RNA extraction & QC:

Promega publications

- "Working with RNA" - http://promega.wordpress.com/2012/01/24/working-with-rna/
- "RNase Contamination Happens; Recombinant RNasin® Inhibitor Can Safeguard Your Samples. http://www.promega.com/resources/articles/pubhub/rnase-contamination-happens-recombinant-rnasin-inhibitor-can-safeguard-your-samples/
- "RNA Purification Kit Comparison: Yield, Quality and Real-Time RT-PCR Performance" - http://www.promega.com/resources/articles/pubhub/promega-notes-2008/rna-purification-kit-comparison-yield-quality-and-real-time-rt-pcr-performance/

RT-qPCR Resources (including those referenced in the webinar):

General

Gene Quantification - http://www.gene-quantification.info/

A-Z of quantitative PCR (Editor: S.A. Bustin), International University Line (IUL), La Jolla, CA, USA

Design, validation, controls; normalization -

- MIQE Guidelines - Minimum information for publication of quantitative real-time PCR experiments - http://www.rdml.org/miqe.php (Bustin et al., Clinical Chemistry, 2009)
- Sean Taylor, "A MIQE Case Study — Effect of RNA Sample Quality and Reference Gene Stability on Gene Expression Data" 2011, Bio-Rad tech note 6245
- Garbarino-Pico, E., Rollag, M.D., Strayer, C.A., Niu, S., Besharse, J.C., and Green, C.B. (2007) Immediate early response of the circadian polyA ribonuclease nocturnin to two extracellular stimuli. RNA 13: 745-755. PMID:17400819

Pre-designed qPCR Primers:

- *Primer Bank - http://pga.mgh.harvard.edu/primerbank/*
- *RTPrimerDB - http://www.rtprimerdb.org/*

Primer design software:

- *Primer3 - http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi*
- *Primer-BLAST - http://www.ncbi.nlm.nih.gov/tools/primer-blast/*

Real-Time qPCR Analysis Software:

geNORM - http://medgen.ugent.be/~jvdesomp/genorm/
Reference gene normalization software

QPCR - https://rtpcr.genome.tugraz.at/rtpcr/
Web-based Real-Time PCR data management and analysis

Real-Time PCR Terms:

ΔR – baseline-corrected fluorescence - R *minus* average R over baseline region

Passive reference – free dye (unassociated with amplification product) used for normalization (controls for experimental/optical variation)

R_n – normalized fluorescence (R divided by passive reference)

ΔR_n – baseline-corrected, normalized fluorescence.

Experimental Target – target gene/transcript/channel/dye of interest

Control Target – normalizer, endogenous control, reference gene/transcript/channel/dye, housekeeping gene

Experimental Sample – unknown, treated, e.g., tumor, mutant, +2 hours, etc.

Control Sample – calibrator, reference sample, e.g., untreated control, normal, time zero, wild type, etc.