

qPCR protocol (for 1 plate)

- 1) Dilute primers (calculate how much nuclease-free water to add from the information you get from the supplier) → this will be your stock primers!
 - a. For the qPCR, dilute the stock primers 10x.
- 2) Make standards
 - a. Calculate plasmid dilution for the DNA standard you received from the supplier. Add nuclease-free water to reach target concentration 2.00E+0.9. This will be the stock solution.
 - b. For the qPCR, dilute your stock solution 10x → this will be your STx10 (diluted stock dilution).
 - c. For the 7 standards, dilute STx10 by 10 seven times:

Standard	Dilution	Standard used for dilution	Add standard [μL]	Add water [μL]
St1	x10	STx10	2	18
St2	x100	St1	2	18
St3	x1000	St2	2	18
St4	x10000	St3	2	18
St5	x100000	St4	2	18
St6	x1000000	St5	2	18
St7	x10000000	St6	2	18

3) Master mix

	μL needed for 1 reaction
Water	6
Fwd primer (GS2-F)	0.75
Rev primer (GS5-R)	0.75
SYBR Green	12.5
DNA or standard	5

Calculate how many reactions (=wells) you are using on the plate, then calculate how much master mix you need. Add some extra for pipetting loss with the multi-pipette.

- 4) Prepare qPCR plate
 - Vortex all liquids before using! Spin afterwards to get liquid to the bottom of the tubes.
 - Add 20 μL Master mix to all wells
 - Add 5 μL of standard to 1A-G, 2A-G and 3A-G (add in the post-PCR room)
 - Add 5 μL of water to H1-3 (blank)
 - Add 5 μL of diluted samples to other wells

- When adding everything, go all the way down with the pipette tip into the wells. Don't do the extra-pressing to get air out of the pipette, because we don't want any splashing or bubbles! When releasing the liquid, slowly move the tip upward to avoid bubbles.
- Check from below if all wells are filled. Close the plate with the plastic cover sticker. You can use a bank card or similar to get it on evenly, then stretch it out with it. Close the sides with the card.
- Centrifuge the covered plate until there are no more bubbles in any of the wells.

5) Run the qPCR plate

- Go to the qPCR room. Start the computer to the right of the printer.
- Start and open the program Bio-Rad CFX.
- Cycle conditions:
 - o 2 minutes at 90 degrees
 - o 40 cycles at 95 degrees for 15 seconds
 - o 40 cycles at 60 degrees for 15 seconds
 - o 40 cycles at 72 degrees for 15 seconds
- Save your protocol in your folder.