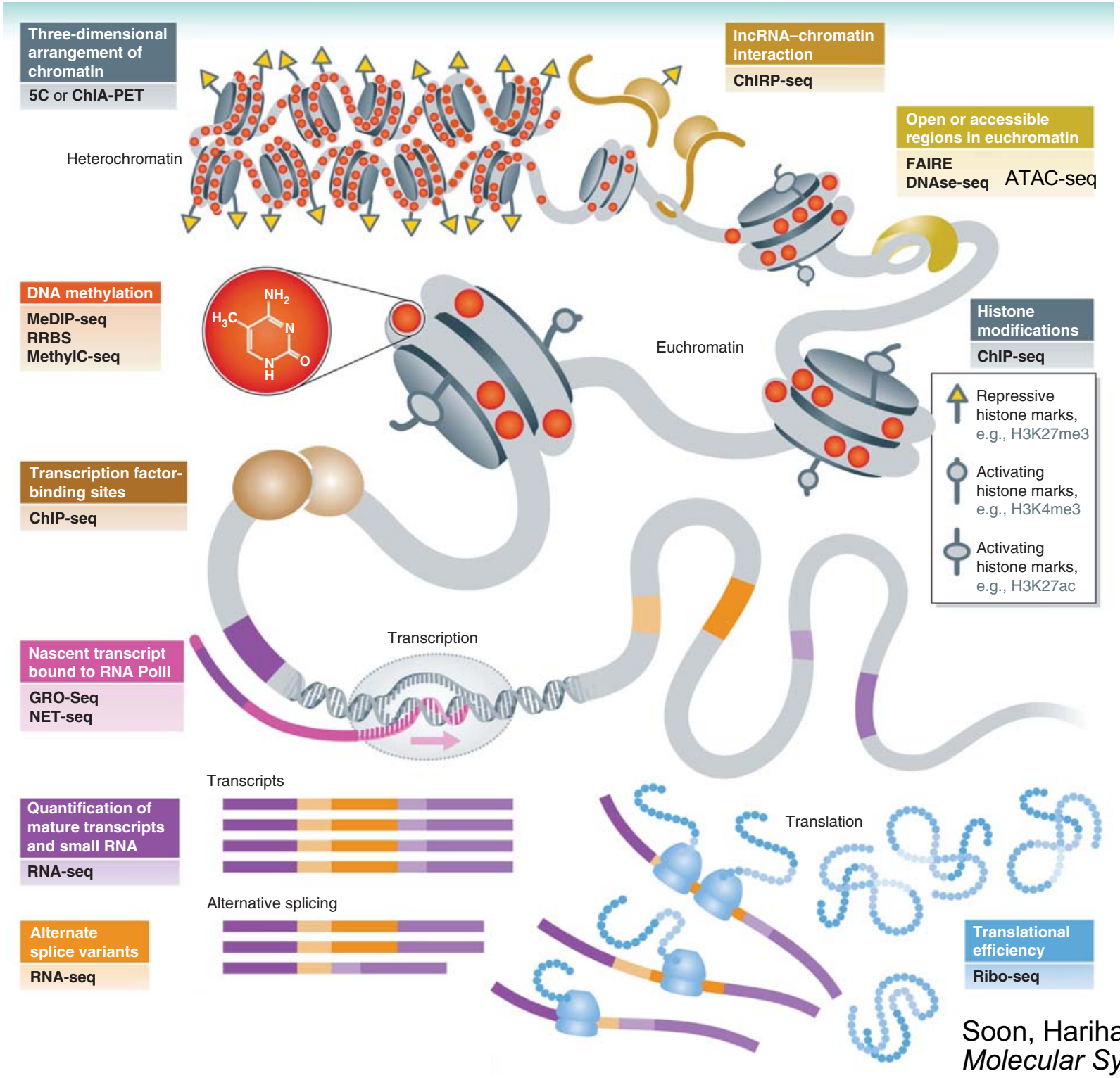
Short Read Alignment Algorithms

Raluca Gordân

Department of Biostatistics and Bioinformatics
Department of Computer Science
Department of Molecular Genetics and Microbiology
Center for Genomic and Computational Biology
Duke University

June 21, 2021



Soon, Hariharan, Snyder
Molecular Systems Biology 2012

Sequencing technologies

Method	Read length	Accuracy (single read not consensus)	Reads per run	Time per run	Cost per 1 million bases (in US$)	Advantages	Disadvantages
Single-molecule real-time sequencing (Pacific Biosciences)	10,000 bp to 15,000 bp avg (14,000 bp N50); maximum read length >40,000 bases[65][66][67]	87% single-read accuracy[68]	50,000 per SMRT cell, or 500–1000 megabases[69][70]	30 minutes to 4 hours[71]	$0.13–$0.60	Longest read length. Fast. Detects 4mC, 5mC, 6mA.[72]	Moderate throughput. Equipment can be very expensive.
Ion semiconductor (Ion Torrent sequencing)	up to 600 bp[73]	98%	up to 80 million	2 hours	$1	Less expensive equipment. Fast.	Homopolymer errors.
Pyrosequencing (454)	700 bp	99.9%				Long read size. Fast.	Runs are expensive. Homopolymer errors.
Sequencing by synthesis (Illumina)	MiniSeq, NextSeq: 75-300 bp; MiSeq: 50-600 bp; HiSeq 2500: 50-500 bp; HiSeq 3/4000: 50-300 bp; HiSeq X: 300 bp	99.9% (Phred30)	MiniSeq/MiSeq: 1-25 Million; NextSeq: 130-00 Million, HiSeq 2500: 300 million - 2 billion, HiSeq 3/4000 2.5 billion, HiSeq X: 3 billion	1 to 11 days, depending upon sequencer and specified read length[74]	$0.05 to $0.15	Potential for high sequence yield, depending upon sequencer model and desired application.	Equipment can be very expensive. Requires high concentrations of DNA.
Sequencing by ligation (SOLiD sequencing)	50+35 or 50+50 bp	99.9%	1.2 to 1.4 billion	1 to 2 weeks	$0.13	Low cost per base.	Slower than other methods. Has issues sequencing palindromic sequences.[75]
Nanopore Sequencing[76])	Dependent on library prep, not the device, so user chooses read length. (up to 500 kb reported)	~92–97% single read (up to 99.96% consensus)	dependent on read length selected by user	data streamed in real time. Choose 1 min to 48 hrs	$500–999 per Flow Cell, base cost dependent on expt	Very long reads, Portable (Palm sized)	Lower throughput than other machines, Single read accuracy in 90s.
Chain termination (Sanger sequencing)	400 to 900 bp	99.9%	N/A	20 minutes to 3 hours	$2400	Long individual reads. Useful for many applications.	More expensive and impractical for larger sequencing projects. This method also requires the time consuming step of plasmid cloning or PCR.

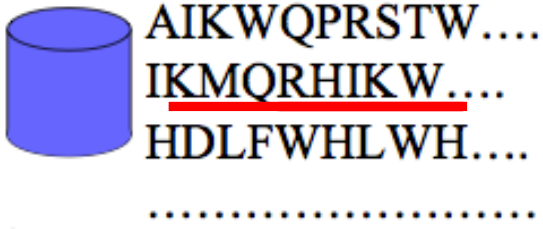
TECHNICAL BIASES!

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

Sequence alignment

Heuristic local alignment (**BLAST**)

- INPUT:
  - Database



  - Query: PSKMQRGIKWLLP
- OUTPUT:
  - sequences similar to query

Global/local alignment (Needleman-Wunsch, **Smith-Waterman**)

- INPUT:
  - Two sequences

$$X = x_1 x_2 \ldots x_m$$

$$Y = y_1 y_2 \ldots y_n$$

- OUTPUT:
  - Optimal alignment between X and Y (or substrings of X and Y)

Short read alignment

- INPUT:
  - A few million short reads, with certain error characteristics (specific to the sequencing platform)
    - Illumina: few errors, mostly substitutions
  - A reference genome
- OUTPUT:
  - Alignments of the reads to the reference genome

- Can we use BLAST?
  - Assuming BLAST returns the result for a read in 1 sec
  - For 10 million reads: 10 million seconds = 116 days
- Algorithms for exact string matching are more appropriate

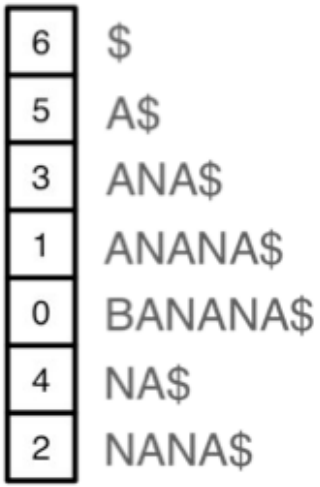
Algorithms for exact string matching

- Search for the substring ANA in the string BANANA

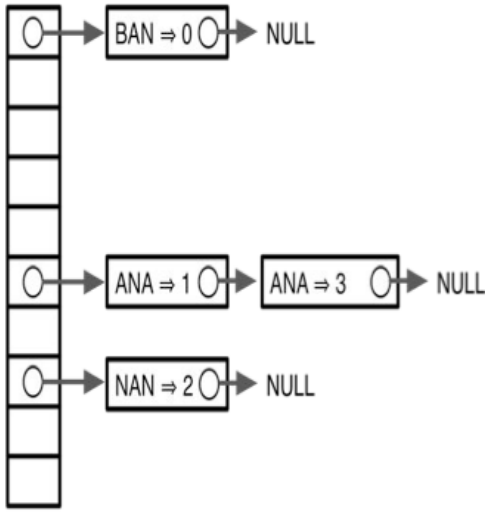
Brute Force

```
BANANA
BAN
  ANA
    NAN
      ANA
```

Suffix Array



Hash (Index) Table



Naive

Slow & Easy

Binary Search

PacBio Aligner (BLASR); Bowtie

Seed-and-extend

BLAST, BLAT

Time complexity versus space complexity

Brute force search for GATTACA

- Where is GATTACA in the human genome?

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	...
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	...
G	A	T	T	A	C	A									

No match at offset 1

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	...
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	...
	G	A	T	T	A	C	A								

Match at offset 2

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	...
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	...
		G	A	T	T	A	C	A	...						

No match at offset 3...

Brute force search for GATTACA

- Simple, easy to understand
- Analysis
  - Genome length = n = 3,000,000,000
  - Query length = m = 7
  - Comparisons: (n-m+1) * m = 21,000,000,000
- Assuming each comparison takes 1/1,000,000 of a second…
- … the total running time is 21,000 seconds = 0.24 days
- … for one 7-bp read

Suffix arrays

- Preprocess the genome
  - Sort all the suffixes of the genome

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	...
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	...
	G	A	T	T	A	C	A								

Split into suffixes

Sort suffixes alphabetically

Suffix array

6	$
5	A$
3	ANA$
1	ANANA$
0	BANANA$
4	NA$
2	NANA$

- Use binary search

Suffix arrays

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	...
T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	...

Lo = 1; Hi = 15

Mid = (1+15)/2 = 8

Middle = Suffix[8] = CC

Compare GATTACA to CC => Higher

Lo = Mid + 1

Lo →

Hi →

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Suffix arrays - search for GATTACA

Lo = 9; Hi = 15

Mid = (9+15)/2 = 12

Middle = Suffix[12] = TACC

Compare GATTACA to TACC => Lower

Hi = Mid - 1

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Lo →

Hi →

Suffix arrays - search for GATTACA

Lo = 9; Hi = 11

Mid = (9+11)/2 = 10

Middle = Suffix[10] = GATTACC

Compare GATTACA to GATTACC => Lower

Hi = Mid - 1

Lo →

Hi →

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
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11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Suffix arrays - search for GATTACA

Lo = 9; Hi = 9

Mid = (9+9)/2 = 9

Middle = Suffix[9] = GATTACAG…

Compare GATTACA to GATTACAG… => Match

Return: match at position 2

What if there are multiple matches?

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
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Lo
Hi

Suffix arrays - analysis

#	Sequence	Pos
1	ACAGATTACC…	6
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3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
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12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

- Word (query) of size **m = 7**
- Genome of size **n = 3,000,000,000**
- Bruce force:
  - approx. **m x n = 21,000,000,000** comparisons
- Suffix arrays:
  - approx. **m x $\log_2$(n) = 7 x 32 = 224** comparisons

- Assuming each comparison takes 1/1,000,000 of a second…
- … the total running time is **0.000224 seconds** for one 7-bp read
- Compared to **0.24 days** for one 7-bp read in the case of brute force search

- For 10 million reads, the suffix array search would take 2240 seconds = **37 minutes**

Suffix arrays - analysis

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
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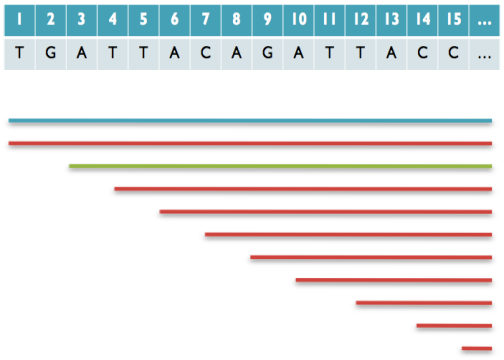
- Word (query) of size **m = 7**
- Genome of size **n = 3,000,000,000**

- For 10 million reads, the suffix array search would take 2240 seconds = **37 minutes**

- Problem? Time complexity versus space complexity

- Total characters in all suffixes combined:
  1+2+3+…+n = n(n+1)/2

- For the human genome:
  4.5 billion billion characters!!!



Software

Open Access

Ultrafast and memory-efficient alignment of short DNA sequences to the human genome

Ben Langmead, Cole Trapnell, Mihai Pop and Steven L Salzberg

Address: Center for Bioinformatics and Computational Biology, Institute for Advanced Computer Studies, University of Maryland, College Park, MD 20742, USA.

Correspondence: Ben Langmead. Email: langmead@cs.umd.edu

Published: 4 March 2009

Genome Biology 2009, 10:R25 (doi:10.1186/gb-2009-10-3-r25)

Bowtie is an ultrafast, memory-efficient alignment program for aligning short DNA sequence reads to large genomes. For the human genome, Burrows-Wheeler indexing allows Bowtie to **align more than 25 million reads per CPU hour with a memory footprint of approximately 1.3 gigabytes**. Bowtie extends previous Burrows-Wheeler techniques with a novel quality-aware backtracking algorithm that permits **mismatches**. Multiple processor cores can be used simultaneously to achieve even greater alignment speeds. Bowtie is open source http://bowtie.cbcb.umd.edu.

Fast gapped-read alignment with Bowtie 2

Ben Langmead[1,2] & Steven L Salzberg[1–3]

As the rate of sequencing increases, greater throughput is demanded from read aligners. The full-text minute index is often used to make alignment very fast and memory-efficient, but the approach is ill-suited to finding longer, gapped alignments. Bowtie 2 combines the strengths of the full-text minute index with the flexibility and speed of hardware-accelerated dynamic programming algorithms to achieve a combination of high speed, sensitivity and accuracy.

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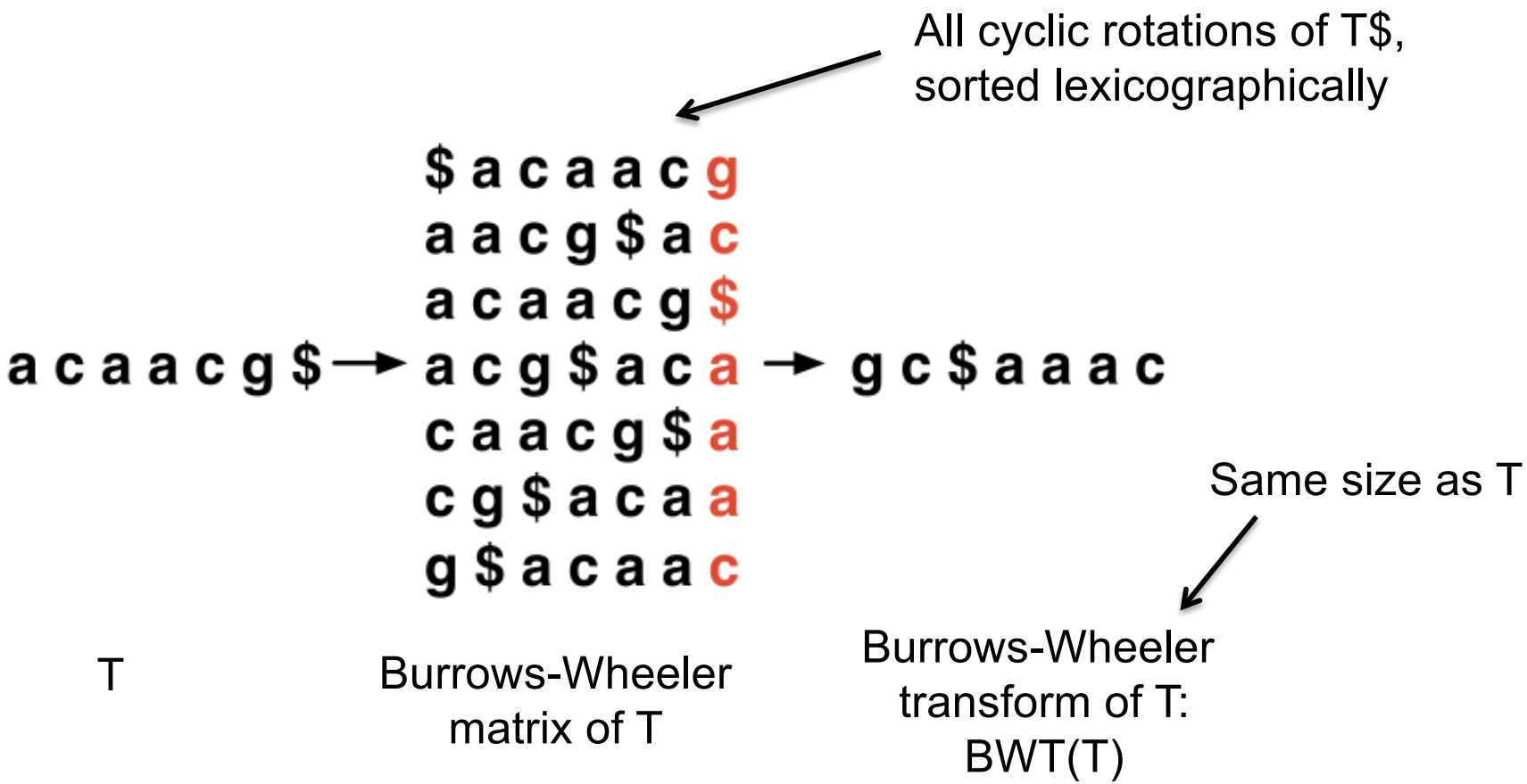
- Bowtie indexes the genome using a scheme based on the Burrows-Wheeler transform (**BWT**) and the Ferragina-Manzini (**FM**) index

Burrows-Wheeler transform

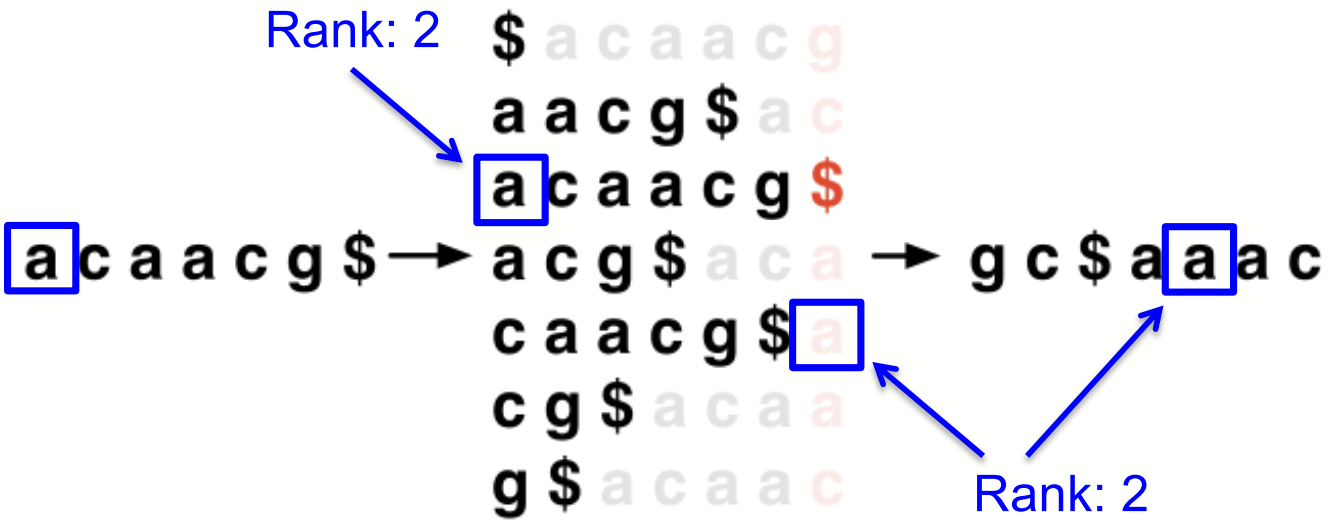
- The BWT is a reversible permutation of the characters in a text
- BWT-based indexing allows large texts to be searched efficiently in a small memory footprint



Last first (LF) mapping

- The BW matrix has a property called **last first (LF) mapping**:

  The i^{th} occurrence of character X in the last column corresponds to the same text character as the i^{th} occurrence of X in the first column
- This property is at the core of algorithms that use the BWT index to search the text



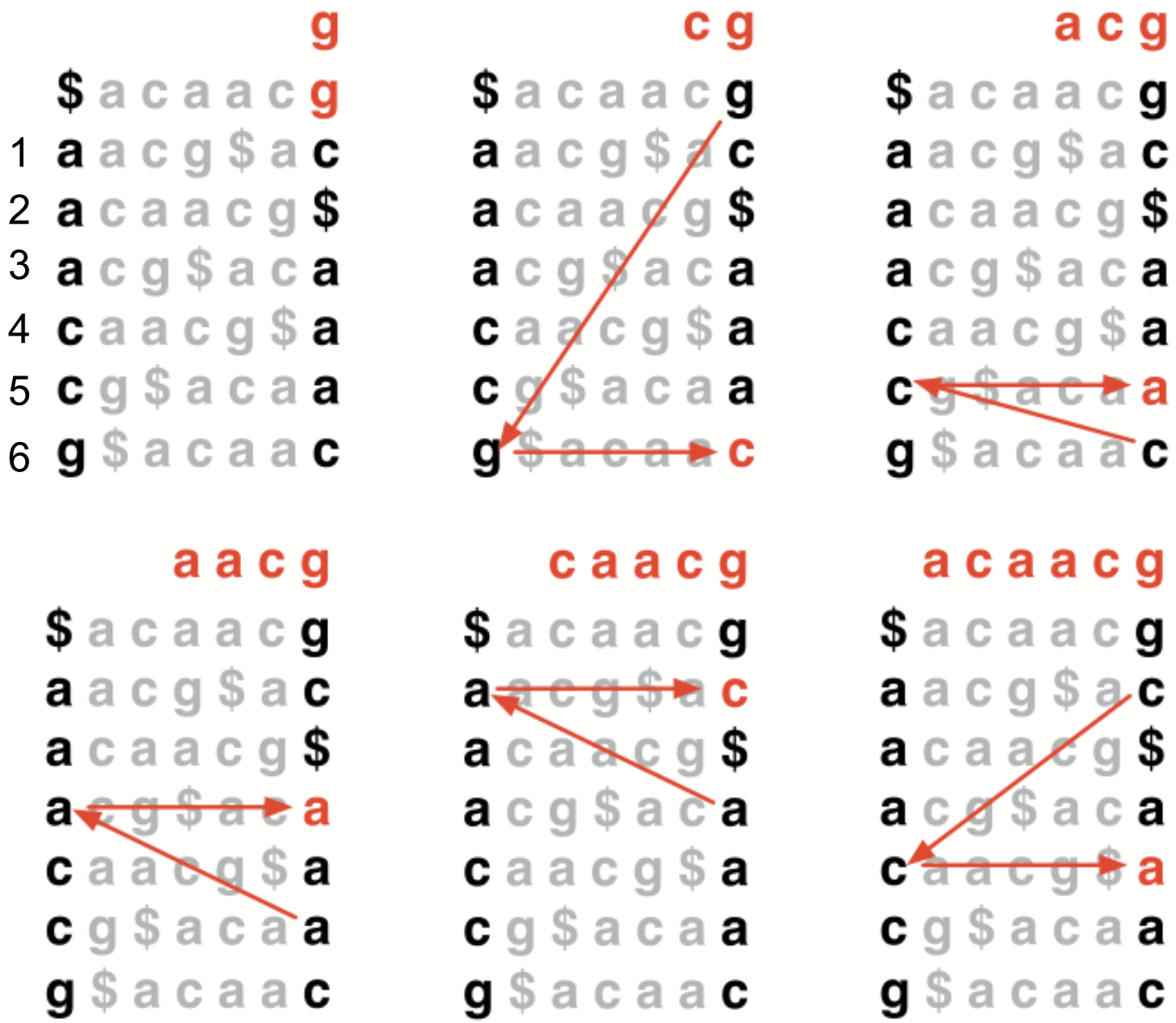
LF property implicitly encodes the Suffix Array

Last first (LF) mapping

We can repeatedly apply LF mapping to **reconstruct T from BWT(T)**

UNPERMUTE algorithm

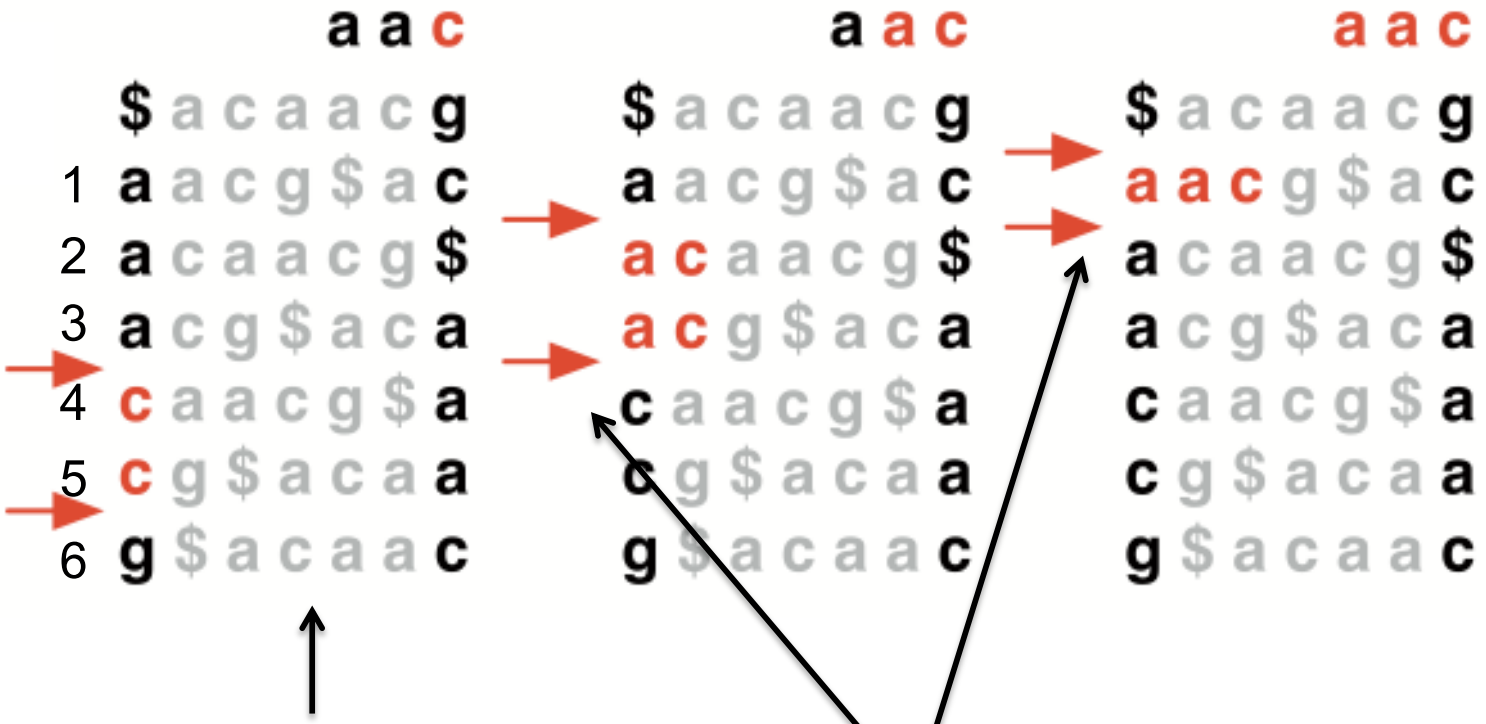
(Burrows and Wheeler, 1994)



LF mapping and exact matching

EXACTMATCH algorithm (Ferragina and Manzini, 2000) - calculates the range of matrix rows beginning with successively longer suffixes of the query

Reference: acaacg. Query: aac



the matrix is sorted lexicographically

rows beginning with a given sequence appear consecutively

At each step, the size of the range either shrinks or remains the same

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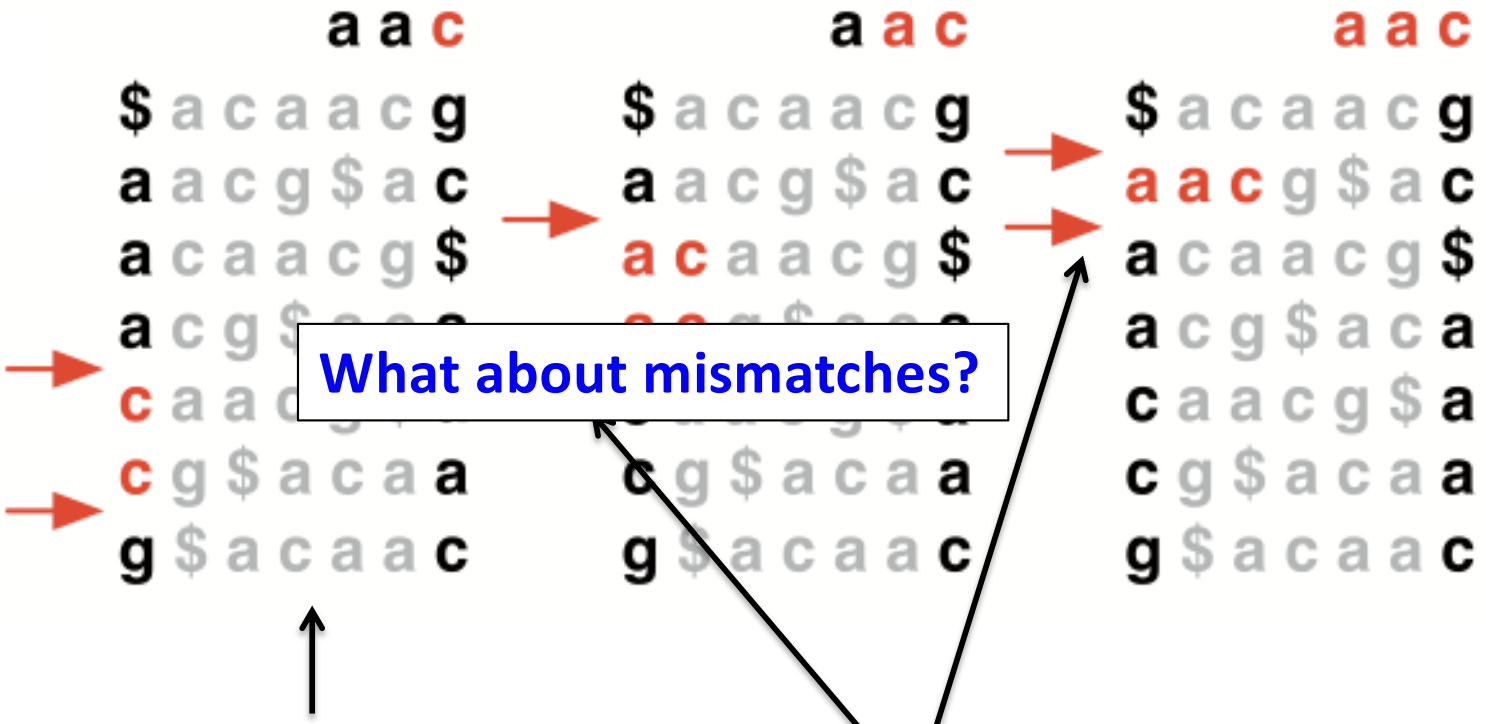

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Mismatches?

- EXACTMATCH is insufficient for short read alignment because alignments may contain mismatches
- What are the main causes for mismatches?

  - sequencing errors

  - differences between reference and query organisms

Bowtie – mismatches and backtracking search

- Bowtie conducts a **backtracking search** to quickly find alignments that satisfy a specified alignment policy
- Each character in a read has a **numeric quality value**, with lower values indicating a higher likelihood of a sequencing error
- Example: Illumina uses Phred quality scoring

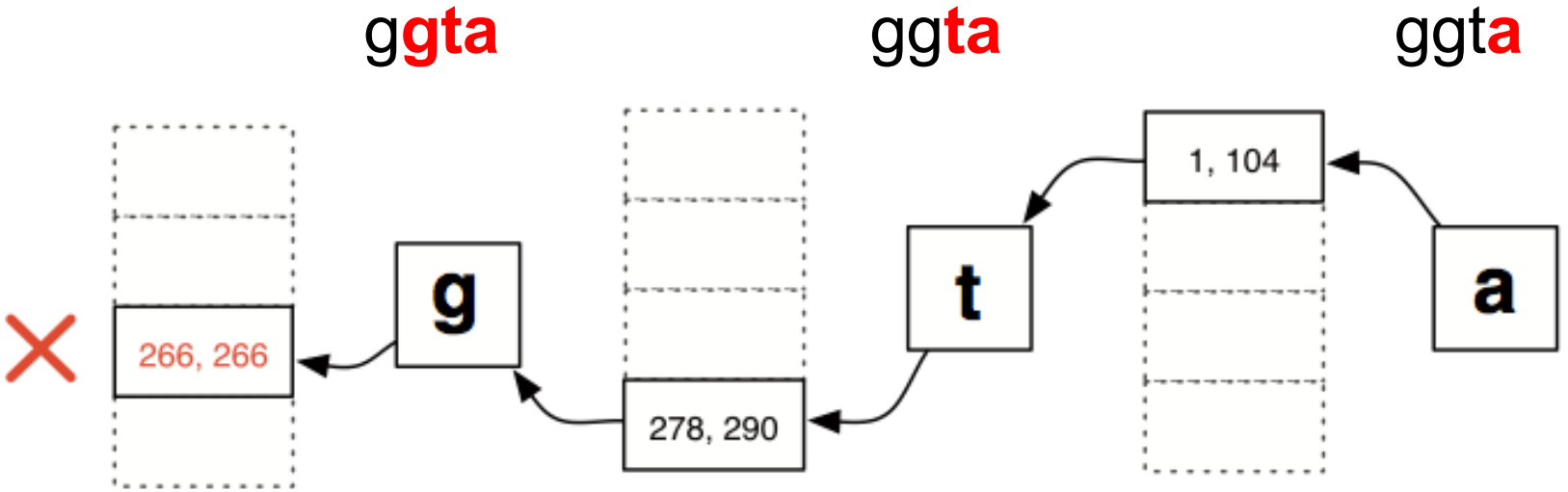
  Phred score of a base is: $Q_{phred} = -10*\log_{10}(e)$ where e is the estimated probability of a base being wrong
- Bowtie alignment policy allows a **limited number of mismatches** and prefers alignments where the **sum of the quality values at all mismatched positions is low**

$e=10\%=0.1 \Rightarrow Q_{phred} = 10$

$e=1\%=0.01 \Rightarrow Q_{phred} = 20$

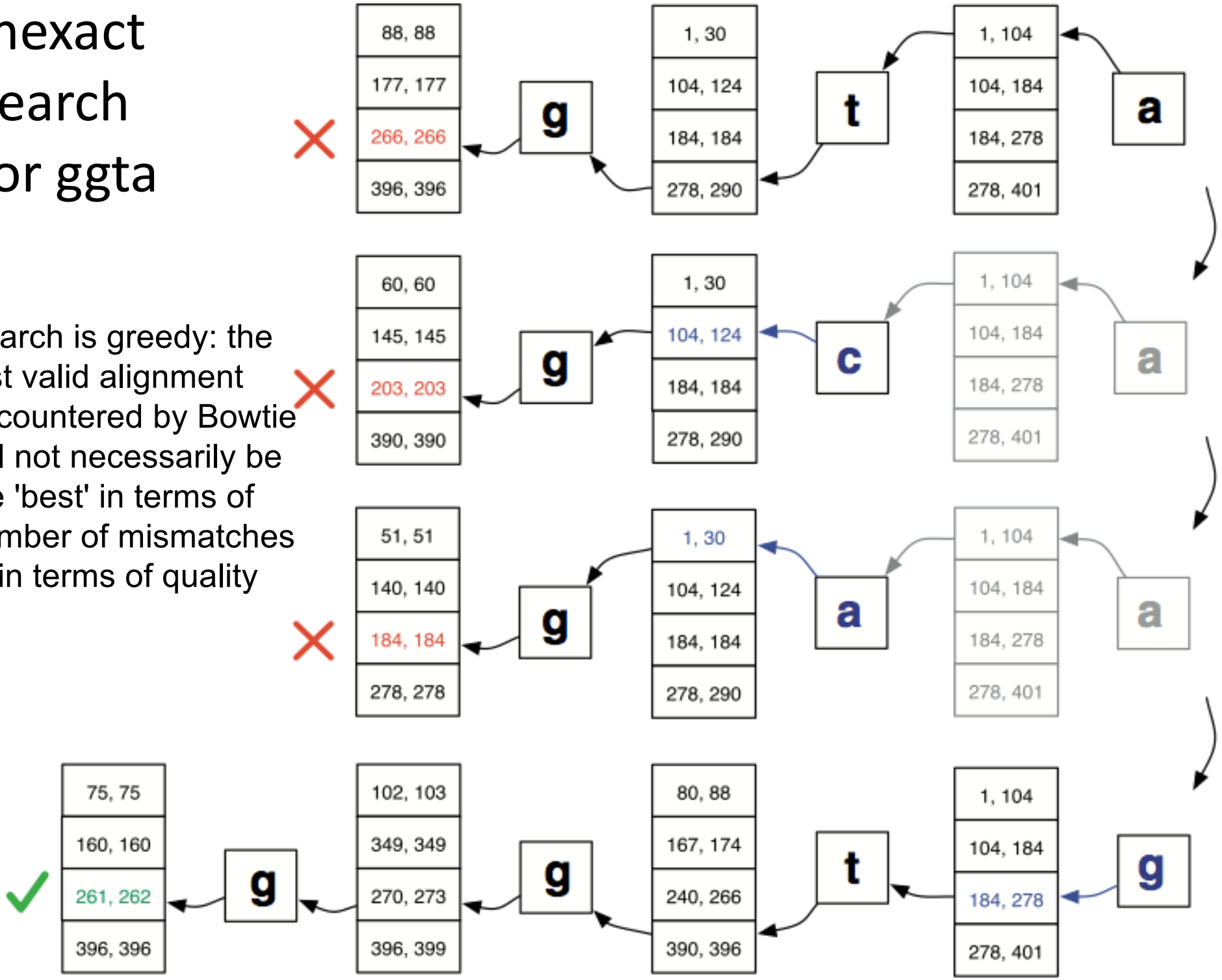
$e=0.1\%=0.001 \Rightarrow Q_{phred} = 30$

Exact search for ggta



Inexact search for ggta

Search is greedy: the first valid alignment encountered by Bowtie will not necessarily be the 'best' in terms of number of mismatches or in terms of quality



Aligning 2 million reads to the human genome

Length	Program	CPU time	Wall clock time	Peak virtual memory footprint (megabytes)	Bowtie speed-up	Reads aligned (%)
36 bp	Bowtie	6 m 15 s	6 m 21 s	1,305	-	62.2
	Maq	3 h 52 m 26 s	3 h 52 m 54 s	804	36.7×	65.0
	Bowtie -v 2	4 m 55 s	5 m 00 s	1,138	-	55.0
	SOAP	16 h 44 m 3 s	18 h 1 m 38 s	13,619	216×	55.1
50 bp	Bowtie	7 m 11 s	7 m 20 s	1,310	-	67.5
	Maq	2 h 39 m 56 s	2 h 40 m 9 s	804	21.8×	67.9
	Bowtie -v 2	5 m 32 s	5 m 46 s	1,138	-	56.2
	SOAP	48 h 42 m 4 s	66 h 26 m 53 s	13,619	691×	56.2
76 bp	Bowtie	18 m 58 s	19 m 6 s	1,323	-	44.5
	Maq 0.7.1	4 h 45 m 7 s	4 h 45 m 17 s	1,155	14.9×	44.9
	Bowtie -v 2	7 m 35 s	7 m 40 s	1,138	-	31.7

Maq: Mapping and Assembly with Qualities

Mapping short DNA sequencing reads and calling variants using mapping quality scores

Heng Li, Jue Ruan and Richard Durbin

Genome Res. 2008 18: 1851-1858 originally published online August 19, 2008

SOAP = Short Oligonucleotide Analysis Package

BIOINFORMATICS APPLICATIONS NOTE Vol. 24 no. 5 2008, pages 713–714 doi:10.1093/bioinformatics/btn025

Sequence analysis

SOAP: short oligonucleotide alignment program

Ruiqiang Li[1,2], Yingrui Li[1], Karsten Kristiansen[2] and Jun Wang[1,2,*]

[1]Beijing Genomics Institute at Shenzhen, Shenzhen 518083, China and [2]Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense M, DK-5230, Denmark

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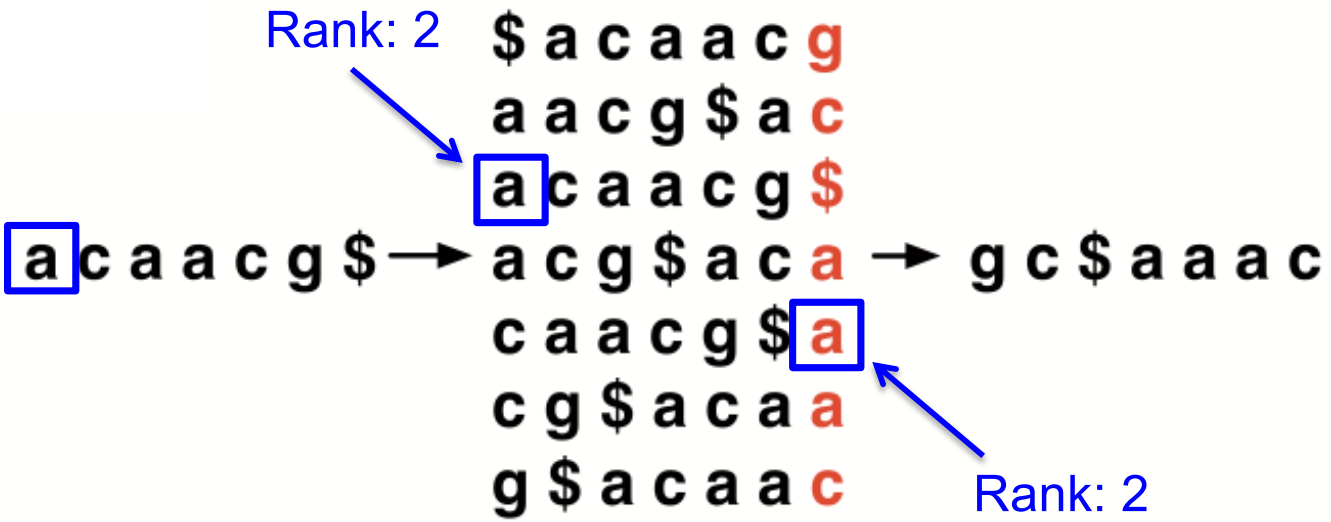

$$BWT[i] = \begin{cases} T[SA[i]-1] & SA[i] \neq 0 \\ \$ & SA[i] = 0 \end{cases}$$

LF property implicitly encodes the Suffix Array

Software

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Published: 4 March 2009

Genome ***Biology*** 2009, **10**:R25 (doi:10.1186/gb-2009-10-3-r25)

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BIOINFORMATICS **ORIGINAL PAPER**

Vol. 25 no. 14 2009, pages 1754–1760
doi:10.1093/bioinformatics/btp324

Sequence analysis

Fast and accurate short read alignment with Burrows–Wheeler transform

Heng Li and Richard Durbin*

Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Cambridge, CB10 1SA, UK

BWA

BIOINFORMATICS ORIGINAL PAPER

Vol. 25 no. 9 2009, pages 1105–1111
doi:10.1093/bioinformatics/btp120

Sequence analysis

TopHat: discovering splice junctions with RNA-Seq

Cole Trapnell[1,*], Lior Pachter[2] and Steven L. Salzberg[1]

[1]Center for Bioinformatics and Computational Biology, University of Maryland, College Park, MD 20742 and [2]Department of Mathematics, University of California, Berkeley, CA 94720, USA

BIOINFORMATICS ORIGINAL PAPER

Vol. 29 no. 1 2013, pages 15–21
doi:10.1093/bioinformatics/bts635

Sequence analysis

Advance Access publication October 25, 2012

STAR: ultrafast universal RNA-seq aligner

Alexander Dobin[1,*], Carrie A. Davis[1], Felix Schlesinger[1], Jorg Drenkow[1], Chris Zaleski[1], Sonali Jha[1], Philippe Batut[1], Mark Chaisson[2] and Thomas R. Gingeras[1]

[1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA and [2]Pacific Biosciences, Menlo Park, CA, USA

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Alexander Dobin[1,*], Carrie A. Davis[1], Felix Schlesinger[1], Jorg Drenkow[1], Chris Zaleski[1], Sonali Jha[1], Philippe Batut[1], Mark Chaisson[2] and Thomas R. Gingeras[1]

[1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA and [2]Pacific Biosciences, Menlo Park, CA, USA

"Accurate alignment of high-throughput RNA-seq data is a challenging and yet unsolved problem because of the

- non-contiguous transcript structure,
- relatively short read lengths and
- constantly increasing throughput of the sequencing technologies."

"Currently available RNA-seq aligners suffer from

- high mapping error rates,
- low mapping speed,
- read length limitation and
- mapping biases."

Solution:

- sequential maximum mappable seed search in uncompressed suffix arrays
- followed by seed clustering and stitching procedure.

Suffix arrays - search for GATTACA

Lo = 9; Hi = 9

Mid = (9+9)/2 = 9

Middle = Suffix[9] = GATTACAG…

Compare GATTACA to GATTACAG… => Match

Return: match at position 2

#	Sequence	Pos
1	ACAGATTACC…	6
2	ACC…	13
3	AGATTACC…	8
4	ATTACAGATTACC…	3
5	ATTACC…	10
6	C…	15
7	CAGATTACC…	7
8	CC…	14
9	GATTACAGATTACC…	2
10	GATTACC…	9
11	TACAGATTACC…	5
12	TACC…	12
13	TGATTACAGATTACC…	1
14	TTACAGATTACC…	4
15	TTACC…	11

Lo
Hi

Maximum Mappable Prefix (MMP) search

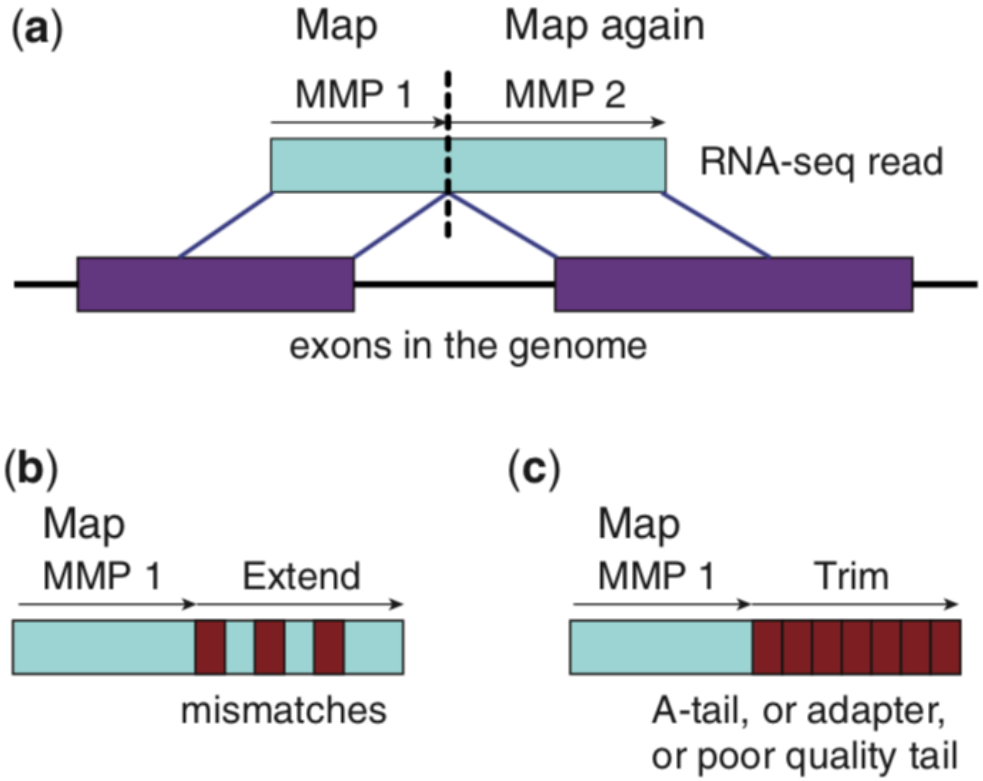

Fig. 1. Schematic representation of the Maximum Mappable Prefix search in the STAR algorithm for detecting (a) splice junctions, (b) mis-matches and (c) tails

- Using uncompressed suffix arrays leads to increased speed (compared to BWT)
- This speed advantage is traded off against the increased memory usage

Maximum Mappable Prefix (MMP) search

Table 1. Mapping speed and RAM benchmarks on the experimental RNA-seq dataset

Aligner	Mapping speed: million read pairs/hour		Peak physical RAM, GB	
	6 threads	12 threads	6 threads	12 threads
STAR	309.2	549.9	27.0	28.4
STAR sparse	227.6	423.1	15.6	16.0
TopHat2	8.0	10.1	4.1	11.3
RUM	5.1	7.6	26.9	53.8
MapSplice	3.0	3.1	3.3	3.3
GSNAP	1.8	2.8	25.9	27.0