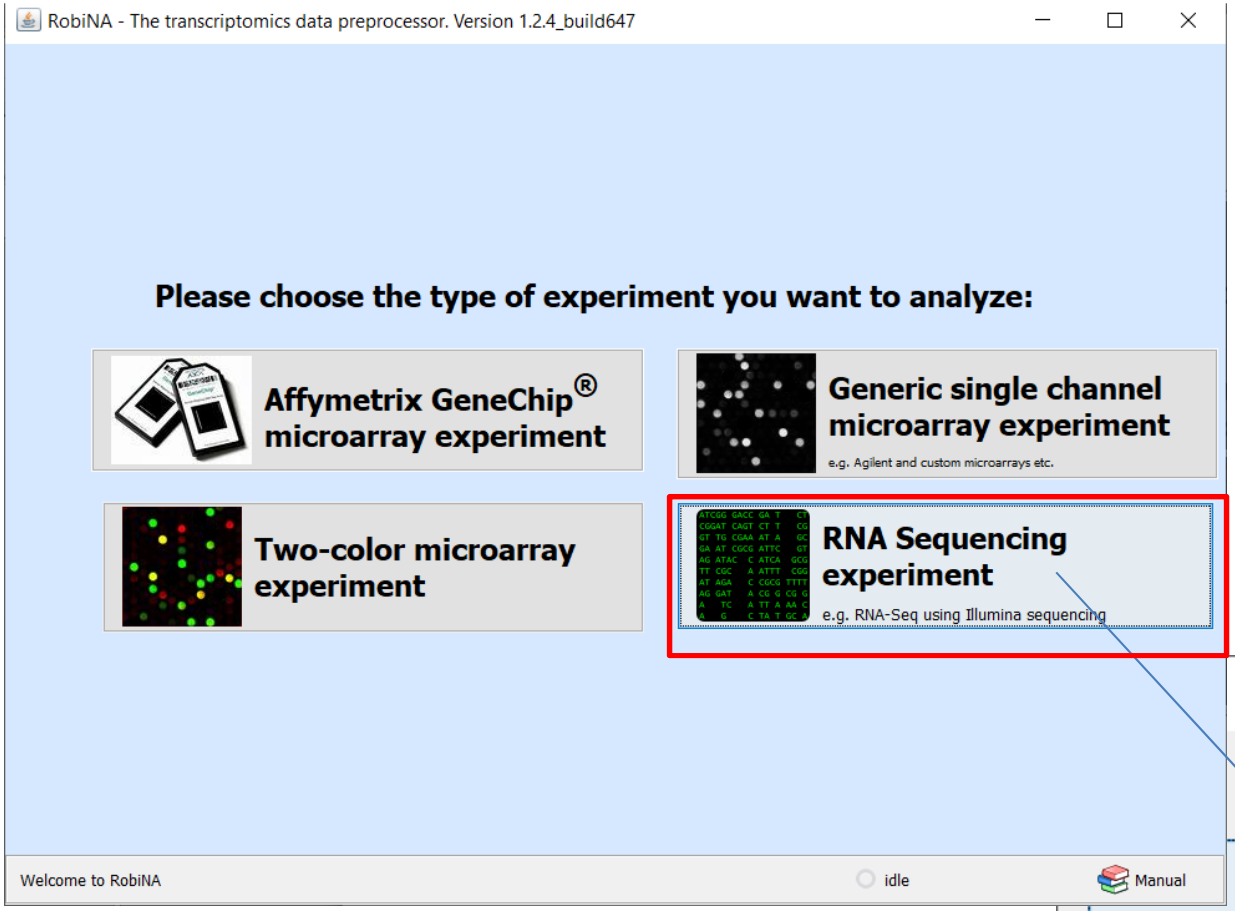
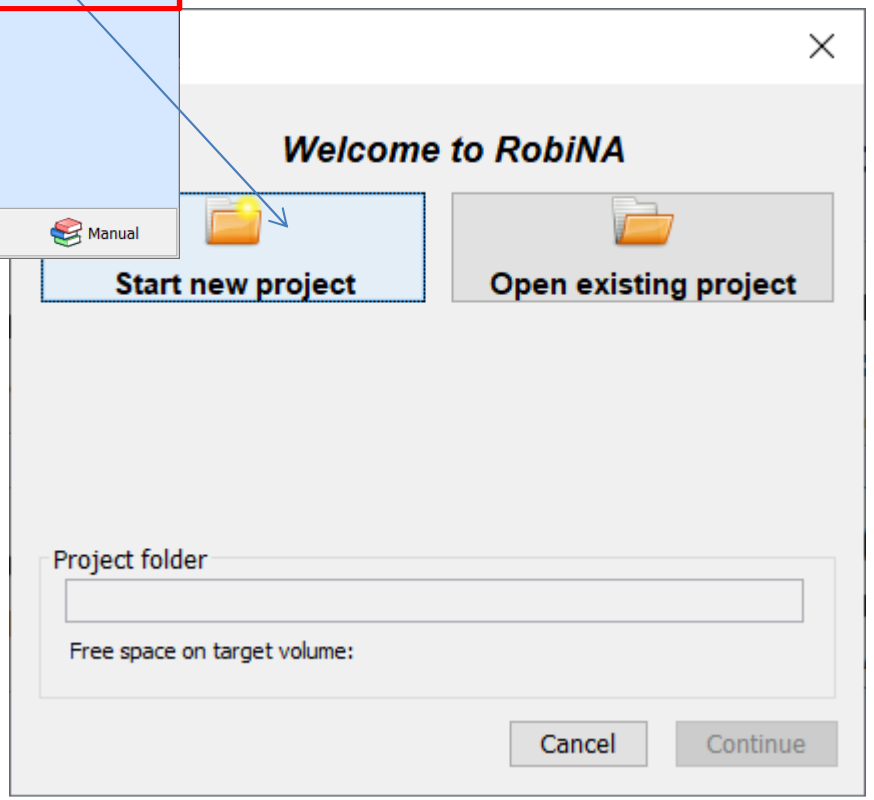
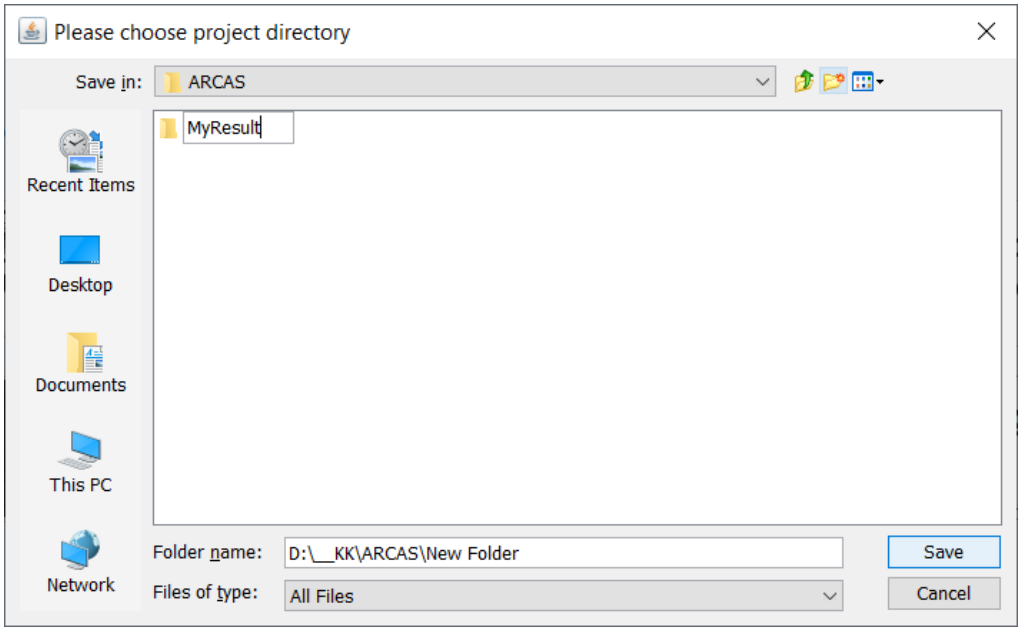


Choose RNA Sequencing Experiment.

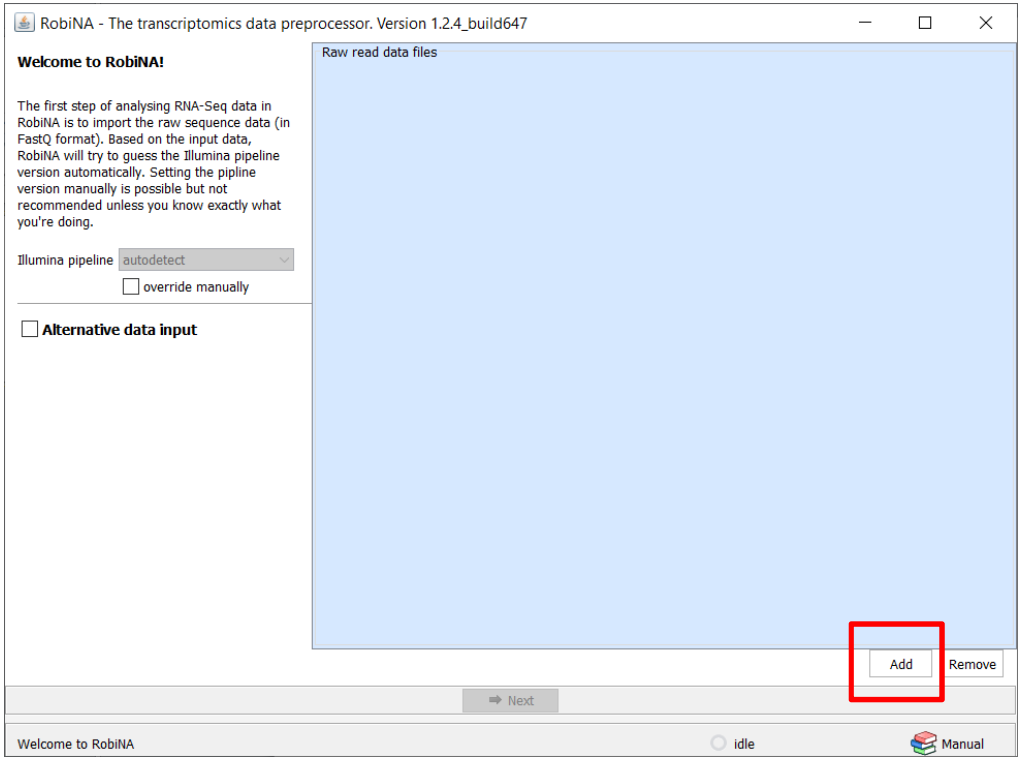


Select *Start New Projectc*

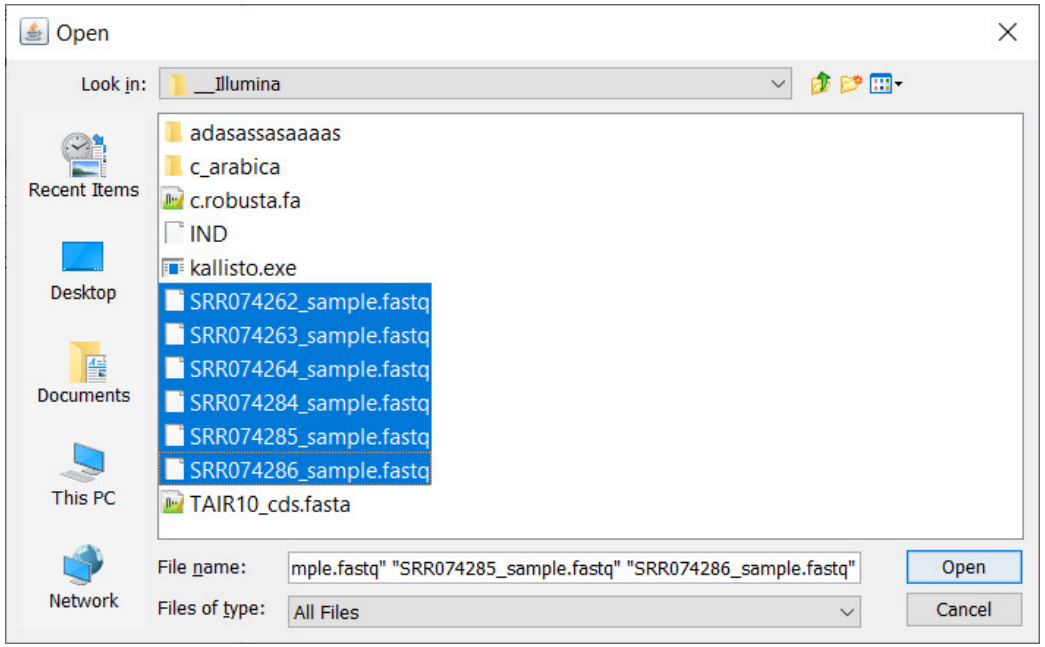
Microarray support in Robina-Arcas branch will be discontinued and be replaced by DNASeq and single cell based analysis methods.



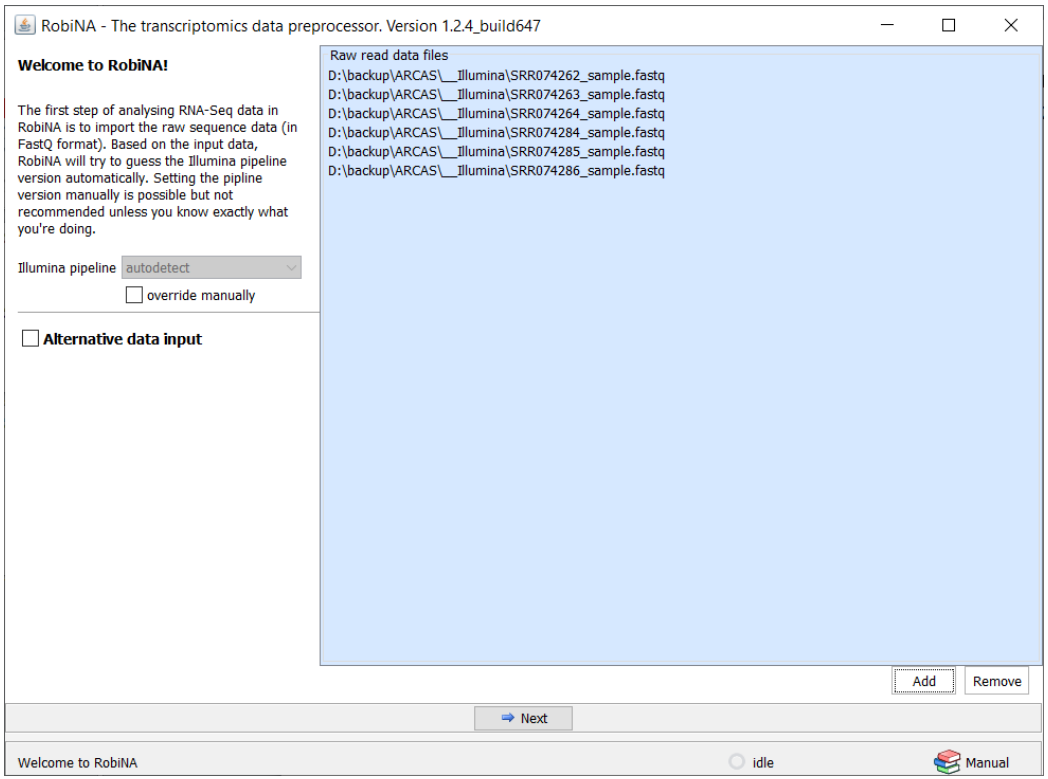
Select an empty Folder for the project or create a new empty folder



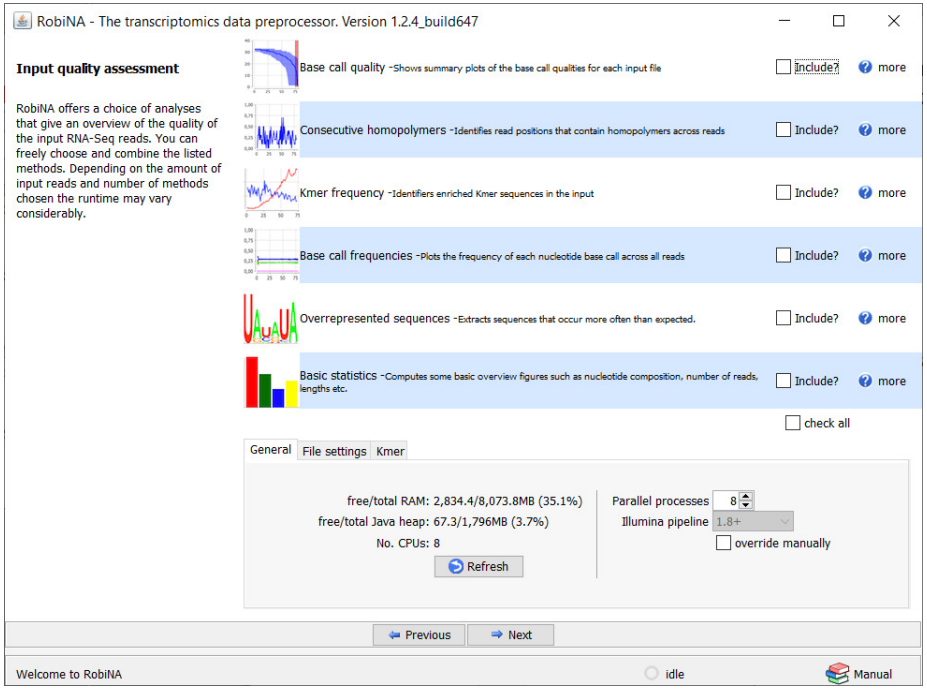
Press add to add Illumina files
(or alternative input for sam/bam)

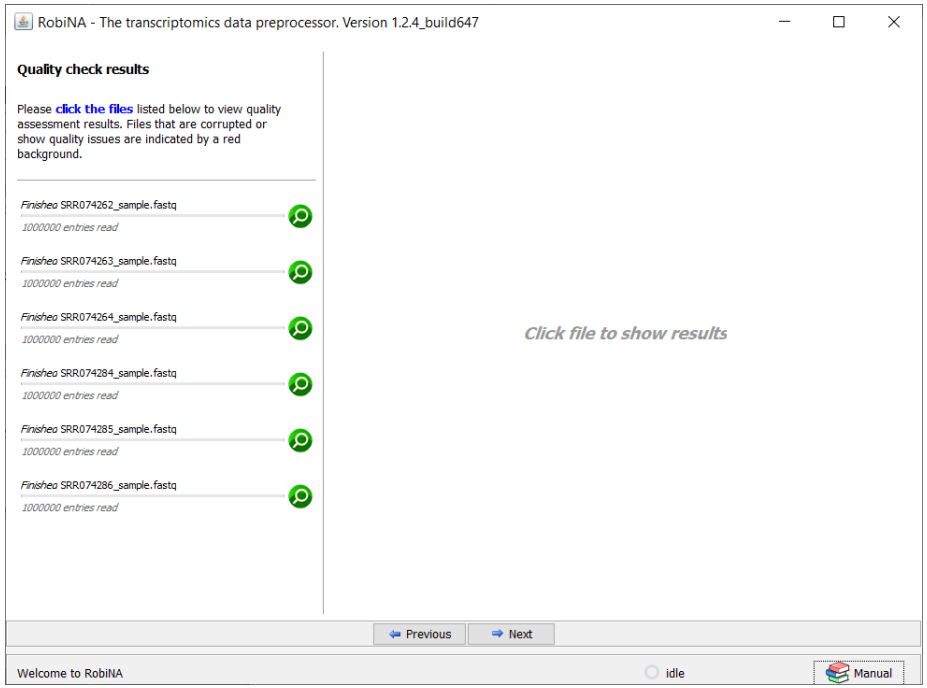


Select raw Illumina files. You can use e.g. shift-click to batch select multiple files



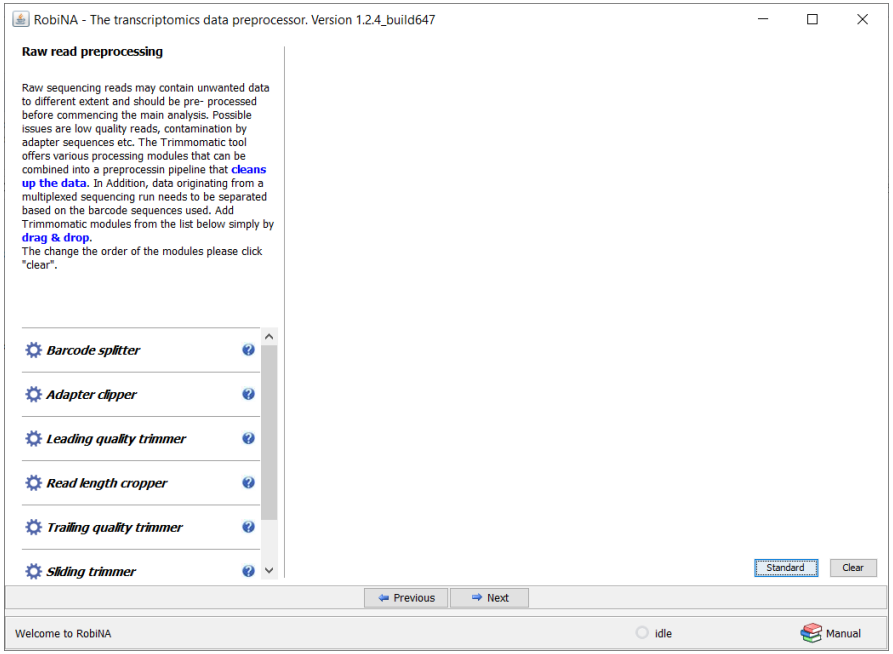
The files will appear in your main window





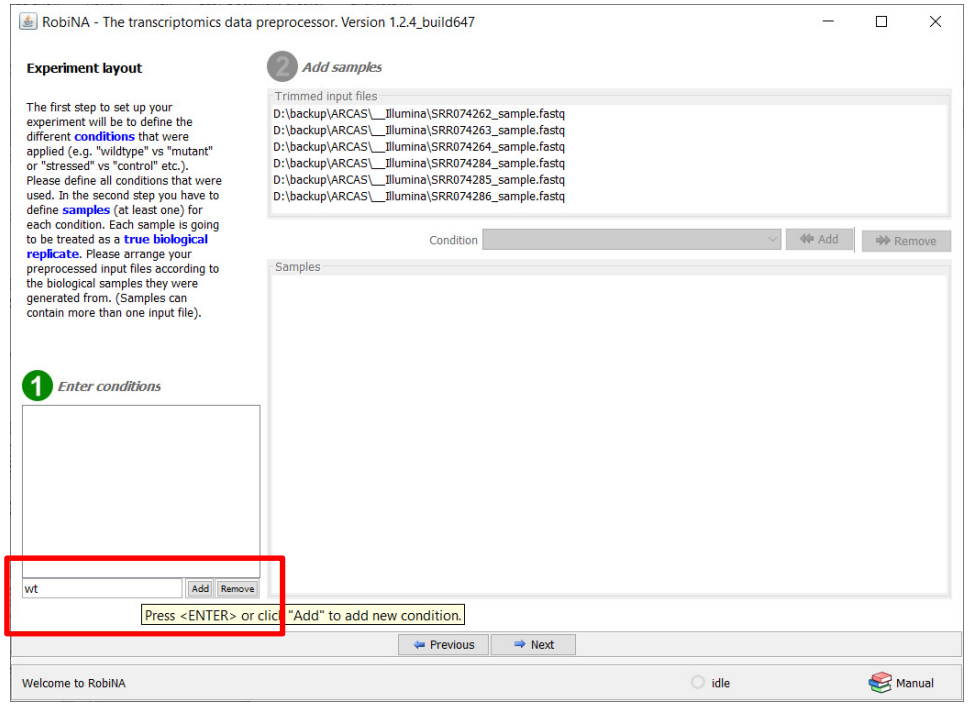
Select QCs you want to perform. If you didn't QC your files before do it here. We recommend at least base call quality and base call frequencies. At the bottom you find machine settings that Robina autodetected. (e.g. logical CPUs)

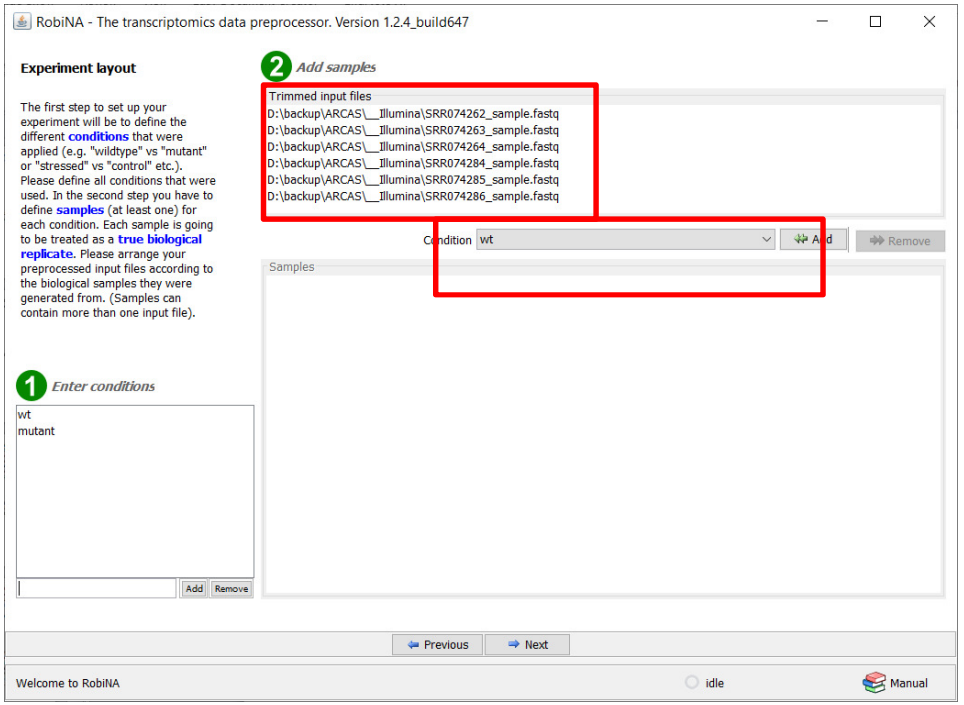
As Robina goes through you see a progress bar. After a while you can check QC file by file.



This is a pathological example. You can see base enrichments per base here! Basically this means you sequenced one biomolecule (e.g. an adapater)
Base quality is around 30 which is good.

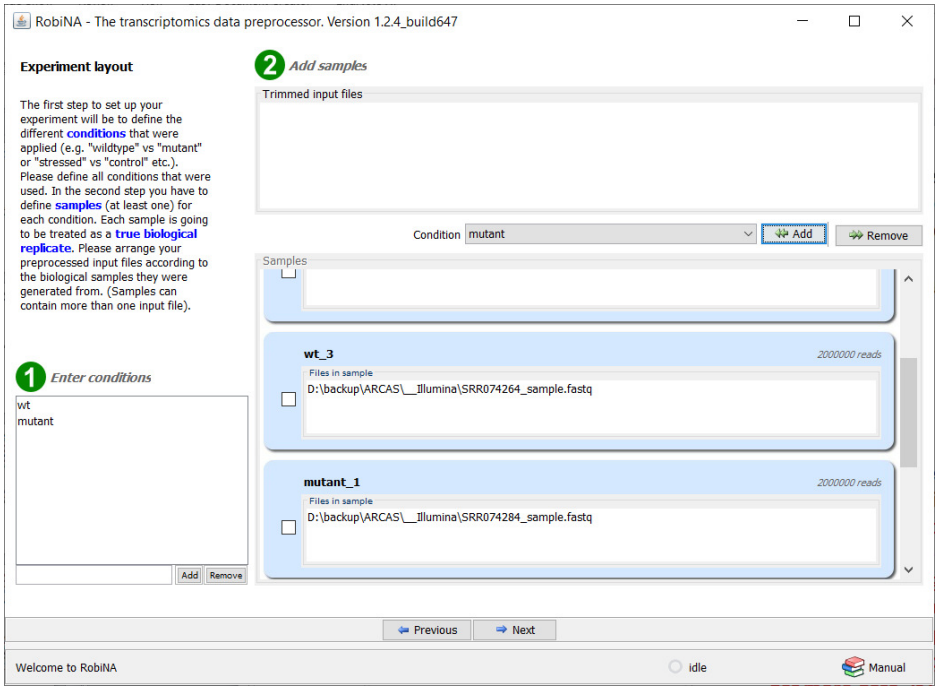
You can now trimm the data files using Trimmomatic. Arcas does not need quality trimming anymore, this is in general not recommended.
You can even forgo adapter clipping if you are in a hurry and your QC looks reasonable. (not like the one above)



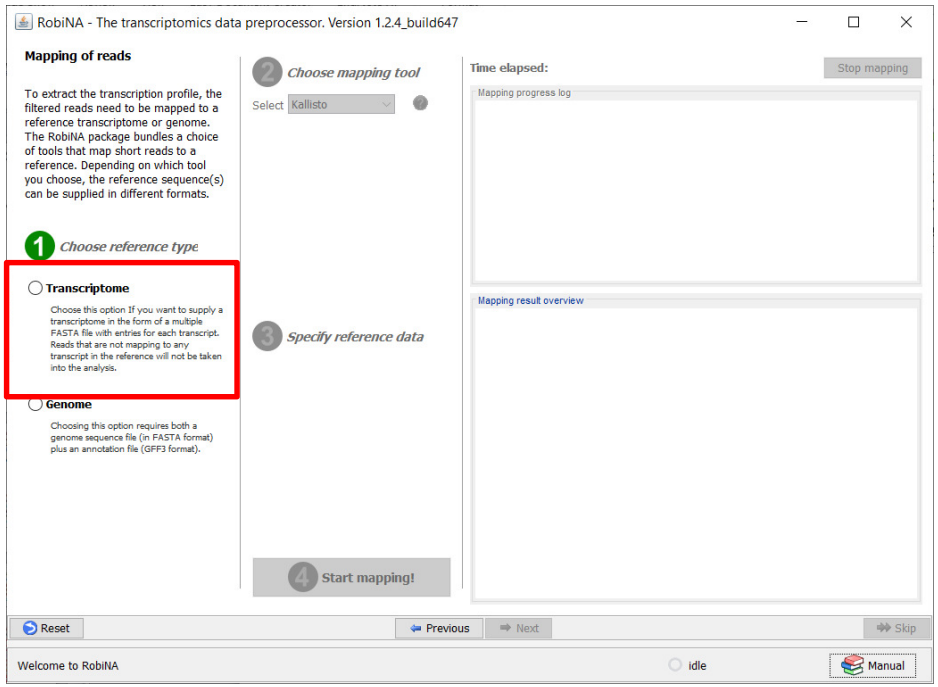


Add your conditions here. In case you have multiple factors Robinas follows the edgeR preferred analysis way to ask for contrasts of interest only, i.e. please add them e.g. if you have wt and mutant and 0h and 3h use wt_0h wt3_h mut_0h and mut_3h

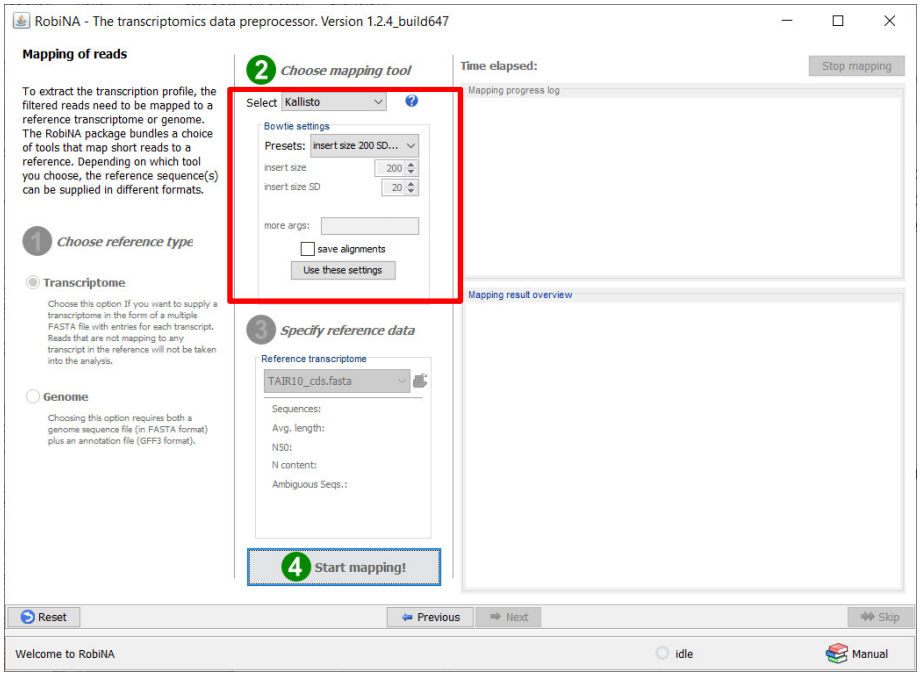
Now add files one by into the appropiate condition. If you select mutiple files and once for a condition this means you consider these as part of the same biological replicate! This happens e.g. if your samples are spread over multiple lanes or if you have to resequence your samples to get more data. If your files represent multiple biological replicates do not batch select them.



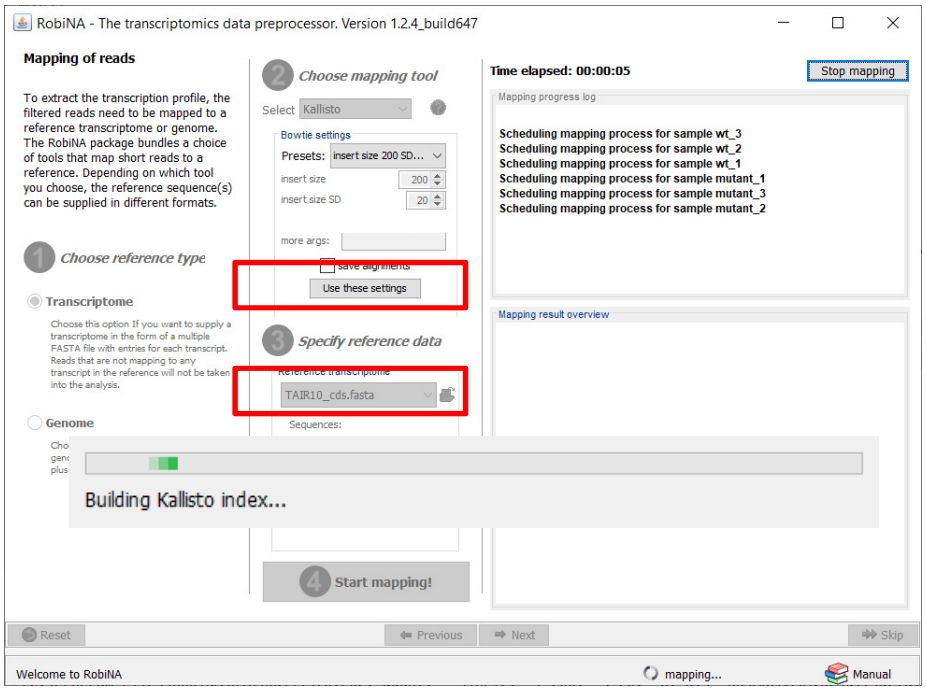
Your files are added into the replicate groups and biological replicates numbers are marked with _1 _2 _3 …



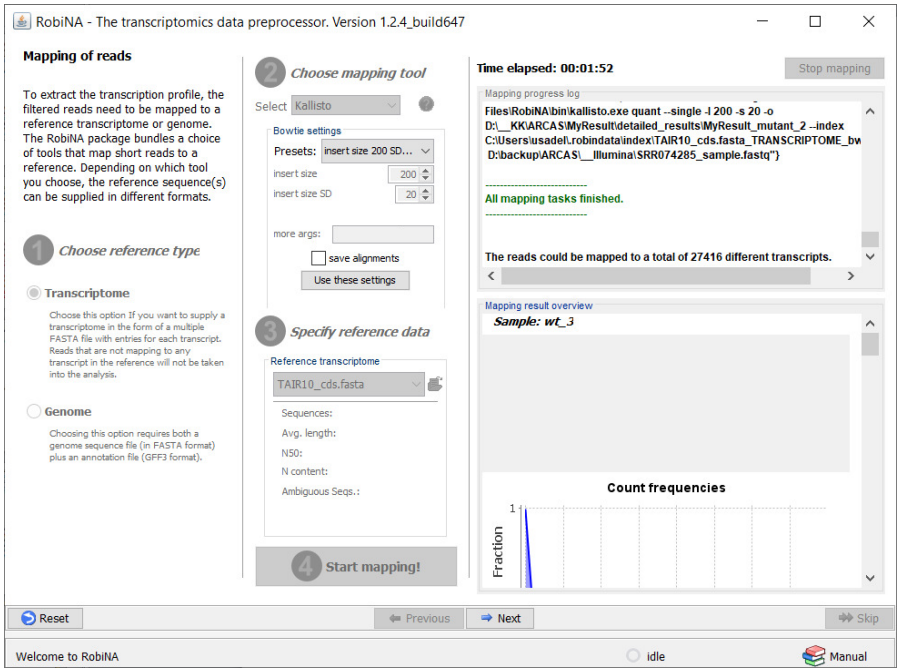
Choose Transcriptome



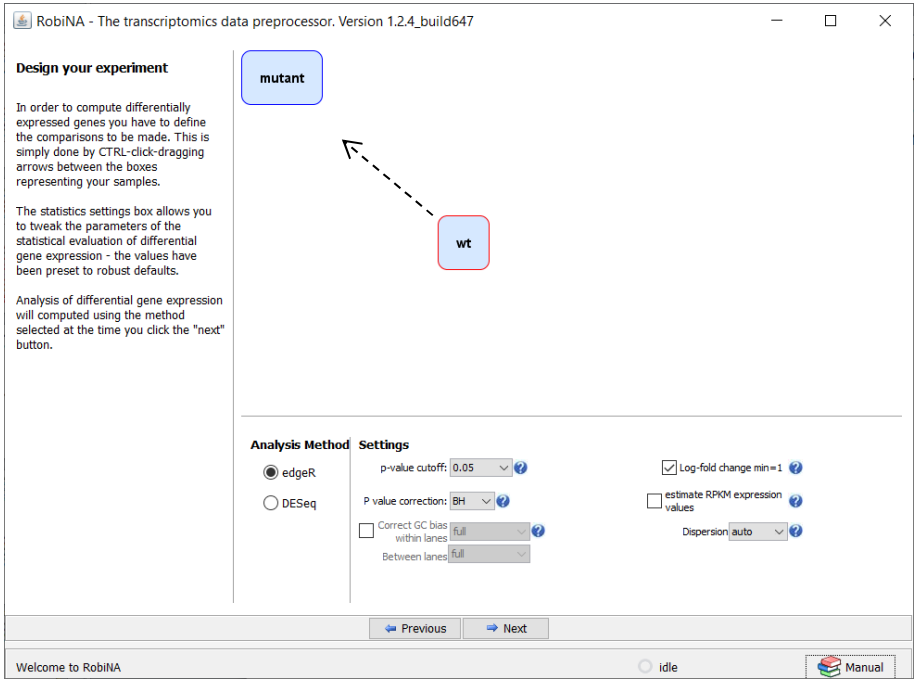
Add in insert size and insert size standard deviation (SD) from your data sets. You can obtain this e.g. from the QC gels prior to running your samples. By default we supply 200/20.



The first time you work with a transcriptome you need to build an index by pressing “Use these settings” in box2 and selecting the transcript data file in box 3 (Use full length mRNA including UTRs)



You can see the progress and check the kallisto command in the upper box. Once finished you get a count frequency diagram in the lower box



Now you can formulate your query by Ctrl-click-dragging arrows

Your analysis is finished you can find all data sets in the folder your selected in the beginning