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## 🌐 ONT Sequencing IT/Compute Pop!\_OS 22.04 Setup V.3

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### ABSTRACT

The IT requirements for processing MinION data should be carefully reviewed before purchasing a MinION device. You will want to go with a Linux system. [System76](#) is really the primary/best vendor for laptops. Pay careful attention to the GPU. It is probably the most important component for fast processing of the data. [Here is a link](#) to a Facebook thread of some discussion when first considering the specs required.

Setting up all of the programs/dependencies, particularly for utilizing the GPU during the analytical workflows is the next important step. You will want to get all of this in place before you start with the lab workflows, as there are many things that could go wrong or that you will need to work through in order for you to be able to actually begin a run.

**Protocol status:** Working

We use this protocol and it's working


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## Preparing a new CPU for MinION Sequencing

- 1 The final setup I went with can be found below. It was expensive (around  4000 for the laptop in early 2022), but should be able to achieve live basecalling for two MinION devices at the same time. Overall specs of my laptop:

Pop!_OS 21.10 (64-bit) with full disk-encryption	
4.6 GHz i7-11800H - up to 4.6 GHz - 24MB Cache - 8 Cores - 16 Threads)	
64 GB Dual Channel DDR4 at 3200 MHz (2x 32GB)	\$549.00
1 TB NVMe <i>Seq Read: 7,000 MB/s, Seq Write: 5,000 MB/s</i>	\$329.00
No Additional Storage	
1 Year Limited Parts and Labor Warranty	
Normal Assembly Service	
16 GB RTX 3080 W/ 6144 CUDA Cores	\$649.00
17.3" Matte 144Hz Full HD 1080p	\$79.00
United States QWERTY Keyboard	
WiFi + Bluetooth	

Specs of the System 76 Oryx Pro laptop this protocol uses for ONT sequencing.

Minimum IT requirements for MinION from ONT:



- 2 The remainder of this protocol assumes you have completed all of the preliminary setup steps that are common with any new CPU.

Install CUDA toolkit - <https://developer.nvidia.com/cuda-toolkit> :

#### Command

```
wget
https://developer.download.nvidia.com/compute/cuda/repos/ubuntu2204/x86_64/cuda-ubuntu2204.pin
sudo mv cuda-ubuntu2204.pin /etc/apt/preferences.d/cuda-repository-pin-600
wget
https://developer.download.nvidia.com/compute/cuda/11.7.0/local_installers/cuda-repo-ubuntu2204-11-7-local_11.7.0-515.43.04-1_amd64.deb
sudo dpkg -i cuda-repo-ubuntu2204-11-7-local_11.7.0-515.43.04-1_amd64.deb
sudo cp /var/cuda-repo-ubuntu2204-11-7-local/cuda-*-keyring.gpg
/usr/share/keyrings/
sudo apt-get update
sudo apt-get -y install cuda
```

- 3 Install Boost

#### Command

```
sudo apt install libboost-all-dev
```

- 4 The process at this link was instrumental to this protocol. It is recreated and simplified here. [ORIGINAL PROTOCOL](#). It was written for Pop!\_OS 21.04. The following protocols also work with Pop!\_OS 22.04. I would

follow the steps at the link rather than here so you get a broader context of the actions you are performing on your system.

#### Command

##### Add ONT Focal Repository (Pop!\_OS 22.04)

```
# update packages list
sudo apt-get update
# check for and install wget if needed
if [ $(dpkg-query -W -f='${Status}' wget 2>/dev/null | grep -c "ok installed") -
eq 0 ];
then
    sudo apt --yes install wget;
fi
# add the key
wget -O- https://mirror.oxfordnanoportal.com/apt/ont-repo.pub | sudo apt-key
add -
# add the focal repo
echo "deb http://mirror.oxfordnanoportal.com/apt focal-stable non-free" | sudo
tee /etc/apt/sources.list.d/nanoporetech.sources.list
```

#### Command

##### sudo apt update (Pop!\_OS 22.04)

```
sudo apt update
```

## Command

### Check for access to ONT files (Pop!\_OS 22.04)

```
apt policy minknow-core-minion-nc
```

## Expected result

```
minknow-core-minion-nc:
  Installed: 4.3.4-focal
  Candidate: 4.3.4-focal
  Version table:
   4.3.4-focal 100
   10 http://mirror.oxfordnanoportal.com/apt focal-stable/non-free amd64 Packages
   100 /var/lib/dpkg/status
```

## 5 Add the Focal repos:

## Command

### Create a new file and edit in nano

```
sudo nano /etc/apt/sources.list.d/system-focal.sources
```

Copy and paste the following into your file:

```
X-Repolib-Name: Pop_OS System Sources
Enabled: yes
Types: deb deb-src
URIs: http://us.archive.ubuntu.com/ubuntu/
Suites: focal focal-security focal-updates focal-backports
Components: main restricted universe multiverse
X-Repolib-Default-Mirror: http://us.archive.ubuntu.com/ubuntu/
```

#### Command

**Check that the file exists and contains the right information. (Pop!\_OS 22.04)**

```
cat system-focal.sources
```

#### Expected result

```
X-Repolib-Name: Pop_OS System Sources
Enabled: yes
Types: deb deb-src
URIs: http://us.archive.ubuntu.com/ubuntu/
Suites: focal focal-security focal-updates focal-backports
Components: main restricted universe multiverse
X-Repolib-Default-Mirror: http://us.archive.ubuntu.com/ubuntu/
```

**6** Pin the Focal repos. Start by creating another new file with nano:

#### Command

```
sudo nano /etc/apt/preferences.d/focal-default-settings
```

Copy and paste the following into your file:

```
Package: *  
Pin: release n=focal*  
Pin-Priority: 10
```

Check that it was created correctly

#### Command

```
cat focal-default-settings
```

#### Expected result

```
Package: *  
Pin: release n=focal*  
Pin-Priority: 10
```



**Command**

```
sudo apt update
```

## 7 Install MinKNOW and required packages

**Command**

```
sudo apt install \  
  minknow-core-minion-nc \  
  ont-kingfisher-ui-minion \  
  ont-bream4-minion \  
  ont-configuration-customer-minion \  
  ont-jwt-auth \  
  ont-vbz-hdf-plugin
```

## 8 Install ONT Guppy

**Command**

```
sudo apt install ont-guppy
```

#### Command

##### Check the paths once installed

```
which guppy_basecaller
```

#### Expected result

```
/usr/bin/guppy_basecaller
```

#### Command

```
guppy_basecaller --version
```

#### Expected result

```
: Guppy Basecalling Software, (C) Oxford Nanopore Technologies, Limited.  
Version 5.0.11+2b6dbff
```

## 9 Setup the MinKnow service

### Command

```
sudo /opt/ont/minknow/bin/config_editor --conf application \
--filename /opt/ont/minknow/conf/app_conf \
--set guppy.server_executable="/opt/ont/guppy/bin/guppy_basecall_server" \
--set guppy.client_executable="/opt/ont/guppy/bin/guppy_basecall_client" \
--set guppy.gpu_calling=1 \
--set guppy.num_threads=16 \
--set guppy.ipc_threads=2
```

### Command

```
systemctl restart minknow.service
```

### Command

```
systemctl status minknow.service
```

## Command

```
sudo nano /lib/systemd/system/guppyd.service
```

Copy the following to your new file:

```
[Unit]
Description=Service to manage the guppy basecall server.
Documentation=https://community.nanoporetech.com/protocols/Guppy-protocol/v/GPB_2003_v1_revQ_14Dec2018

[Service]
Type=simple
ExecStart=/opt/ont/guppy/bin/guppy_basecall_server --log_path /var/log/guppy
--config dna_r9.4.1_450bps_fast.cfg --port 5555 -x cuda:all
Restart=always
User=root
MemoryLimit=8G
MemoryHigh=8G
CPUQuota=200%

[Install]
Alias=guppyd.service
WantedBy=multi-user.target
```

Check the file:

#### Command

```
cat /lib/systemd/system/guppyd.service
```

#### Command

```
systemctl enable guppyd.service
```

#### Command

```
systemctl restart guppyd.service
```

MinKNOW GUI should now be available in your programs. Validate that it opens correctly.

## 10

Changes to MinKnow file permissions at the bottom here:

<https://gringer.gitlab.io/presentation-notes/2021/10/08/gpu-calling-in-minknow/>

"For my computer, there's an issue with MinKNOW not being able to access or create files. As a "nuclear" option, Miles Benton suggested changing the user and group for the minknow service to root"

## Command

```
sudo service minknow stop
sudo perl -i -pe 's/(User|Group)=minknow/$1=root/'
/lib/systemd/system/minknow.service
sudo systemctl daemon-reload
sudo service minknow start
```

- 11** Per this document: [https://denbi-nanopore-training-course.readthedocs.io/en/latest/read\\_qc/MinionQC.html](https://denbi-nanopore-training-course.readthedocs.io/en/latest/read_qc/MinionQC.html)  
 Install R: <https://cran.r-project.org/>  
 Install MinionQC: [https://github.com/roblanf/minion\\_qc](https://github.com/roblanf/minion_qc)

Install R:

## Command

```
# update indices
sudo apt update -qq
# install two helper packages we need
sudo apt install --no-install-recommends software-properties-common dirmngr
# add the signing key (by Michael Rutter) for these repos
# To verify key, run gpg --show-keys /etc/apt/trusted.gpg.d/cran_ubuntu_key.asc
# Fingerprint: E298A3A825C0D65DFD57CBB651716619E084DAB9
wget -qO- https://cloud.r-project.org/bin/linux/ubuntu/marutter_pubkey.asc |
sudo tee -a /etc/apt/trusted.gpg.d/cran_ubuntu_key.asc
# add the R 4.0 repo from CRAN -- adjust 'focal' to 'groovy' or 'bionic' as
needed
sudo add-apt-repository "deb https://cloud.r-project.org/bin/linux/ubuntu
$(lsb_release -cs)-cran40/"
```

## Command

```
sudo apt install --no-install-recommends r-base
```

#### Command

```
install.packages(c("data.table",
                  "futile.logger",
                  "ggplot2",
                  "optparse",
                  "plyr",
                  "readr",
                  "reshape2",
                  "scales",
                  "viridis",
                  "yaml"))
```

## 12 Install Bioconductor:

In an R command window:

#### Command

```
if (!require("BiocManager", quietly = TRUE))
  install.packages("BiocManager")
BiocManager::install(version = "3.15")
```

## 13 Install Anaconda:

from: <https://www.digitalocean.com/community/tutorials/how-to-install-the-anaconda-python-distribution-on-ubuntu-22-04>



#### Command

```
cd /tmp
```

#### Command

```
curl https://repo.anaconda.com/archive/Anaconda3-2022.05-Linux-x86_64.sh --  
output anaconda.sh
```

#### Command

**You can now verify the data integrity of the installer with cryptographic hash verification through the SHA-256 checksum. You'll use the sha256sum command along with the filename of the script:**

```
sha256sum anaconda.sh
```

### Expected result

You'll receive output that looks similar to this:

```
fedf9e340039557f7b5e8a8a86affa9d299f5e9820144bd7b92ae9f7ee08ac60
anaconda.sh
```

### Command

```
bash anaconda.sh
```

### Expected result

Press ENTER/yes as needed

```
Welcome to Anaconda3 2021.11
```

```
In order to continue the installation process, please review the license
agreement.
```

```
Please, press ENTER to continue
```

```
>>>
```

### Command

```
source ~/.bashrc
```

### Command

```
conda list
```

### Expected result

```
# packages in environment at /home/user/anaconda3:
#
# Name                                Version                                Build      Channel
_ipyw_jlab_nb_ext_conf               0.1.0                                py39h06a4308_0
_libgcc_mutex                         0.1                                  main
_openmp_mutex                         4.5                                  1_gnu
alabaster                             0.7.12                              pyhd3eb1b0_0
anaconda                             2022.05                              py39_0
```

### Command

```
conda search "^python$"
```

#### Command

```
conda create --name my_env python=3
```

#### Command

```
conda activate my_env
```

#### Command

#### Verify Python is installed

```
python --version
```

#### Command

```
conda install --name my_env35 numpy
```

## 14

Install NGSspeciesID: <https://github.com/ksahlin/NGSpeciesID>

**Command**

```
conda create -n NGSspeciesID python=3.6 pip
conda activate NGSspeciesID
```

**Command**

```
conda install --yes -c conda-forge -c bioconda medaka==0.11.5 openblas==0.3.3
spoa racon minimap2
pip install NGSspeciesID
```

**Command**

```
conda activate NGSspeciesID
```

## Command

### Test the install

```
mkdir test_ngspeciesID
cd test_ngspeciesID
```

## Command

### Download the test fastq file called "sample\_h1.fastq" (filesize 390kb)

```
curl -LO
https://raw.githubusercontent.com/ksahlin/NGSpeciesID/master/test/sample_h1.fastq
```

## Command

Run the NGSpecies command on test file. Outputs will be saved in **"/test\_ngspeciesID/sample\_h1/"**, where the final polished consensus file ("consensus.fasta") is located in the **"/test\_ngspeciesID/sample\_h1/medaka\_cl\_id\_"** directory.

```
NGSpeciesID --ont --fastq sample_h1.fastq --outfolder ./sample_h1 --consensus --medaka
```

15 You should now be ready to begin sequencing runs.

16

## Updating MinKnow & Installing Dorado

17 Since this protocol was first written, MinKnow needed an update for the 10.4.1 Flongle cells and then again to bring in Dorado basecalling. The steps to perform the update can be found below:

```

sudo apt install ont-python=3.10.7-9
sudo apt install ont-bream4-minion=7.8.2-1~focal
sudo apt install ont-configuration-customer-minion=5.8.6-1~focal
sudo apt install ont-kingfisher-ui-minion=5.8.13-1~focal
sudo apt install minknow-core-minion-nc=5.8.3

sudo apt update
sudo apt install wget
wget -O- https://cdn.oxfordnanoportal.com/apt/ont-repo.pub | sudo apt-key
add -
echo "deb http://cdn.oxfordnanoportal.com/apt focal-stable non-free" | sudo
tee /etc/apt/sources.list.d/nanoporetech.sources.list

sudo apt update
sudo apt install ont-standalone-minknow-gpu-release

Carryover code from last update:

sudo service minknow stop
sudo perl -i -pe 's/((User|Group)=minknow/$1=root/'
/lib/systemd/system/minknow.service
sudo systemctl daemon-reload
sudo service minknow start

sudo apt install cuda
conda install -c bioconda seqkit

python -m venv venv --prompt duplex
. venv/bin/activate
pip install duplex_tools

```

These dependency versions will likely keep changing with time.

## 18 Installing Dorado:



```
sudo -i

sudo apt-get update && apt-get install -y --no-install-recommends \
    curl \
    git \
    ca-certificates \
    build-essential \
    nvidia-cuda-toolkit \
    libhdf5-dev \
    libssl-dev \
    libzstd-dev \
    cmake \
    autoconf \
    automake

git clone https://github.com/nanoporetech/dorado.git dorado
cd dorado
cmake -S . -B cmake-build
cmake --build cmake-build --config Release -j
ctest --test-dir cmake-build

pip install pre-commit
pre-commit install
```