

Mycobacterium Chimera SJ_42 Assembly Method Summary

Files:

- MiSeq Reads:
 - /projects/rwarren_prj2/ntm/MinION/SJ_42/daniel/15A114_S13_L001_R1_001.fastq
 - /projects/rwarren_prj2/ntm/MinION/SJ_42/daniel/15A114_S13_L001_R2_001.fastq
- ONT Reads:
 - /projects/labinstruments/MinION_Run/170130_A66989/reads/downloads/pass/
 - /projects/labinstruments/MinION_Run/170130_A66989/reads/downloads/fail/

Software:

- Unicycler (v0.3.0b)
- LINKS (v1.8.5)
- Nanopolish (v0.5.0)
- Sealer (v1.5.2)
- Pilon (v1.2.1)
- Racon (v0.5.0)
- Minimap (v0.2)
- BWA (v0.7.15)
- Freebayes (v1.1.0-9-g09d4ecf)
- Bowtie2 (v2.3.0)
- Samtools (v1.3.1)
- Java (v1.8.0_92)
- Mummer (v3.23)

Process:

- Extract the reads

```
PASS='/projects/labinstruments/MinION_Run/170130_A66989/reads/download
s/pass/'
FAIL='/projects/labinstruments/MinION_Run/170130_A66989/reads/download
s/fail/'
nanopolish extract -q -t template ${PASS} -o template.pass.fq
nanopolish extract -q -t template ${FAIL} -o template.fail.fq
/nanopolish extract -q -t complement ${PASS} -o complement.pass.fq
nanopolish extract -q -t complement ${FAIL} -o complement.fail.fq
nanopolish extract -q -t 2d ${PASS} -o 2d.pass.fq
nanopolish extract -q -t 2d ${FAIL} -o 2d.fail.fq
```

- Create single file with only 1D (template and complement reads)

```
cat template*fq complement*fq > 1d.pass.fail.fq
```

- Generate Unicycler assembly

```
R1='/projects/rwarren_prj2/ntm/MinION/SJ_42/daniel/15A114_S13_L001_R1_001.fastq'  
R2='/projects/rwarren_prj2/ntm/MinION/SJ_42/daniel/15A114_S13_L001_R2_001.fastq'  
unicycler -1 ${R1} -2 ${R2} -l 1d.pass.fail.fq -o output_unicycler
```

- Polish Unicycler assembly (you may have to use --bwa, --racon, etc. to specify software to use if not in path)

```
unicycler_polish -1 ${R1} -2 ${R2} --long_reads 1d.pass.fail.fq -a output_unicycler/assembly.fasta
```

- Re-scaffold using LINKS

```
mkdir links  
echo $(pwd)/1d.pass.fail.fq > minionALL.fof  
{LINKS} -f output_unicycler/*final_polish.fasta -s minionALL.fof -d 500,1000,2000,3000,4000,5000,6000,7000,8000,9000,10000,12000,14000,16000,18000,20000 -b links/links -l 10 -t 1
```

- Seal gaps with Sealer

```
abyss-sealer -o sealed --print-flanks -S links/links.scaffolds.fa -v -j 12 -B 1000 -F 700 -P 10 -k120 -k110 -k100 -k90 -k80 -k70 -k60 -k50 -k40 -k30 ${R1} ${R2}
```

Done!