Convert a Plink PFD file to TreeMix:

- 1. Convert Genepop or whatever file to PED and MAP files for use in plink. These should have the same name, but different extensions (i.e. .ped and .map)
- 2. Convert this ped file to a binary ped file in Plink: ./plink --file InputFile --make-bed --out binaryOutput
- 3. Now make a frequency cluster file sorting data into clusters (probably your sampling sites):
 - ./plink --noweb --bfile binaryOutput --freq --within clusters.clust --out SNPSforTreemix

The clusters.clust file should be a modified version of the .fam file that is created when you make the binary ped file. Open this .fam file in a text editor, remove all columns except 1 and 2, and add a third column with the cluster name you want to assign for every individual. In the end, column 1 is the 'Family' column, 2 is the individual ID, and 3 is the cluster. There cannot be any trailing spaces, and these numbers/strings should be separated by spaces only, not tabs. Also, not sure if it needs to be .clust when you're putting it into Plink, .txt should be fine too.

- 4. Now gzip this created output file.
- 5. Run the python script that came with TreeMix. Put the Plink output gzipped file in the same directory as the python script, and in the terminal type: plink2treemix.py SNPSforTreemix.frq.gz treemixInput.frq.gz
- 6. This output gzipped file can be run directly in TreeMix now!

***TreeMix is fairly easy to use, the manual is helpful. Everything is run from the terminal, and then use R or RStudio to plot the resulting tree and residuals for migration events if you wish. Plotting functions for R are included in TreeMix and are also listed in the manual.