



Version 2

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# Plasmid Sequence Assembly from Long Reads V.2

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In Development

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## ABSTRACT

This protocol demonstrates how to assemble reads from plasmid DNA, and generate a circularised and non-repetitive consensus sequence

At the moment, this protocol uses Canu to de-novo assemble high-quality single-cut reads.

## Input(s):

- demultiplexed fastq files (see protocol [Demultiplexing Nanopore reads with LAST](#)). I've noticed that the default demultiplexing carried out by Guppy (at least up to v4.2.2, as used in the first version of this protocol) has issues with [chimeric reads](#), which can affect assembly.

## Output(s):

- Consensus sequence per barcode as a fasta file

## DOI

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## WHAT'S NEW

Changed from Guppy demultiplexing to LAST demultiplexing

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#### Read file preparation

- 1 Demultiplex reads as per protocol [Demultiplexing Nanopore reads with LAST](#).

If this has been done, then the following command should produce output without errors:

```
for bc in $(awk '{print $2}' barcode_counts.txt);
do ls demultiplexed/reads_${bc}.fq.gz;
done
```

Example output:

```
demultiplexed/reads_BC02.fq.gz
demultiplexed/reads_BC03.fq.gz
demultiplexed/reads_BC04.fq.gz
demultiplexed/reads_BC05.fq.gz
demultiplexed/reads_BC07.fq.gz
demultiplexed/reads_BC09.fq.gz
```

If the barcode\_counts.txt file is missing, the output will look like this:

```
awk: fatal: cannot open file `barcode_counts.txt' for reading (No such file or directory)
```

If one or more of the barcode-demultiplexed files are missing, the output will look something like this:

```
demultiplexed/reads_BC02.fq.gz
demultiplexed/reads_BC03.fq.gz
demultiplexed/reads_BC04.fq.gz
ls: cannot access 'demultiplexed/reads_BC05.fq.gz': No such file or directory
ls: cannot access 'demultiplexed/reads_BC07.fq.gz': No such file or directory
demultiplexed/reads_BC09.fq.gz
```

- 2 Create a directory to store results files

```
mkdir results
```

- 3 Determine the N50/L50 read length for each barcode. This will be used as the initial guess at the assembly size.

```
(for bc in $(awk '{print $2}' barcode_counts.txt);
do echo -n ${bc};
fastx-length.pl demultiplexed/reads_${bc}.fq.gz 2>&1 > /dev/null | \
grep L50 | awk '{print "\t"$5$6}' | perl -pe 's/b$//';
done) > results/read_L50.txt
```

This file can be viewed to confirm the assembly lengths:

```
cat results/read_L50.txt
```

Example output:

```
BC02    347
BC03    8.904k
BC04    8.888k
BC05    10.262k
BC07    11.076k
BC09    11.093k
```

*Note: this file will be used for subsequent downstream processing. If any of these barcodes shouldn't be processed further, feel free to remove the corresponding lines from this file*

#### Read filtering

- 4 Filter out any reads that are less than half of the target read length, and determine the average quality of the remainder, keeping information on (at most) 200 of the highest-quality reads:

```
cat results/read_L50.txt | while read bc len;
do echo ${bc} ${len};
~/scripts/fastx-compstats.pl demultiplexed/reads_${bc}.fq.gz | \
sort -t ',' -k 16rn,16 | \
awk -F ',' -v "len=${len}" \
  'BEGIN{if(match(len, "k") > 0){sub("k","",len); len=len*1000}}
  {if($18 > (len / 2)){print}}' | \
  head -n 200 | grep -v '^name' > results/bestLong_200X_${bc}.csv;
done
```

Check how successful the sequencer was at getting 200X coverage by counting lines:

```
wc -l results/bestLong_200X_*.csv
```

Example output. In this case BC02 has fewer than 200 reads, so the assembly is less likely to be high-quality:

```
90 results/bestLong_200X_BC02.csv
200 results/bestLong_200X_BC03.csv
200 results/bestLong_200X_BC04.csv
200 results/bestLong_200X_BC05.csv
200 results/bestLong_200X_BC07.csv
200 results/bestLong_200X_BC09.csv
1090 total
```

- 5 Subset the original read sets to only include the high-quality long reads:

```
cat results/read_L50.txt | while read bc len;
do echo ${bc} ${len};
~/scripts/fastx-fetch.pl -i results/bestLong_200X_${bc}.csv \
  demultiplexed/reads_${bc}.fq.gz > results/bestLong_200X_${bc}.fastq; done
```

#### Canu Assembly

- 6 Run a Canu assembly on each read set, using default options:

```
cat results/read_L50.txt | while read bc len;
do canu -nanopore results/bestLong_200X_${bc}.fastq \
  -p ${bc} -d results/canu_${bc} genomeSize=${len};
done
```

## 7 Trim the assembled contigs based on Canu's trim recommendations:

```
cat results/read_L50.txt | while read bc len;
do echo ${bc} ${len};
samtools faidx results/canu_${bc}/${bc}.contigs.fasta \
$(grep '^>' results/canu_${bc}/${bc}.contigs.fasta | \
perl -pe 's/^>(.*?) .*trim=(.*)/$1:$2/') > results/circTrimmed_${bc}.fasta;
done
```