**Expanded analysis using Bowtie, Rsubread and NOISeq**

**PART ONE – Bowtie**

1. First, one must run Bowtie in order to align the raw sequencing reads to P. infestans genome T30-4 from NCBI by only eliminating tRNAs as contamination.
2. This alignment must be done for each isolate, biological replicate and condition.
3. To do it, one must have some files in one same folder: the script to run Bowtie, raw sequencing reads fastq file, txt with tRNA sequences from *P. infestans, P. infestans* fastagenome from NCBI.
4. Use command qusb to run the script.
5. The output for each alignment will be a SAM file calles Isolate\_0-0\_BR\_final or Isolate\_100-100\_BR\_final, depending on the treatment.

**PART TWO – Rsubread**

1. Then, we are going to generate read count files from these alignments.
2. Load R using command: module load R/3.3.2mro
3. Write R to enter the console.
4. Download the following:
   1. source("https://bioconductor.org/biocLite.R")
   2. biocLite("Rsubread")
   3. library(Rsubread)
5. Run the following command for each resulting alignment:

fc<-featureCounts(files="Isolate\_0-0\_BR\_final",annot.ext="Pinfestans.gtf", isGTFAnnotationFile=TRUE,GTF.featureType="exon",GTF.attrType="gene\_id")

1. The annotation file Pinfestans.gtf must be in that same folder.
2. Run this to save the counts to a txt: write.table(x=data.frame(fc$annotation[,c("GeneID","Length")],fc$counts,stringsAsFactors=FALSE),file="counts.txt",quote=FALSE,sep="\t",row.names=FALSE)
3. Download each count txt and unite the ones that belong to one isolate into one excel file with the following headers

GeneID Isolate0BR1 Isolate0BR2 Isolate0BR3 Isolate100BR1 Isolate100BR2 Isolate100BR3

**PART THREE - NOISeq**

1. In order to analyze if this count files indicate DEG, we used the package NOISeq in R Studio.
2. Upload the final count files (one for isolate) to R Studio as well as the txt containing the Gene IDs and lengths.
3. Run script named Script\_NOIseq-R.R and you will end up with three files per isolate, one with all DEG, one with upregulated ones, one with downregulated ones.

**Note:** As in the re-analysis, those genes known only as conserved hypothetical proteins were ran in Blast2Go to try to annotate them. The Blast2Go results table is also included in the Final\_results folder.