Alignment (II)

Bioinformatics Applications (PLPTH813)

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3/2/2021

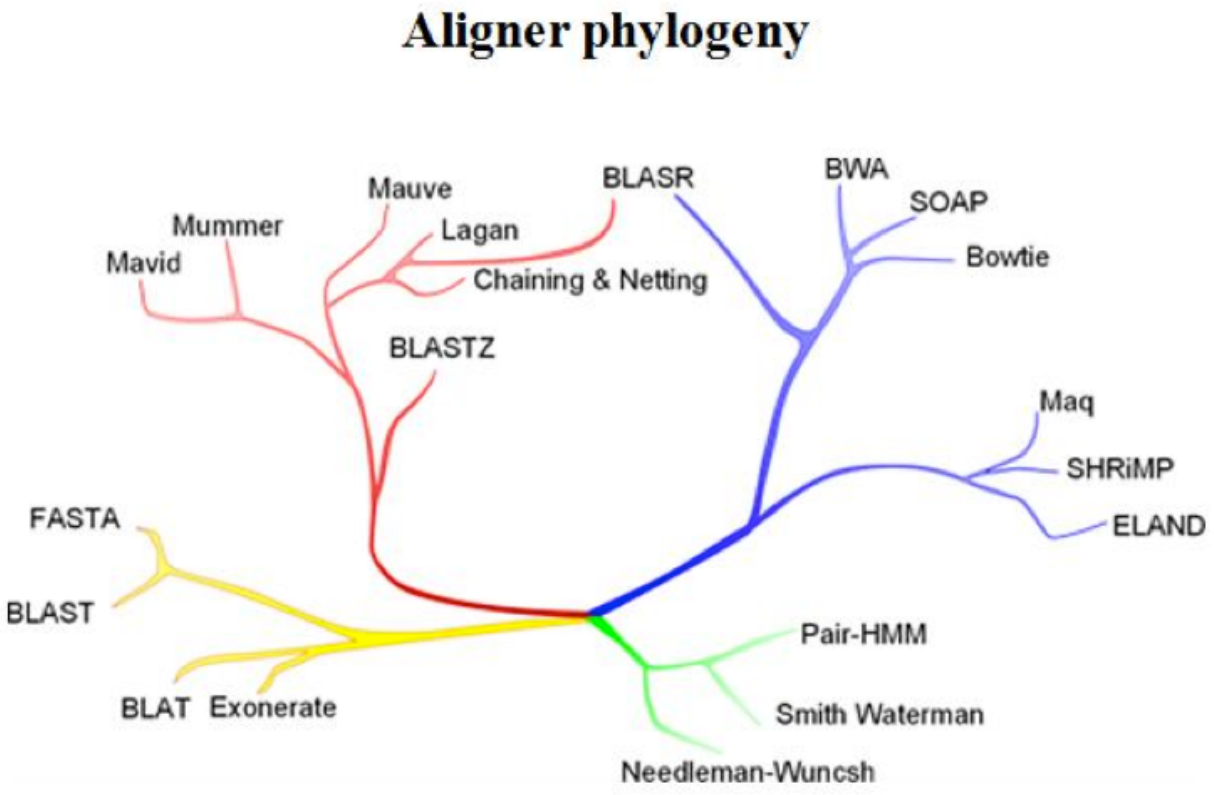
Alignment I - review

- The local alignment algorithm, **Smith–Waterman algorithm**, ensures the best performance on accuracy and the most precise results with respect to its scoring scheme but it is very time-consuming.

- **BLAST** is a seed-extension algorithm for database searching.
  1. Identify a match for the seed sequence
  2. Extend match using a local alignment

- However, BLAST is still not fast enough for efficient alignments of millions of NGS reads.

Relationship among different algorithms



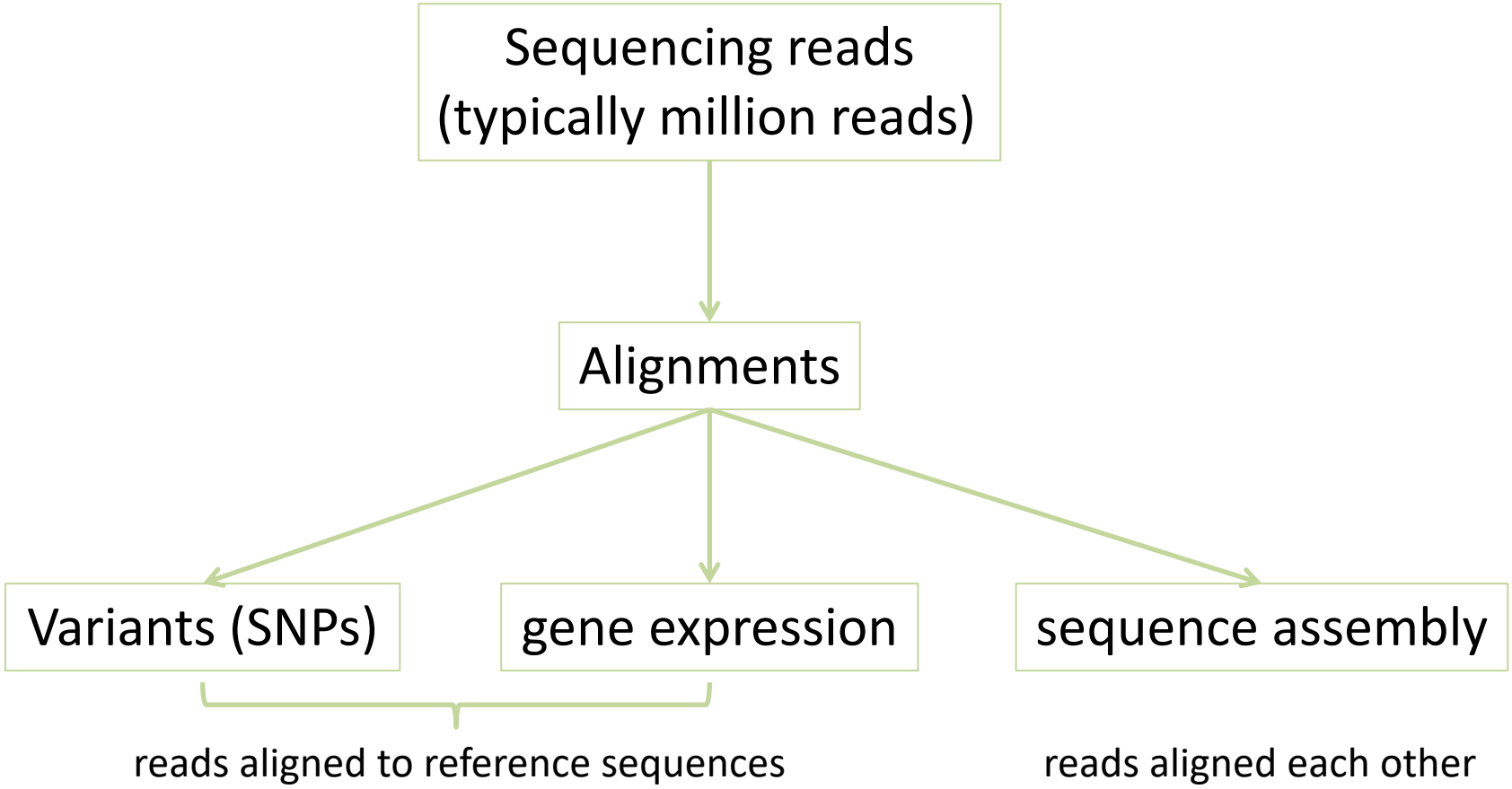
Long sequences	Short reads
Pairwise heuristic	Sensitive aligners

http://evomicsorg.wpengine.netdna-cdn.com/wp-content/uploads/2014/01/alignCompare2.jpg

Outline

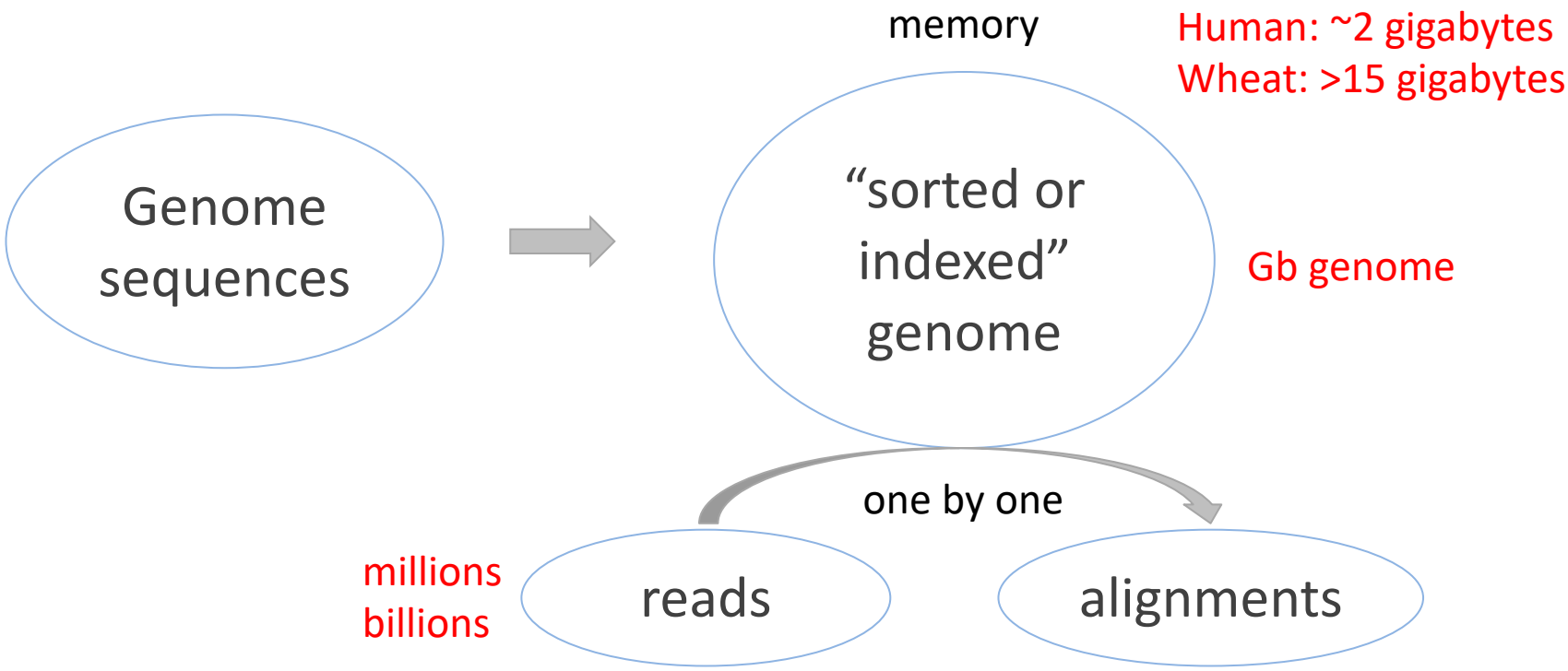
- One of short-read alignment algorithms – BWT
- An short-read aligner - BWA
- Alignment standard format – SAM and BAM
- A tool to handle alignments - SAMtools
- An alignment visualization tool - IGV

Alignment is a central step for most NGS analyses



Short-read alignments

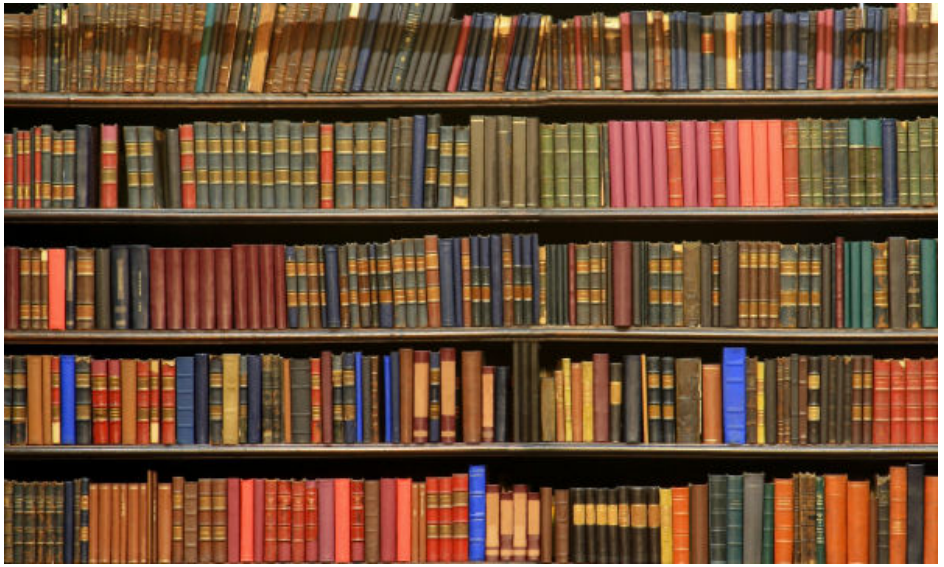
1. “Sorting or indexing” genome sequences in a smart way to accelerate the alignment
2. Keeping the “Sorting or indexing” data at a reasonable size



3. A good aligner needs to find a good tradeoff between Speed and Accuracy.

Indexing speeds up searching

un-indexed library



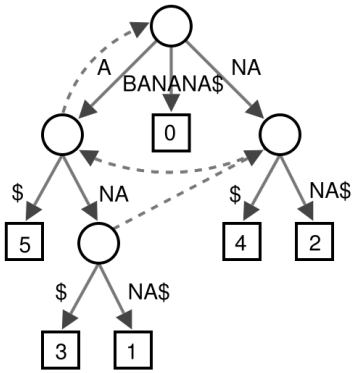
indexed library

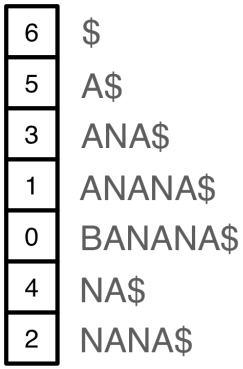
Indexing algorithms

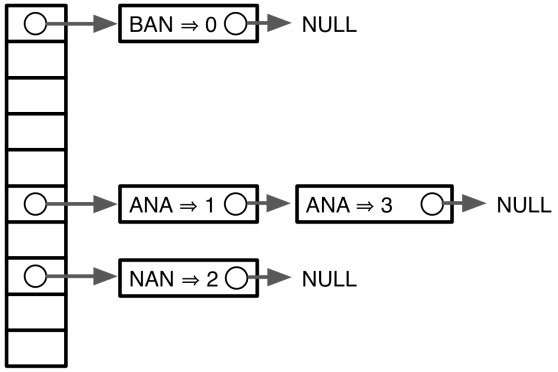
A benefit of string indexing is to avoid scanning the whole reference. Therefore alignment searching is much faster.

BWT | Suffix Tree | Suffix Array | Hash table

Input	BWT
^BANANA|	BNN^AA|A







<2 gigabytes >35 gigabytes >12 gigabytes >12 gigabytes

Human genome memory footprint*

* From Bowtie poster

Burrows–Wheeler transform

Transformation algorithm*

Input	All Rotations	Sorting all rows	Taking last character	BWT output
acaacg$	acaacg$	$acaacg	$acaac**g**	gc$aaac
	$acaacg	aacg$ac	aacg$a**c**	
	g$acaac	acaacg$	acaacg**$**	
	cg$acaa	acg$aca	acg$ac**a**	
	acg$aca	caacg$a	caacg$**a**	
	aacg$ac	cg$acaa	cg$aca**a**	
	caacg$a	g$acaac	g$acaa**c**	

a c a a c g $

$ a c a a c **g**
a a c g $ a **c**
a c a a c g **$**
a c g $ a c **a**
c a a c g $ **a**
c g $ a c a **a**
G $ a c a a **c**

g c $ a a a c

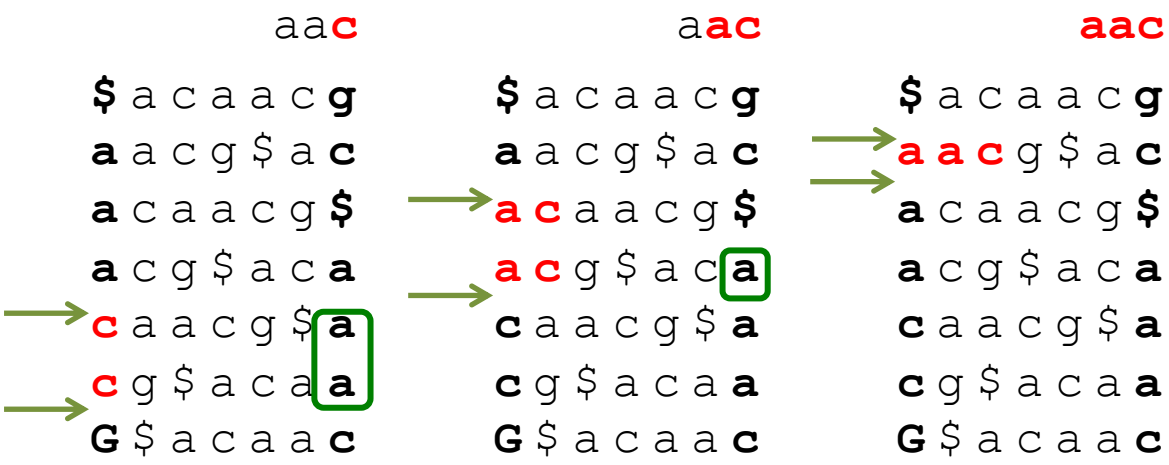
- The transformation is **reversible**, without needing to store any additional data.
- BWT is a **compression** technique to find repeated patterns and encoding the duplications more compactly. (“bzip” is based on this compression)

* wikipedia.com

Matching algorithms in Bowtie and BWA

Find exact match of `aac` in the sequence of `acaacg`

1. BWT(`acaacg`) is `gc$aaac`
2. searching



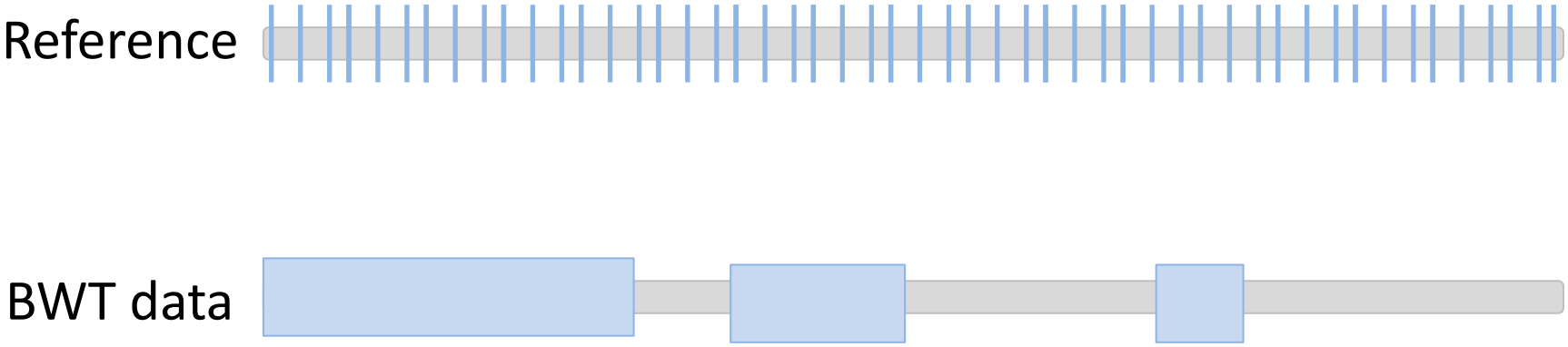
Ultrafast and memory-efficient alignment of short DNA sequences to the human genome
Ben Langmead, Cole Trapnell, Mihai Pop and Steven L Salzberg

Bowtie

Fast and accurate short read alignment with Burrows–Wheeler transform
Heng Li and Richard Durbin*

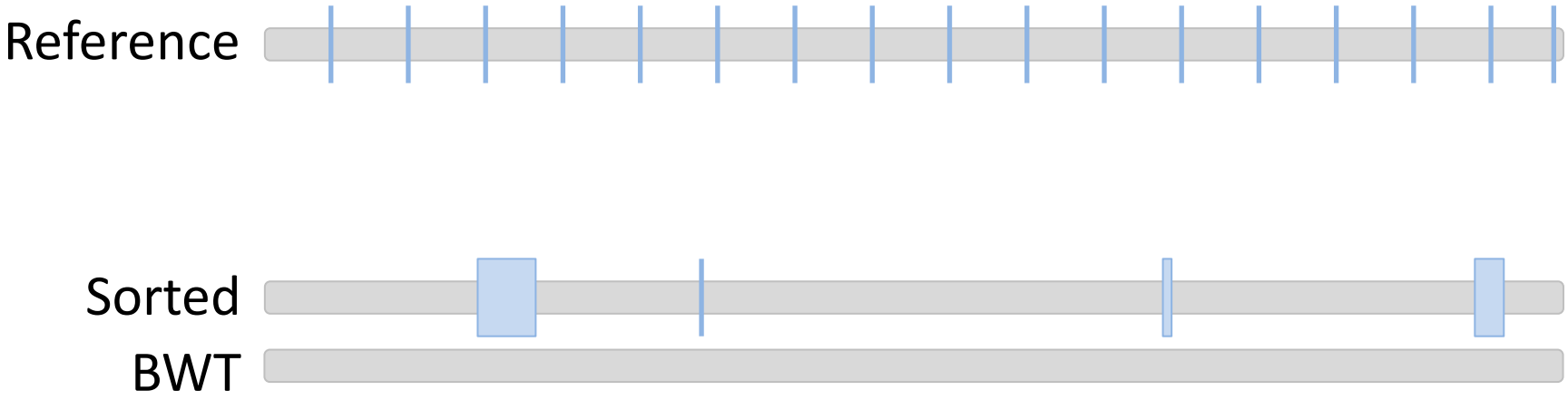
BWA

BWA searching



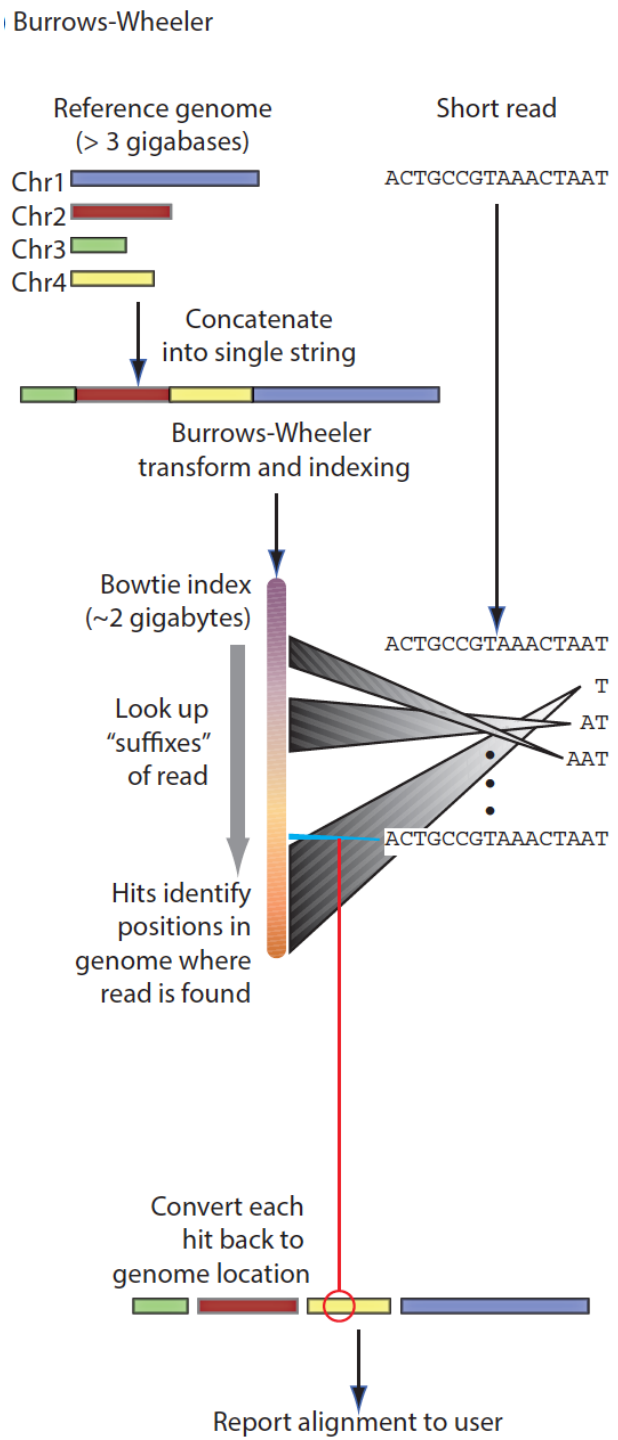
5’ CGTAGCA 3’

backward searching



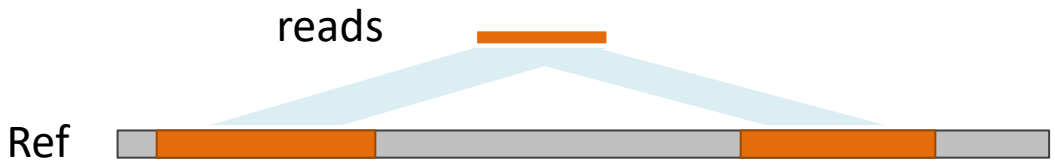
5’ CGTAGCA 3’

Working flow



Alignment issues

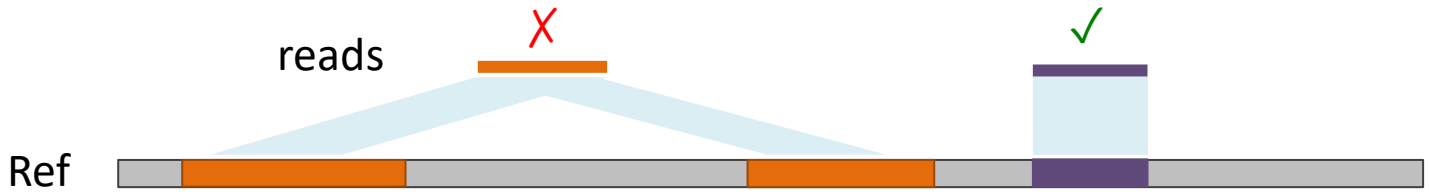
- Repeats



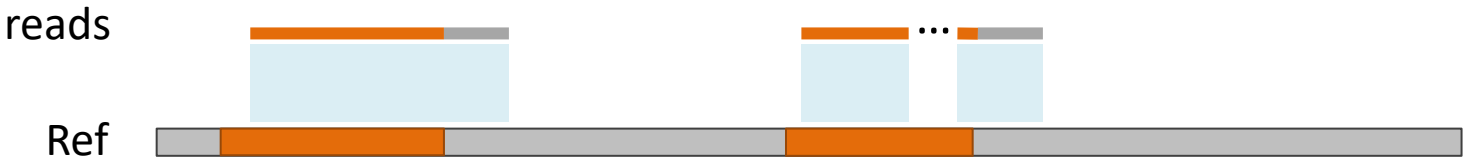
- Sequencing errors
- Polymorphisms (reference and sequenced sample)
- Quality of reference genomes (mis-assembly and incomplete genome)

Solutions

- Unique mapped reads



- Longer reads or Paired-end reads

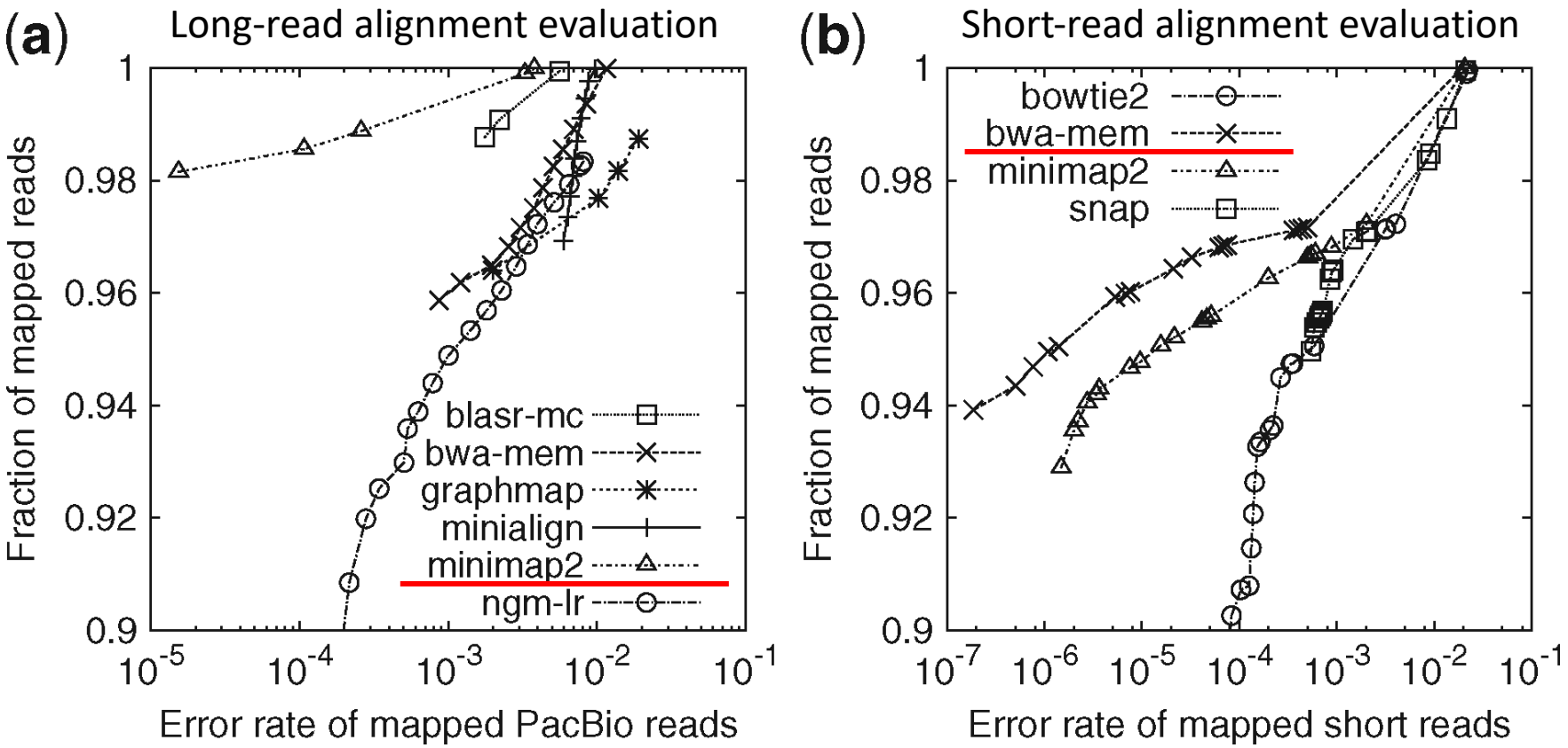


- Tolerance of mismatches or gaps for each alignment

The aligner: BWA

- BWA is a software package for mapping low-divergent sequences against a large reference genome, such as the human genome.
- It consists of three algorithms: BWA-backtrack, BWA-SW and BWA-MEM.
- BWA-backtrack is designed for Illumina sequence reads up to 100bp, while BWA-SW and BWA-MEM are for longer sequences ranged from 70bp to 1Mbp.
- **BWA-MEM**, which is the latest, is generally recommended for high-quality queries as it is faster and more accurate.

bwa, bowtie2, minimap2



Bioinformatics, 34:3094–3100, https://doi.org/10.1093/bioinformatics/bty191

Align queries (reads) to the reference with BWA-MEM

- Alignment using “mem”

```
bwa mem ref.fa reads.fq > se.sam
bwa mem ref.fa pair1.fq pair2.fq > pe.sam
```

Build a BWA sequence index database

- Index reference sequences

```
bwa index ref.fa
```

example

```
bwa index MG1655.fasta
```

--------output------------

```
MG1655.fasta.amb
MG1655.fasta.ann
MG1655.fasta.bwt
MG1655.fasta.pac
MG1655.fasta.sa
```

Question

What parameters/items are needed to describe an alignment between a read and a reference?

And how about an alignment of a paired-end read to the reference?

Alignment format: SAM

- SAM stands for **S**equence **A**lignment/**M**ap format.

- The Sequence Alignment/Map (SAM) format is a generic alignment format for storing read alignments against reference sequence.

- The SAM/BAM format, together with SAMtools, enables a generic and modular approach to the analysis of genomic sequencing data.

More about SAM/BAM format and samtools

Heng Li's blog Archive Categories Pages Tags

SAM/BAM/samtools is 10 years old

21 December 2018

Feature	Year first available	Target	Feature descriptions
1	2009	BAM	End-of-file marker to detect truncated files
2	2009	SAM	"="/"X" CIGAR operators to encode sequence matches or mismatches
3	2011	SAM	"B" tag type to efficiently store binary arrays
4	2012	BAM	CSI index for chromosomes longer than 512Mbp
5	2012	htslib	BCFv2 as an efficient binary representation of VCF files
6	2013	SAM	Supplementary alignment to encode split alignment
7	2014	htslib	CRAMv3 as a more compact binary representation of SAM
8	2015	htslib	Direct access to remote files over internet
9	2017	BAM	BAM extension to support CIGARs with >65535 operators
10	2017	htslib	Htsget protocol for efficient database access
11	2017	htslib	CRC error checking to detect internally corrupted BAM files

Examples of SAM alignments

@SQ SN:1 LN:307041717

@SQ SN:2 LN:244442276

HISEQ:446:C9KP3ANXX:3:1102:8028:81847 99 1 162 40 63M1I61M = 285 234
AATGGTCGTTCATGGCTATTTTCGACAAAAATGGGGGTTGTGTGGCAATTGATCATCGACCAGAAGCT
CATACACCTCACCCCACATATGTTTCCTTGCCATAGATCACATTCTTGGATTTCTGG
BBCBBGDEGGGGGG>FGGGGGGGGGFGGGGGEGEGGG9C<FGGGDGBGGGGGGGFG>@GGGD
GGGGC@GDGGGG0CG>GBG0<CCGGGGGFGGGCFGC0CCGGGG=8800:8@CDD/88@C8DDG
NM:i:2 MD:Z:46C77 AS:i:112 XS:i:112 RG:Z:A188-1

HISEQ:446:C9KP3ANXX:3:1305:17136:31643 99 1 205 40 20M1I104M = 379 252
GGCCACTGATCATCGACCAGAAGCTCATACACCTCACCCCACATATGTTTCCTTGCCATAGATCACATT
CTTGGATTTCTGGTGGAGACCATTTCTTGGTCAAAAATCCGTAGGTGTTAGCCTTC
CCCCCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
GGGGGGGGGGGGGGGGGGGGGGGCFF/::FGGGGG00>0FGGGGGGFBFGBCGGD<F>FGGEG=G8
8 NM:i:2 MD:Z:5T118 AS:i:112 XS:i:112 RG:Z:A188-1

HISEQ:446:C9KP3ANXX:3:1202:12503:18962 163 1 464 40 125M = 586 247
GTGGAGACCATTTCTTGGTCAAAAATCCGTAGGTGCTAGCCTTCGGTATTATTGAAAATGGTCGTTCAT
GGCTATTTTCGACAAAATGGGGGTTGGGTGGCAATTGATCATCGACCAGAGGCTCA
BBCCCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
GGGGGGGGGCGFBDF>11<<E0/=C0:00=DGG<.<.;CCCBG@0FF=F86EDDDDCG<DD@B668
NM:i:3 MD:Z:35T59T5C23 AS:i:110 XS:i:115 RG:Z:A188-1

SAM section 1: headers

In the header section, the line starts with '@'

- @SQ Reference sequence dictionary.
- @RG Read group.
- …

SAM section 2: the alignment section

In SAM, each alignment line has 11 mandatory fields and a variable number of optional fields.

```
EAS600_70:5:1:3215:930  99    REF   2767401     60    100M  =     2767797     498
      NTGATATTAACTTGTCCAATATGATCAAATAGCATTAACCCCCCCTCACAACGTCCTGCATAGGGAACACGTTTTCCCCTGTGCACCCACGACTAAATTT
      !+++*+87777@@@@@@@@@@@@@@@@@@@@@<:::<<99989::32222298&)--28888589179@@@@@##############################
      NM:i:1      MD:Z:0A99   AS:i:99     XS:i:0
```

No.	Name	Description	
1	QNAME	Query NAME of the read or the read pair	EAS600_70:5:1:3215:930
2	FLAG	Bitwise FLAG (pairing, strand, mate strand, etc.)	99
3	RNAME	Reference sequence NAME	REF
4	POS 1	1-Based leftmost POSition of clipped alignment	2767401
5	MAPQ	MAPping Quality (Phred-scaled)	60
6	CIGAR	Extended CIGAR string (operations: MIDNSHP)	100M
7	MRNM	Mate Reference NaMe ('=' if same as RNAME)	=
8	MPOS	1-Based leftmost Mate POSition	2767797
9	ISIZE	Inferred Insert SIZE	498
10	SEQ	Query SEQuence on the same strand as the reference	NTGATA…
11	QUAL	Query QUALity (ASCII-33=Phred base quality)	!+++*+8…

optional field

FLAG (for single-end or paired-end reads)

Bitwise flag values

Hexadecimal Value	Decimal Value	Description
0x0001	1	The read is paired in sequencing, no matter whether it is mapped in a pair
0x0002	2	The read is mapped in a proper pair (depends on the protocol, normally inferred during alignment)
0x0004	4	The query sequence itself is unmapped
0x0008	8	The second read is unmapped
0x0010	16	The read is reverse complemented
0x0020	32	The second read is reversed
0x0040	64	The read is the first read in a pair
0x0080	128	The read is the second read in a pair
0x0100	256	The alignment is not primary (a read having split hits may have multiple primary alignment records)
0x0200	512	The read fails platform/vendor quality checks
0x0400	1024	The read is either a PCR duplicate or an optical duplicate

16 Reverse complemented

1 + 4 + 128 = 133 paired; segment unmapped;

* http://support.illumina.com/help/SequencingAnalysisWorkflow/Content/Vault/Informatics/Sequencing_Analysis/CASAVA/swSEQ_mCA_BitwiseFlagValues.htm

Problem: explain these decimal FLAG numbers

73

81

192

Hexadecimal Value	Decimal Value	Description
0x0001	1	The read is paired in sequencing, no matter whether it is mapped in a pair
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MAPQ: Mapping quality

- MAPQ = $-10 \log_{10}$ Prob{error mapping}, rounded to the nearest integer.

0: Prob(error mapping) = $10^{0/(-10)} = 1$

10: Prob(error mapping) = $10^{10/(-10)} = 0.1$

30: Prob(error mapping) = $10^{30/(-10)} = 0.001$

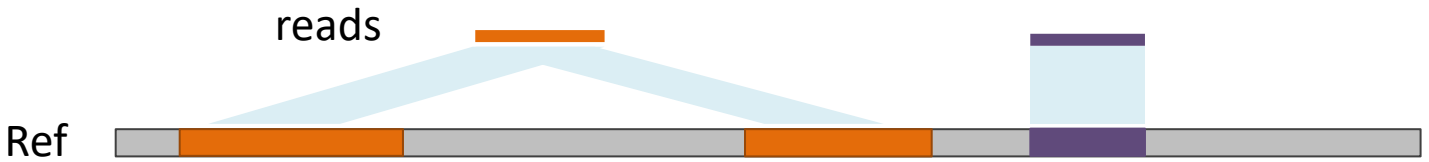
40: Prob(error mapping) = $10^{40/(-10)} = 0.0001$

Factors influencing mapping quality

- Alignment quality (matches, mismatches, gaps)

```
s: CTGTTGCT
    ||||||
t: ATGCTGCA
```

- Mapping ambiguity



Mapping quality ≠ Alignment quality

A read with a high mapping score

- A read alignment with a mapping quality 30 or above usually implies:

The read is mapped at the location with **a high alignment score** and the read is not mapped to anywhere else or alignments at other locations are not as good as this alignment.

CIGAR

Example 1: 100M
Example 2: 24M188N83M
Example 3: 80M1I20M2D60M
Example 4: 98M2S

Operation	Description
M	alignment match (can be a sequence match or mismatch)
I	insertion to the reference
D	deletion from the reference
N	skipped region from the reference
S	soft clipping (clipped sequences present in SEQ)
H	hard clipping (clipped sequences NOT present in SEQ)
P	padding (silent deletion from padded reference)
=	sequence match
X	sequence mismatch

For mRNA-to-genome alignment, an N operation represents an intron.

P, =, and X are rarely used.

Binary Alignment/Map format (BAM)

- To improve the performance, a companion format Binary Alignment/Map (BAM) was designed.
- BAM is the binary representation of SAM and keeps exactly the same information as SAM.

Table. File size of two example files

File type	Storage usage
SAM	313 M
BAM	97 M

SAMtools

- SAMtools is a popular open-source software package for sequence alignment manipulation. It is a reliable tool for almost all bioinformatists who work on NGS data.

```
-- indexing
   faidx       index/extract FASTA
   index       index alignment
-- editing
   calmd       recalculate ...
   fixmate     fix mate information
   reheader    replace BAM header
   rmdup       remove PCR duplicates
   targetcut   cut fosmid regions
-- file operations
   bamshuf     shuffle and group ...
   cat         concatenate BAMs
   merge       merge sorted alignments
   mpileup     multi-way pileup
   sort        sort alignment file
   split       splits a file by read group
   bam2fq      converts a BAM to a FASTQ
```

```
-- stats
   bedcov      read depth per BED region
   depth       compute the depth
   flagstat    simple stats
   idxstats    BAM index stats
   phase       phase heterozygotes
   stats       generate stats (former bamcheck)
-- viewing
   flags       explain BAM flags
   tview       text alignment viewer
   view        SAM<->BAM<->CRAM conversion
```

Conversion between SAM and BAM

For efficient computation and storage, SAM is often converted to BAM. Sorting and indexing the alignment facilitate the following analyzing procedures.

- **converting:**

```
samtools view aln.sam -o aln.bam
```

- **sorting:**

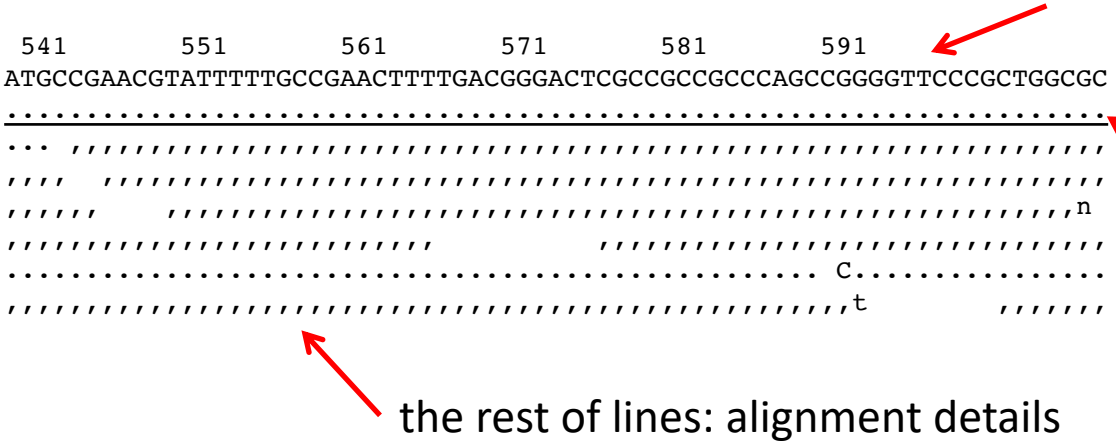
```
samtools sort aln.bam alnsort
```

- **indexing:**

```
samtools index alnsort.bam
```

Visualization

```
samtools tview alnsort.bam ref.fasta
```



1st line: the genome coordinates

2nd line: the reference sequence

3rd line: the consensus sequence, . indicates the identical sequence to the reference genome

the rest of lines: alignment details

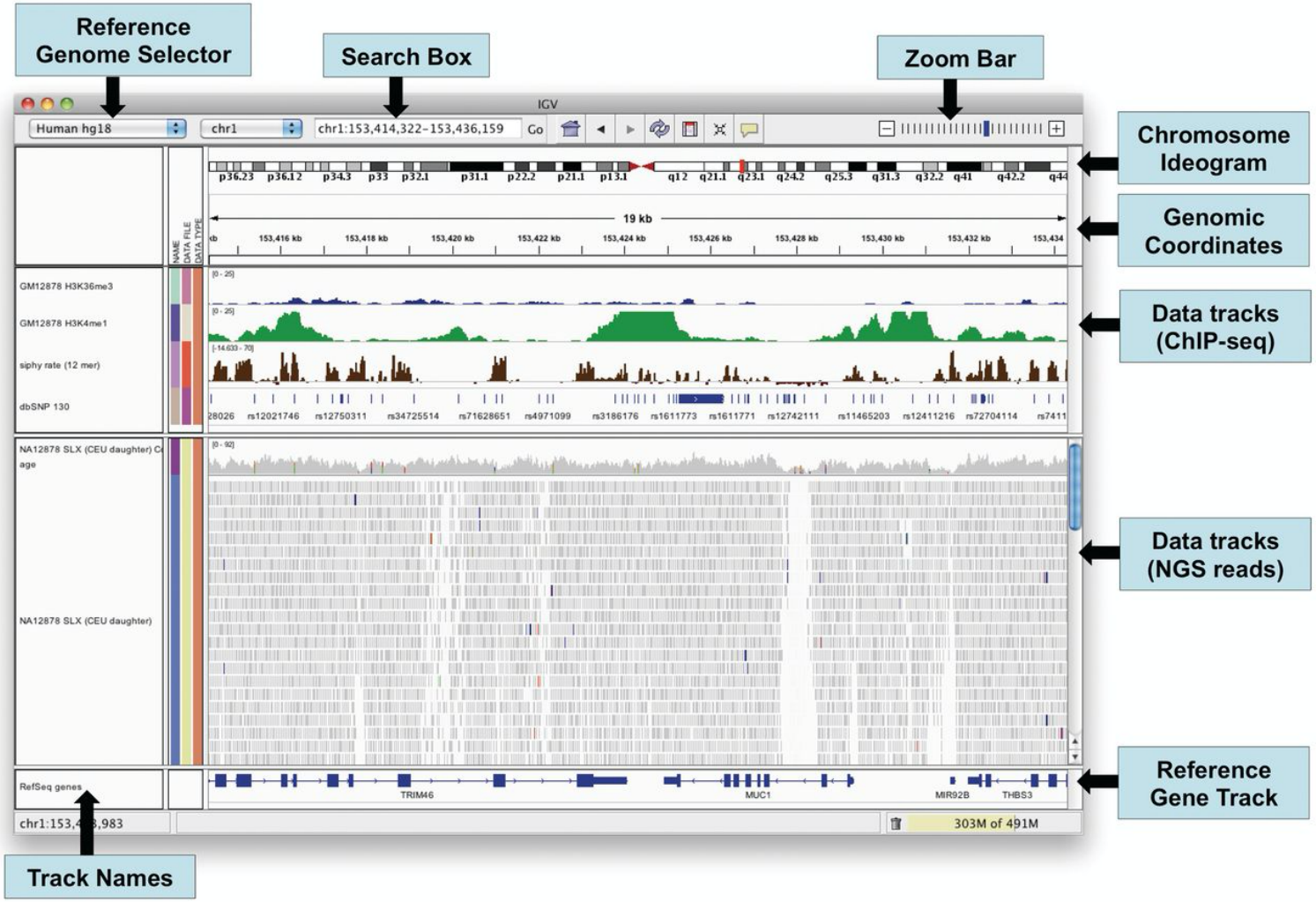
Integrative Genomics Viewer (IGV)

- The Integrative Genomics Viewer (IGV) is a high-performance visualization tool for interactive exploration of large, integrated genomic datasets.

- IGV focus on the integrative nature of genomic studies, with support for both array-based and next-generation sequencing data, and the integration of phenotypic data.

James T. Robinson, et al., Nature Biotechnology 29:24–26 (2011)
Helga Thorvaldsdóttir, et al., Briefings in Bioinformatics 14:178-192 (2013)
www.broadinstitute.org/igv

The IGV application window.



Data for IGV

- Reference genomes (build-in or external references)
- Data format:

1. Non-indexed formats: GFF, BED and WIG.
2. Indexed formats: BAM (sequence alignments)

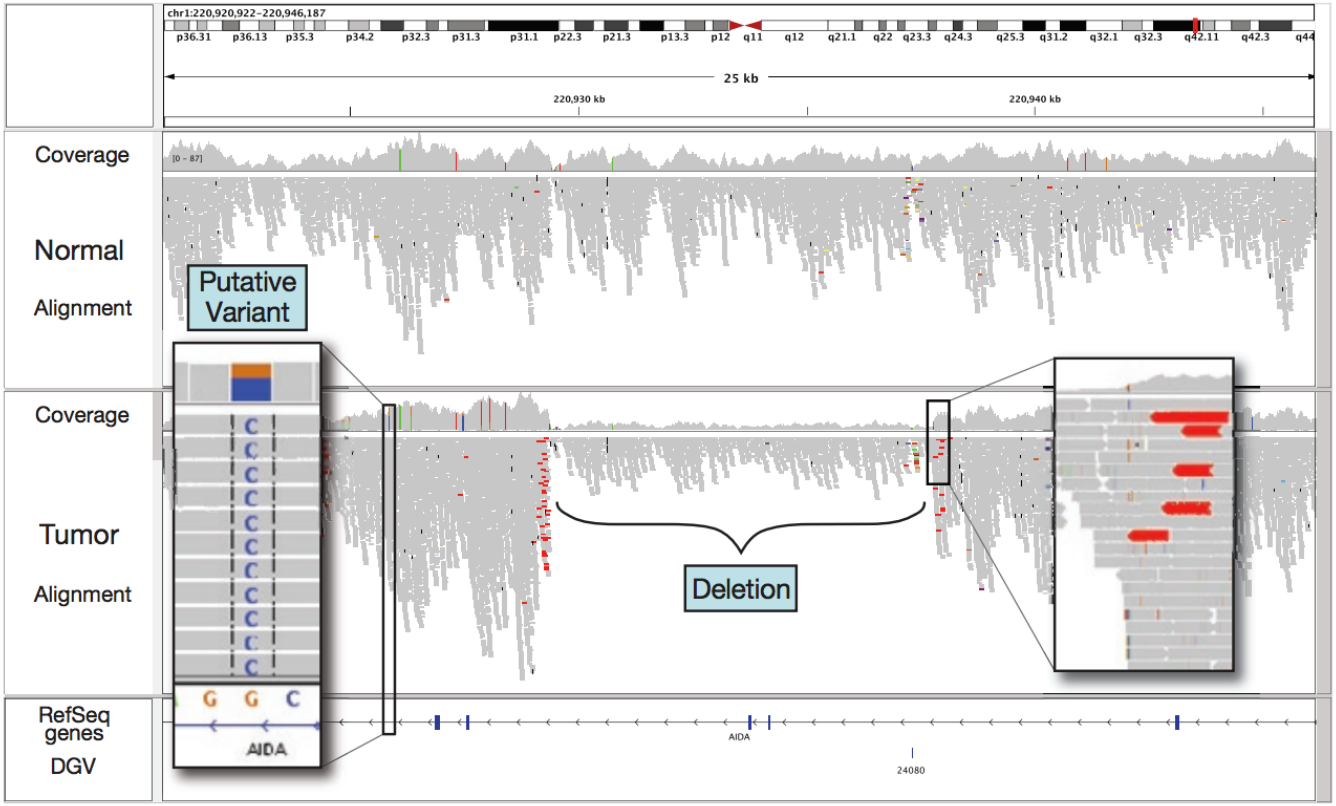
For example:

A reference genome: .fasta

A genome annotation file: .gff3 (optional)

An alignment file: .bam (plus .bai index file)

IGV example



1. Loci with a large percentage of mismatches relative to the reference are flagged in the coverage plot as color-coded bars.
2. Alignments with unexpected inferred insert sizes are indicated by color.
3. Evidence for a ~10-kb deletion in the tumor sample not present in the normal.

Review

- One of short-read alignment algorithms – BWT
- An aligner - BWA
- Alignment standard format – SAM and BAM
- A tool to handle alignments - SAMtools
- An alignment visualization tool - IGV