Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

RT-qPCR is a method by which one can quantify (relative to a control sample) how many copies of an RNA there are in a cell. This protocol is useful if you are interested in seeing whether the gene expression is altered within cells. This protocol is a three step procedure that involves RNA isolation, reverse transcription and real-time PCR.

RNA isolation

For this protocol it is vitally important to wear gloves while isolating RNA and use filtered tips some to decrease the possibility of contamination. RNA isolation is easiest with the RNeasy Mini Kit from Qiagen. This kit is able to isolate RNA from cells or tissue using a column purification method where you can obtain high quality RNA. If you are interested in isolating small RNAs, such as micro RNAs, Ambion's mirVana RNA isolation kit can be used to isolate these RNAs. The protocols for each kit are attached. Follow these protocols to successfully obtain mRNAs or microRNAs.

RT reactions

Once you have isolated your RNA, store these samples at -80°C at all times. RNA is easily degraded so you should only thaw your samples once or twice when performing these steps. If you want to keep a stock of RNA samples for an extended period of time, make small aliquots of each sample and store them at -80°C.

You will need to quantify how much RNA is present in each sample. To quantify RNA you must obtain a 260/280 nm value for each sample. This ratio (for good and not degraded RNA) should be between 1.8 and 2.0. You will also need to determine the concentration of RNA in terms of µg/µL.

For the RT reaction each tube should contain 1 to 2 µg of RNA per sample. The amount of RNA must be equal in all the reactions so that the efficiency of the RT step is equal between samples. The RT reactions for mRNA can be completed using the High Capacity cDNA Reverse Transcription Kit from Life Technologies. This kit uses a random set of primers to RT all mRNAs. This random primer is part of the kit. Alternatively if you want to RT micro RNAs then you will use the TaqMan® MicroRNA
Reverse Transcription Kit from Life Technologies. This kit uses a specific primer to RT a single micro RNA. This primer is NOT part of the kit and must be purchased separately.

An excel spreadsheet has been provided so that you can calculate how much of each reagent is needed for the RT step. Once the RT reaction has been completed (usually in a total of 20 microliters) this cDNA can be stored at -20°C.

qPCR reactions

Once you have finished the RT reaction you will have 20 microliters of cDNA. Add 80 microliters of RNase free water to each RT sample prior to doing the qPCR. (This dilutes the cDNA so that your PCR will run correctly). An excel spreadsheet has been provided so that you can calculate how much of each reagent is needed for the qPCR step. For qPCR to work, you

need to have 2 different primer sets – one that amplifies a housekeeping gene such as actin, 18S,