DNA Alignment

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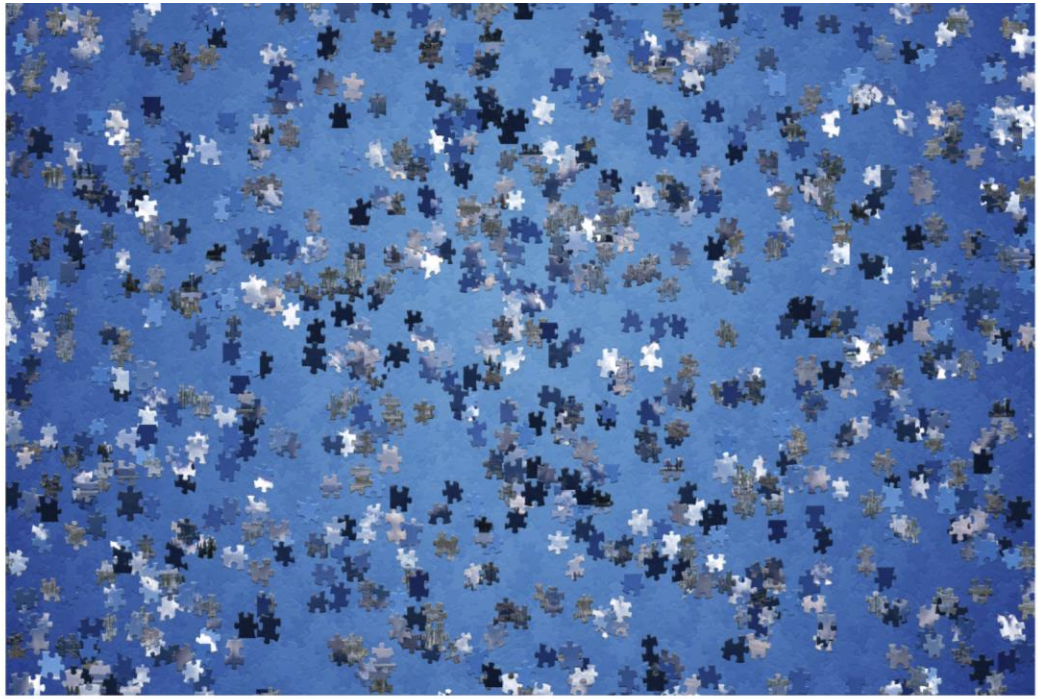
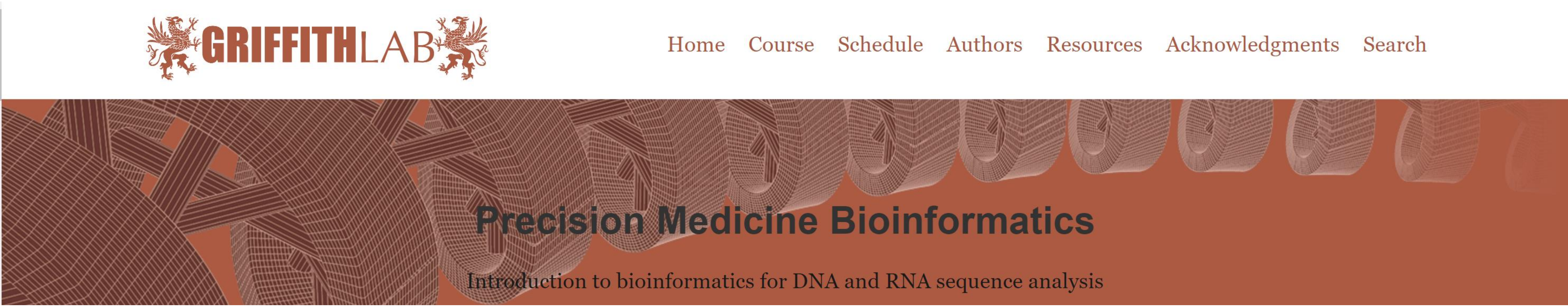


Slide courtesy of Andrew Farrell (University of Utah)

Learning Objectives

- Understand the process of obtaining and indexing raw DNA sequence data for alignment
- Perform a quality assessment using the aligned data and understand clean-up steps
- Prepare and visualize alignment results using genome viewers



Precision Medicine Bioinformatics

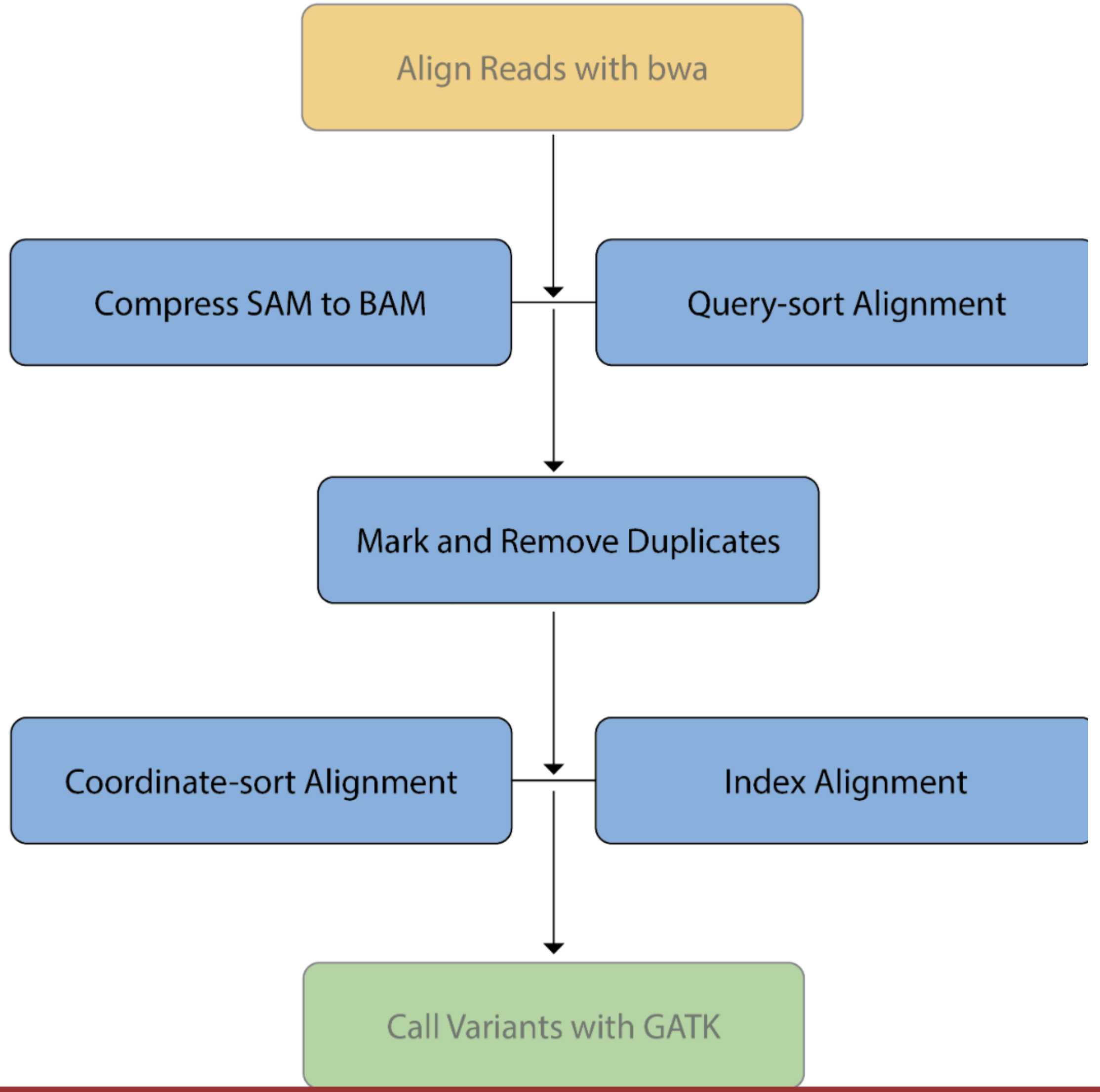
Introduction to bioinformatics for DNA and RNA sequence analysis

Based on: https://pmbio.org/module-03-align/0003/02/01/Alignment/

For our exercise:

https://gist.github.com/MariamKhanfar/8eae80dfc5bd9011d2ccbab27458ca04

Main QC steps in DNA alignment



Align Reads with bwa

Compress SAM to BAM

Query-sort Alignment

Mark and Remove Duplicates

Coordinate-sort Alignment

Index Alignment

Call Variants with GATK

Overview of main steps

- Create directories
- Download the data
- Download the reference
- Index Reference with BWA
- Align the data to the reference → Result in SAM
- Convert SAM to BAM
- Sort by query name
- Mark duplicates
- Sort by coordinates
- Index the final BAM
- Generate a flagstat report for the BAM

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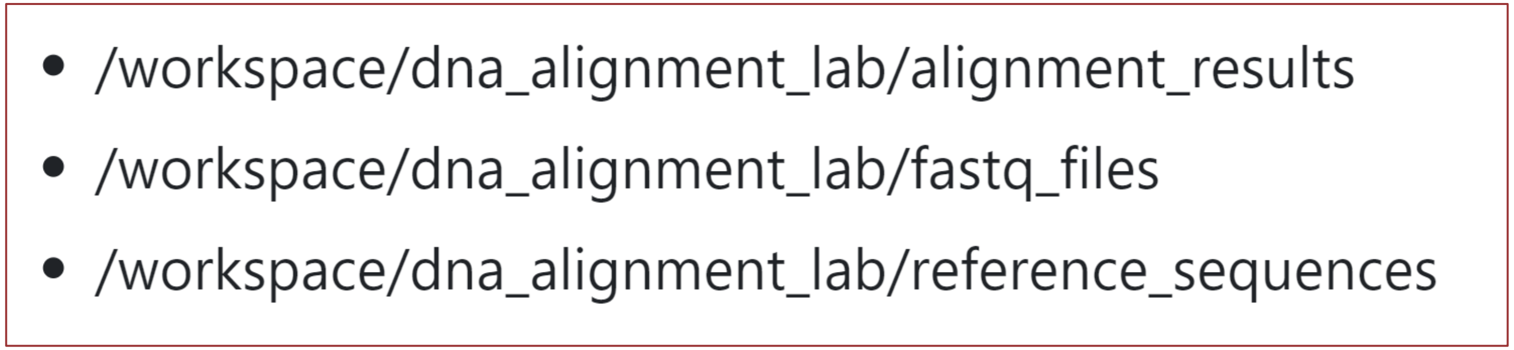
Prepare directories for analysis and obtain data

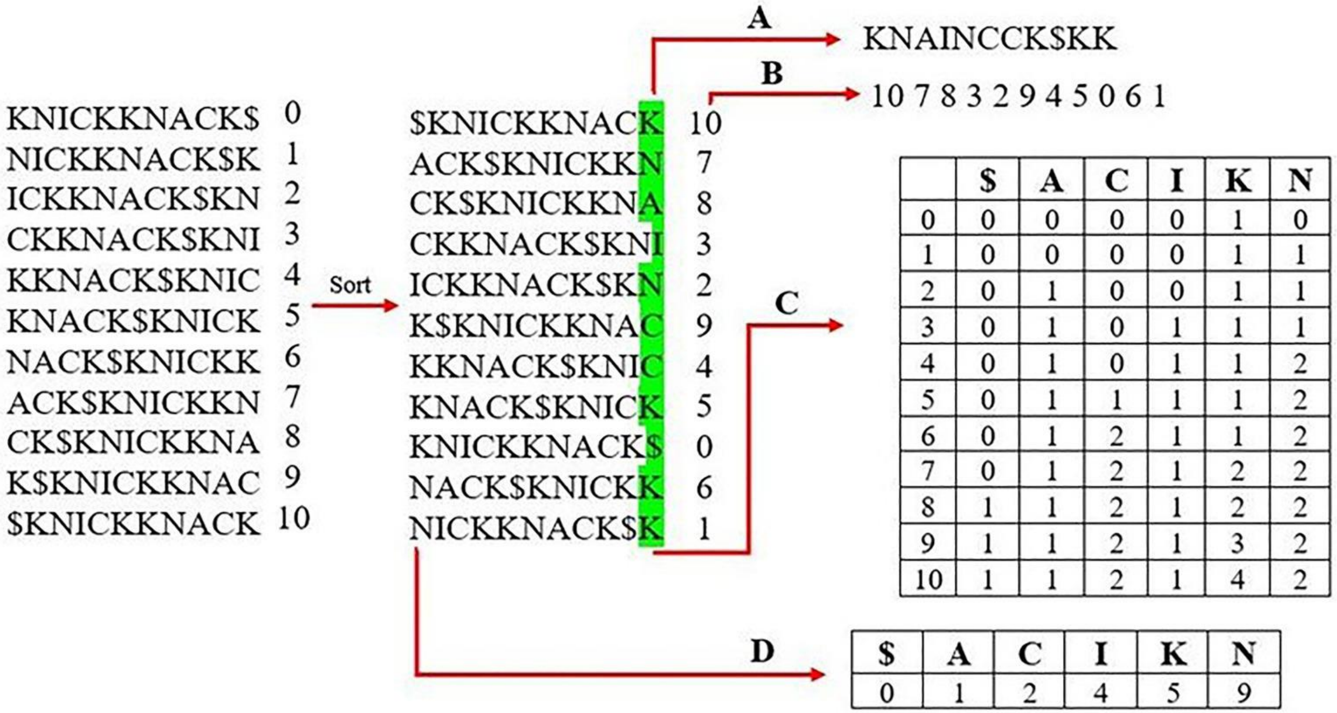
- Create the main directory “*dna_alignment_lab*”
- Create subdirectories within “*dna_alignment_lab*” for organizing data



- /workspace/dna_alignment_lab/alignment_results
- /workspace/dna_alignment_lab/fastq_files
- /workspace/dna_alignment_lab/reference_sequences

Index reference genome + DNA alignment

- Indexing is like creating a map of the reference genome, allowing the aligner to quickly locate where a sequence might fit; finds information without reading the entire text



KNICKKNACK$	0
NICKKNACK$K	1
ICKKNACK$KN	2
CKKNACK$KNI	3
KKNACK$KNIC	4
KNACK$KNICK	5
NACK$KNICKK	6
ACK$KNICKKN	7
CK$KNICKKNA	8
K$KNICKKNAC	9
$KNICKKNACK	10

$KNICKKNACK	10
ACK$KNICKKN	7
CK$KNICKKNA	8
CKKNACK$KNI	3
ICKKNACK$KN	2
K$KNICKKNAC	9
KKNACK$KNIC	4
KNACK$KNICK	5
KNICKKNACK$	0
NACK$KNICKK	6
NICKKNACK$K	1

KNAINCCK$KK

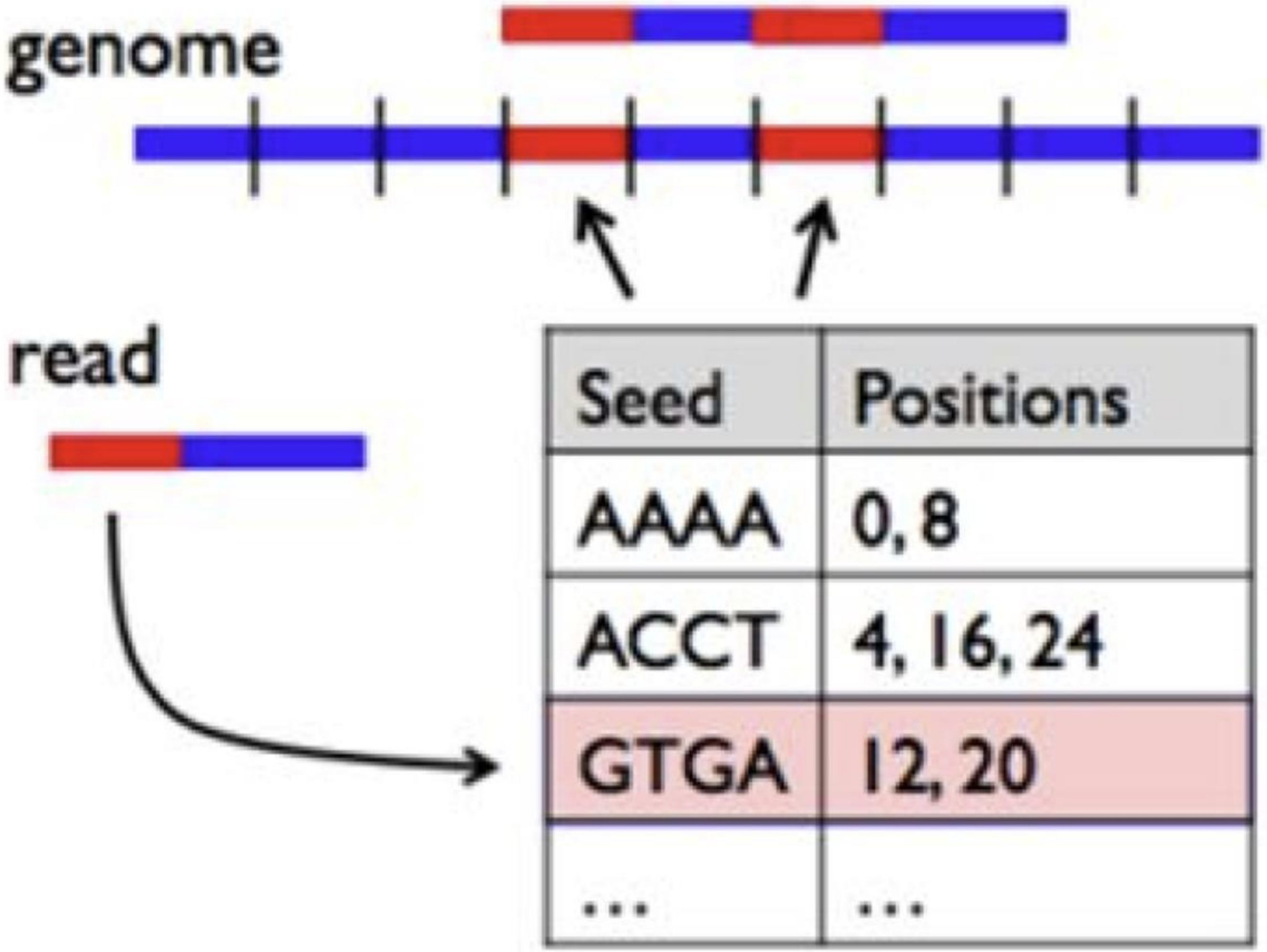
10 7 8 3 2 9 4 5 0 6 1

	$	A	C	I	K	N
0	0	0	0	0	1	0
1	0	0	0	0	1	1
2	0	1	0	0	1	1
3	0	1	0	1	1	1
4	0	1	0	1	1	2
5	0	1	1	1	1	2
6	0	1	2	1	1	2
7	0	1	2	1	2	2
8	1	1	2	1	2	2
9	1	1	2	1	3	2
10	1	1	2	1	4	2

$	A	C	I	K	N
0	1	2	4	5	9

https://www.frontiersin.org/articles/10.3389/fpls.2021.657240/full

DNA alignment with BWA



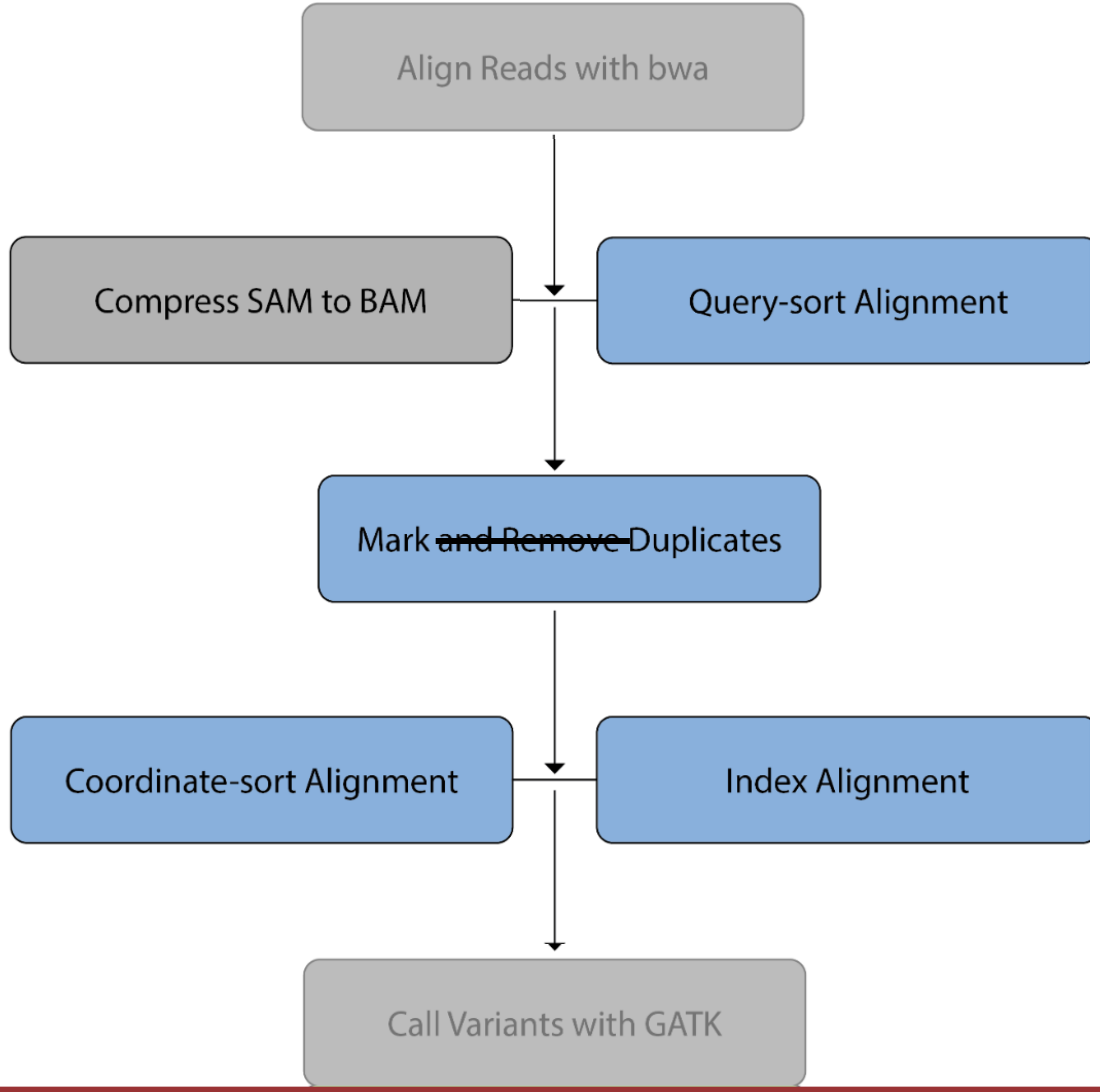
Seed	Positions
AAAA	0, 8
ACCT	4, 16, 24
GTGA	12, 20
...	...

https://rbatorsky.github.io/intro-to-ngs-bioinformatics/lessons/03_Alignment.html

Convert SAM to BAM

- SAM files are human-readable alignments, but they're large and unwieldy.
- BAM files are the binary version of SAM, much smaller and faster for computers to process.

Main QC steps in DNA alignment



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https://hbctraining.github.io/variant_analysis/lessons/06_alignment_file_processing.html

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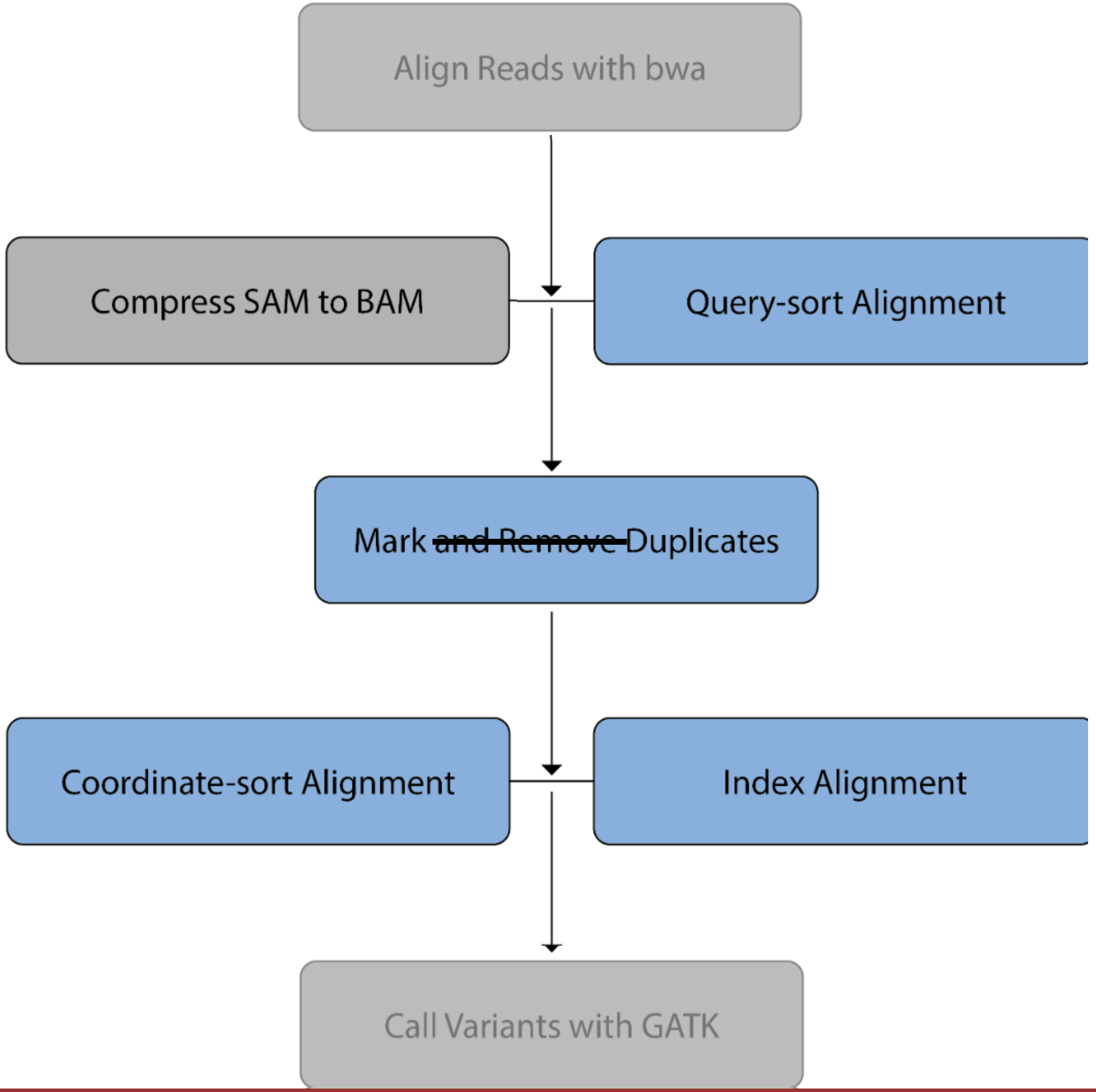
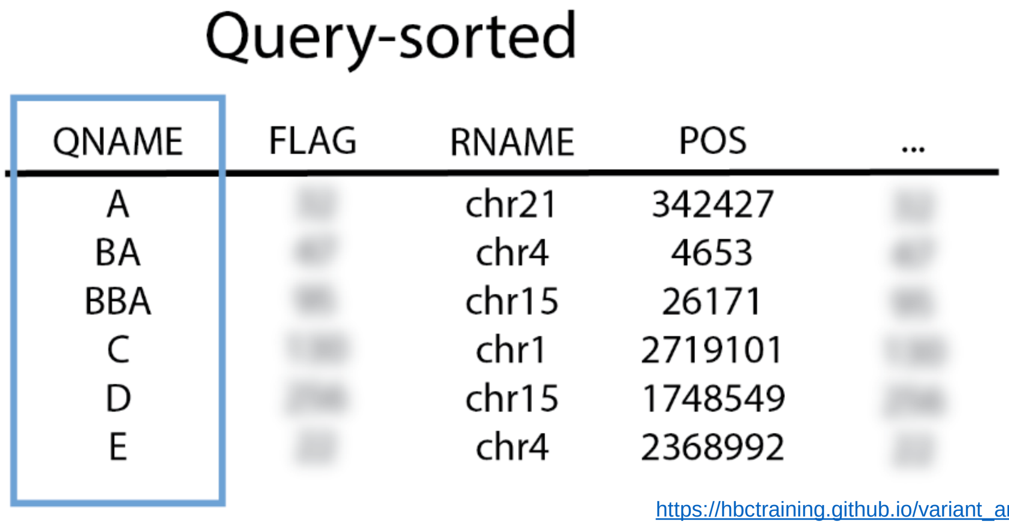


Can be done on:
SAM, BAM, CRAM

https://hbctraining.github.io/variant_analysis/lessons/06_alignment_file_processing.html

Sort by query name

- Grouping together reads that came from the same DNA fragment (i.e. identifier given to each read during sequencing)
- This is important for the following step; Marking duplicates, as it is essential to account for biases in sequencing where some fragments are overrepresented.



Query-sorted

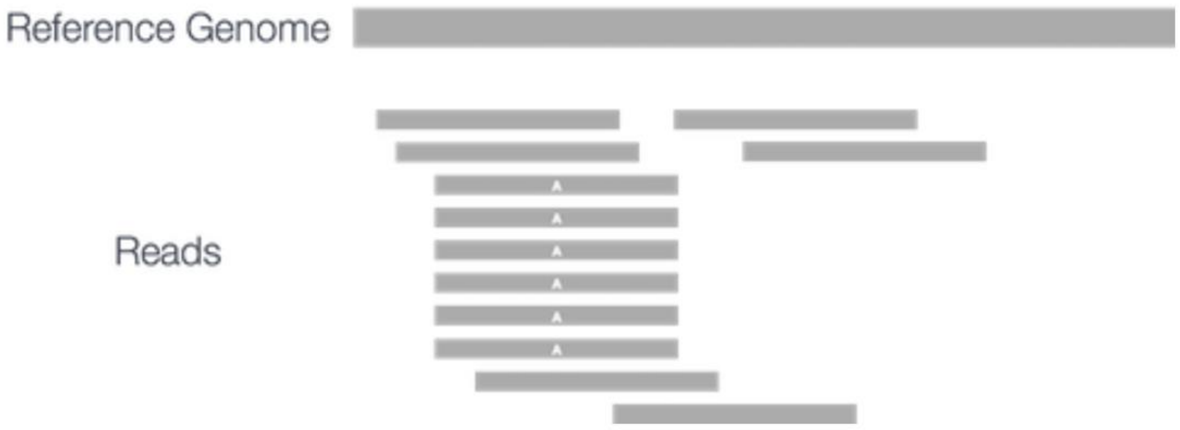
QNAME	FLAG	RNAME	POS	...
A	[illegible]	chr21	342427	[illegible]
BA	[illegible]	chr4	4653	[illegible]
BBA	[illegible]	chr15	26171	[illegible]
C	[illegible]	chr1	2719101	[illegible]
D	[illegible]	chr15	1748549	[illegible]
E	[illegible]	chr4	2368992	[illegible]

https://hbctraining.github.io/variant_analysis/lessons/06_alignment_file_processing.html

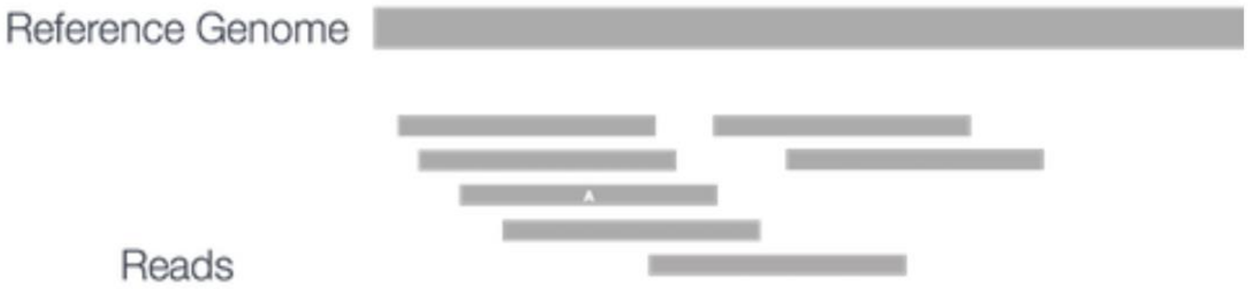
Mark Duplicates

- To assure proper pair alignment, must be used on a name sorted BAM (i.e. mark secondary alignments)
- Locate and tag duplicate reads (both PCR and optical/sequencing-driven) , duplicate reads are defined as originating from the same original fragment of DNA.
- Produces a metrics file indicating the numbers of duplicates for both single- and paired-end reads.

Mark Duplicates



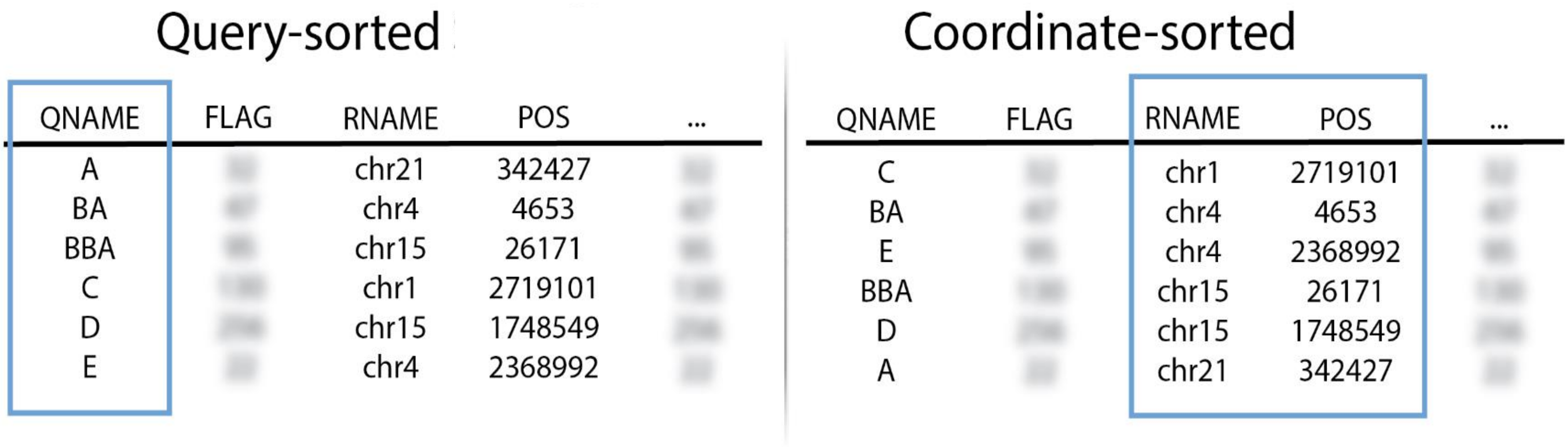
Mark Duplicates →



Sort by position

- Position sorting is performed to organize the reads by their location on the genome
- This is essential for downstream analysis, which require reads to be in order based on their *chromosomal coordinates.*
- Ensure that the subsequent analysis (like variant calling) do not mistakenly count extra copies of the same fragment, which could distort the results.
- Position sorting facilitates the identification of regions with genuine high coverage due to biological reasons rather than technical artifacts.

Sort by query name vs position



Query-sorted

QNAME	FLAG	RNAME	POS	...
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D	[illegible]	chr15	1748549	[illegible]
E	[illegible]	chr4	2368992	[illegible]

Coordinate-sorted

QNAME	FLAG	RNAME	POS	...
C	[illegible]	chr1	2719101	[illegible]
BA	[illegible]	chr4	4653	[illegible]
E	[illegible]	chr4	2368992	[illegible]
BBA	[illegible]	chr15	26171	[illegible]
D	[illegible]	chr15	1748549	[illegible]
A	[illegible]	chr21	342427	[illegible]

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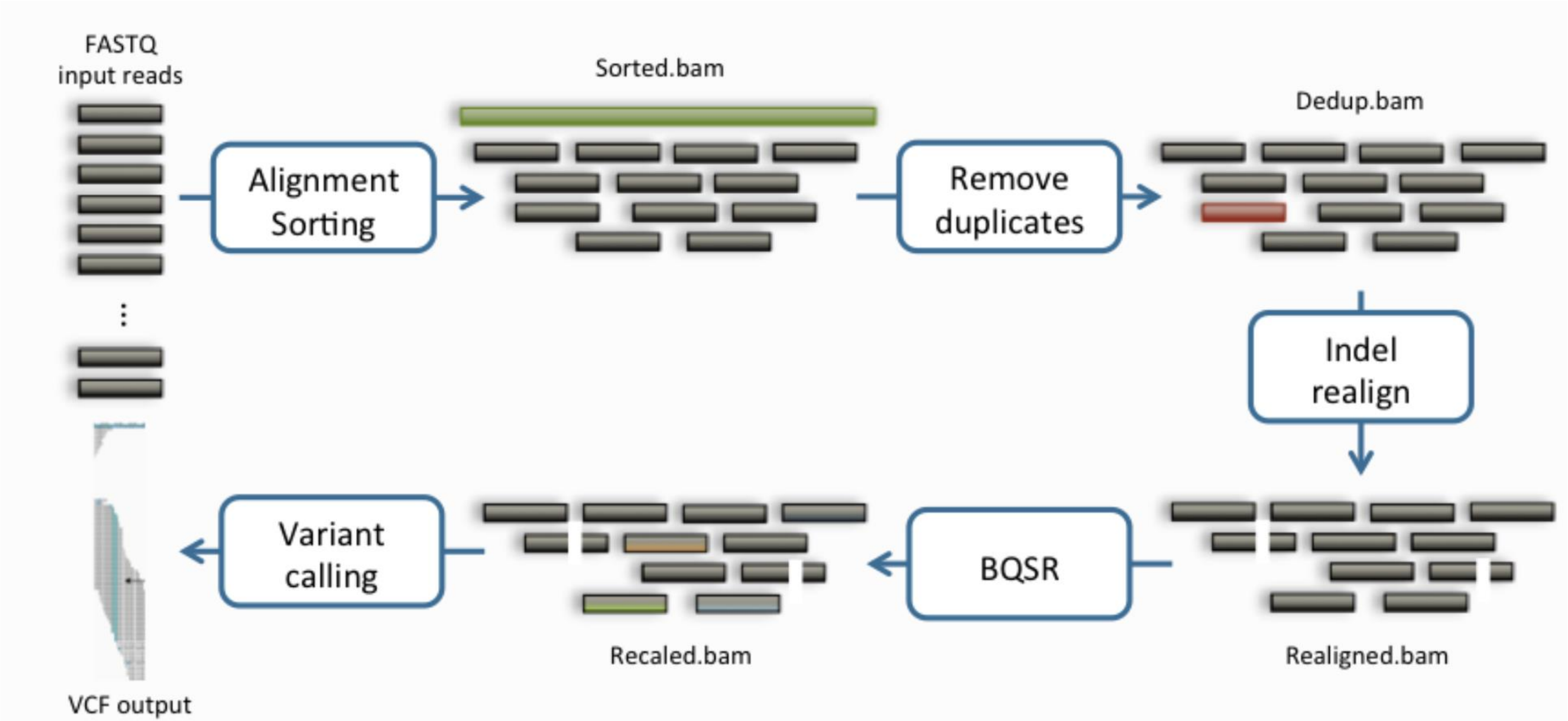
Index your final BAM

- In order to efficiently load and search a bam file, downstream applications typically require an index

SAM Flagstat

- Extract and count the number of reads that are: aligned, primary, not duplicate, and properly paired …etc, based on FLAG field.
  https://samtools.github.io/hts-specs/SAMv1.pdf
  https://broadinstitute.github.io/picard/explain-flags.html

Indel Realign or BQSR?



https://support.sentieon.com/versions/201808.01/manual/DNAseq_usage/dnaseq/