Realtime PCR Assay Selection

Sources and Design Guidelines

- Predesigned and validated assays are available from:
    - Thermo Fisher (Applied Biosystems)
    - Qiagen (SABiosciences)
    - Bio-Rad

You can search for the genes of interest in the species of choice through the database interface. SAbiosciencs has a library of primers for SYBR green detection methods and sells them in preset array format as well as individually. You must use the SuperArray master mix for PCR. This option is most cost effective for instrument run level service. GRC does not stock the SuperArray mastermix and extra charges may apply. Sigma-Aldrich has products for reverse transcription and real time PCR.

- Primers or primer probe sets can be designed using any PCR primer design software.
- Amplicon length should be 50-150 bp.
- Primer length can be 20-25 bp with Tm 58-60°C Probes should be about 20-25 bp and have a Tm of 68-70°C
- When possible, probes should be designed to span an exon-exon boundary to minimize the potential for amplifying genomic DNA.
- When SYBR green chemistry is to be used, design primers to span an exon boundary if possible, for the same reason.
- Possible design web sites include:
    - GenScript
    - Primer3
    - IDT
    - Sigma-Aldrich