Introduction to Sequence Alignment

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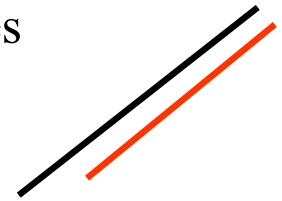
Outline

1. Global vs. local approaches to aligning sequences
    1. Dot Plots
2. BLAST
    1. Dynamic Programming
3. Hash Tables
    1. BLAT
4. BWT (Burrow Wheeler's Transformation)
    1. Bowtie, BWA
    2. Some slides borrowed from Stratos
5. Trimming sequences
    1. Trimmomatic

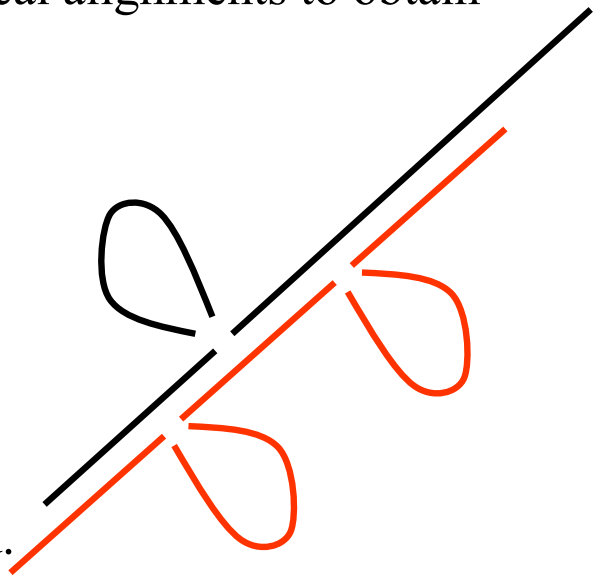
Global and local approaches to aligning sequences

GLOBAL: Attempt to "match" and assess similarity between two entire sequences



LOCAL: Find subsequences of high similarity

… and then possibly "stick" (chain, net, thread) together local alignments to obtain an overall comparison of the original sequences.



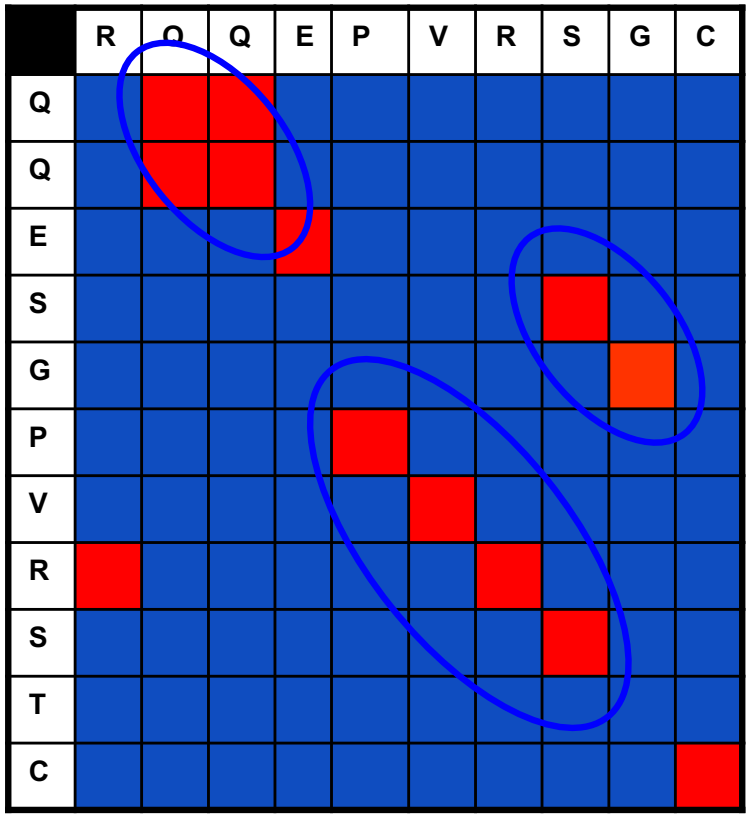
The second approach is more meaningful

(especially for long sequences, of different lengths, like whole genomes)

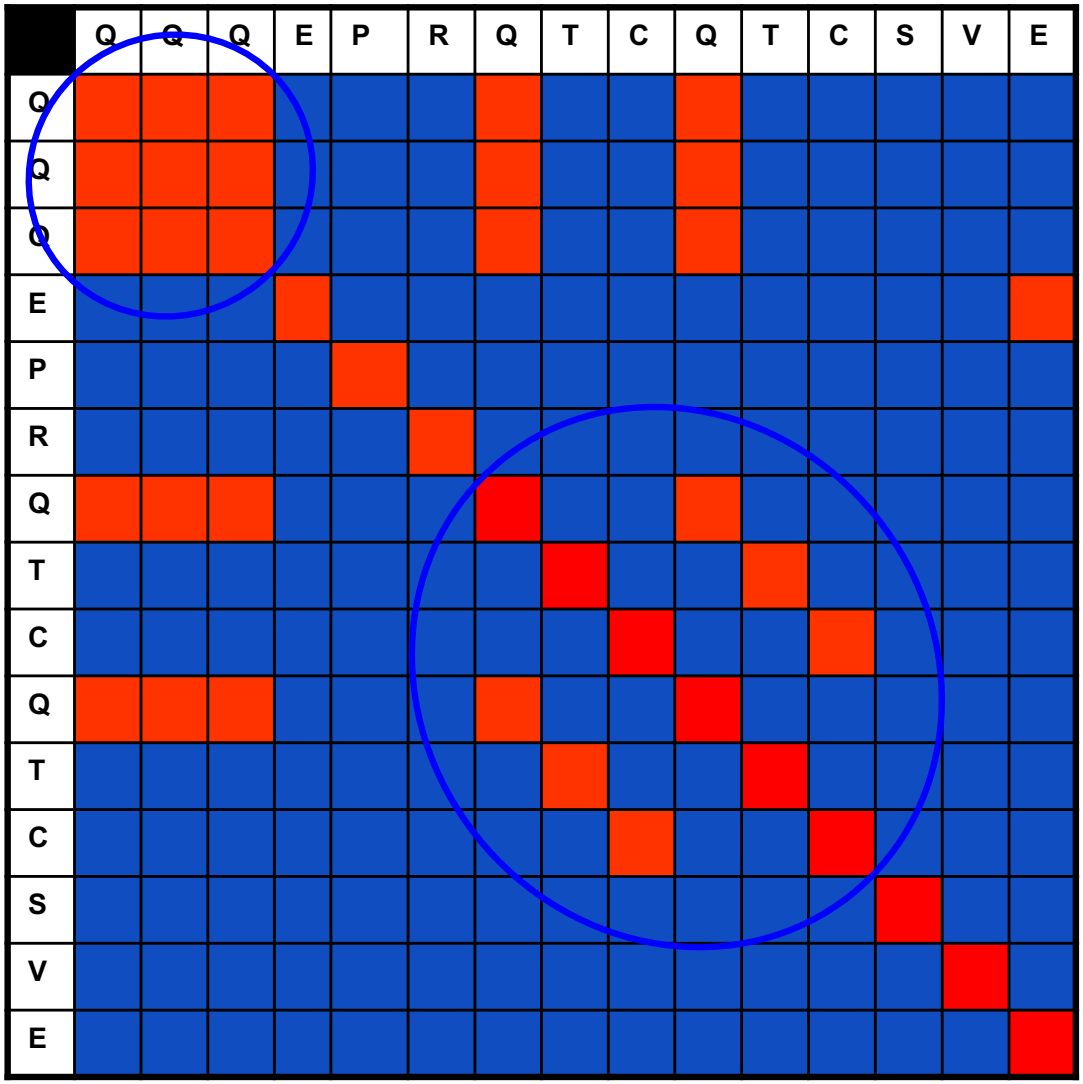
Two protein or DNA sequences are unlikely to present a straightforward overall "match", even if they are closely related.

Why? Substitutions are not the only process by which they diverge: insertions, deletions and rearrangements

Visualizing pair-wise alignments: Dot plots (matrices)



A dot for every match… visualize regions of alignment between two sequences, even when not in same position.

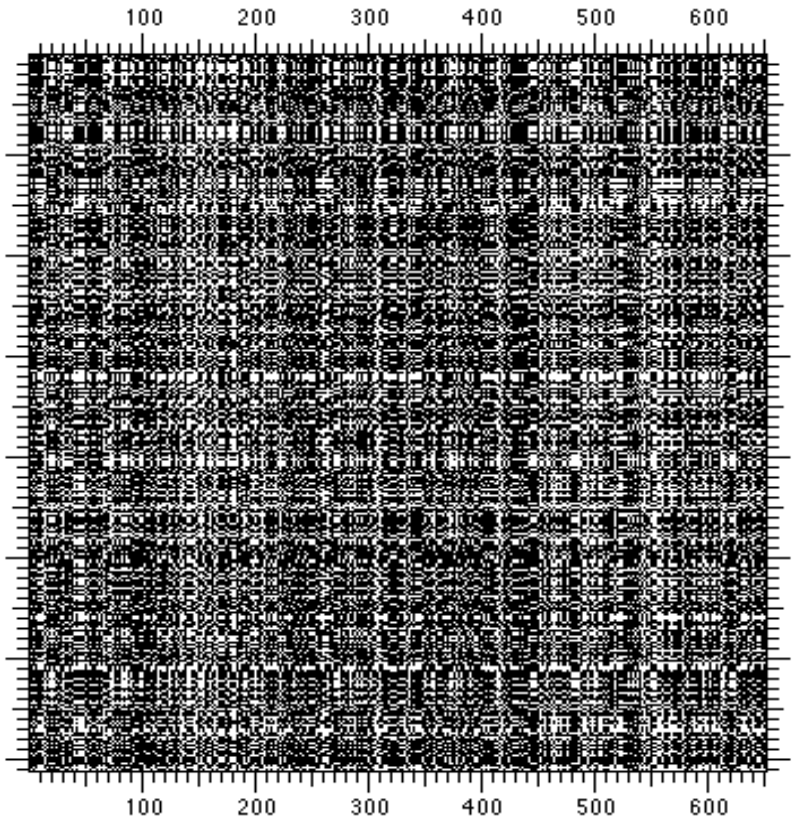


A sequence against itself… low complexity regions; repeats (may want to "mask out" prior to aligning the sequence with other sequences).

A real example: (dot-plots with tuning parameters)

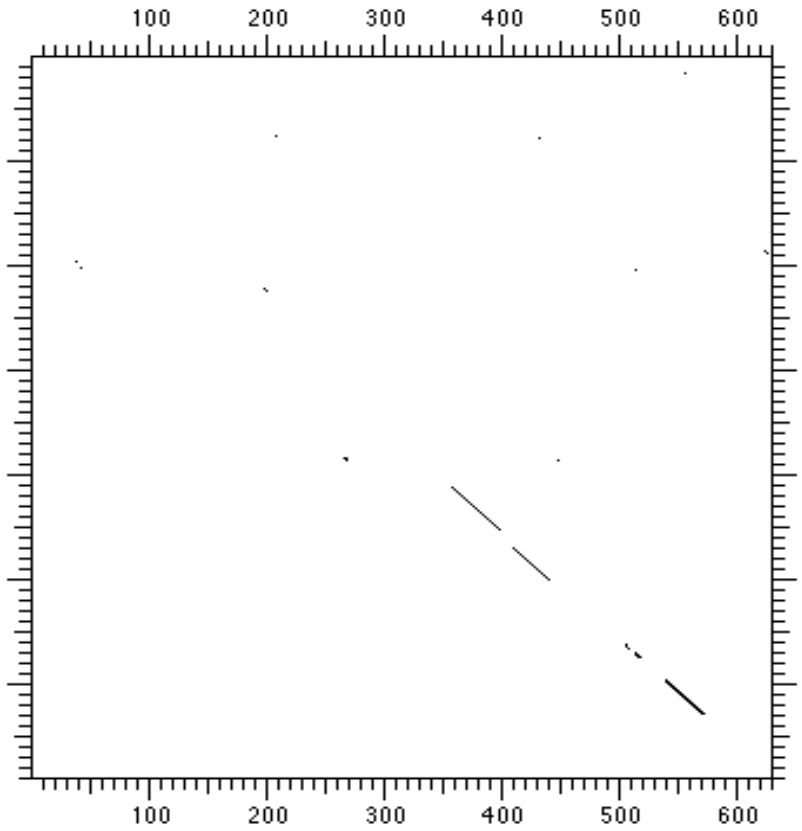
- every point corresponds to a window on the sequence, of size **W**
- a dot is drawn if there are at least **S** (stringency) matches in the window

W=1 (single position); S=1



too much detail to detect structure visually…

W=23; S=15



… appropriately tuning the parameters the alignment structure appears

Dynamic programming: Pairwise sequence alignment

Create a matrix table for comparing sequences with one sequence along each axis (size m+1, n+1)

Fill in partial alignment scores until score for entire sequence has been calculated:

- **Assign score for each position *i,j*** , progressing from top left to bottom right

- **Score rule**: take maximum of the three choices

1. Take value from left, assign gap penalty (gap along left axis)
2. Take value from top, assign gap penalty (gap along top axis)
3. Take value from diagonal above left, assign match/mismatch score

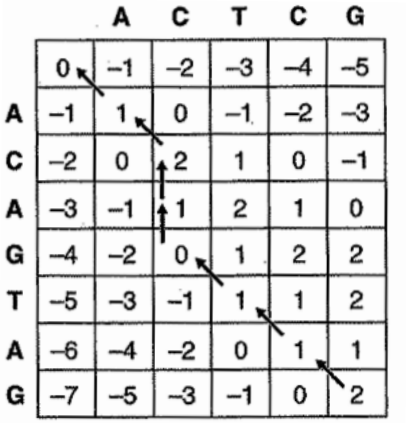
- **Repeat** until table is completed.

Use **trace-back** to obtain full alignment:

AC--TCG
ACAGTAG

		A	C	T	C	G
	0	–1	–2	–3	–4	–5
A	–1	1				
C	–2					
A	–3					
G	–4					
T	–5					
A	–6					
G	–7					

		A	C	T	C	G
	0	–1	–2	–3	–4	–5
A	–1	1	0	–1	–2	–3
C	–2	0	2	1	0	–1
A	–3	–1	1	2	1	0
G	–4	–2	0	1	2	2
T	–5	–3	–1	1	1	2
A	–6	–4	–2	0	1	1
G	–7	–5	–3	–1	0	2



Heuristic (vs. optimal) methods for pair-wise alignment: the BLAST family of algorithms

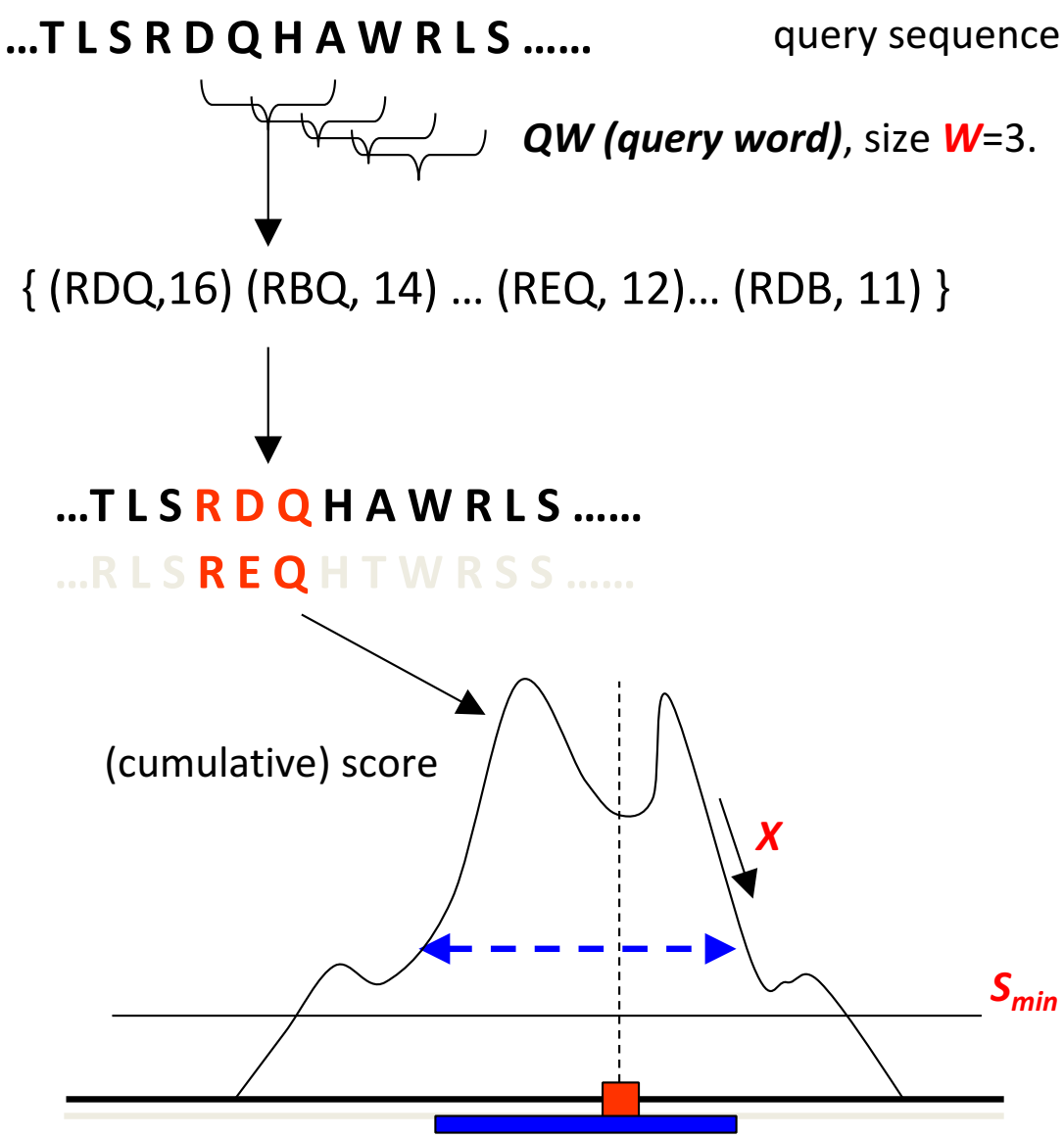
Given a ***scoring system*** (scoring matrix and gap penalties), alignment can be evaluated ***quantitatively***, and ***optimal*** alignments can be sought with ***Dynamic Programming*** algorithms:

- ***GLOBAL***: Needleman-Wunsch-Gotoh algorithm
- ***LOCAL***: Smith-Waterman algorithm

These have high ***algorithmic complexity ($O(N^2)$)***, and are replaced by ***heuristic procedures*** in most practical applications.

Most commonly used: ***BLAST family of algorithms*** (local alignment).

BLAST: heuristic database search using local alignment
Basic Local Alignment Search Tool



A cartoon for BLAST

(parameters)

For each ***QW***, use the **scoring matrix** to form a ***neighborhood NB*** = {all words of size ***W*** with a score ≥ ***T***=11}

Find match(es) to words belonging to NB in the subject (target) sequence

For each match, use the **scoring matrix** and **gap penalties** to produce an **HSP** (High scoring Segment Pair), then extend alignment on both sides, until

- drop is > ***X***, or
- score goes below S_{min} (minimum hit score)

How likely is it to find a match by chance?

"Given a set of sequences NOT related to the query sequence (or even random sequences), what is the probability of finding a match with alignment score *S* simply by chance?"

Score will depend on:

- Length and composition of the query and target sequence
- Scoring matrix

Statistical significance *is given by* ***probability*** *and* ***expectation*** ***values:***

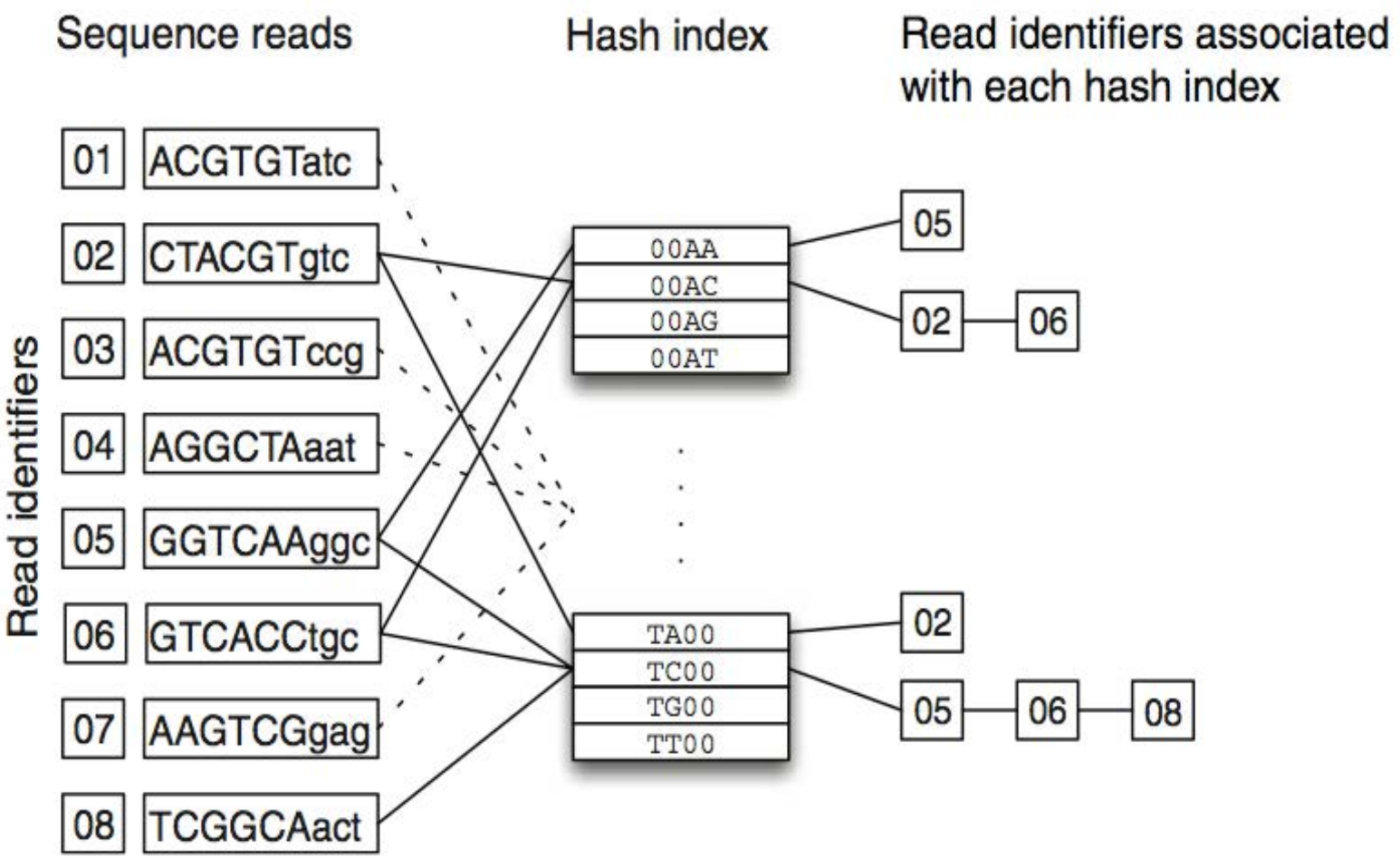
P-value: probability of finding *one or more sequences* of score >=S by random chance

E-value: *expected number of sequences* of score >=S that would be found by random chance

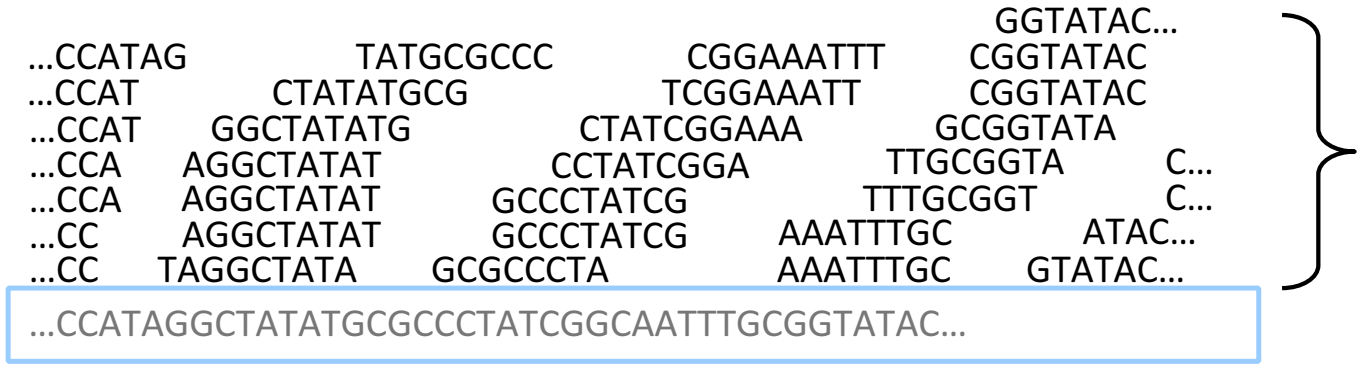
About Blat (from genome.ucsc.edu)

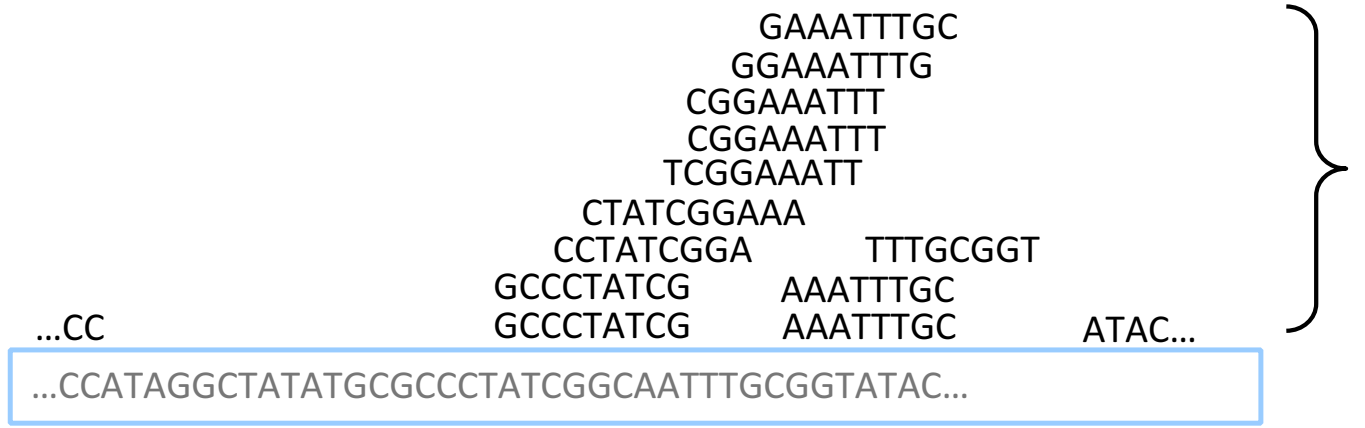
- “BLAT on DNA is designed to quickly find sequences of 95% and greater similarity of length 25 bases or more. “
- “It may miss more divergent or shorter sequence alignments. It will find perfect sequence matches of 20 bases. “
- “BLAT is not BLAST.”
- “DNA BLAT works by keeping an index of the entire genome in memory. The index consists of all overlapping 11-mers stepping by 5 except for those heavily involved in repeats.”
- “The index takes up about 2 gigabytes of RAM. The genome itself is not kept in memory, allowing BLAT to deliver high performance on a reasonably priced Linux box. “
- “The index is used to find areas of probable homology, which are then loaded into memory for a detailed alignment.”

Hash Table (BLAT)



Short Read Applications



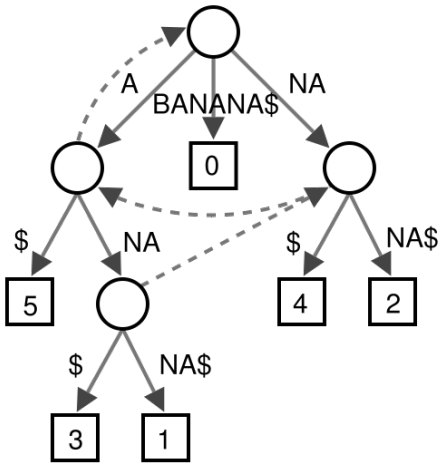


Finding the alignments is typically the performance bottleneck

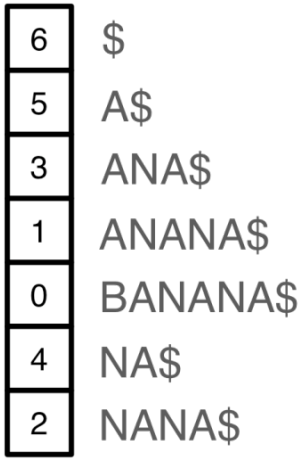
- New alignment algorithms must address the requirements and characteristics of NGS reads
  - Millions of reads per run (30x of genome coverage)
  - Short Reads (as short as 36bp)
  - Different types of reads (single-end, paired-end, mate-pair, etc.)
  - Base-calling quality factors
  - Sequencing errors (~ 1%)
  - Repetitive regions
  - Sequencing organism vs. reference genome
  - Must adjust to evolving sequencing technologies and data formats

Indexing

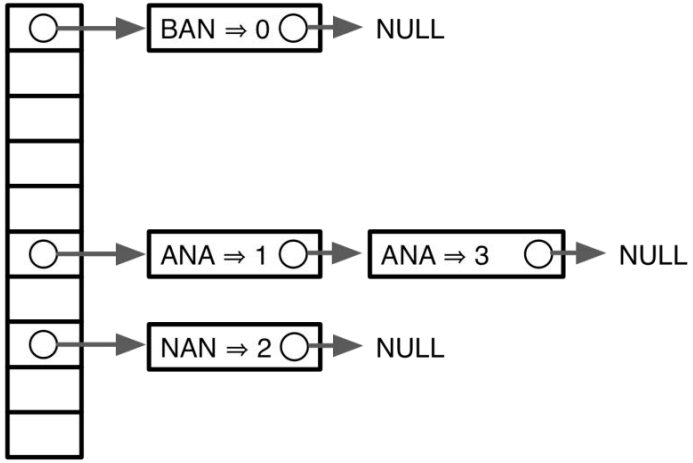
- Genomes and reads are too large for direct approaches like dynamic programming
- *Indexing* is required



Suffix tree



Suffix array



Seed hash tables

Many variants, incl. spaced seeds

- Choice of index is key to performance

Suffix Array

[00] gggtaaagctataactattgatcaggcgtt
[01] ggtaaagctataactattgatcaggcgtt
[02] gtaaagctataactattgatcaggcgtt
[03] taaagctataactattgatcaggcgtt
[04] aaagctataactattgatcaggcgtt
[05] aagctataactattgatcaggcgtt
[06] agctataactattgatcaggcgtt
[07] gctataactattgatcaggcgtt
[08] ctataactattgatcaggcgtt
[09] tataactattgatcaggcgtt
[10] ataactattgatcaggcgtt
[11] taactattgatcaggcgtt
[12] aactattgatcaggcgtt
[13] actattgatcaggcgtt
[14] ctattgatcaggcgtt
[15] tattgatcaggcgtt
[16] attgatcaggcgtt
[17] ttgatcaggcgtt
[18] tgatcaggcgtt
[19] gatcaggcgtt
[20] atcaggcgtt
[21] tcaggcgtt
[22] caggcgtt
[23] aggcgtt
[24] ggcgtt
[25] gcgtt
[26] cgtt
[27] gtt
[28] tt
[29] t

Find "ctat" in the reference

[04] aaagctataactattgatcaggcgtt
[12] aactattgatcaggcgtt
[05] aagctataactattgatcaggcgtt
[13] actattgatcaggcgtt
[06] agctataactattgatcaggcgtt
[23] aggcgtt
[10] ataactattgatcaggcgtt
[20] atcaggcgtt
[16] attgatcaggcgtt
[22] caggcgtt
[26] cgtt
[08] ctataactattgatcaggcgtt
[14] ctattgatcaggcgtt
[19] gatcaggcgtt
[25] gcgtt
[07] gctataactattgatcaggcgtt
[24] ggcgtt
[00] gggtaaagctataactattgatcaggcgtt
[01] ggtaaagctataactattgatcaggcgtt
[02] gtaaagctataactattgatcaggcgtt
[27] gtt
[29] t
[03] taaagctataactattgatcaggcgtt
[11] taactattgatcaggcgtt
[09] tataactattgatcaggcgtt
[15] tattgatcaggcgtt
[21] tcaggcgtt
[18] tgatcaggcgtt
[28] tt
[17] ttgatcaggcgtt

NGS Read Alignment
Burrows Wheeler Transformation (BWT)

- Invented by David Wheeler in 1983 (Bell Labs). Published in 1994.
  "A Block Sorting Lossless Data Compression Algorithm"
  *Systems Research Center Technical Report No **124**. Palo Alto, CA: Digital Equipment Corporation,* Burrows M, Wheeler DJ. 1994

- Originally developed for compressing large files (*bzip2*, etc.)

- Lossless, Fully Reversible

- Alignment Tools based on BWT: *bowtie*, BWA, *SOAP2*, etc.

- Approach:
  - Align reads on the *transformed* reference genome, using an efficient index (FM index)
  - Solve the simple problem first (align one character) and then build on that solution to solve a slightly harder problem (two characters) etc.

- Results in great speed and efficiency gains (a few GigaByte of RAM for the entire H. Genome). Other approaches require tens of GigaBytes of memory and are much slower.

Burrows Wheeler Transformation

Text =

c	t	g	a	a	a	c	t	g	g	t	$
1	2	3	4	5	6	7	8	9	10	11	0

➢ Introduce $ at the end and construct all cyclic permutations of Text

c	t	g	a	a	a	c	t	g	g	t	$
t	g	a	a	a	c	t	g	g	t	$	c
g	a	a	a	c	t	g	g	t	$	c	t
a	a	a	c	t	g	g	t	$	c	t	g
a	a	c	t	g	g	t	$	c	t	g	a
a	c	t	g	g	t	$	c	t	g	a	a
c	t	g	g	t	$	c	t	g	a	a	a
t	g	g	t	$	c	t	g	a	a	a	c
g	g	t	$	c	t	g	a	a	a	c	t
g	t	$	c	t	g	a	a	a	c	t	g
t	$	c	t	g	a	a	a	c	t	g	g
$	c	t	g	a	a	a	c	t	g	g	t

Burrows Wheeler Transformation

- Sort rows alphabetically, keeping of which row went where

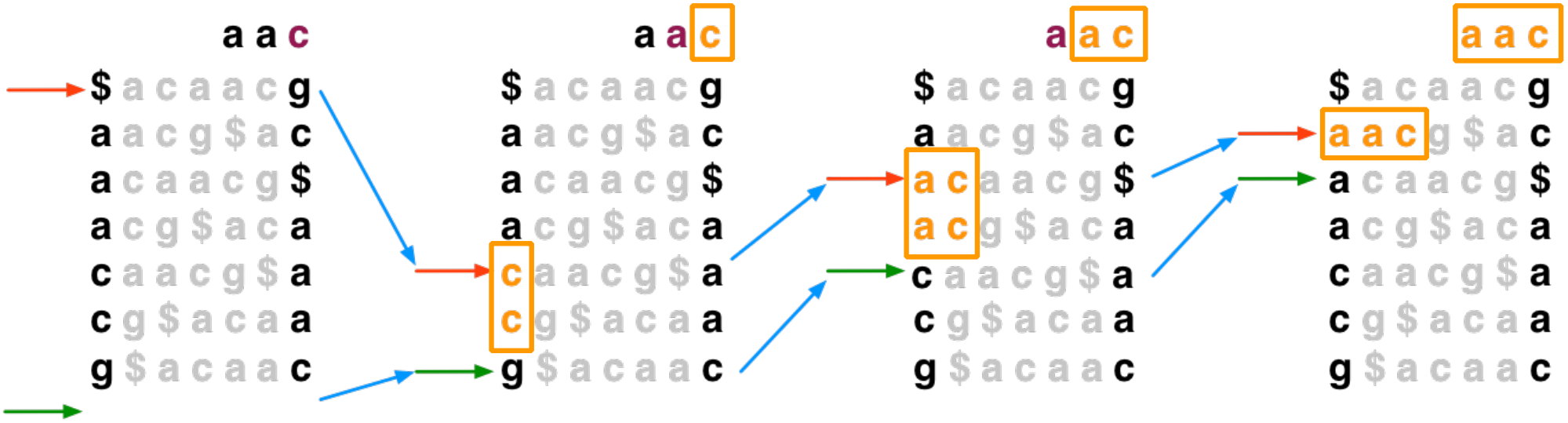
$	c	t	g	a	a	a	c	t	g	g	t
a	a	a	c	t	g	g	t	$	c	t	g
a	a	c	t	g	g	t	$	c	t	g	a
a	c	t	g	g	t	$	c	t	g	a	a
c	t	g	a	a	a	c	t	g	g	t	$
c	t	g	g	t	$	c	t	g	a	a	a
g	a	a	a	c	t	g	g	t	$	c	t
g	g	t	$	c	t	g	a	a	a	c	t
g	t	$	c	t	g	a	a	a	c	t	g
t	$	c	t	g	a	a	a	c	t	g	g
t	g	a	a	a	c	t	g	g	t	$	c
t	g	g	t	$	c	t	g	a	a	a	c

Burrows Wheeler Matrix

BWT (Text) =

t	g	a	a	$	a	t	t	g	g	c	c
11	3	4	5	0	6	2	8	9	10	1	7

Exact Matching with FM Index



- In progressive rounds, **top** & **bot** delimit the range of rows beginning with progressively longer suffixes of Q

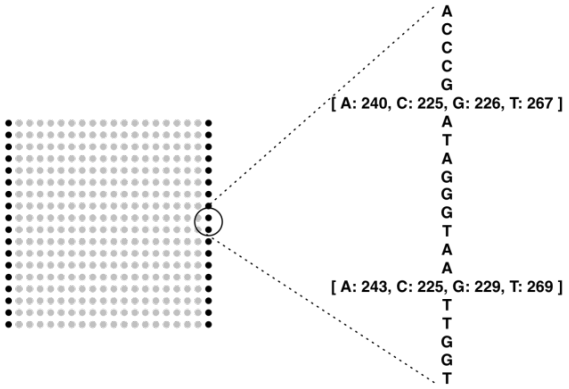
FM Index is Small

- Entire FM Index on DNA reference consists of:
  - BWT (same size as T)
  - Checkpoints (~15% size of T)
  - SA sample (~50% size of T)
- Total: ~1.65x the size of T

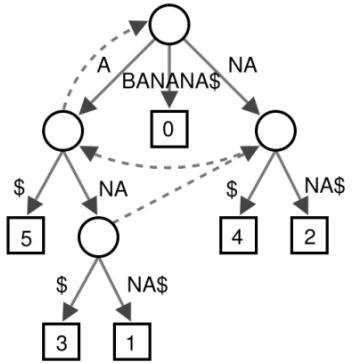
Assuming 2-bit-per-base encoding and no compression, as in Bowtie

Assuming a 16-byte checkpoint every 448 characters, as in Bowtie

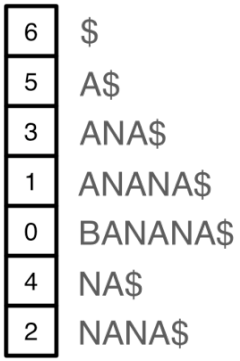
Assuming Bowtie defaults for suffix-array sampling rate, etc



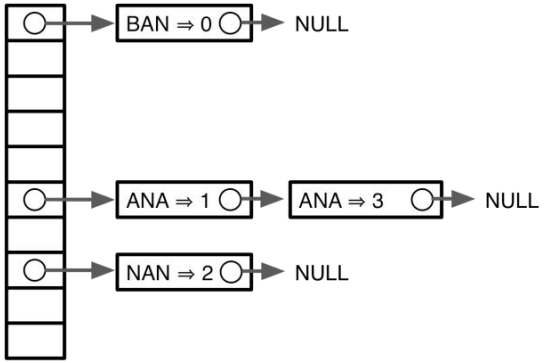
~1.65x



>45x



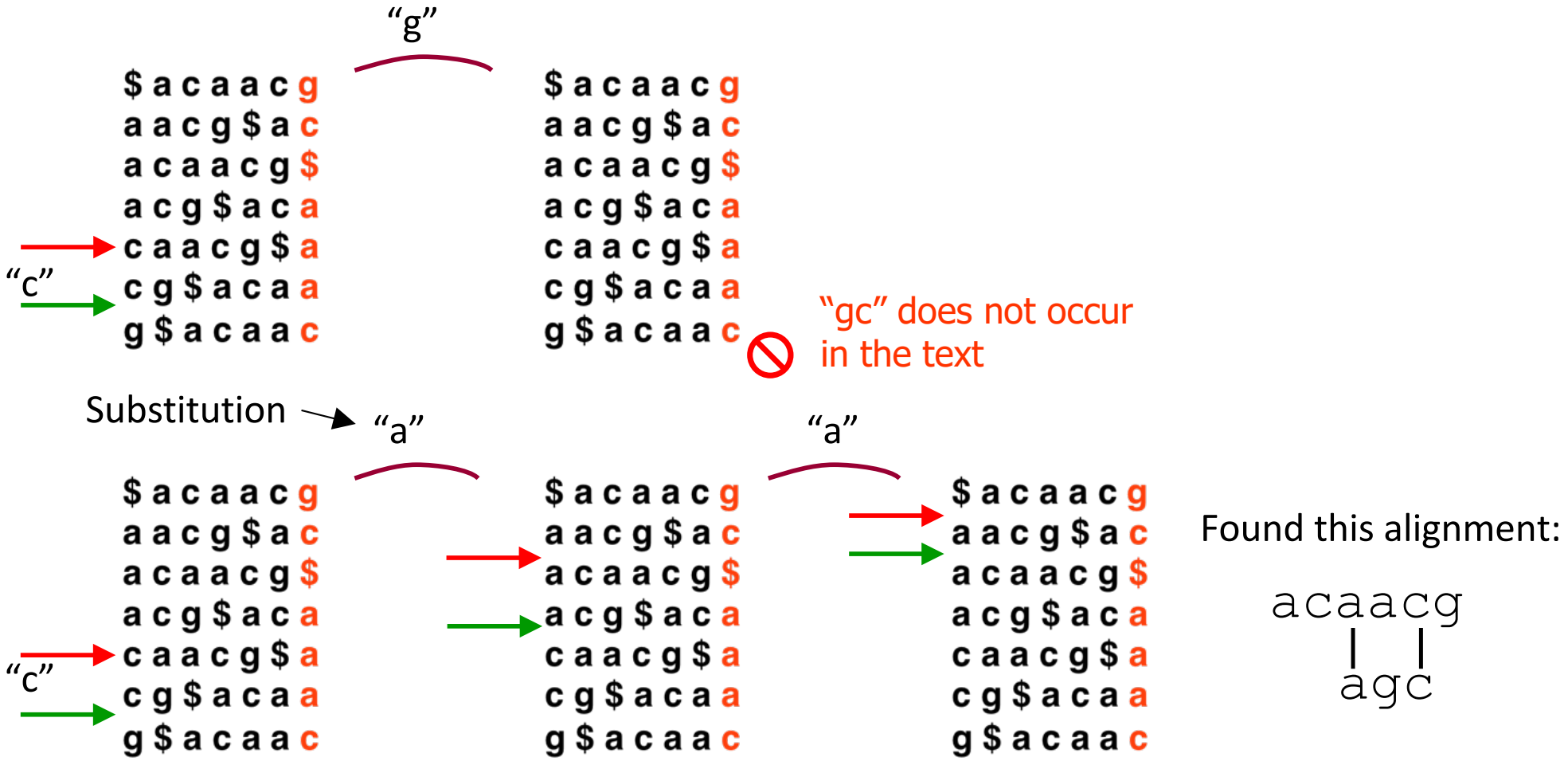
>15x

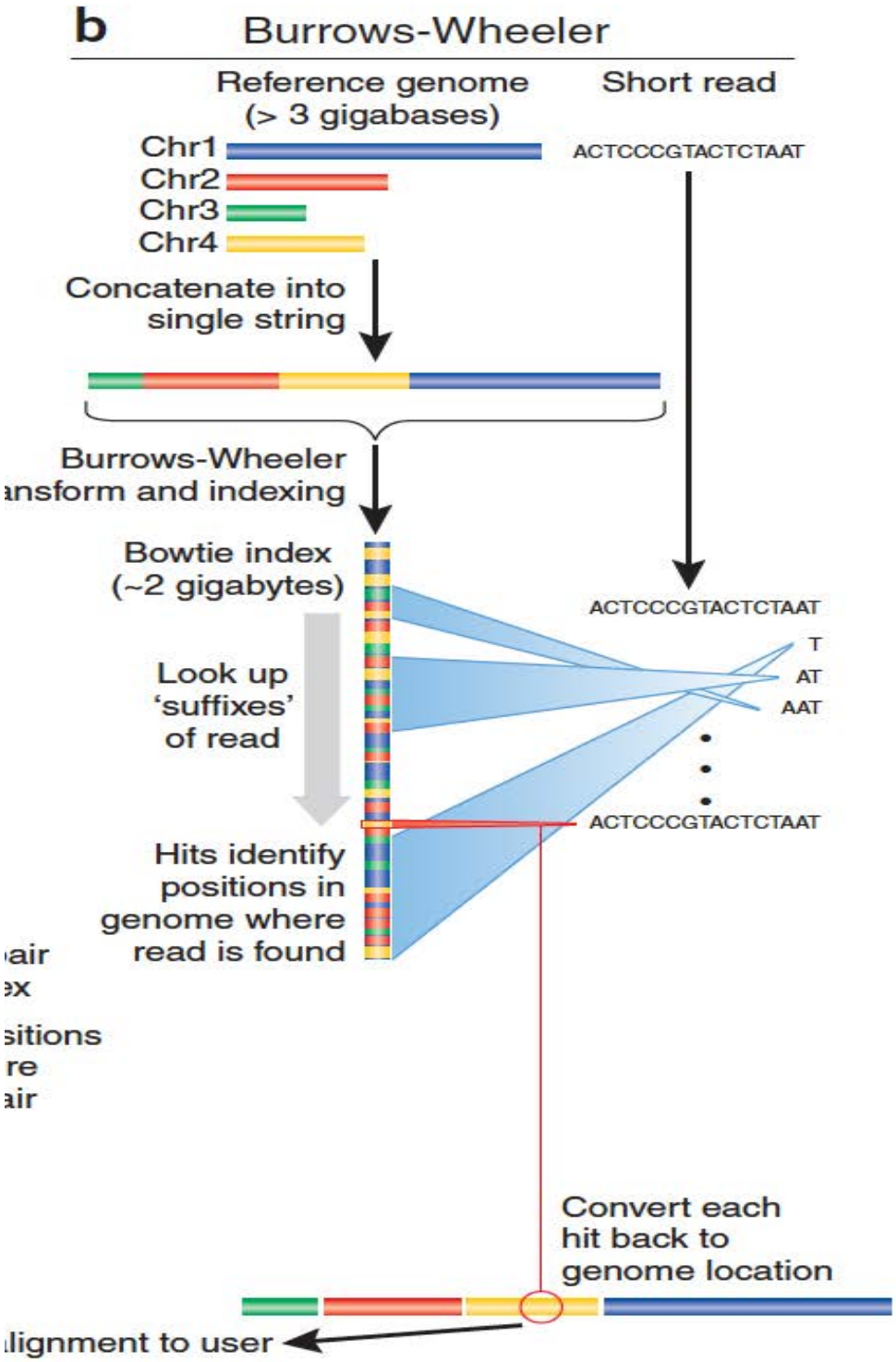


>15x

Backtracking

- Backtracking attempt for Q = “agc”, T = “acaacg”:




b
Burrows-Wheeler
Reference genome
(> 3 gigabases)
Short read
Chr1
Chr2
Chr3
Chr4
ACTCCCGTACTCTAAT
Concatenate into
single string
Burrows-Wheeler
ansform and indexing
Bowtie index
(~2 gigabytes)
ACTCCCGTACTCTAAT
T
AT
AAT
Look up
'suffixes'
of read
ACTCCCGTACTCTAAT
Hits identify
positions in
genome where
read is found
air
ex
sitions
re
air
Convert each
hit back to
genome location
lignment to user

Mapping Reads from RNA molecules

- What is the advantage of mapping reads from RNA to the genome sequenced instead of a database of all predicted RNA molecules?
  - We are not depending on the quality of annotation.
  - We are not assuming that we know about all of the RNA molecules in the cell.
- How can we find reads mapping to spliced junctions?
  - Create a separate database of all possible spliced junctions
  - Split reads in half and map them separately.

Bowtie & TopHat

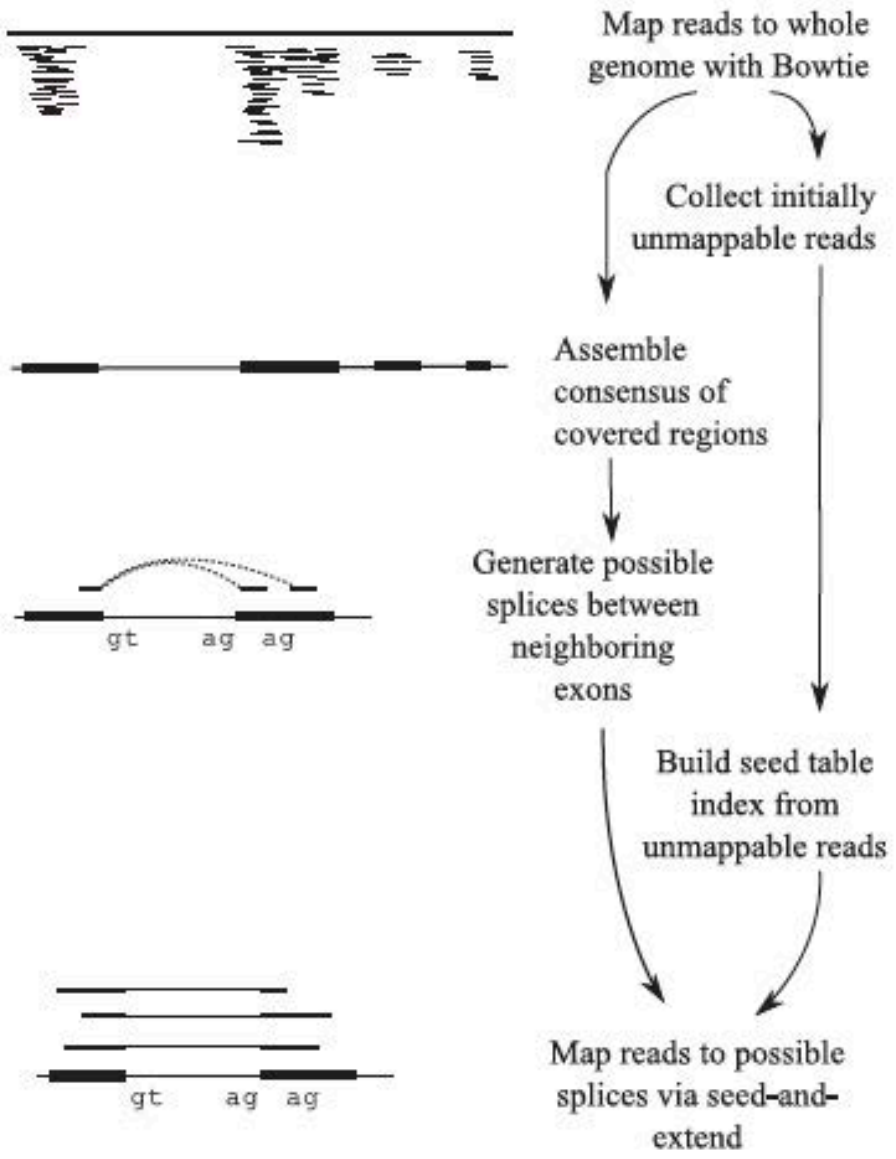


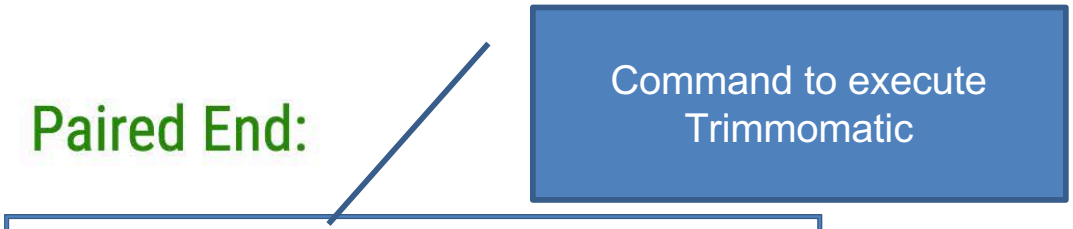
Fig. 1. The TopHat pipeline. RNA-Seq reads are mapped against the whole reference genome, and those reads that do not map are set aside. An initial consensus of mapped regions is computed by Maq. Sequences flanking potential donor/acceptor splice sites within neighboring regions are joined to form potential splice junctions. The IUM reads are indexed and aligned to these splice junction sequences.

TRIMMING: Why do we want to trim?

- Low quality sequences
- Adapter sequences
- Crop head or tail of the sequence
- Remove reads that end up to be too small
- For pair-end sequences, make sure the pairs are both in the files.

Trimmomatic

http://www.usadellab.org/cms/?page=trimmomatic



Paired End:

```
java -jar trimmomatic-0.35.jar PE -phred33 input_forward.fq.gz
input_reverse.fq.gz output_forward_paired.fq.gz
output_forward_unpaired.fq.gz output_reverse_paired.fq.gz
output_reverse_unpaired.fq.gz ILLUMINACLIP:TruSeq3-PE.fa:2:30:10
LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36
```

This will perform the following:

- Remove adapters (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10)
- Remove leading low quality or N bases (below quality 3) (LEADING:3)
- Remove trailing low quality or N bases (below quality 3) (TRAILING:3)
- Scan the read with a 4-base wide sliding window, cutting when the average quality per base drops below 15 (SLIDINGWINDOW:4:15)
- Drop reads below the 36 bases long (MINLEN:36)

Trimmomatic

Paired End:


```
java -jar trimmomatic-0.35.jar PE -phred33 input_forward.fq.gz
input_reverse.fq.gz output_forward_paired.fq.gz
output_forward_unpaired.fq.gz output_reverse_paired.fq.gz
output_reverse_unpaired.fq.gz ILLUMINACLIP:TruSeq3-PE.fa:2:30:10
LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36
```

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Paired End:


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input_reverse.fq.gz output_forward_paired.fq.gz
output_forward_unpaired.fq.gz output_reverse_paired.fq.gz
output_reverse_unpaired.fq.gz ILLUMINACLIP:TruSeq3-PE.fa:2:30:10
LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36
```

This will perform the following:

- Remove adapters (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10)
- Remove leading low quality or N bases (below quality 3) (LEADING:3)
- Remove trailing low quality or N bases (below quality 3) (TRAILING:3)
- Scan the read with a 4-base wide sliding window, cutting when the average quality per base drops below 15 (SLIDINGWINDOW:4:15)
- Drop reads below the 36 bases long (MINLEN:36)

Trimmomatic

Paired End:

```
java -jar trimmomatic-0.35.jar PE -phred33 input_forward.fq.gz
input_reverse.fq.gz output_forward_paired.fq.gz
output_forward_unpaired.fq.gz output_reverse_paired.fq.gz
output_reverse_unpaired.fq.gz ILLUMINACLIP:TruSeq3-PE.fa:2:30:10
LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36
```

The input sequences, each file represents one member of the pair.

This will perform the following:

- Remove adapters (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10)
- Remove leading low quality or N bases (below quality 3) (LEADING:3)
- Remove trailing low quality or N bases (below quality 3) (TRAILING:3)
- Scan the read with a 4-base wide sliding window, cutting when the average quality per base drops below 15 (SLIDINGWINDOW:4:15)
- Drop reads below the 36 bases long (MINLEN:36)

Trimmomatic

Paired End:

```
java -jar trimmomatic-0.35.jar PE -phred33 input_forward.fq.gz
input_reverse.fq.gz output_forward_paired.fq.gz
output_forward_unpaired.fq.gz output_reverse_paired.fq.gz
output_reverse_unpaired.fq.gz ILLUMINACLIP:TruSeq3-PE.fa:2:30:10
LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36
```

If both members of the pair survive the filtering then put them here.

This will perform the following:

- Remove adapters (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10)
- Remove leading low quality or N bases (below quality 3) (LEADING:3)
- Remove trailing low quality or N bases (below quality 3) (TRAILING:3)
- Scan the read with a 4-base wide sliding window, cutting when the average quality per base drops below 15 (SLIDINGWINDOW:4:15)
- Drop reads below the 36 bases long (MINLEN:36)

Trimmomatic

Paired End:

```
java -jar trimmomatic-0.35.jar PE -phred33 input_forward.fq.gz
input_reverse.fq.gz output_forward_paired.fq.gz
output_forward_unpaired.fq.gz output_reverse_paired.fq.gz
output_reverse_unpaired.fq.gz ILLUMINACLIP:TruSeq3-PE.fa:2:30:10
LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36
```

Reads that survive filtering but the mate did not.

This will perform the following:

- Remove adapters (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10)
- Remove leading low quality or N bases (below quality 3) (LEADING:3)
- Remove trailing low quality or N bases (below quality 3) (TRAILING:3)
- Scan the read with a 4-base wide sliding window, cutting when the average quality per base drops below 15 (SLIDINGWINDOW:4:15)
- Drop reads below the 36 bases long (MINLEN:36)

Trimmomatic

Paired End:

```
java -jar trimmomatic-0.35.jar PE -phred33 input_forward.fq.gz
input_reverse.fq.gz output_forward_paired.fq.gz
output_forward_unpaired.fq.gz output_reverse_paired.fq.gz
output_reverse_unpaired.fq.gz ILLUMINACLIP:TruSeq3-PE.fa:2:30:10
LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36
```

This will perform the following:

- Remove adapters (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10)
- Remove leading low quality or N bases (below quality 3) (LEADING:3)
- Remove trailing low quality or N bases (below quality 3) (TRAILING:3)
- Scan the read with a 4-base wide sliding window, cutting when the average quality per base drops below 15 (SLIDINGWINDOW:4:15)
- Drop reads below the 36 bases long (MINLEN:36)

Trimmomatic

Paired End:

```
java -jar trimmomatic-0.35.jar PE -phred33 input_forward.fq.gz
input_reverse.fq.gz output_forward_paired.fq.gz
output_forward_unpaired.fq.gz output_reverse_paired.fq.gz
output_reverse_unpaired.fq.gz ILLUMINACLIP:TruSeq3-PE.fa:2:30:10
LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36
```

This will perform the following:

- Remove adapters (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10)
- Remove leading low quality or N bases (below quality 3) (LEADING:3)
- Remove trailing low quality or N bases (below quality 3) (TRAILING:3)
- Scan the read with a 4-base wide sliding window, cutting when the average quality per base drops below 15 (SLIDINGWINDOW:4:15)
- Drop reads below the 36 bases long (MINLEN:36)

Trimmomatic

Paired End:

```
java -jar trimmomatic-0.35.jar PE -phred33 input_forward.fq.gz
input_reverse.fq.gz output_forward_paired.fq.gz
output_forward_unpaired.fq.gz output_reverse_paired.fq.gz
output_reverse_unpaired.fq.gz ILLUMINACLIP:TruSeq3-PE.fa:2:30:10
LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36
```

This will perform the following:

- Remove adapters (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10)
- Remove leading low quality or N bases (below quality 3) (LEADING:3)
- Remove trailing low quality or N bases (below quality 3) (TRAILING:3)
- Scan the read with a 4-base wide sliding window, cutting when the average quality per base drops below 15 (SLIDINGWINDOW:4:15)
- Drop reads below the 36 bases long (MINLEN:36)

Trimmomatic

Paired End:

```
java -jar trimmomatic-0.35.jar PE -phred33 input_forward.fq.gz
input_reverse.fq.gz output_forward_paired.fq.gz
output_forward_unpaired.fq.gz output_reverse_paired.fq.gz
output_reverse_unpaired.fq.gz ILLUMINACLIP:TruSeq3-PE.fa:2:30:10
LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36
```

This will perform the following:

- Remove adapters (ILLUMINACLIP:TruSeq3-PE.fa:2:30:10)
- Remove leading low quality or N bases (below quality 3) (LEADING:3)
- Remove trailing low quality or N bases (below quality 3) (TRAILING:3)
- Scan the read with a 4-base wide sliding window, cutting when the average quality per base drops below 15 (SLIDINGWINDOW:4:15)
- Drop reads below the 36 bases long (MINLEN:36)