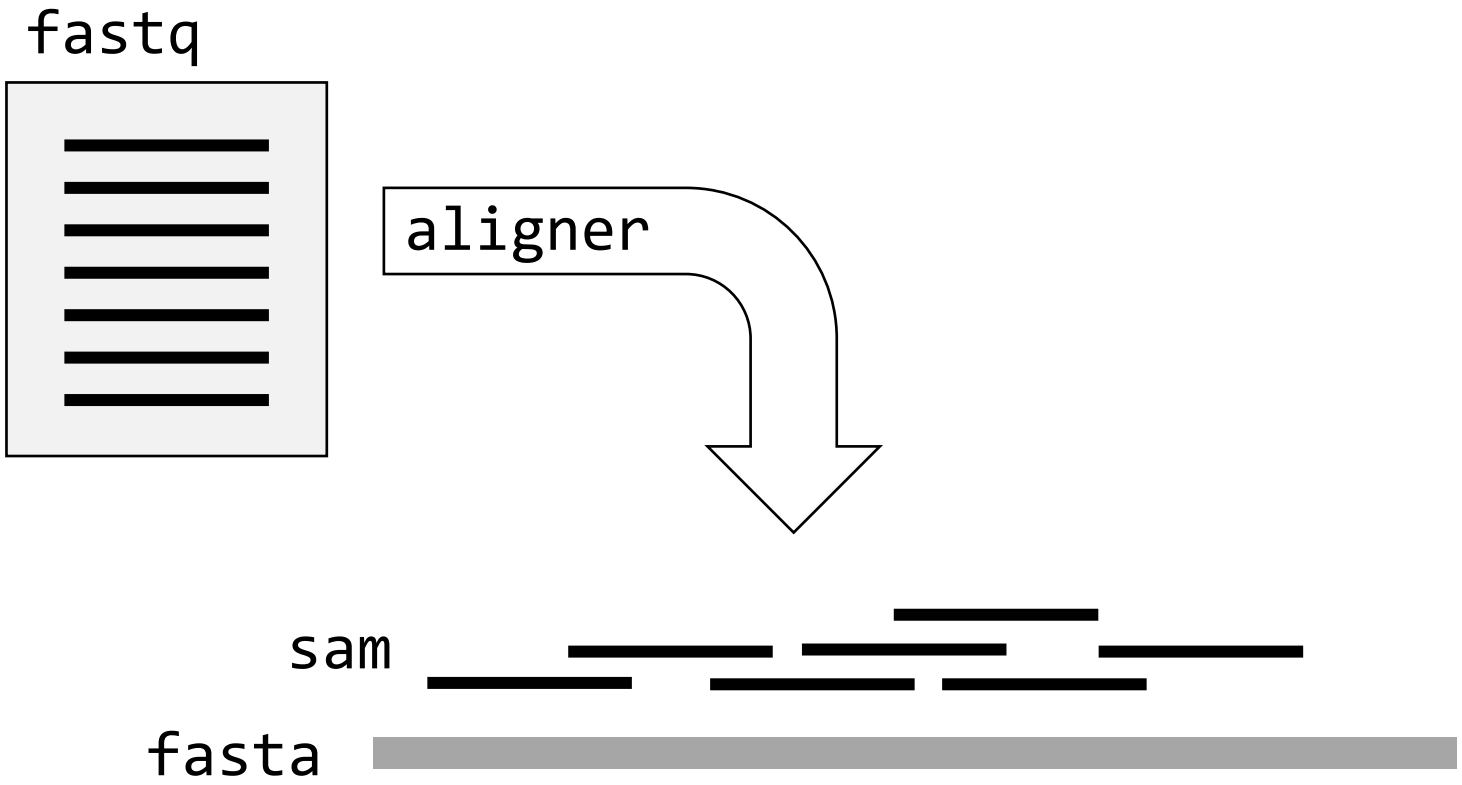
NGS - quality control, alignment, visualisation

Read alignment


fastq
aligner
sam
fasta

How do aligners work?

Aim: find **substrings** in **large string**

Typically:

- Millions of substrings (reads)

- In string of tens of millions of characters (genome)

Indexing

Aim: generate a ‘phonebook’ for fast searches

Reference: TAATA$

EOF

0	T	A	A	T	A	$
1	A	A	T	A	$	
2	A	T	A	$		
3	T	A	$			
4	A	$				
5	$					

sort

suffix array

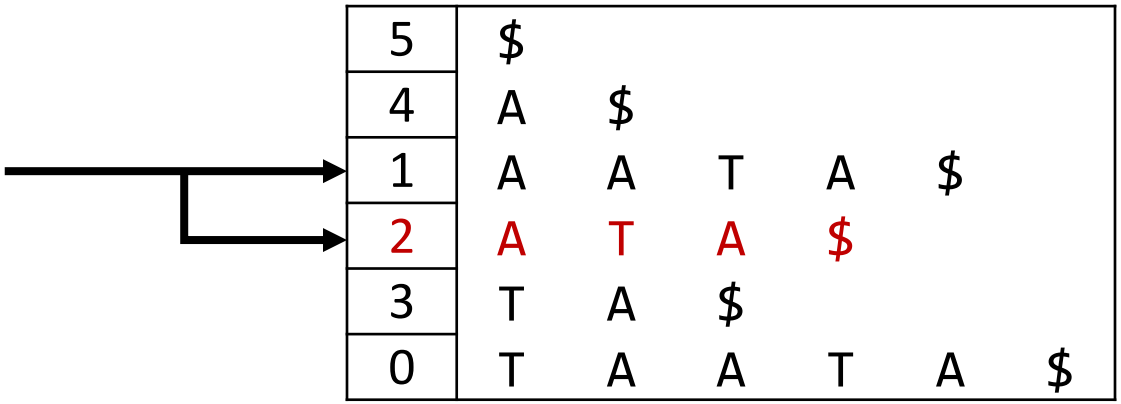
5	$					
4	A	$				
1	A	A	T	A	$	
2	A	T	A	$		
3	T	A	$			
0	T	A	A	T	A	$

Querying

Reference: TAATA$

Query: ATA

Can use binary search:



Indexing and querying

- Suffix array: large, same sequence stored multiple times
- BWT: only first and last columns are stored -> still enables fast querying

suffix array

5	$
4	A $
1	A A T A $
2	A T A $
3	T A $
0	T A A T A $

Burrows-**W**heeler **T**ransformation

$	T	A	A	T	A
A	$	T	A	A	T
A	A	T	A	$	T
A	T	A	$	T	A
T	A	$	T	A	A
T	A	A	T	A	$

Global vs local

- Global (end-to-end)

```
Read:      GACTGGGCGATCTCGACTTCG
           |||||  |||||||||| |||
Reference: GACTG--CGATCTCGACATCG
```

- Local (allows for ‘clipping’)

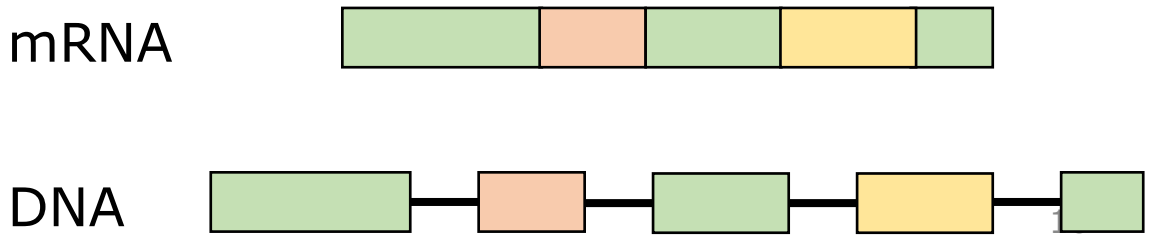
```
Read:      ACGGTTGCGTTAA-TCCGCCACG
               |||||||| ||||||
Reference: TAACTTGCGTTAAATCCGCCTGG
```


examples from: http://bowtie-bio.sourceforge.net/bowtie2/manual.shtml#end-to-end-alignment-versus-local-alignment

Question 8

Software

- Basic alignment:
  - bowtie2 (BWT; default = global)
  - bwa-mem (BWT; default = local)
- Splice-aware (RNA-seq):
  - hisat2
  - STAR
- Long reads + short reads + splice-aware:
  - minimap2



Mapping quality

$$MAPQ$$
$$= -10log_{10}\,\Pr\{mapping\ position\ is\ wrong\}$$

$$-10log_{10}\,(0.01) = 20$$
$$-10log_{10}\,(0.5) = 3$$