**Errors In Computer set up.**

**From Gurdeep 11th Feb 2022**

1. Live basecalling is not working – it does not produce fastq when ruuning live basecalling, rather the data are saved as queued reads and Fast5\_skips folders.

2. Guppy basecalling- [guppy/error] main: CUDA error at /builds/ofan/ont\_core\_cpp/ont\_core/common/cuda\_common.cpp:233: CUDA\_ERROR\_OUT\_OF\_MEMORY

[guppy/warning] main: An error has occurred. Aborting.

Response: these errors (1 and 2) are related and due to a problem with the link between MinKNOW and gpu-powered Guppy. This link facilitates live basecalling in MinKNOW using a laptop with an NVIDIA GPU. Updates often break the link, requiring it to be set-up again. Instructions are [here](https://community.nanoporetech.com/protocols/experiment-companion-minknow/v/mke_1013_v1_revbz_11apr2016/installing-gpu-version-of-guppy-with-minknow-for-minion).

However, sometimes these instructions don’t work because nanopore only supports particular NVIDIA models so it often takes a bit of a workaround to get ours to work properly! In this case, searching the forum using keywords like “guppy basecalling”, “GPU”, specific errors you are getting or name of your nvidia model etc will help to find solutions; or you can post in the forum yourself (give details).

3. Demulplexing – error- Unexpected option '--arrangements\_files' found on command line.

Response: This error is due to an update to the Guppy software. From guppy v6.0 onward, the --arrangements\_file option has been replaced with –barcode\_kits option. So in order to run demultiplexing in guppy the command would be:

guppy\_barcoder --require\_barcodes\_both\_ends -i run\_name -s output\_directory --barcode\_kits "EXP-NBD104"

where the bit within the quotes should reflect the barcode kit used to generate the data. If you used barcode kits 1-12 and 13-24 it would be “EXP-NBD104 EXP-NBD114”, 96 barcode kit would be “EXP-NBD196” etc. It’s now updated in the bioinformatics instructions on the artic-rabv repo. (Remember if your guppy version is <6 then you will use --arrangements\_files "barcode\_arrs\_nb12.cfg barcode\_arrs\_nb24.cfg" instead of --barcode\_kits)

4. When running demultiplexing without arrangement - [guppy/error] guppy::ReadProcessor::run: CUDA error at /builds/ofan/ont\_core\_cpp/ont\_core/common/cuda\_common.cpp:233:

CUDA\_ERROR\_OUT\_OF\_MEMORY. Exiting.

Response: This problem is actually related to 1 and 2. The cuda error is because the stand-alone gpu guppy is not linked to the gpu properly.

5. Rampart – add ea-rabv, still runs under Tanzania ref only.

Response: Ensure you are directing rampart to the correct protocol! The rampart protocols are in the artic-rabv repo in the rampart folder. There are different protocols for each primer scheme i.e. peru, Philippines, east Africa so you need to choose the relevant one and use that in the rampart command. Example commands:

rampart --protocol ~/Github/artic-rabv/rampart/tza\_protocol/rabv\_ea --basecalledPath <minknow\_data\_path>/<run\_name>/fastq\_pass

would run the rampart files for the rabv\_ea primer scheme (i.e. the rabv\_ea primer positions and reference file)

rampart --protocol ~/Github/artic-rabv/rampart/phl\_protocol --basecalledPath <minknow\_data\_path>/<run\_name>/fastq\_pass

would run the Philippines relevant protocol