

?? ?? ????? (??)

Bacterial Genome data ??

?? ? ?? ?? ?? ?? ?? .

bwa, **samtools** ?? ?? ?? .

which bwa

??? bwa? ?? ?? .

which samtools

??? samtools? ?? ?? .

Basic Bacterial Genome Sequence Analysis

1. Get a reference sequence:

```
mkdir -p /tmp/outbreaks/SG-M1
```

```
cd /tmp/outbreaks/SG-M1
```

```
wget
```

```
ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/001/275/545/GCF_001275545.2_ASM127554v2/GCF_001275545.2_ASM127554v2_genomic.fna.gz
```

```
gunzip GCF_001275545.2_ASM127554v2_genomic.fna.gz
```

```
mv GCF_001275545.2_ASM127554v2_genomic.fna SG-M1.fna
```

2. Map and call SNPs:

Note: For an annotation of the programs used below and other bioinformatics tools, check out our course [github page](#).

Reference indexing

```
bwa index SG-M1.fna
```

Mapping

```
bwa mem SG-M1.fna /tmp/fastq/SRR6327950/SRR6327950_1.fastq.gz  
/tmp/fastq/SRR6327950/SRR6327950_2.fastq.gz | samtools view -bS - > SRR6327950.bam
```

BAM Sorting

```
samtools sort SRR6327950.bam -o SRR6327950-sort.bam
```

BAM Indexing

```
samtools index SRR6327950-sort.bam
```

Variant calling

```
lofreq faidx SG-M1.fna
```

```
lofreq call -f SG-M1.fna -r NZ_CP012419.2:400000-500000 SRR6327950-sort.bam > SRR6327950-400k.vcf
```

Mapping takes ~5 min on a t2.medium. Sorting takes ~2 min. Running lofreq on this limited section of the genome takes ~1 min.

3. Assembly (runs ~4 min then will run out of RAM if you're on a t2.medium):

```
spades.py -t 2 -1 /tmp/fastq/SRR6327950/SRR6327950_1.fastq.gz -2  
/tmp/fastq/SRR6327950/SRR6327950_2.fastq.gz -o SRR6327950_spades
```

NOTE: This assembly above will complete on a t3a.large and takes about 5 hours.

!!!! ! [] AWS EC2 !!!!! [] [] [] [] !!!!! . 3!!!!
!!!! [] [] !!!!! , [] [] [] [] [] [] . RAM[] []
[] [] [] [] [] !!!!! . !!!!! [] [] !!!!! [] EBS [] []
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