Sequence Mapping/Alignment

BCB 504: Applied Bioinformatics

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March 20, 2013

Outline

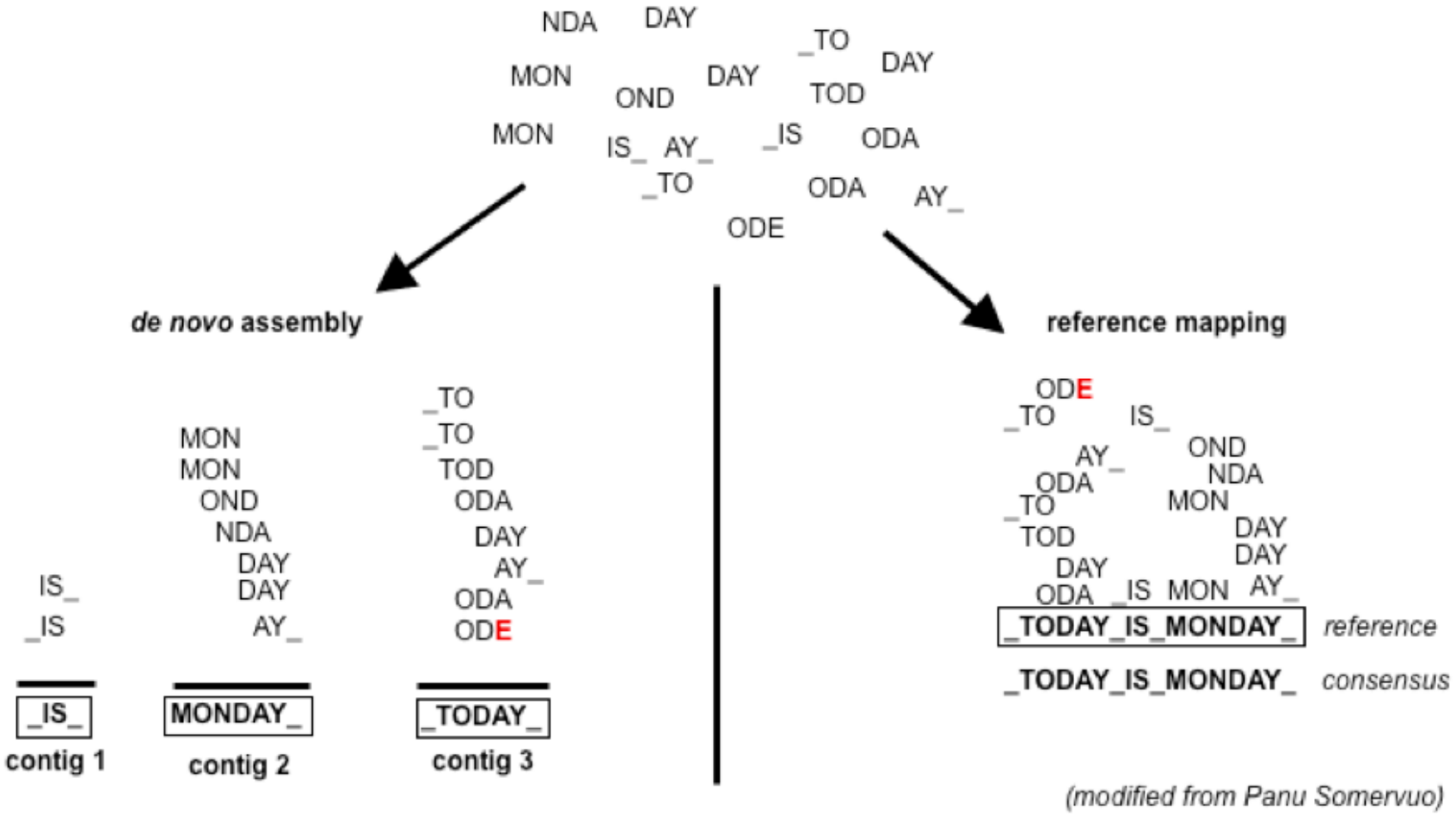
Mapping/Alignment

Given sequence data,

Assembly seeks to put together the puzzle without knowing what the picture is

Mapping tries to put together the puzzle pieces directly onto an image of the picture

In mapping the question is more, given a small chunk of sequence, where in the genome did this piece most likely come from.


NDA
DAY
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_IS
ODA
_TO
ODA
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de novo assembly
reference mapping
IS_
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_IS
MON
AY_
_IS_
MONDAY_
TODAY
contig 1
contig 2
contig 3
_TODAY_IS_MONDAY_
reference
_TODAY_IS_MONDAY_
consensus
(modified from Panu Somervuo)

The Mapping Problem I

The mapping problem:

Given a string S over a finite alphabet $\Sigma = \{A, C, T, G\}$, $|S|$ is used to denote the length of S, $S[i]$ is the ith character of S and $S[i : j]$ is the substring of S which starts at position i and ends at position j. A k-mer of S is a substring of S of length $q > 0$.

The unit cost edit distance between two strings S_1 and S_2 is the minimum number of substitutions, insertions and deletions required to convert S_1 to S_2 refered to as edist(S_1, S_2).

Every genomic sequence can be then be represented as a string over the alphabet $\Sigma = \{A, C, T, G\}$. Given a genome database G of subject sequences $\{S_1, S_2, ..., S_n\}$, a query sequence (read) Q of length l and an integer n, it is required to find all substrings

The Mapping Problem II

from G, such that for each substring α, edist(α,Q) $< n$. We refer to the integer n as the maxEditDist parameter.

The goal then is to find the match(es) with either the "best" edit distance (smallest), or all maches with edit distance less than maxEditDist. Main issues:

- Large search space
- Regions of similarity (aka repeats)
- Gaps
- Complexity (RNA, transcripts)

Basic Local Alignment Search Tool (BLAST)

Some say the first bioinformatics tool, developed at NIH and published in 1990.
Problem:

- Exact algorithms like Smith-Waterman and Needleman-Wunsch (dynamic programming) are slow, when the search space becomes large.
- With the advent of automated DNA sequencing technology, the database of possible matches was becoming increasingly larger.

the BLAST algorithm emphasizes speed over sensitivity, and does not guarantee an optimal alignment.
BLAST is a few-to-many - performs gapped alignment

BLAST Like Alignment Tool (BLAT)

Blat (Jim Kent, UCSC, 2002) was designed to solve the problem of performing comparisons between large genomes and was one of the first algorithms to efficiently search many query sequences against a large database (a genome). Blat also performs a gapped-alignment for searching RNA sequences against a genome and handling splice junctions.

gapped-alignment alignment allowing for insertions and deletions greater than a few base pairs. Gapped alignment are less efficient, but more accurate.

BLAT is a many-to-many algorithm - performs gapped alignments

Improving Algorithms

Many additional algorithms have been developed since BLAST and BLAT, mainly improving on either speed or accuracy, or both.

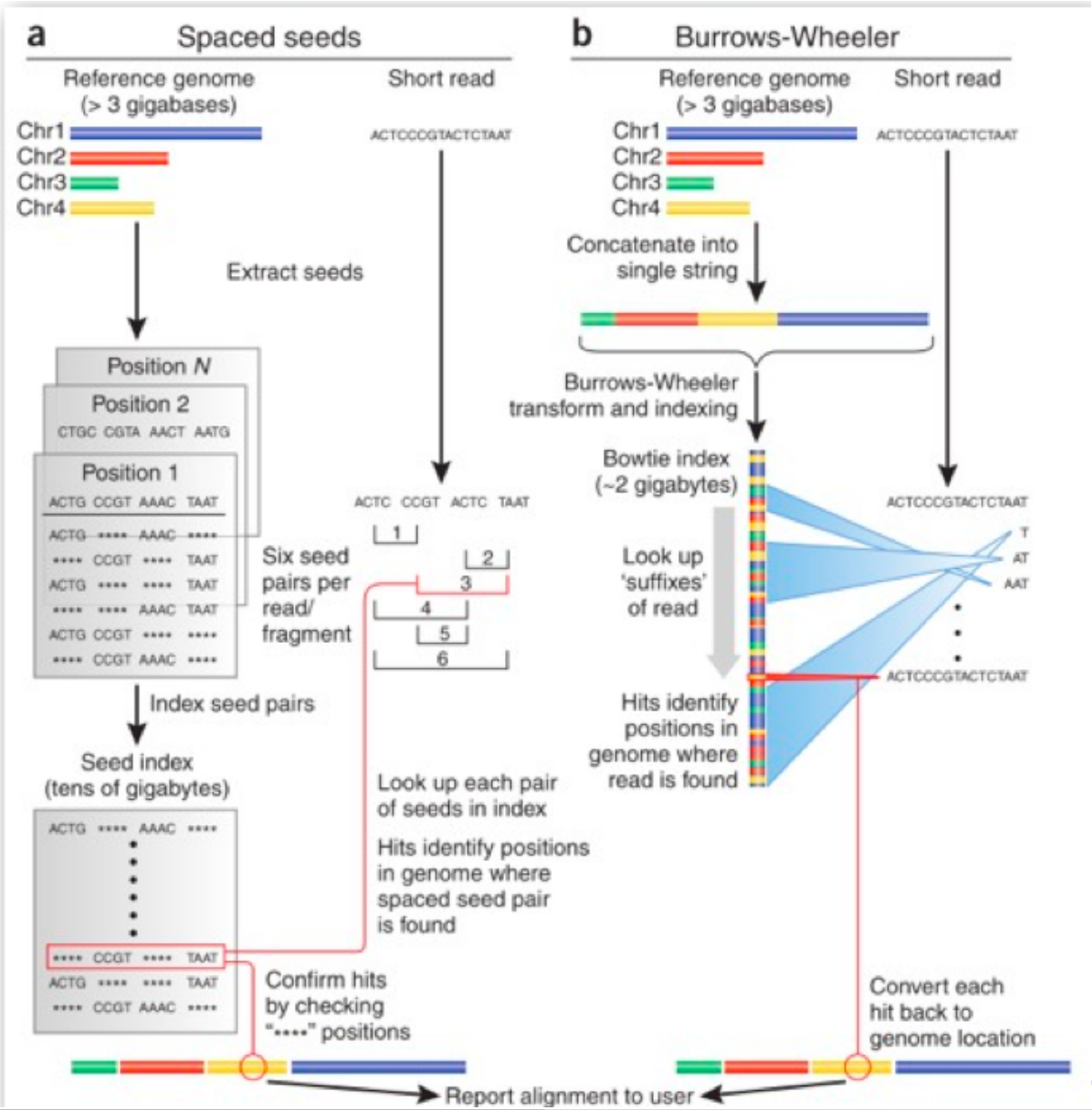
and then came Illumina data -
New Problem:

- We still have a large search space (aka genome)
- Very small pieces, many possible close matches
- Millions or tens of millions of query sequences

Types

Hash based First generation of alignment algorithms relied on hashes (Eland [Illumina], RMAP, MAQ, SHRiMP, SOAP)

Burrows-Wheeler Second generation algorithms with a reduced memory footprint (BWA, SOAP2, Bowtie)


a
Spaced seeds
Reference genome
(> 3 gigabases)
Short read
Chr1
Chr2
Chr3
Chr4
ACTCCCGTACTCTAAT
Extract seeds
Position N
Position 2
CTGC CGTA AACT AATG
Position 1
ACTG CCGT AAAC TAAT
ACTC CCGT ACTC TAAT
Six seed pairs per read/ fragment
Index seed pairs
Seed index
(tens of gigabytes)
Look up each pair of seeds in index
Hits identify positions in genome where spaced seed pair is found
Confirm hits by checking "••••" positions
b
Burrows-Wheeler
Concatenate into single string
Burrows-Wheeler transform and indexing
Bowtie index
(~2 gigabytes)
Look up 'suffixes' of read
Hits identify positions in genome where read is found
Convert each hit back to genome location
Report alignment to user

hash based example: MAQ

- Index reference genome (or sequence reads) => creates hash index
  - Big file: >50GB
  - takes a long time (hours or overnight), but only need to do once
- Divide each read into segments (seeds) and look up in table
  - Search stage finds regions in the genome that can potentially be homologous to the read.
  - Alignment stage verifies these regions to check if they are indeed homologous. More computationally intensive

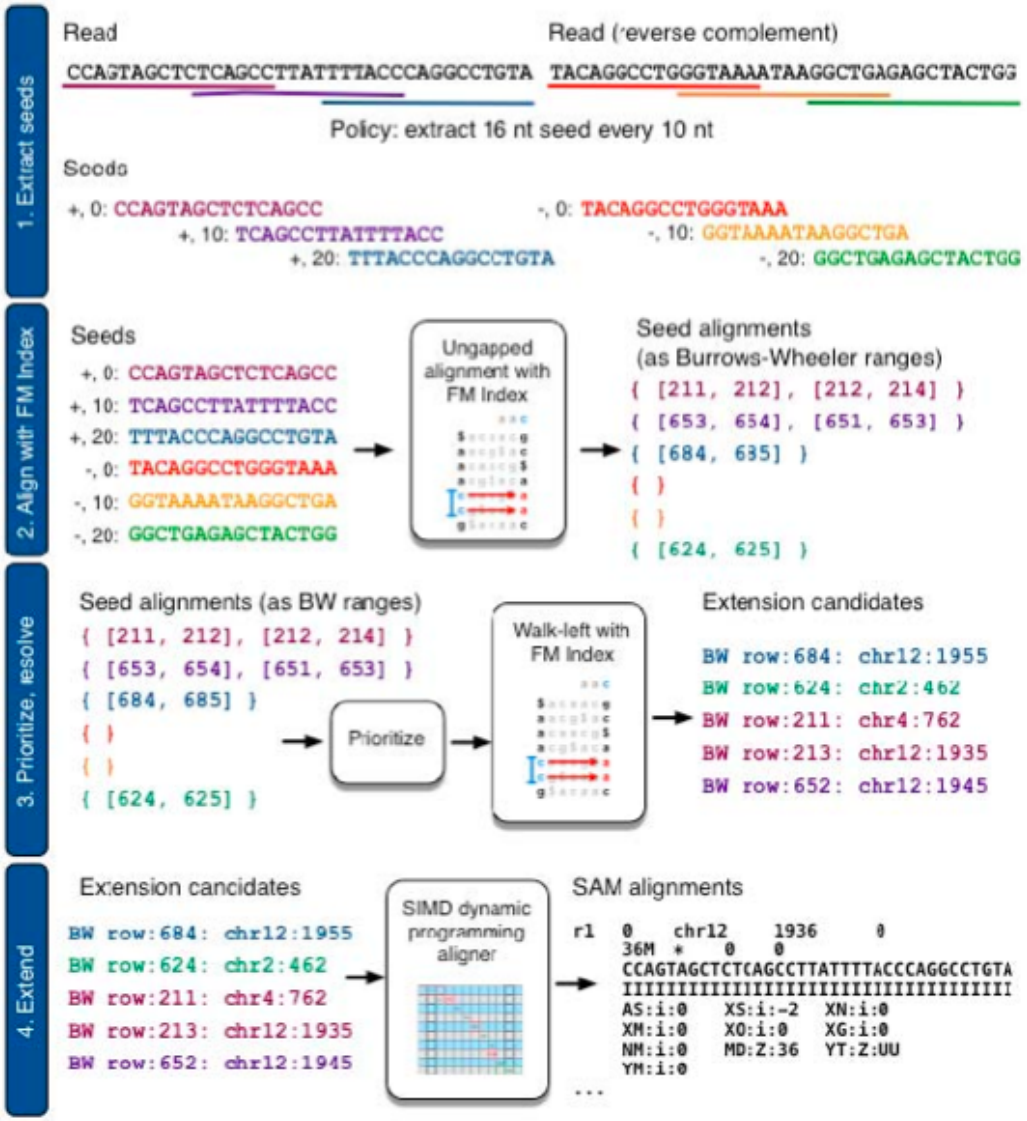
burrows-wheeler example: Bowtie2

Used in data compression (e.g. bzip) => index: much smaller than hash-based index (<2GB)

- Alignment speed: 30x faster than MAQ

Steps:

- Create BWT index of genome
- Align read 1 character at a time to BWT-transformed genome


1. Extract seeds
Read
Read (reverse complement)
CCAGTAGCTCTCAGCCTTATTTTACCCAGGCCTGTA
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
...

considerations

- Placing reads in regions that do not exist in the reference genome (reads extend off the end).
- Sequencing errors and variations: alignment between read and true source in genome may have more differences than alignment with some other copy of repeat.
- What if the closest fully sequenced genome is too divergent? (3% is a common alignment capability)
- Placing reads in repetitive regions: Some algorithms only return 1 mapping; If multiple: map quality = 0
- Algorithms that use paired-end information => might prefer correct distance over correct alignment.

SAM/BAM format

SAM (Sequence Alignment/Map) format = unified format for storing read alignments to a reference genome (Consistant since Sept. 2011).
See http://samtools.sourceforge.net/SAM1.pdf

BAM = binary version of SAM for fast querying

SAM format

SAM files contain two regions

- The header section
  - Each header line begins with character '@' followed by a two-letter record type code
- The alignment section
  - Each alignment line has 11 mandatory fields. These fields always appear in the same order and must be present, but their values can be '0' or '*', if the cooresponding information if unavailable, or not applicable.

SAM Alignment lines

SAM aligmenment lines:

```
7172283   163   chr9   139389330   60   90M   =   139389482   242   TAGGAGG...   EHHHHHH...
7705896    83   chr9   139389513   60   90M   =   139389512   -91   GCTGGGG...   EBCHHFC...
7705896   163   chr9   139389512   60   90M   =   139389513    91   AGCTGGG...   HHHHHHH...
```

1	QNAME	query template name
2	FLAG	bitwise flag
3	RNAME	reference sequence name
4	POS	1-based leftmost mapping position
5	MAPQ	mapping quality
6	CIGAR	CIGAR string
7	RNEXT	reference name of mate
8	PNEXT	position of mate
9	TLEN	observed template length
10	SEQ	sequence
11	QUAL	ASCII of Phred-scaled base quality

SAM flags

SAM flag field:

Flag	Description
0x0001	the read is paired in sequencing, no matter whether it is mapped in a pair
0x0002	the read is mapped in a proper pair (depends on the protocol, normally inferred during alignment) [1]
0x0004	the query sequence itself is unmapped
0x0008	the mate is unmapped [1]
0x0010	strand of the query (0 for forward; 1 for reverse strand)
0x0020	strand of the mate [1]
0x0040	the read is the first read in a pair [1,2]
0x0080	the read is the second read in a pair [1,2]
0x0100	the alignment is not primary (a read having split hits may have multiple primary alignment records)
0x0200	the read fails platform/vendor quality checks
0x0400	the read is either a PCR duplicate or an optical duplicate

MAPQ explained I

MAPQ, contains the "phred-scaled posterior probability that the mapping position" is wrong.

In a probabilistic view, each read alignment is an estimate of the true alignment and is therefore also a random variable. It can be wrong. The error probability is scaled in the Phred. For example, given 1000 read alignments with mapping quality being 30, one of them will be wrong pn average. Understanding Mapping Qualities

The calculation of mapping qualities is simple, but this simple calculation considers all the factors below:

- The repeat structure of the reference. Reads falling in repetitive regions usually get very low mapping quality.

MAPQ explained II

- The base quality of the read. Low quality means the observed read sequence is possibly wrong, and wrong sequence may lead to a wrong alignment.
- The sensitivity of the alignment algorithm. The true hit is more likely to be missed by an algorithm with low sensitivity, which also causes mapping errors.
- Paired end or not. Reads mapped in pairs are more likely to be correct.

When you see a read alignment can get a mapping quality 30, it usually implies:

- The overall base quality of the read is good.
- The best alignment has few mismatches.

MAPQ explained III

- The read has few or just one 'good' hit on the reference, which means the current alignment is still the best even if one or two bases are actually mutations or sequencing errors.

In principle however, each mapper seems to compute the MAPQ in their own way.