Introduction to Read-Based Alignment

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Workshop on Genomics
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Aligning to a Reference

- Aligning sequences is a classic problem
  - Early bioinformatic problem
  - Very similar to older text matching problems
- Several algorithms exist
  - Tradeoffs of speed versus accuracy, sensitivity
- Sequencing throughput creates new problems
  - Short reads have less information than long seqs
  - Data volume requires faster processing per read

Example of alignment

Read:

TCAACTCTGCCAACACCTTCCTCCTCCAGGAAGCACTCCTGGATTTCCCTCTTGCCAACAAGATTCTGGGAGGGCA

Genome:

ATAAAATGGCCAAAATTAACTAGAAGGTGAGTAGAAACTTAAATAAACTAATTACCATTGATGAGAAAAAAAATC
TGCCACTGAAAAAGGCACCCGGTCCAGAGGGTTTCATGAGCGGGAACTGTAGAAACCTTTCGAATTCAACTCTGC
CAACACCTTCCTCCTCCAGGAAGCACTCCTGGATTTCCCTCTTGCCAACAAGATTCTGGGAGGGCAGCTCCTCCA
ACATGCCCCCAACAGCTCTCTGCAGACATATCATATCATATCATATCTTCCATACCATAACTGCCATGCCATACA

Example of alignment

Read:

TCAACTCTGCCAACACCTTCCTCCTCCAGGAAGCACTCCTGGATTTCCCTCTTGCCAACAAGATTCTGGGAGGGCA

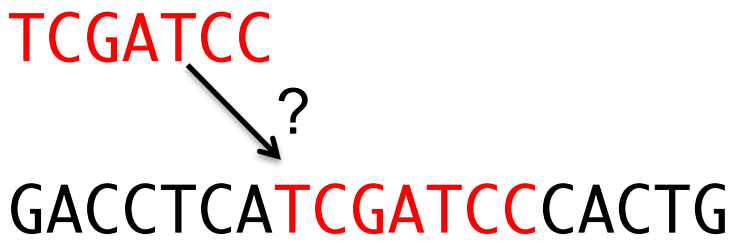
Genome:

ATAAAATGGCCAAAATTAACTAGAAGGTGAGTAGAAACTTAAATAAACTAATTACCATTGATGAGAAAAAAAATC
TGCCACTGAAAAAGGCACCCGGTCCAGAGGGTTTCATGAGCGGGAACTGTAGAAACCTTTCGAATTCAACTCTGC
CAACACCTTCCTCCTCCAGGAAGCACTCCTGGATTTCCCTCTTGCCAACAAGATTCTGGGAGGGCAGCTCCTCCA
ACATGCCCCCAACAGCTCTCTGCAGACATATCATATCATATCATATCTTCCATACCATAACTGCCATGCCATACA

How Would You Find That?

- Brute force comparison
- Smith-Waterman
- Suffix Tree
- Burrows-Wheeler Transform

Brute Force Method



Brute Force Method

TCGATCC
x
GACCTCATCGATCCCACTG

Brute Force Method

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 TCGATCC
 x
GACCTCATCGATCCCACTG
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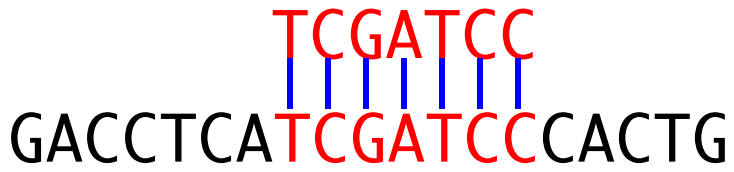
Brute Force Method

Brute Force Method



Smith-Waterman

Simplistic Scoring Scheme:

+1 match if moving diagonally
-1 mismatch if moving diagonally
-1 gap if moving hor. or vert.

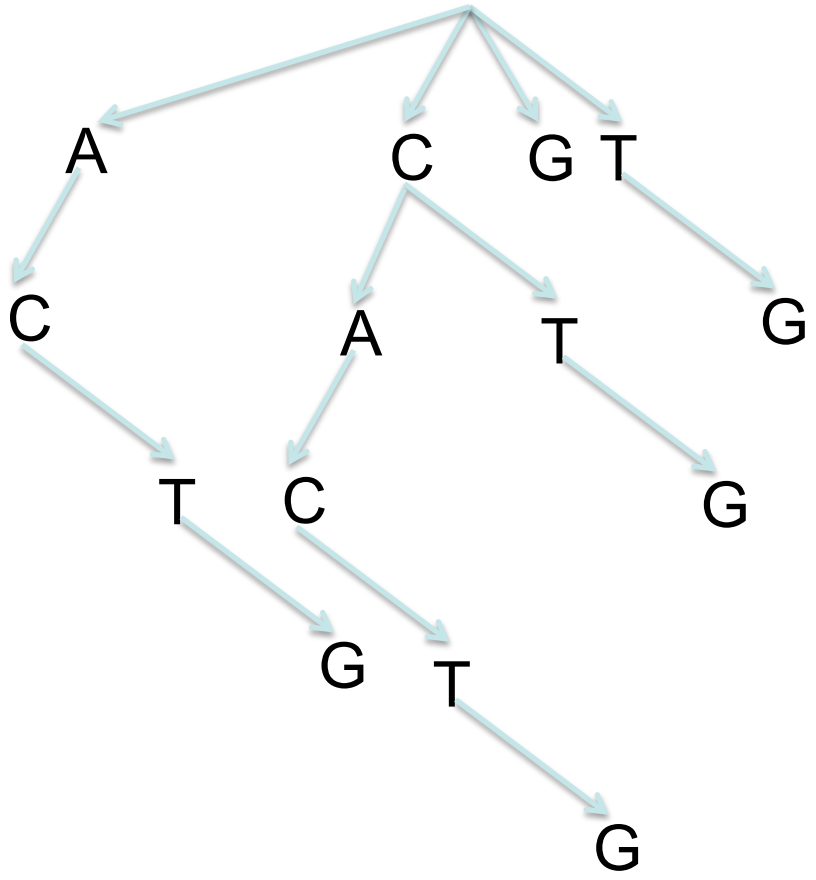
} take the highest of these

(no penalty for terminal gaps)

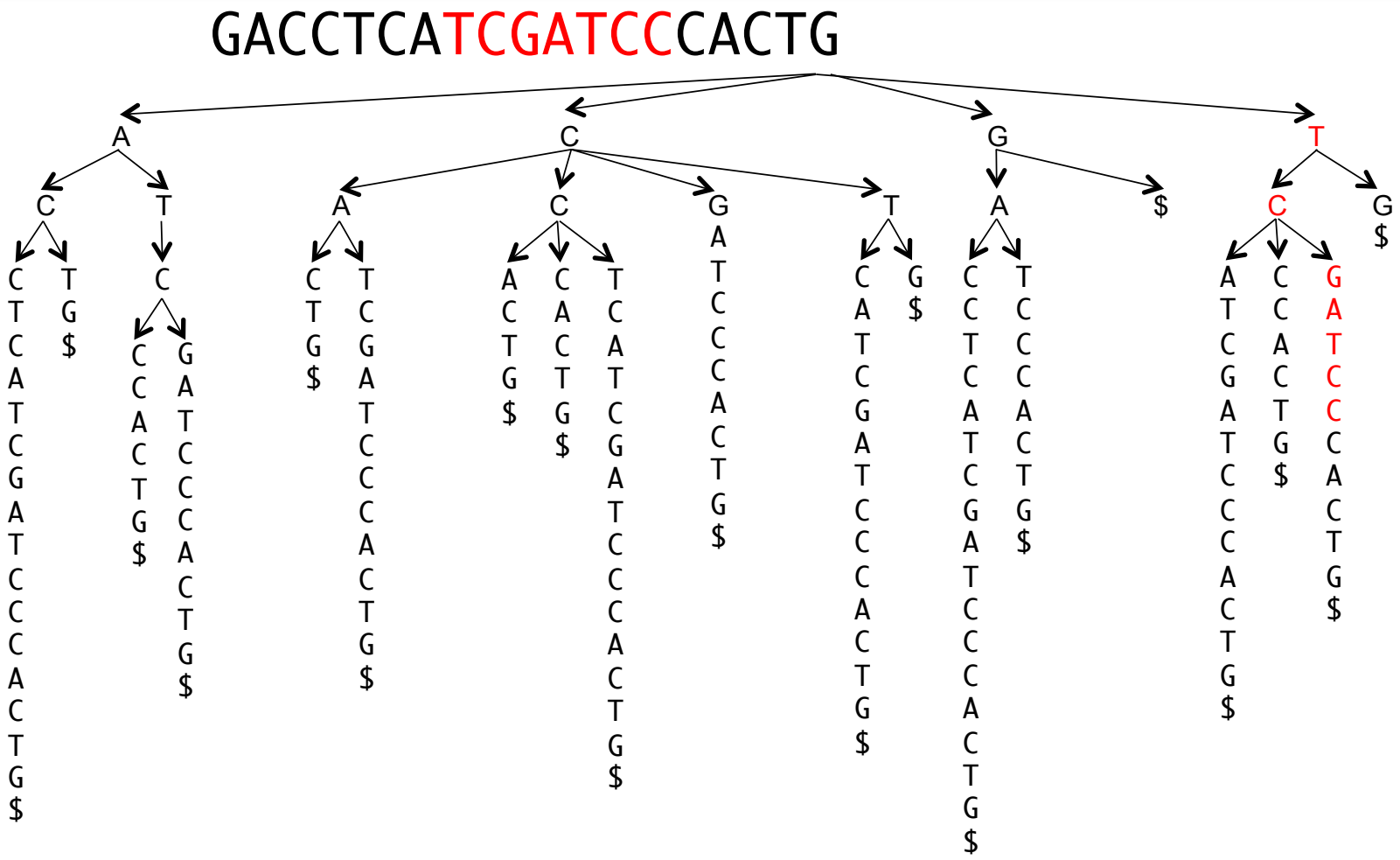
		^	G	A	C	C	T	C	A	T	C	G	A	T	C	C
	^	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T		0	-1	-1	-1	-1	1	0	-1	1	0	-1	-1	1	0	-1	-1	-1	-1	1	0
C		0	-1	0	-1	0	0	2	1	0	2	1	0	0	2	1	0	-1	0	0	0
G		0	1	0	-1	-2	-1	1	1	0	1	3	2	1	1	1	0	-1	-2	-1	0
A		0	0	1	0	-1	-2	0	2	1	0	2	4	3	2	1	0	1	0	-1	-2
T		0	-1	0	0	-1	0	-1	-1	3	2	1	1	5	4	3	2	1	0	1	0
C		0	-1	-1	1	1	0	1	0	2	4	3	2	4	6	5	4	3	2	1	0
C		0	-1	-2	0	2	1	1	0	1	3	3	2	3	5	7	6	5	4	3	2

Suffix Tree

GACCTCATCGATCCCACTG
G
TG
CTG
ACTG
CACTG



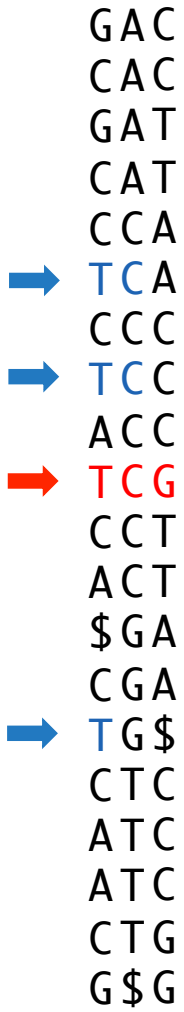
Suffix Tree


GACCTCATCGATCCCACTG
A
C
G
T
C
T
A
C
G
T
$
C
G
$
CTCATCGATCCCACTG$
TG$
CCACTG$
GATCCCACTG$
CTG$
TCGATCCCACTG$
ACTG$
CACTG$
TCATCGATCCCACTG$
GATCCCACTG$
CATCGATCCCACTG$
G$
CCTCATCGATCCCACTG$
TCCCACTG$
ATCGATCCCACTG$
CCACTG$
GATCCCACTG$

Burrows-Wheeler Transform

GACCTCATCGATCCCACTG$	ACCTCATCGATCCCACTG$G
ACCTCATCGATCCCACTG$G	ACTG$GACCTCATCGATCCC
CCTCATCGATCCCACTG$GA	ATCCCACTG$GACCTCATCG
CTCATCGATCCCACTG$GAC	ATCGATCCCACTG$GACCTC
TCATCGATCCCACTG$GACC	CACTG$GACCTCATCGATCC
CATCGATCCCACTG$GACCT	CATCGATCCCACTG$GACCT
ATCGATCCCACTG$GACCTC	CCACTG$GACCTCATCGATC
TCGATCCCACTG$GACCTCA	CCCACTG$GACCTCATCGAT
CGATCCCACTG$GACCTCAT	CCTCATCGATCCCACTG$GA
GATCCCACTG$GACCTCATC	CGATCCCACTG$GACCTCAT
ATCCCACTG$GACCTCATCG	CTCATCGATCCCACTG$GAC
TCCCACTG$GACCTCATCGA	CTG$GACCTCATCGATCCCA
CCCACTG$GACCTCATCGAT	GACCTCATCGATCCCACTG$
CCACTG$GACCTCATCGATC	GATCCCACTG$GACCTCATC
CACTG$GACCTCATCGATCC	G$GACCTCATCGATCCCACT
ACTG$GACCTCATCGATCCC	TCATCGATCCCACTG$GACC
CTG$GACCTCATCGATCCCA	TCCCACTG$GACCTCATCGA
TG$GACCTCATCGATCCCAC	TCGATCCCACTG$GACCTCA
G$GACCTCATCGATCCCACT	TG$GACCTCATCGATCCCAC
$GACCTCATCGATCCCACTG	$GACCTCATCGATCCCACTG

How Do We Use This To Align?



- Start with the transform column
- My read starts with a T, so I want rows with Ts in them
- This column gives me all the single nucleotide counts
- Sort the single nucleotide counts to get the alphabetically first column
- Now these two columns give me all the dinucleotide counts
- Sort those to get the alphabetically first two columns
- Now there is only one place my read can match

FM Index

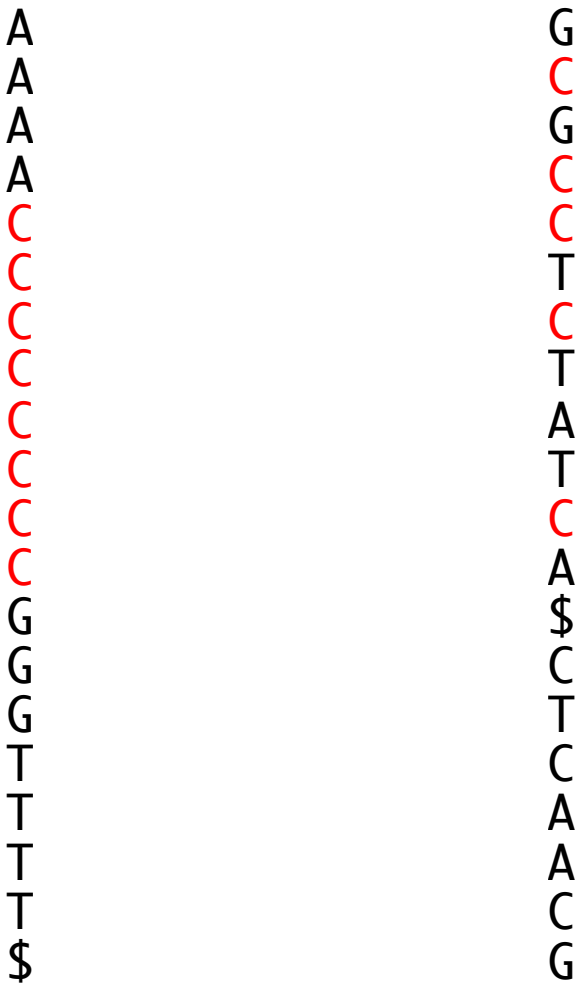
GCGCCTCTATCA$CTCAACG

- Start with the transform column
- Count all the characters, sort them, and store the count of lower characters

A	C	G	T	$
0	4	12	15	19

- This gives the positions of all the bases in the first column (because it's sorted)

FM Index



- Take the query sequence TCGATCC
- Start at the end and use the count table to look up the position of the *last base* in the *first column*

A	C	G	T	$
0	4	12	15	19

- The last column comes immediately before the first column
- Find all the rows of the last column with the next to last base

FM Index

AC	G
AC	C
AT	G
AT	C
CA	C
CA	T
CC	C
CC	T
CC	A
CG	T
CT	C
CT	A
GA	$
GA	C
G$	T
TC	C
TC	A
TC	A
TG	C
$G	G

- Take the query sequence TCGATCC
- The order of a given character in the last column matches the order of the same instance of that character in the first column
- The 3rd-5th Cs in the last column precede Cs in the first column, so we now want the 3rd-5th Cs in column 1

A	C	G	T	$
0	4	12	15	19

- Now we take the next character and look for Ts in the last column (the 2nd T)

FM Index

ACC	G
ACT	C
ATC	G
ATC	C
CAC	C
CAT	T
CCA	C
CCC	T
CCT	A
CGA	T
CTC	C
CTG	A
GAC	$
GAT	C
G$G	T
TCA	C
TCC	A
TCG	A
TG$	C
$GA	G

- Take the query sequence TCGATCC
- The second T is preceded by the 3rd A

A	C	G	T	$
0	4	12	15	19

FM Index

ACCT	G
ACTG	C
ATCC	G
ATCG	C
CACT	C
CATC	T
CCAC	C
CCCA	T
CCTC	A
CGAT	T
CTCA	C
CTG$	A
GACC	$
GATC	C
G$GA	T
TCAT	C
TCCC	A
TCGA	A
TG$G	C
$GAC	G

- Take the query sequence TCGATCC
- The third A is preceded by the 2nd G

A	C	G	T	$
0	4	12	15	19

FM Index

ACCTC	G
ACTG$	C
ATCCC	G
ATCGA	C
CACTG	C
CATCG	T
CCACT	C
CCCAC	T
CCTCA	A
CGATC	T
CTCAT	C
CTG$G	A
GACCT	$
GATCC	C
G$GAC	T
TCATC	C
TCCCA	A
TCGAT	A
TG$GA	C
$GACC	G

- Take the query sequence TCGATCC
- The second G is preceded by the 6th C

A	C	G	T	$
0	4	12	15	19

FM Index

ACCTCA	G
ACTG$G	C
ATCCCA	G
ATCGAT	C
CACTG$	C
CATCGA	T
CCACTG	C
CCCACT	T
CCTCAT	A
CGATCC	T
CTCATC	C
CTG$GA	A
GACCTC	$
GATCCC	C
G$GACC	T
TCATCG	C
TCCCAC	A
TCGATC	A
TG$GAC	C
$GACCT	G

- Take the query sequence TCGATCC
- The sixth C is preceded by the 3rd T

A	C	G	T	$
0	4	12	15	19

FM Index

ACCTCAT	G
ACTG$GA	C
ATCCCAC	G
ATCGATC	C
CACTG$G	C
CATCGAT	T
CCACTG$	C
CCCACTG	T
CCTCATC	A
CGATCCC	T
CTCATCG	C
CTG$GAC	A
GACCTCA	$
GATCCCA	C
G$GACCT	T
TCATCGA	C
TCCCACT	A
TCGATCC	A
TG$GACC	C
$GACCTC	G

- Take the query sequence TCGATCC
- And we're done

A	C	G	T	$
0	4	12	15	19

- To find the position in the genome, we keep a separate index of positions for a sparse set of rows in the table and then just walk through the transform to the nearest indexed row

Heuristic Improvements

- Seeding
  - Use a hash of exact matches to limit searches
  - Use varying hash types
- Limiting mismatches or indels
  - Constrain poor quality searches
  - Known as ‘banded’ in Smith-Waterman
- Using base quality to guide backtracking

Common Short Read Aligners

- Seed and Smith-Waterman extend
  - Mosaik, BFAST, Novoalign
- BWA align gap-free
  - Bowtie
- BWA align with gaps
  - BWA aln, Bowtie2
- BWA Seed and Smith-Waterman extend
  - BWA mem
- Seed clustering and stitching
  - Blasr, STAR

Blasr

- Designed for long error-prone reads (PacBio)
- Combines multiple methods
- Starts by finding short exact matches using suffix or B-W
- Next locally identifies a linear chain of shorter exact matches
- Performs banded Smith-Waterman constrained by the shorter exact matches