



Nanopore Sequencing Methods

Sample libraries were prepared using Oxford Nanopore Technologies (ONT) Native Barcoding Kit 24 V14 (SQK-NBD114.24) to manufacturer's specifications. All samples were run on Nanopore R10.4.1 flow cells on either a MinION, Mk1B or GridION. ONT's Guppy (v6.4.6) was used to demultiplex samples [1]. Samples were demultiplexed using Guppy's Super Accurate basecalling model (dna_r10.4.1_e8.2_400bps_modbases_5mc_cg_sup.cfg).

Each sample comes with a FASTQ file, BAM file, and FAST5 files. The FASTQ file contains the demultiplexed reads and their accompanying quality scores. The BAM file contains the demultiplexed reads, their accompanying quality scores, and 5mC methylation (CG - context) data encoded in each reads' 'MM' and 'ML' tags. [FAST5](#) is a hierarchical data format 5 ([HDF5](#)) file with a specific schema as defined by ONT. These FAST5 files are how native nanopore data is stored. The files do not contain demultiplexed reads, however, they do include raw signal intensities that can be used with third-party software for bespoke methylation analysis. Because FAST5 files are large, we have split them into multiple files each containing 5000 entries. You can find a md5 checksum for each file in the '*checksums.txt*' file

Sequencing statistics are included in the '*DNA Sequencing Stats.xlsx*' file.

What is an md5sum?

The md5sum functions as a file's compact digital fingerprint. md5sums are used to verify the integrity of files between two servers. If you calculate the md5sum of the file on your server, it should match that of the file listed in the '*DNA Sequencing Stats.xlsx*' file. If it does not match, the file was corrupted either being uploaded or downloaded to box, please let SeqCenter know if this is the case.

Calculating the md5sum

Windows users, please see [this tutorial](#).

MacOS users, please see [this tutorial](#).

Linux users, please see [this tutorial](#).

References

- [1] Analysis solutions for nanopore sequencing data. [Nanopore sequencing data analysis \(nanoporetech.com\)](#)