

# Short Read Alignment Algorithms

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# Sequencing applications

RNA-seq – this course

ChIP-seq: identify and measure significant peaks

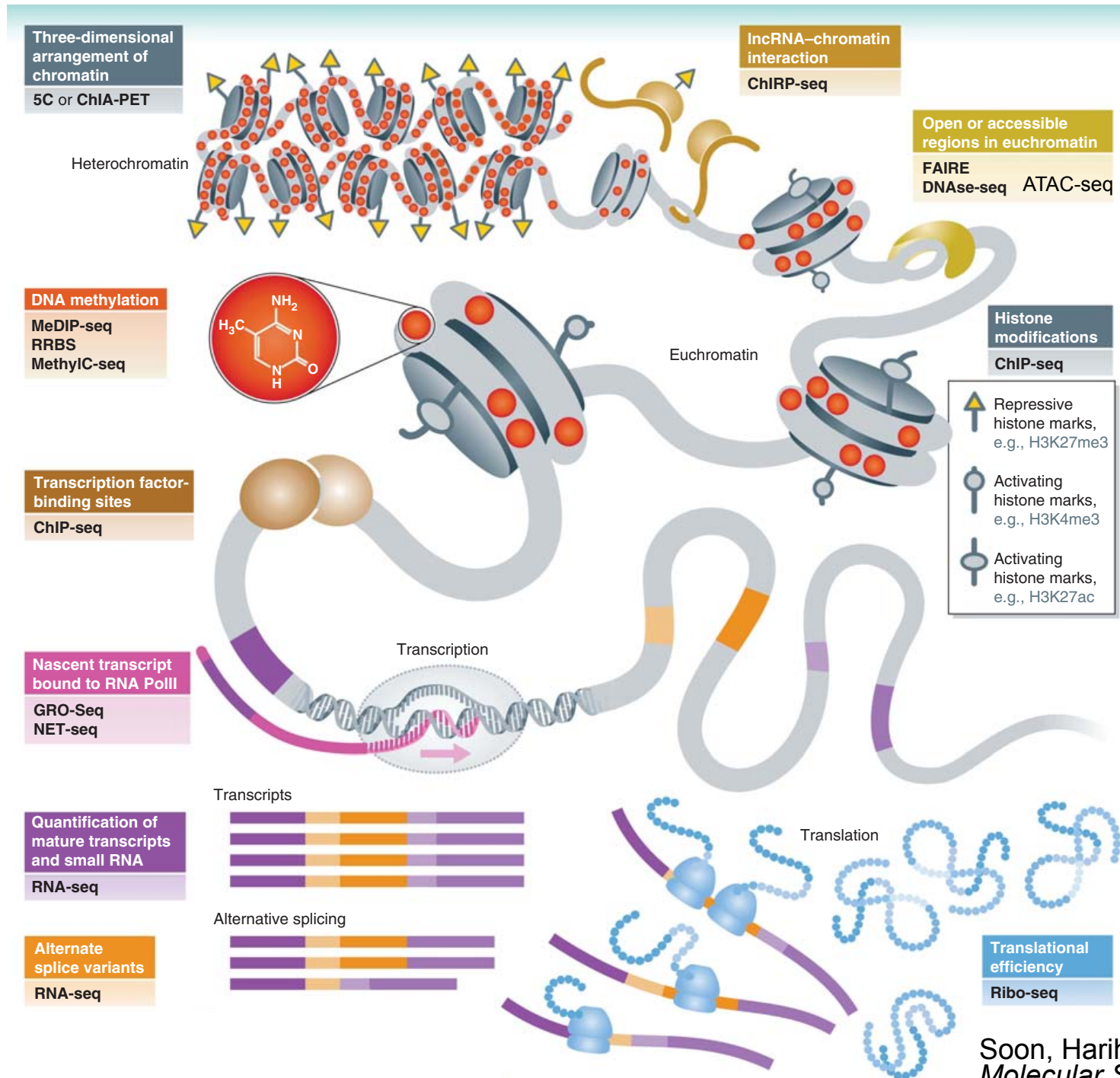
```

                                GAAATTTGC
                                GGAAATTTG
                                CGGAAATTT
                                CGGAAATTT
                                TCGGAAATT
                                CTATCGGAAA
                                CCTATCGGA  TTTGCGGT
                                GCCCTATCG  AAATTTGC
                                GCCCTATCG  AAATTTGC  ATAC...
...CC
...CCATAGGCTATATGCGGCCCTATCGGAAATTTGCGGTATAC...
```

Genotyping: identify variations

```

                                GGTATAC...
...CCATAG  TATGCGCCC  CGGAAATTT  CGGTATAC
...CCAT  CTATATGCG  TCGGAAATT  CGGTATAC
...CCAT  GGCTATATG  CTATCGGAAA  GCGGTATA
...CCA  AGGCTATAT  CCTATCGGA  TTGCGGTA  C...
...CCA  AGGCTATAT  GCCCTATCG  TTTGCGGT  C...
...CC  AGGCTATAT  GCCCTATCG  AAATTTGC  ATAC...
...CC  TAGGCTATA  GCGCCCTA  AAATTTGC  GTATAC...
...CCATAGGCTATATGCGGCCCTATCGGCAATTTGCGGTATAC...
```



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 <sup>[65][66][67]</sup>	87% single-read accuracy <sup>[68]</sup>	50,000 per SMRT cell, or 500–1000 megabases <sup>[69][70]</sup>	30 minutes to 4 hours <sup>[71]</sup>	\$0.13–\$0.60	Longest read length. Fast. Detects 4mC, 5mC, 6mA. <sup>[72]</sup>	Moderate throughput. Equipment can be very expensive.
Ion semiconductor (Ion Torrent sequencing)	up to 600 bp <sup>[73]</sup>	98%	up to 80 million	2 hours	\$1	Less expensive equipment. Fast.	Homopolymer errors.
Pyrosequencing (454)	700 bp	99.9%	TECHNICAL BIASES!			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)				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. <sup>[75]</sup>
Nanopore Sequencing <sup>[76]</sup>	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.

# Sequence alignment

## Heuristic local alignment (**BLAST**)

- INPUT:

- Database



AIKWQPRSTW....  
IKMQRHIKW....  
HDLFWHLWH....  
.....

- Query: PSKMQRGIKWLLP

- OUTPUT:

- sequences similar to query

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

- INPUT:

- Two sequences

$$X = x_1x_2\cdots x_m$$

$$Y = y_1y_2\cdots y_n$$

- OUTPUT:

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

# Short read alignment

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- 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**

Naive

Slow & Easy

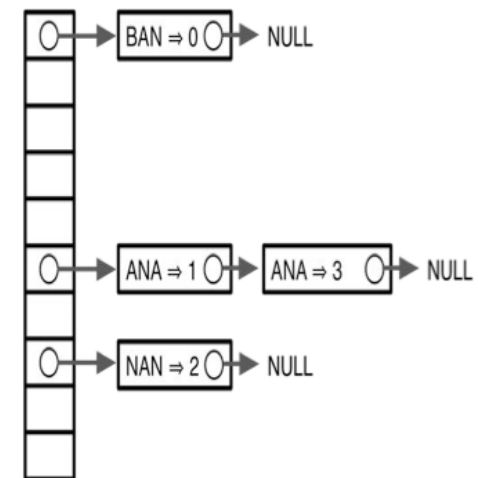
## Suffix Array

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

Binary Search

PacBio Aligner  
(BLASR); Bowtie

## Hash (Index) Table



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

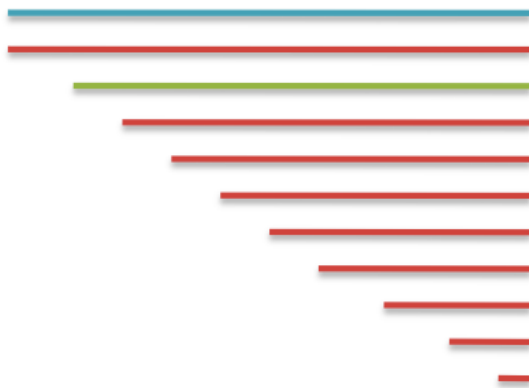


- Simple, easy to understand
- Analysis
  - Genome length =  $n = 3,000,000,000$
  - Query length =  $m = 7$
  - Comparisons:  $(n-m+1) * m = 21,000,000,000$
- Assuming each comparison takes  $1/1,000,000$  of a second...
- ... the total running time is 21,000 seconds = 0.24 days
- ... for one 7-bp read

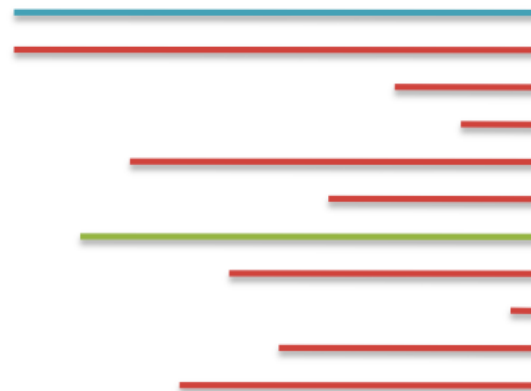
# Suffix arrays

- 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	#	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
Hi	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

#	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 = 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
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11	TACAGATTACC...	5
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13	TGATTACAGATTACC...	1
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15	TTACC...	11

# Suffix arrays - analysis

#	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

- Word (query) of size  $m = 7$
- Genome of size  $n = 3,000,000,000$
- Brute force:
  - approx.  $m \times n = 21,000,000,000$  comparisons
- Suffix arrays:
  - approx.  $m \times \log_2(n) = 7 \times 32 = 224$  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
4	ATTACAGATTACC...	3
5	ATTACC...	10
6	C...	15
7	CAGATTACC...	7
8	CC...	14
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11	TACAGATTACC...	5
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**
- 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:  
 $1+2+3+\dots+n = n(n+1)/2$
- For the human genome:  
4.5 billion billion characters!!!



# Algorithms for exact string matching

- Search for the substring ANA in the string BANANA

## Brute Force

**BANANA**  
**BAN**  
  **ANA**  
    **NAN**  
      **ANA**

Naive

Slow & Easy

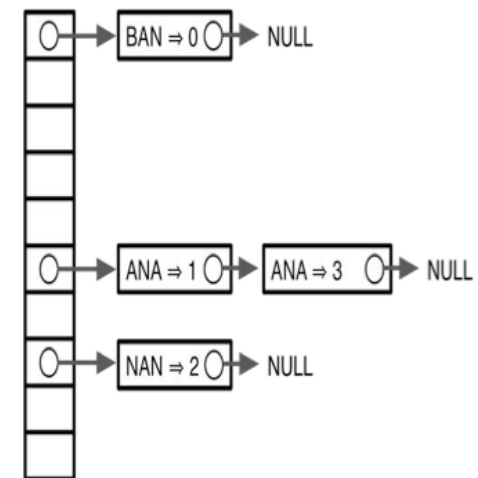
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6	\$
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1	ANANA\$
0	BANANA\$
4	NA\$
2	NANA\$

Binary Search

PacBio Aligner  
(BLASR); Bowtie

## Hash (Index) Table



Seed-and-extend

BLAST, BLAT

Time complexity versus space complexity

# Hashing

- Where is GATTACA in the human genome?
  - Build an inverted index of every k-mer in the genome

- How do we access the table?
  - We can only use numbers to index
  - Encode sequences as numbers

Simple: A=0,C=1,G=2,T=3  $\Rightarrow$  GATTACA=2,033,010

Smart: A =  $00_2$ , C =  $01_2$ , G =  $10_2$ , T =  $11_2$   
 $\Rightarrow$  GATTACA =  $10001111000100_2 = 9156_{10}$

- Lookup: very fast
- But constructing an optimal hash is tricky

AAAAAAA	→	...
AAAAAAC	→	...
AAAAAAG	→	...
...		
GATTAAT		
GATTACA	→	2
GATTACC		5000
...		32000000
TTTTTTG		...
TTTTTTT		

# Hashing

- Number of possible sequences of length  $k$  is  $4^k$
- $K=7 \Rightarrow 4^7 = 16,384$  (easy to store)
- $K=20 \Rightarrow 4^{20} = 1,099,511,627,776$  (impossible to store directly in RAM)
  - There are only 3B 20-mers in the genome
  - Even if we could build this table, 99.7% will be empty
  - But we don't know which cells are empty until we try

AAAAAAA	→	...
AAAAAAC	→	...
AAAAAAG	→	...
...		
GATTAAT		
GATTACA	→	2
GATTACC		5000
...		32000000
TTTTTTG		...
TTTTTTT		

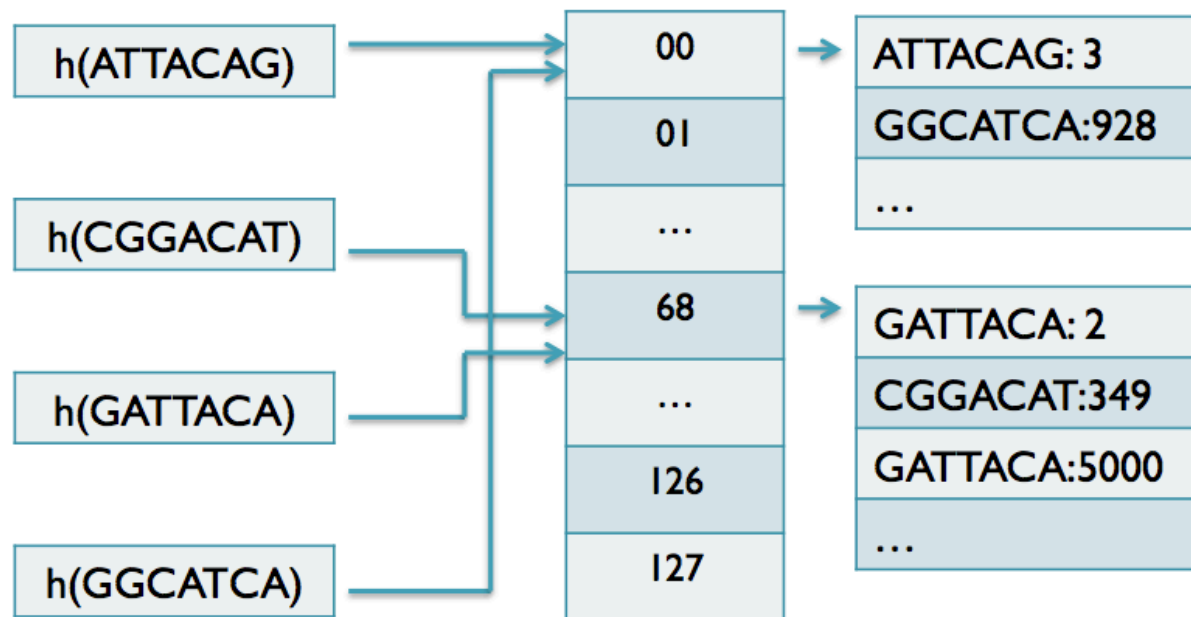
# Hashing

- Number of possible sequences of length  $k$  is  $4^k$
- $K=7 \Rightarrow 4^7 = 16,384$  (easy to store)
- $K=20 \Rightarrow 4^{20} = 1,099,511,627,776$  (impossible to store directly in RAM)
  - There are only 3B 20-mers in the genome
  - Even if we could build this table, 99.7% will be empty
  - But we don't know which cells are empty until we try
- Use a **hash function** to shrink the possible range
  - Maps a number  $n$  in  $[0, R]$  to  $h$  in  $[0, H]$ 
    - Use **128** buckets instead of **16,384**
  - Division:  $\text{hash}(n) = H * n / R$ 
    - $\text{hash}(\text{GATTACA}) = 128 * 9156 / 16384 = 71$
  - Modulo:  $\text{hash}(n) = n \% H$ 
    - $\text{hash}(\text{GATTACA}) = 9156 \% 128 = 68$

AAAAAAA	→	...
AAAAAAC	→	...
AAAAAAG	→	...
...		
GATTAAT		
GATTACA	→	2
GATTACC		5000
...		32000000
TTTTTTG		...
TTTTTTT		

# Hashing

- By construction, **multiple keys have the same hash value**
  - Store elements with the same key in a bucket chained together
    - A good hash evenly distributes the values: R/H have the same hash value
  - Looking up a value scans the entire bucket



# Algorithms for exact string matching

- Search for the substring GATTACA in the genome

## Brute Force

I	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															
I	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															
I	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...															

Easy

Slow

## Suffix Array

#	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

Fast (binary search)

High space complexity

## Hash (Index) Table

AAAAAAA	→	...
AAAAAAC	→	...
AAAAAAG	→	...
...		
GATTAAT		
GATTACA	→	2
GATTACC		5000
...		32000000
TTTTTTG		...
TTTTTTT		

Fast

Tricky to develop hash function

Software

# 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<sup>1,2</sup> & Steven L Salzberg<sup>1-3</sup>

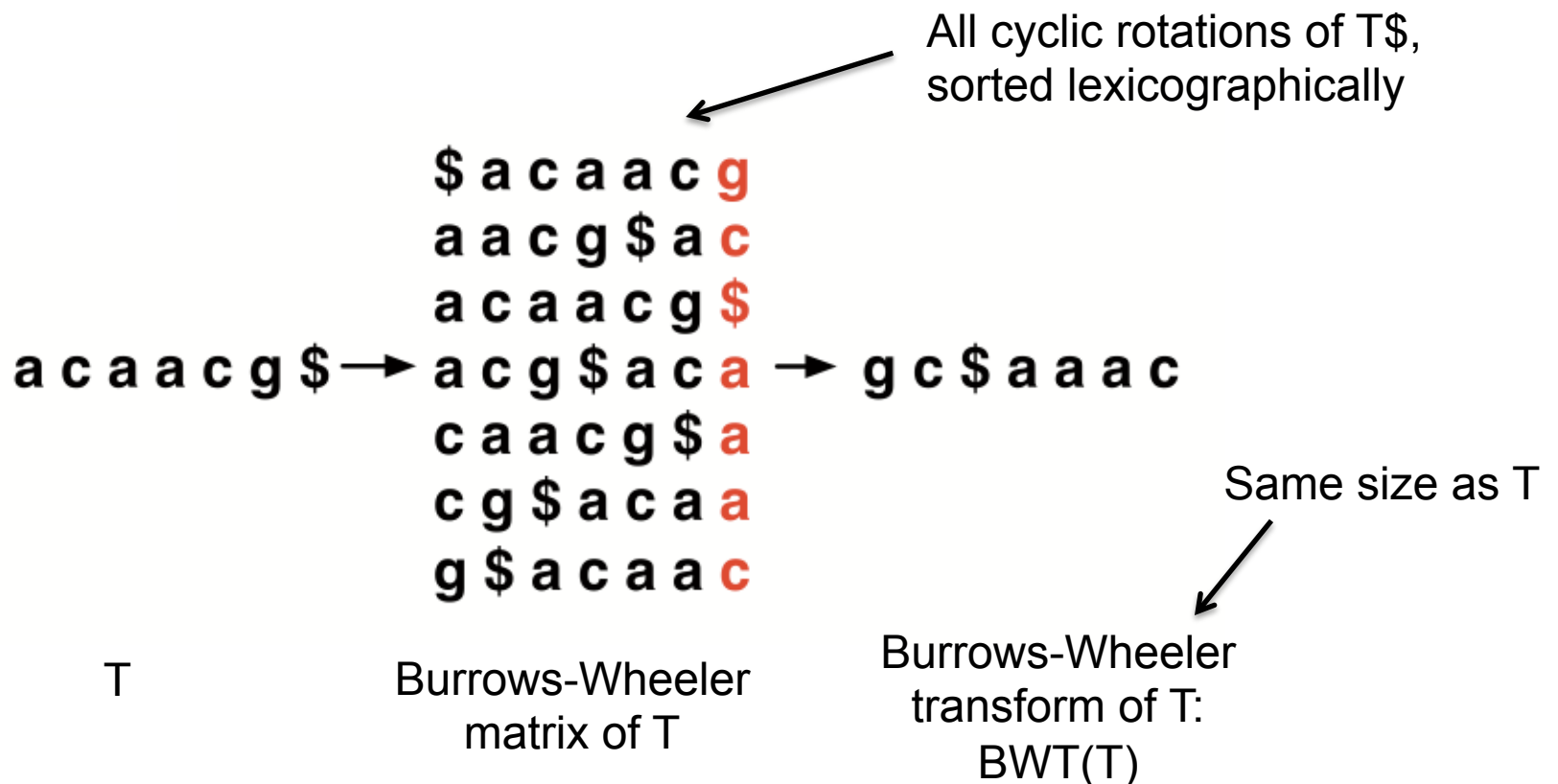
As the rate of sequencing increases, greater throughput is demanded from read aligners. The full-text minute index is often used to make alignment very fast and memory-efficient, but the approach is ill-suited to finding longer, gapped alignments. Bowtie 2 combines the strengths of the full-text minute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

**NATURE METHODS** | VOL.9 NO.4 | APRIL 2012 | 357

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

# Burrows-Wheeler transform

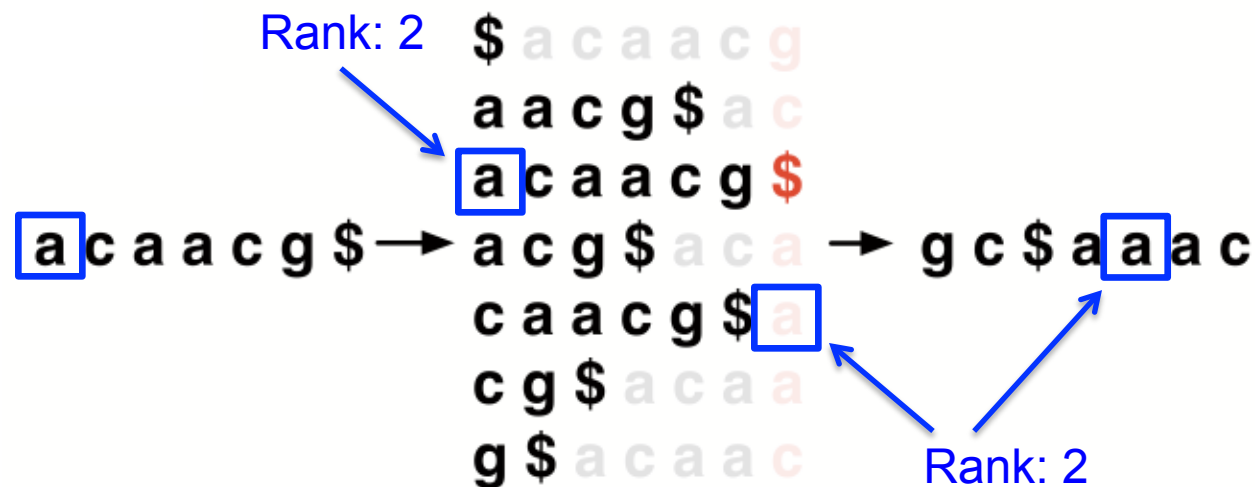
- The BWT is a reversible permutation of the characters in a text
- BWT-based indexing allows large texts to be searched efficiently in a small memory footprint





# Last first (LF) mapping

- The BW matrix has a property called **last first (LF) mapping**:  
The  $i^{\text{th}}$  occurrence of character X in the last column corresponds to the same text character as the  $i^{\text{th}}$  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)

**g**

	\$	a	c	a	a	c	<b>g</b>
1	a	a	c	g	\$	a	c
2	a	c	a	a	c	g	\$
3	a	c	g	\$	a	c	a
4	c	a	a	c	g	\$	a
5	c	g	\$	a	c	a	a
6	g	\$	a	c	a	a	c

**c g**

	\$	a	c	a	a	c	<b>g</b>
1	a	a	c	g	\$	a	c
2	a	c	a	a	c	g	\$
3	a	c	g	\$	a	c	a
4	c	a	a	c	g	\$	a
5	c	g	\$	a	c	a	a
6	g	\$	a	c	a	a	<b>c</b>

**a c g**

	\$	a	c	a	a	c	<b>g</b>
1	a	a	c	g	\$	a	c
2	a	c	a	a	c	g	\$
3	a	c	g	\$	a	c	a
4	c	a	a	c	g	\$	a
5	c	g	\$	a	c	a	<b>a</b>
6	g	\$	a	c	a	a	c

**a a c g**

	\$	a	c	a	a	c	<b>g</b>
1	a	a	c	g	\$	a	c
2	a	c	a	a	c	g	\$
3	a	c	g	\$	a	c	<b>a</b>
4	c	a	a	c	g	\$	a
5	c	g	\$	a	c	a	a
6	g	\$	a	c	a	a	c

**c a a c g**

	\$	a	c	a	a	c	<b>g</b>
1	a	a	c	g	\$	a	<b>c</b>
2	a	c	a	a	c	g	\$
3	a	c	g	\$	a	c	a
4	c	a	a	c	g	\$	a
5	c	g	\$	a	c	a	a
6	g	\$	a	c	a	a	c

**a c a a c g**

	\$	a	c	a	a	c	<b>g</b>
1	a	a	c	g	\$	a	c
2	a	c	a	a	c	g	\$
3	a	c	g	\$	a	c	a
4	c	a	a	c	g	\$	<b>a</b>
5	c	g	\$	a	c	a	a
6	g	\$	a	c	a	a	c

# 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



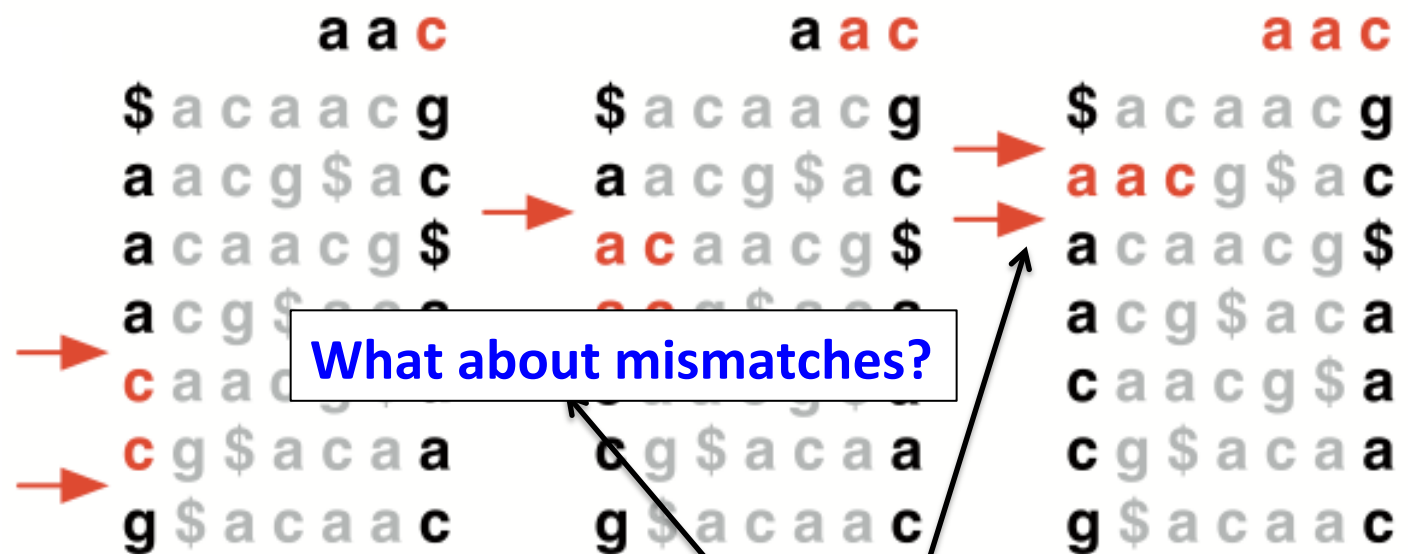
the matrix is sorted lexicographically  
rows beginning with a given sequence  
appear consecutively

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

# 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

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the matrix is sorted lexicographically  
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appear consecutively

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

# 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:  $Q_{\text{phred}} = -10 \cdot \log_{10}(e)$  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**

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

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

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

# Bowtie - backtracking search

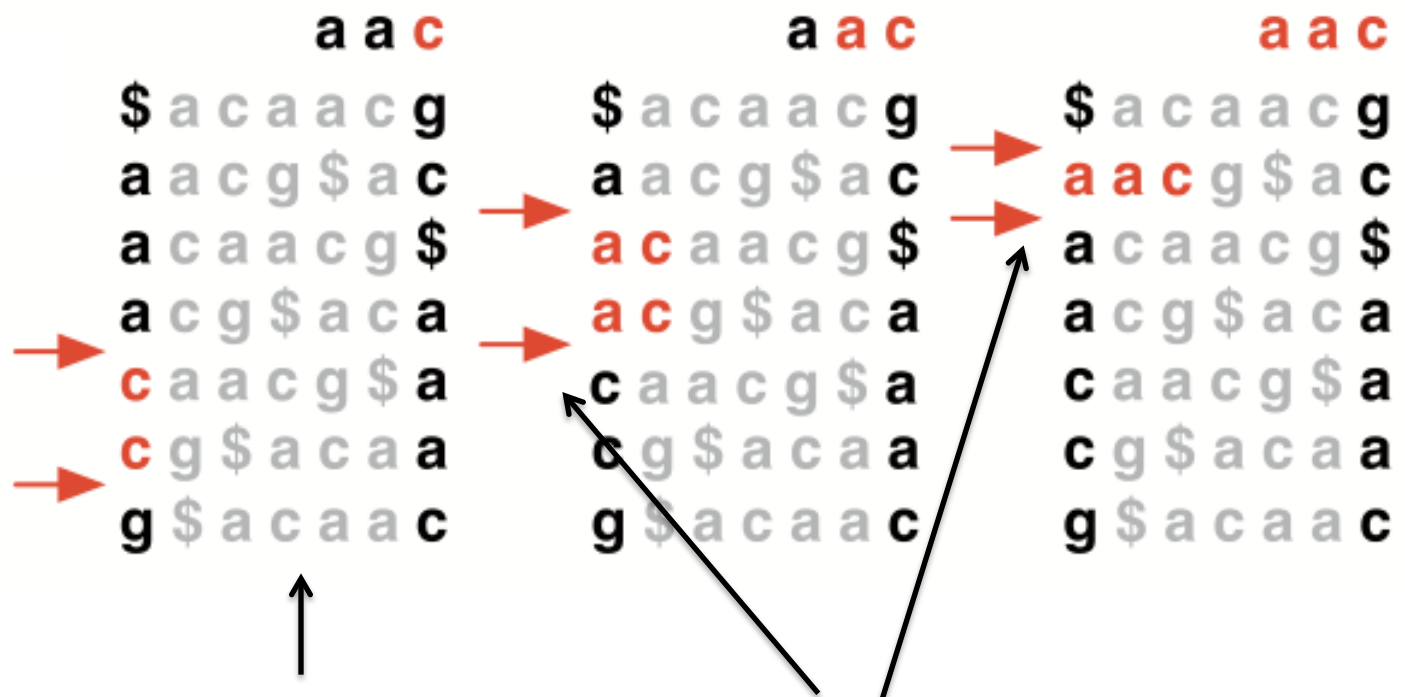
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- The search is similar to EXACTMATCH
- It calculates matrix ranges for successively longer query suffixes

# 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

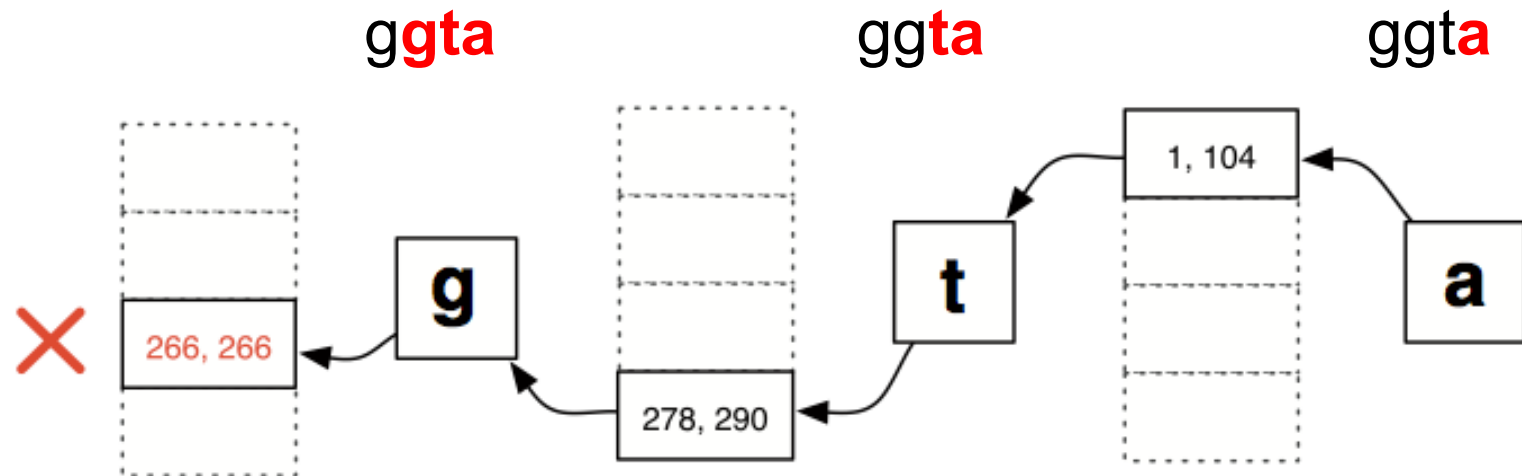


# Bowtie - backtracking search

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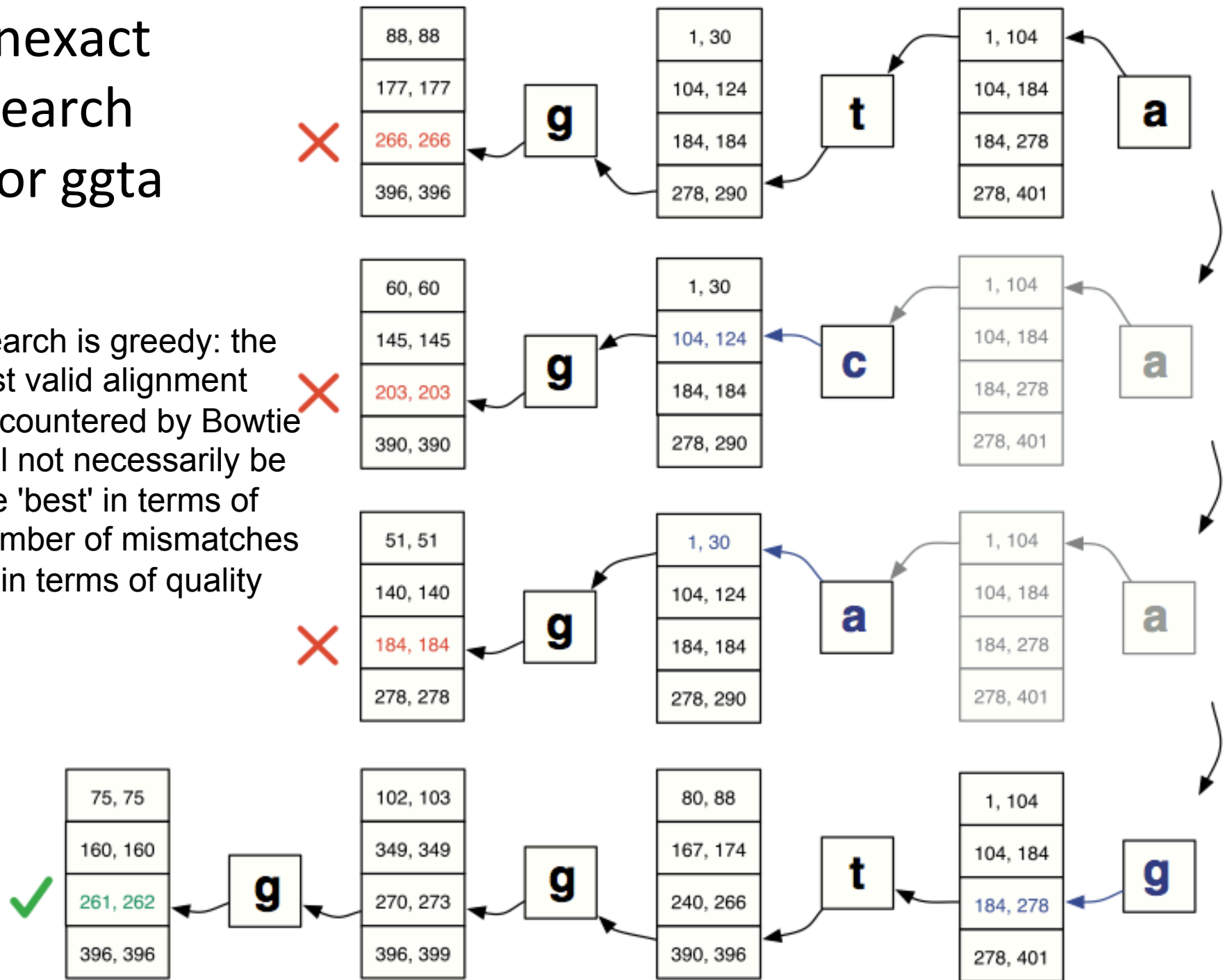
- The search is similar to EXACTMATCH
- It calculates matrix ranges for successively longer query suffixes
- **If the range becomes empty** (a suffix does not occur in the text), then the algorithm may select an already-matched query position and substitute a different base there, introducing a **mismatch** into the alignment
- The EXACTMATCH search resumes from just after the substituted position
- The algorithm selects only those substitutions that are **consistent with the alignment policy**

# 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



# Bowtie - backtracking search

---

- This standard aligner can, in some cases, encounter sequences that cause excessive backtracking
- Bowtie mitigates excessive backtracking with the novel technique of **double indexing**
  - Idea: create 2 indices of the genome: one containing the BWT of the genome, called the **forward index**, and a second containing the BWT of the genome with its sequence reversed (not reverse complemented) called the **mirror index**.
- Let's consider a matching policy that allows one mismatch in the alignment (either in the first half or in the second half)
- Bowtie proceeds in two phases:
  1. load the **forward index** into memory and invoke the aligner with the constraint that it may *not* substitute at positions in the query's **right half**
  2. load the **mirror index** into memory and invoke the aligner on the *reversed query*, with the constraint that the aligner may *not* substitute at positions in the reversed query's right half (the original query's **left half**).
- The constraints on backtracking into the right half prevent excessive backtracking, whereas the use of two phases and two indices maintains full sensitivity

# Bowtie - backtracking search

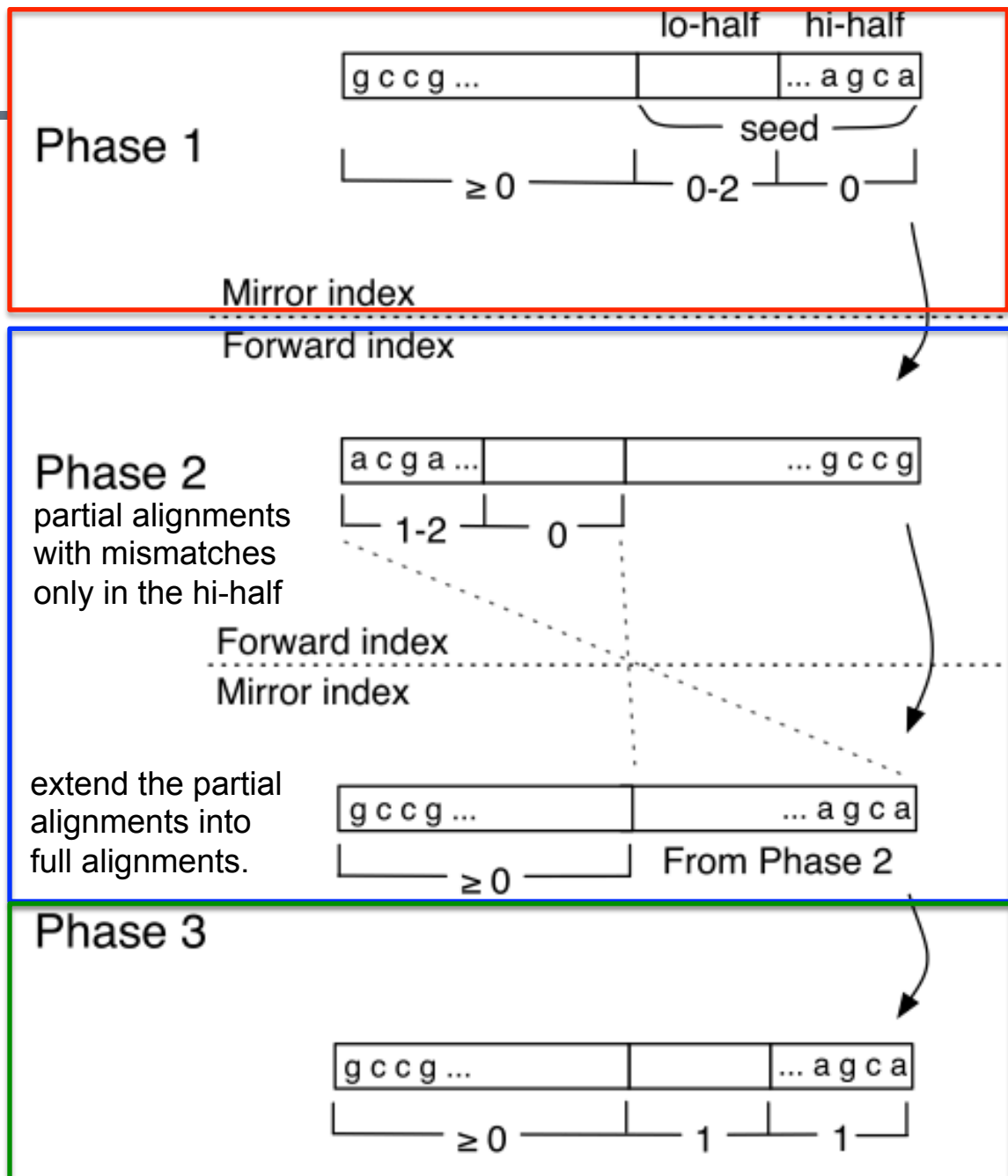
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- Base quality varies across the read
- Bowtie allows the user to select
  - the number of mismatches permitted in the high-quality end of a read (default: 2 mismatches in the first 28 bases)
  - maximum acceptable quality of mismatched positions over the alignment (default: 70 PHRED score)
- The first 28 bases on the high-quality end of the read are termed the **seed**
- The seed consists of two halves:
  - the 14 bp on the high-quality end (usually the 5' end) = the **hi-half**
  - the 14 bp on the low-quality end = **lo-half**
- Assuming 2 mismatches permitted in the seed, a reportable alignment will fall into one of four cases:
  1. no mismatches in seed;
  2. no mismatches in hi-half, one or two mismatches in lo-half
  3. no mismatches in lo-half, one or two mismatches in hi-half
  4. one mismatch in hi-half, one mismatch in lo- half

# Bowtie

- The Bowtie algorithm consists of three phases that alternate between using the forward and mirror indices

1. no mismatches in seed
2. no mismatches in hi-half, one or two mismatches in lo-half
3. no mismatches in lo-half, one or two mismatches in hi-half
4. one mismatch in hi-half, one mismatch in lo-half



## 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

SOAP = Short Oligonucleotide Analysis Package

### 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

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Sequence analysis

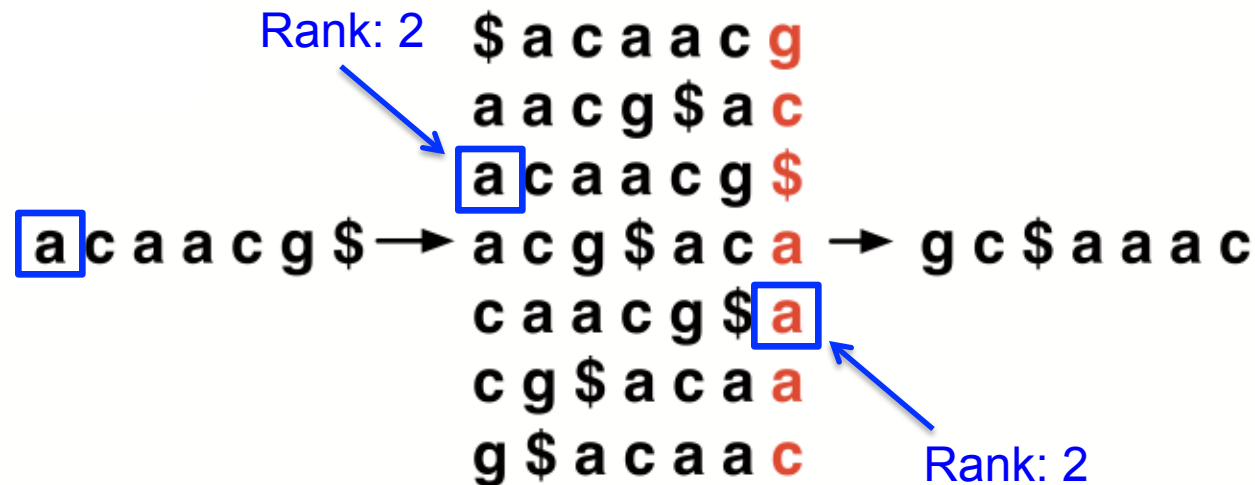
### SOAP: short oligonucleotide alignment program

Ruiqiang Li<sup>1,2</sup>, Yingrui Li<sup>1</sup>, Karsten Kristiansen<sup>2</sup> and Jun Wang<sup>1,2,\*</sup>

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

# Last first (LF) mapping

- The BW matrix has a property called **last first (LF) mapping**:  
The  $i^{\text{th}}$  occurrence of character X in the last column corresponds to the same text character as the  $i^{\text{th}}$  occurrence of X in the first column
- This property is at the core of algorithms that use the BWT index to search the text



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

LF property implicitly encodes the Suffix Array



# Constructing the index

- How do we construct a BWT index?
- Calculating the BWT is closely related to building a suffix array
- Each element of the **BWT** can be derived from the corresponding element of the **suffix array**:

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

- One could generate all suffixes, sort them to obtain the SA, then calculate the BWT in a single pass over the suffix array
- However, constructing the entire suffix array in memory requires at least **~12 gigabytes** for the human genome
- Instead, Bowtie uses a **block-wise strategy**: builds the suffix array and the BWT block-by-block, discarding suffix array blocks once the corresponding BWT block has been built
- Bowtie can build the full index for the human genome in about **24 hours** in less than **1.5 gigabytes of RAM**
- If **16 gigabytes of RAM** or more is available, Bowtie can exploit the additional RAM to produce the same index in about **4.5 hours**

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# Storing the index

---

- The largest single component of the Bowtie index is the BWT sequence. Bowtie stores the BWT in a **2-bit-per-base** format
- A Bowtie index for the assembled human genome sequence is about **1.3 gigabytes**
- A full Bowtie index actually consists of pair of equal-size indexes, the **forward** and **mirror** indexes, for any given genome, but it can be run such that only one of the two indexes is ever resident in memory at once (using the `-z` option)
- What about gaps?

# Bowtie 2

---

- Bowtie: very efficient **ungapped** alignment of short reads based on BWT index
- Index-based alignment algorithms can be quite inefficient when gaps are allowed
- Gaps can results from
  - sequencing errors
  - true insertions and deletions
- Bowtie 2 extends the index-based approach of Bowtie to permit gapped alignment
- It divides the algorithm into two stages
  1. an initial, ungapped **seed-finding stage** that benefits from the speed and memory efficiency of the full-text index
  2. a **gapped extension stage** that uses dynamic programming and benefits from the efficiency of single-instruction multiple-data (SIMD) parallel processing available on modern processors

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## Fast gapped-read alignment with Bowtie 2

Ben Langmead<sup>1,2</sup> & Steven L Salzberg<sup>1-3</sup>

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.

**NATURE METHODS** | VOL.9 NO.4 | APRIL 2012 | 357

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

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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

*Sequence analysis***TopHat: discovering splice junctions with RNA-Seq**Cole Trapnell<sup>1,\*</sup>, Lior Pachter<sup>2</sup> and Steven L. Salzberg<sup>1</sup><sup>1</sup>Center for Bioinformatics and Computational Biology, University of Maryland, College Park, MD 20742 and<sup>2</sup>Department of Mathematics, University of California, Berkeley, CA 94720, USA*Sequence analysis*

Advance Access publication October 25, 2012

**STAR: ultrafast universal RNA-seq aligner**Alexander Dobin<sup>1,\*</sup>, Carrie A. Davis<sup>1</sup>, Felix Schlesinger<sup>1</sup>, Jorg Drenkow<sup>1</sup>, Chris Zaleski<sup>1</sup>, Sonali Jha<sup>1</sup>, Philippe Batut<sup>1</sup>, Mark Chaisson<sup>2</sup> and Thomas R. Gingeras<sup>1</sup><sup>1</sup>Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA and <sup>2</sup>Pacific Biosciences, Menlo Park, CA, USA

*Sequence analysis*

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“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

# 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

#	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

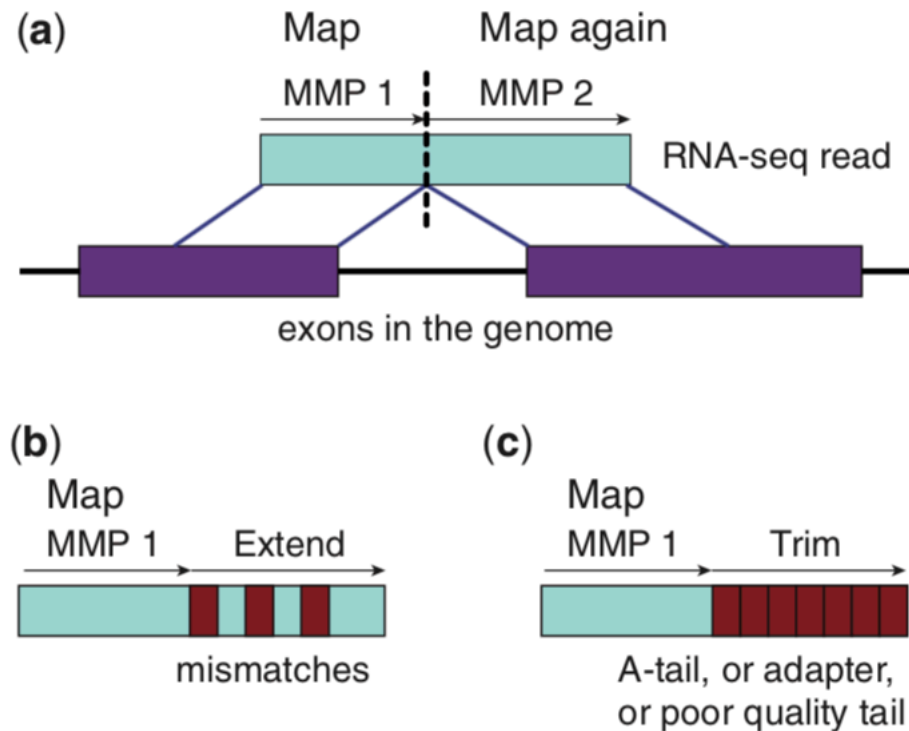


Fig. 1. Schematic representation of the Maximum Mappable Prefix search in the STAR algorithm for detecting (a) splice junctions, (b) mis-matches and (c) tails

- 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





## Exercises:

(a) Perform, by hand, the Burrows-Wheeler Transform on the sequence GCACGCTGC\$.

(b) Suppose you are given a sequence TGTTGCCC\$A, which has been permuted by way of the Burrows-Wheeler Transform. Perform, by hand, the reverse of the BWT in order to obtain the original sequence from which it was generated. Hint: If you wish to check your result, you may employ the BWT in the forward direction (i.e., the algorithm you employed in part (a)) on the answer you obtained here. Assuming you have performed the reversal correctly, you should find that your "original sequence" generates the permuted sequence given in this problem: TGTTGCCC\$A.

(c) Consider the sequence you had to transform in part (a): GCACGCTGC\$. Using this exact matching protocol, determine whether the following subsequences are contained within the sequence from part (a): CACG, AATG. CTGC.