OneRoof: Base-, Variant-, and Consensus-calling under One Proverbial Roof

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Overview

`oneroof` is a pipeline designed to take a common series of bioinformatic tasks (see below) and put them under "one roof". We mean this quite literally: the pipeline will perform at its best when run on networked devices in the same building.

`oneroof` was originally developed in the early stages of the United States Bovine Highly Pathogenic Avian Influenza (HPAI) outbreak of 2024, when we wanted one, configurable, easy-to-run pipeline that would do all of the following:

1. Handle super-accuracy basecalling with GPU acceleration on pod5-formatted Nanopore signal files, working on GCP or AWS if need be.
2. Demultiplex BAM-formatted reads that come out of basecalling.
3. Perform the above two steps as signal files become available, either locally or remotely via a TCP stream.
4. Accept raw read BAMs or FASTQs if basecalling and demultiplexing have already been performed elsewhere.
5. Accept paired Illumina reads in addition to Nanopore reads.
6. Use forward and reverse primer sequences to select only those reads that represent complete amplicons.
7. Trim away primers—and also any bases that are upstream of the forward primer or downstream of the reverse primer.
8. Align to a custom reference with the proper presets for the provided data.
9. Call variants and consensus sequences with appropriate settings for the provided data.
10. Perform tree building with `nextclade`, quality introspection with `multiQC`, and error correction based on the input sequence platform.

Though many excellent pipelines currently exist, e.g. `nf-core/viralrecon`, `epi2me-labs/wf-amplicon`, and `nf-core/nanoseq`, none of these pipelines quite handled all of the above. `oneroof`

seeks to handle these requirements while remaining highly configurable for users, highly modular for developers, and easy to control in the command line for both.

Overall, `oneroof` can be summarized as a variant-calling pipeline written in and managed by Nextflow. Its software dependencies are provided through containers or through an environment assembled by `pixi`. To run it on your own Nanopore pod5s, simply run something like:

```
nextflow run nrminor/oneroof \
--pod5_dir my_pod5_dir \
--primer_bed my_primers.bed \
--refseq my_ref.fasta \
--ref_gbk my_ref.gbk \
--kit "SQK-NBD114-24"
```

These are the core elements required to run on Nanopore data: a directory of pod5 files, a BED file of primer coordinates, a reference sequence in FASTA and Genbank format, and the Nanopore barcoding kit used.

And for Illumina paired-end reads, it's even simpler:

```
nextflow run nrminor/oneroof \
--illumina_fastq_dir my_illumina_reads/
```

Quick Start

For most users, `oneroof` will have two core requirements: The Docker container engine, available here, and Nextflow, available here. For users interested in super-accuracy basecalling Nanopore signal files, an on-board GPU supported by the Dorado basecaller is also required.

All remaining software dependencies will be supplied through the pipeline's Docker image, which will be pulled and used to launch containers automatically.

From there, the pipeline's three data dependencies are sequence data in BAM, FASTQ, or POD5 format, a BED file of primer coordinates, and a reference sequence in FASTA and Genbank format. For Nanopore data, a barcoding kit identifier is also required. Simply plug in these files to a command like the above and hit enter!

Configuration

Most users should configure `oneroof` through the command line via the following parameters:

Command line argument	Default value	Explanation
`--primer_bed`	None	A bed file of primer coordinates relative to the reference provided with the parameters `refseq` and `ref_gbk`.
`--refseq`	None	The reference sequence to be used for mapping in FASTA format.
`--ref_gbk`	None	The reference sequence to be used for variant annotation in Genbank format.
`--remote_pod5_location`	None	A remote location to use with a TCP stream to watch for pod5 files in realtime as they are generated by the sequencing instrument.
`--pod5_dir`	None	If a remote pod5 location isn't given, users may provide a local, on-device directory where pod5 files have been manually transferred.
`--prepped_data`	None	If pod5 files have already been basecalled and demultiplexed, users can specify their location with prepped data.
`--illumina_fastq_dir`	None	If users have Illumina data to be processed, they may specify their paired-end FASTQ files' location with `illumina_fastq_dir`.
`--model`	sup@latest	the Nanopore basecalling model to apply to the provided pod5 data (defaults to the latest super-accuracy version)
`--kit`	None	The Nanopore barcoding kit used to prepare sequencing libraries.
`--pod5_batch_size`	all pod5s	How many pod5 files to basecall at once. With a single available GPU, all pod5 files should be basecalled together, so this parameter defaults to telling Nextflow to take all pod5 files at once.
`--basecall_max`	1	If basecalling pod5 files is to be parallelized across multiple available GPUs, this parameter tells Nextflow how many parallel instances of the basecaller to run at once (defaults to 1).
`--max_mismatch`	0	The maximum number of mismatches to allow when finding primers
[illegible]	[illegible]	[illegible]

Developer Setup

`oneroof` depends on software packages supplied through various `conda` registries as well as through `PyPI`, the Python Package Index. To unify these various channels, we used the relatively new `pixi` package and environment manager, which stores all dependencies from both locations in the file `pyproject.toml`.

To reproduce the environment required by this pipeline, make sure you are on a Mac with an x86_64 (Intel) processor, a 64-bit linux machine, or a 64-bit Windows machine using Windows Subsystem for Linux (Apple Silicon coming soon!). Then, to reproduce the environment, install pixi with:

```
curl -fsSL https://pixi.sh/install.sh | bash
```

Download the pipeline with:

```
git clone https://github.com/nrminor/oneroof.git && cd oneroof
```

And then open a `pixi` subshell within your terminal with:

```
pixi shell
```

As long as you are using a supported system, the pipeline should run within that subshell. You can also run the pipeline within that subshell without containers using the "containerless" profile:

```
nextflow run . \
-profile containerless \
--pod5_dir my_pod5_dir \
--primer_bed my_primers.bed \
--refseq my_ref.fasta \
--ref_gbk my_ref.gbk \
--kit "SQK-NBD114-24"
```

Especially on Macs, this will reduce the overhead of using the Docker Virtual Machine and allow the pipeline to invoke tools installed directly within the local project environment.

Note also that more information on the repo's files is available in our developer guide.

Contributing

Contributions, feature requests, improvement suggests, and bug reports via GitHub issues are all welcome! For more information on how to work with the project and what all the repo files are, see our developer guide.

Is it any good?

Yes.

Citation

Coming soon!