Burrows-Wheeler Transform (BWT)

Slides from Eleazar Eskin and Ben Langmead

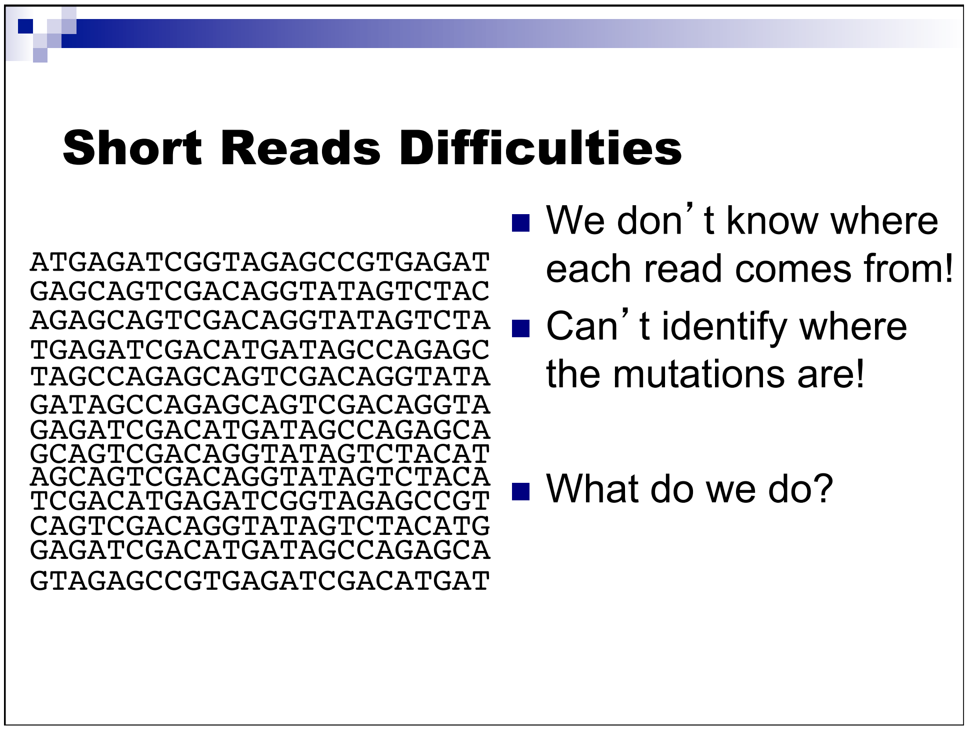
Short Read Sequencing Problem (A Computer Science Problem)

Full DNA Sequence

AGAGC**A**GTCGAC
A**G**GTATAG**T**CTA
CATGAGATC**G**AC
ATGAGATC**G**GTA
GAGC**C**GTGAGAT
C**G**ACATGATAG**C**
CAGAGC**A**GTCGA
CA**G**GTATAG**T**CT
ACATGAGATC**G**A
CATGAGATC**G**GT
AGAGC**C**GTGAGA
TC**G**ACATGATAG
CCAGAGC**A**GTCG
ACA**G**GTATAG**T**C
TACATGAGATC**G**
ACATGAGATC**G**G
TAGAG**C**CGTGAG
ATC**G**ACATGATA
G**C**CAGAG**C**AGTC
GACA**G**GTATAG**T**
CTACATGAGATC

- Short read sequencers generate random short substrings from the DNA sequence of a certain length.

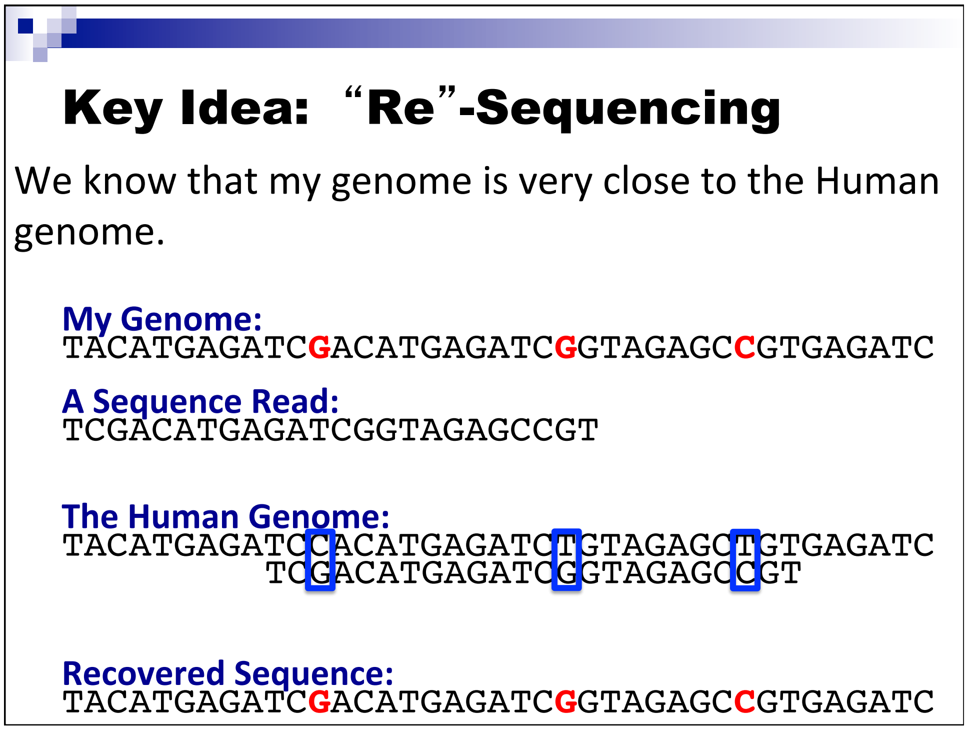
```
ATGAGATCGGTAGAGCCGTGAGAT
GAGCAGTCGACAGGTATAGTCTAC
AGAGCAGTCGACAGGTATAGTCTA
TGAGATCGACATGATAGCCAGAGC
TAGCCAGAGCAGTCGACAGGTATA
GATAGCCAGAGCAGTCGACAGGTA
GAGATCGACATGATAGCCAGAGCA
GCAGTCGACAGGTATAGTCTACAT
AGCAGTCGACAGGTATAGTCTACA
TCGACATGAGATCGGTAGAGCCGT
CAGTCGACAGGTATAGTCTACATG
GAGATCGACATGATAGCCAGAGCA
GTAGAGCCGTGAGATCGACATGAT
```



Short Reads Difficulties

```
ATGAGATCGGTAGAGCCGTGAGAT
GAGCAGTCGACAGGTATAGTCTAC
AGAGCAGTCGACAGGTATAGTCTA
TGAGATCGACATGATAGCCAGAGC
TAGCCAGAGCAGTCGACAGGTATA
GATAGCCAGAGCAGTCGACAGGTA
GAGATCGACATGATAGCCAGAGCA
GCAGTCGACAGGTATAGTCTACAT
AGCAGTCGACAGGTATAGTCTACA
TCGACATGAGATCGGTAGAGCCGT
CAGTCGACAGGTATAGTCTACATG
GAGATCGACATGATAGCCAGAGCA
GTAGAGCCGTGAGATCGACATGAT
```

- We don't know where each read comes from!
- Can't identify where the mutations are!
- What do we do?



Key Idea: "Re"-Sequencing

We know that my genome is very close to the Human genome.

My Genome:

```
TACATGAGATCGACATGAGATCGGTAGAGCCGTGAGATC
```

A Sequence Read:

```
TCGACATGAGATCGGTAGAGCCGT
```

The Human Genome:

```
TACATGAGATCCACATGAGATCTGTAGAGCTGTGAGATC
          TCGACATGAGATCGGTAGAGCCGT
```

Recovered Sequence:

```
TACATGAGATCGACATGAGATCGGTAGAGCCGTGAGATC
```

“Re”-Sequencing Output

Resequencing provides a list of changes to make from the reference to change it to the target. Similar to unix “diff”.

My Genome:
```
TACATGAGATCGACATGAGATCGGTAGAGCCGTGAGATC
```

The Human Genome:
```
TACATGAGATCCACATGAGATCTGTAGAGCTGTGAGATC
           G          G       C
```

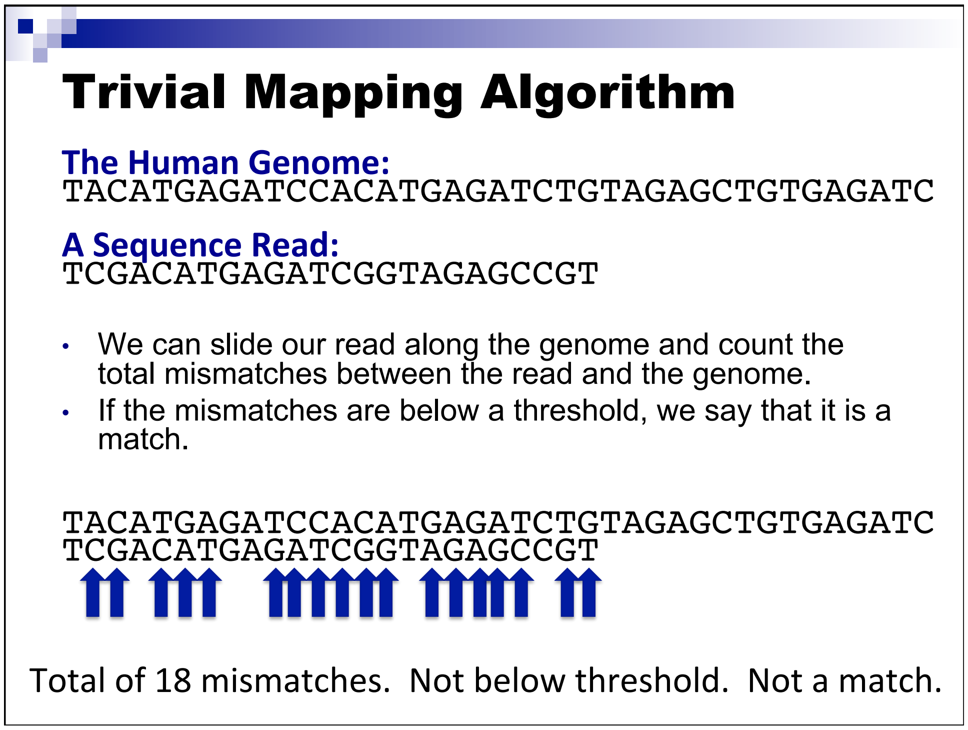
A list of changes

Recovered Sequence:
```
TACATGAGATCGACATGAGATCGGTAGAGCCGTGAGATC
```

Algorithmic “Re”-Sequencing Challenges

- Sequences are long!
  - **Human Genome is 3,000,000,000 long.**
- Sequencers generate many reads!
  - **A sequencer generates over 1,000,000,000 reads.**
- We need efficient algorithms to “map” each read to its location in the genome.

There are other challenges which we are not mentioning.



Trivial Mapping Algorithm

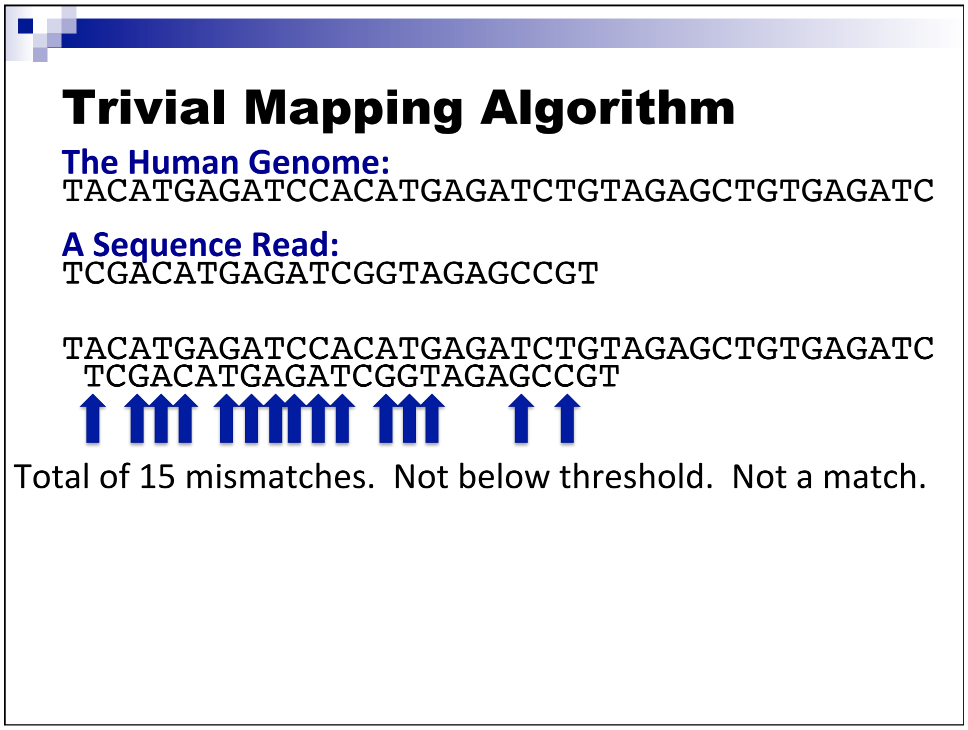
The Human Genome:
TACATGAGATCCACATGAGATCTGTAGAGCTGTGAGATC

A Sequence Read:
TCGACATGAGATCGGTAGAGCCGT

- We can slide our read along the genome and count the total mismatches between the read and the genome.
- If the mismatches are below a threshold, we say that it is a match.

```
TACATGAGATCCACATGAGATCTGTAGAGCTGTGAGATC
TCGACATGAGATCGGTAGAGCCGT
 ↑↑ ↑↑↑  ↑↑↑↑↑↑ ↑↑↑↑↑ ↑↑
```

Total of 18 mismatches. Not below threshold. Not a match.



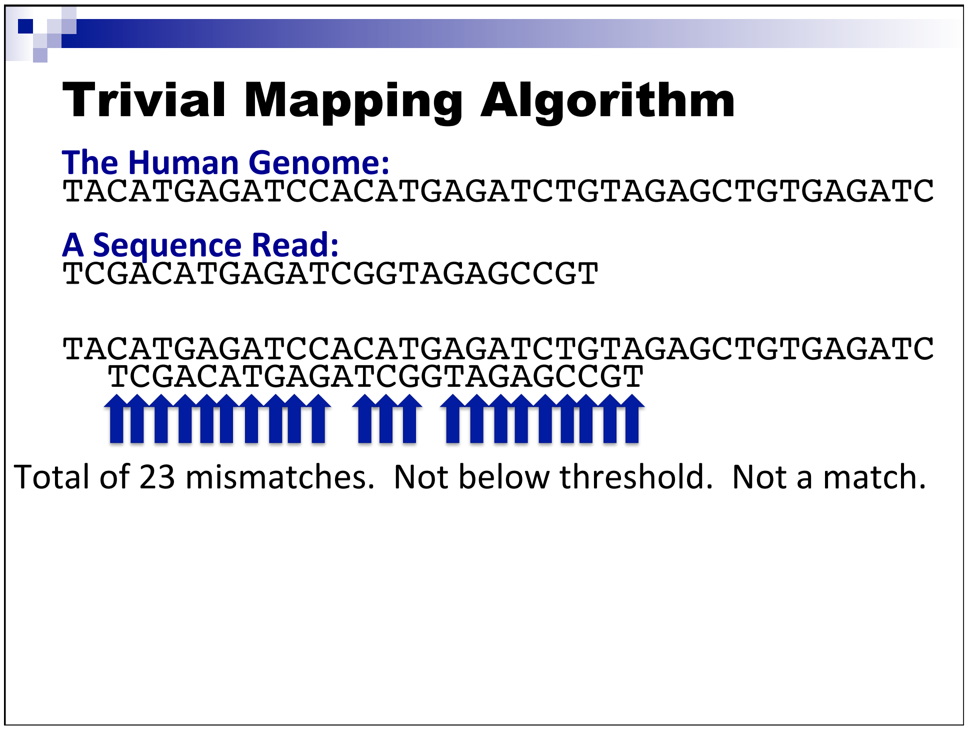
Trivial Mapping Algorithm

The Human Genome:
TACATGAGATCCACATGAGATCTGTAGAGCTGTGAGATC

A Sequence Read:
TCGACATGAGATCGGTAGAGCCGT

```
TACATGAGATCCACATGAGATCTGTAGAGCTGTGAGATC
 TCGACATGAGATCGGTAGAGCCGT
 ↑ ↑↑↑ ↑↑↑↑↑↑ ↑↑↑   ↑ ↑
```

Total of 15 mismatches. Not below threshold. Not a match.



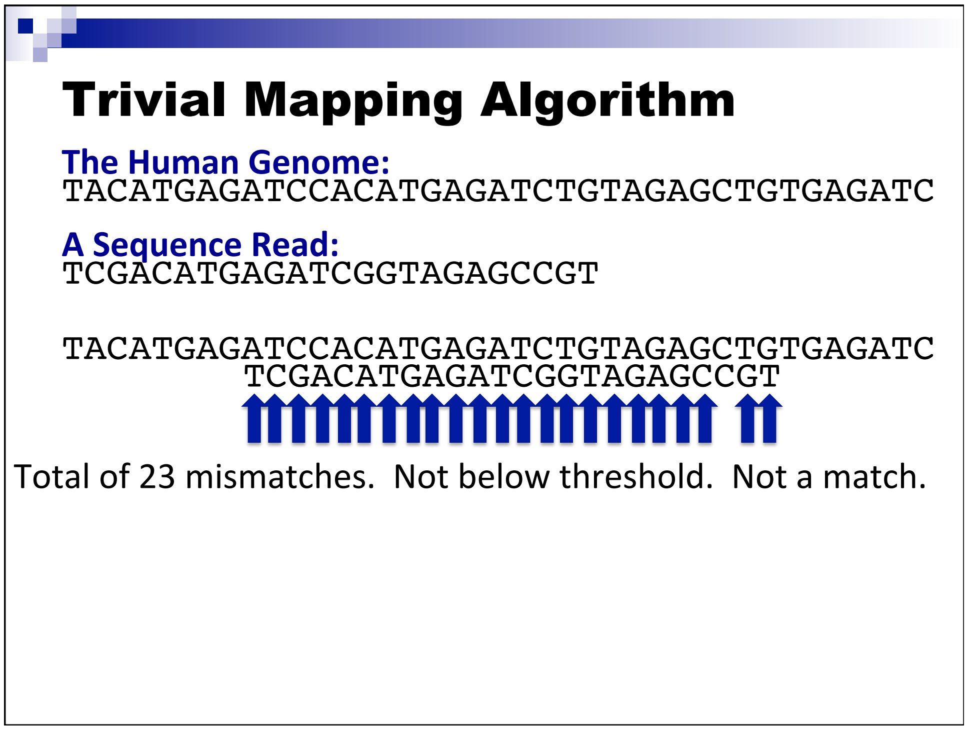
Trivial Mapping Algorithm

The Human Genome:
TACATGAGATCCACATGAGATCTGTAGAGCTGTGAGATC

A Sequence Read:
TCGACATGAGATCGGTAGAGCCGT

```
TACATGAGATCCACATGAGATCTGTAGAGCTGTGAGATC
  TCGACATGAGATCGGTAGAGCCGT
  ↑↑↑↑↑↑↑↑↑↑ ↑↑↑ ↑↑↑↑↑↑↑↑↑
```

Total of 23 mismatches. Not below threshold. Not a match.



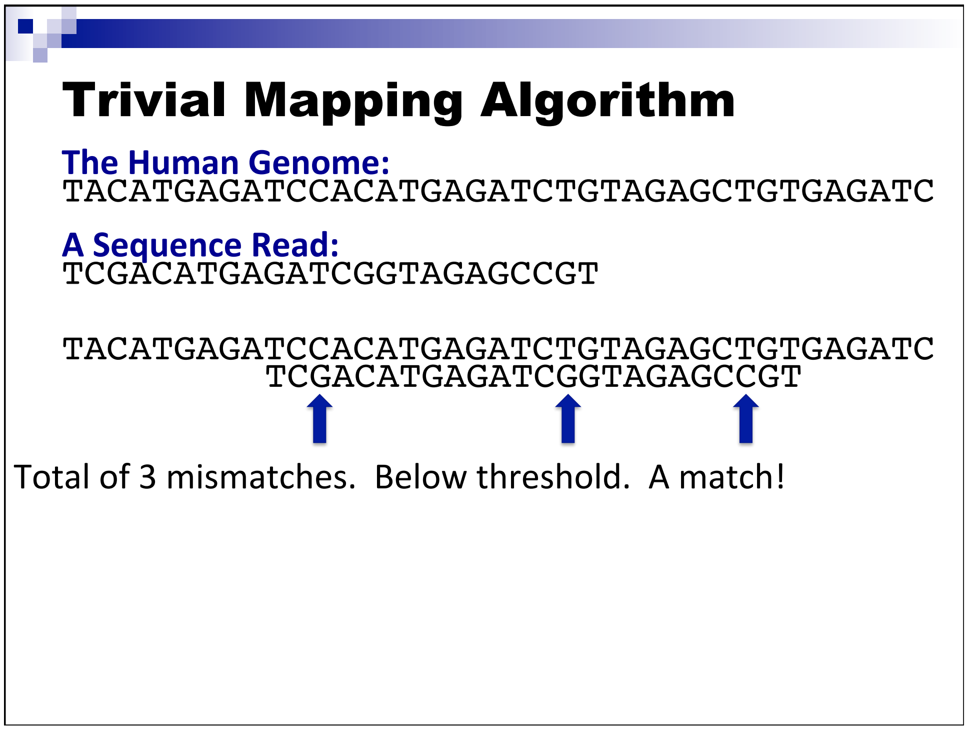
Trivial Mapping Algorithm

The Human Genome:
TACATGAGATCCACATGAGATCTGTAGAGCTGTGAGATC

A Sequence Read:
TCGACATGAGATCGGTAGAGCCGT

```
TACATGAGATCCACATGAGATCTGTAGAGCTGTGAGATC
        TCGACATGAGATCGGTAGAGCCGT
        ↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑↑ ↑↑
```

Total of 23 mismatches. Not below threshold. Not a match.



Trivial Mapping Algorithm

The Human Genome:

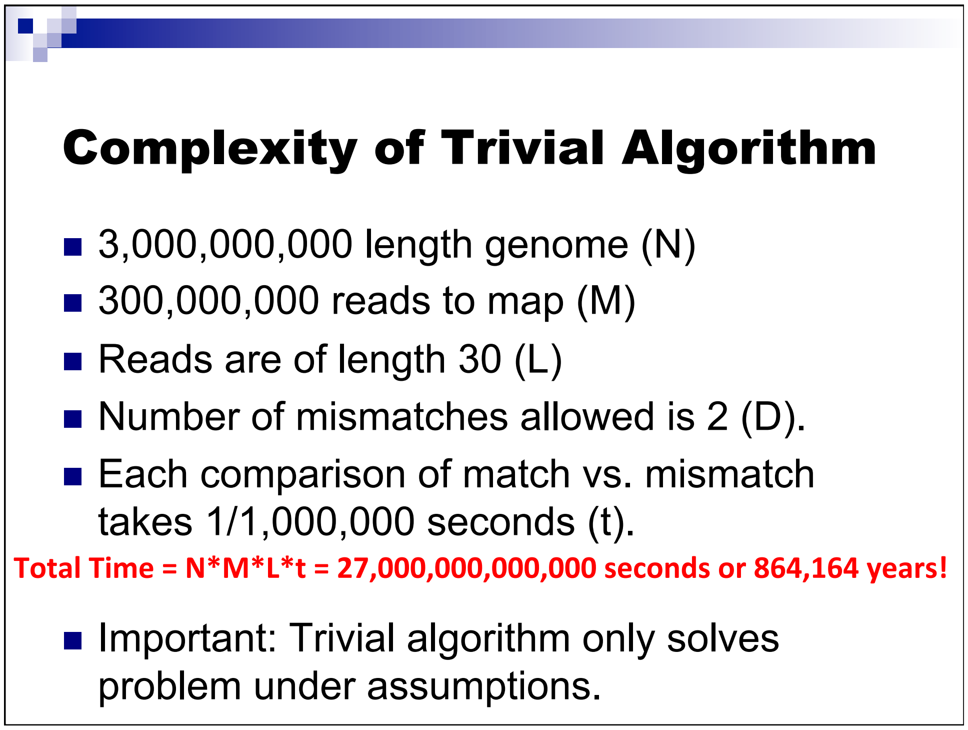
```
TACATGAGATCCACATGAGATCTGTAGAGCTGTGAGATC
```

A Sequence Read:

```
TCGACATGAGATCGGTAGAGCCGT
```

```
TACATGAGATCCACATGAGATCTGTAGAGCTGTGAGATC
           TCGACATGAGATCGGTAGAGCCGT
              ⬆            ⬆         ⬆
```

Total of 3 mismatches. Below threshold. A match!



Complexity of Trivial Algorithm

- 3,000,000,000 length genome (N)
- 300,000,000 reads to map (M)
- Reads are of length 30 (L)
- Number of mismatches allowed is 2 (D).
- Each comparison of match vs. mismatch takes 1/1,000,000 seconds (t).

Total Time = N*M*L*t = 27,000,000,000,000 seconds or 864,164 years!

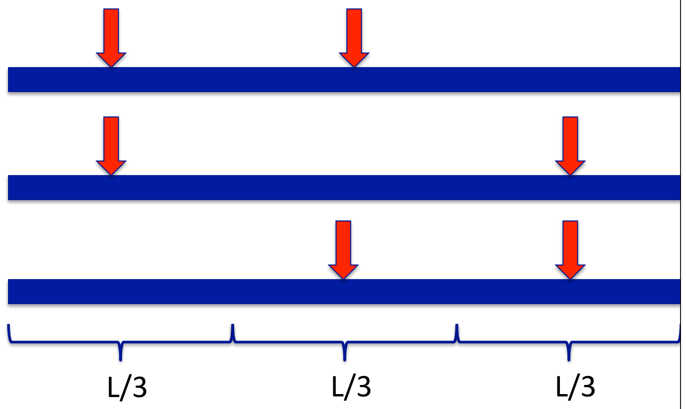
- Important: Trivial algorithm only solves problem under assumptions.

Some observations

- Most positions in the genome match very poorly.
- We are looking for only a few mismatches. (D is small)
- A substring of our read will match perfectly.

Perfect Matching Read Substrings

Three "worst" possible cases for placement of mutations.



- In each case, there is a perfect match of L/3.

Finding a perfect match of length L/3

- Intuition: Create an index (or phone book) for the genome.
- We can look up an entry quickly.

Sequence	Positions
AAAAAAAAAA	32453, 64543, 76335
AAAAAAAAAC	64534, 84323, 96536
AAAAAAAAAG	12352, 32534, 56346
AAAAAAAAAT	23245, 54333, 75464
AAAAAAAACA	
AAAAAAAACC	43523, 67543
…	
CAAAAAAAAA	32345, 65442
CAAAAAAAAC	34653, 67323, 76354
…	
TCGACATGAG	54234, 67344, 75423
TCGACATGAT	11213, 22323
…	
TTTTTTTTTG	64252
TTTTTTTTTT	64246, 77355, 78453

If L=30, each entry will have a key of length 10. Each entry will contain on average $N/4^{10}$ positions. (Approximately 3,000).

If L=45, each entry will have a key of length 15. Each entry will contain on average 3 positions.

Complexity of Indexing Algorithm

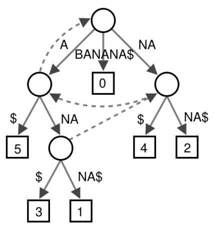
- We need to look up each third of the read in the index.
- For L=30, our index will contain entries of length 10. Each entry will contain on average $N/(4^{L/3})$ or 3,000 positions.
- For each position, we need to compute the number of mismatches.
- Our running time is $L^* M^*3^*N/(4^{L/3})^*T$=81,000,000 seconds or 937 days.
- If L=45, then the time is 81,000 seconds or 22.5 hours.

Indexing a genome

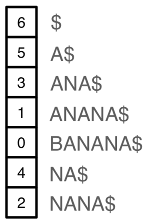
- To find exactly matching substrings, we need to build an index for the whole genome.
- Problem: The genome is BIG!

Indexing

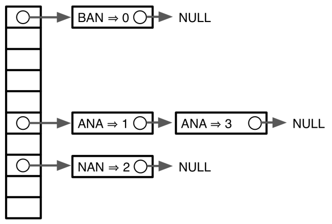
- Genome indices can be big. For human:



> 35 GBs



> 12 GBs



> 12 GBs

- Large memory requirement implications
  - **Requires large memory machine (expensive)**
  - **Partition genome and index each part (slow)**

Burrows-Wheeler Transform

- http://en.wikipedia.org/wiki/Burrows-Wheeler_transform
- Reversible permutation used originally in compression

```
                 $ a c a a c g      $ a c a a c g
                 a a c g $ a c      a a c g $ a c
                 a c a a c g $      a c a a c g $
a c a a c g $ →  a c g $ a c a  →   a c g $ a c a  →  g c $ a a a c
      T          c a a c g $ a      c a a c g $ a       BWT(T)
                 c g $ a c a a      c g $ a c a a
                 g $ a c a a c      g $ a c a a c
                    Burrows          Last column
                    Wheeler
                    Matrix
```

- Once BWT(T) is built, *all else shown here is discarded*
  - **Matrix will be shown for illustration only**

Burrows M, Wheeler DJ: A block sorting lossless data compression algorithm. *Digital Equipment Corporation, Palo Alto, CA* 1994, Technical Report 124; 1994

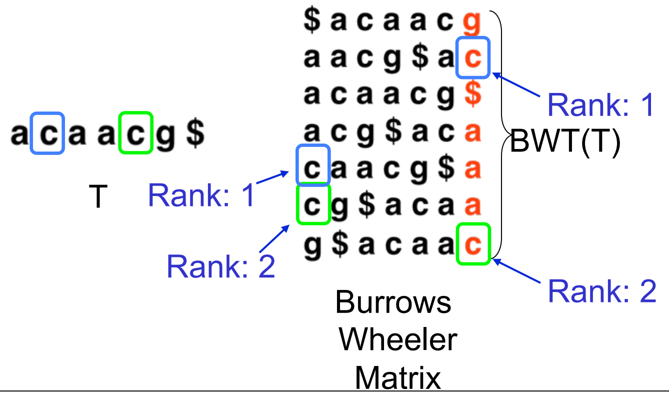
Burrows-Wheeler Transform

- Store only last column
- First column can be recovered by counting symbols in last column because it is sorted

```
                  $ a c a a c g                              $
                  a a c g $ a c                              a
                  a c a a c g $                              a
a c a a c g $     a c g $ a c a  } BWT(T)  [A:3 C:2 G:1 T:0]  a
                  c a a c g $ a                              c
      T           c g $ a c a a                              c
                  g $ a c a a c                              g
                     Burrows
                     Wheeler
                     Matrix
```

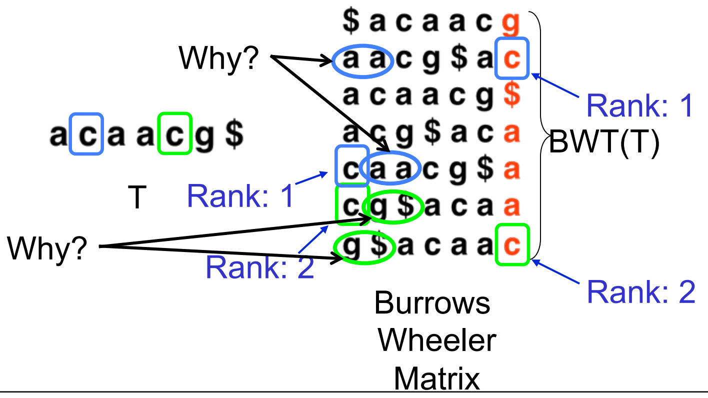
Burrows-Wheeler Transform

- Property that makes BWT(T) reversible is "LF Mapping"
  - ith occurrence of a character in Last column is same ***text*** **occurrence as the ith occurrence in First column**



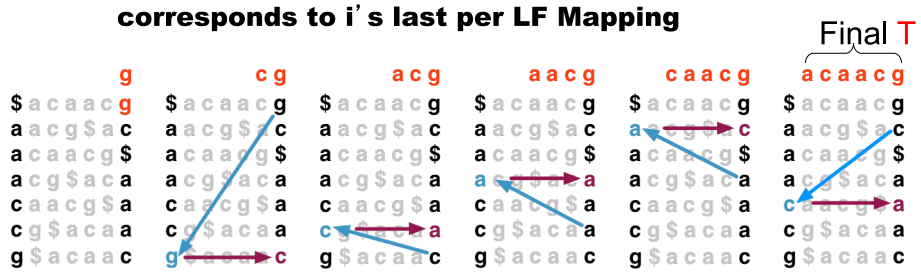
Burrows-Wheeler Transform

- Property that makes BWT(T) reversible is "LF Mapping"
  - **ith occurrence of a character in Last column is same** ***text*** **occurrence as the ith occurrence in First column**



Burrows-Wheeler Transform

- To recreate T from BWT(T), repeatedly apply rule:
  - **T = BWT[LF(i)] + T; i = LF(i)**
    - **Where LF(i) maps row i to row whose first character corresponds to i's last per LF Mapping**



- Could be called "unpermute" or "walk-left" algorithm

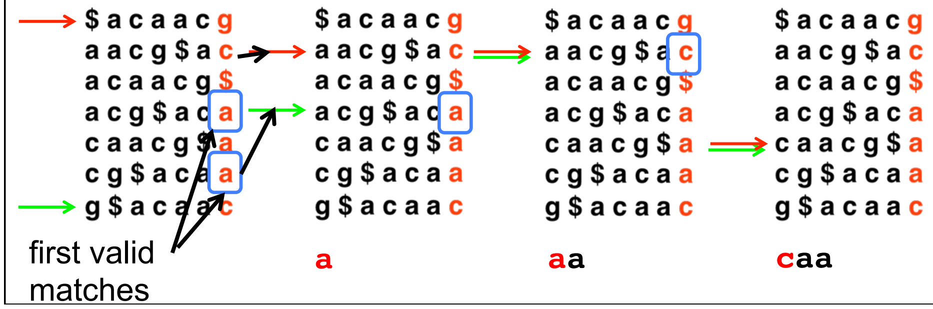
FM Index

- Ferragina & Manzini propose "FM Index" based on BWT
- Observed:
  - **LF Mapping also allows *exact matching* within T**
  - **LF(i) can be made fast with *checkpointing***
  - **...and more (see FOCS paper)**

- Ferragina P, Manzini G: Opportunistic data structures with applications. *FOCS. IEEE Computer Society; 2000.*
- Ferragina P, Manzini G: An experimental study of an opportunistic index. *SIAM symposium on Discrete algorithms*. Washington, D.C.; 2001.

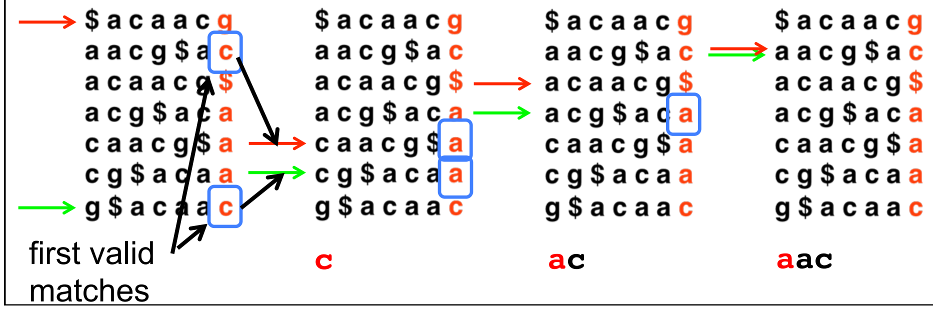
Exact Matching with FM Index

- Look up pattern in reverse.
- Use 2 pointers to represent range of matches.
  - **All matches will be next to each other in matrix.**
- Find first valid match for next symbol in range.
  - **Example: searching for "caa"**



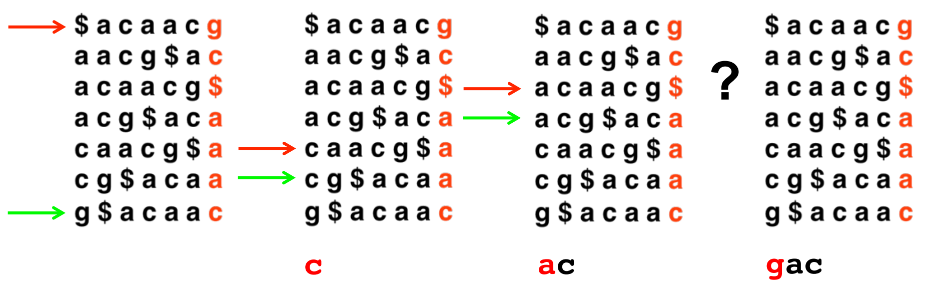
Exact Matching with FM Index

- Look up pattern in reverse.
- Use 2 pointers to represent range of matches.
- Find first valid match for next symbol in range.
  - **Example: searching for "aac"**



Exact Matching with FM Index

- If no match…
  - **Example: searching for “gac”**

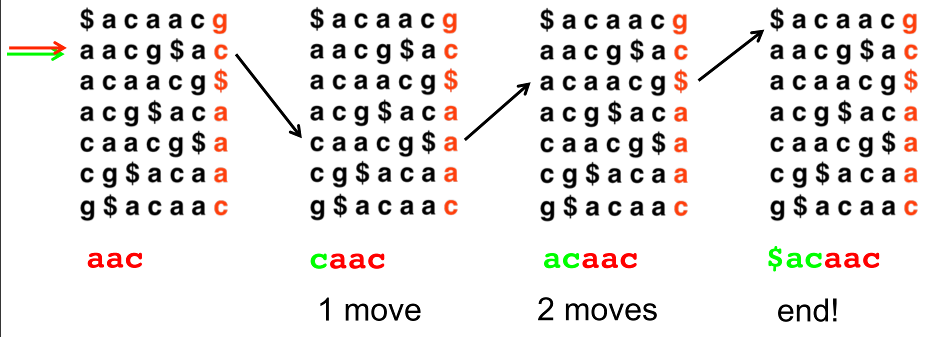


```
$acaacg   $acaacg   $acaacg     $acaacg
aacg$ac   aacg$ac   aacg$ac  ?  aacg$ac
acaacg$   acaacg$   acaacg$     acaacg$
acg$aca   acg$aca   acg$aca     acg$aca
caacg$a   caacg$a   caacg$a     caacg$a
cg$acaa   cg$acaa   cg$acaa     cg$acaa
g$acaac   g$acaac   g$acaac     g$acaac

          c         ac          gac
```

- Pointers will get lost.
- FM index can quickly check for a match.

Where in sequence is the match?

- Use “walk-left” to build sequence to start
- Count number of sequences
  - **Example: searching for “aac”**

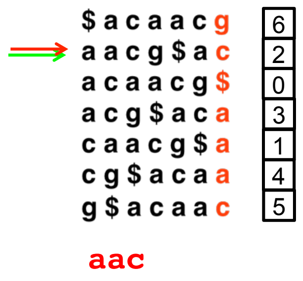


```
$acaacg   $acaacg   $acaacg   $acaacg
aacg$ac   aacg$ac   aacg$ac   aacg$ac
acaacg$   acaacg$   acaacg$   acaacg$
acg$aca   acg$aca   acg$aca   acg$aca
caacg$a   caacg$a   caacg$a   caacg$a
cg$acaa   cg$acaa   cg$acaa   cg$acaa
g$acaac   g$acaac   g$acaac   g$acaac

aac       caac      acaac     $acaac

          1 move    2 moves   end!
```

- Number of moves back is start position of match
  - **Example: “aac” is in position 2.**

Where in sequence is match?

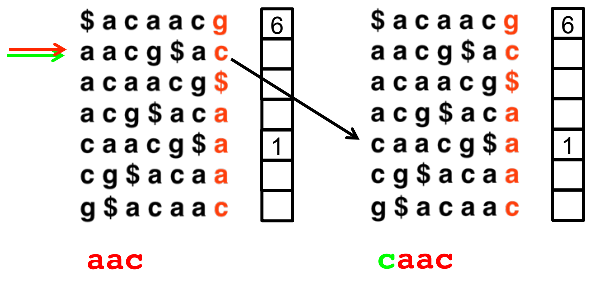
- "walk-left" to start of sequence is slow
- Requires on average N/2 steps to reach start.
- Alternate strategy: keep index of positions.
  - **Example: searching for "aac"**



- Problem: requires as much storage as hashtable!

Where in sequence is match?

- Key Idea: Store fraction of array (sampling)
- Only store some positions and "walk-left"
- Combines two previous strategies
  - **Example: searching for "aac"**



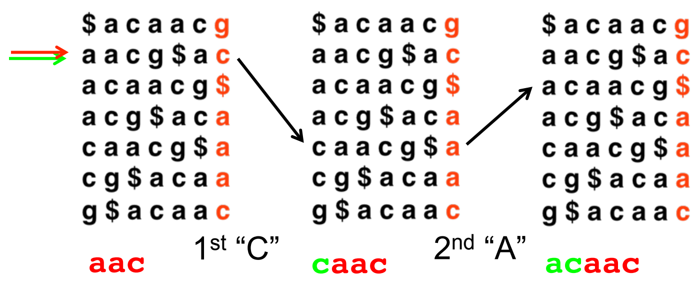
Position =
number of moves
+ position in array

For "aac" = 1 + 1 = 2

- How many values to store provides defines time/space tradeoff.

"walk-left" optimization

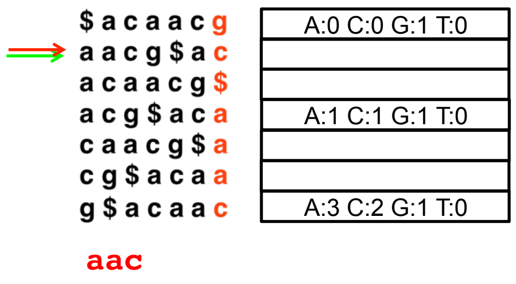
- Each "walk-left" requires counting previous occurrences of symbol in BWT
  - Example: searching for "aac"



- Requires counting occurrences in N/2 length string
- Really slow!

"walk-left" optimization

- Idea: use checkpoints to store previous counts
  - Example: searching for "aac"



A:0 C:0 G:1 T:0
A:1 C:1 G:1 T:0
A:3 C:2 G:1 T:0

- Requires counting occurrences only until checkpoint.
- Really fast!

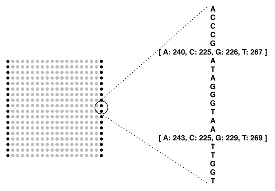
FM Index is Small (Bowtie)

- 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

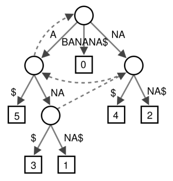
Assuming 2-bit-per-base encoding and no compression, as in Bowtie

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

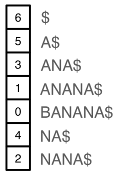
Assuming Bowtie defaults for suffix-array sampling rate, etc


[A: 240, C: 225, G: 226, T: 267]
[A: 243, C: 225, G: 229, T: 269]

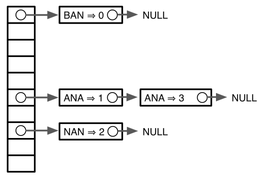
~1.65x



>45x



>15x



>15x

Reference Paper

- Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biology 10:R25.
  - (Some slides from paper)