Pt. 1 - Intro to Transcriptomics and Transcriptome Assembly

Programming for Biologists

CSHL 2019

Brian Haas

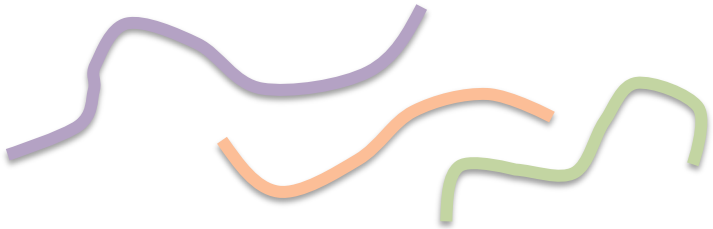
Broad Institute

Transcriptomics Lecture Overview

- Overview of RNA-Seq
- Transcript reconstruction methods
- Expression quantitation

RNA-Seq Empowers Transcriptome Studies

Extract RNA, convert to cDNA

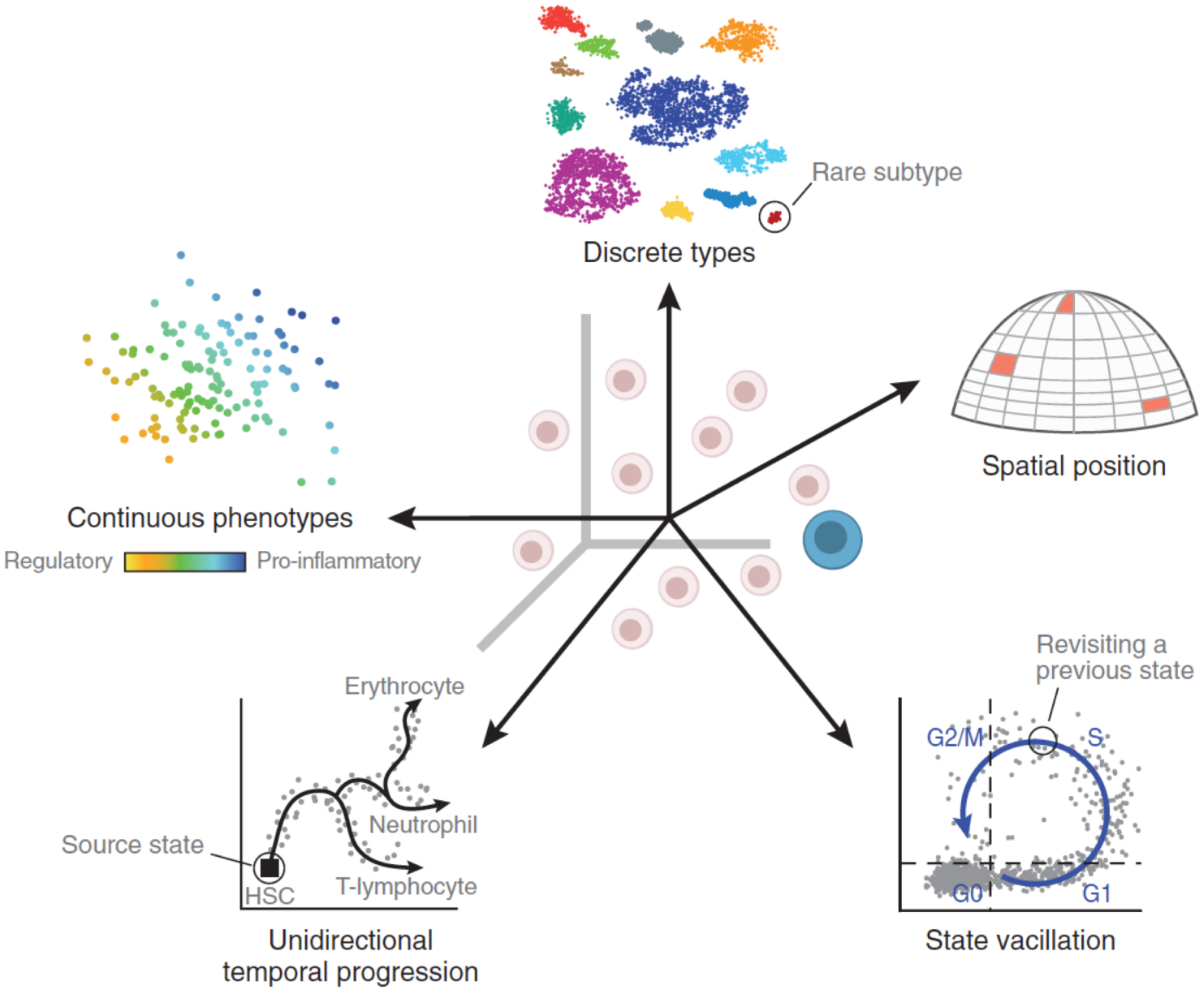


Next-gen Sequencer
(pick your favorite)

RNA-Seq Empowers Many Facets of Biological Investigations

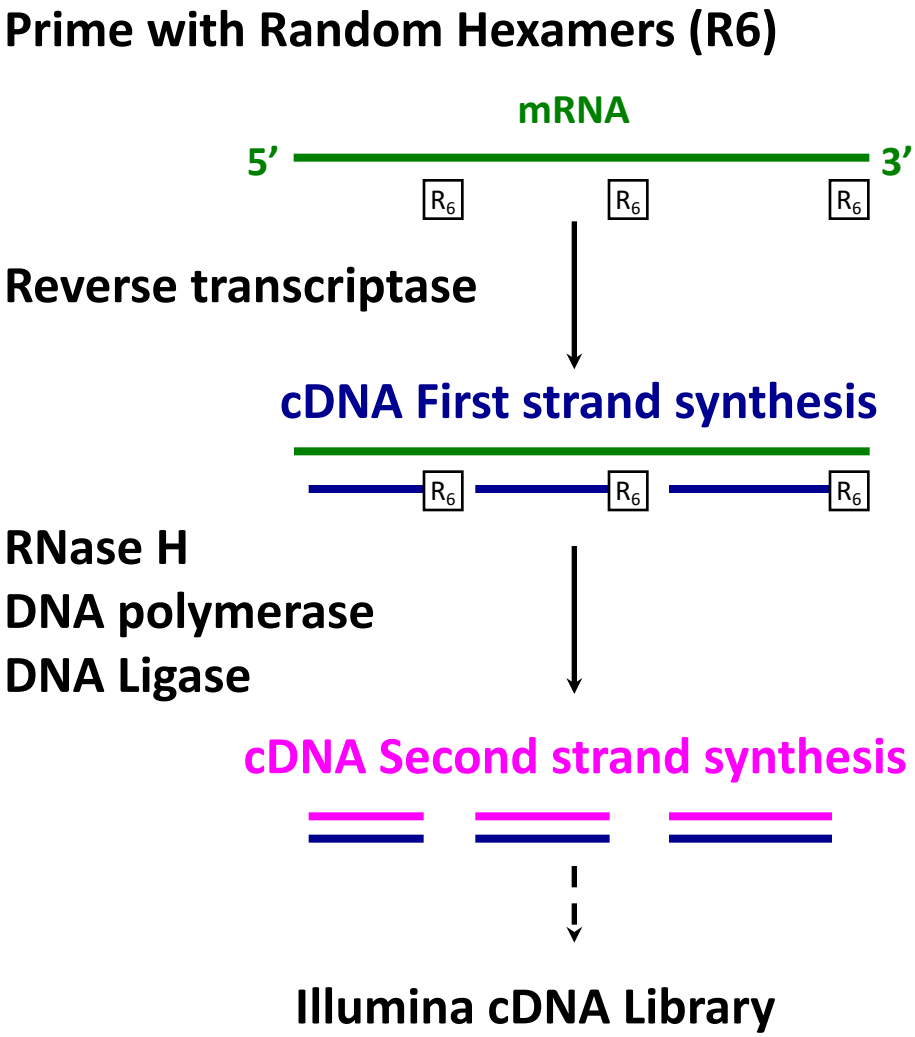
- Transcript identification (ie. which genes active)
- Expression Levels
- Alternative splicing isoforms
- Allelic variants
- Mutations
- Fusion Transcripts
- RNA-editing

RNA-Seq is Empowering Discovery at Single Cell Resolution



Wagner, Regev, and Yosef. NBT 2016

RNA-Seq: How do we make cDNA?



Slide courtesy of Joshua Levin, Broad Institute.

Generating RNA-Seq: *How to Choose?*

Many different instruments hit the scene in the last decade

Illumina

454

SOLiD

Helicos

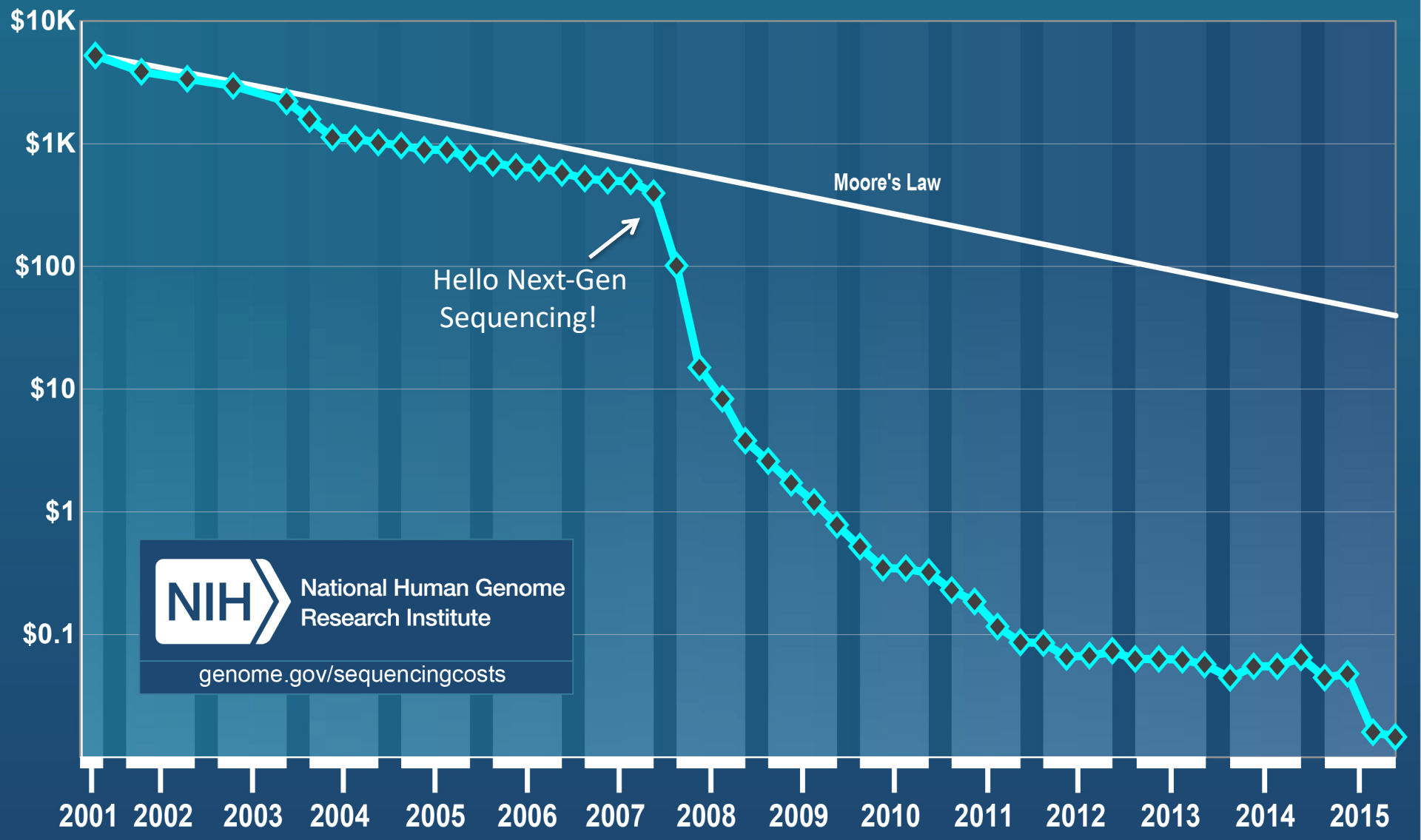
Ion Torrent

Pacific Biosciences

Oxford Nanopore

Slide courtesy of Joshua Levin, Broad Institute.

Cost per Raw Megabase of DNA Sequence



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

RNA-Seq: *How to Choose?*

Illumina

Helicos

Ion Torrent

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

Oxford Nanopore

Slide courtesy of Joshua Levin, Broad Institute.

Generating RNA-Seq: *How to Choose?*

Popular choices for RNA-Seq today

Illumina

454

SOLiD

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Oxford Nanopore

Generating RNA-Seq: *How to Choose?*

Popular choices for RNA-Seq today

[Current RNA-Seq workhorse]

Illumina

Ion Torrent

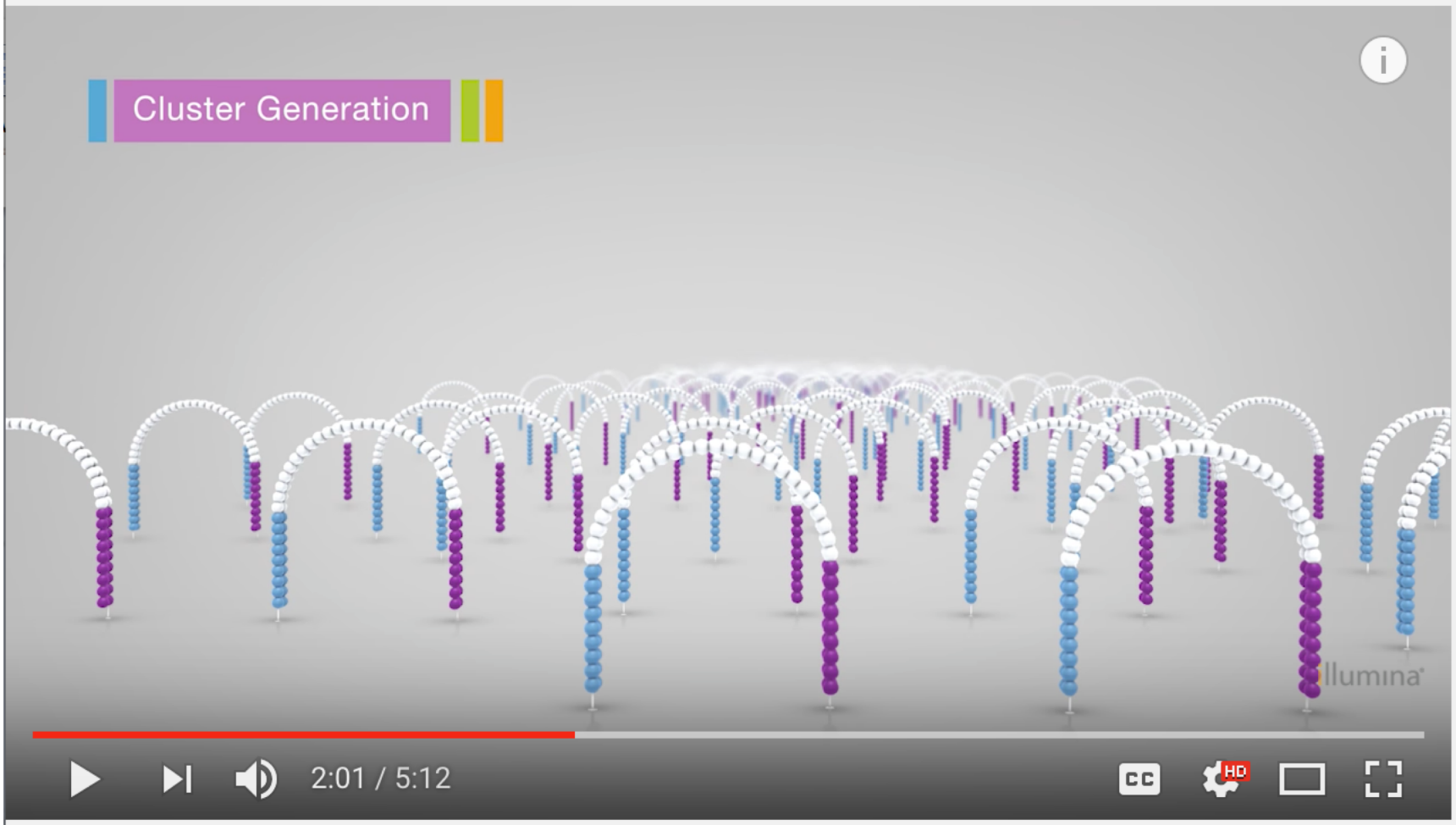
[Full-length single molecule sequencing]

Pacific Biosciences

[Newly emerging technology for full-length single molecule sequencing]

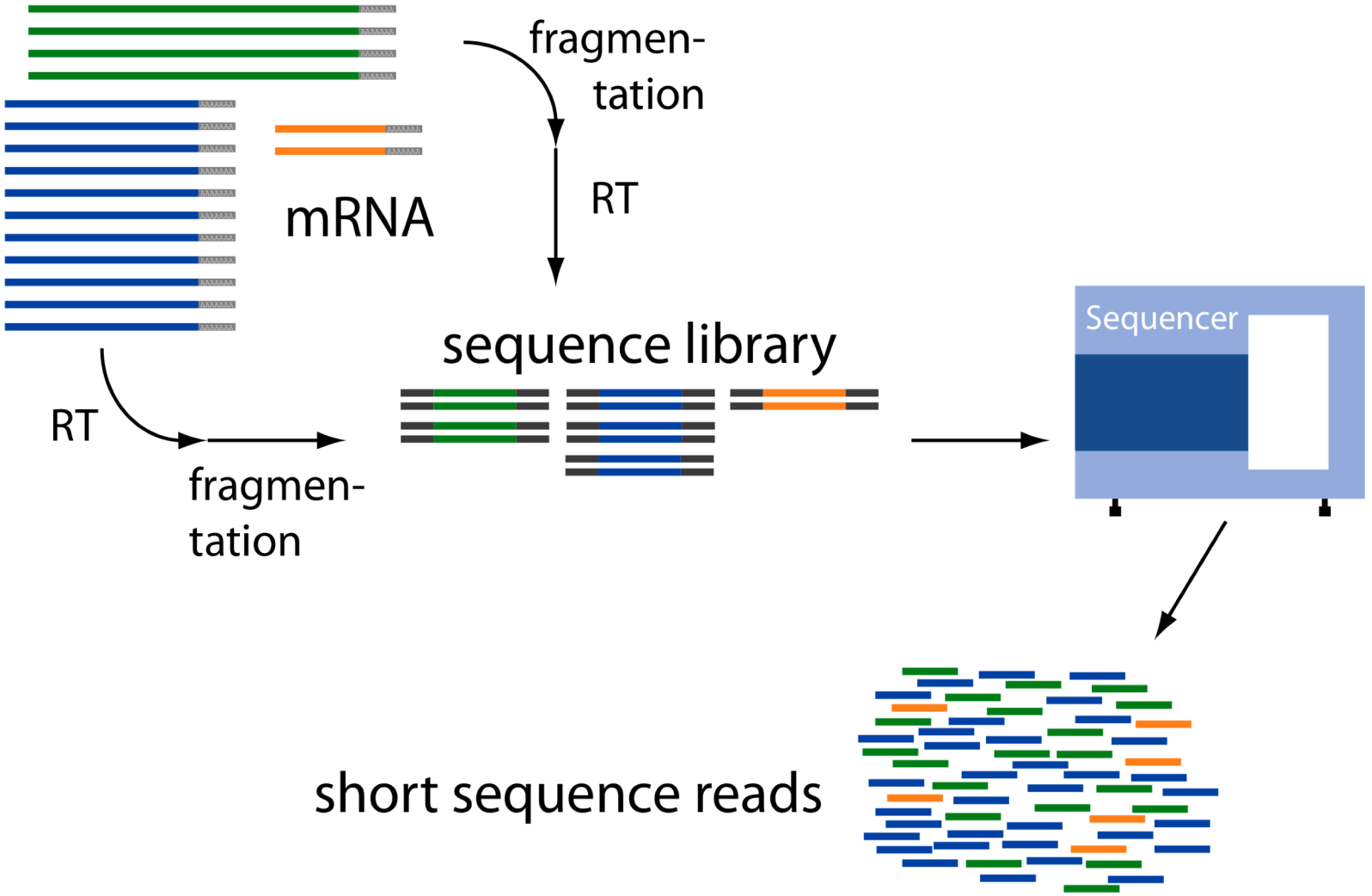
Oxford Nanopore

Illumina Sequencing by Synthesis



Illumina Inc https://www.youtube.com/watch?v=fCd6B5HRaZ8

Overview of RNA-Seq



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

Common Data Formats for RNA-Seq

FASTA format:

```
>61DFRAAXX100204:1:100:10494:3070/1
AAACAACAGGGCACATTGTCACTCTTGTATTTGAAAAACACTTTCCGGCCAT
```

FASTQ format:

```
@61DFRAAXX100204:1:100:10494:3070/1
AAACAACAGGGCACATTGTCACTCTTGTATTTGAAAAACACTTTCCGGCCAT
+
ACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCBC?CCCCCCCCC@@CACCCCCA
```

Read

Quality values

AsciiEncodedQual(x) = -10 * log10(Pwrong(x)) + 33

AsciiEncodedQual (‘C’) = **64**

So, Pwrong(‘C’) = 10^((**64**-33/ (-10)) = 10^-3.4 = **0.0004**

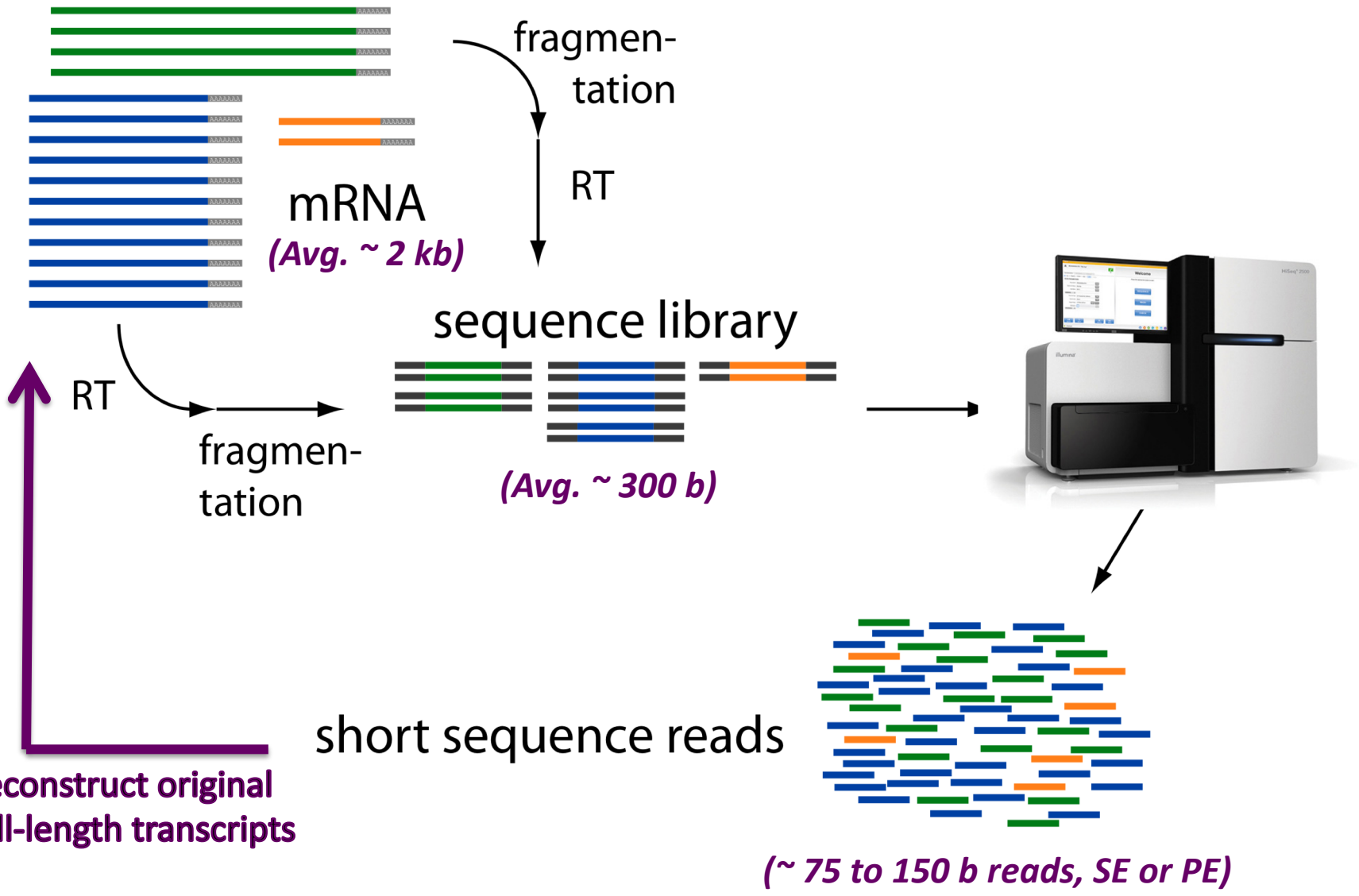
Paired-end Sequences

Two FastQ files, read name indicates left (/1) or right (/2) read of paired-end

```
@61DFRAAXX100204:1:100:10494:3070/1
AAACAACAGGGCACATTGTCACTCTTGTATTTGAAAAACACTTTCCGGCCAT
+
ACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCBC?CCCCCCCCC@@CACCCCCA
```

```
@61DFRAAXX100204:1:100:10494:3070/2
CTCAAATGGTTAATTCTCAGGCTGCAAATATTCGTTCAGGATGGAAGAACA
+
C<CCCCCCCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCBCCCC
```

RNA-Seq Challenge: Transcript Reconstruction



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

Transcript Reconstruction from RNA-Seq Reads

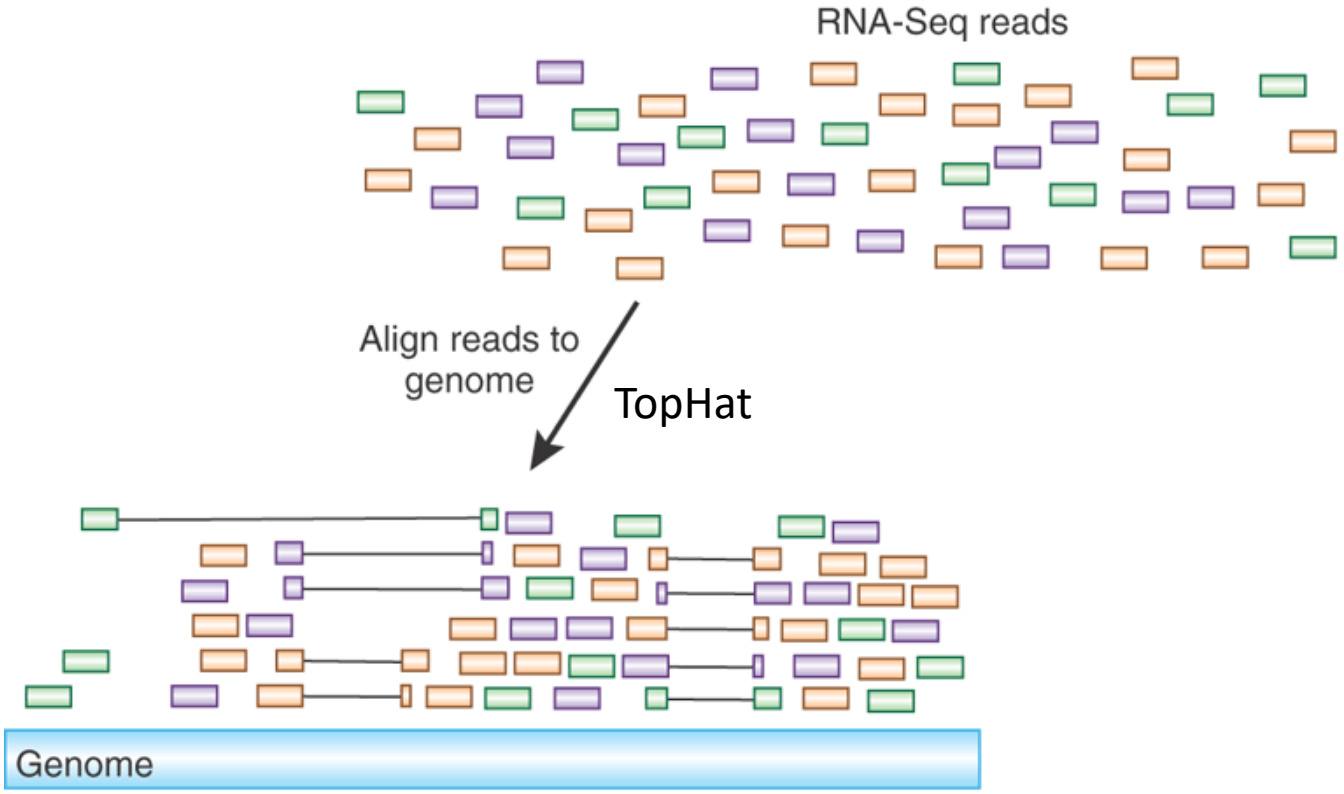
Advancing RNA-Seq analysis

Brian J Haas & Michael C Zody

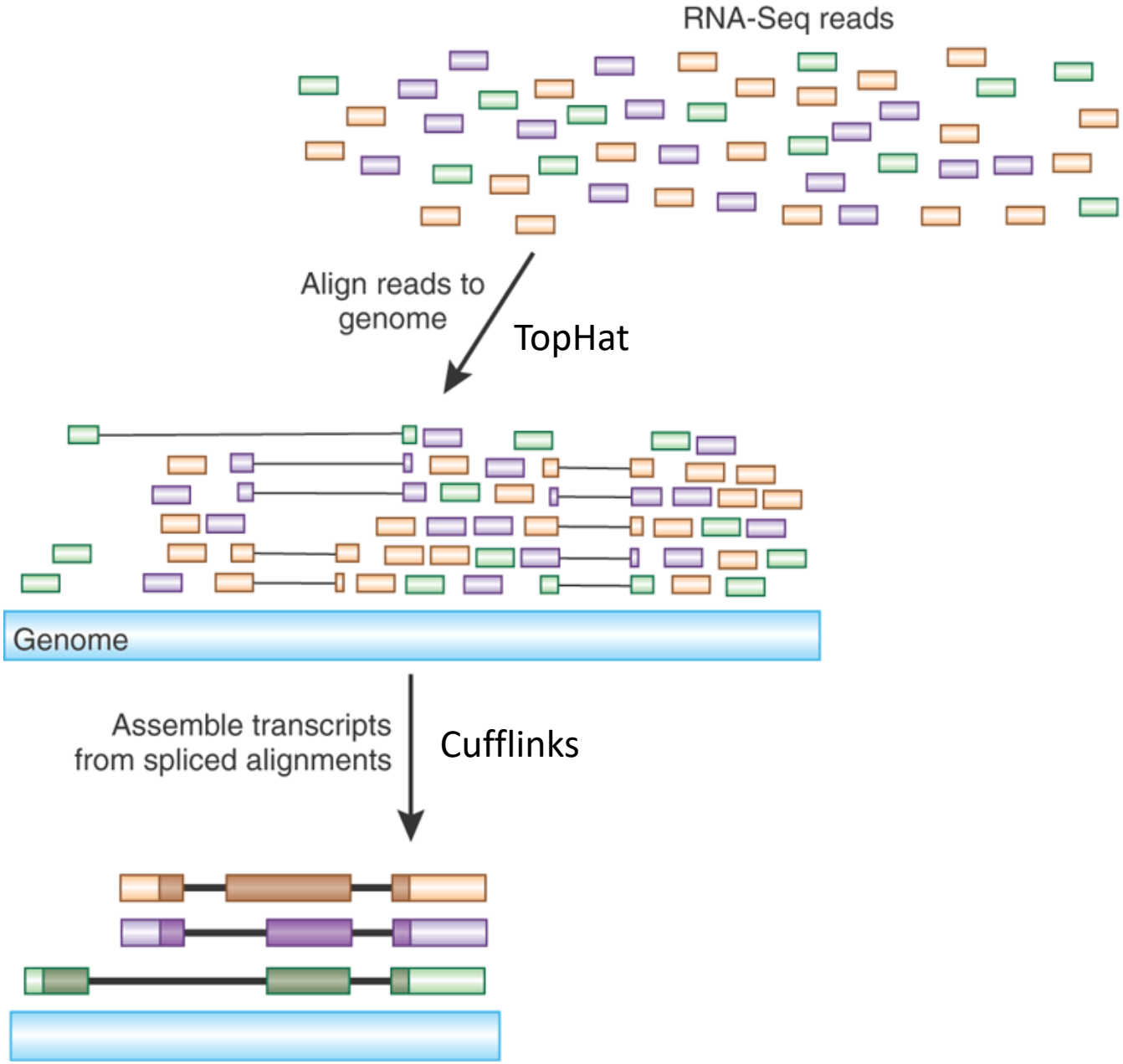
Nature Biotech, 2010

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

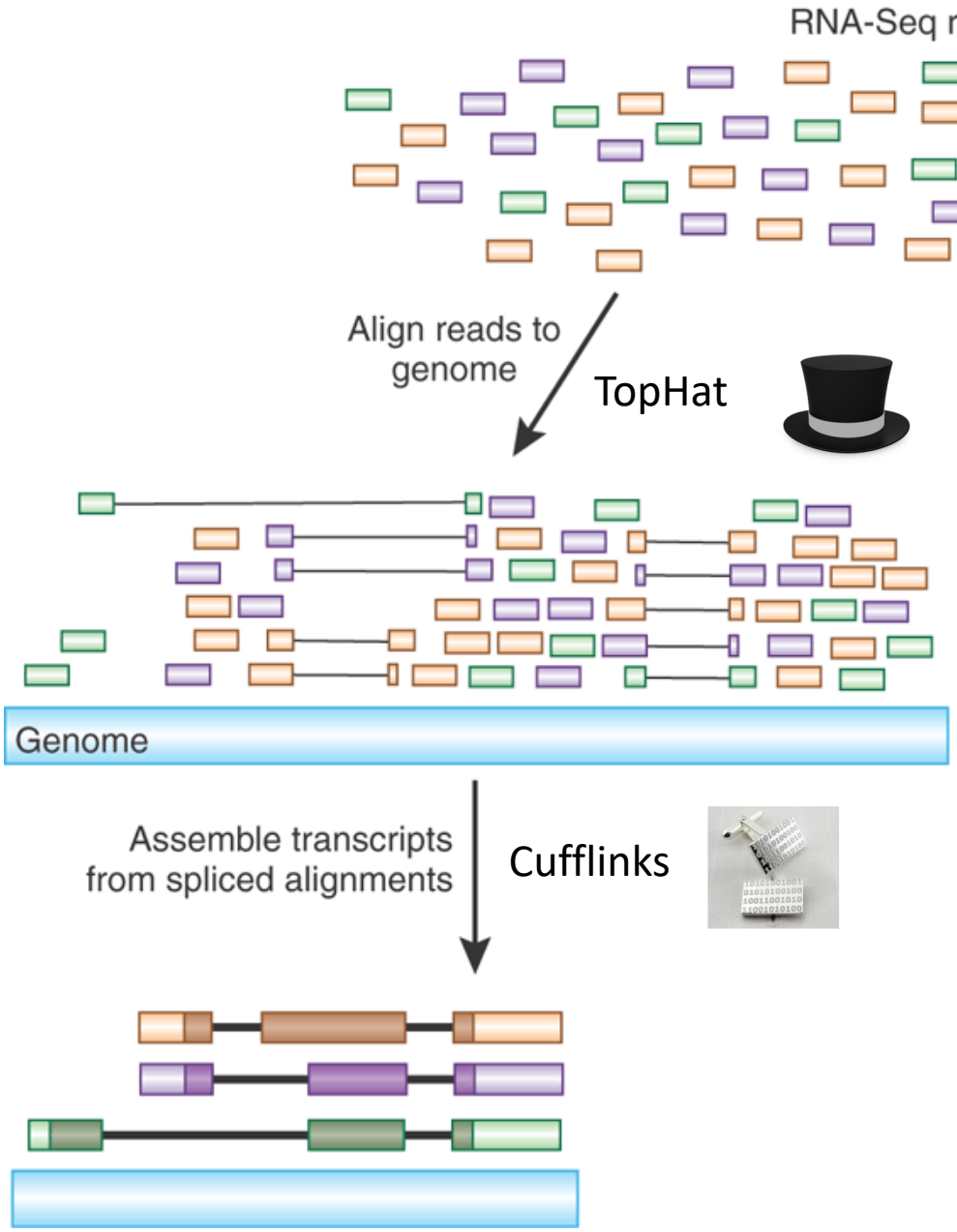
Transcript Reconstruction from RNA-Seq Reads



Transcript Reconstruction from RNA-Seq Reads



Transcript Reconstruction from RNA-Seq Reads



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

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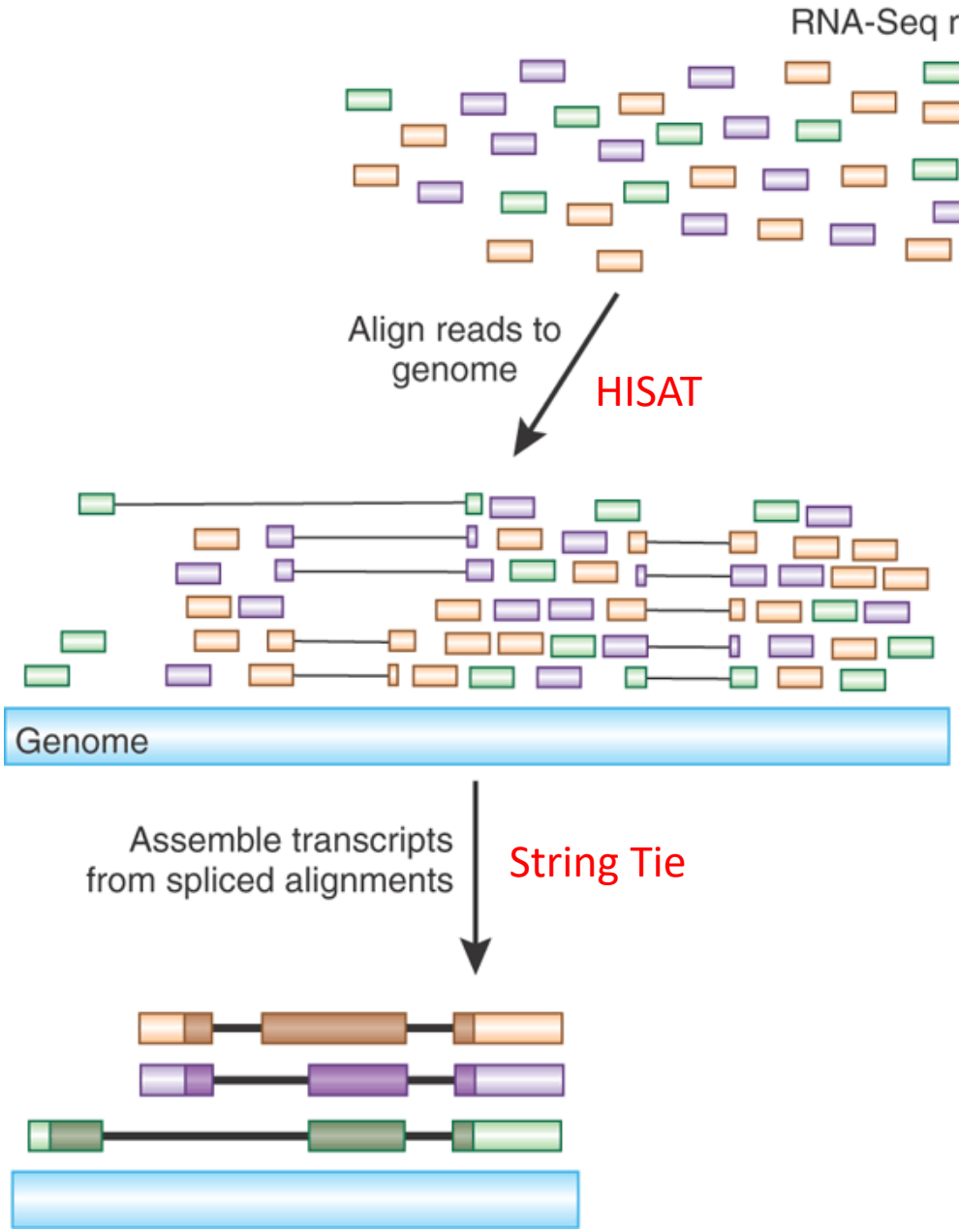

The "New Tuxedo" Suite:

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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

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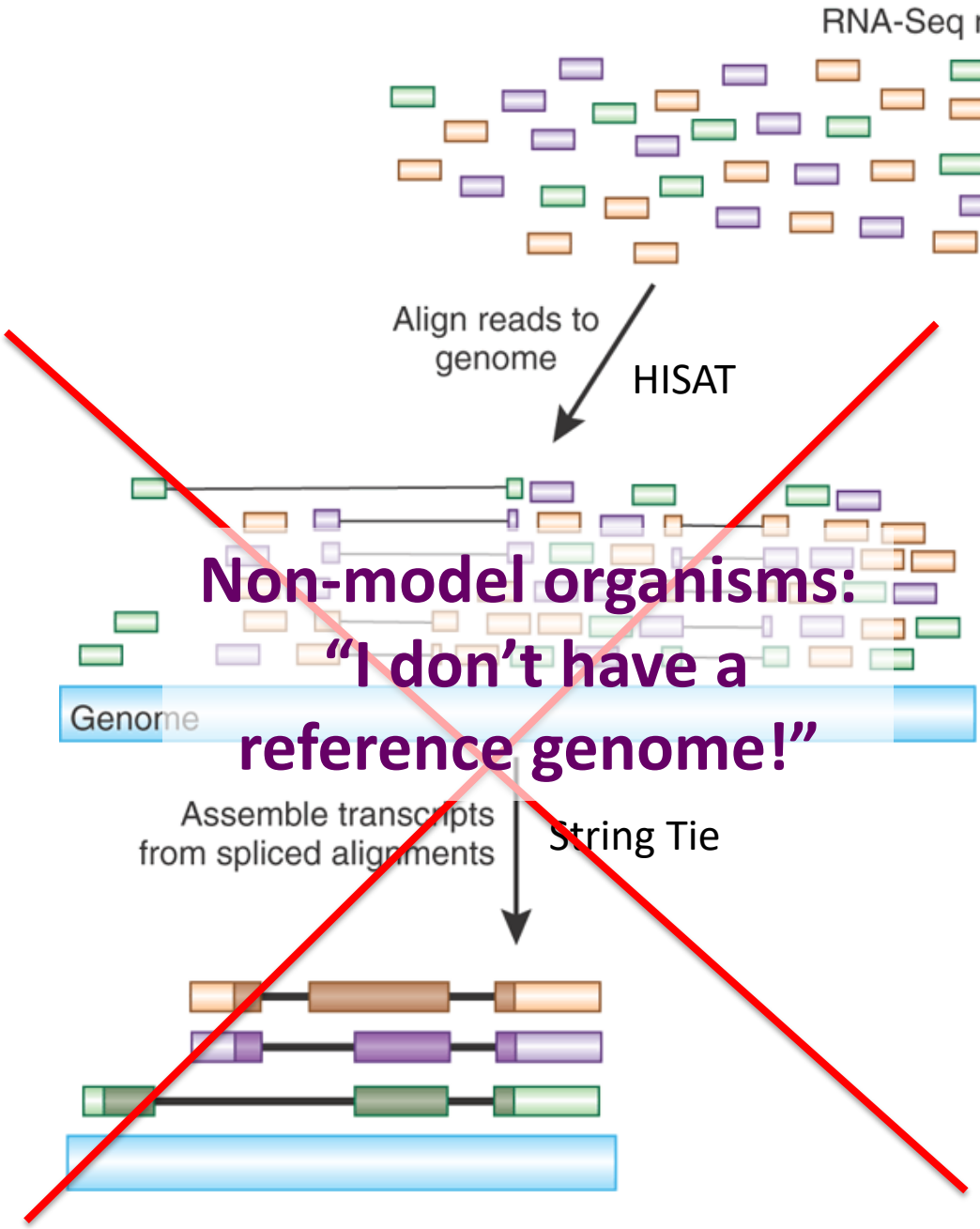

Non-model organisms: "I don't have a reference genome!"

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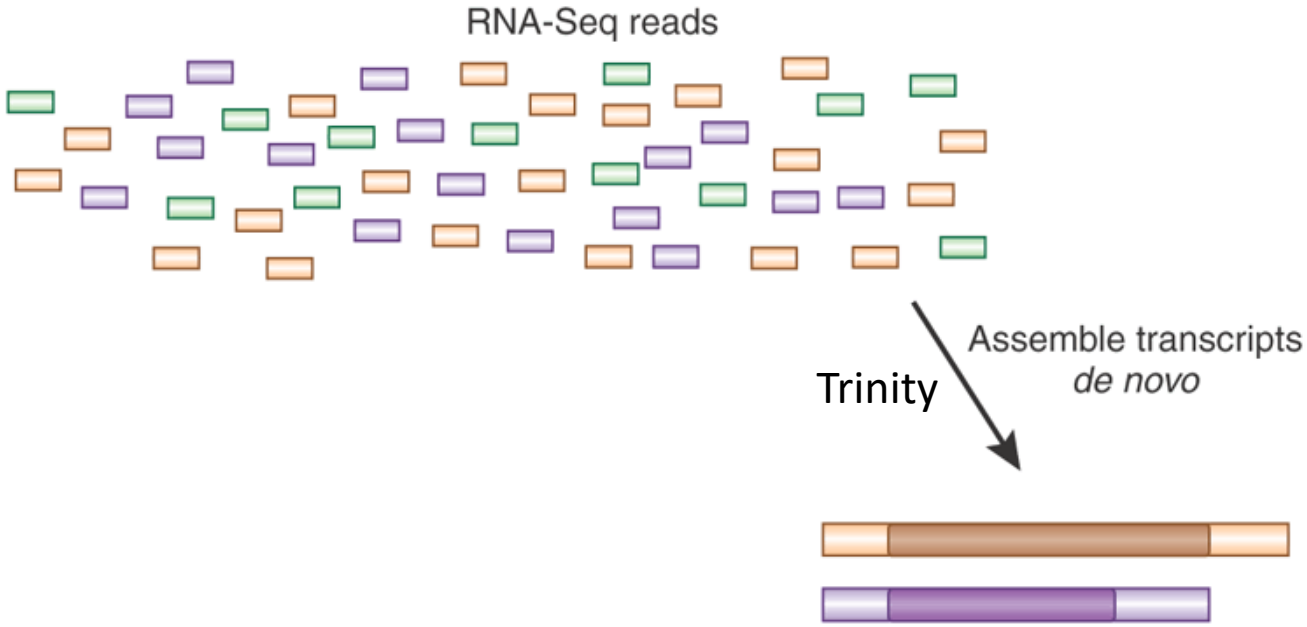

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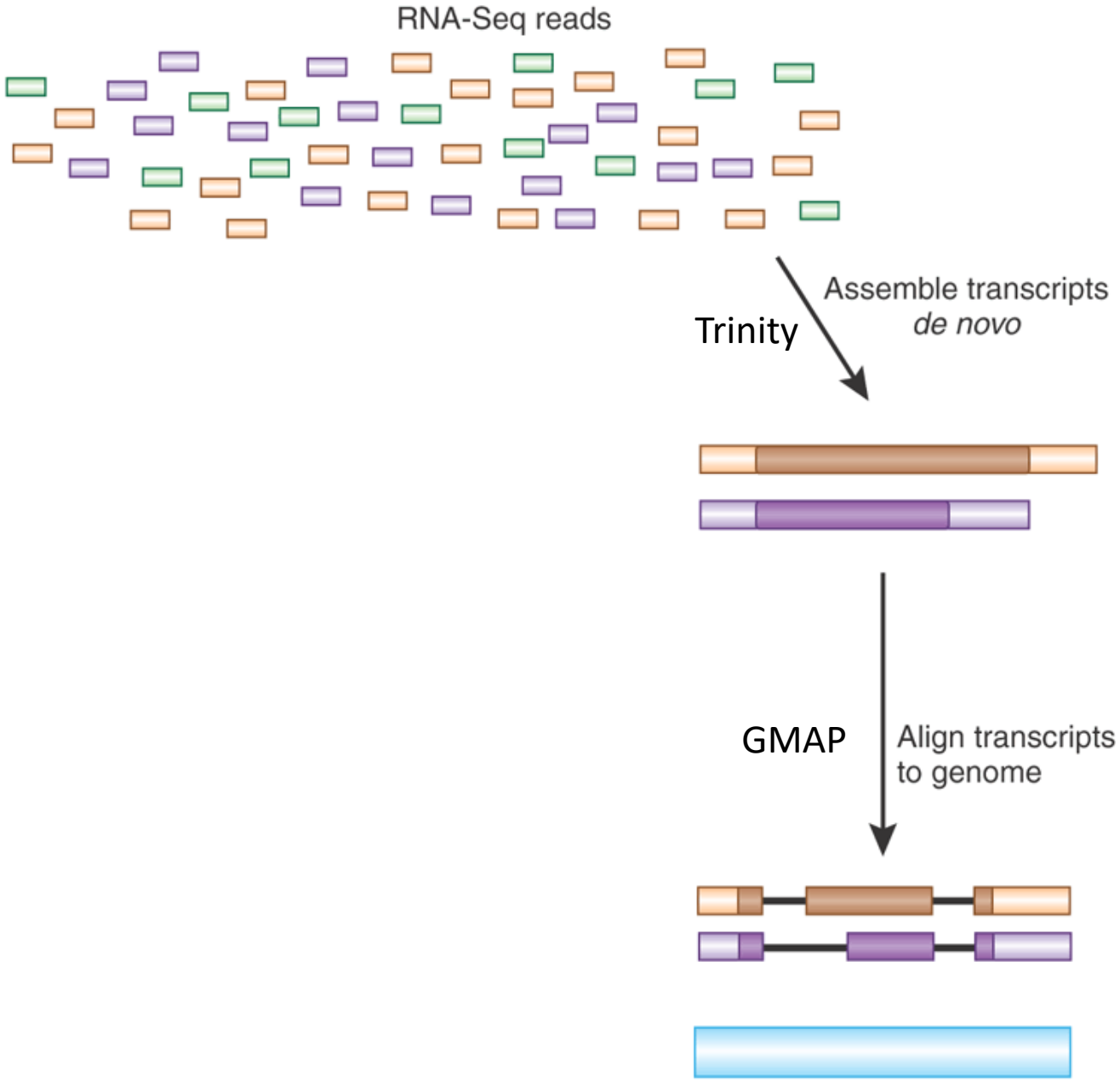


Transcript Reconstruction from RNA-Seq Reads

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

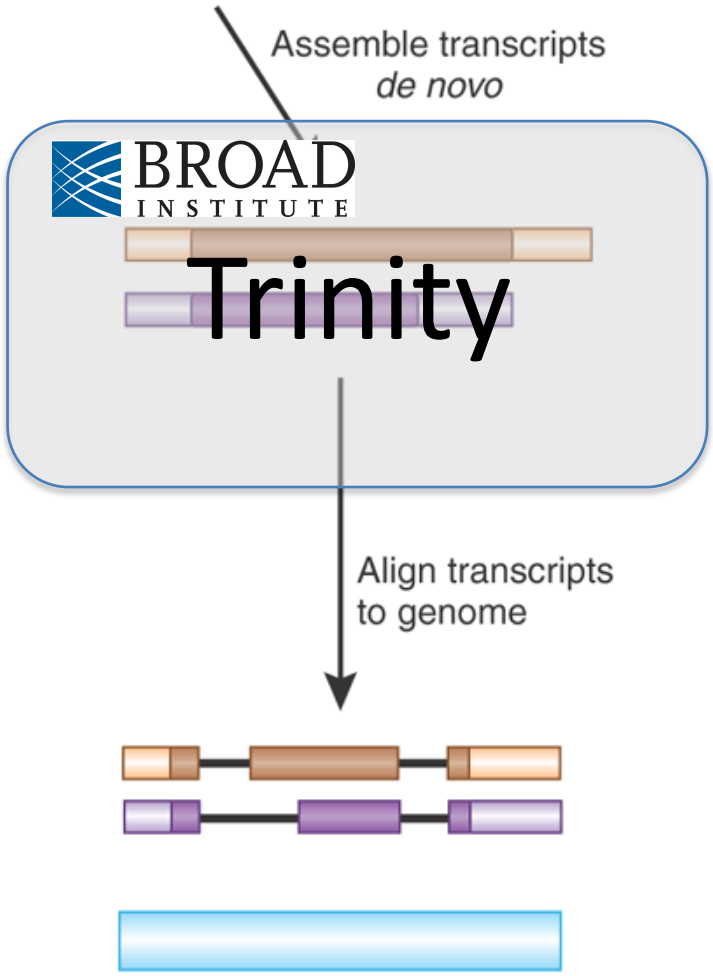
NATURE PROTOCOLS | PROTOCOL

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



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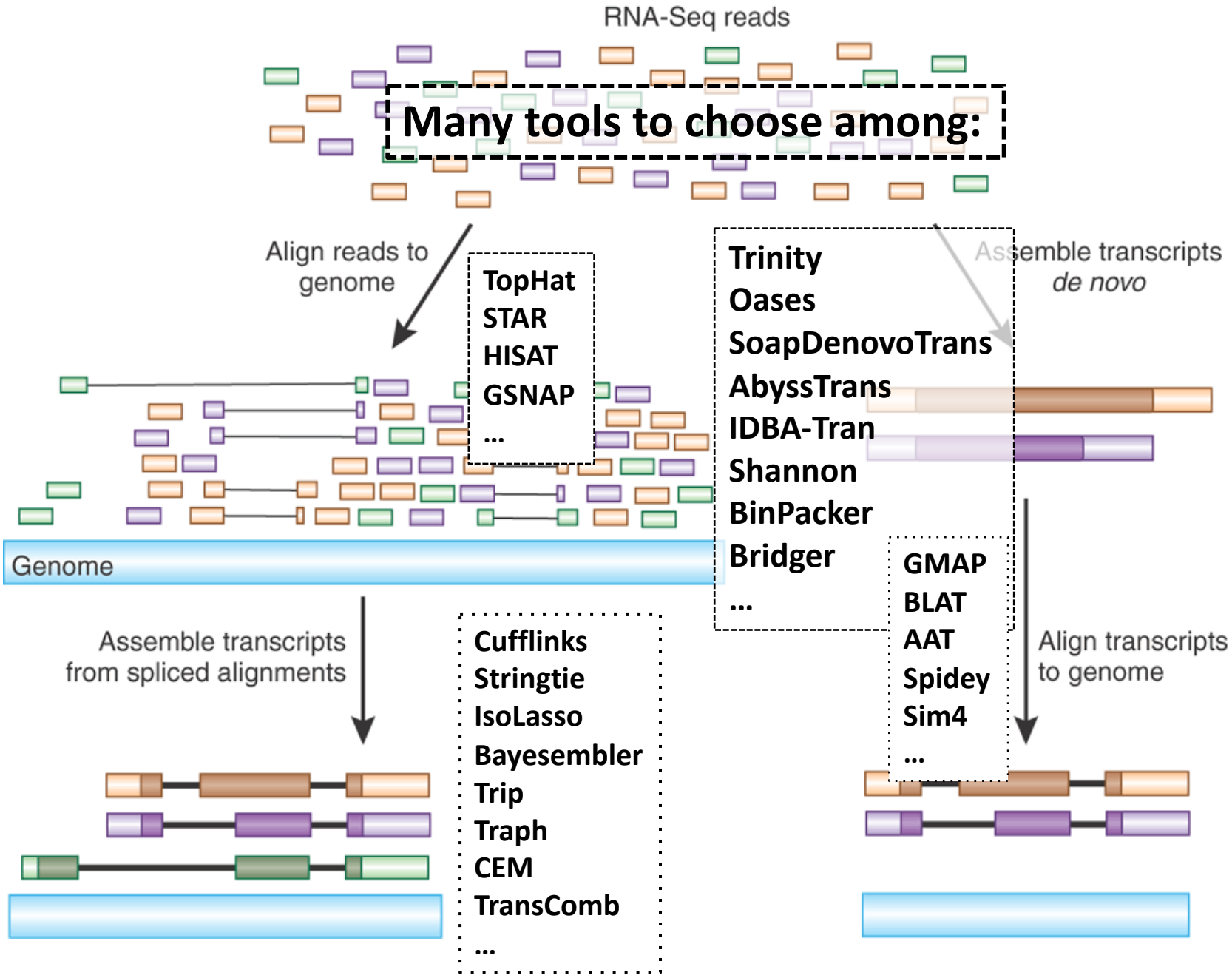
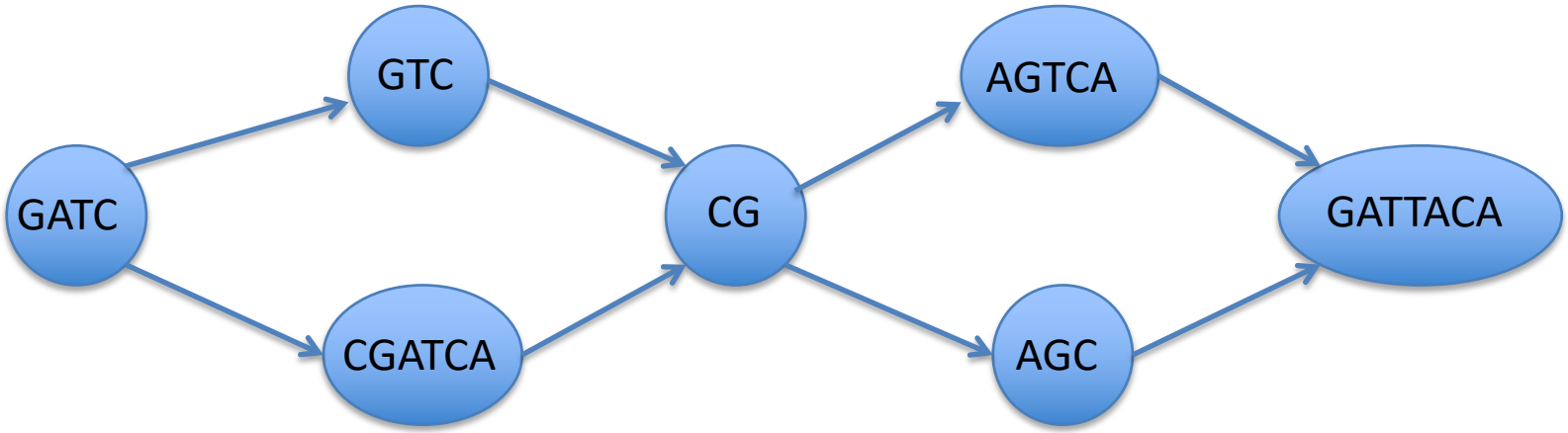


Graph Data Structures Commonly Used For Assembly

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

Reads to Graph



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

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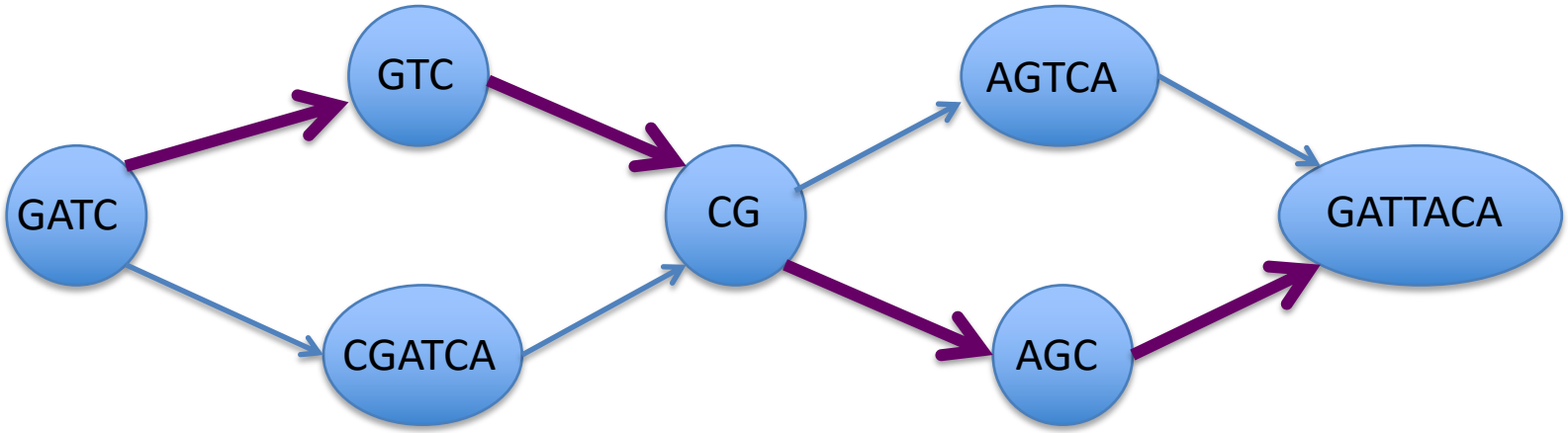
Reads to Graph



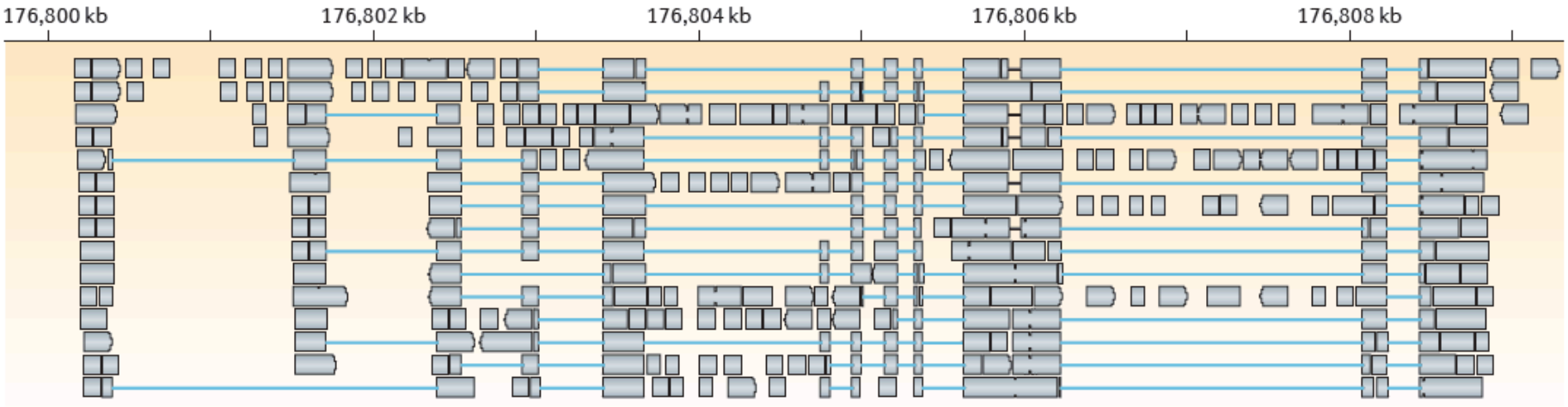
GATCGTCCGAGCGATTACA

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

The General Approach to *De novo* RNA-Seq Assembly Using De Bruijn Graphs

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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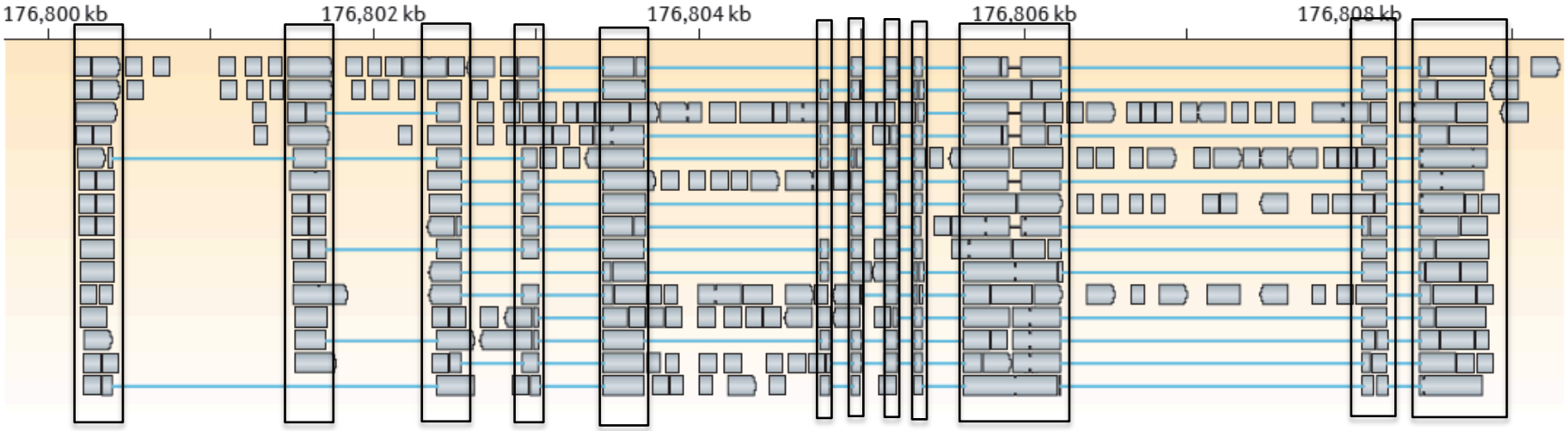
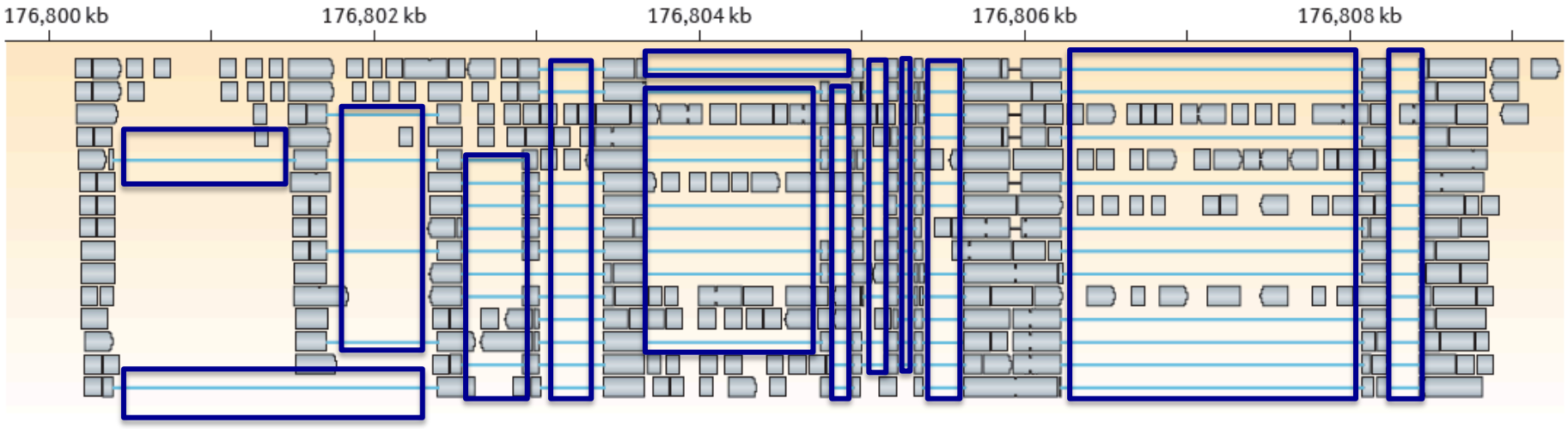


Alignment segment piles => exon regions

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

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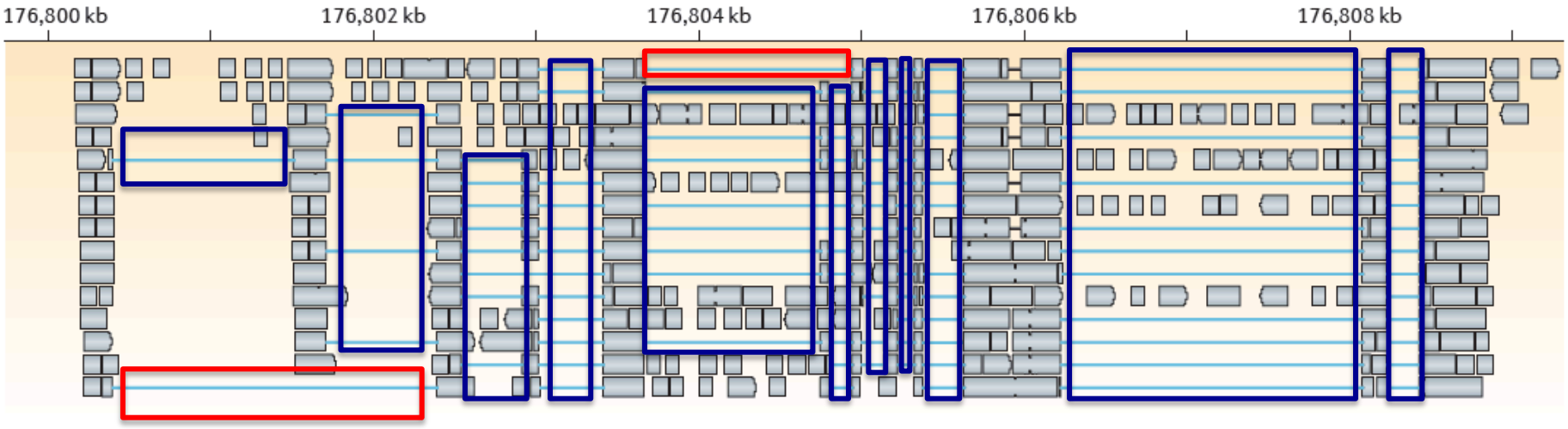
Splice-align reads to the genome

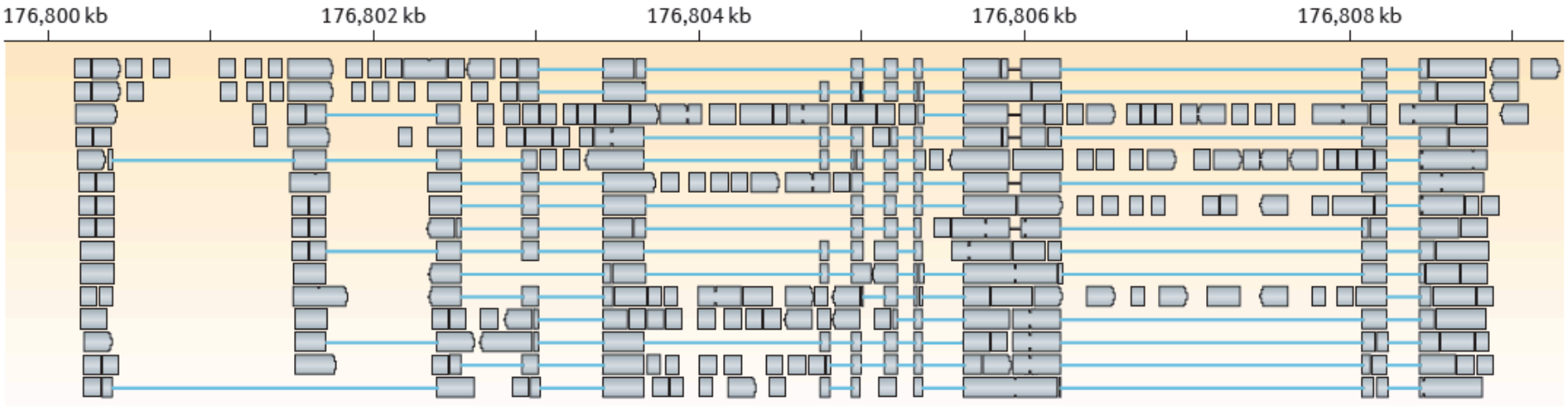


Overlapping but different introns = evidence of alternative splicing

From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome



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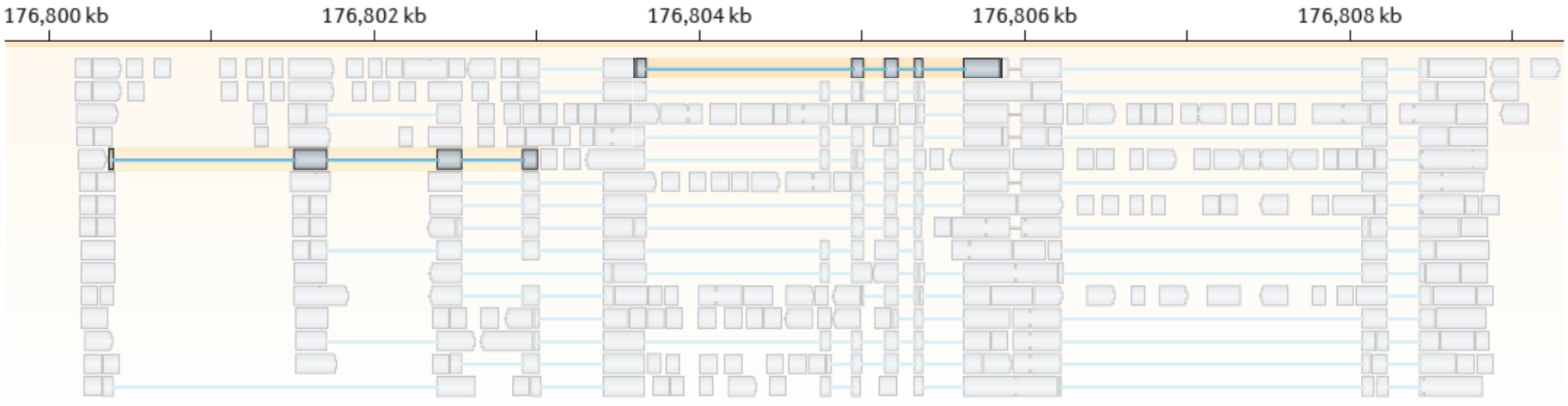
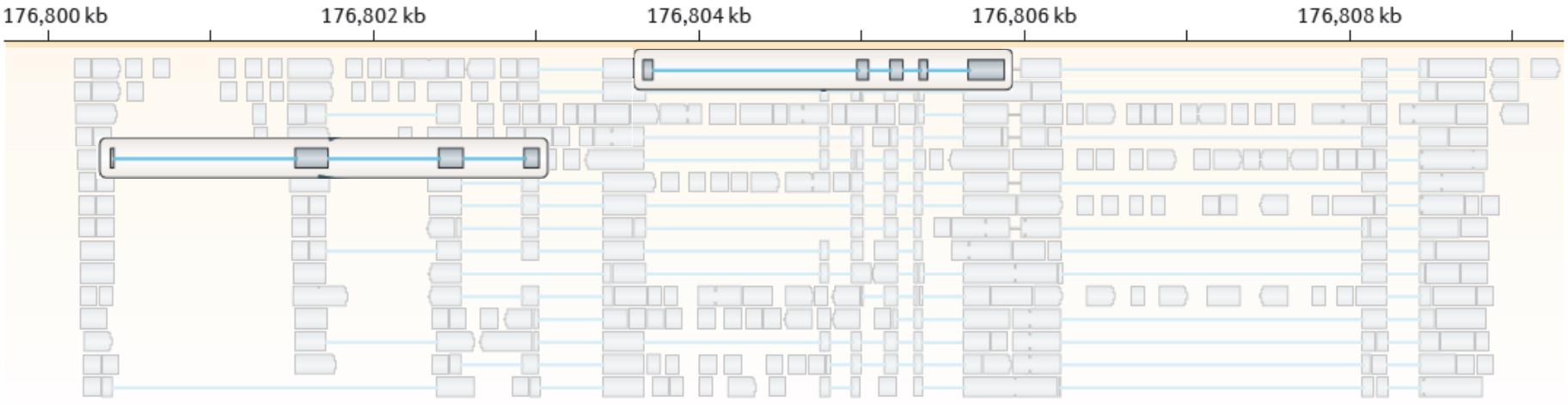


Individual reads can yield multiple exon and intron segments (splice patterns)

From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome

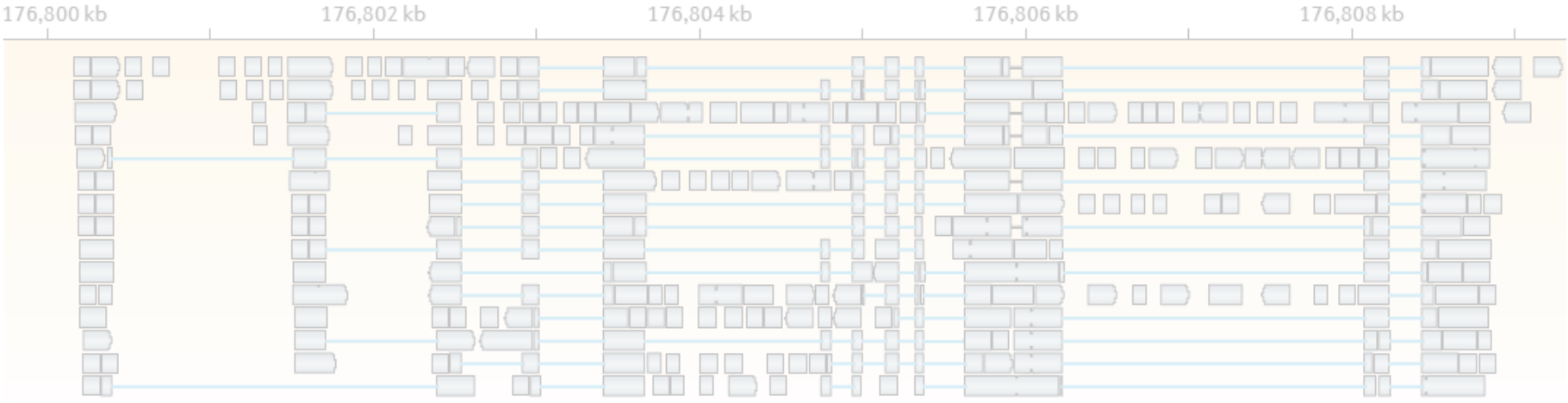


Nodes = unique splice patterns

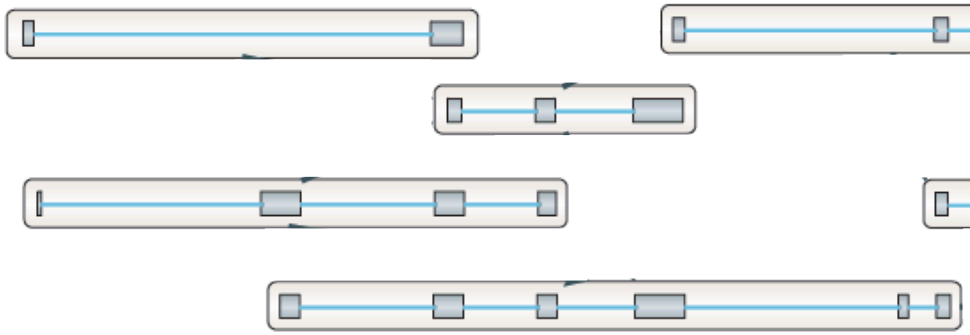
From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome



Construct graph from unique splice patterns of aligned reads.



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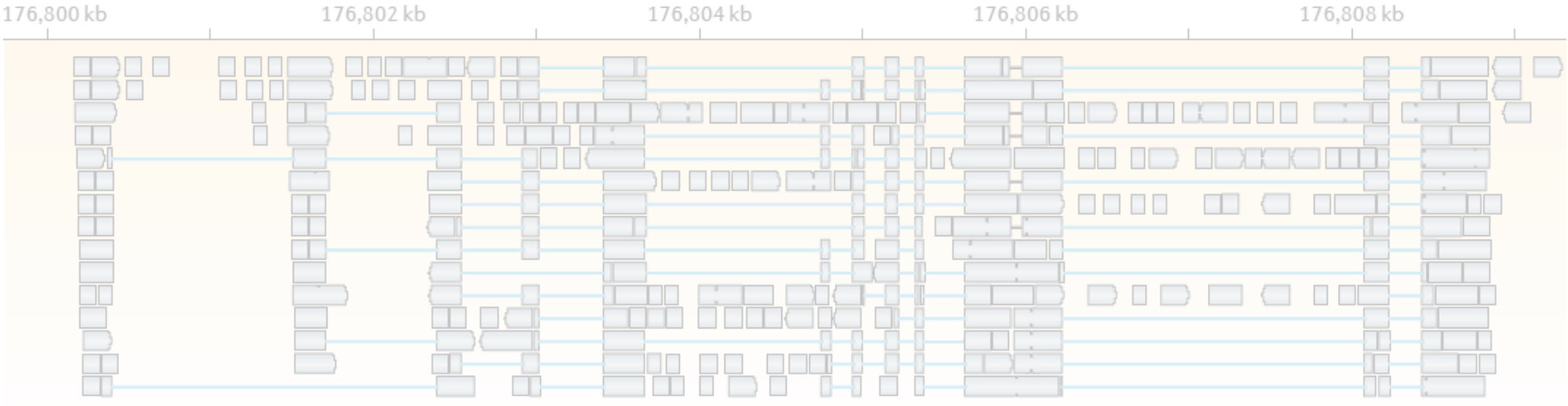

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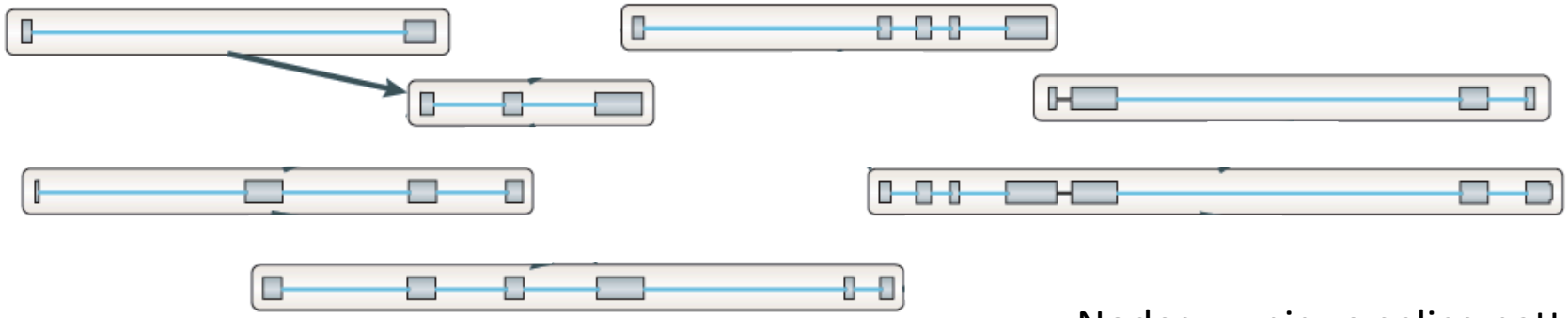
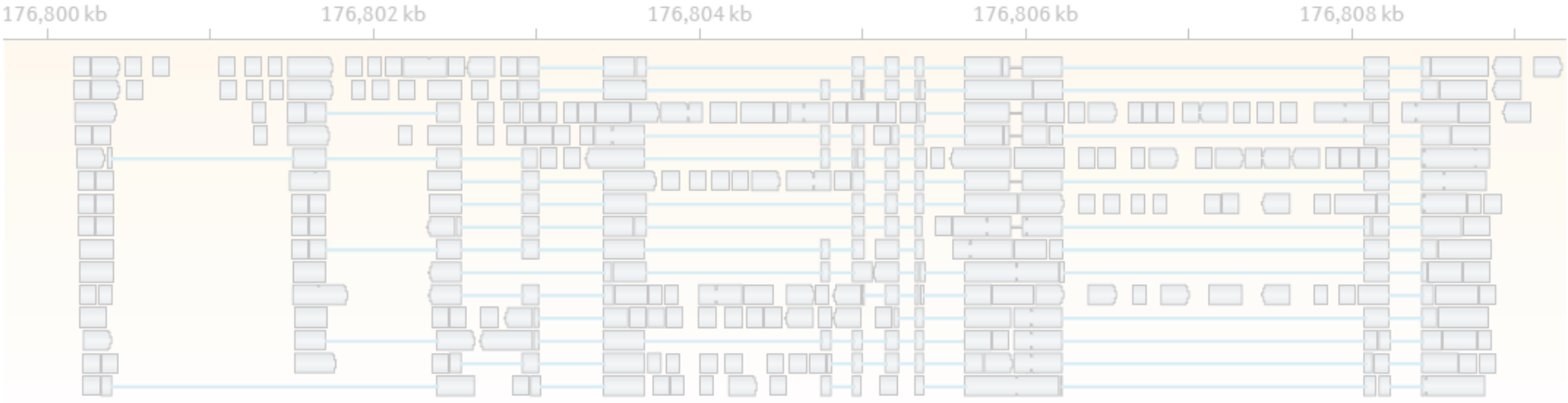


Nodes = unique splice patterns
Edges = compatible patterns

From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome



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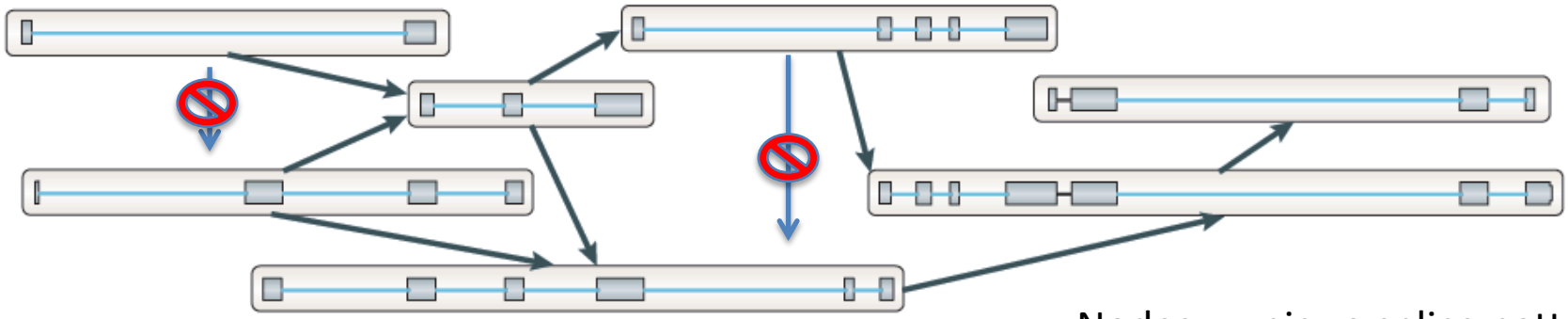

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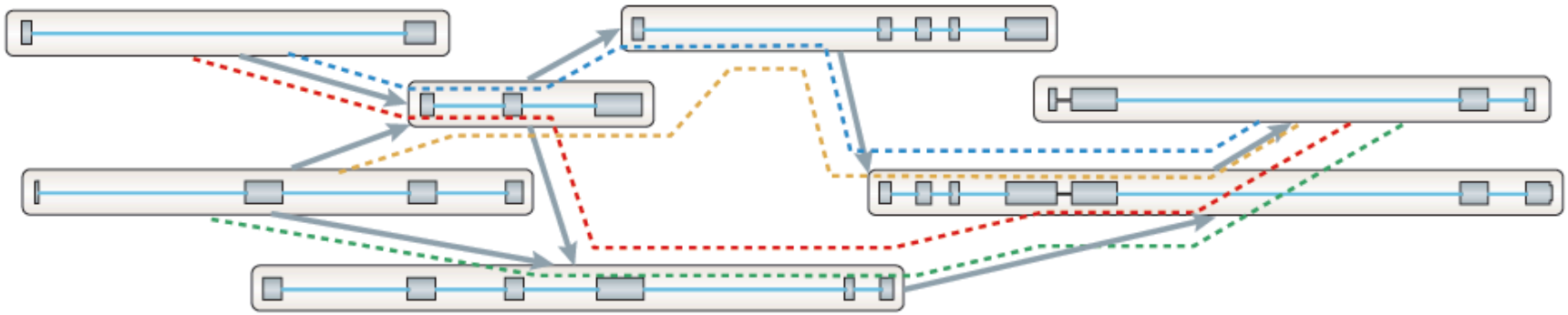
From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Traverse paths through the graph to assemble transcript isoforms



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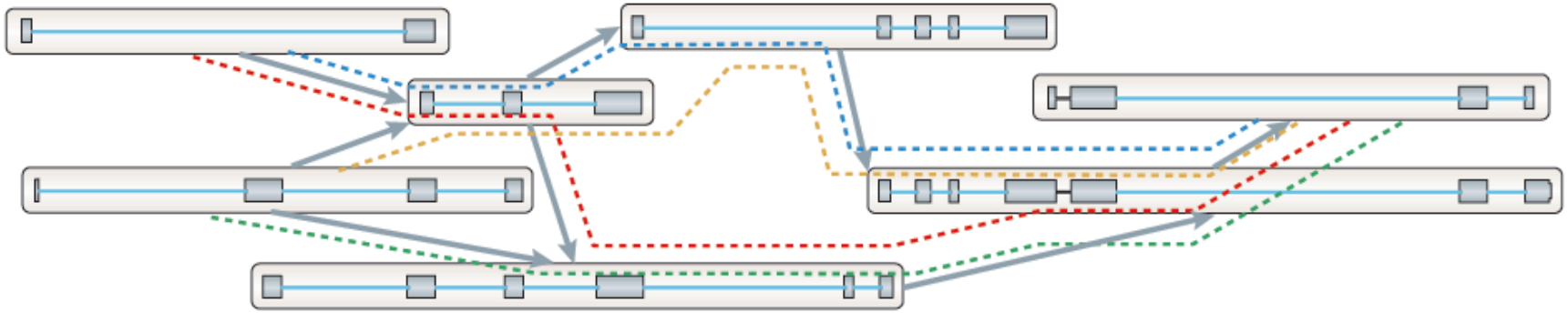
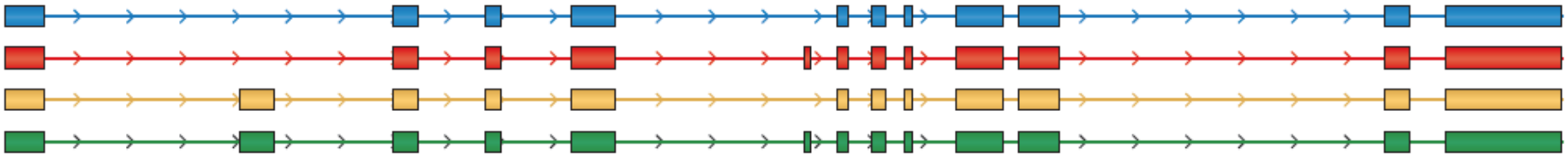


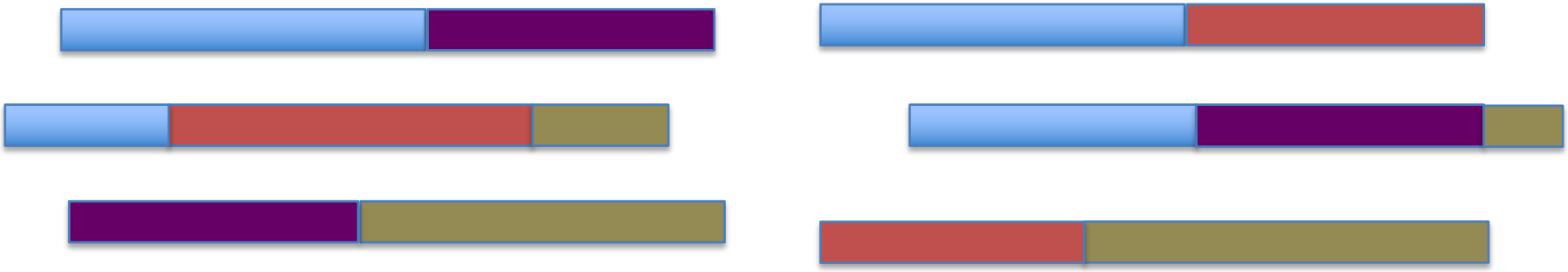
Reconstructed isoforms



From Martin & Wang. Nature Reviews in Genetics. 2011

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

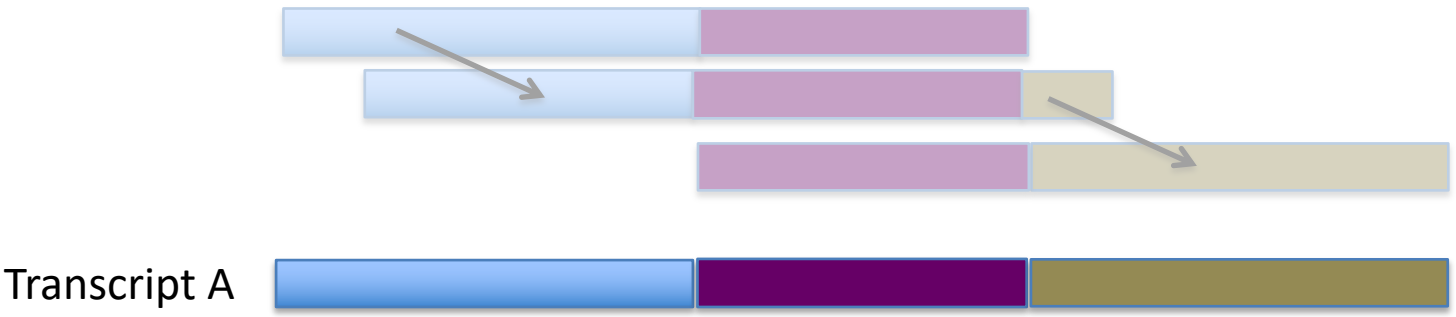
Read Overlap Graph: Reads as nodes, overlaps as edges



Read Overlap Graph: Reads as nodes, overlaps as edges

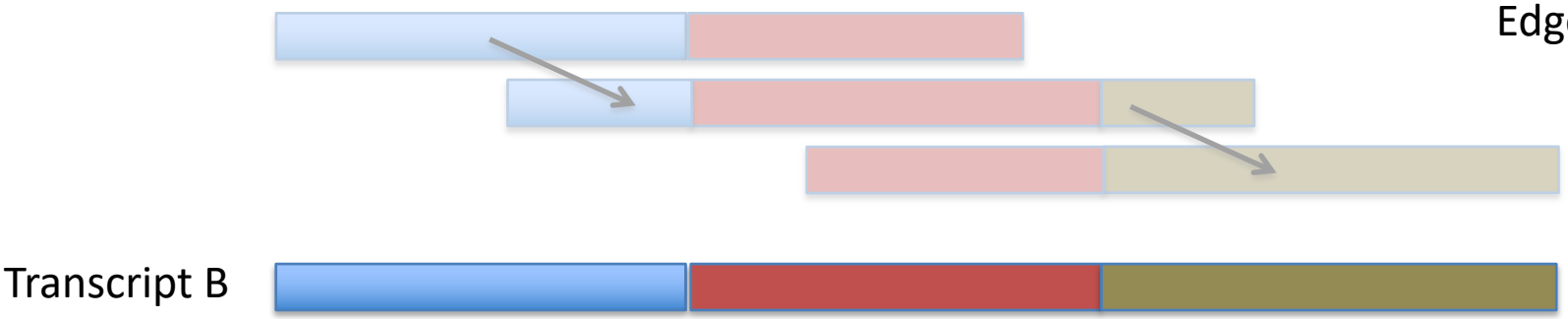
Node = read
Edge = overlap

Read Overlap Graph: Reads as nodes, overlaps as edges

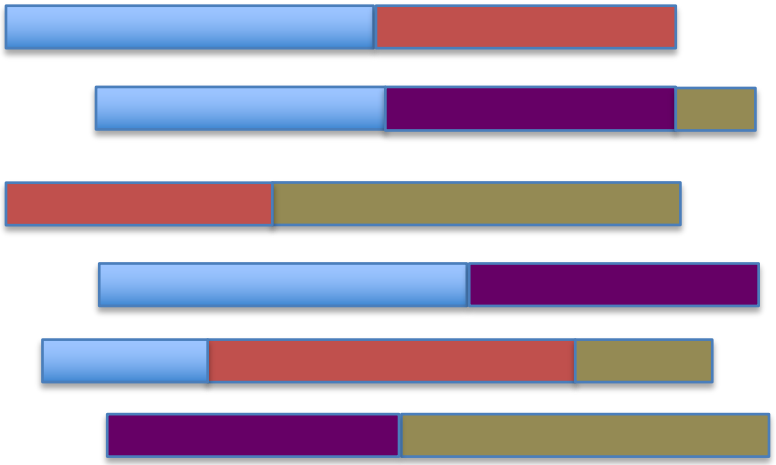


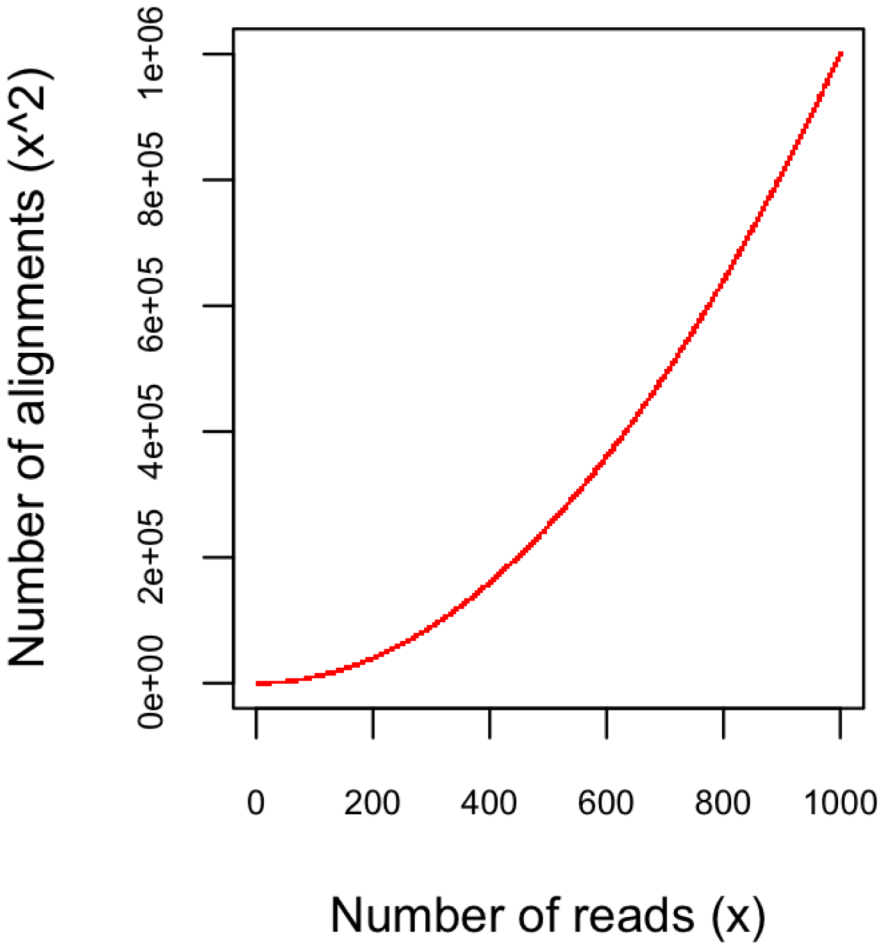
Generate consensus sequence where reads overlap

Node = read
Edge = overlap



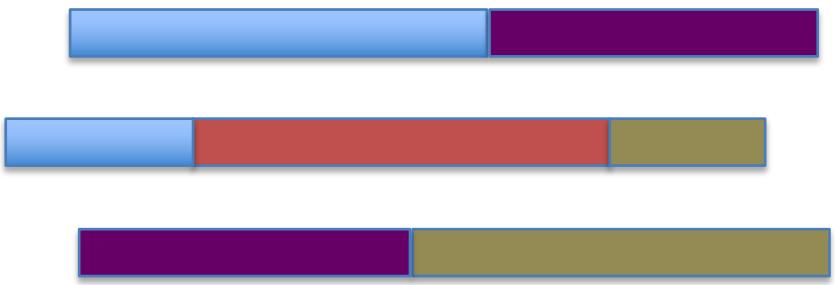
Finding pairwise overlaps between *n* reads involves ~ n^2 comparisons.



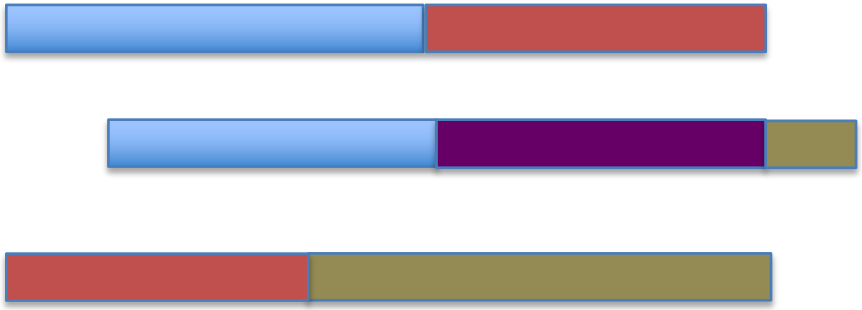


Impractical for typical RNA-Seq data (50M reads)

No genome to align to…



De novo assembly required

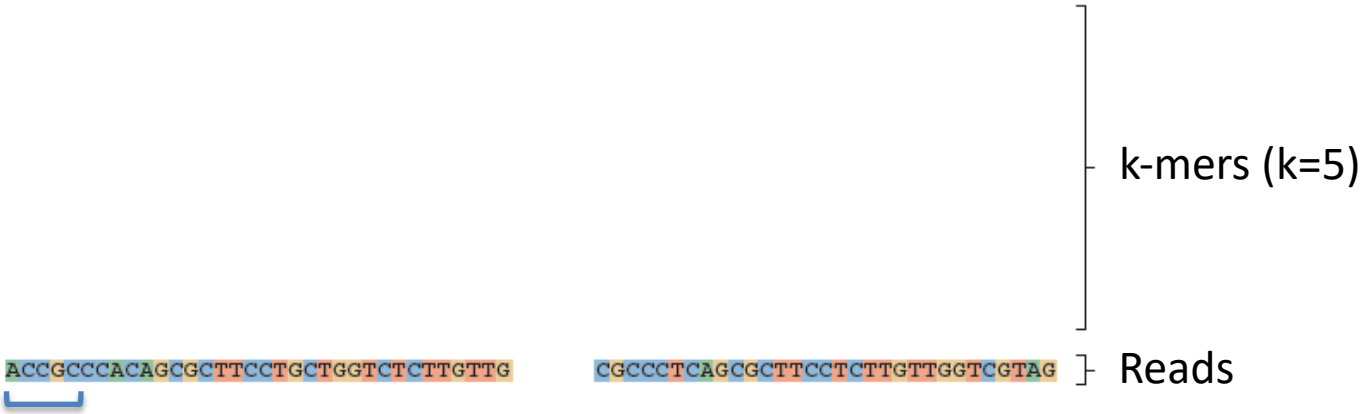


Want to avoid n^2 read alignments to define overlaps

Use a de Bruijn graph

Sequence Assembly via de Bruijn Graphs

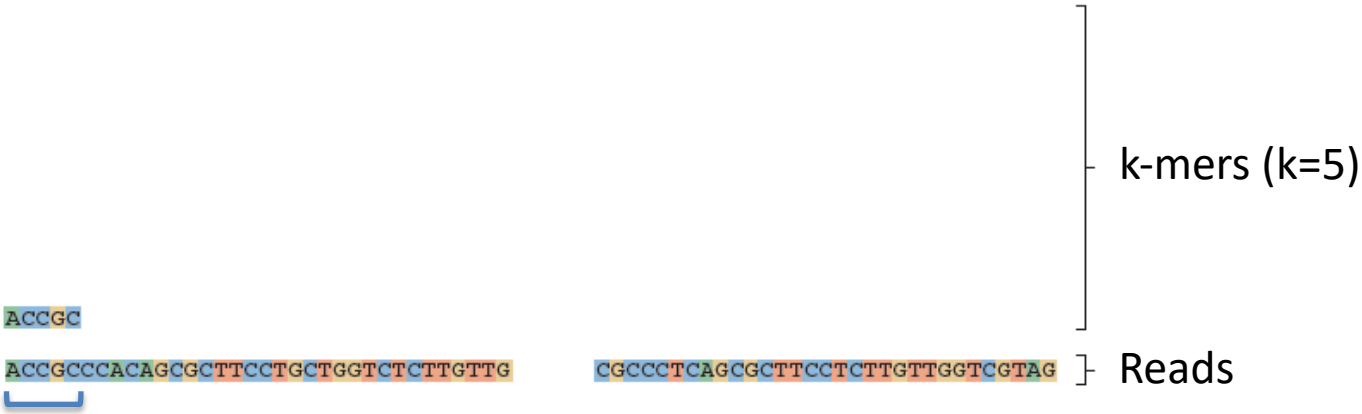
Generate all substrings of length k from the reads



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

Sequence Assembly via De Bruijn Graphs

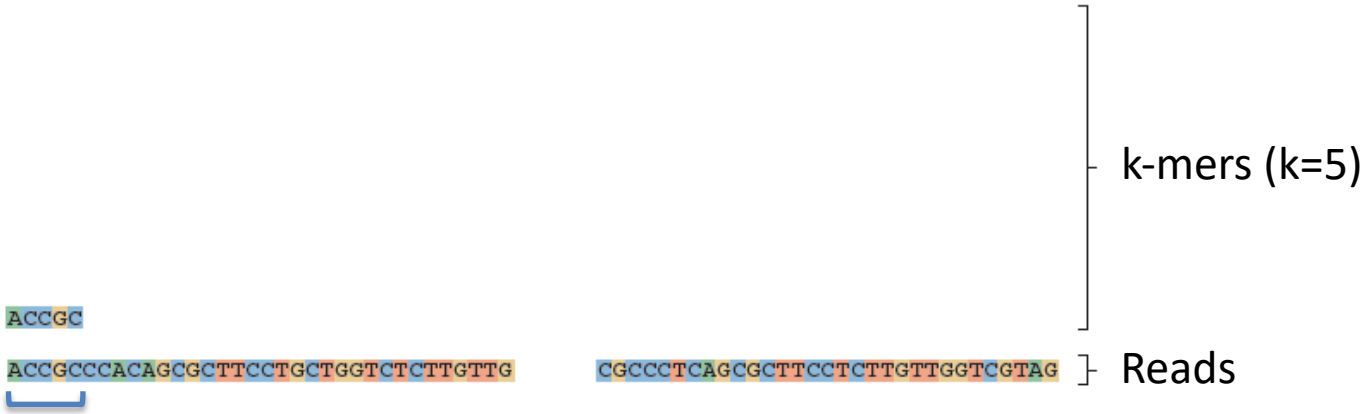
Generate all substrings of length k from the reads



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

Sequence Assembly via De Bruijn Graphs

Generate all substrings of length k from the reads



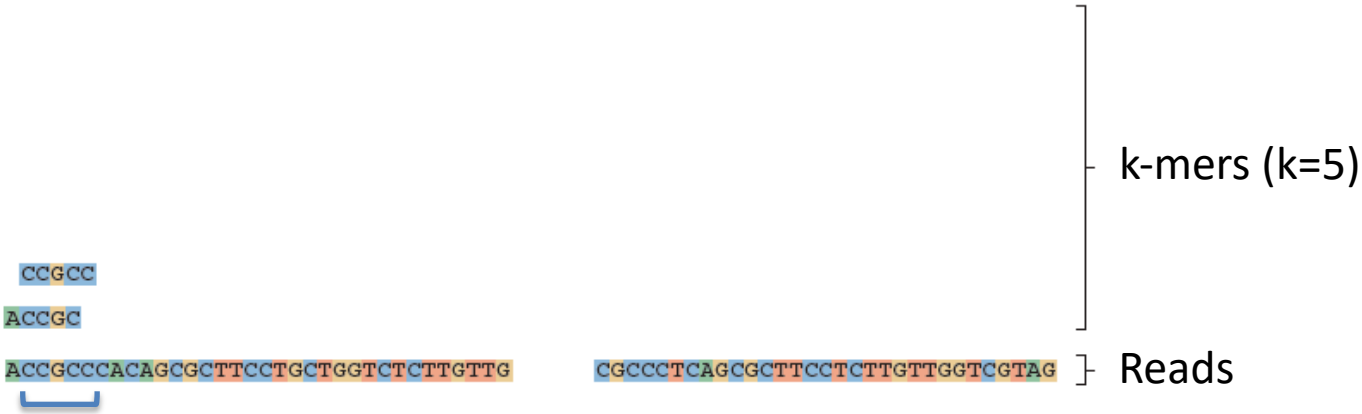
Construct the de Bruijn graph

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



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

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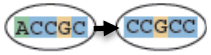

From Martin & Wang, Nat. Rev. Genet. 2011

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Sequence Assembly via De Bruijn Graphs

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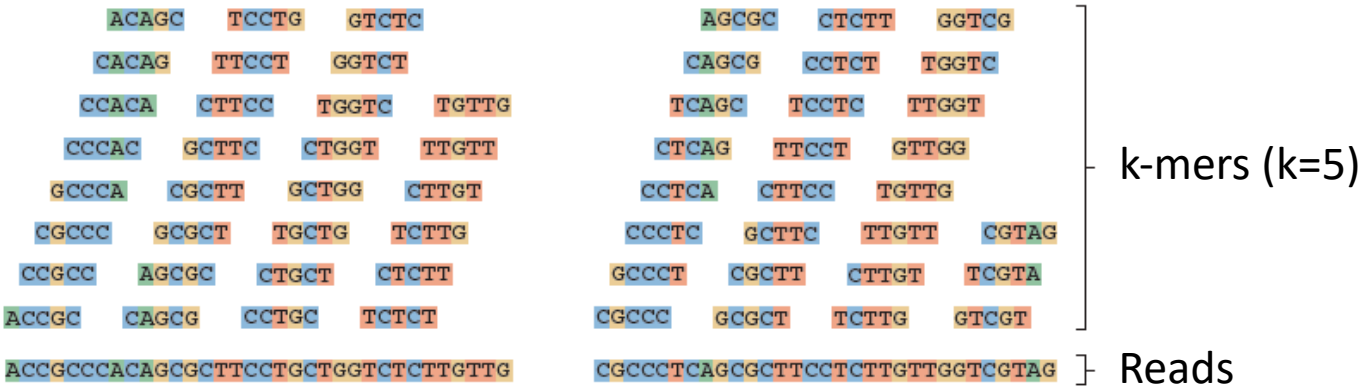
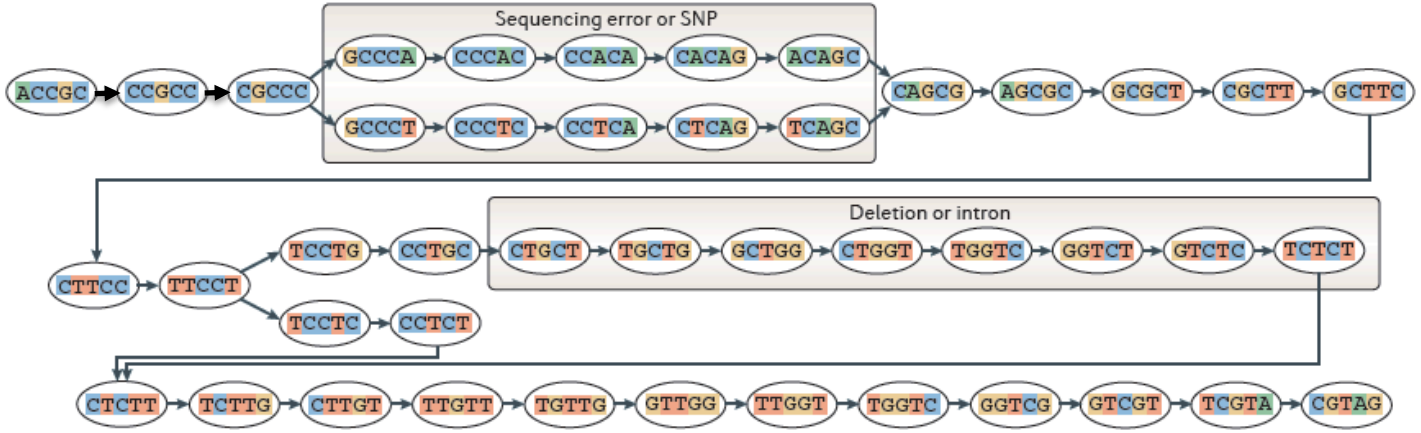


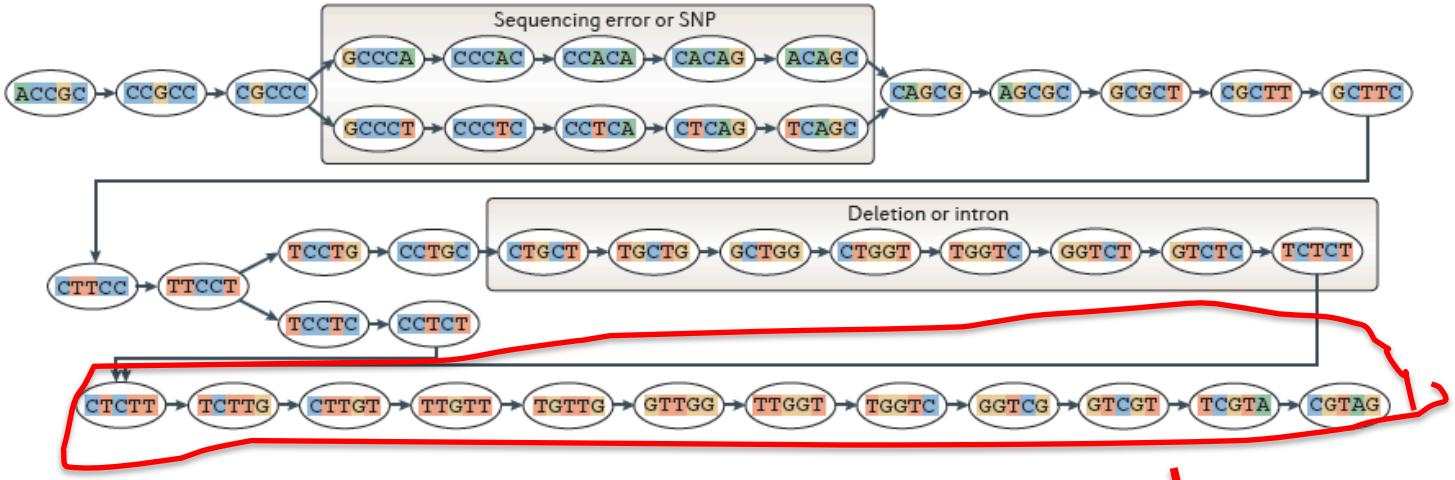
Construct the de Bruijn graph



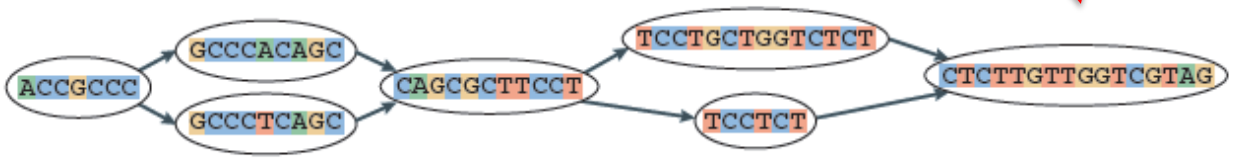
Nodes = unique k-mers
Edges = overlap by (k-1)

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

Construct the de Bruijn graph

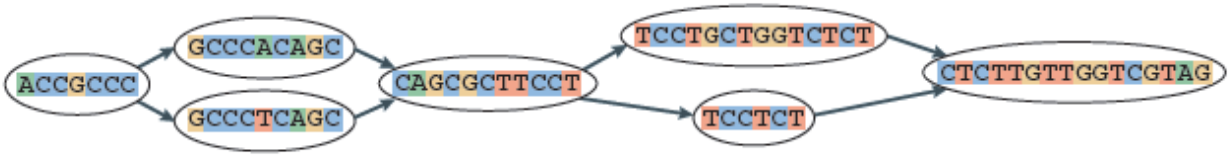


Collapse the de Bruijn graph

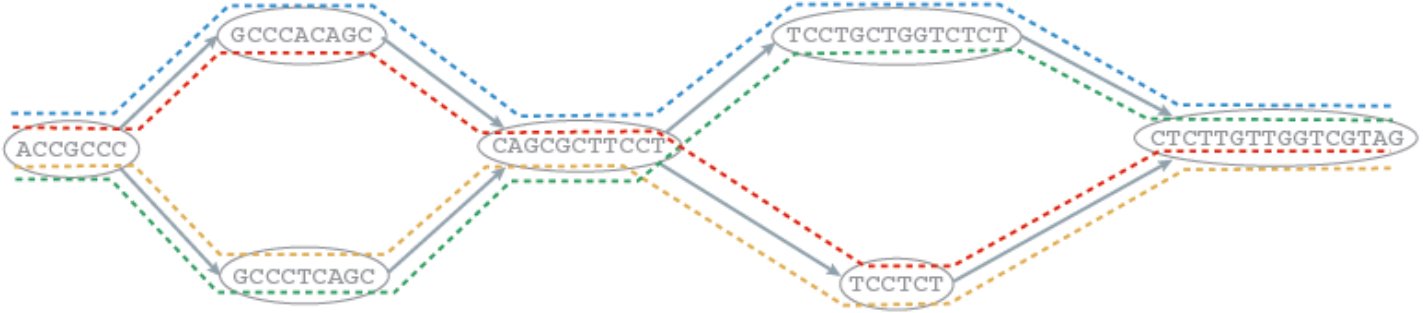


From Martin & Wang, Nat. Rev. Genet. 2011

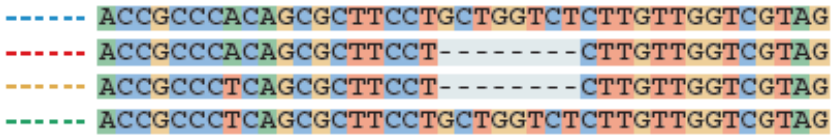
Collapse the de Bruijn graph



Traverse the graph



Assemble Transcript Isoforms



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

Contrasting Genome and Transcriptome *De novo* Assembly

Genome Assembly

- Uniform coverage
- Single contig per locus
- Assemble small numbers of large Mb-length chromosomes
- Double-stranded data

Transcriptome Assembly

- Exponentially distributed coverage levels
- Multiple contigs per locus (alt splicing)
- Assemble many thousands of Kb-length transcripts
- Strand-specific data available

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

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.

GATTACA
9

Kmer Catalog (hashtable)

Kmer	Count among all reads
AATGTGA	4
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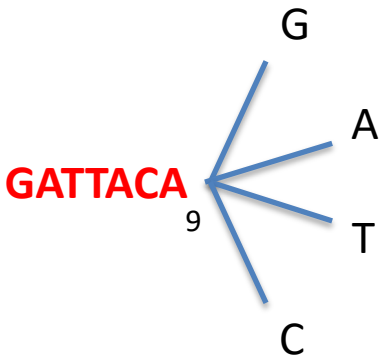
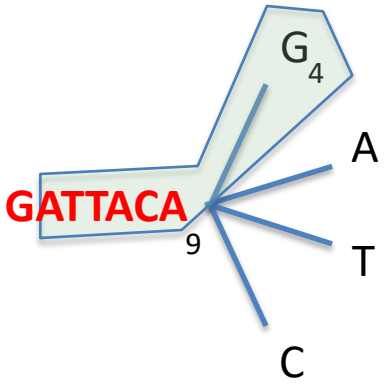
TGTGAAA	1
GATTACA	9

Inchworm Algorithm

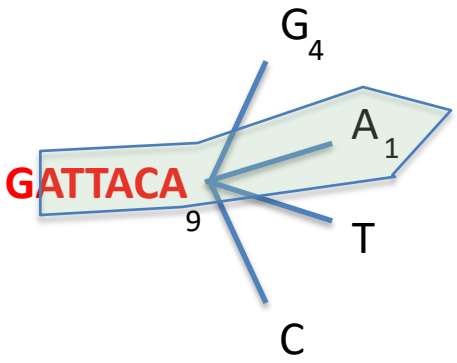
- Decompose all reads into overlapping Kmers => hashtable(kmer, count)
- Identify seed kmer as most abundant Kmer, ignoring low-complexity kmers.
- Extend kmer at 3’ end, guided by coverage.



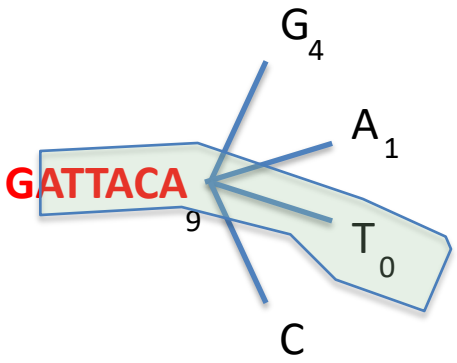
Inchworm Algorithm



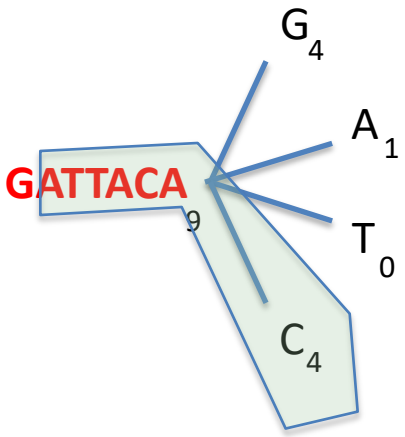
Inchworm Algorithm



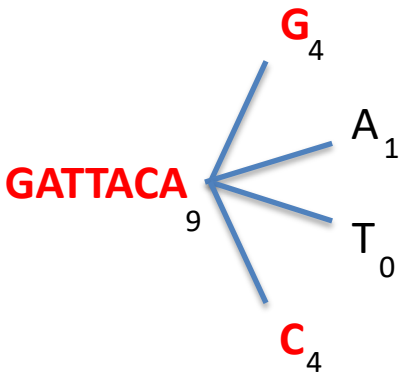
Inchworm Algorithm



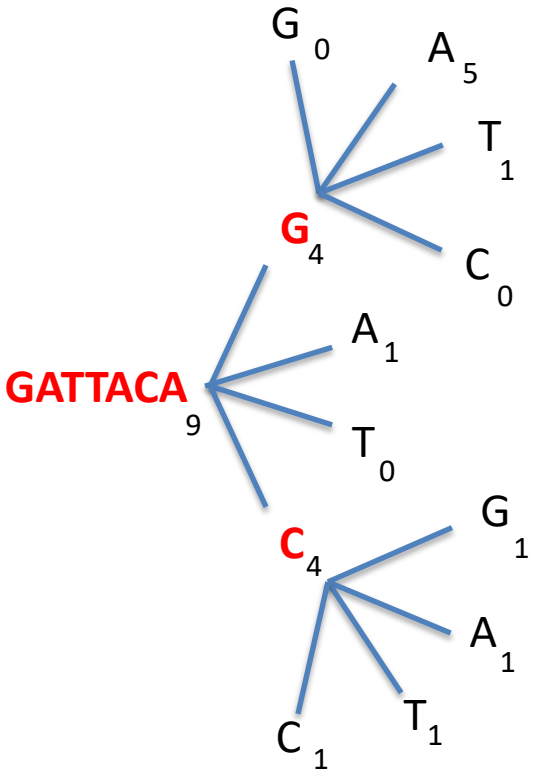
Inchworm Algorithm



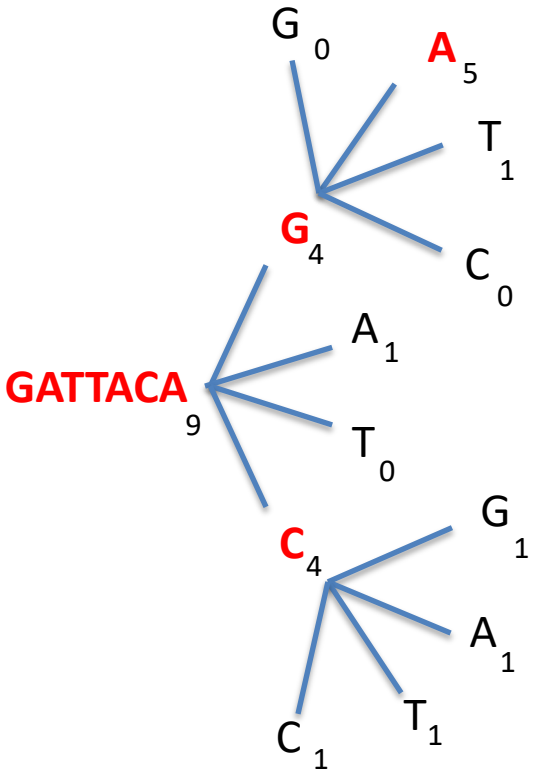
Inchworm Algorithm



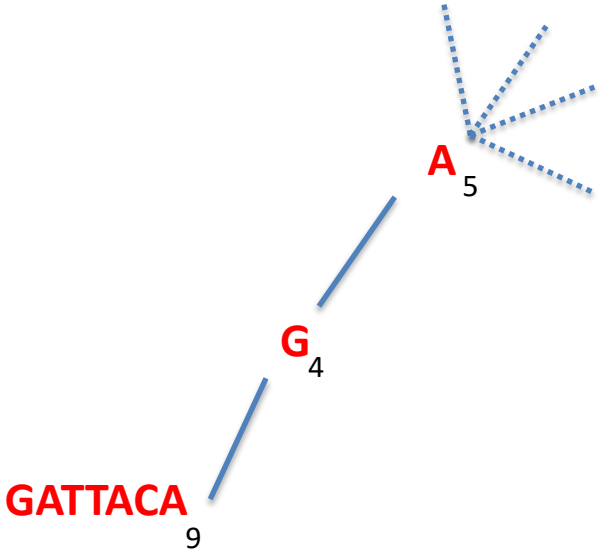
Inchworm Algorithm



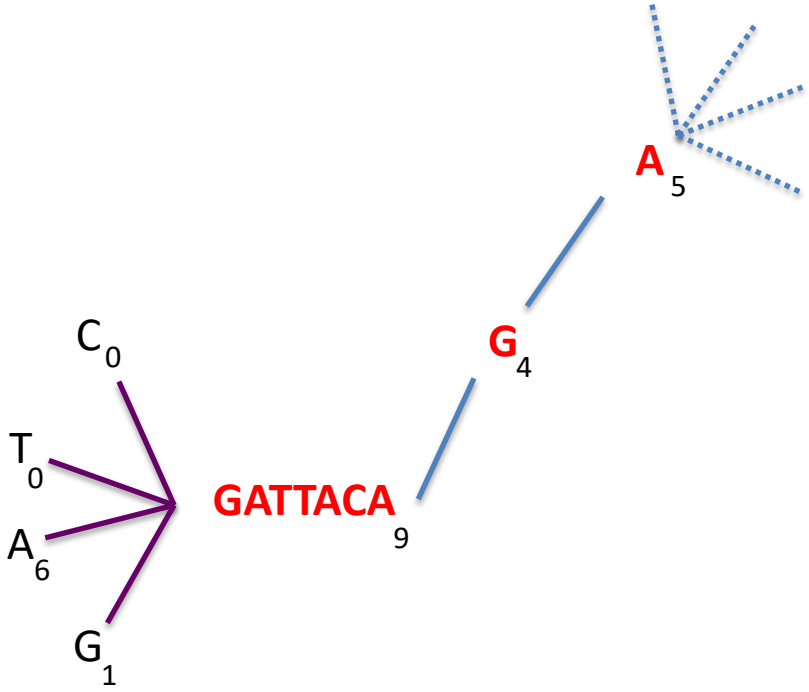
Inchworm Algorithm



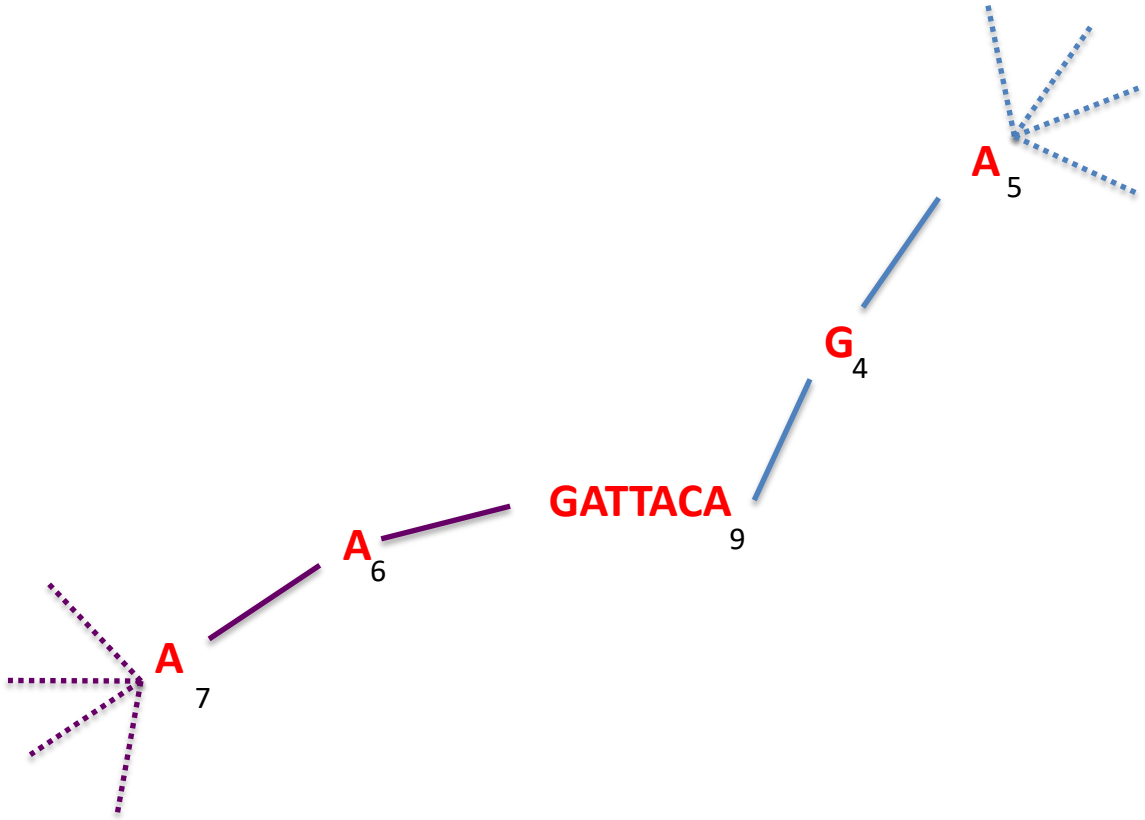
Inchworm Algorithm



Inchworm Algorithm



Inchworm Algorithm



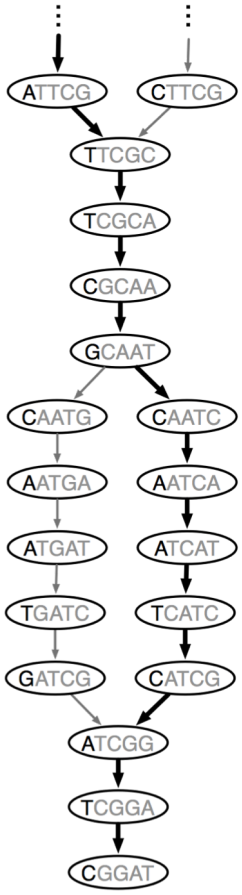
Report contig: ….AAGATTACAGA….

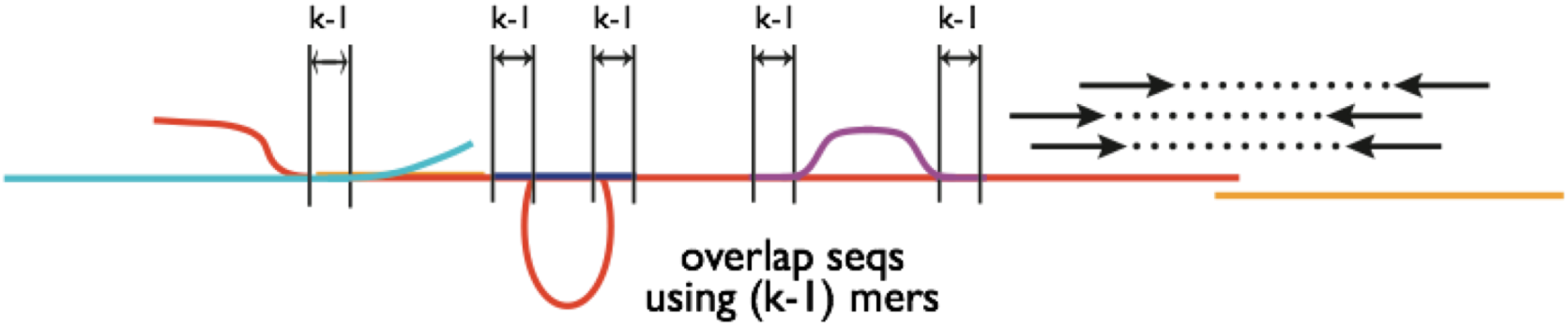
Remove assembled kmers from catalog, then repeat the entire process.

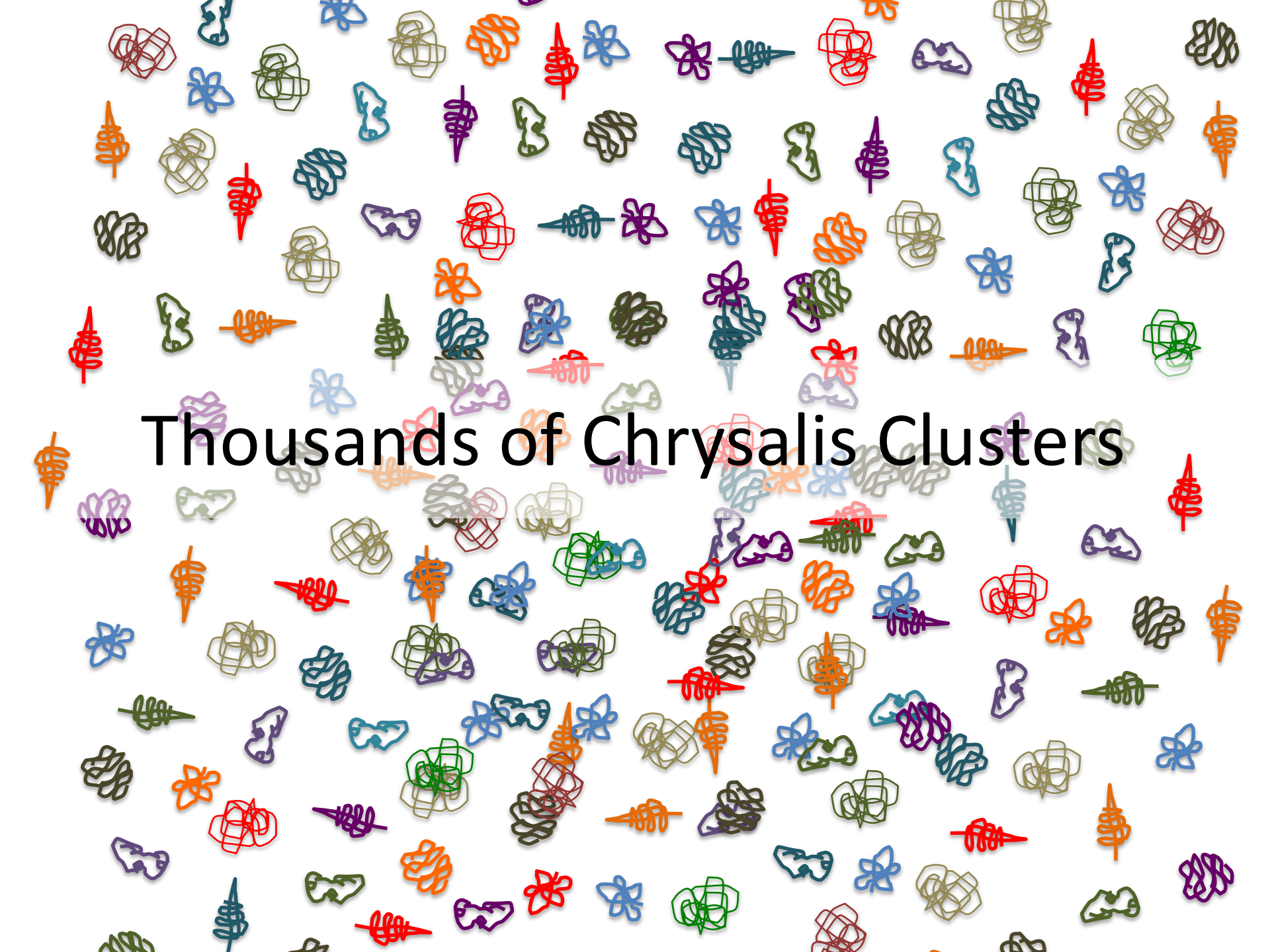
Chrysalis

Integrate isoforms via k-1 overlaps

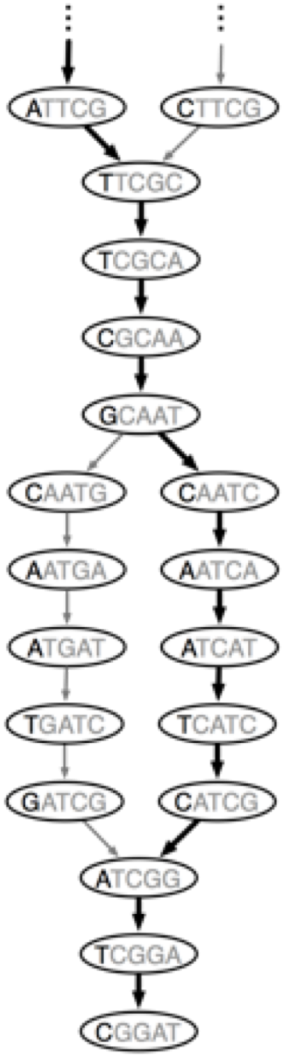
Build de Bruijn Graphs (ideally, one per gene)





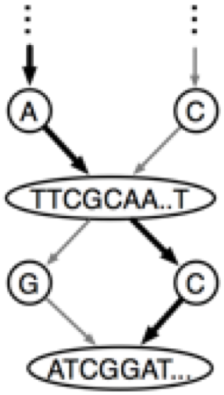

Thousands of Chrysalis Clusters

Butterfly



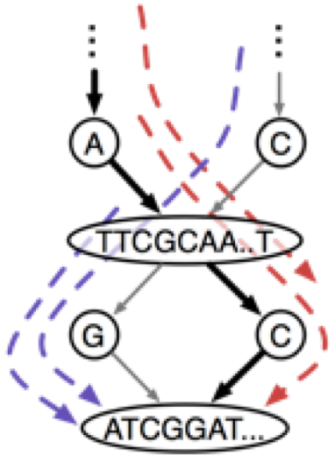
de Bruijn graph

compacting



compact graph

finding paths



compact graph with reads

extracting sequences

..CTTCGCAA..TGATCGGAT...

..ATTCGCAA..TCATCGGAT...

sequences (isoforms and paralogs)

Trinity output: A multi-fasta file

nature protocols

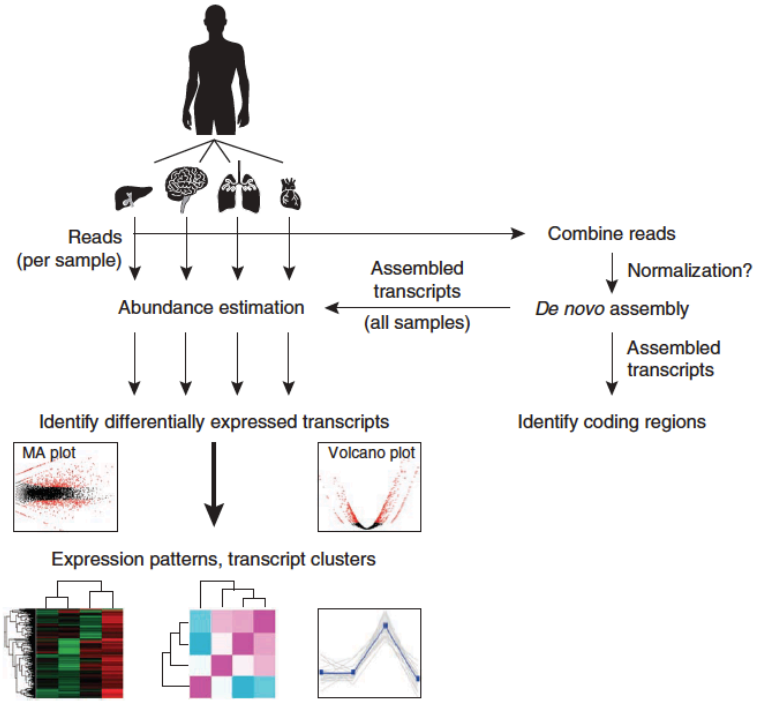
NATURE PROTOCOLS | PROTOCOL

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



RNA-Seq De novo Assembly Using Trinity

Quick Guide for the Impatient

Trinity assembles transcript sequences from Illumina RNA-Seq data.

Download Trinity here.

Build Trinity by typing 'make' in the base installation directory.

Assemble RNA-Seq data like so:

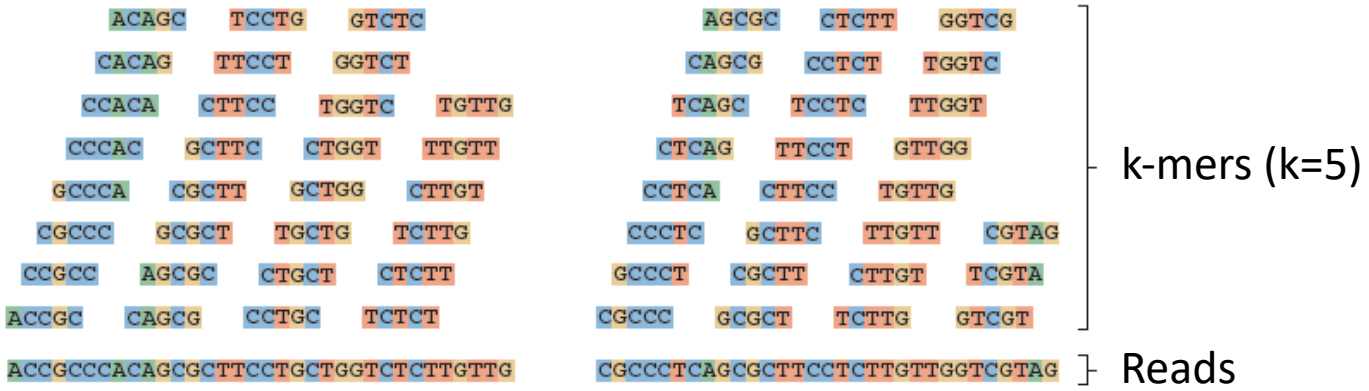
```
Trinity --seqType fq --left reads_1.fq --right reads_2.fq --CPU 6 --max_memory 20G
```

Find assembled transcripts as: 'trinity_out_dir/Trinity.fasta'

Use the documentation links in the right-sidebar to navigate this documentation, and contact our Google group for technical support.

ProgForBio Exercise 1: Build a program that counts k-mers

Generate all substrings of length k from the reads



Using a kmer size of 8 and reporting the top 10 kmers and their counts:

```
kmer_counter.py 8 reads.left.fq 10
```

.

```
TTTTTTTT        1743
AAAAAAAA        1204
CCAGCCTT        666
GAAGCTGG        627
CAGGCAGG        549
TTTGCTGT        536
GCCTGCTG        533
CTTGGTCT        525
AAGCTGGA        518
TTTTATTT        504
```

https://github.com/trinityrnaseq/CSHLProgForBiol2019/tree/master/Exercise_1-counting_kmers