viral-ngs Documentation

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Broad Institute Viral Genomics

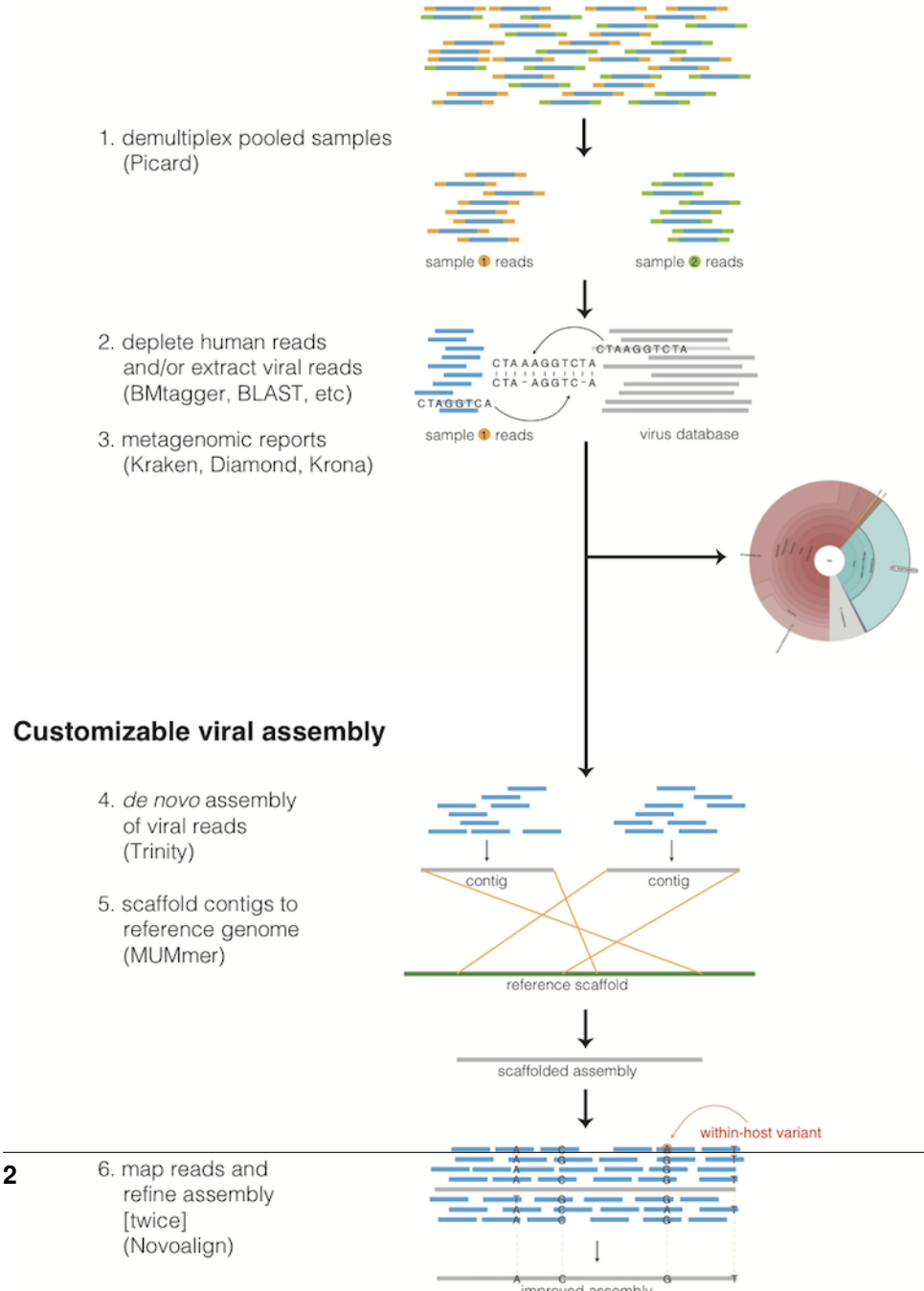
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CHAPTER 1

Contents

Description of the methods



Taxonomic read filtration

Human, contaminant, and duplicate read removal

The assembly pipeline begins by depleting paired-end reads from each sample of human and other contaminants using BMTAGGER and BLASTN, and removing PCR duplicates using M-Vicuna (a custom version of Vicuna).

Taxonomic selection

Reads are then filtered to to a genus-level database using LASTAL, quality-trimmed with Trimmomatic, and further deduplicated with PRINSEQ.

Viral genome analysis

Viral genome assembly

The filtered and trimmed reads are subsampled to at most 100,000 pairs. *de novo* assemby is performed using Trinity. SPAdes is also offered as an alternative *de novo* assembler. Reference-assisted assembly improvements follow (contig scaffolding, orienting, etc.) with MUMMER and MUSCLE or MAFFT. Gap2Seq is used to seal gaps between scaffolded *de novo* contigs with sequencing reads.

Each sample's reads are aligned to its *de novo* assembly using Novoalign and any remaining duplicates were removed using Picard MarkDuplicates. Variant positions in each assembly were identified using GATK IndelRealigner and UnifiedGenotyper on the read alignments. The assembly was refined to represent the major allele at each variant site, and any positions supported by fewer than three reads were changed to N.

This align-call-refine cycle is iterated twice, to minimize reference bias in the assembly.

Intrahost variant identification

Intrahost variants (iSNVs) were called from each sample's read alignments using V-Phaser2 and subjected to an initial set of filters: variant calls with fewer than five forward or reverse reads or more than a 10-fold strand bias were eliminated. iSNVs were also removed if there was more than a five-fold difference between the strand bias of the variant call and the strand bias of the reference call. Variant calls that passed these filters were additionally subjected to a 0.5% frequency filter. The final list of iSNVs contains only variant calls that passed all filters in two separate library preparations. These files infer 100% allele frequencies for all samples at an iSNV position where there was no intra-host variation within the sample, but a clear consensus call during assembly. Annotations are computed with snpEff.

Taxonomic read identification

Metagenomic classifiers include Kraken and Diamond. In each case, results are visualized with Krona.

Installation

Cloud compute implementations

Docker Images

To facilitate cloud compute deployments, we publish a complete Docker image with associated dependencies to the Docker registry at quay.io. Simply `docker pull quay.io/broadinstitute/viral-ngs` for the latest stable version.

DNAnexus

This assembly pipeline is also available via the DNAnexus cloud platform. RNA paired-end reads from either HiSeq or MiSeq instruments can be securely uploaded in FASTQ or BAM format and processed through the pipeline using graphical and command-line interfaces. Instructions for the cloud analysis pipeline are available at https://github.com/dnanexus/viral-ngs/wiki

Google Cloud Platform: dsub

All of the command line functions in viral-ngs are accessible from the docker image and can be invoked directly using dsub.

Here is an example invocation of `illumina.py illumina_demux` (replace the project with your GCP project, and the input, output-recursive, and logging parameters with URIs within your GCS buckets):

```
dsub --project broad-sabeti-lab --zones "us-east1-*" \
  --image quay.io/broadinstitute/viral-ngs \
  --name illumina_demux-test \
  --logging gs://sabeti-temp-30d/dpark/test-demux/logs \
  --input FC_TGZ=gs://sabeti-sequencing/flowcells/broad-walkup/160907_M04004_0066_
↪000000000-AJH8U.tar.gz \
  --output-recursive OUTDIR=gs://sabeti-temp-30d/dpark/test-demux \
  --command 'illumina.py illumina_demux ${FC_TGZ} 1 ${OUTDIR}' \
  --min-ram 30 \
  --min-cores 8 \
  --disk-size 2000
```

The speed of disk write and read operations is linearly proportional to the disk size, hitting the maximum disk speed somewhere around 1-8TB (depending on your I/O pattern). See GCE documentation.

Manual Installation

Install Conda

To use viral-ngs, you need to install the Conda package manager which is most easily obtained via the Miniconda Python distribution. Miniconda can be installed on your system without admin priviledges.

After installing Miniconda for your platform, be sure to update it:

```
conda update -y conda
```

Configure Conda

The viral-ngs software and its dependencies are distributed through the a channel of the conda package manager. It is necessary to add this channel to the conda config:

```
conda config --add channels r
conda config --add channels defaults
conda config --add channels conda-forge
conda config --add channels bioconda
conda config --add channels broad-viral
```

Make a conda environment and install viral-ngs

It is recommended to install viral-ngs into its own conda environment. This ensures its dependencies do not interfere with other conda packages installed on your system. A new conda environment can be created with the following command, which will also install conda:

```
conda create -n viral-ngs-env viral-ngs
```

Activate the viral-ngs environment and complete the install

In order to finish installing viral-ngs, you will need to activate its conda environment:

```
source activate viral-ngs-env
```

Due to license restrictions, the viral-ngs conda package cannot distribute and install GATK directly. To fully install GATK, you must download a licensed copy of GATK v3.6 from the Broad Institute, and call "gatk-register," which will copy GATK into your viral-ngs conda environment:

```
mkdir -p /path/to/gatk_dir
wget -O - 'https://software.broadinstitute.org/gatk/download/auth?package=GATK-
↪archive&version=3.6-0-g89b7209' | tar -xjvC /path/to/gatk_dir
gatk-register /path/to/gatk_dir/GenomeAnalysisTK.jar
```

The single-threaded version of Novoalign is installed by default. If you have a license for Novoalign to enable multi-threaded operation, viral-ngs will copy it to the viral-ngs conda environment if the `NOVOALIGN_LICENSE_PATH` environment variable is set. Alternatively, the conda version of Novoalign can be overridden if the `NOVOALIGN_PATH` environment variable is set. If you obtain a Novoalign license after viral-ngs has already been installed, it can be added to the conda environment by calling:

```
# obtain a Novoalign license file: novoalign.lic
novoalign-license-register /path/to/novoalign.lic
```

Activating viral-ngs once installed

After viral-ngs has been installed, only one command is needed to load the environment and all of its dependencies. This is the command that must be run each time before using viral-ngs:

```
source activate viral-ngs-env
```

To deactivate the conda environment:

```
source deactivate
```

Easy deployment script for viral-ngs

viral-ngs can be deployed with help of a shell script, `easy-deploy/easy-deploy-viral-ngs.sh` . This script will install an independent copy of viral-ngs from the latest source, install all dependencies, and make it simple to activate the viral-ngs environment and create projects. The script is available from the repository broadinstitute/viral-ngs-deploy.

One-line install command

After downloading the easy-install shell script, this one-line command will install viral-ngs on a 64-bit macOS or Linux system:

```
./easy-deploy-script/easy-deploy-viral-ngs.sh setup
```

One-line install command for Broad Institute users

This one-line command will download the `easy-deploy-viral-ngs.sh` script and setup viral-ngs in the current working directory. Simply ssh to one of the Broad login nodes and paste this command:

```
wget https://raw.githubusercontent.com/broadinstitute/viral-ngs/master/easy-deploy-
↪script/easy-deploy-viral-ngs.sh && chmod a+x ./easy-deploy-viral-ngs.sh && reuse␣
↪UGER && qrsh -l h_vmem=10G -cwd -N "viral-ngs_deploy" -q interactive ./easy-deploy-
↪viral-ngs.sh setup
```

Note: The script will run the install on a UGER interactive node, so you must have the ability to create to start a new interactive session. A project can be specified via `qrsh -P "<project_name>"`

Usage

Installation

- `./easy-deploy-viral-ngs.sh setup` Installs a fresh copy of viral-ngs, installs all dependencies, and creates a directory, `viral-ngs-etc/` , in the current working directory.

Resulting directories:

```
viral-ngs-etc/
    conda-env/
    viral-ngs/
    mc3/
```

Activating the environment

- `source ./easy-deploy-viral-ngs.sh load` Loads the dotkits needed by viral-ngs and activates the Python virtual environment

Creating a project directory

- `./easy-deploy-viral-ngs.sh create-project <project_name>` Creates a directory for a new Snakemake-compatible project, with data directories and symlinked run scripts. Copies in the files `Snakefile` and `config.yaml`

Resulting directories:

```
viral-ngs-analysis-software/
    projects/
        <project_name>/
            Snakefile
            bin/ (symlink)
            config.yaml
            data/
            log/
            reports/
            run-pipe_LSF.sh (symlink)
            run-pipe_UGER.sh (symlink)
            samples-assembly-failures.txt
            samples-assembly.txt
            samples-depletion.txt
            samples-runs.txt
            tmp/
            venv/ (symlink)
            [...other project files...]
```

Command line tools

metagenomics.py - metagenomic analyses

This script contains a number of utilities for metagenomic analyses.

```
usage: metagenomics.py subcommand
```

Sub-commands:

subset_taxonomy

Generate a subset of the taxonomy db files filtered by the whitelist. The whitelist taxids indicate specific taxids plus their parents to add to taxonomy while whitelistTreeTaxids indicate specific taxids plus both parents and all children taxa. Whitelist GI and accessions can only be provided in file form and the resulting gi/accession2taxid files will be filtered to only include those in the whitelist files. Finally, taxids + parents for the gis/accessions will also be included.

```
usage: metagenomics.py subset_taxonomy [-h]
                                       [--whitelistTaxids WHITELISTTAXIDS␣
↪[WHITELISTTAXIDS ...]]
                                       [--whitelistTaxidFile␣
↪WHITELISTTAXIDFILE]
                                       [--whitelistTreeTaxids␣
↪WHITELISTTREETAXIDS [WHITELISTTREETAXIDS ...]]
                                       [--whitelistTreeTaxidFile␣
↪WHITELISTTREETAXIDFILE]
                                       [--whitelistGiFile WHITELISTGIFILE]
                                       [--whitelistAccessionFile␣
↪WHITELISTACCESSIONFILE]
                                       [--skipGi] [--skipAccession]
                                       [--skipDeadAccession]
                                       [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                       [--version] [--tmp_dir TMP_DIR]
```

```
                                        [--tmp_dirKeep]
                                        taxDb outputDb
```

Positional arguments:

taxDb Taxonomy database directory (containing nodes.dmp, parents.dmp etc.)

outputDb Output taxonomy database directory

Options:

--whitelistTaxids List of taxids to add to taxonomy (with parents)

--whitelistTaxidFile File containing taxids - one per line - to add to taxonomy with parents.

--whitelistTreeTaxids List of taxids to add to taxonomy (with parents and children)

--whitelistTreeTaxidFile File containing taxids - one per line - to add to taxonomy with parents and children.

--whitelistGiFile File containing GIs - one per line - to add to taxonomy with nodes.

--whitelistAccessionFile File containing accessions - one per line - to add to taxonomy with nodes.

--skipGi=False Skip GI to taxid mapping files

--skipAccession=False Skip accession to taxid mapping files

--skipDeadAccession=False Skip dead accession to taxid mapping files

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

kraken

Classify reads by taxon using Kraken

```
usage: metagenomics.py kraken [-h] [--outReports OUTREPORTS [OUTREPORTS ...]]
                              [--outReads OUTREADS [OUTREADS ...]]
                              [--lockMemory]
                              [--filterThreshold FILTERTHRESHOLD]
                              [--threads THREADS]
                              [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                              [--version] [--tmp_dir TMP_DIR] [--tmp_dirKeep]
                              db inBams [inBams ...]
```

Positional arguments:

db Kraken database directory.

inBams Input unaligned reads, BAM format.

Options:

--outReports Kraken summary report output file. Multiple filenames space separated.

--outReads Kraken per read classification output file. Multiple filenames space separated.

--lockMemory=False Lock kraken database in RAM. Requires high ulimit -l.

--filterThreshold=0.05 Kraken filter threshold (default %(default)s)

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

krona

Create an interactive HTML report from a tabular metagenomic report

```
usage: metagenomics.py krona [-h] [--queryColumn QUERYCOLUMN]
                             [--taxidColumn TAXIDCOLUMN]
                             [--scoreColumn SCORECOLUMN]
                             [--magnitudeColumn MAGNITUDECOLUMN] [--noHits]
                             [--noRank]
                             [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                             [--version]
                             inTsv db outHtml
```

Positional arguments:

inTsv Input tab delimited file.

db Krona taxonomy database directory.

outHtml Output html report.

Options:

--queryColumn=2 Column of query id. (default %(default)s)

--taxidColumn=3 Column of taxonomy id. (default %(default)s)

--scoreColumn Column of score. (default %(default)s)

--magnitudeColumn Column of magnitude. (default %(default)s)

--noHits=False Include wedge for no hits.

--noRank=False Include no rank assignments.

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

diamond

Classify reads by the taxon of the Lowest Common Ancestor (LCA)

```
usage: metagenomics.py diamond [-h] [--outReads OUTREADS] [--threads THREADS]
                               [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                               [--version] [--tmp_dir TMP_DIR] [--tmp_dirKeep]
                               inBam db taxDb outReport
```

Positional arguments:

inBam Input unaligned reads, BAM format.

db Diamond database directory.

taxDb Taxonomy database directory.

outReport Output taxonomy report.

Options:

--outReads Output LCA assignments for each read.

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

diamond_fasta

Classify fasta sequences by the taxon of the Lowest Common Ancestor (LCA)

```
usage: metagenomics.py diamond_fasta [-h] [--memLimitGb MEMLIMITGB]
                                     [--threads THREADS]
                                     [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                     [--version] [--tmp_dir TMP_DIR]
                                     [--tmp_dirKeep]
                                     inFasta db taxDb outFasta
```

Positional arguments:

inFasta Input sequences, FASTA format, optionally gzip compressed.

db Diamond database file.

taxDb Taxonomy database directory.

outFasta Output sequences, same as inFasta, with taxid|###| prepended to each sequence identifier.

Options:

--memLimitGb approximate memory usage in GB

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

build_diamond_db

```
usage: metagenomics.py build_diamond_db [-h] [--threads THREADS]
                                        [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                        [--version] [--tmp_dir TMP_DIR]
                                        [--tmp_dirKeep]
                                        protein_fastas [protein_fastas ...] db
```

Positional arguments:

protein_fastas Input protein fasta files

db Output Diamond database file

Options:

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

align_rna

Align to metagenomics bwa index, mark duplicates, and generate LCA report

```
usage: metagenomics.py align_rna [-h] [--dupeReport DUPEREPORT] [--sensitive]
                                 [--outBam OUTBAM] [--outReads OUTREADS]
                                 [--dupeReads DUPEREADS]
                                 [--JVMmemory JVMMEMORY] [--threads THREADS]
                                 [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                 [--version] [--tmp_dir TMP_DIR]
                                 [--tmp_dirKeep]
                                 inBam db taxDb outReport
```

Positional arguments:

inBam Input unaligned reads, BAM format.

db Bwa index prefix.

taxDb Taxonomy database directory.

outReport Output taxonomy report.

Options:

--dupeReport Generate report including duplicates.

--sensitive=False Use sensitive instead of default BWA mem options.

--outBam Output aligned, indexed BAM file. Default is to write to temp.

--outReads Output LCA assignments for each read.

--dupeReads Output LCA assignments for each read including duplicates.

--JVMmemory=2g JVM virtual memory size (default: %(default)s)

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

report_merge

Merge multiple metegenomic reports into a single metagenomic report. Any Krona input files created by this

```
usage: metagenomics.py report_merge [-h]
                                    [--outSummaryReport OUT_KRAKEN_SUMMARY]
                                    [--krakenDB KRAKEN_DB]
                                    [--outByQueryToTaxonID OUT_KRONA_INPUT]
                                    [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                    [--version] [--tmp_dir TMP_DIR]
                                    [--tmp_dirKeep]
                                    metagenomic_reports
                                    [metagenomic_reports ...]
```

Positional arguments:

metagenomic_reports Input metagenomic reports with the query ID and taxon ID in the 2nd and 3rd columns (Kraken format)

Options:

--outSummaryReport Path of human-readable metagenomic summary report, created by kraken-report

--krakenDB Kraken database (needed for outSummaryReport)

--outByQueryToTaxonID Output metagenomic report suitable for Krona input.

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

taxlevel_summary

Aggregates taxonomic abundance data from multiple Kraken-format summary files. It is intended to report information on a particular taxonomic level (–taxlevelFocus; ex. 'species'), within a higher-level grouping (–taxHeading; ex. 'Viruses'). By default, when –taxHeading is at the same level as –taxlevelFocus a summary with lines for each sample is emitted. Otherwise, a histogram is returned. If per-sample information is desired, –noHist can be specified. In per-sample data, the suffix "-pt" indicates percentage, so a value of 0.02 is 0.0002 of the total number of reads for the sample. If –topN is specified, only the top N most abundant taxa are included in the histogram count or per-sample output. If a number is specified for –countThreshold, only taxa with that number of reads (or greater) are included. Full data returned via –jsonOut (filtered by –topN and –countThreshold), whereas -csvOut returns a summary.

```
usage: metagenomics.py taxlevel_summary [-h] [--jsonOut JSON_OUT]
                                        [--csvOut CSV_OUT]
                                        [--taxHeading TAX_HEADINGS [TAX_
↪HEADINGS ...]]
                                        [--taxlevelFocus
↪{species,genus,family,order,class,phylum,kingdom,superkingdom}]
                                        [--topN TOP_N_ENTRIES]
                                        [--countThreshold COUNT_THRESHOLD]
                                        [--zeroFill] [--noHist]
                                        [--includeRoot]
                                        [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                        [--version] [--tmp_dir TMP_DIR]
                                        [--tmp_dirKeep]
                                        summary_files_in
                                        [summary_files_in ...]
```

Positional arguments:

summary_files_in Kraken-format summary text file with tab-delimited taxonomic levels.

Options:

--jsonOut The path to a json file containing the relevant parsed summary data in json format.

--csvOut The path to a csv file containing sample-specific counts.

--taxHeading=Viruses The taxonomic heading to analyze (default: %(default)s). More than one can be specified.

--taxlevelFocus=species The taxonomic heading to summarize (totals by Genus, etc.) (default: %(default)s).

Possible choices: species, genus, family, order, class, phylum, kingdom, superkingdom

--topN=100 Only include the top N most abundant taxa by read count (default: %(default)s)

--countThreshold=1 Minimum number of reads to be included (default: %(default)s)

--zeroFill=False When absent from a sample, write zeroes (rather than leaving blank).

--noHist=False Write out a report by-sample rather than a histogram.

--includeRoot=False Include the count of reads at the root level and the unclassified bin.

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

kraken_build

Builds a kraken database from library directory of fastas and taxonomy db directory. The –subsetTaxonomy option allows shrinking the taxonomy to only include taxids associated with the library folders. For this to work, the library fastas must have the standard id names such as '>NC1234.1' accessions, '>gi|123456789|ref|XXXX||', or custom kraken name '>kraken:taxid|1234|'.

Setting the –minimizerLen (default: 16) small, such as 10, will drastically shrink the db size for small inputs, which is useful for testing.

The built db may include symlinks to the original –library / –taxonomy directories. If you want to build a static archiveable version of the library, simply use the –clean option, which will also remove any unnecessary files.

```
usage: metagenomics.py kraken_build [-h] [--library LIBRARY]
                                    [--taxonomy TAXONOMY] [--subsetTaxonomy]
                                    [--minimizerLen MINIMIZERLEN]
                                    [--kmerLen KMERLEN]
                                    [--maxDbSize MAXDBSIZE] [--clean]
                                    [--workOnDisk] [--threads THREADS]
                                    [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                    [--version] [--tmp_dir TMP_DIR]
                                    [--tmp_dirKeep]
                                    db
```

Positional arguments:

db Kraken database output directory.

Options:

--library Input library directory of fasta files. If not specified, it will be read from the "library" subdirectory of "db".

--taxonomy Taxonomy db directory. If not specified, it will be read from the "taxonomy" subdirectory of "db".

--subsetTaxonomy=False Subset taxonomy based on library fastas.

--minimizerLen Minimizer length (kraken default: 15)

--kmerLen k-mer length (kraken default: 31)

--maxDbSize Maximum db size in GB (will shrink if too big)

--clean=False Clean by deleting other database files after build

--workOnDisk=False Work on disk instead of RAM. This is generally much slower unless the "db" directory lives on a RAM disk.

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

taxon_filter.py - tools for taxonomic removal or filtration of reads

This script contains a number of utilities for filtering NGS reads based on membership or non-membership in a species / genus / taxonomic grouping.

```
usage: taxon_filter.py subcommand
```

Sub-commands:

deplete

Run the entire depletion pipeline: bwa, bmtagger, mvicuna, blastn.

```
usage: taxon_filter.py deplete [-h] [--bwaDbs [BWADBS [BWADBS ...]]]
                               [--bmtaggerDbs [BMTAGGERDBS [BMTAGGERDBS ...]]]
                               [--blastDbs [BLASTDBS [BLASTDBS ...]]]
                               [--srprismMemory SRPRISM_MEMORY]
                               [--chunkSize CHUNKSIZE] [--clearTags]
                               [--tagsToClear TAGS_TO_CLEAR [TAGS_TO_CLEAR ...
↪]]
                               [--JVMmemory JVMMEMORY] [--threads THREADS]
                               [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                               [--version] [--tmp_dir TMP_DIR] [--tmp_dirKeep]
                               inBam [revertBam] bwaBam bmtaggerBam rmdupBam
                               blastnBam
```

Positional arguments:

inBam Input BAM file.

revertBam Output BAM: read markup reverted with Picard.

bwaBam Output BAM: depleted of reads with BWA.

bmtaggerBam Output BAM: depleted of reads with BMTagger.

rmdupBam Output BAM: bmtaggerBam run through M-Vicuna duplicate removal.

blastnBam Output BAM: rmdupBam run through another depletion of reads with BLASTN.

Options:

--bwaDbs=() Reference databases for blast to deplete from input.

--bmtaggerDbs=() Reference databases to deplete from input. For each db, requires prior creation of db.bitmask by bmtool, and db.srprism.idx, db.srprism.map, etc. by srprism mkindex.

--blastDbs=() Reference databases for blast to deplete from input.

--srprismMemory=7168 Memory for srprism.

--chunkSize=1000000 blastn chunk size (default: %(default)s)

--clearTags=False When supplying an aligned input file, clear the per-read attribute tags

--tagsToClear=['XT', 'X0', 'X1', 'XA', 'AM', 'SM', 'BQ', 'CT', 'XN', 'OC', 'OP'] A space-separated list of tags to remove from all reads in the input bam file (default: %(default)s)

--JVMmemory=4g JVM virtual memory size for Picard FilterSamReads (default: %(default)s)

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

deplete_human

A wrapper around 'deplete'; deprecated but preserved for legacy compatibility.

```
usage: taxon_filter.py deplete_human [-h] [--bwaDbs [BWADBS [BWADBS ...]]]
                                     [--bmtaggerDbs [BMTAGGERDBS [BMTAGGERDBS␣
↪...]]]
                                     [--blastDbs [BLASTDBS [BLASTDBS ...]]]
                                     [--srprismMemory SRPRISM_MEMORY]
                                     [--chunkSize CHUNKSIZE] [--clearTags]
                                     [--tagsToClear TAGS_TO_CLEAR [TAGS_TO_
↪CLEAR ...]]
                                     [--JVMmemory JVMMEMORY]
                                     [--threads THREADS]
                                     [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                     [--version] [--tmp_dir TMP_DIR]
                                     [--tmp_dirKeep]
                                     inBam [revertBam] bwaBam bmtaggerBam
                                     rmdupBam blastnBam
```

Positional arguments:

inBam Input BAM file.

revertBam Output BAM: read markup reverted with Picard.

bwaBam Output BAM: depleted of reads with BWA.

bmtaggerBam Output BAM: depleted of reads with BMTagger.

rmdupBam Output BAM: bmtaggerBam run through M-Vicuna duplicate removal.

blastnBam Output BAM: rmdupBam run through another depletion of reads with BLASTN.

Options:

--bwaDbs=() Reference databases for blast to deplete from input.

--bmtaggerDbs=() Reference databases to deplete from input. For each db, requires prior creation of db.bitmask by bmtool, and db.srprism.idx, db.srprism.map, etc. by srprism mkindex.

--blastDbs=() Reference databases for blast to deplete from input.

--srprismMemory=7168 Memory for srprism.

--chunkSize=1000000 blastn chunk size (default: %(default)s)

--clearTags=False When supplying an aligned input file, clear the per-read attribute tags

--tagsToClear=['XT', 'X0', 'X1', 'XA', 'AM', 'SM', 'BQ', 'CT', 'XN', 'OC', 'OP'] A space-separated list of tags to remove from all reads in the input bam file (default: %(default)s)

--JVMmemory=4g JVM virtual memory size for Picard FilterSamReads (default: %(default)s)

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

filter_lastal_bam

Restrict input reads to those that align to the given reference database using LASTAL.

```
usage: taxon_filter.py filter_lastal_bam [-h]
                                         [-n MAX_GAPLESS_ALIGNMENTS_PER_
↪POSITION]
                                         [-l MIN_LENGTH_FOR_INITIAL_MATCHES]
                                         [-L MAX_LENGTH_FOR_INITIAL_MATCHES]
                                         [-m MAX_INITIAL_MATCHES_PER_POSITION]
                                         [--JVMmemory JVMMEMORY]
                                         [--threads THREADS]
                                         [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                         [--version] [--tmp_dir TMP_DIR]
                                         [--tmp_dirKeep]
                                         inBam db outBam
```

Positional arguments:

inBam Input reads

db Database of taxa we keep

outBam Output reads, filtered to refDb

Options:

-n=1 maximum gapless alignments per query position (default: %(default)s)

-l=5 minimum length for initial matches (default: %(default)s)

-L=50 maximum length for initial matches (default: %(default)s)

-m=100 maximum initial matches per query position (default: %(default)s)

--JVMmemory=4g JVM virtual memory size (default: %(default)s)

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

deplete_bam_bmtagger

Use bmtagger to deplete input reads against several databases.

```
usage: taxon_filter.py deplete_bam_bmtagger [-h]
                                            [--srprismMemory SRPRISM_MEMORY]
                                            [--JVMmemory JVMMEMORY]
                                            [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                            [--version] [--tmp_dir TMP_DIR]
                                            [--tmp_dirKeep]
                                            inBam refDbs [refDbs ...] outBam
```

Positional arguments:

inBam Input BAM file.

refDbs Reference databases (one or more) to deplete from input. For each db, requires prior creation of db.bitmask by bmtool, and db.srprism.idx, db.srprism.map, etc. by srprism mkindex.

outBam Output BAM file.

Options:

--srprismMemory=7168 Memory for srprism.

--JVMmemory=4g JVM virtual memory size (default: %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

deplete_blastn_bam

Use blastn to remove reads that match at least one of the specified databases.

```
usage: taxon_filter.py deplete_blastn_bam [-h] [--chunkSize CHUNKSIZE]
                                          [--JVMmemory JVMMEMORY]
                                          [--threads THREADS]
                                          [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                          [--version] [--tmp_dir TMP_DIR]
                                          [--tmp_dirKeep]
                                          inBam refDbs [refDbs ...] outBam
```

Positional arguments:

inBam Input BAM file.

refDbs One or more reference databases for blast. An ephemeral database will be created if a fasta file is provided.

outBam Output BAM file with matching reads removed.

Options:

--chunkSize=1000000 FASTA chunk size (default: %(default)s)

--JVMmemory=4g JVM virtual memory size (default: %(default)s)

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

deplete_bwa_bam

Use BWA to remove reads that match at least one of the specified databases.

```
usage: taxon_filter.py deplete_bwa_bam [-h] [--threads THREADS]
                                       [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                       [--version] [--tmp_dir TMP_DIR]
                                       [--tmp_dirKeep]
                                       inBam refDbs [refDbs ...] outBam
```

Positional arguments:

inBam Input BAM file.

refDbs One or more reference databases for bwa. An ephemeral database will be created if a fasta file is provided.

outBam Ouput BAM file with matching reads removed.

Options:

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

lastal_build_db

build a database for use with last based on an input fasta file

```
usage: taxon_filter.py lastal_build_db [-h]
                                       [--outputFilePrefix OUTPUTFILEPREFIX]
                                       [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                       [--version] [--tmp_dir TMP_DIR]
                                       [--tmp_dirKeep]
                                       inputFasta outputDirectory
```

Positional arguments:

inputFasta Location of the input FASTA file

outputDirectory Location for the output files (default is cwd: %(default)s)

Options:

--outputFilePrefix Prefix for the output file name (default: inputFasta name, sans ".fasta" extension)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

bwa_build_db

Create a database for use with bwa from an input reference FASTA file

```
usage: taxon_filter.py bwa_build_db [-h] [--outputFilePrefix OUTPUTFILEPREFIX]
                                    [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                    [--version] [--tmp_dir TMP_DIR]
                                    [--tmp_dirKeep]
                                    inputFasta outputDirectory
```

Positional arguments:

inputFasta Location of the input FASTA file

outputDirectory Location for the output files

Options:

--outputFilePrefix Prefix for the output file name (default: inputFasta name, sans ".fasta" extension)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

blastn_build_db

Create a database for use with blastn from an input reference FASTA file

```
usage: taxon_filter.py blastn_build_db [-h]
                                       [--outputFilePrefix OUTPUTFILEPREFIX]
                                       [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                       [--version] [--tmp_dir TMP_DIR]
                                       [--tmp_dirKeep]
                                       inputFasta outputDirectory
```

Positional arguments:

inputFasta Location of the input FASTA file

outputDirectory Location for the output files

Options:

--outputFilePrefix Prefix for the output file name (default: inputFasta name, sans ".fasta" extension)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

bmtagger_build_db

Create a database for use with Bmtagger from an input FASTA file.

```
usage: taxon_filter.py bmtagger_build_db [-h]
                                         [--outputFilePrefix OUTPUTFILEPREFIX]
                                         [--word_size WORD_SIZE]
                                         [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                         [--version] [--tmp_dir TMP_DIR]
```

```
                                        [--tmp_dirKeep]
                                        inputFasta outputDirectory
```

Positional arguments:

inputFasta	Location of the input FASTA file
outputDirectory	Location for the output files (Where *.bitmask and *.srprism files will be stored)

Options:

--outputFilePrefix	Prefix for the output file name (default: inputFasta name, sans ".fasta" extension)
--word_size=18	Database word size (default: %(default)s)
--loglevel=INFO	Verboseness of output. [default: %(default)s] Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit
--tmp_dir=/tmp	Base directory for temp files. [default: %(default)s]
--tmp_dirKeep=False	Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

assembly.py - *de novo* assembly

This script contains a number of utilities for viral sequence assembly from NGS reads. Primarily used for Lassa and Ebola virus analysis in the Sabeti Lab / Broad Institute Viral Genomics.

```
usage: assembly.py subcommand
```

Sub-commands:

trim_rmdup_subsamp

Take reads through Trimmomatic, Prinseq, and subsampling. This should probably move over to read_utils.

```
usage: assembly.py trim_rmdup_subsamp [-h] [--n_reads N_READS]
                                      [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                      [--version] [--tmp_dir TMP_DIR]
                                      [--tmp_dirKeep]
                                      inBam clipDb outBam
```

Positional arguments:

inBam	Input reads, unaligned BAM format.
clipDb	Trimmomatic clip DB.
outBam	Output reads, unaligned BAM format (currently, read groups and other header information are destroyed in this process).

Options:

--n_reads=100000 Subsample reads to no more than this many individual reads. Note that paired reads are given priority, and unpaired reads are included to reach the count if there are too few paired reads to reach n_reads. (default %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

assemble_trinity

This step runs the Trinity assembler. First trim reads with trimmomatic, rmdup with prinseq, and random subsample to no more than 100k reads.

```
usage: assembly.py assemble_trinity [-h] [--n_reads N_READS]
                                    [--outReads OUTREADS] [--always_succeed]
                                    [--JVMmemory JVMMEMORY]
                                    [--threads THREADS]
                                    [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                    [--version] [--tmp_dir TMP_DIR]
                                    [--tmp_dirKeep]
                                    inBam clipDb outFasta
```

Positional arguments:

inBam Input unaligned reads, BAM format.

clipDb Trimmomatic clip DB.

outFasta Output assembly.

Options:

--n_reads=100000 Subsample reads to no more than this many pairs. (default %(default)s)

--outReads Save the trimmomatic/prinseq/subsamp reads to a BAM file

--always_succeed=False If Trinity fails (usually because insufficient reads to assemble), emit an empty fasta file as output. Default is to throw a DenovoAssemblyError.

--JVMmemory=4g JVM virtual memory size (default: %(default)s)

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

assemble_spades

De novo RNA-seq assembly with the SPAdes assembler.

```
usage: assembly.py assemble_spades [-h] [--contigsTrusted CONTIGS_TRUSTED]
                                   [--contigsUntrusted CONTIGS_UNTRUSTED]
                                   [--nReads N_READS] [--outReads OUTREADS]
                                   [--filterContigs]
                                   [--spadesOpts SPADES_OPTS]
                                   [--memLimitGb MEM_LIMIT_GB]
                                   [--threads THREADS]
                                   [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                   [--version] [--tmp_dir TMP_DIR]
                                   [--tmp_dirKeep]
                                   in_bam clip_db out_fasta
```

Positional arguments:

in_bam Input unaligned reads, BAM format. May include both paired and unpaired reads.

clip_db Trimmomatic clip db

out_fasta Output assembled contigs. Note that, since this is RNA-seq assembly, for each assembled genomic region there may be several contigs representing different variants of that region.

Options:

--contigsTrusted Optional input contigs of high quality, previously assembled from the same sample

--contigsUntrusted Optional input contigs of high medium quality, previously assembled from the same sample

--nReads=10000000 Before assembly, subsample the reads to at most this many

--outReads Save the trimmomatic/prinseq/subsamp reads to a BAM file

--filterContigs=False only output contigs SPAdes is sure of (drop lesser-quality contigs from output)

--spadesOpts= (advanced) Extra flags to pass to the SPAdes assembler

--memLimitGb=4 Max memory to use, in GB (default: %(default)s)

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

gapfill_gap2seq

This step runs the Gap2Seq tool to close gaps between contigs in a scaffold.

```
usage: assembly.py gapfill_gap2seq [-h] [--memLimitGb MEM_LIMIT_GB]
                                   [--timeSoftLimitMinutes TIME_SOFT_LIMIT_
↪MINUTES]
                                   [--maskErrors] [--gap2seqOpts GAP2SEQ_OPTS]
                                   [--randomSeed RANDOM_SEED]
                                   [--threads THREADS]
                                   [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                   [--version] [--tmp_dir TMP_DIR]
                                   [--tmp_dirKeep]
                                  in_scaffold in_bam out_scaffold
```

Positional arguments:

in_scaffold	FASTA file containing the scaffold. Each FASTA record corresponds to one segment (for multi-segment genomes). Contigs within each segment are separated by Ns.
in_bam	Input unaligned reads, BAM format.
out_scaffold	Output assembly.

Options:

--memLimitGb=4.0	Max memory to use, in gigabytes %(default)s
--timeSoftLimitMinutes=60.0	Stop trying to close more gaps after this many minutes (default: %(default)s); this is a soft/advisory limit
--maskErrors=False	In case of any error, just copy in_scaffold to out_scaffold, emulating a successful run that simply could not fill any gaps.
--gap2seqOpts=	(advanced) Extra command-line options to pass to Gap2Seq
--randomSeed=0	Random seed; 0 means use current time
--threads	Number of threads (default: all available cores)
--loglevel=INFO	Verboseness of output. [default: %(default)s] Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit
--tmp_dir=/tmp	Base directory for temp files. [default: %(default)s]
--tmp_dirKeep=False	Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

order_and_orient

This step cleans up the de novo assembly with a known reference genome. Uses MUMmer (nucmer or promer) to create a reference-based consensus sequence of aligned contigs (with runs of N's in between the de novo contigs).

```
usage: assembly.py order_and_orient [-h]
                                    [--outAlternateContigs␣
↪OUTALTERNATECONTIGS]
                                    [--nGenomeSegments N_GENOME_SEGMENTS]
```

```
                                    [--outReference OUTREFERENCE]
                                    [--outStats OUTSTATS]
                                    [--breaklen BREAKLEN] [--maxgap MAXGAP]
                                    [--minmatch MINMATCH]
                                    [--mincluster MINCLUSTER]
                                    [--min_pct_id MIN_PCT_ID]
                                    [--min_contig_len MIN_CONTIG_LEN]
                                    [--min_pct_contig_aligned MIN_PCT_CONTIG_
↪ALIGNED]
                                    [--threads THREADS]
                                    [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                    [--version] [--tmp_dir TMP_DIR]
                                    [--tmp_dirKeep]
                                    inFasta inReference [inReference ...]
                                    outFasta
```

Positional arguments:

inFasta Input de novo assembly/contigs, FASTA format.

inReference Reference genome for ordering, orienting, and merging contigs, FASTA format. Multiple filenames may be listed, each containing one reference genome. Alternatively, multiple references may be given by specifying a single filename, and giving the number of reference segments with the nGenomeSegments parameter. If multiple references are given, they must all contain the same number of segments listed in the same order.

outFasta Output assembly, FASTA format, with the same number of chromosomes as inReference, and in the same order.

Options:

--outAlternateContigs Output sequences (FASTA format) from alternative contigs that mapped, but were not chosen for the final output.

--nGenomeSegments=0 Number of genome segments. If 0 (the default), the 'inReference' parameter is treated as one genome. If positive, the 'inReference' parameter is treated as a list of genomes of nGenomeSegments each.

--outReference Output the reference chosen for scaffolding to this file

--outStats Output stats used in reference selection

--breaklen, -b Amount to extend alignment clusters by (if –extend). nucmer default 200, promer default 60.

--maxgap=200, -g=200 Maximum gap between two adjacent matches in a cluster. Our default is %(default)s. nucmer default 90, promer default 30. Manual suggests going to 1000.

--minmatch=10, -l=10 Minimum length of an maximal exact match. Our default is %(default)s. nucmer default 20, promer default 6.

--mincluster, -c Minimum cluster length. nucmer default 65, promer default 20.

--min_pct_id=0.6, -i=0.6 show-tiling: minimum percent identity for contig alignment (0.0 - 1.0, default: %(default)s)

--min_contig_len=200 show-tiling: reject contigs smaller than this (default: %(default)s)

--min_pct_contig_aligned=0.3, -v=0.3 show-tiling: minimum percent of contig length in alignment (0.0 - 1.0, default: %(default)s)

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

impute_from_reference

This takes a de novo assembly, aligns against a reference genome, and imputes all missing positions (plus some of the chromosome ends) with the reference genome. This provides an assembly with the proper structure (but potentially wrong sequences in areas) from which we can perform further read-based refinement. Two steps: filter_short_seqs: We then toss out all assemblies that come out to < 15kb or < 95% unambiguous and fail otherwise. modify_contig: Finally, we trim off anything at the end that exceeds the length of the known reference assembly. We also replace all Ns and everything within 55bp of the chromosome ends with the reference sequence. This is clearly incorrect consensus sequence, but it allows downstream steps to map reads in parts of the genome that would otherwise be Ns, and we will correct all of the inferred positions with two steps of read-based refinement (below), and revert positions back to Ns where read support is lacking. FASTA indexing: output assembly is indexed for Picard, Samtools, Novoalign.

```
usage: assembly.py impute_from_reference [-h] [--newName NEWNAME]
                                         [--minLengthFraction␣
↪MINLENGTHFRACTION]
                                         [--minUnambig MINUNAMBIG]
                                         [--replaceLength REPLACELENGTH]
                                         [--aligner {muscle,mafft,mummer}]
                                         [--index]
                                         [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                         [--version] [--tmp_dir TMP_DIR]
                                         [--tmp_dirKeep]
                                        inFasta inReference outFasta
```

Positional arguments:

inFasta Input assembly/contigs, FASTA format, already ordered, oriented and merged with inReference.

inReference Reference genome to impute with, FASTA format.

outFasta Output assembly, FASTA format.

Options:

--newName rename output chromosome (default: do not rename)

--minLengthFraction=0.5 minimum length for contig, as fraction of reference (default: %(default)s)

--minUnambig=0.5 minimum percentage unambiguous bases for contig (default: %(default)s)

--replaceLength=0 length of ends to be replaced with reference (default: %(default)s)

--aligner=muscle which method to use to align inFasta to inReference. "muscle" = MUSCLE, "mafft" = MAFFT, "mummer" = nucmer. [default: %(default)s]

Possible choices: muscle, mafft, mummer

--index=False Index outFasta for Picard/GATK, Samtools, and Novoalign.

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

refine_assembly

This a refinement step where we take a crude assembly, align all reads back to it, and modify the assembly to the majority allele at each position based on read pileups. This step considers both SNPs as well as indels called by GATK and will correct the consensus based on GATK calls. Reads are aligned with Novoalign, then PCR duplicates are removed with Picard (in order to debias the allele counts in the pileups), and realigned with GATK's IndelRealigner (in order to call indels). Output FASTA file is indexed for Picard, Samtools, and Novoalign.

```
usage: assembly.py refine_assembly [-h]
                                   [--already_realigned_bam ALREADY_REALIGNED_
↪BAM]
                                   [--outBam OUTBAM] [--outVcf OUTVCF]
                                   [--min_coverage MIN_COVERAGE]
                                   [--major_cutoff MAJOR_CUTOFF]
                                   [--novo_params NOVO_PARAMS]
                                   [--chr_names [CHR_NAMES [CHR_NAMES ...]]]
                                   [--keep_all_reads] [--JVMmemory JVMMEMORY]
                                   [--GATK_PATH GATK_PATH]
                                   [--NOVOALIGN_LICENSE_PATH NOVOALIGN_
↪LICENSE_PATH]
                                   [--threads THREADS]
                                   [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                   [--version] [--tmp_dir TMP_DIR]
                                   [--tmp_dirKeep]
                                   inFasta inBam outFasta
```

Positional arguments:

inFasta Input assembly, FASTA format, pre-indexed for Picard, Samtools, and Novoalign.

inBam Input reads, unaligned BAM format.

outFasta Output refined assembly, FASTA format, indexed for Picard, Samtools, and Novoalign.

Options:

--already_realigned_bam BAM with reads that are already aligned to inFasta. This bypasses the alignment process by novoalign and instead uses the given BAM to make an assembly. When set, outBam is ignored.

--outBam Reads aligned to inFasta. Unaligned and duplicate reads have been removed. GATK indel realigned.

--outVcf GATK genotype calls for genome in inFasta coordinate space.

--min_coverage=3 Minimum read coverage required to call a position unambiguous.

--major_cutoff=0.5 If the major allele is present at a frequency higher than this cutoff, we will call an unambiguous base at that position. If it is equal to or below this cutoff, we will call an ambiguous base representing all possible alleles at that position. [default: %(default)s]

--novo_params=-r Random -l 40 -g 40 -x 20 -t 100 Alignment parameters for Novoalign.

--chr_names=[] Rename all output chromosomes (default: retain original chromosome names)

--keep_all_reads=False Retain all reads in BAM file? Default is to remove unaligned and duplicate reads.

--JVMmemory=2g JVM virtual memory size (default: %(default)s)

--GATK_PATH A path containing the GATK jar file. This overrides the GATK_ENV environment variable or the GATK conda package. (default: %(default)s)

--NOVOALIGN_LICENSE_PATH A path to the novoalign.lic file. This overrides the NOVOALIGN_LICENSE_PATH environment variable. (default: %(default)s)

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

filter_short_seqs

Check sequences in inFile, retaining only those that are at least minLength

```
usage: assembly.py filter_short_seqs [-h] [-f FORMAT] [-of OUTPUT_FORMAT]
                                     [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                     [--version]
                                     inFile minLength minUnambig outFile
```

Positional arguments:

inFile input sequence file

minLength minimum length for contig

minUnambig minimum percentage unambiguous bases for contig

outFile output file

Options:

-f=fasta, --format=fasta Format for input sequence (default: %(default)s)

-of=fasta, --output-format=fasta Format for output sequence (default: %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

modify_contig

Modifies an input contig. Depending on the options selected, can replace N calls with reference calls, replace ambiguous calls with reference calls, trim to the length of the reference, replace contig ends with reference calls, and trim leading and trailing Ns. Author: rsealfon.

```
usage: assembly.py modify_contig [-h] [-n NAME] [-cn] [-t] [-r5] [-r3]
                                 [-l REPLACE_LENGTH] [-f FORMAT] [-r] [-rn]
                                 [-ca] [--tmp_dir TMP_DIR] [--tmp_dirKeep]
                                 [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                 [--version]
                                 input output ref
```

Positional arguments:

input input alignment of reference and contig (should contain exactly 2 sequences)

output Destination file for modified contigs

ref reference sequence name (exact match required)

Options:

-n, --name fasta header output name (default: existing header)

-cn=False, --call-reference-ns=False should the reference sequence be called if there is an N in the contig and a more specific base in the reference (default: %(default)s)

-t=False, --trim-ends=False should ends of contig.fasta be trimmed to length of reference (default: %(default)s)

-r5=False, --replace-5ends=False should the 5'-end of contig.fasta be replaced by reference (default: %(default)s)

-r3=False, --replace-3ends=False should the 3'-end of contig.fasta be replaced by reference (default: %(default)s)

-l=10, --replace-length=10 length of ends to be replaced (if replace-ends is yes) (default: %(default)s)

-f=fasta, --format=fasta Format for input alignment (default: %(default)s)

-r=False, --replace-end-gaps=False Replace gaps at the beginning and end of the sequence with reference sequence (default: %(default)s)

-rn=False, --remove-end-ns=False Remove leading and trailing N's in the contig (default: %(default)s)

-ca=False, --call-reference-ambiguous=False should the reference sequence be called if the contig seq is ambiguous and the reference sequence is more informative & consistant with the ambiguous base (ie Y->C) (default: %(default)s)

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

vcf_to_fasta

Take input genotypes (VCF) and construct a consensus sequence (fasta) by using majority-read-count alleles in the VCF. Genotypes in the VCF will be ignored–we will use the allele with majority read support (or an ambiguity base if there is no clear majority). Uncalled positions will be emitted as N's. Author: dpark.

```
usage: assembly.py vcf_to_fasta [-h] [--trim_ends] [--min_coverage MIN_DP]
                                [--major_cutoff MAJOR_CUTOFF]
                                [--min_dp_ratio MIN_DP_RATIO]
                                [--name [NAME [NAME ...]]]
                                [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                [--version]
                                inVcf outFasta
```

Positional arguments:

inVcf Input VCF file

outFasta Output FASTA file

Options:

--trim_ends=False If specified, we will strip off continuous runs of N's from the beginning and end of the sequences before writing to output. Interior N's will not be changed.

--min_coverage=3 Specify minimum read coverage (with full agreement) to make a call. [default: %(default)s]

--major_cutoff=0.5 If the major allele is present at a frequency higher than this cutoff, we will call an unambiguous base at that position. If it is equal to or below this cutoff, we will call an ambiguous base representing all possible alleles at that position. [default: %(default)s]

--min_dp_ratio=0.0 The input VCF file often reports two read depth values (DP)–one for the position as a whole, and one for the sample in question. We can optionally reject calls in which the sample read count

	is below a specified fraction of the total read count. This filter will not apply to any sites unless both DP values are reported. [default: %(default)s]
--name=[]	output sequence names (default: reference names in VCF file)
--loglevel=INFO	Verboseness of output. [default: %(default)s]
	Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit

trim_fasta

Take input sequences (fasta) and trim any continuous sections of N's from the ends of them. Write trimmed sequences to an output fasta file.

```
usage: assembly.py trim_fasta [-h]
                              [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                              [--version]
                              inFasta outFasta
```

Positional arguments:

inFasta	Input fasta file
outFasta	Output (trimmed) fasta file

Options:

--loglevel=INFO	Verboseness of output. [default: %(default)s]
	Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit

deambig_fasta

Take input sequences (fasta) and replace any ambiguity bases with a random unambiguous base from among the possibilities described by the ambiguity code. Write output to fasta file.

```
usage: assembly.py deambig_fasta [-h]
                                 [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                 [--version]
                                 inFasta outFasta
```

Positional arguments:

inFasta	Input fasta file
outFasta	Output fasta file

Options:

--loglevel=INFO	Verboseness of output. [default: %(default)s]
	Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit

interhost.py - species and population-level genetic variation

This script contains a number of utilities for SNP calling, multi-alignment, phylogenetics, etc.

```
usage: interhost.py subcommand
```

Sub-commands:

snpEff

Annotate variants in VCF file with translation consequences using snpEff.

```
usage: interhost.py snpEff [-h] [--tmp_dir TMP_DIR] [--tmp_dirKeep]
                           [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                           [--version]
                           inVcf genomes [genomes ...] outVcf emailAddress
```

Positional arguments:

inVcf	Input VCF file
genomes	genome name (snpEff db name, or NCBI accessions)
outVcf	Output VCF file
emailAddress	Your email address. To access the Genbank CoreNucleotide database, NCBI requires you to specify your email address with each request. In case of excessive usage of the E-utilities, NCBI will attempt to contact a user at the email address provided before blocking access.

Options:

--tmp_dir=/tmp	Base directory for temp files. [default: %(default)s]
--tmp_dirKeep=False	Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.
--loglevel=INFO	Verboseness of output. [default: %(default)s] Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit

align_mafft

Run the mafft alignment on the input FASTA file.

```
usage: interhost.py align_mafft [-h] [--localpair | --globalpair]
                                [--preservecase] [--reorder]
                                [--gapOpeningPenalty GAPOPENINGPENALTY]
                                [--ep EP] [--verbose] [--outputAsClustal]
                                [--maxiters MAXITERS] [--threads THREADS]
                                [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                [--version] [--tmp_dir TMP_DIR]
                                [--tmp_dirKeep]
                                inFastas [inFastas ...] outFile
```

Positional arguments:

inFastas Input FASTA files.

outFile Output file containing alignment result (default format: FASTA)

Options:

--localpair All pairwise alignments are computed with the Smith-Waterman algorithm.

--globalpair All pairwise alignments are computed with the Needleman-Wunsch algorithm.

--preservecase Preserve base or aa case, as well as symbols.

--reorder Output is ordered aligned rather than in the order of the input (default: %(default)s).

--gapOpeningPenalty=1.53 Gap opening penalty (default: %(default)s).

--ep Offset (works like gap extension penalty).

--verbose=False Full output (default: %(default)s).

--outputAsClustal Write output file in Clustal format rather than FASTA

--maxiters=0 Maximum number of refinement iterations (default: %(default)s). Note: if "–localpair" or "–globalpair" is specified this defaults to 1000.

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

multichr_mafft

Run the mafft alignment on a series of chromosomes provided in sample-partitioned FASTA files. Output as FASTA. (i.e. file1.fasta would contain chr1, chr2, chr3; file2.fasta would also contain chr1, chr2, chr3)

```
usage: interhost.py multichr_mafft [-h] [--localpair | --globalpair]
                                   [--preservecase] [--reorder]
                                   [--gapOpeningPenalty GAPOPENINGPENALTY]
                                   [--ep EP] [--verbose] [--outputAsClustal]
                                   [--maxiters MAXITERS]
                                   [--outFilePrefix OUTFILEPREFIX]
                                   [--sampleRelationFile SAMPLERELATIONFILE]
                                   [--sampleNameListFile SAMPLENAMELISTFILE]
                                   [--threads THREADS]
                                   [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                   [--version] [--tmp_dir TMP_DIR]
                                   [--tmp_dirKeep]
                                   inFastas [inFastas ...] outDirectory
```

Positional arguments:

inFastas Input FASTA files.

outDirectory Location for the output files (default is cwd: %(default)s)

Options:

--localpair All pairwise alignments are computed with the Smith-Waterman algorithm.

--globalpair All pairwise alignments are computed with the Needleman-Wunsch algorithm.

--preservecase Preserve base or aa case, as well as symbols.

--reorder Output is ordered aligned rather than in the order of the input (default: %(default)s).

--gapOpeningPenalty=1.53 Gap opening penalty (default: %(default)s).

--ep Offset (works like gap extension penalty).

--verbose=False Full output (default: %(default)s).

--outputAsClustal Write output file in Clustal format rather than FASTA

--maxiters=0 Maximum number of refinement iterations (default: %(default)s). Note: if "–localpair" or "–globalpair" is specified this defaults to 1000.

--outFilePrefix=aligned Prefix for the output file name (default: %(default)s)

--sampleRelationFile If the parameter sampleRelationFile is specified (as a file path), a JSON file will be written mapping sample name to sequence position in the output.

--sampleNameListFile If the parameter sampleRelationFile is specified (as a file path), a file will be written mapping sample names in the order of their sequence positions in the output.

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

intrahost.py - within-host genetic variation (iSNVs)

This script contains a number of utilities for intrahost variant calling and annotation for viral genomes.

```
usage: intrahost.py subcommand
```

Sub-commands:

vphaser_one_sample

Input: a single BAM file, representing reads from one sample, mapped to its own consensus assembly. It may contain multiple read groups and libraries. Output: a tab-separated file with no header containing filtered V Phaser-2 output variants with additional column for sequence/chrom name, and library counts and p-values appended to the counts for each allele.

```
usage: intrahost.py vphaser_one_sample [-h]
                                       [--vphaserNumThreads VPHASERNUMTHREADS]
                                       [--minReadsEach MINREADSEACH]
                                       [--maxBias MAXBIAS]
                                       [--removeDoublyMappedReads]
                                       [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                       [--version]
                                       inBam inConsFasta outTab
```

Positional arguments:

inBam	Input Bam file.
inConsFasta	Consensus assembly fasta.
outTab	Tab-separated headerless output file.

Options:

--vphaserNumThreads	Number of threads in call to V-Phaser 2.
--minReadsEach=5	Minimum number of reads on each strand (default: %(default)s).
--maxBias=10	Maximum allowable ratio of number of reads on the two strands (default: %(default)s). Ignored if minReadsEach = 0.
--removeDoublyMappedReads=False	When calling V-Phaser, remove reads mapping to more than one contig. Default is to keep the reads.
--loglevel=INFO	Verboseness of output. [default: %(default)s] Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit

vphaser

Run V-Phaser 2 on the input file without any additional filtering. Combine the non-header lines of the CHROM.var.raw.txt files it produces, adding CHROM as the first field on each line.

```
usage: intrahost.py vphaser [-h] [--numThreads NUMTHREADS]
                            [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                            [--version]
                            inBam outTab
```

Positional arguments:

inBam	Input Bam file.
outTab	Tab-separated headerless output file.

Options:

--numThreads	Number of threads in call to V-Phaser 2.

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

tabfile_rename

Take input tab file and copy to an output file while changing the values in a specific column based on a mapping file. The first line will pass through untouched (it is assumed to be a header).

```
usage: intrahost.py tabfile_rename [-h] [--col_idx COL]
                                   [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                   [--version]
                                   inFile mapFile outFile
```

Positional arguments:

inFile Input flat file

mapFile Map file. Two-column headerless file that maps input values to output values. This script will error if there are values in inFile that do not exist in mapFile.

outFile Output flat file

Options:

--col_idx=0 Which column number to replace (0-based index). [default: %(default)s]

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

merge_to_vcf

Combine and convert vPhaser2 parsed filtered output text files into VCF format. Assumption: consensus assemblies used in creating alignments do not extend beyond ends of reference. the number of alignment files equals the number of chromosomes / segments

```
usage: intrahost.py merge_to_vcf [-h] --samples SAMPLES [SAMPLES ...] --isnvs
                                 ISNVS [ISNVS ...] --alignments ALIGNMENTS
                                 [ALIGNMENTS ...] [--strip_chr_version]
                                 [--naive_filter] [--parse_accession]
                                 [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                 [--version]
                                 refFasta outVcf
```

Positional arguments:

refFasta The target reference genome. outVcf will use these chromosome names, coordinate spaces, and reference alleles

outVcf Output VCF file containing all variants

Options:

--samples A list of sample names

--isnvs A list of file names from the output of vphaser_one_sample These must be in the SAME ORDER as samples.

--alignments a list of fasta files containing multialignment of input assemblies, with one file per chromosome/segment. Each alignment file will contain a line for each sample, as well as the reference genome to which they were aligned.

--strip_chr_version=False If set, strip any trailing version numbers from the chromosome names. If the chromosome name ends with a period followed by integers, this is interepreted as a version number to be removed. This is because Genbank accession numbers are often used by SnpEff databases downstream, but without the corresponding version number. Default is false (leave chromosome names untouched).

--naive_filter=False If set, keep only the alleles that have at least two independent libraries of support and allele freq > 0.005. Default is false (do not filter at this stage).

--parse_accession=False If set, parse only the accession for the chromosome name. Helpful if snpEff has to create its own database

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

Fws

Compute the Fws statistic on iSNV data. See Manske, 2012 (Nature)

```
usage: intrahost.py Fws [-h]
                        [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                        [--version]
                        inVcf outVcf
```

Positional arguments:

inVcf Input VCF file

outVcf Output VCF file

Options:

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

iSNV_table

Convert VCF iSNV data to tabular text

```
usage: intrahost.py iSNV_table [-h]
                               [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
```

```
                                    [--version]
                                    inVcf outFile
```

Positional arguments:

inVcf	Input VCF file
outFile	Output text file

Options:

--loglevel=INFO	Verboseness of output. [default: %(default)s]
	Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit

iSNP_per_patient

Aggregate tabular iSNP data per patient x position (all time points averaged)

```
usage: intrahost.py iSNP_per_patient [-h]
                                     [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                     [--version]
                                     inFile outFile
```

Positional arguments:

inFile	Input text file
outFile	Output text file

Options:

--loglevel=INFO	Verboseness of output. [default: %(default)s]
	Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit

read_utils.py - utilities that manipulate bam and fastq files

Utilities for working with sequence reads, such as converting formats and fixing mate pairs.

```
usage: read_utils.py subcommand
```

Sub-commands:

purge_unmated

Use mergeShuffledFastqSeqs to purge unmated reads, and put corresponding reads in the same order. Corresponding sequences must have sequence identifiers of the form SEQID/1 and SEQID/2.

```
usage: read_utils.py purge_unmated [-h] [--regex REGEX]
                                   [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                   [--version] [--tmp_dir TMP_DIR]
                                   [--tmp_dirKeep]
                                   inFastq1 inFastq2 outFastq1 outFastq2
```

Positional arguments:

inFastq1 Input fastq file; 1st end of paired-end reads.

inFastq2 Input fastq file; 2nd end of paired-end reads.

outFastq1 Output fastq file; 1st end of paired-end reads.

outFastq2 Output fastq file; 2nd end of paired-end reads.

Options:

--regex=^@(\S+)/[1|2]$ Perl regular expression to parse paired read IDs (default: %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

index_fasta_samtools

Index a reference genome for Samtools.

```
usage: read_utils.py index_fasta_samtools [-h]
                                          [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                          [--version]
                                          inFasta
```

Positional arguments:

inFasta Reference genome, FASTA format.

Options:

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

index_fasta_picard

Create an index file for a reference genome suitable for Picard/GATK.

```
usage: read_utils.py index_fasta_picard [-h] [--JVMmemory JVMMEMORY]
                                        [--picardOptions [PICARDOPTIONS␣
↪[PICARDOPTIONS ...]]]
                                        [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                        [--version] [--tmp_dir TMP_DIR]
                                        [--tmp_dirKeep]
                                        inFasta
```

Positional arguments:

inFasta Input reference genome, FASTA format.

Options:

--JVMmemory=512m JVM virtual memory size (default: %(default)s)

--picardOptions=[] Optional arguments to Picard's CreateSequenceDictionary, OPTIONNAME=value ...

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

mkdup_picard

Mark or remove duplicate reads from BAM file.

```
usage: read_utils.py mkdup_picard [-h] [--outMetrics OUTMETRICS] [--remove]
                                  [--JVMmemory JVMMEMORY]
                                  [--picardOptions [PICARDOPTIONS␣
↪[PICARDOPTIONS ...]]]
                                  [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                  [--version] [--tmp_dir TMP_DIR]
                                  [--tmp_dirKeep]
                                  inBams [inBams ...] outBam
```

Positional arguments:

inBams Input reads, BAM format.

outBam Output reads, BAM format.

Options:

--outMetrics Output metrics file. Default is to dump to a temp file.

--remove=False Instead of marking duplicates, remove them entirely (default: %(default)s)

--JVMmemory=2g JVM virtual memory size (default: %(default)s)

--picardOptions=[] Optional arguments to Picard's MarkDuplicates, OPTIONNAME=value ...

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

revert_bam_picard

Revert BAM to raw reads

```
usage: read_utils.py revert_bam_picard [-h] [--JVMmemory JVMMEMORY]
                                       [--picardOptions [PICARDOPTIONS␣
↪[PICARDOPTIONS ...]]]
                                       [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                       [--version] [--tmp_dir TMP_DIR]
                                       [--tmp_dirKeep]
                                       inBam outBam
```

Positional arguments:

inBam	Input reads, BAM format.
outBam	Output reads, BAM format.

Options:

--JVMmemory=2g	JVM virtual memory size (default: %(default)s)
--picardOptions=[]	Optional arguments to Picard's RevertSam, OPTIONNAME=value ...
--loglevel=INFO	Verboseness of output. [default: %(default)s] Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit
--tmp_dir=/tmp	Base directory for temp files. [default: %(default)s]
--tmp_dirKeep=False	Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

picard

Generic Picard runner.

```
usage: read_utils.py picard [-h] [--JVMmemory JVMMEMORY]
                            [--picardOptions [PICARDOPTIONS [PICARDOPTIONS ...
↪]]]
                            [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                            [--version] [--tmp_dir TMP_DIR] [--tmp_dirKeep]
                            command
```

Positional arguments:

command	picard command

Options:

--JVMmemory=2g	JVM virtual memory size (default: %(default)s)
--picardOptions=[]	Optional arguments to Picard, OPTIONNAME=value ...
--loglevel=INFO	Verboseness of output. [default: %(default)s] Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit
--tmp_dir=/tmp	Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

sort_bam

Sort BAM file

```
usage: read_utils.py sort_bam [-h] [--index] [--md5] [--JVMmemory JVMMEMORY]
                              [--picardOptions [PICARDOPTIONS [PICARDOPTIONS .
↪..]]]
                              [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                              [--version] [--tmp_dir TMP_DIR] [--tmp_dirKeep]
                              inBam outBam {unsorted,queryname,coordinate}
```

Positional arguments:

inBam Input bam file.

outBam Output bam file, sorted.

sortOrder How to sort the reads. [default: %(default)s]

Possible choices: unsorted, queryname, coordinate

Options:

--index=False Index outBam (default: %(default)s)

--md5=False MD5 checksum outBam (default: %(default)s)

--JVMmemory=2g JVM virtual memory size (default: %(default)s)

--picardOptions=[] Optional arguments to Picard's SortSam, OPTIONNAME=value ...

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

merge_bams

Merge multiple BAMs into one

```
usage: read_utils.py merge_bams [-h] [--JVMmemory JVMMEMORY]
                                [--picardOptions [PICARDOPTIONS␣
↪[PICARDOPTIONS ...]]]
                                [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                [--version] [--tmp_dir TMP_DIR]
                                [--tmp_dirKeep]
                                inBams [inBams ...] outBam
```

Positional arguments:

inBams Input bam files.

outBam Output bam file.

Options:

--JVMmemory=2g JVM virtual memory size (default: %(default)s)

--picardOptions=[] Optional arguments to Picard's MergeSamFiles, OPTION-NAME=value ...

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

filter_bam

Filter BAM file by read name

```
usage: read_utils.py filter_bam [-h] [--exclude] [--JVMmemory JVMMEMORY]
                                [--picardOptions [PICARDOPTIONS␣
↪[PICARDOPTIONS ...]]]
                                [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                [--version] [--tmp_dir TMP_DIR]
                                [--tmp_dirKeep]
                                inBam readList outBam
```

Positional arguments:

inBam Input bam file.

readList Input file of read IDs.

outBam Output bam file.

Options:

--exclude=False If specified, readList is a list of reads to remove from input. Default behavior is to treat readList as an inclusion list (all unnamed reads are removed).

--JVMmemory=4g JVM virtual memory size (default: %(default)s)

--picardOptions=[] Optional arguments to Picard's FilterSamReads, OPTION-NAME=value ...

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

fastq_to_bam

Convert a pair of fastq paired-end read files and optional text header to a single bam file.

```
usage: read_utils.py fastq_to_bam [-h]
                                  (--sampleName SAMPLENAME | --header HEADER)
                                  [--JVMmemory JVMMEMORY]
                                  [--picardOptions [PICARDOPTIONS␣
↪[PICARDOPTIONS ...]]]
                                  [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                  [--version] [--tmp_dir TMP_DIR]
                                  [--tmp_dirKeep]
                                  inFastq1 inFastq2 outBam
```

Positional arguments:

inFastq1	Input fastq file; 1st end of paired-end reads.
inFastq2	Input fastq file; 2nd end of paired-end reads.
outBam	Output bam file.

Options:

--sampleName	Sample name to insert into the read group header.
--header	Optional text file containing header.
--JVMmemory=2g	JVM virtual memory size (default: %(default)s)
--picardOptions=[]	Optional arguments to Picard's FastqToSam, OPTION-NAME=value ... Note that header-related options will be overwritten by HEADER if present.
--loglevel=INFO	Verboseness of output. [default: %(default)s] Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit
--tmp_dir=/tmp	Base directory for temp files. [default: %(default)s]
--tmp_dirKeep=False	Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

join_paired_fastq

Join paired fastq reads into single reads with Ns

```
usage: read_utils.py join_paired_fastq [-h] [--outFormat OUTFORMAT]
                                       [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                       [--version] [--tmp_dir TMP_DIR]
                                       [--tmp_dirKeep]
                                       output inFastqs [inFastqs ...]
```

Positional arguments:

output	Output file.
inFastqs	Input fastq file (2 if paired, 1 if interleaved)

Options:

--outFormat=fastq	Output file format.

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

split_bam

Split BAM file equally into several output BAM files.

```
usage: read_utils.py split_bam [-h]
                               [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                               [--version] [--tmp_dir TMP_DIR] [--tmp_dirKeep]
                               inBam outBams [outBams ...]
```

Positional arguments:

inBam Input BAM file.

outBams Output BAM files

Options:

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

reheader_bam

Copy a BAM file (inBam to outBam) while renaming elements of the BAM header. The mapping file specifies which (key, old value, new value) mappings. For example: LB lib1 lib_one SM sample1 Sample_1 SM sample2 Sample_2 SM sample3 Sample_3 CN broad BI

```
usage: read_utils.py reheader_bam [-h]
                                  [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                  [--version] [--tmp_dir TMP_DIR]
                                  [--tmp_dirKeep]
                                  inBam rgMap outBam
```

Positional arguments:

inBam Input reads, BAM format.

rgMap Tabular file containing three columns: field, old, new.

outBam Output reads, BAM format.

Options:

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

reheader_bams

Copy BAM files while renaming elements of the BAM header. The mapping file specifies which (key, old value, new value) mappings. For example: LB lib1 lib_one SM sample1 Sample_1 SM sample2 Sample_2 SM sample3 Sample_3 CN broad BI FN in1.bam out1.bam FN in2.bam out2.bam

```
usage: read_utils.py reheader_bams [-h]
                                   [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                   [--version] [--tmp_dir TMP_DIR]
                                   [--tmp_dirKeep]
                                   rgMap
```

Positional arguments:

rgMap Tabular file containing three columns: field, old, new.

Options:

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

rmdup_cdhit_bam

Remove duplicate reads from BAM file using cd-hit-dup.

```
usage: read_utils.py rmdup_cdhit_bam [-h] [--JVMmemory JVM_MEMORY]
                                     [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                     [--version] [--tmp_dir TMP_DIR]
                                     [--tmp_dirKeep]
                                     inBam outBam
```

Positional arguments:

inBam Input reads, BAM format.

outBam Output reads, BAM format.

Options:

--JVMmemory=4g JVM virtual memory size (default: %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

rmdup_mvicuna_bam

Remove duplicate reads from BAM file using M-Vicuna. The primary advantage to this approach over Picard's MarkDuplicates tool is that Picard requires that input reads are aligned to a reference, and M-Vicuna can operate on unaligned reads.

```
usage: read_utils.py rmdup_mvicuna_bam [-h] [--JVMmemory JVMMEMORY]
                                       [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                       [--version] [--tmp_dir TMP_DIR]
                                       [--tmp_dirKeep]
                                       inBam outBam
```

Positional arguments:

inBam Input reads, BAM format.

outBam Output reads, BAM format.

Options:

--JVMmemory=4g JVM virtual memory size (default: %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

rmdup_prinseq_fastq

Run prinseq-lite's duplicate removal operation on paired-end reads. Also removes reads with more than one N.

```
usage: read_utils.py rmdup_prinseq_fastq [-h] [--includeUnmated]
                                         [--unpairedOutFastq1␣
↪UNPAIREDOUTFASTQ1]
                                         [--unpairedOutFastq2␣
↪UNPAIREDOUTFASTQ2]
                                         [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                         [--version] [--tmp_dir TMP_DIR]
                                         [--tmp_dirKeep]
                                         inFastq1 inFastq2 outFastq1 outFastq2
```

Positional arguments:

inFastq1 Input fastq file; 1st end of paired-end reads.

inFastq2 Input fastq file; 2nd end of paired-end reads.

outFastq1 Output fastq file; 1st end of paired-end reads.

outFastq2 Output fastq file; 2nd end of paired-end reads.

Options:

--includeUnmated=False Include unmated reads in the main output fastq files (default: %(default)s)

--unpairedOutFastq1 File name of output unpaired reads from 1st end of paired-end reads (independent of –includeUnmated)

--unpairedOutFastq2 File name of output unpaired reads from 2nd end of paired-end reads (independent of –includeUnmated)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

filter_bam_mapped_only

Samtools to reduce a BAM file to only reads that are aligned (-F 4) with a non-zero mapping quality (-q 1) and are not marked as a PCR/optical duplicate (-F 1024).

```
usage: read_utils.py filter_bam_mapped_only [-h]
                                            [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                            [--version] [--tmp_dir TMP_DIR]
                                            [--tmp_dirKeep]
                                            inBam outBam
```

Positional arguments:

inBam Input aligned reads, BAM format.

outBam Output sorted indexed reads, filtered to aligned-only, BAM format.

Options:

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

novoalign

Align reads with Novoalign. Sort and index BAM output.

```
usage: read_utils.py novoalign [-h] [--options OPTIONS] [--min_qual MIN_QUAL]
                               [--JVMmemory JVMMEMORY]
                               [--NOVOALIGN_LICENSE_PATH NOVOALIGN_LICENSE_
↪PATH]
                               [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                               [--version] [--tmp_dir TMP_DIR] [--tmp_dirKeep]
                               inBam refFasta outBam
```

Positional arguments:

inBam Input reads, BAM format.

refFasta Reference genome, FASTA format, pre-indexed by Novoindex.

outBam Output reads, BAM format (aligned).

Options:

--options=-r Random Novoalign options (default: %(default)s)

--min_qual=0 Filter outBam to minimum mapping quality (default: %(default)s)

--JVMmemory=2g JVM virtual memory size (default: %(default)s)

--NOVOALIGN_LICENSE_PATH A path to the novoalign.lic file. This overrides the NOVOALIGN_LICENSE_PATH environment variable. (default: %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

novoindex

```
usage: read_utils.py novoindex [-h]
                               [--NOVOALIGN_LICENSE_PATH NOVOALIGN_LICENSE_
↪PATH]
                               [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                               [--version]
                               refFasta
```

Positional arguments:

refFasta Reference genome, FASTA format.

Options:

--NOVOALIGN_LICENSE_PATH A path to the novoalign.lic file. This overrides the NOVOALIGN_LICENSE_PATH environment variable. (default: %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

gatk_ug

Call genotypes using the GATK UnifiedGenotyper.

```
usage: read_utils.py gatk_ug [-h] [--options OPTIONS] [--JVMmemory JVMMEMORY]
                             [--GATK_PATH GATK_PATH]
                             [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                             [--version] [--tmp_dir TMP_DIR] [--tmp_dirKeep]
                             inBam refFasta outVcf
```

Positional arguments:

inBam Input reads, BAM format.

refFasta Reference genome, FASTA format, pre-indexed by Picard.

outVcf Output calls in VCF format. If this filename ends with .gz, GATK will BGZIP compress the output and produce a Tabix index file as well.

Options:

--options=–min_base_quality_score 15 -ploidy 4 UnifiedGenotyper options (default: %(default)s)

--JVMmemory=2g JVM virtual memory size (default: %(default)s)

--GATK_PATH A path containing the GATK jar file. This overrides the GATK_ENV environment variable or the GATK conda package. (default: %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

gatk_realign

Local realignment of BAM files with GATK IndelRealigner.

```
usage: read_utils.py gatk_realign [-h] [--JVMmemory JVMMEMORY]
                                  [--GATK_PATH GATK_PATH]
                                  [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                  [--version] [--tmp_dir TMP_DIR]
                                  [--tmp_dirKeep] [--threads THREADS]
                                  inBam refFasta outBam
```

Positional arguments:

inBam Input reads, BAM format, aligned to refFasta.

refFasta Reference genome, FASTA format, pre-indexed by Picard.

outBam Realigned reads.

Options:

--JVMmemory=2g JVM virtual memory size (default: %(default)s)

--GATK_PATH A path containing the GATK jar file. This overrides the GATK_ENV environment variable or the GATK conda package. (default: %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

--threads Number of threads (default: all available cores)

align_and_fix

Take reads, align to reference with Novoalign, optionally mark duplicates with Picard, realign indels with GATK, and optionally filters final file to mapped/non-dupe reads.

```
usage: read_utils.py align_and_fix [-h] [--outBamAll OUTBAMALL]
                                   [--outBamFiltered OUTBAMFILTERED]
                                   [--aligner_options ALIGNER_OPTIONS]
                                   [--aligner {novoalign,bwa}]
                                   [--JVMmemory JVMMEMORY] [--threads THREADS]
                                   [--skipMarkDupes] [--GATK_PATH GATK_PATH]
                                   [--NOVOALIGN_LICENSE_PATH NOVOALIGN_
↪LICENSE_PATH]
                                   [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                   [--version] [--tmp_dir TMP_DIR]
                                   [--tmp_dirKeep]
                                   inBam refFasta
```

Positional arguments:

inBam Input unaligned reads, BAM format.

refFasta Reference genome, FASTA format; will be indexed by Picard and Novoalign.

Options:

--outBamAll Aligned, sorted, and indexed reads. Unmapped and duplicate reads are retained. By default, duplicate reads are marked. If "–skipMarkDupes" is specified duplicate reads are included in outout without being marked.

--outBamFiltered Aligned, sorted, and indexed reads. Unmapped reads are removed from this file, as well as any marked duplicate reads. Note

that if "–skipMarkDupes" is provided, duplicates will be not be marked and will be included in the output.

--aligner_options aligner options (default for novoalign: "-r Random", bwa: "-T 30"

--aligner=novoalign aligner (default: %(default)s)

Possible choices: novoalign, bwa

--JVMmemory=4g JVM virtual memory size (default: %(default)s)

--threads Number of threads (default: all available cores)

--skipMarkDupes=False If specified, duplicate reads will not be marked in the resulting output file.

--GATK_PATH A path containing the GATK jar file. This overrides the GATK_ENV environment variable or the GATK conda package. (default: %(default)s)

--NOVOALIGN_LICENSE_PATH A path to the novoalign.lic file. This overrides the NOVOALIGN_LICENSE_PATH environment variable. (default: %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

bwamem_idxstats

Take reads, align to reference with BWA-MEM and perform samtools idxstats.

```
usage: read_utils.py bwamem_idxstats [-h] [--outBam OUTBAM]
                                     [--outStats OUTSTATS]
                                     [--minScoreToFilter MIN_SCORE_TO_FILTER]
                                     [--alignerOptions ALIGNER_OPTIONS]
                                     [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                     [--version] [--tmp_dir TMP_DIR]
                                     [--tmp_dirKeep]
                                     inBam refFasta
```

Positional arguments:

inBam Input unaligned reads, BAM format.

refFasta Reference genome, FASTA format, pre-indexed by Picard and Novoalign.

Options:

--outBam Output aligned, indexed BAM file

--outStats Output idxstats file

--minScoreToFilter Filter bwa alignments using this value as the minimum allowed alignment score. Specifically, sum the alignment scores across all alignments for each query (including reads in a pair, supplementary and secondary alignments) and then only include, in the output, queries whose summed alignment score is at least this value. This is only applied when the aligner is 'bwa'. The filtering on a summed alignment score is sensible for reads in a pair and supplementary alignments, but may not be reasonable if bwa outputs secondary alignments (i.e., if '-a' is in the aligner options). (default: not set - i.e., do not filter bwa's output)

--alignerOptions bwa options (default: bwa defaults)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

extract_tarball

Extract an input .tar, .tgz, .tar.gz, .tar.bz2, .tar.lz4, or .zip file to a given directory (or we will choose one on our own). Emit the resulting directory path to stdout.

```
usage: read_utils.py extract_tarball [-h]
                                     [--compression {gz,bz2,lz4,zip,none,auto}
↪]
                                     [--pipe_hint PIPE_HINT]
                                     [--threads THREADS]
                                     [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                     [--version] [--tmp_dir TMP_DIR]
                                     [--tmp_dirKeep]
                                     tarfile out_dir
```

Positional arguments:

tarfile Input tar file. May be "-" for stdin.

out_dir Output directory

Options:

--compression=auto Compression type (default: %(default)s). Auto-detect is incompatible with stdin input unless pipe_hint is specified.

Possible choices: gz, bz2, lz4, zip, none, auto

--pipe_hint If tarfile is stdin, you can provide a file-like URI string for pipe_hint which ends with a common compression file extension if you want to use compression=auto.

--threads Number of threads (default: all available cores)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

reports.py - produce various metrics and reports

Functions to create reports from genomics pipeline data.

```
usage: reports.py subcommand
```

Sub-commands:

assembly_stats

Fetch assembly-level statistics for a given sample

```
usage: reports.py assembly_stats [-h]
                                 [--cov_thresholds COV_THRESHOLDS [COV_
↪THRESHOLDS ...]]
                                 [--assembly_dir ASSEMBLY_DIR]
                                 [--assembly_tmp ASSEMBLY_TMP]
                                 [--align_dir ALIGN_DIR]
                                 [--reads_dir READS_DIR]
                                 [--raw_reads_dir RAW_READS_DIR]
                                 samples [samples ...] outFile
```

Positional arguments:

samples Sample names.

outFile Output report file.

Options:

--cov_thresholds=(1, 5, 20, 100) Genome coverage thresholds to report on. (default: %(default)s)

--assembly_dir=data/02_assembly Directory with assembly outputs. (default: %(default)s)

--assembly_tmp=tmp/02_assembly Directory with assembly temp files. (default: %(default)s)

--align_dir=data/02_align_to_self Directory with reads aligned to own assembly. (default: %(default)s)

--reads_dir=data/01_per_sample Directory with unaligned filtered read BAMs. (default: %(default)s)

--raw_reads_dir=data/00_raw Directory with unaligned raw read BAMs. (default: %(default)s)

coverage_only

```
usage: reports.py coverage_only [-h]
                                [--cov_thresholds COV_THRESHOLDS [COV_
↪THRESHOLDS ...]]
                                [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                [--version]
                                mapped_bams [mapped_bams ...] out_report
```

Positional arguments:

mapped_bams	Aligned-to-self mapped bam files.
out_report	Output report file.

Options:

--cov_thresholds=(1, 5, 20, 100)	Genome coverage thresholds to report on. (default: %(default)s)
--loglevel=INFO	Verboseness of output. [default: %(default)s] Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit

alignment_summary

Write or print pairwise alignment summary information for sequences in two FASTA files, including SNPs, ambiguous bases, and indels.

```
usage: reports.py alignment_summary [-h] [--outfileName OUTFILENAME]
                                    [--printCounts]
                                    inFastaFileOne inFastaFileTwo
```

Positional arguments:

inFastaFileOne	First fasta file for an alignment
inFastaFileTwo	First fasta file for an alignment

Options:

--outfileName	Output file for counts in TSV format
--printCounts=False	Undocumented

consolidate_fastqc

Consolidate multiple FASTQC reports into one.

```
usage: reports.py consolidate_fastqc [-h] inDirs [inDirs ...] outFile
```

Positional arguments:

inDirs	Input FASTQC directories.
outFile	Output report file.

consolidate_spike_count

Consolidate multiple spike count reports into one.

```
usage: reports.py consolidate_spike_count [-h] inDir outFile
```

Positional arguments:

inDir Input spike count directory.

outFile Output report file.

plot_coverage

Generate a coverage plot from an aligned bam file

```
usage: reports.py plot_coverage [-h] [--plotFormat] [--plotDataStyle]
                                [--plotStyle] [--plotWidth PLOT_WIDTH]
                                [--plotHeight PLOT_HEIGHT]
                                [--plotDPI PLOT_DPI] [--plotTitle PLOT_TITLE]
                                [--plotXLimits PLOT_X_LIMITS PLOT_X_LIMITS]
                                [--plotYLimits PLOT_Y_LIMITS PLOT_Y_LIMITS]
                                [-q BASE_Q_THRESHOLD] [-Q MAPPING_Q_THRESHOLD]
                                [-m MAX_COVERAGE_DEPTH]
                                [-l READ_LENGTH_THRESHOLD]
                                [--outSummary OUT_SUMMARY]
                                [--plotOnlyNonDuplicates]
                                [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                [--version] [--tmp_dir TMP_DIR]
                                [--tmp_dirKeep]
                                in_bam out_plot_file
```

Positional arguments:

in_bam Input reads, BAM format.

out_plot_file The generated chart file

Options:

--plotFormat File format of the coverage plot. By default it is inferred from the file extension of out_plot_file, but it can be set explicitly via –plotFormat. Valid formats include: tif, png, svgz, pgf, eps, pdf, raw, tiff, jpg, svg, ps, rgba, jpeg

Possible choices: tif, png, svgz, pgf, eps, pdf, raw, tiff, jpg, svg, ps, rgba, jpeg

--plotDataStyle=filled The plot data display style. Valid options: filled, line, dots (default: %(default)s)

Possible choices: filled, line, dots

--plotStyle=ggplot The plot visual style. Valid options: seaborn-dark-palette, seaborn-colorblind, seaborn-pastel, bmh, seaborn-whitegrid, seaborn-ticks, seaborn-paper, dark_background, seaborn-deep, classic, fivethirtyeight, seaborn-talk, ggplot, seaborn-notebook, seaborn-dark, seaborn-poster, seaborn-darkgrid, seaborn-muted, grayscale, seaborn-white, seaborn-bright (default: %(default)s)

Possible choices: seaborn-dark-palette, seaborn-colorblind, seaborn-pastel, bmh, seaborn-whitegrid, seaborn-ticks, seaborn-paper, dark_background, seaborn-deep, classic, fivethirtyeight, seaborn-talk, ggplot, seaborn-notebook, seaborn-dark, seaborn-poster, seaborn-darkgrid, seaborn-muted, grayscale, seaborn-white, seaborn-bright

--plotWidth=880 Width of the plot in pixels (default: %(default)s)

--plotHeight=680 Width of the plot in pixels (default: %(default)s)

--plotDPI=80.0 dots per inch for rendered output, more useful for vector modes (default: %(default)s)

--plotTitle=Coverage Plot The title displayed on the coverage plot (default: '%(default)s')

--plotXLimits Limits on the x-axis of the coverage plot; args are '<min> <max>'

--plotYLimits Limits on the y-axis of the coverage plot; args are '<min> <max>'

-q The minimum base quality threshold

-Q The minimum mapping quality threshold

-m The max coverage depth (default: %(default)s)

-l Read length threshold

--outSummary Coverage summary TSV file. Default is to write to temp.

--plotOnlyNonDuplicates=False Plot only non-duplicates (samtools -F 1024), coverage counted by bedtools rather than samtools.

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

align_and_plot_coverage

Take reads, align to reference with BWA-MEM, and generate a coverage plot

```
usage: reports.py align_and_plot_coverage [-h] [--plotFormat]
                                          [--plotDataStyle] [--plotStyle]
                                          [--plotWidth PLOT_WIDTH]
                                          [--plotHeight PLOT_HEIGHT]
                                          [--plotDPI PLOT_DPI]
                                          [--plotTitle PLOT_TITLE]
                                          [--plotXLimits PLOT_X_LIMITS PLOT_X_
↪LIMITS]
                                          [--plotYLimits PLOT_Y_LIMITS PLOT_Y_
↪LIMITS]
                                          [-q BASE_Q_THRESHOLD]
                                          [-Q MAPPING_Q_THRESHOLD]
                                          [-m MAX_COVERAGE_DEPTH]
                                          [-l READ_LENGTH_THRESHOLD]
                                          [--outSummary OUT_SUMMARY]
                                          [--outBam OUT_BAM] [--sensitive]
                                          [--excludeDuplicates]
                                          [--JVMmemory JVMMEMORY]
                                          [--picardOptions [PICARDOPTIONS␣
↪[PICARDOPTIONS ...]]]
```

```
                                          [--minScoreToFilter MIN_SCORE_TO_
↪FILTER]
                                          [--aligner {novoalign,bwa}]
                                          [--aligner_options ALIGNER_OPTIONS]
                                          [--NOVOALIGN_LICENSE_PATH NOVOALIGN_
↪LICENSE_PATH]
                                          [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                          [--version] [--tmp_dir TMP_DIR]
                                          [--tmp_dirKeep]
                                          in_bam out_plot_file ref_fasta
```

Positional arguments:

in_bam	Input reads, BAM format.
out_plot_file	The generated chart file
ref_fasta	Reference genome, FASTA format.

Options:

--plotFormat	File format of the coverage plot. By default it is inferred from the file extension of out_plot_file, but it can be set explicitly via –plotFormat. Valid formats include: tif, png, svgz, pgf, eps, pdf, raw, tiff, jpg, svg, ps, rgba, jpeg Possible choices: tif, png, svgz, pgf, eps, pdf, raw, tiff, jpg, svg, ps, rgba, jpeg
--plotDataStyle=filled	The plot data display style. Valid options: filled, line, dots (default: %(default)s) Possible choices: filled, line, dots
--plotStyle=ggplot	The plot visual style. Valid options: seaborn-dark-palette, seaborn-colorblind, seaborn-pastel, bmh, seaborn-whitegrid, seaborn-ticks, seaborn-paper, dark_background, seaborn-deep, classic, fivethirtyeight, seaborn-talk, ggplot, seaborn-notebook, seaborn-dark, seaborn-poster, seaborn-darkgrid, seaborn-muted, grayscale, seaborn-white, seaborn-bright (default: %(default)s) Possible choices: seaborn-dark-palette, seaborn-colorblind, seaborn-pastel, bmh, seaborn-whitegrid, seaborn-ticks, seaborn-paper, dark_background, seaborn-deep, classic, fivethirtyeight, seaborn-talk, ggplot, seaborn-notebook, seaborn-dark, seaborn-poster, seaborn-darkgrid, seaborn-muted, grayscale, seaborn-white, seaborn-bright
--plotWidth=880	Width of the plot in pixels (default: %(default)s)
--plotHeight=680	Width of the plot in pixels (default: %(default)s)
--plotDPI=80.0	dots per inch for rendered output, more useful for vector modes (default: %(default)s)
--plotTitle=Coverage Plot	The title displayed on the coverage plot (default: '%(default)s')
--plotXLimits	Limits on the x-axis of the coverage plot; args are '<min> <max>'

--plotYLimits	Limits on the y-axis of the coverage plot; args are '<min> <max>'
-q	The minimum base quality threshold
-Q	The minimum mapping quality threshold
-m	The max coverage depth (default: %(default)s)
-l	Read length threshold
--outSummary	Coverage summary TSV file. Default is to write to temp.
--outBam	Output aligned, indexed BAM file. Default is to write to temp.
--sensitive=False	Equivalent to giving bwa: '-k 12 -A 1 -B 1 -O 1 -E 1'. Only relevant if the bwa aligner is selected (the default).
--excludeDuplicates=False	MarkDuplicates with Picard and only plot non-duplicates
--JVMmemory=2g	JVM virtual memory size (default: %(default)s)
--picardOptions=[]	Optional arguments to Picard's MarkDuplicates, OPTION-NAME=value ...
--minScoreToFilter	Filter bwa alignments using this value as the minimum allowed alignment score. Specifically, sum the alignment scores across all alignments for each query (including reads in a pair, supplementary and secondary alignments) and then only include, in the output, queries whose summed alignment score is at least this value. This is only applied when the aligner is 'bwa'. The filtering on a summed alignment score is sensible for reads in a pair and supplementary alignments, but may not be reasonable if bwa outputs secondary alignments (i.e., if '-a' is in the aligner options). (default: not set - i.e., do not filter bwa's output)
--aligner=bwa	aligner (default: %(default)s) Possible choices: novoalign, bwa
--aligner_options	aligner options (default for novoalign: "-r Random -l 40 -g 40 -x 20 -t 100 -k", bwa: bwa defaults
--NOVOALIGN_LICENSE_PATH	A path to the novoalign.lic file. This overrides the NOVOALIGN_LICENSE_PATH environment variable. (default: %(default)s)
--loglevel=INFO	Verboseness of output. [default: %(default)s] Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit
--tmp_dir=/tmp	Base directory for temp files. [default: %(default)s]
--tmp_dirKeep=False	Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

fastqc

```
usage: reports.py fastqc [-h] inBam outHtml
```

Positional arguments:

inBam	Input reads, BAM format.
outHtml	Output report, HTML format.

illumina.py - for raw Illumina outputs

Utilities for demultiplexing Illumina data.

```
usage: illumina.py subcommand
```

Sub-commands:

illumina_demux

Read Illumina runs & produce BAM files, demultiplexing to one bam per sample, or for simplex runs, a single bam will be produced bearing the flowcell ID. Wraps together Picard's ExtractBarcodes (for multiplexed samples) and IlluminaBasecallsToSam while handling the various required input formats. Also can read Illumina BCL directories, tar.gz BCL directories.

```
usage: illumina.py illumina_demux [-h] [--outMetrics OUTMETRICS]
                                  [--commonBarcodes COMMONBARCODES]
                                  [--sampleSheet SAMPLESHEET]
                                  [--flowcell FLOWCELL]
                                  [--read_structure READ_STRUCTURE]
                                  [--max_mismatches MAX_MISMATCHES]
                                  [--minimum_base_quality MINIMUM_BASE_
↪QUALITY]
                                  [--min_mismatch_delta MIN_MISMATCH_DELTA]
                                  [--max_no_calls MAX_NO_CALLS]
                                  [--minimum_quality MINIMUM_QUALITY]
                                  [--compress_outputs COMPRESS_OUTPUTS]
                                  [--sequencing_center SEQUENCING_CENTER]
                                  [--adapters_to_check [ADAPTERS_TO_CHECK␣
↪[ADAPTERS_TO_CHECK ...]]]
                                  [--platform PLATFORM]
                                  [--max_reads_in_ram_per_tile MAX_READS_IN_
↪RAM_PER_TILE]
                                  [--max_records_in_ram MAX_RECORDS_IN_RAM]
                                  [--apply_eamss_filter APPLY_EAMSS_FILTER]
                                  [--force_gc FORCE_GC]
                                  [--first_tile FIRST_TILE]
                                  [--tile_limit TILE_LIMIT]
                                  [--include_non_pf_reads INCLUDE_NON_PF_
↪READS]
                                  [--run_start_date RUN_START_DATE]
                                  [--read_group_id READ_GROUP_ID]
                                  [--compression_level COMPRESSION_LEVEL]
                                  [--JVMmemory JVMMEMORY] [--threads THREADS]
                                  [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                  [--version] [--tmp_dir TMP_DIR]
                                  [--tmp_dirKeep]
                                  inDir lane outDir
```

Positional arguments:

inDir	Illumina BCL directory (or tar.gz of BCL directory). This is the top-level run directory.

lane Lane number.

outDir Output directory for BAM files.

Options:

--outMetrics Output ExtractIlluminaBarcodes metrics file. Default is to dump to a temp file.

--commonBarcodes Write a TSV report of all barcode counts, in descending order. Only applicable for read structures containing "B"

--sampleSheet Override SampleSheet. Input tab or CSV file w/header and four named columns: barcode_name, library_name, barcode_sequence_1, barcode_sequence_2. Default is to look for a SampleSheet.csv in the inDir.

--flowcell Override flowcell ID (default: read from RunInfo.xml).

--read_structure Override read structure (default: read from RunInfo.xml).

--max_mismatches=0 Picard ExtractIlluminaBarcodes MAX_MISMATCHES (default: %(default)s)

--minimum_base_quality=25 Picard ExtractIlluminaBarcodes MINIMUM_BASE_QUALITY (default: %(default)s)

--min_mismatch_delta Picard ExtractIlluminaBarcodes MIN_MISMATCH_DELTA (default: %(default)s)

--max_no_calls Picard ExtractIlluminaBarcodes MAX_NO_CALLS (default: %(default)s)

--minimum_quality Picard ExtractIlluminaBarcodes MINIMUM_QUALITY (default: %(default)s)

--compress_outputs Picard ExtractIlluminaBarcodes COMPRESS_OUTPUTS (default: %(default)s)

--sequencing_center Picard IlluminaBasecallsToSam SEQUENCING_CENTER (default: %(default)s)

--adapters_to_check=('PAIRED_END', 'NEXTERA_V1', 'NEXTERA_V2') Picard IlluminaBasecallsToSam ADAPTERS_TO_CHECK (default: %(default)s)

--platform Picard IlluminaBasecallsToSam PLATFORM (default: %(default)s)

--max_reads_in_ram_per_tile=200000 Picard IlluminaBasecallsToSam MAX_READS_IN_RAM_PER_TILE (default: %(default)s)

--max_records_in_ram=1000000 Picard IlluminaBasecallsToSam MAX_RECORDS_IN_RAM (default: %(default)s)

--apply_eamss_filter Picard IlluminaBasecallsToSam APPLY_EAMSS_FILTER (default: %(default)s)

--force_gc=False Picard IlluminaBasecallsToSam FORCE_GC (default: %(default)s)

--first_tile Picard IlluminaBasecallsToSam FIRST_TILE (default: %(default)s)

--tile_limit Picard IlluminaBasecallsToSam TILE_LIMIT (default: %(default)s)

--include_non_pf_reads=False Picard IlluminaBasecallsToSam INCLUDE_NON_PF_READS (default: %(default)s)

--run_start_date Picard IlluminaBasecallsToSam RUN_START_DATE (default: %(default)s)

--read_group_id Picard IlluminaBasecallsToSam READ_GROUP_ID (default: %(default)s)

--compression_level=7 Picard IlluminaBasecallsToSam COMPRESSION_LEVEL (default: %(default)s)

--JVMmemory=7g JVM virtual memory size (default: %(default)s)

--threads=0 Number of threads (default: 0)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

lane_metrics

Write out lane metrics to a tsv file.

```
usage: illumina.py lane_metrics [-h] [--read_structure READ_STRUCTURE]
                                [--JVMmemory JVMMEMORY]
                                [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                [--version] [--tmp_dir TMP_DIR]
                                [--tmp_dirKeep]
                                inDir outPrefix
```

Positional arguments:

inDir Illumina BCL directory (or tar.gz of BCL directory). This is the top-level run directory.

outPrefix Prefix path to the *.illumina_lane_metrics and *.illumina_phasing_metrics files.

Options:

--read_structure Override read structure (default: read from RunInfo.xml).

--JVMmemory=8g JVM virtual memory size (default: %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

common_barcodes

Extract Illumina barcodes for a run and write a TSV report of the barcode counts in descending order

```
usage: illumina.py common_barcodes [-h] [--truncateToLength TRUNCATETOLENGTH]
                                   [--omitHeader] [--includeNoise]
                                   [--outMetrics OUTMETRICS]
                                   [--sampleSheet SAMPLESHEET]
                                   [--flowcell FLOWCELL]
                                   [--read_structure READ_STRUCTURE]
                                   [--max_mismatches MAX_MISMATCHES]
                                   [--minimum_base_quality MINIMUM_BASE_
↪QUALITY]
                                   [--min_mismatch_delta MIN_MISMATCH_DELTA]
                                   [--max_no_calls MAX_NO_CALLS]
                                   [--minimum_quality MINIMUM_QUALITY]
                                   [--compress_outputs COMPRESS_OUTPUTS]
                                   [--JVMmemory JVMMEMORY]
                                   [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                   [--version] [--tmp_dir TMP_DIR]
                                   [--tmp_dirKeep]
                                   inDir lane outSummary
```

Positional arguments:

inDir Illumina BCL directory (or tar.gz of BCL directory). This is the top-level run directory.

lane Lane number.

outSummary Path to the summary file (.tsv format). It includes several columns: (barcode1, likely_index_name1, barcode2, likely_index_name2, count), where likely index names are either the exact match index name for the barcode sequence, or those Hamming distance of 1 away.

Options:

--truncateToLength If specified, only this number of barcodes will be returned. Useful if you only want the top N barcodes.

--omitHeader=False If specified, a header will not be added to the outSummary tsv file.

--includeNoise=False If specified, barcodes with periods (".") will be included.

--outMetrics Output ExtractIlluminaBarcodes metrics file. Default is to dump to a temp file.

--sampleSheet Override SampleSheet. Input tab or CSV file w/header and four named columns: barcode_name, library_name, barcode_sequence_1, barcode_sequence_2. Default is to look for a SampleSheet.csv in the inDir.

--flowcell Override flowcell ID (default: read from RunInfo.xml).

--read_structure Override read structure (default: read from RunInfo.xml).

--max_mismatches=0 Picard ExtractIlluminaBarcodes MAX_MISMATCHES (default: %(default)s)

--minimum_base_quality=25 Picard ExtractIlluminaBarcodes MINIMUM_BASE_QUALITY (default: %(default)s)

--min_mismatch_delta Picard ExtractIlluminaBarcodes MIN_MISMATCH_DELTA (default: %(default)s)

--max_no_calls Picard ExtractIlluminaBarcodes MAX_NO_CALLS (default: %(default)s)

--minimum_quality Picard ExtractIlluminaBarcodes MINIMUM_QUALITY (default: %(default)s)

--compress_outputs Picard ExtractIlluminaBarcodes COMPRESS_OUTPUTS (default: %(default)s)

--JVMmemory=8g JVM virtual memory size (default: %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

miseq_fastq_to_bam

Convert fastq read files to a single bam file. Fastq file names must conform to patterns emitted by Miseq machines. Sample metadata must be provided in a SampleSheet.csv that corresponds to the fastq filename. Specifically, the _S##_ index in the fastq file name will be used to find the corresponding row in the SampleSheet

```
usage: illumina.py miseq_fastq_to_bam [-h] [--inFastq2 INFASTQ2]
                                      [--runInfo RUNINFO]
                                      [--sequencing_center SEQUENCING_CENTER]
                                      [--JVMmemory JVMMEMORY]
                                      [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                      [--version] [--tmp_dir TMP_DIR]
                                      [--tmp_dirKeep]
                                     outBam sampleSheet inFastq1
```

Positional arguments:

outBam Output BAM file.

sampleSheet Input SampleSheet.csv file.

inFastq1 Input fastq file; 1st end of paired-end reads if paired.

Options:

--inFastq2 Input fastq file; 2nd end of paired-end reads.

--runInfo Input RunInfo.xml file.

--sequencing_center Name of your sequencing center (default is the sequencing machine ID from the RunInfo.xml)

--JVMmemory=2g JVM virtual memory size (default: %(default)s)

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

extract_fc_metadata

Extract RunInfo.xml and SampleSheet.csv from the provided Illumina directory

```
usage: illumina.py extract_fc_metadata [-h]
                                       [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                       [--version] [--tmp_dir TMP_DIR]
                                       [--tmp_dirKeep]
                                       flowcell outRunInfo outSampleSheet
```

Positional arguments:

flowcell Illumina directory (possibly tarball)

outRunInfo Output RunInfo.xml file.

outSampleSheet Output SampleSheet.csv file.

Options:

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

broad_utils.py - for data generated at the Broad Institute

Utilities for getting sequences out of the Broad walk-up sequencing pipeline. These utilities are probably not of much use outside the Broad.

```
usage: broad_utils.py subcommand
```

Sub-commands:

get_bustard_dir

Find the basecalls directory from a Picard directory

```
usage: broad_utils.py get_bustard_dir [-h]
                                      [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                      inDir
```

Positional arguments:

inDir	Picard directory

Options:

--loglevel=ERROR	Verboseness of output. [default: %(default)s]
	Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

get_run_date

Find the sequencing run date from a Picard directory

```
usage: broad_utils.py get_run_date [-h]
                                   [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                   inDir
```

Positional arguments:

inDir	Picard directory

Options:

--loglevel=ERROR	Verboseness of output. [default: %(default)s]
	Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

get_all_names

Get all samples

```
usage: broad_utils.py get_all_names [-h]
                                    [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                    {samples,libraries,runs} runfile
```

Positional arguments:

type	Type of name
	Possible choices: samples, libraries, runs
runfile	File with seq run information

Options:

--loglevel=ERROR	Verboseness of output. [default: %(default)s]
	Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

ncbi.py - utilities to interact with NCBI

This script contains a number of utilities for submitting our analyses to NCBI's Genbank and SRA databases, as well as retreiving records from Genbank.

```
usage: ncbi.py subcommand
```

Sub-commands:

tbl_transfer

This function takes an NCBI TBL file describing features on a genome (genes, etc) and transfers them to a new genome.

```
usage: ncbi.py tbl_transfer [-h] [--oob_clip] [--tmp_dir TMP_DIR]
                            [--tmp_dirKeep]
                            [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                            [--version]
                            ref_fasta ref_tbl alt_fasta out_tbl
```

Positional arguments:

ref_fasta	Input sequence of reference genome
ref_tbl	Input reference annotations (NCBI TBL format)
alt_fasta	Input sequence of new genome
out_tbl	Output file with transferred annotations

Options:

--oob_clip=False	Out of bounds feature behavior. False: drop all features that are completely or partly out of bounds True: drop all features completely out of bounds but truncate any features that are partly out of bounds
--tmp_dir=/tmp	Base directory for temp files. [default: %(default)s]
--tmp_dirKeep=False	Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.
--loglevel=INFO	Verboseness of output. [default: %(default)s] Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit

tbl_transfer_prealigned

This breaks out the ref and alt sequences into separate fasta files, and then creates unified files containing the reference sequence first and the alt second. Each of these unified files is then passed as a cmap to tbl_transfer_common.

This function expects to receive one fasta file containing a multialignment of a single segment/chromosome along with the respective reference sequence for that segment/chromosome. It also expects a reference containing all reference segments/chromosomes, so that the reference sequence can be identified in the input file by name. It also expects a list of reference tbl files, where each file is named according to the ID present for its corresponding sequence in the refFasta. For each non-reference sequence present in the inputFasta, two files are written: a fasta containing the segment/chromosome for the same, along with its corresponding feature table as created by tbl_transfer_common.

```
usage: ncbi.py tbl_transfer_prealigned [-h] [--oob_clip] [--tmp_dir TMP_DIR]
                                       [--tmp_dirKeep]
                                       [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                       [--version]
                                       inputFasta refFasta refAnnotTblFiles
                                       [refAnnotTblFiles ...] outputDir
```

Positional arguments:

inputFasta	FASTA file containing input sequences, including pre-made alignments and reference sequence
refFasta	FASTA file containing the reference genome
refAnnotTblFiles	Name of the reference feature tables, each of which should have a filename comrised of [refId].tbl so they can be matched against the reference sequences
outputDir	The output directory

Options:

--oob_clip=False	Out of bounds feature behavior. False: drop all features that are completely or partly out of bounds True: drop all features completely out of bounds but truncate any features that are partly out of bounds
--tmp_dir=/tmp	Base directory for temp files. [default: %(default)s]
--tmp_dirKeep=False	Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.
--loglevel=INFO	Verboseness of output. [default: %(default)s] Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit

fetch_fastas

This function downloads and saves the FASTA files from the Genbank CoreNucleotide database given a given list of accession IDs.

```
usage: ncbi.py fetch_fastas [-h] [--forceOverwrite]
                            [--combinedFilePrefix COMBINEDFILEPREFIX]
                            [--fileExt FILEEXT] [--removeSeparateFiles]
                            [--chunkSize CHUNKSIZE] [--tmp_dir TMP_DIR]
                            [--tmp_dirKeep]
                            [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                            [--version]
                            emailAddress destinationDir accession_IDs
                            [accession_IDs ...]
```

Positional arguments:

emailAddress	Your email address. To access the Genbank CoreNucleotide database, NCBI requires you to specify your email address with each request. In case of excessive usage of the E-utilities, NCBI

will attempt to contact a user at the email address provided before blocking access. This email address should be registered with NCBI. To register an email address, simply send an email to eutilities@ncbi.nlm.nih.gov including your email address and the tool name (tool='https://github.com/broadinstitute/viral-ngs').

destinationDir Output directory with where .fasta and .tbl files will be saved

accession_IDs List of Genbank nuccore accession IDs

Options:

--forceOverwrite=False Overwrite existing files, if present.

--combinedFilePrefix The prefix of the file containing the combined concatenated results returned by the list of accession IDs, in the order provided.

--fileExt The extension to use for the downloaded files

--removeSeparateFiles=False If specified, remove the individual files and leave only the combined file.

--chunkSize=1 Causes files to be downloaded from GenBank in chunks of N accessions. Each chunk will be its own combined file, separate from any combined file created via –combinedFilePrefix (default: %(default)s). If chunkSize is unspecified and >500 accessions are provided, chunkSize will be set to 500 to adhere to the NCBI guidelines on information retreival.

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

fetch_feature_tables

This function downloads and saves feature tables from the Genbank CoreNucleotide database given a given list of accession IDs.

```
usage: ncbi.py fetch_feature_tables [-h] [--forceOverwrite]
                                    [--combinedFilePrefix COMBINEDFILEPREFIX]
                                    [--fileExt FILEEXT]
                                    [--removeSeparateFiles]
                                    [--chunkSize CHUNKSIZE]
                                    [--tmp_dir TMP_DIR] [--tmp_dirKeep]
                                    [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                    [--version]
                                    emailAddress destinationDir accession_IDs
                                    [accession_IDs ...]
```

Positional arguments:

emailAddress Your email address. To access the Genbank CoreNucleotide database, NCBI requires you to specify your email address with

each request. In case of excessive usage of the E-utilities, NCBI will attempt to contact a user at the email address provided before blocking access. This email address should be registered with NCBI. To register an email address, simply send an email to eutilities@ncbi.nlm.nih.gov including your email address and the tool name (tool='https://github.com/broadinstitute/viral-ngs').

destinationDir Output directory with where .fasta and .tbl files will be saved

accession_IDs List of Genbank nuccore accession IDs

Options:

--forceOverwrite=False Overwrite existing files, if present.

--combinedFilePrefix The prefix of the file containing the combined concatenated results returned by the list of accession IDs, in the order provided.

--fileExt The extension to use for the downloaded files

--removeSeparateFiles=False If specified, remove the individual files and leave only the combined file.

--chunkSize=1 Causes files to be downloaded from GenBank in chunks of N accessions. Each chunk will be its own combined file, separate from any combined file created via –combinedFilePrefix (default: %(default)s). If chunkSize is unspecified and >500 accessions are provided, chunkSize will be set to 500 to adhere to the NCBI guidelines on information retreival.

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

fetch_genbank_records

This function downloads and saves full flat text records from Genbank CoreNucleotide database given a given list of accession IDs.

```
usage: ncbi.py fetch_genbank_records [-h] [--forceOverwrite]
                                     [--combinedFilePrefix COMBINEDFILEPREFIX]
                                     [--fileExt FILEEXT]
                                     [--removeSeparateFiles]
                                     [--chunkSize CHUNKSIZE]
                                     [--tmp_dir TMP_DIR] [--tmp_dirKeep]
                                     [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                     [--version]
                                    emailAddress destinationDir accession_IDs
                                     [accession_IDs ...]
```

Positional arguments:

emailAddress Your email address. To access the Genbank CoreNucleotide database, NCBI requires you to specify your email address with each request. In case of excessive usage of the E-utilities, NCBI will attempt to contact a user at the email address provided before blocking access. This email address should be registered with NCBI. To register an email address, simply send an email to eutilities@ncbi.nlm.nih.gov including your email address and the tool name (tool='https://github.com/broadinstitute/viral-ngs').

destinationDir Output directory with where .fasta and .tbl files will be saved

accession_IDs List of Genbank nuccore accession IDs

Options:

--forceOverwrite=False Overwrite existing files, if present.

--combinedFilePrefix The prefix of the file containing the combined concatenated results returned by the list of accession IDs, in the order provided.

--fileExt The extension to use for the downloaded files

--removeSeparateFiles=False If specified, remove the individual files and leave only the combined file.

--chunkSize=1 Causes files to be downloaded from GenBank in chunks of N accessions. Each chunk will be its own combined file, separate from any combined file created via –combinedFilePrefix (default: %(default)s). If chunkSize is unspecified and >500 accessions are provided, chunkSize will be set to 500 to adhere to the NCBI guidelines on information retreival.

--tmp_dir=/tmp Base directory for temp files. [default: %(default)s]

--tmp_dirKeep=False Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.

--loglevel=INFO Verboseness of output. [default: %(default)s]

Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION

--version, -V show program's version number and exit

prep_genbank_files

Prepare genbank submission files. Requires .fasta and .tbl files as input, as well as numerous other metadata files for the submission. Creates a directory full of files (.sqn in particular) that can be sent to GenBank.

```
usage: ncbi.py prep_genbank_files [-h] [--comment COMMENT]
                                  [--sequencing_tech SEQUENCING_TECH]
                                  [--master_source_table MASTER_SOURCE_TABLE]
                                  [--organism ORGANISM] [--mol_type MOL_TYPE]
                                  [--biosample_map BIOSAMPLE_MAP]
                                  [--coverage_table COVERAGE_TABLE]
                                  [--tmp_dir TMP_DIR] [--tmp_dirKeep]
                                  [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                                  [--version]
                                  templateFile fasta_files [fasta_files ...]
                                  annotDir
```

Positional arguments:

templateFile	Submission template file (.sbt) including author and contact info
fasta_files	Input fasta files
annotDir	Output directory with genbank submission files (.tbl files must already be there)

Options:

--comment	comment field
--sequencing_tech	sequencing technology (e.g. Illumina HiSeq 2500)
--master_source_table	source modifier table
--organism	species name
--mol_type	molecule type
--biosample_map	A file with two columns and a header: sample and BioSample. This file may refer to samples that are not included in this submission.
--coverage_table	A genome coverage report file with a header row. The table must have at least two columns named sample and aln2self_cov_median. All other columns are ignored. Rows referring to samples not in this submission are ignored.
--tmp_dir=/tmp	Base directory for temp files. [default: %(default)s]
--tmp_dirKeep=False	Keep the tmp_dir if an exception occurs while running. Default is to delete all temp files at the end, even if there's a failure.
--loglevel=INFO	Verboseness of output. [default: %(default)s] Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit

prep_sra_table

This is a very lazy hack that creates a basic table that can be pasted into various columns of an SRA submission spreadsheet. It probably doesn't work in all cases.

```
usage: ncbi.py prep_sra_table [-h]
                              [--loglevel
↪{DEBUG,INFO,WARNING,ERROR,CRITICAL,EXCEPTION}]
                              [--version]
                              lib_fname biosampleFile md5_fname outFile
```

Positional arguments:

lib_fname	A file that lists all of the library IDs that will be submitted in this batch
biosampleFile	A file with two columns and a header: sample and BioSample. This file may refer to samples that are not included in this submission.
md5_fname	A file with two columns and no header. Two columns are MD5 checksum and filename. Should contain an entry for every bam

	file being submitted in this batch. This is typical output from "md5sum *.cleaned.bam".
outFile	Output table that contains most of the variable columns needed for SRA submission.

Options:

--loglevel=INFO	Verboseness of output. [default: %(default)s] Possible choices: DEBUG, INFO, WARNING, ERROR, CRITICAL, EXCEPTION
--version, -V	show program's version number and exit

Using the Snakemake pipelines

Rather than chaining together viral-ngs pipeline steps as series of tool commands called in isolation, it is possible to execute them as a complete automated pipeline, from processing raw sequencer output to creating files suitable for GenBank submission. This utilizes Snakemake, which is documented at: https://snakemake.readthedocs.io/en/stable/

Here is an overview of the Snakemake rule graph:


download_lastal_sources
build_lastal_db
depletion
filter_to_taxon
spikein_report
merge_one_per_sample
assemble_trinity
download_reference_genome
orient_and_impute
consolidate_spike_count
refine_assembly_1
refine_assembly_2
map_reads_to_self
multi_align_mafft
fastqc_report
isnvs_per_sample
consolidate_fastqc
isnvs_vcf
all

Installation instructions

It is recommended to install the viral-ngs conda package from the `broad-viral` channel, as detailed in the installation section of this documentation.

The script `easy-deploy-viral-ngs.sh` can be used to install conda and the viral-ngs package on a standard Linux system, as well as to create new project analysis directories. Project directories can also be created manually as described below.

Setting up an analysis directory

Copying and creating project directories and files

The Snakemake pipline is intended to be run on an input one or more sequencer bam files, each having a filename represending a sample name. The output files are named with the same sample names, and are organized into folders corresponding to the steps of the pipeline in which they were created.

To get started, create an analysis directory somewhere in your compute environment to contain the pipeline input and output files.

Into this directory, copy the following file from the `viral-ngs/pipes` directory:

```
config.yaml
Snakefile
```

Since the file `config.yaml` is project-specific, you will need to make changes to it as approprate for your usage. The config file changes are described in greater detail below.

Next, `cd` to the analysis directory and create symbolic links to the following:

- The viral-ngs virtual environment:

  ```
  ln -s /path/to/venv-viral-ngs venv
  ```

- The viral-ngs project, checked out from GitHub or extracted from a version-tagged archive:

  ```
  ln -s /path/to/viral-ngs bin
  ```

Within the analysis directory, create the directories and files used by the Snakemake pipeline:

```
data/
    00_raw/
    01_cleaned/
    01_per_sample/
    02_align_to_self/
    02_assembly/
    03_align_to_ref/
    03_interhost/
    04_intrahost/
log/
reports/
tmp/
```

The directory structure created needs to match the locations specified in `config.yaml` .

Adding input data

- Copy each of the raw sample bam files to the `00_raw/` directory and ensure the file names follow the convention of `{sample}.bam`.
- Create a file, `samples-depletion.txt`, to list all of the samples that should be run through the depletion pipeline, with one samplename per line as {sample}, following the format of the input bam file: `{sample}.bam`. For example, if you copied a file called "G1190.bam" into `00_raw/`, then then `samples-depletion.txt` would contain the line:

  ```
  G1190
  ```

- Create a file, `samples-assembly.txt`, to list all of the samples that should be run through the assembly pipeline.
- Create a file, `samples-runs.txt`, to list all of the samples that should be run through the int**er**host analysis pipeline.
- Create a blank file, `samples-assembly-failures.txt`, that may be filled in later.

Modifying the `config.yaml` file

Minimal modification to the config file is necessary, though there are a few things you need to specify:

An email address for when the pipeline fetches reference data from the NCBI via their Entrez API:

```
email_point_of_contact_for_ncbi: "someone@example.com"
```

The path to the depletion databases to be used by BMTagger, along with the file prefixes of the specific databases to use. The process for creating BMTagger depletion databases is described in the NIH BMTagger docs.

```
bmtagger_db_dir: "/path/to/depletion_databases"
bmtagger_dbs_remove:
  - "hg19"
  - "GRCh37.68_ncRNA-GRCh37.68_transcripts-HS_rRNA_mitRNA"
  - "metagenomics_contaminants_v3"
```

Pre-built depletion databases are available in both *.tar.gz and *.lz4 format, for removing human reads and common metagenomic contaminants:

- GRCh37.68_ncRNA-GRCh37.68_transcripts-HS_rRNA_mitRNA.tar.gz (*.lz4)
- hg19.tar.gz (*.lz4)
- metagenomics_contaminants_v3.tar.gz (*.lz4)

Note that these databases must be extracted prior to use.

In addition to the databases used by BMTagger, you will need to specify the location and file prefix of the BLAST database to be used for depletion. The process for creating the BLAST database is described in the NIH BLAST docs, and on this website from the University of Oxford.

```
blast_db_dir: "/path/to/depletion_databases"
blast_db_remove: "metag_v3.ncRNA.mRNA.mitRNA.consensus"
```

A pre-built depletion database is also available for BLAST:

- metag_v3.ncRNA.mRNA.mitRNA.consensus.tar.gz (*.lz4)

Note that this database must be extracted prior to use.

Additional databases are needed to perform metagenomic classification using Kraken, Diamond, or Krona.

```
kraken_db: "/path/to/kraken_full_20150910"

diamond_db: "/path/to/diamond_db/nr"

krona_db: "/path/to/krona"
```

Pre-built databases for Kraken, Diamond, and Krona are available:

- kraken_ercc_db_20160718.tar.gz including ERCC spike-in RNA seqs (*.lz4)
- kraken_db.tar.gz (*.lz4)
- krona_taxonomy_20160502.tar.gz (*.lz4)
- nr.dmnd.gz (*.lz4)

Note that these databases must be extracted prior to use.

An array of the NCBI GenBank CoreNucleotide accessions for the sequences comprising the reference genome to be used for contig assembly as well as for int**er**host and int**ra**host variant analysis. In addition, you will need to specify a file prefix to be used to represent the full reference genome file used downstream.

```
accessions_for_ref_genome_build:
  - "KJ660346.2"
```

An optional file containing a list of accessions may be specified for filtering reads via LAST. This is intended to narrow to a genus. If this file is not provided, viral-ngs defaults to using the accessions specified for the reference genome.

```
accessions_file_for_lastal_db_build: "/path/to/lastal_accessions.txt"
```

A FASTA file to be used by Trimmomatic during assembly to remove contaminents from reads:

```
trim_clip_db: "/path/to/depletion_databases/contaminants.fasta"
```

Pre-built databases for Trimmomatic:

- contaminants.fasta.tar.gz (*.lz4)

A FASTA file containing spike-ins to be reported:

```
spikeins_db: "/path/to/references/ercc_spike-ins.fasta"
```

Modifying the `Snakefile`

Depending on the state of your input data, and where in the pipeline it may enter, it may be necessary to omit certain processing steps. For example, if your sequencing center has already demultiplexed your data and no demultiplexing is needed, you can comment out the following line in the `Snakefile` :

```
include: os.path.join(pipesDir, 'demux.rules')
```

Running the pipeline

Configuring for your compute platform

Running the pipeline directly

After the above setup is complete, run the pipeline directly by calling `snakemake` within the analysis directory.

Running the pipeline on GridEngine (UGER)

Within `config.yaml`, set the "project" to one that exists on the cluster system.

Inside the analysis directory, run the job submission command. Ex.:

```
use UGER
qsub -cwd -b y -q long -l m_mem_free=4G ./bin/pipes/Broad_UGER/run-pipe.sh
```

To kill all jobs that exited (qstat status "Eqw") with an error:

```
qdel $(qstat | grep Eqw | awk '{print $1}')
```

Running the pipeline on LSF

Inside the analysis directory, run the job submission command. Ex.:

```
bsub -o log/run.out -q forest ./bin/pipes/Broad_LSF/run-pipe.sh
```

If you notice jobs hanging in the **PEND** state, an upstream job may have failed. Before killing such jobs, verify that the jobs are pending due to their dependency:

```
bjobs -al | grep -A 1 "PENDING REASONS" | grep -v "PENDING REASONS" | grep -v '^--$'
```

To kill all **PEND**ing jobs:

```
bkill `bjobs | grep PEND | awk '{print $1}'` > /dev/null
```

When things go wrong

The pipeline may fail with errors during execution, usually while generating assemblies with Trinity. If this occurs, examine the output, add the failing sample names to `samples-assembly-failures.txt`, keeping the good ones in `samples-assembly.txt`, and re-run the pipeline. Due to sample degradation prior to sequencing in the wet lab, not all samples have the integrity to complete the pipeline, and it may necessary to skip over these samples by adding them to the `samples-assembly-failures.txt`.

Assembly of pre-filtered reads

Taxonomic filtration of raw reads

Starting from Illumina BCL directories

When starting from Illumina run directories, the viral-ngs Snakemake pipeline can demultiplex raw BCL files, and merge samples from multiple flowcell lanes or libraries. To use viral-ngs in this way, create the following files:

`flowcells.txt` (example below): A tab-delimited file describing the flowcells to demultiplex, as well as the lane to use, a path to the file listing the barcodes used in the lane, the `bustard_dir` (the run directory as written by an Illumina sequencer), and an optional column for `max_mismatches`, which specifies how many bases are allowed to

differ for a read to be assigned to a particular barcode (default: 0). The column `max_mismatches` may be omitted, including its header.

```
flowcell        lane    barcode_file    bustard_dir     max_mismatches
H32G3ADXY       1       /path/to/barcodes.txt    /path/to/illumina/run/directory/run_
↪BH32G3ADXY 1
H32G3ADXY       2       /path/to/barcodes.txt    /path/to/illumina/run/directory/run_
↪BH32G3ADXY 1
AKJ6R   1       /path/to/barcodes.txt      /path/to/illumina/run/directory/run_AKJ6R␣
↪    1
```

`barcodes.txt` (example below): A tab-delimited file describing the barcodes used for a given sample, along with a library ID.

```
sample  barcode_1       barcode_2       library_id_per_sample
41C     TAAGGCGA        TATCCTCT        AP2
21P     CGTACTAG        TATCCTCT        AP2
42C     AGGCAGAA        TATCCTCT        AP2
41P     TCCTGAGC        TATCCTCT        AP2
42P     GGACTCCT        TATCCTCT        AP2
61C     TAGGCATG        TATCCTCT        AP2
61P     CTCTCTAC        AGAGTAGA        AP2
62C     CAGAGAGG        AGAGTAGA        AP2
62P     GCTACGCT        AGAGTAGA        AP2
142C    CGAGGCTG        AGAGTAGA        AP2
WATERCTL        AAGAGGCA        AGAGTAGA        AP2
```

`samples-depletion.txt` : the list of sample names to deplete *as described above*.

Developing viral-ngs

Testing easy_deploy with Vagrant and Ansible

By default, easy_deploy installs a version of viral-ngs by cloning the github repository, which makes it difficult to test local changes to the playbook. Testing the easy_deploy setup locally can be done using Vagrant and the Ansible playbook with some custom commands. To do so, we need to take the following steps:

1. Install Vagrant
2. Install Ansible
3. Change to the easy_deploy directory

```
$ cd easy_deploy
```

4. Run the easy deploy script to bootstrap the VM and answer the prompts

```
$ ./run.sh
```

5. From now on, run Ansible playbook manually for provisioning: http://docs.ansible.com/ansible/guide_vagrant.html#running-ansible-manually.
6. Add *deploy=sync* or *deploy=archive* to the *–extra-vars* of the Ansible playbook command

```
$ ansible-playbook ... --extra-vars=deploy=sync
```

To test playbook changes in a local repository, the choice of *deploy=sync* or *deploy=archive* changes the strategy used to "deploy" viral-ngs into the Vagrant VM.

The *deploy=sync* option creates a symlink to the synced folder containing the root of your viral-ngs git repo. Therefore, any tool installation or other changes will be reflected on both the host and the guest machine. This is desirable for fast iteration of changes, but makes it difficult to isolate the host's viral-ngs installation from the installation on the guest VM.

The *deploy=archive* option performs a *git archive* on the host's viral-ngs repository and untars it into the project directory. This is a clean install each time, which can be time consuming due to the need to reinstall all dependencies, and only the current HEAD commit will be reflected in the guest. No uncommitted/dirty changes will be picked up using this method. This is more ideally suited for a finalized clean test of the playbook.