Supplemental File 1

Linkage Disequilibrium Prune

```
./plink --vcf MyGenotypes.vcf --vcf-require-gt --allow-extra-chr --chr-set 20 no-y no-xy
    no-mt -indep-pairwise 500kb 1 0.99  -out SNPsInLD
```

Map Reads.

```
bowtie2 --end-to-end -x tifrunner -f -U FRT-markers.fasta --rg-id FRT-markers --rg
    SM:FRTmarkers -S FRT-markers.sam
```

Install Anaconda, and create Conda environment.

```
#Install Conda
wget https://repo.anaconda.com/archive/Anaconda3-2021.05-Linux-x86_64.sh

#Run Bash Script to Install
bash Anaconda3-5.3.1-Linux-x86_64.sh

#Close Ubuntu WSL – Reload to Source .bashrc file

#Create Conda Environment
create -n bioenv python=3.7
```

Activate bioenv environment

```
conda activate bioenv
```

Install channels and dependencies

```
conda config --add channels defaults
conda config --add channels bioconda
conda config --add channels conda-forge
conda install bowtie2
conda install samtools
#If not updated samtools
conda install samtools==1.12
conda install freebayes
```

Untar, unzip and shorten file names

```
#Untar tarball
tar -xvf *.tar

#Unzip fastq.gz
gzip -d *.fastq.gz

#Python line used to shorten file names
import os
path = os.getcwd()
[os.rename('{}'.format(file), '{}.fastq'.format(file.split('_')[0] + "-" + file.split('_')[1])) for
    file in os.listdir(path) if "fastq" in file]
```

Align reads to Tifrunner gnm2.

```
 for i in `ls -1 FRT*.fastq | sed 's/\.fastq//'`
do
bowtie2 --local -x ../Bioinformatics/Tifrunner -q -U $i.fastq --rg-id $I --rg SM:$i -S
        $i.sam
done
```

Filter SAM file to remove multi-mapping reads and sorting

```
#Filter using AS > XS
for i in `ls -1 FRT*.sam | sed 's/\.sam//'`
do
samtools view –h –e "[AS]!=[XS]" –o ${i}_F.bam ${i}.sam
done &

 #Sort Bam Files for Freebayes
for i in `ls -1 FRT*.bam | sed 's/\.bam//'`
do
samtools sort ${i}.bam –o ${i}S.bam
done &
```

Call SNPs with Freebayes using Ubuntu on Windows

```
for i in {1..6}
do
freebayes –f ./Bioinformatics/arahy.Tifrunner.gnm2.J5KF.genome_main.fna
        FRT$i*_FS.bam > FRT$i-NovSeq.vcf
done
```

Input to Henry 2 cluster to call SNPs with Freebayes.

```
more FRT.csh
#!/bin/tcsh
#BSUB -n 8
#BSUB -W 18720
#BSUB -J FRT-NovSeq
#BSUB -R span[hosts=1]
#BSUB -R select[qc]
#BSUB -o stdout.%J
#BSUB -e stderr.%J
conda activate /usr/local/usrapps/peanutbreeding/bioenv
freebayes -f arahy.Tifrunner.gnm2.J5K5.genome_main.fna FRT-NovSeq.bam >
        FRTNovSeq.vcf
conda deactivate
```

Filter VCF for only targeted markers.

```
vcftools --vcf FRT-MiSeq.vcf --snps FRT-markers.txt --recode --recode-INFO-all --out
        FRTMiSeq-filter
```