SYBR Green qPCR Protocol

Per well:

5 ul SYBR

0.05 ul primer 1 (stock at 100uM – dilute 1:10 to reach 10uM working stock)

0.05 ul primer 2 (stock at 100uM – dilute 1:10 to reach 10uM working stock)

* if using Qiagen primer sets, add 0.1ul of primer set per well (stock at 100uM – dilute 1:10 to reach 10uM working stock)

1.9 ul nuclease-free water

Make a master mix for the number of wells desired (plus a little extra – ~10%). Add 3 ul of 2.5 ng/ul cDNA to each well. Add 7 ul of master mix. Carefully cover with optical plate cover. Take care to avoid touching the top of the cover as this can interfere with the reading. Vortex and spin down for 1 min. at 1000rpm to remove bubbles.

Run thermocycler: 384 well format, Sybr-green, ΔΔCt analysis, standard run, with melt curve.