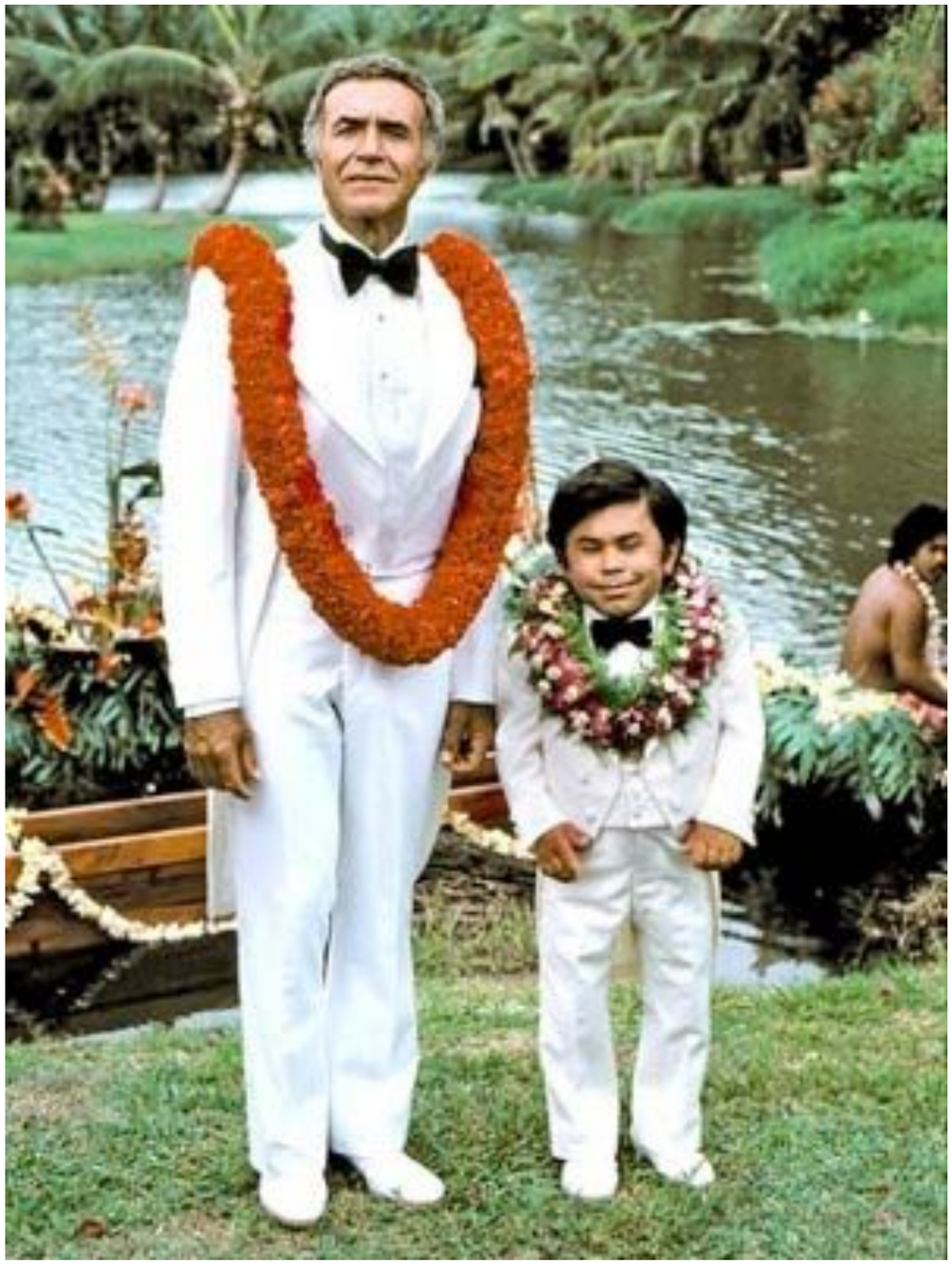
Exact String Matching and searching for SNPs

CMSC423

What makes them different?



Much human variation is due to difference in ~ 6 million base pairs (0.1 % of genome) referred to as SNPs

Single Nucleotide Polymorphism (SNP)

Genomic DNA:

TACATAGCCATCGGTA[A/G]GTACTCAATGATGATA

Three genotypes

AA

Mother

TACATAGCCATCGGTAAGTACTCAATGATGATA
ATGTATCGGTAGCCATTCATGAGTTACTACTAT

Father

TACATAGCCATCGGTAAGTACTCAATGATGATA
ATGTATCGGTAGCCATTCATGAGTTACTACTAT

AG

Mother

TACATAGCCATCGGTAAGTACTCAATGATGATA
ATGTATCGGTAGCCATTCATGAGTTACTACTAT

Father

TACATAGCCATCGGTAGGTACTCAATGATGATA
ATGTATCGGTAGCCATCCATGAGTTACTACTAT

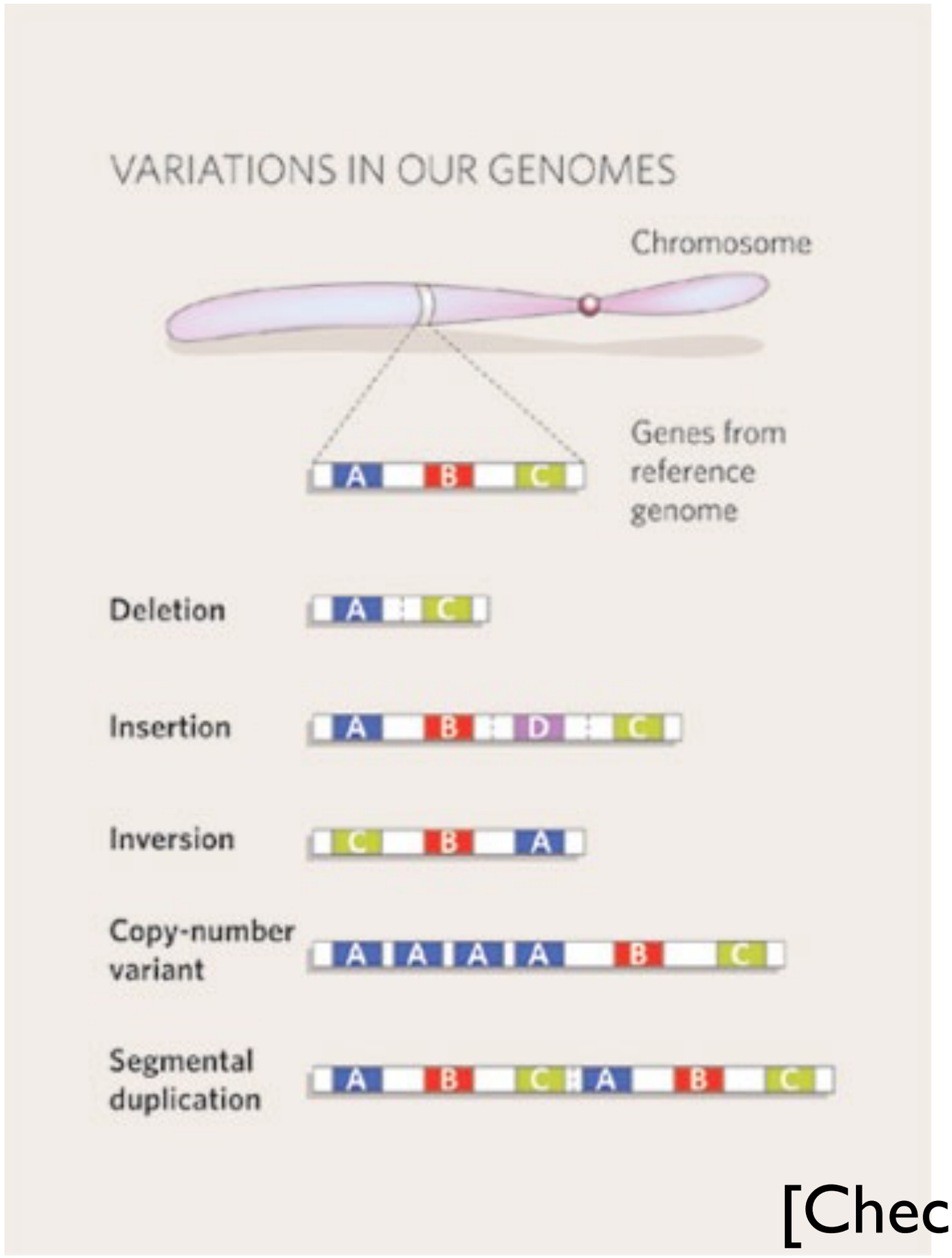
GG

Mother

TACATAGCCATCGGTAGGTACTCAATGATGATA
ATGTATCGGTAGCCATCCATGAGTTACTACTAT

Father

TACATAGCCATCGGTAGGTACTCAATGATGATA
ATGTATCGGTAGCCATCCATGAGTTACTACTAT



[Check, Nature 437]

DNA Sequence Variation in a Gene Can Change the Protein Produced by the Genetic Code

Gene A from Person 1

GCA AGA GAT AAT TGT...

Ala (1) Arg (2) Asp (3) Asn (4) Cys (5) ...

Protein Products

Gene A from Person 2

Codon change made no difference in amino acid sequence

GCG AGA GAT AAT TGT...

Ala (1) Arg (2) Asp (3) Asn (4) Cys (5) ...

Gene A from Person 3

Codon change resulted in a different amino acid at position 2

GCA AAA GAT AAT TGT...

Ala (1) Lys (2) Asp (3) Asn (4) Cys (5) ...

OR

Y-GA 98-649

Health or Disease?

DNA Sequence

Person 1 A A A T T T

Normal protein

Some DNA variations have no negative effects

Person 2 A A T T T T

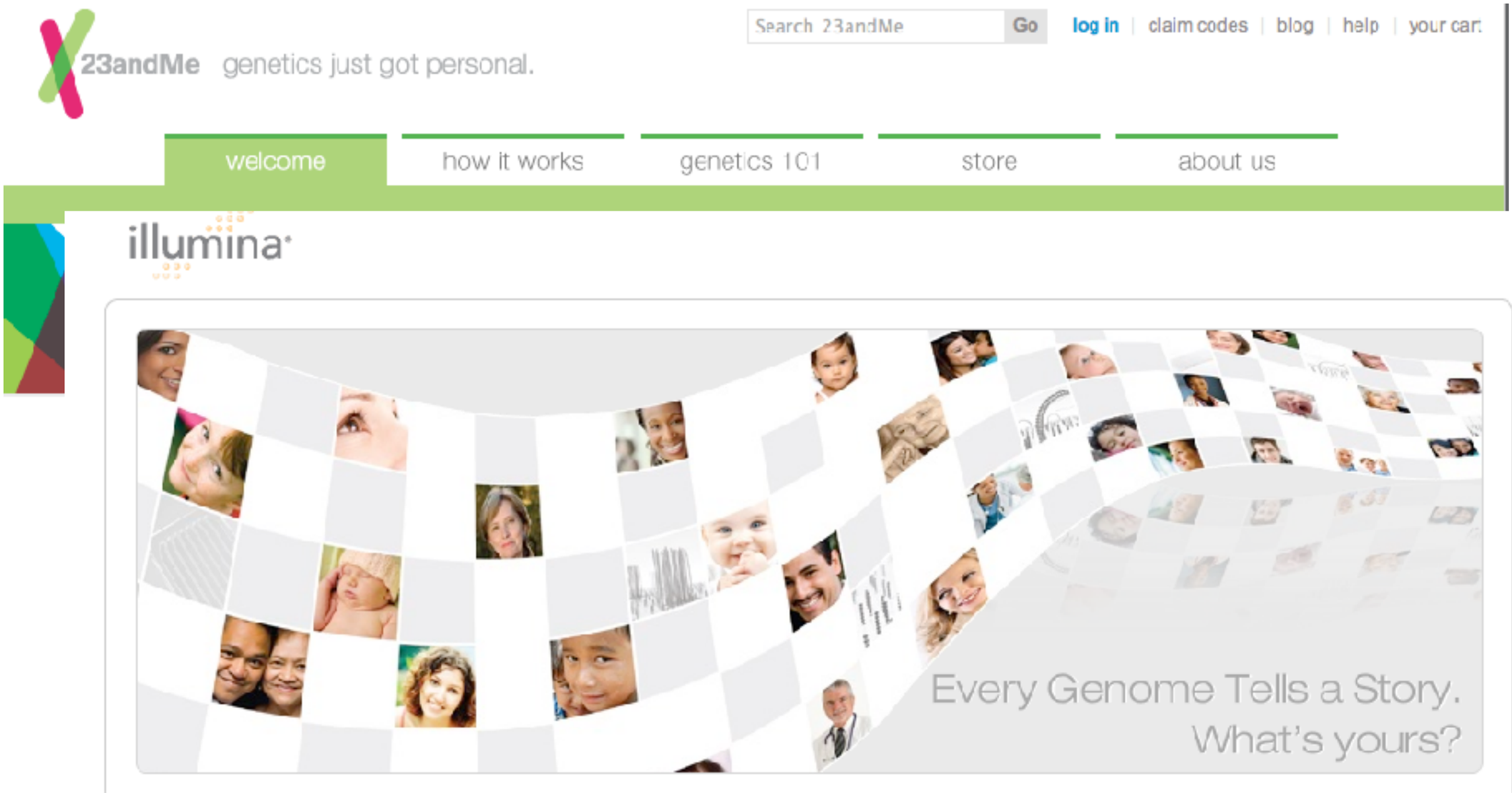
Low or nonfunctioning protein

Person 3 A A C T T T

Other variations lead to disease (e.g., sickle cell) or increased susceptibility to disease (e.g., lung cancer)

YGG-00-0480

Personal Genomics



Next-gen Sequencing

illumina

life technologies

Semiconductor Sequencing for Life™

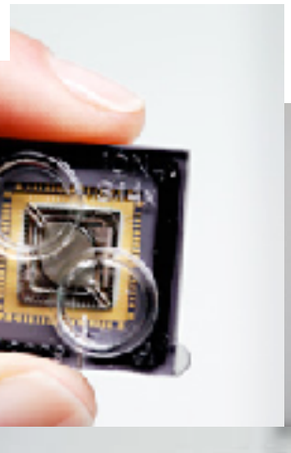
ion torrent

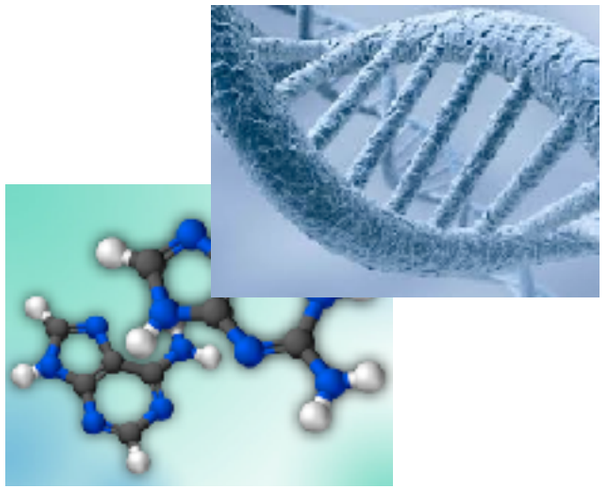
NANOPORE

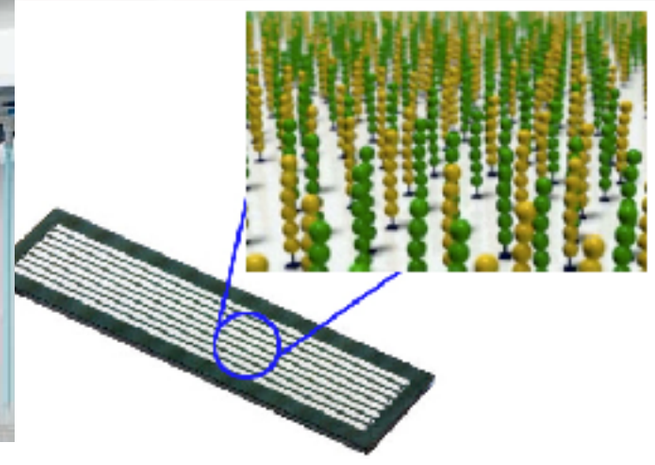
454 SEQUENCING

Roche

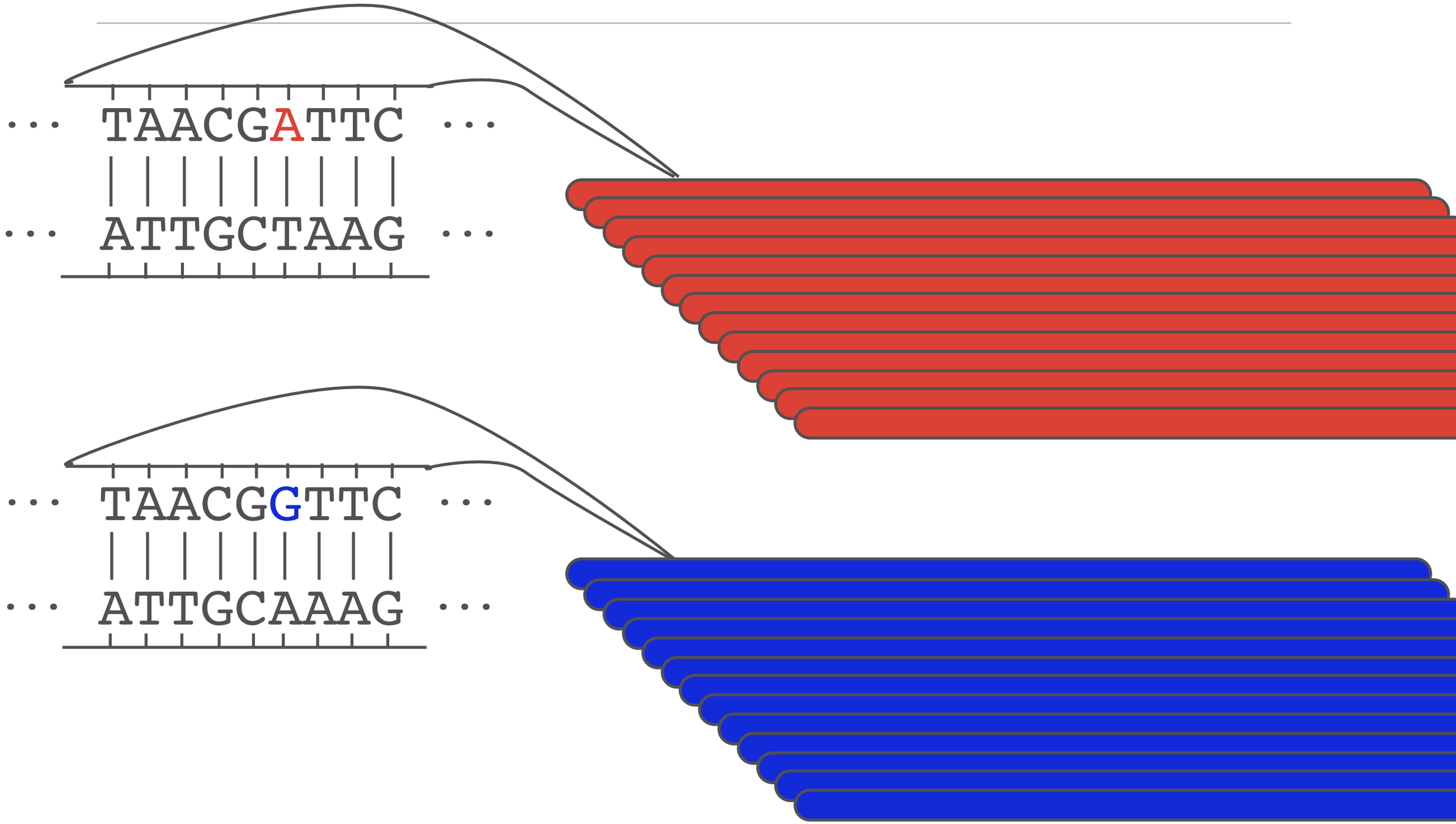
Applied Biosystems



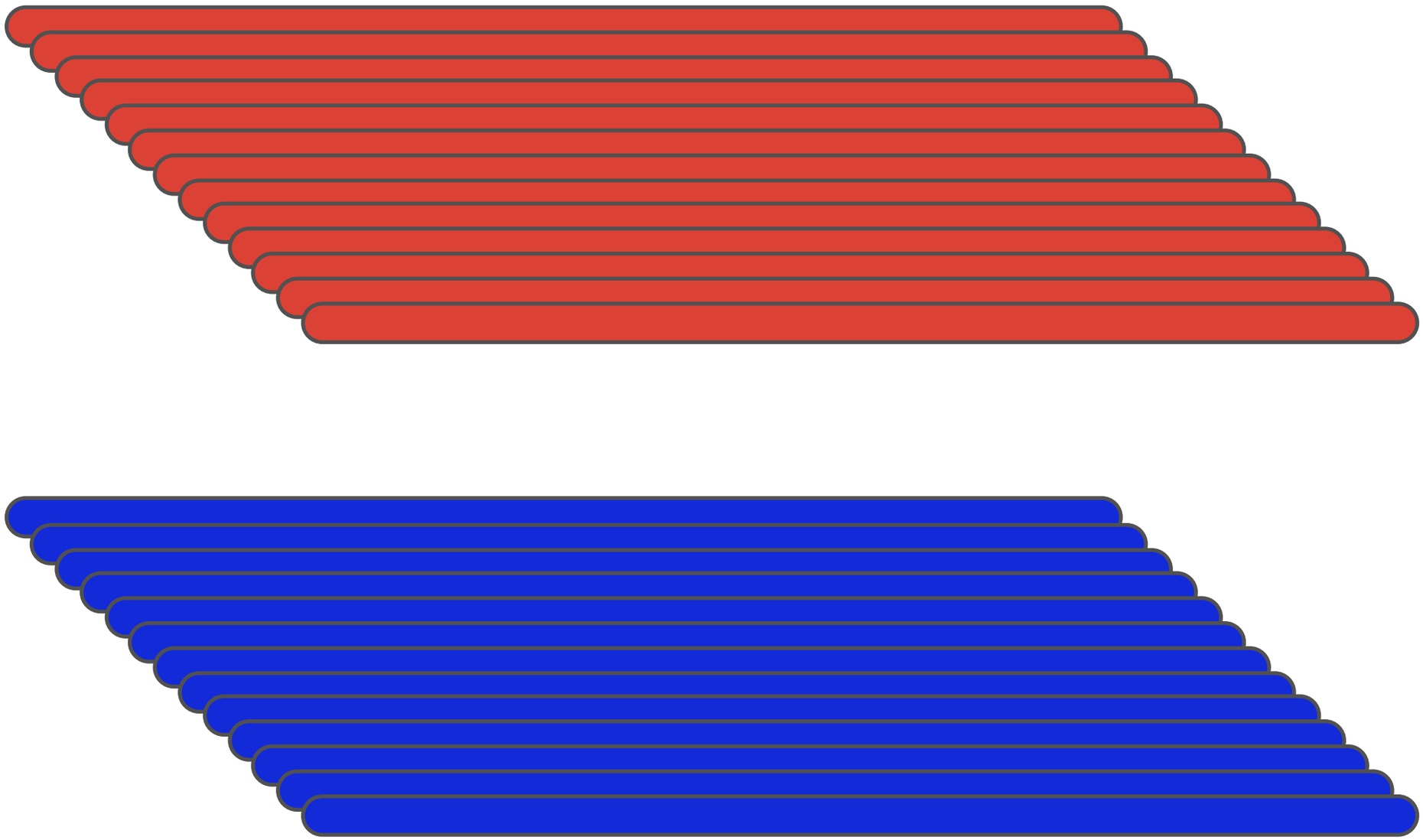




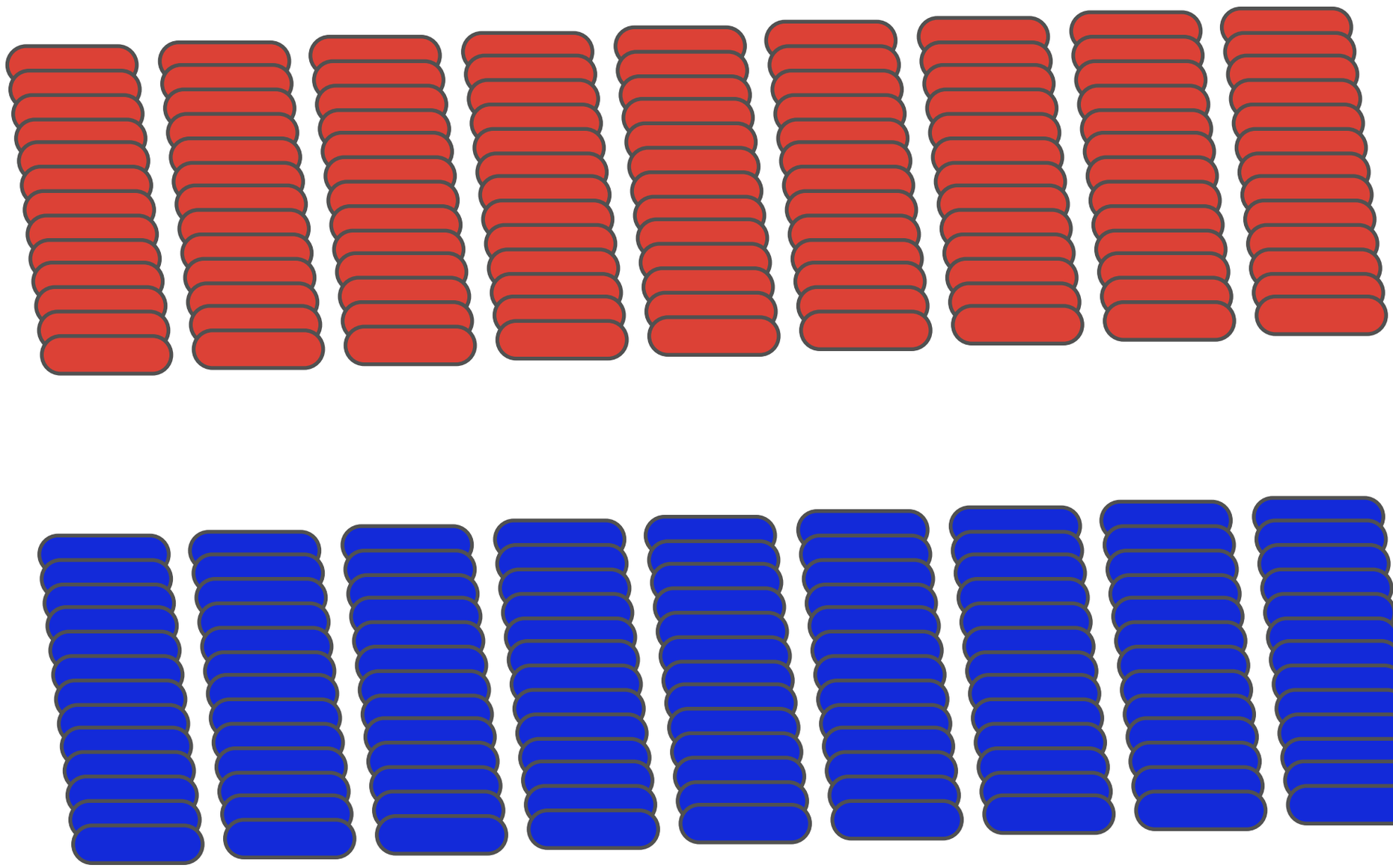
Sec-gen Sequencing for SNPs



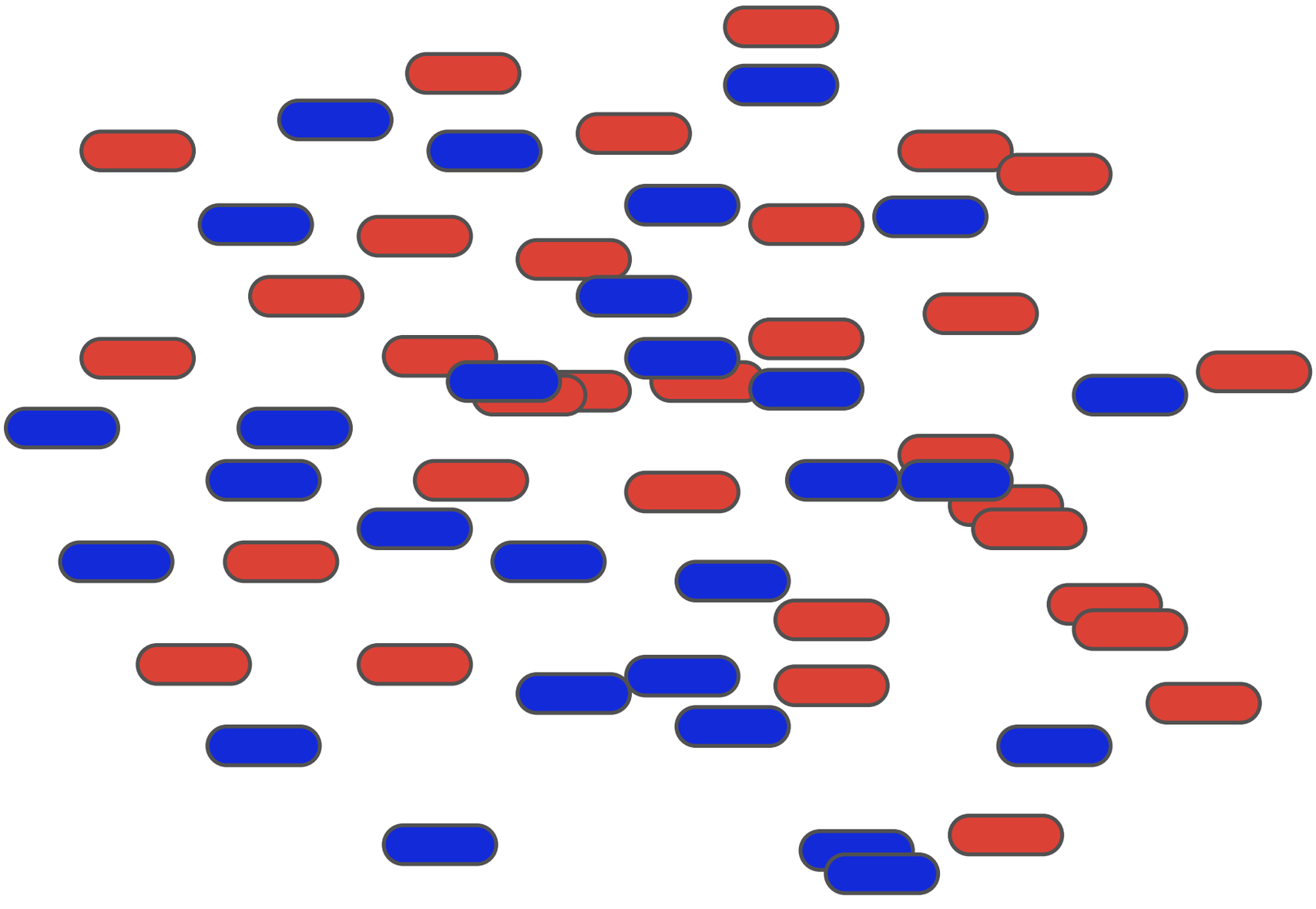
Sec-gen Sequencing for SNPs



Sec-gen Sequencing for SNPs



Sec-gen Sequencing for SNPs



Sec-gen Sequencing for SNPs

Align

```
GTCGCAGTANCTGTCT
|||||||||| ||||||
GTCGCAGTATCTGTCT

GGATCTGCGATATACC
|||||| ||||||||||
GGATCT-CGATATACC

AATCTGATCTTATTTT
||||||||||||||||
AATCTGATCTTATTTT

ATATATATATATATAT
||||||||||||||||
ATATATATATATATAT

TCTCTCCCANNAGAGC
||||||||| |||||
TCTCTCCCAGGAGAGC
```

"Pileup" or "Coverage plot"

"Depth of coverage" = 14

Aggregate

Statistics

```
GTCGCAGTATCTG CT
GTCGCAGTATCTGTNN
TGTCGCAGTATCTGTC
TATGTCGCAGTATCTG
TATATCGCAGTATCTT
TATATCGCAGTATCTG
NATATCGCAGTATNTG
CCCTATATCGCAGTAT
ACACCCTATGTCGCA
ACACCCTATCTCGCA
ACACCCTATGTCGCA
GA-CACCCTATGTCGC
CCGGA-CACCCTATAT
CCGGA-CACCCTATAT
GCCGGA-CACCCTATG
```

Call: HET A, G
p-value: 0.0023

"Coverage"

```
TGGTATTTTCGTCTGGGGGGTATGCACGCGATAGCATTGCGAGACGCTGGAGCCGGAGCACCCTATGTCGCAGTATCTGTCTTTGATTCCTGCCTCATCCTATTAT
```

Reference

(slide courtesy of Ben Langmead)

The problem

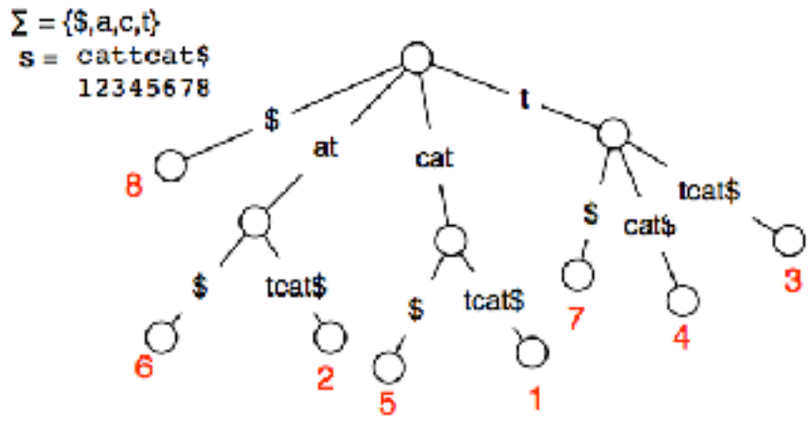
- Given:
  - 100's of millions of short reads: 100-200bp reads
  - A long reference genome (~3Bbp for human)
- Do:
  - Find high scoring scoring (fitting) alignments for each read
- What we know:
  - Dynamic programming solution for fitting alignment:
    - 1e8 * 1e9 * 1e2 operations, 1e9 * 1e2 memory

Strategies

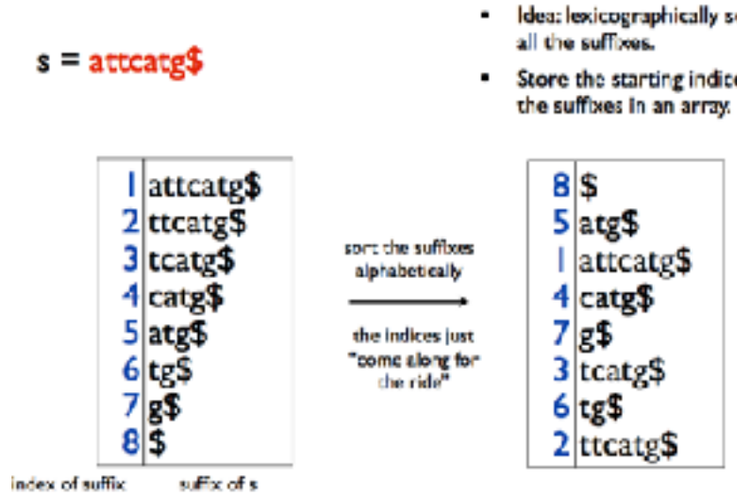
- What if we only allow a small number of substitutions?
  - Let's first try to find *exact* matches and work from those (the d+1 trick in the midterm)
- We are aligning to the same reference 100's of millions of time
  - Is there preprocessing we can do to amortize time?
- Genomes are repetitive
  - Can we search for matches in the genome in a smart way?
  - Can we compress the genome, and search over the compressed representation?

Exact Matching

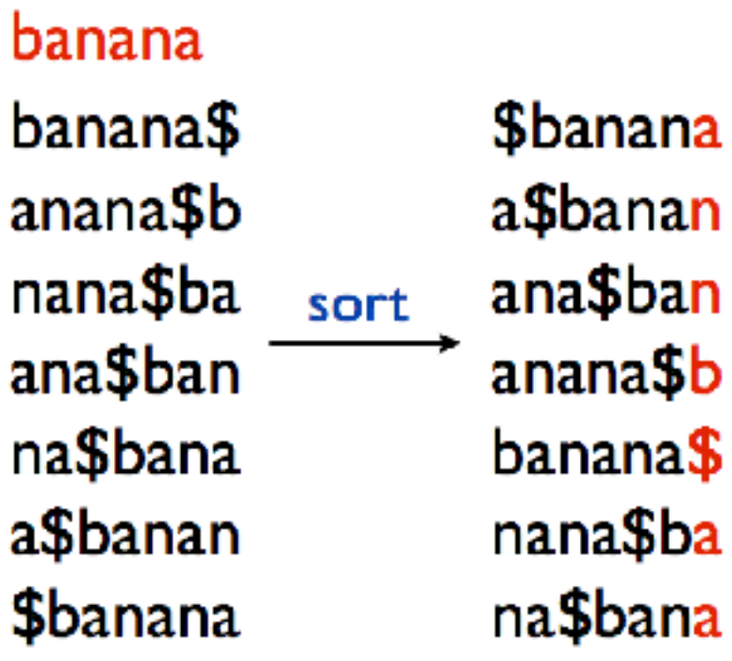
- Suffix Tries/Trees



- Suffix Arrays

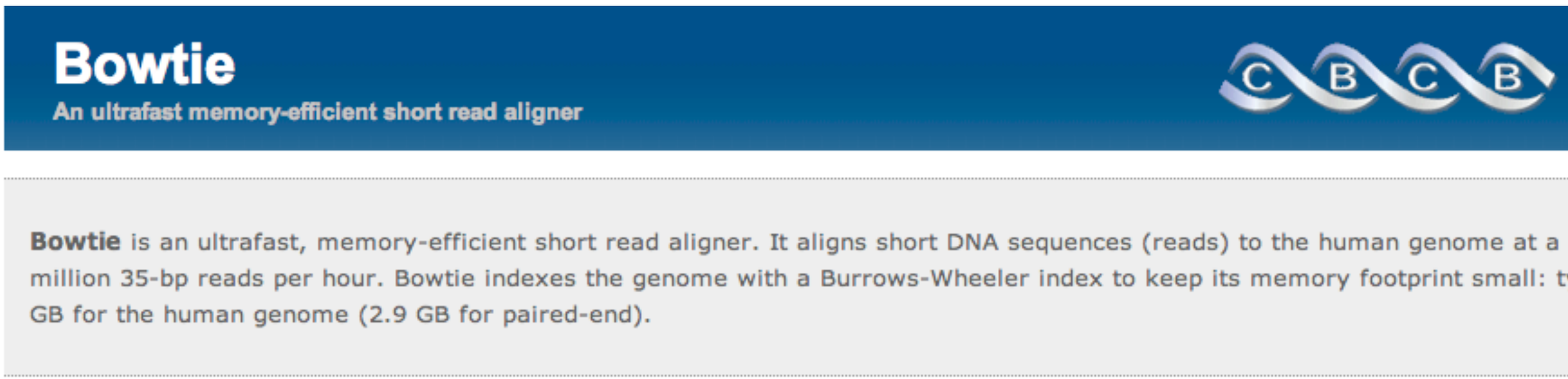


- The Burrows-Wheeler transform



State of the Art

- Bowtie: ultra-fast mapping of short reads to reference genome

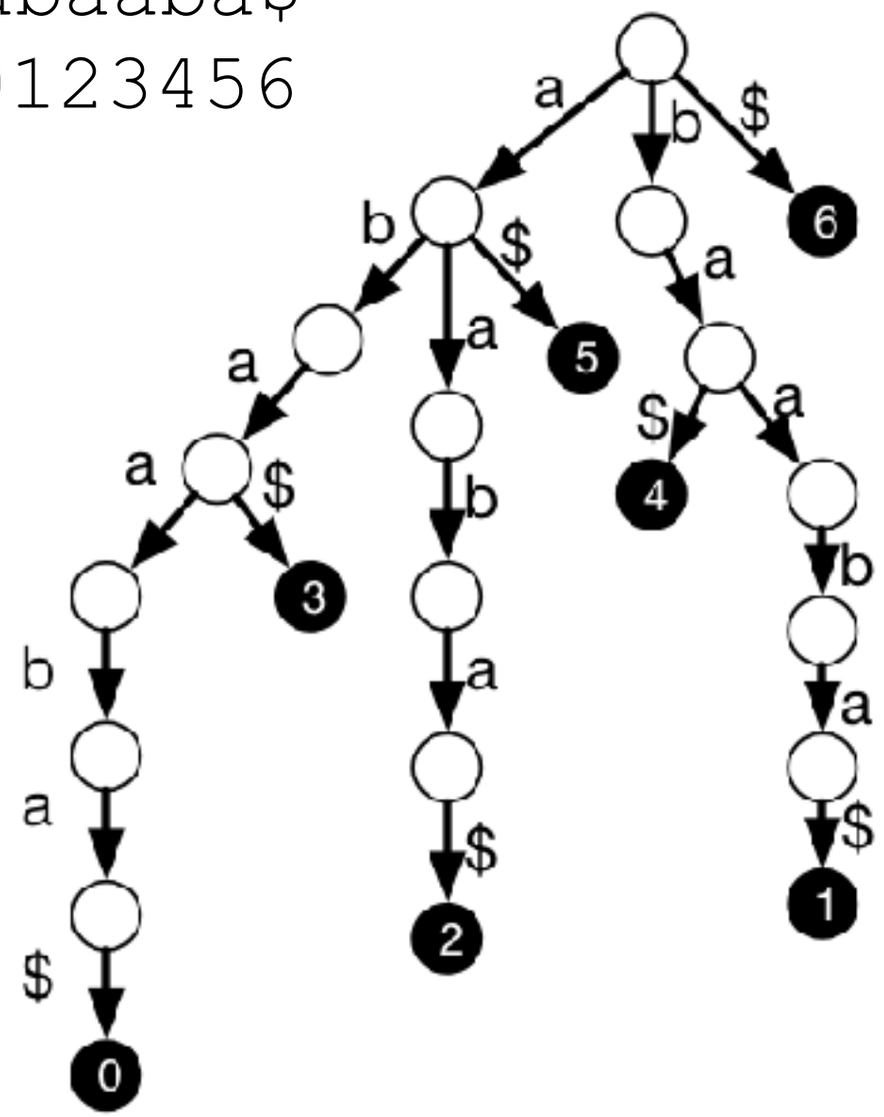


- http://bowtie-bio.sourceforge.net

First, we get some inspiration from KMP algorithm and fundamental preprocessing

Suffix Trie

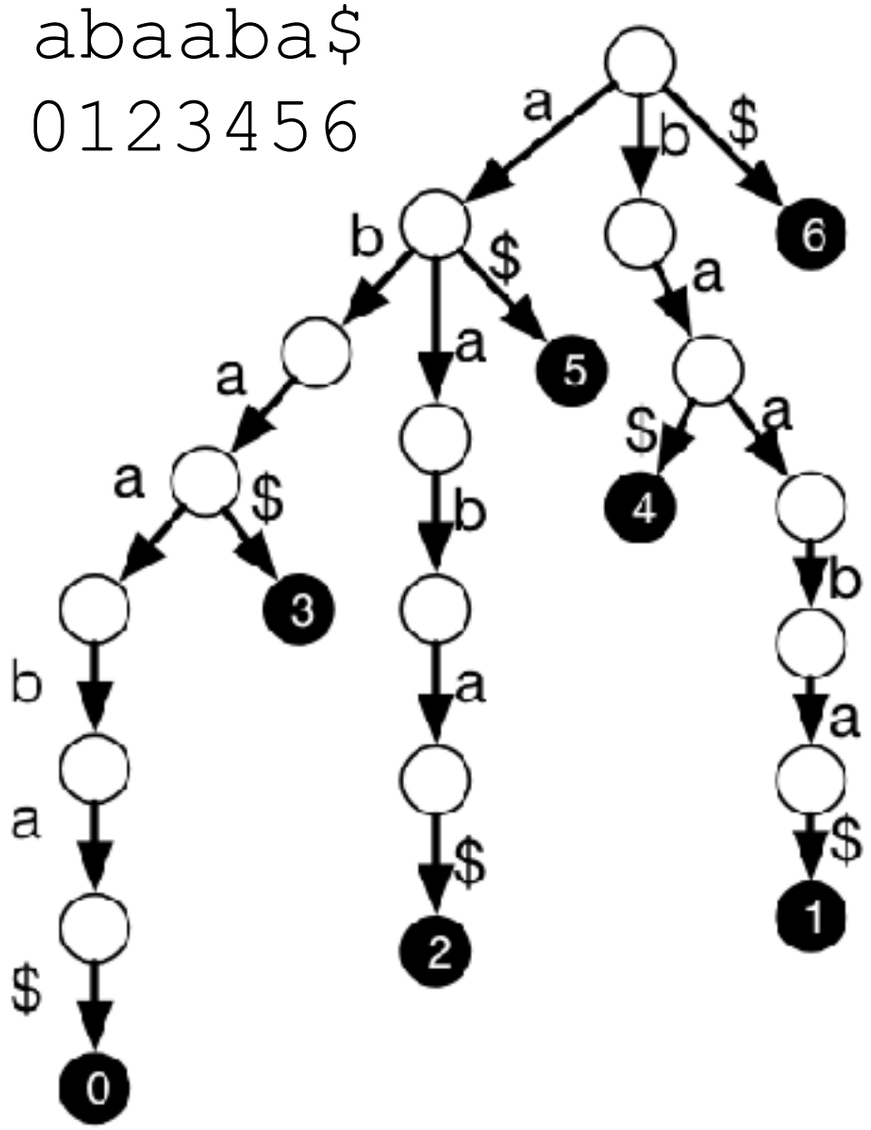
T: abaaba$
0123456



- Edges labeled with characters from alphabet.
- Each path from root to leaf corresponds to a *suffix* of T.

Suffix Trie

T: abaaba$
0123456



- Naive construction algorithm is O(|T|²)
  - There are linear time construction algorithms (see Gusfield)
- Memory requirement is also O(|T|²)
- Time to find matches O(|P|)

1) Compute Suffix Trie of

abbaaba$

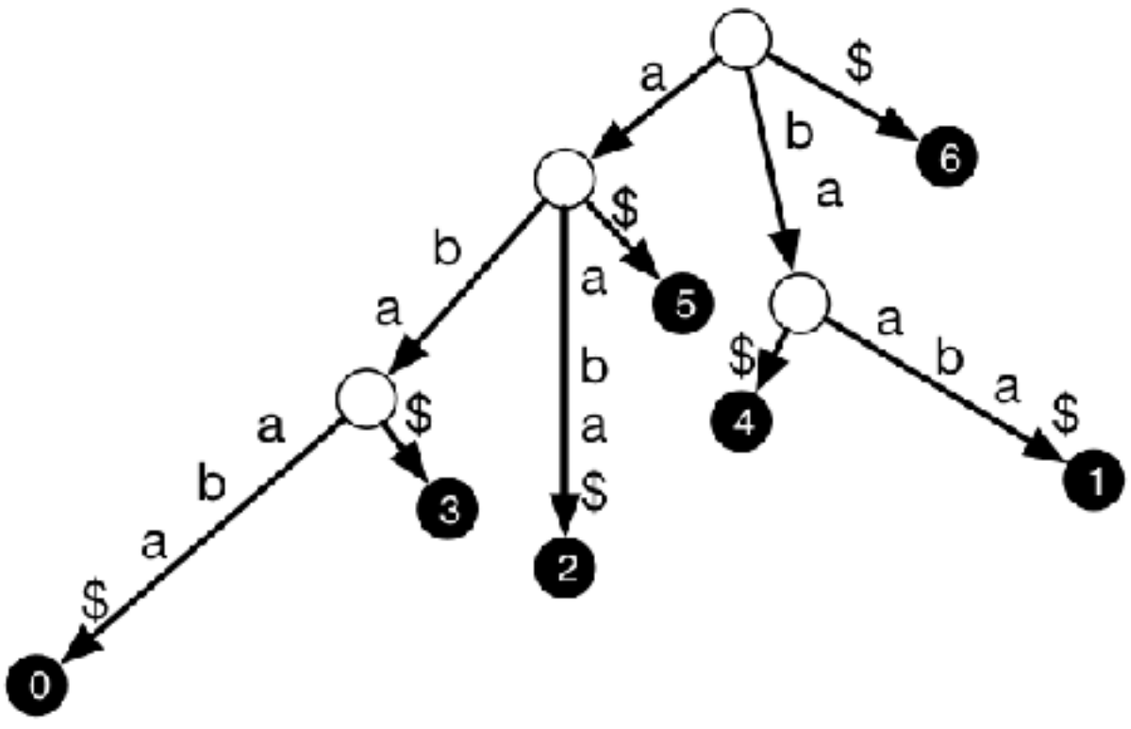
2) Mark search path for pattern baab

3) Algorithm: given string S, and k-mer length k, uses a suffix trie to compute (sk, i) for **every** k-mer sk in S, where i is the number of times it occurs in S

4) Compute Suffix Tree of #1

Suffix Tree

T: abaaba$
0123456

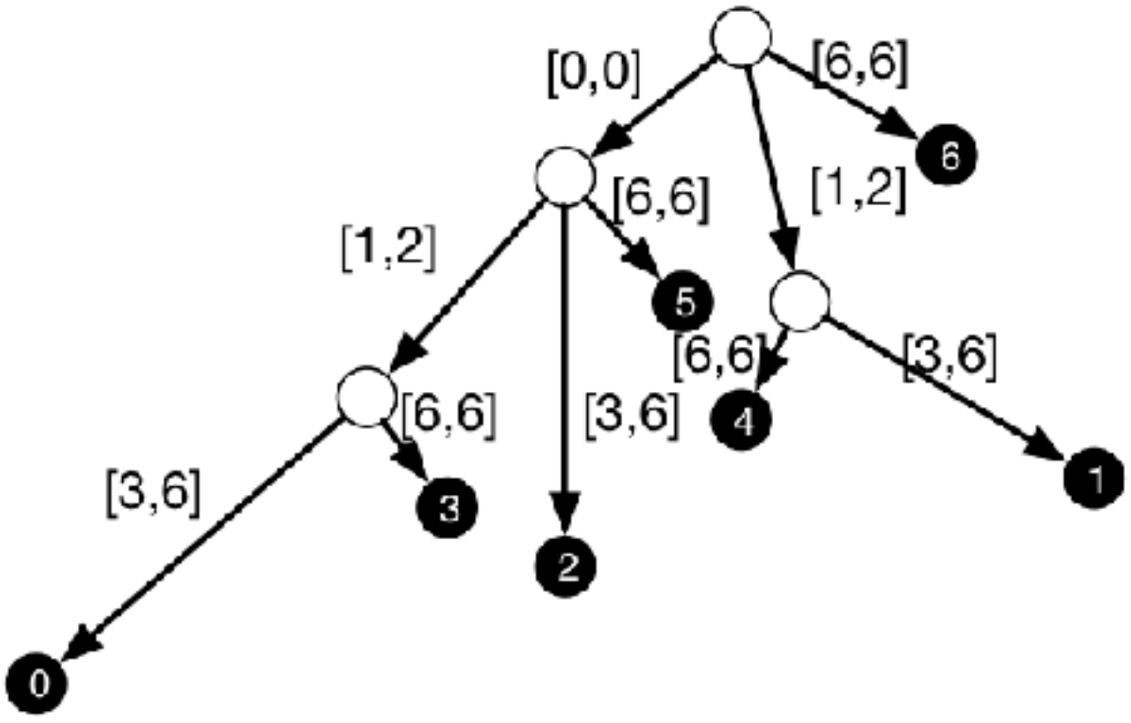


- Collapse non-branching nodes
  - #nodes O(|T|)

- Memory requirement is *not* O(|T|)
  - In the worst case, space required for edge labels is O(|T|)

Suffix Tree

T: abaaba$
0123456



- Collapse non-branching nodes
  - #nodes O(|T|)
- Label edges with substring *[start,end]*
  - O(1) per edge
- Memory now O(|T|)
- Construction algorithm O(|T|) (see Gusfield)

Suffix Arrays

- Even though Suffix Trees are O(n) space, the constant hidden by the big-Oh notation is somewhat "big": ≈ 20 bytes / character in good implementations.

- If you have a 10Gb genome, 20 bytes / character = 200Gb to store your suffix tree. "Linear" but large.

- Suffix arrays are a more efficient way to store the suffixes that can do most of what suffix trees can do, but just a bit slower.

- Slight space vs. time tradeoff.

Example Suffix Array

s = attcatg$

- Idea: lexicographically sort all the suffixes.
- Store the starting indices of the suffixes in an array.

1	attcatg$
2	ttcatg$
3	tcatg$
4	catg$
5	atg$
6	tg$
7	g$
8	$

index of suffix suffix of s

sort the suffixes alphabetically

the indices just "come along for the ride"

8	$
5	atg$
1	attcatg$
4	catg$
7	g$
3	tcatg$
6	tg$
2	ttcatg$

Example Suffix Array

s = attcatg$

- Idea: lexicographically sort all the suffixes.
- Store the starting indices of the suffixes in an array.

1	attcatg$
2	ttcatg$
3	tcatg$
4	catg$
5	atg$
6	tg$
7	g$
8	$

index of suffix suffix of s

sort the suffixes alphabetically

the indices just "come along for the ride"

8
5
1
4
7
3
6
2

Another Example Suffix Array

s = cattcat$

- Idea: lexicographically sort all the suffixes.
- Store the starting indices of the suffixes in an array.

1	cattcat$
2	attcat$
3	ttcat$
4	tcat$
5	cat$
6	at$
7	t$
8	$

index of suffix suffix of s

sort the suffixes alphabetically

the indices just "come along for the ride"

8	$
6	at$
2	attcat$
5	cat$
1	cattcat$
7	t$
4	tcat$
3	ttcat$

Another Example Suffix Array

s = cattcat$

- Idea: lexicographically sort all the suffixes.
- Store the starting indices of the suffixes in an array.

1	cattcat$
2	attcat$
3	ttcat$
4	tcat$
5	cat$
6	at$
7	t$
8	$

index of suffix suffix of s

sort the suffixes alphabetically

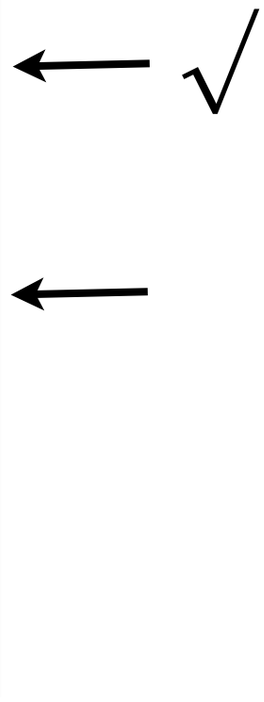
the indices just "come along for the ride"

8
6
2
5
1
7
4
3

Search via Suffix Arrays

s = cattcat$

8	$
6	at$
2	attcat$
5	cat$
1	cattcat$
7	t$
4	tcat$
3	ttcat$



- Does string "at" occur in s?
- Binary search to find "at".

- What about "tt"?

Counting via Suffix Arrays

s = cattcat$

8	$
6	at$
2	attcat$
5	cat$
1	cattcat$
7	t$
4	tcat$
3	ttcat$

- How many times does “at” occur in the string?
- All the suffixes that start with “at” will be next to each other in the array.
- Find one suffix that starts with “at” (using binary search).
- Then count the neighboring sequences that start with at.

Constructing Suffix Arrays

- Easy $O(n^2 \log n)$ algorithm:

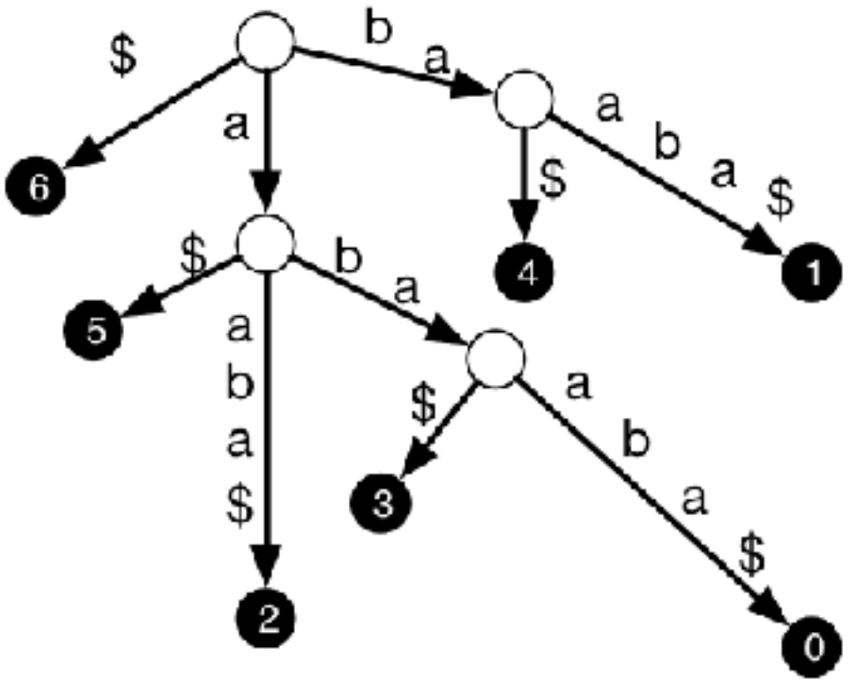
  sort the n suffixes, which takes O(n log n) comparisons, where each comparison takes O(n).

- There are several direct O(n) algorithms for constructing suffix arrays that use very little space.

- An simple O(n) algorithm: build the suffix tree, and exploit the relationship between suffix trees and suffix arrays (next slide)

Relationship between Suffix Arrays and Suffix Trees

T: abaaba$
0123456



6	$
5	a$
2	aaba$
3	aba$
0	abaaba$
4	ba$
1	baaa$

Build suffix trees with edge labels sorted lexicographically
Order of leaves: 6,5,2,3,0,4,1

Recap

Structure	Processing Time	Memory	Search
Suffix Trie	O(|T|)	O(|T|^2)	O(|P|)
Suffix Tree	O(|T|)	O(|T|)*	O(|P|)
Suffix Array	O(|T|)	O(|T|) (but **much** smaller than Suffix Tree)	O(|P|log$_2$|T|)

*In best implementations about 20 bytes per character (as opposed to 4 bytes for suffix array)