**SmallncRNAs – Instructions**

**PART ONE**

1. In order to process the small ncRNAs raw data, you need to have certain files in one same folder:
   1. Txt with tRNA sequences.
   2. Txt with rRNA sequences.
   3. Fasta with *P. infestans* fasta genome.
   4. Gtf file of *P. infestans.*
2. With those files in the same folder use qsub command to run the script called SmallRNAsARPipeline.sh
3. That will result in many files, we are more interested in the ones named, for example, US23-0-1-count, which are the count files for each isolate, biological replicate and treatment.
4. 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 TWO**

1. In order to analyze if this count files indicate DE small ncRNAs, 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 SmallRNAsNOISeq.R and you will end up with three files per isolate, one with all DEG, one with upregulated ones, one with downregulated ones.