MinION workshop - Getting to know your data

pandoc brachy.md –smart –standalone –bibliography test.bib -o brachy.pdf

Section Goals

- Understand the initial data that is coming from your MinION run
- What is contained within the `fast5` datafiles
- What we can, and cannot, do with the data at this point

General pipeline so far:

- The MinKNOW software on the sequencing laptop facilitates the collection and parsing of data from the MinION flowcell.
- This 'raw' data is voltage traces that are measured at 5khz (5 thousands times per second).
- The voltage across the membrane changes as molecules enter, move through, and exit each sequencing pore.
- This entire measurement is called an 'event' which will be the collected signal measurements for that molecule.
- The software recognizes this initial voltage change and begins writing out the trace to a new `fast5` file.
- There is **one file per DNA molecule** that passes through each pore. Therefore many files are created!
- Hence, the requirement for high quality transfer speeds on your laptop to facilitate this (SSDs)
- This is a fundamental shift from Illumina platforms in which most analysis is based off of fewer, but much larger, files.

The initial data is NOT basecalled

- At this point, we do not have nucleotide information, just voltage traces.
- We can look at this information within the fast5 files that are created.

What are `fast5` files?

- Each file contains signal trace for a single DNA molecule
- `fast5` files are stored in the `HDF5` file format.
- `HDF5` acts as a larger organizational framework (box) in which different types of data can be stored in a hierarchical way.
- Like directories, sub-directories, and files/data on your computer.
- The `HDF5` format is quite flexable in being able to contain a wide variety of data types internally (strings,int,float,arrays, etc) within the single 'HDF5' format.
- The `fast5` files are therefore able to not only hold the signal trace, but also a wide variety of metadata related to how it was created, timestamps, software versions, etc.
- The format allows for new items to be added to it over time, creating a single container from raw information onwards
- We will see this shortly.
- We can investigate any `fast5` file using an `HDF5` 'viewer' which will show the internal structure and datasets in an easy-to-examine GUI.
- The group that created the `HDF5` format provides a java-based viewing software to download:
- HDFView for windows
- HDFView for OSX
- HDFView for Linux
- There are also command-line tools to viewing HDF5 files for those interested

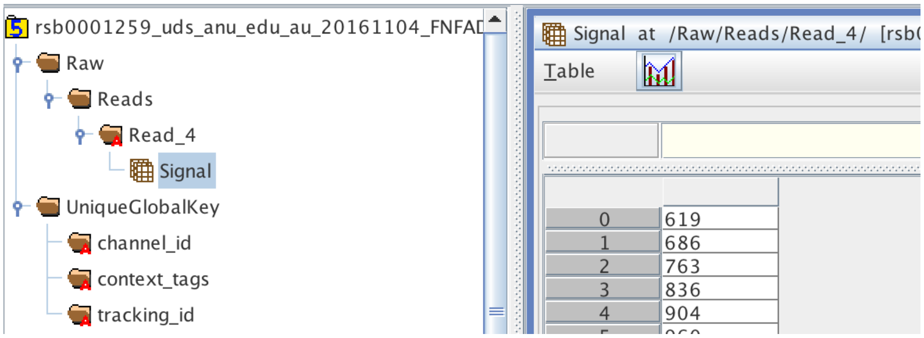
Where are the `fast5` files located?

- As sequencing begins, data beings to be filled in to the `./reads` directory within your MinKNOW install folder:

As of MinKNOW v1.6.11:

Windows: OSX: `/Library/MinKNOW/data/reads/` Linux:

Let's take a look at a `fast5` file


rsb0001259_uds_anu_edu_au_20161104_FNFAD
Raw
Reads
Read_4
Signal
UniqueGlobalKey
channel_id
context_tags
tracking_id
Signal at /Raw/Reads/Read_4/ [rsb(
Table

0	619
1	686
2	763
3	836
4	904

Figure 1: screenshot from hdfview