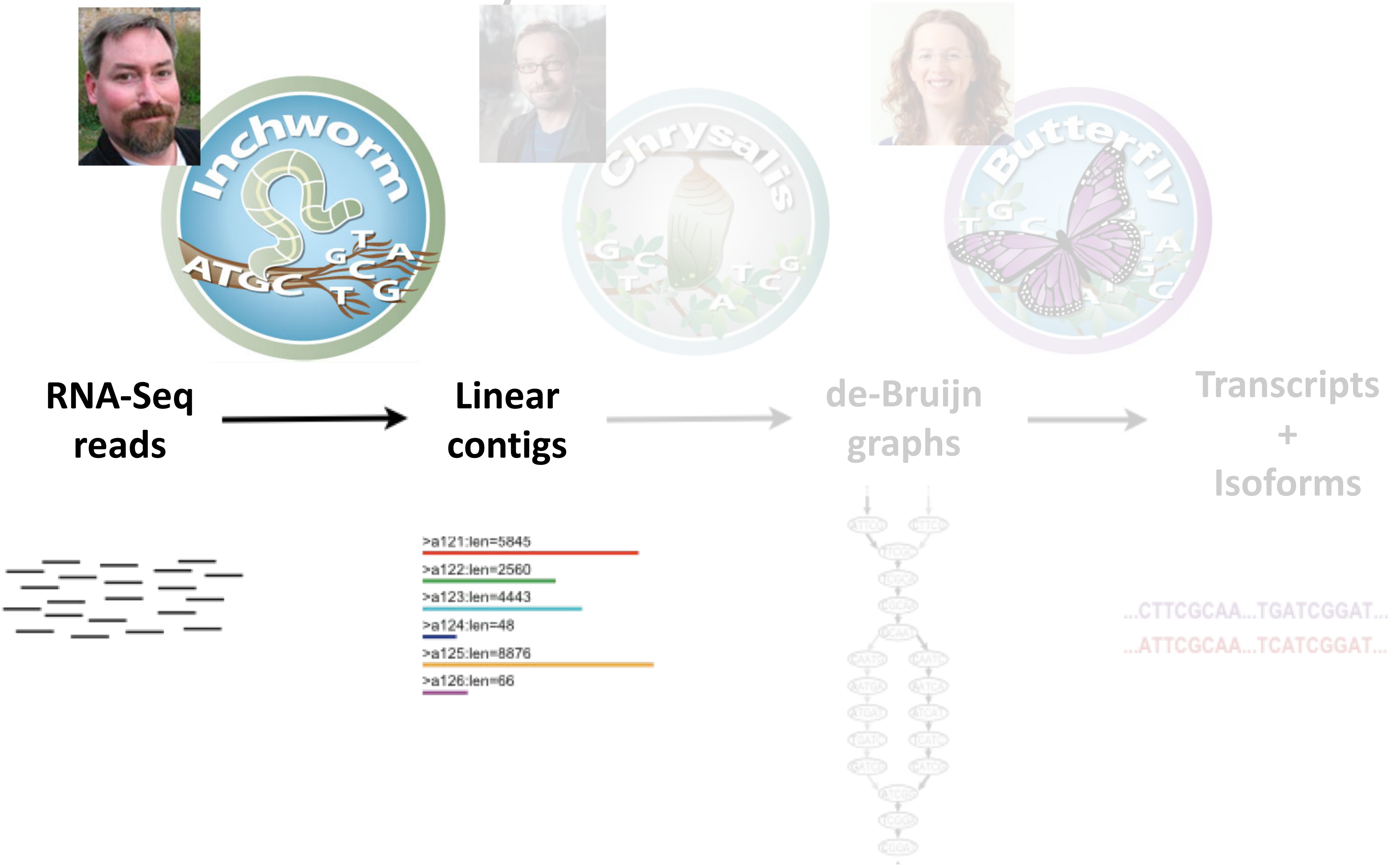
>a124:len=48
>a125:len=8876
>a126:len=66



...CTTCGCAA...TGATCGGAT...
...ATTCGCAA...TCATCGGAT...

Thousands of disjoint graphs

Trinity – How it works:



RNA-Seq reads → **Linear contigs** → de-Bruijn graphs → Transcripts + Isoforms

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...CTTCGCAA...TGATCGGAT...
...ATTCGCAA...TCATCGGAT...

Thousands of disjoint graphs

Inchworm Algorithm

- Decompose all reads into overlapping Kmers => hashtable(kmer, count)

Read: **AATGTGAAAACTGGATTACATGCTGGTATGTC…**

AATGTGA

ATGTGAA Overlapping kmers of length (k)

TGTGAAA

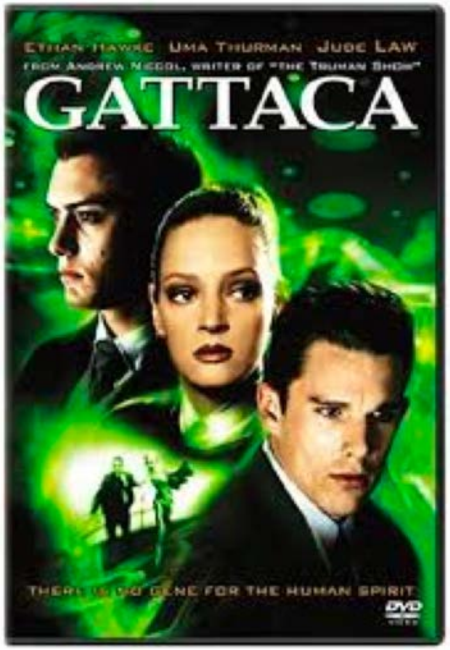
…

Kmer Catalog (hashtable)

Kmer	Count among all reads
AATGTGA	**4**
ATGTGAA	**2**
TGTGAAA	**1**
GATTACA	**9**

Inchworm Algorithm

- Decompose all reads into overlapping Kmers => hashtable(kmer, count)
- Identify seed kmer as most abundant Kmer, ignoring low-complexity kmers.


ETHAN HAWKE UMA THURMAN JUDE LAW
FROM ANDREW NICCOL, WRITER OF "THE TRUMAN SHOW"
GATTACA
THERE IS NO GENE FOR THE HUMAN SPIRIT
DVD

https://en.wikipedia.org/wiki/Gattaca

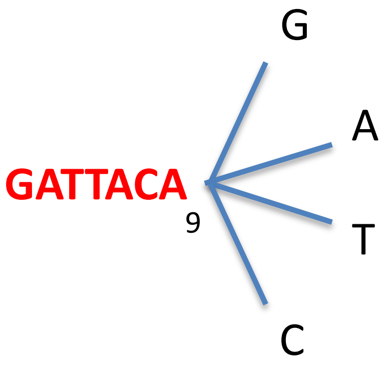
GATTACA
9

Kmer Catalog (hashtable)

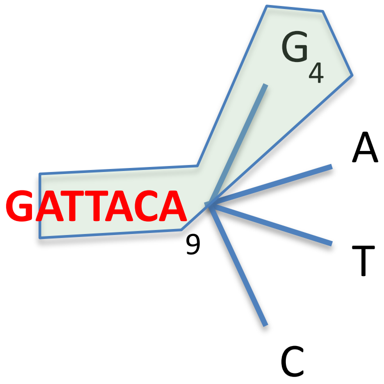
Kmer	Count among all reads
AATGTGA	4
ATGTGAA	2
TGTGAAA	1
GATTACA	9

Inchworm Algorithm

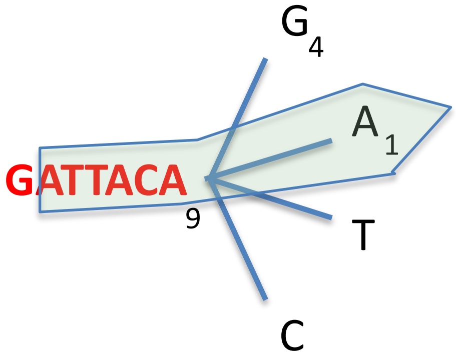
- Decompose all reads into overlapping Kmers => hashtable(kmer, count)
- Identify seed kmer as most abundant Kmer, ignoring low-complexity kmers.
- Extend kmer at 3’ end, guided by coverage.



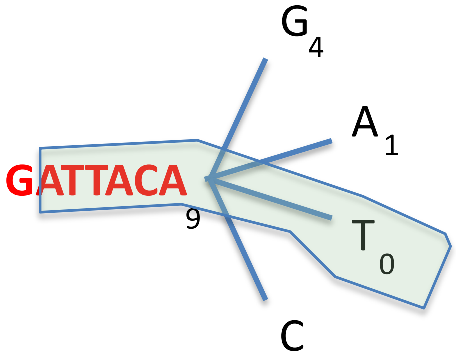
Inchworm Algorithm



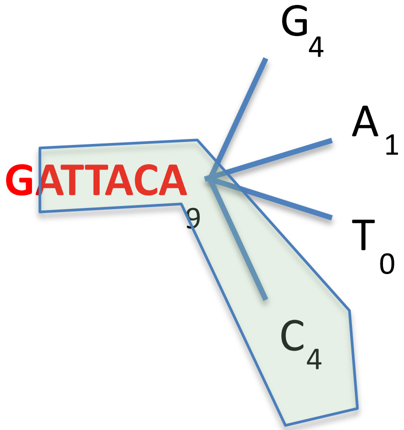
Inchworm Algorithm



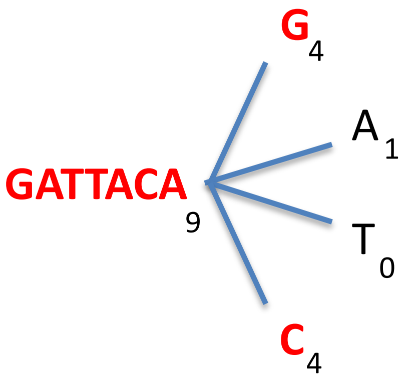
Inchworm Algorithm



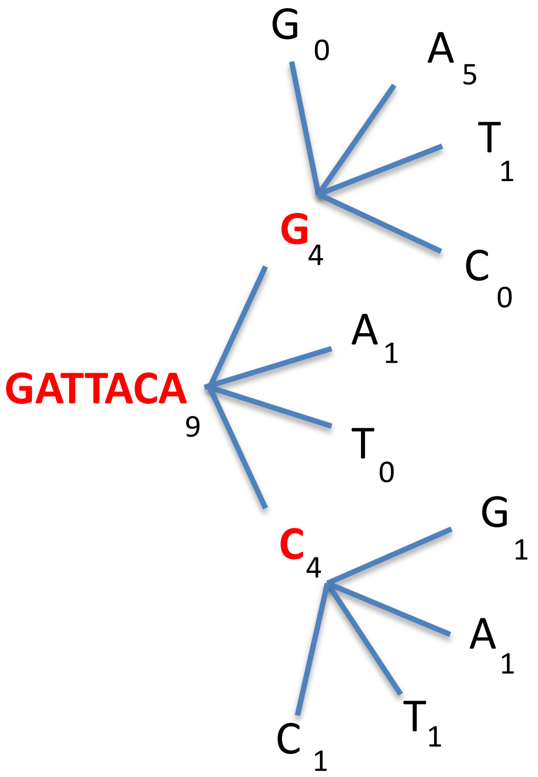
Inchworm Algorithm



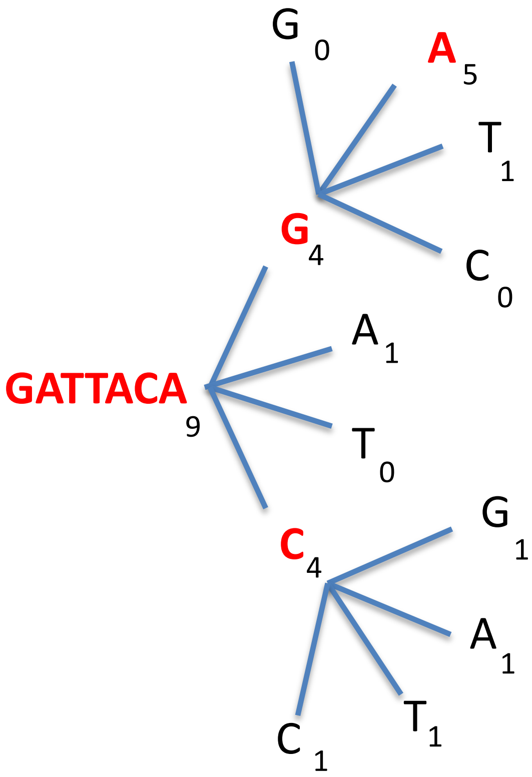
Inchworm Algorithm



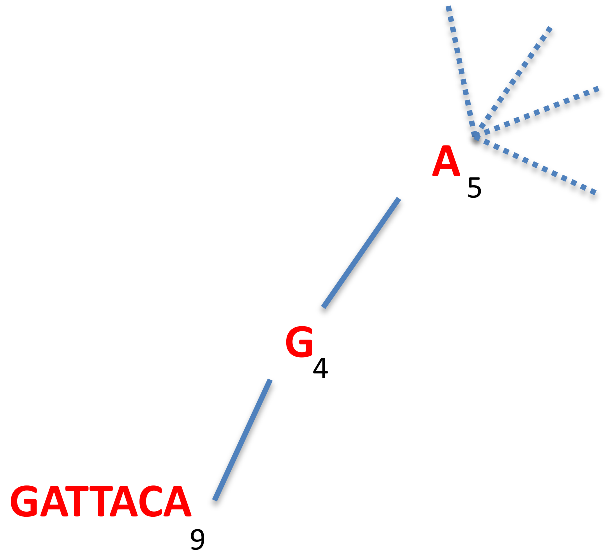
Inchworm Algorithm



Inchworm Algorithm



Inchworm Algorithm

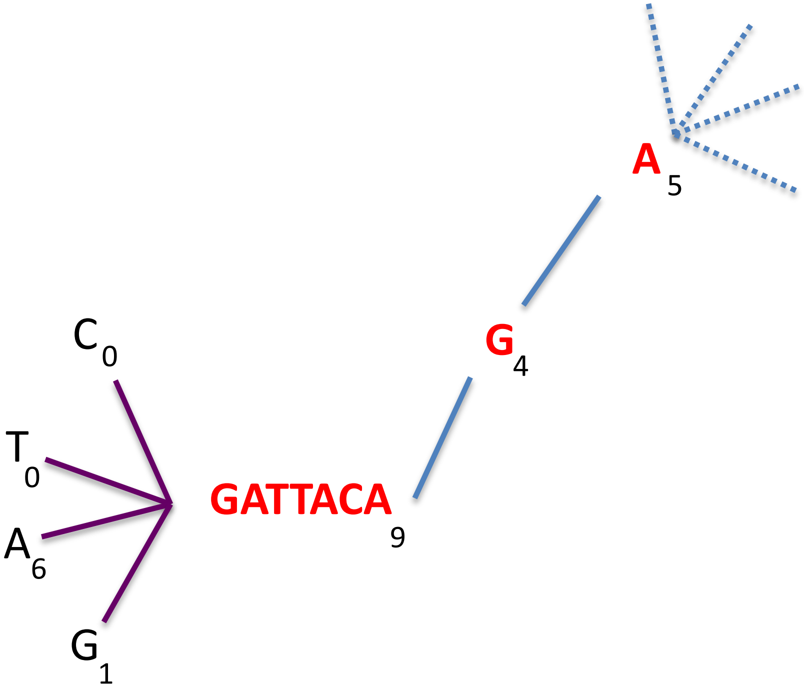


A_5

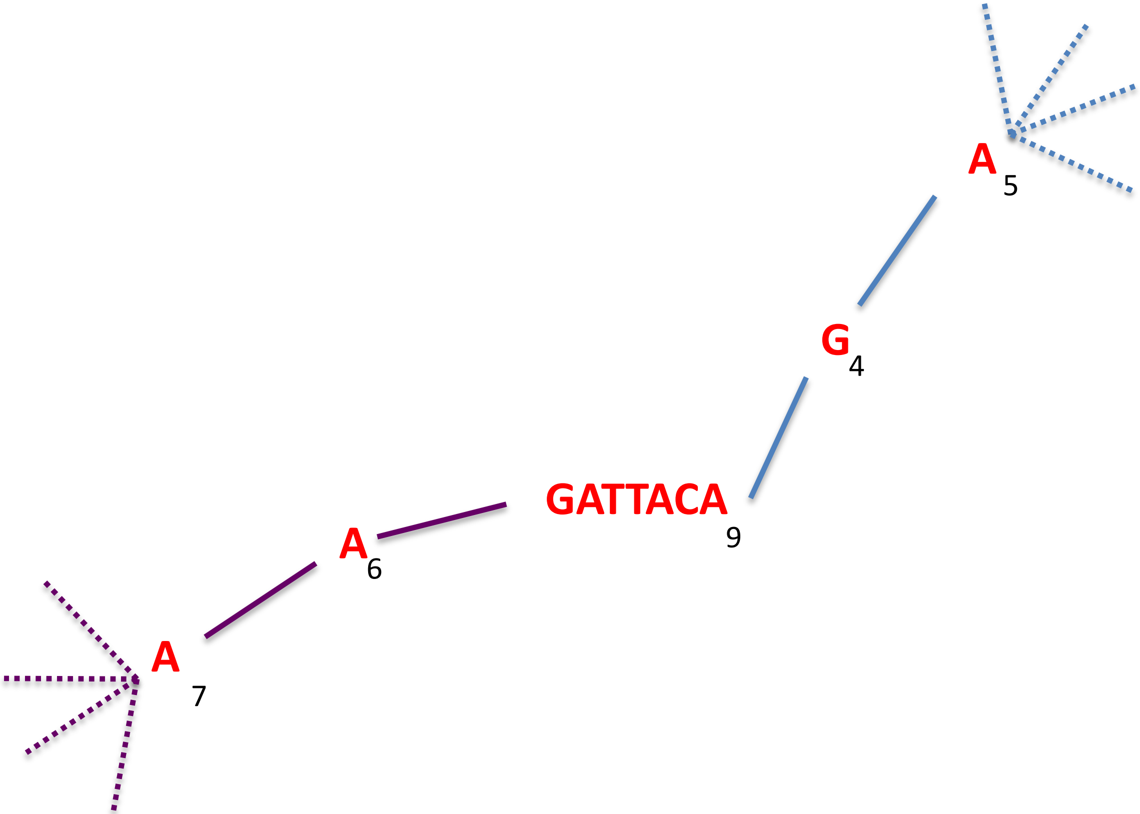
G_4

GATTACA_9

Inchworm Algorithm



Inchworm Algorithm

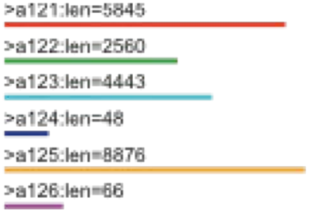


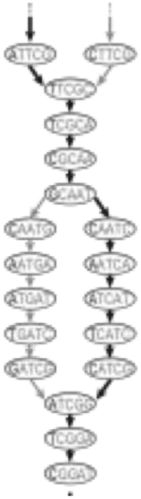
Report contig: ....AAGATTACAGA....

Remove assembled kmers from catalog, then repeat the entire process.

Trinity – How it works:

RNA-Seq reads → **Linear contigs** → **de-Bruijn graphs** → Transcripts + Isoforms





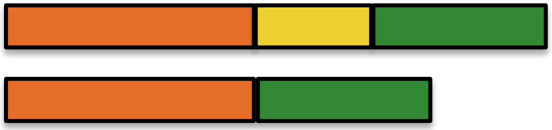
Thousands of disjoint graphs

...CTTCGCAA...TGATCGGAT...

...ATTCGCAA...TCATCGGAT...

Inchworm Contigs from Alt-Spliced Transcripts

Expressed isoforms



Isoform A

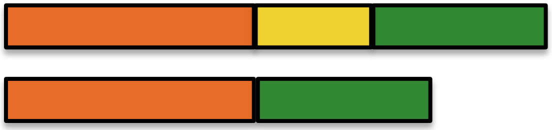
Isoform B

Inchworm Contigs from Alt-Spliced Transcripts

Expressed isoforms

Expression

Isoform A

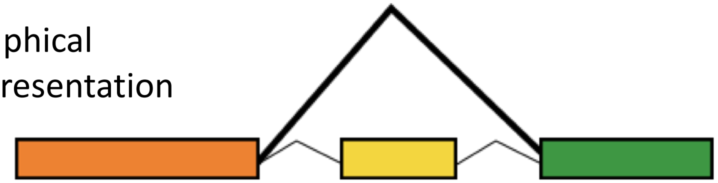


(low)

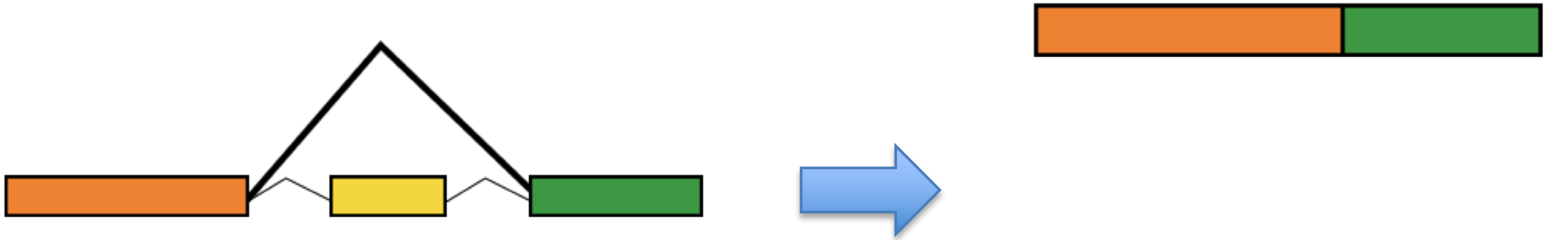
Isoform B

(high)

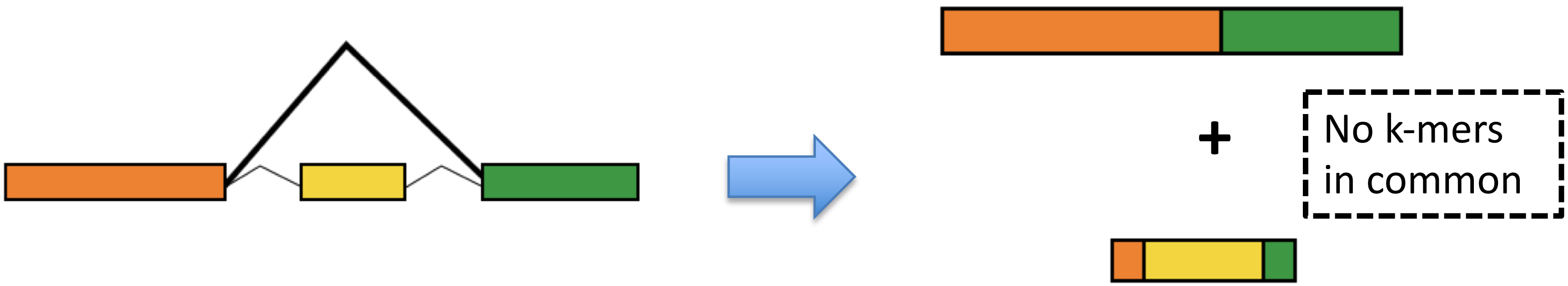
Graphical representation



Inchworm Contigs from Alt-Spliced Transcripts

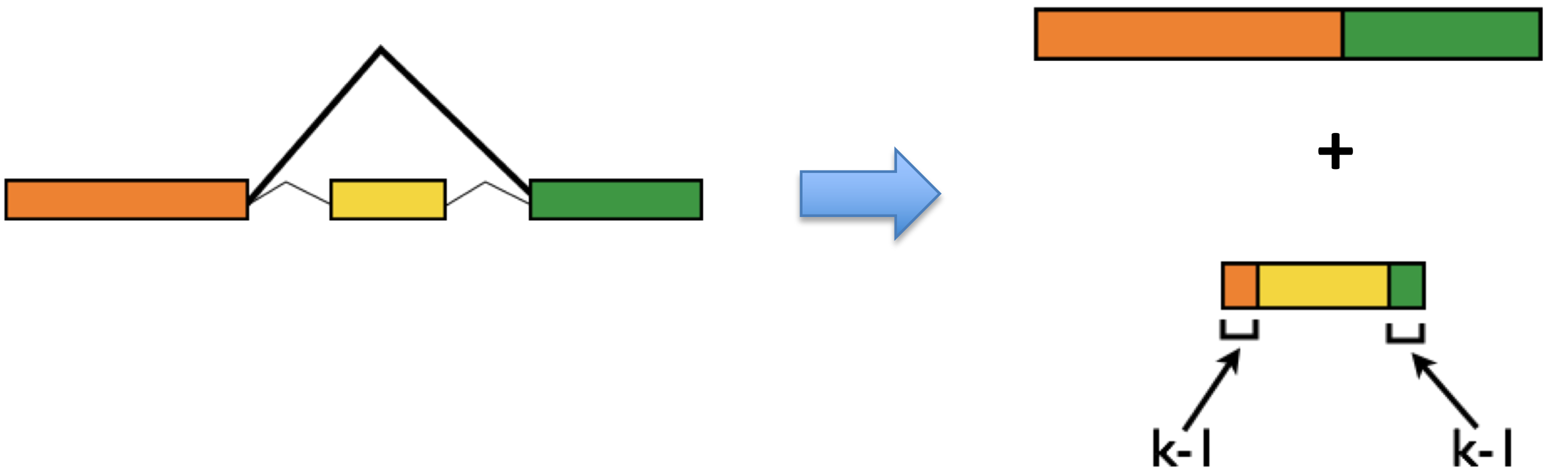


Inchworm Contigs from Alt-Spliced Transcripts

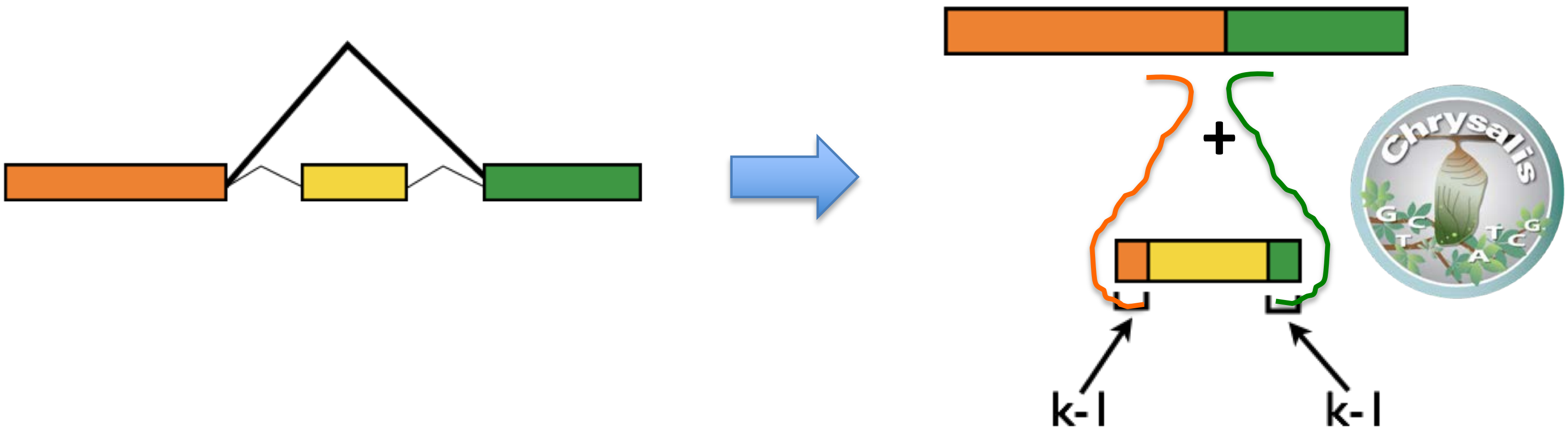


No k-mers in common

Inchworm Contigs from Alt-Spliced Transcripts

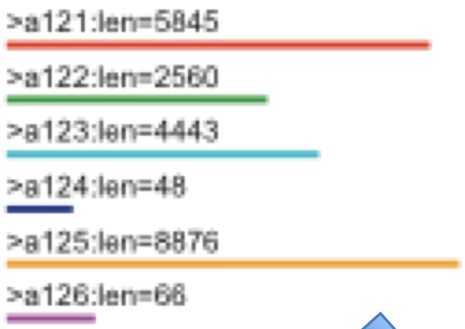


Chrysalis Re-groups Related Inchworm Contigs



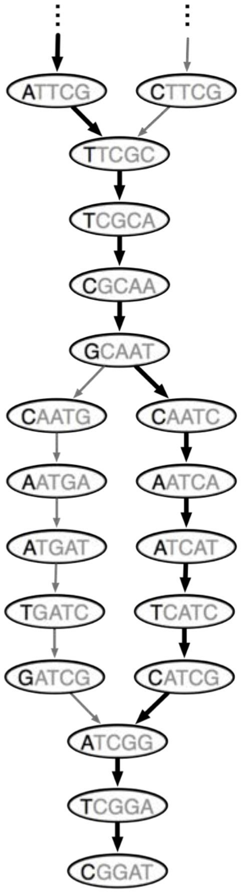
Chrysalis uses (k-1) overlaps and read support to link related Inchworm contigs

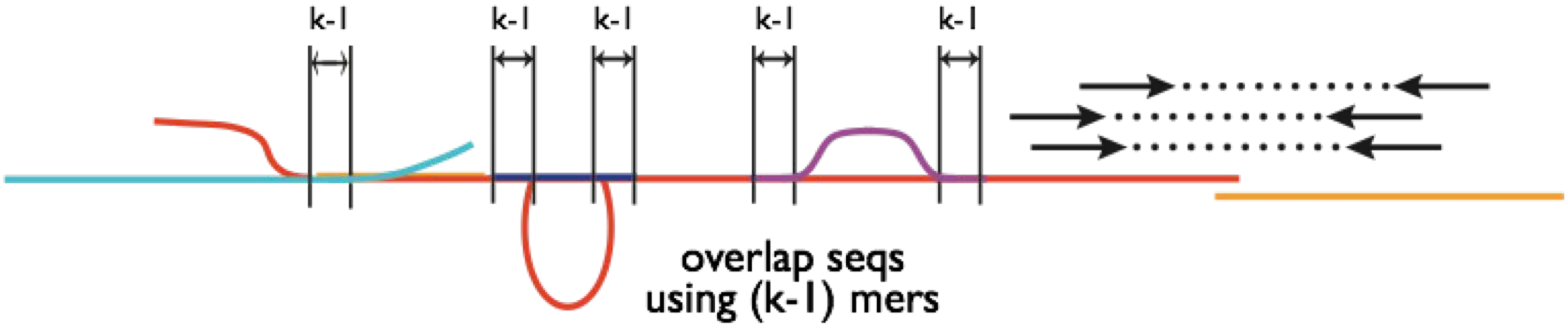
Chrysalis



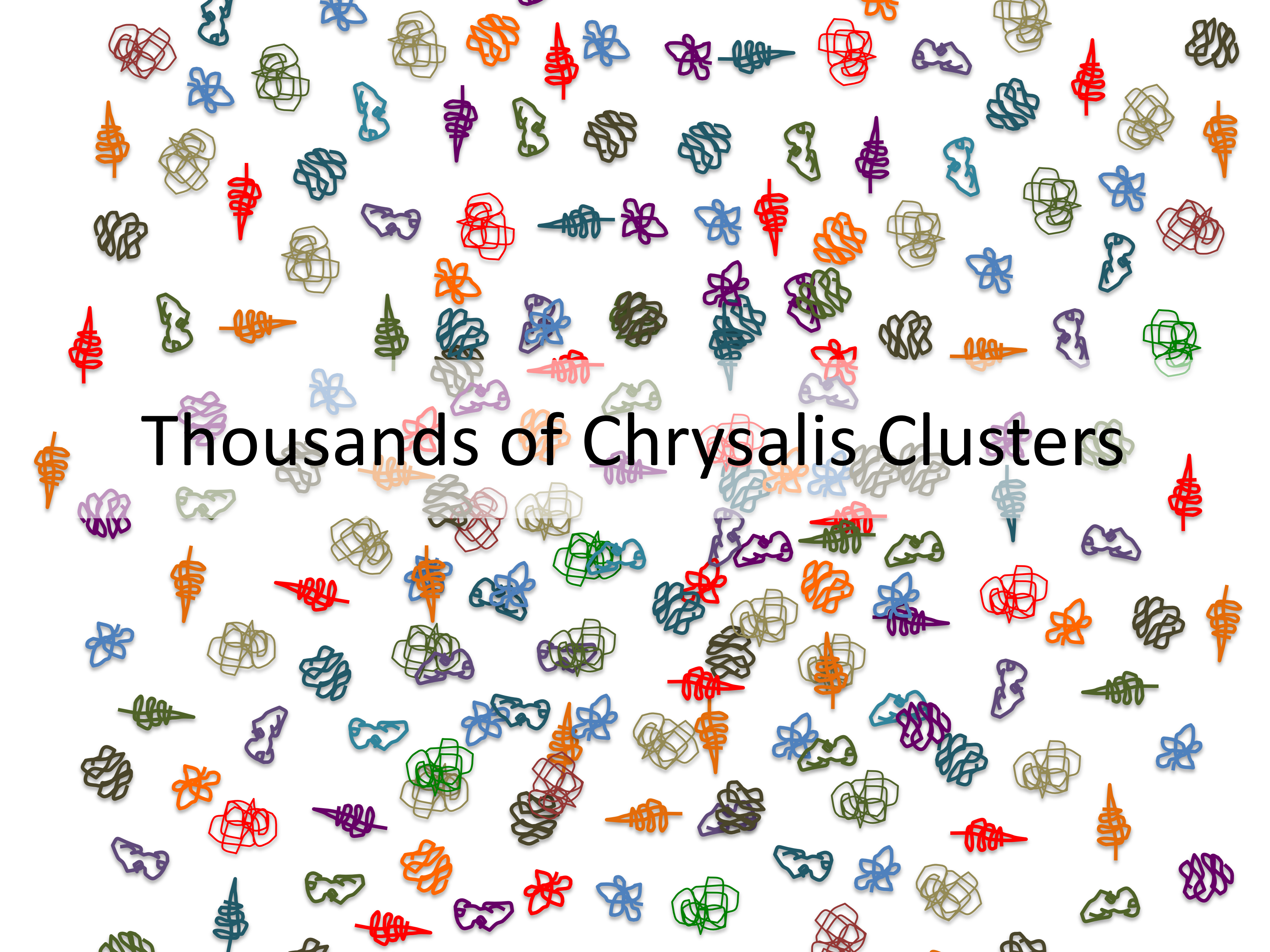
Integrate isoforms via k-1 overlaps

Build de Bruijn Graphs (ideally, one per gene)





Thousands of Chrysalis Clusters



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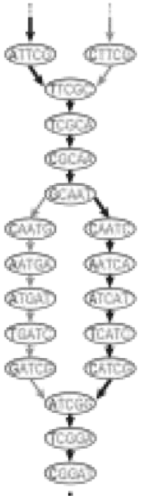
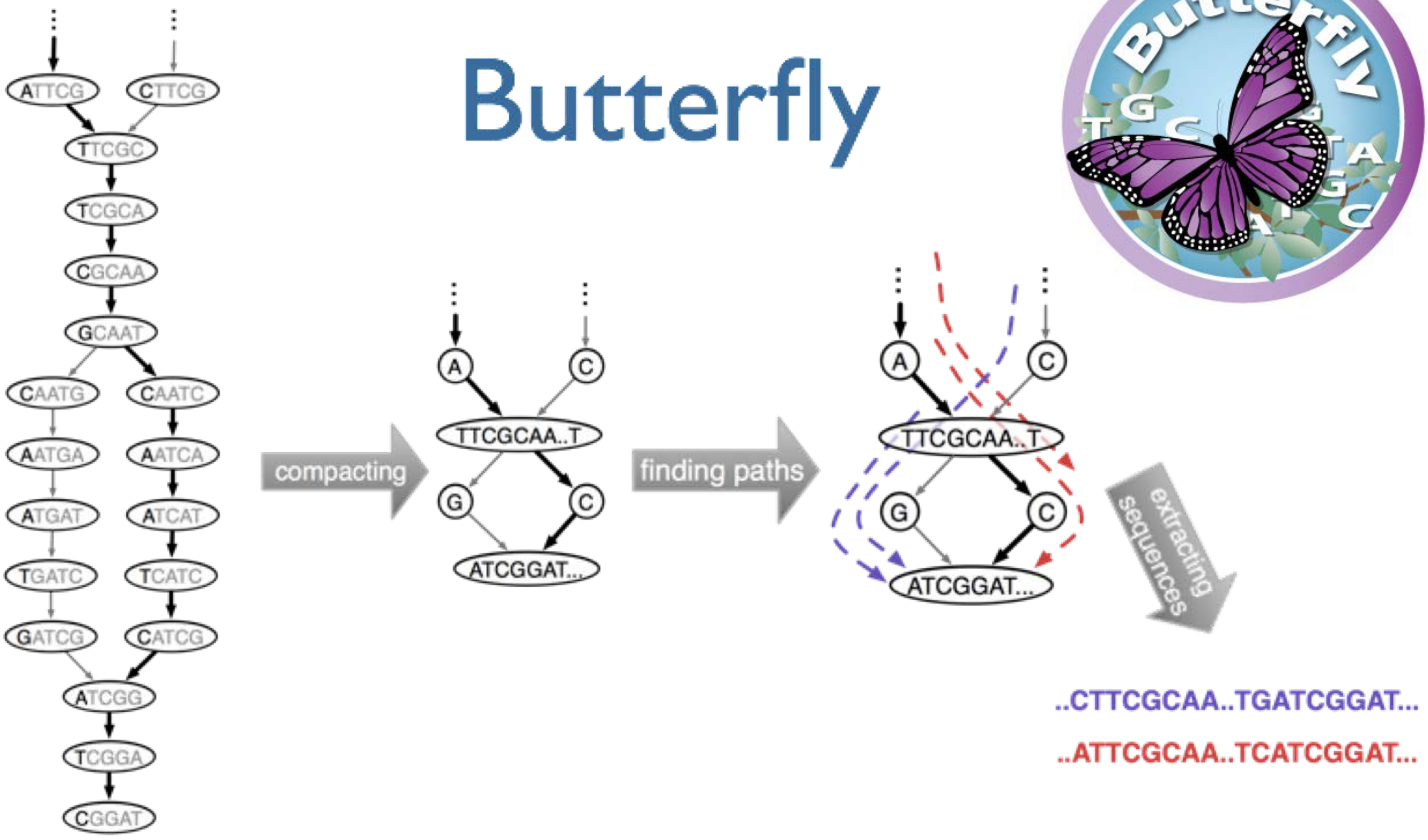


...CTTCGCAA...TGATCGGAT...

...ATTCGCAA...TCATCGGAT...

Thousands of disjoint graphs

Butterfly



de Bruijn graph

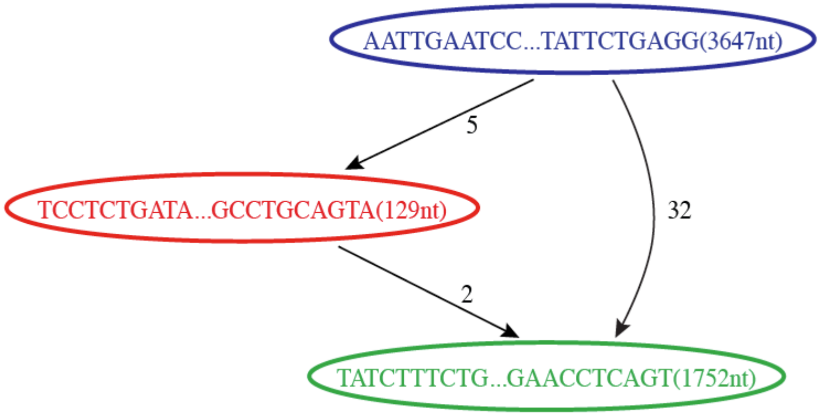
compact graph

compact graph with reads

sequences
(isoforms and paralogs)

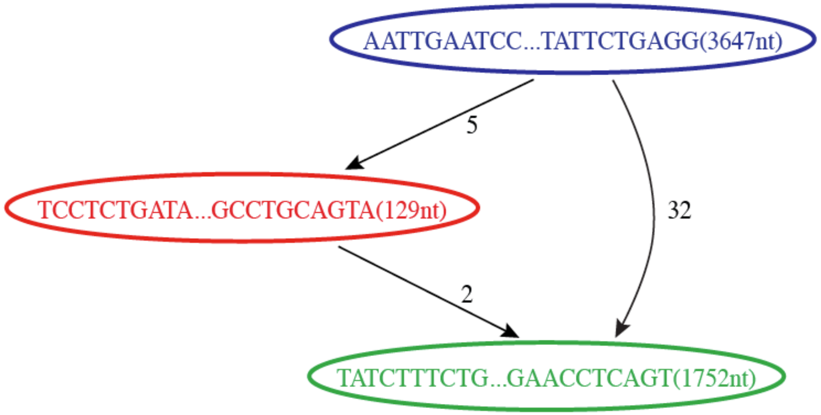
Butterfly Example 1:
Reconstruction of Alternatively Spliced Transcripts

Butterfly's Compacted Sequence Graph

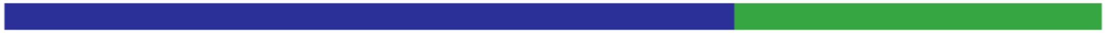

AATTGAATCC...TATTCTGAGG(3647nt)
5
32
TCCTCTGATA...GCCTGCAGTA(129nt)
2
TATCTTTCTG...GAACCTCAGT(1752nt)

Reconstruction of Alternatively Spliced Transcripts

Butterfly's Compacted Sequence Graph

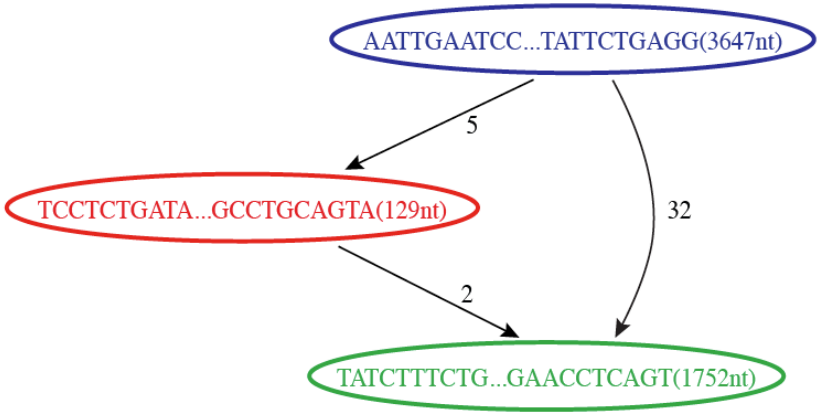

AATTGAATCC...TATTCTGAGG(3647nt)
5
32
TCCTCTGATA...GCCTGCAGTA(129nt)
2
TATCTTTCTG...GAACCTCAGT(1752nt)

Reconstructed Transcripts

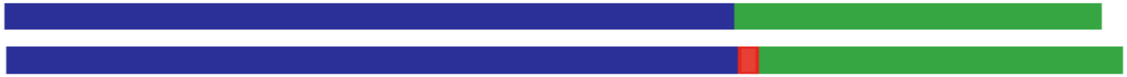


Reconstruction of Alternatively Spliced Transcripts

Butterfly’s Compacted Sequence Graph

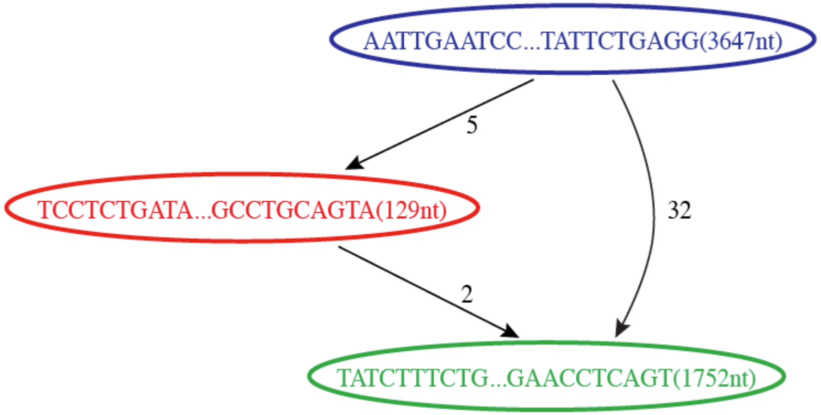

AATTGAATCC...TATTCTGAGG(3647nt)
5
32
TCCTCTGATA...GCCTGCAGTA(129nt)
2
TATCTTTCTG...GAACCTCAGT(1752nt)

Reconstructed Transcripts

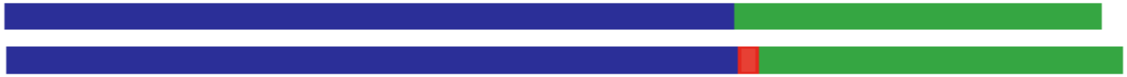


Reconstruction of Alternatively Spliced Transcripts

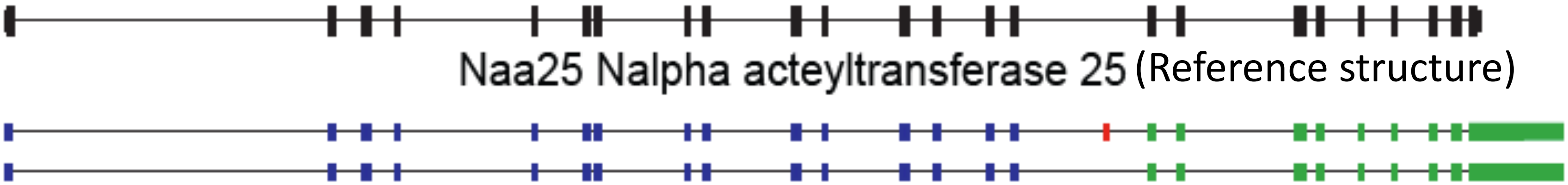
Butterfly's Compacted Sequence Graph


AATTGAATCC...TATTCTGAGG(3647nt)
5
32
TCCTCTGATA...GCCTGCAGTA(129nt)
2
TATCTTTCTG...GAACCTCAGT(1752nt)

Reconstructed Transcripts

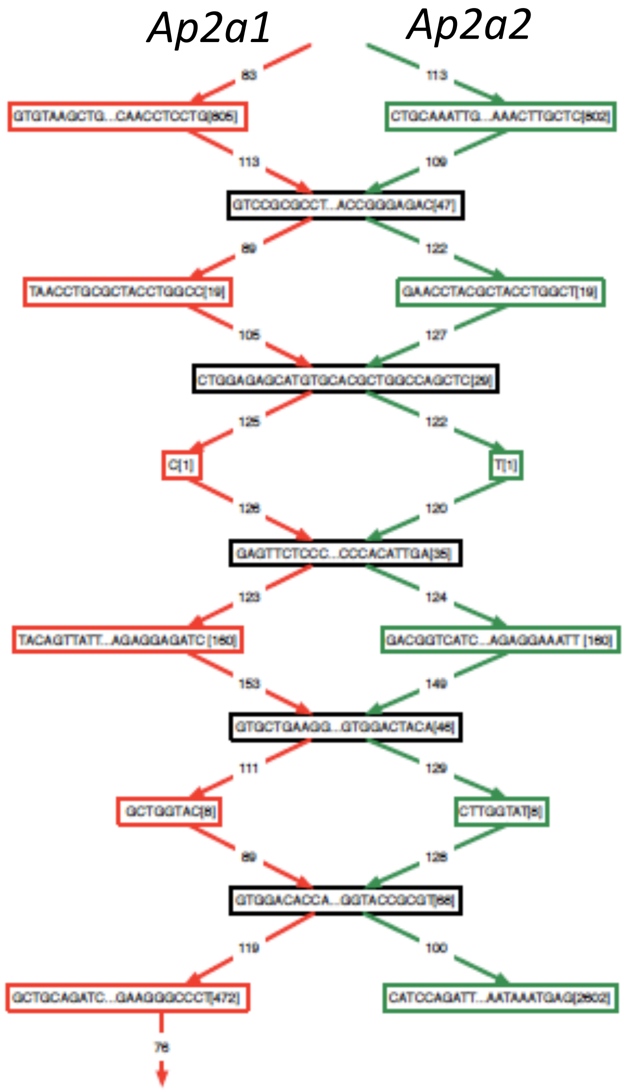


Aligned to Mouse Genome

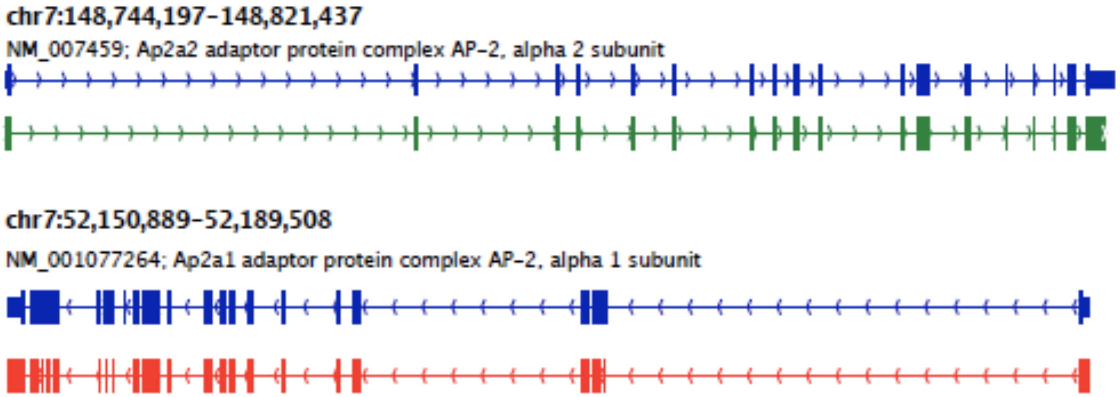


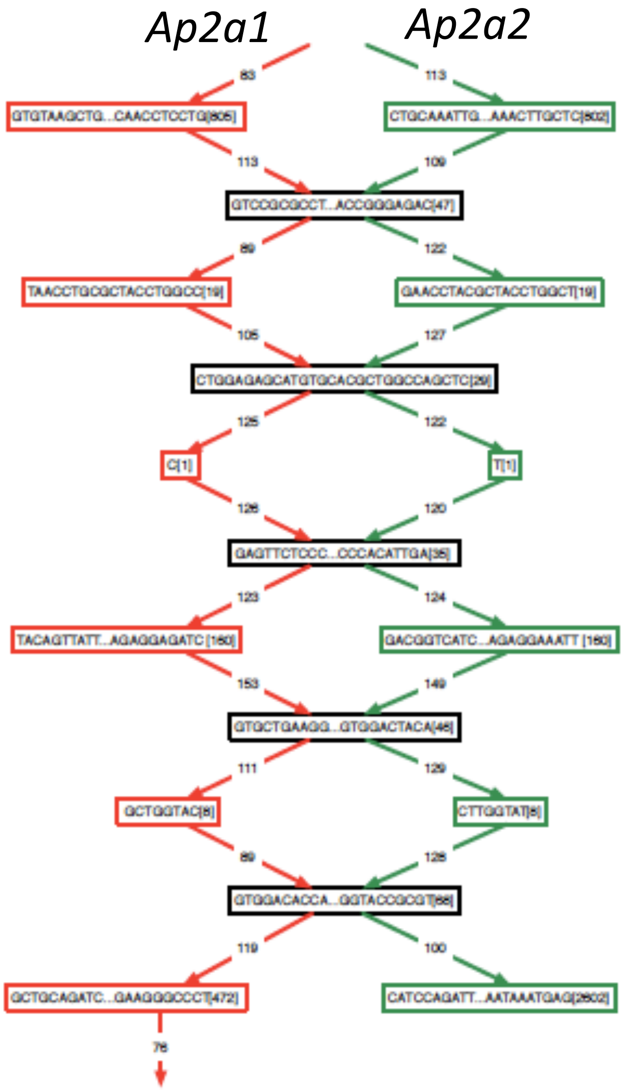
(Reference structure)

Butterfly Example 2:
Teasing Apart Transcripts of Paralogous Genes


Ap2a1
Ap2a2
83
113
GTGTAAGCTG...CAACCTCCTG[805]
CTGCAAATTG...AAACTTGCTC[802]
113
109
GTCCGCGCCT...ACCGGGAGAC[47]
89
122
TAACCTGCGCTACCTGGCC[19]
GAACCTACGCTACCTGGCT[19]
105
127
CTGGAGAGCATGTGCACGCTGGCCAGCTC[29]
125
122
C[1]
T[1]
128
120
GAGTTCTCCC...CCCACATTGA[35]
123
124
TACAGTTATT...AGAGGAGATC [160]
GACGGTCATC...AGAGGAAATT [160]
153
149
GTGCTGAAGG...GTGGACTACA[46]
111
129
GCTGGTAC[8]
CTTGGTAT[8]
89
128
GTGGACACCA...GGTACCGCGT[68]
119
100
GCTGCAGATC...GAAGGGCCCT[472]
CATCCAGATT...AATAAATGAG[2602]
78

Teasing Apart Transcripts of Paralogous Genes


chr7:148,744,197-148,821,437
NM_007459; Ap2a2 adaptor protein complex AP-2, alpha 2 subunit
chr7:52,150,889-52,189,508
NM_001077264; Ap2a1 adaptor protein complex AP-2, alpha 1 subunit


Ap2a1
Ap2a2
83
113
GTGTAAGCTG...CAACCTCCTG[806]
CTGCAAATTG...AAACTTGCTC[802]
113
109
GTCCGCGCCT...ACCGGGAGAC[47]
89
122
TAACCTGCGCTACCTGGCC[19]
GAACCTACGCTACCTGGCT[19]
105
127
CTGGAGAGCATGTGCACGCTGGCCAGCTC[29]
125
122
C[1]
T[1]
126
120
GAGTTCTCCC...CCCACATTGA[35]
123
124
TACAGTTATT...AGAGGAGATC [160]
GACGGTCATC...AGAGGAAATT [160]
153
149
GTGCTGAAGG...GTGGACTACA[46]
111
129
GCTGGTAC[8]
CTTGGTAT[8]
89
128
GTGGACACCA...GGTACCGCGT[68]
119
100
GCTGCAGATC...GAAGGGCCCT[472]
CATCCAGATT...AATAAATGAG[2602]
76

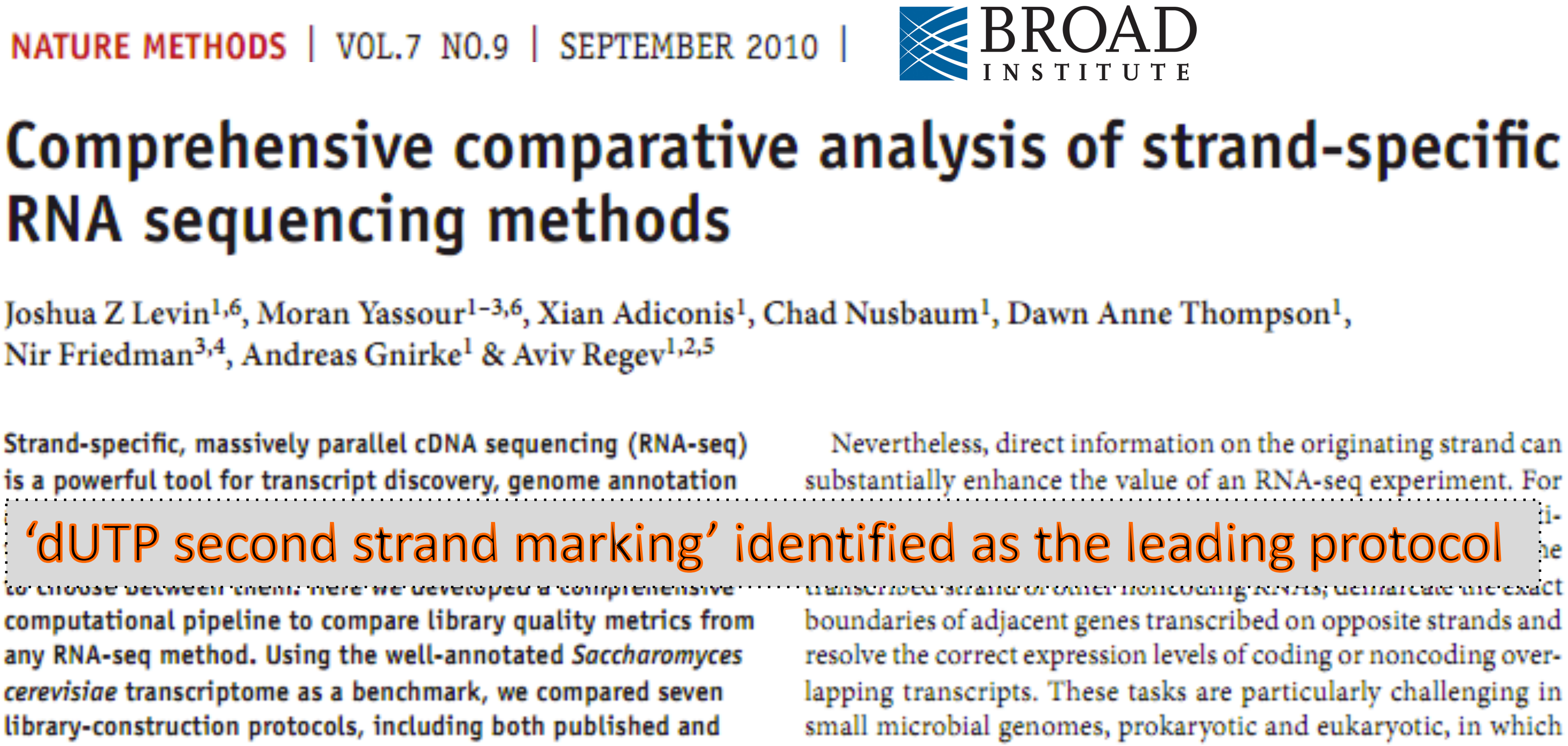
Strand-specific RNA-Seq is Preferred

Computationally: fewer confounding graph structures in de novo assembly:

ex. Forward != reverse complement

(GGAA != TTCC)

Biologically: separate sense vs. antisense transcription



NATURE METHODS | VOL.7 NO.9 | SEPTEMBER 2010 |

BROAD INSTITUTE

Comprehensive comparative analysis of strand-specific RNA sequencing methods

Joshua Z Levin[1,6], Moran Yassour[1–3,6], Xian Adiconis[1], Chad Nusbaum[1], Dawn Anne Thompson[1], Nir Friedman[3,4], Andreas Gnirke[1] & Aviv Regev[1,2,5]

Strand-specific, massively parallel cDNA sequencing (RNA-seq) is a powerful tool for transcript discovery, genome annotation

to choose between them. Here we developed a comprehensive computational pipeline to compare library quality metrics from any RNA-seq method. Using the well-annotated *Saccharomyces cerevisiae* transcriptome as a benchmark, we compared seven library-construction protocols, including both published and

Nevertheless, direct information on the originating strand can substantially enhance the value of an RNA-seq experiment. For

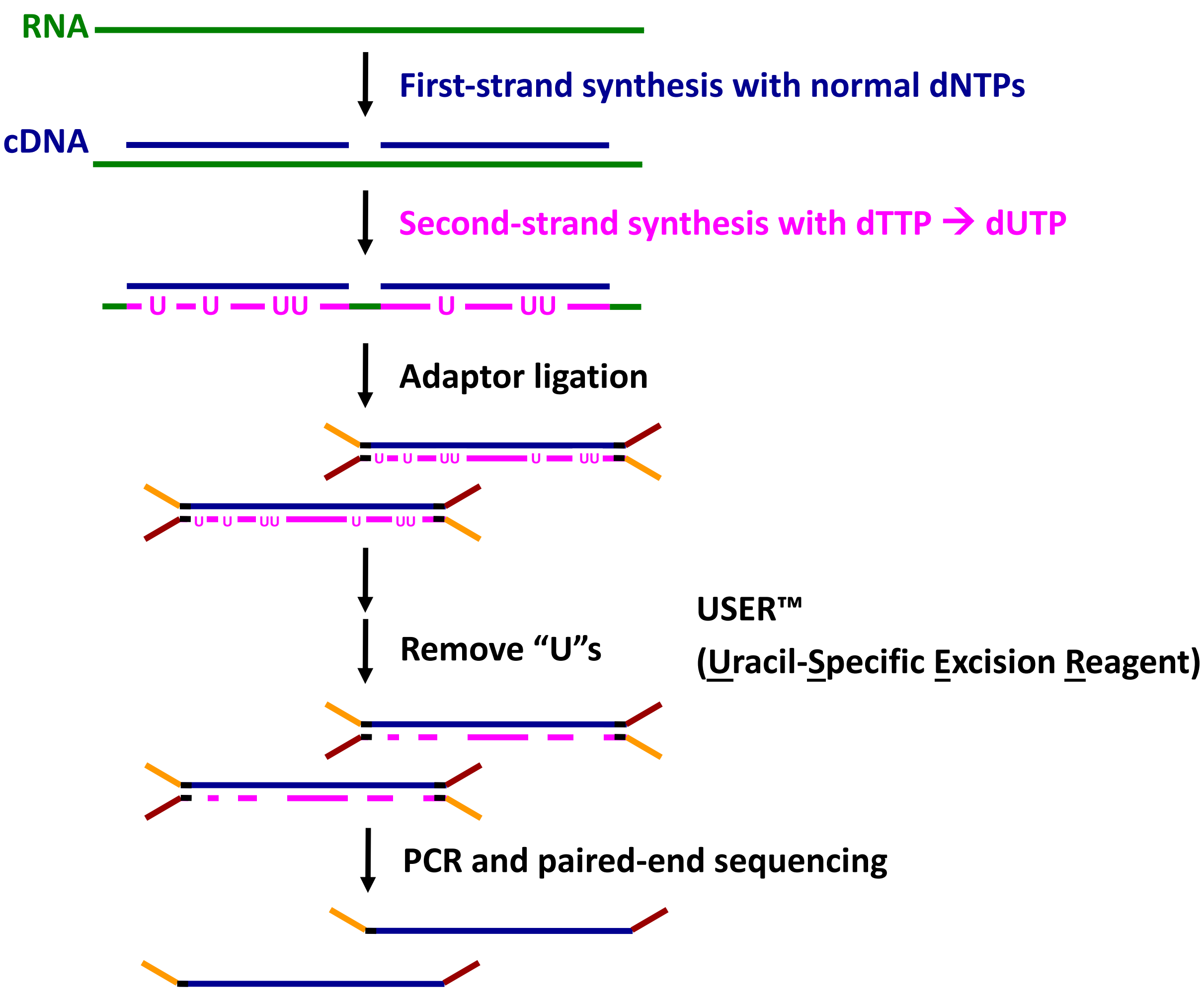
ti-

he

transcribed strand of other noncoding RNAs, demarcate the exact boundaries of adjacent genes transcribed on opposite strands and resolve the correct expression levels of coding or noncoding overlapping transcripts. These tasks are particularly challenging in small microbial genomes, prokaryotic and eukaryotic, in which

‘dUTP second strand marking’ identified as the leading protocol

dUTP 2nd Strand Method: Our Favorite



RNA

First-strand synthesis with normal dNTPs

cDNA

Second-strand synthesis with dTTP → dUTP

Adaptor ligation

Remove "U"s

USER™

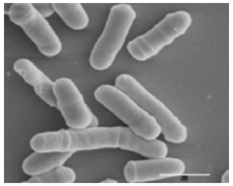
(Uracil-Specific Excision Reagent)

PCR and paired-end sequencing

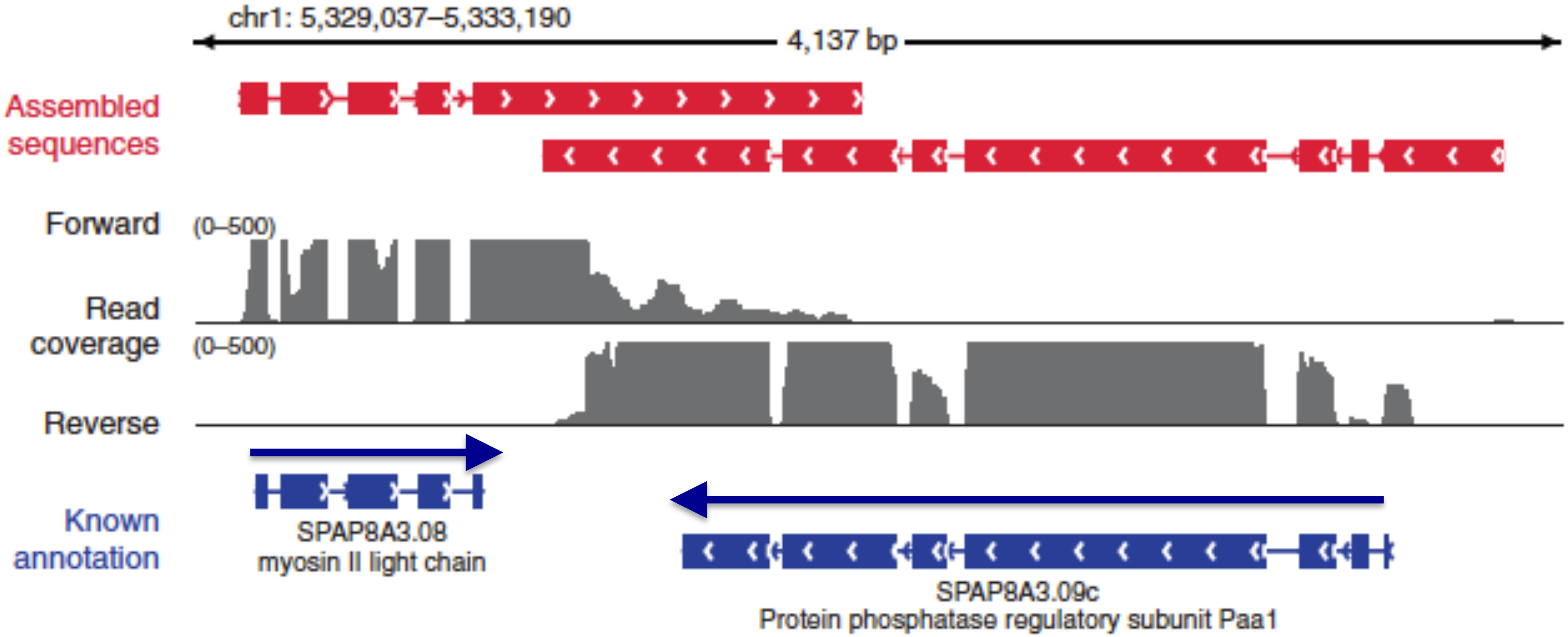
Modified from Parkhomchuk *et al.* (2009) *Nucleic Acids Res.* 37:e123

Slide courtesy of Joshua Levin, Broad Institute.

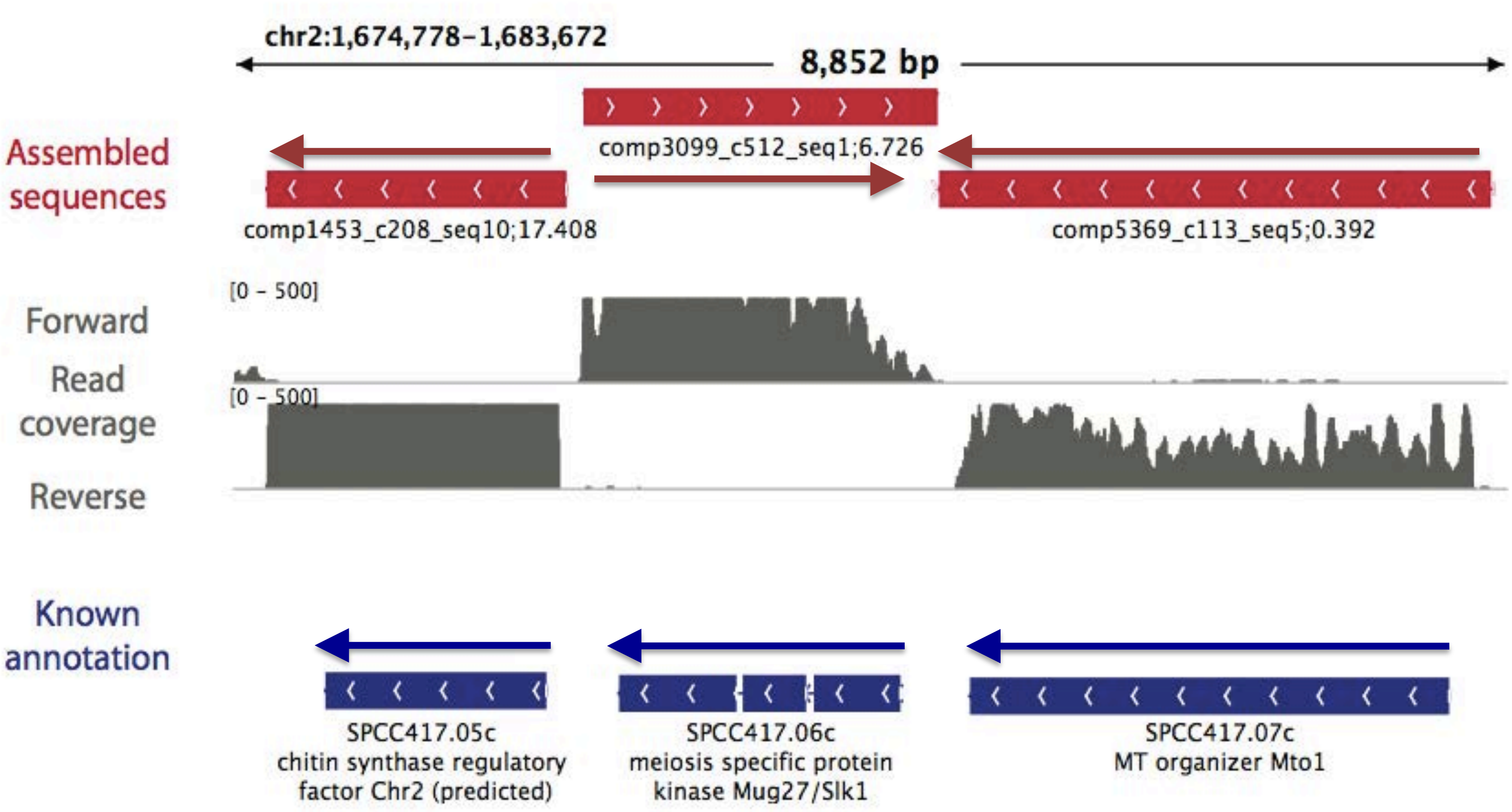
Overlapping UTRs from Opposite Strands

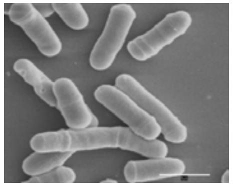


Schizosacharomyces pombe
(fission yeast)



Antisense-dominated Transcription





Trinity is a Highly Effective and Highly Popular RNA-Seq Assembler

Nature Biotechnology, 2011

Thousands of routine users.

~9k literature citations

Freely available, well-supported, open source software

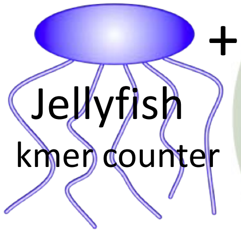
GitHub

http://trinityrnaseq.github.io

Trinity – Today, Many More Components

(off-the-shelf and into the Trinity ecosystem)

Rob Patro



Jellyfish
kmer counter

+

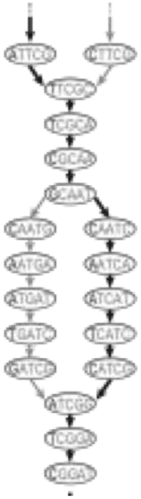
RNA-Seq reads → **Linear contigs** → **de-Bruijn graphs** → **Transcripts + Isoforms**

>a121:len=5845
>a122:len=2560
>a123:len=4443
>a124:len=48
>a125:len=8876
>a126:len=66

+

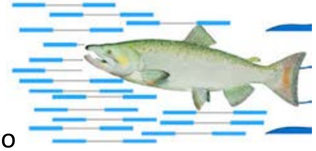
(Capture paired-end links between inchworm contigs)

Ben Langmead



...CTTCGCAA...TGATCGGAT...
...ATTCGCAA...TCATCGGAT...

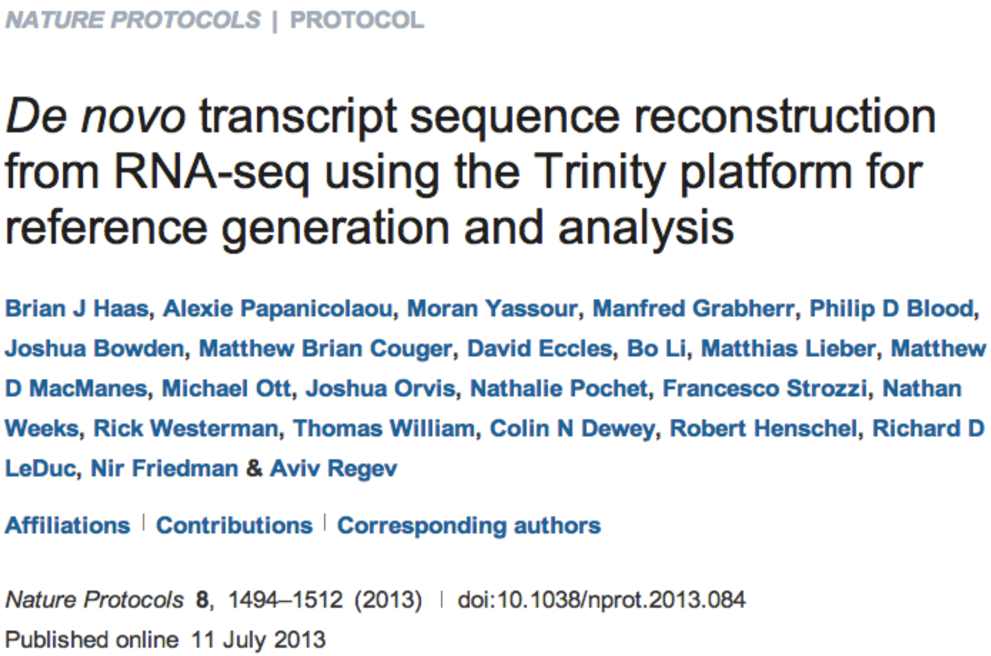
+



Rob Patro

Salmon expression quantification (eliminate assembly artifacts) nature protocols

Transcriptome Assembly is Just the End of the Beginning…



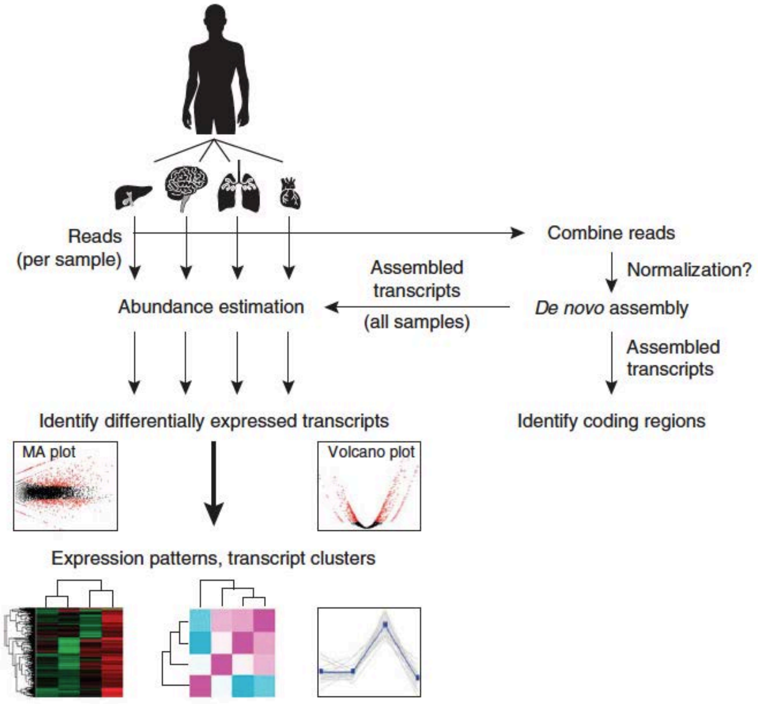
NATURE PROTOCOLS | PROTOCOL

De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis

Brian J Haas, Alexie Papanicolaou, Moran Yassour, Manfred Grabherr, Philip D Blood, Joshua Bowden, Matthew Brian Couger, David Eccles, Bo Li, Matthias Lieber, Matthew D MacManes, Michael Ott, Joshua Orvis, Nathalie Pochet, Francesco Strozzi, Nathan Weeks, Rick Westerman, Thomas William, Colin N Dewey, Robert Henschel, Richard D LeDuc, Nir Friedman & Aviv Regev

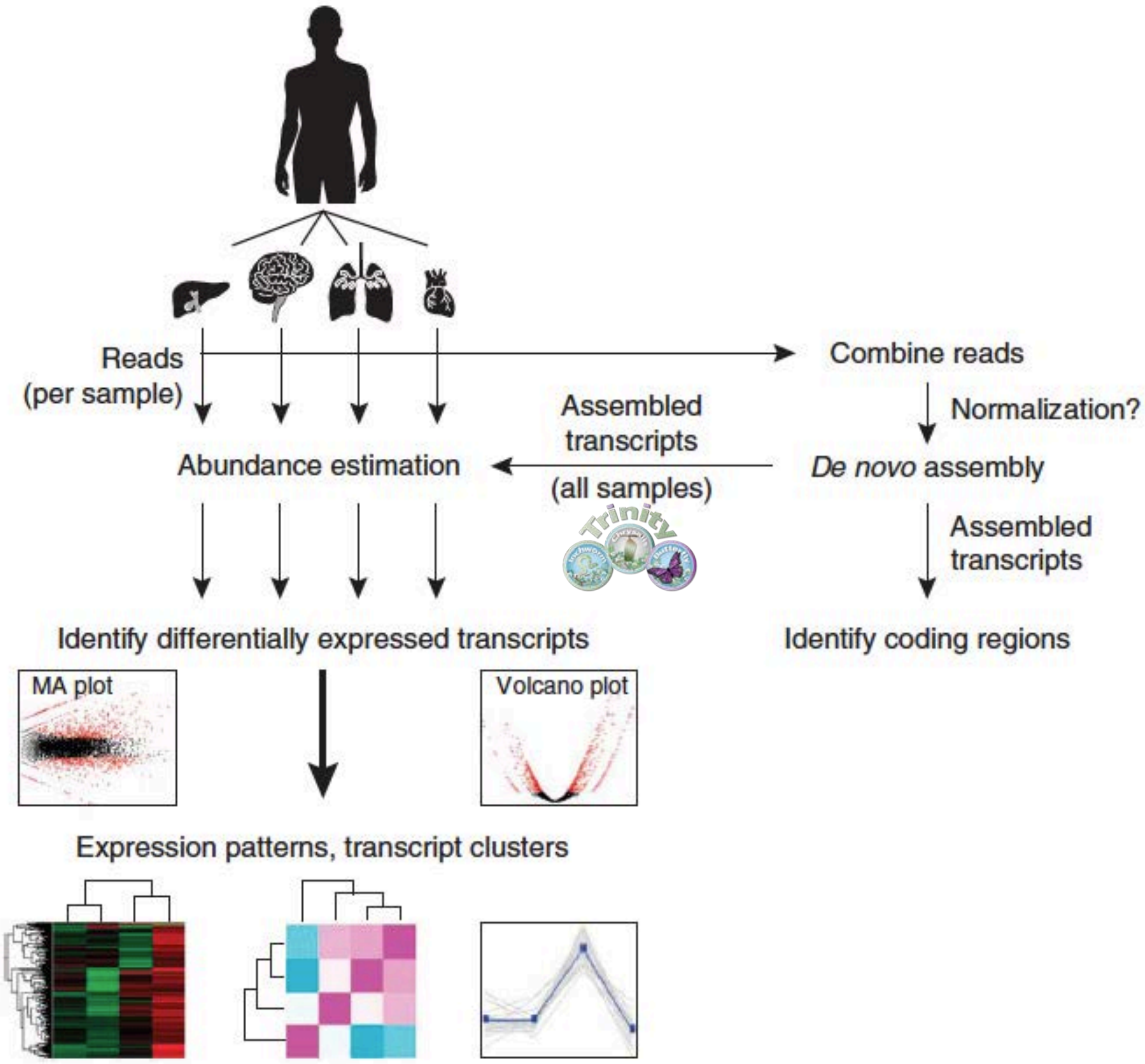
Affiliations | Contributions | Corresponding authors

Nature Protocols **8**, 1494–1512 (2013) | doi:10.1038/nprot.2013.084
Published online 11 July 2013



Trinity Framework for De novo Transcriptome Assembly and Analysis

(focus of the transcriptomics lab)



Reads (per sample)

Combine reads

Normalization?

De novo assembly

Assembled transcripts (all samples)

Abundance estimation

Assembled transcripts

Identify differentially expressed transcripts

Identify coding regions

MA plot

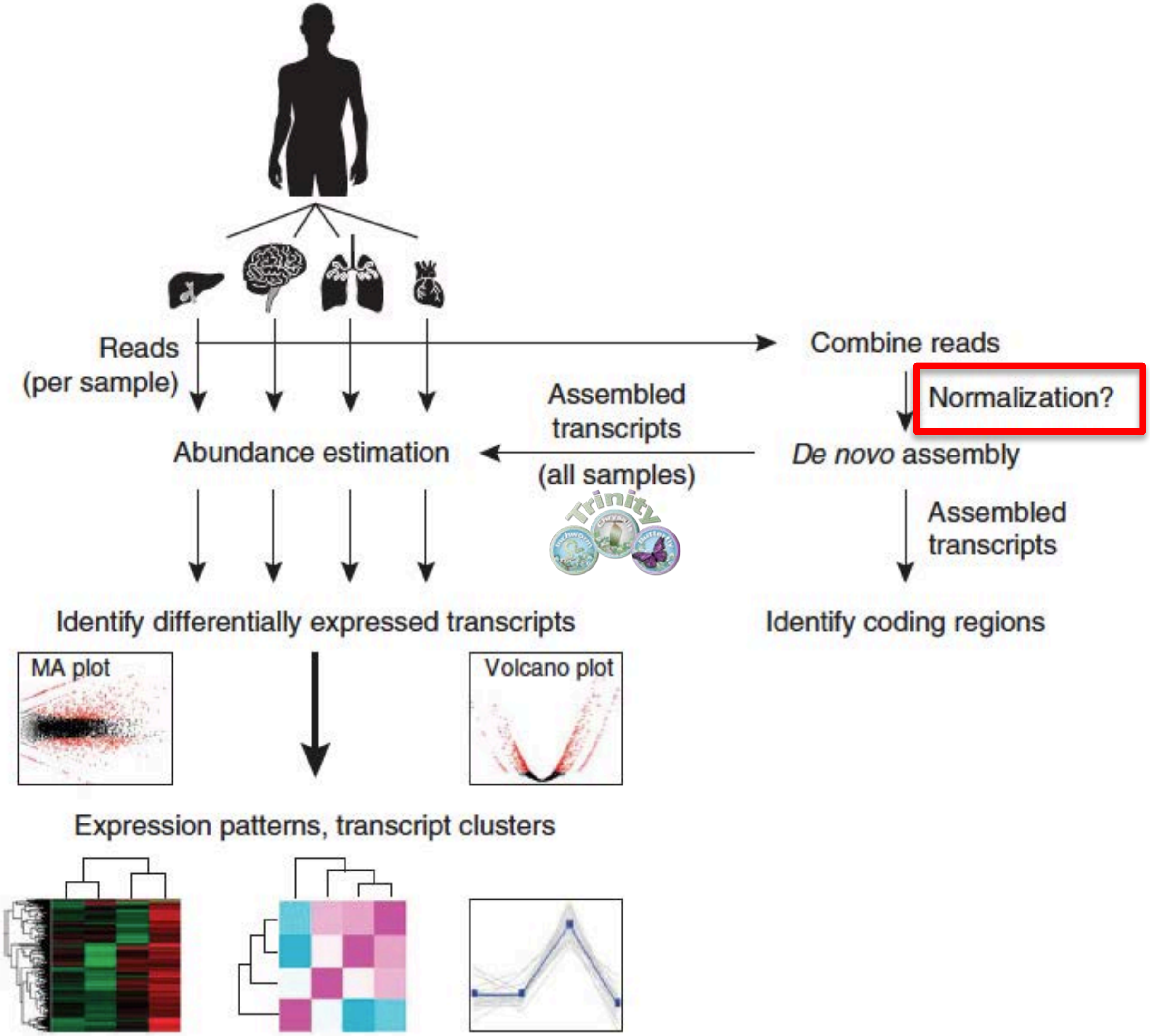
Volcano plot

Expression patterns, transcript clusters

Bioconductor,
& Trinity

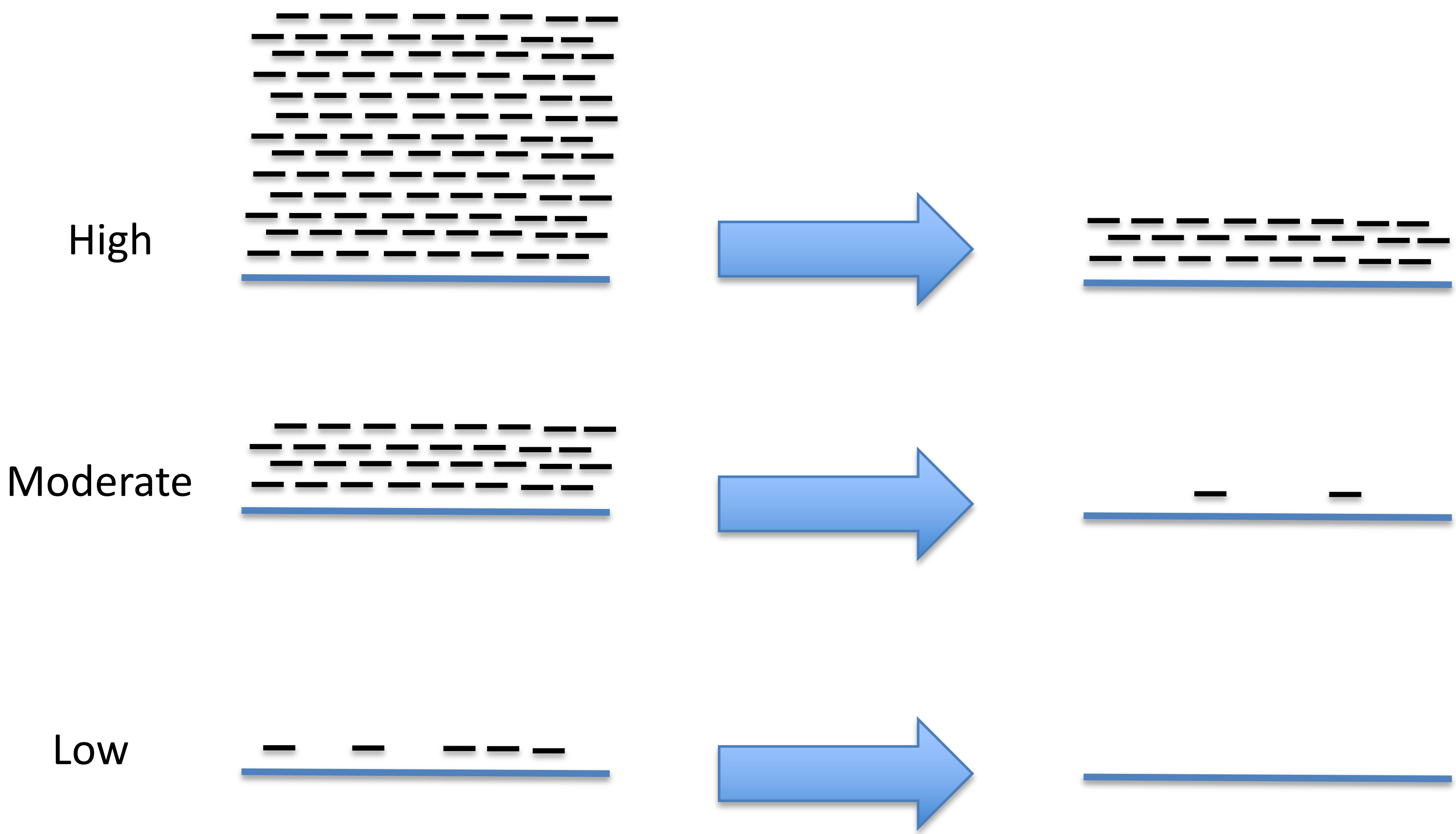
Trinity Framework for De novo Transcriptome Assembly and Analysis

(focus of the transcriptomics lab)

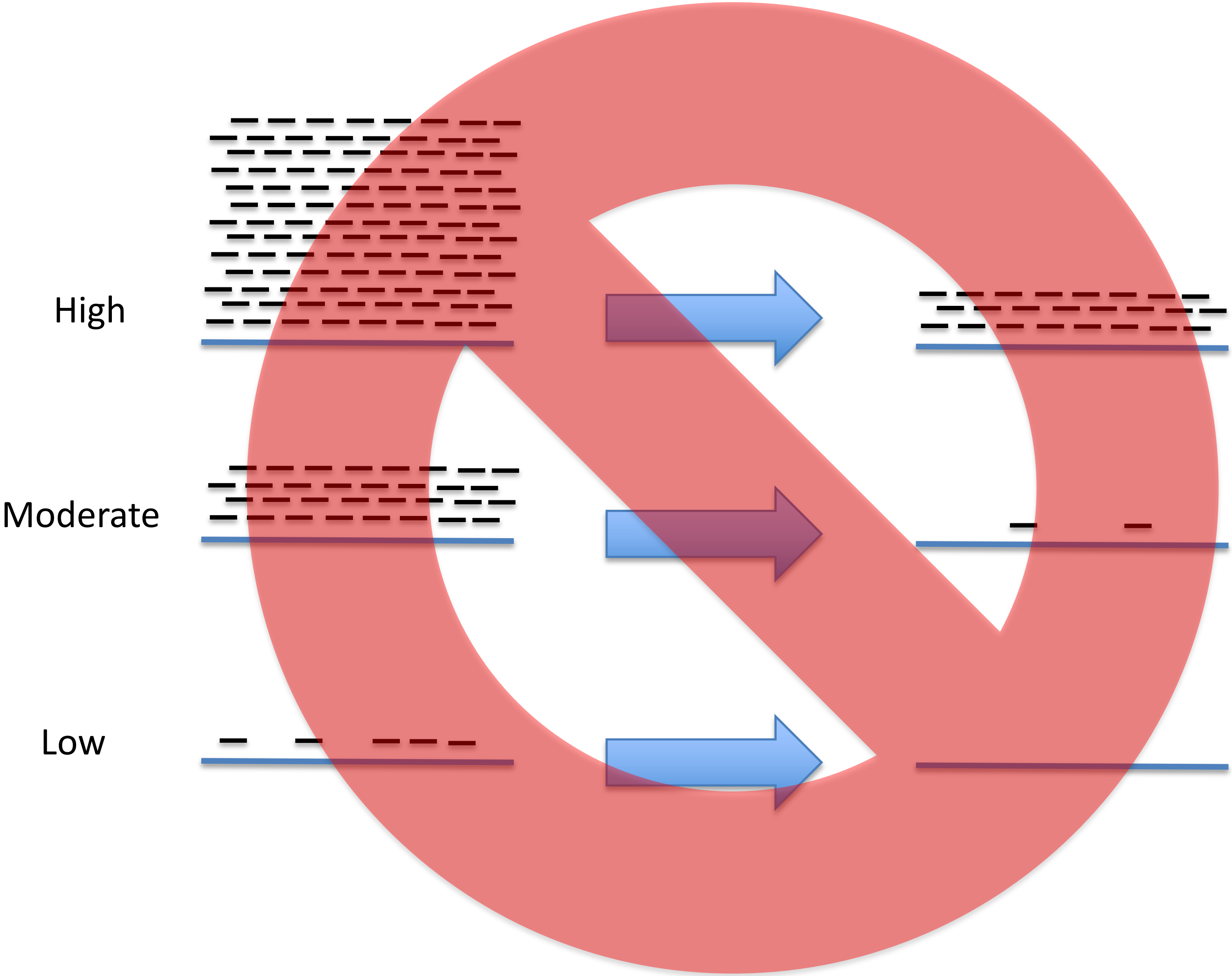


Bioconductor,
& Trinity

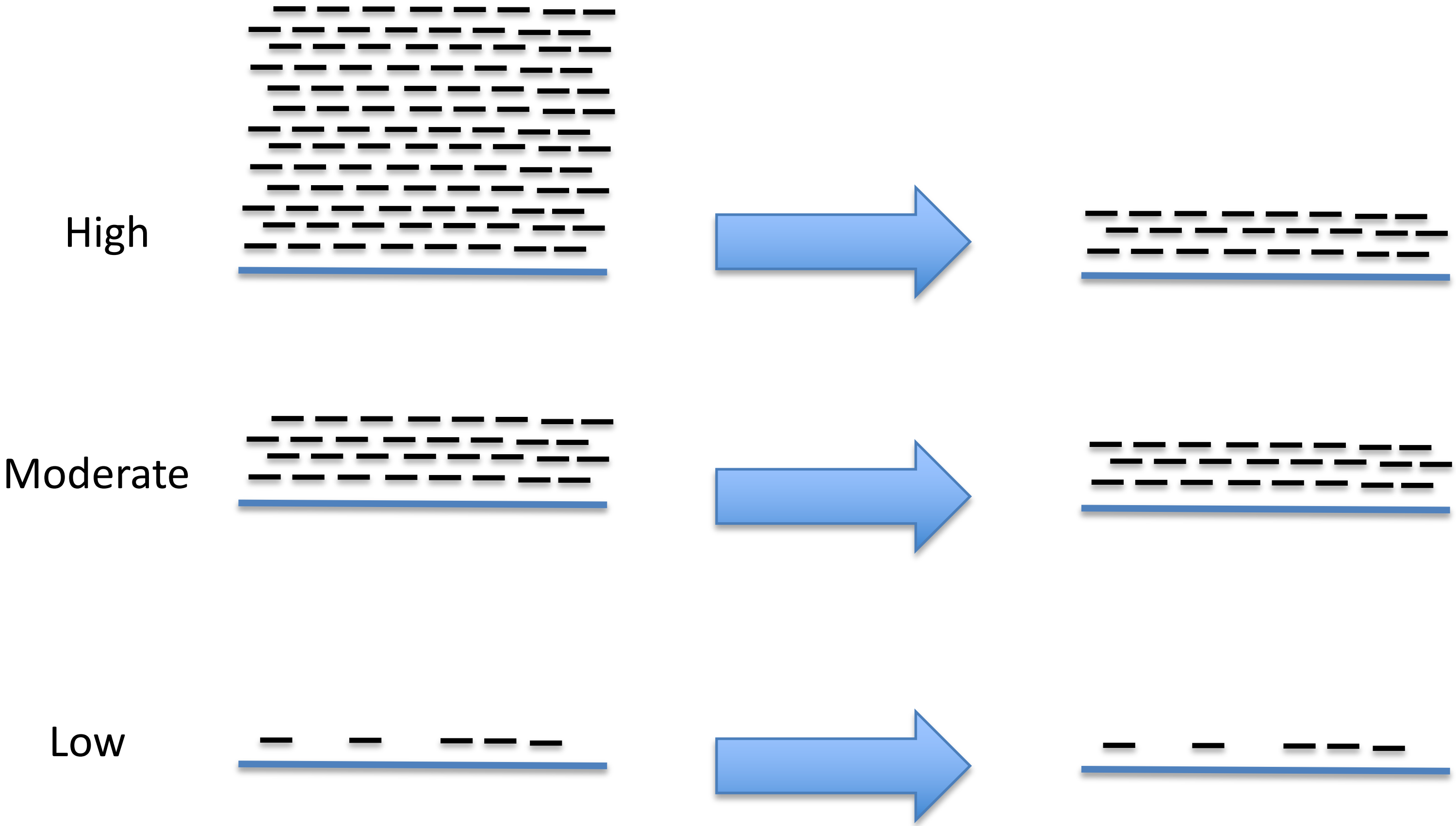
Could sub-sample the reads



Could sub-sample the reads



In silico normalization of reads

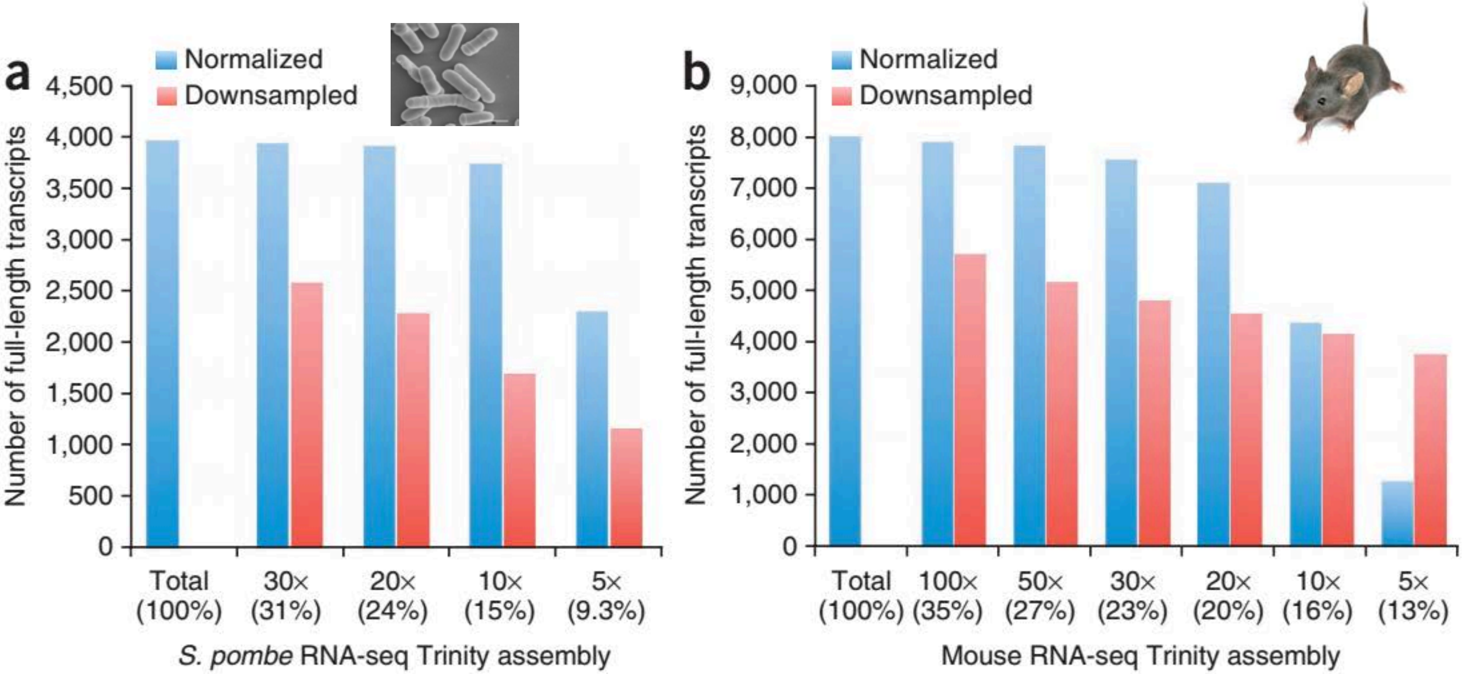


Select reads according to the probability:

$$P(\text{select read}) = \text{Min}\left(\frac{\text{target\_coverage(read)}}{\text{observed\_coverage(read)}}, 1\right)$$

Inspired by C. Titus Brown's Diginorm

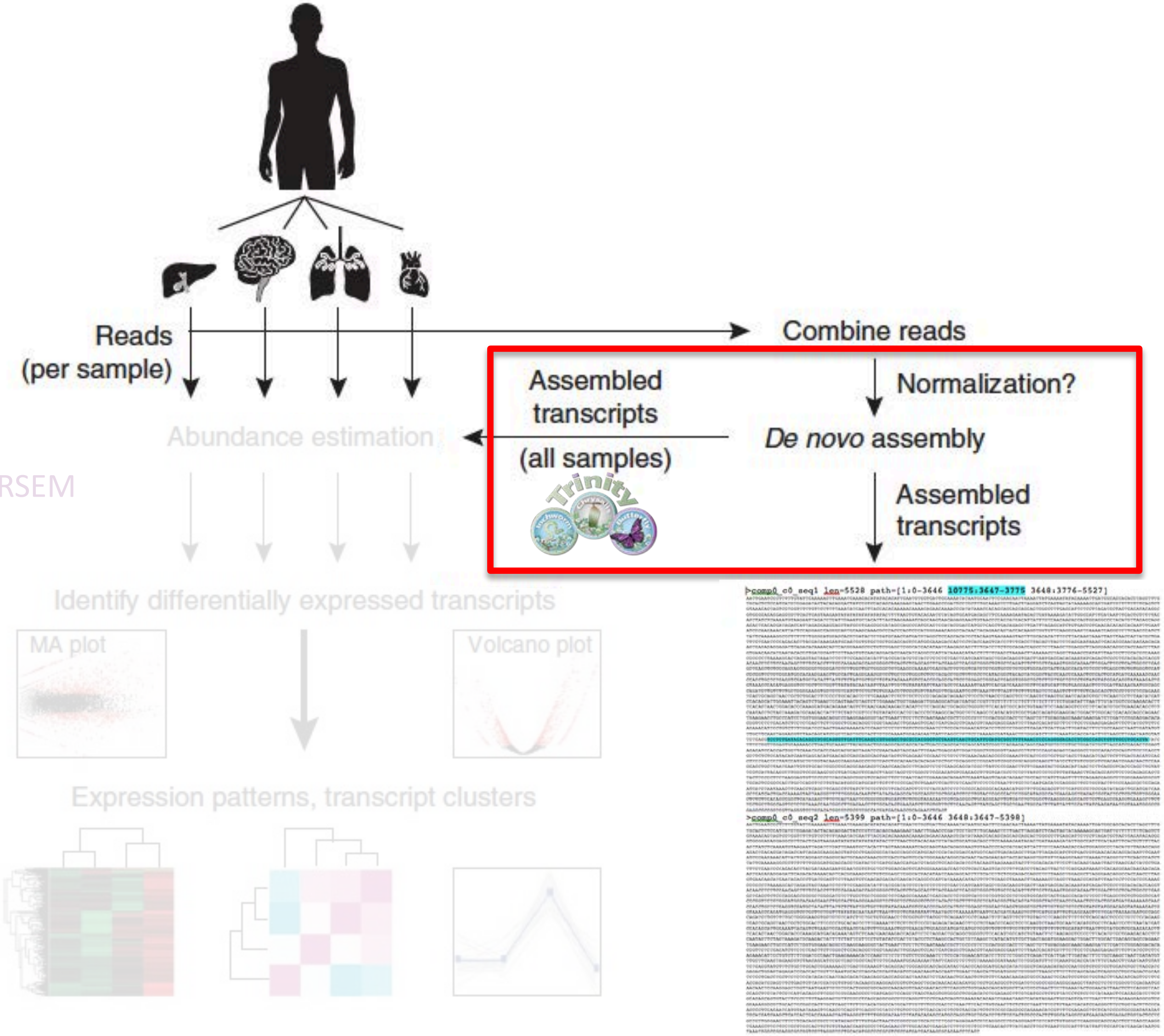
Impact of Normalization on *De novo* Full-length Transcript Reconstruction



Largely retain full-length reconstruction, but use less RAM and assemble much faster.

Haas et al., 2013

The product of Trinity: a Fasta file of assembled transcripts

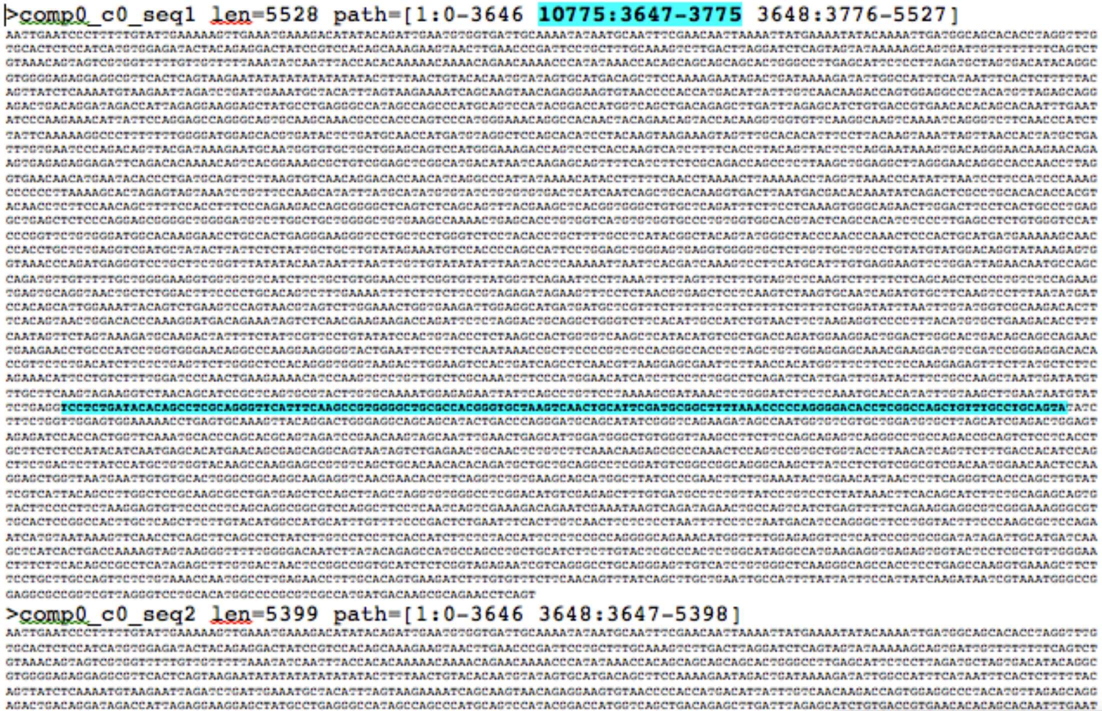


```
>comp0_c0_seq1 len=5528 path=[1:0-3646 10775:3647-3775 3648:3776-5527]
[illegible]
>comp0_c0_seq2 len=5399 path=[1:0-3646 3648:3647-5398]
[illegible]
```

Bowtie & RSEM

Bioconductor,
& Trinity

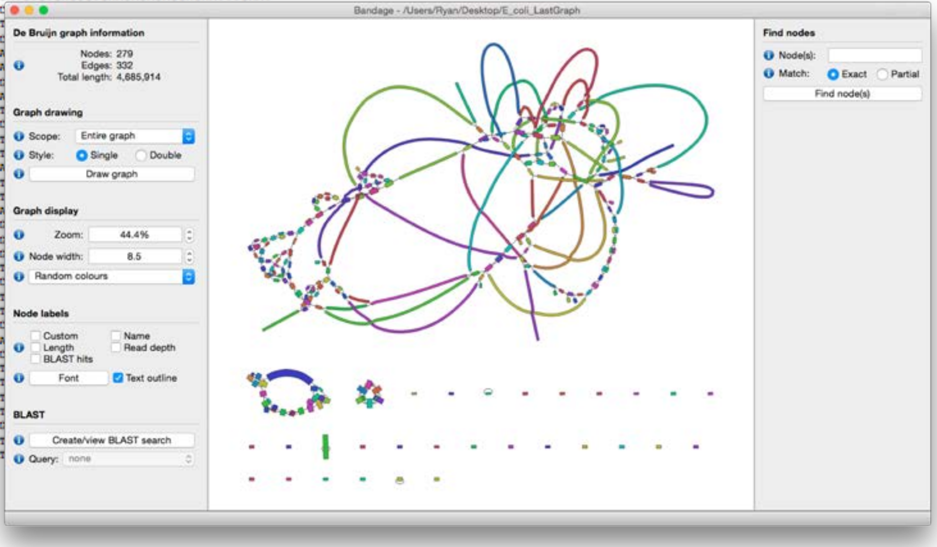
Trinity output: A multi-fasta file



```
>comp0_c0_seq1 len=5528 path=[1:0-3646 10775:3647-3775 3648:3776-5527]
[illegible]
>comp0_c0_seq2 len=5399 path=[1:0-3646 3648:3647-5398]
[illegible]
```

Can visualize using Bandage

https://rrwick.github.io/Bandage/



Bandage - /Users/Ryan/Desktop/E_coli_LastGraph

De Bruijn graph information

Nodes: 279
Edges: 332
Total length: 4,685,914

Graph drawing

Scope: Entire graph
Style: ● Single ○ Double

Graph display

Zoom: 44.4%
Node width: 8.5
Random colours

Node labels

□ Custom □ Name
□ Length □ Read depth
□ BLAST hits
☑ Text outline

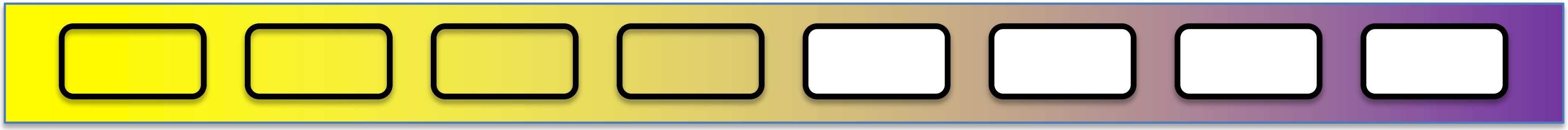
BLAST

Query: none

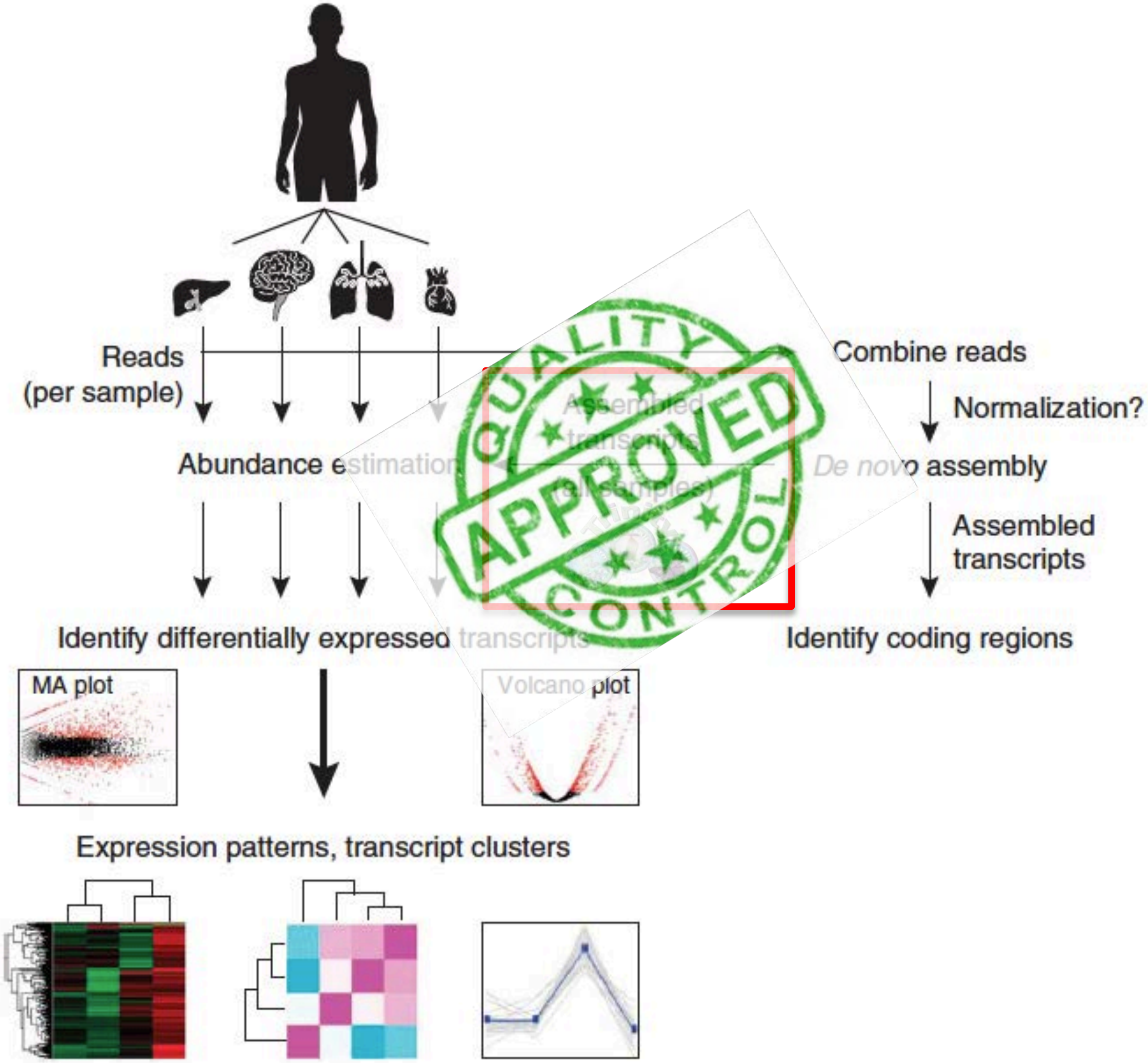
Find nodes

Node(s):
Match: ● Exact ○ Partial

Part 4. Transcriptome Quality Assessment

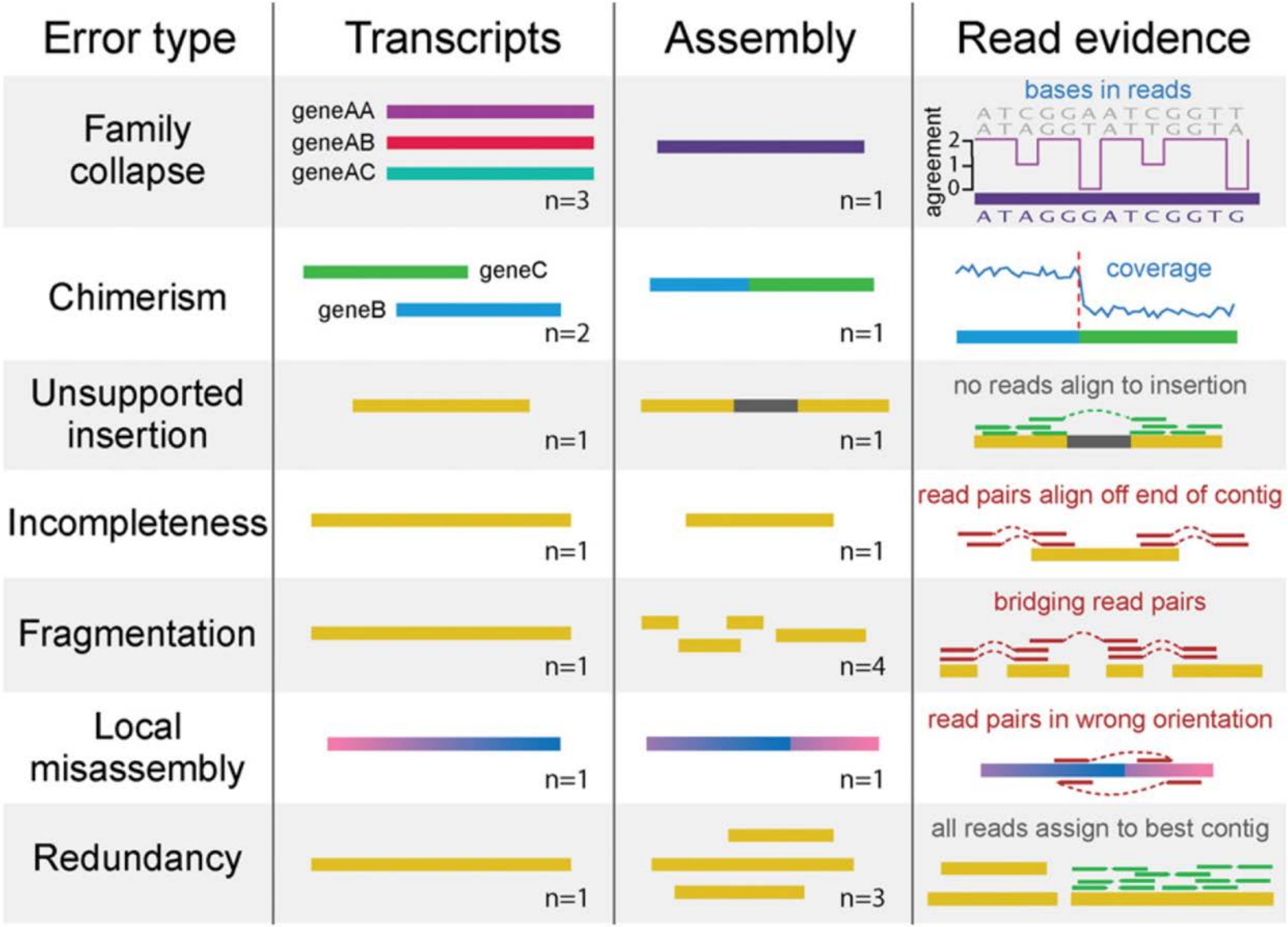


Evaluating the quality of your transcriptome assembly



Bioconductor,
& Trinity

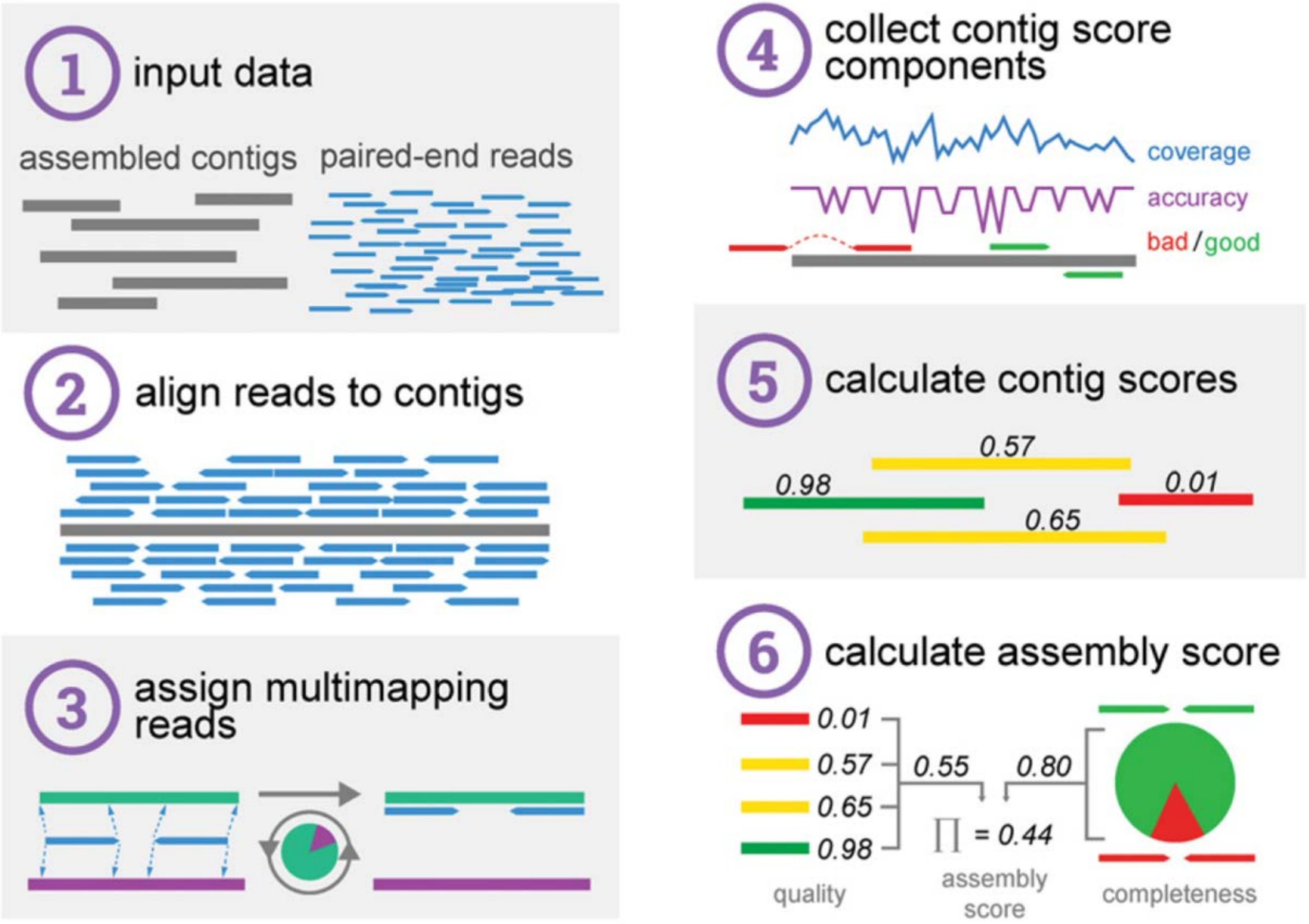
De novo Transcriptome Assembly is Prone to Certain Types of Errors



Error type	Transcripts	Assembly	Read evidence
Family collapse	geneAA geneAB geneAC n=3	n=1	bases in reads ATCGGAATCGGTT ATAGGTATTGGTA agreement 2 1 0 ATAGGGATCGGTG
Chimerism	geneC geneB n=2	n=1	coverage
Unsupported insertion	n=1	n=1	no reads align to insertion
Incompleteness	n=1	n=1	read pairs align off end of contig
Fragmentation	n=1	n=4	bridging read pairs
Local misassembly	n=1	n=1	read pairs in wrong orientation
Redundancy	n=1	n=3	all reads assign to best contig

Smith-Unna et al. Genome Research, 2016

TransRate



1 input data

2 align reads to contigs

3 assign multimapping reads

4 collect contig score components

5 calculate contig scores

6 calculate assembly score

Smith-Unna et al. Genome Research, 2016

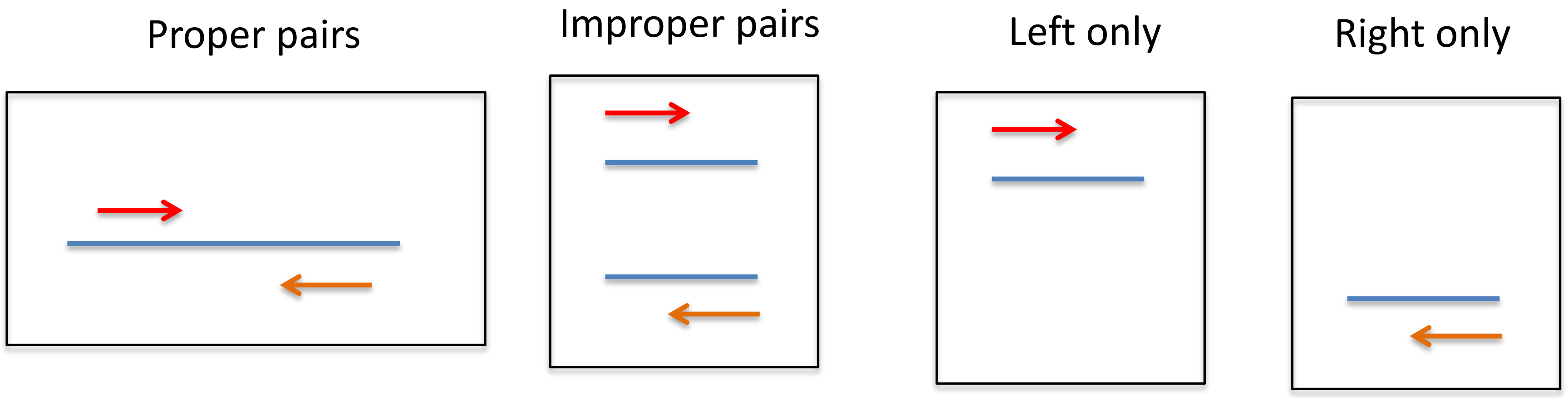
Simple Quantitative and Qualitative Assembly Metrics

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:



Proper pairs

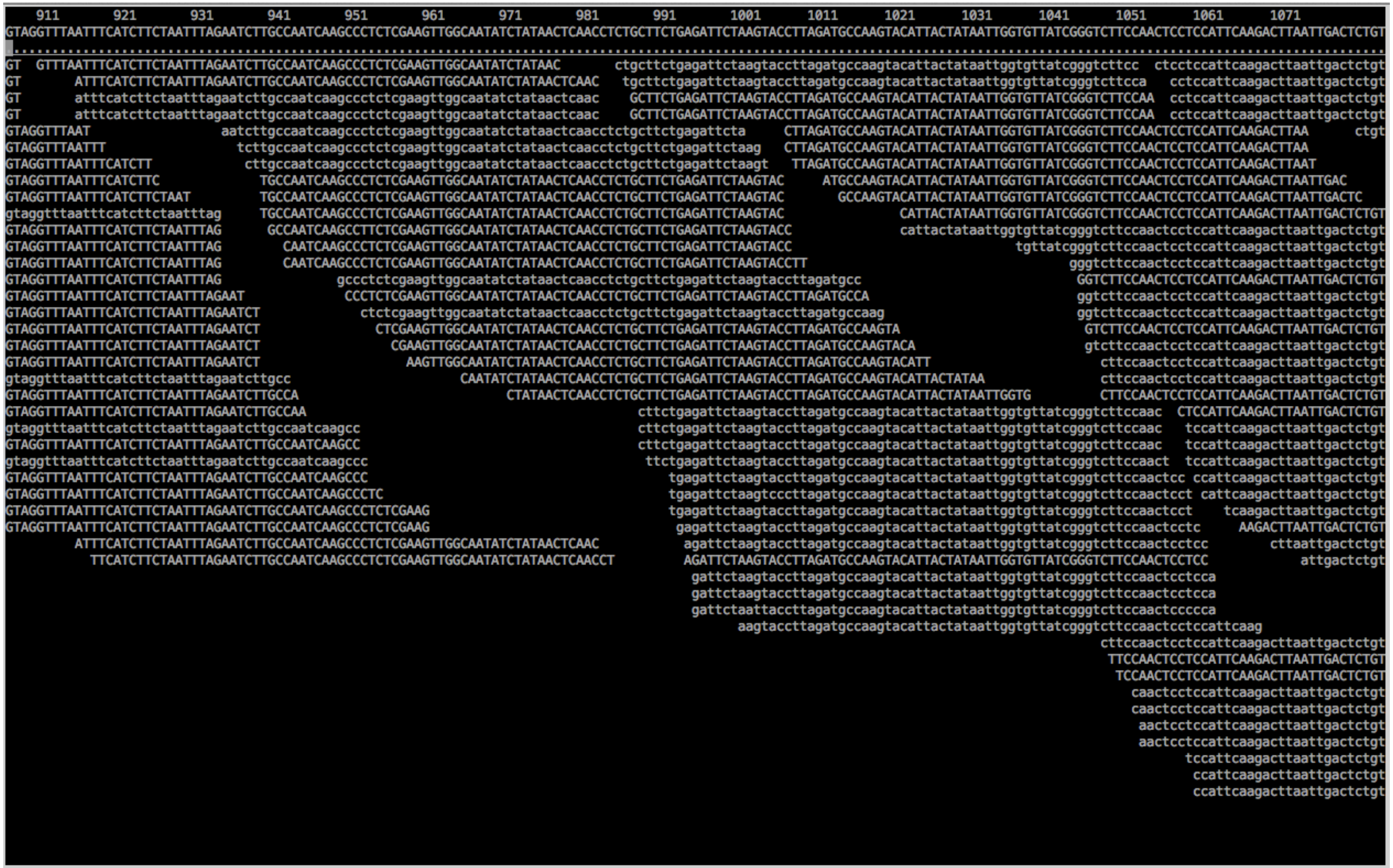
Improper pairs

Left only

Right only

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

% samtools tview alignments.bam target.fasta



```
    911       921       931       941       951       961       971       981       991       1001      1011      1021      1031      1041      1051      1061      1071
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...................................................................................................................................................................................
GT  GTTTAATTTCATCTTCTAATTTAGAATCTTGCCAATCAAGCCCTCTCGAAGTTGGCAATATCTATAAC       ctgcttctgagattctaagtaccttagatgccaagtacattactataattggtgttatcgggtcttcc  ctcctccattcaagacttaattgactctgt
GT       ATTTCATCTTCTAATTTAGAATCTTGCCAATCAAGCCCTCTCGAAGTTGGCAATATCTATAACTCAAC   tgcttctgagattctaagtaccttagatgccaagtacattactataattggtgttatcgggtcttcca   cctccattcaagacttaattgactctgt
GT       atttcatcttctaatttagaatcttgccaatcaagccctctcgaagttggcaatatctataactcaac    GCTTCTGAGATTCTAAGTACCTTAGATGCCAAGTACATTACTATAATTGGTGTTATCGGGTCTTCCAA  cctccattcaagacttaattgactctgt
GT       atttcatcttctaatttagaatcttgccaatcaagccctctcgaagttggcaatatctataactcaac    GCTTCTGAGATTCTAAGTACCTTAGATGCCAAGTACATTACTATAATTGGTGTTATCGGGTCTTCCAA  cctccattcaagacttaattgactctgt
GTAGGTTTAAT                 aatcttgccaatcaagccctctcgaagttggcaatatctataactcaacctctgcttctgagattcta     CTTAGATGCCAAGTACATTACTATAATTGGTGTTATCGGGTCTTCCAACTCCTCCATTCAAGACTTAA      ctgt
GTAGGTTTAATTT                 tcttgccaatcaagccctctcgaagttggcaatatctataactcaacctctgcttctgagattctaag   CTTAGATGCCAAGTACATTACTATAATTGGTGTTATCGGGTCTTCCAACTCCTCCATTCAAGACTTAA
GTAGGTTTAATTTCATCTT            cttgccaatcaagccctctcgaagttggcaatatctataactcaacctctgcttctgagattctaagt   TTAGATGCCAAGTACATTACTATAATTGGTGTTATCGGGTCTTCCAACTCCTCCATTCAAGACTTAAT
GTAGGTTTAATTTCATCTTC             TGCCAATCAAGCCCTCTCGAAGTTGGCAATATCTATAACTCAACCTCTGCTTCTGAGATTCTAAGTAC     ATGCCAAGTACATTACTATAATTGGTGTTATCGGGTCTTCCAACTCCTCCATTCAAGACTTAATTGAC
GTAGGTTTAATTTCATCTTCTAAT         TGCCAATCAAGCCCTCTCGAAGTTGGCAATATCTATAACTCAACCTCTGCTTCTGAGATTCTAAGTAC       GCCAAGTACATTACTATAATTGGTGTTATCGGGTCTTCCAACTCCTCCATTCAAGACTTAATTGACTC
gtaggtttaatttcatcttctaatttag     TGCCAATCAAGCCCTCTCGAAGTTGGCAATATCTATAACTCAACCTCTGCTTCTGAGATTCTAAGTAC               CATTACTATAATTGGTGTTATCGGGTCTTCCAACTCCTCCATTCAAGACTTAATTGACTCTGT
GTAGGTTTAATTTCATCTTCTAATTTAG      GCCAATCAAGCCTTCTCGAAGTTGGCAATATCTATAACTCAACCTCTGCTTCTGAGATTCTAAGTACC              cattactataattggtgttatcgggtcttccaactcctccattcaagacttaattgactctgt
GTAGGTTTAATTTCATCTTCTAATTTAG        CAATCAAGCCCTCTCGAAGTTGGCAATATCTATAACTCAACCTCTGCTTCTGAGATTCTAAGTACC                             tgttatcgggtcttccaactcctccattcaagacttaattgactctgt
GTAGGTTTAATTTCATCTTCTAATTTAG        CAATCAAGCCCTCTCGAAGTTGGCAATATCTATAACTCAACCTCTGCTTCTGAGATTCTAAGTACCTT                                  gggtcttccaactcctccattcaagacttaattgactctgt
GTAGGTTTAATTTCATCTTCTAATTTAG               gccctctcgaagttggcaatatctataactcaacctctgcttctgagattctaagtaccttagatgcc                            GGTCTTCCAACTCCTCCATTCAAGACTTAATTGACTCTGT
GTAGGTTTAATTTCATCTTCTAATTTAGAAT             CCCTCTCGAAGTTGGCAATATCTATAACTCAACCTCTGCTTCTGAGATTCTAAGTACCTTAGATGCCA                           ggtcttccaactcctccattcaagacttaattgactctgt
GTAGGTTTAATTTCATCTTCTAATTTAGAATCT             ctctcgaagttggcaatatctataactcaacctctgcttctgagattctaagtaccttagatgccaag                         ggtcttccaactcctccattcaagacttaattgactctgt
GTAGGTTTAATTTCATCTTCTAATTTAGAATCT               CTCGAAGTTGGCAATATCTATAACTCAACCTCTGCTTCTGAGATTCTAAGTACCTTAGATGCCAAGTA                        GTCTTCCAACTCCTCCATTCAAGACTTAATTGACTCTGT
GTAGGTTTAATTTCATCTTCTAATTTAGAATCT                 CGAAGTTGGCAATATCTATAACTCAACCTCTGCTTCTGAGATTCTAAGTACCTTAGATGCCAAGTACA                      gtcttccaactcctccattcaagacttaattgactctgt
GTAGGTTTAATTTCATCTTCTAATTTAGAATCT                   AAGTTGGCAATATCTATAACTCAACCTCTGCTTCTGAGATTCTAAGTACCTTAGATGCCAAGTACATT                      cttccaactcctccattcaagacttaattgactctgt
gtaggtttaatttcatcttctaatttagaatcttgcc                      CAATATCTATAACTCAACCTCTGCTTCTGAGATTCTAAGTACCTTAGATGCCAAGTACATTACTATAA               cttccaactcctccattcaagacttaattgactctgt
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GTAGGTTTAATTTCATCTTCTAATTTAGAATCTTGCCAATCAAGCC                                    cttctgagattctaagtaccttagatgccaagtacattactataattggtgttatcgggtcttccaac   tccattcaagacttaattgactctgt
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                                                                                                                                              cttccaactcctccattcaagacttaattgactctgt
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                                                                                                                                                TCCAACTCCTCCATTCAAGACTTAATTGACTCTGT
                                                                                                                                                  caactcctccattcaagacttaattgactctgt
                                                                                                                                                  caactcctccattcaagacttaattgactctgt
                                                                                                                                                   aactcctccattcaagacttaattgactctgt
                                                                                                                                                   aactcctccattcaagacttaattgactctgt
                                                                                                                                                         tccattcaagacttaattgactctgt
                                                                                                                                                          ccattcaagacttaattgactctgt
                                                                                                                                                          ccattcaagacttaattgactctgt
```
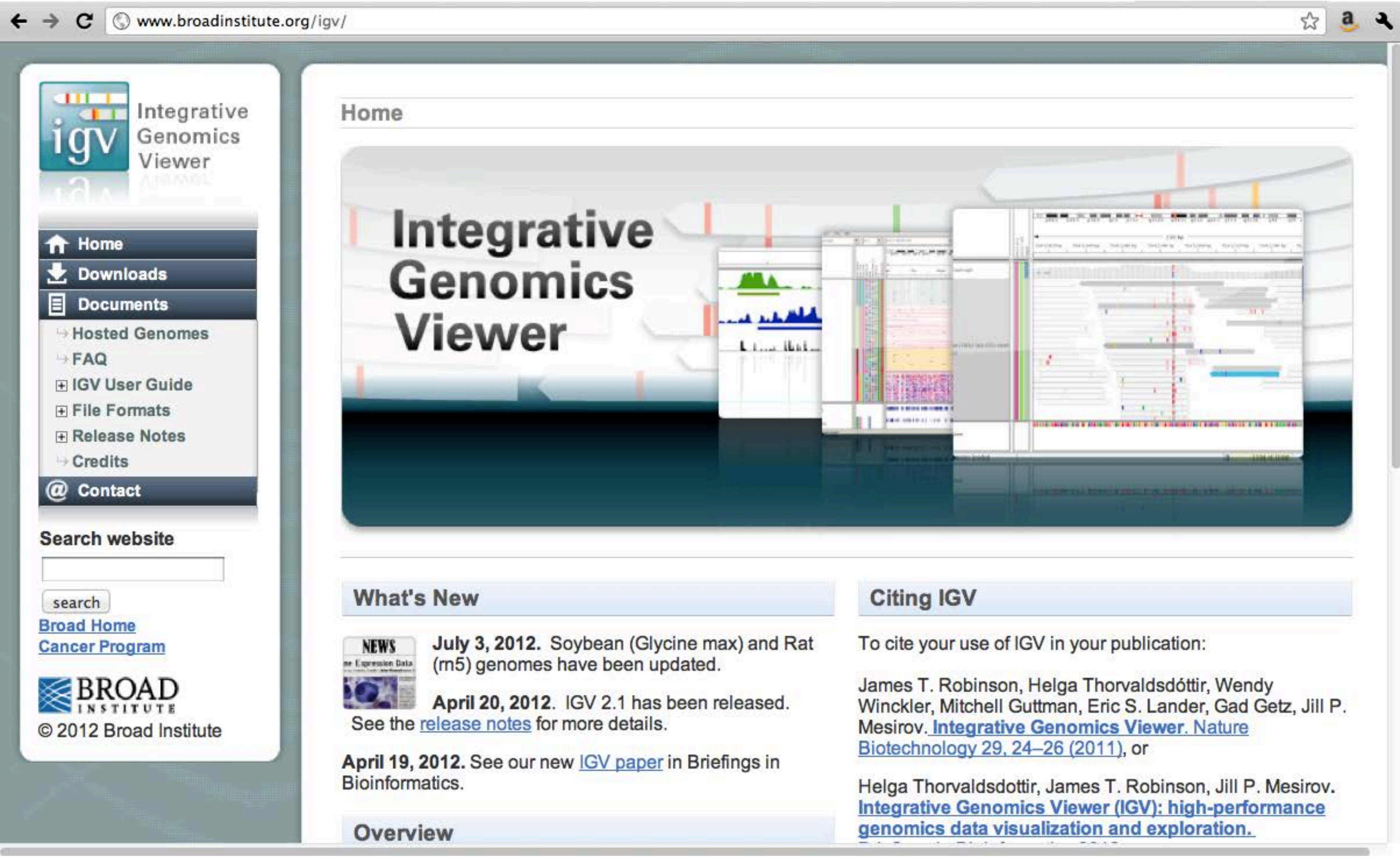

IGV



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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

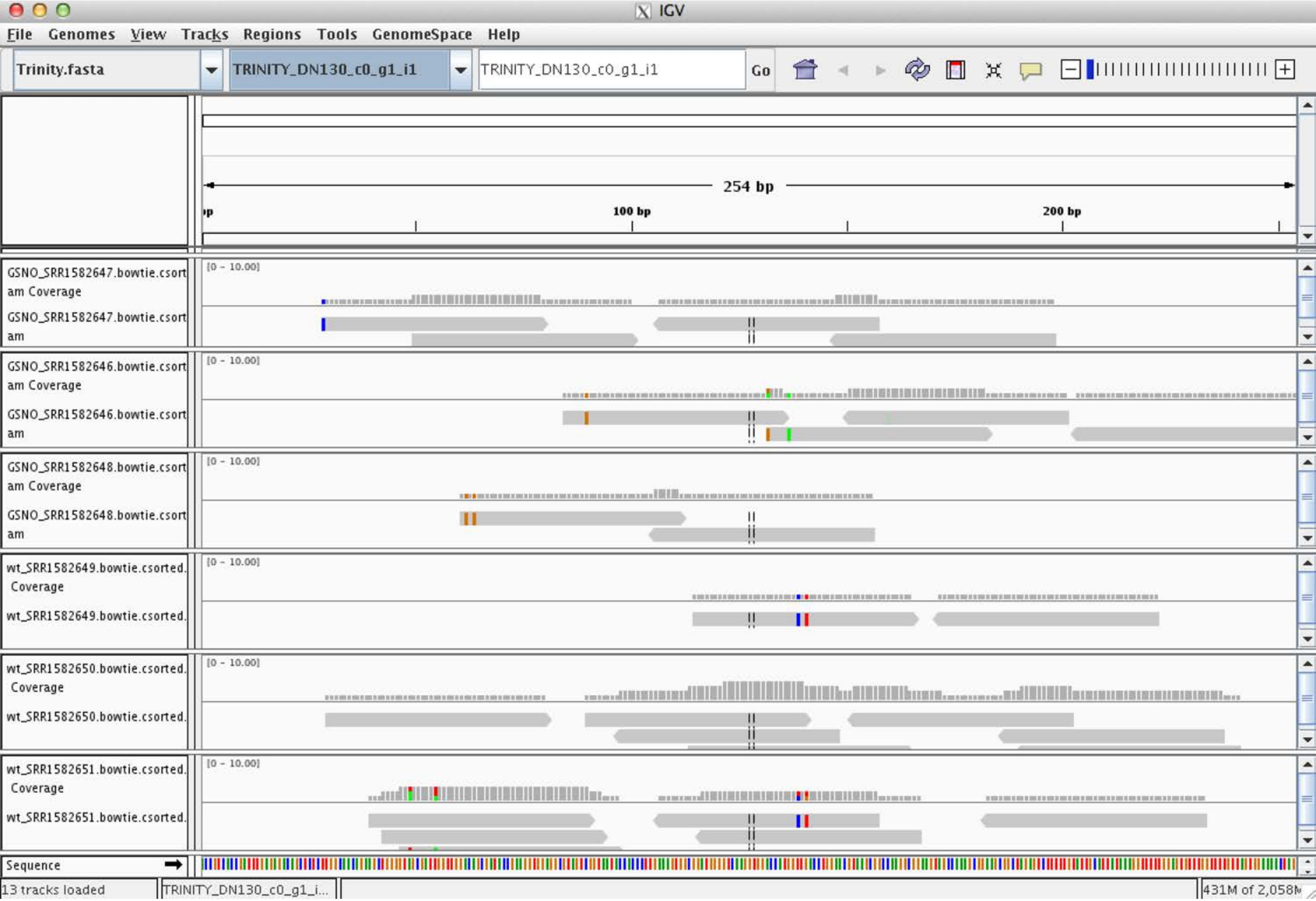
Citing IGV

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


IGV
File Genomes View Tracks Regions Tools GenomeSpace Help
Trinity.fasta
TRINITY_DN130_c0_g1_i1
TRINITY_DN130_c0_g1_i1
Go
254 bp
100 bp
200 bp
GSNO_SRR1582647.bowtie.csortam Coverage
GSNO_SRR1582647.bowtie.csortam
GSNO_SRR1582646.bowtie.csortam Coverage
GSNO_SRR1582646.bowtie.csortam
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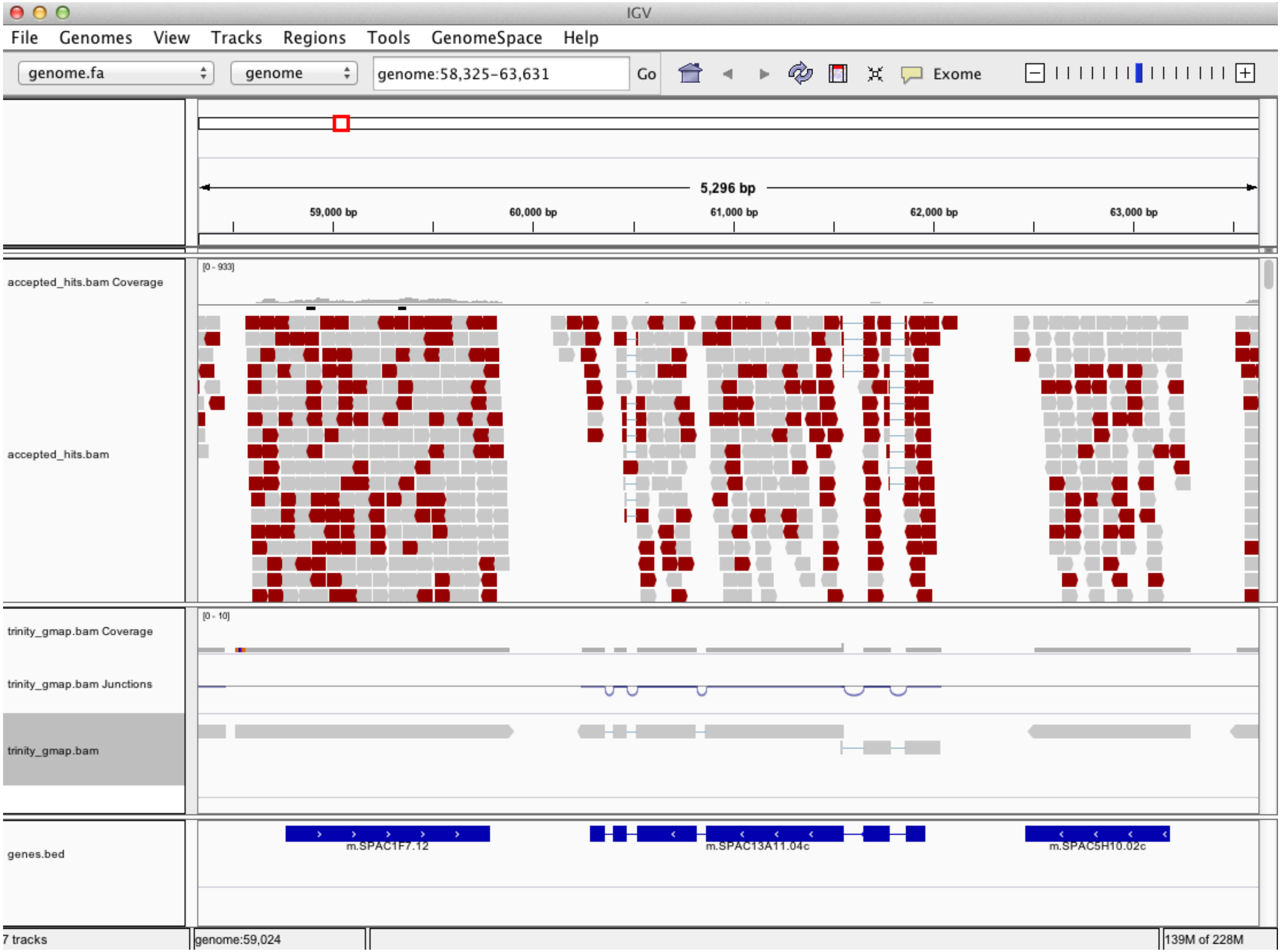
wt_SRR1582650.bowtie.csorted.
wt_SRR1582651.bowtie.csorted. Coverage
wt_SRR1582651.bowtie.csorted.
[0 - 10.00]
Sequence
13 tracks loaded
TRINITY_DN130_c0_g1_i...
431M of 2,058M

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

(Trinity transcripts aligned to the genome using GMAP)

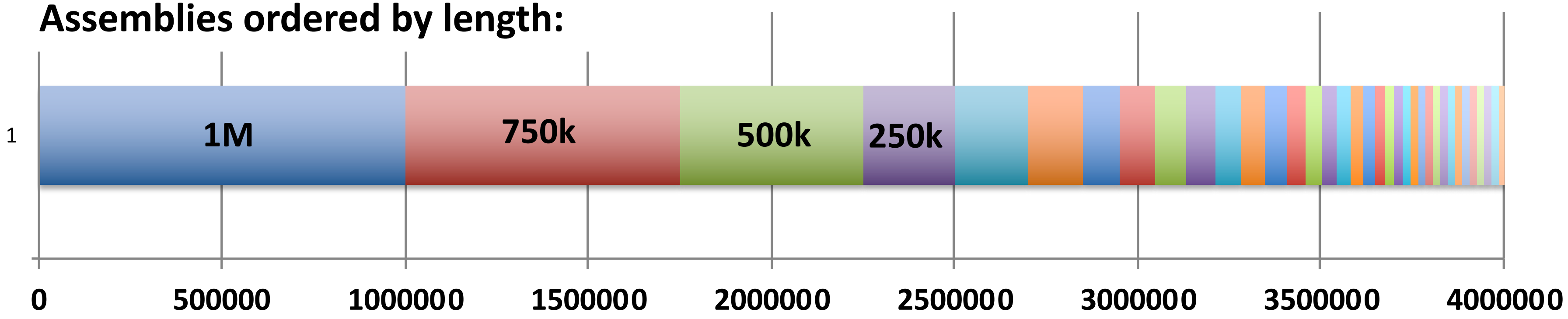


The Contig N50 statistic

“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’

Assemblies ordered by length:



N50 contig length = 500k

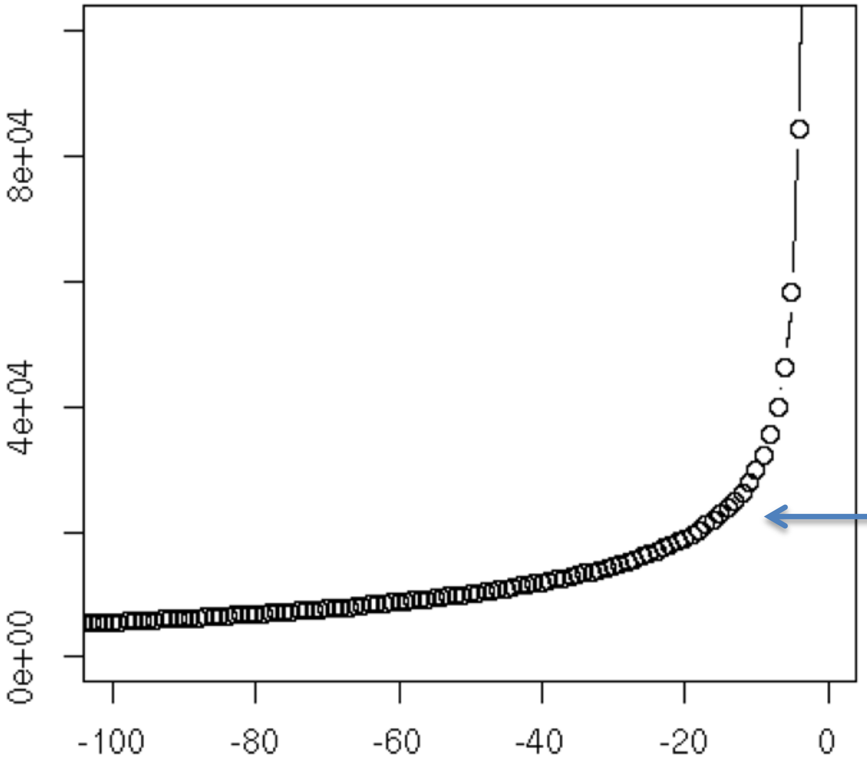
Often, most assembled transcripts are *very* lowly expressed

(How many ‘transcripts & genes’ are there really?)

1.4 million Trinity transcript contigs

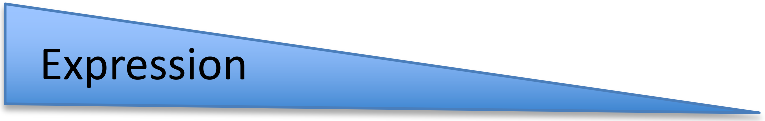
N50 ~ 500 bases

Cumulative # of Transcripts



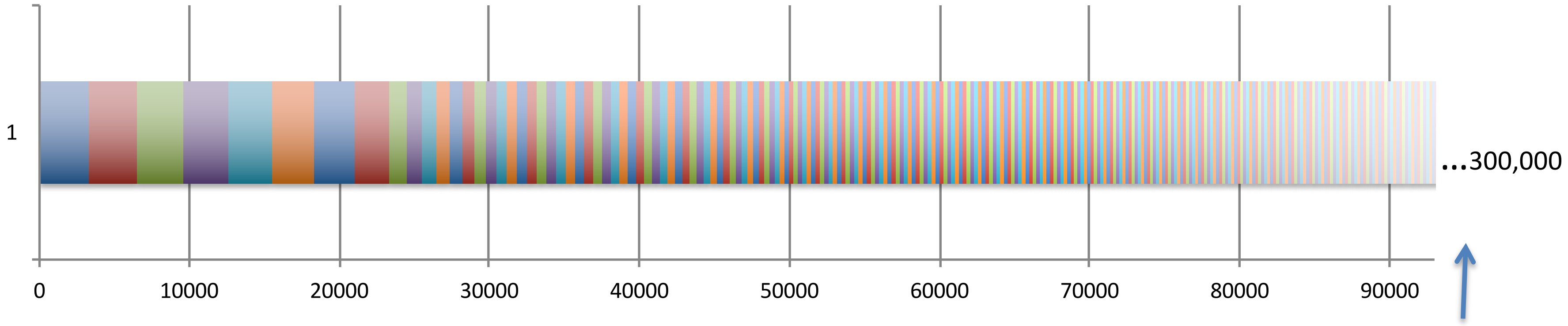
20k transcripts

-1 * minimum TPM



* Salamander transcriptome

N50 Calculation for *Transcriptome* Assemblies??



N50 length?
(small)

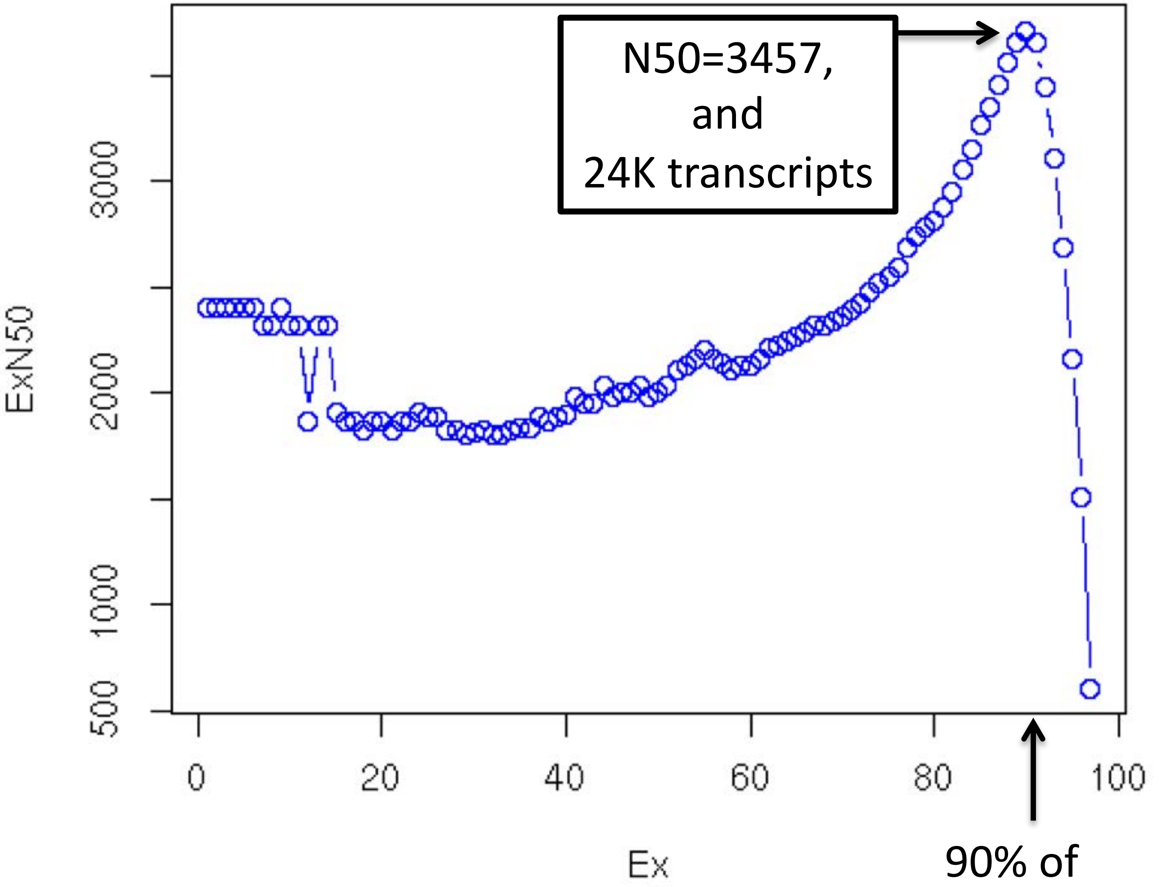
In transcriptome assemblies – N50 is ***not*** very useful.

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

Expression-informed N50 Calculation for Transcriptome Assemblies (ExN50)

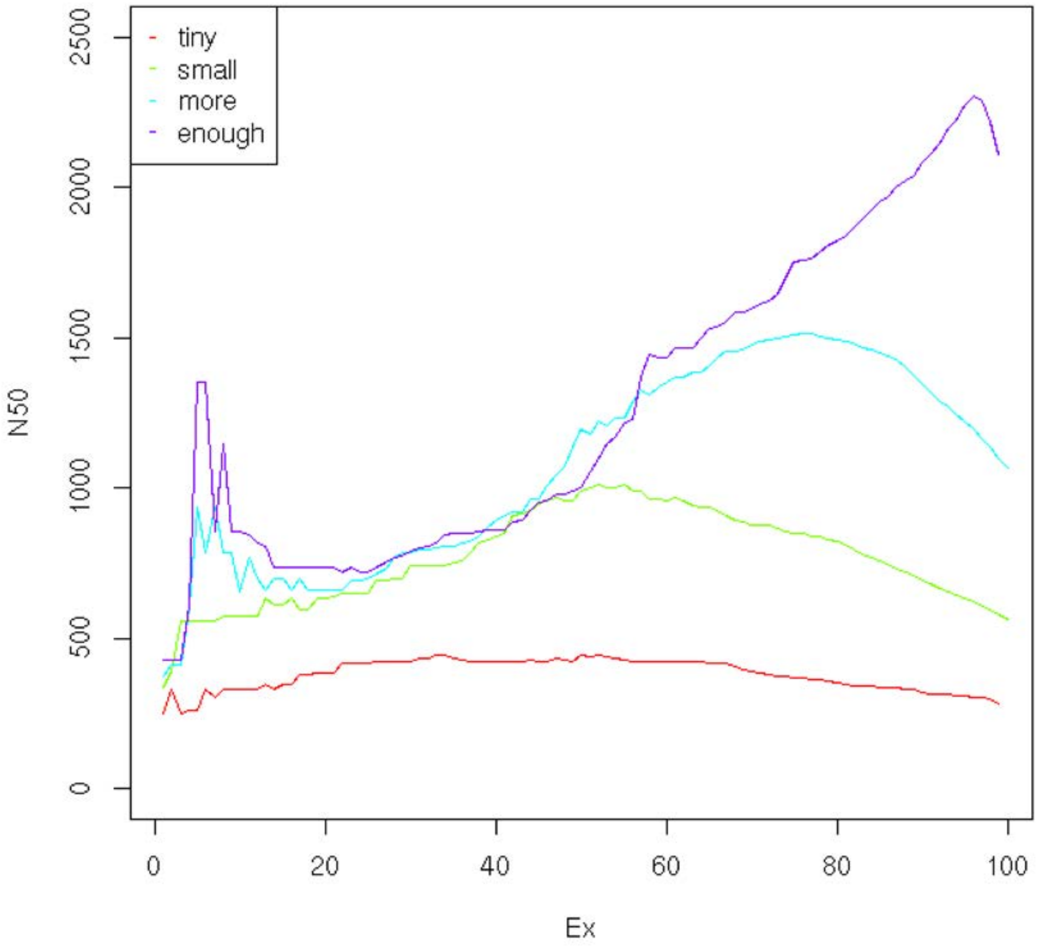
Compute N50 Based on the Top-most Highly Expressed Transcripts

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



90% of expression data

ExN50 Profiles for Different Trinity Assemblies Using Different Read Depths



Millions of Reads

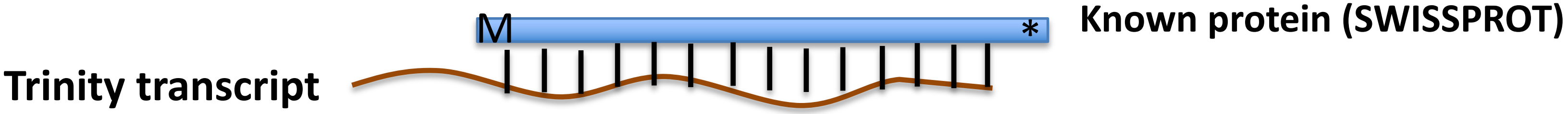
Thousands of Reads

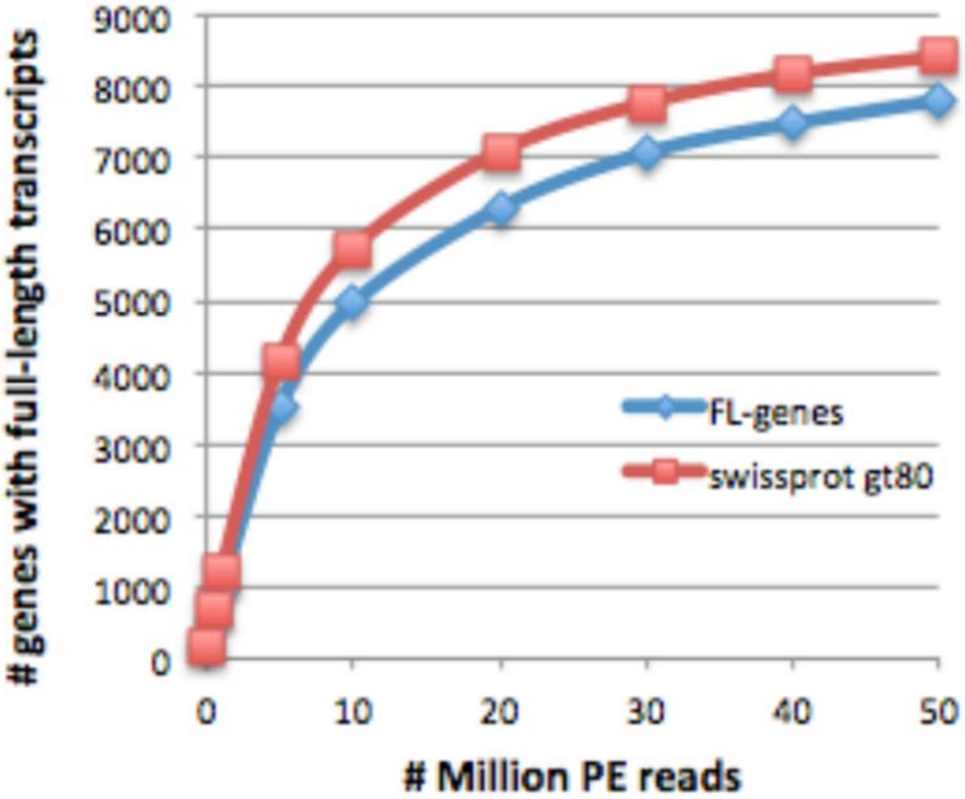
Note shift in ExN50 profiles as you assemble more and more reads.

* Candida transcriptome

Evaluating the quality of your transcriptome assembly

Full-length Transcript Detection via BLASTX

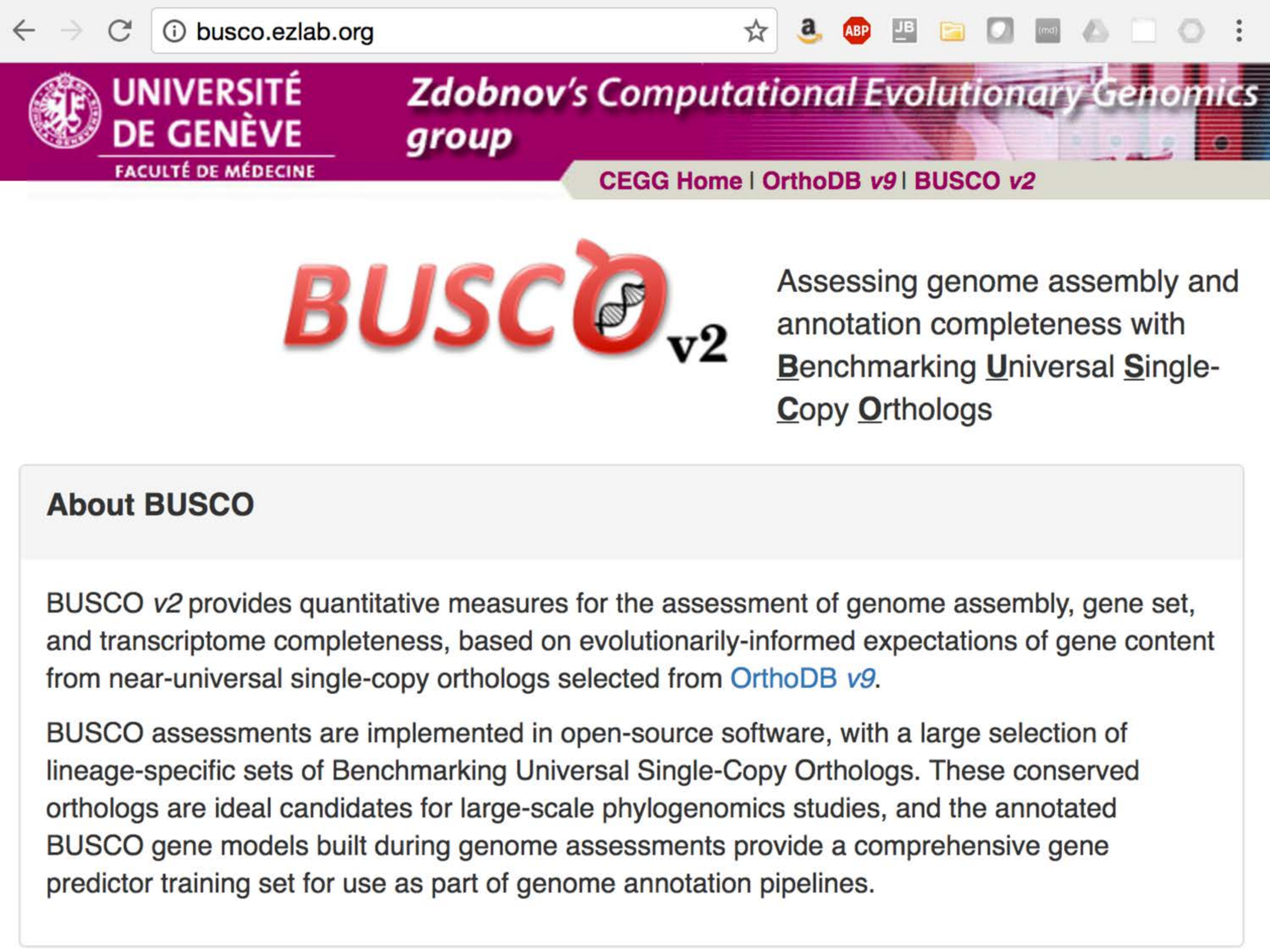




Have you sequenced deeply enough?

Haas et al. Nat. Protoc. 2013

* Mouse transcriptome



UNIVERSITÉ DE GENÈVE
FACULTÉ DE MÉDECINE

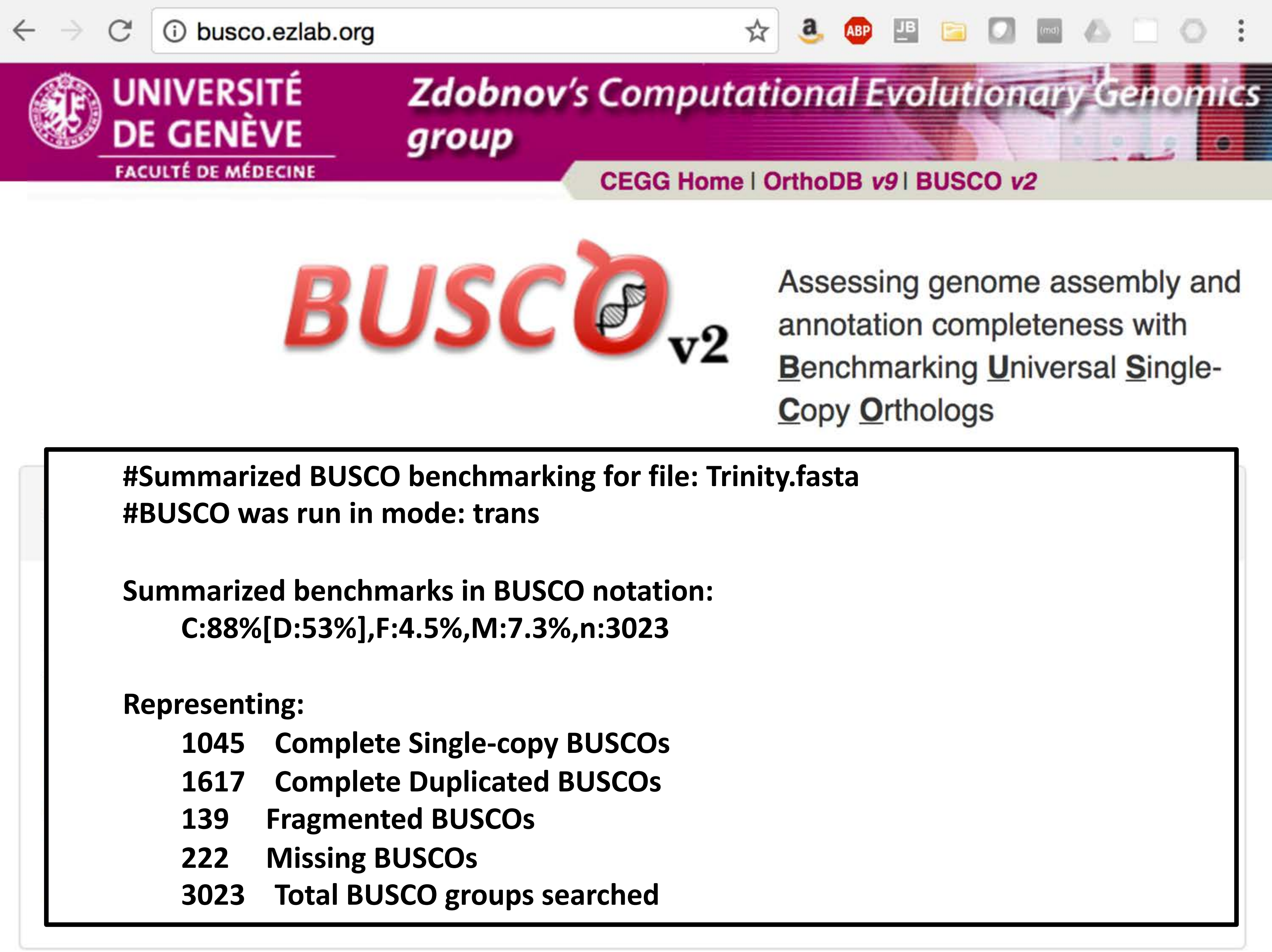
Zdobnov's Computational Evolutionary Genomics group

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?

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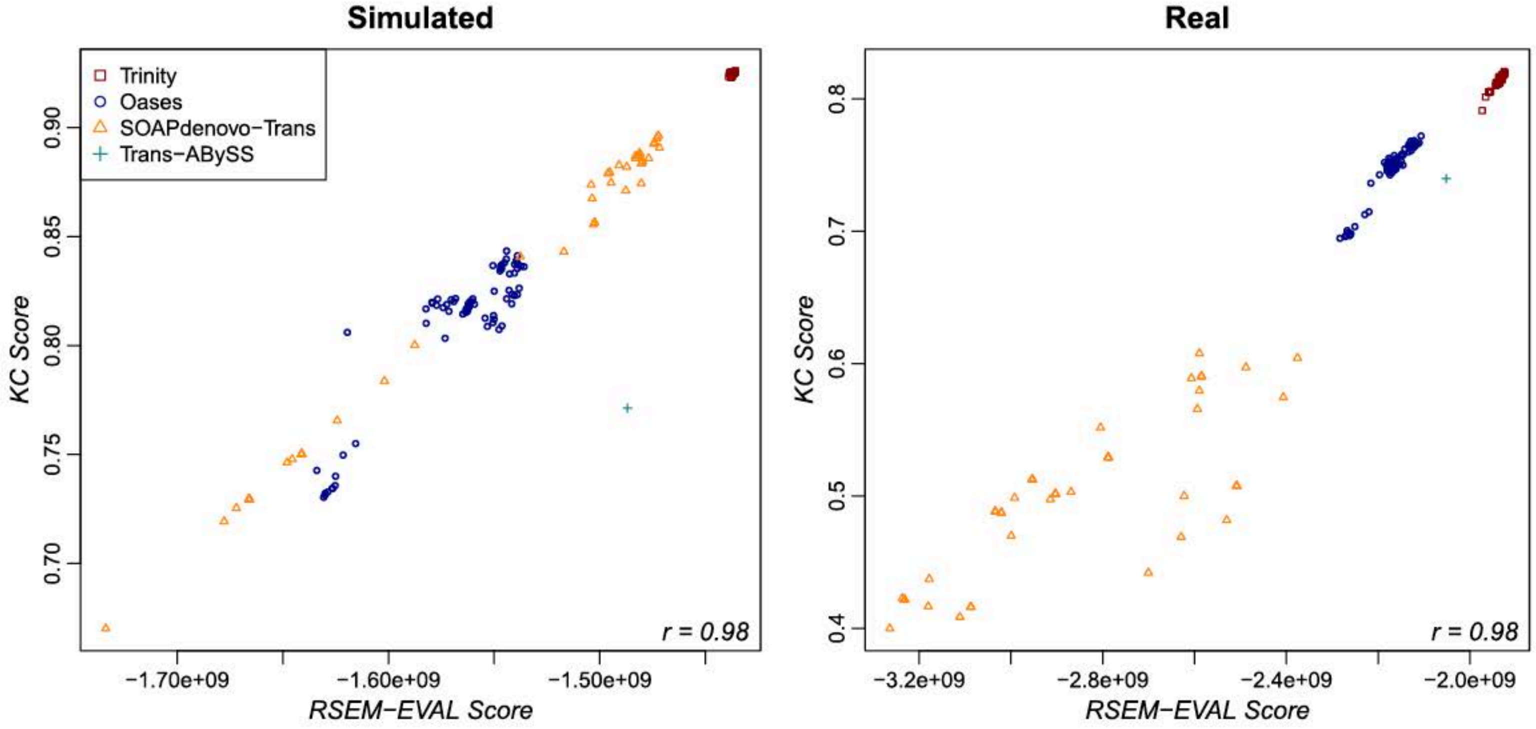
Bigger Score = Better Assembly

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.”

Ref Genome –based metric

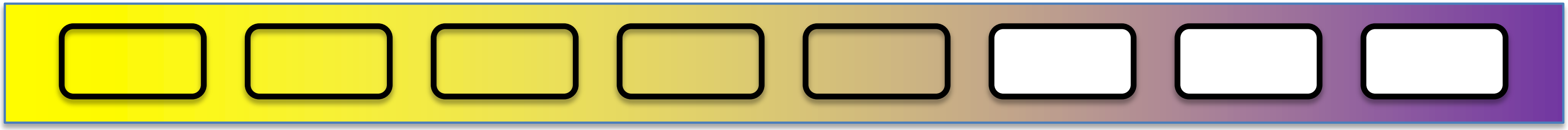


Yay Trinity!!

RSEM-EVAL Genome-free metric

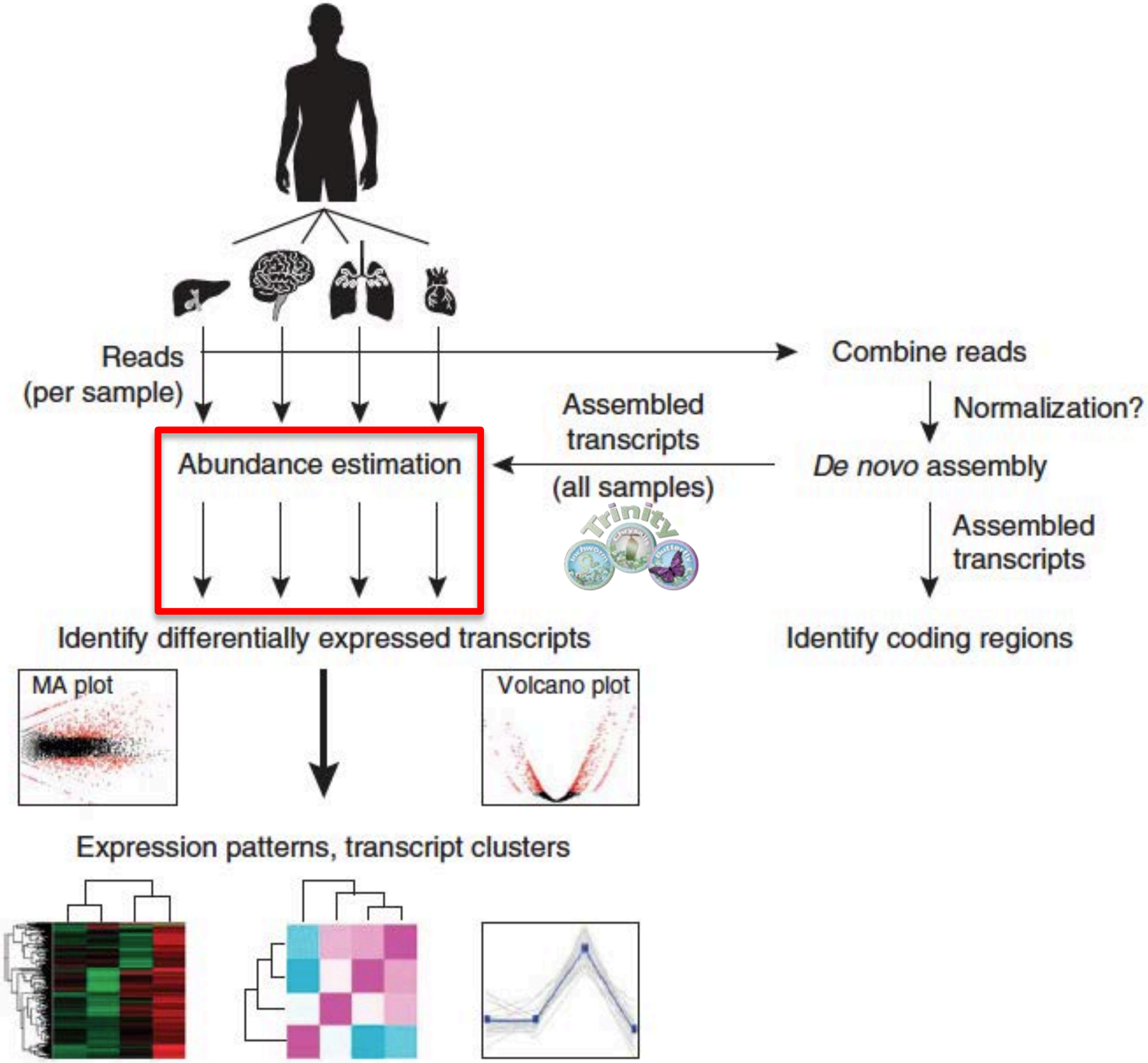
Li et al. **Evaluation of de novo transcriptome assemblies from RNA-Seq data**, Genome Biology 2014

Part 5. Expression Quantification



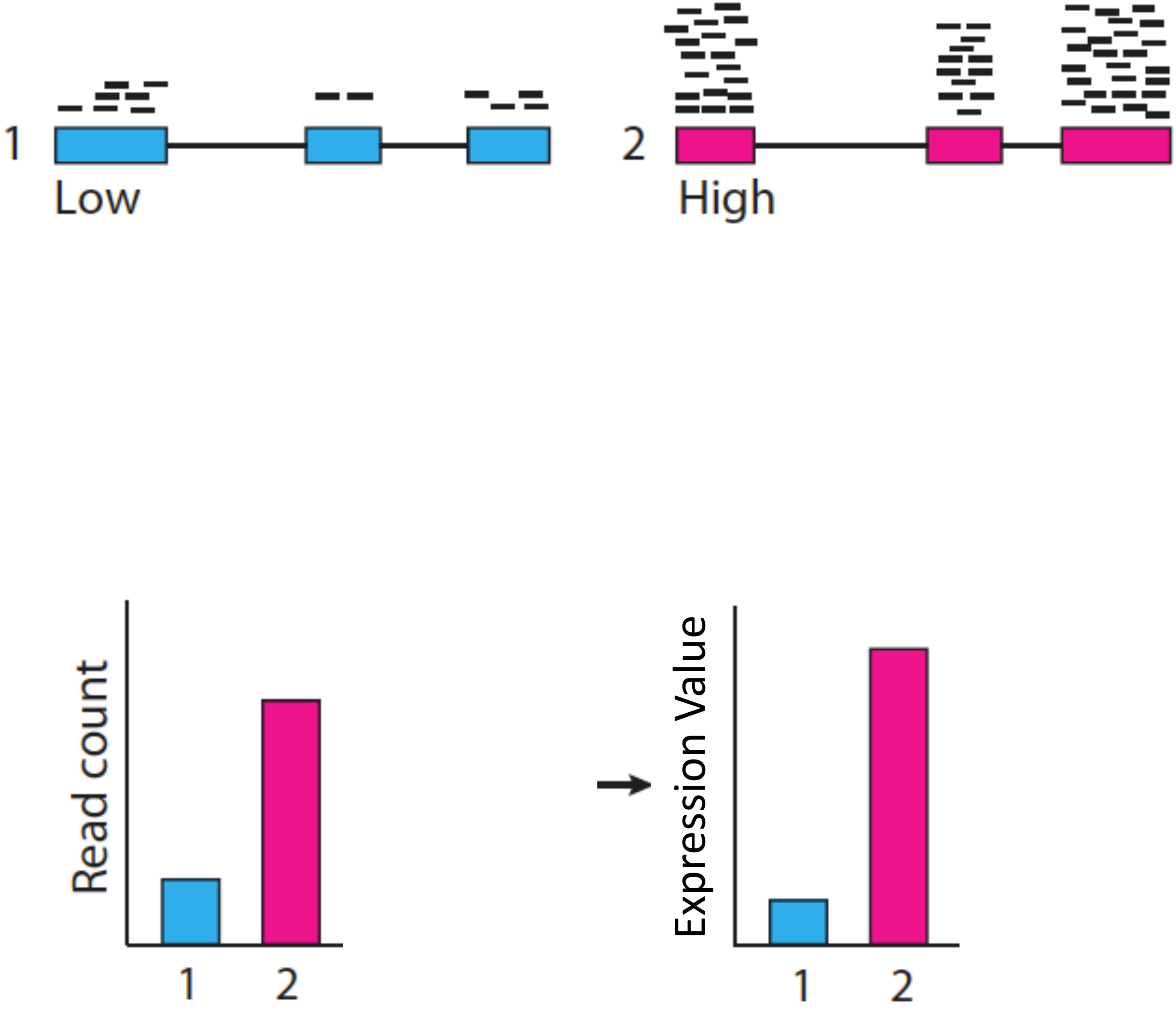
Abundance Estimation

(Aka. Computing Expression Values)



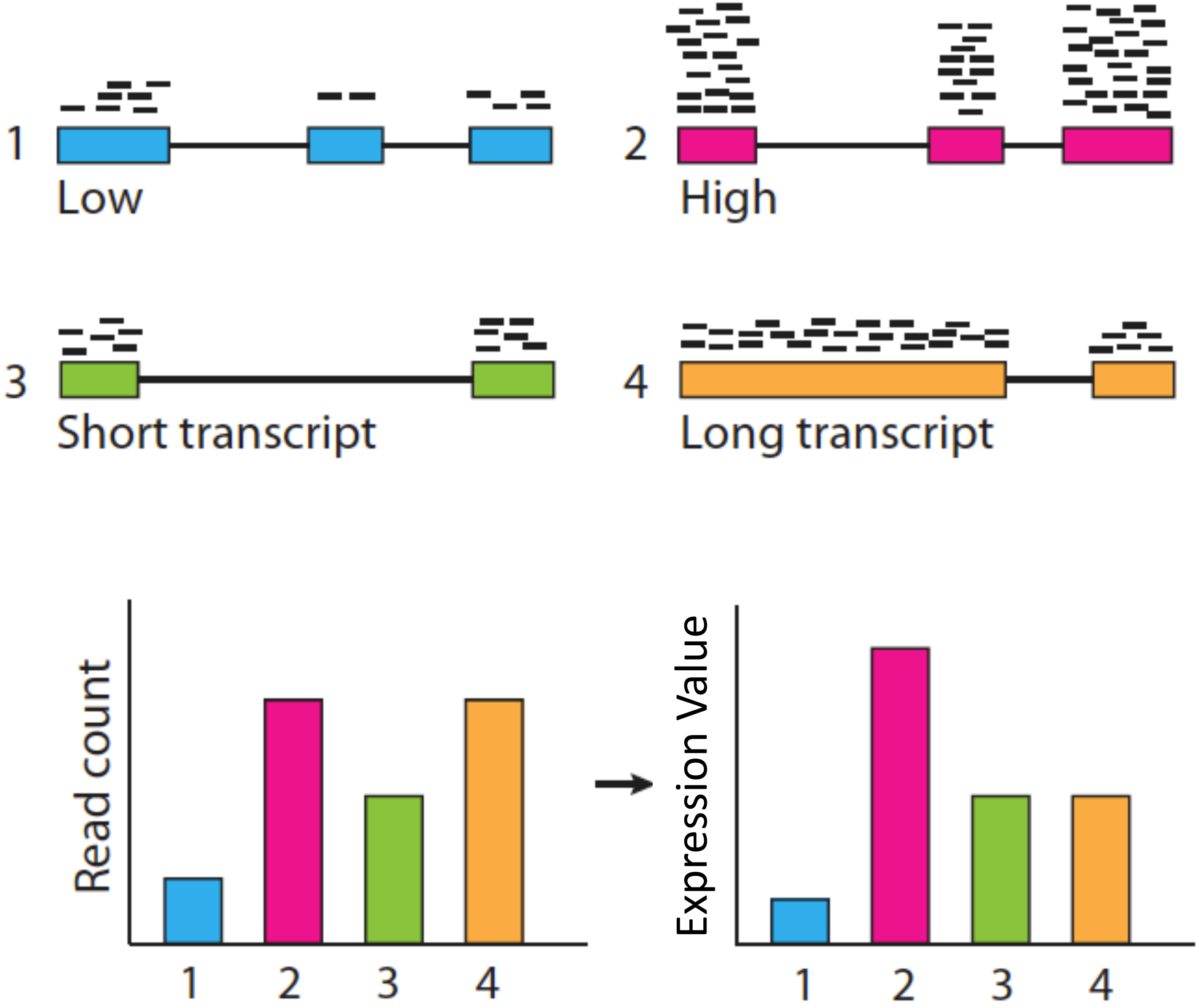
Bioconductor,
& Trinity

Calculating expression of genes and transcripts



Slide courtesy of Cole Trapnell

Calculating expression of genes and transcripts



Slide courtesy of Cole Trapnell

Normalized Expression Values

- Transcript-mapped read counts are normalized for both length of the transcript and total depth of sequencing.

- Reported as: Number of RNA-Seq **F**ragments

  Per **K**ilobase of transcript

  per total **M**illion fragments mapped

  FPKM

RPKM (reads per kb per M) used with Single-end RNA-Seq reads
FPKM used with Paired-end RNA-Seq reads.

Transcripts per Million (TPM)

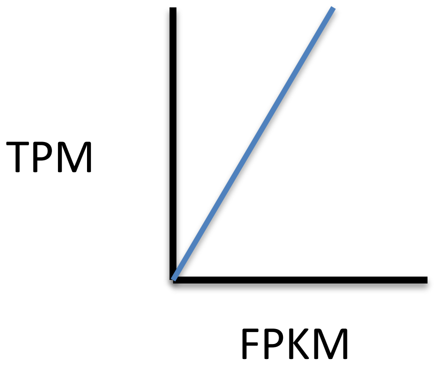
$$TPM_i = \frac{FPKM_i}{\sum_j FPKM} * 1e6$$

Preferred metric for measuring expression

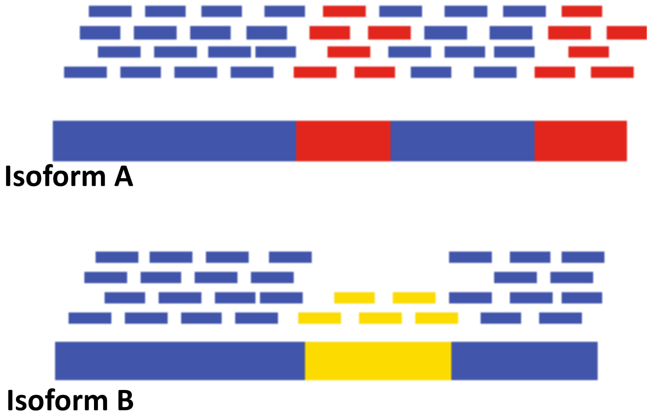
- Better reflects transcript concentration in the sample.
- Nicely sums to 1 million

Linear relationship between TPM and FPKM values.

Both are valid metrics, but best to be consistent.

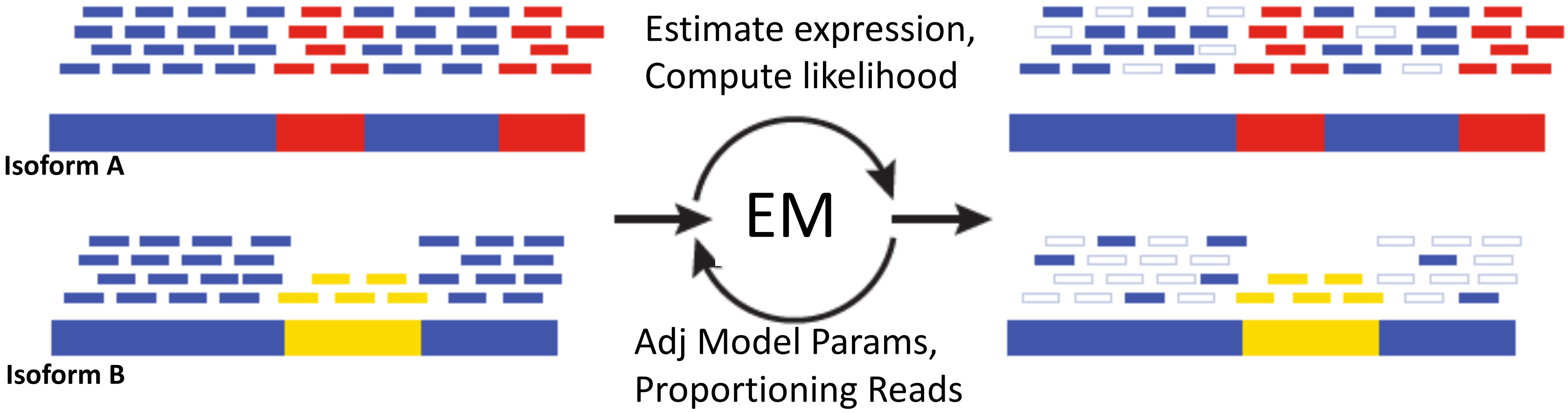


Multiply-mapped Reads Confound Abundance Estimation



Blue = multiply-mapped reads
Red, Yellow = uniquely-mapped reads

Multiply-mapped Reads Confound Abundance Estimation



Blue = multiply-mapped reads
Red, Yellow = uniquely-mapped reads

Use Expectation Maximization (EM) to find the most likely assignment of reads to transcripts.

Performed by:

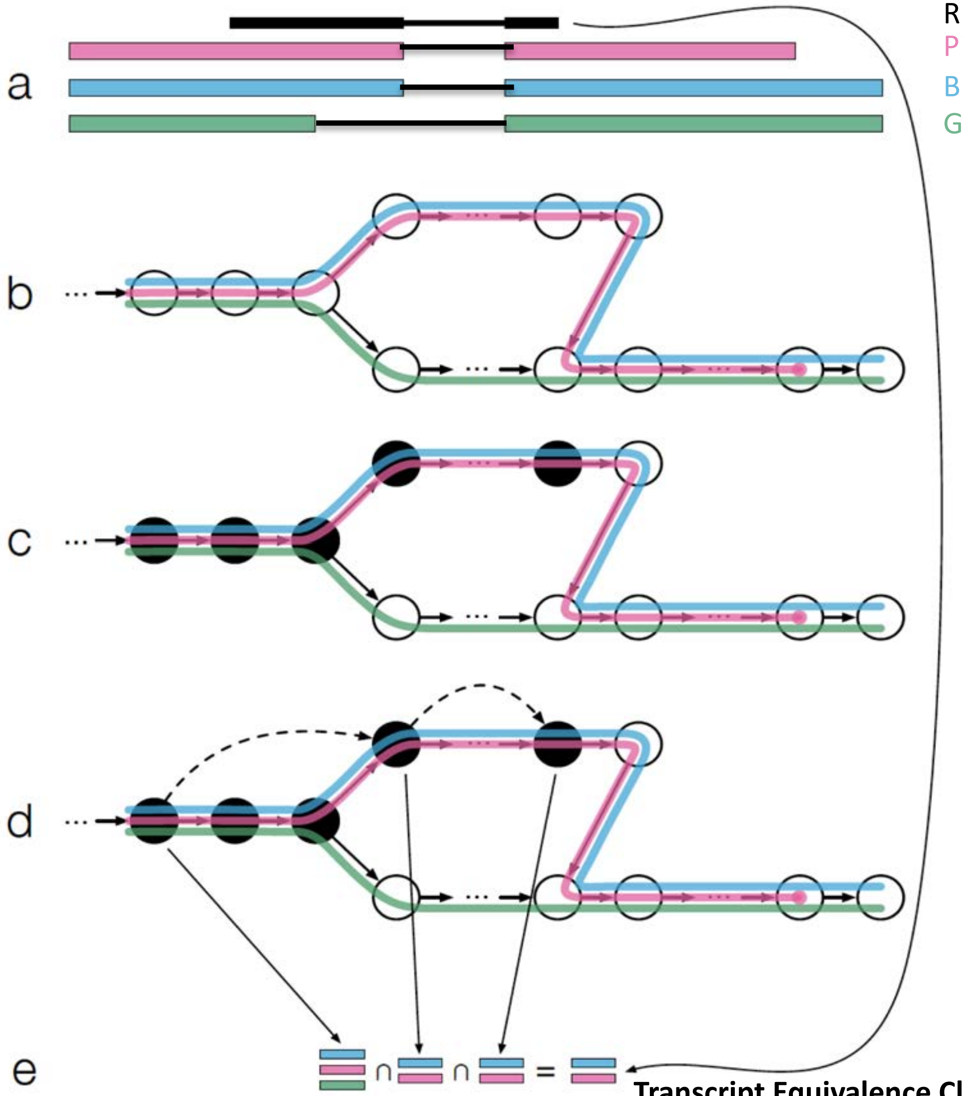
- Cufflinks, String Tie (Tuxedo)
- RSEM, eXpress (genome-free)
- Kallisto, Salmon (alignment-free)

Fast Abundance Estimation Using Pseudo-alignments and Equivalence Classes

(Kallisto software, Bray et al., NBT 2016)

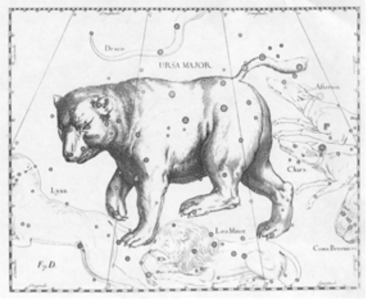
Alignment-Free!

De bruijn graph for isoforms, not reads



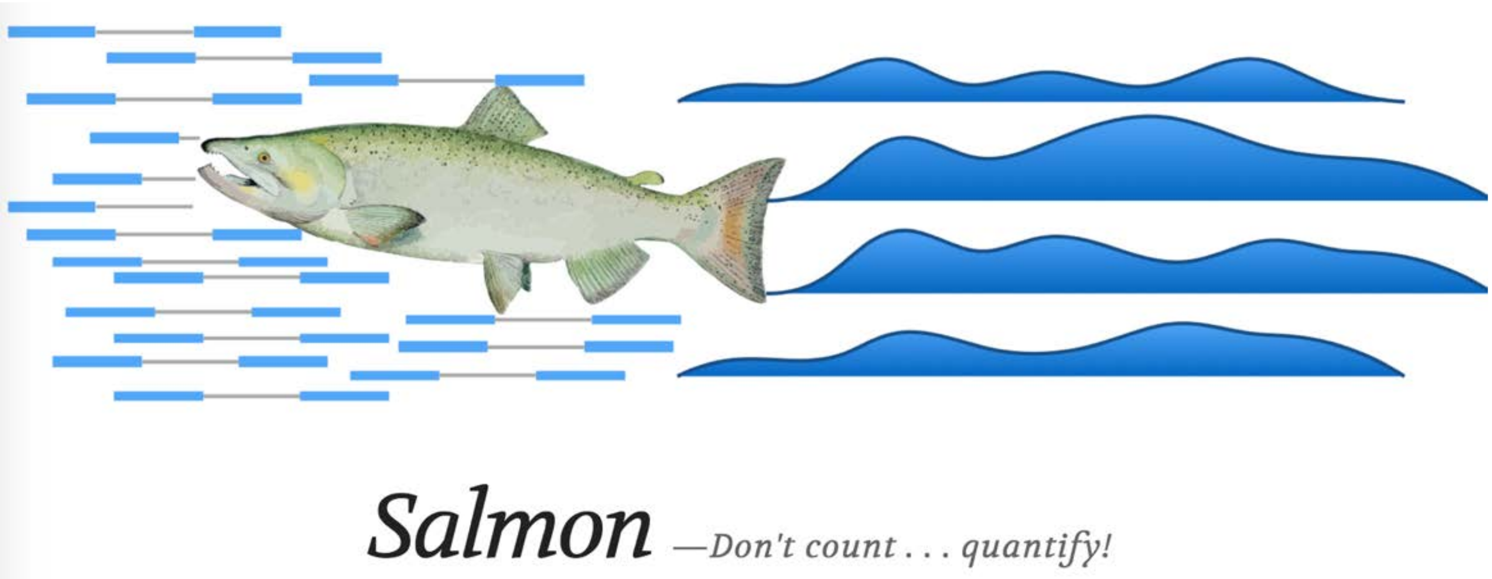
RNA-seq read
Pink_isoform
Blue_isoform
Green_isoform

Transcript Equivalence Class
aka Transcript Compatibility Class

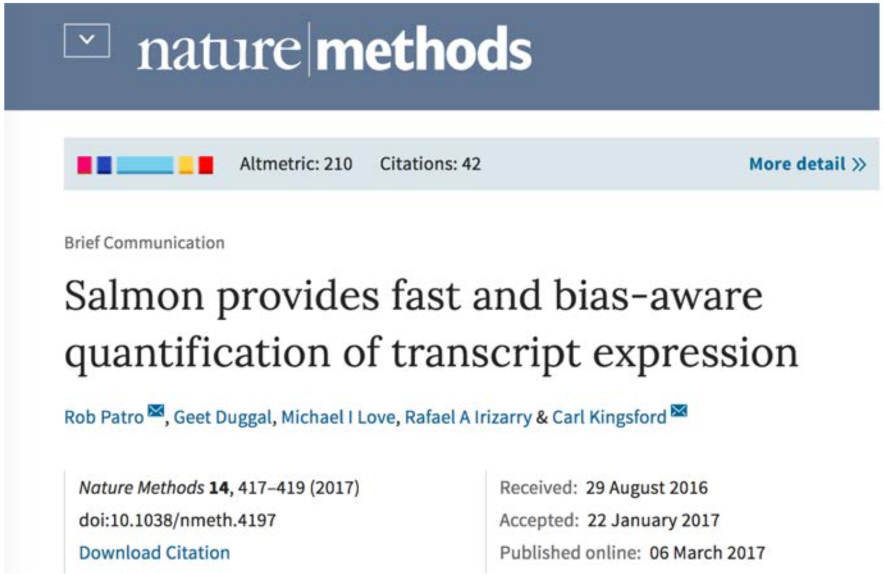


https://pachterlab.github.io/kallisto/

Adapted from Fig 1 from Bray et al.



Uses a suffix array
instead of the
de Bruijn graph



Altmetric: 210 Citations: 42 More detail »

Brief Communication

Salmon provides fast and bias-aware quantification of transcript expression

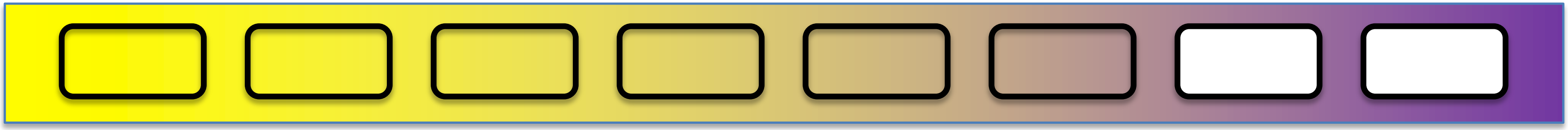
Rob Patro, Geet Duggal, Michael I Love, Rafael A Irizarry & Carl Kingsford

Nature Methods **14**, 417–419 (2017)
doi:10.1038/nmeth.4197
Download Citation

Received: 29 August 2016
Accepted: 22 January 2017
Published online: 06 March 2017

https://combine-lab.github.io/salmon/

Part 6. Differential Expression



Differential Expression Analysis

Thx, Charlotte Soneson! ☺

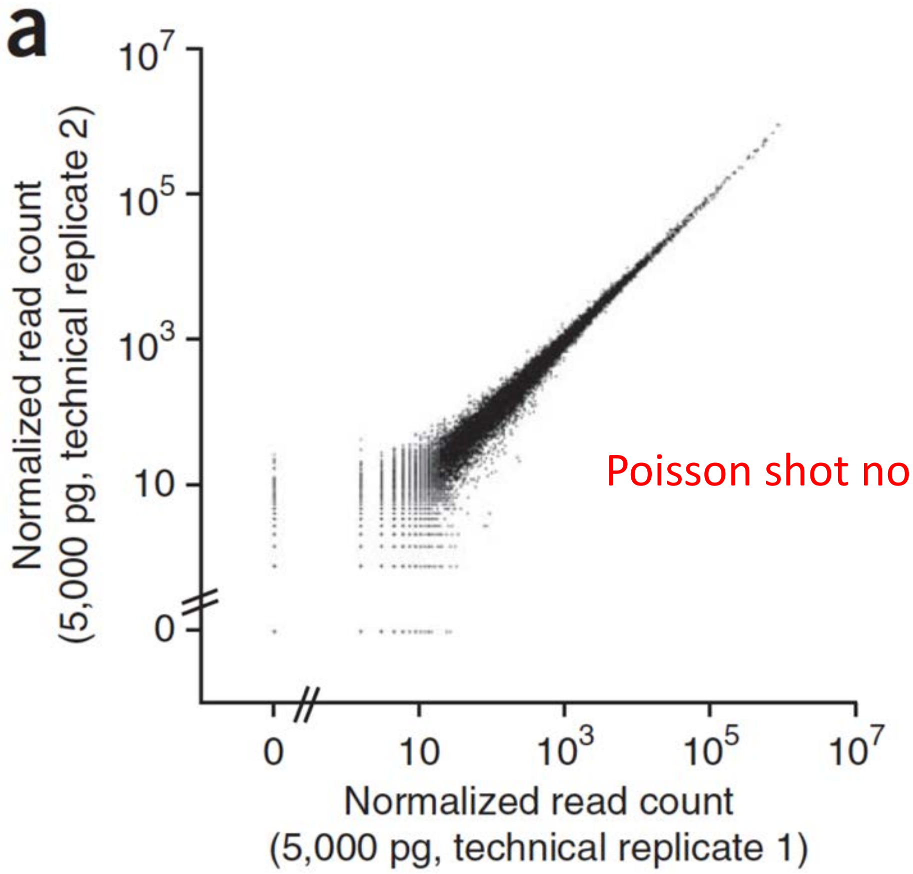
Differential Expression Analysis Involves

- Counting reads mapped to features
- Statistical significance testing

Beware of small counts leading to notable fold changes

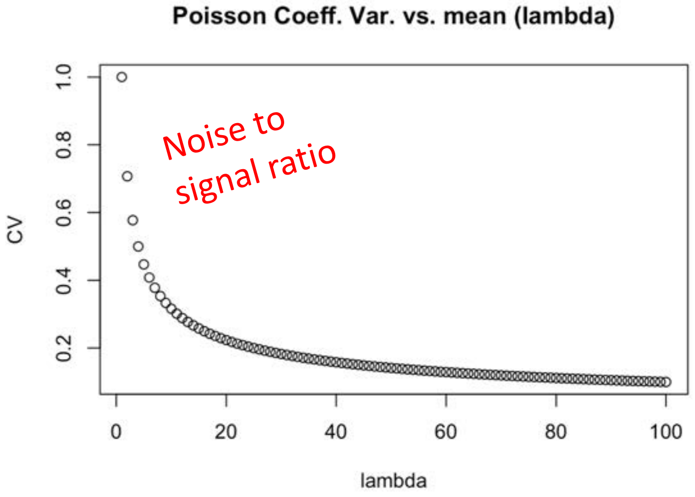
	Sample_A	**Sample_B**	**Fold_Change**	**Significant?**
Gene A	1	2	2-fold	No
Gene B	100	200	2-fold	Yes

Variation Observed Between Technical Replicates



Variation observed is well described by models of random sampling (Poisson Distribution)

Poisson shot noise is high for small counts.

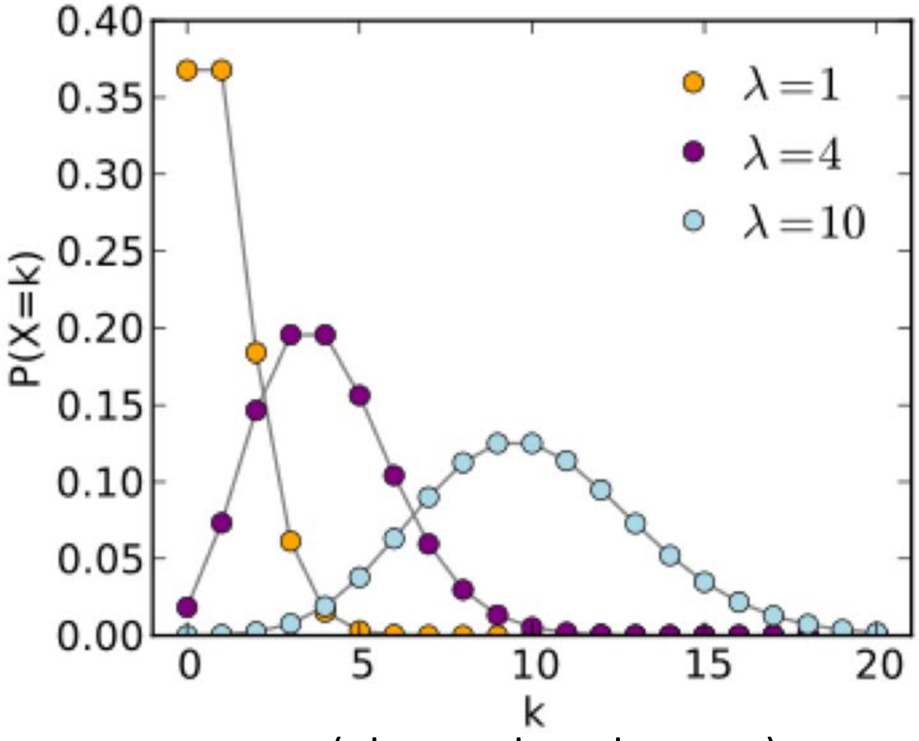


Noise to signal ratio

* plot from Brennecke, et al. Nature Methods, 2013

Observed RNA-Seq Counts Result from Random Sampling of the Population of Reads

Technical variation in RNA-Seq counts per feature is well modeled by the Poisson distribution



Mean # fragments

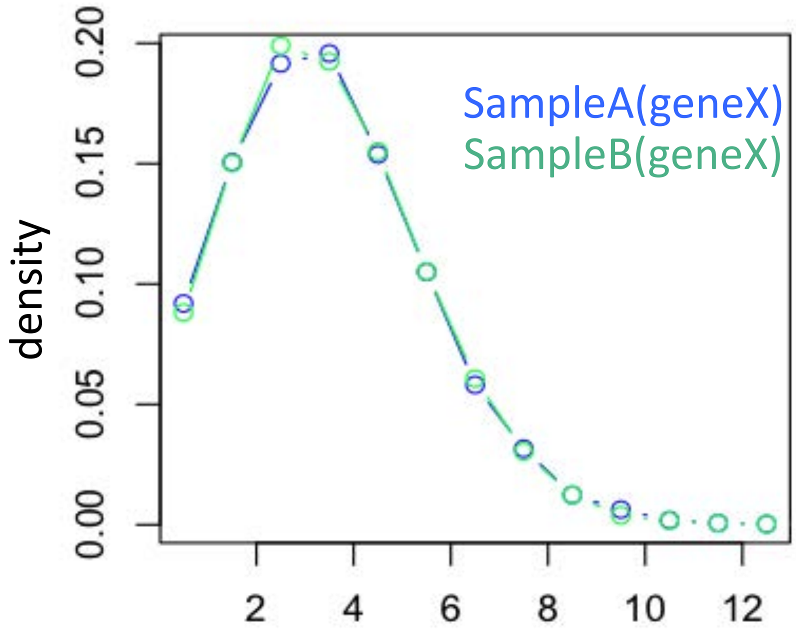
(observed read counts)

See: http://en.wikipedia.org/wiki/Poisson_distribution

Example: One gene*not* differentially expressed

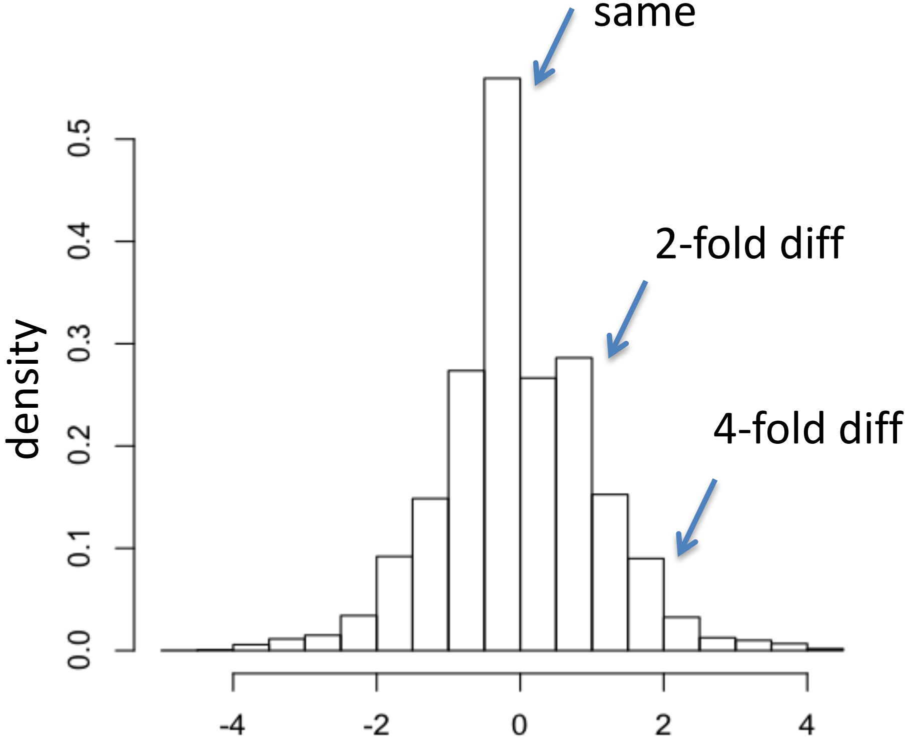
Example: SampleA(gene) = SampleB(gene) = 4 reads

Distribution of observed counts for single gene (under Poisson model)



(k) number of reads observed

Dist. of $\log_2$(fold change) values

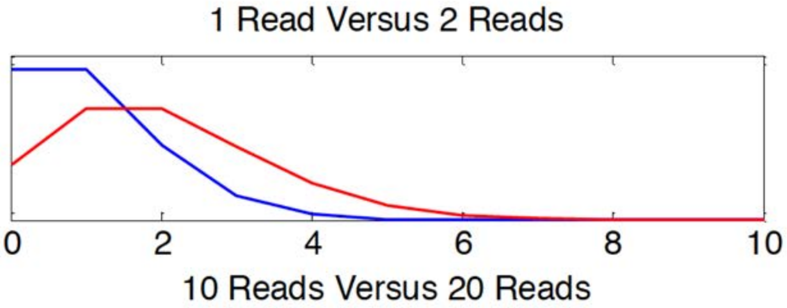


x = $\log_2$(SampleA/SampleB)

Sequencing Depth Matters

Poisson distributions for counts based on **2-fold** expression differences

P(x=k)



No confidence in 2-fold difference. Likely observed by chance.

From: http://gkno2.tumblr.com/post/24629975632/thinking-about-rna-seq-experimental-design-for
and from supplementary text of Busby et al., Bioinformatics, 2013

Sequencing Depth Matters

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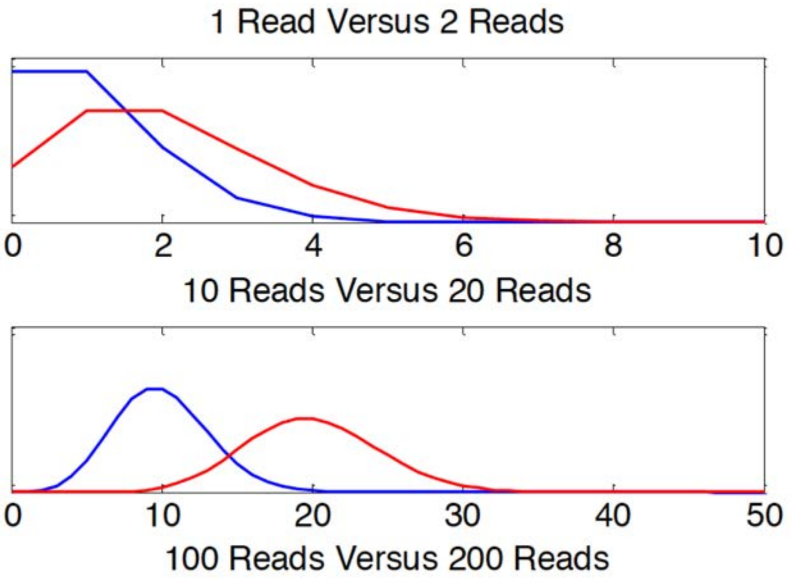
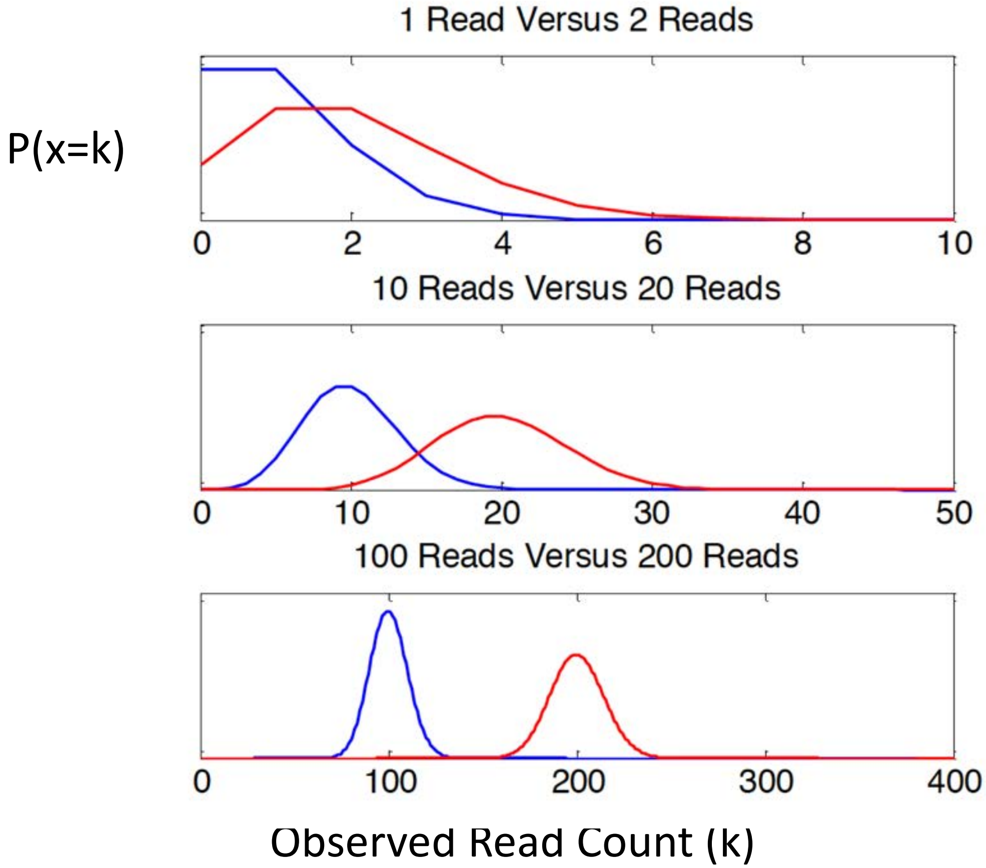


No confidence in 2-fold difference. Likely observed by chance.

From: http://gkno2.tumblr.com/post/24629975632/thinking-about-rna-seq-experimental-design-for
and from supplementary text of Busby et al., Bioinformatics, 2013

Sequencing Depth Matters

Poisson distributions for counts based on **2-fold** expression differences



No confidence in 2-fold difference. Likely observed by chance.

High confidence in 2-fold difference. Unlikely observed by chance.

From: http://gkno2.tumblr.com/post/24629975632/thinking-about-rna-seq-experimental-design-for
and from supplementary text of Busby et al., Bioinformatics, 2013

Greater Depth = More Statistical Power

Example: Single gene, reads sampled at different sequencing depths

Reads per sample	Sample A Number of reads	Sample B Number of reads	P-value (Fishers Exact Test)
100,000	1	2	1
1,000,000	10	20	0.099
10,000,000	100	200	**8.0e-09**

Technical vs. Biological Replicates

RNA-Seq Technical replicates aren’t essential

(Technical variation is well-modeled by the Poisson distribution)

“We find that the Illumina sequencing data are highly replicable, with relatively little technical variation, and thus, for many purposes, it may suffice **to sequence each mRNA sample only once**” *Marioni et al., Genome Research, 2008*

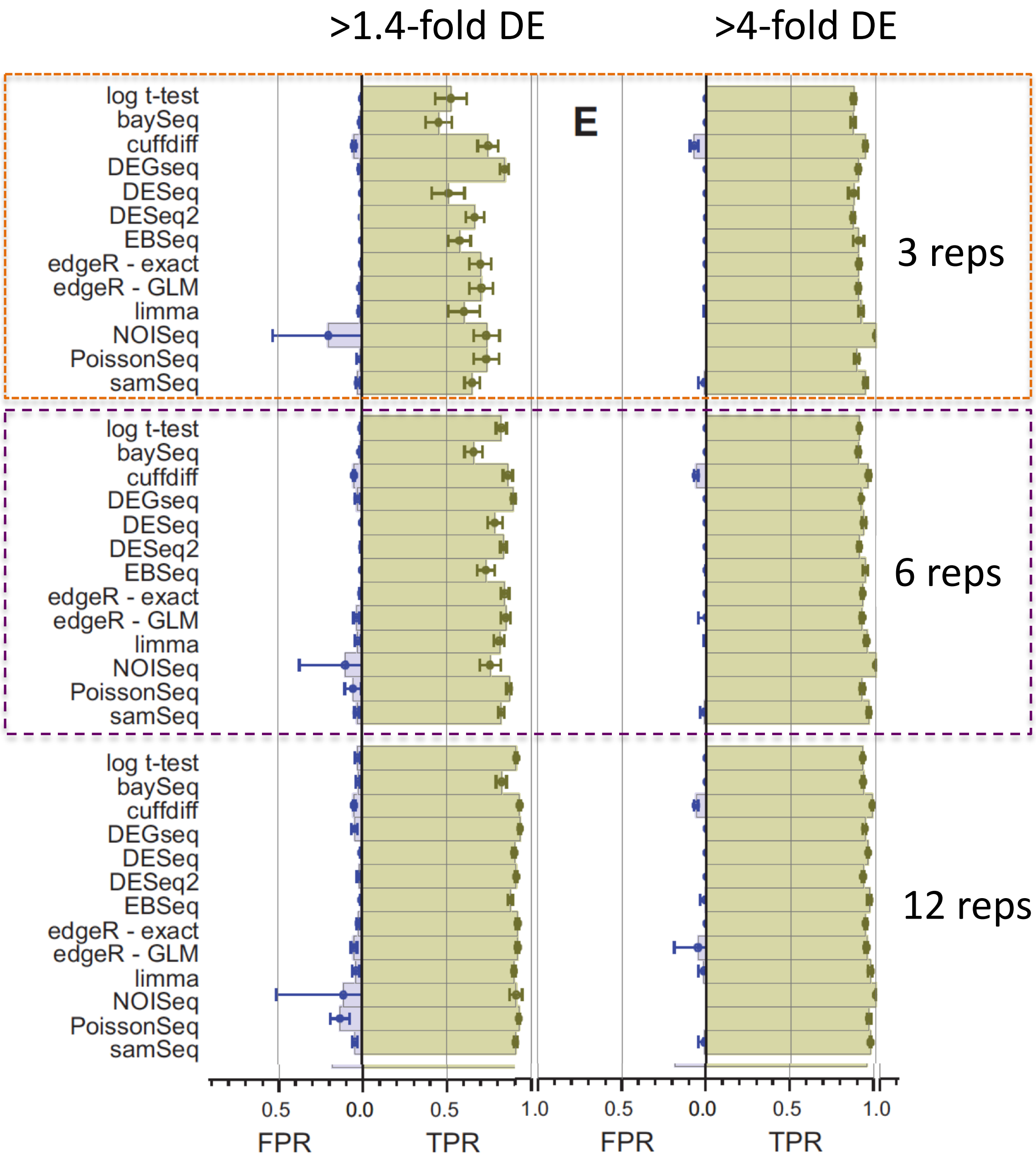
However, biological replicates *ARE* essential

total_variance = technical_variance + biological_variance

(Total variance well-modeled by negative binomial distribution)

“… **at least six biological replicates should be used**, rising to at least 12 when it is important to identify SDE genes for all fold changes.” *Schurch et al., RNA, 2016*

DE Accuracy Improves with Higher Biological Replication



At a minimum, do 3 bio replicates

Respectable goal

*Figure taken and adapted from Schurch et al., RNA, 2016

Tools for DE analysis with RNA-Seq

Bioconductor

OPEN SOURCE SOFTWARE FOR BIOINFORMATICS

edgeR
ShrinkSeq
DESeq
baySeq
Vsf
Limma/Voom
mmdiff
cuffdiff

ROTS
TSPM
DESeq2
EBSeq
NBPSeq
SAMseq
NoiSeq
Sleuth

(italicized not in R/Bioconductor but stand-alone)

See: http://www.biomedcentral.com/1471-2105/14/91
A comparison of methods for differential expression analysis of RNA-seq data
Soneson & Delorenzi, 2013

Typical output from DE analysis

```
                          logFC                 logCPM               PValue                FDR
TRINITY_DN876_c0_g1_i1    -7.15049572793027     10.6197708379285     0                     0
TRINITY_DN6470_c0_g1_i1   -7.26777912190146     7.03987604865422     1.687485656951e-287   6.46813252309319e-284
TRINITY_DN5186_c0_g1_i1   -7.85623682454322     9.18570464327063     1.17049180235068e-278 2.99099671894011e-275
TRINITY_DN768_c0_g1_i1    7.72884741150304      9.7514619195169      4.32504881419265e-272 8.28895605240022e-269
TRINITY_DN70_c0_g1_i1     -12.7646078189688     7.86482982471445     3.92853491279431e-253 6.02322972829624e-250
TRINITY_DN1587_c0_g1_i1   -5.89392061881667     9.07366563894607     6.32919557933429e-243 8.08660221852944e-240
TRINITY_DN3236_c0_g1_i1   -7.27029815068473     8.02209568234202     3.64955175271959e-235 3.99678053376405e-232
TRINITY_DN4631_c0_g1_i1   -7.45310693639574     6.91664918183241     4.30540921272851e-229 4.1256583780971e-226
TRINITY_DN5082_c0_g5_i1   -5.33154406167545     10.6977538760467     2.74243356676259e-225 2.33594396920022e-222
TRINITY_DN1789_c0_g3_i1   10.2032564835076      7.32607652700285     1.44273728647186e-213 1.10600240380933e-210
TRINITY_DN4204_c0_g1_i1   4.81030233739325      9.88844409410644     9.27180216086162e-205 6.46160321501501e-202
TRINITY_DN799_c0_g1_i1    -4.22044475626154     6.9937398638711      1.24746518421083e-197 7.96922341846683e-195
TRINITY_DN196_c0_g2_i1    4.60597918494257      9.86878463857276     1.9819997623131e-192  1.16877001368402e-189
TRINITY_DN5041_c0_g1_i1   -4.27126549355785     9.70894399883        1.8930437900069e-185  1.03657669244235e-182
TRINITY_DN1619_c0_g1_i1   -4.47156415953777     9.22535948721718     1.76766063029526e-181 9.03392426122899e-179
TRINITY_DN899_c0_g1_i1    -4.90914328409143     7.93768691394594     1.11054513767547e-180 5.32089939088761e-178
TRINITY_DN324_c0_g2_i1    4.87160837667488      6.84850312231775     2.20092562166991e-179 9.92487989160089e-177
TRINITY_DN3241_c0_g1_i1   -4.77760618069256     7.94111259715689     1.60585457735621e-173 6.83915621667372e-171
TRINITY_DN4379_c0_g1_i1   3.85133572453294      7.23712813663389     3.48140532848425e-164 1.4046554341137e-161
TRINITY_DN1919_c0_g1_i1   4.05998814332136      6.95937301668582     1.8588621194715e-161  7.12501850393425e-159
TRINITY_DN2504_c0_g1_i1   -6.92417817059644     6.20370039359785     2.42022459856956e-160 8.83497227268296e-158
…
```

Up vs. Down regulated

Avg. expression level

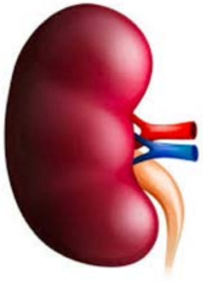
Significance

-- Before Comparing RNA-Seq Samples --

Some Cross-sample Normalization May Be Required

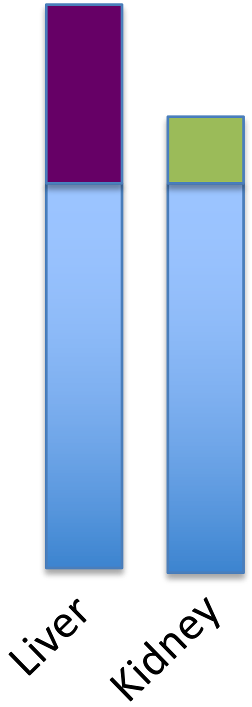
eg.

Vs.

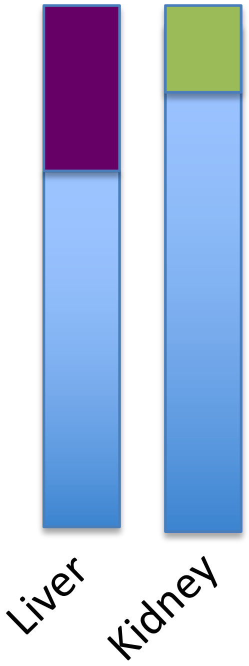


Why cross-sample normalization is important

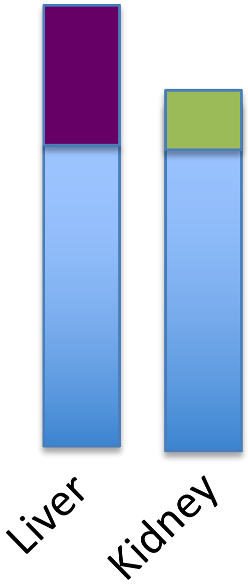
Absolute RNA quantities per cell



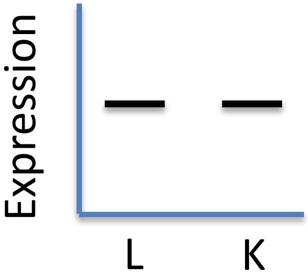
Measured relative abundance via RNA-Seq

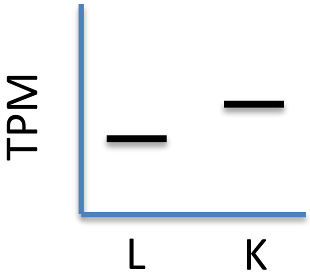


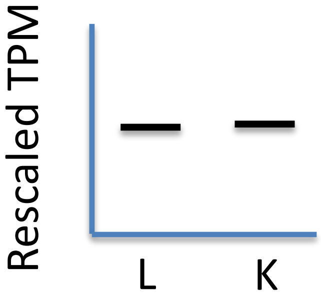
Cross-sample normalized (rescaled) relative abundance



eg. Some housekeeping gene's expression level:

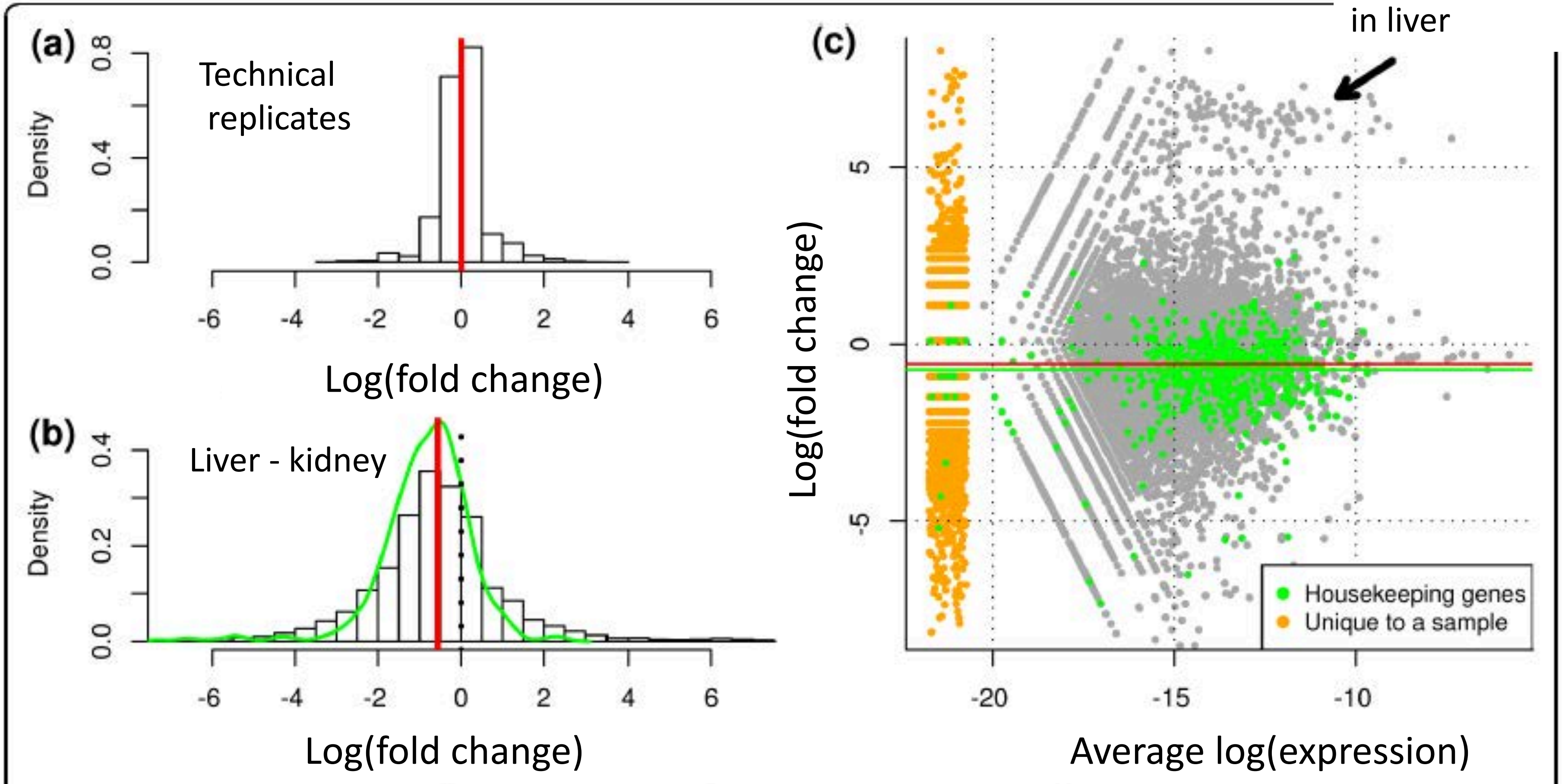






Cross-sample Normalization Required
Otherwise, housekeeping genes look diff expressed due to sample composition differences

Subset of genes highly expressed in liver



Technical replicates

Log(fold change)

Liver - kidney

Log(fold change)

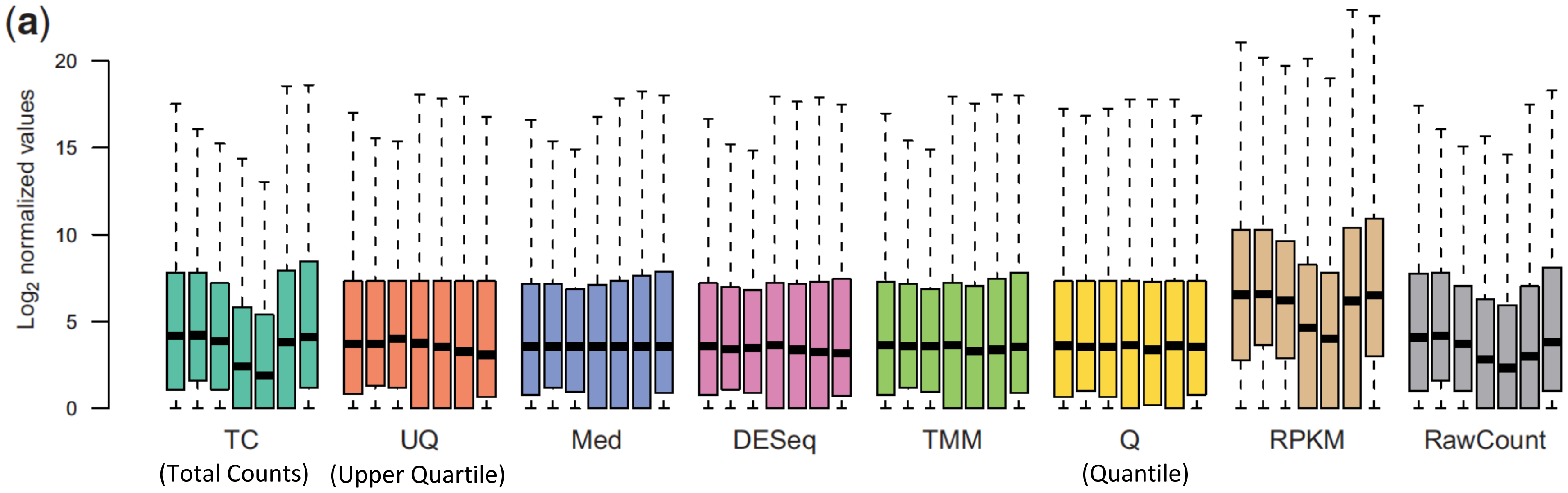
Log(fold change)

Average log(expression)

Figure 1 Normalization is required for RNA-seq data. Data from [6] comparing log ratios of **(a)** technical replicates and **(b)** liver versus kidney expression levels, after adjusting for the total number of reads in each sample. The green line shows the smoothed distribution of log-fold-changes of the housekeeping genes. **(c)** An M versus A plot comparing liver and kidney shows a clear offset from zero. Green points indicate 545 housekeeping genes, while the green line signifies the median log-ratio of the housekeeping genes. The red line shows the estimated TMM normalization factor. The smear of orange points highlights the genes that were observed in only one of the liver or kidney the overall bias in log-fold-changes.

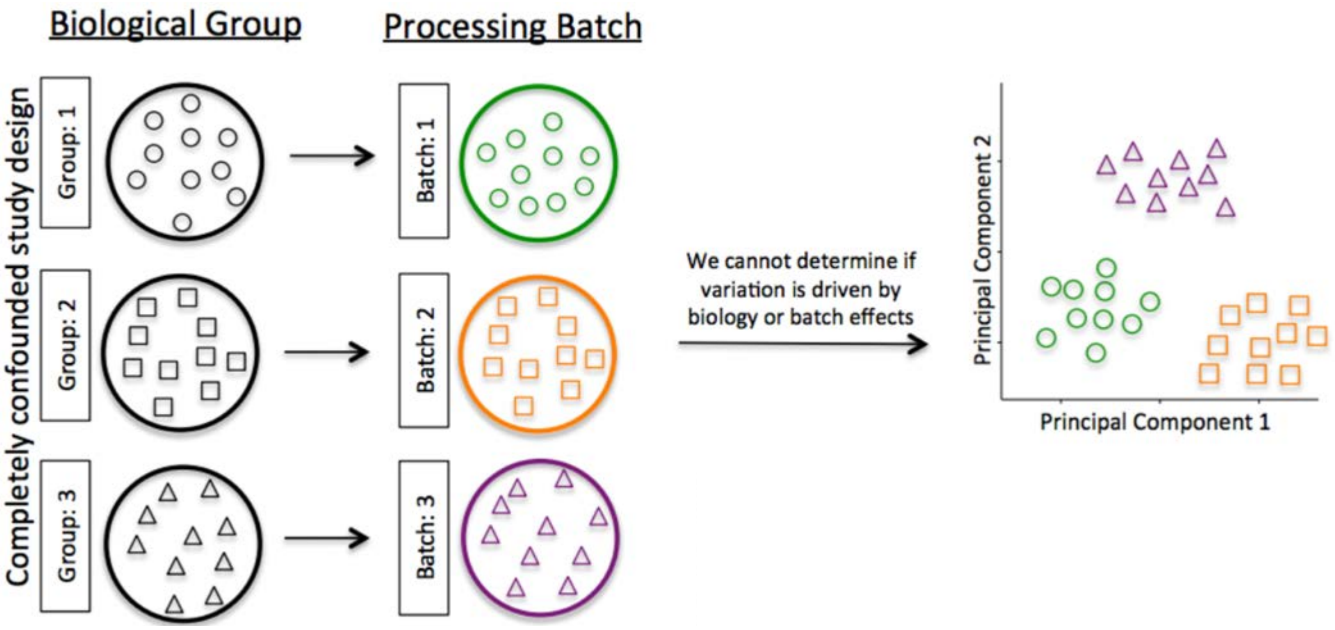
Adapted from: Robinson and Oshlack, Genome Biology, 2010

Normalization methods for Illumina high-throughput RNA sequencing data analysis.



From “A comprehensive evaluation of normalization methods for Illumina high throughput RNA sequencing data analysis” Brief Bioinform. 2013 Nov;14(6):671-83
http://www.ncbi.nlm.nih.gov/pubmed/22988256

Avoid Batch Effects



We cannot determine if variation is driven by biology or batch effects

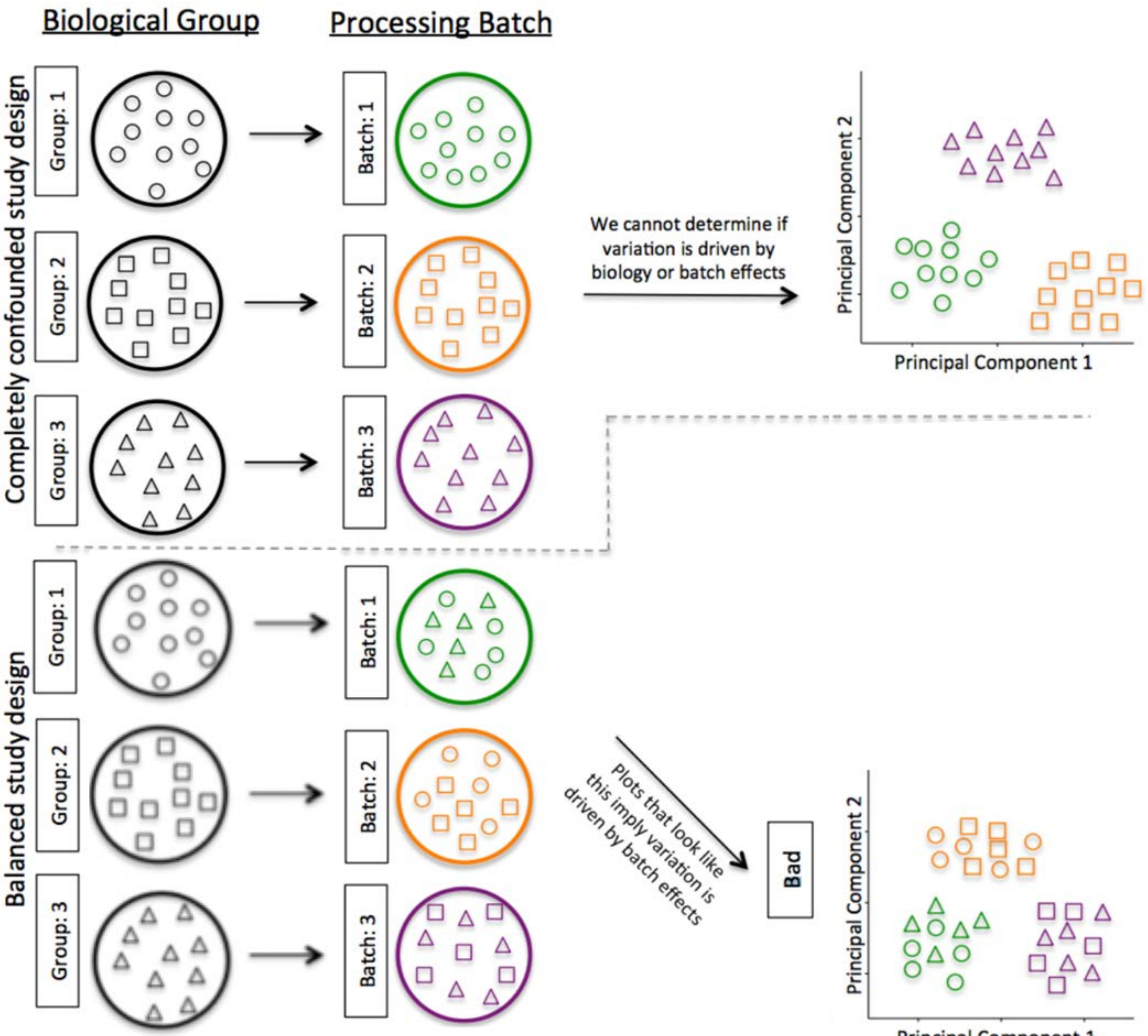
Grouping by Study or Batch?

Batch variable types:

- Times and dates
- Technician processing the samples
- Sequencing machine, or flow cell lane (Illumina)

Adapted from: Stephanie C. Hicks, Mingxiang Teng, Rafael A. Irizarry.
https://www.biorxiv.org/content/early/2015/09/04/025528
On the widespread and critical impact of systematic bias and batch effects in single-cell RNA-Seq data.

Avoid Batch Effects



Grouping by Study or Batch?

Grouping by Batch

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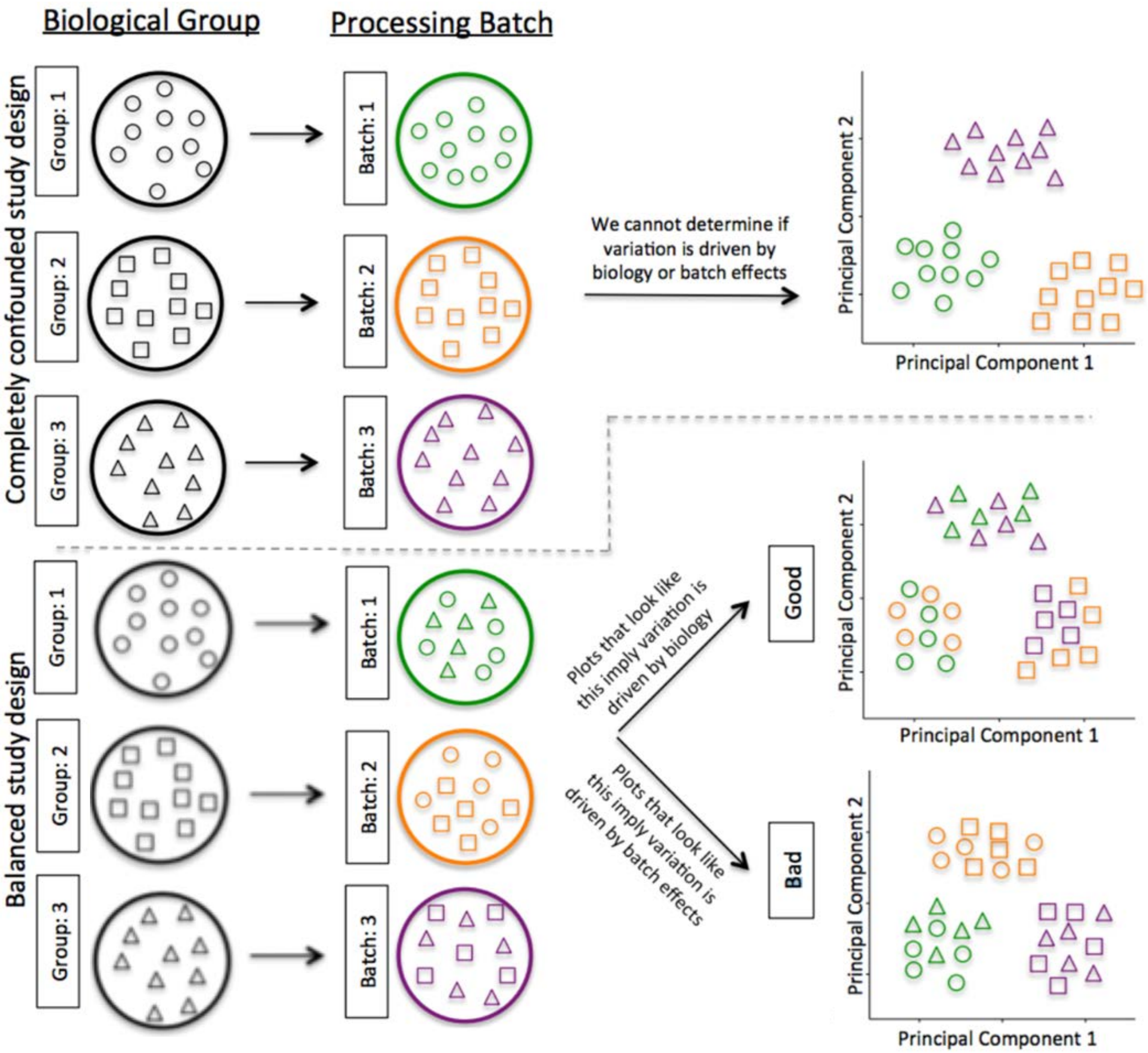
https://www.biorxiv.org/content/early/2015/09/04/025528
On the widespread and critical impact of systematic bias and batch effects in single-cell RNA-Seq data.

(Explore Batch Removal Techniques)

Avoid Batch Effects



Grouping by Study or Batch?

Grouping by Study

Grouping by Batch

(Explore Batch Removal Techniques)

Adapted from: Stephanie C. Hicks, Mingxiang Teng, Rafael A. Irizarry.
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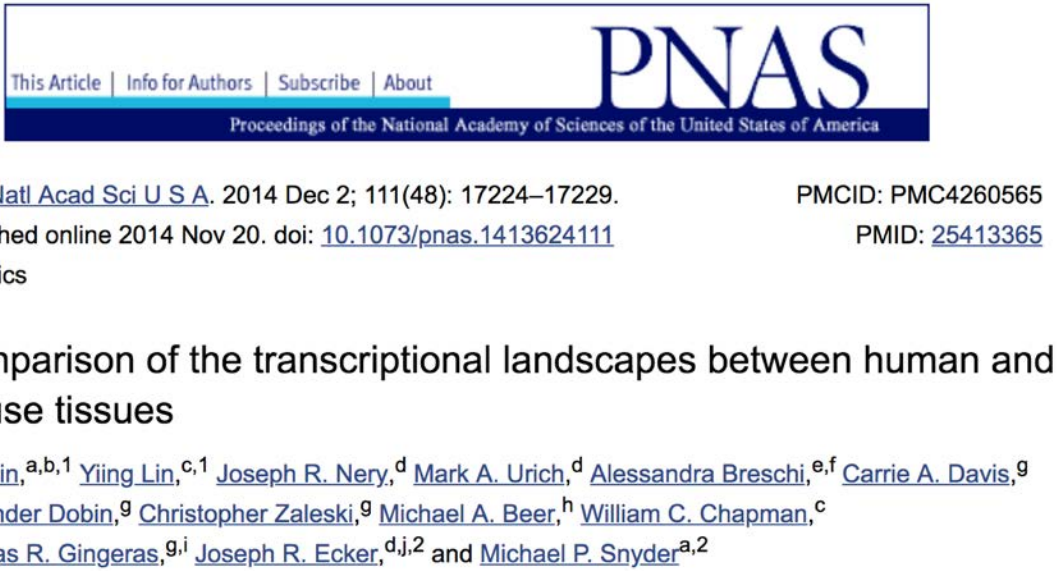
On the widespread and critical impact of systematic bias and batch effects in single-cell RNA-Seq data.

Mouse and human tissue expression more similar within than between species. *?!?!?*



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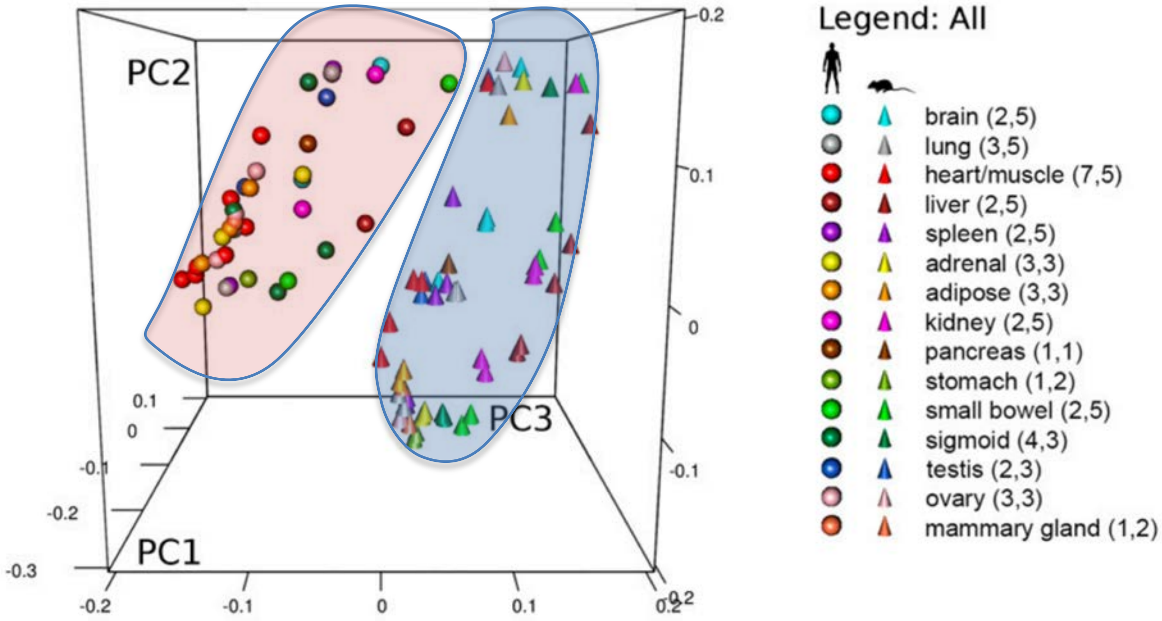
Proceedings of the National Academy of Sciences of the United States of America

Proc Natl Acad Sci U S A. 2014 Dec 2; 111(48): 17224–17229.
Published online 2014 Nov 20. doi: 10.1073/pnas.1413624111
Genetics

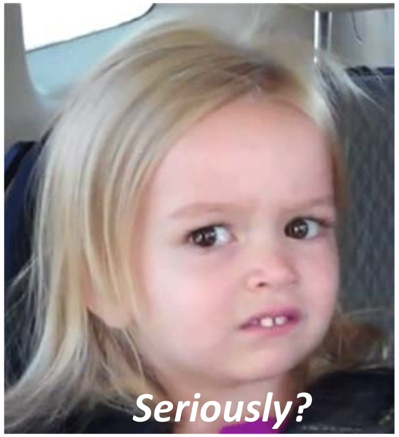
PMCID: PMC4260565
PMID: 25413365

Comparison of the transcriptional landscapes between human and mouse tissues

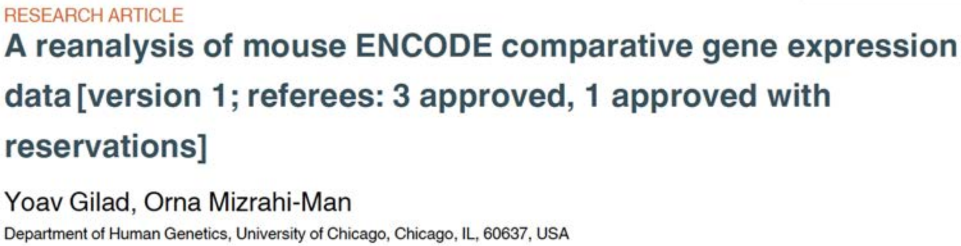
Shin Lin,[a,b,1] Yiing Lin,[c,1] Joseph R. Nery,[d] Mark A. Urich,[d] Alessandra Breschi,[e,f] Carrie A. Davis,[g] Alexander Dobin,[g] Christopher Zaleski,[g] Michael A. Beer,[h] William C. Chapman,[c] Thomas R. Gingeras,[g,i] Joseph R. Ecker,[d,j,2] and Michael P. Snyder[a,2]



"… our results indicate that for the human–mouse comparison, tissues appear more similar to one another within the same species than to the comparable organs of other species …"



~6 months later



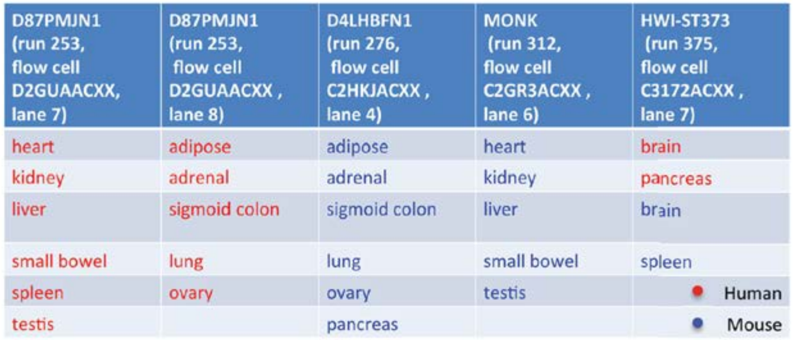
RESEARCH ARTICLE

A reanalysis of mouse ENCODE comparative gene expression data [version 1; referees: 3 approved, 1 approved with reservations]

Yoav Gilad, Orna Mizrahi-Man

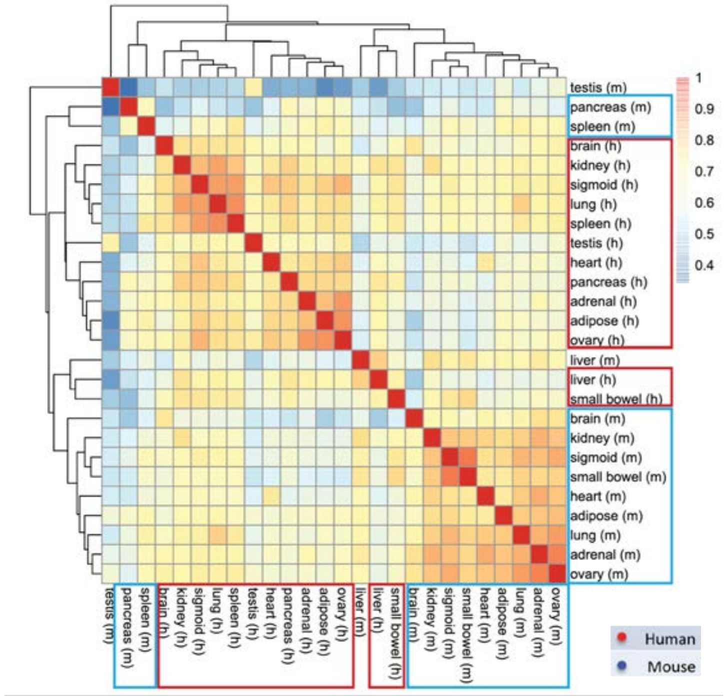
Department of Human Genetics, University of Chicago, Chicago, IL, 60637, USA

Grouping of samples by Sequencing Batch

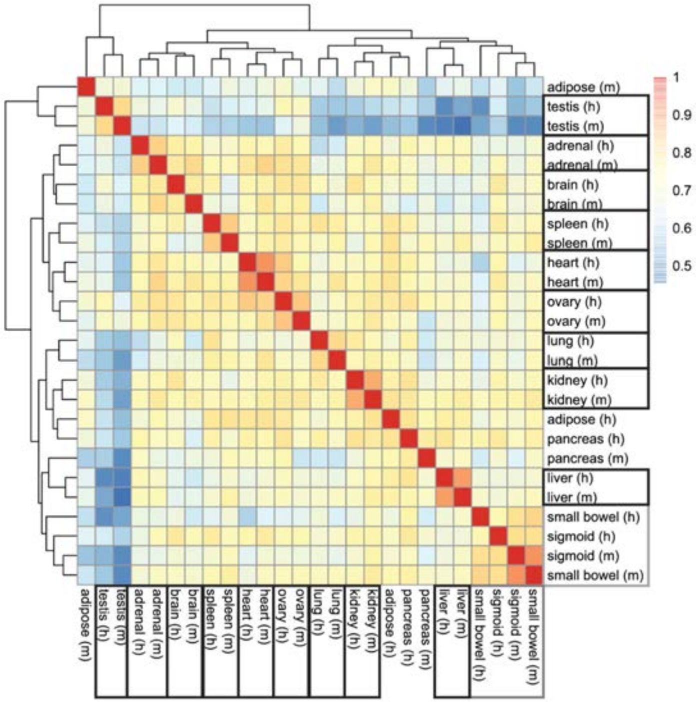


D87PMJN1 (run 253, flow cell D2GUAACXX, lane 7)	D87PMJN1 (run 253, flow cell D2GUAACXX , lane 8)	D4LHBFN1 (run 276, flow cell C2HKJACXX , lane 4)	MONK (run 312, flow cell C2GR3ACXX , lane 6)	HWI-ST373 (run 375, flow cell C3172ACXX , lane 7)
heart	adipose	adipose	heart	brain
kidney	adrenal	adrenal	kidney	pancreas
liver	sigmoid colon	sigmoid colon	liver	brain
small bowel	lung	lung	small bowel	spleen
spleen	ovary	ovary	testis	● Human
testis		pancreas		● Mouse

Yes, tissue expression patterns within species more similar than between species, but doesn't make sense and maybe due to a batch effect?



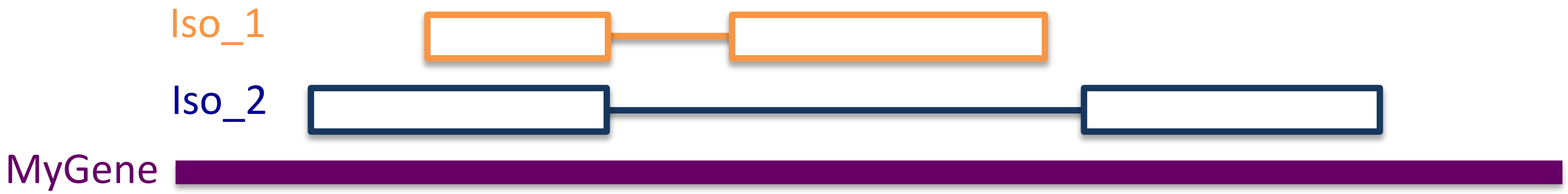
Post Batch Correction:
Tissue patterns more similar than by species



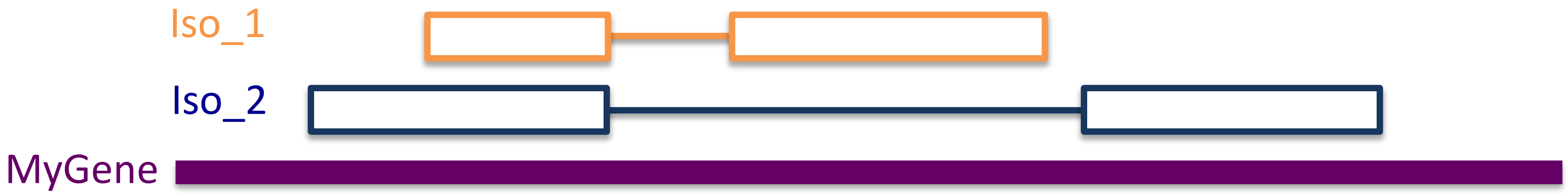
Flavors of Differential Expression Analyses

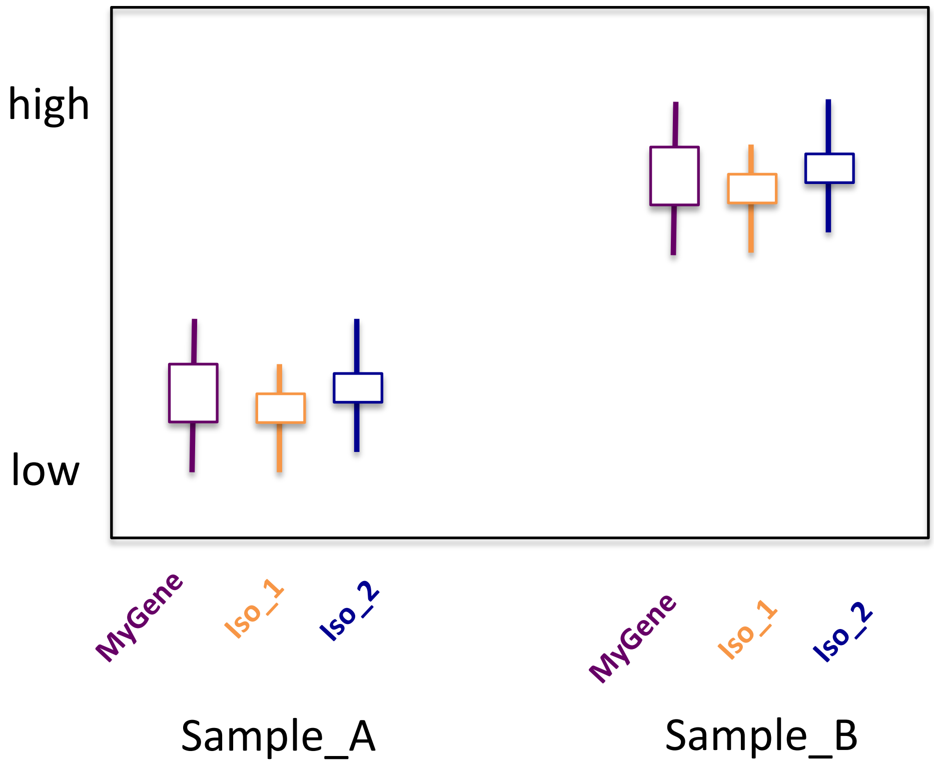
- Transcripts:
  - Differential Transcript Expression (DTE)
  - Differential Transcript Usage (DTU)
  - Differential Exon Usage (DEU)
- Gene:
  - Differential Gene Expression (DGE) ?
  - Gene Differential Expression (GDE)

Differential Gene Expression (DGE) and Differential Transcript Expression (DTE) (Example 1)



Differential Gene Expression (DGE) and Differential Transcript Expression (DTE) (Example 1)

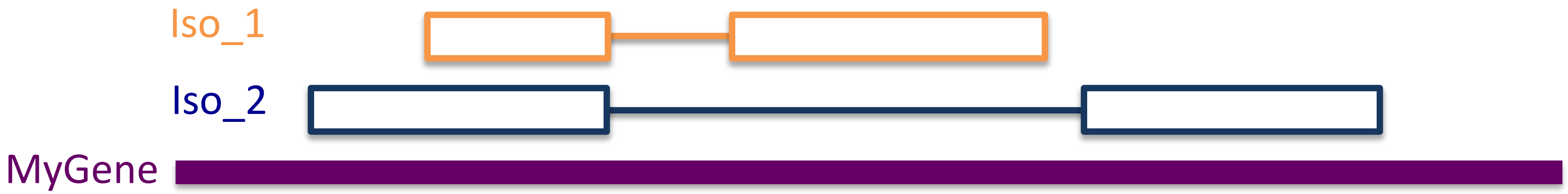


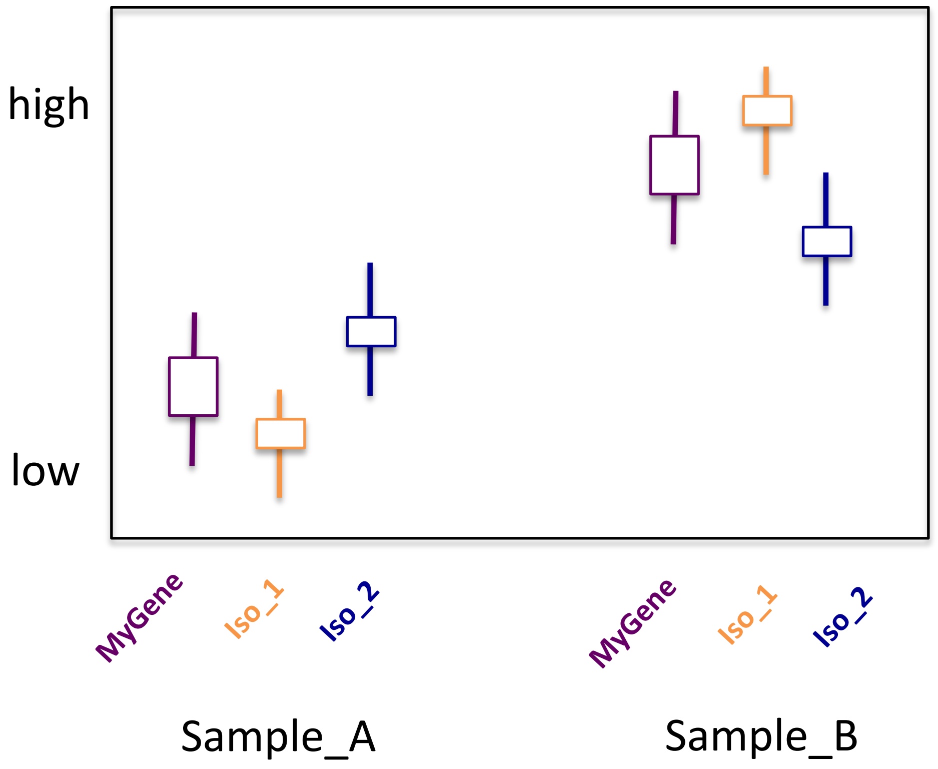


Feature	Diff Expressed?
MyGene	Yes
Iso_1	Yes
Iso_2	Yes

Diff. Transcript Usage ?
(eg. Isoform switching) No

Differential Gene Expression (DGE) and Differential Transcript Expression (DTE) (Example 2)

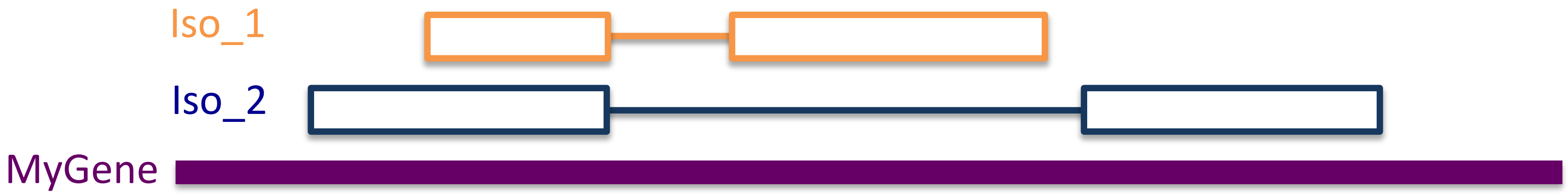


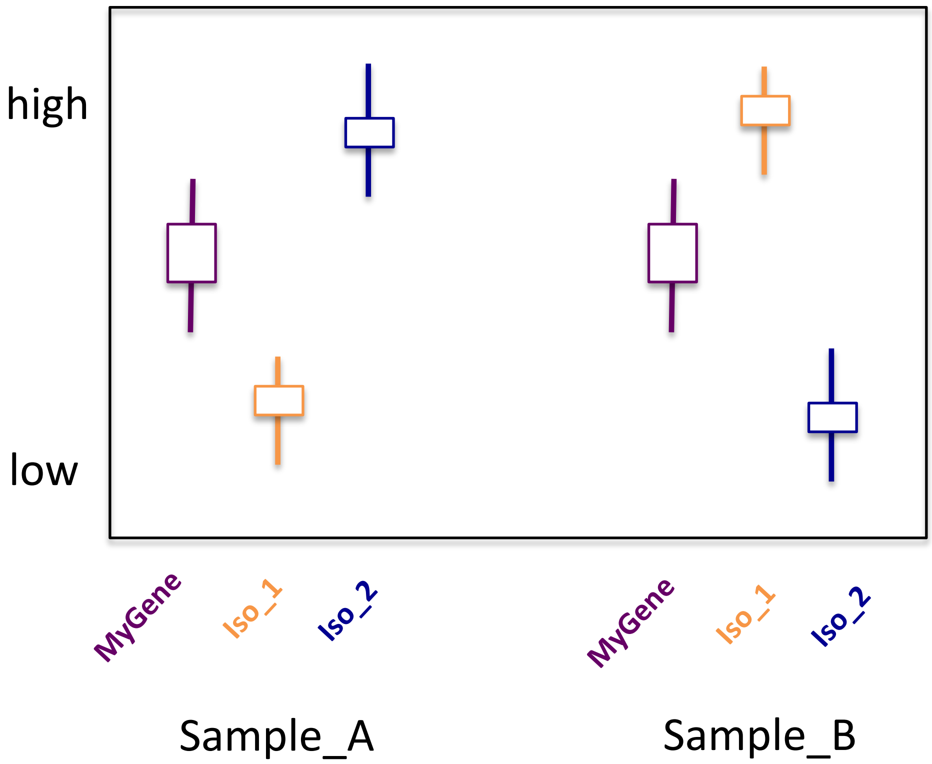


Feature	Diff Expressed?
MyGene	Yes
Iso_1	Yes
Iso_2	Yes

Diff. Transcript Usage ?
(eg. Isoform switching) Yes

Differential Gene Expression (DGE) and Differential Transcript Expression (DTE) (Example 3)





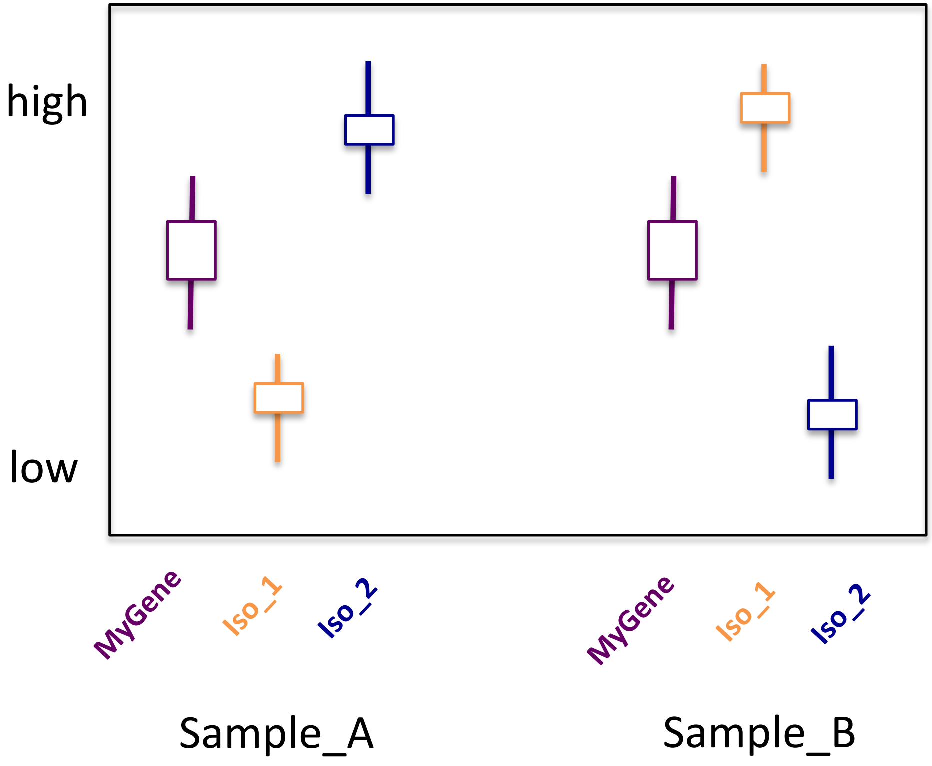
Feature	Diff Expressed?
MyGene	No
Iso_1	Yes
Iso_2	Yes

Diff. Transcript Usage ?
(eg. Isoform switching) Yes

Differential Gene Expression (DGE) and Differential Transcript Expression (DTE) (Example 3)

From Gene-level view (DGE): not apparent
From Transcript-level view (GDE): Yes, gene should be acknowledged as having changed.

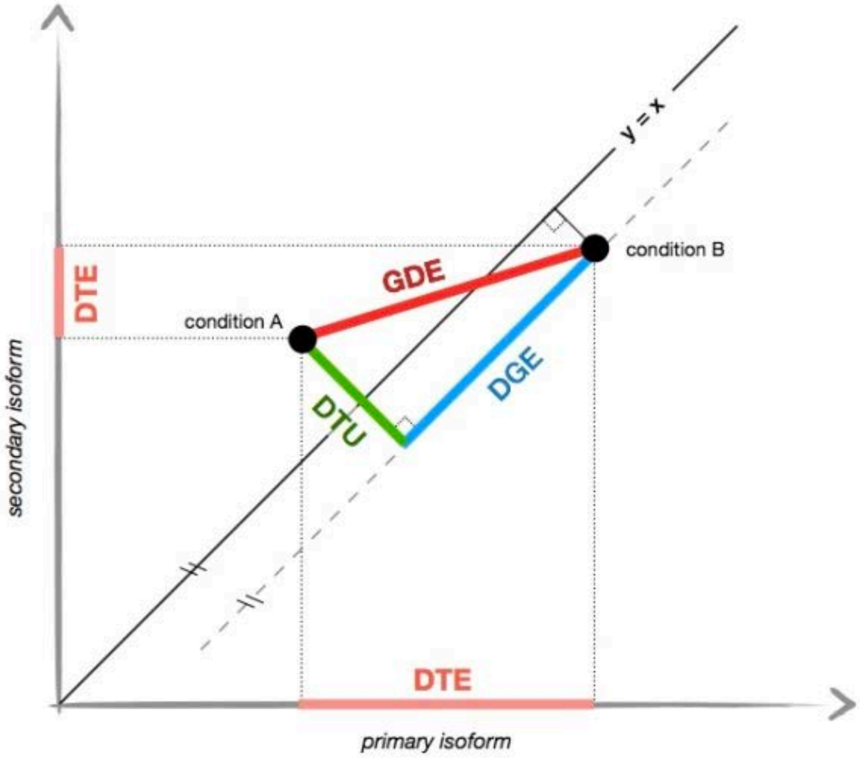
Prevailing viewpoint:
DTE or DTU -> Gene Diff Expressed (GDE)



Feature	Diff Expressed?
MyGene	No?
Iso_1	Yes
Iso_2	Yes

Diff. Transcript Usage ? (eg. Isoform switching)	Yes

Clarifying view: (DTE or DTU or DGE) as special cases of Gene Differential Expression (DGE)

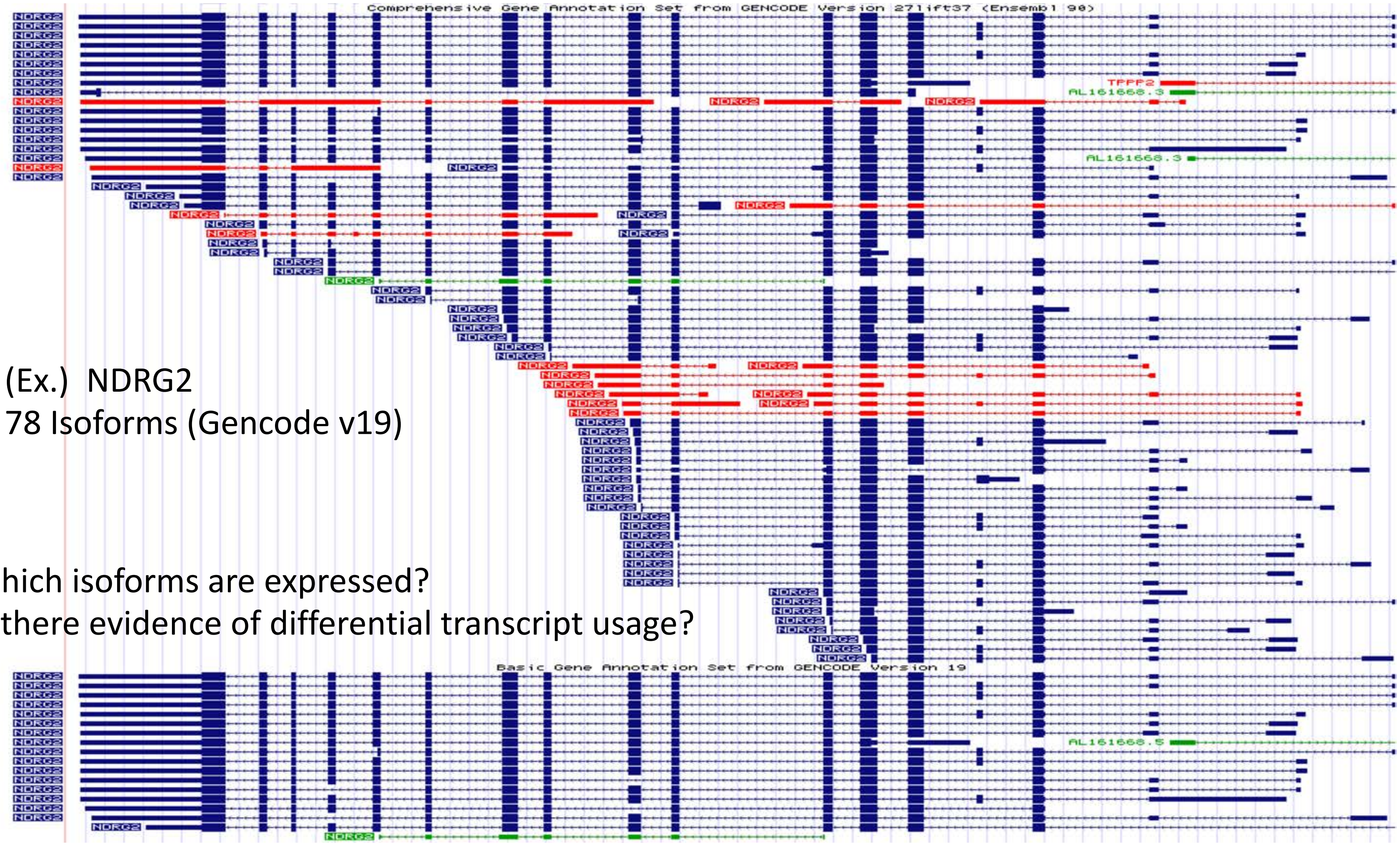


DTE: differential transcript expression
DTU: differential transcript usage
DGE: differential gene expression (gene-level analysis)
GDE: gene differential expression (transcript-level analysis)

Ntranos, Yi, et al., 2018 – see supp.

See Lior Pachter's blog post: https://liorpachter.wordpress.com/2019/01/07/fast-and-accurate-gene-differential-expression-by-testing-transcript-compatibility-counts/

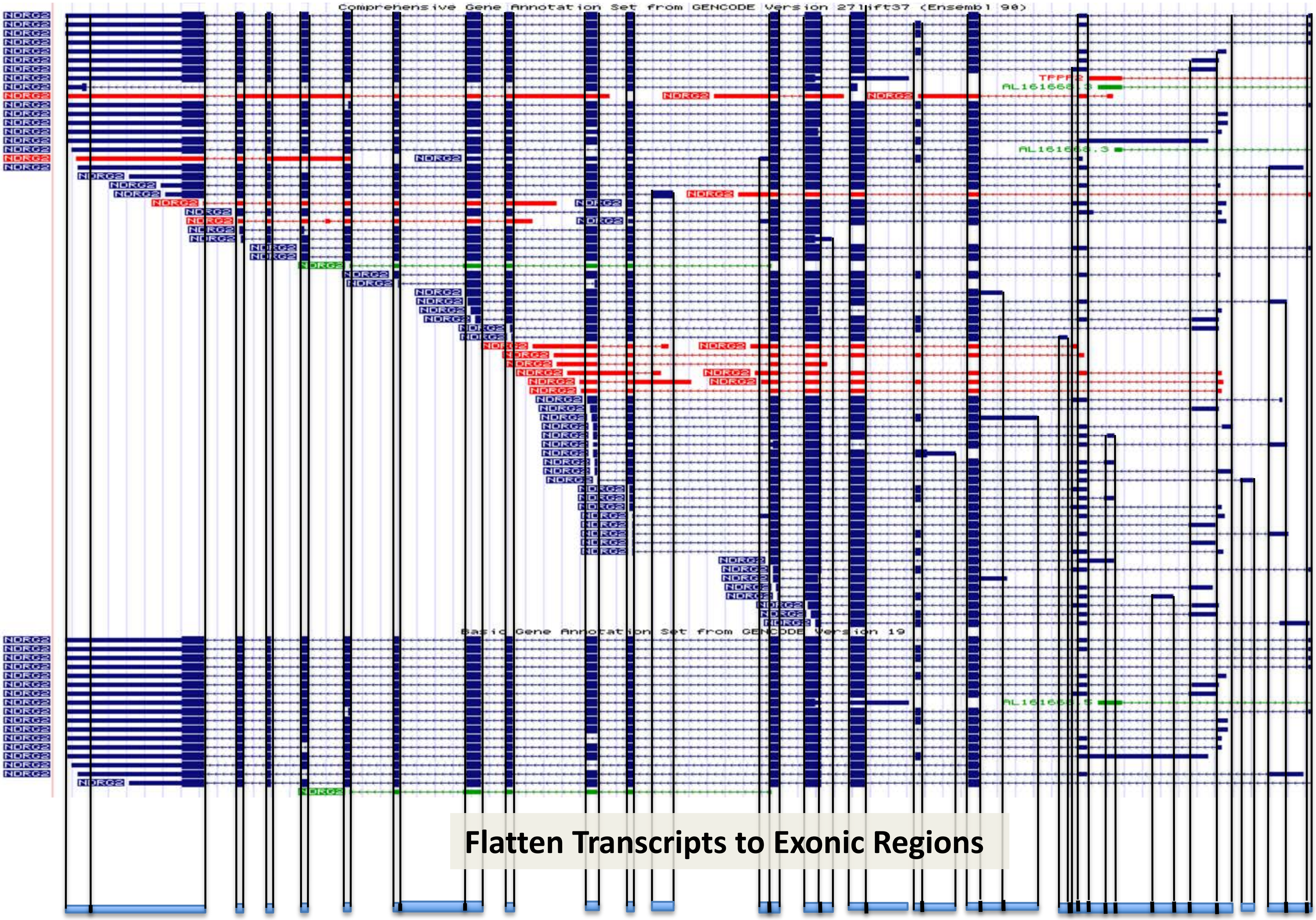
High Confidence Differential Transcript Expression is Difficult to Attain With Many Candidate Isoforms


Comprehensive Gene Annotation Set from GENCODE Version 27lift37 (Ensembl 90)
NDRG2
TPPP2
AL161668.3
Basic Gene Annotation Set from GENCODE Version 19
AL161668.5

(Ex.) NDRG2
78 Isoforms (Gencode v19)

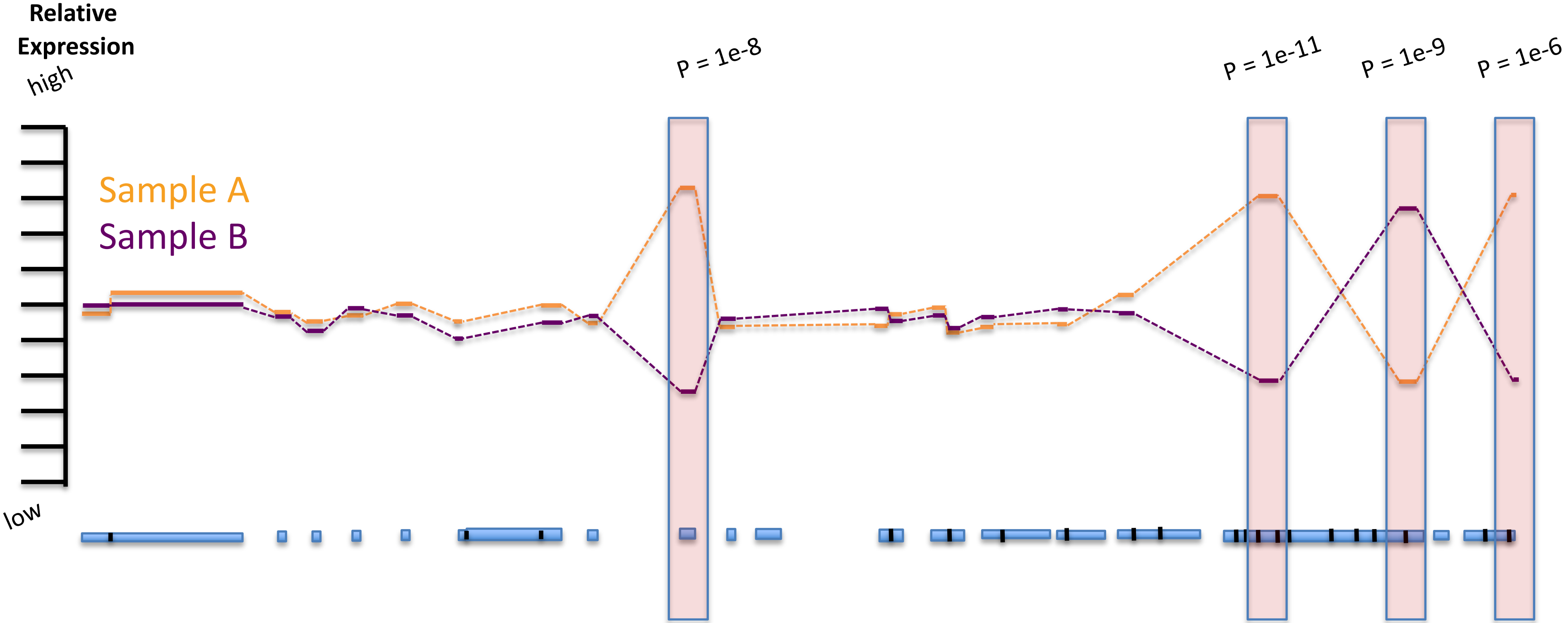
Which isoforms are expressed?
Is there evidence of differential transcript usage?

Measure Differential Transcript Usage (DTU) via Differential Exon Usage (DEU)


Comprehensive Gene Annotation Set from GENCODE Version 27lift37 (Ensembl 90)
NDRG2
TPPP2
AL161668.3
Basic Gene Annotation Set from GENCODE Version 19
AL161668.5

Flatten Transcripts to Exonic Regions

Measure Differential Transcript Usage (DTU) via Differential Exon Usage (DEU)



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Genome Res. 2012 Oct; 22(10): 2008–2017. PMCID: PMC3460195
doi: 10.1101/gr.133744.111

Detecting differential usage of exons from RNA-seq data

Simon Anders,[1,2] Alejandro Reyes,[1] and Wolfgang Huber

Averaged Replicates

Each Replicate

Flattened gene structure:

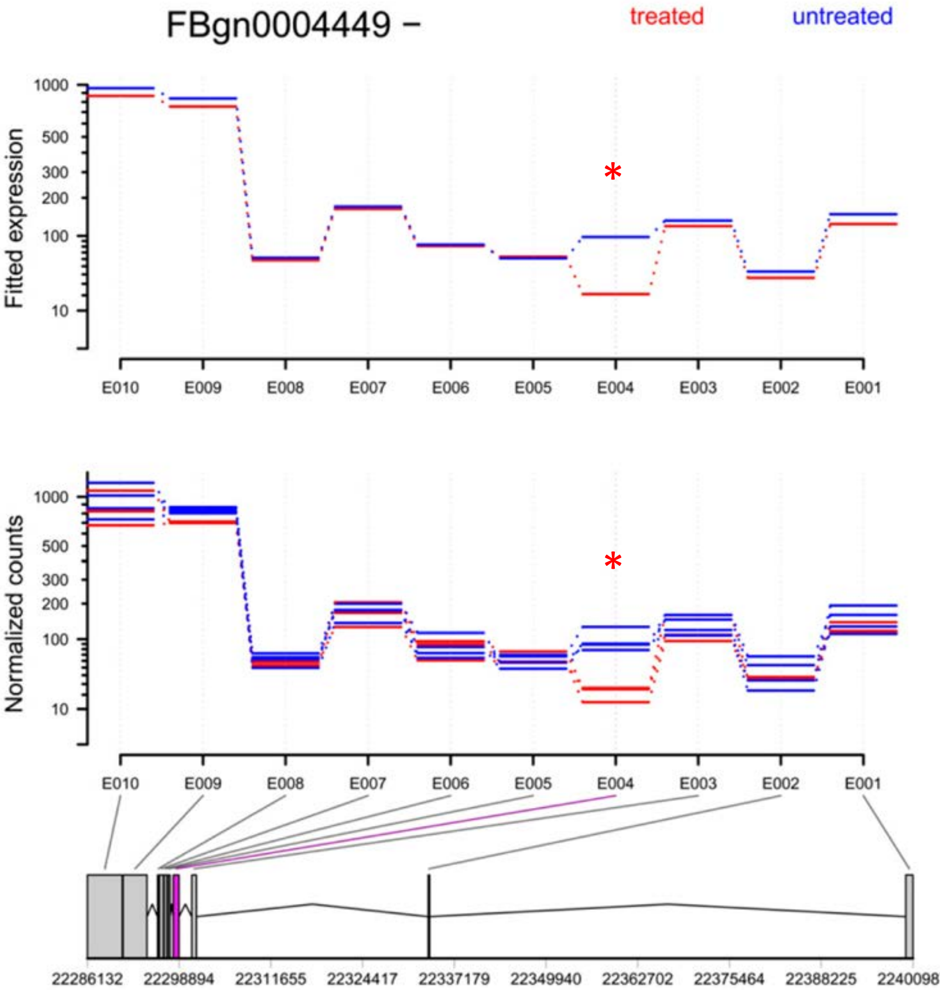
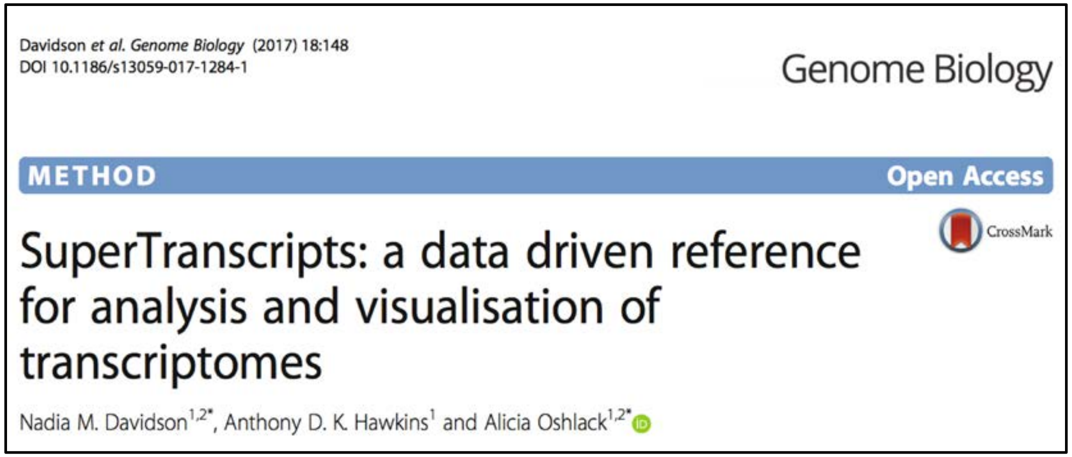


Figure 3. The treatment of knocking down the splicing factor *pasilla* affects the fourth exon (counting bin E004) of the gene *Ten-m* (CG5723). (*Top* panel) Fitted values according to the linear model; (*middle* panel) normalized counts for each sample; (*bottom* panel) flattened gene model. (Red) Data for knockdown samples; (blue) control.

Enabling Differential Transcript Usage Analysis for De novo Transcriptome Assemblies



Davidson *et al. Genome Biology* (2017) 18:148
DOI 10.1186/s13059-017-1284-1

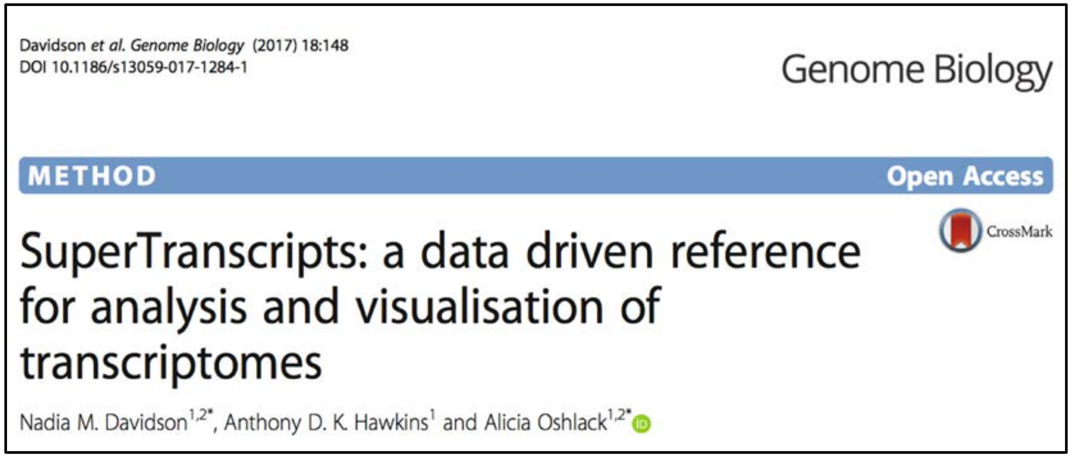
Genome Biology

METHOD Open Access

SuperTranscripts: a data driven reference for analysis and visualisation of transcriptomes

Nadia M. Davidson[1,2*], Anthony D. K. Hawkins[1] and Alicia Oshlack[1,2*]

Enabling Differential Transcript Usage Analysis for De novo Transcriptome Assemblies



Davidson *et al. Genome Biology* (2017) 18:148
DOI 10.1186/s13059-017-1284-1

Genome Biology

METHOD Open Access

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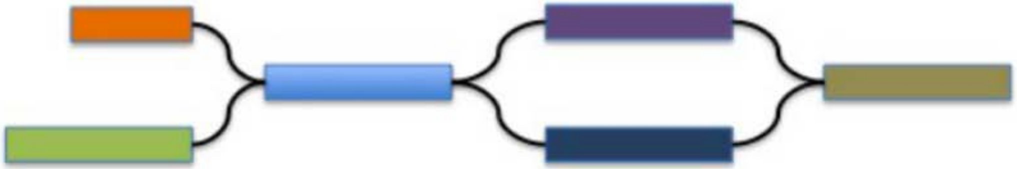
SuperTranscripts: a data driven reference for analysis and visualisation of transcriptomes

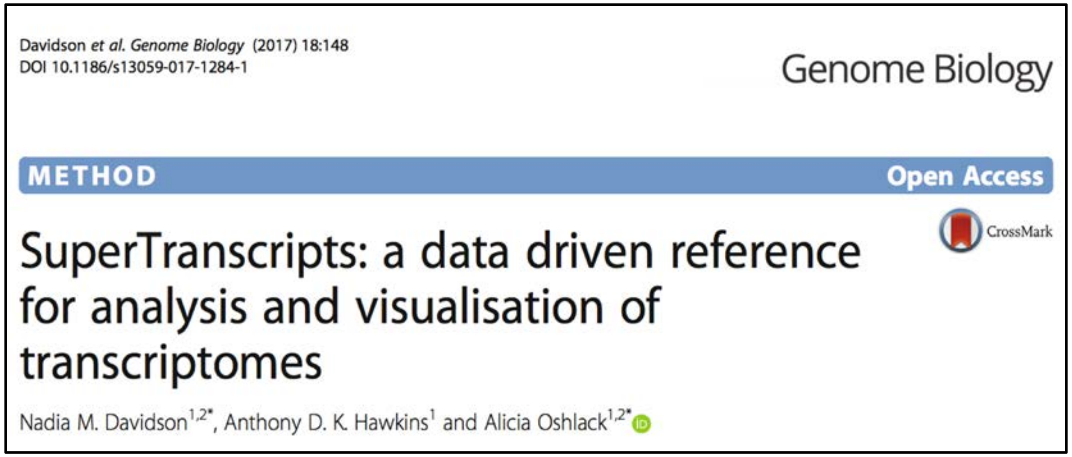
Nadia M. Davidson[1,2*], Anthony D. K. Hawkins[1] and Alicia Oshlack[1,2*]

Transcript splice graph:



Similar method and protocols now integrated into Trinity:
https://github.com/trinityrnaseq/trinityrnaseq/wiki/SuperTranscripts

Enabling Differential Transcript Usage Analysis for De novo Transcriptome Assemblies



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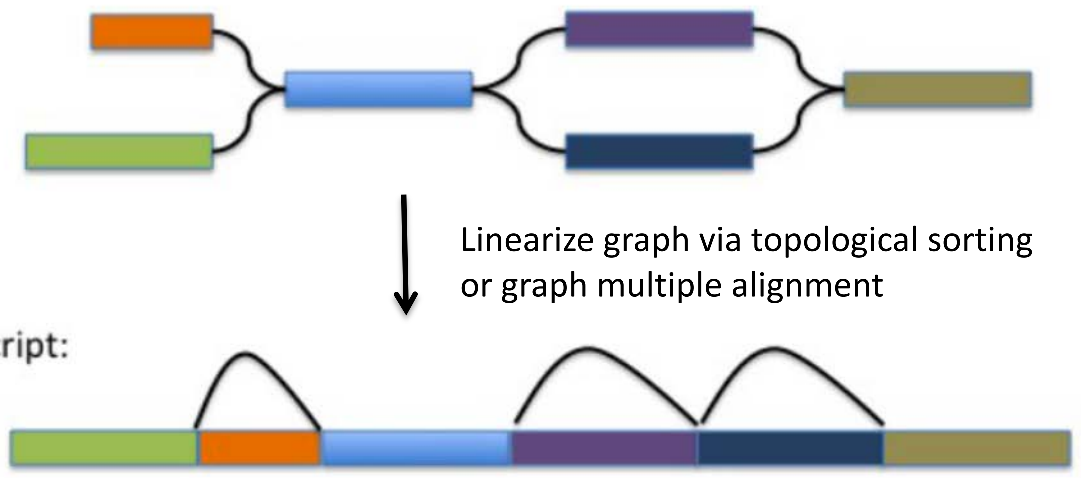
Transcript splice graph:



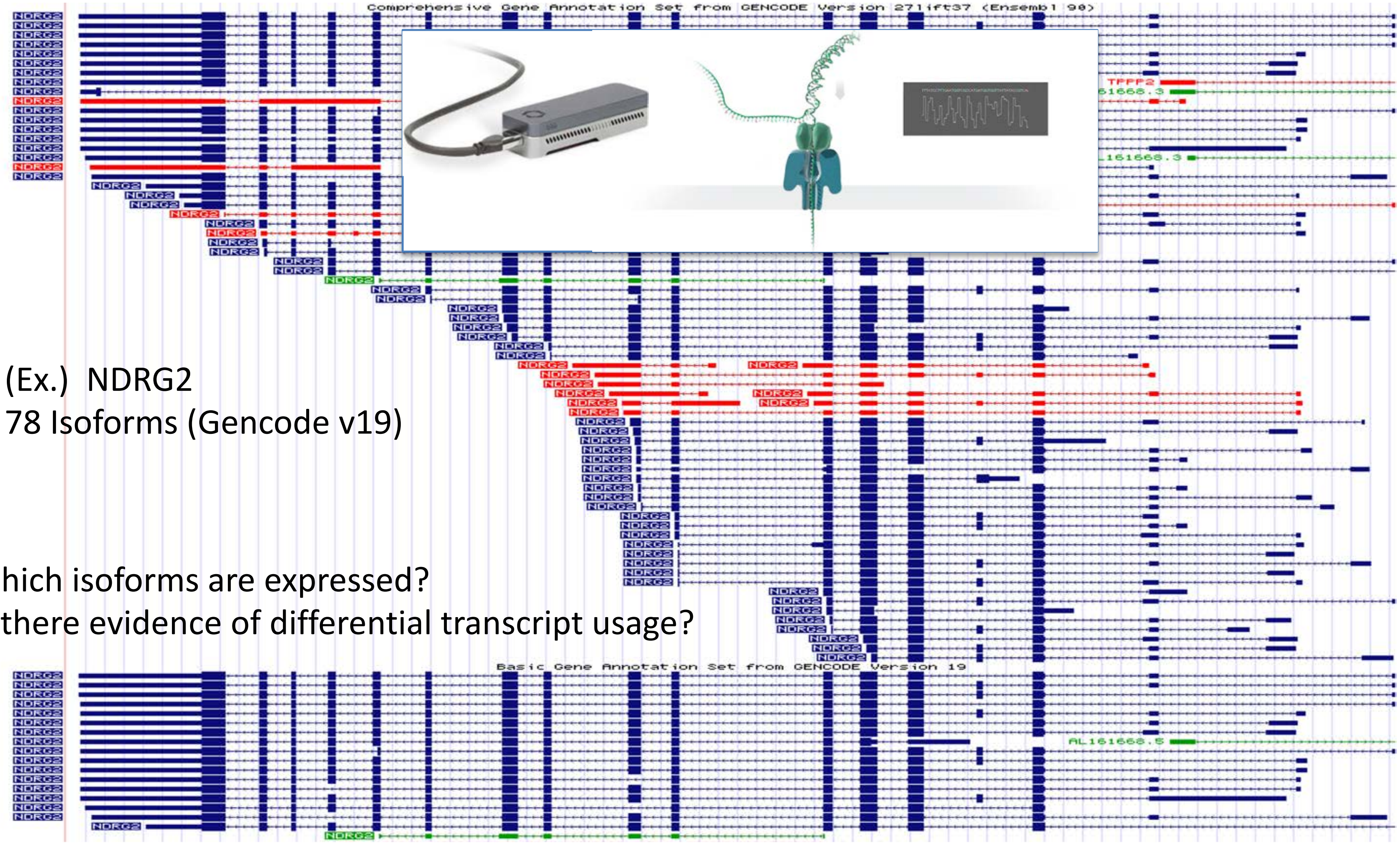
Linearize graph via topological sorting or graph multiple alignment

SuperTranscript:

DEXseq for DTU,
GATK for Variant Detection

Similar method and protocols now integrated into Trinity:
https://github.com/trinityrnaseq/trinityrnaseq/wiki/SuperTranscripts

Too complex… don’t guess from short reads, use long reads.


Comprehensive Gene Annotation Set from GENCODE Version 27lift37 (Ensembl 90)
NDRG2
TPPP2
AL161668.3
Basic Gene Annotation Set from GENCODE Version 19
AL161668.5

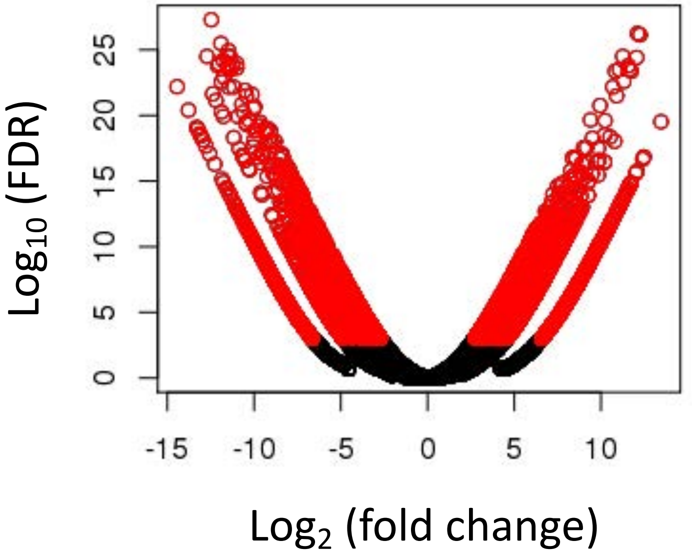
(Ex.) NDRG2
78 Isoforms (Gencode v19)

Which isoforms are expressed?
Is there evidence of differential transcript usage?

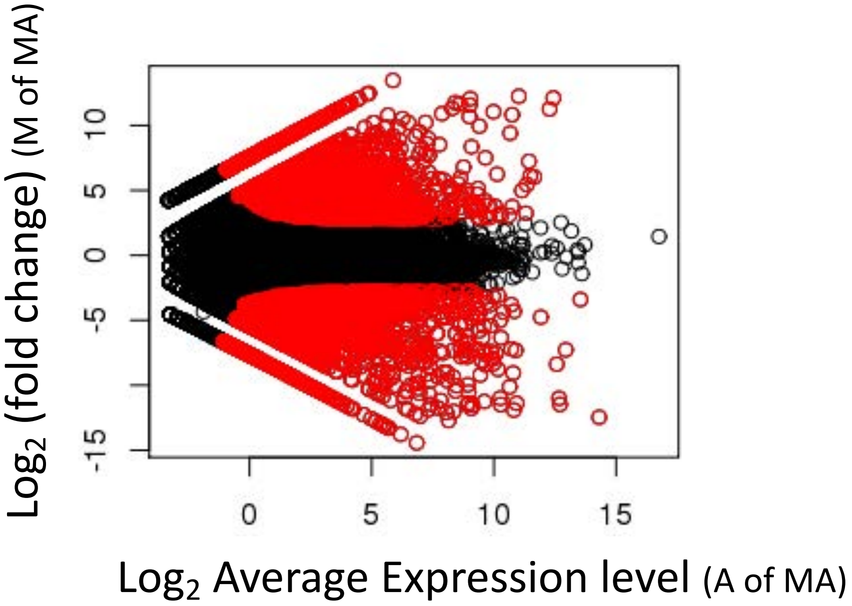
Visualization of DE results and Expression Profiling

Plotting Pairwise Differential Expression Data

Volcano plot
(fold change vs. significance)

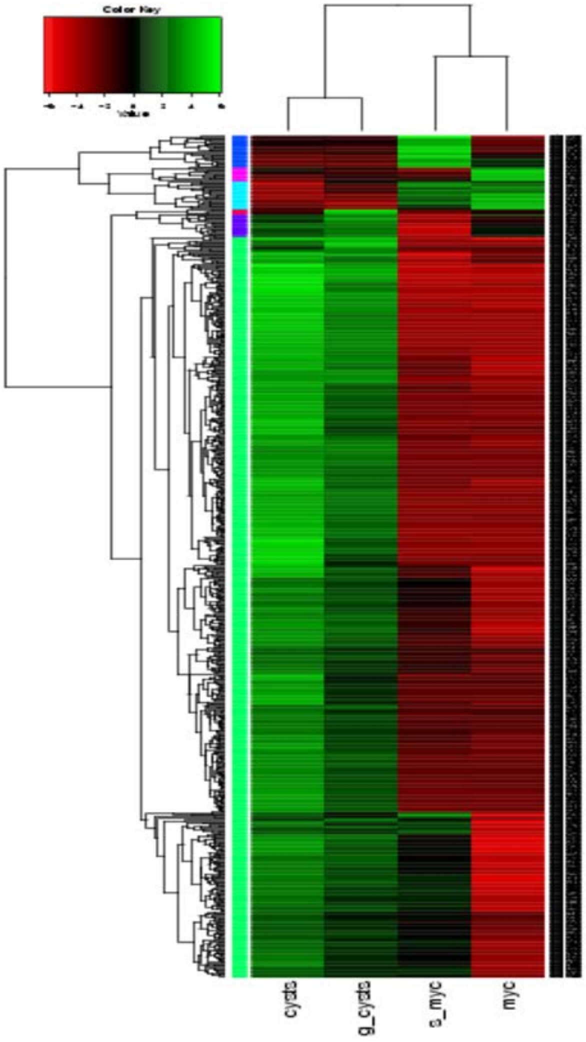


MA plot
(abundance vs. fold change)



Significantly differently expressed transcripts have FDR <= 0.001
(shown in red)

Comparing Multiple Samples



Heatmaps provide an effective tool for navigating differential expression across multiple samples.

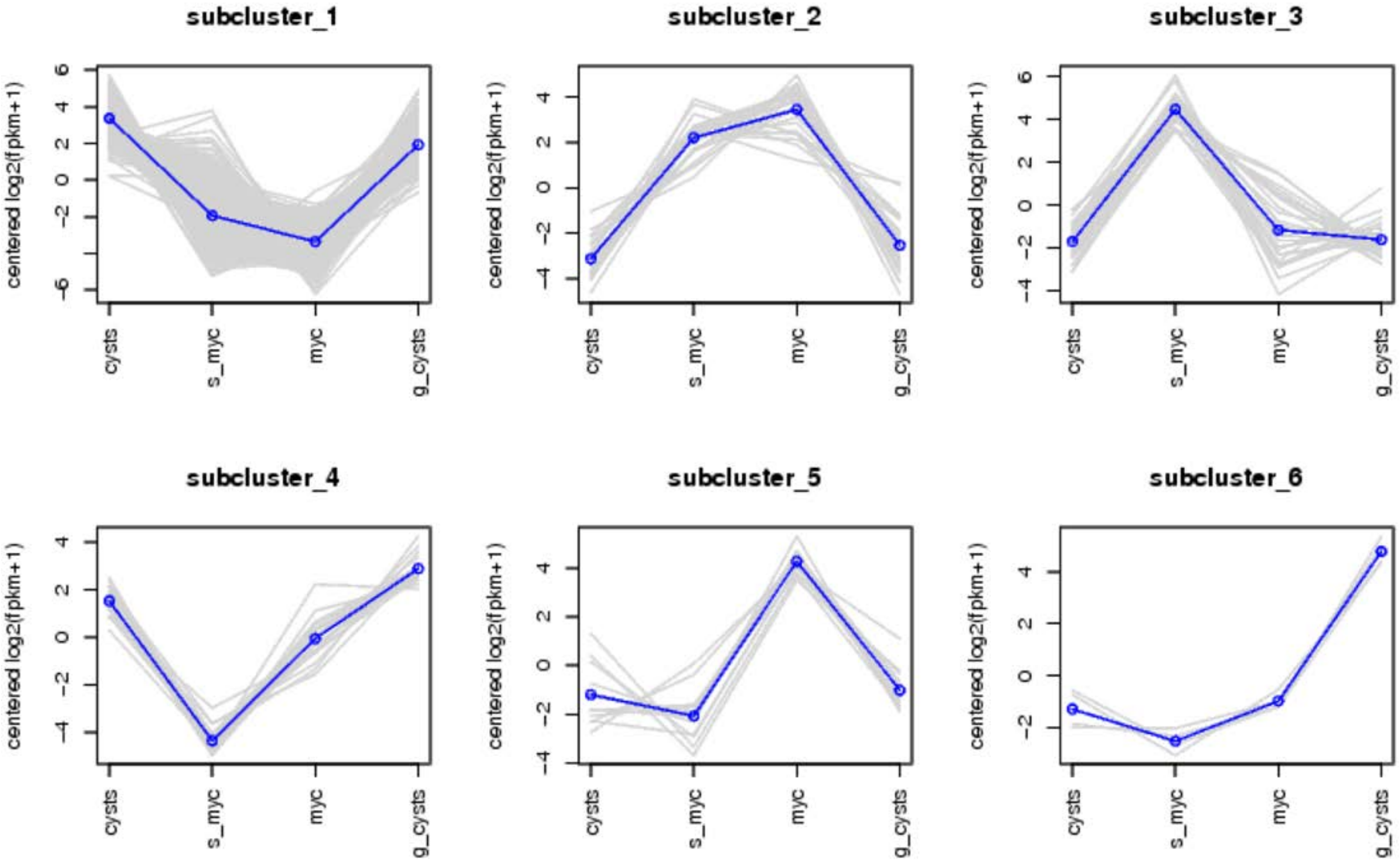
Clustering can be performed across both axes:

-cluster transcripts with similar expression patters.

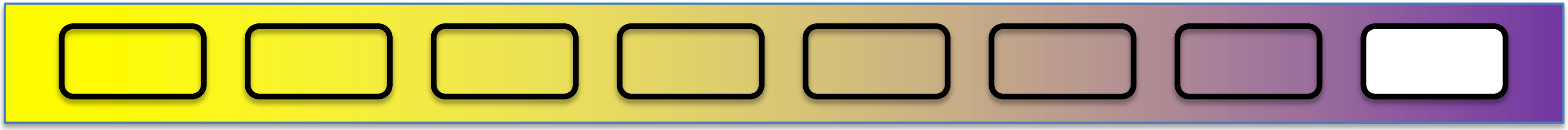
-cluster samples according to similar expression values among transcripts.

Examining Patterns of Expression Across Samples

Can extract clusters of transcripts and examine them separately.



Part 7. Functional Annotation



Transcript Functional Annotation

```
GGAGCTGGAGGCCCCCAGGCAACTACACCGTCCACGTACCCAGAGGGGCTGGCCCTCCC
ACCAGAGACCACGCCCTGGTGTGCCTTAGGGGCCCTGGTTTGTTAGTCTCTGAGTGTGCA
GTTGCTGCACATGGGGCCCTGGCGCTTGCTGCACCAACTTCCTGTTGGGCCCGTGGTCCT
TGGAGGCATGCAGTTCAGCAGACAGTGACTCAGCCATCCACCCAACATGCGGAACGTGTC
TCTTCTGCAGGTCCCGGTCCACAGCAGGATTCCCCCTCTGTGAAAAGGCACGCTGATCTG
TCTGGA                                                 TCGAC
TCTCCC                                                 TCCCA
AAAGAC                                                 CCTGG
GGCTTG                                                 CCTAA
TGACCT                                                 TGCTG
GAAAAG                                                 CAGCC
TTGTCA                                                 TTCCA
GGAAGCACATAATTGAAGGACTGAAAGCGTCCCTGGAGCGGCTGCAGCTGGAGTACGTGG
ATGTGGTTTTTGCCAACCGCCCAGACCCCAACACGCCCATGGAAGAGACCGTGCGGCCA
TGACCCATGTCATCAACCAGGGGATGGCCATGTACTGGGGCACATCACGCTGGAGCTCCA
TGGAGATCATGGAGGCCTACTCGGTGGCTCGGCAGTTCAACCTGATCCCGCCCATCTGCG
AGCAAGCGGAATATCACATGTTCCAGAGGGAGAAGGTGGAGGTCCAGCTGCCAGAGCTGT
TCCACAAGATAGGAGTAGGTGCCATGACCTGGTCCCCTCTGGCGTGCGGCATCGTCTCAG
GGAAGTATGACAGCGGGATCCCACCCTACTCCAGAGCCTCCCTGAAGGGCTACCAGTGGT
TGAAGGACAAGATCCTGAGTGAGGAGGGTCGCCGCCAGCAGGCCAAGCTGAAGGAACTGC
AGGCCATTGCCGAACGCCTGGGCTGCACCCTACCCCAGCTGGCCATAGCCTGGTGCCTGA
GGAATGAGGGTGTCAGCTCCGTGCTTCTGGGTGCTTCCAATGCAGAACAACTTATGGAGA
```

Can we gather hints of biological function from sequence?

Methods used to predict function from sequence

- Sequence homology

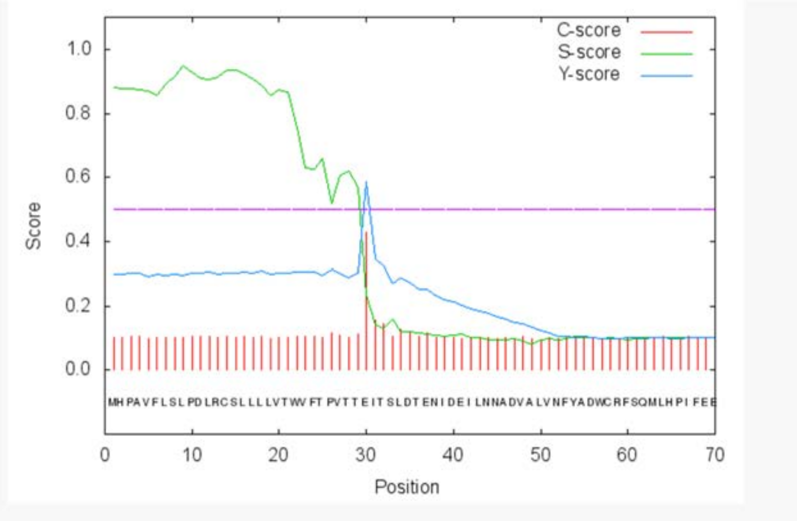
Searching protein database for sequence similarity

```
Query           THVHRPYNEHKSLSGTARYMSINTHLGREQSRRDDLESMGHVFMYFLRGSLPW--QGLKA
                T    P + K   GT  Y S + HLG    RR DLE +G      L   LPW  Q L A
Database Match  TGDFKP-DPKKMHNGTIEYTSRDAHLG-VPTRRADLEILGYNLIEWLGAELPWVTQKLLA
```

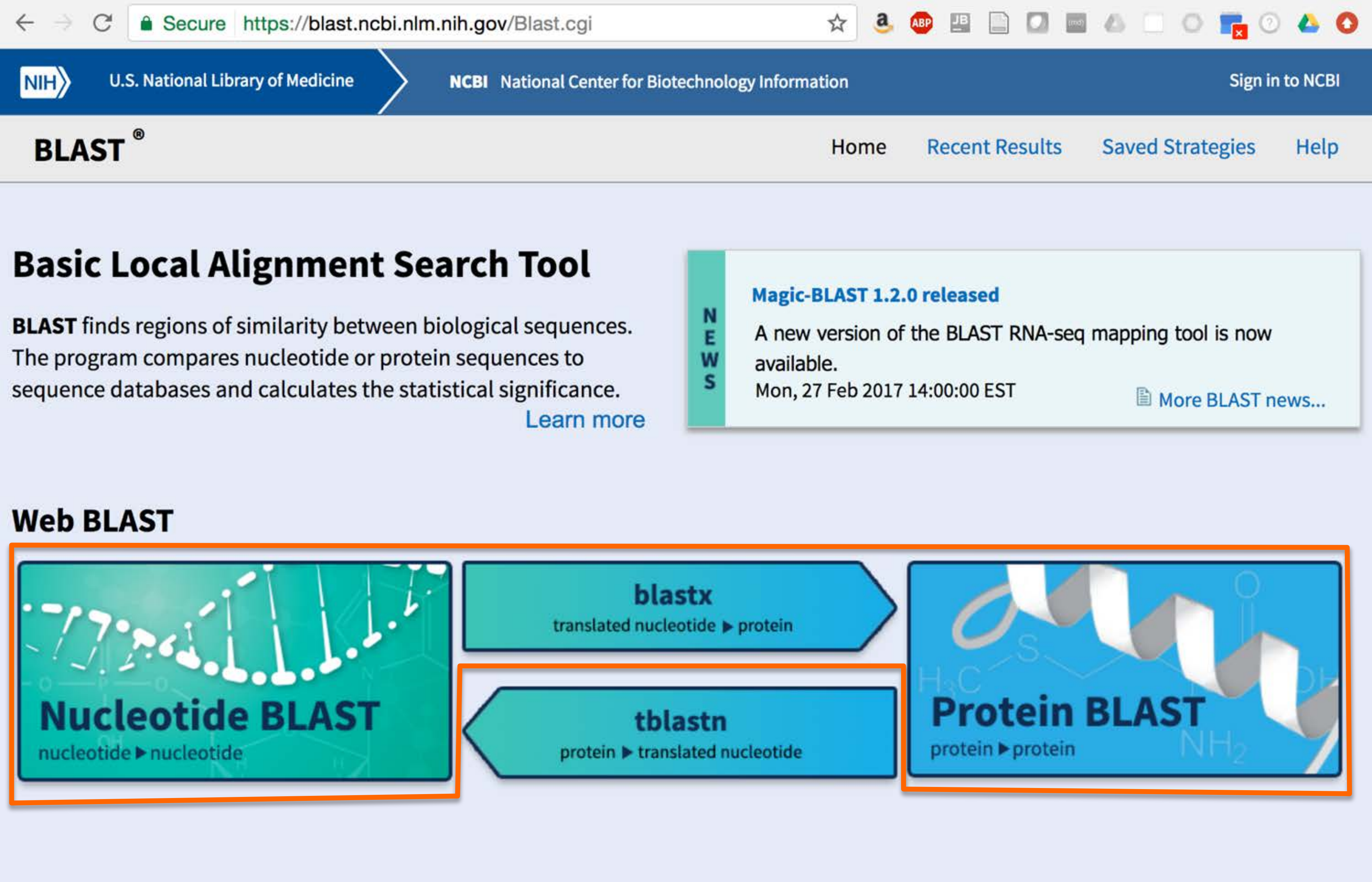
- Sequence composition

Predict functions of sequence using machine learning methods for pattern recognition.

- Neural Networks
- Hidden Markov Models



Use BLAST to search for sequence similarity to known proteins



https://blast.ncbi.nlm.nih.gov/Blast.cgi

U.S. National Library of Medicine

NCBI National Center for Biotechnology Information

Sign in to NCBI

BLAST®

Home Recent Results Saved Strategies Help

Basic Local Alignment Search Tool

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance.

Learn more

NEWS

Magic-BLAST 1.2.0 released

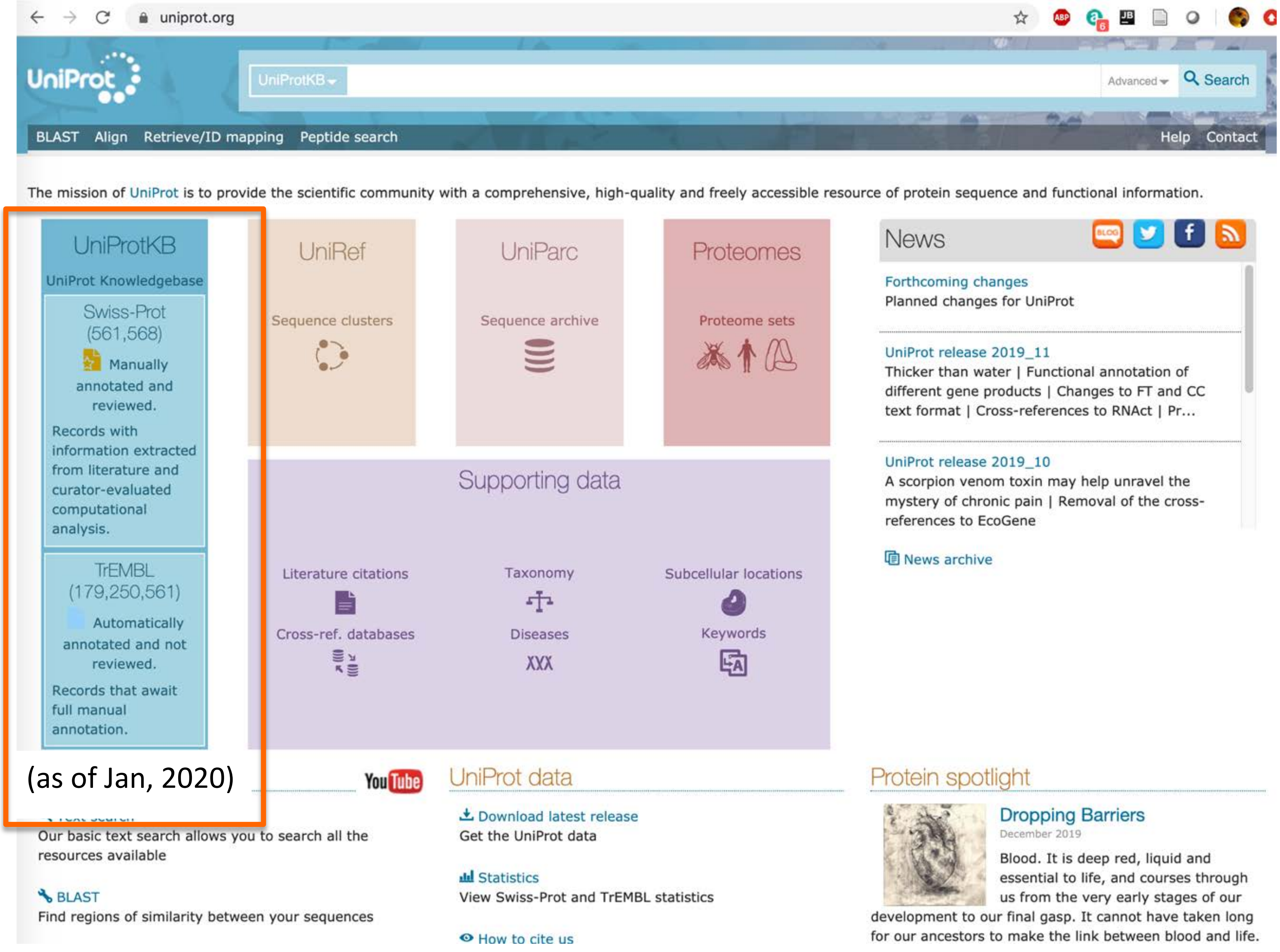
A new version of the BLAST RNA-seq mapping tool is now available.

Mon, 27 Feb 2017 14:00:00 EST

More BLAST news...

Web BLAST

The Swiss-Prot database is a valuable source of proteins with known functions



The mission of UniProt is to provide the scientific community with a comprehensive, high-quality and freely accessible resource of protein sequence and functional information.

UniProtKB

UniProt Knowledgebase

Swiss-Prot (561,568)

Manually annotated and reviewed.

Records with information extracted from literature and curator-evaluated computational analysis.

TrEMBL (179,250,561)

Automatically annotated and not reviewed.

Records that await full manual annotation.

(as of Jan, 2020)

UniRef

Sequence clusters

UniParc

Sequence archive

Proteomes

Proteome sets

Supporting data

Literature citations

Taxonomy

Subcellular locations

Cross-ref. databases

Diseases

Keywords

News

Forthcoming changes

Planned changes for UniProt

UniProt release 2019_11

Thicker than water | Functional annotation of different gene products | Changes to FT and CC text format | Cross-references to RNAct | Pr...

UniProt release 2019_10

A scorpion venom toxin may help unravel the mystery of chronic pain | Removal of the cross-references to EcoGene

News archive

Text search

Our basic text search allows you to search all the resources available

BLAST

Find regions of similarity between your sequences

UniProt data

Download latest release

Get the UniProt data

Statistics

View Swiss-Prot and TrEMBL statistics

How to cite us

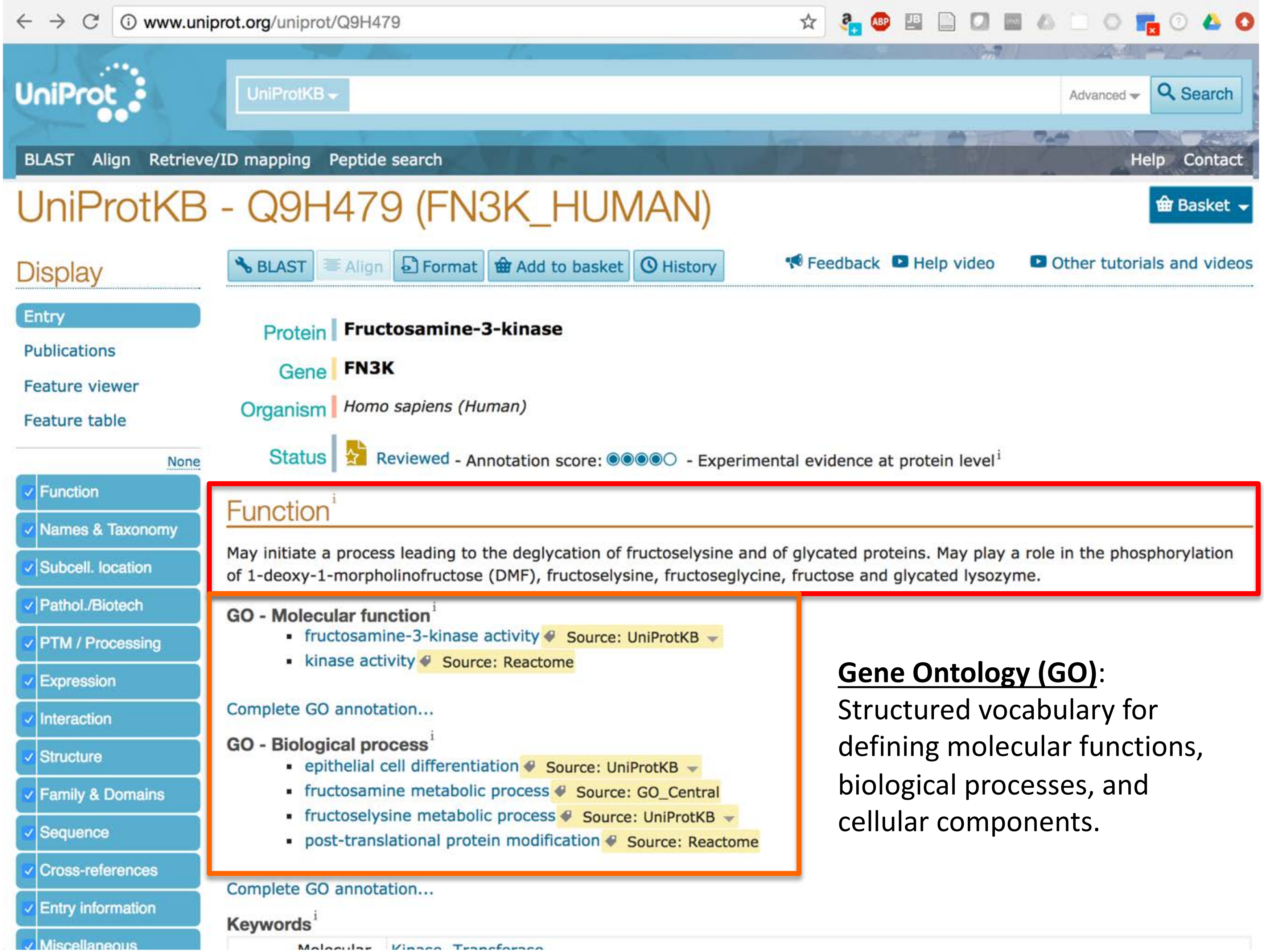
Protein spotlight

Dropping Barriers

December 2019

Blood. It is deep red, liquid and essential to life, and courses through us from the very early stages of our development to our final gasp. It cannot have taken long for our ancestors to make the link between blood and life.

Example of a Swiss-Prot Record



UniProtKB - Q9H479 (FN3K_HUMAN)

Protein **Fructosamine-3-kinase**

Gene **FN3K**

Organism *Homo sapiens (Human)*

Status Reviewed - Annotation score: ●●●●○ - Experimental evidence at protein level[i]

Function[i]

May initiate a process leading to the deglycation of fructoselysine and of glycated proteins. May play a role in the phosphorylation of 1-deoxy-1-morpholinofructose (DMF), fructoselysine, fructoseglycine, fructose and glycated lysozyme.

GO - Molecular function[i]

- fructosamine-3-kinase activity Source: UniProtKB
- kinase activity Source: Reactome

Complete GO annotation...

GO - Biological process[i]

- epithelial cell differentiation Source: UniProtKB
- fructosamine metabolic process Source: GO_Central
- fructoselysine metabolic process Source: UniProtKB
- post-translational protein modification Source: Reactome

Complete GO annotation...

Keywords[i]

Molecular Kinase, Transferase

Gene Ontology (GO): Structured vocabulary for defining molecular functions, biological processes, and cellular components.

No significant sequence similarity… What else?

```
GGAGCTGGAGGCCCCCAGGCAACTACACCGTCCACGTACCCAGAGGGGCTGGGCCCTCCC
ACCAGAGACCACGCCCTGGTGTGCCTTAGGGGCCCTGGTTTGTTAGTCTCTGAGTGTGCA
GTTGCTGCACATGGGGCCCTGGCGCTTGCTGCACCAACTTCCTGTTGGGCCCGTGGTCCT
TGGAGGCATGCAGTTCAGCAGACAGTGACTCAGCCATCCACCCAACATGCGGAACGTGTC
TCTTCTGCAGGTCCCGGTCCACAGCAGGATTCCCCCTCTGTGAAAAGGCACGCTGATCTG
TCTGGATAAGTGTGGCCGGCCCCATGTATCCGGAATCAACCACGGGGTCCCCAGCTCGAC
TCTCCCTGCGGCAGACAGGCTCCCCCGGGATGATCTACAGTACTCGTTATGGGAGTCCCA
AAAGACAGCTCCAGTTTTACAGGAATCTGGGCAAATCTGGCCTTCGGGTCTCCTGCCTGG
GGCTTGGAACATGGGTGACCTTCGGGGGCCAGATCACGGATGAGATGGCAGAGCACCTAA
TGACCTTGGCCTACGATAATGGCATCAACCTGTTCGATACGGCGGAGGTCTACGCTGCTG
GAAAAGCTGAAGTGGTATTAGGGAACATCATTAAGAAGAAGGGATGGAGACGGTCCAGCC
TTGTCATCACCACCAAGATCTTCTGGGGTGGAAAAGCGGAGACTGAGAGAGGCCTTTCCA
GGAAGCACATAATTGAAGGACTGAAAGCGTCCCTGGAGCGGCTGCAGCTGGAGTACGTGG
ATGTGGTTTTTGCCAACCGCCCAGACCCCAACACGCCCATGGAAGAGACCGTGCGGGCCA
TGACCCATGTCATCAACCAGGGGATGGCCATGTACTGGGGCACATCACGCTGGAGCTCCA
TGGAGATCATGGAGGCCTACTCGGTGGCTCGGCAGTTCAACCTGATCCCGCCCATCTGCG
AGCAAGCGGAATATCACATGTTCCAGAGGGAGAAGGTGGAGGTCCAGCTGCCAGAGCTGT
TCCACAAGATAGGAGTAGGTGCCATGACCTGGTCCCCTCTGGCGTGCGGCATCGTCTCAG
GGAAGTATGACAGCGGGATCCCACCCTACTCCAGAGCCTCCCTGAAGGGCTACCAGTGGT
TGAAGGACAAGATCCTGAGTGAGGAGGGTCGCCGCCAGCAGGCCAAGCTGAAGGAACTGC
AGGCCATTGCCGAACGCCTGGGCTGCACCCTACCCCAGCTGGCCATAGCCTGGTGCCTGA
GGAATGAGGGTGTCAGCTCCGTGCTTCTGGGTGCTTCCAATGCAGAACAACTTATGGAGA
```

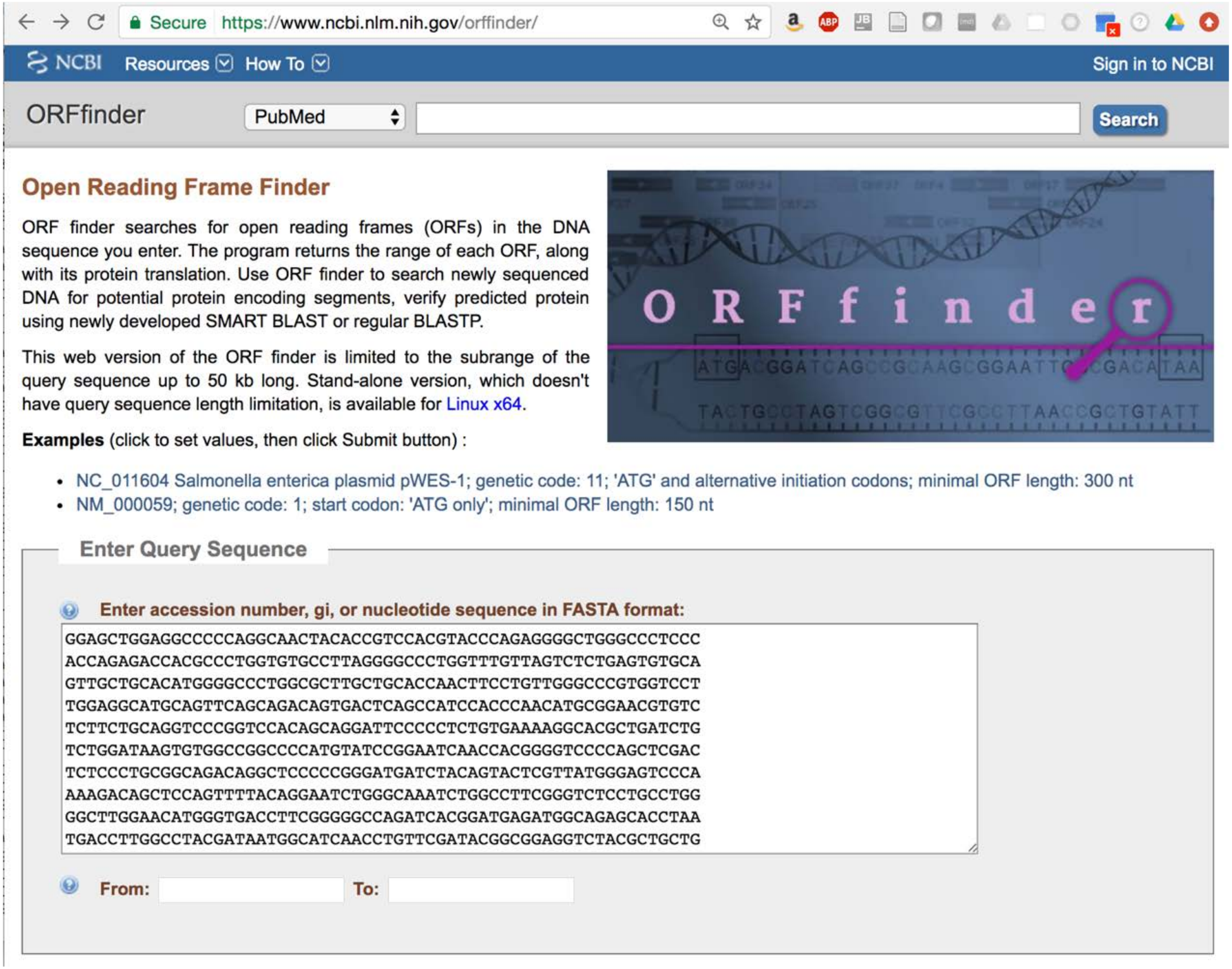
Is there an ORF for a potential Coding Region?

```
GGAGCTGGAGGCCCCCAGGCAACTACACCGTCCACGTACCCAGAGGGGCTGGGCCCTCCC
ACCAGAGACCACGCCCTGGTGTGCCTTAGGGGCCCTGGTTTGTTAGTCTCTGAGTGTGCA
GTTGCTGCACATGGGGCCCTGGCGCTTGCTGCACCAACTTCCTGTTGGGCCCGTGGTCCT
TGGAGGCATGCAGTTCAGCAGACAGTGACTCAGCCATCCACCCAACATGCGGAACGTGTC
TCTTCTGCAGGTCCCGGTCCACAGCAGGATTCCCCCTCTGTGAAAAGGCACGCTGATCTG
TCTGGATAAGTGTGGCCGGCCCCATGTATCCGGAATCAACCACGGGGTCCCCAGCTCGAC
TCTCCCTGCGGCAGACAGGCTCCCCCGGGATGATCTACAGTACTCGTTATGGGAGTCCCA
AAAGACAGCTCCAGTTTTACAGGAATCTGGGCAAATCTGGCCTTCGGGTCTCCTGCCTGG
GGCTTGGAACATGGGTGACCTTCGGGGGCCAGATCACGGATGAGATGGCAGAGCACCTAA
TGACCTTGGCCTACGATAATGGCATCAACCTGTTCGATACGGCGGAGGTCTACGCTGCTG
GAAAAGCTGAAGTGGTATTAGGGAACATCATTAAGAAGAAGGGATGGAGACGGTCCAGCC
TTGTCATCACCACCAAGATCTTCTGGGGTGGAAAAGCGGAGACTGAGAGAGGCCTTTCCA
GGAAGCACATAATTGAAGGACTGAAAGCGTCCCTGGAGCGGCTGCAGCTGGAGTACGTGG
ATGTGGTTTTTGCCAACCGCCCAGACCCCAACACGCCCATGGAAGAGACCGTGCGGGCCA
TGACCCATGTCATCAACCAGGGGATGGCCATGTACTGGGGCACATCACGCTGGAGCTCCA
TGGAGATCATGGAGGCCTACTCGGTGGCTCGGCAGTTCAACCTGATCCCGCCCATCTGCG
AGCAAGCGGAATATCACATGTTCCAGAGGGAGAAGGTGGAGGTCCAGCTGCCAGAGCTGT
TCCACAAGATAGGAGTAGGTGCCATGACCTGGTCCCCTCTGGCGTGCGGCATCGTCTCAG
GGAAGTATGACAGCGGGATCCCACCCTACTCCAGAGCCTCCCTGAAGGGCTACCAGTGGT
TGAAGGACAAGATCCTGAGTGAGGAGGGTCGCCGCCAGCAGGCCAAGCTGAAGGAACTGC
AGGCCATTGCCGAACGCCTGGGCTGCACCCTACCCCAGCTGGCCATAGCCTGGTGCCTGA
GGAATGAGGGTGTCAGCTCCGTGCTTCTGGGTGCTTCCAATGCAGAACAACTTATGGAGA
```

Is there an ORF for a potential Coding Region?

GGAGCTGGAGGCCCCCAGGCAACTACACCGTCCACGTACCCAGAGGGGCTGGGCCCTCCC
ACCAGAGACCACGCCCTGGTGTGCCTTAGGGGCCCTGGTTTGTTAGTCTCTGAGTGTGCA
GTTGCTGCAC**ATGGGGCCCTGGCGCTTGCTGCACCAACTTCCTGTTGGGCCCGTGGTCCT**
TGGAGGCATGCAGTTCAGCAGACAGTGACTCAGCCATCCACCCAACATGCGGAACGTGTC
TCTTCTGCAGGTCCCGGTCCACAGCAGGATTCCCCCTCTGTGAAAAGGCACGCTGATCTG
TCTGGATAAGTGTGGCCGGCCCCATGTATCCGGAATCAACCACGGGGTCCCCAGCTCGAC
TCTCCCTGCGGCAGACAGGCTCCCCCGGGATGATCTACAGTACTCGTTATGGGAGTCCCA
AAAGACAGCTCCAGTTTTACAGGAATCTGGGCAAATCTGGCCTTCGGGTCTCCTGCCTGG
GGCTTGGAACATGGGTGACCTTCGGGGGCCAGATCACGGATGAGATGGCAGAGCACCTAA
TGACCTTGGCCTACGATAATGGCATCAACCTGTTCGATACGGCGGAGGTCTACGCTGCTG
GAAAAGCTGAAGTGGTATTAGGGAACATCATTAAGAAGAAGGGATGGAGACGGTCCAGCC
TTGTCATCACCACCAAGATCTTCTGGGGTGGAAAAGCGGAGACTGAGAGAGGCCTTTCCA
GGAAGCACATAATTGAAGGACTGAAAGCGTCCCTGGAGCGGCTGCAGCTGGAGTACGTGG
ATGTGGTTTTTGCCAACCGCCCAGACCCCAACACGCCCATGGAAGAGACCGTGCGGGCCA
TGACCCATGTCATCAACCAGGGGATGGCCATGTACTGGGGCACATCACGCTGGAGCTCCA
TGGAGATCATGGAGGCCTACTCGGTGGCTCGGCAGTTCAACCTGATCCCGCCCATCTGCG
AGCAAGCGGAATATCACATGTTCCAGAGGGAGAAGGTGGAGGTCCAGCTGCCAGAGCTGT
TCCACAAGATAGGAGTAGGTGCCATGACCTGGTCCCCTCTGGCGTGCGGCATCGTCTCAG
GGAAGTATGACAGCGGGATCCCACCCTACTCCAGAGCCTCCCTGAAGGGCTACCAGTGGT
TGAAGGACAAGATCCTGAGTGAGGAGGGTCGCCGCCAGCAGGCCAAGCTGAAGGAACTGC
AGGCCATTGCCGAACGCCTGGGCTGCACCCTACCCCAGCTGGCCATAGCCTGGTGCCTGA
GGAATGAGGGTGTCAGCTCCGTGCTTCTGGGTGCTTCCAATGCAGAACAACTTATGGAGA

Find all ORFs using ORFfinder



Open Reading Frame Finder

ORF finder searches for open reading frames (ORFs) in the DNA sequence you enter. The program returns the range of each ORF, along with its protein translation. Use ORF finder to search newly sequenced DNA for potential protein encoding segments, verify predicted protein using newly developed SMART BLAST or regular BLASTP.

This web version of the ORF finder is limited to the subrange of the query sequence up to 50 kb long. Stand-alone version, which doesn't have query sequence length limitation, is available for Linux x64.

Examples (click to set values, then click Submit button) :

- NC_011604 Salmonella enterica plasmid pWES-1; genetic code: 11; 'ATG' and alternative initiation codons; minimal ORF length: 300 nt
- NM_000059; genetic code: 1; start codon: 'ATG only'; minimal ORF length: 150 nt

Enter Query Sequence

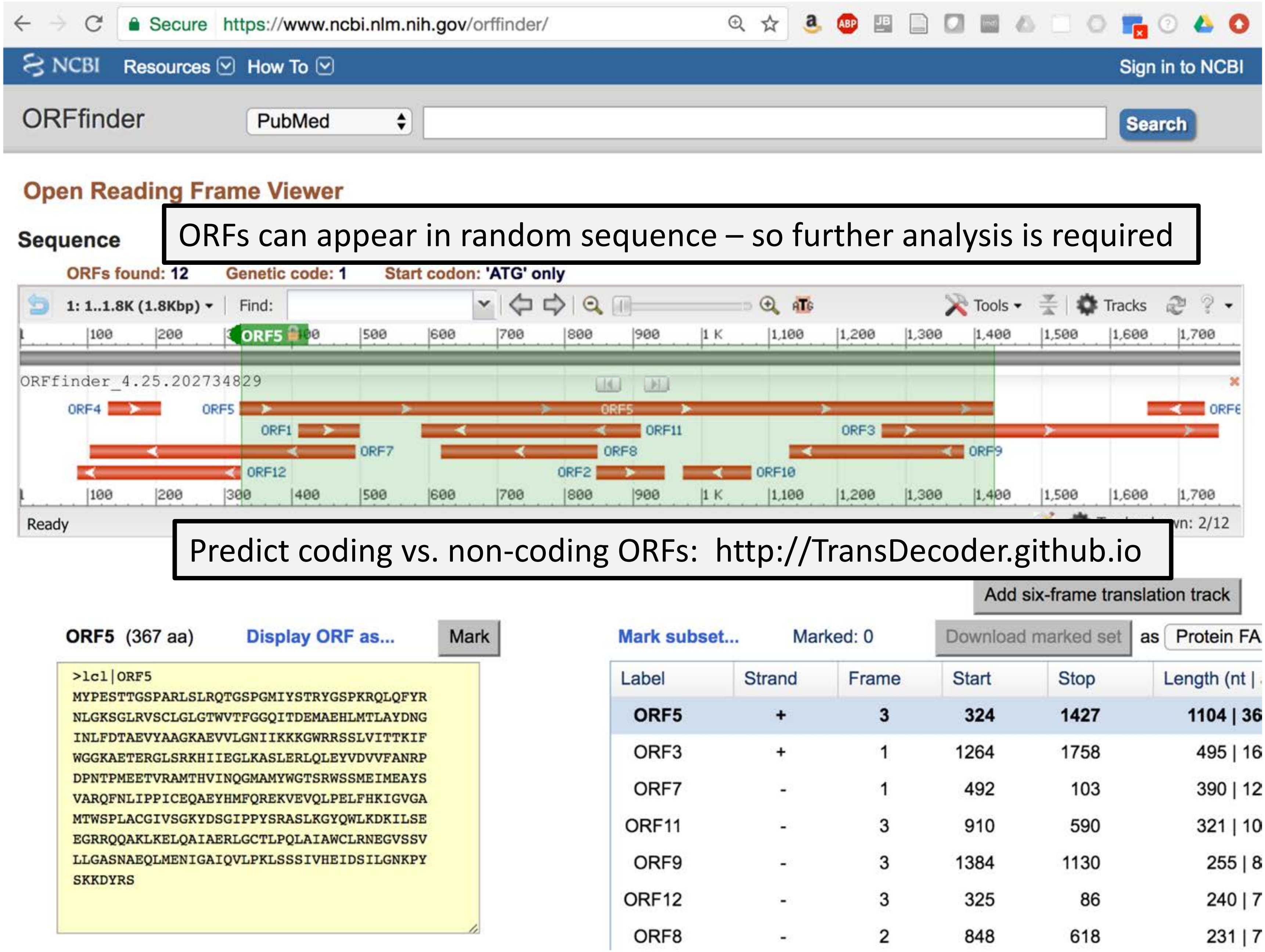
Enter accession number, gi, or nucleotide sequence in FASTA format:

```
GGAGCTGGAGGCCCCCAGGCAACTACACCGTCCACGTACCCAGAGGGGCTGGGCCCTCCC
ACCAGAGACCACGCCCTGGTGTGCCTTAGGGGCCCTGGTTTGTTAGTCTCTGAGTGTGCA
GTTGCTGCACATGGGGCCCTGGCGCTTGCTGCACCAACTTCCTGTTGGGCCCGTGGTCCT
TGGAGGCATGCAGTTCAGCAGACAGTGACTCAGCCATCCACCCAACATGCGGAACGTGTC
TCTTCTGCAGGTCCCGGTCCACAGCAGGATTCCCCCTCTGTGAAAAGGCACGCTGATCTG
TCTGGATAAGTGTGGCCGGCCCCATGTATCCGGAATCAACCACGGGGTCCCCAGCTCGAC
TCTCCCTGCGGCAGACAGGCTCCCCCGGGATGATCTACAGTACTCGTTATGGGAGTCCCA
AAAGACAGCTCCAGTTTTACAGGAATCTGGGCAAATCTGGCCTTCGGGTCTCCTGCCTGG
GGCTTGGAACATGGGTGACCTTCGGGGGCCAGATCACGGATGAGATGGCAGAGCACCTAA
TGACCTTGGCCTACGATAATGGCATCAACCTGTTCGATACGGCGGAGGTCTACGCTGCTG
```

From:

To:

ORFfinder finds all open reading frames and provides translations



ORFs can appear in random sequence – so further analysis is required

ORFs found: 12 Genetic code: 1 Start codon: 'ATG' only

Predict coding vs. non-coding ORFs: http://TransDecoder.github.io

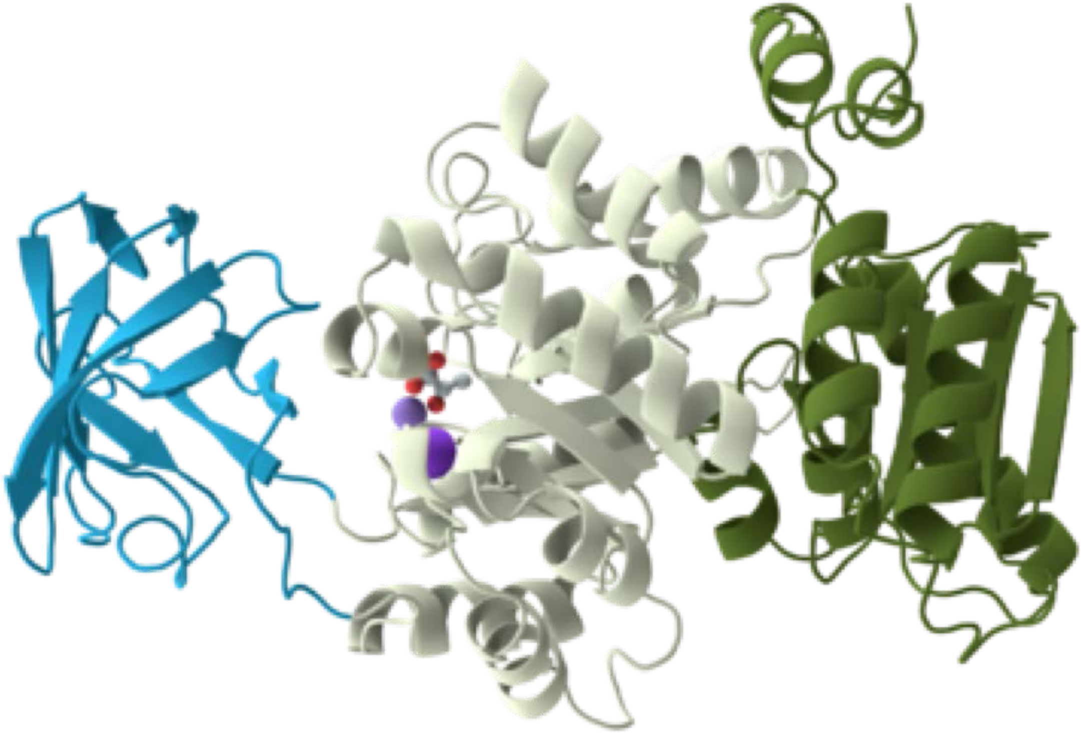
ORF5 (367 aa)

```
>lcl|ORF5
MYPESTTGSPARLSLRQTGSPGMIYSTRYGSPKRQLQFYR
NLGKSGLRVSCLGLGTWVTFGGQITDEMAEHLMTLAYDNG
INLFDTAEVYAAGKAEVVLGNIIKKKGWRRSSLVITTKIF
WGGKAETERGLSRKHIIEGLKASLERLQLEYVDVVFANRP
DPNTPMEETVRAMTHVINQGMAMYWGTSRWSSMEIMEAYS
VARQFNLIPPICEQAEYHMFQREKVEVQLPELFHKIGVGA
MTWSPLACGIVSGKYDSGIPPYSRASLKGYQWLKDKILSE
EGRRQQAKLKELQAIAERLGCTLPQLAIAWCLRNEGVSSV
LLGASNAEQLMENIGAIQVLPKLSSSIVHEIDSILGNKPY
SKKDYRS
```

Marked: 0

Label	Strand	Frame	Start	Stop	Length (nt |
ORF5	**+**	**3**	**324**	**1427**	**1104 | 36**
ORF3	+	1	1264	1758	495 | 16
ORF7	-	1	492	103	390 | 12
ORF11	-	3	910	590	321 | 10
ORF9	-	3	1384	1130	255 | 8
ORF12	-	3	325	86	240 | 7
ORF8	-	2	848	618	231 | 7

Can we recognize functional domains in putative coding regions?

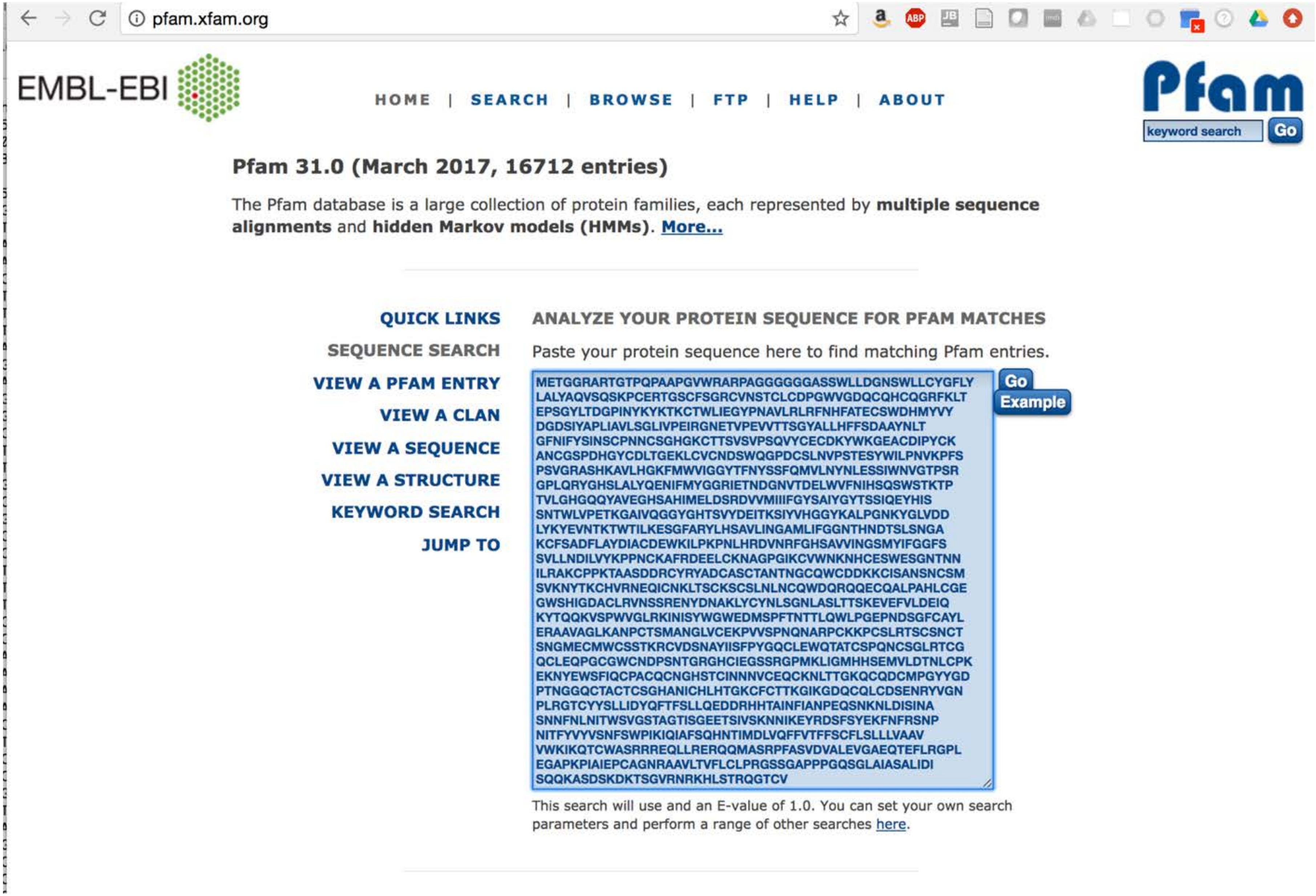


Hints at substrate binding or catalytic activity

DNA, RNA, calcium, phoshate, etc.

Glycoslase, methylase, kinase, nuclease, lipase, protease, etc.

Search the Pfam library of HMMs to identify potential functional domains



Pfam 31.0 (March 2017, 16712 entries)

The Pfam database is a large collection of protein families, each represented by **multiple sequence alignments** and **hidden Markov models (HMMs)**. More...

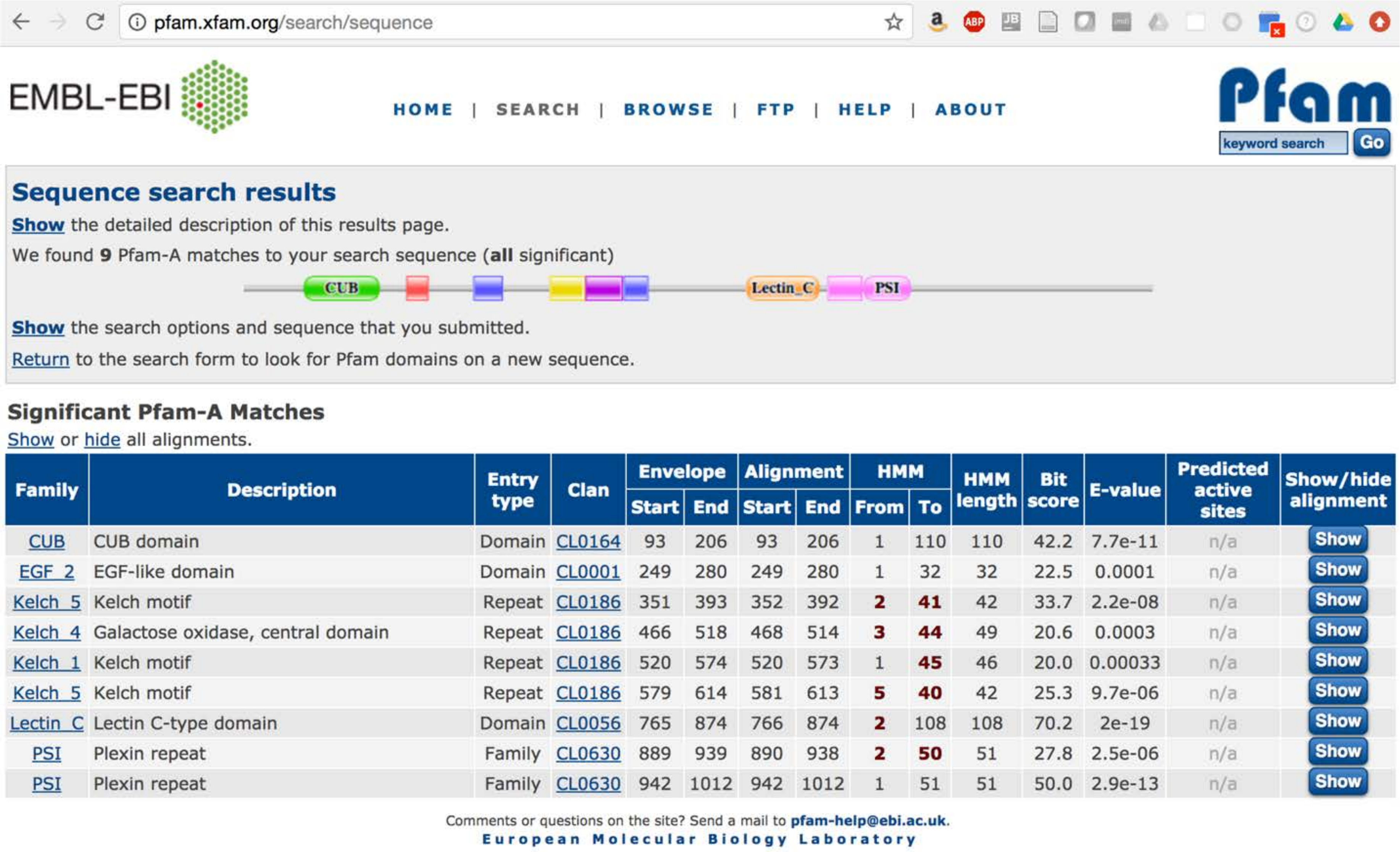
ANALYZE YOUR PROTEIN SEQUENCE FOR PFAM MATCHES

Paste your protein sequence here to find matching Pfam entries.

```
METGGRARTGTPQPAAPGVWRARPAGGGGGGASSWLLDGNSWLLCYGFLY
LALYAQVSQSKPCERTGSCFSGRCVNSTCLCDPGWVGDQCQHCQGRFKLT
EPSGYLTDGPINYKYKTKCTWLIEGYPNAVLRLRFNHFATECSWDHMYVY
DGDSIYAPLIAVLSGLIVPEIRGNETVPEVVTTSGYALLHFFSDAAYNLT
GFNIFYSINSCPNNCSGHGKCTTSVSVPSQVYCECDKYWKGEACDIPYCK
ANCGSPDHGYCDLTGEKLCVCNDSWQGPDCSLNVPSTESYWILPNVKPFS
PSVGRASHKAVLHGKFMWVIGGYTFNYSSFQMVLNYNLESSIWNVGTPSR
GPLQRYGHSLALYQENIFMYGGRIETNDGNVTDELWVFNIHSQSWSTKTP
TVLGHGQQYAVEGHSAHIMELDSRDVVMIIIFGYSAIYGYTSSIQEYHIS
SNTWLVPETKGAIVQGGYGHTSVYDEITKSIYVHGGYKALPGNKYGLVDD
LYKYEVNTKTWTILKESGFARYLHSAVLINGAMLIFGGNTHNDTSLSNGA
KCFSADFLAYDIACDEWKILPKPNLHRDVNRFGHSAVVINGSMYIFGGFS
SVLLNDILVYKPPNCKAFRDEELCKNAGPGIKCVWNKNHCESWESGNTNN
ILRAKCPPKTAASDDRCYRYADCASCTANTNGCQWCDDKKCISANSNCSM
SVKNYTKCHVRNEQICNKLTSCKSCSLNLNCQWDQRQQECQALPAHLCGE
GWSHIGDACLRVNSSRENYDNAKLYCYNLSGNLASLTTSKEVEFVLDEIQ
KYTQQKVSPWVGLRKINISYWGWEDMSPFTNTTLQWLPGEPNDSGFCAYL
ERAAVAGLKANPCTSMANGLVCEKPVVSPNQNARPCKKPCSLRTSCSNCT
SNGMECMWCSSTKRCVDSNAYIISFPYGQCLEWQTATCSPQNCSGLRTCG
QCLEQPGCGWCNDPSNTGRGHCIEGSSRGPMKLIGMHHSEMVLDTNLCPK
EKNYEWSFIQCPACQCNGHSTCINNNVCEQCKNLTTGKQCQDCMPGYYGD
PTNGGQCTACTCSGHANICHLHTGKCFCTTKGIKGDQCQLCDSENRYVGN
PLRGTCYYSLLIDYQFTFSLLQEDDRHHTAINFIANPEQSNKNLDISINA
SNNFNLNITWSVGSTAGTISGEETSIVSKNNIKEYRDSFSYEKFNFRSNP
NITFYVYVSNFSWPIKIQIAFSQHNTIMDLVQFFVTFFSCFLSLLLVAAV
VWKIKQTCWASRRREQLLRERQQMASRPFASVDVALEVGAEQTEFLRGPL
EGAPKPIAIEPCAGNRAAVLTVFLCLPRGSSGAPPPGQSGLAIASALIDI
SQQKASDSKDKTSGVRNRKHLSTRQGTCV
```

This search will use and an E-value of 1.0. You can set your own search parameters and perform a range of other searches here.

Example Pfam report illustrating modular domain architecture



pfam.xfam.org/search/sequence
EMBL-EBI
HOME | SEARCH | BROWSE | FTP | HELP | ABOUT
Pfam
keyword search
Go
CUB
Lectin_C
PSI

Sequence search results

Show the detailed description of this results page.

We found **9** Pfam-A matches to your search sequence (**all** significant)

Show the search options and sequence that you submitted.

Return to the search form to look for Pfam domains on a new sequence.

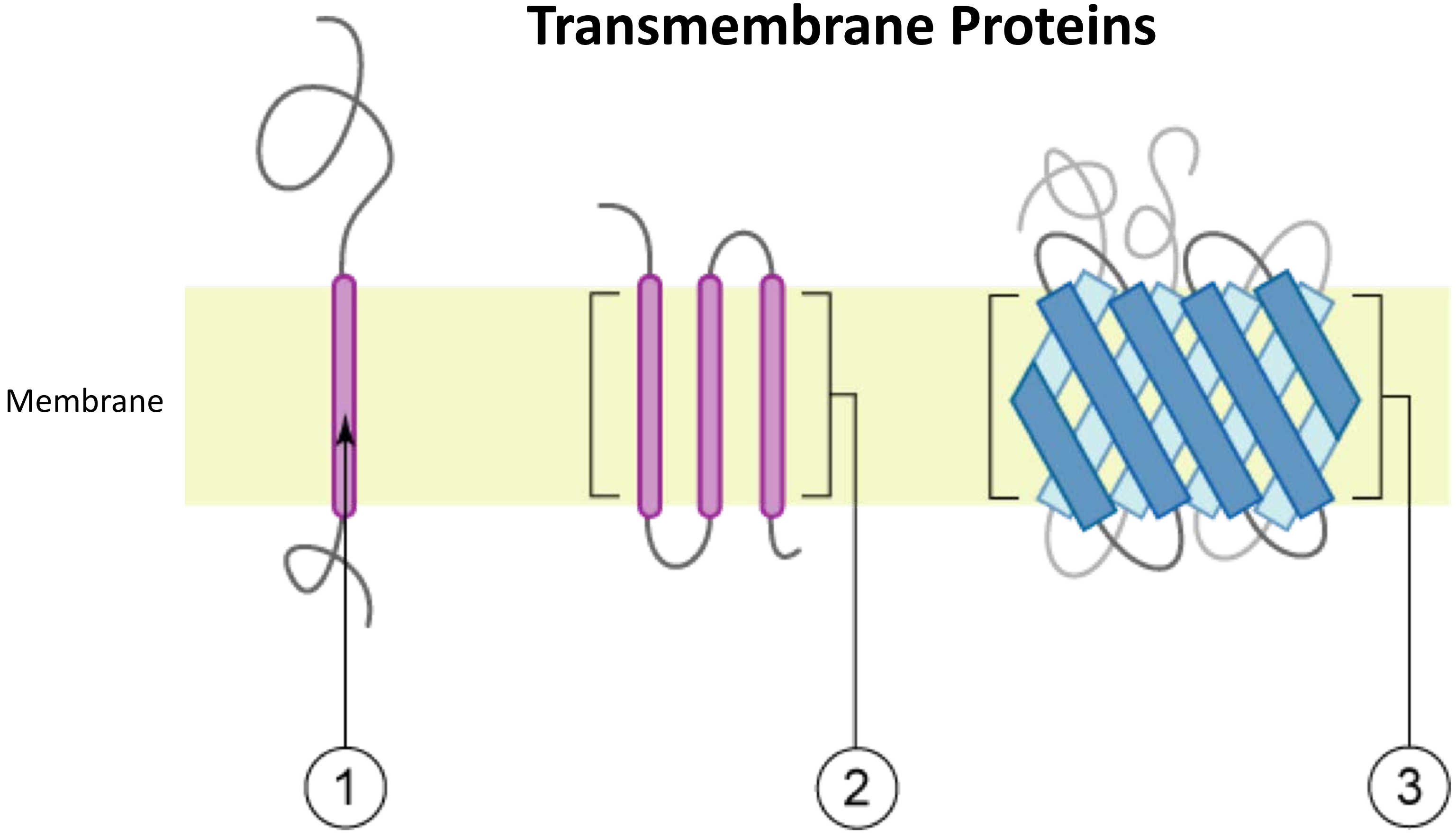
Significant Pfam-A Matches

Show or hide all alignments.

Family	Description	Entry type	Clan	Envelope		Alignment		HMM		HMM length	Bit score	E-value	Predicted active sites	Show/hide alignment
				Start	End	Start	End	From	To					
CUB	CUB domain	Domain	CL0164	93	206	93	206	1	110	110	42.2	7.7e-11	n/a	Show
EGF_2	EGF-like domain	Domain	CL0001	249	280	249	280	1	32	32	22.5	0.0001	n/a	Show
Kelch_5	Kelch motif	Repeat	CL0186	351	393	352	392	**2**	**41**	42	33.7	2.2e-08	n/a	Show
Kelch_4	Galactose oxidase, central domain	Repeat	CL0186	466	518	468	514	**3**	**44**	49	20.6	0.0003	n/a	Show
Kelch_1	Kelch motif	Repeat	CL0186	520	574	520	573	1	**45**	46	20.0	0.00033	n/a	Show
Kelch_5	Kelch motif	Repeat	CL0186	579	614	581	613	**5**	**40**	42	25.3	9.7e-06	n/a	Show
Lectin_C	Lectin C-type domain	Domain	CL0056	765	874	766	874	**2**	108	108	70.2	2e-19	n/a	Show
PSI	Plexin repeat	Family	CL0630	889	939	890	938	**2**	**50**	51	27.8	2.5e-06	n/a	Show
PSI	Plexin repeat	Family	CL0630	942	1012	942	1012	1	51	51	50.0	2.9e-13	n/a	Show

Comments or questions on the site? Send a mail to **pfam-help@ebi.ac.uk**.
European Molecular Biology Laboratory

Transmembrane Proteins



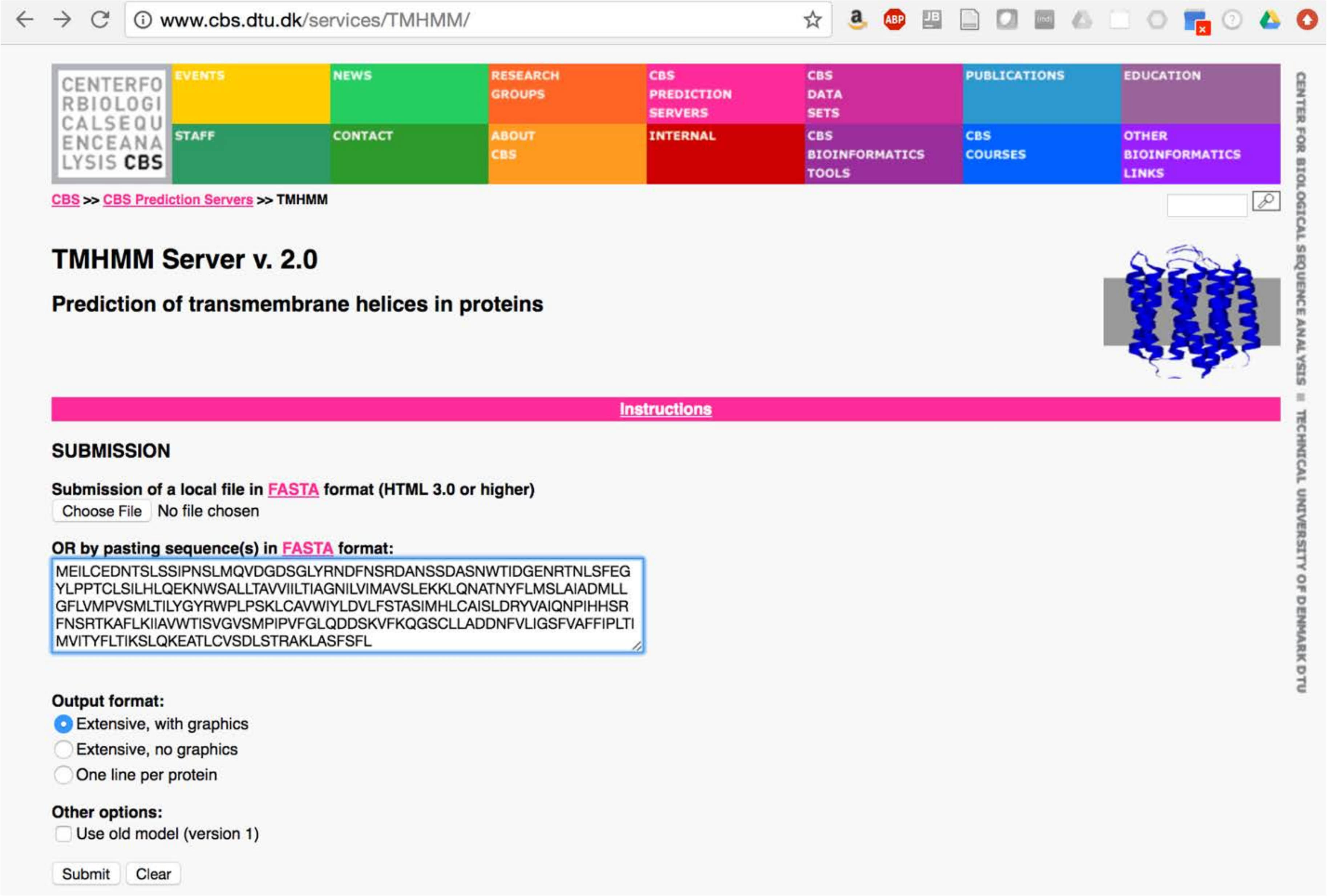
Single transmembrane α-helix (bitopic membrane protein)

Polytopic transmembrane α-helical protein

Polytopic transmembrane β-sheet protein

From: https://en.wikipedia.org/wiki/Transmembrane_protein

Using TMHMM to identify putative transmembrane proteins


www.cbs.dtu.dk/services/TMHMM/
CENTERFORBIOLOGICALSEQUENCEANALYSIS CBS
EVENTS
NEWS
RESEARCH GROUPS
CBS PREDICTION SERVERS
CBS DATA SETS
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OTHER BIOINFORMATICS LINKS
CENTER FOR BIOLOGICAL SEQUENCE ANALYSIS ▪ TECHNICAL UNIVERSITY OF DENMARK DTU

CBS >> CBS Prediction Servers >> TMHMM

TMHMM Server v. 2.0

Prediction of transmembrane helices in proteins

Instructions

SUBMISSION

Submission of a local file in FASTA format (HTML 3.0 or higher)

Choose File No file chosen

OR by pasting sequence(s) in FASTA format:

```
MEILCEDNTSLSSIPNSLMQVDGDSGLYRNDFNSRDANSSDASNWTIDGENRTNLSFEG
YLPPTCLSILHLQEKNWSALLTAVVIILTIAGNILVIMAVSLEKKLQNATNYFLMSLAIADMLL
GFLVMPVSMLTILYGYRWPLPSKLCAVWIYLDVLFSTASIMHLCAISLDRYVAIQNPIHHSR
FNSRTKAFLKIIAVWTISVGVSMPIPVFGLQDDSKVFKQGSCLLADDNFVLIGSFVAFFIPLTI
MVITYFLTIKSLQKEATLCVSDLSTRAKLASFSFL
```

Output format:

● Extensive, with graphics

○ Extensive, no graphics

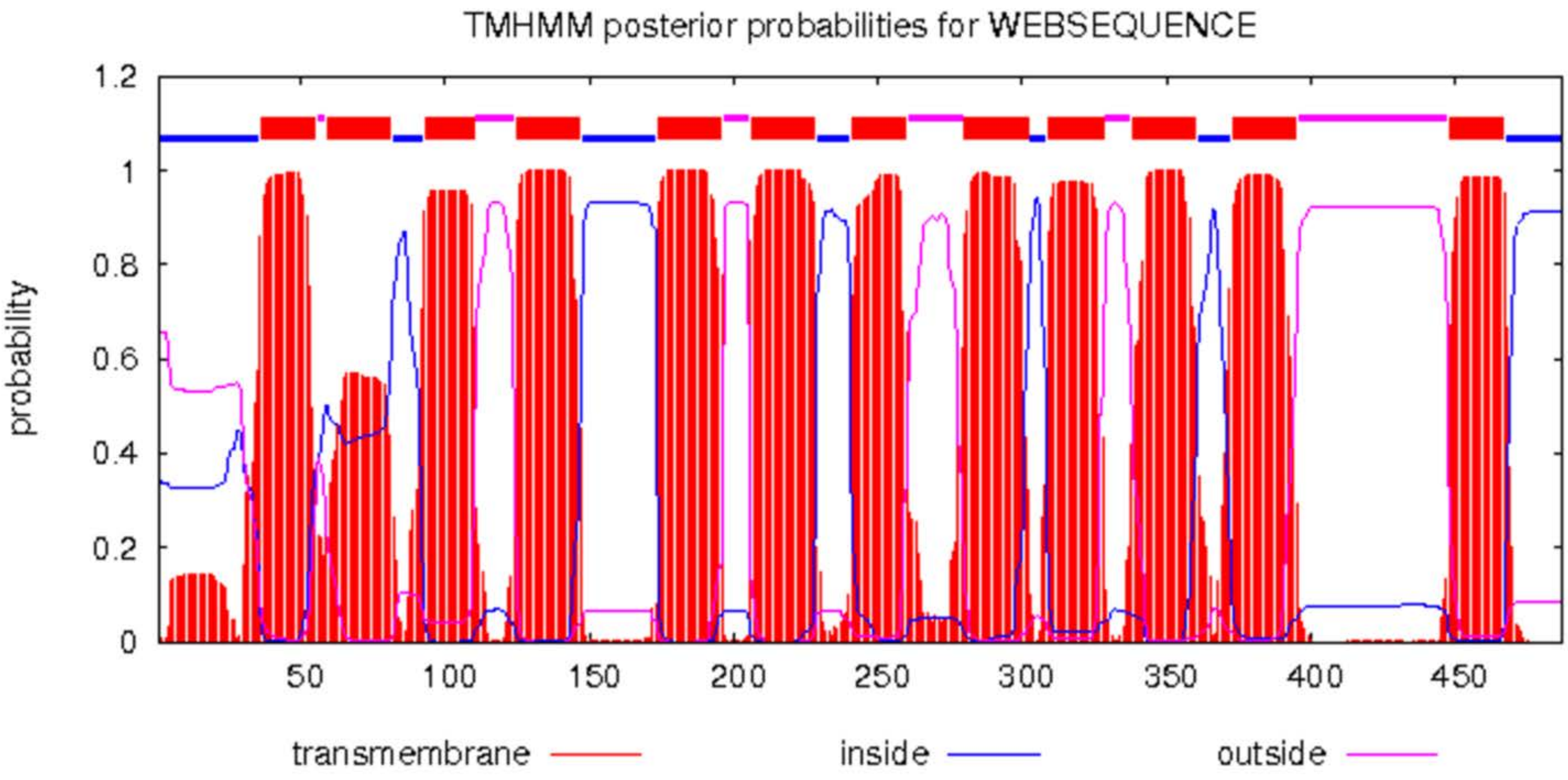
○ One line per protein

Other options:

□ Use old model (version 1)

Submit Clear

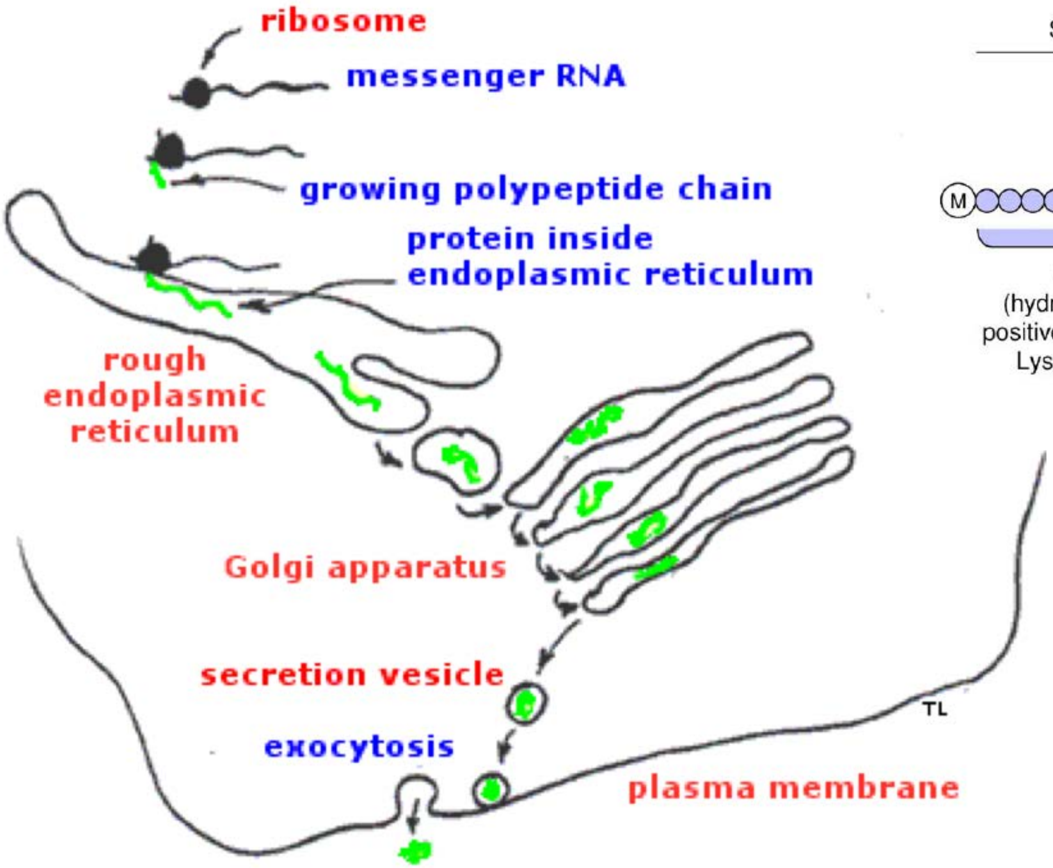
Trans-membrane Domains via TmHMM



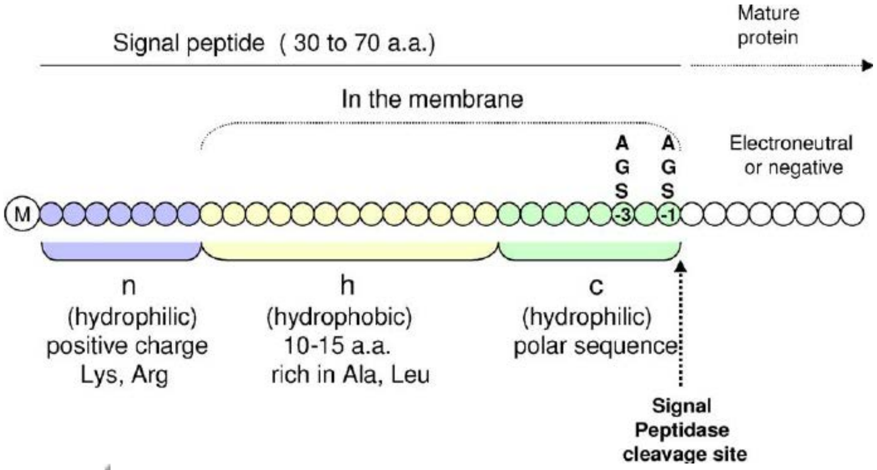
Topology=i36-55o59-81i93-110o125-147i174-196o206-228i241-260o280-302i309-328o338-360i373-395o448-467i

http://www.cbs.dtu.dk/services/TMHMM/

Predicting Secreted Proteins



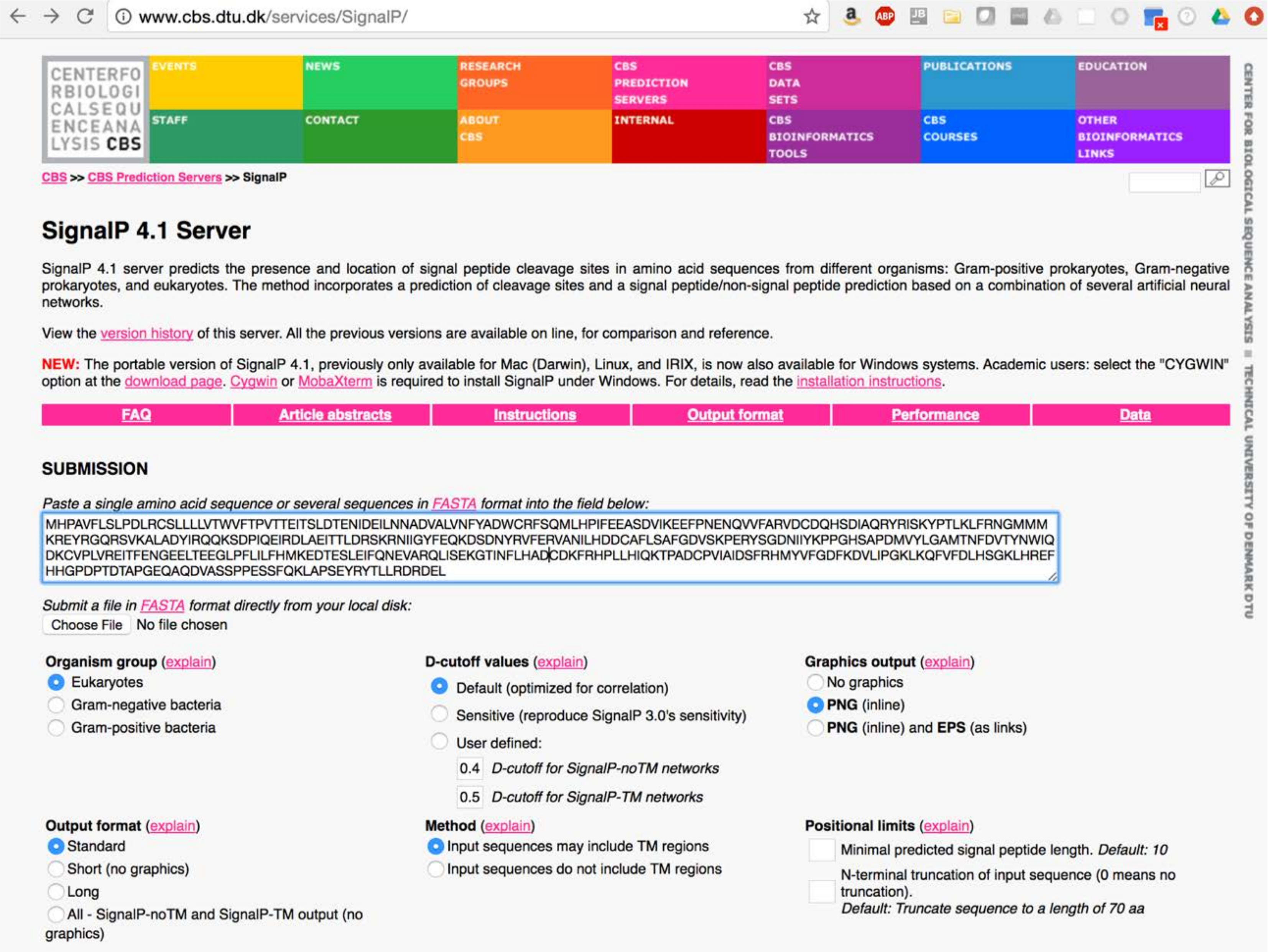
(from: https://courses.washington.edu/conj/cell/secretion.htm)



(from: Vaccine 23(15):1770-8)

SignalP: Prediction of N-terminal signal peptides

(predict secreted proteins)


www.cbs.dtu.dk/services/SignalP/
CENTERFORBIOLOGICALSEQUENCEANALYSIS CBS
EVENTS
NEWS
RESEARCH GROUPS
CBS PREDICTION SERVERS
CBS DATA SETS
PUBLICATIONS
EDUCATION
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OTHER BIOINFORMATICS LINKS
CENTER FOR BIOLOGICAL SEQUENCE ANALYSIS ≡ TECHNICAL UNIVERSITY OF DENMARK DTU

CBS >> CBS Prediction Servers >> SignalP

SignalP 4.1 Server

SignalP 4.1 server predicts the presence and location of signal peptide cleavage sites in amino acid sequences from different organisms: Gram-positive prokaryotes, Gram-negative prokaryotes, and eukaryotes. The method incorporates a prediction of cleavage sites and a signal peptide/non-signal peptide prediction based on a combination of several artificial neural networks.

View the version history of this server. All the previous versions are available on line, for comparison and reference.

NEW: The portable version of SignalP 4.1, previously only available for Mac (Darwin), Linux, and IRIX, is now also available for Windows systems. Academic users: select the "CYGWIN" option at the download page. Cygwin or MobaXterm is required to install SignalP under Windows. For details, read the installation instructions.

SUBMISSION

Paste a single amino acid sequence or several sequences in FASTA format into the field below:

```
MHPAVFLSLPDLRCSLLLLVTWVFTPVTTEITSLDTENIDEILNNADVALVNFYADWCRFSQMLHPIFEEASDVIKEEFPNENQVVFARVDCDQHSDIAQRYRISKYPTLKLFRNGMMM
KREYRGQRSVKALADYIRQQKSDPIQEIRDLAEITTLDRSKRNIIGYFEQKDSDNYRVFERVANILHDDCAFLSAFGDVSKPERYSGDNIIYKPPGHSAPDMVYLGAMTNFDVTYNWIQ
DKCVPLVREITFENGEELTEEGLPFLILFHMKEDTESLEIFQNEVARQLISEKGTINFLHADCDKFRHPLLHIQKTPADCPVIAIDSFRHMYVFGDFKDVLIPGKLKQFVFDLHSGKLHREF
HHGPDPTDTAPGEQAQDVASSPPESSFQKLAPSEYRYTLLRDRDEL
```

Submit a file in FASTA format directly from your local disk:

Choose File No file chosen

Organism group (explain)

- ● Eukaryotes
- ○ Gram-negative bacteria
- ○ Gram-positive bacteria

D-cutoff values (explain)

- ● Default (optimized for correlation)
- ○ Sensitive (reproduce SignalP 3.0's sensitivity)
- ○ User defined:
  - 0.4 *D-cutoff for SignalP-noTM networks*
  - 0.5 *D-cutoff for SignalP-TM networks*

Graphics output (explain)

- ○ No graphics
- ● **PNG** (inline)
- ○ **PNG** (inline) and **EPS** (as links)

Output format (explain)

- ● Standard
- ○ Short (no graphics)
- ○ Long
- ○ All - SignalP-noTM and SignalP-TM output (no graphics)

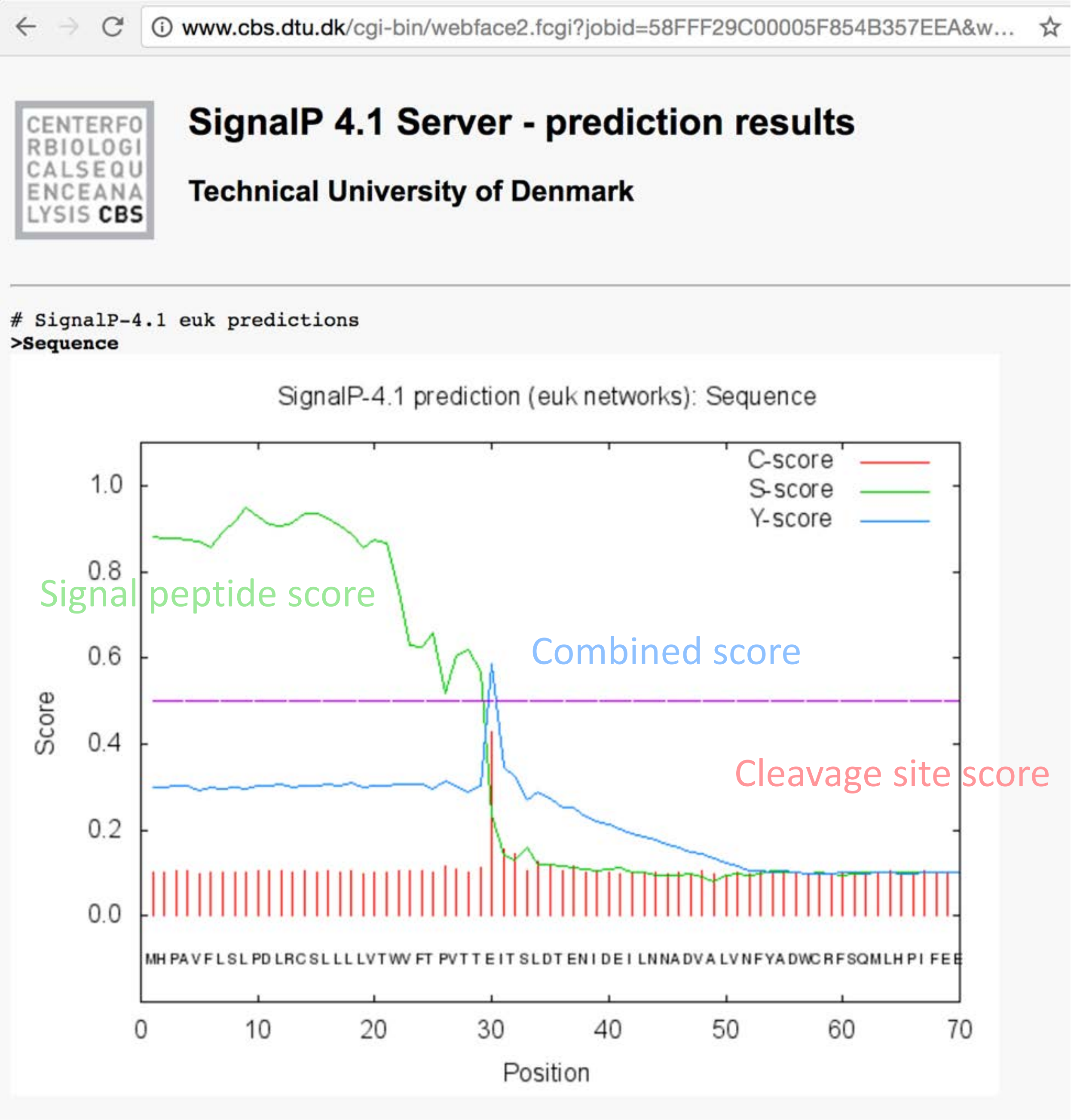
Method (explain)

- ● Input sequences may include TM regions
- ○ Input sequences do not include TM regions

Positional limits (explain)

- Minimal predicted signal peptide length. *Default: 10*
- N-terminal truncation of input sequence (0 means no truncation). *Default: Truncate sequence to a length of 70 aa*

Example SignalP predicted signal peptide



www.cbs.dtu.dk/cgi-bin/webface2.fcgi?jobid=58FFF29C00005F854B357EEA&w…
CENTERFORBIOLOGICALSEQUENCEANALYSIS CBS

SignalP 4.1 Server - prediction results

Technical University of Denmark

```
# SignalP-4.1 euk predictions
>Sequence
```

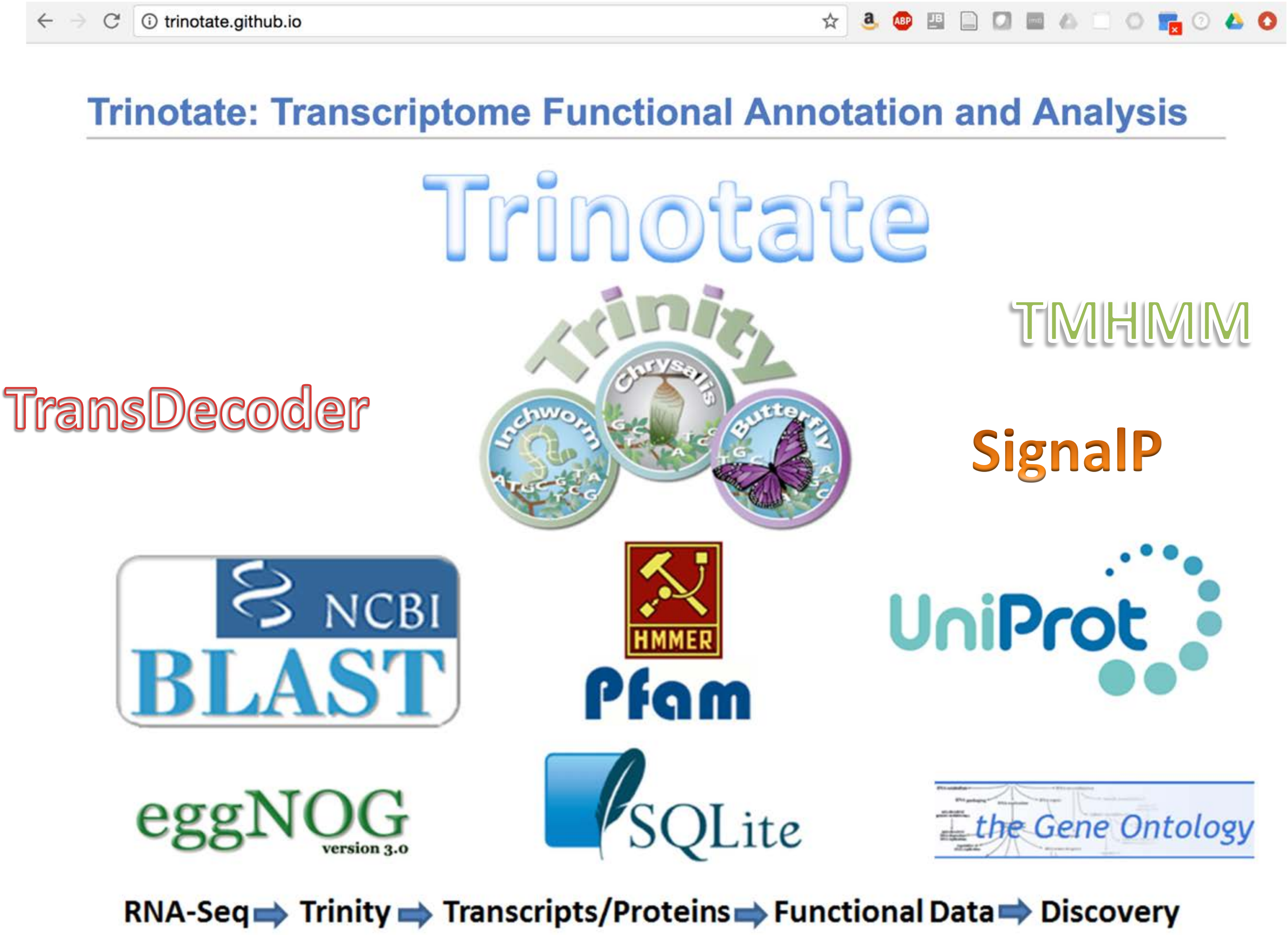
SignalP-4.1 prediction (euk networks): Sequence
C-score
S-score
Y-score
1.0
0.8
0.6
0.4
0.2
0.0
Score
MH PA V F L S L PD L RC S L L L L V T WV FT PV T T E I T S L D T E N I D E I L N N A D V A L V N F Y A D W C R F S Q M L H P I F E E
0
10
20
30
40
50
60
70
Position

Signal peptide score

Combined score

Cleavage site score

Transcriptome-scale functional annotation using Trinotate

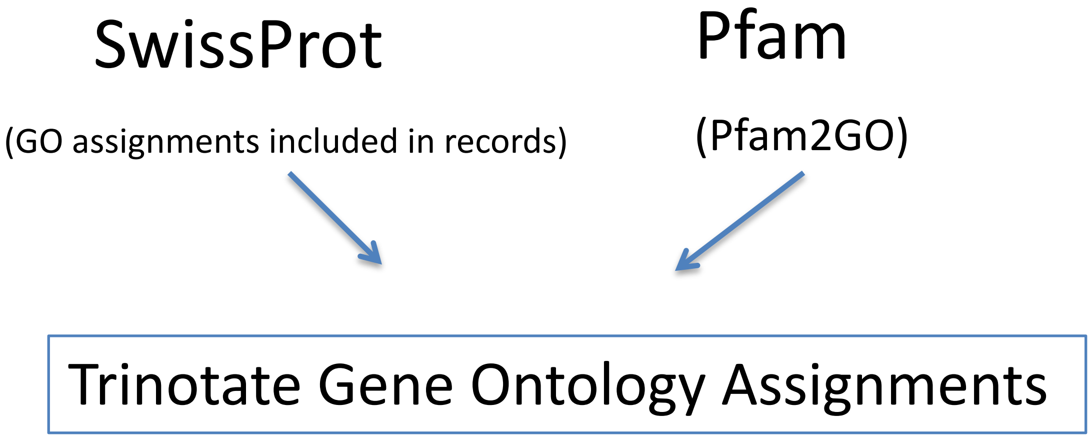


trinotate.github.io

Trinotate: Transcriptome Functional Annotation and Analysis

RNA-Seq ➡ Trinity ➡ Transcripts/Proteins ➡ Functional Data ➡ Discovery

GoSeq for Functional Enrichment Testing



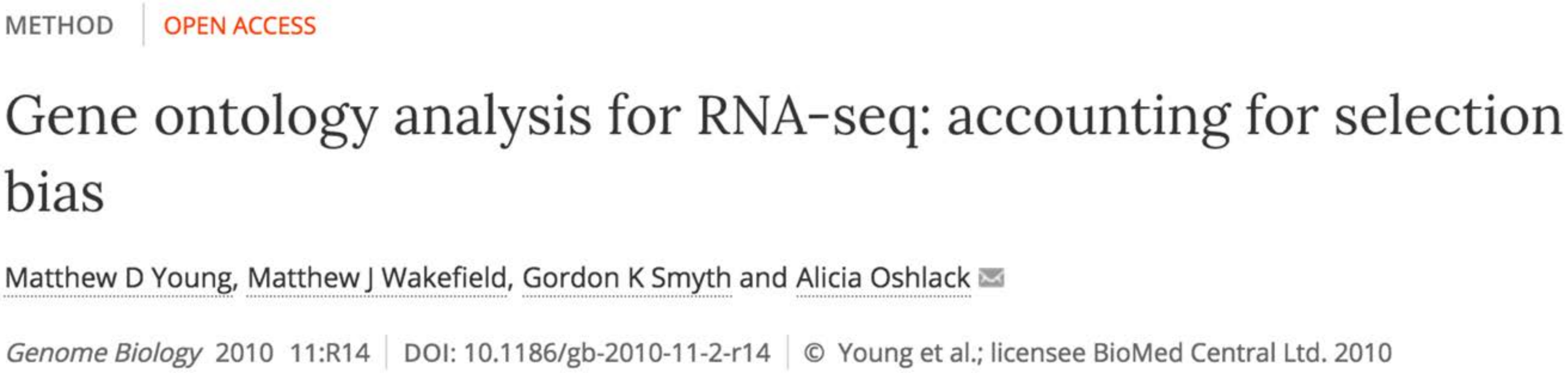
SwissProt

(GO assignments included in records)

Pfam

(Pfam2GO)

Trinotate Gene Ontology Assignments



METHOD | OPEN ACCESS

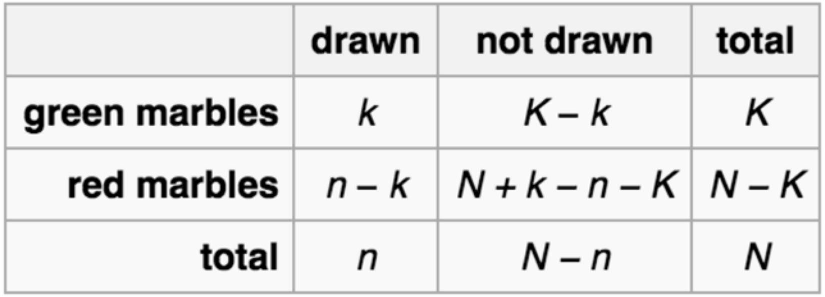
Gene ontology analysis for RNA-seq: accounting for selection bias

Matthew D Young, Matthew J Wakefield, Gordon K Smyth and Alicia Oshlack

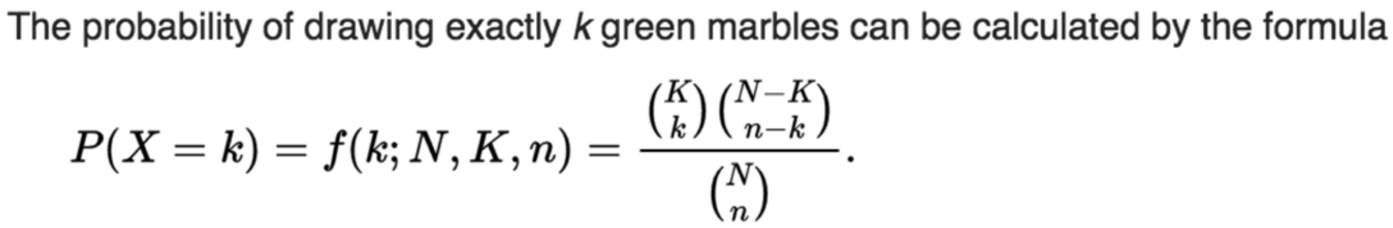
Genome Biology 2010 11:R14 | DOI: 10.1186/gb-2010-11-2-r14 | © Young et al.; licensee BioMed Central Ltd. 2010

Gene ontology functional enrichment

	(+) Differentially Expressed	(-) Not Differentially Expressed	Totals
+ Gene Ontology	50	200	250
- Gene Ontology	1950	17800	19750
Totals	2000	18000	20000



	drawn	not drawn	total
green marbles	k	$K - k$	K
red marbles	$n - k$	$N + k - n - K$	$N - K$
total	n	$N - n$	N

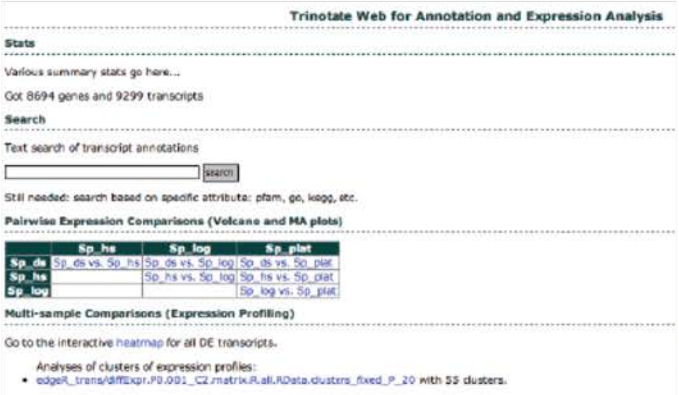


The probability of drawing exactly *k* green marbles can be calculated by the formula

$$P(X = k) = f(k; N, K, n) = \frac{\binom{K}{k}\binom{N-K}{n-k}}{\binom{N}{n}}.$$

Trinotate Web for Interactive Analysis

TrinotateWeb Entry Point



Trinotate Web for Annotation and Expression Analysis

Stats

Various summary stats go here...

Got 8694 genes and 9299 transcripts

Search

Text search of transcript annotations

search

Still needed: search based on specific attribute: pfam, go, kegg, etc.

Pairwise Expression Comparisons (Volcano and MA plots)

	Sp_hs	Sp_log	Sp_plat
Sp_ds	Sp_ds vs. Sp_hs	Sp_ds vs. Sp_log	Sp_ds vs. Sp_plat
Sp_hs		Sp_hs vs. Sp_log	Sp_hs vs. Sp_plat
Sp_log			Sp_log vs. Sp_plat

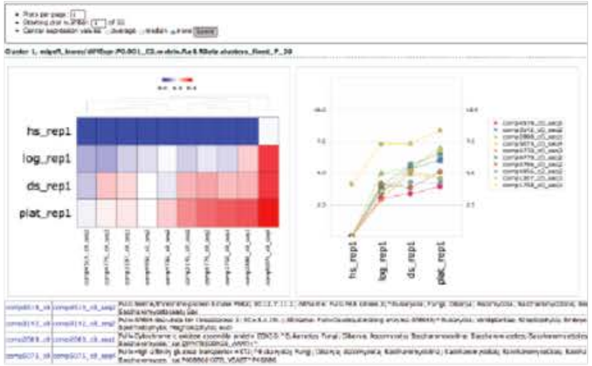
Multi-sample Comparisons (Expression Profiling)

Go to the interactive heatmap for all DE transcripts.

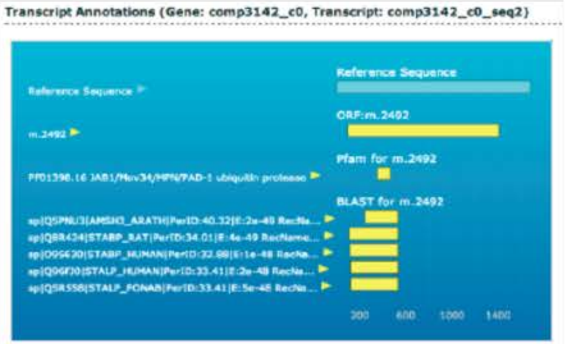
Analyses of clusters of expression profiles:

- edgeR_trans/diffExpr.P0.001_C2.matrix.R.all.RData.clusters_fixed_P_20 with 55 clusters.

Clustered Expression Profiles

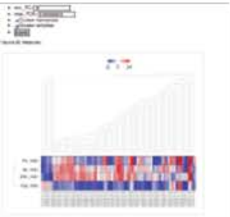


Transcript/Protein Annotation Report
Blast Hits, Pfam Domains, etc.

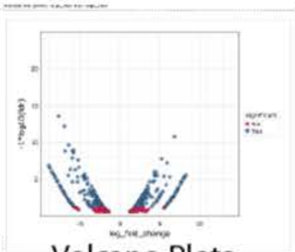


Transcript Annotations (Gene: comp3142_c0, Transcript: comp3142_c0_seq2)

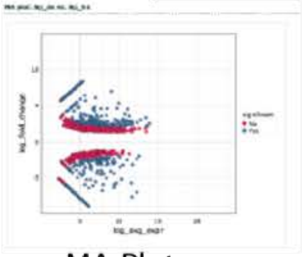
Reference Sequence
Reference Sequence
m.2492
ORF:m.2492
PF01398.16 JAB1/Mov34/MPN/PAD-1 ubiquitin protease
Pfam for m.2492
BLAST for m.2492
sp|Q5PNU3|AMSH3_ARATH|PerID:40.32|E:2e-49 RecNa...
sp|Q8R424|STABP_RAT|PerID:34.01|E:4e-49 RecName...
sp|O95630|STABP_HUMAN|PerID:32.88|E:1e-48 RecNa...
sp|Q96FJ0|STALP_HUMAN|PerID:33.41|E:2e-48 RecNa...
sp|Q5R558|STALP_PONAB|PerID:33.41|E:5e-48 RecNa...
200
600
1000
1400



Heatmaps

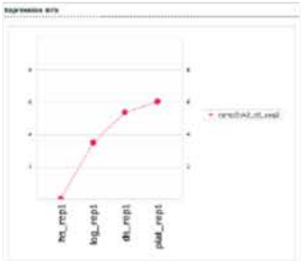


Volcano Plots



MA-Plots

Very Early Release and Just Scratching the Surface



Individual Transcript Expression Profiles

Transcript and Protein Sequences

Part 8. Case study: salamander transcriptome

Deciphering the Cell Circuitry of Limb Regeneration Via Single Cell Transcriptome Studies

Work done in collaboration with Jessica Whited's lab

BWH

BRMC

Brigham Regenerative Medicine Center

Axolotl (*Ambystoma mexicanum*) Transcriptomics

Axolotl "water monster”, aka Mexican salamander or Mexican walking fish.

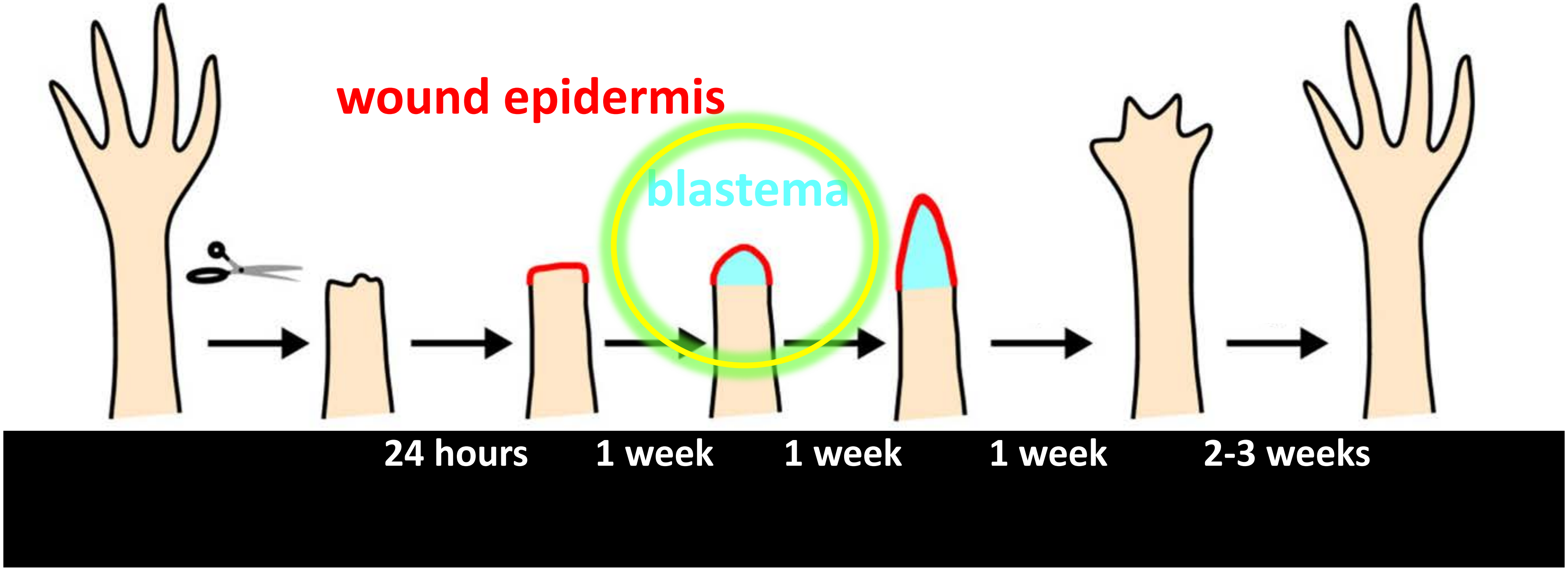
- Model for vertebrate studies of tissue regeneration
- Short generation time
- Can fully regenerate a severed limb in just weeks.
- Genome estimated at ~30 Gb (not yet sequenced)

Google Anonymous Axolotl Icon

Lovable Pets, Too!

Rayan Chikhi's
pet axolotls

Key morphological steps during limb regeneration



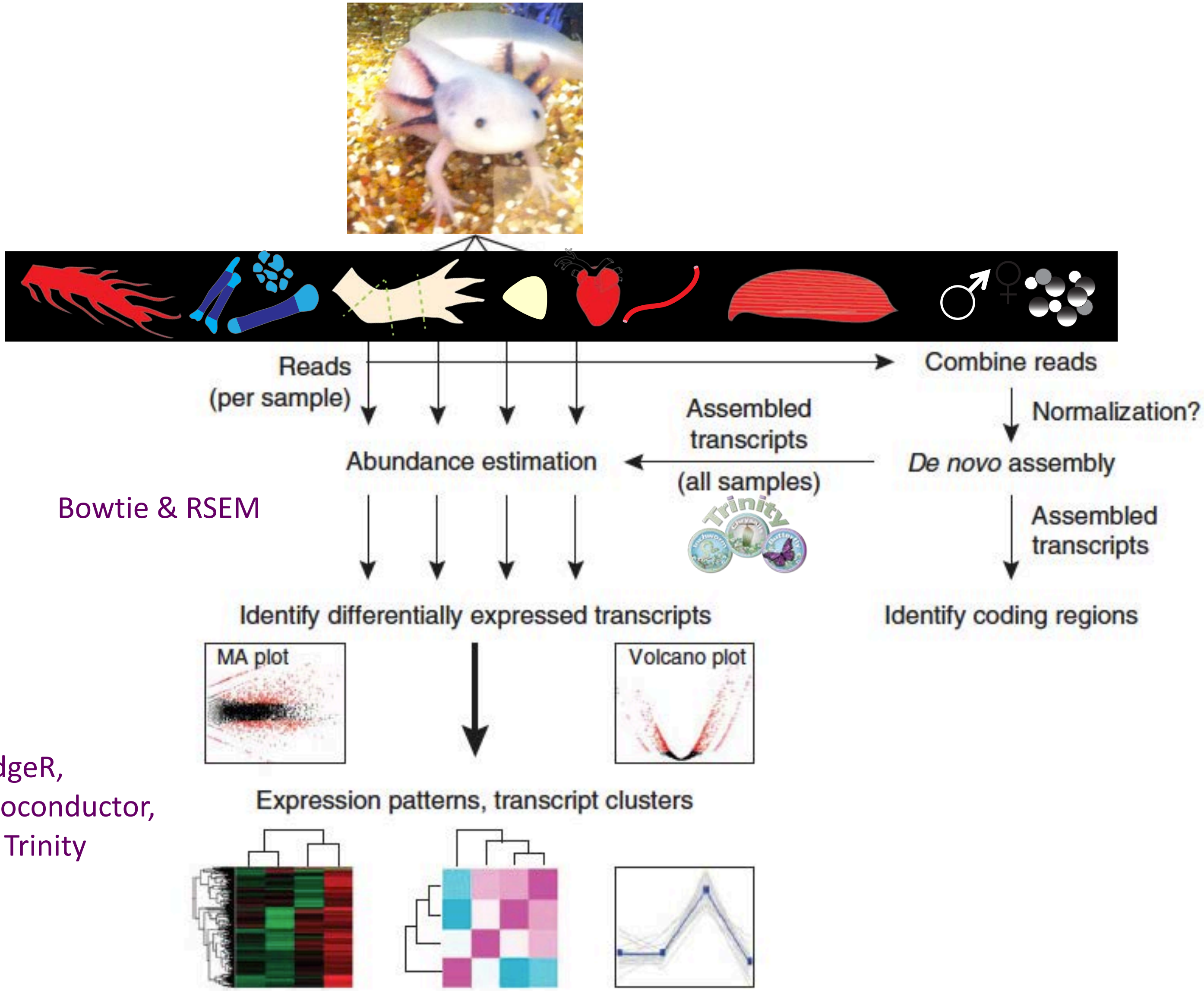
Jessica Whited, Mark Mannucci, Ari Haberberg

1. Building a reference Axolotl transcriptome

limb tissues and select other tissues with biological replicates

1.3 billion of 100 bp paired-end Illumina reads

Framework for De novo Transcriptome Assembly and Analysis



Reads (per sample)

Combine reads

Normalization?

Assembled transcripts

(all samples)

Abundance estimation

De novo assembly

Assembled transcripts

Identify differentially expressed transcripts

Identify coding regions

Expression patterns, transcript clusters

1.3 Billion Total Reads

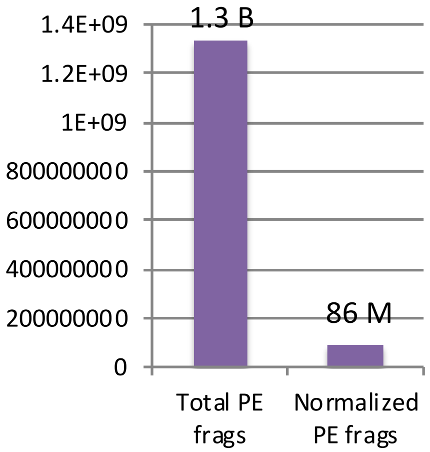
86 Million Normalized Reads

Bowtie & RSEM

EdgeR, Bioconductor, & Trinity

Axolotl Transcriptome De novo Assembly Statistics And Quality Assessment

In silico Normalization

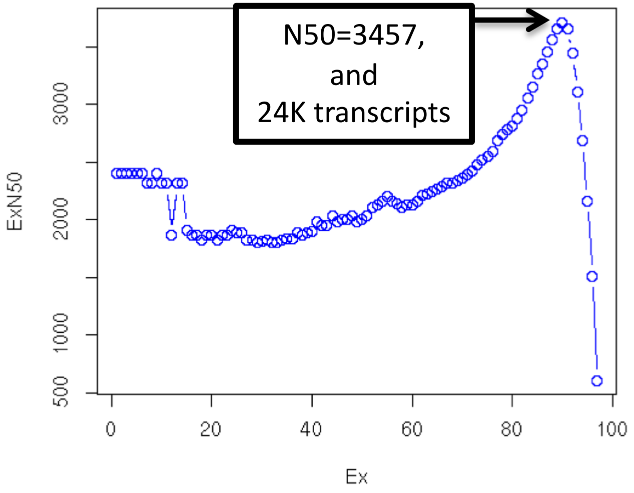


Counts of Transcripts

Trinity contigs (transcripts)	**1,554,055**
Trinity components (genes)	**1,388,798**

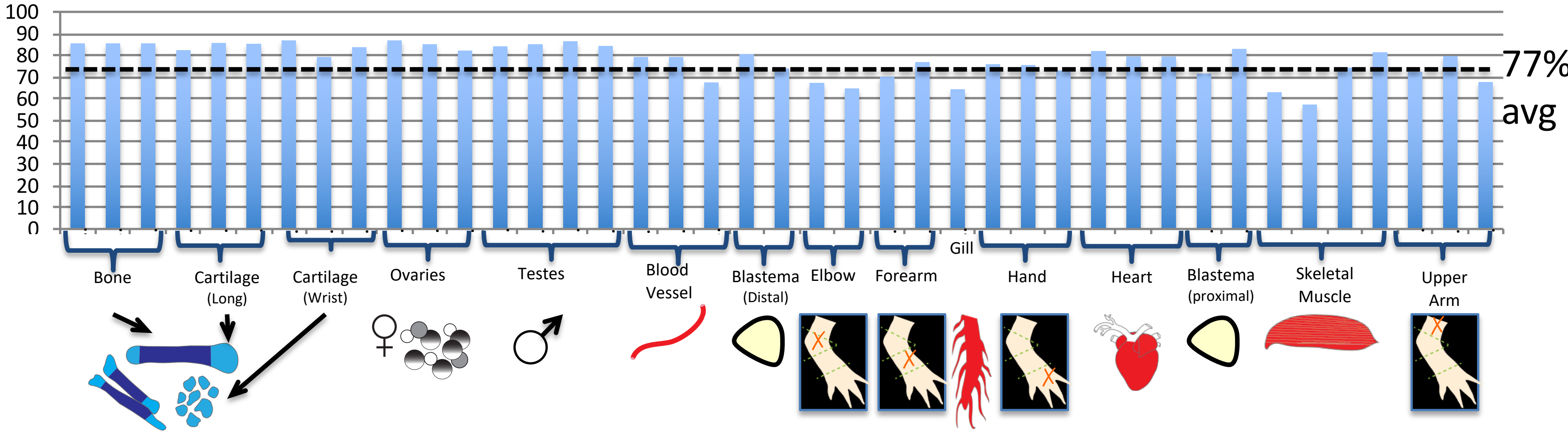
Min. length 200 bases

ExN50 looks good!

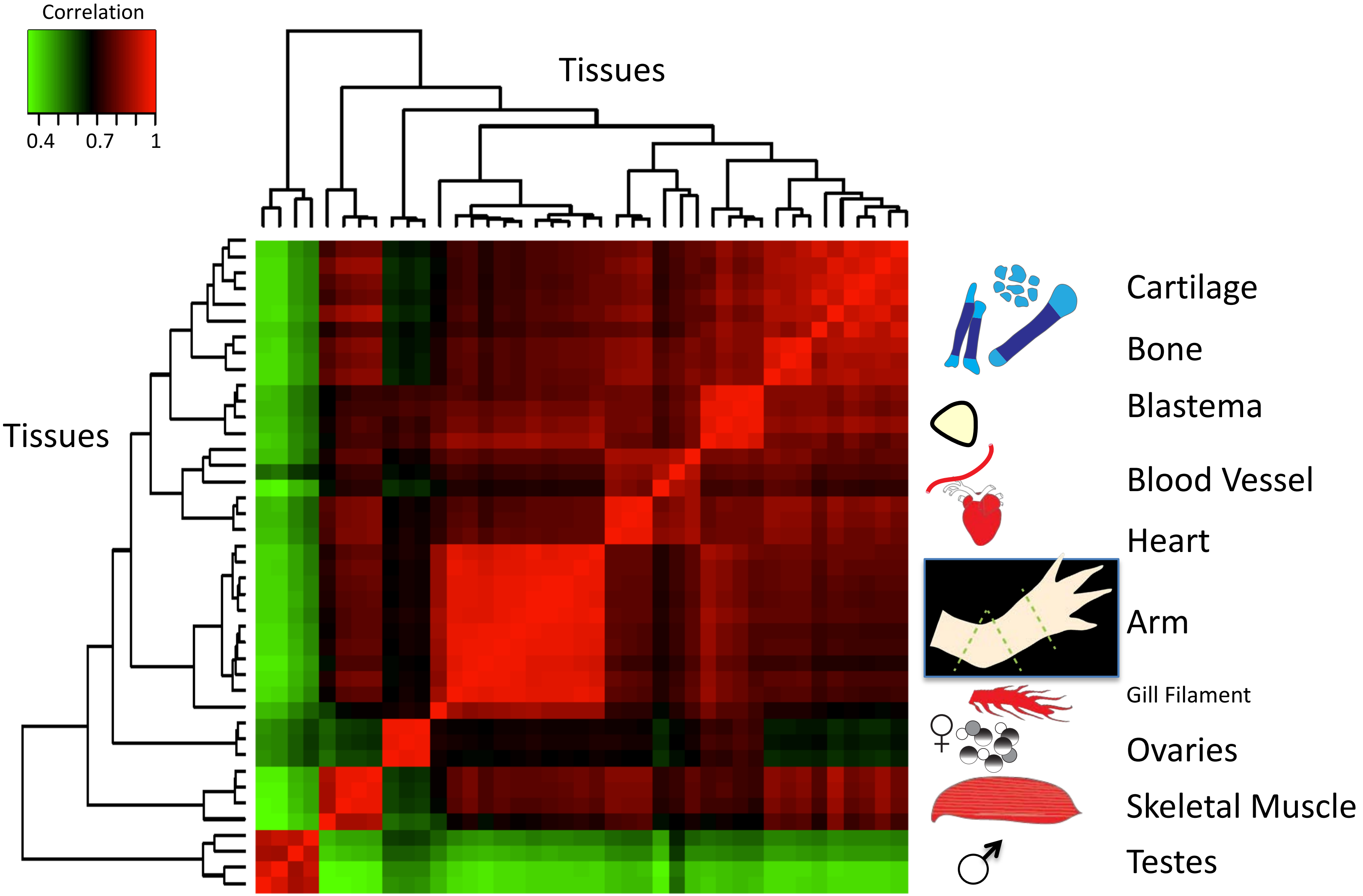


N50=3457, and 24K transcripts

Percent of Non-normalized Fragments Mapping as Properly Paired to Transcriptome

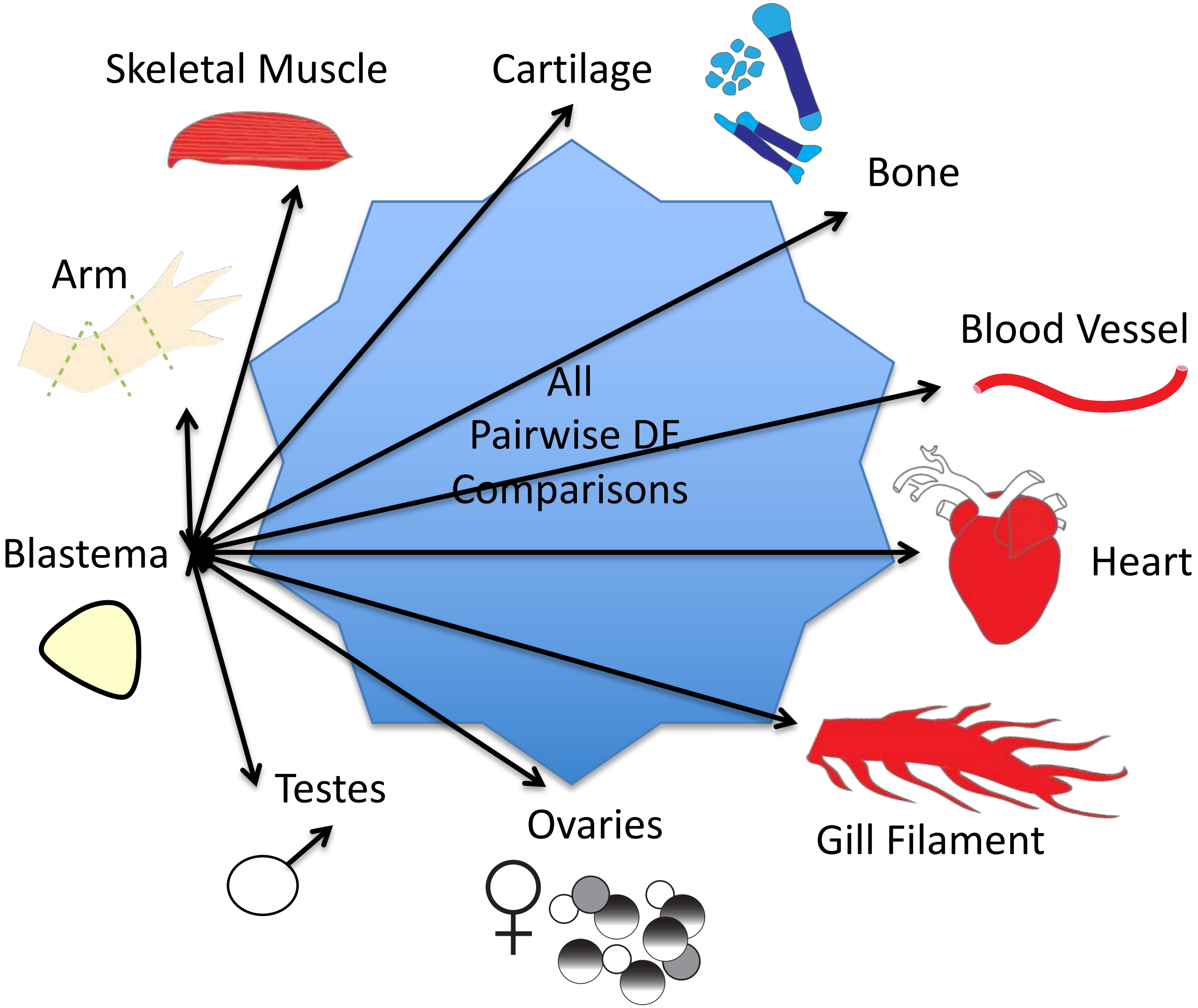


Biological Replicates Cluster According to Sample



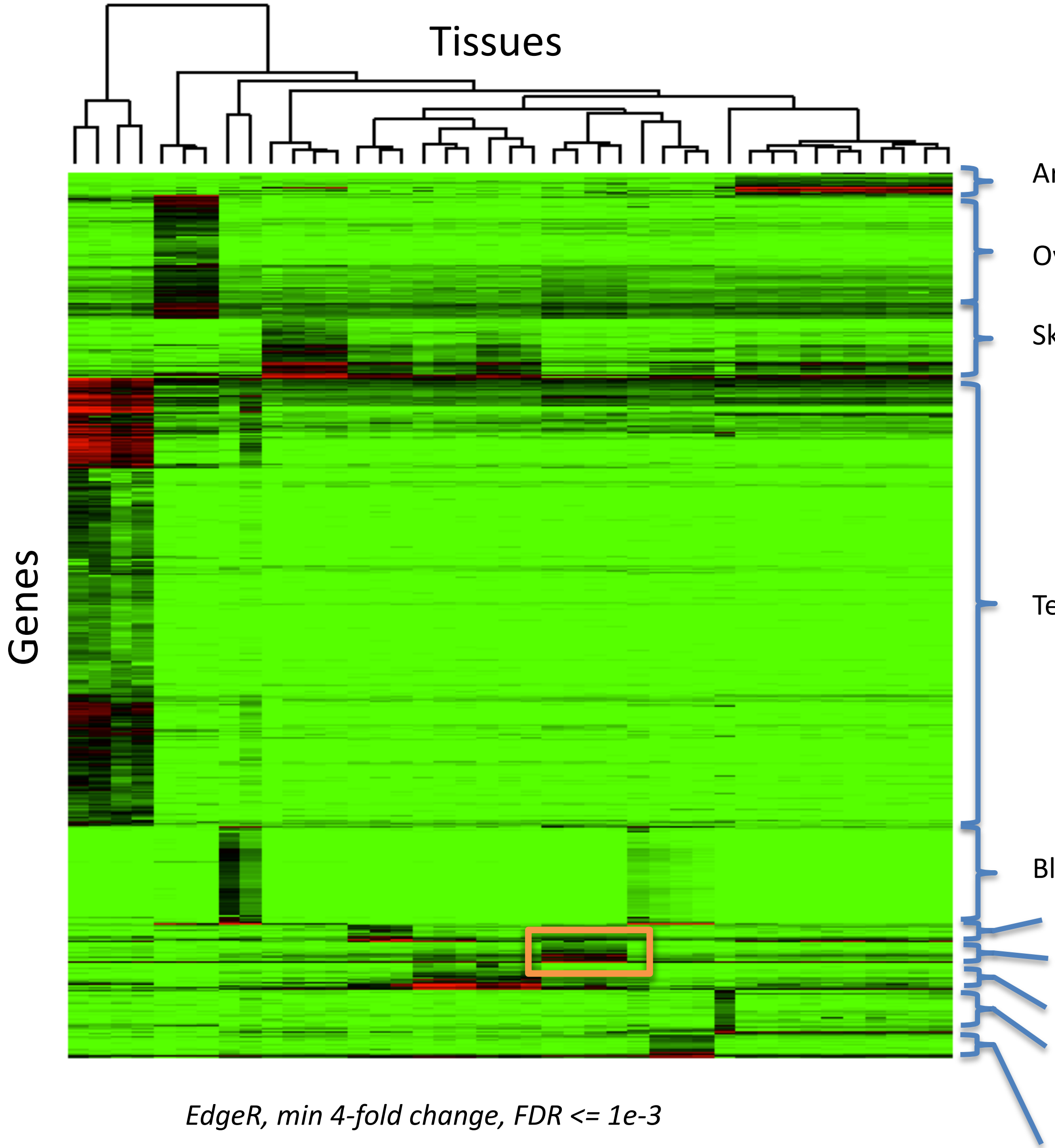
Pearson Correlation Matrix for Tissue Replicates

2. Identification of Tissue-enriched Expression



EdgeR, min 4-fold change, FDR <= 1e-3

Identification of Tissue-enriched Gene Expression



Arm (193), GO: thick ascending limb development [8.8e-5]

Ovaries (1225)

Skeletal Muscle (539)

Testes (4113), GO: spermatogenesis [2.5e-14]

Blood Vessel (939)

Bone (272), GO: myeloid leukocyte differentiation [2.2e-3]

Blastema (202): limb morphogenesis [2.5e-5]

Cartilage (255), GO: collagen fibril organization [4.5e-10]

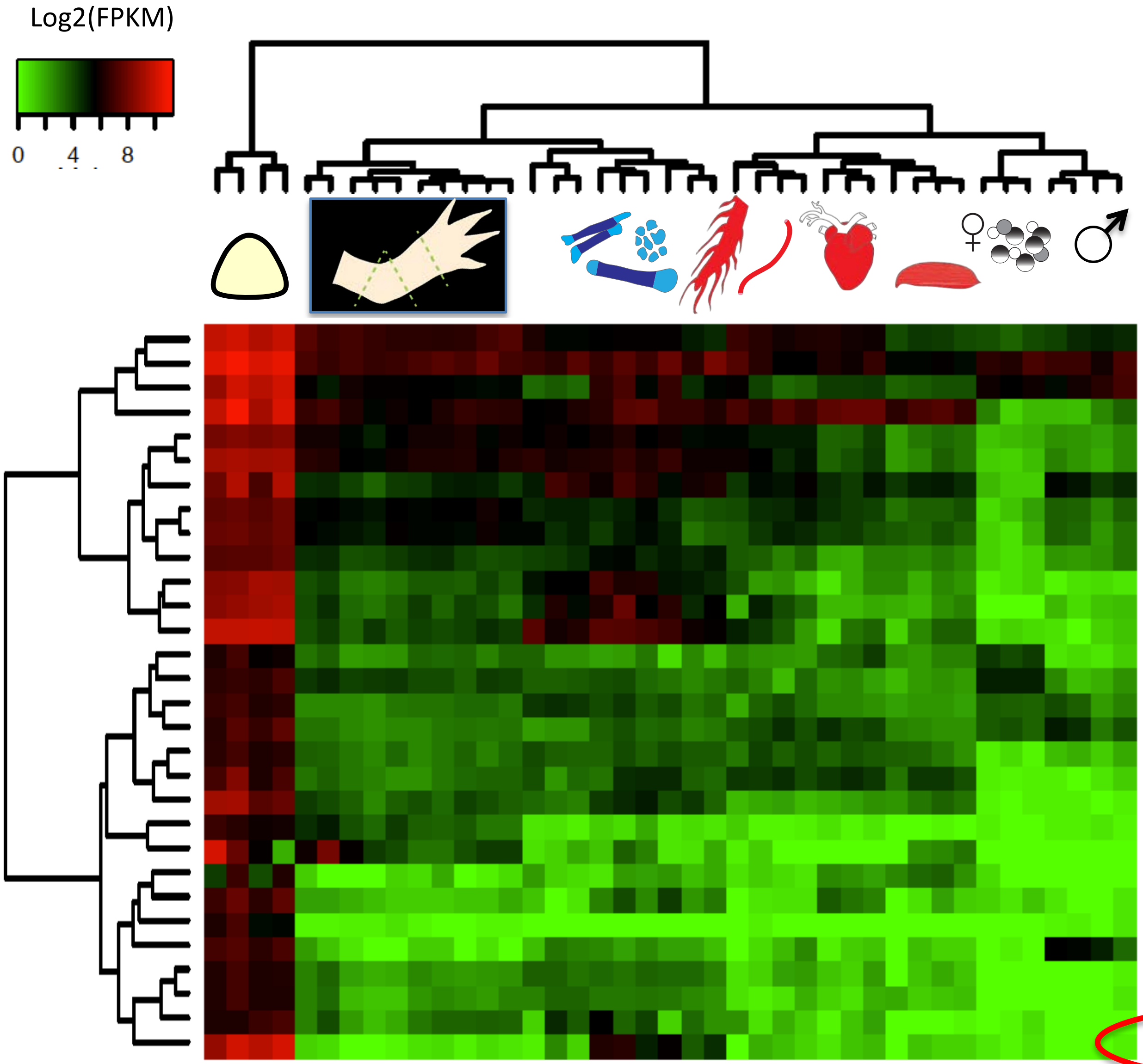
Gill Filament (765)

Heart (238),
GO: vascular process in circulatory system [2.6e-3]

EdgeR, min 4-fold change, FDR <= 1e-3

Functional enrichment using GO-Seq

Most Highly Expressed Blastema-enriched Genes



CIRBP (cold-inducible) RNA-binding protein

RABP2 Retinoic Acid Binding Protein 2
MFAP2: Microfibrillar-associated protein 2

MKA: Pleiotrophic factor-alpha-1

GPC6: Glypican
FBN2: Fibrillin
TENA: Tenascin
HES1: transcription factor
CXG1: connexin
RAI4: cytoskeleton & cell-cell adhesion

VWDE: von Willebrand factor D and EGF
KERA: Keratacan
K2C6A: Keratin, cytoskeletal

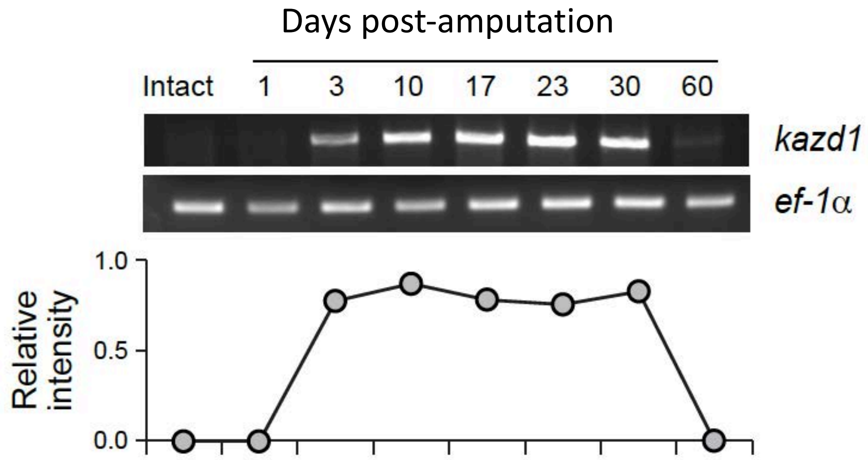
TWIST: transcription factor (pt. 2 of 2)
TWIST: transcription factor (pt. 1 of 2)

KAZD1: growth factor binding protein

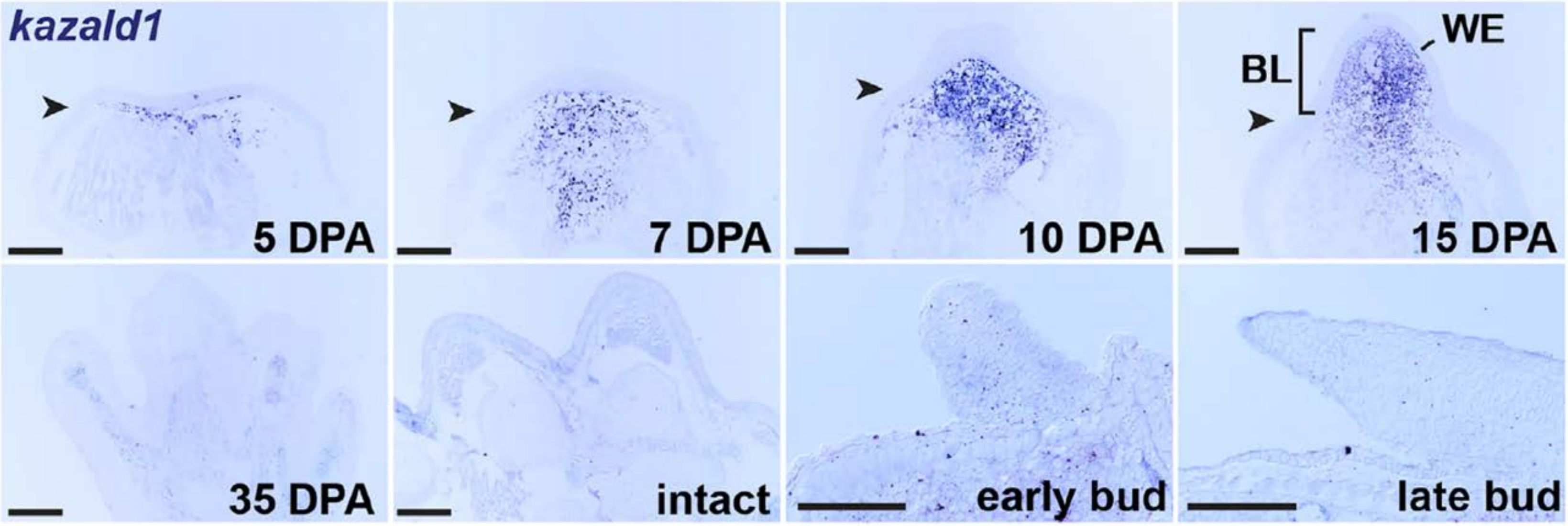
Color key: Regulator Signaling Structure and Extracellular Matrix

Functional Characterization of Blastema-enriched KAZD1

RT-PCR Timecourse of Kazald1 Expression

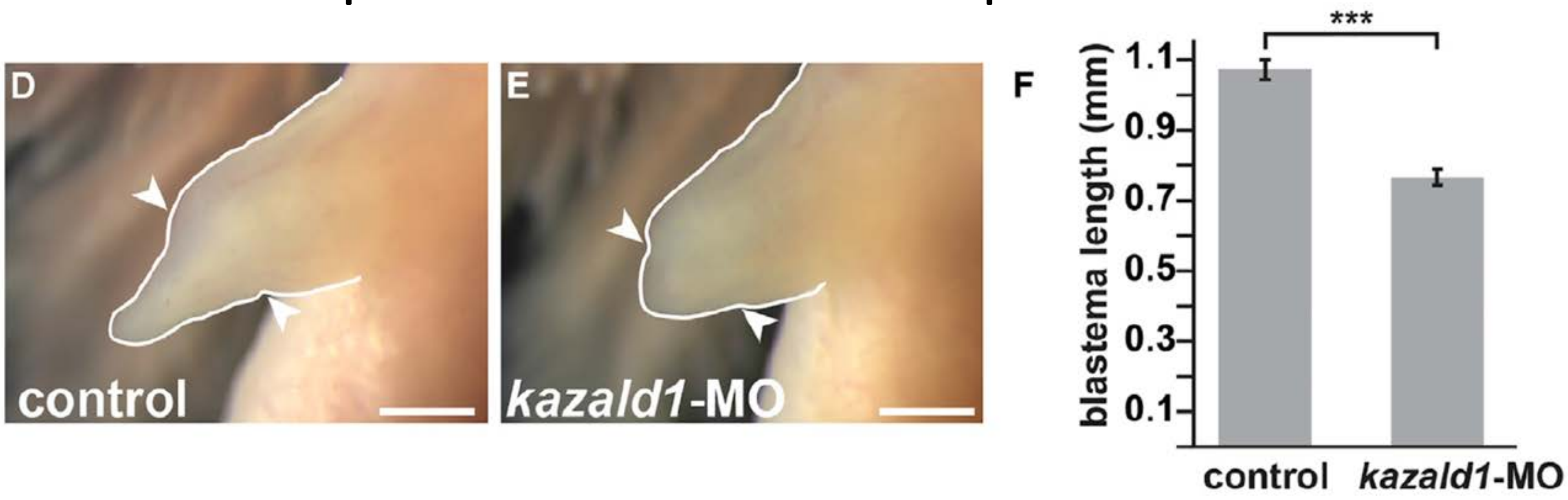


In situ hybridization of kazald1 over course of regeneration

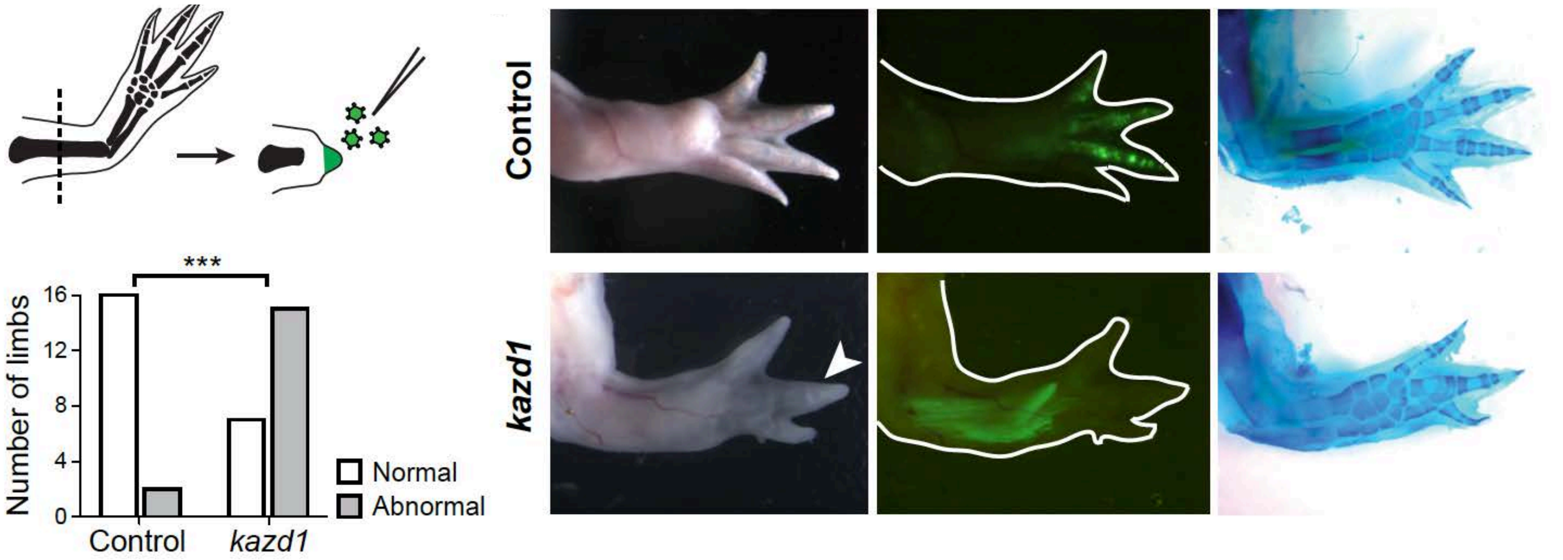


Work by Jessica Whited's group, Cell Reports, 2017

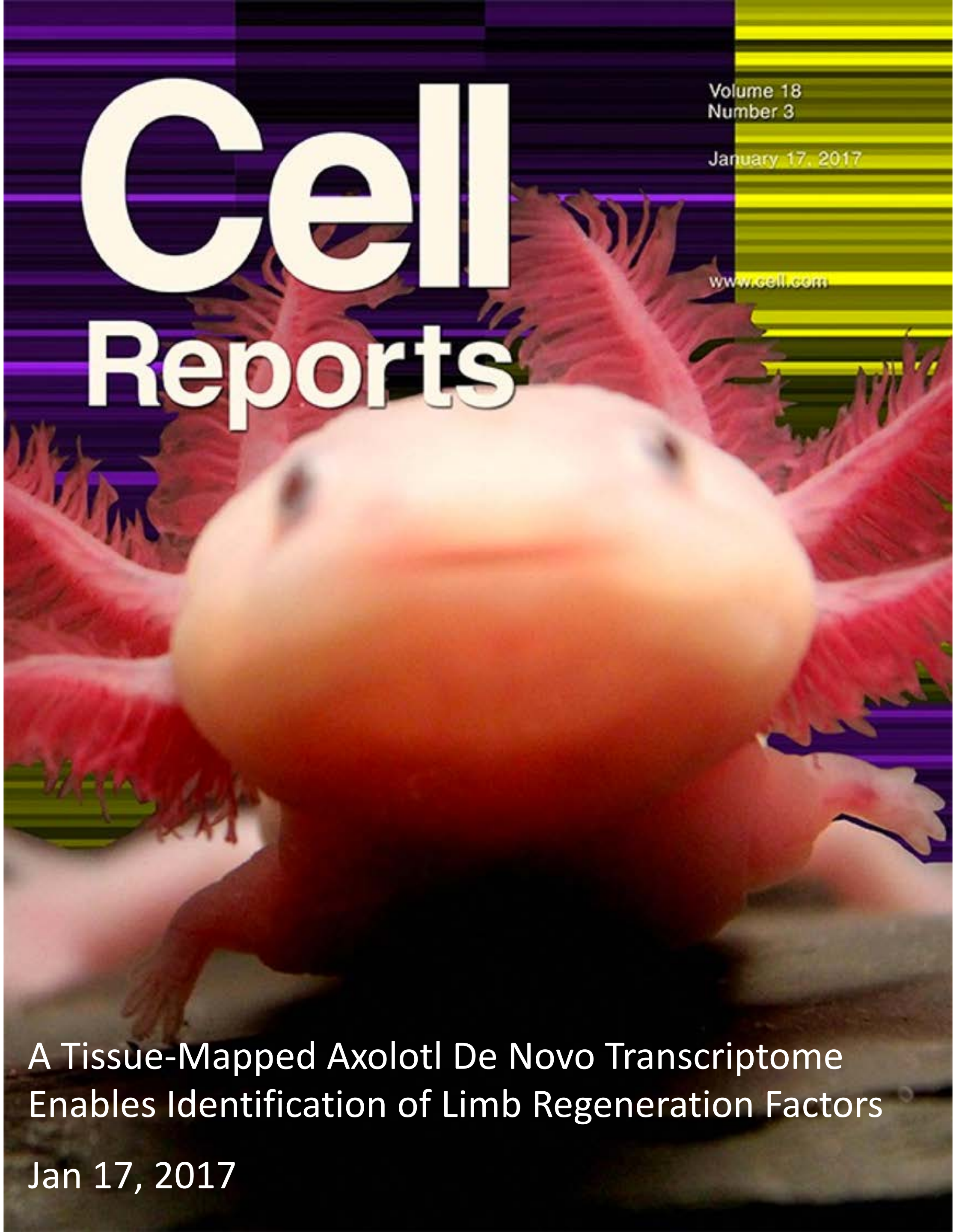
Morpholino Knockdown of Kazald1 Expression



Viral-based Delivered Over-expression of KAZD1 Leads to Regeneration Defects



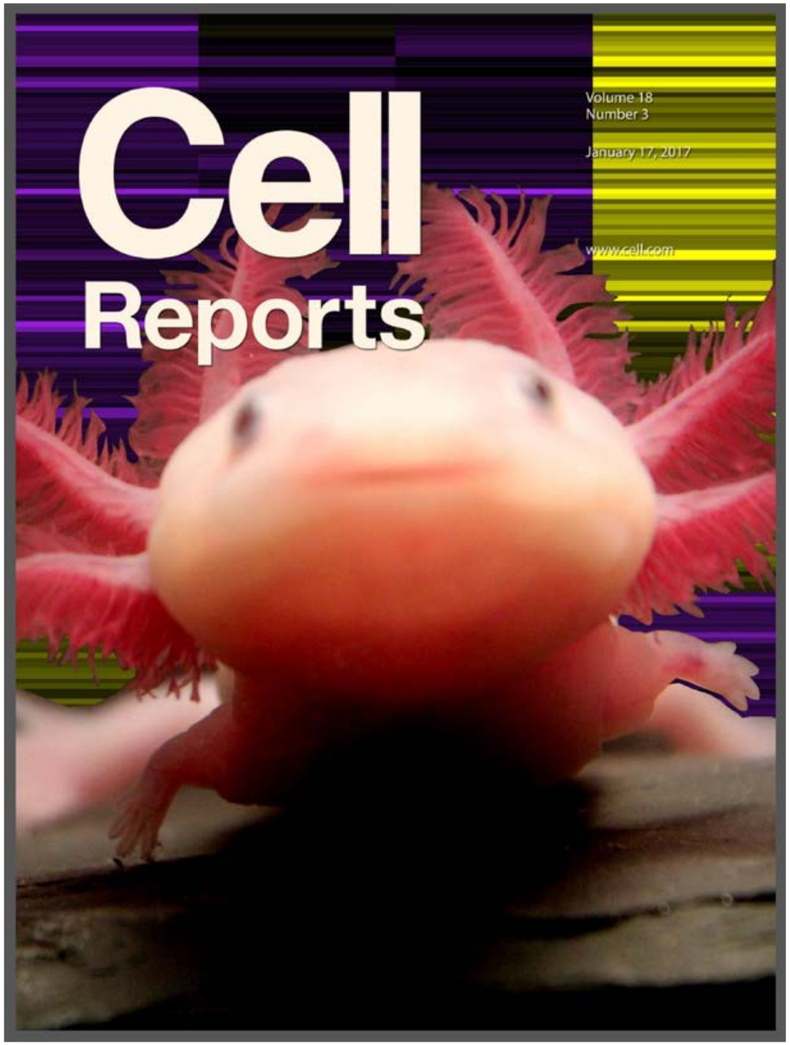
Work by Jessica Whited's group, Cell Reports, 2017



A Tissue-Mapped Axolotl De Novo Transcriptome
Enables Identification of Limb Regeneration Factors

Jan 17, 2017

Example Applications of the Trinity RNA-Seq Protocol



Resource

A Tissue-Mapped Axolotl De Novo Transcriptome Enables Identification of Limb Regeneration Factors

Donald M. Bryant[1, 6], Kimberly Johnson[1, 6], Tia DiTommaso[1], Timothy Tickle[2], Matthew Brian Couger[3], Duygu Payzin-Dogru[1], Tae J. Lee[1], Nicholas D. Leigh[1], Tzu-Hsing Kuo[1], Francis G. Davis[1], Joel Bateman[1], Sevara Bryant[1], Anna R. Guzikowski[1], Stephanie L. Tsai[4], Steven Coyne[1], William W. Ye[1], Robert M. Freeman Jr.[5], Leonid Peshkin[5], Clifford J. Tabin[4], Aviv Regev[2], Brian J. Haas[2], ✉, Jessica L. Whited[1, 7]

Original Article

Loggerhead sea turtle embryos (*Caretta caretta*) regulate expression of stress response and developmental genes when exposed to a biologically realistic heat stress

Blair P. Bentley ✉, Brian J. Haas, Jamie N. Tedeschi, Oliver Berry

Summary of Key Points

- RNA-Seq is a versatile method for transcriptome analysis enabling quantification and novel transcript discovery.
- Expression quantification is based on sampling and counting reads derived from transcripts
- Fold changes based on few read counts lack statistical significance – need deeper sequencing and more replicates.
- Trinity assembly and supported downstream computational analysis tools facilitate transcriptome studies.
- The Trinity framework can empower transcriptome studies for organisms lacking reference genome sequences (ex. Axolotl) or suboptimal references (ex. cancer).

Summary of Current Trends

- Quantification without read alignment (pseudalignment – kallisto, salmon).

- Differential expression w/o expression estimation (transcript equivalence classes)

- Leverage longer reads (no assembly required?) (pacbio, nanopore)

Acknowledgements

BROAD INSTITUTE

Current and Former Trinity Contributors

Aviv Regev
* Brian Haas
Moran Yassour
Manfred Grabherr
Tim Tickle
Asma Bankapur
Christophe Georgescu
Vrushali Fangal
Maxwell Brown

Trinotate & TrinoateWeb
Brian Couger
Leonardo Gonzalez

HSCI | HARVARD STEM CELL INSTITUTE
1000 scientists. One goal. Discovering cures.

Salamander Transcriptomics
Jessica Whited
Nick Leigh

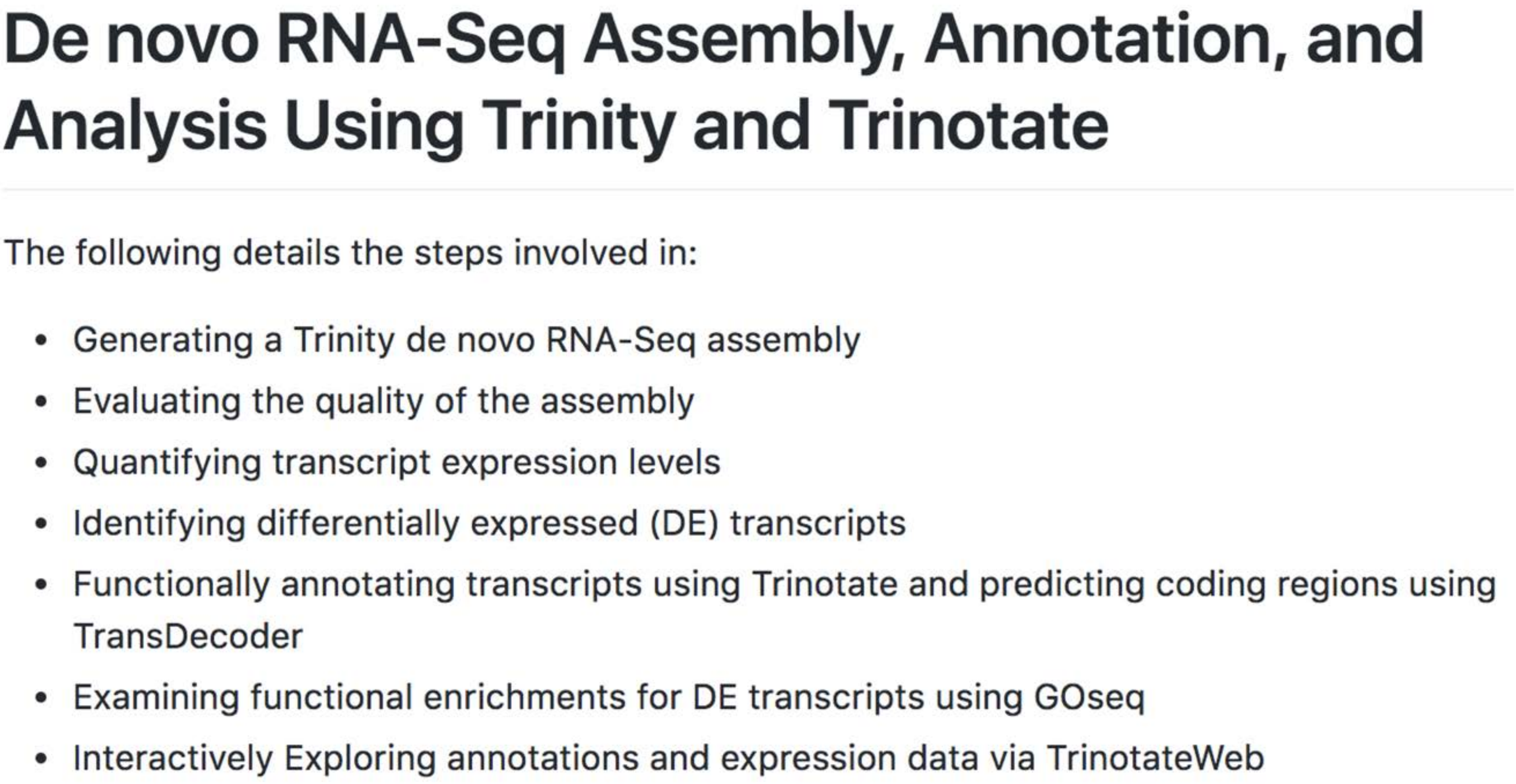
Trinity is funded by:

ITCR Informatics Technology for Cancer Research

NIH NATIONAL CANCER INSTITUTE

Transcriptomics Lab

(Krumlov Prelate, 2-5pm and 7-10pm)

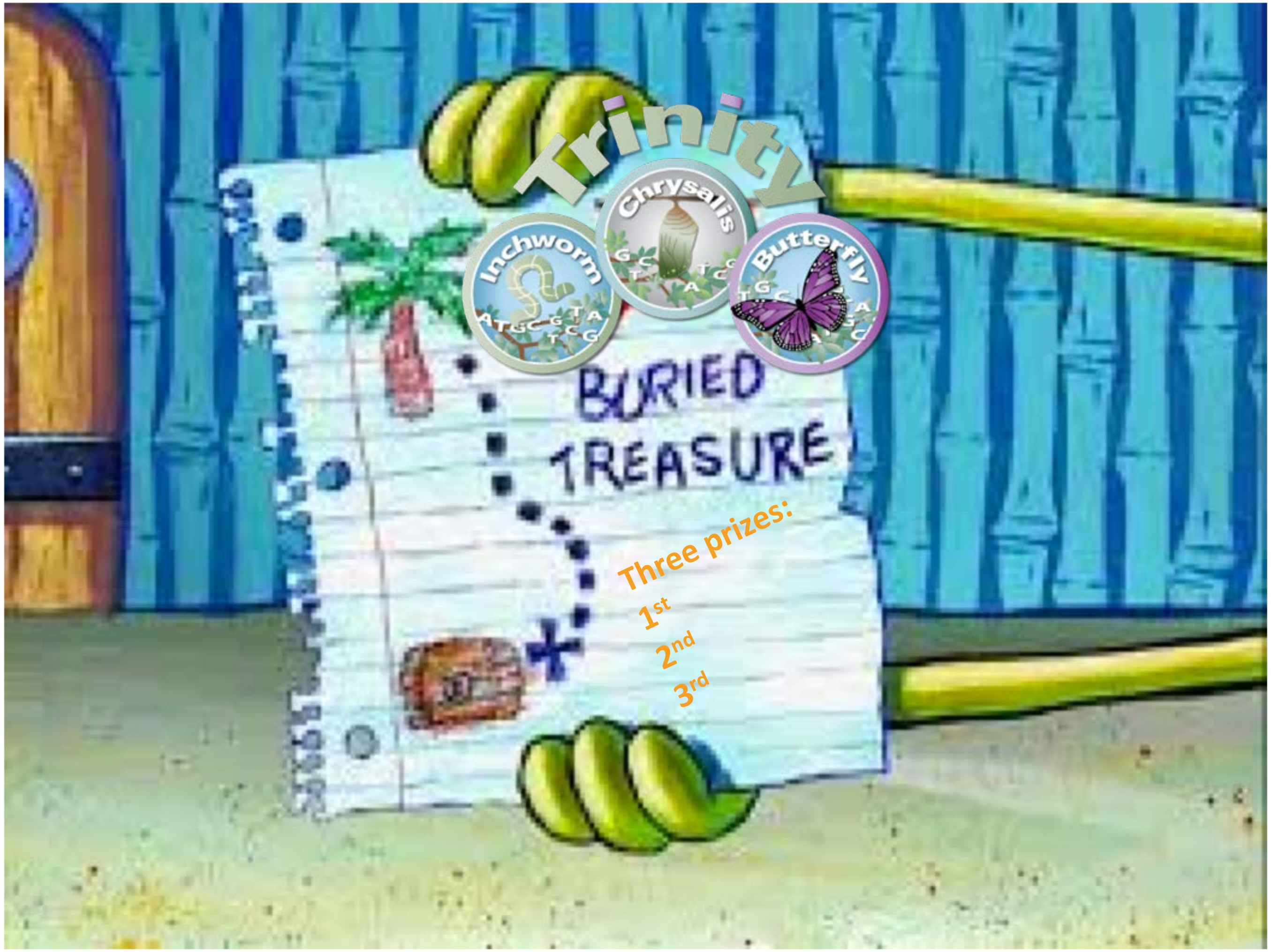


De novo RNA-Seq Assembly, Annotation, and Analysis Using Trinity and Trinotate

The following details the steps involved in:

- Generating a Trinity de novo RNA-Seq assembly
- Evaluating the quality of the assembly
- Quantifying transcript expression levels
- Identifying differentially expressed (DE) transcripts
- Functionally annotating transcripts using Trinotate and predicting coding regions using TransDecoder
- Examining functional enrichments for DE transcripts using GOseq
- Interactively Exploring annotations and expression data via TrinotateWeb

Trinity Treasure Hunt!!! ☺



Will provide link to the challenge via slack – stay tuned, will start ~ 8pm

Slack channel: #transcriptomicslab