Office Hours

Thursday office hours:

- Moved to 12:30 (12:30-1:30pm)

Read Alignment

Adapted from Mäkinen, *et al.* Chapter 10

Computational Problem

Given

- a reference genome G, and
- a set of reads $R = (r_1, r_2, r_3, \ldots, r_k) \in (\Sigma^n)^k$ where each read r is a subsequence of G with a small number changes

Output

- the semi-global alignment of r_i and G for all $r_i \in R$

Computational Problem

Given

- a reference genome G, and
- a set of reads $R = (r_1, r_2, r_3, \ldots, r_k) \in (\Sigma^n)^k$ where each read r is a subsequence of G with a small number changes

Output

- the semi-global alignment of r_i and G for all $r_i \in R$ with $<k$ changes

call these k-error mappings

Read Filtering

If a read is long enough, it should not align well to a random region of *G*

This assumes that the sequence was read correctly

Sequencing machines output a *quality score* for each position of a read

- this can be interpreted as a probability *P(r[j])* that the character is correct
- in other words with probability *P(r[j]),* position *r[j]* is a random character

This means that a given sequence will match a random sequence with probability

$$\mathbb{P}(r) = \prod_{1 \leq i \leq n} \left(\mathbb{P}\left(r\left[j\right] \right) q_{r[j]} + \left(1 - \mathbb{P}\left(r\left[j\right] \right) \right) \right)$$

- where q_c is the probability of c in a random sequence

Read Filtering

We expect the number of random matches between *r* and a given string *T* such that $|T| = m$ to be $\mathbb{E}(r, T) = (m - n + 1)\mathbb{P}(r)$

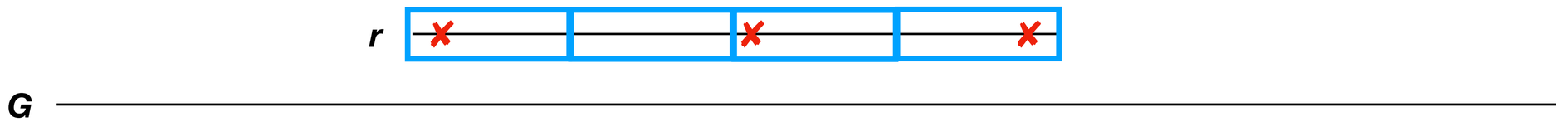
We can then threshold the reads that are too low quality by this expectation

Pigeonhole Principle

Assume for now we're not dealing with insertions or deletions

The **pigeonhole** principle in this case says that if the read is partitioned into *k+1* pieces, one must appear in the genome exactly if the read has a *k*-error mapping.

All *k*-error mappings will have at least one exact match, not all exact matches lead to *k*-error mappings



Initial ideas to read mapping

Construct an index of *G* (say a succinct suffix array)

Search for each of the pieces of the read in the index

Verify each occurrence's alignment against that region of *G*

$O(m \log \sigma + (\log^{(1+\varepsilon)} n + m^2/w)\, c)$ time

number of candidates

Aligning reads

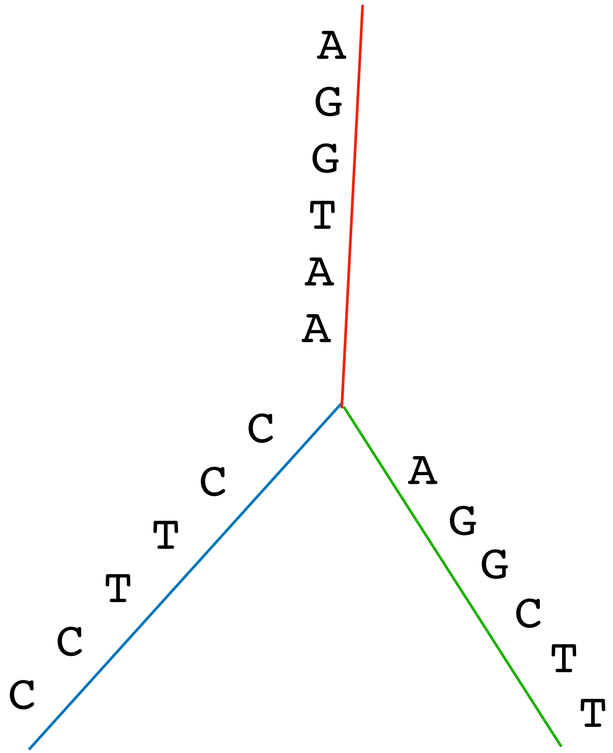
We mentioned that we want the semi-global alignment of each read to the genome, ignoring any deletions (from the genome) at the start or end of the alignment

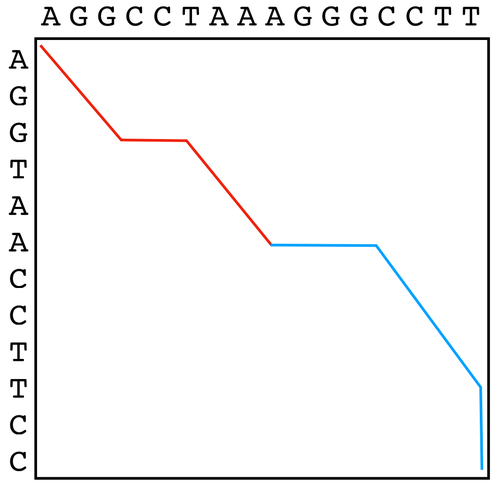
We can align the read along the suffix tree of the genome, where each row is a position in the genome

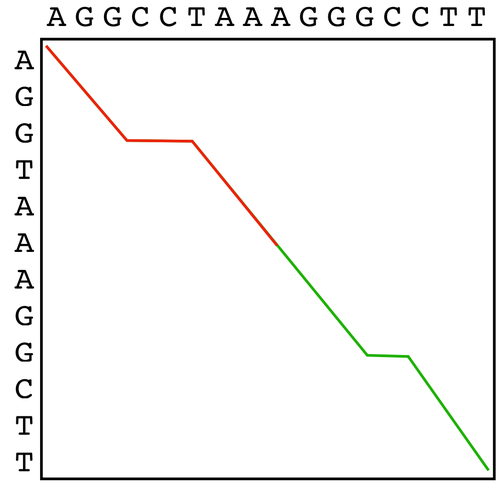
parts of the alignment matrix will be shared.

Aligning reads

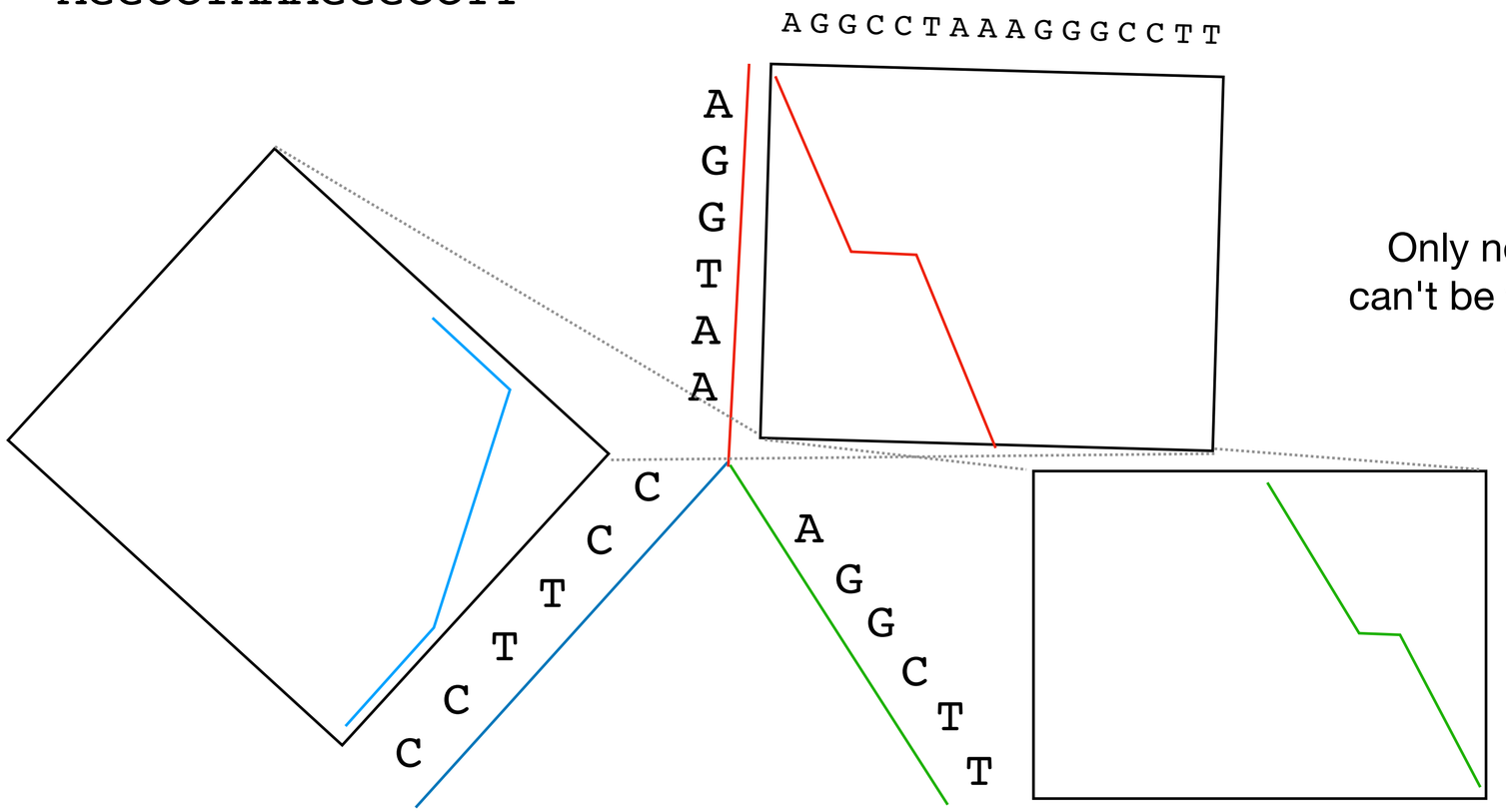
AGGCCTAAAGGGCCTT







Aligning reads



Only need to go to a depth of *2m* since the best alignment can't be worse than deleting one string and inserting the other.

We don't have the suffix tree!

Dynamic Programming using a BWT

Since we want to save the previous rows

- we can read the characters one-by-one from the sequence
- when you reach the max depth, backtack up the tree to the last branch
- overwrite the new rows with the characters read down the new branch

How do we backtrack on a BWT?

Dynamic Programming using a BWT

```
define Branch(d,[i...j]):
    for c ∈ idx.enumerateRight(i,j) do
        process (c,d)
        if d = 2m and score > threshold do
            output alignment
        if d < 2m do
            Branch(d+1,idx.extendRight(c, [i,j]))
```

compute the dynamic programming table row using character *c* in row *d*

$O(m\sigma)$-time

$O(m^2+m\sigma)$-space

Prefix Pruning

The full dynamic program is still slow

What if we go back to hamming distance, but still use the BWT

Prefix Pruning

```
define Branch(d,k,[i...j]):
    for c ∈ idx.enumerateRight(i,j) do
        if p[d] ≠ c do
            k = k-1
        if k ≥ 0 do
            if d = m do
                output locations in [i,j]
            else
                Branch(d+1, k, idx.extendRight(c,[i,j]))
```

O(mσ)-time

O(mσ)-space

Approximate Overlaps

If we're not given a reference genome, we are left to do *de novo* assembly.

The first step is known as overlap mapping.

Given a set of reads $\boldsymbol{R} = \{R^1, R^2, \ldots, R^d\}$ find the set of suffix-prefix overlaps (R^i, R^j, o^{ij}).

- Search is performed using the **reverse** BWT for $T = R^1\$^1R^2\$^2R^3\$^3 \ldots R^d\$^d\$$

Paired-end reads

We mentioned previously that many times reads come in pairs from the sequencer

These pairs can be used to determine exactly where a read maps (in the case of reads that can be placed in multiple locations)

Mapping can be done independently (useful for large scale change detection), but can also be performed together

- Search performed using the suffix array of the genome, which is large

Split alignment of reads

In RNA-Sequencing (RNA-Seq) the reads will have the introns removed, meaning there will be large gaps in the mapping position on the genome

If we have a complete *transcriptome* (all possible spliced transcripts), we could map reads to that, but we may not have it

There is no clean solution to this, possibilities include:

- find all error free regions of a read, piece them together if the distances are reasonable
- predict exon boundaries in the read, then align those contiguous regions

Bowtie

Langmead, Trapnell, Pop, Salzberg 2009

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

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)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2009/10/3/R25

Received: 21 October 2008
Revised: 19 December 2008
Accepted: 4 March 2009

FM-Index

The "BWT Index" discussed previously is also called the "FM Index"

- Originally defined by Ferragina and Manzini in 2000/2005

Reminder that the BWT/FM-index is:

- A data structure for a sting *T* containing
  - $BWT_{T\$}$ encoded as a wavelet tree
  - an integer array *C* continuing the counts of each character from Σ in T

Refresher on BWTs

Can be constructed using the last character of the lexicographic order of all cyclic rotations of the text

Encodes the original text, which can be recovered by a walk in the sequence

Searching for patterns is done back to front using similar techniques to sequence recovery

Using a BWT to Align Reads

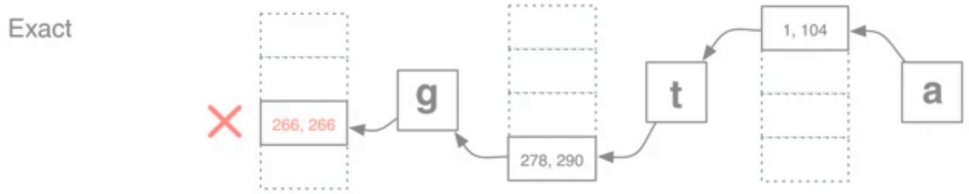
The BWT and FM-Index are insufficient for aligning reads since it doesn't allow for errors

Previously mentioned some method to overcome this

Bowtie assumes all changes are single point changes (i.e. mismatches only)

- They use a backtracking search to find matching locations
- The quality scores are used to prioritize alignments
- Other speed-ups are included to ensure all matching locations are found

Backtracking



Start by matching the exact sequence

If the algorithm reaches a point with no matches swap out characters already matched and restart search from that there

When ties occur, start with the character with the lowest quality score, keep the rest in a stack

Keep track of how many changes are made

"*Bowtie conducts a quality-aware, greedy, randomized, depth-first search through the space of possible alignments.*"

Backtracking Options

The user specifies the sum of the quality scores that can be changed

- this means that a mapping can have lots of low quality replacements, or
- one medium quality change

Bowtie outputs the first valid alignment by default (within the specified constraints)

- can be modified to complete the backtracking and return the "best" alignment
- 2x-3x slower to do this

User can specify a number of alignments to consider

- default is to use only one
- might want the two best alignments
- 2 alignments is ~2x slower than using only 1

Excessive Backtracking

In low quality reads, lots of time may be spent backtracking since there are many possible changes at low quality positions.

They mitigate this by creating two indexes (as we saw previously), one for the forward and one for the reverse of the string

- the backtracking is performed somewhat simultaneously on both index as we will see next

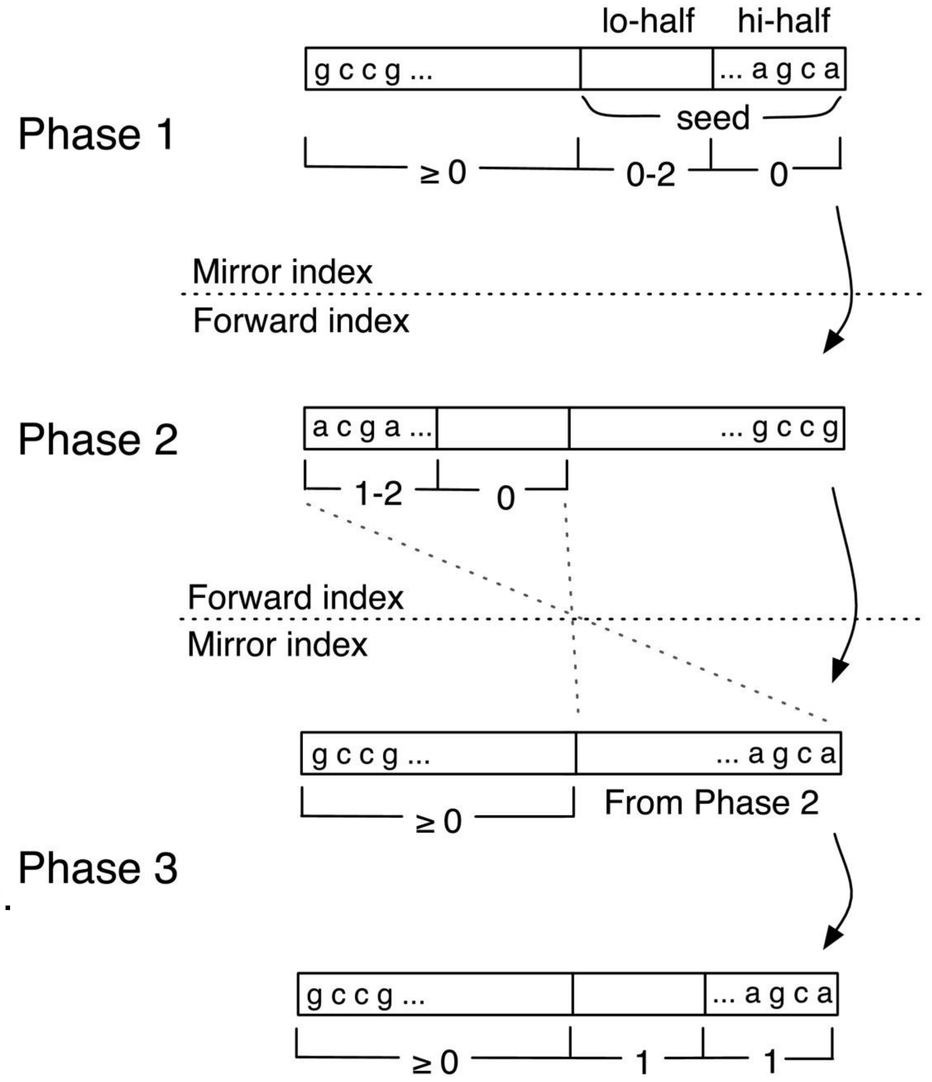
One other step they take is to concentrate on the "high-quality" end of a read (the first 28 characters read) which is most reliable

Phased Search

Split the seed (first 28 bases) into two parts, hi-half and lo-half

Assume we're allowing 2 changes in the seed, a good alignment will have either:

1. no mismatches
2. no mismatches in hi-half, 1 or 2 mismatches in lo-half
3. 1 or 2 mismatches in hi-half, no mismatches in lo-half
4. 1 mismatch in hi-half, 1 mismatch in lo-half

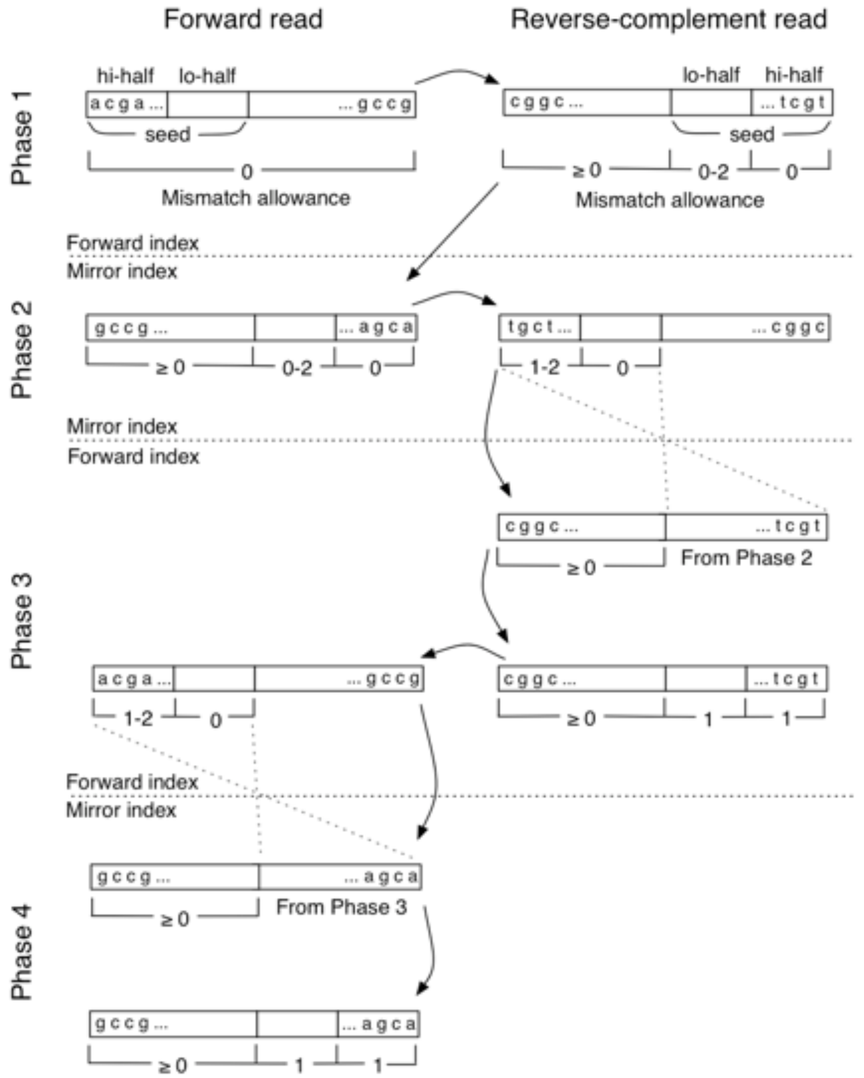


Phase 1 uses the mirror index and invokes the aligner to find alignments for cases 1 & 2.
Phases 2 and 3 cooperate to find alignments for case 3:
Phase 2 finds partial alignments with mismatches only in the hi-half, and phase 3 attempts to extend those partial alignments into full alignments.
Finally, phase 3 invokes the aligner to find alignments for case 4.

Phased search with reverse strand

Since both the read and its reverse complement are possibilities for ma match, need to consider both.

Phases 2-4 here map to phases 1-3 previously.



Performance

Maq (Li, Ruan, Durban 2008), SOAP (Li, Li, Kristiansen, Wang 2008) the leading competitors at the time

Both used hashing to find potential mapping locations

Performance

	Platform	CPU time	Wall clock time	Reads mapped per hour (millions)	Peak virtual memory footprint (megabytes)	Bowtie speed-up	Reads aligned (%)
Bowtie -v 2	Server	**15 m 7 s**	**15 m 41 s**	**33.8**	**1,149**	351×	**67.4**
SOAP		91 h 57 m 35 s	91 h 47 m 46 s	0.10	13,619		67.3
Bowtie	PC	**16 m 41 s**	**17 m 57 s**	**29.5**	**1,353**	59.8×	**71.9**
MAQ		17 h 46 m 35 s	17 h 53 m 7 s	0.49	804		74.7
Bowtie	Server	**17 m 58 s**	**18 m 26 s**	**28.8**	**1,353**	107×	**71.9**
MAQ		32 h 56 m 53 s	32 h 58 m 39 s	0.27	804		74.7

Take Aways

Bowtie was (at the time) the fastest short read aligniner

Used a one-time index based on a BWT that could be reused (novel at the time)

Is able to run on a standard PC

When first published didn't use mate-pair information

Bowtie2

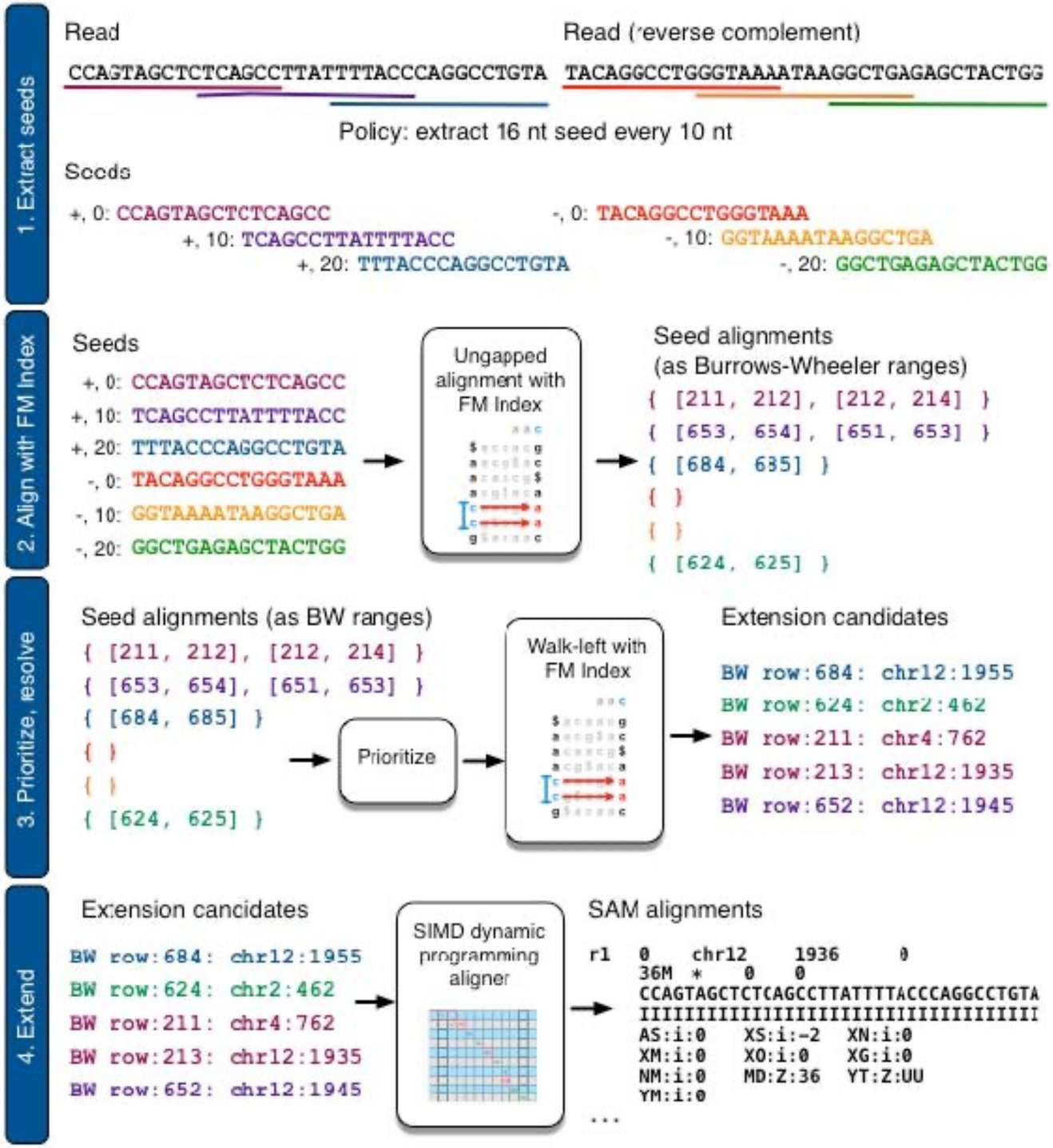
Brief Communication | Published: 04 March 2012

Fast gapped-read alignment with Bowtie 2

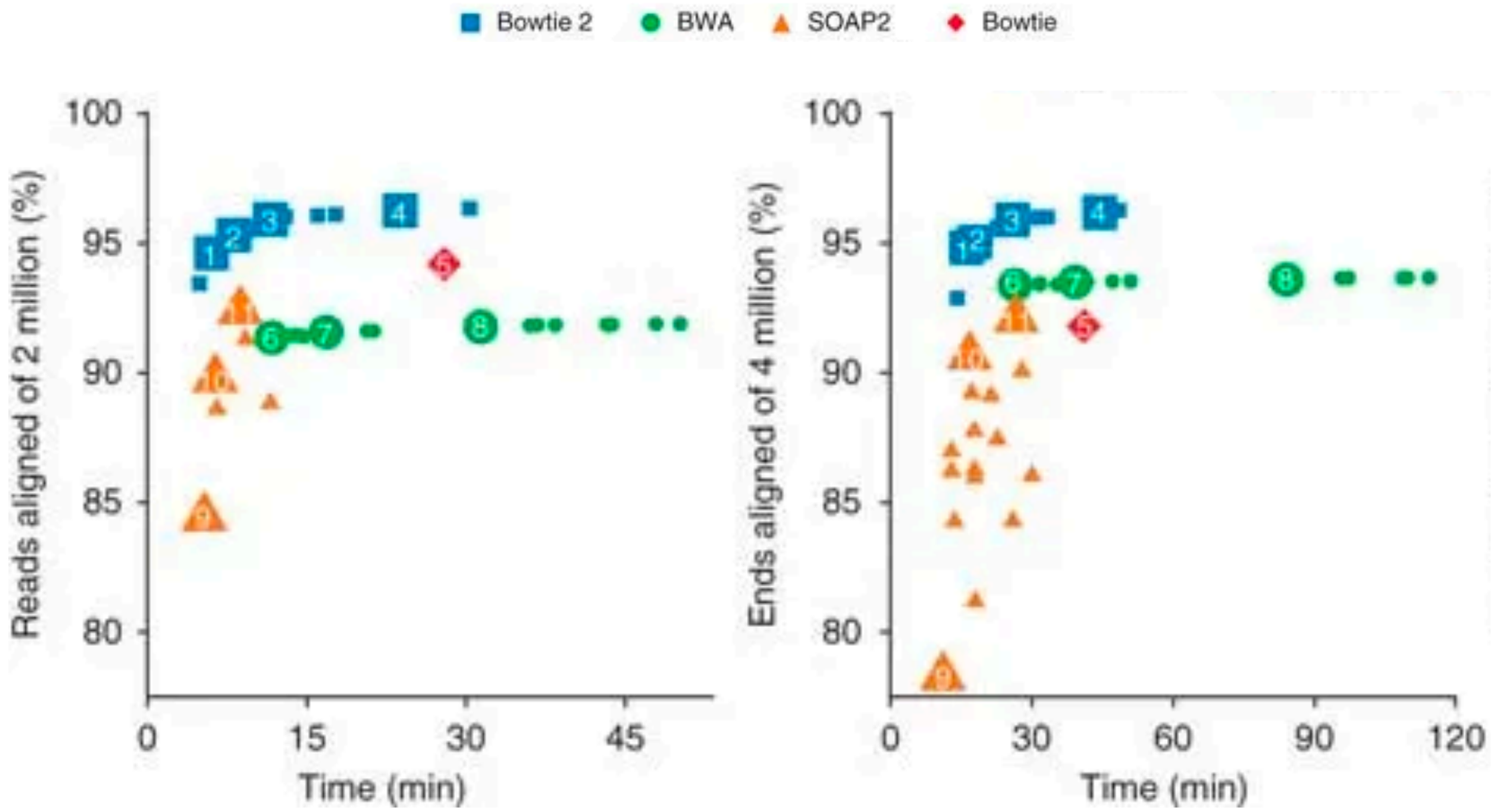
Ben Langmead & Steven L Salzberg

Nature Methods **9**, 357–359(2012) | Cite this article

Bowtie2


1. Extract seeds
Read
CCAGTAGCTCTCAGCCTTATTTTACCCAGGCCTGTA
Read (reverse complement)
TACAGGCCTGGGTAAAATAAGGCTGAGAGCTACTGG
Policy: extract 16 nt seed every 10 nt
Seeds
+, 0: CCAGTAGCTCTCAGCC
+, 10: TCAGCCTTATTTTACC
+, 20: TTTACCCAGGCCTGTA
-, 0: TACAGGCCTGGGTAAA
-, 10: GGTAAAATAAGGCTGA
-, 20: GGCTGAGAGCTACTGG
2. Align with FM Index
Seeds
+, 0: CCAGTAGCTCTCAGCC
+, 10: TCAGCCTTATTTTACC
+, 20: TTTACCCAGGCCTGTA
-, 0: TACAGGCCTGGGTAAA
-, 10: GGTAAAATAAGGCTGA
-, 20: GGCTGAGAGCTACTGG
Ungapped alignment with FM Index
Seed alignments (as Burrows-Wheeler ranges)
{ [211, 212], [212, 214] }
{ [653, 654], [651, 653] }
{ [684, 685] }
{ }
{ }
{ [624, 625] }
3. Prioritize, resolve
Seed alignments (as BW ranges)
{ [211, 212], [212, 214] }
{ [653, 654], [651, 653] }
{ [684, 685] }
{ }
{ }
{ [624, 625] }
Prioritize
Walk-left with FM Index
Extension candidates
BW row:684: chr12:1955
BW row:624: chr2:462
BW row:211: chr4:762
BW row:213: chr12:1935
BW row:652: chr12:1945
4. Extend
Extension candidates
BW row:684: chr12:1955
BW row:624: chr2:462
BW row:211: chr4:762
BW row:213: chr12:1935
BW row:652: chr12:1945
SIMD dynamic programming aligner
SAM alignments
r1 0 chr12 1936 0
36M * 0 0
CCAGTAGCTCTCAGCCTTATTTTACCCAGGCCTGTA
IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII
AS:i:0 XS:i:-2 XN:i:0
XM:i:0 XO:i:0 XG:i:0
NM:i:0 MD:Z:36 YT:Z:UU
YM:i:0
...

Bowtie2


Bowtie 2
BWA
SOAP2
Bowtie
Reads aligned of 2 million (%)
100
95
90
85
80
0
15
30
45
Time (min)
Ends aligned of 4 million (%)
100
95
90
85
80
0
30
60
90
120
Time (min)