

GENOMICS RESEARCH CORE**Full Service Realtime PCR Methods****Reverse Transcription**

The Genomics Research Core uses the High Capacity RNA to cDNA Reverse Transcription kit from Thermo Fisher according to manufacturer's instructions. Our standard methods are to use 800 ng total RNA in an RT reaction and to perform both a standard RT with enzyme and a negative control without enzyme.

Realtime PCR

Thermo Fisher Gene Expression Master Mix, for Taqman or SYBR Green as appropriate, is used at final 1X concentration in a 20 μ l reaction volume on a 96 well plate. Assays on Demand are also used at a final 1X concentration as defined by the manufacturer. Custom designed primers are added at 250 nM final concentration and probes at 100 nM. Genomics Research Core standard procedure uses 20 ng cDNA equivalents per PCR reaction.

Genomics Research Core routinely sets up 3 wells of PCR for each gene using each RT positive reaction and a single well for each gene using the corresponding RT negative control reaction.

Cycling conditions:

Standard cycling conditions for realtime PCR are:

95°C for 12 minutes

40 cycles of:

95°C for 15 seconds

60°C for 1 minute

Please note clearly on the drop off form if other cycling conditions are required for your assays.