
reveals

SevenBridges

Short read alignment

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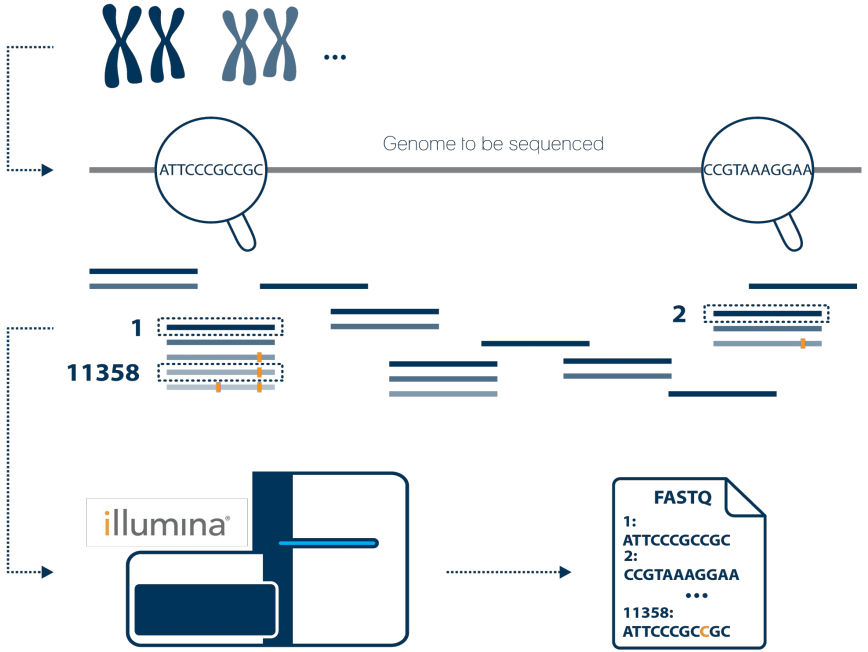
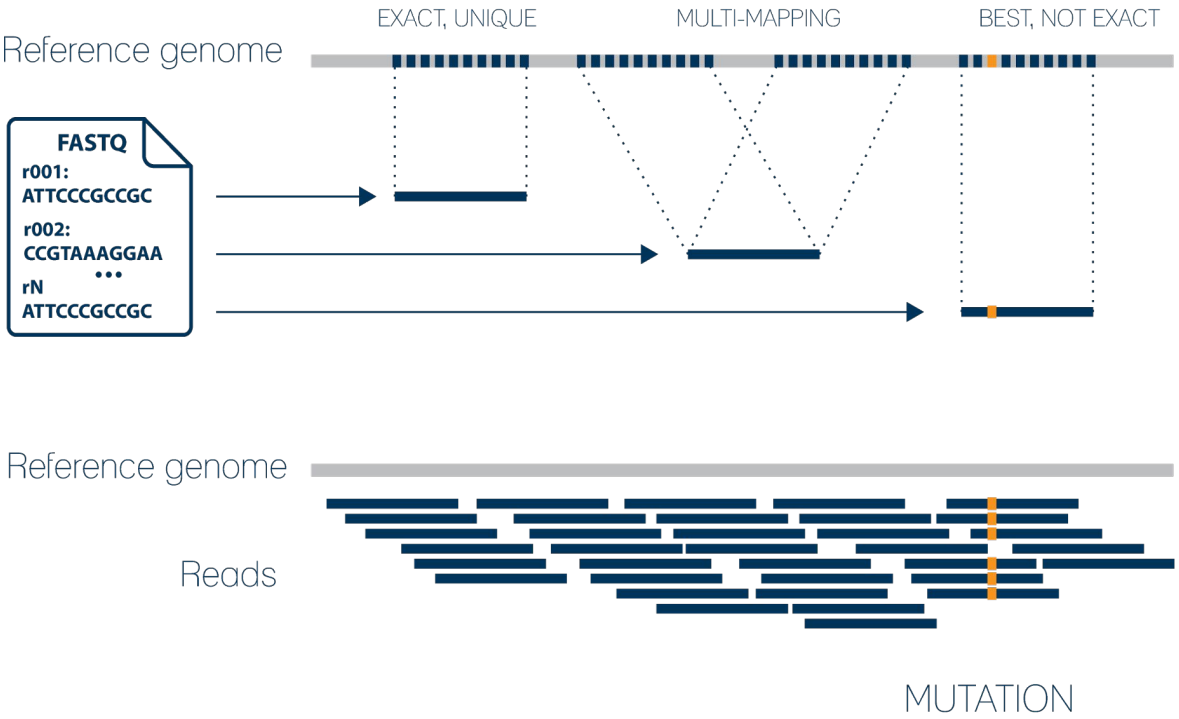
DNA Sequencing - Reminder

- We got a FASTQ files with the “reads” - little pieces of the genome



Alignment



How to align reads against the reference?

Problem description

- Scale
  - 1 billion reads for one human genome in average
  - Human Genome fasta has 3.000.000.000 bp
  - Computationally and memory intensive problem
- Let’s look at simplified situation - only two sequences
  - Read
  - Reference genome
- We need to find where is this read coming from
  - Starting position of the read on the reference genome
  - How does it align
- What are possible approaches?

Brute Force

Brute Force: At every possible offset in the reference check if all of the characters of the query match

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

Brute Force

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Ref.	T	G	A	T	T	A	C	A	G	A	T	T	A	C	C	...
Query		G	A	T	T	A	C	A								

Brute Force

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

Brute Force

This is too slow: For the reference length **n** and query length **m** we would have **(n - m + 1) * m** comparisons.

Run time: **O(nm)**

How can we improve it?

Suffix arrays

Split the reference into n suffixes and sort them alphabetically.

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

#	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

Where is ‘GATTACA’?

#	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

Where is ‘GATTACA’? - **Binary search!**

Step 1
Lo = 1; Hi = 15; Mid = (1+15)/2 = 8
Suffix[8] = CC…
‘GATTACA’ is higher than ‘CC…’
=> Lo = Mid + 1

Step 2
Lo = 9; Hi = 15; Mid = (9+15)/2 = 12
Suffix[12] = TACC…
‘GATTACA’ is lower than ‘TACC…’
=> Hi = Mid - 1

Step 3
Lo = 9; Hi = 11; Mid = (9+11)/2 = 10
Suffix[10] = GATTACC…
‘GATTACA’ is lower than ‘GATTACC…’
=> Hi = Mid - 1

Step 4
Lo = 9; Hi = 9; Mid = (9+9)/2 = 9
Suffix[9] = GATTACA…

=> **Match at position 2!**

Suffix arrays

This is more complicated approach for implementation and takes a lot of memory, but much faster!

Looking up a query 32 times instead of 3 billion as with Brute Force (for the reference with 3 billion base pairs).

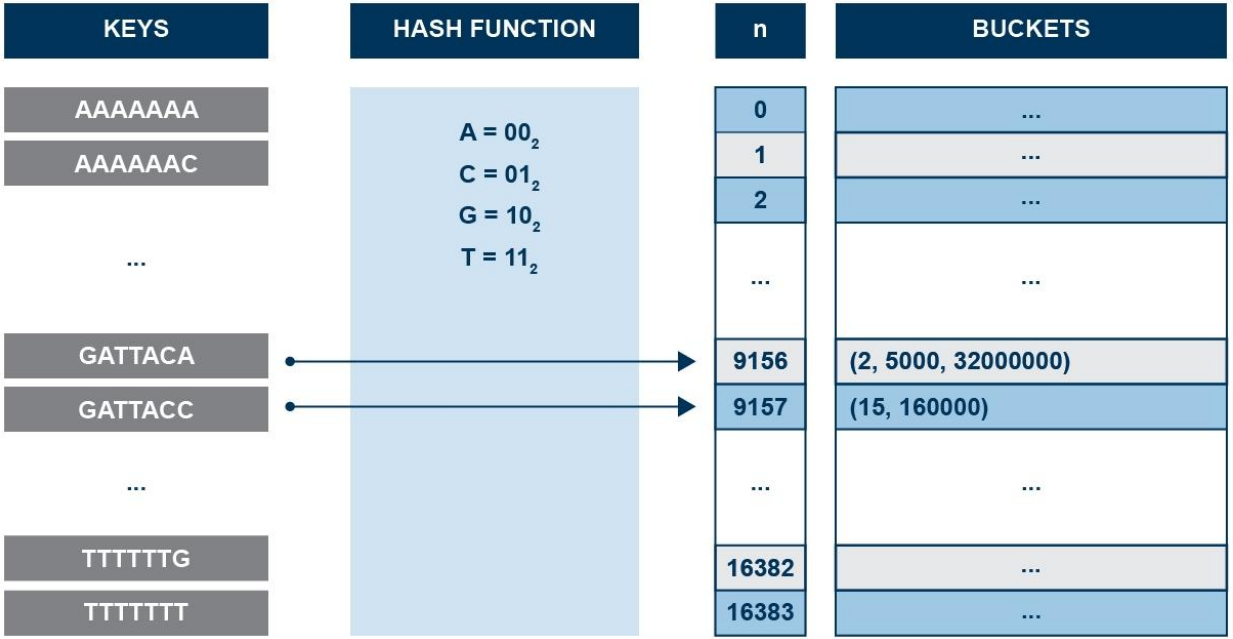
Run time: **$O(m \log_2 n)$**

Can we do it any better?

It would be great if would know in advance the index at which the query is located in the array.

Hash map

Idea: Build a table with every k-mer in the reference.



Hash map

Number of possible sequences of length k?

4^k

$4^7 = 16{,}384$ (easy to store)

$4^{20} = 1{,}099{,}511{,}627{,}776$ (impossible to directly store in RAM!)

But! There are only 3 billion 20-mers in the reference - 99.7% of the table would be empty anyways.

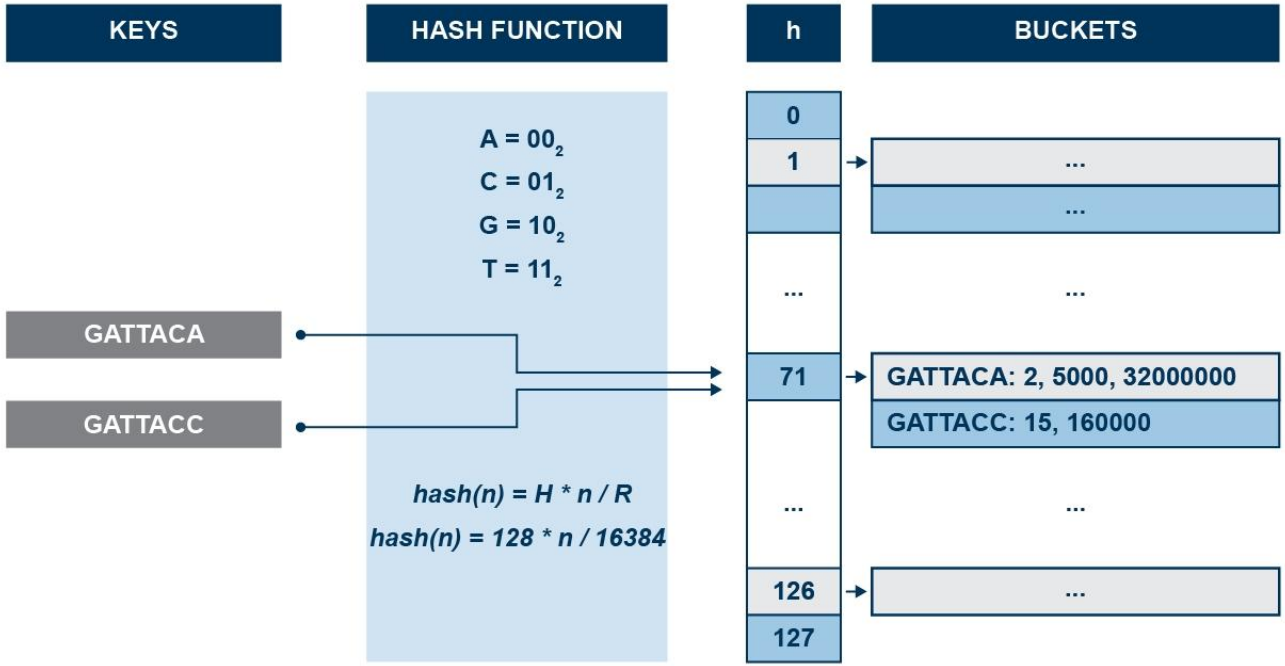
Hash map

- 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 1 billion instead of 1 trillion
  - Division: hash(n) = H * n / R ,
    - H - # of buckets
    - n is sequence number
    - R - total # of sequences
    - hash(GATTACA) = 128 * 9156 / 16384 = 71
  - Modulo: hash(n) = n % H
    - hash(GATTACA) = 9156 % 128 = 68

- By construction, multiple keys have the same hash value

Hash map

- Store elements with the same key in a bucket chained together
- Looking up a value scans the entire bucket

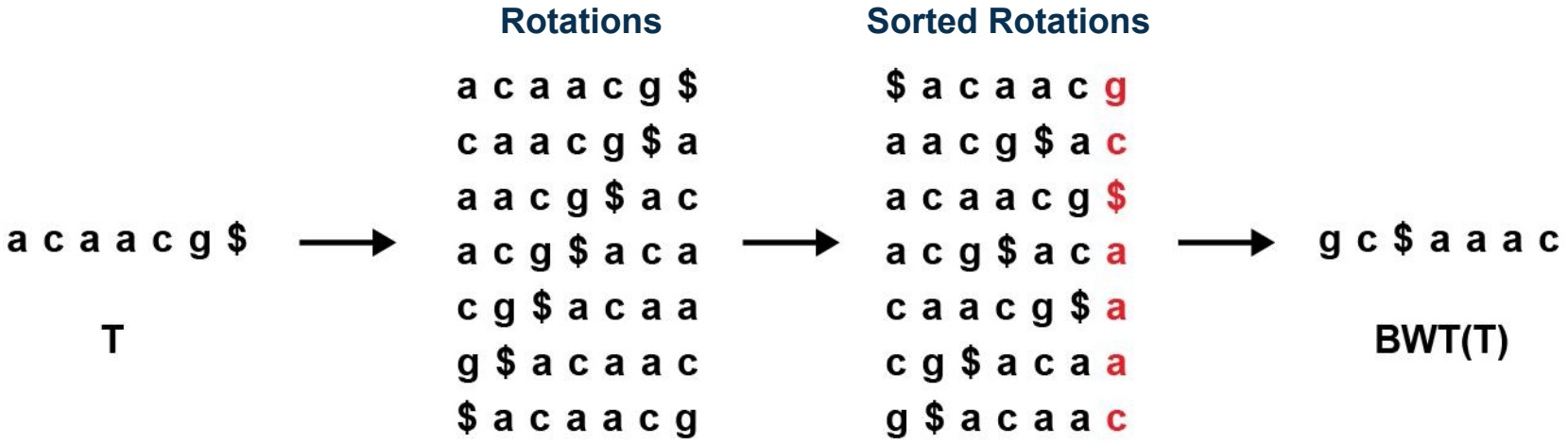


- Run time:
  - Construction: O(n)
  - Lookup: O(1)
  - Sorting the genome mers in linear time

Burrows-Wheeler Transformation

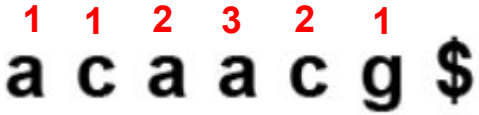
- Reversible lossless transformation algorithm which permutes an input string into a new string

- **BWT** string lends itself to an **effective compression**



Burrows-Wheeler Transformation

- Rank preserving property - needed for **LF** (Last-First) mapping



$$
\begin{array}{l}
\$\ a_1\ c_1\ a_2\ a_3\ c_2\ g_1 \\
a_2\ a_3\ c_2\ g_1\ \$\ a_1\ c_1 \\
a_1\ c_1\ a_2\ a_3\ c_2\ g_1\ \$ \\
a_3\ c_2\ g_1\ \$\ a_1\ c_1\ a_2 \\
c_1\ a_2\ a_3\ c_2\ g_1\ \$\ a_1 \\
c_2\ g_1\ \$\ a_1\ c_1\ a_2\ a_3 \\
g_1\ \$\ a_1\ c_1\ a_2\ a_3\ c_2
\end{array}
$$

Burrows-Wheeler Transformation

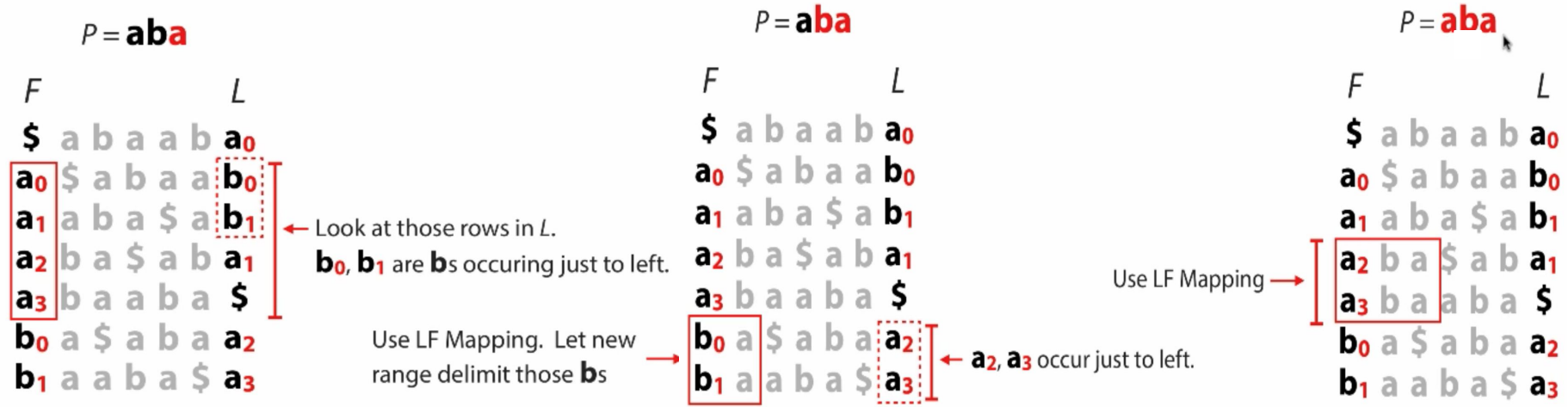
- BWT is reversable
- Recreating T from BWT(T): Start in the first row and apply **LF** repeatedly, accumulating predecessors along the way:

FM index

- Ferragina & Manzini proposed “**FM Index**” based on BWT
- Observed:
  - LF Mapping also allows ***exact matching*** within T
  - LF(i) can be made fast with ***checkpointing***

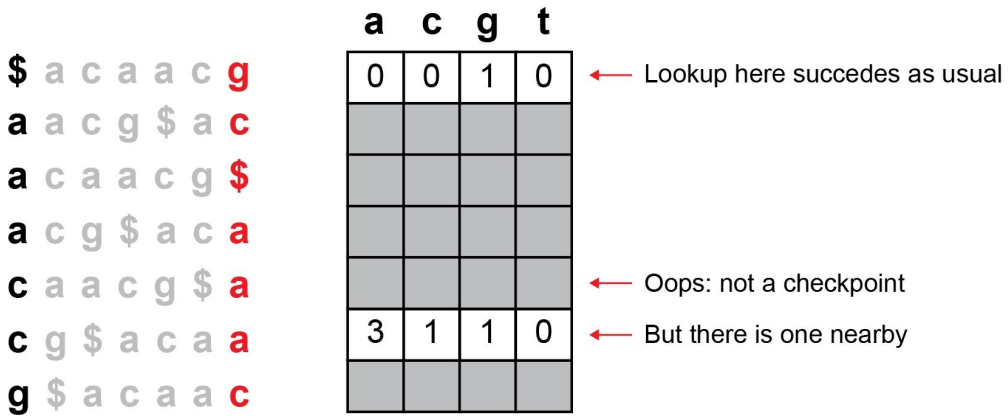
FM index - Idea

- Algorithm that finds all occurrences of a pattern P in text T by using BWT(T)
- Look for range of rows of BW(T) with P as prefix
- Do this for P's shortest suffix, then extend to successively longer suffixes until range becomes empty or we exhausted P



FM index - checkpoints

- **LF**(i, **qc**) must determine the rank of **qc** in row i
  - Naïve solution: Count occurrences of **qc** in all previous rows
- Better solution: Pre-calculate occurrences of A, C, G and T in L up to periodic **checkpoints**:



FM index - **Position in reference - Suffix array sample**

- Once we know a row contains a legal alignment, how do we determine its position in the reference?
- Hybrid solution: Store sample of **suffix array**; "walk left" to next sampled ("marked") row to the left:

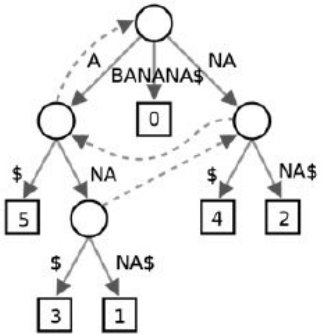
Default: every 32nd row

FM index

- Entire FM Index of the reference consists of:
  - Reference BWT (same size as reference)
  - Suffix array sample (50% size of the reference)
  - Checkpoints (15% size of the reference for every 448th row)

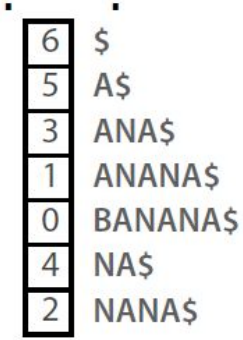
- Example: DNA alphabet (2 bits per nucleotide), human genome (3 billion nucleotides), SA sample - every 32nd row, checkpoints - every 128th row:
  - First column: 16 bytes
  - Last column: 2 bits x 3 billion chars ~ 750MB
  - SA sample: 3 billion x 4 bytes / 32 ~ 400MB
  - Checkpoints: 3 billion x 4 bytes x 4 / 128 ~ 400MB

Comparison of different seeding techniques



Suffix tree

≥ 45 GB



Suffix array

≥ 12 GB

FM Index

~ 1 GB

Score-based alignment

- So far we mentioned only exact match alignments
- Some of the reads will match perfectly to the reference
- Many reads will not:
  - Genomic variations (‘mutations’, SNP, Indel, etc.)
  - Sequencing errors
- Aligners commonly take a score-based approach:
  - Calculate a score, related to the distance between the read and the local reference sequence
  - Place the read so that the score in maximised
- Many algorithms exist, there is a speed/precision tradeoff

Two-step aligners

- Most modern aligners take a two-step approach (also called “seed and extend”)
- First find “coarse” alignments or seeds
- Than do fine detailed alignments in the vicinity of the seeds
- Choose the best scoring fine grain alignment

Seeding step

- Find a set of possible coordinates
- Many false-positives, but very is usually fast
- Usually aligners use one of mentioned data structures to create index for the references
  - Suffix arrays
  - Hash maps
  - BWT
- A common tradeoff is speed vs RAM footprint

Seeding step example

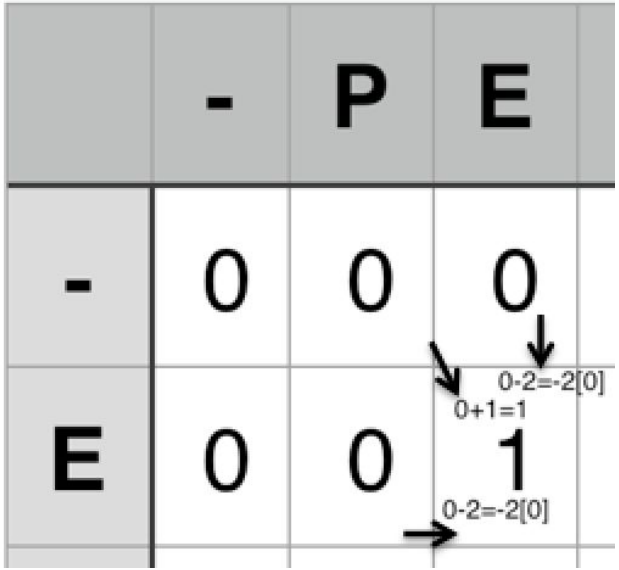
- Example: a simple hash-based scheme
- Create a hash-table which holds the positions, where each kmer in the genome occurs
- For each kmer in the read find the list of locations
- Regions with hits from many different kmers are hits
- Some kmers will have no hits, some many

Extending step

- Calculates a precise sequences match score
- Often use a lot of RAM (related to sequence size)
- The extend step also needs to produce the information on **how** the sequences match
- Mostly based on **dynamic programming**

Smith-Waterman aligner

- Dynamic programming local alignment algorithm



$$\text{Value}_{i,j} = \begin{cases} 0 \\ \text{Value}_{i-1,j-1} + M \\ \text{Value}_{i-1,j} + G \\ \text{Value}_{i,j-1} + G \end{cases}$$

M = *Match score* if letters match, otherwise *mismatch penalty* (+/- 1 in this example)
G = *Gap penalty* (-2 in this example)

Smith-Waterman aligner

	-	P	E	R	I	C	A	A
-	0	0	0	0	0	0	0	0
E	0	0	1	0	0	0	0	0
R	0	0	0	2	0	0	0	0
C	0	0	0	0	1	1	0	0
A	0	0	0	0	0	0	2	1
A	0	0	0	0	0	0	1	3

P E R I C A A
- E R - C A A

- Calculate scores for each field
- Remember the path to each field
- Backtrack from maximum to zero
- Diagonal step is match/mismatch
- Horizontal step is a deletion
- Vertical step in an insertion

Match=1, Mismatch=-1, Gap =-2

CIGAR strings

- Run length encoding:

  AAAABBAAAD = 4A2B3A1D

- CIGAR Codes:
  - **M** - alignment match (Match or Mismatch)
  - **I** - insertion, **D** - Deletion, **S** - soft clip
  - H, X, =, P, N - rare in DNA Seq

Exercise 1 - a simple seeding aligner step

- We will create seed part of alignment algorithm
- This will be done in 4 steps
- Data we are going to use for tests
  - Read: 'ACATACACATGTCCTGTTTTGATGTCCTATAATTAATTTTCTCTCCGTTTTTAACTTTTATCTATCTTATTAATGT'
  - Reference sequence: “example_reference.fasta” located in the project
- Seed phase of alignment algorithm
  - Implement a simple hash-based aligner in Python
    - A dict can be used to create the index
    - Create an index for each kmer in a sequence
  - To map a read, find locations for each kmer in the read
    - Find the region with most kmers mapping to it
    - Mind the offset from the beginning of the read

Exercise 1 - a simple seeding aligner step

Step 1.

- Create index for provided fasta file, chromosome 20
- def create_index(fasta, k)
- Function should return dict
  - Keys - kmers present in reference
  - Values - list of kmer positions in the reference

Step 2.

- Analyse different k-mer sizes (e.g. k=10 and k=6)
  - Number of unique k-mers
  - Number of collisions

Exercise 1 - a simple seeding aligner step

Step 3.

- Create seed function
- def seed_read(index, k, read)
- Returns dict with
  - reference positions as keys
  - number of supporting kmers as values

Step 4.

- Possible improvements?
  - Filter out all kmers that have more than n mapping positions
  - def seed_read2_with_improvements(index, k, read, n=2)

Exercise 2 - Smith Waterman by hand

- Sequence1 - "attcagct"
- Sequence2 - "atcagtct”

Scoring:

- indel_score = -2
- match_score = 2
- mismatch_score = -1

Exercise 2 - Smith Waterman by hand

		A	T	T	C	A	G	C	T
	0	0	0	0	0	0	0	0	0
A	0	2							
T	0								
C	0								
A	0								
G	0								
T	0								
C	0								
T	0								

Exercise 2 - Smith Waterman by hand

		A	T	T	C	A	G	C	T
	0	0	0	0	0	0	0	0	0
A	0	2	0	0	0	2	0	0	0
T	0	0	4	2	0	0	1	0	2
C	0	0	2	3	4	2	0	3	1
A	0	2	0	1	2	6	4	2	2
G	0	0	1	0	0	4	8	6	4
T	0	0	2	3	1	2	6	7	8
C	0	0	0	1	5	3	4	8	6
T	0	0	2	2	3	4	2	6	10

Exercise 3 - Smith Waterman simplified implementation

Implement algorithm that will fill in score table for two sequences

Let’s test it on previous example:

- Sequence1 - "attcagct"
- Sequence2 - "atcagtct”

Scoring:

- indel_score = -2
- match_score = 2
- mismatch_score = -1

BWA aligner

- A widely used aligner for DNA Sequencing
- http://bio-bwa.sourceforge.net for more info
- BWA requires an index to be built
  - It uses BWT transformation for reference index creation
- Seed and extend approach

BAM file format

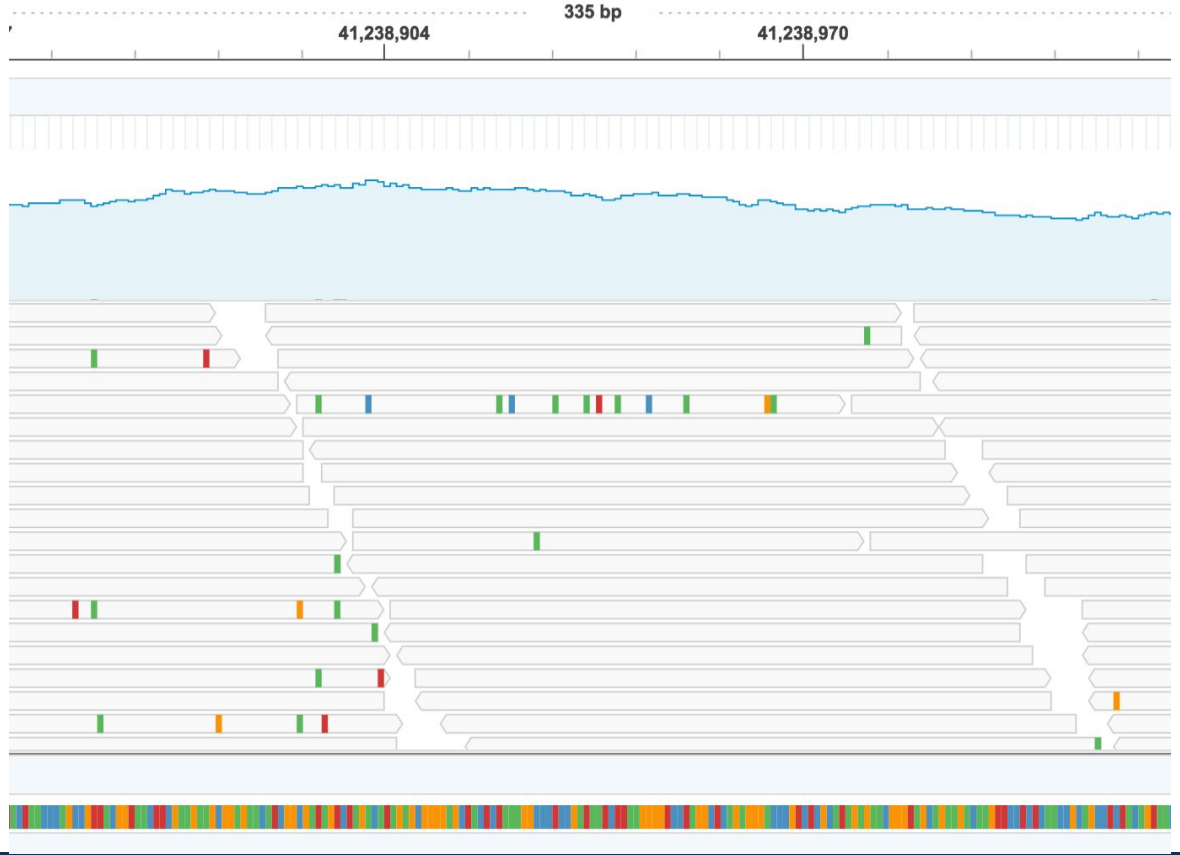
- Standardized format for holding aligned reads
  - The read sequence and qualities
  - Position (chromosome and the first matching base)
  - CIGAR string
  - Flag (various info, like *read has a pair*, *read is aligned*, etc.)
  - Read pair position
  - Other optional tags
- Bgzip compression (roughly ⅓ of raw text)
- Besides BAM, SAM (plain text) also exists
- Specification: https://samtools.github.io/hts-specs/SAMv1.pdf

BAM file format

- BAM is 0-based, SAM is 1-based
- BAM files can be sorted:
  - Coordinate sort, by read position
  - Query name (read name), read pairs are placed together
- Coordinate sorted BAM files can be *indexed*
- By convention, BAM index files:
  - Have .bai appended to the name
  - Are placed in the same folder as the BAM
  - Usually not explicitly passed to tools
  - Majority of the tools requires .bai file

Genome browser

- A visualization tool for genomic data
- See how the reads actually align to the reference



Pysam

- Pysam can be used to process BAM files
- pysam.AlignmentFile
  - AlignmentFile(path_to_file)
  - Iteration through reads in BAM file:
    - for read in AlignmentFile(path_to_file):
- Reads are wrapped in AlignedSegment objects
  - AlignedSegment provides access to read fields and helpers
- pysam.AlignmentFile supports fetching regions
  - The BAM file needs to be sorted and indexed!

Exercise 5 - Pysam

- Create an AlignmentFile object for “merged-tumor.bam”
  - Take the first read from the AlignmentFile
  - Inspect the fields in the AlignedSegment
  - Inspect the flag field
    - Check out the flag for some reads using: https://broadinstitute.github.io/picard/explain-flags.html
- Calculate:
  - How many unmapped reads are in the file?
  - Total number of reads
  - Number of reads with mapping quality 0
  - Average mapping quality for all the reads
  - Average mapping quality if reads with 0 mapp quality are filtered out

Questions?