Short Sequencing Read Maping

BWA for short read alignment

Index genome

It is necessary to index the genome in preparation of alignement.

```
#!/usr/bin/bash -l
#SBATCH -p short -N 1 -n 1 -c 2 --mem 2gb
module load bwa
GENOME=S_enterica_CT18.fasta
bwa index $GENOME
```

Align reads

```
#!/usr/bin/bash -l
#SBATCH -p short -N 1 -n 1 -c 16 --mem 4gb

module load bwa
module load samtools
CPU=16
mkdir -p ~/bigdata/Short_read_aligning
cd ~/bigdata/Short_read_aligning
mkdir -p fastq
ln -s /bigdata/gen220/shared/data/S_enterica/*.fastq.gz fastq
ln -s /bigdata/gen220/shared/data/S_enterica/S_enterica_CT18.fasta
ln -s /bigdata/gen220/shared/data/S_enterica/acc.txt
GENOME=S_enterica_CT18.fasta
if [ ! -f $GENOME.sa ]; then
   bwa index $GENOME
fi

for acc in $(cat acc.txt)
do
    FWDREAD=fastq/${acc}_1.fastq.gz
    REVREAD=fastq/${acc}_2.fastq.gz

    bwa mem -t $CPU $GENOME $FWDREAD $REVREAD > ${acc}.sam
    samtools fixmate -O bam ${acc}.sam ${acc}_fixmate.bam
    samtools sort --threads $CPU -O BAM -o ${acc}.bam ${acc}_fixmate.bam
    samtools index ${acc}.bam
done
```

Visualizing depth of coverage

Interactively - you can use `samtools`

```
module load samtools
samtools tview SRR10574912.bam
# to see the reference genome loaded as well add this option
samtools tview SRR10574912.bam --reference S_enterica_CT18.fasta
```

SNP calling

There are many standardized SNP calling pipelines. GATK provides a robust pipeline that can be used.

Samtools/BCFTools are also useful and straight forward.

freebayes is another very useful pipeline for non-model systems.

Samtools/BCFTools SNP and INDEL calling

Workflows from the htslib

```
#!/usr/bin/bash -l
#SBATCH -p short -N 1 -n 1 -c 4 --mem 16gb
module load samtools
module load bcftools
GENOME=S_enterica_CT18.fasta

# need to make a string which is all the bam files you want to process
# but if we do *.bam it will catch the intermediate bam files that are in the folder
for a in $(cat acc.txt)
do
  m="$a.bam $m"
done

VCF=Salmonella.vcf.gz
VCFFILTER=Salmonella.filtered.vcf.gz
bcftools mpileup -Ou -f $GENOME $m | bcftools call -vmO z -o $VCF
tabix -p vcf $VCF
bcftools stats -F $GENOME -s - $VCF > $VCF.stats
mkdir -p plots
plot-vcfstats -p plots/ $VCF.stats
bcftools filter -O z -o $VCFFILTER -s LOWQUAL -i'%QUAL>10' $VCF
```

Genome Browsers

We will do more on genome browsers later in the course. But if you want to see how to visualize genome you can see some of these tools.

IGV

IGV - High-performance visualization tool for interactive exploration of large, integrated genomic datasets. It supports a wide variety of data types, including array-based and next-generation sequence data, and genomic annotations.

JBrowse

JBrowse2 provides google-maps style interface to genomes

Public genome browsers

Many browsers allow upload of aligned data (bam files) to integrate local data with public genome resources.

- Ensembl, Ensembl Genomes
- UCSC Genome Browser
- WormBase, FlyBase
- TAIR - Arabidopsis, Phytozome
- EuPathDB, JGI Genomes
- IMG/M - JGI

Displaying data in EnsEMBL

Go to Ensembl Site for Salmonella enterica subsp. enterica serovar Typhi str. CT18

See the EnsEMBL tutorial on how to add a BAM file track (note this only works if you have aligned reads to the SAME ASSEMBLY that is in Ensembl).

Click on "Display your data in Ensembl Bacteria"

Make a link on the web for your data. Follow directions on HPCC site

```
mkdir -p ~/.html/share
cd ~/.html/share
ln -s ~/bigdata/Short_read_aligning . # or wherever you were doning
```

Now you can use the URL `http://cluster.hpcc.ucr.edu/~YOURLOGIN/share/Short_read_aligning` and the `.bam` files that are in there.