

Quantitative RT-PCR

Follow standard protocol to make cDNA (see RT-PCR protocol).

- Combine 2 μ L from each cDNA reaction to make concentrated standard. Dilute conc. Standard 1:10 for standard 1 (St 1). Make 5-fold serial dilutions for the other standards to make at total of 4 standards. (St 1, St 2, St 3, St 4) These standards must be run for each primer on a plate to generate a standard curve.
- Dilute an aliquot of each cDNA sample 1:50.

Make a master PCR mix for each set of primers.

qPCR master mix	1X
SYBR (AB 4309155)	12.5 μ L
F primer (10 μ M)	1 μ L
R primer (10 μ M)	1 μ L
H ₂ O	5.5 μ L

- Add 20 μ L of qPCR master mix into each well on a 96well optical reaction plate (AB 4306737). Most consistent results are obtained using a single beveled tip for each well.
- Add 5 μ L of diluted cDNA to each reaction.
- Spin down @ ~1800rpm.

Set up reactions in ABI Prism 7700 Sequence Detector (R. Johnson Lab)

Open SDS 1.9.1 program, a new plate will automatically open.

qPCR RUN

In set up mode:

- Set dye layer to **SYBR** on dropdown menu.
- Sample Type → **SETUP**
 - Quencher → **NONE** & unclick box.
 - STND → **SYBR**
 - UNKN → **SYBR**
 - NTC → **SYBR**

Thermal Cycler Conditions

95°C 10m
95°C 15s } 40 cycles
60°C 1m

Press **show data collection** to ensure that data will be collected during 60°C. (it will normally collect at all steps)

All steps should now be set up. To start run:

Show Analysis

File → Save As → select file location and name.

RUN

The status will indicate it is waiting, the machine will beep, and start running the program. After program is done, status will indicate idle & sample temp will be @ 25°C.

SAVE RUN!!!!!!

- Analysis → **Analyze**
 - Set baseline so curves look linear.
 - Set Threshold @ linear part of graph.
 - **OK**

- File → Export
 - **Results**
 - **Clipped**
 - **Report**

Dissociation Curve

Exit and re-open SDS 1.9.1 program to open a new plate.

In set up mode:

- Set dye layer to **SYBR** on dropdown menu.
- Sample Type → **SETUP**
 - Quencher → **NONE** & unclick box.
 - UNKN → **SYBR**

Thermal Cycler Conditions

95°C 1m

65°C 1m

→ set ramp time to 5m

95°C 5s

File → Save As → select file location and name.

RUN

Analysis → Analyze → **OK**

File → Export → **Multicomponent**.

Press **show data collection** to ensure that data will be collected during the 5m ramp.

Drag spreadsheet into Dissociation Curves 1.0 software.