

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
...CC                            GCCCTATCG AAATTTGC  ATAC...
...CCATAGGCTATATGCGCCCTATCGGAAATTTGCGGTATAC...

```

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...
...CCATAGGCTATATGCGCCCTATCGGCAATTTGCGGTATAC...

```

Comparison of next-generation sequencing methods^{[57][58]}

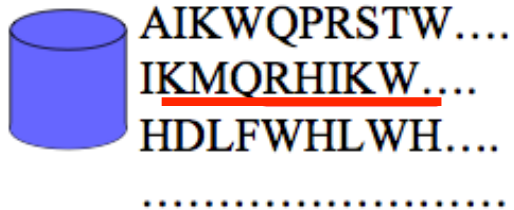
| Method | Read length | Accuracy (single read not consensus) | Reads per run | Time per run | Cost per 1 million bases (in US\$) | Advantages | Disadvantages |
|--|---|--|---|--|------------------------------------|--|--|
| Single-molecule real-time sequencing (Pacific Biosciences) | 10,000 bp to 15,000 bp avg (14,000 bp N50); maximum read length >40,000 bases ^{[59][60][61]} | 87% single-read accuracy ^[62] | 50,000 per SMRT cell, or 500–1000 megabases ^{[63][64]} | 30 minutes to 4 hours ^[65] | \$0.13–\$0.60 | Longest read length. Fast. Detects 4mC, 5mC, 6mA. ^[66] | Moderate throughput. Equipment can be very expensive. |
| Ion semiconductor (Ion Torrent sequencing) | up to 400 bp | 98% | up to 80 million | 2 hours | \$1 | Less expensive equipment. Fast. | Homopolymer errors. |
| Pyrosequencing (454) | 700 bp | 99.9% | 1 million | 24 hours | \$10 | Long read size. Fast. | Runs are expensive. Homopolymer errors. |
| Sequencing by synthesis (Illumina) | 50 to 300 bp | 99.9% (Phred30) | up to 6 billion (TruSeq paired-end) | 1 to 11 days, depending upon sequencer and specified read length ^[67] | \$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. Have issue sequencing palindromic sequence. ^[68] |
| 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. |

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

$$X = x_1x_2\cdots x_m$$

$$Y = y_1y_2\cdots y_n$$

- OUTPUT:

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

Short read alignment

- 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

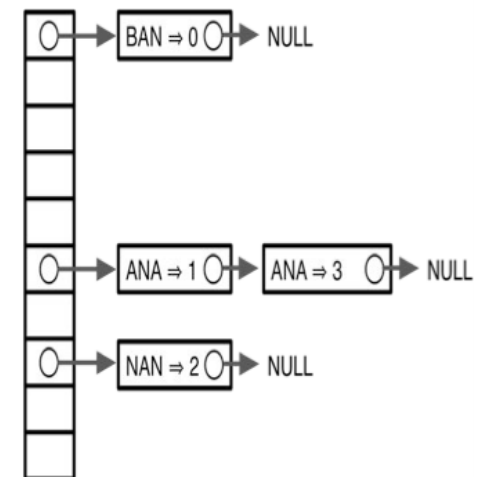
Suffix Array

| | |
|---|----------|
| 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

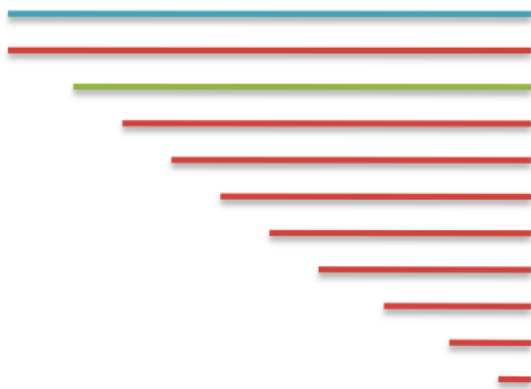


- Simple, easy to understand
- Analysis
 - Genome length = $n = 3,000,000,000$
 - Query length = $m = 7$
 - Comparisons: $(n-m+1) * m = 21,000,000,000$
- Assuming each comparison takes $1/1,000,000$ of a second...
- ... the total running time is 21,000 seconds = 0.24 days
- ... for one 7-bp read

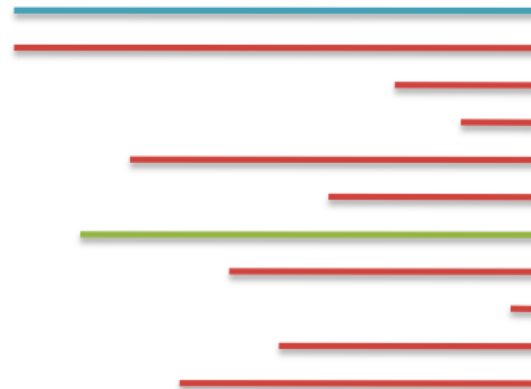
Suffix arrays

- 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 - search for GATTACA

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 |
| 15 | TTACC... | 11 |

Hi
→

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 |
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| 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 |
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| 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 |
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| 12 | TACC... | 12 |
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| 14 | TTACAGATTACC... | 4 |
| 15 | TTACC... | 11 |

Lo →
Hi →

Suffix arrays - analysis

| # | Sequence | Pos |
|----|--------------------|-----|
| 1 | ACAGATTACC... | 6 |
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| 12 | TACC... | 12 |
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| 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 |
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| 5 | ATTACC... | 10 |
| 6 | C... | 15 |
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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:
 $1+2+3+\dots+n = n(n+1)/2$
- 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

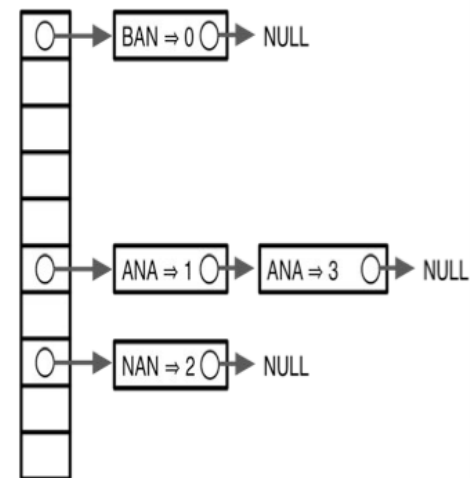
Suffix Array

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|---|----------|
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Binary Search

PacBio Aligner
 (BLASR); Bowtie

Hash (Index) Table



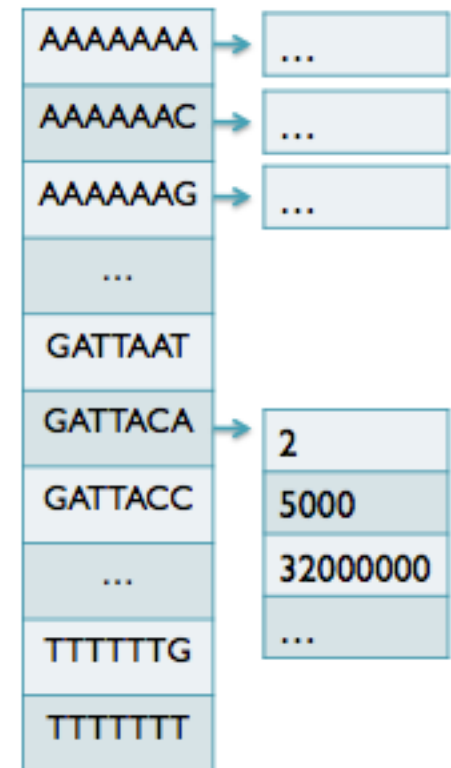
Seed-and-extend

BLAST, BLAT

Time complexity versus space complexity

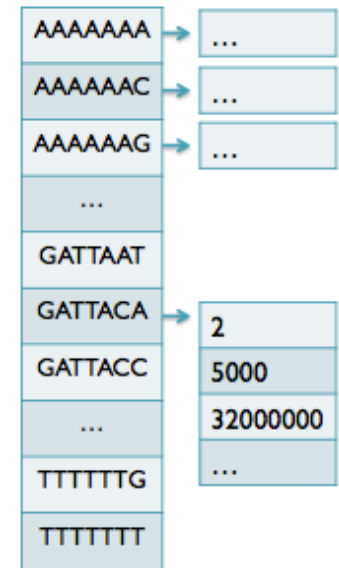
Hashing

- 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



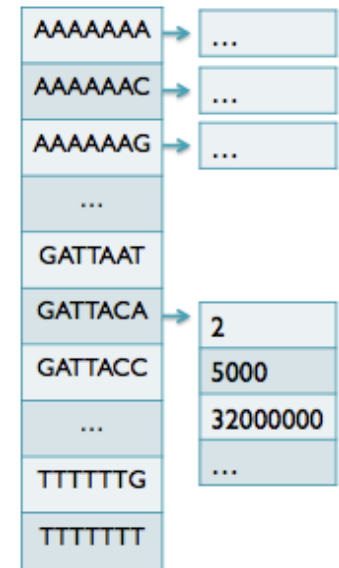
Hashing

- 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



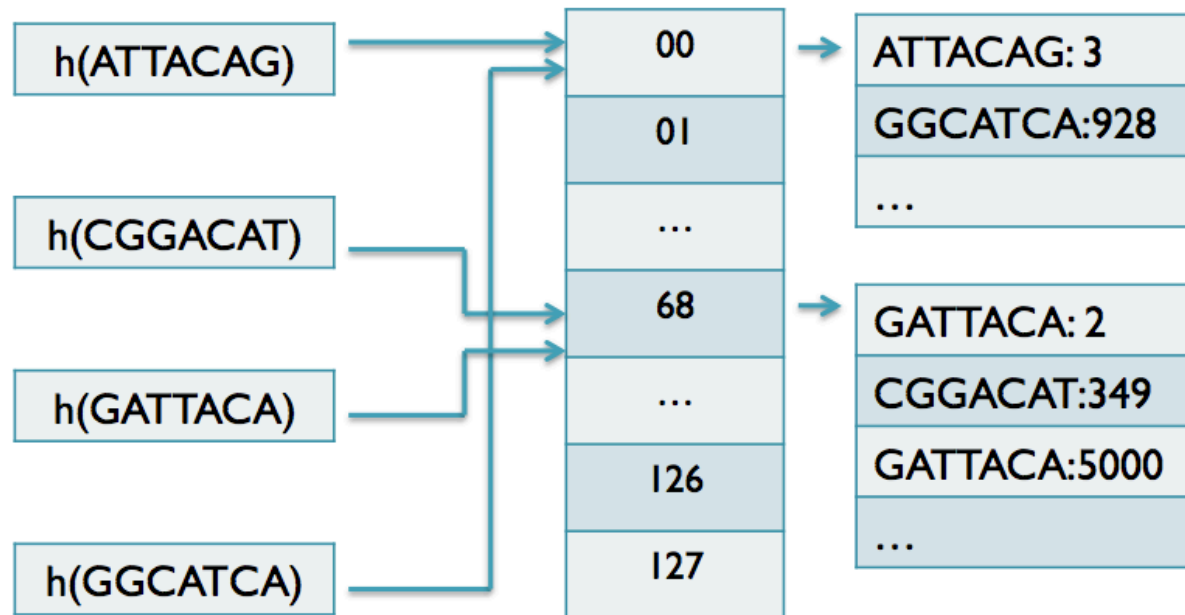
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- $K=20 \Rightarrow 4^{20} = 1,099,511,627,776$ (impossible to store directly in RAM)
 - There are only 3B 20-mers in the genome
 - Even if we could build this table, 99.7% will be empty
 - But we don't know which cells are empty until we try
- Use 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**, or **1B** instead of **1T**
 - Division: $\text{hash}(n) = H * n / R$
 - $\text{hash}(\text{GATTACA}) = 128 * 9156 / 16384 = 71$
 - Modulo: $\text{hash}(n) = n \% H$
 - $\text{hash}(\text{GATTACA}) = 9156 \% 128 = 68$



Hashing

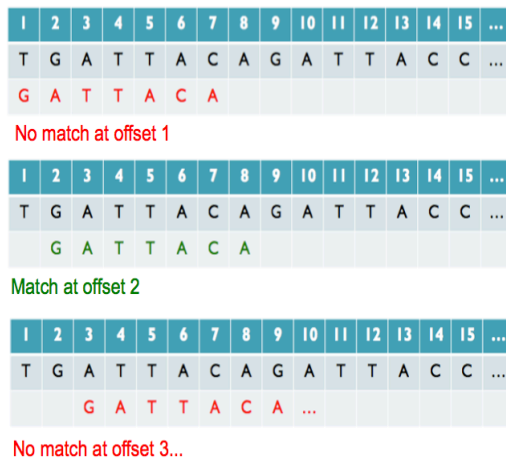
- By construction, **multiple keys have the same hash value**
 - Store elements with the same key in a bucket chained together
 - A good hash evenly distributes the values: R/H have the same hash value
 - Looking up a value scans the entire bucket



Algorithms for exact string matching

- Search for the substring GATTACA in the genome

Brute Force



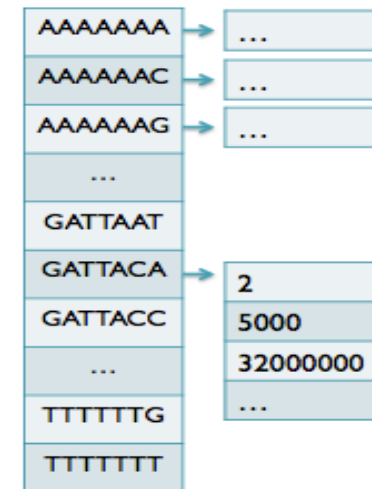
Easy
Slow

Suffix Array

| # | Sequence | Pos |
|----|--------------------|-----|
| 1 | ACAGATTACC... | 6 |
| 2 | ACC... | 13 |
| 3 | AGATTACC... | 8 |
| 4 | ATTACAGATTACC... | 3 |
| 5 | ATTACC... | 10 |
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| 12 | TACC... | 12 |
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| 15 | TTACC... | 11 |

Fast (binary search)
High space complexity

Hash (Index) Table



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^{1,2} & Steven L Salzberg¹⁻³

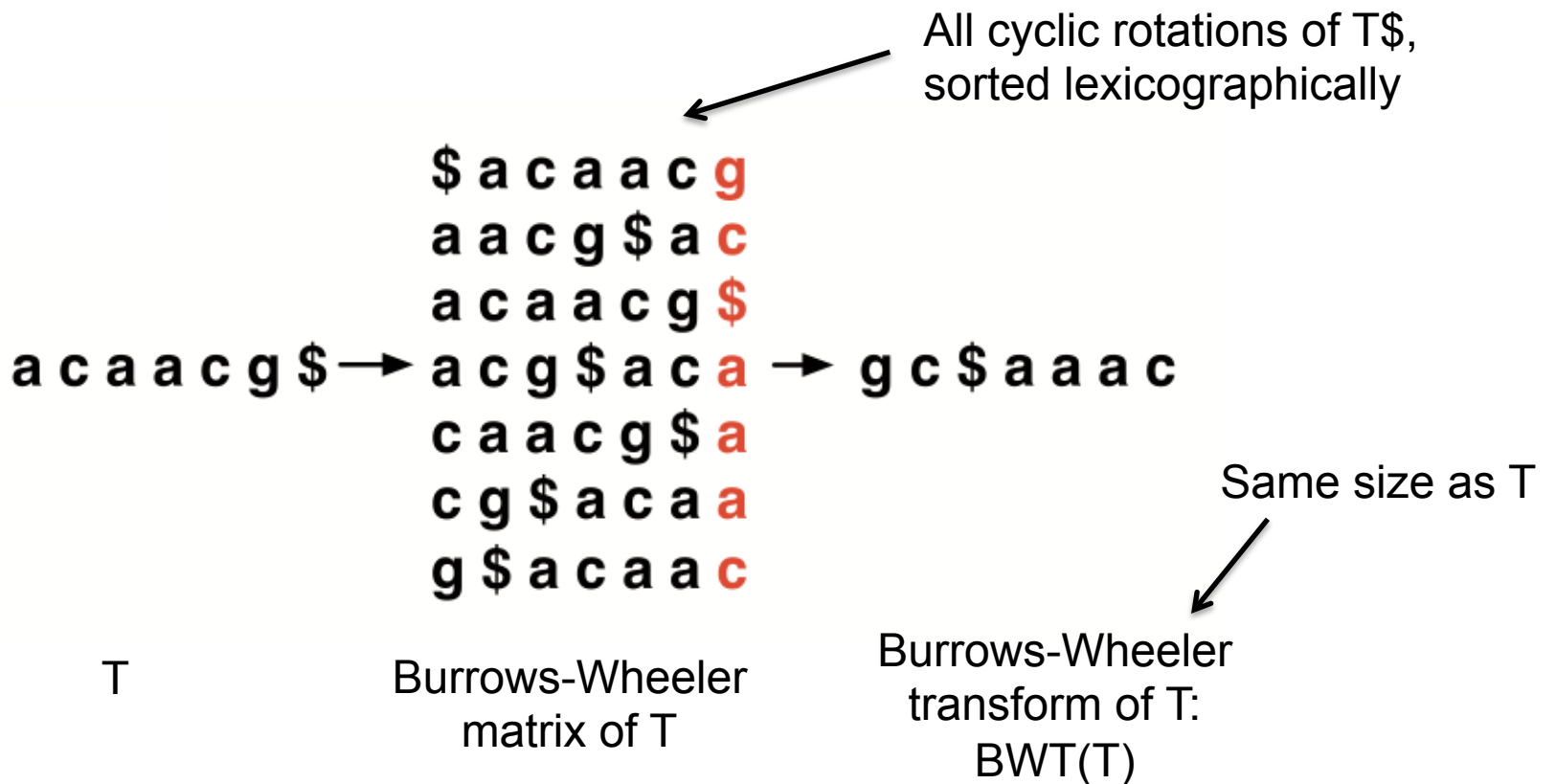
As the rate of sequencing increases, greater throughput is demanded from read aligners. The full-text minute index is often used to make alignment very fast and memory-efficient, but the approach is ill-suited to finding longer, gapped alignments. Bowtie 2 combines the strengths of the full-text minute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

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

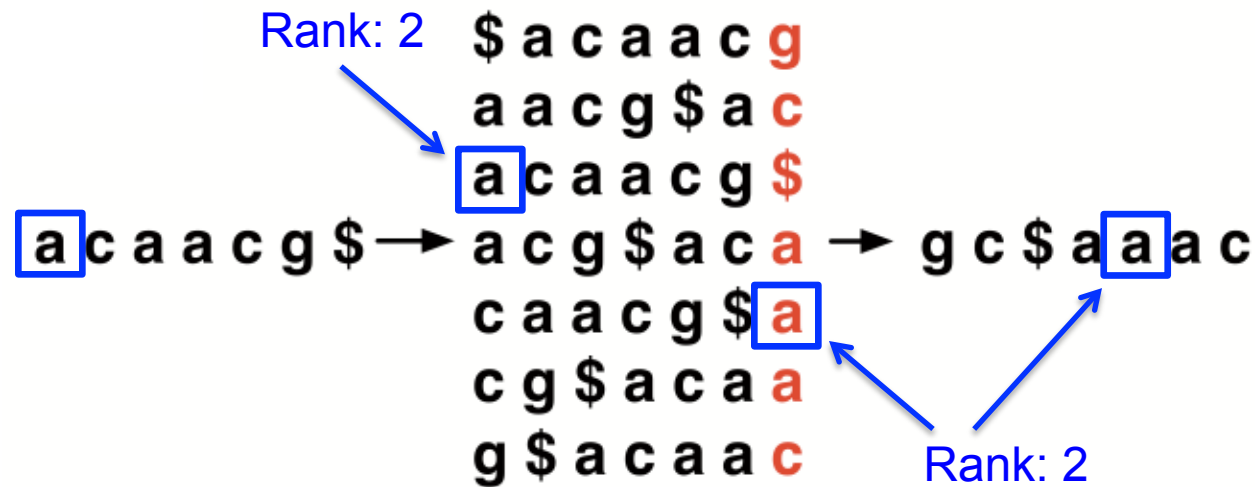
Burrows-Wheeler transform

- 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^{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



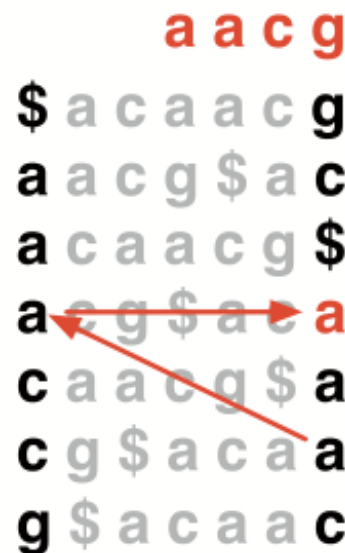
$$BWT[i] = \begin{cases} T[SA[i] - 1] & SA[i] \neq 0 \\ \$ & SA[i] = 0 \end{cases} \quad \text{LF property implicitly encodes the Suffix Array}$$

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



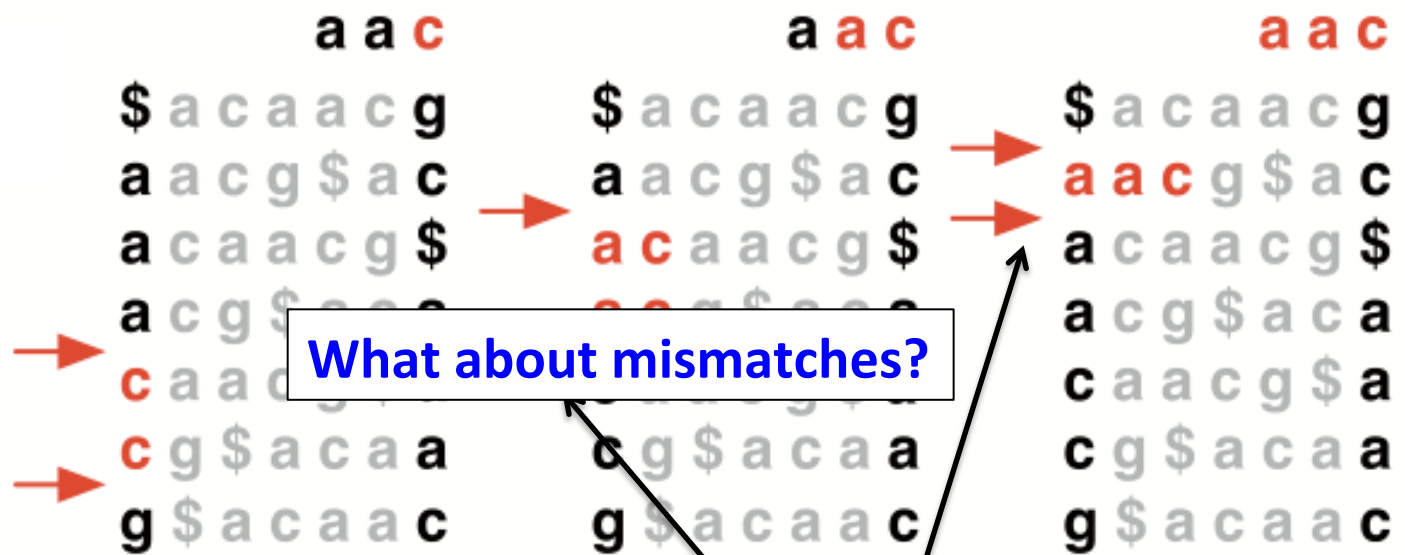
the matrix is sorted lexicographically
rows beginning with a given sequence
appear consecutively

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

LF mapping and exact matching

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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

- EXACTMATCH is insufficient for short read alignment because alignments may contain mismatches
- 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**

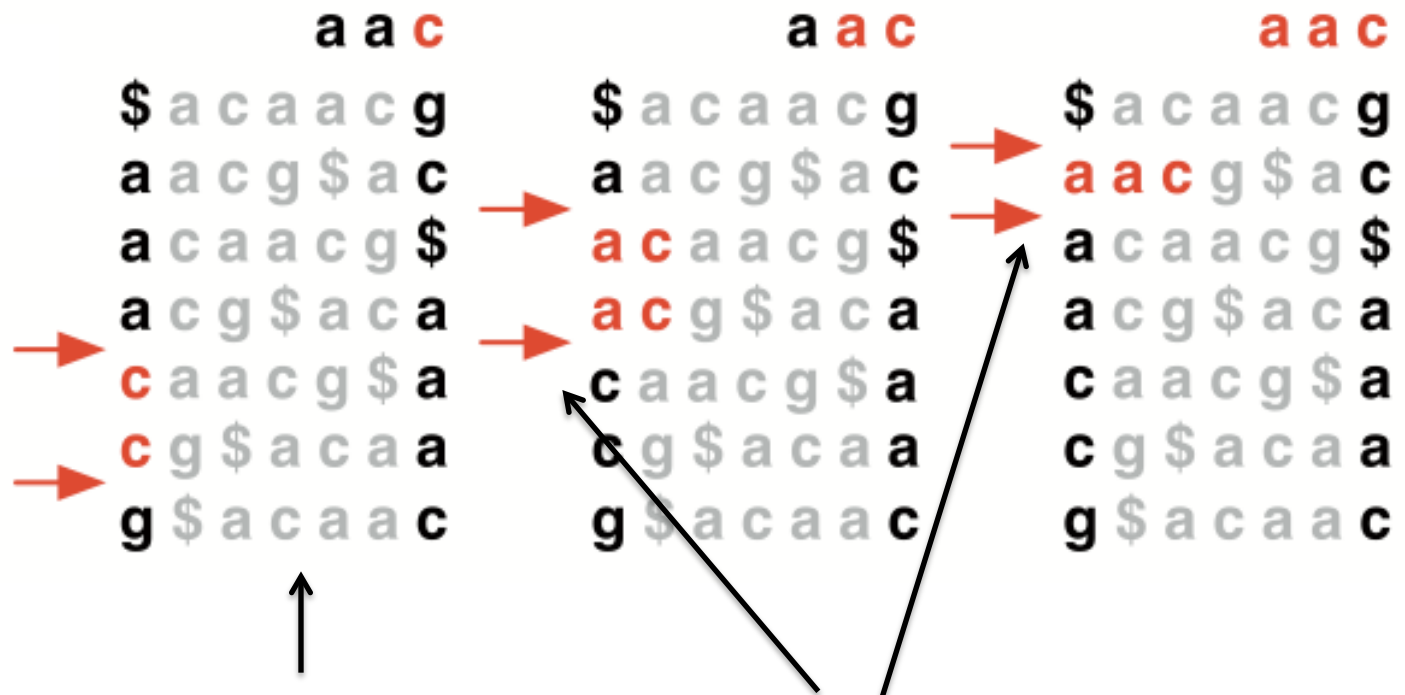
Bowtie - backtracking search

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

LF mapping and exact matching

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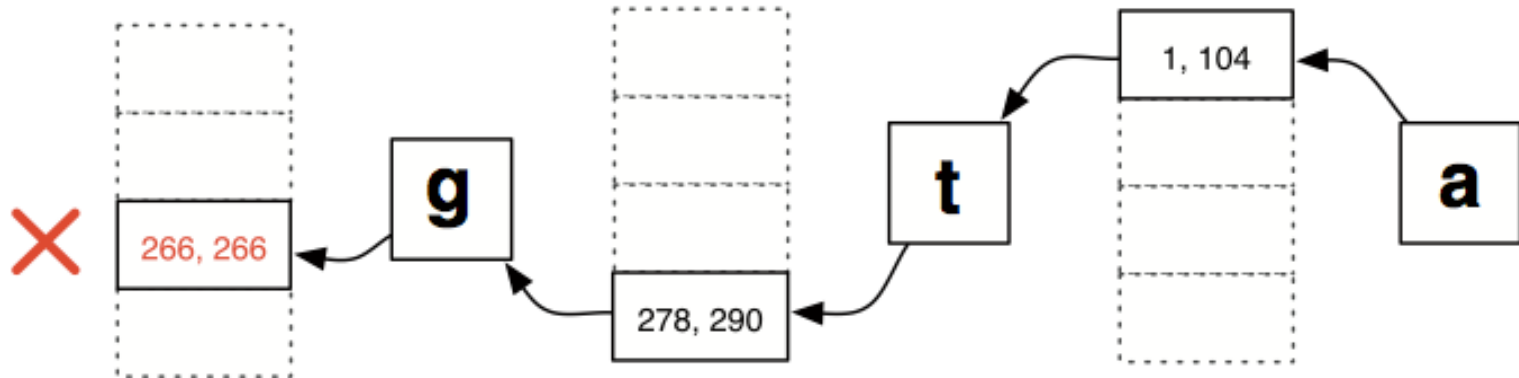
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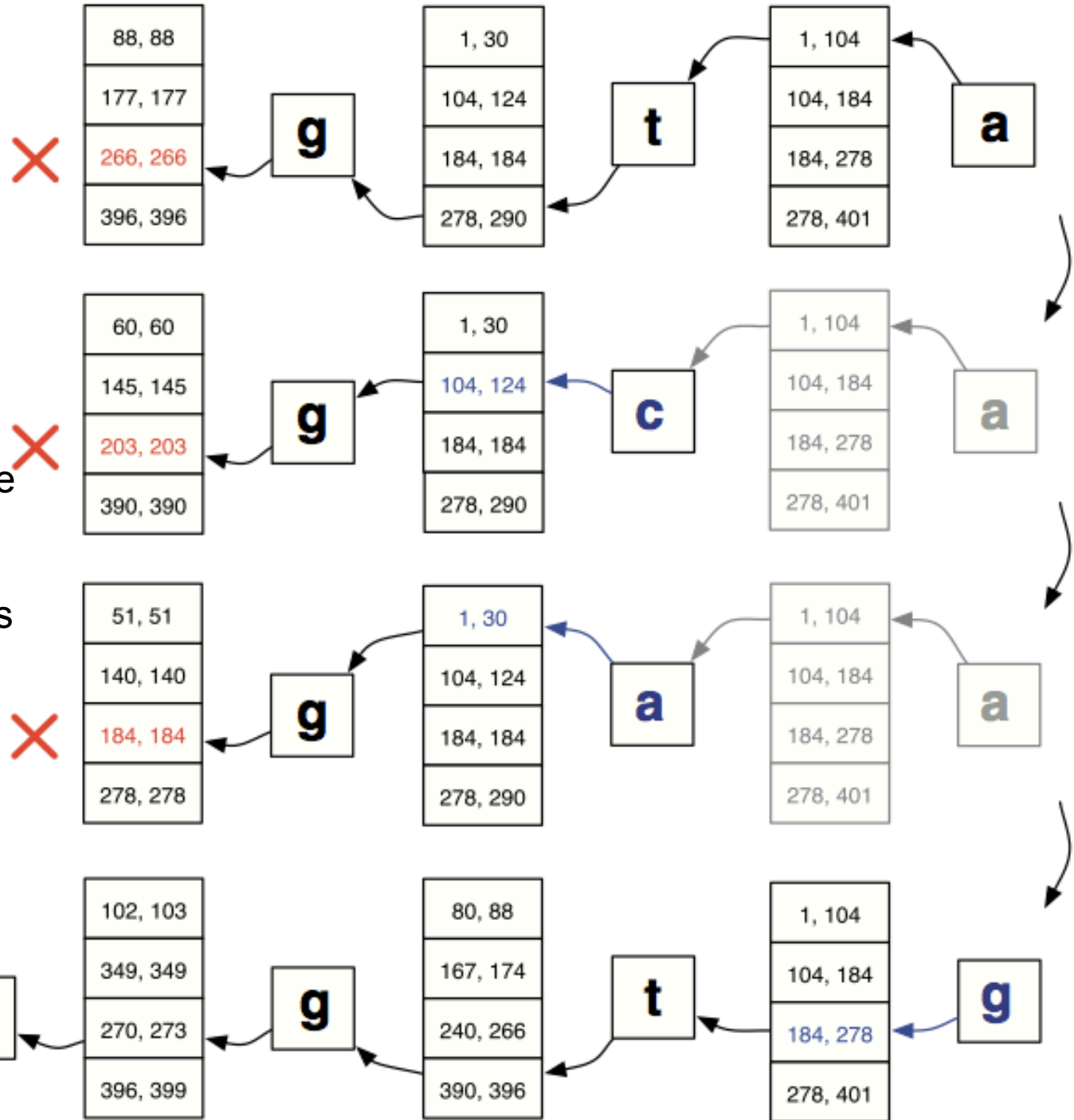
Bowtie - backtracking search

- 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** and which yield a modified suffix that occurs at least once in the text
- If there are multiple candidate substitution positions, then the algorithm **greedily** selects a position with a minimal quality value

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

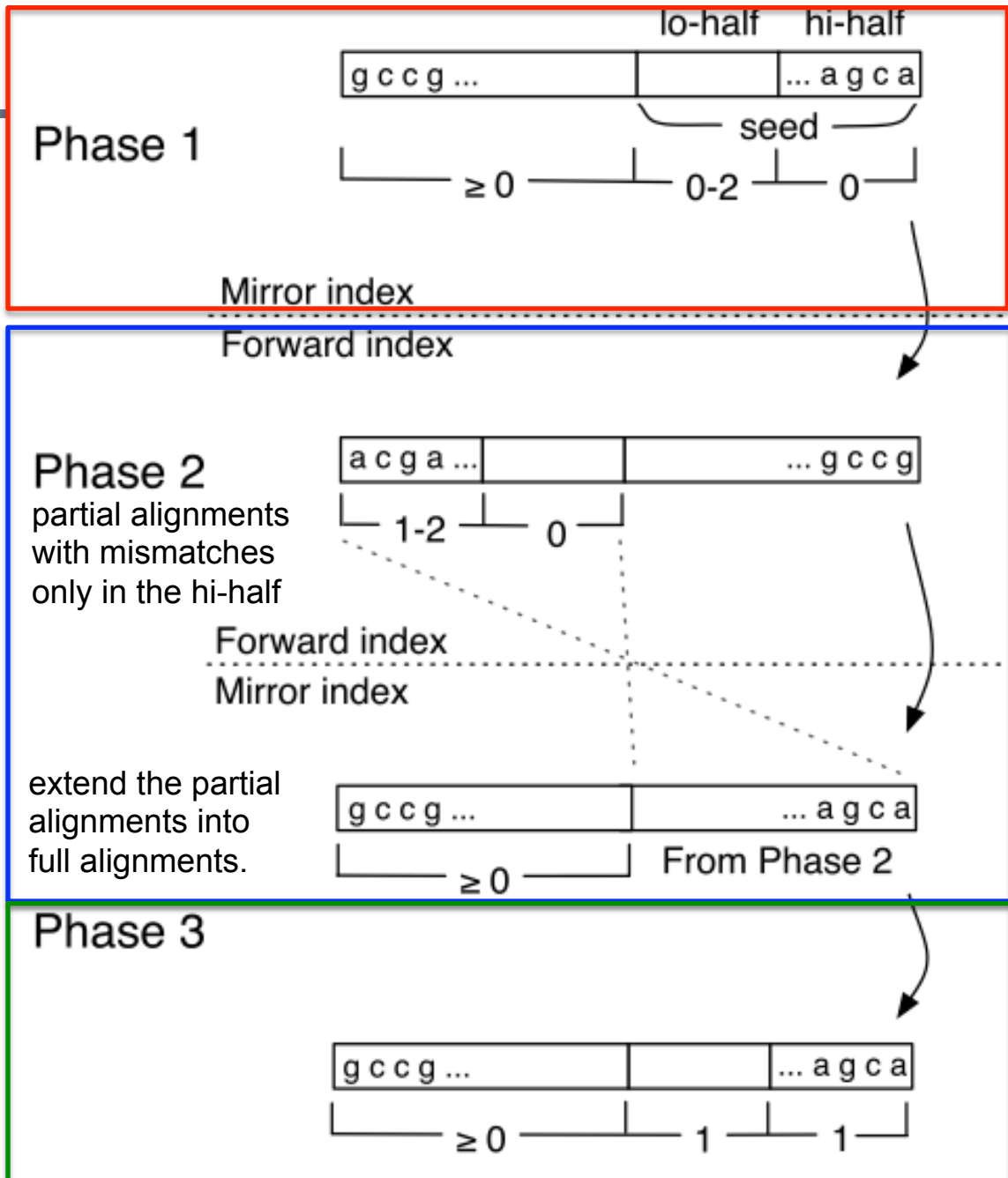
Bowtie - backtracking search

- 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 the overall 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

BIOINFORMATICS APPLICATIONS NOTE

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Sequence analysis

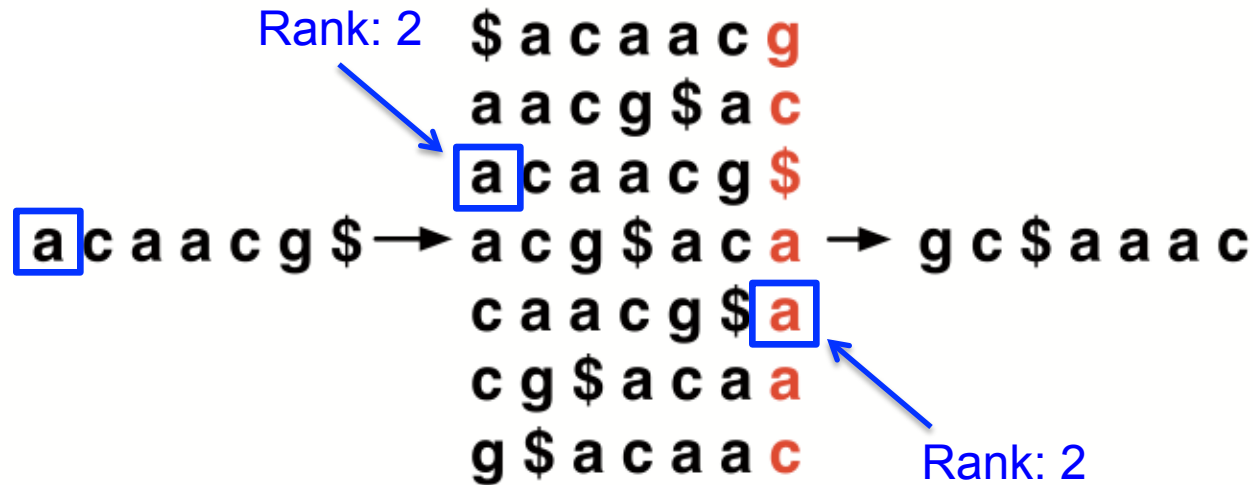
SOAP: short oligonucleotide alignment program

Ruiqiang Li^{1,2}, Yingrui Li¹, Karsten Kristiansen² and Jun Wang^{1,2,*}

¹Beijing Genomics Institute at Shenzhen, Shenzhen 518083, China and ²Department 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^{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



$$BWT[i] = \begin{cases} T[SA[i] - 1] & SA[i] \neq 0 \\ \$ & SA[i] = 0 \end{cases} \quad \text{LF property implicitly encodes the Suffix Array}$$

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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Bowtie index building performance

| Physical memory target (GB) | Actual peak memory footprint (GB) | Wall clock time |
|-----------------------------|-----------------------------------|-----------------|
| 16 | 14.4 | 4 h 36 m |
| 8 | 5.84 | 5 h 5 m |
| 4 | 3.39 | 7 h 40 m |
| 2 | 1.39 | 21 h 30 m |

additional RAM to produce the same index in about **4.5 hours**

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 result 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^{1,2} & Steven L Salzberg¹⁻³

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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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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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

Software

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Sequence analysis

TopHat: discovering splice junctions with RNA-Seq

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TopHat