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

**Citation:**

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**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 in “wgfast.py” 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 8.1.7. 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* (see build directions in the standard-RAxML directory. 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*.

**Citations for all dependencies.**

**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). For the sub-sampling routine to complete, a genome must be present in your dataset that is called ‘Reference’.
3. Phylogeny. A script is included with *WG-FAST* that can generate an appropriate phylogeny from a NASP matrix (see below). This script also generates a ‘Parameters’ file, which can be used with *WG-FAST* and cuts down on the computational time required for each subsequent run.
4. Reference genome in FASTA format. This should be the same FASTA that was used to call SNPs with NASP.

**Complete list of 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 created 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 due to mixed SNP positions.
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 nodes, the more trusted the placement, although caveats exist (see Manuscript).
5. If sub-sample routine is invoked, information is also available for how often the sub-sample was placed correctly. A sub-sample is considered to be “correct” by comparing the patristic distance from the sub-sampled genome to the “Reference”, then comparing that to the distance between the un-sub-sampled genome to the “Reference”. If this ratio falls within the “fudge-factor” range, then it is considered to be correct. The number of times that the sub-sample falls within this range is divided by the total number of iterations and a p-value is reported.

Other scripts:

1. wgfast\_prep.py

-What does it do? Given a NASP matrix, this script will generate a maximum likelihood phylogeny with RAxML and will also generate a “parameters” file that can be used for future *WG-FAST* runs.

-What do you need for the script to run? Requirements include:

* Python > 2.7 < 3.0
* NASP matrix
* RAxML in your $PATH as “raxmlHPC-SSE3”

-What does the output look like? Two files are produced:

1. “nasp\_raxml.tree”. Your tree. Names have been fixed to work with WG-FAST
2. “nasp.PARAMS”. Parameters file. Use this with the “-X” flag described above.

$python wgfast\_prep.py -m nasp.matrix

1. subsample\_snps\_pearson.py

-What does it do? Given a NASP matrix, the script generates a new matrix over a given number of iterations at a given level of SNP sampling.

-What do you need for the script to run? Requirements include:

* Python > 2.7 < 3.0
* NASP matrix
* ‘mothur’ executable in your PATH. Mothur can be freely obtained from: <http://www.mothur.org/wiki/Download_mothur>

-What does the output look like? One file is generated “results.txt”, that is new-line delimited, with each line containing a Pearson correlation value (0 to 1).

$python subsample\_snps\_pearson.py -m nasp.matrix -s 100

3. Subsample\_