Transcriptomics, Weekly Notes

Week 1 (18/6-24/6)

Lab was prepped. Inoculating and incubating E.coli cultures. Running practise TotalRNA purifications to get a feel for the work we will be doing. Also performed one rRNA-depletion and precipitation with poor results - some minor troubleshooting is done.

Week 2 (25/6-1/7)

Running poly(A)-tailing reactions + cDNA conversion. Neither of them works well, poly(A)-tails seem to be degraded and little to no cDNA yield. Another rRNA depletion is done with good results. A new totalRNA purification was also done. Plenty of nucleic acid material for practise next week.

Week 3 (2/7-8/7)

Troubleshooting Poly(A)-tailing reaction by trying to remove the incubation step. Another cDNA conversion done with poor results. PCR amplification is done on the cDNA product using the same primers, with good yields - proves that the cDNA protocol works. Our spectrophotometers are troubleshooted due to them giving strange OD600 results - the absorbance is measured and cultures are plated for CFU counting next week.

Week 4 (9/7-15/7)

CFU counting shows illogical results - we will redo our M9 media as things don't seem to want to grow in it. Practising RNA precipitation protocol, we seem to have an overall loss of 20% of nucleic acids after doing the step. The rRNA step is done with control RNA to check if any nucleic acids are lost in the process. New M9 is made and a new batch of CFU plates are done - an equation is made to determine total cell amount at a certain OD600 value.

Week 5 (16/7-22/7)

First totalRNA purification with live strongyles is done with good results. rRNA depetion is done on the RNA yield with good results. Poly(A)-tailing step is also done with good results. A cDNA conversion is done with a good yield as well - despite measurements showing that about 20% of the cDNA is lost during the purification step. The fact the cDNA has been successfully converted proves that the poly(A)-tailing step must work.

Week 6 (23/7-29/7)

A new cDNA conversion step is done on poly(A)-stocks from last week - sequencing is coming up and we want things to be right. A new rRNA depletion is done on totalRNA stocks from last week to have backup material. Yet another E.coli worm culture is grown and another totalRNA purification is done with alright results. cDNA was end-prepped. Samples sent for sequencing did not give a good gene expression profile. Possible salt contamination could be the issue. Low pore occupancy in the nanopore sequencing device.

Week 7 (30/7-5/8)

A new rRNA depletion was done on the totalRNA from last week. The following precipitation step resulted in 75% of the RNA being lost. Some precipitation practise was done due to this. More worm cultures were incubated and a totalRNA purification was done with poor results - probably due to old lysozyme used. Poor precipitation results is believed to be due to old ethanol used. Another totalRNA purification was done with better results, followed by rRNA depletion with poor results, the step is redone the following day. A new poly(A)-tailing step and cDNA conversion is done in anticipation of a second sequencing, but the cDNA conversion step fails with no yield.

Week 8 (6/8-12/8)

Yet another rRNA depletion was done with totalRNA stocks and the pipeline was followed to the cDNA conversion step, which had an okay yield this time. cDNA was end-prepped and sequenced, again with poor results. The DNA might be getting fragmented in the steps leading up to the sequencing. Another rRNA depletion all the way to cDNA conversion was done with good results. They will be end-prepped next week and sequenced. A new worm culture was set up for totalRNA purification next week to replenish our stocks.

Week 9 (13/8-19/8)

All reagents are checked to determine how many runs we have left. End-prepping of last weeks cDNA samples was done. A third sequencing run was done, still with too poor results to be used. Worm culture from last week is ready and we do two totalRNA purifications, because one of them failed. The second one worked well. An rRNA depetion was done on this material, good results. Poly(A)-tailing and cDNA conversions steps were also done with good results. These will be end-prepped for another try at sequencing next week, the error may be our performance doing the protocol.

Week 10 (20/8-26/8)

The cDNA from last week was end-prepped and sequenced, still with bad results. DNA appears to be heavily fragmented and also contaminated with RNA. Using totalRNA stock, we do another rRNA depletion + Poly(A)-tailing and cDNA conversion. This cDNA is also end-prepped and sequenced - same results. Troubleshooting starts by measuring RNA amounts in sample after cDNA step, we’re also testing the efficacy of our RNA degrading enzymes.

Week 11 (27/8-31/8)

We continue our cDNA troubleshooting and attempt for one final sequencing using our remaining materials. However, the final cDNA conversion fails and we have ran our of reagents. We conclude that we do not have enough time left to figure out why our sequencing material is of poor quality.