Tutorial 4 – BWA, SAMtools & BCFtools Part 2

MICB405 – BIOINFORMATICS – 2021W-T1

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AXEL HAUDUC

BWA MEM vs BWA ALN

bwa mem syntax

bwa mem ref.fasta read1.fastq read2.fastq > alignment.sam

bwa aln (“base BWA”) syntax

bwa aln ref.fasta read1.fastq > read1.sai

bwa aln ref.fasta read2.fastq > read2.sai

bwa sampe ref.fasta read1.sai read2.sai read1.fastq read2.fastq > alignment.sam

Recap of BWA ALN

Older, original algorithm that created SAM files

Somewhat better for read lengths below 70bp

- Tailored to older sequencers that produced ≈ 36bp reads

SAI file = “suffix array index”

- Intermediate file containing Burrows-Wheeler transformed intervals of reference where each read matches
- Converted to SAM taking into account paired-end reads with secondary step “bwa sampe”

Getting more detailed statistics on BAMs

samtools stats file.bam| grep “^SN”

samtools stats contains sets of information in lines beginning with a set of codes to denote different sections

CHK	Checksum
SN	Summary numbers
FFQ	First fragment qualities
LFQ	Last fragment qualities
GCF	GC content of first fragments
GCL	GC content of last fragments
GCC	ACGT content per cycle
GCT	ACGT content per cycle, read oriented
FBC	ACGT content per cycle for first fragments only
FTC	ACGT raw counters for first fragments
LBC	ACGT content per cycle for last fragments only
LTC	ACGT raw counters for last fragments
BCC	ACGT content per cycle for BC barcode
CRC	ACGT content per cycle for CR barcode
OXC	ACGT content per cycle for OX barcode
RXC	ACGT content per cycle for RX barcode
QTQ	Quality distribution for BC barcode
CYQ	Quality distribution for CR barcode
BZQ	Quality distribution for OX barcode
QXQ	Quality distribution for RX barcode
IS	Insert sizes
RL	Read lengths
FRL	Read lengths for first fragments only
LRL	Read lengths for last fragments only
ID	Indel size distribution
IC	Indels per cycle
COV	Coverage (depth) distribution
GCD	GC-depth

Very quick comparison of BAMs

```
diff --side-by-side \
<(samtools stats aln/bordetella.final.bam | grep "^SN") \
<(samtools stats mem/bordetella.final.bam | grep "^SN")
```

Process substitution:

<(COMMAND) = insert output of COMMAND as a file here

- Useful when you need to pipe in 2+ items into your command

Samtools tview

This is a commandline option to viewing read alignments

- Less flexible than IGV, but easy for quick comparisons

samtools tview syntax

samtools tview file.bam --reference reference.fasta

Jumping to a specific locus

samtools tview file.bam --reference reference.fasta -p chrom:position

samtools view file.bam chrom:start-end

Filtering your BAM file

Select reads with a mapping quality above 40

- samtools view file.bam -b -h -q 40 > file.filtered.bam

Keep reads w/ matching flags (4 = UNMAPPED)

- samtools view file.bam -b -h -f 4 > file.filtered.bam

Discard reads w/ matching flags

- samtools view file.bam -b -h -F 4 > file.filtered.bam

View alignments and vcfs together

scp reference, bam, bam.bai, vcf, and vcf.bai into your computer and load into IGV