RNA Quality
Determine RNA quality with use of Agilent Bioanalyzer 2100.

Turbo DNA-*free*:
Add the following to a RNase-free, 0.5-ml microcentrifuge tube on ice:
Up to 10µg RNA
0.1 Volume Reaction Buffer
1 µl Turbo DNase
Molecular Biology Grade Water to 10 µl
Incubate 30 minutes at 37°C
Add 1 µl Turbo DNase and incubate an additional 30 minutes at 37°C
Add 2 µl of DNase Inactivation Reagent
Incubate 2 min. room temp.
Centrifuge 10,000 x g for 2 min.
Remove supernatant to new tube.

cDNA Synthesis Using SuperScript III

1. Prepare Master Mix

Oligo$(dT)_{20}$	1 µL
dNTP, 10 mM	1 µL
RNA	1-10 µg (no more than 10 µL)
RNase-free Water	Up to 12 µL

2. Heat mixture to 65°C for 5 minutes
3. Place on ice for 2 minutes.
4. Prepare Master Mix

5x First Strand Buffer	4 µL
0.1 M DTT	1 µL
RNaseOUT	1 µL
SuperScript III	2 µL

5. Mix by pipetting.
6. Incubate at 50°C for 60 min
7. Inactivate reaction at 70°C for 15 min
8. Cool to 4°C.

The cDNA can now be used as a template for amplification in PCR. However, amplification of some PCR targets (those >1 kb) may require the removal of RNA complementary to the cDNA. To remove RNA complementary to the cDNA, add 1 µl (2 units) of *E. coli* RNase H and incubate at 37°C for 20 min.

Cost per reaction:

TurboDNase	$3.32
Oligo(dT)$_{20}$	$2.00
dNTP, 10 mM	$0.50
5x First Strand Buffer	-------
0.1 M DTT	-------
RNaseOUT	$0.80
SuperScript III	$9.00
RNase H	$6.00

qRT-PCR Conditions and Analysis

PCR reactions are performed in an optical 384-well plate with an ABI PRISM 7900 HT sequence detection system (Applied Biosystems)

For 10 µl reaction volume:
2 µl of primer pair (1 uM)
2 µl of 1:20 dilution of cDNA
5 µl of 2X Power Sybr Green MASTER MIX (Cat no. 4368708**)**
1 µl Molecular Biology Grade Water

Turn on 7900HT
Open the "template 5uL" located in the microarray facility inbox
Change the Sample Volume to 10 µl
Click connect
Click Open/Close open door for placement of sample plate
Click Start

Data analysis:
Data is collected and analyzed using the SDS 2.2.1 software (Applied Biosystems).
Click the for automated data analysis.

Cost per reaction:

Power Sybr Green Master Mix	$0.27
384-Well Plate	$1.80
Optical Cover	$1.00