

## Protocol for Primer Testing

Reaction Mixture: for use in 96 well plates, reaction volume 20 uL

<b>Reagent</b>	<b>Volume (uL)</b>
cDNA_____	5
1.25 uM Primer pair_____	5
10 X Master Mix_____	10.0
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<b>Total_____</b>	<b>20.0</b>

1. Make cDNA. I have been using iScript from Bio-Rad. I start with 1ug of RNA in a 20ul reaction. If I need a lot of cDNA, then I will scale up to 2ug in 40ul.
2. Dilute cDNA with water from 20 to 52ul or from 40 to 104ul. This is your "concentration 1." You will serial dilute from this.
3. Serial dilute cDNA starting from concentration 1. Dilute by 5-fold, or 2-fold if you suspect your primers amplify a low abundance gene. Make 8 serial dilutions.
4. Add the serial diluted cDNA to the plate. Be sure to do in duplicate or triplicate.
5. Add primers to plate (primer concentration remains the same)
6. Add master mix.
7. Seal plate and proceed with qPCR run.

To calculate primer efficiency:

The CFX software can do this for you if you label your serial dilutions on the plate documents as standards. Tell the plate document what your starting concentration is (which doesn't matter, it's an arbitrary number), your dilution factor, and whether the dilutions are increasing or decreasing. When the run is finished, it automatically calculates the efficiency.

To calculate in excel:

1. export your Cq values to excel.
2. Plot Cq value on y-axis vs. Log(relative concentration) on x-axis
3. Add a trendline.
4. Calculate the efficiency from the slope of your trendline as:
  - a. Efficiency =  $((\text{dilution factor})^{(1/-\text{slope})})-1$
  - b. Multiply this number by 100 if you want percent