SAT_mix manual

SAT_mix = SNPhylo + Admixture + Treemix

Original script of SAT_mix is SNPhylo’s which was customized, and modified for PAPGI study by JaeJin Choi, KOBIC 2014

Purpose: Integrate three different methods and provide “Big picture”

SNPhylo + Admixture + Treemix

Requirements/Pre-installation
1. Interpreter(compiler): R, Python, Perl
2. External program: MUSCLE, DNAML, Admixture, Treemix, Plink, (SNPhylo)

Run ./setup.sh for configuration

Input file formats: VCF, Hapmap, PED, GDS, simple SNP file; Contain AGCT, not integer

Primary parameters:

Linkage Disequilibrium(LD)
Minor Allele Frequency(MAF)
MISS, PNSS – recommend to set = 0

Function specific parameters
1. SNPhylo

Prefixed; Support 3 options based on the length of SNP sequence

2. Admixture

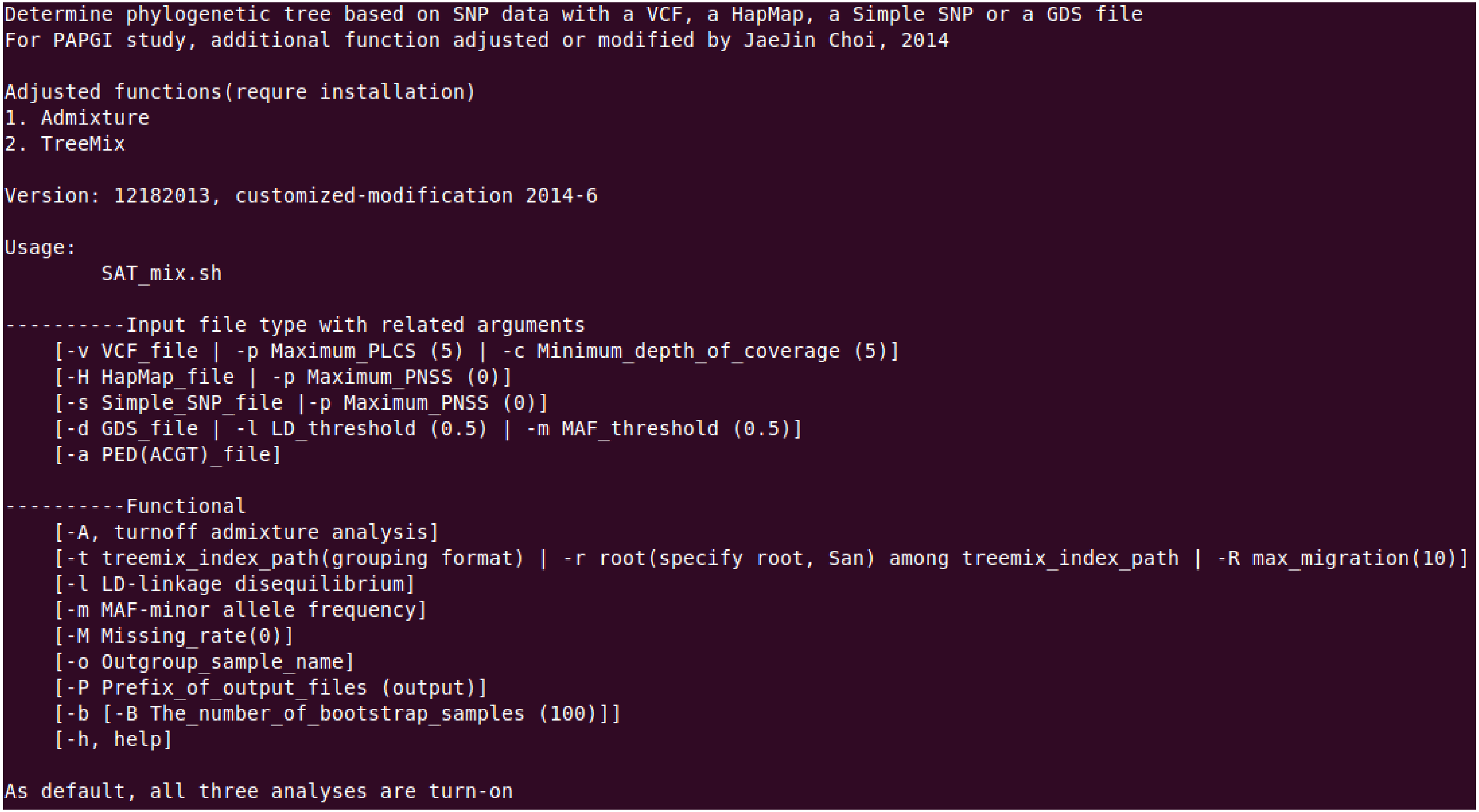
Prefixed; ancestor k = 2 ~ 7

3. Treemix

-t group index
-R number of migration
-r root (is in group index)

SAT_mix manual

For more detail; -h for help
Original script is “SNPhylo”

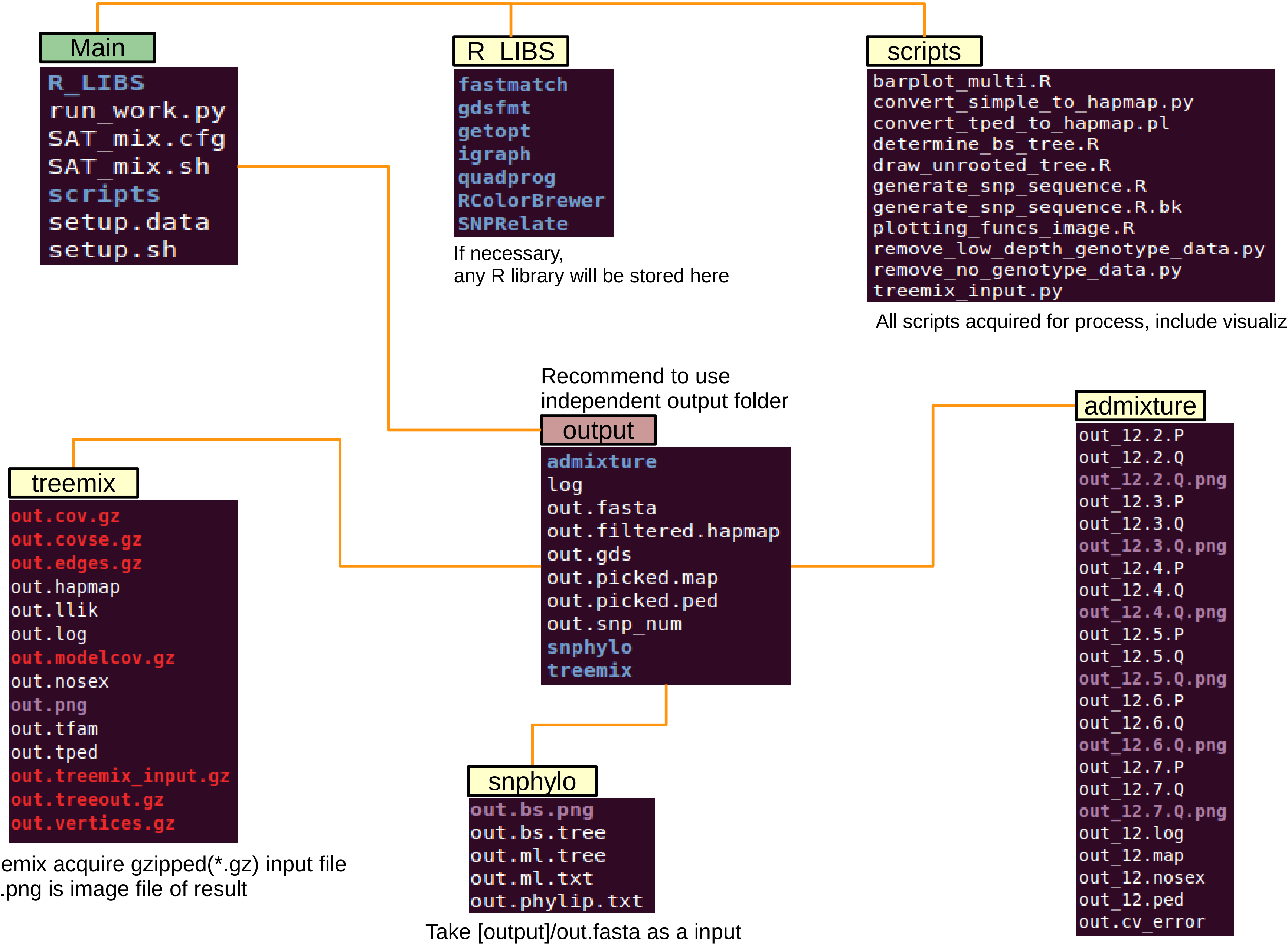


```
Determine phylogenetic tree based on SNP data with a VCF, a HapMap, a Simple SNP or a GDS file
For PAPGI study, additional function adjusted or modified by JaeJin Choi, 2014

Adjusted functions(requre installation)
1. Admixture
2. TreeMix

Version: 12182013, customized-modification 2014-6

Usage:
        SAT_mix.sh

----------Input file type with related arguments
    [-v VCF_file | -p Maximum_PLCS (5) | -c Minimum_depth_of_coverage (5)]
    [-H HapMap_file | -p Maximum_PNSS (0)]
    [-s Simple_SNP_file |-p Maximum_PNSS (0)]
    [-d GDS_file | -l LD_threshold (0.5) | -m MAF_threshold (0.5)]
    [-a PED(ACGT)_file]

----------Functional
    [-A, turnoff admixture analysis]
    [-t treemix_index_path(grouping format) | -r root(specify root, San) among treemix_index_path | -R max_migration(10)]
    [-l LD-linkage disequilibrium]
    [-m MAF-minor allele frequency]
    [-M Missing_rate(0)]
    [-o Outgroup_sample_name]
    [-P Prefix_of_output_files (output)]
    [-b [-B The_number_of_bootstrap_samples (100)]]
    [-h, help]

As default, all three analyses are turn-on
```

Any file path should be direct in absolute path(full length path)

Example; sh [root of]/SAT_mix.sh -l 0.05 -m 0.01 -p 0 -M 0 -P [root of]/out -b -H [root of]/any.hapmap -t [root of]/group_index -R 10 -r San

SAT_mix file structure



Main

```
R_LIBS
run_work.py
SAT_mix.cfg
SAT_mix.sh
scripts
setup.data
setup.sh
```

R_LIBS

```
fastmatch
gdsfmt
getopt
igraph
quadprog
RColorBrewer
SNPRelate
```

If necessary,
any R library will be stored here

scripts

```
barplot_multi.R
convert_simple_to_hapmap.py
convert_tped_to_hapmap.pl
determine_bs_tree.R
draw_unrooted_tree.R
generate_snp_sequence.R
generate_snp_sequence.R.bk
plotting_funcs_image.R
remove_low_depth_genotype_data.py
remove_no_genotype_data.py
treemix_input.py
```

All scripts acquired for process, include visualization

Recommend to use
independent output folder

output

```
admixture
log
out.fasta
out.filtered.hapmap
out.gds
out.picked.map
out.picked.ped
out.snp_num
snphylo
treemix
```

treemix

```
out.cov.gz
out.covse.gz
out.edges.gz
out.hapmap
out.llik
out.log
out.modelcov.gz
out.nosex
out.png
out.tfam
out.tped
out.treemix_input.gz
out.treeout.gz
out.vertices.gz
```

Treemix acquire gzipped(*.gz) input file
out.png is image file of result

snphylo

```
out.bs.png
out.bs.tree
out.ml.tree
out.ml.txt
out.phylip.txt
```

Take [output]/out.fasta as a input
1. out.bs.tree in newick format
2. out.bs.png is image file of out.bs.tree

admixture

```
out_12.2.P
out_12.2.Q
out_12.2.Q.png
out_12.3.P
out_12.3.Q
out_12.3.Q.png
out_12.4.P
out_12.4.Q
out_12.4.Q.png
out_12.5.P
out_12.5.Q
out_12.5.Q.png
out_12.6.P
out_12.6.Q
out_12.6.Q.png
out_12.7.P
out_12.7.Q
out_12.7.Q.png
out_12.log
out_12.map
out_12.nosex
out_12.ped
out.cv_error
```

Run k = 2 ~7
Determine optimum ‘k’ from out.cv_error, which
have smallest CV error rate

SAT_mix file; how script run

Assume run;

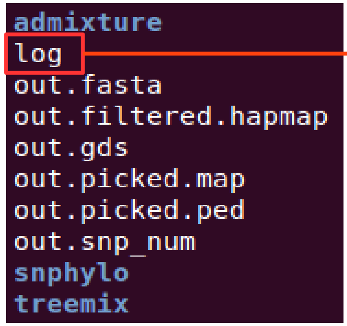
```
sh [root of]/SAT_mix.sh -l 0.05 -m 0.05 -p 0 -M 0 -P [root of]/out -b -H [root pf]/any.hapmap -t [root of]/group_index -R 10 -r San > log
```

LD | -l = 0.05
MAF | -m = 0.05
MISS | -M , and PNSS | -p = 0

-t [group_index]
Root | -R = ‘San’
Maximum migration event | -r = 10

157 Individuals

output



```
admixture
log
out.fasta
out.filtered.hapmap
out.gds
out.picked.map
out.picked.ped
out.snp_num
snphylo
treemix
```

```
Start to remove low quality data.

23669 low quality lines were removed

Start HapMap2GDS ...
            Scanning ...
            file: [root of]/l0.05-m0.05/out.filtered.hapmap
            content: 135018 rows x 168 columns
Wed Jun 25 23:08:02 2014        store sample id, snp id, position, and chromosome.
            start writing: 157 samples, 135017 SNPs ...
            file: [root of]/l0.05-m0.05/out.filtered.hapmap
Wed Jun 25 23:16:08 2014        Done.
Finally picked; 5348 SNPs

--admixture start
Prepare Admixture...
Obtain; [root pof/l0.05-m0.05/admixture/out_12.ped(map), --recode12

Admixture analysis proceed...

(k = 2 ~ 7)

--admixture done


TreeMix analysis proceed...
/San


--treemix start

(obtain treemix input file by several conversion)

--treemix done



--snphylo start
MSA proceed using 5348 SNPs

BS tree draw proceed


Adding species:
   1. M_39
   2. M_40
   3. M_69
.
.
.
 157. M_15

Output written to file "outfile"

Tree also written onto file "outtree"

Done.

--snphylo done
!End without notable errors
```

Remove no genotype SNPs (low quality, and missing)

After LD, MAF, and MISS filtration, we obtain 5348 SNPs

Admixture

‘K’ = 2 ~ 7, prefixed
Output; out_12.’K’.Q.png.

Treemix

Output; out.png → ML tree image with n migration events in arrow

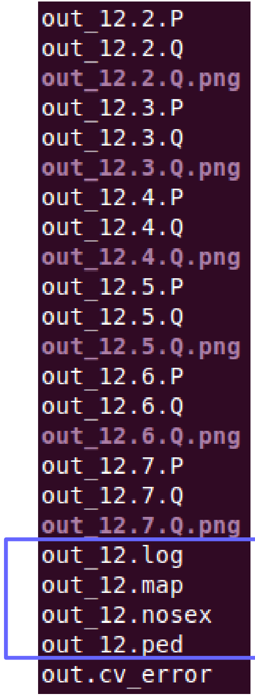
SNPhylo

Output;
1. out.bs.tree → ML tree with bootstrap support in newick format

2. out.bs.png → image file of out.bs.tree

SAT_mix output; admixture

admixture

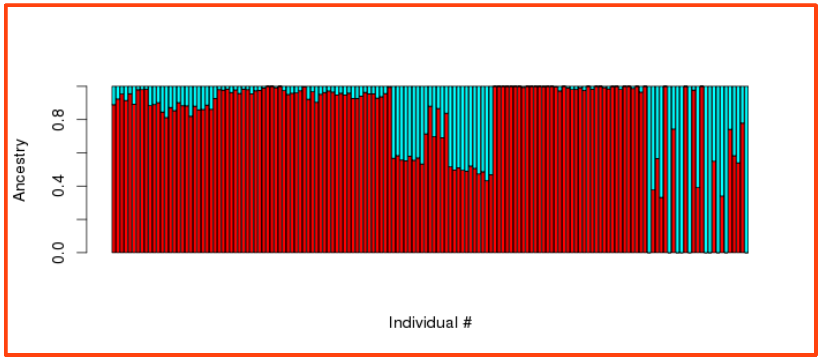


```
out_12.2.P
out_12.2.Q
out_12.2.Q.png
out_12.3.P
out_12.3.Q
out_12.3.Q.png
out_12.4.P
out_12.4.Q
out_12.4.Q.png
out_12.5.P
out_12.5.Q
out_12.5.Q.png
out_12.6.P
out_12.6.Q
out_12.6.Q.png
out_12.7.P
out_12.7.Q
out_12.7.Q.png
out_12.log
out_12.map
out_12.nosex
out_12.ped
out.cv_error
```

```
--admixture start
Prepare Admixture...
Obtain;[root of]/l0.05-m0.05/admixture/out_12.ped(map), --recode12

Admixture analysis proceed...
1- tree k=2
2- obtain figure [root of]/l0.05-m0.05/admixture/out_12.2.Q.png
1- tree k=3
2- obtain figure [root of]/l0.05-m0.05/admixture/out_12.3.Q.png
1- tree k=4
2- obtain figure [root of]/l0.05-m0.05/admixture/out_12.4.Q.png
1- tree k=5
2- obtain figure [root pf]/l0.05-m0.05/admixture/out_12.5.Q.png
1- tree k=6
2- obtain figure [root of]/l0.05-m0.05/admixture/out_12.6.Q.png
1- tree k=7
2- obtain figure [root pf]/l0.05-m0.05/admixture/out_12.7.Q.png
--admixture done
```

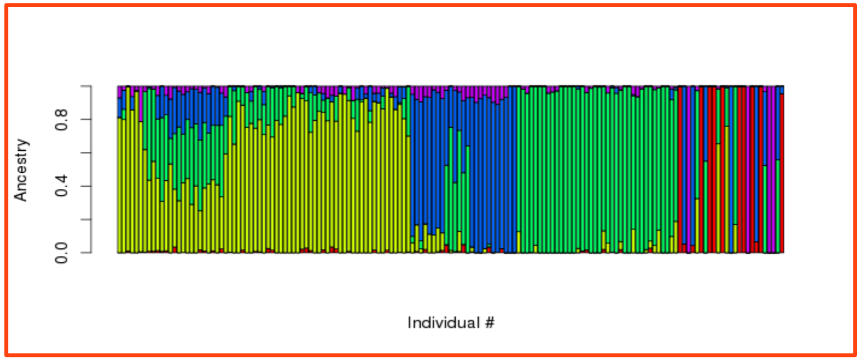
out.cv_error; K with smallest CV is optimal suggested from ‘admixture’

out_12.2.Q.png



……

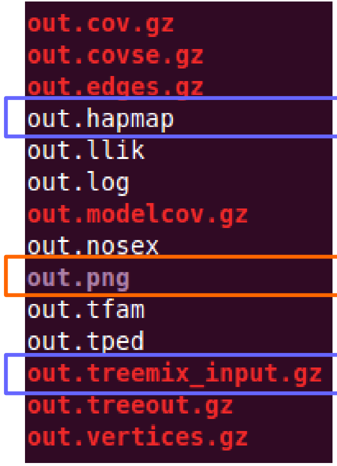
out_12.5.Q.png



….K=7

SAT_mix output; treemix

treemix

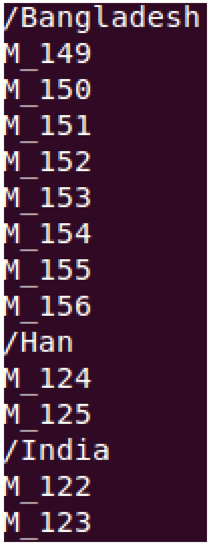


```
out.cov.gz
out.covse.gz
out.edges.gz
out.hapmap
out.llik
out.log
out.modelcov.gz
out.nosex
out.png
out.tfam
out.tped
out.treemix_input.gz
out.treeout.gz
out.vertices.gz
```

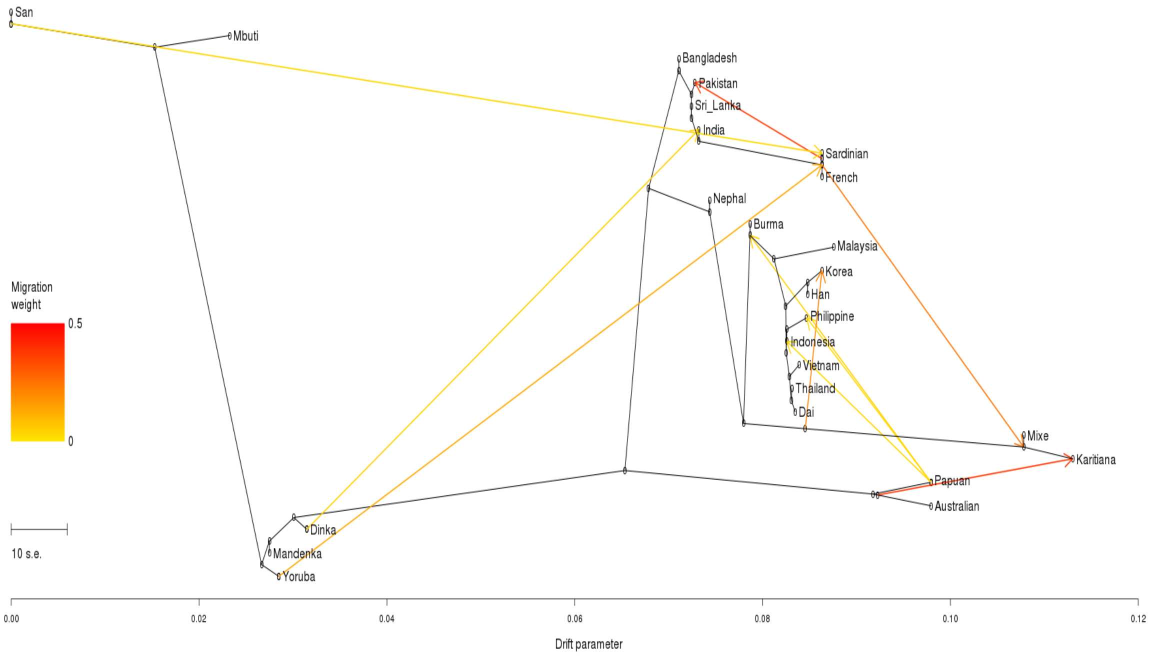
```
TreeMix analysis proceed...
/San

--treemix start
Prepare TreeMix...
Convert [root of]/l0.05-m0.05/out.picked.ped(map) -> [root of]/l0.05-m0.05/treemix/out.hapmap
Obtain;[root of]/l0.05-m0.05/treemix/out.hapmap
1- convert hapmap -> treemix input format
2- gzip compress [root of]/l0.05-m0.05/treemix/out.treemix_input -> [root of]/l0.05-m0.05/treemix/out.treemix_input.gz
3- run treemix, -m 10 -root San
4- obtain figure[root of]/l0.05-m0.05/treemix/out.png
--treemix done
```

group_index in file use with argument '-t'
In this case, grouping is based on individual's nationality

/[name of group] #'/' at the front!
..
… [name of individual]

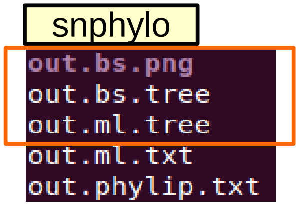


```
/Bangladesh
M_149
M_150
M_151
M_152
M_153
M_154
M_155
M_156
/Han
M_124
M_125
/India
M_122
M_123
```



SAT_mix output; snphylo

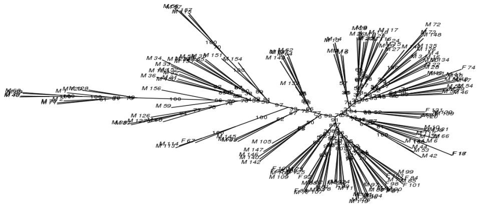
snphylo



```
out.bs.png
out.bs.tree
out.ml.tree
out.ml.txt
out.phylip.txt
```

out.bs.tree; newick tree with bootstrap score

out.bs.png; image file of out.bs.tree



out.ml.tree; newick tree

MUSCLE options; multiple sequence alignment

1. SNP sequence <= 50000
Muscle -phyi -in [input].fasta -out [output]

2. 50000 <= SNP sequence < 100000
Muscle -phyi -in [input].fasta -out [output] -maxiters 2

3. SNP sequence >= 100000
Muscle -phyi -in [input].fasta -out [output] -maxiters 1 -diags -sv

As sequence get longer, alignment accuracy decrease

```
--snphylo start
MSA proceed using 5348 SNPs

BS tree draw proceed

(spaces)

Nucleic acid sequence Maximum Likelihood method, version 3.695

Settings for this run:
  U                 Search for best tree?  Yes
  T        Transition/transversion ratio:  2.0000
  F       Use empirical base frequencies?  Yes
  C                One category of sites?  Yes
  R           Rate variation among sites?  constant rate
  W                       Sites weighted?  No
  S        Speedier but rougher analysis?  Yes
  G                Global rearrangements?  No
  J   Randomize input order of sequences?  No. Use input order
  O                        Outgroup root?  No, use as outgroup species  1
  M           Analyze multiple data sets?  No
  I          Input sequences interleaved?  Yes
  0   Terminal type (IBM PC, ANSI, none)?  ANSI
  1    Print out the data at start of run  No
  2  Print indications of progress of run  Yes
  3                       Print out tree  Yes
  4       Write out trees onto tree file?  Yes
  5   Reconstruct hypothetical sequences?  No

  Y to accept these or type the letter for one to change

Adding species:
   1. M_39
.
.
.
 157. M_15

Output written to file "outfile"

Tree also written onto file "outtree"

Done.

--snphylo done
!End without notable errors
```