MOLab TqPCR Protocol (Using 2x SYBR Green Master Mix)
(TCH @ 08/07/2017)

Preparation of qPCR primers

- Design with Primer3 Plus: http://primer3plus.com/cgi-bin/dev/primer3plus.cgi
- qPCR primer features: 18mers; Tm=60°C; amplicon size: 100-200bps
- **Dilute qPCR primers**: dilute Forward (Fwd) and Reverse (Rev) primers with ddH_2O to make 1µg/µl stock solution; take 5µl from each primer stock solution (total = 10µl) and add 490µl ddH2O; mix well and keep at -20°C or -80°C for qPCR use (i.e., each primer @ 10ng/µl).
- 2x SYBR Green qPCR mix: Bio-Rad, Thermo Fisher brands; We are using Bimake's 2x SYBR Green qPCR Master Mix (Cat# B21204)

qPCR Reaction Setup and TqPCR Cycling Program

RT-PCR (cDNA) template:	2.5µl
Primer mix (each at 10ng/µl):	2.5µl
2x SYBR Green Master Mix:	5.0µl **(or 2.5µl to save $$$)****

- Add 10ul mineral oil/tube or well
- Seal plates with film
- Briefly spin/tap down the contents in each tube/well
- Run **Touchdown-qPCR** program as follows (on Bio-Rad CFX-Connect unit):

1. 95°C × 3' for one cycle
2. 95°C × 20"
3. 66°C × 10"
4. GOTO **Step #2** for 4 cycles by decreasing 3°C per cycle
5. 95°C × 20"
6. 55°C × 10"
7. 70°C× 1"
8. plate read
9. GOTO **Step #5** for 40 cycles

** *We found that **2.5µl** of 2x Master Mix yielded similar Cq values (to that of 5.0µl 2x Master mix) for transcripts with average or above average abundance. Nonetheless, the use of 5.0µl of 2x SYBR Green Master Mix is recommended to detect extremely low abundant transcripts.*

Reference

Zhang Q, Wang J, Deng F, Yan Z, Xia Y, Wang Z, et al. (2015) TqPCR: A Touchdown qPCR Assay with Significantly Improved Detection Sensitivity and Amplification Efficiency of SYBR Green qPCR. PLoS ONE 10(7): e0132666. doi:10.1371/journal.pone.0132666
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0132666