Intro to Transcriptomics and Transcriptome Assembly

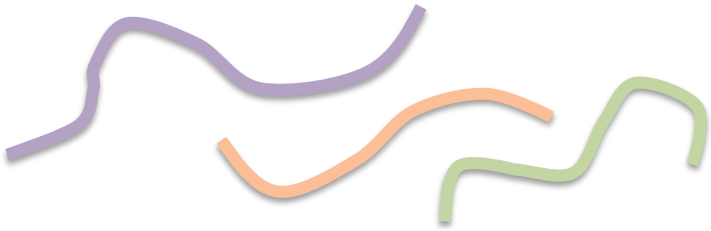
Programming for Biologists
CSHL 2023

Brian Haas

Broad Institute

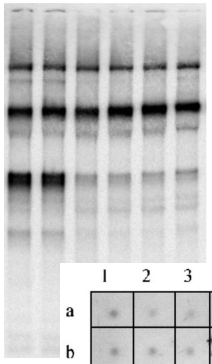
Biological Investigations Empowered by Transcriptomics

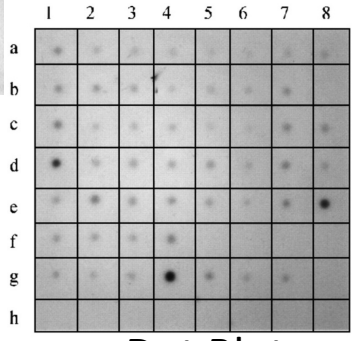
Extract RNA,
… some protocol for processing, ...



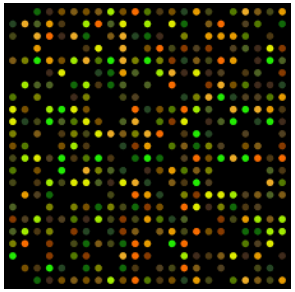
Analysis Method
(pick your favorite)

Northern

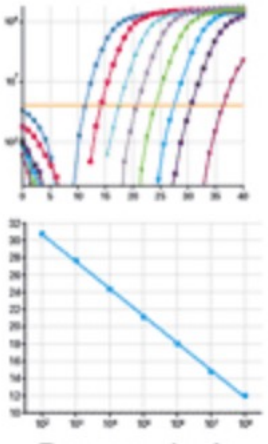




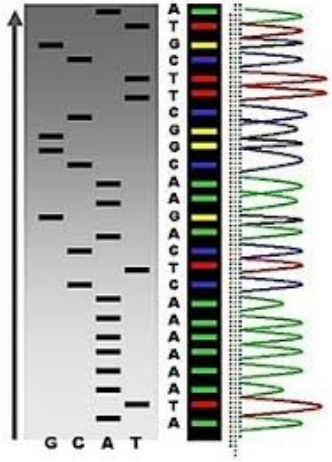
Dot Blot



Microarray



qRT-PCR



Sanger Sequencing

Other…

Minion

MinION

Historical Timeline to Modern Transcriptomics (from 1970)

Reverse Transcription (1970)

Northern Blot
Sanger Sequencing
(1977)

Expressed Sequence Tags (1992)

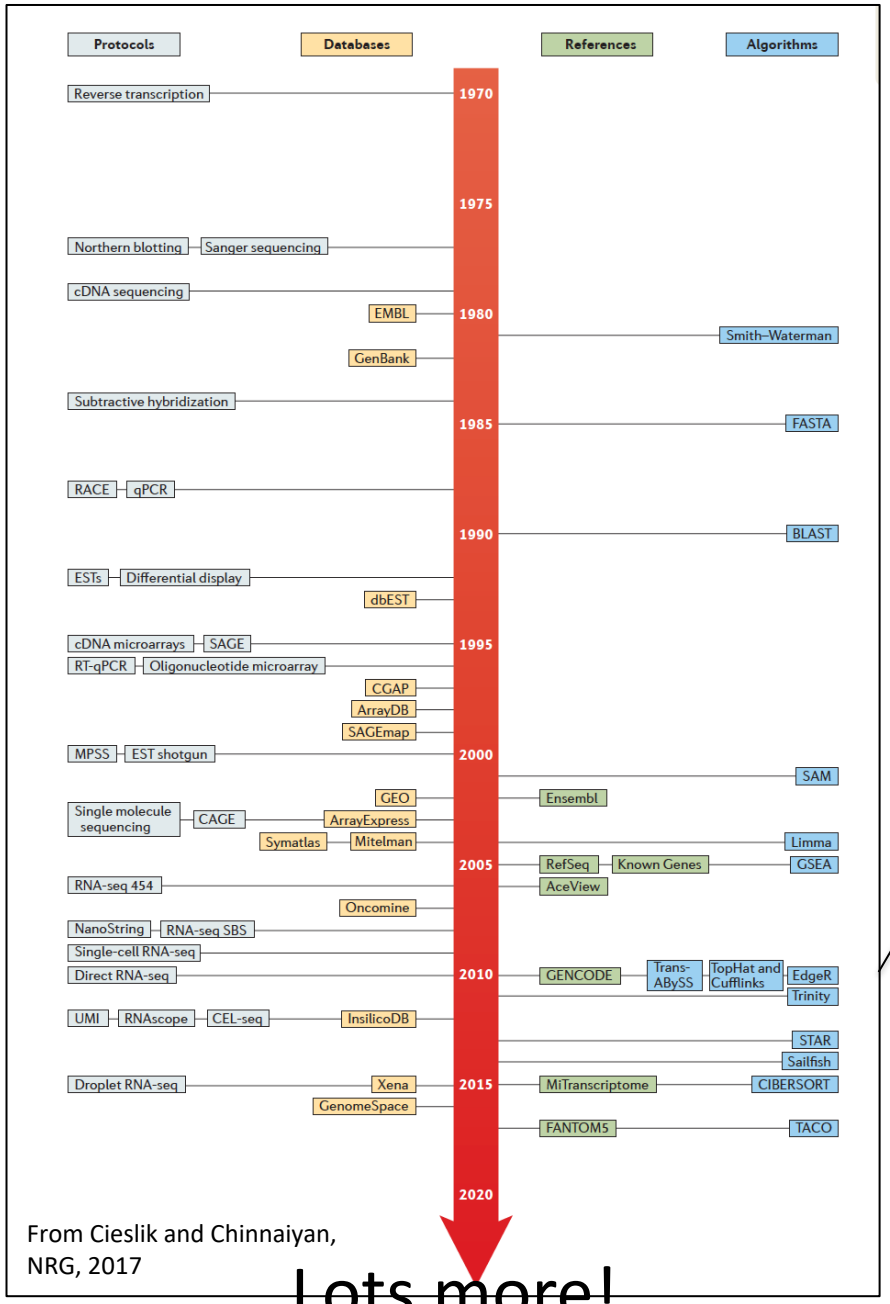
cDNA microarrays (1995)

RNA-Seq (2006-2008)

PacBio IsoSeq (2014)

Droplet single cell RNA-Seq (2015)

Direct RNA Seq Nanopore (2018)



From Cieslik and Chinnaiyan, NRG, 2017

Lots more!

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

Smith Waterman (1981)

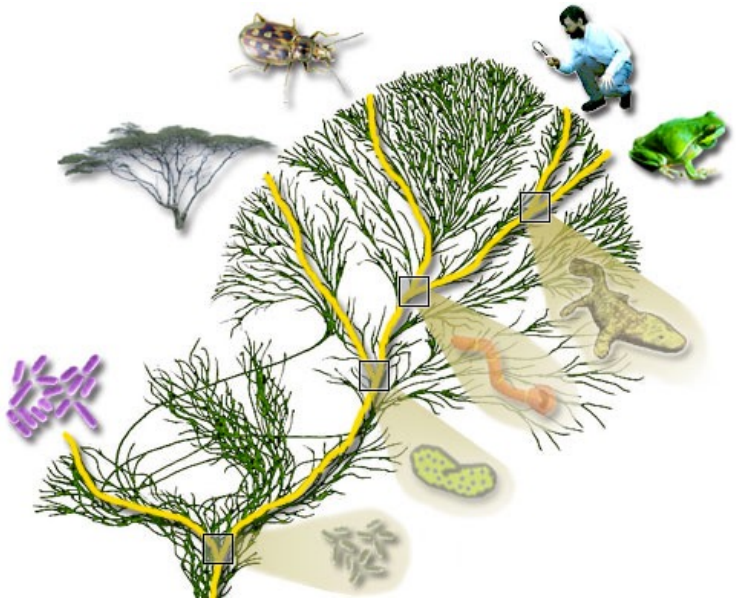
BLAST (1990)

Tophat/Cufflinks (2010)

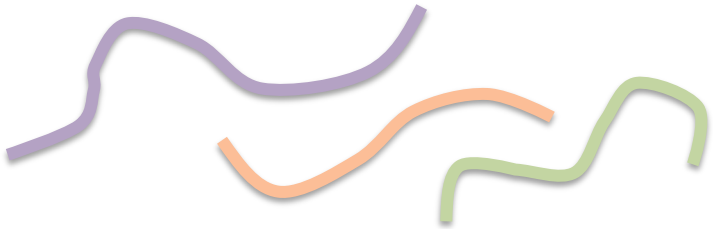
RSEM (2011)

Kallisto (2016)
Salmon (2017)

Modern Transcriptome Studies Empowered by RNA-seq

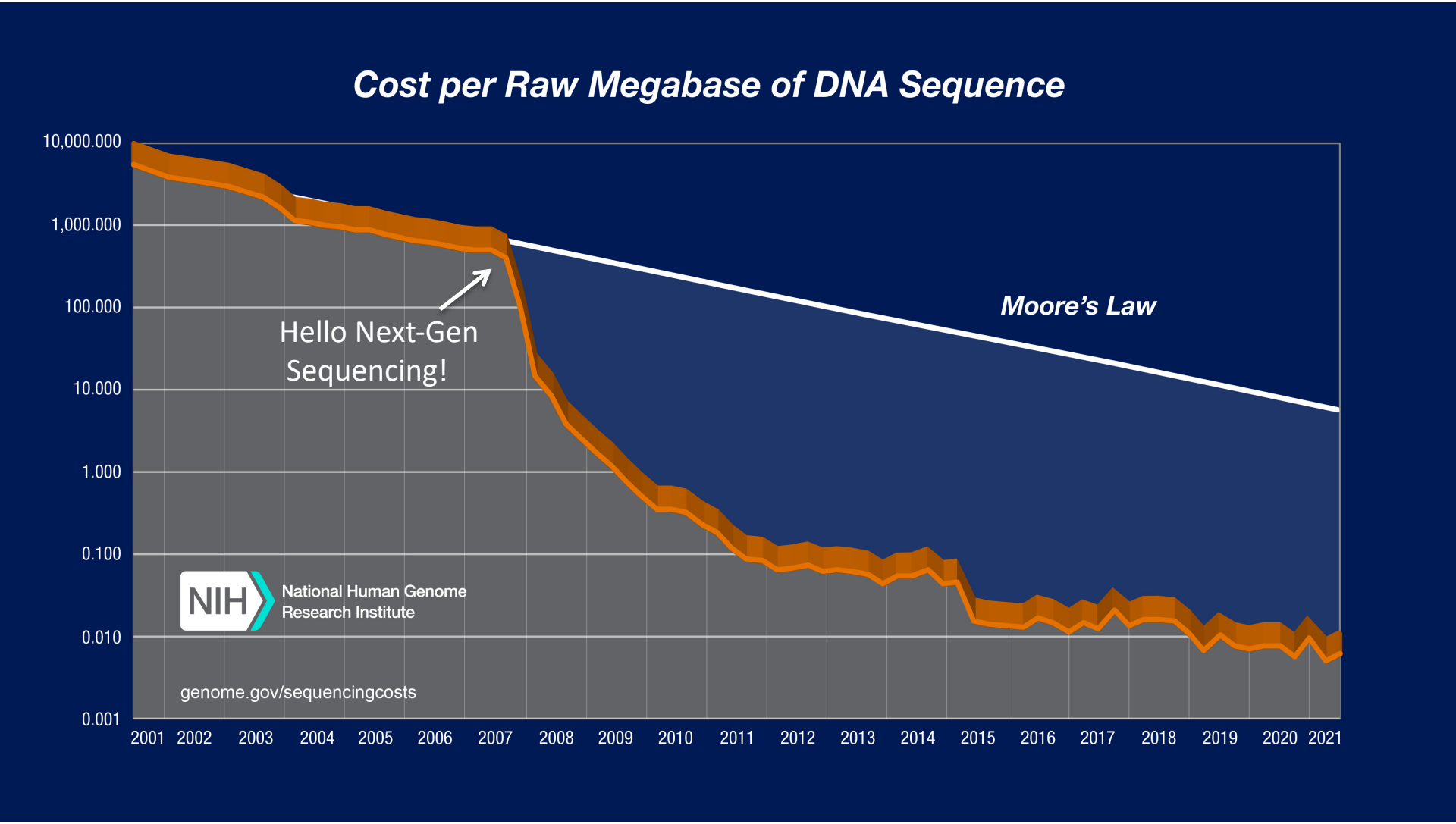


Extract RNA, convert to cDNA



Next-gen Sequencer
(pick your favorite)

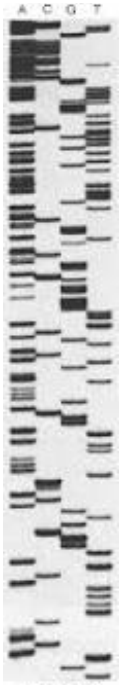
Millions to Billions of Reads

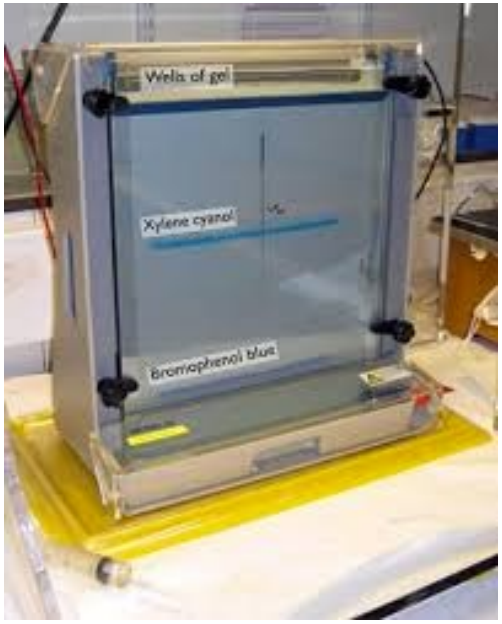


From https://www.genome.gov/sequencingcostsdata/

Personal Reflections...

Circa 1995





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	5500 XL	318 HiQ 520	Ion 530	Ion Proton P1	PGM HiQ 540	RS P6-C4	Sequel	R&D end 2018	Smidg ION RnD	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)	4	25	25	400	600	3000	4000	5000	6000	3300	6600	20000	1400	3-5	15-20	165	60-80	5.5	38.5	--	--	--	--	--	--	400	1600	1600	--
Read length: (paired-end*)	150*	150*	300*	150*	100*	100*	125*	150*	150*	150*	150*	150*	60	200 400	200 400	200	200	15K	12K	32K	--	--	--	--	--	--	100*	50	--
Run time: (d)	0.54	1	2	1.2	1.125	11	6	3.5	3	1.66	1.66	1.66	7	0.37	0.16	--	0.16	4.3	--	--	--	2	2	2	--	--	1	0.4	--
Yield: (Gb)	1	7.5	15	120	120	600	1000	1500	1800	1000	2000	6000	180	1.5	7	10	12	12	5	150	4	8	40	2400	11000	80	200	8	--
Rate: (Gb/d)	1.85	7.5	7.5	100	106.6	55	166	400	600	600	1200	3600	30	5.5	50	--	93.75	2.8	--	--	--	4	20	1200	5500	--	200	20	--
Reagents: ($K)	0.1	1.75	1	5	6.145	23.47	29.9	--	--	--	--	--	10.5	0.6	--	1	1.2	2.4	--	1	--	0.5	1.5	--	--	0.5	--	--	--
per-Gb: ($)	100	233	66	50	51.2	39.1	31.7	20.5	7.08	18	15	5.8	58.33	--	--	100	--	200	80	6.6	--	62.5	37.5	20	4.3	--	--	--	--
hg-30x: ($)	12000	28000	8000	5000	6144	4692	3804	2460	849.6	1800	1564	700	7000	--	--	12000	--	24000	9600	1000	--	7500	4500	2400	500	--	600	--	
Machine: ($)	30K	49.5K	99K	250K	740K	690K	690K	900K	1M	999K	999K	999K	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-q

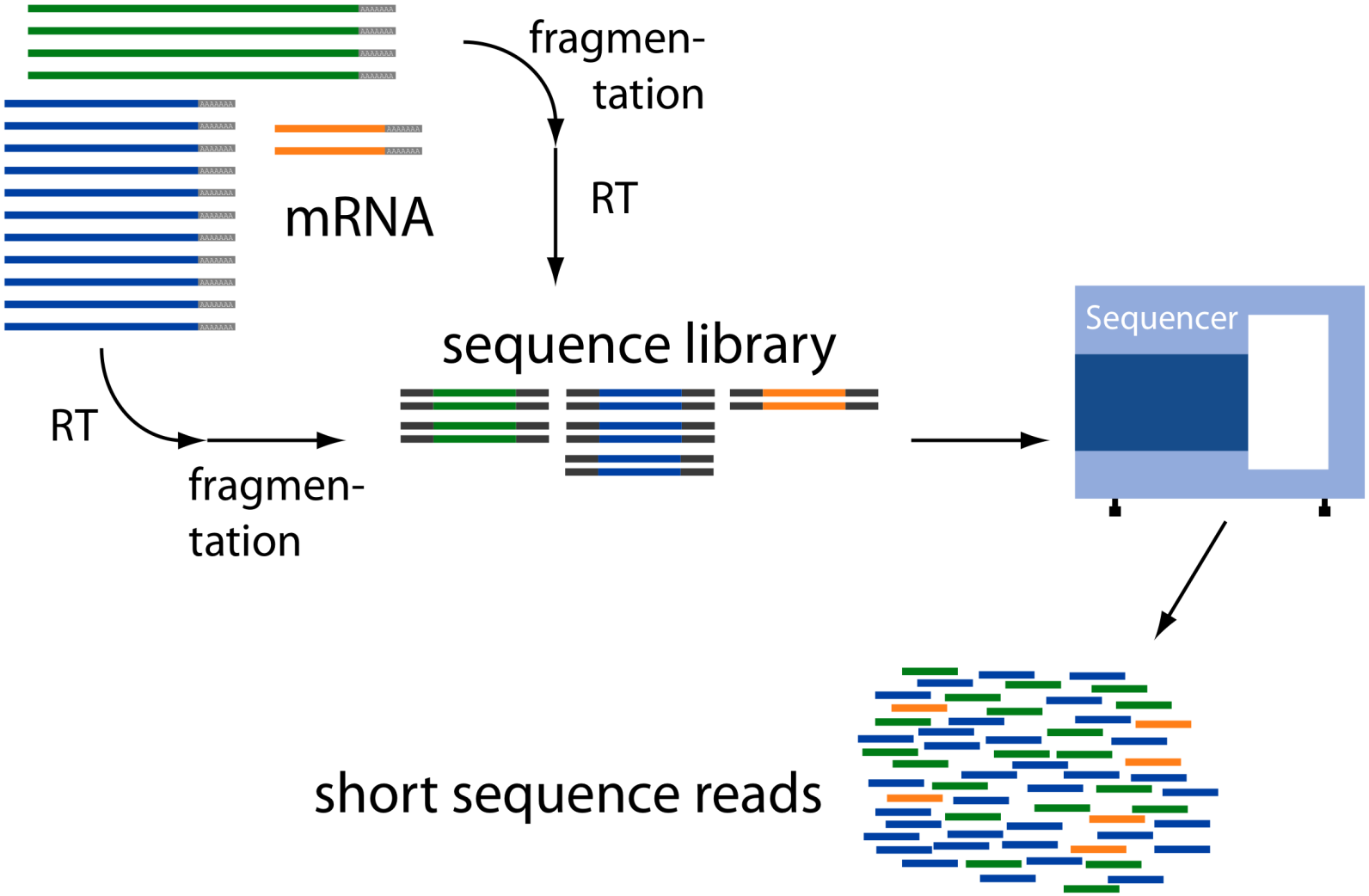
g	Mini ION R9.5	Grid ION X5	Prome thION RnD	Prome thION theor etical	QiaGen Gene Reader	BGI SEQ 500	BGI SEQ 50	#
--	--	--	--	--	400	1600	1600	--
--	--	--	--	--	--	100*	50	--
--	2	2	2	--	--	1	0.4	--
4	8	40	2400	11000	80	200	8	--
--	4	20	1200	5500	--	200	20	--
--	0.5	1.5	--	--	0.5	--	--	--
--	62.5	37.5	20	4.3	--	--	--	--
--	7500	4500	2400	500	--	600	--	
--	--	125K	75K	75K	--	200K	--	--

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

Thx Joshua Levin, for the cartoon. ☺

Each has pros/cons

Overview of RNA-Seq



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

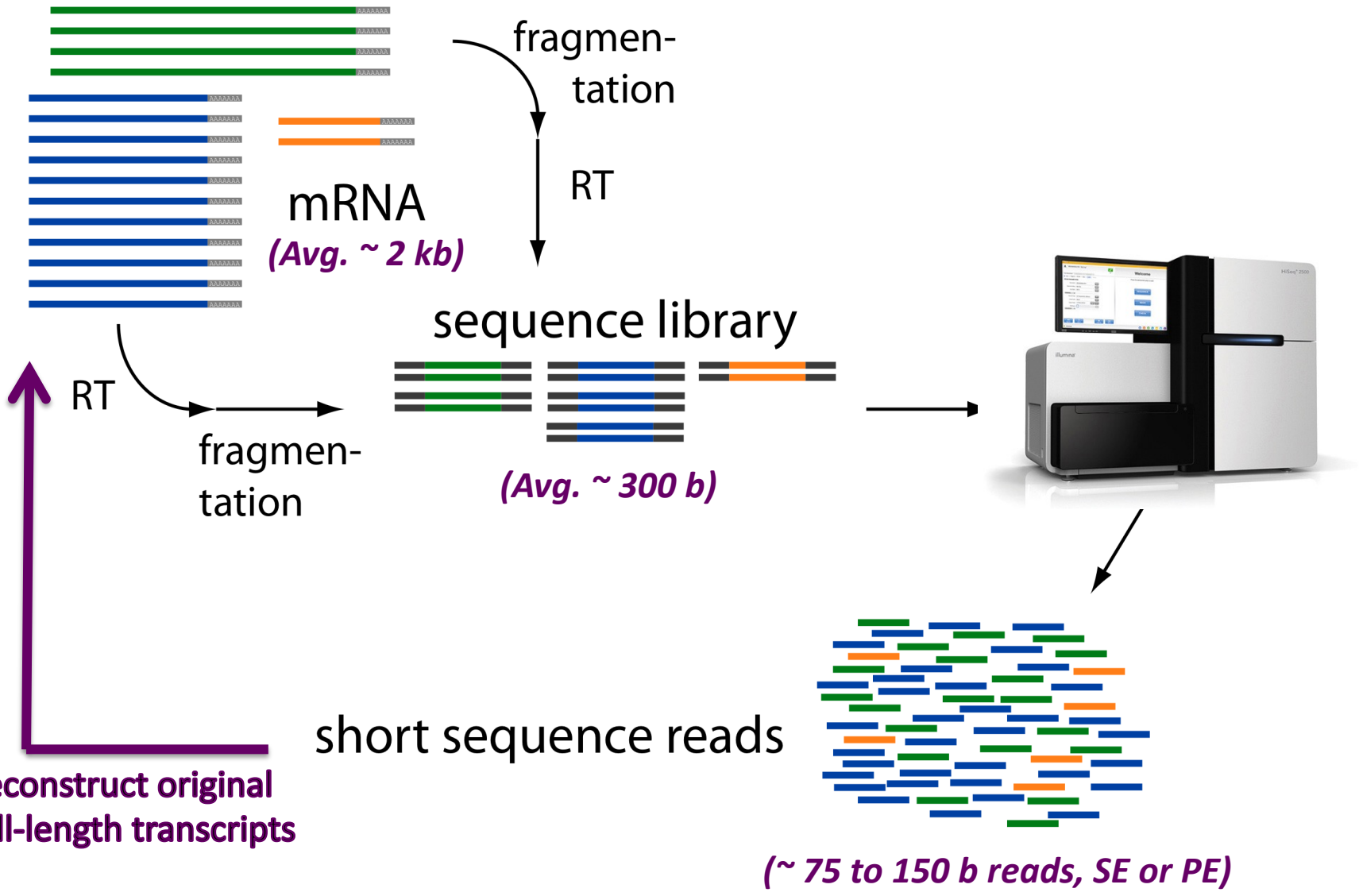
Paired-end Sequences

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

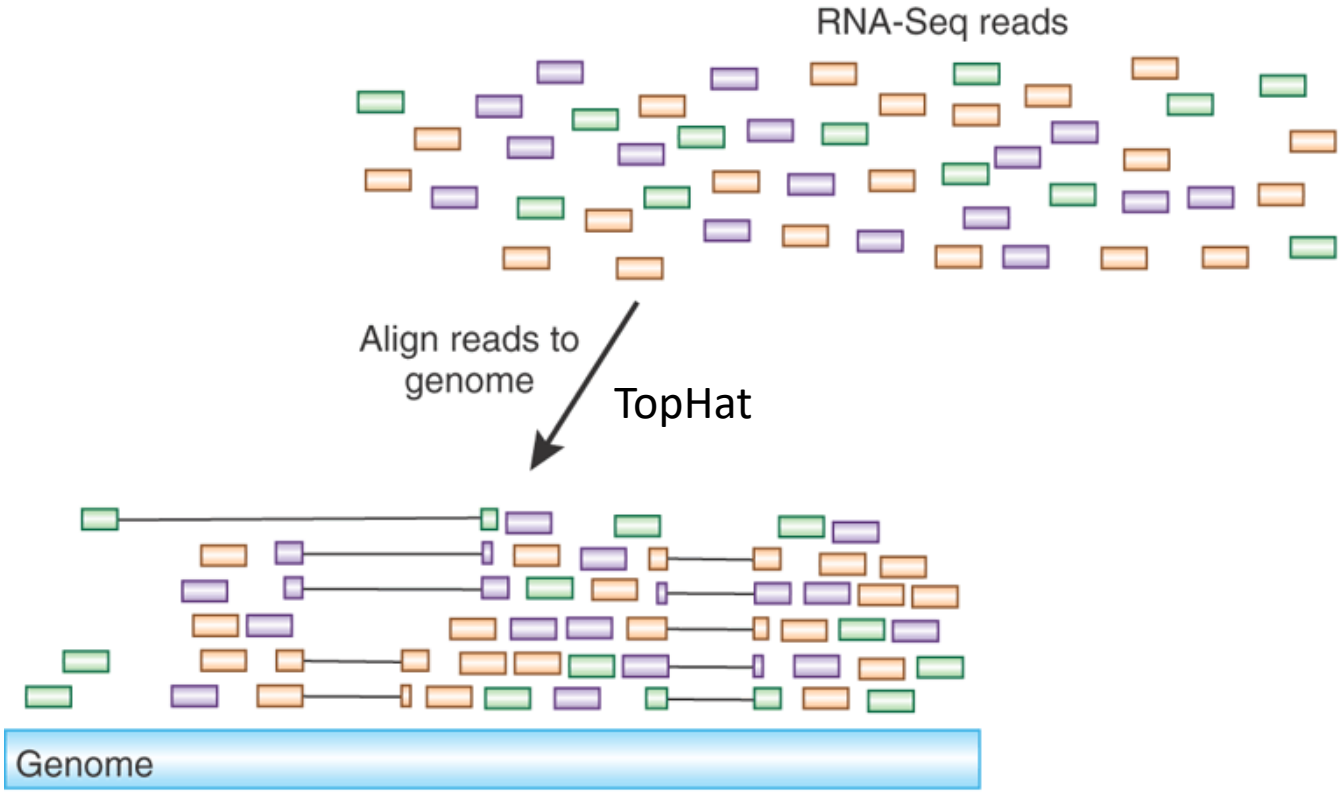
Advancing RNA-Seq analysis

Brian J Haas & Michael C Zody

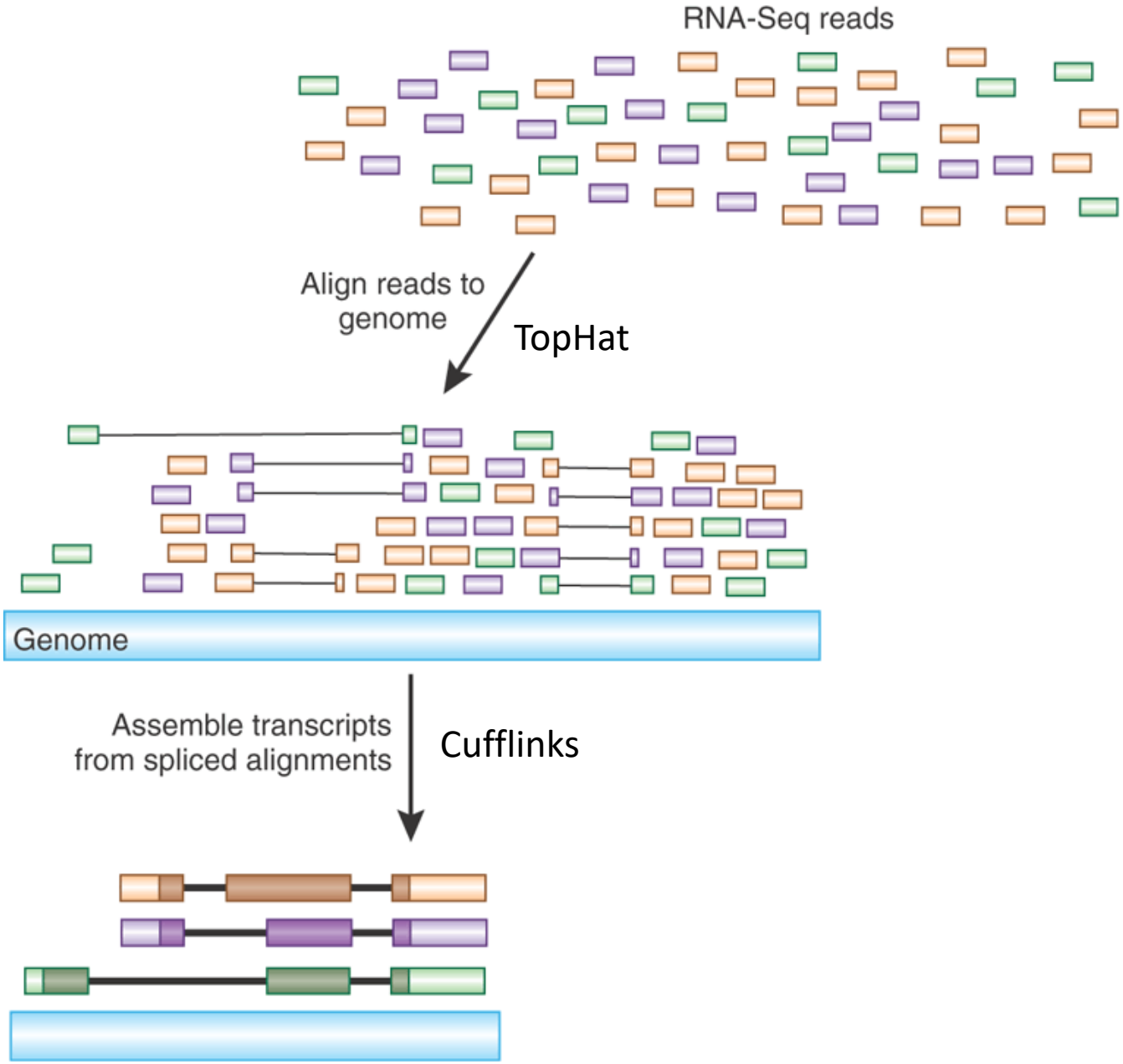
Nature Biotech, 2010

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



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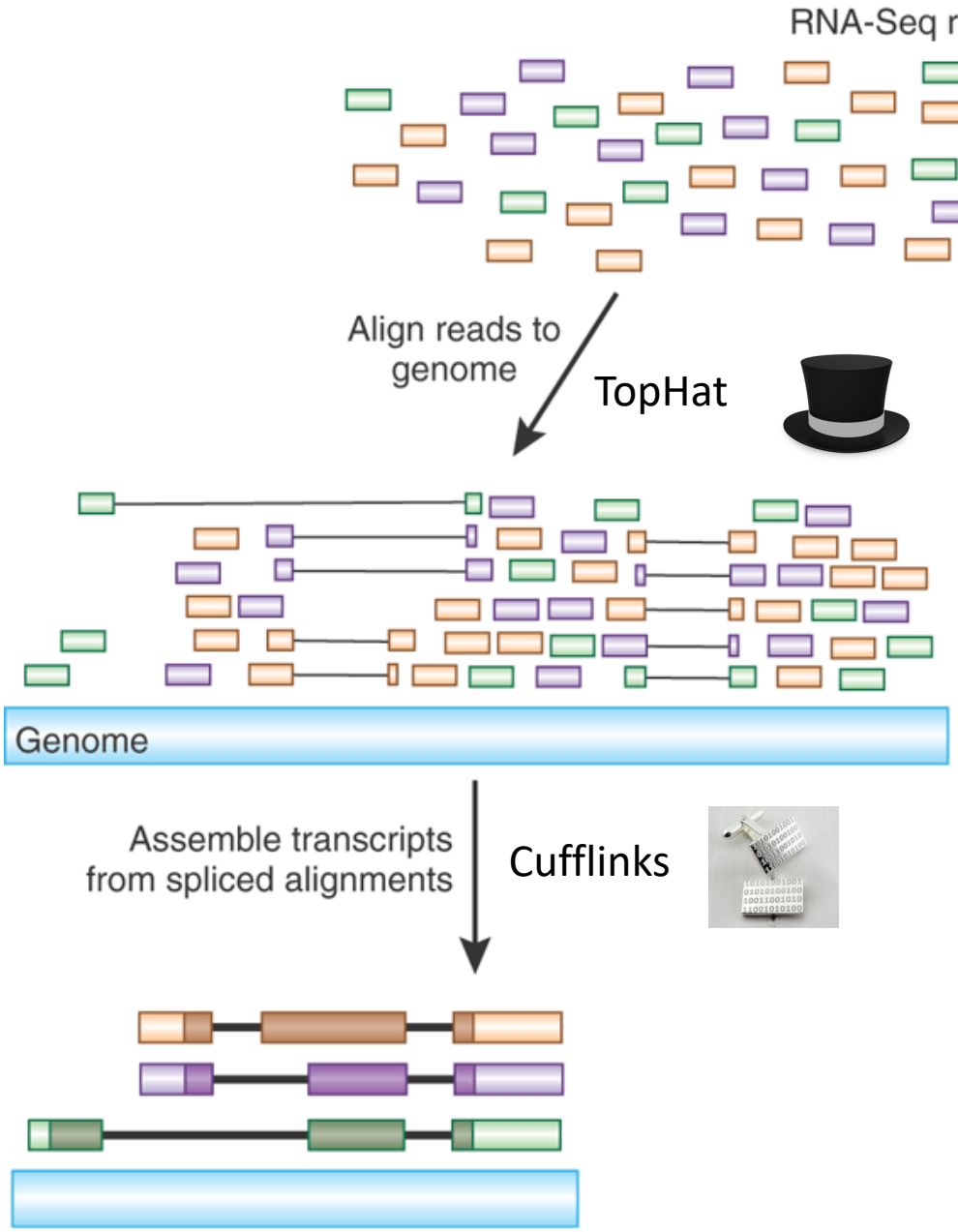


The Tuxedo Suite:

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

NATURE PROTOCOLS | PROTOCOL

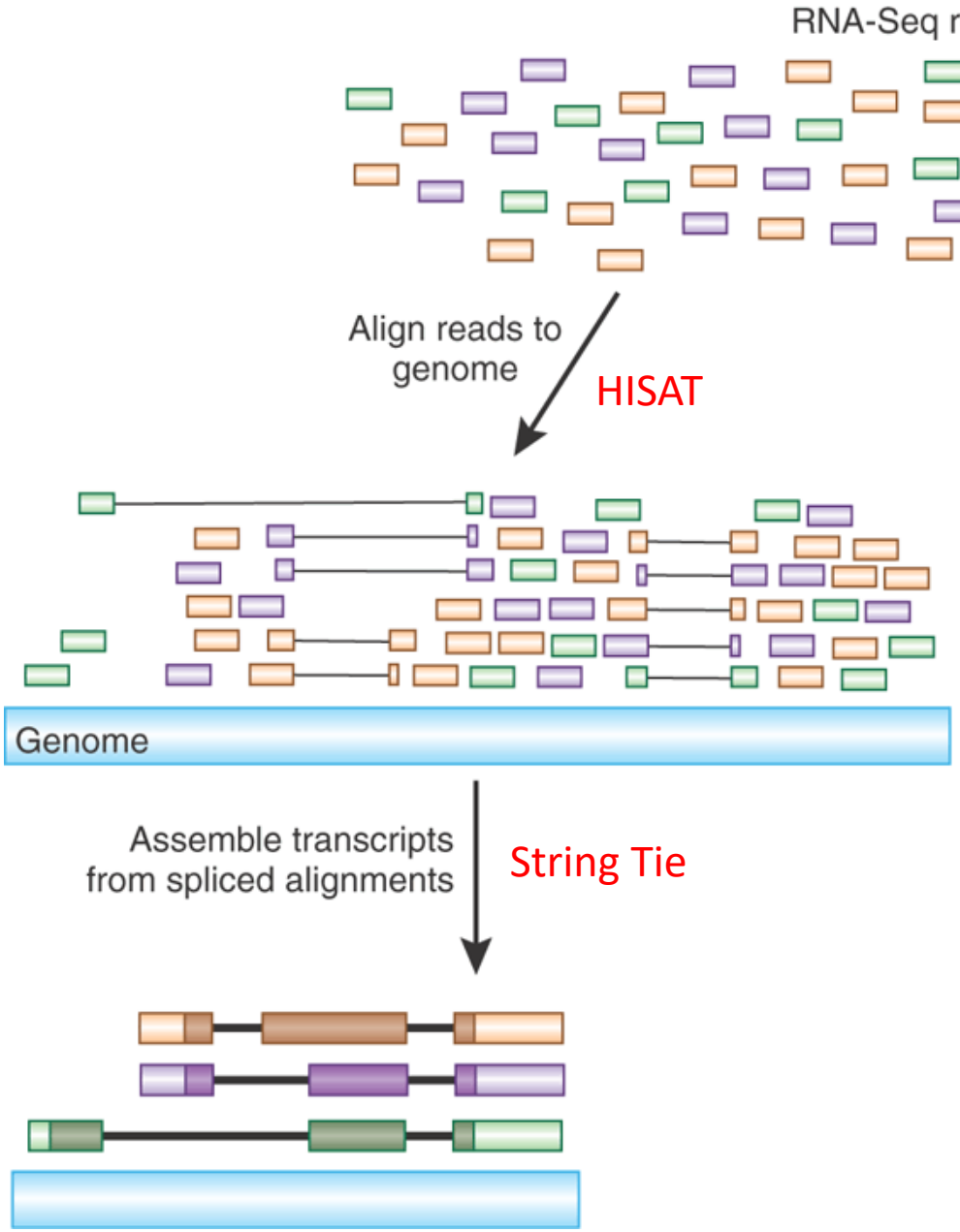
Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks

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



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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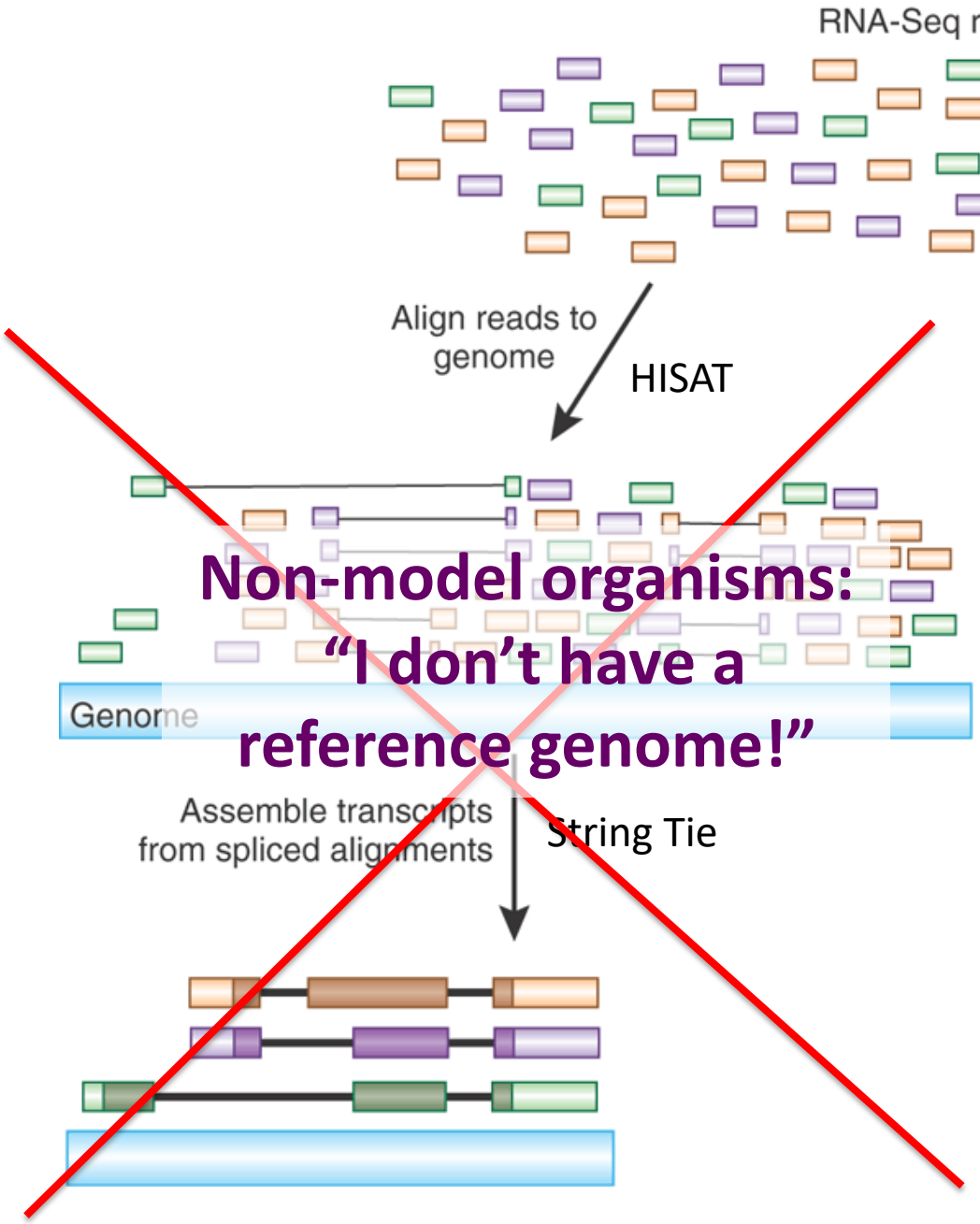

Non-model organisms: "I don't have a reference genome!"

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

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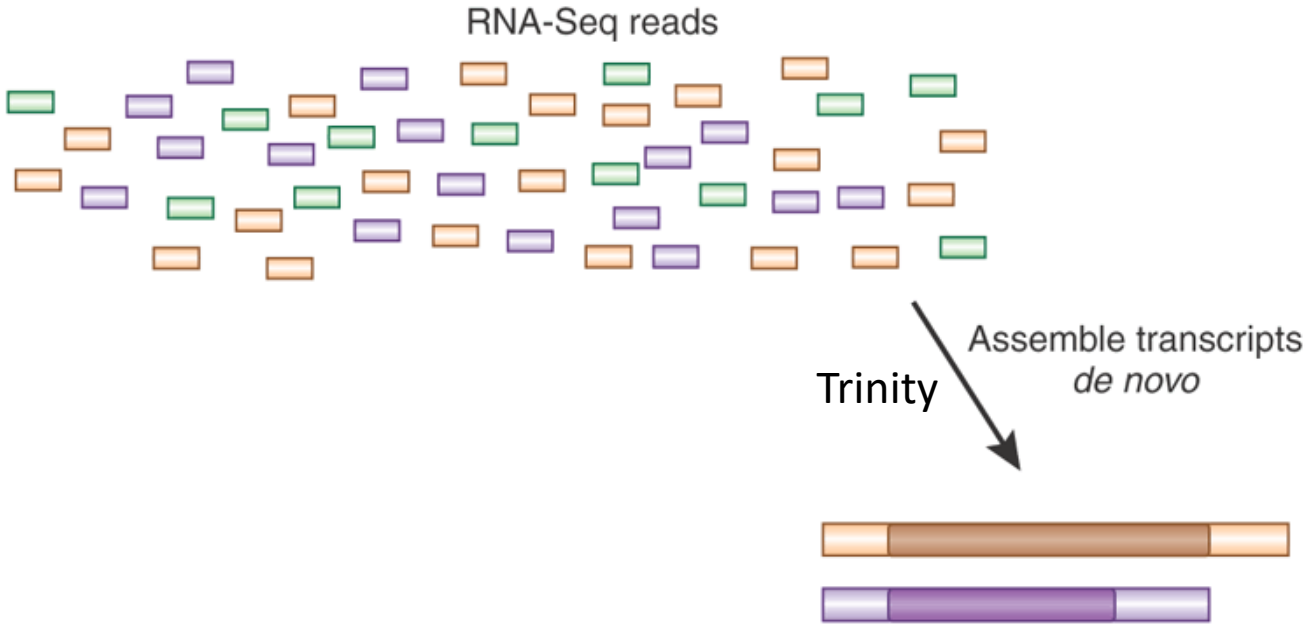
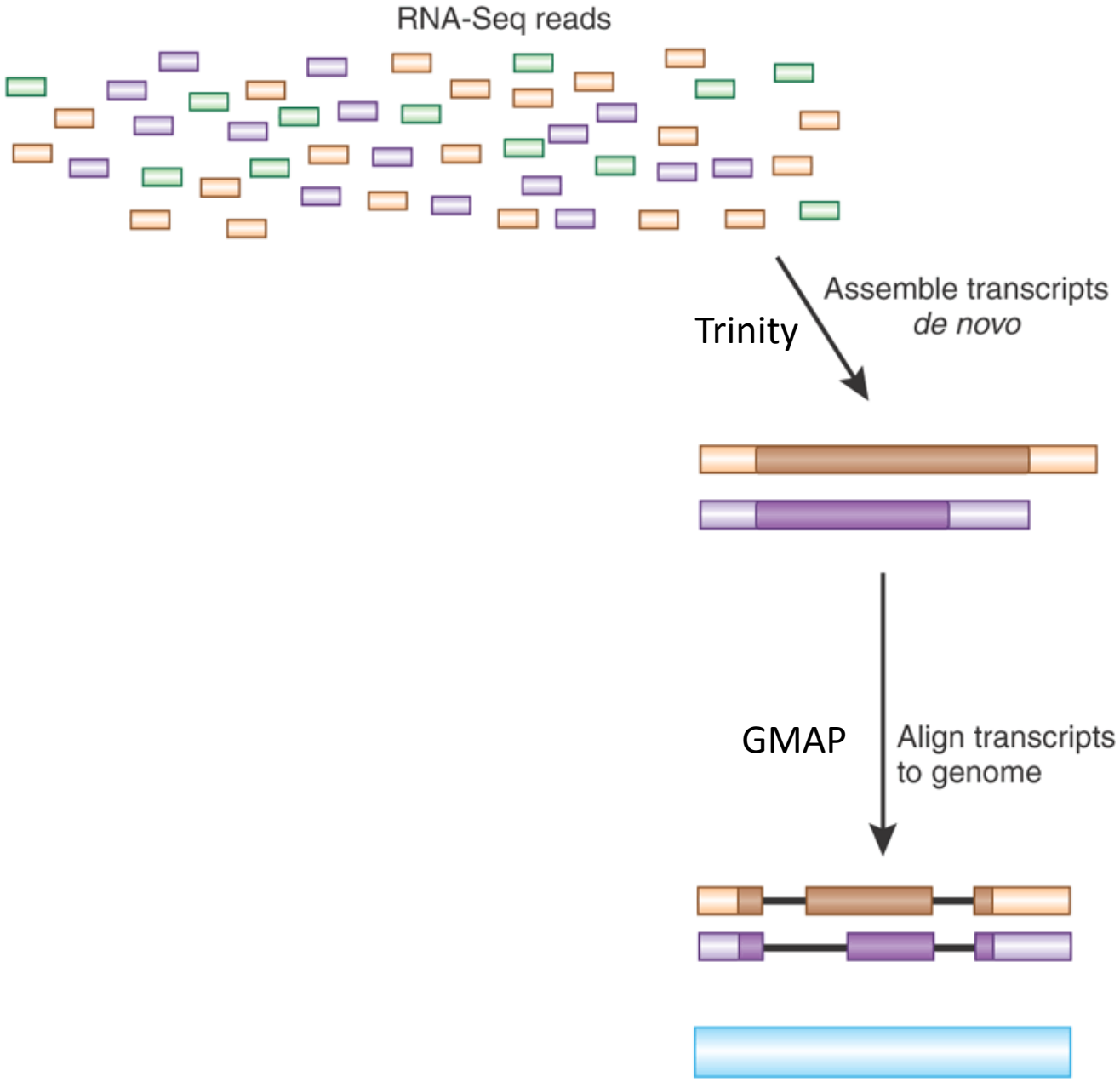


Transcript Reconstruction from RNA-Seq Reads



Transcript Reconstruction from RNA-Seq Reads

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

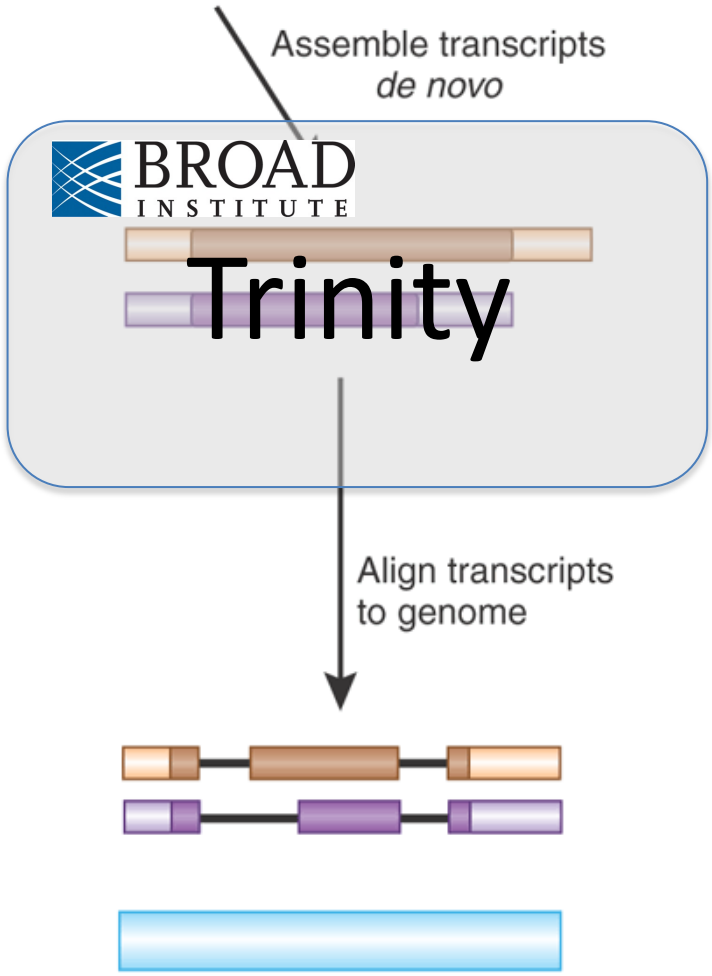
NATURE PROTOCOLS | PROTOCOL

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

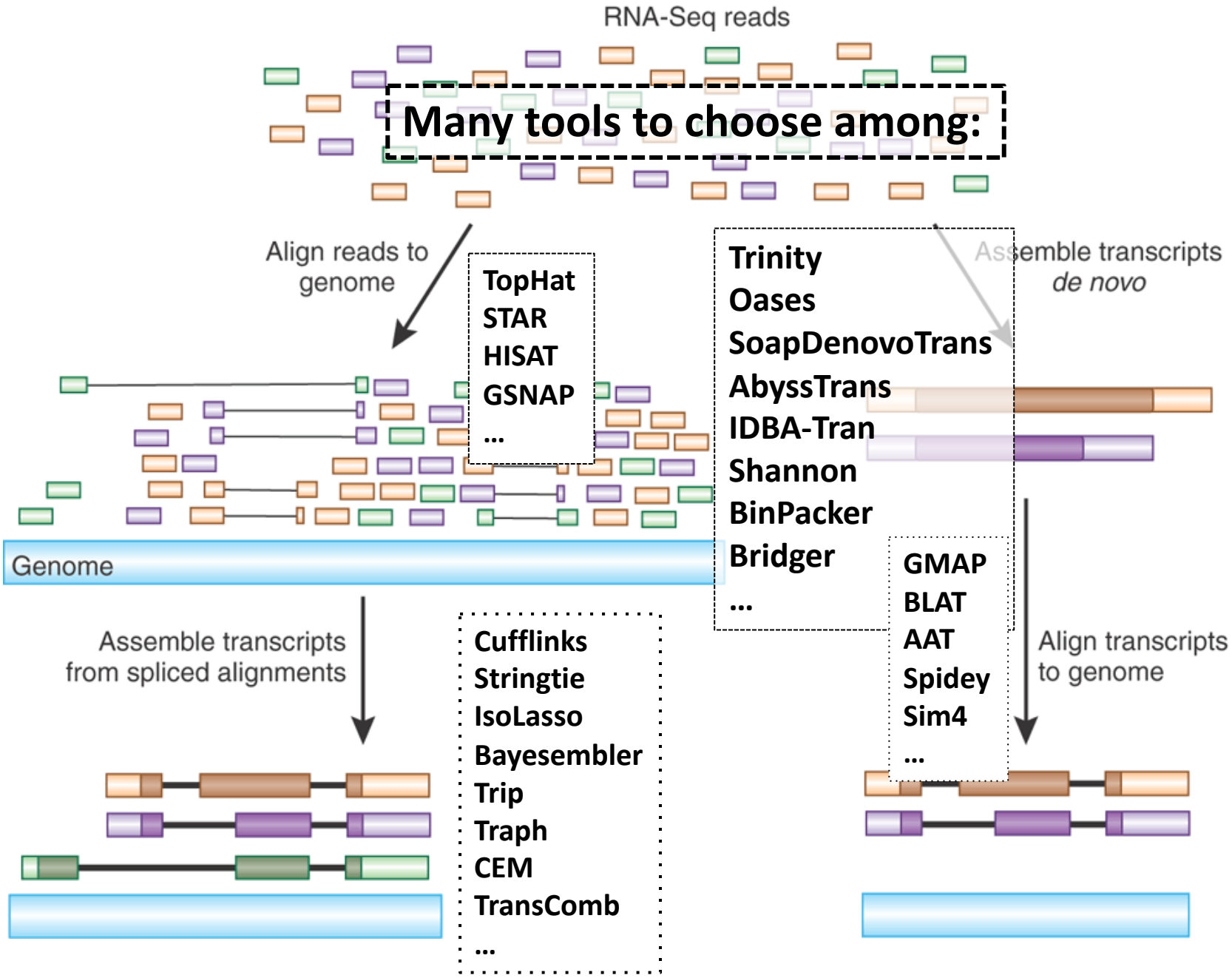
Brian J Haas, Alexie Papanicolaou, Moran Yassour, Manfred Grabherr, Philip D Blood, Joshua Bowden, Matthew Brian Couger, David Eccles, Bo Li, Matthias Lieber, Matthew D MacManes, Michael Ott, Joshua Orvis, Nathalie Pochet, Francesco Strozzi, Nathan Weeks, Rick Westerman, Thomas William, Colin N Dewey,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



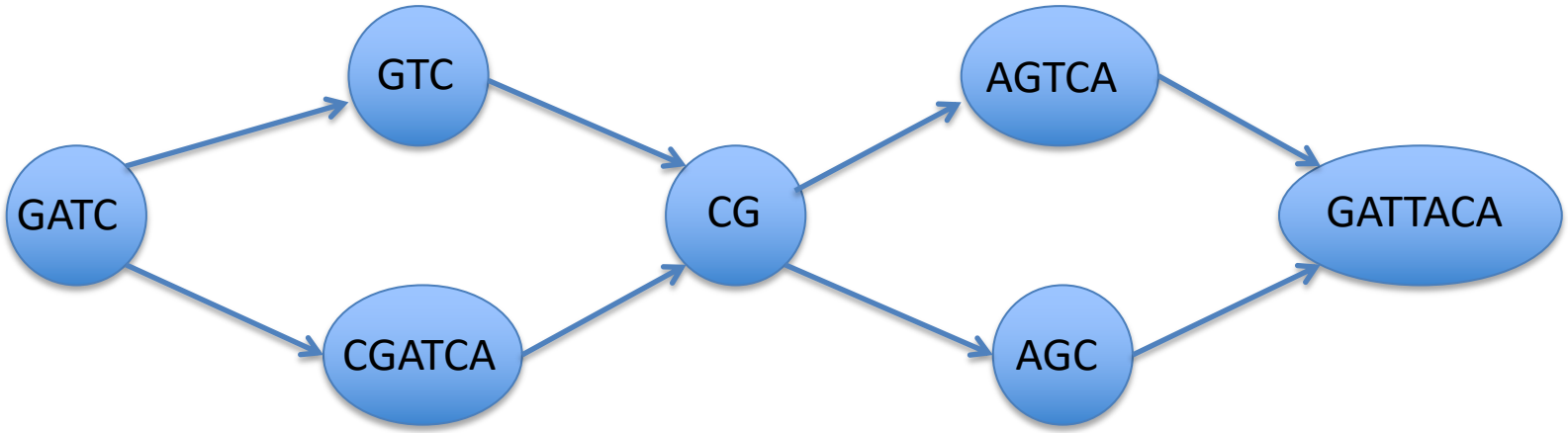
Transcript Reconstruction from RNA-Seq Reads



Graph Data Structures Commonly Used For Assembly

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

Reads to Graph



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

Graph Data Structures Commonly Used For Assembly

- Sequence
- Order
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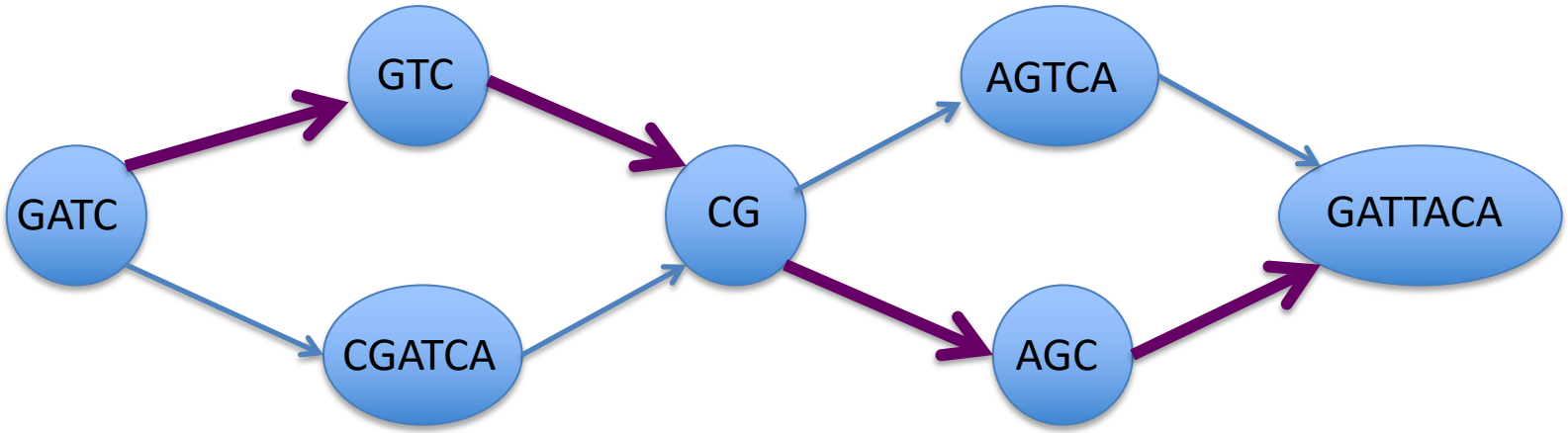
- Overlap

Reads to Graph

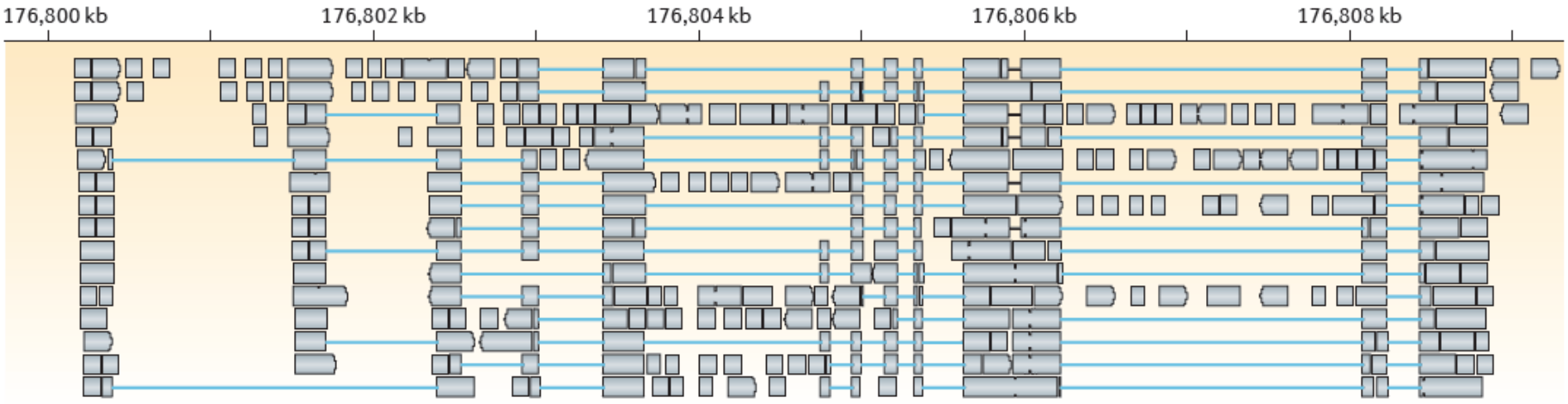


GATCGTCCGAGCGATTACA

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

Genome-Guided Transcript Reconstruction

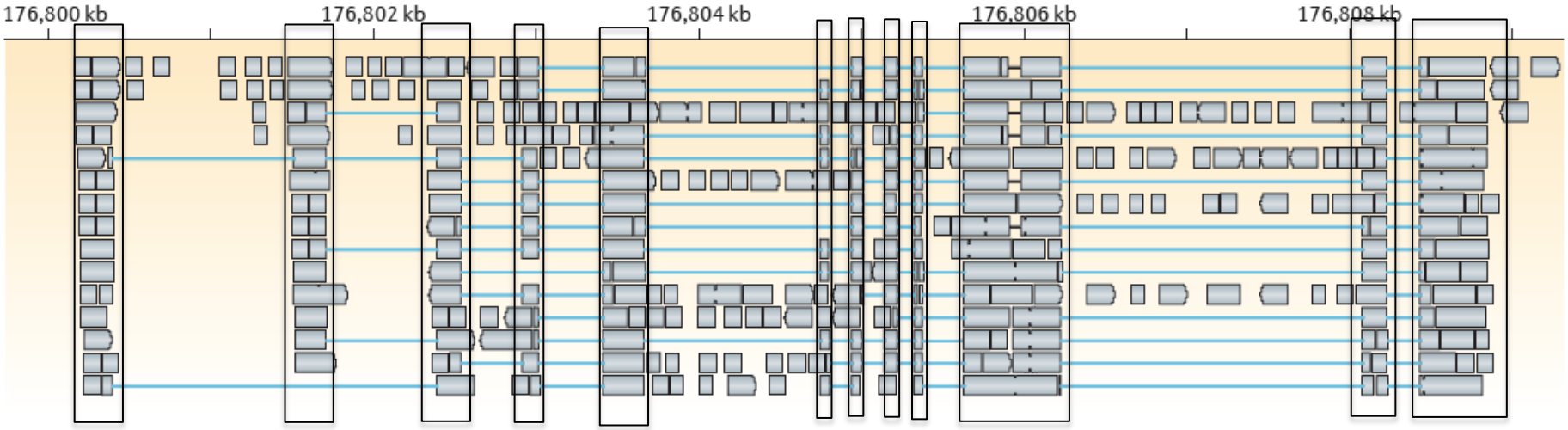
Splice-align reads to the genome



From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome

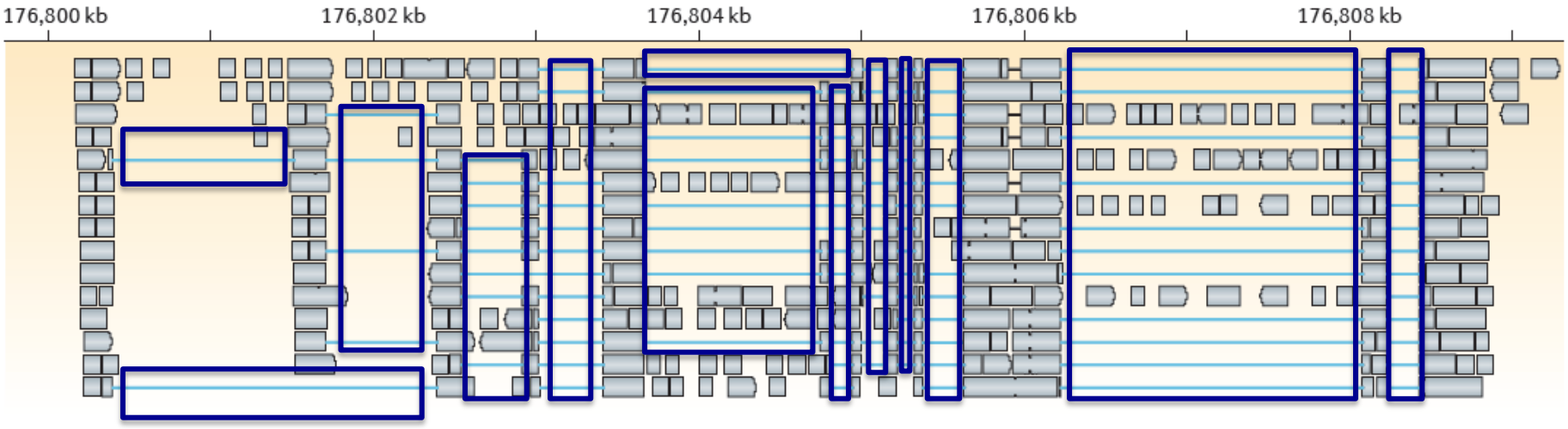


Alignment segment piles => exon regions

From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome

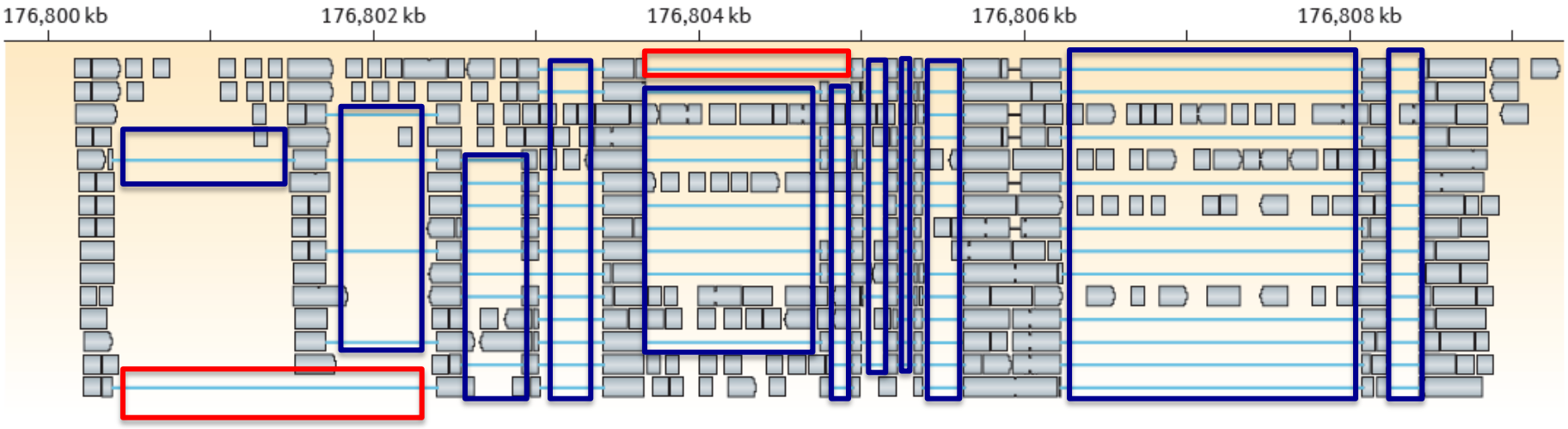


Large alignment gaps => introns

From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome

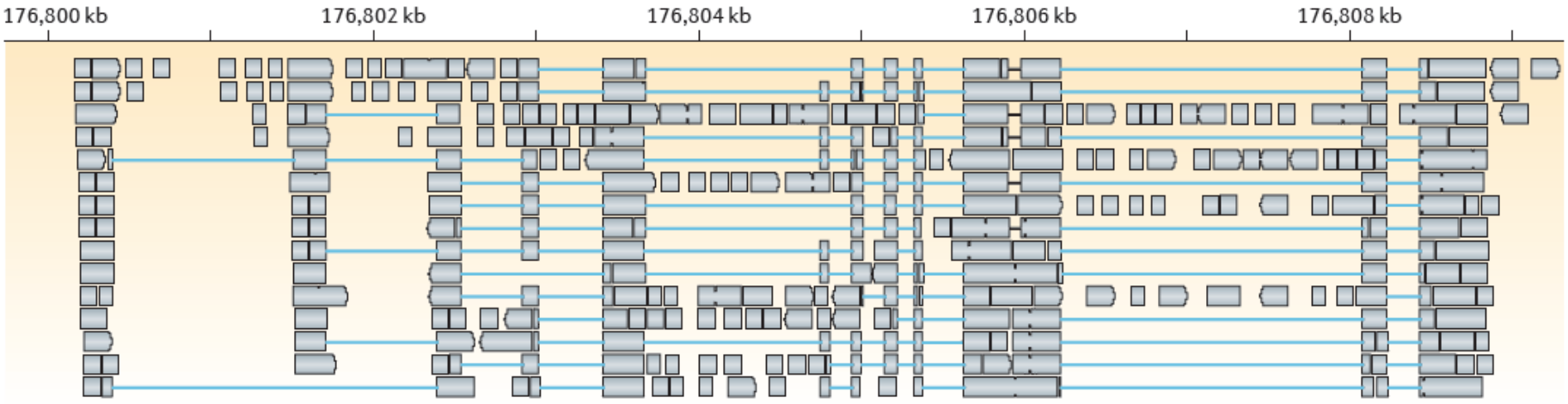


Overlapping but different introns = evidence of alternative splicing

From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Splice-align reads to the genome



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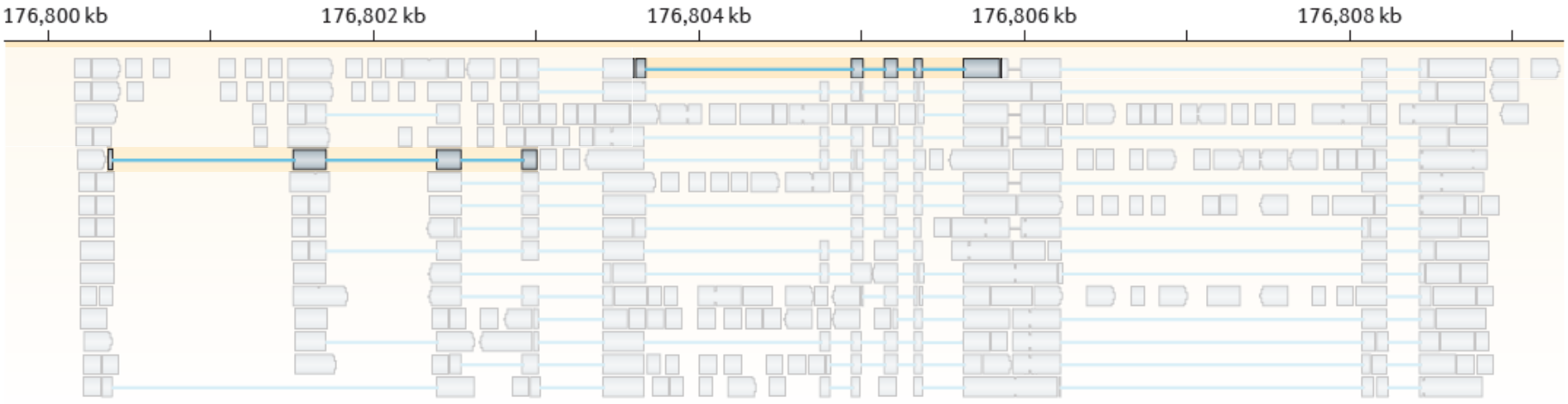
Splice-align reads to the genome

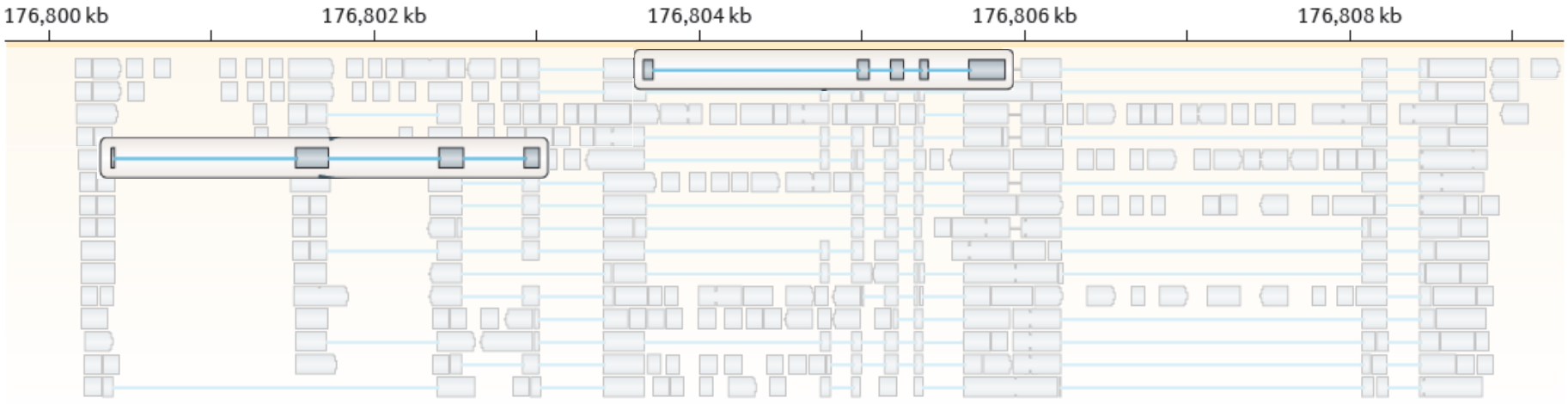


Individual reads can yield multiple exon and intron segments (splice patterns)

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

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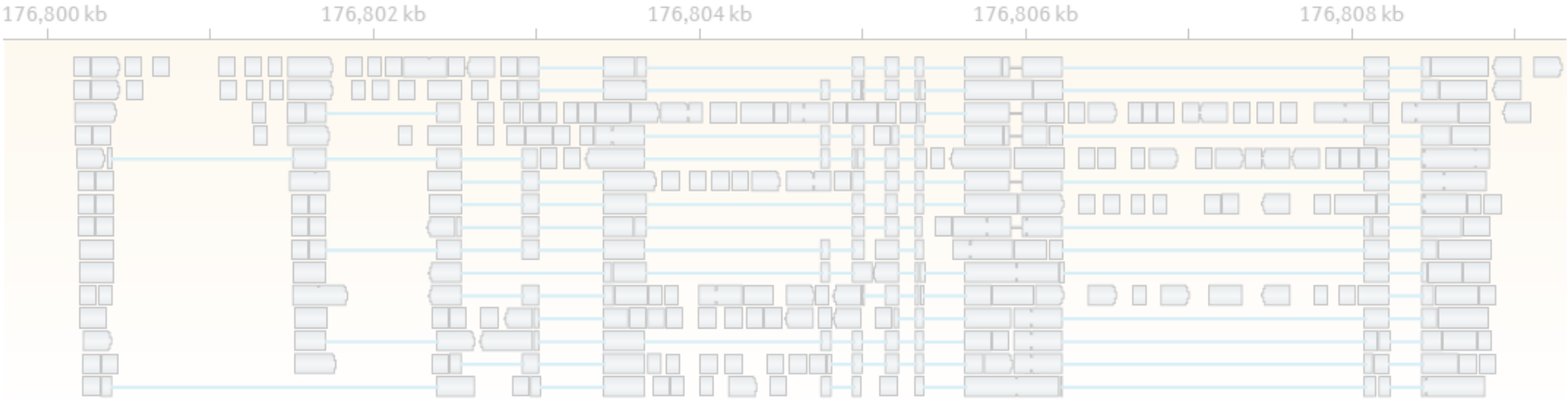
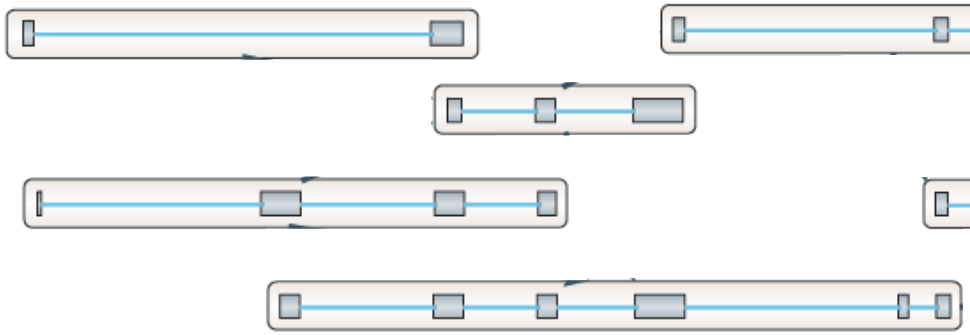


Construct graph from unique splice patterns of aligned reads.



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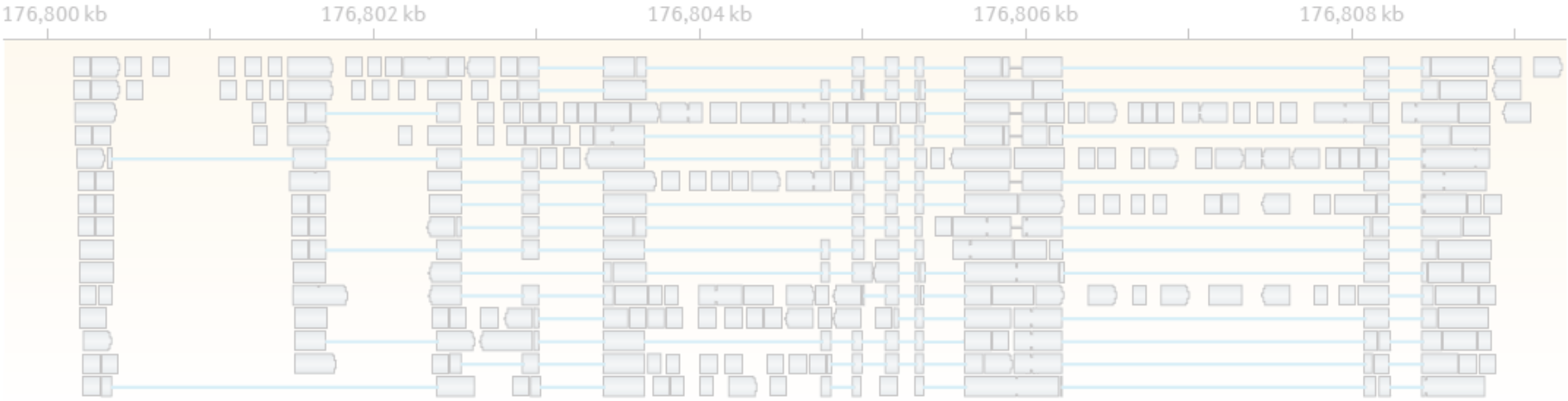

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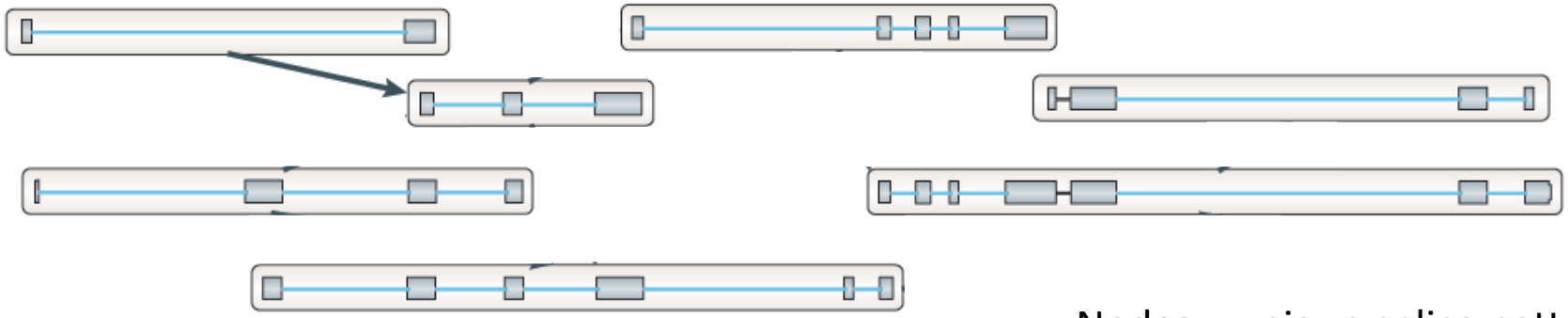

Nodes = unique splice patterns
Edges = compatible patterns

From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

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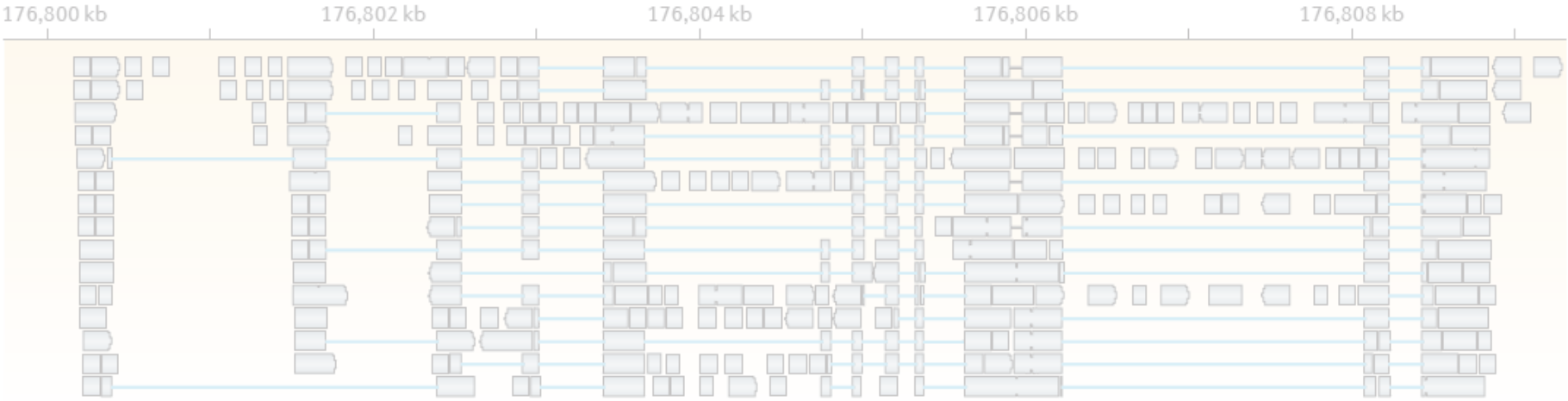

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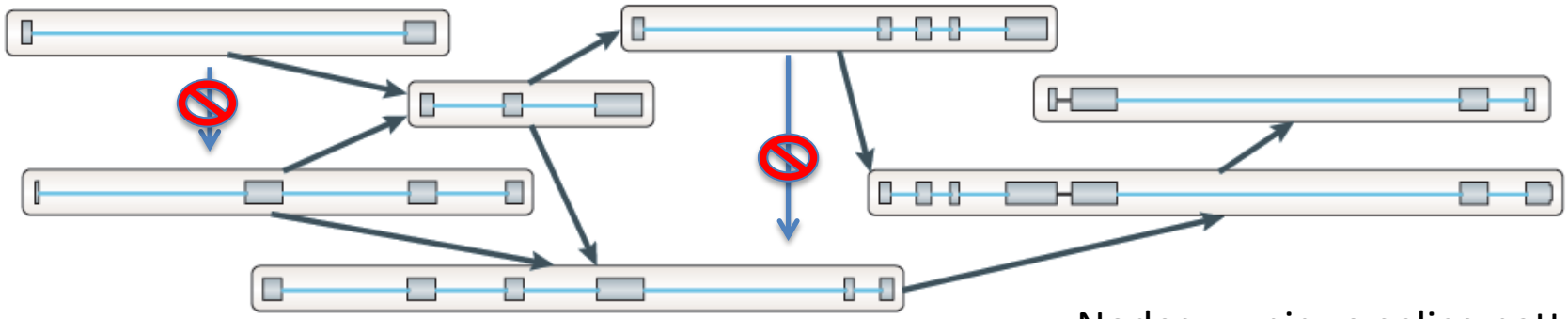

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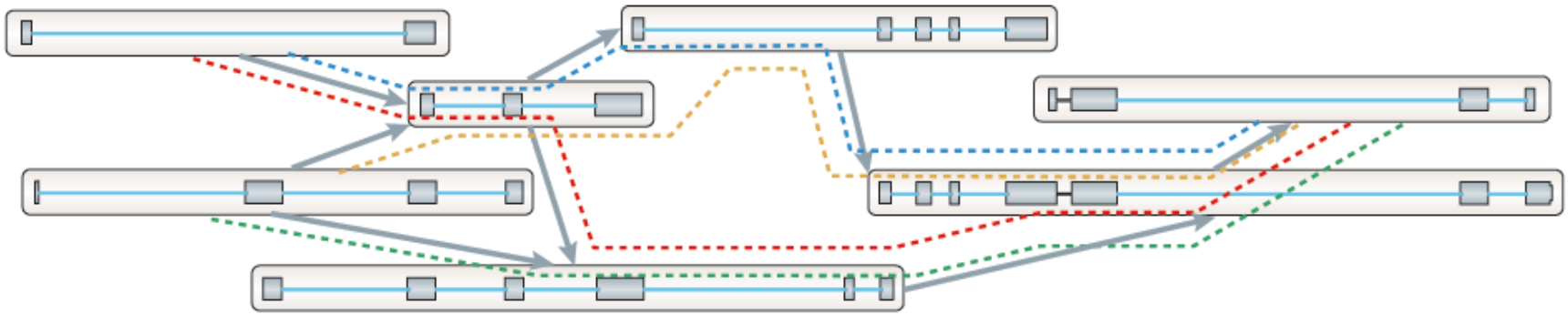
From Martin & Wang. Nature Reviews in Genetics. 2011

Genome-Guided Transcript Reconstruction

Traverse paths through the graph to assemble transcript isoforms



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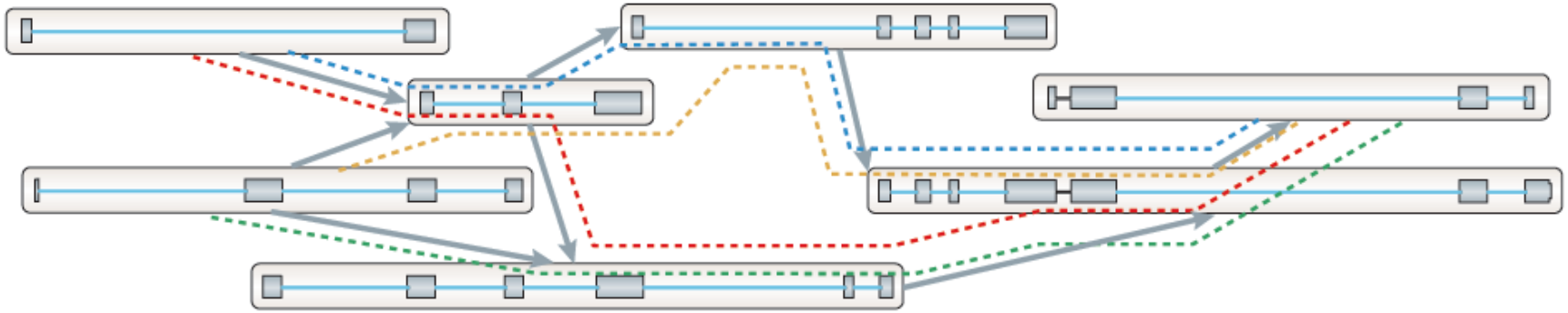
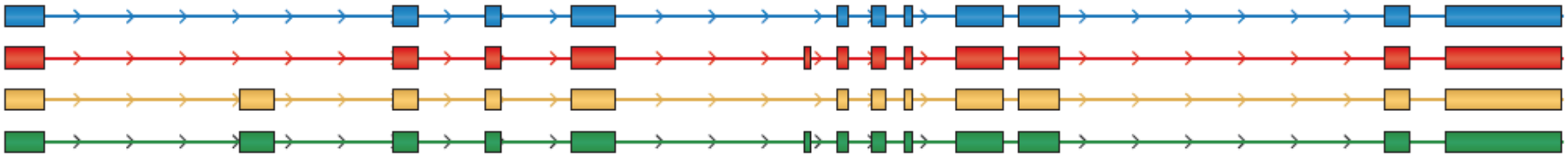


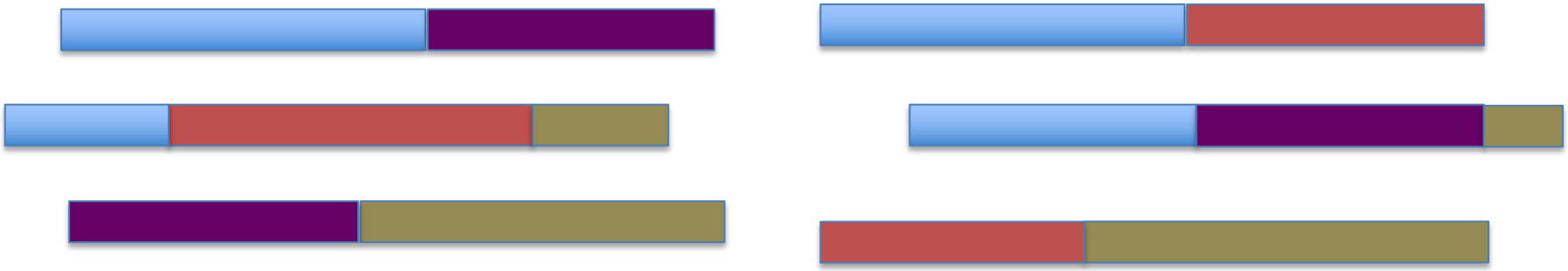
Reconstructed isoforms



From Martin & Wang. Nature Reviews in Genetics. 2011

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

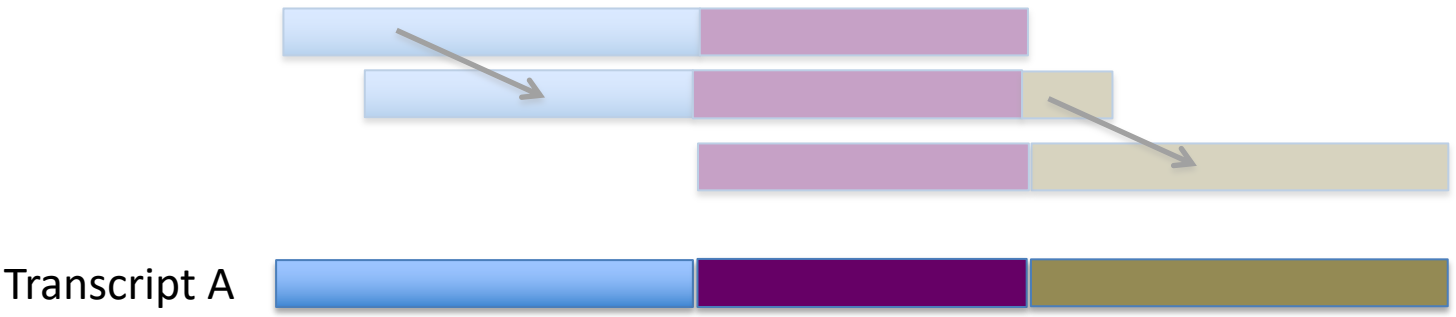
Read Overlap Graph: Reads as nodes, overlaps as edges



Read Overlap Graph: Reads as nodes, overlaps as edges

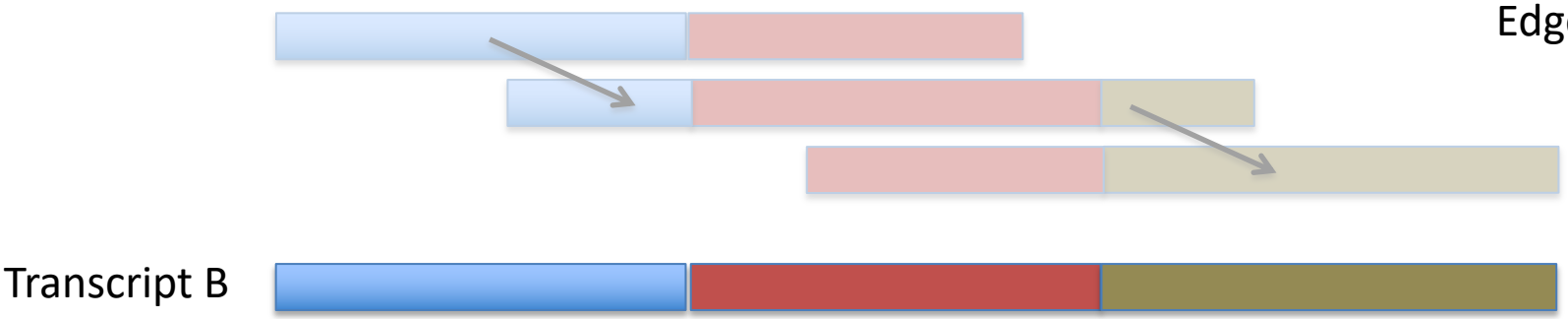
Node = read
Edge = overlap

Read Overlap Graph: Reads as nodes, overlaps as edges

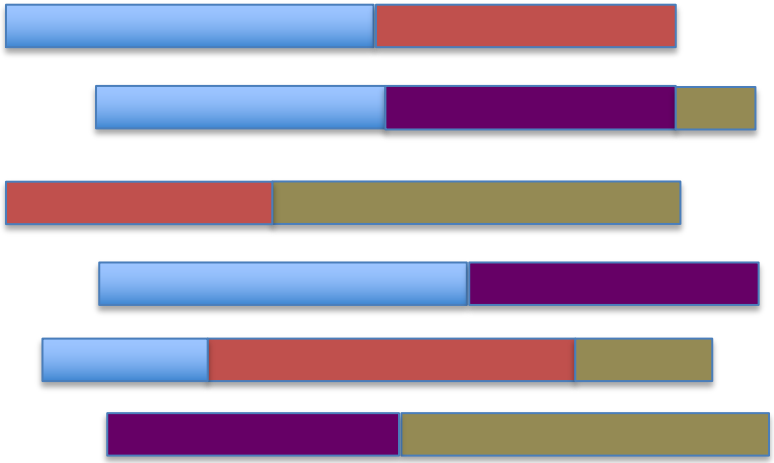


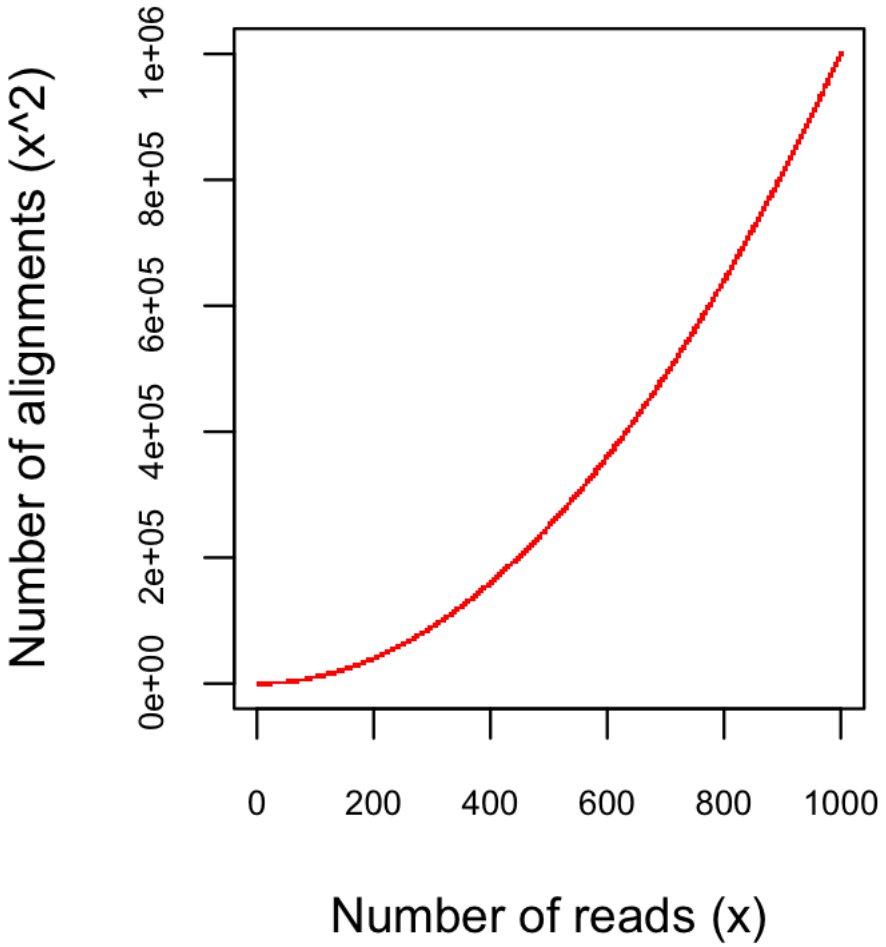
Generate consensus sequence where reads overlap

Node = read
Edge = overlap



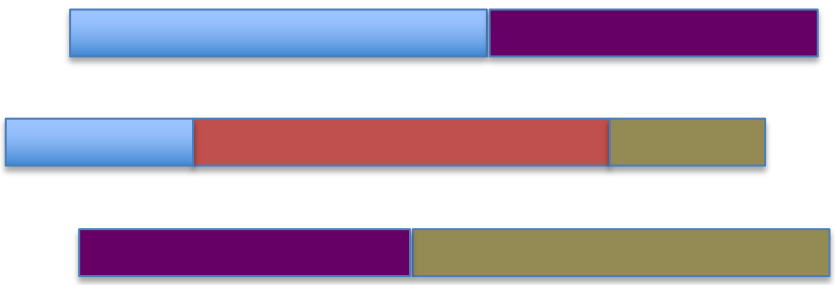
Finding pairwise overlaps between *n* reads involves ~ n^2 comparisons.



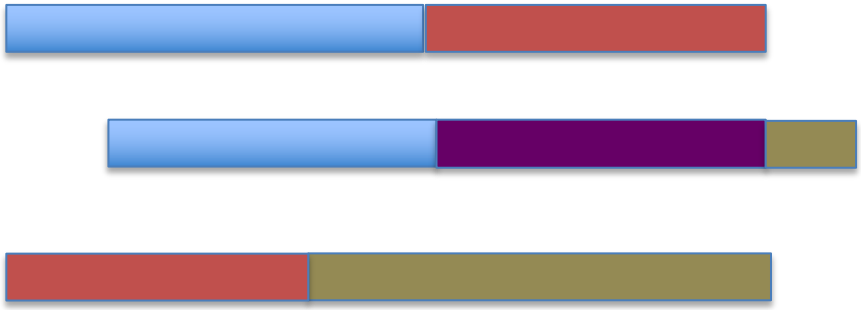


Impractical for typical RNA-Seq data (50M reads)

No genome to align to…



De novo assembly required

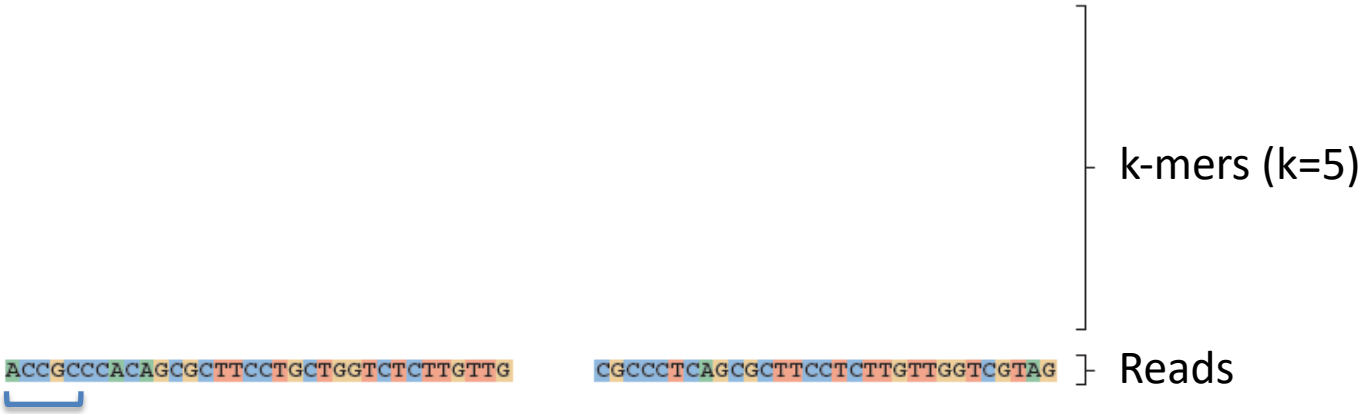


Want to avoid n^2 read alignments to define overlaps

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

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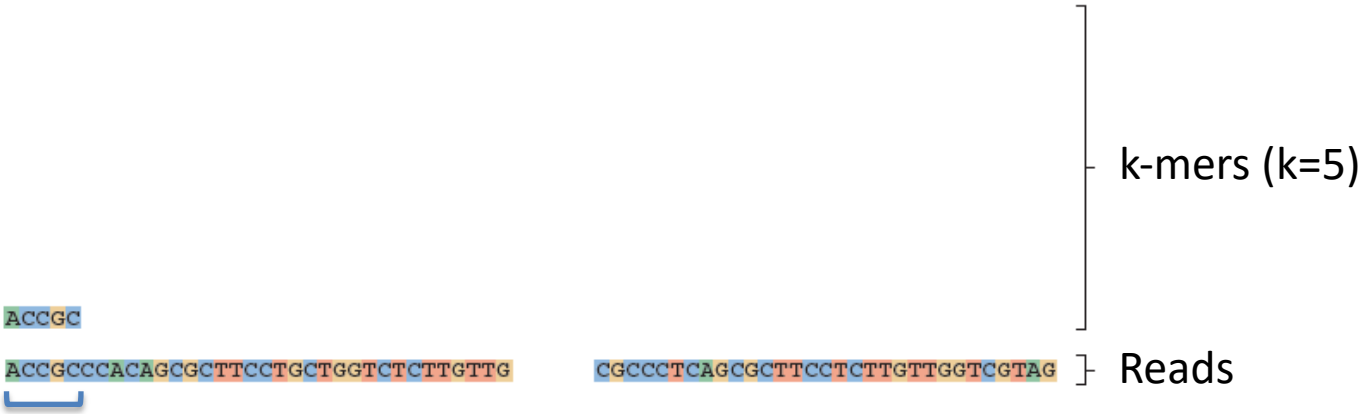

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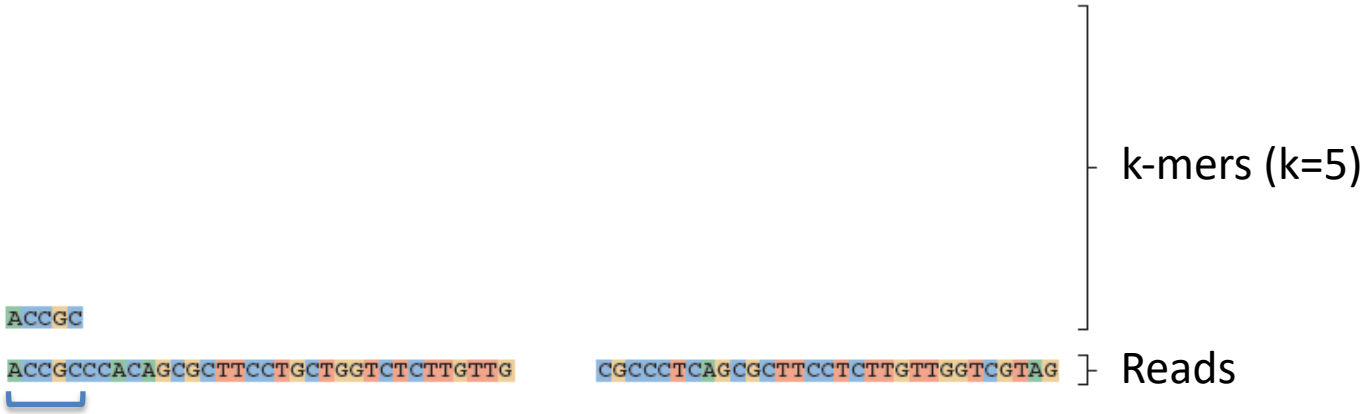

Construct the de Bruijn graph

Nodes = unique k-mers

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

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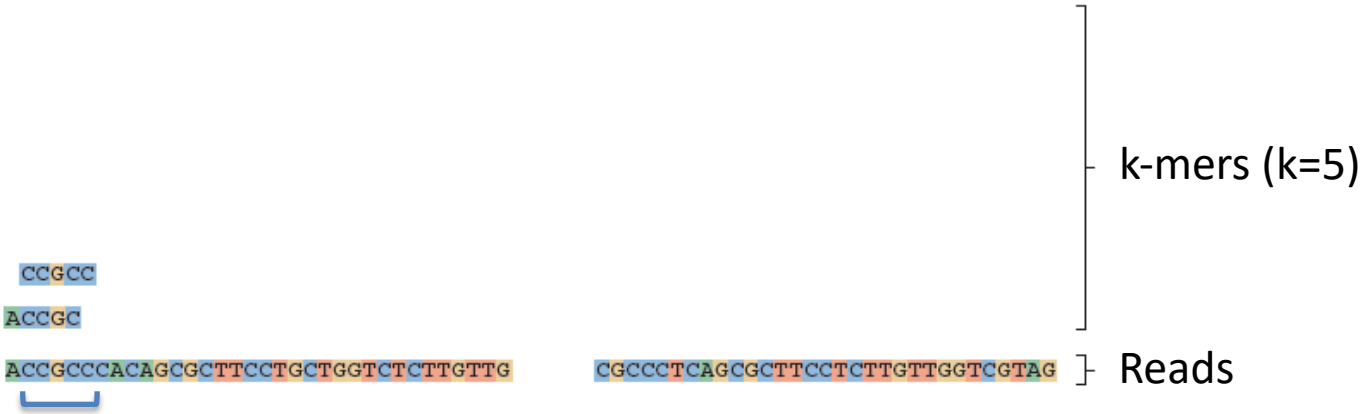

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Edges = overlap by (k-1)

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

Sequence Assembly via De Bruijn Graphs

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Construct the de Bruijn graph

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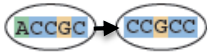

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Sequence Assembly via De Bruijn Graphs

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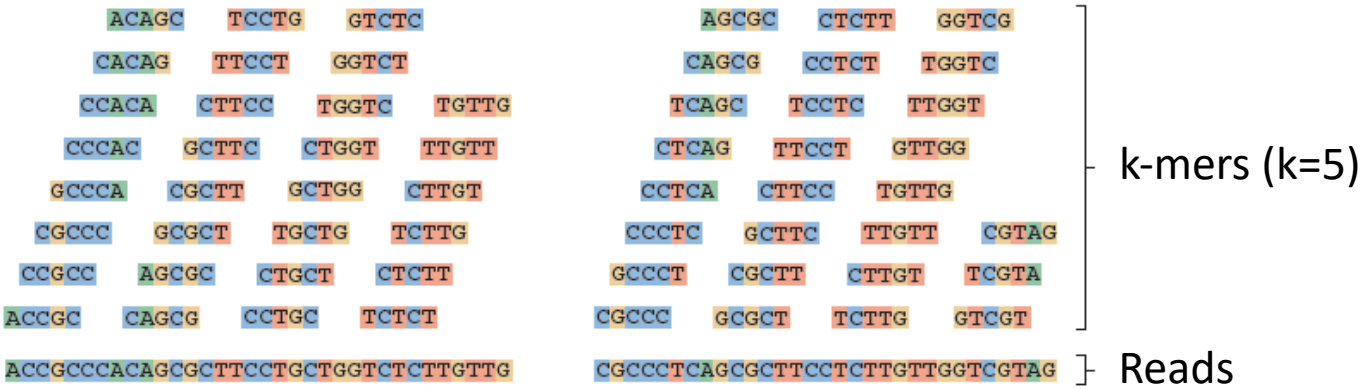

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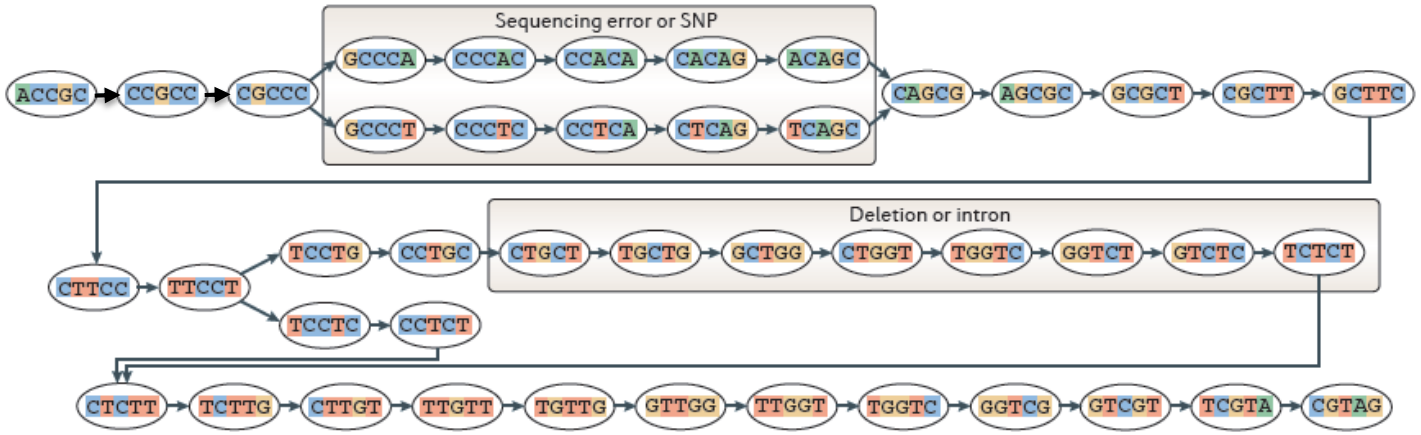

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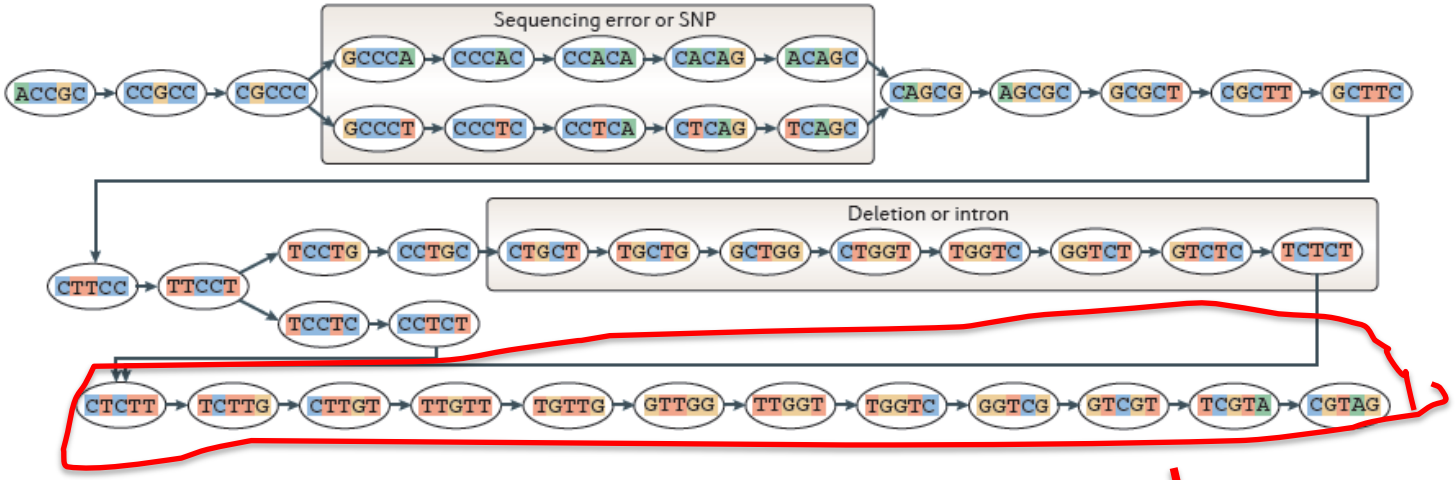
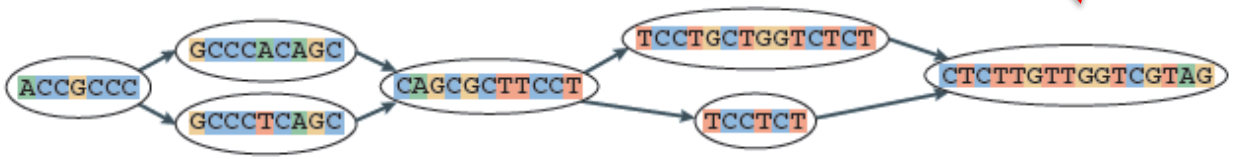


Collapse the de Bruijn graph



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

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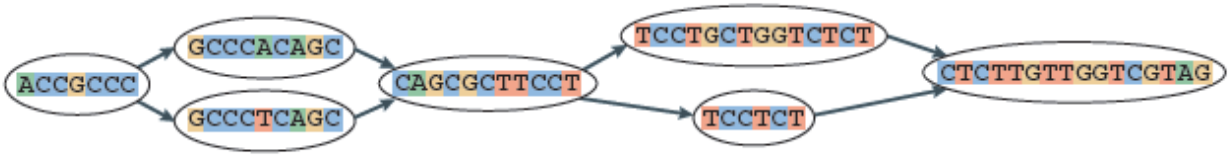
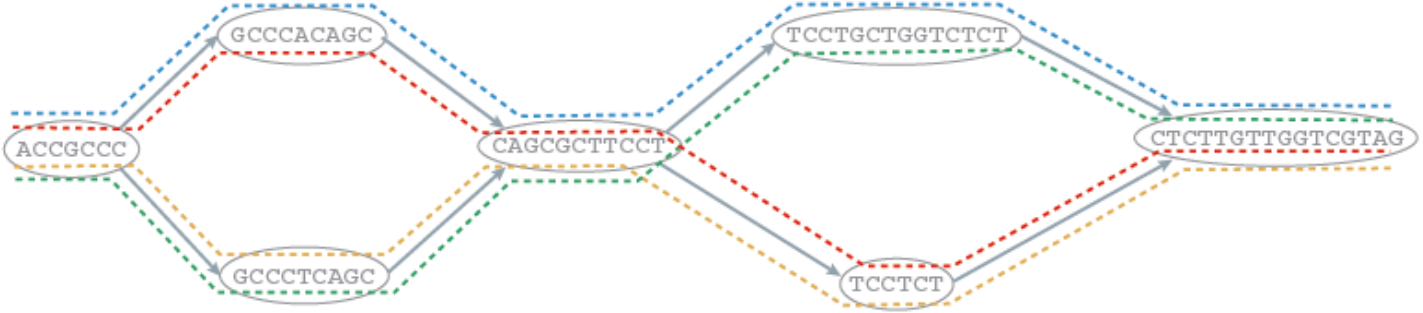
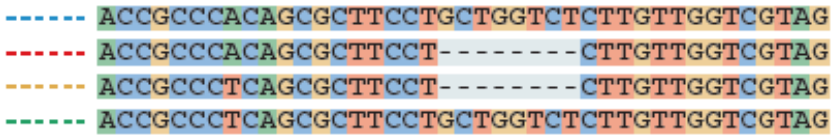


Traverse the graph



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.

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

GATTACA
9

Kmer Catalog (hashtable)

Kmer	Count among all reads
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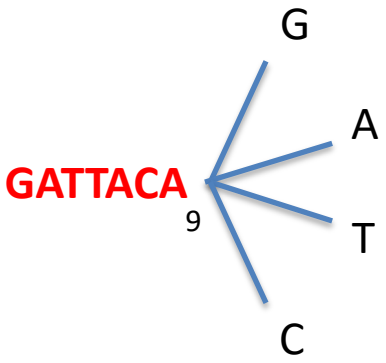
GATTACA	9

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.



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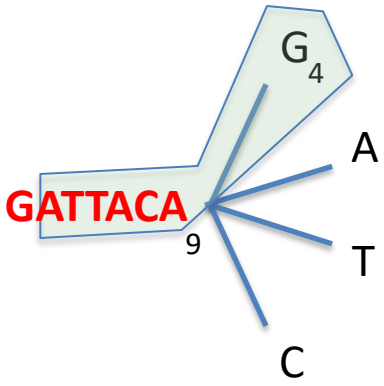

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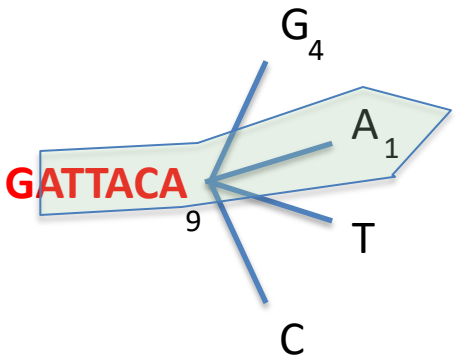
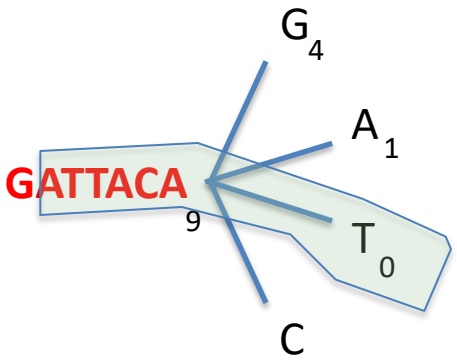


Inchworm Algorithm



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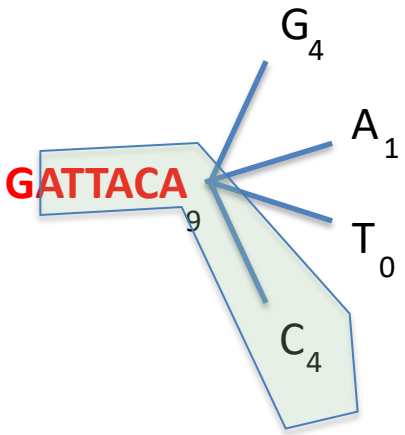
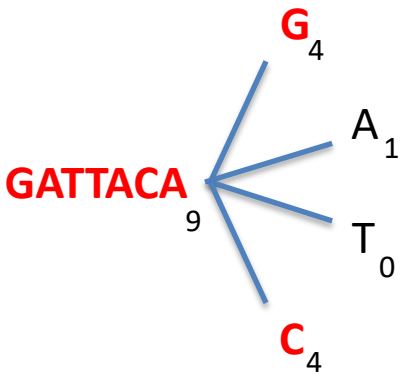
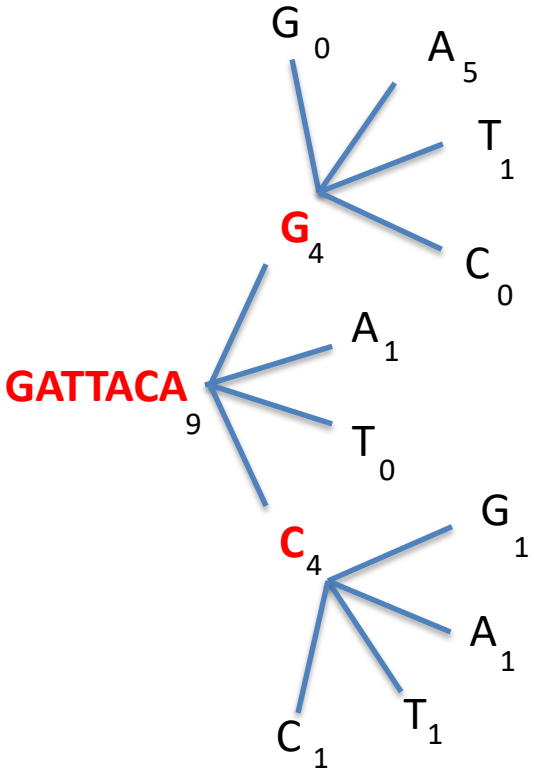


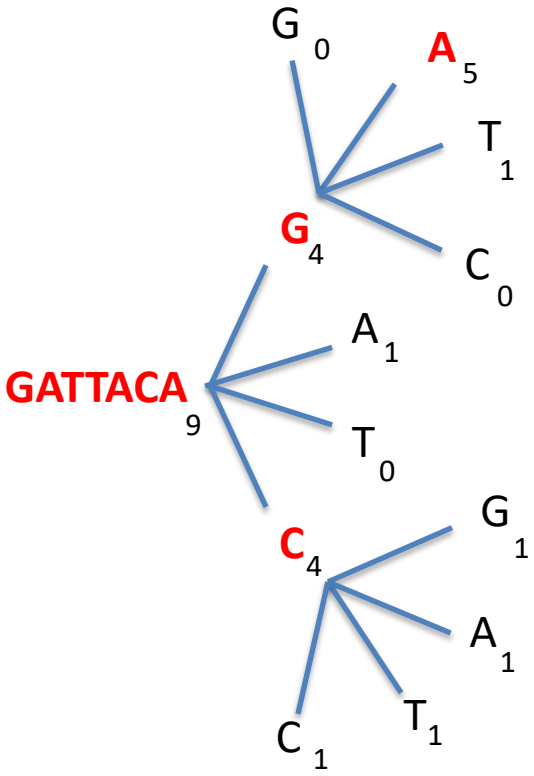
Inchworm Algorithm



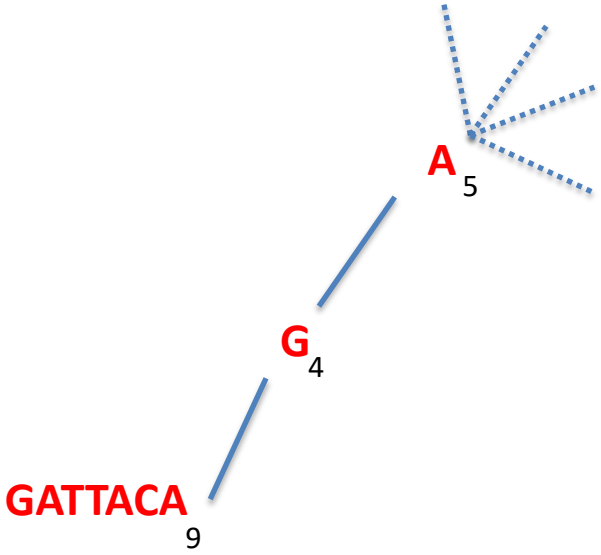
Inchworm Algorithm



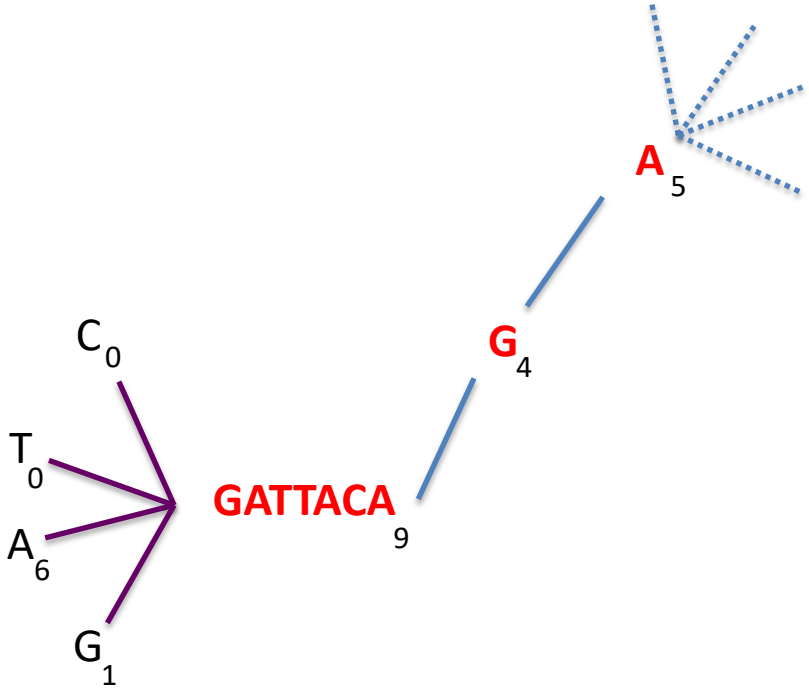
Inchworm Algorithm



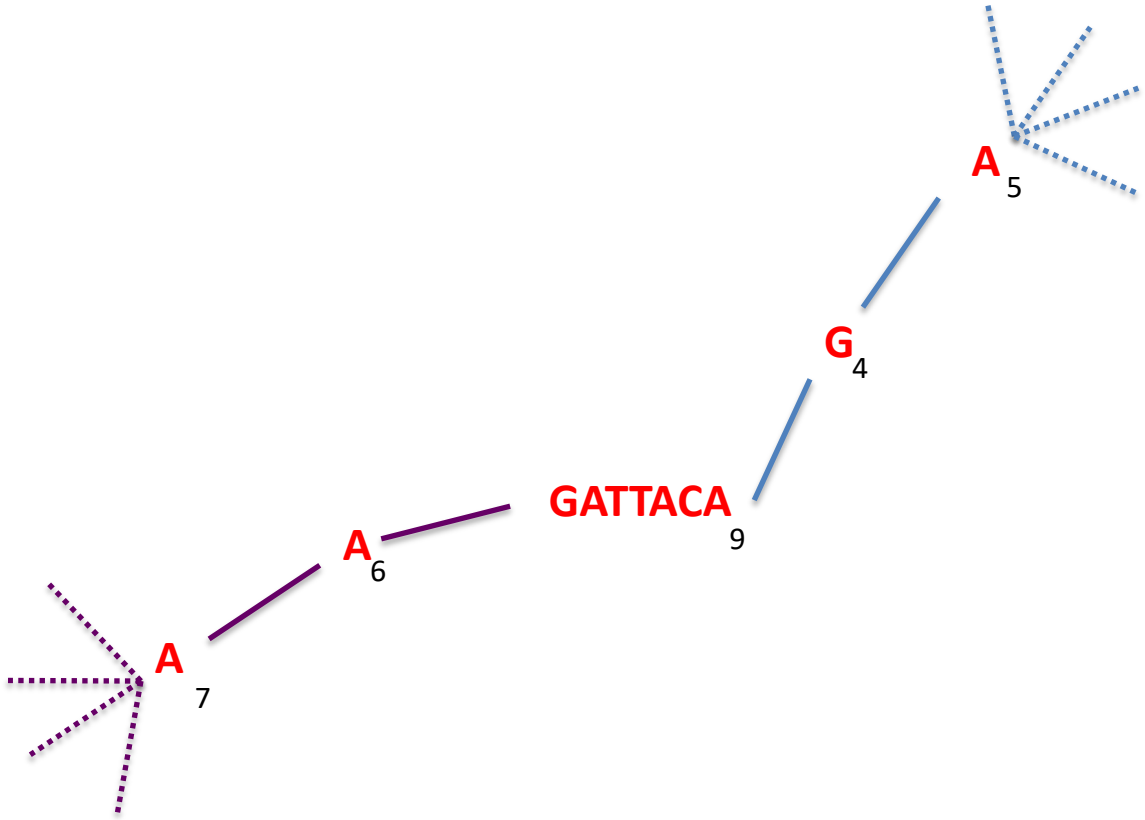
Inchworm Algorithm



Inchworm Algorithm



Inchworm Algorithm



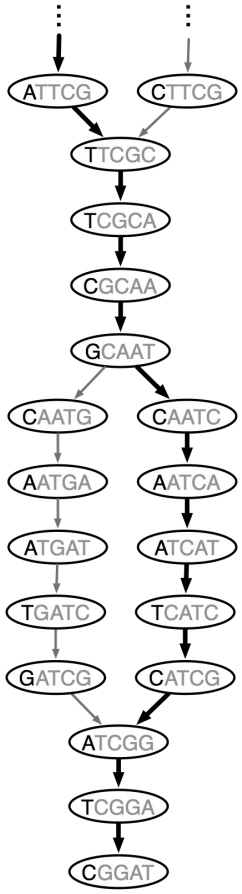
Report contig: ….AAGATTACAGA….

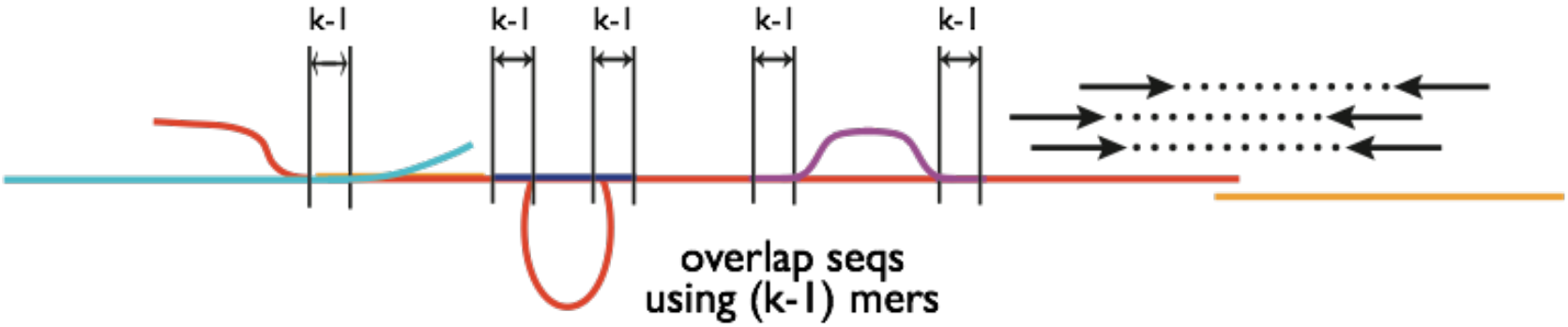
Remove assembled kmers from catalog, then repeat the entire process.

Chrysalis

Integrate isoforms via k-1 overlaps

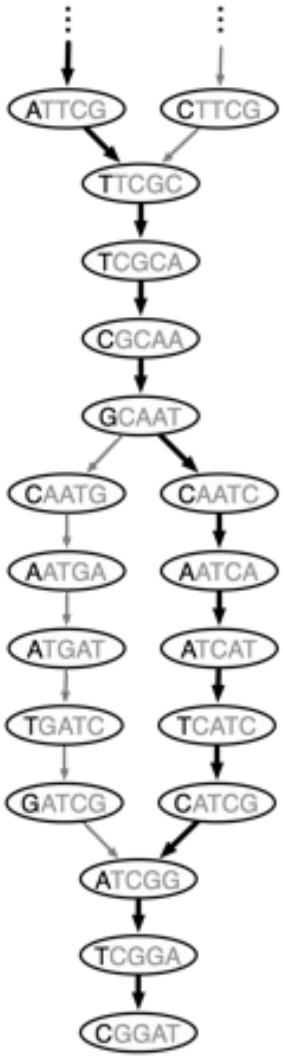
Build de Bruijn Graphs (ideally, one per gene)





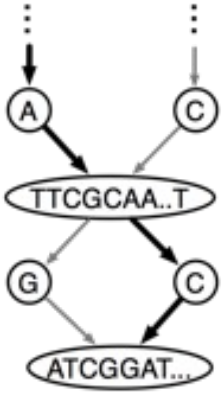

Thousands of Chrysalis Clusters

Butterfly



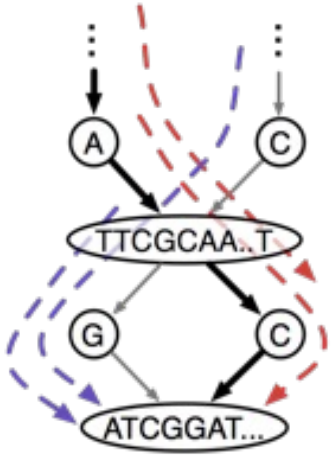
de Bruijn graph

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

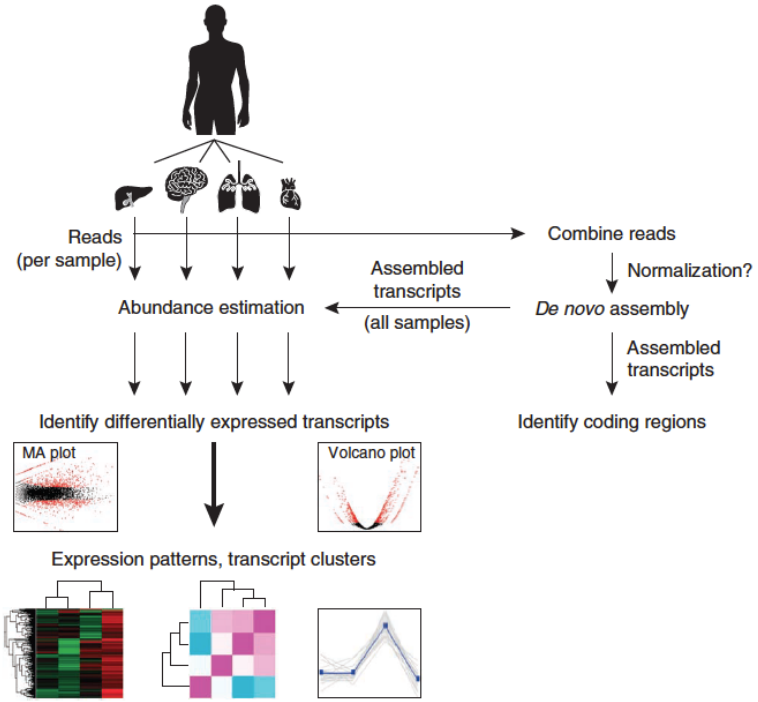
De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis

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:

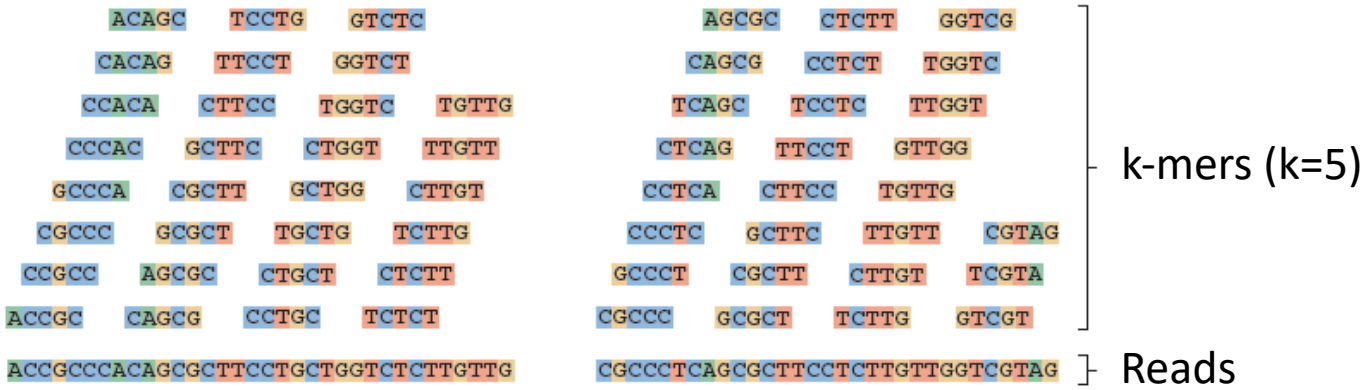
```
Trinity --seqType fq --left reads_1.fq --right reads_2.fq --CPU 6 --max_memory 20G
```

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 : 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:

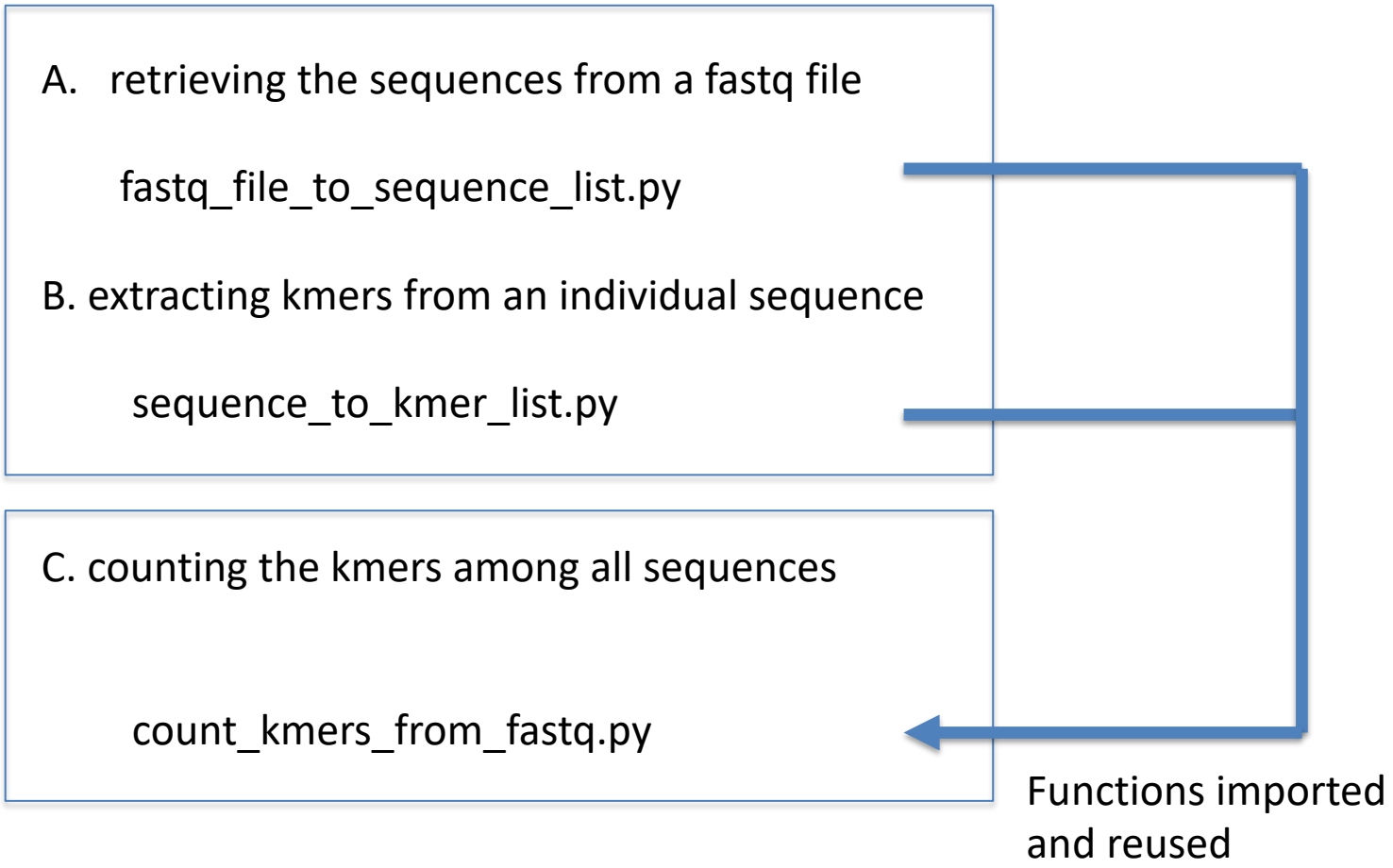
```
kmer_counter.py 8 reads.left.fq 10
```

.

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

https://github.com/trinityrnaseq/CSHLProgForBio/tree/main/Exercise-counting_kmers

Build in 3 phases – demonstrating function reuse



Useful structure of a python script for shareable functions

my_module.py (can run directly or use as a function library)

```
#!/usr/bin/env python3

import sys

def decorate_name_val(name_val):
    decorated = " ".join([get_unicorn(), name_val, get_unicorn()])
    return(decorated)

def get_unicorn():
    # returns unicorn symbol
    return("\U0001f984")


if __name__ == '__main__':
    print("my_module.py running as driver: ", decorate_name_val(__name__))
```

executed

```
(base) wm4ca-d15:whatsupwith__name__? bhaas$ ./my_module.py
my_module.py running as driver:  🦄 __main__ 🦄
```

When a script is executed, the special __name__ variable is automatically set to '__main__' within the scope of that file.

https://github.com/trinityrnaseq/CSHLProgForBio/tree/main/whatsupwith__name__%3F

Useful structure of a python script for shareable functions

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```

```
(base) wm4ca-d15:whatsupwith__name__? bhaas$ ./my_module.py
my_module.py running as driver:  🦄 __main__ 🦄
```

my_script.py *imported*

```
#!/usr/bin/env python

from my_module import *

print("my_script.py: checking __name__ value:", decorate_name_val(__name__))
```

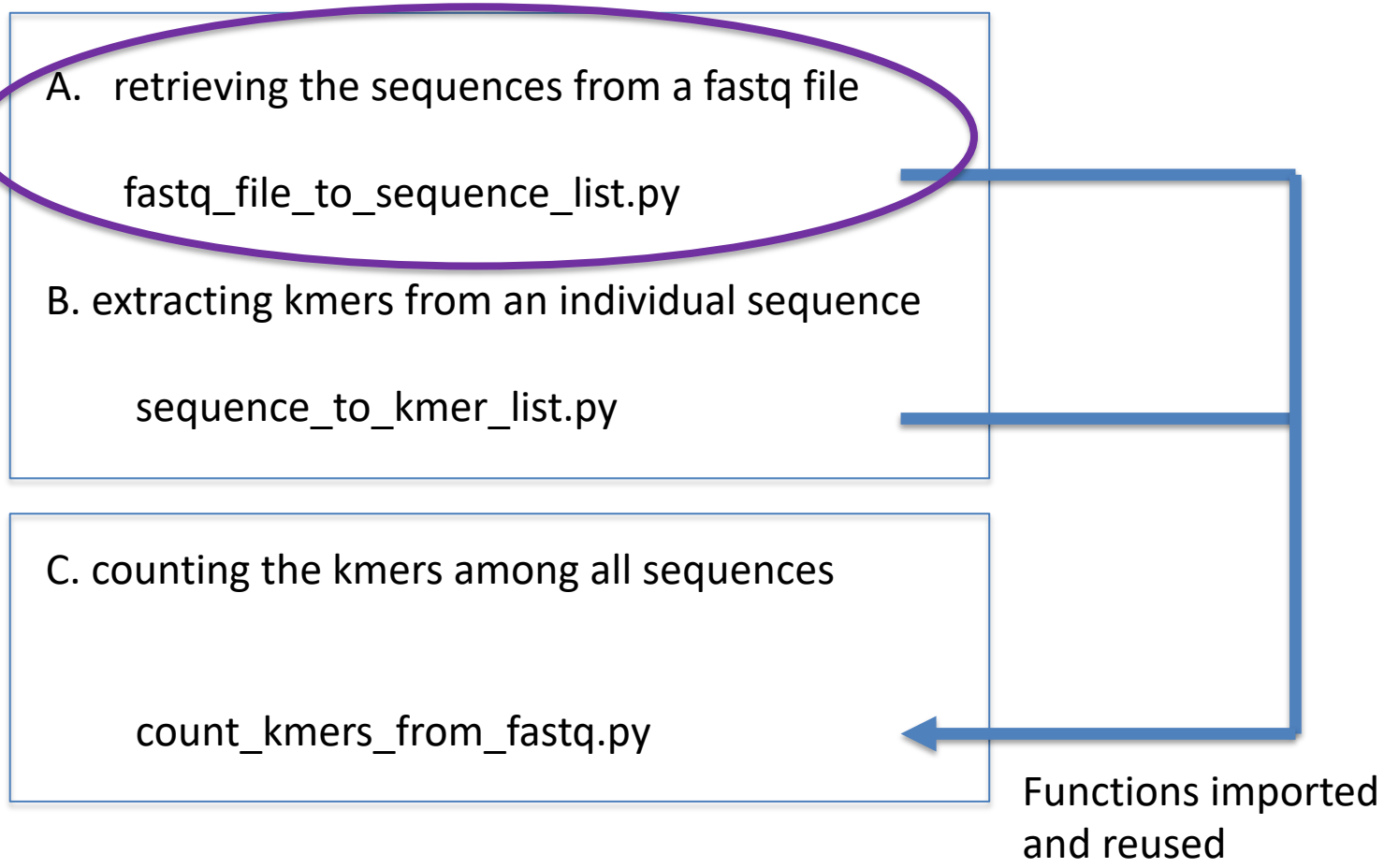
executed

Uses imported decorate_name_val()

```
(base) wm4ca-d15:whatsupwith__name__? bhaas$ ./my_script.py
my_script.py: checking __name__ value: 🦄 __main__ 🦄
```

https://github.com/trinityrnaseq/CSHLProgForBio/tree/main/whatsupwith__name__%3F

Build in 3 phases – demonstrating function reuse



Part A: Retrieve sequences from a fastq file

Write a python script that retrieves a list of all read sequences from a fastq file.

A script fastq_file_to_sequence_list.py is provided as a starting point. Fill in the missing code.

A fastq file <reads.fq> is provided as input.

The script usage is:

```
usage: ./fastq_file_to_sequence_list.py filename.fastq num_seqs_show
```

Running it like so:

```
fastq_file_to_sequence_list.py reads.fq 10
```

Should produce the following output:

['ACTGCATCCTGGAAAGAATCAATGGTGGCCGGAAAGTGTTTTTCAAATACAAGAGTGACAATGTGCCCTGTTGTTT',
'GTAATTTCCGTACCTGCCACAGTGTGGGCTCACCCTGCTTAGAGGACAGGGAAGGACCCTAAAGGTAGGCTGATGC',
'CTGGGCTGCAGCTAAGTTCTCTGCATCCTCCTTCTTGCTTGTGGCTGGGAAGAAGACAATGTTGTCGATGGTCTGG',
'CACGTTTTCTAAGCAGTTTGTACCAGATCGTGCTAACTGCTCATTGTCTTGTTGTACACACCAGTAAAGCTGGGCA',
'TGCTCATTGTCTTGTTGTACACACCAGTAAAGCTGGGCAAAAATATCATCCAAAAGTACATCGCTGAGAACTCCTA',
'CCCACCTGAAAACATTTTCTACATCCACTGTTATATGGAATGCTTGATAAGCTTTTCATTCTAACCATCAGAGCAC',
'TCTGAATAAGTCCTGCCACCAATGTTTTTCATAAGTGTGGCCATATGTTTTCATTATTTCAAACATTACTGTTAAG',
'CTCCGTTTTTTGAGAGTGCAACACATAGATACTGCTTGATAGCATTAATAAACATCTCATTTGTCCTGAAAACAGG',
'GCCTGAGTGTGCAAAAATCTTCAGAGTAAGAATACCATAGTTGCTAAATATCTTTTACCATGAGCAATAATTTTTT',
'TCTGGTGCAGCTAGATGGAATACTGAGAAAATGTTCTTCCATCCTGAACGAATATTTGCAGCCTGAGAATTAACCA']

GitHub link: https://github.com/trinityrnaseq/CSHLProgForBio/tree/main/Exercise-counting_kmers#part-a-retrieve-sequences-from-a-fastq-file

A. fastq_file_to_sequence_list.py

Reusable function part:

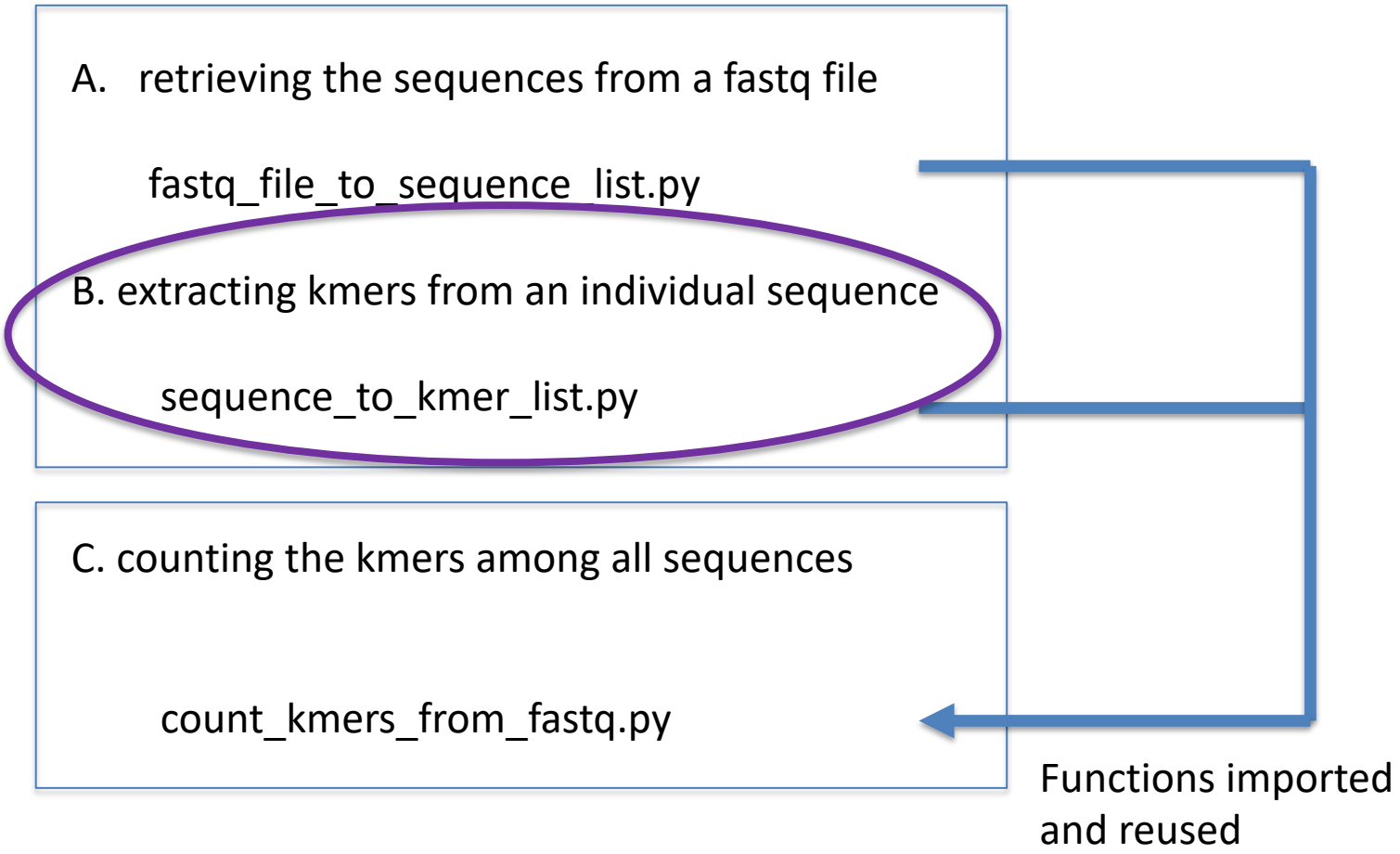
```python

## method: seq_list_from_fastq_file(fastq_filename)
##
##  Extracts the sequence lines from a fastq file and returns a list
##  of the sequence lines
##
##  input parameters:
##
##  fastq_filename :  name of the fastq file (type: string)
##
##  returns seq_list : list of read sequences.
##                   ie.  ["GATCGCATAG", "CGATGCAG", ...]

def seq_list_from_fastq_file(fastq_filename):

    seq_list = list()

    ## begin your code









    ## end your code

    return seq_list

```

Driver part for testing:

```python


def main():

    progname = sys.argv[0]

    usage = "\n\n\tusage: {} filename.fastq num_seqs_show\n\n\n".format(progname)

    if len(sys.argv) < 3:
        sys.stderr.write(usage)
        sys.exit(1)

    # capture command-line arguments
    fastq_filename = sys.argv[1]
    num_seqs_show = int(sys.argv[2])

    seq_list = seq_list_from_fastq_file(fastq_filename)

    print(seq_list[0:num_seqs_show])

    sys.exit(0)  # always good practice to indicate worked ok!



if __name__ == '__main__':
    main()

```

https://github.com/trinityrnaseq/CSHLProgForBio/blob/main/Exercise-counting_kmers/fastq_file_to_sequence_list.py

Build in 3 phases – demonstrating function reuse



Part B: Extracting kmers from a sequence

Write a python script to extract all kmers of a specified length from a nucleotide sequence.

A script fastq_file_to_sequence_list.py is provided as a starting point. Fill in the missing code.

The script usage is:

```
usage: ./sequence_to_kmer_list.py sequence kmer_length
```

Running it like so:

```
sequence_to_kmer_list.py ACTGCATCCTGGAAAGAATCAATGGTGGCCGGAAAGTGTTTTTCAAATACAAGAGTGACAATGTGCCCTGTTGTTT 6
```

Should produce the following output:

['ACTGCA', 'CTGCAT', 'TGCATC', 'GCATCC', 'CATCCT', 'ATCCTG', 'TCCTGG', 'CCTGGA', 'CTGGAA', 'TGGAAA', 'GGAAAG', 'GAAAGA', 'AAAGAA', 'AAGAAT', 'AGAATC', 'GAATCA', 'AATCAA', 'ATCAAT', 'TCAATG', 'CAATGG', 'AATGGT', 'ATGGTG', 'TGGTGG', 'GGTGGC', 'GTGGCC', 'TGGCCG', 'GGCCGG', 'GCCGGA', 'CCGGAA', 'CGGAAA', 'GGAAAG', 'GAAAGT', 'AAAGTG', 'AAGTGT', 'AGTGTT', 'GTGTTT', 'TGTTTT', 'GTTTTT', 'TTTTTC', 'TTTTCA', 'TTTCAA', 'TTCAAA', 'TCAAAT', 'CAAATA', 'AAATAC', 'AATACA', 'ATACAA', 'TACAAG', 'ACAAGA', 'CAAGAG', 'AAGAGT', 'AGAGTG', 'GAGTGA', 'AGTGAC', 'GTGACA', 'TGACAA', 'GACAAT', 'ACAATG', 'CAATGT', 'AATGTG', 'ATGTGC', 'TGTGCC', 'GTGCCC', 'TGCCCT', 'GCCCTG', 'CCCTGT', 'CCTGTT', 'CTGTTG', 'TGTTGT', 'GTTGTT', 'TTGTTT']

B. sequence_to_kmer_list.py

Reusable function part

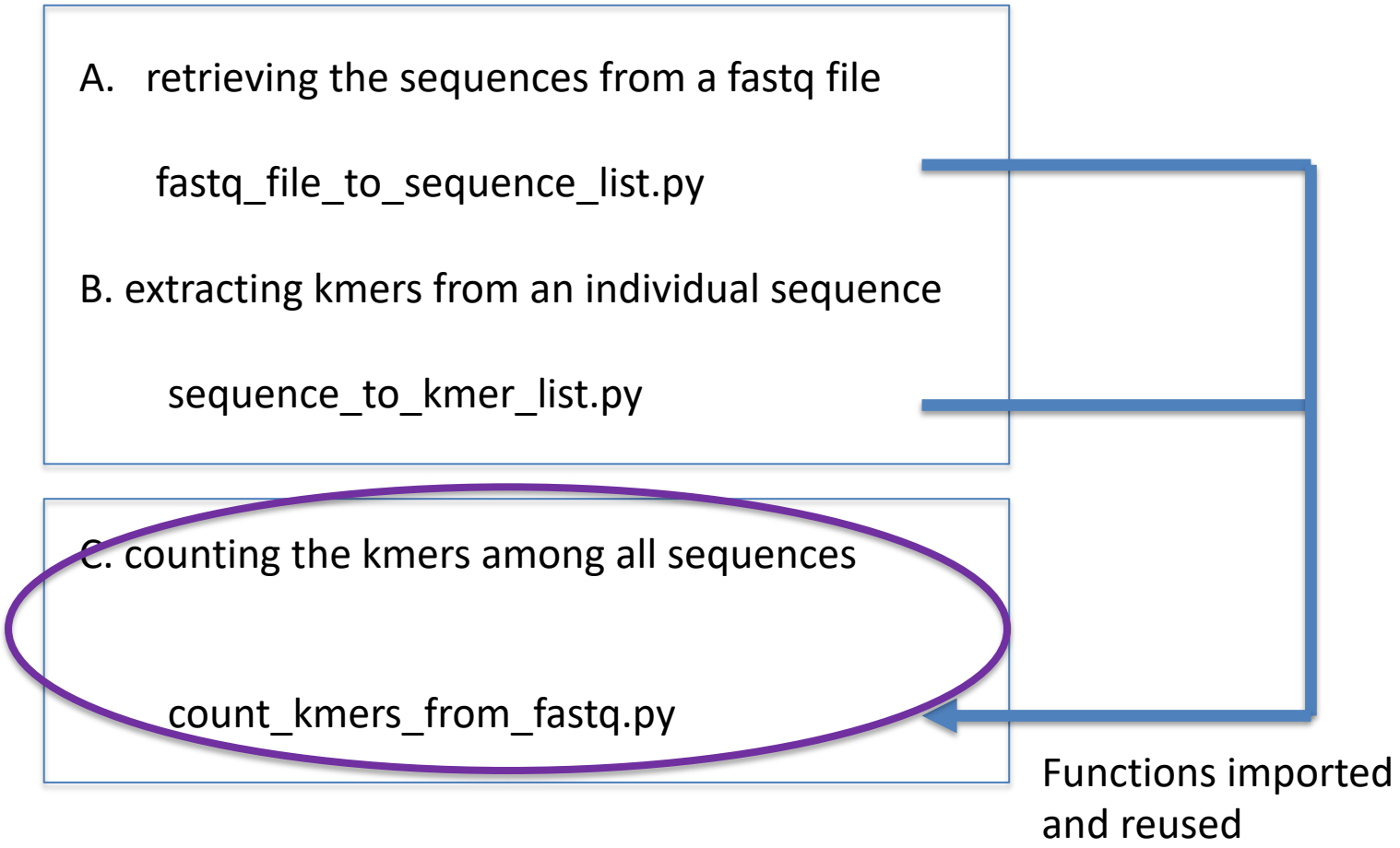
```python

## method: sequence_to_kmer_list(sequence, kmer_length)
##
##  Extracts all kmers of a specified length from a sequence
##
##  ie.  sequence: GATCGATCGATCGA
##   and given kmer_length = 4
##   would yield
##                GATC
##                 ATCG
##                  TCGA
##                   .... and so forth
##
##  input parameters:
##
##  seuqence : nucleotide sequence (type: string)
##
##  returns kmer_list : list of kmer sequences.
##                   ie.  ["GATC", "ATCG", ...]

def sequence_to_kmer_list(sequence, kmer_length):

    kmers_list = list()

    ## begin your code









    ## end your code

    return kmers_list

```

Driver part for testing:

```python

def main():

    progname = sys.argv[0]

    usage = "\n\n\tusage: {} sequence kmer_length\n\n\n".format(progname)

    if len(sys.argv) < 3:
        sys.stderr.write(usage)
        sys.exit(1)

    # capture command-line arguments
    sequence = sys.argv[1]
    kmer_length = int(sys.argv[2])

    kmers  = sequence_to_kmer_list(sequence, kmer_length)

    print(kmers)

    sys.exit(0)  # always good practice to indicate worked ok!



if __name__ == '__main__':
    main()

```

https://github.com/trinityrnaseq/CSHLProgForBio/blob/main/Exercise-counting_kmers/sequence_to_kmer_list.py

Build in 3 phases – demonstrating function reuse



Part C: Counting all kmers from all sequences in a fastq file

Now, let's count all kmers in all sequences. We can leverage each of the methods implemented above. Because of the way we wrote the above scripts, we can leverage them as a code library and simply import them for use in a new script.

Use the script count_kmers_from_fastq.py as the starting point. You'll see at the top of this script:

```
from sequence_to_kmer_list import *
from fastq_file_to_sequence_list import *
```

Those lines import the methods we implemented earlier so that we can just reuse them without having to rewrite or copy/paste any code in this new script.

The usage of our script is:

```
    usage: ./count_kmers_from_fastq.py filename.fastq kmer_length num_top_kmers_show
```

And when we run it like so:

```
    count_kmers_from_fastq.py reads.fq 6 10
```

It should produce the output:

```
TTTTTT: 3085
CTTCTT: 2550
AAAAAA: 2498
CTGCTG: 2446
AGCTGG: 2400
CAGCAG: 2265
CAGCTG: 2243
TCTTCT: 2208
CTGGAG: 2174
TGCTGT: 2156
```

C. count_kmers_from_fastq.py

Import earlier functions

```
#!/usr/bin/env python

import os, sys

from sequence_to_kmer_list import *
from fastq_file_to_sequence_list import *

```

New function to implement

```

## method: count_kmers(kmer_list)
##
##  Counts the frequency of each kmer in the given list of kmers
##
##  input parameters:
##
##  kmer_list : list of kmers (type: list)
##              ie.  ["GATC", "TCGA", "GATC", ...]
##
##
##  returns kmer_counts_dict : dict containing ( kmer : count )
##                   ie.  {  "GATC" : 2,
##                           "TCGA" : 1,
##                            ...       }


def count_kmers(kmer_list):

    kmer_count_dict = dict()

    ##################
    ## Step 2:
    ## begin your code


    Step 2


    ## end your code
    ################

    return kmer_count_dict

```

Main code block

```

def main():

    progname = sys.argv[0]

    usage = "\n\n\tusage: {} filename.fastq kmer_length num_top_kmers_show\n\n\n".format(
        progname
    )

    if len(sys.argv) < 4:
        sys.stderr.write(usage)
        sys.exit(1)

    # capture command-line arguments
    fastq_filename = sys.argv[1]
    kmer_length = int(sys.argv[2])
    num_top_kmers_show = int(sys.argv[3])

    seq_list = seq_list_from_fastq_file(fastq_filename)

    all_kmers = list()

    ######################
    ## Step 1:
    ## begin your code, populate 'all_kmers' list with the
    ## collection of kmers from all sequences

    Step 1

    ## end your code
    ######################

    kmer_count_dict = count_kmers(
        all_kmers
    )  # see step 2 above. You implement this. :-)

    unique_kmers = list(kmer_count_dict.keys())

    ########################
    ## Step 3: sort unique_kmers by abundance descendingly
    ## (Note, you can run and test without first implementing Step 3)
    ## begin your code       hint: see the built-in 'sorted' method documentation


    ## end your code

    Step 3

    ## printing the num top kmers to show
    top_kmers_show = unique_kmers[0:num_top_kmers_show]

    for kmer in top_kmers_show:
        print("{}: {}".format(kmer, kmer_count_dict[kmer]))

    sys.exit(0)  # always good practice to indicate worked ok!


if __name__ == "__main__":
    main()

```

https://github.com/trinityrnaseq/CSHLProgForBio/blob/main/Exercise-counting_kmers/count_kmers_from_fastq.py

Extra Credit

It should produce the output:

```
TTTTTT: 3085
CTTCTT: 2550
AAAAAA: 2498
CTGCTG: 2446
AGCTGG: 2400
CAGCAG: 2265
CAGCTG: 2243
TCTTCT: 2208
CTGGAG: 2174
TGCTGT: 2156
```

Extra credit section:

If you've accomplished the above, here's another challenge!

Note that the top-most kmer is of low complexity. If we are going to perform downstram operations like assembly and want to start with a seed kmer, we might want to avoid low complexity kmers as they would lack specificity.

Challenge: include another method that computes the complexity of each kmer using Shannon's Entropy (example: see: https://en.wikipedia.org/wiki/Sequence_logo#Logo_creation), and picture the kmer as representing one column of the seqlogo for which you would get one entropy calculation.

Add the entropy value as another column in the above printing.