Ultrafast and memory-efficient alignment of short DNA sequences to the human genome

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We are in 2009

Outline of Bowtie

- Burrows-Wheeler Transform(Indexing)
- Exact and inexact alignment
- Excessive backtracking

Burrows- Wheeler Transform: Forward Transform

Let $T = \texttt{BANANA}$. BWT(T) will be:

$$\begin{bmatrix}
\$ & B & A & N & A & N & A \\
A & \$ & B & A & N & A & N \\
N & A & \$ & B & A & N & A \\
A & N & A & \$ & B & A & N \\
N & A & N & A & \$ & B & A \\
A & N & A & N & A & \$ & B \\
B & A & N & A & N & A & \$
\end{bmatrix}$$

Burrows- Wheeler Transform: Forward Transform

Let $T = \texttt{BANANA}$. BWT(T) will be:

$$\begin{bmatrix} \$ & B & A & N & A & N & A \\ A & \$ & B & A & N & A & N \\ N & A & \$ & B & A & N & A \\ A & N & A & \$ & B & A & N \\ N & A & N & A & \$ & B & A \\ A & N & A & N & A & \$ & B \\ B & A & N & A & N & A & \$ \end{bmatrix} \rightarrow \begin{bmatrix} \$ & B & A & N & A & N & A \\ A & \$ & B & A & N & A & N \\ A & N & A & \$ & B & A & N \\ A & N & A & N & A & \$ & B \\ B & A & N & A & N & A & \$ \\ N & A & \$ & B & A & N & A \\ N & A & N & A & \$ & B & A \end{bmatrix}$$

$$BWT(T) \rightarrow \texttt{ANNB\$AA}$$

BWT: Reverse Transform(UNPERMUTE)

$	A
A	N
A	N
A	B
B	$
N	A
N	A

BWT: Reverse Transform(UNPERMUTE)

$$\begin{tikzcd}
\mathtt{\$} \arrow[r] & \mathtt{A} \\
\mathtt{A} & \mathtt{N} \\
\mathtt{A} & \mathtt{N} \\
\mathtt{A} & \mathtt{B} \\
\mathtt{B} & \mathtt{\$} \\
\mathtt{N} & \mathtt{A} \\
\mathtt{N} & \mathtt{A}
\end{tikzcd}$$

BWT: Reverse Transform(UNPERMUTE)

$$\begin{tikzcd}
\mathtt{\$} \arrow[r] & \mathtt{A} \arrow[dl] \\
\mathtt{A} & \mathtt{N} \\
\mathtt{A} & \mathtt{N} \\
\mathtt{A} & \mathtt{B} \\
\mathtt{B} & \mathtt{\$} \\
\mathtt{N} & \mathtt{A} \\
\mathtt{N} & \mathtt{A}
\end{tikzcd}$$

BWT: Reverse Transform(UNPERMUTE)

$$\begin{tikzcd}
\texttt{\$} \arrow[r] & \texttt{A} \arrow[dl] \\
\texttt{A} \arrow[r] & \texttt{N} \\
\texttt{A} & \texttt{N} \\
\texttt{A} & \texttt{B} \\
\texttt{B} & \texttt{\$} \\
\texttt{N} & \texttt{A} \\
\texttt{N} & \texttt{A}
\end{tikzcd}$$

BWT: Reverse Transform(UNPERMUTE)

$$\begin{tikzcd}
\$ \arrow[r] & A \arrow[dl] \\
A \arrow[r] & N \arrow[ddddl] \\
A \arrow[r] & N \arrow[ddddl] \\
A \arrow[r] & B \arrow[dl] \\
B \arrow[r] & \$ \\
N \arrow[r] & A \arrow[uuul] \\
N \arrow[r] & A \arrow[uuul]
\end{tikzcd}$$

BWT: Reverse Transform(UNPERMUTE)

$$\begin{tikzcd}
\mathtt{\$} \arrow[r] & \mathtt{A} \arrow[dl] \\
\mathtt{A} \arrow[r] & \mathtt{N} \arrow[ddddl] \\
\mathtt{A} \arrow[r] & \mathtt{N} \arrow[ddddl] \\
\mathtt{A} \arrow[r] & \mathtt{B} \arrow[dl] \\
\mathtt{B} \arrow[r] & \mathtt{\$} \\
\mathtt{N} \arrow[r] & \mathtt{A} \arrow[uuul] \\
\mathtt{N} \arrow[r] & \mathtt{A} \arrow[uuul]
\end{tikzcd}$$

follow the tip of blue arrows to get back the initial message:

`BANANA$`

EXACTMATCH algoritham

$\Sigma = \mathtt{B}_1\mathtt{A}_1\mathtt{N}_1\mathtt{A}_2\mathtt{N}_2\mathtt{A}_3$.
Pattern to find, $P = \mathtt{NAN}$

$	$\mathtt{A}_1$
$\mathtt{A}_1$	$\mathtt{N}_1$
$\mathtt{A}_2$	$\mathtt{N}_2$
$\mathtt{A}_3$	$\mathtt{B}_1$
$\mathtt{B}_1$	$
$\mathtt{N}_1$	$\mathtt{A}_2$
$\mathtt{N}_2$	$\mathtt{A}_3$

EXACTMATCH algoritham

$\Sigma = \mathtt{B}_1\mathtt{A}_1\mathtt{N}_1\mathtt{A}_2\mathtt{N}_2\mathtt{A}_3$.
Pattern to find, $P = \mathtt{NAN}$

0	\$	$\mathtt{A}_1$	1
1	$\mathtt{A}_1$	$\mathtt{N}_1$	5
2	$\mathtt{A}_2$	$\mathtt{N}_2$	6
3	$\mathtt{A}_3$	$\mathtt{B}_1$	4
4	$\mathtt{B}_1$	\$	0
5	$\mathtt{N}_1$	$\mathtt{A}_2$	2
6	$\mathtt{N}_2$	$\mathtt{A}_3$	3

EXACTMATCH algoritham

$\Sigma = \mathtt{B}_1\mathtt{A}_1\mathtt{N}_1\mathtt{A}_2\mathtt{N}_2\mathtt{A}_3$.
Pattern to find, $P = \mathtt{NAN}$

top$\rightarrow$	0	\$	$\mathtt{A}_1$	1
	1	$\mathtt{A}_1$	$\mathtt{N}_1$	5
	2	$\mathtt{A}_2$	$\mathtt{N}_2$	6
	3	$\mathtt{A}_3$	$\mathtt{B}_1$	4
	4	$\mathtt{B}_1$	\$	0
	5	$\mathtt{N}_1$	$\mathtt{A}_2$	2
bot$\rightarrow$	6	$\mathtt{N}_2$	$\mathtt{A}_3$	3

EXACTMATCH algoritham

$\Sigma = \mathtt{B}_1\mathtt{A}_1\mathtt{N}_1\mathtt{A}_2\mathtt{N}_2\mathtt{A}_3$.
Pattern to find, $P = \mathtt{NAN}$

	0	\$	$\mathtt{A}_1$	1
	1	$\mathtt{A}_1$	$\mathtt{N}_1$	5
	2	$\mathtt{A}_2$	$\mathtt{N}_2$	6
	3	$\mathtt{A}_3$	$\mathtt{B}_1$	4
	4	$\mathtt{B}_1$	\$	0
$\mathtt{N}_1 \rightarrow$	5	$\mathtt{N}_1$	$\mathtt{A}_2$	2
$\mathtt{N}_2 \rightarrow$	6	$\mathtt{N}_2$	$\mathtt{A}_3$	3

EXACTMATCH algoritham

$\Sigma = \mathtt{B}_1\mathtt{A}_1\mathtt{N}_1\mathtt{A}_2\mathtt{N}_2\mathtt{A}_3$.
Pattern to find, $P = \mathtt{NAN}$

	0	\$	$\mathtt{A}_1$	1
	1	$\mathtt{A}_1$	$\mathtt{N}_1$	5
$\mathtt{A}_2\mathtt{N}_1 \rightarrow$	2	$\mathtt{A}_2$	$\mathtt{N}_2$	6
$\mathtt{A}_3\mathtt{N}_2 \rightarrow$	3	$\mathtt{A}_3$	$\mathtt{B}_1$	4
	4	$\mathtt{B}_1$	\$	0
	5	$\mathtt{N}_1$	$\mathtt{A}_2$	2
	6	$\mathtt{N}_2$	$\mathtt{A}_3$	3

EXACTMATCH algoritham

$\Sigma = \mathtt{B}_1\mathtt{A}_1\mathtt{N}_1\mathtt{A}_2\mathtt{N}_2\mathtt{A}_3$.
Pattern to find, $P = \mathtt{NAN}$

	0	\$	$\mathtt{A}_1$	1
	1	$\mathtt{A}_1$	$\mathtt{N}_1$	5
	2	$\mathtt{A}_2$	$\mathtt{N}_2$	6
	3	$\mathtt{A}_3$	$\mathtt{B}_1$	4
$\mathtt{B}_1\mathtt{A}_3\mathtt{N}_2\rightarrow$	4	$\mathtt{B}_1$	\$	0
	5	$\mathtt{N}_1$	$\mathtt{A}_2$	2
$\mathtt{N}_2\mathtt{A}_2\mathtt{N}_1\rightarrow$	6	$\mathtt{N}_2$	$\mathtt{A}_3$	3

Exact vs Inexact matching

- Exact matching performs poorly for DNA short read alignment because of mismatches from sequencing errors and other reasons.
- New alignment algorithm which conducts a backtracking search to quickly find alignments that satisfy a specified alignment policy.
- Each character in a read has a numeric quality value(m_i), with lower values indicating a higher likelihood of a sequencing error.
- We allows a limited number of mismatches, while trying to minimize $\sum_i m_i$, where i spans over all mismatches.

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

Excessive backtracking

If a particular suffix doesn’t occur in the text, the algorithm can backtrack. Backtracking involves **potentially substituting a different base at an already-matched query position, introducing a mismatch, and then resuming the search.**

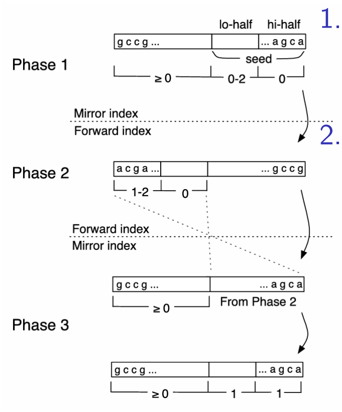
Excessive backtracking occurs when too many alternative paths are explored during the alignment process due to mismatches. Bowtie uses double indexing to deal with this problem. Excessive backtracking is significant only when a read has many low-quality positions and does not align or aligns poorly to the reference.

Mismatch cases

- The high-quality base-pairs at 3' is called Seed(default: 28).
- Seed is further divided into hi-half and lo-half each with 14bps.
- Assuming the default mismatches(2), a reportable alignment can occur in 4 different ways.
  1. No mismatches in seed
  2. No mismatches in hi-half, one or two mismatches in lo-half
  3. No mismatches in lo-half, one or two mismatches in hi-half
  4. O ne mismatch in hi-half, one mismatch in lo-half

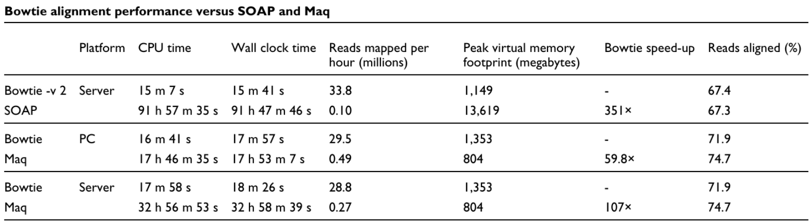
Phased Maq-like search

Bowtie uses a 3 phase approach:



1. Phase 1 uses the mirror index and invokes the aligner to find alignments for cases 1 and 2.
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 align ments. Finally, phase 3 invokes the aligner to find alignments for case 4.

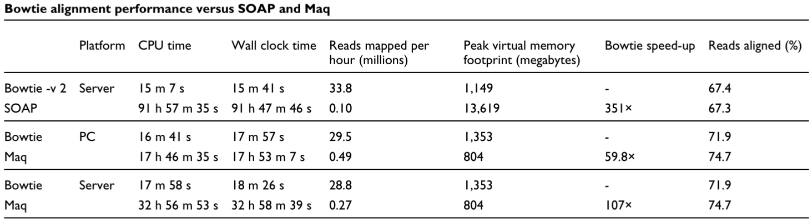
Perfomance results



Bowtie alignment performance versus SOAP and Maq

	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	-	67.4
SOAP		91 h 57 m 35 s	91 h 47 m 46 s	0.10	13,619	351×	67.3
Bowtie	PC	16 m 41 s	17 m 57 s	29.5	1,353	-	71.9
Maq		17 h 46 m 35 s	17 h 53 m 7 s	0.49	804	59.8×	74.7
Bowtie	Server	17 m 58 s	18 m 26 s	28.8	1,353	-	71.9
Maq		32 h 56 m 53 s	32 h 58 m 39 s	0.27	804	107×	74.7

Perfomance results



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	Platform	CPU time	Wall clock time	Reads mapped per hour (millions)	Peak virtual memory footprint (megabytes)	Bowtie speed-up	Reads aligned (%)
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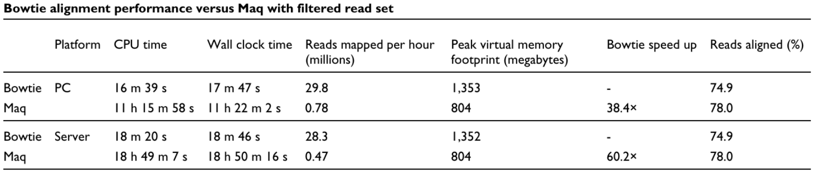
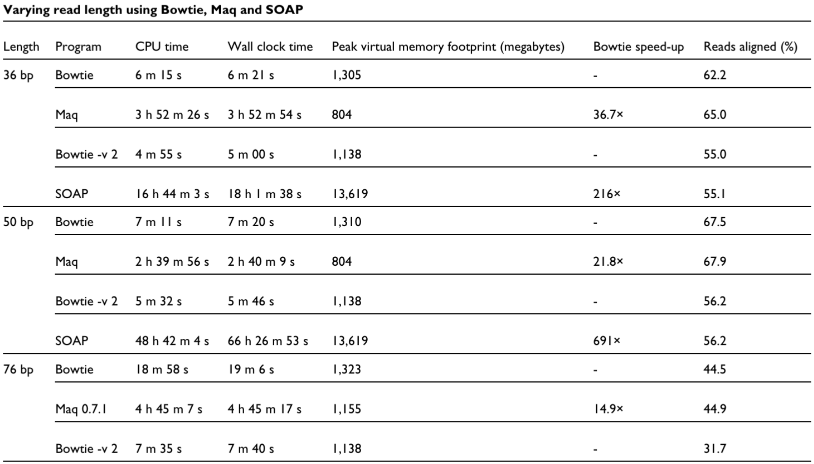


Bowtie alignment performance versus Maq with filtered read set

	Platform	CPU time	Wall clock time	Reads mapped per hour (millions)	Peak virtual memory footprint (megabytes)	Bowtie speed up	Reads aligned (%)
Bowtie	PC	16 m 39 s	17 m 47 s	29.8	1,353	-	74.9
Maq		11 h 15 m 58 s	11 h 22 m 2 s	0.78	804	38.4×	78.0
Bowtie	Server	18 m 20 s	18 m 46 s	28.3	1,352	-	74.9
Maq		18 h 49 m 7 s	18 h 50 m 16 s	0.47	804	60.2×	78.0

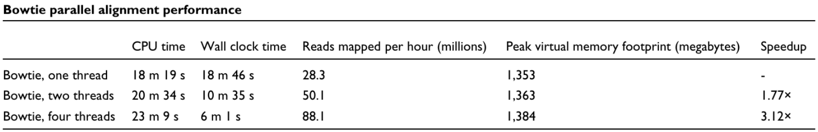
Perfomance Results-II



Varying read length using Bowtie, Maq and SOAP

Length	Program	CPU time	Wall clock time	Peak virtual memory footprint (megabytes)	Bowtie speed-up	Reads aligned (%)
36 bp	Bowtie	6 m 15 s	6 m 21 s	1,305	-	62.2
	Maq	3 h 52 m 26 s	3 h 52 m 54 s	804	36.7×	65.0
	Bowtie -v 2	4 m 55 s	5 m 00 s	1,138	-	55.0
	SOAP	16 h 44 m 3 s	18 h 1 m 38 s	13,619	216×	55.1
50 bp	Bowtie	7 m 11 s	7 m 20 s	1,310	-	67.5
	Maq	2 h 39 m 56 s	2 h 40 m 9 s	804	21.8×	67.9
	Bowtie -v 2	5 m 32 s	5 m 46 s	1,138	-	56.2
	SOAP	48 h 42 m 4 s	66 h 26 m 53 s	13,619	691×	56.2
76 bp	Bowtie	18 m 58 s	19 m 6 s	1,323	-	44.5
	Maq 0.7.1	4 h 45 m 7 s	4 h 45 m 17 s	1,155	14.9×	44.9
	Bowtie -v 2	7 m 35 s	7 m 40 s	1,138	-	31.7

Perfomance Results-III



Bowtie parallel alignment performance

	CPU time	Wall clock time	Reads mapped per hour (millions)	Peak virtual memory footprint (megabytes)	Speedup
Bowtie, one thread	18 m 19 s	18 m 46 s	28.3	1,353	-
Bowtie, two threads	20 m 34 s	10 m 35 s	50.1	1,363	1.77×
Bowtie, four threads	23 m 9 s	6 m 1 s	88.1	1,384	3.12×

Perfomance Results-III

Bowtie parallel alignment performance

	CPU time	Wall clock time	Reads mapped per hour (millions)	Peak virtual memory footprint (megabytes)	Speedup
Bowtie, one thread	18 m 19 s	18 m 46 s	28.3	1,353	-
Bowtie, two threads	20 m 34 s	10 m 35 s	50.1	1,363	1.77×
Bowtie, four threads	23 m 9 s	6 m 1 s	88.1	1,384	3.12×

Bowtie index building performance

Physical memory target (GB)	Actual peak memory footprint (GB)	Wall clock time
16	14.4	4 h 36 m
8	5.84	5 h 5 m
4	3.39	7 h 40 m
2	1.39	21 h 30 m

Summary

- Bowtie's speed and small memory footprint are due chiefly to its use of the Burrows-Wheeler index in combination with the novel, quality-aware, backtracking algorithm introduced here. Double indexing is used to avoid the performance penalty of excessive backtracking.
- Bowtie exhibits a large performance advantage over both Maq and SOAP when mapping reads to the human genome.
- Unlike many other short-read aligners, Bowtie creates a per manent index of the reference that may be re-used across alignment runs.