**The whole genome focused array SNP typing (*WG-FAST*) pipeline**

**Citation:**

**Contact:** Please address queries, concerns, improvements to jasonsahl at gmail dot com

**What does *WG-FAST* do?**

*WG-FAST* was designed as a tool to phylogenetically genotype unknown samples, even those with extremely low read coverage, in the context of a well-studied dataset.

**What does *WG-FAST* not do?**

*WG-FAST* is not intended to identify new SNPs in a dataset. If too many samples are processed with *WG-FAST*, a phylogenetic discovery bias can most certainly exist.

**Installation**

-The code is housed here: https://github.com/jasonsahl/wgfast.git

-Install the code with:

>git clone <https://github.com/jasonsahl/wgfast.git>

-The following line must be edited to reflect your installation directory:

WGFAST\_PATH**=**"/Users/jsahl/wgfast"

**Dependencies**

1. GATK – tested version is 2.72. This version requires Java 1.7. Should be back compatible with older versions. Download Jar file and place in WGFAST\_PATH/bin. Can be obtained from: <https://www.broadinstitute.org/gatk/download>
2. Samtools – tested version is 0.1.19. Must be in PATH as “samtools”. Can be obtained from: <http://samtools.sourceforge.net/>
3. BWA-MEM – tested version is 0.7.5a. Must be in PATH as “bwa”. Can be obtained from: <http://bio-bwa.sourceforge.net/>
4. Picard tools – tested version is 1.79. Included in “binary” directory and does not need to be independently installed.
5. RAxML – tested version is 8.1.3. Must be in PATH as “raxmlHPC-SSE3” and “raxmlHPC-PTHREADS-SSE3”. Can be obtained from: <https://github.com/stamatak/standard-RAxML>. The PTHREADS version does not support the ASC substitution models. Because RAxML is under constant development, a stable version is included with *WG-FAST*. As improvements are made to RAxML, the version will be updated in *WG-FAST*, as long as changes don’t affect performance.
6. DendroPy – tested version is 3.12.0, must be installed in PYTHONPATH. Can be obtained from: <https://github.com/jeetsukumaran/DendroPy>. Dendropy is included with *WG-FAST* with the following included information:

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**Additional dependencies included with WG-FAST**.

1. BioPython – must be in PYTHONPATH. Can be obtained from: <https://github.com/biopython/biopython>
2. Trimmomatic. Included with WG-FAST.

**Required input files**

1. Directory of sequence reads. The reads must be named according to Illumina HiSeq or MiSeq conventions. Reads must be in the Illumina 1.9+ FastQ format. If you have old Illumina FastQ encodings, they must be converted before running WG-FAST.
2. SNP matrix. The easiest way to generate this is by using NASP (<https://github.com/TGenNorth/NASP>). If other SNP matrix formats are used, they must conform to having the first column including (contig::coordinate) and the column following the SNP calls must be (#SNPcall).
3. Phylogeny. A script is included with WG-FAST that can generate an appropriate phylogeny from a NASP matrix.
4. Reference genome in FASTA format. This should be the same FASTA that was used to call SNPs with NASP.

**Arguments:**

-h, --help show this help message and exit

-m MATRIX, --snp\_matrix=MATRIX

path to NASP snp\_matrix [REQUIRED]

-t TREE, --tree=TREE path to input tree [REQUIRED]

-r REFERENCE, --reference\_fasta=REFERENCE

path to reference fasta [REQUIRED]

-d DIRECTORY, --directory=DIRECTORY

path to directory of fastq.gz files [REQUIRED]

-x PARAMETERS, --parameters\_file=PARAMETERS

parameters for RAxML insertion, defaults to NULL

-p PROCESSORS, --processors=PROCESSORS

# of processors to use - defaults to 2

-c COVERAGE, --coverage=COVERAGE

minimum SNP coverage required to be called a SNP -

defaults to 3

-o PROPORTION, --proportion=PROPORTION

proportion of alleles to be called a SNP, defaults to

0.9

-k KEEP, --keep=KEEP keep temp files? Defaults to F

-s SUBSAMPLE, --subsample=SUBSAMPLE

Run subsample routine? Defaults to T

-n SUBNUMS, --subnums=SUBNUMS

number of subsamples to process, defaults to 100

-g DOC, --doc=DOC run depth of coverage on all files? Defaults to T

-e TMP\_DIR, --temp=TMP\_DIR

temporary directory for GATK analysis, defaults to

/tmp

-z INSERTION\_METHOD, --method=INSERTION\_METHOD

method to insert unknown genomes (MP or ML), defaults

to ML

-f FUDGE, --fudge\_factor=FUDGE

How close does a subsample have to be from true

placement? Defaults to 0.1

-y ONLY\_SUBS, --only\_subs=ONLY\_SUBS

Only run sub-sample routine and exit? Defaults to F

-j MODEL, --model=MODEL

which model to run with raxml, GTRGAMMA, ASC\_GTRGAMMA,

GTRCAT, ASC\_GTRCAT

**Test Data:**

1. To give WG-FAST a run, to make sure everything is installed correctly, check out the test\_data directory.
2. The following command was run from a run directory within the WG-FAST installation:

python ../wgfast.py -m ../test\_data/nasp\_matrix.tsv –t ../test\_data/nasp\_raxml.tree \ -r ../test\_data/reference.fasta -d ../test\_data/reads -x ../test\_data/nasp.PARAMS \ -c 1

**Output printed to screen**

1. Number of callable positions. These are all positions called in each sample, compared to the reference. This is the number prior to any filtering
2. Number of SNPs. These are the number of observed polymorphisms, based on calls made by GATK.
3. Number of discarded SNPs. These are polymorphisms that were called by GATK, but were thrown out because they failed to meet the depth and/or proportion filters.
4. Insertion likelihood values. The higher the likelihood value and the fewer the number of possible insertion sites, the more trusted the placement.