Alignment

RCN Bioinformatics Mentoring 2021

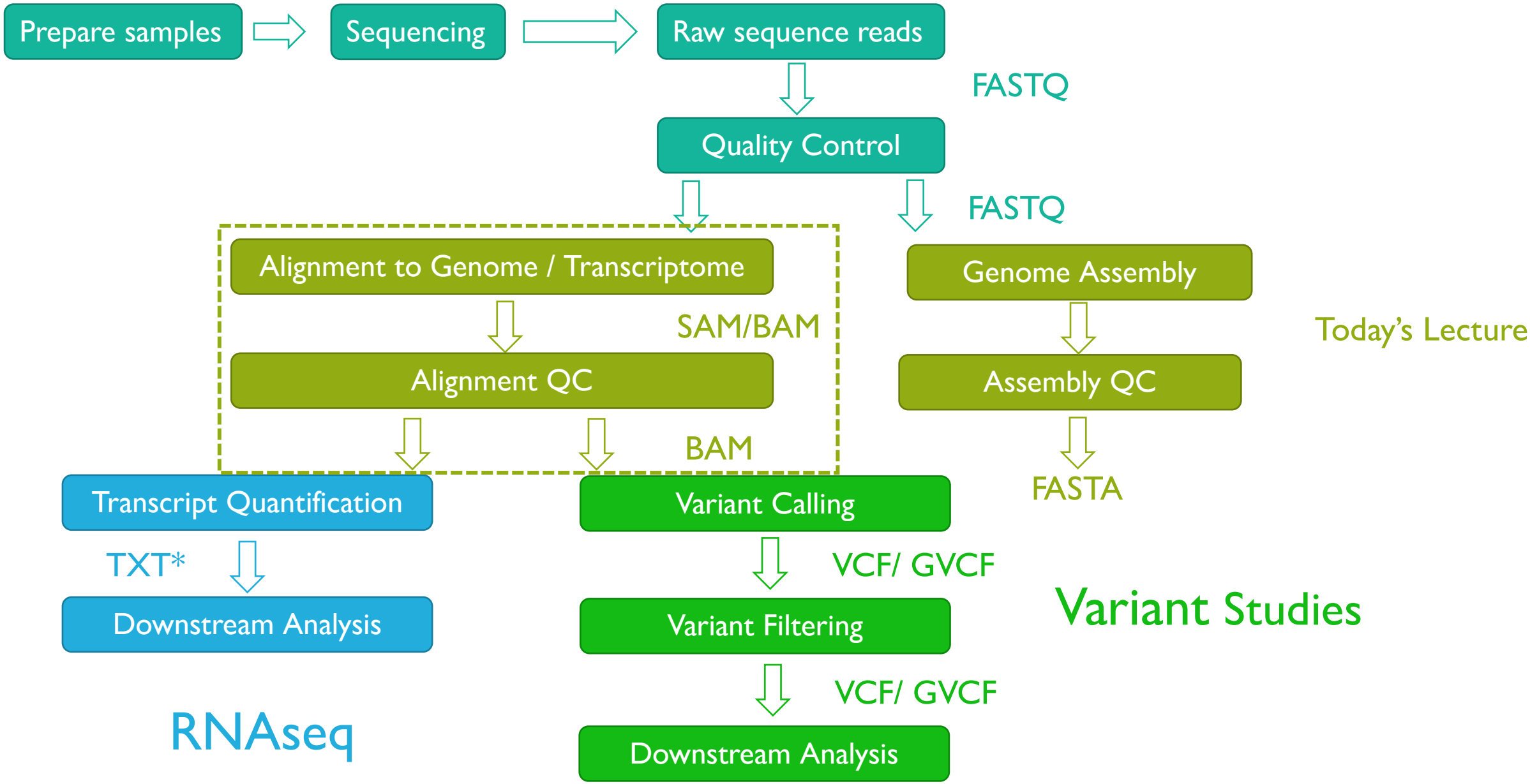
OUTLINE

10am Lecture

- Alignment strategies
- File formats
- QC

11am Tutorial

- Short-read mapping with BWA
- Splice-aware mapping with STAR


Prepare samples
Sequencing
Raw sequence reads
FASTQ
Quality Control
FASTQ
Alignment to Genome / Transcriptome
SAM/BAM
Alignment QC
BAM
Genome Assembly
Assembly QC
FASTA
Today's Lecture
Transcript Quantification
TXT*
Downstream Analysis
RNAseq
Variant Calling
VCF/ GVCF
Variant Filtering
VCF/ GVCF
Downstream Analysis
Variant Studies

SEQUENCE ALIGNMENT

FASTQ

FASTA

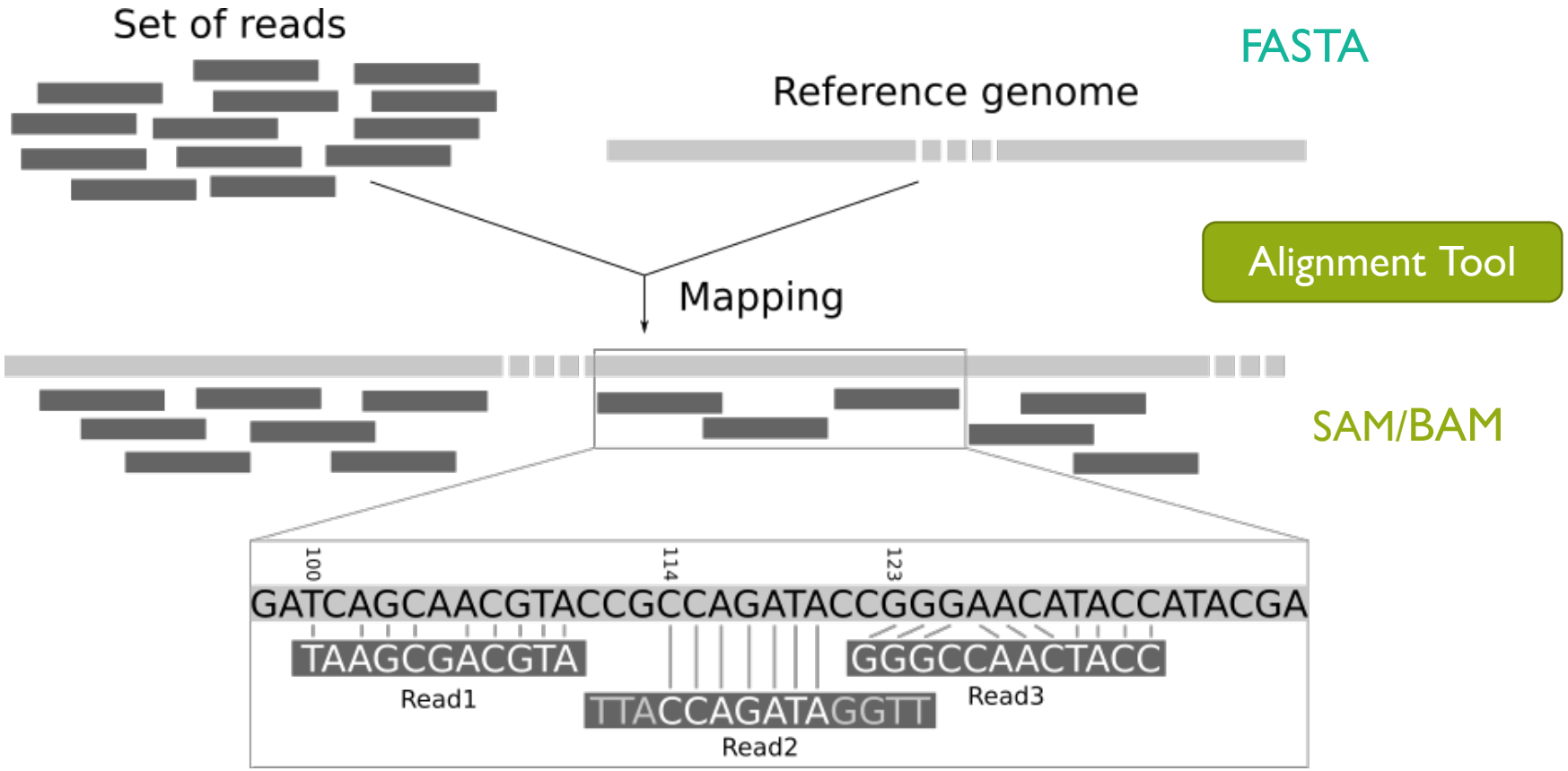


Figure from Joachim Wolff, Bérénice Batut, Helena Rasche, 2020 **Mapping (Galaxy Training Materials)**. /training-material/topics/sequence-analysis/tutorials/mapping/tutorial.html Online; accessed Wed Dec 09 2020

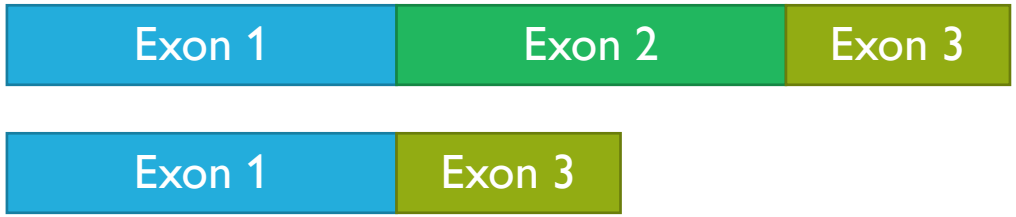
GENERAL CHALLENGES

- Accommodating indels
- Distinguishing sequencing error from real variation
- Short reads (50-150bp) can map to multiple parts of the genome
- Millions of reads
- Large reference genome (huge search space)
- Potentially incomplete or low quality reference genome
- Repetitive sequences

RNASEQ CHALLENGES

Gene model in a reference genome

Transcript isoforms



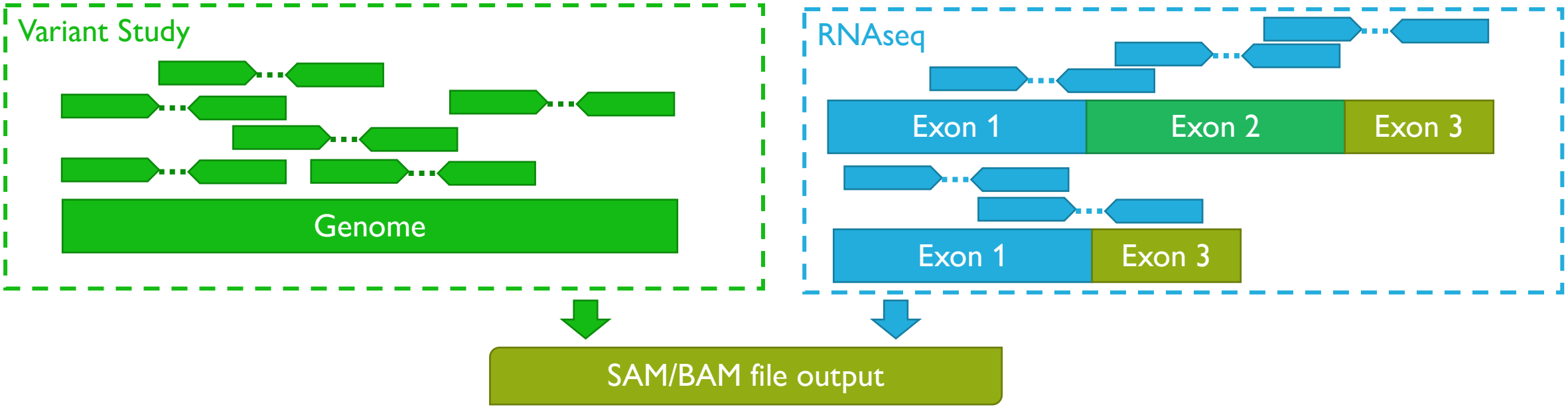
A BIT ABOUT REFERENCES

- Model with versions
- Annotated vs Not (check your version!)

- Annotations different on different databases (Chris' Lecture)
- Transcriptome = fasta with all known transcripts

SHORT-READ ALIGNMENT

- Includes a gap penalty
- Standard alignment software: **Bowtie2** or **bwa** (also **stampy** and lots of others)



SPLICE-AWARE ALIGNMENT

- For RNAseq
- Align to genome (and sometimes annotation)
- Reads can be split up to accommodate very long introns & other splice junctions
- Standards are **HISAT2** and **STAR**

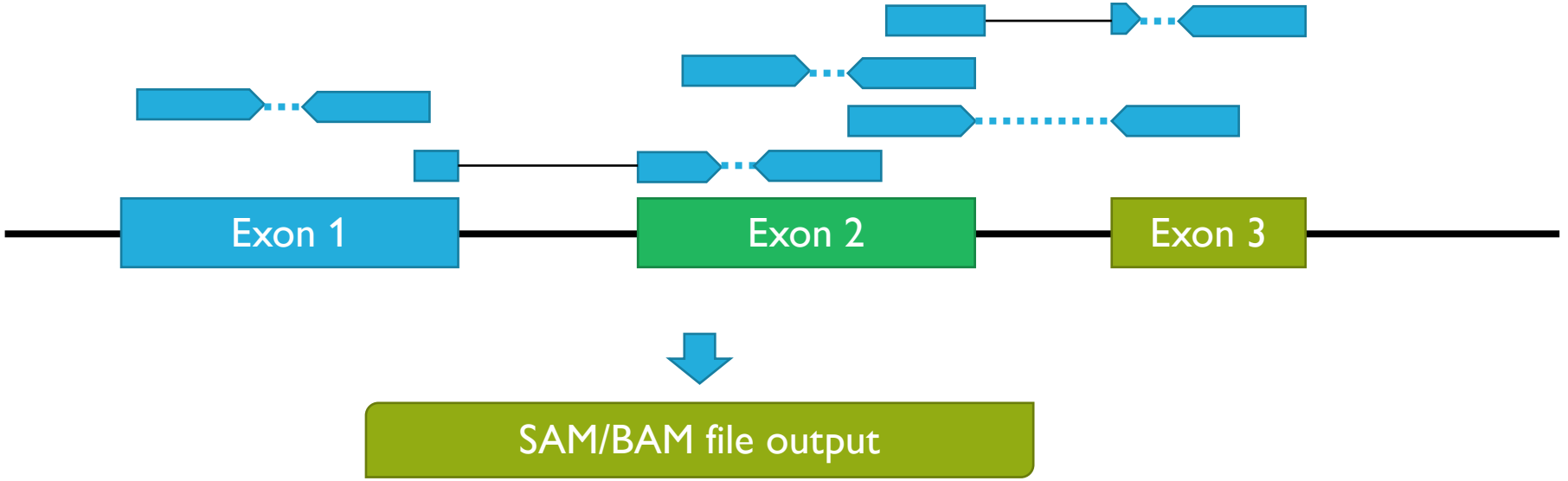


Figure from Van den Berge 2019. Ann. Rev. Biomed. Data Sci. 2: 139-73

ALIGNMENT STEP 1: INDEX

- Index your reference prior to commencing
- Index allows alignment programs to efficiently search
- Ways of breaking the reference into bits

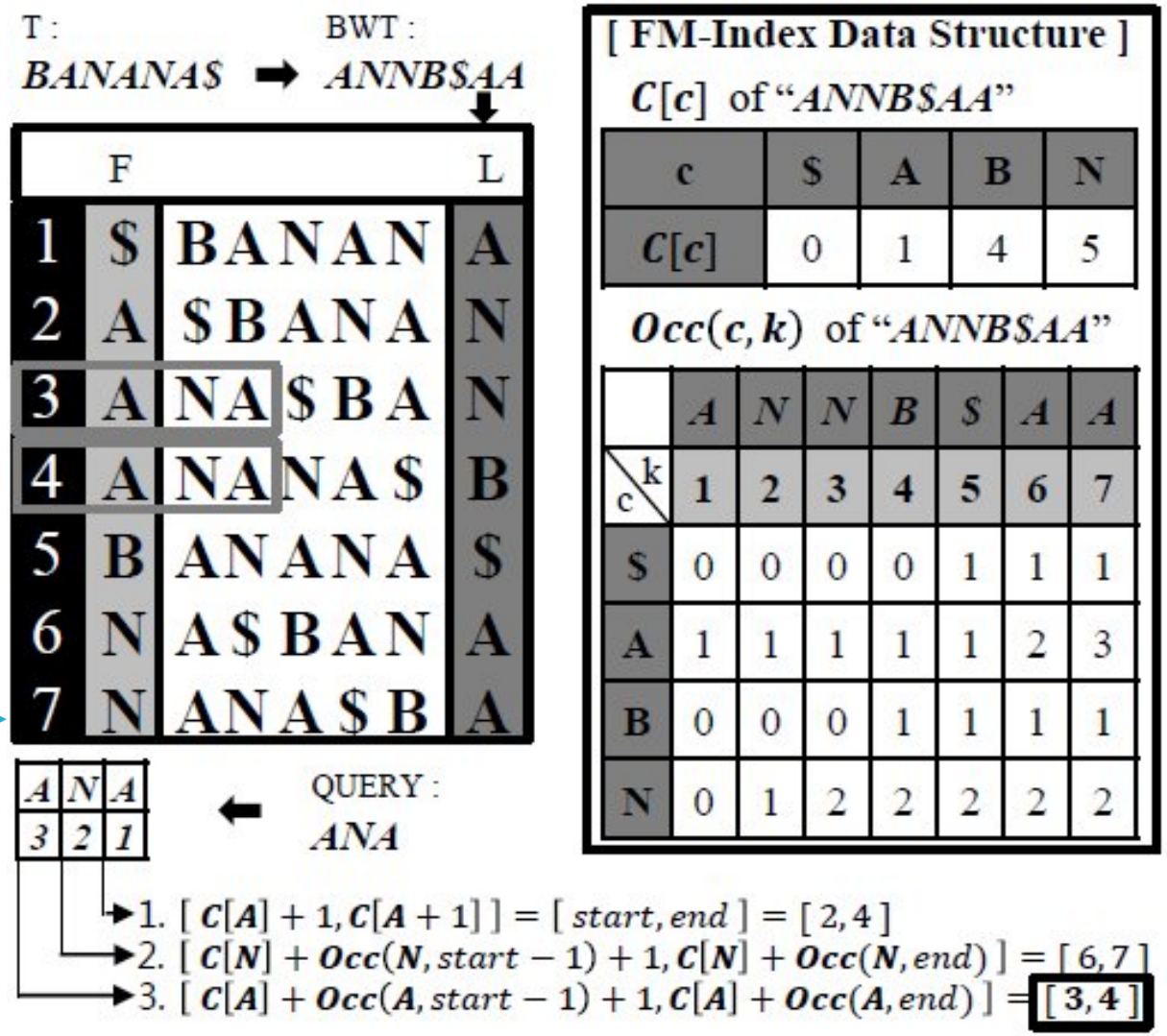
	Hash-based	Suffix-Arrays	Burrows-Wheeler (FM index)
Programs	BLAST, (Kallisto, Salmon)	STAR, Salmon	BWA, Bowtie2
Method	K-mer based	Sorted table of suffixes of a string	Sorted rearrangements of string
	Can be slow	Large memory requirements to index	Lower memory req. but reduced search efficiency

Check out this lecture for more info on indices:
https://hbctraining.github.io/Intro-to-rnaseq-hpc-salmon/lectures/alignment_quantification.pdf

BWA

- bwa index <INFILE.FA>
- creates FM-index
- creates a bunch of files, .pac, .sa, .bwt., .ann .amb (burrows wheeler indices + suffix arrays)

Burrows Wheeler Transform



Li & Durbin (2009);
FM figure from SeokOck et al (2013) A Very Fast Algorithm for Detecting Partially Plagiarized Documents Using FM-Index. IICCE

STEP 2: ALIGN READS TO GENOME

- "bwa mem" (for short read applications), "bwa swa" for >200bp (Smith-Waterman)
- Finds matches to query in index.
- Assigns them a MQ (map quality) score based on PHRED base score + mismatches + gaps


```
Chr10:1020    AGTACCAGAAGTATCTCGACTCTAAGGAATTGAGTCA
                  |||| ||| | | | | | |
                  AGTAACTCGACTCTA          Mismatches 1        MQ=10

Chr2:20231    TCATATGGTAGTAACTGCACGTTAAGTAAATTAGCAAT
                  | | | | | | | | | |    | |
                  AGTAACTCGACTCTA          Mismatches 2        MQ=1
```

Figure from McGill iGEM (2020) https://www.youtube.com/watch?v=P_YKQKFI4Lk: 18:5

SAM FILE FORMAT

- **Flags:** alignment information (pairs mapped? Secondary alignment? Or both?)
  https://broadinstitute.github.io/picard/explain-flags.html
- **MAPQ:** Reflects -10log10(probability the mapping is wrong)
  - Different aligners score a bit differently – don't compare MAPQ between aligners.
  - Perfect mapping in bwa mem=60, perfect mapping in bowtie2=42
  - Unique mapping in STAR is 255
  - 0 is ambiguous mapping – equal quality in multiple locations.

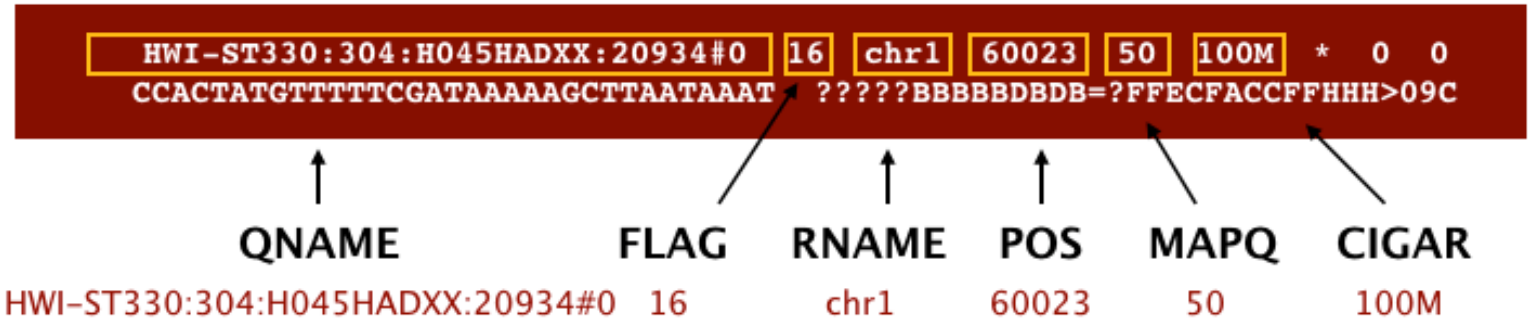


Figure from https://hbctraining.github.io/Intro-to-rnaseq-hpc-salmon/lessons/03_QC_STAR_and_Qualimap_run.html

SAM FILE FORMAT

- 1 SAM/BAM per FASTQ
- SAM format is the FASTQ information plus additional information about position of read and its mapping quality
- BAM is a binary compressed version of a SAM file (much smaller file size!)
- **samtools** software can convert SAM to BAM
- **STAR** can give you BAM directly

CHECK IT OUT

Integrative Genomics Viewer

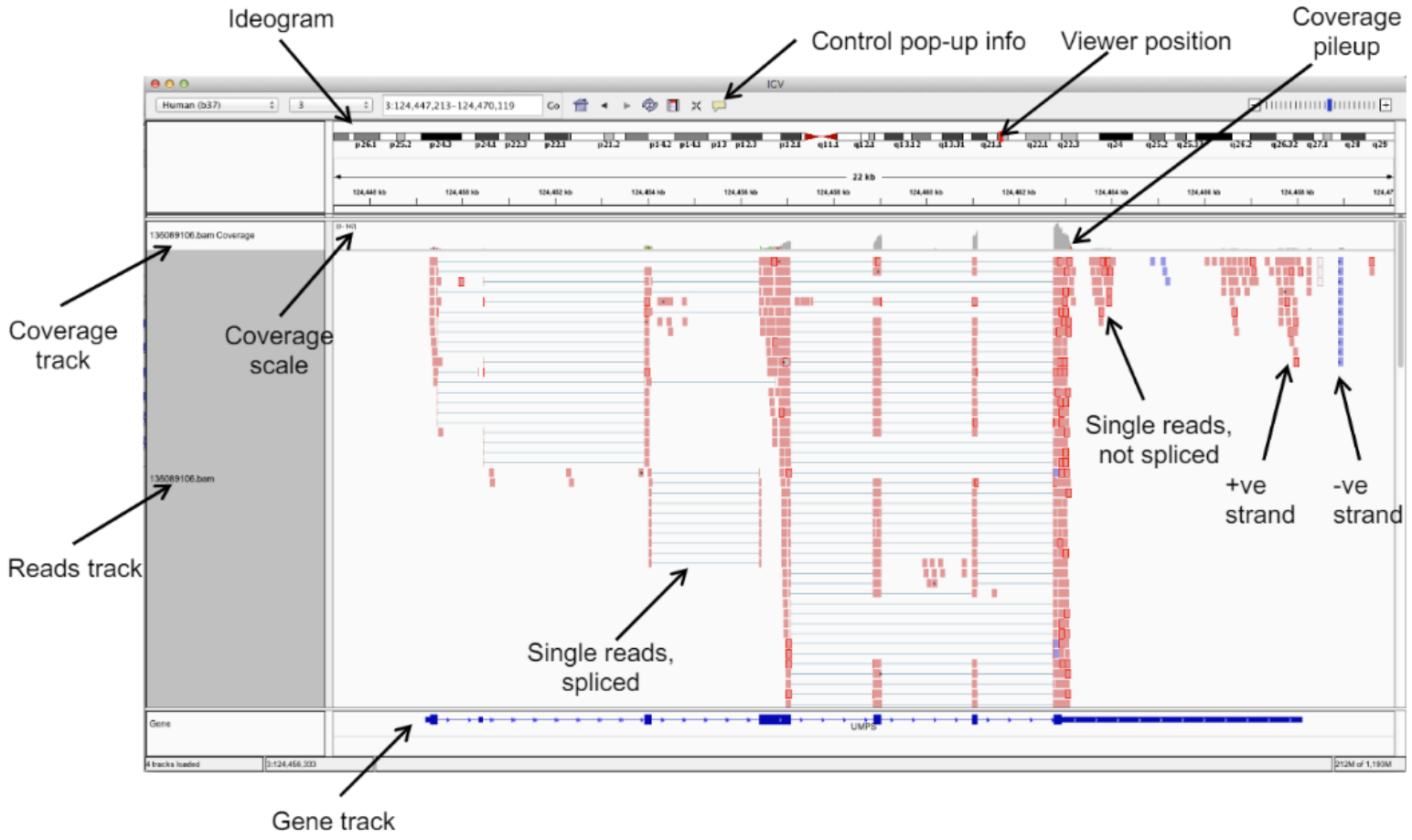


Figure from https://github.com/griffithlab/rnabio.org/blob/master/assets/lectures/cshl/2020/mini/RNASeq_MiniLecture_02_04_alignmentQC.pdf

STEP 3: SORT AND INDEX BAM

- Convert sam to bam

samtools view -b -S -o <OUTFILE.bam> <INFILE.sam>

- Sort sam file

samtools sort -o <OUTFILE.bam.sorted> <INFILE.bam>

- Index bam file (creates a .bai)

samtools index <INFILE.bam.sorted>

- If PCR step in library prep – Mark PCR duplicates (molecules with same UMI OR exact length and sequence)

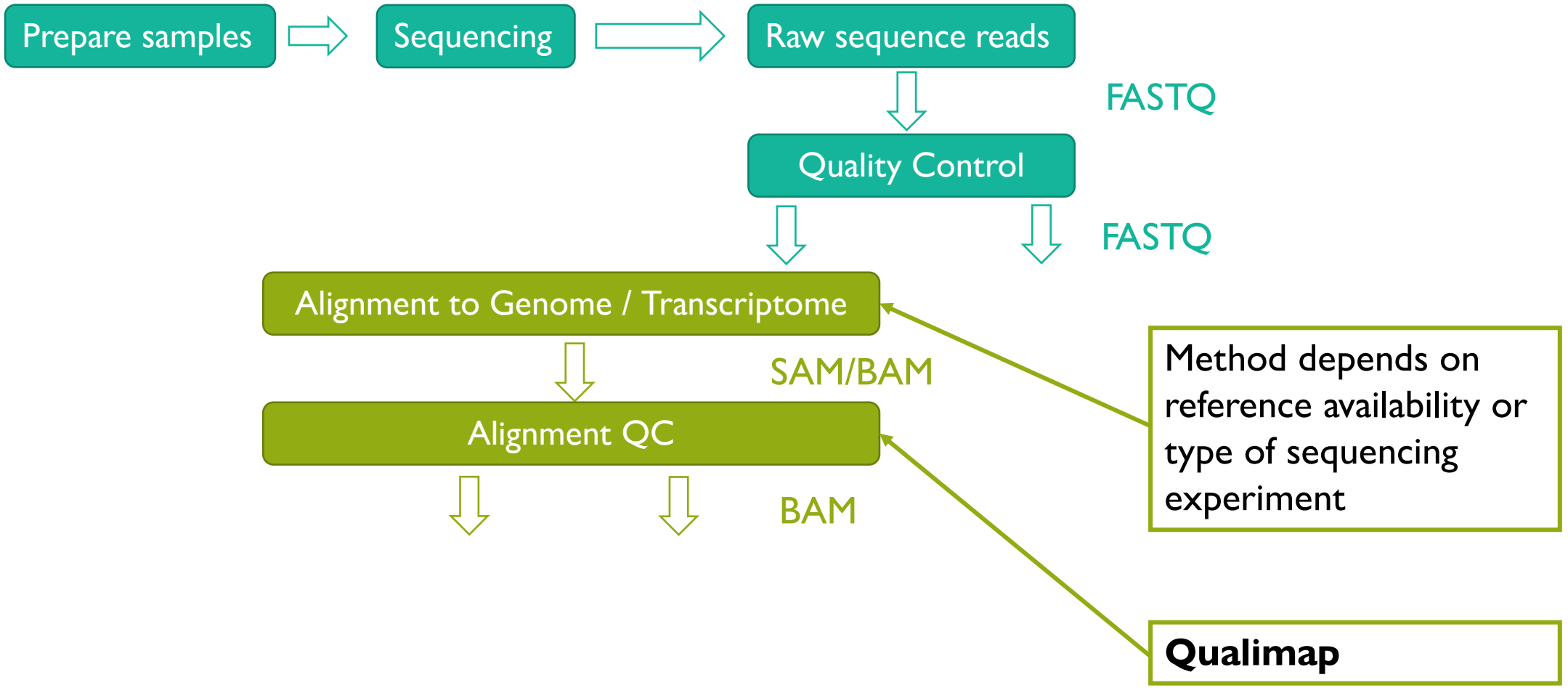
java –jar picard.jar MarkDuplicates I=input.bam O=marked_duplicates.bam, M=marked_dup_metrics.txt

ALIGNMENT QUALITY

- **Qualimap** program
  - Can assess short-read alignments "qualmap bamqc" and RNAseq alignments "qualimap rnaseq"
- Should have similar mapping rates across all samples – may need to remove a sample
- If mapping to a heterospecific reference can use to adaptively explore good mapping parameters to increase number of mapped reads or correct for other biases.
- Repetitive regions = way more mapped reads (not RNAseq)

For RNAseq

- Good quality mapped to conspecific reference > 60% uniquely mapped reads (will vary depending on species and genome quality)!
- Want lots of reads mapping to exons – if not might be DNA contamination or new transcripts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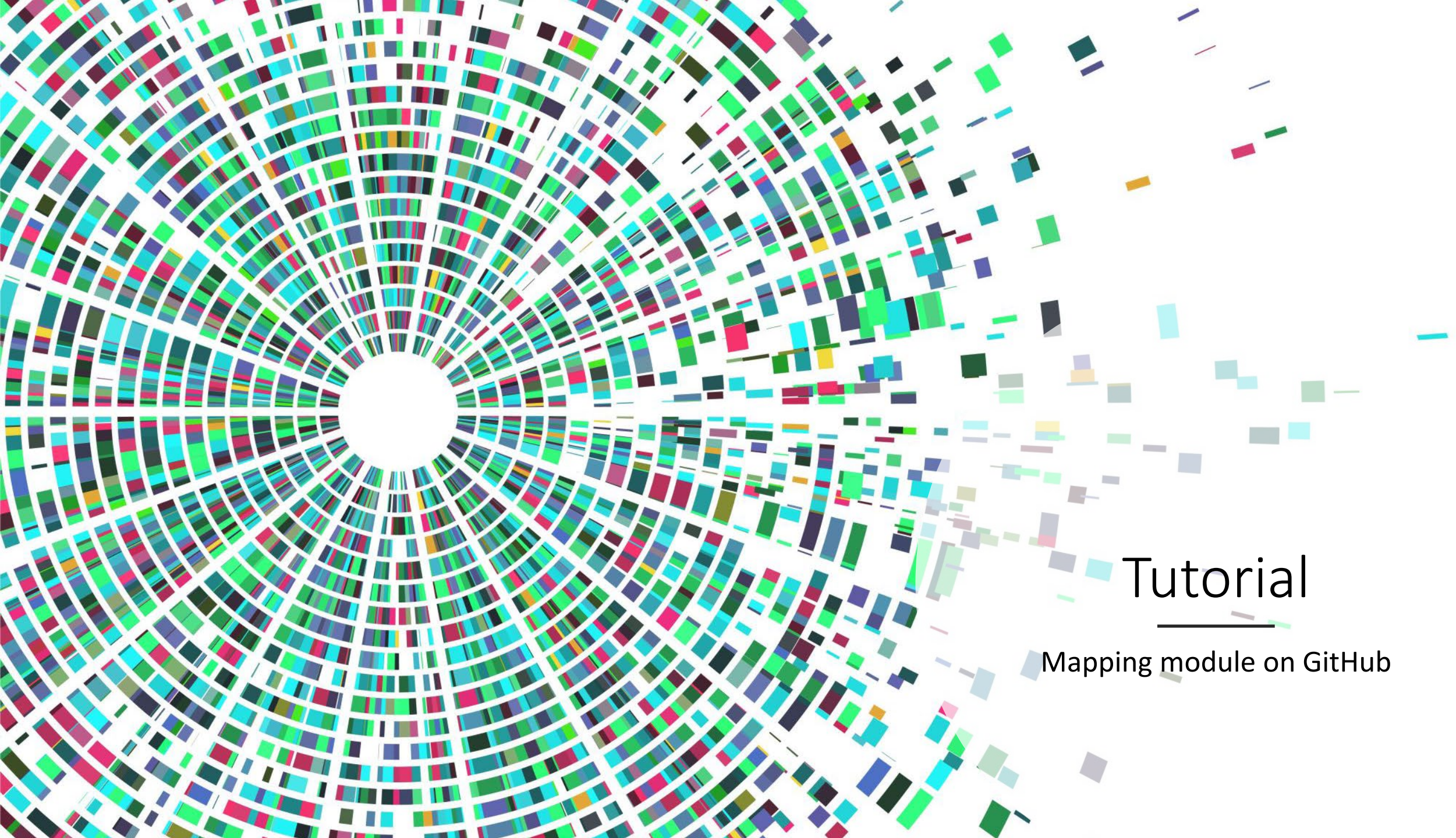
BAM
Method depends on reference availability or type of sequencing experiment
Qualimap

FURTHER READING & REFERENCES

- Li and Durbin (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics.* 25: 1754-1760
- Sheng et al (2017) Multi-perspective quality control of Illumina RNA sequencing data analysis. *Briefings in Functional Genomics*
- McGill iGEM (2020) Introduction to Burrows-Wheeler Alignment and Samtools for Cancer Mutation Calling Bioinformatics. https://www.youtube.com/watch?v=P_YKQKFI4Lk
- Griffith Lab RNA-seq Bioinformatics Course Lecture (https://rnabio.org/course/)
- HBC training Tutorials
  - https://hbctraining.github.io/Intro-to-rnaseq-hpc-salmon/lectures/alignment_quantification.pdf
  - https://hbctraining.github.io/Intro-to-rnaseq-hpc-salmon/lessons/03_QC_STAR_and_Qualimap_run.html
- http://www.acgt.me/blog/2014/12/16/understanding-mapq-scores-in-sam-files-does-37-42
- https://sequencing.qcfail.com/articles/mapq-values-are-really-useful-but-their-implementation-is-a-mess/
- https://samtools.github.io/hts-specs/SAMv1.pdf



Tutorial

Mapping module on GitHub