LABORATORY OF MOLECULAR PATHOLOGY

Amplicon quantification

Using QuantiFLuor dsDNA system from Promega on Qubit or Roche LightCycler 480II

Prep:

☐ 01 Thaw the QuantiFluor dye. Quick vortex and quick spin. Keep it in the dark.

☐ 02 Quick vortex and quick spin the Standard DNA.

☐ 03 Prepare standards for quantification from the concentrated DNA standard.
You will need just 2 µL per standard. Don't waste it. Use a two-fold serial dilution.

100 ng/µL → 50 ng/µL → **25 ng/µL** → **12.5 ng/µL** → **6.25 ng/µL** → **3.125 ng/µL** → **0 ng/µL**

☐ 04 Calculate how much of the working solution you need for your samples. Don't forget to include the five standards.

- ☐ For **Qubit,** you need **200 µL** total per sample or standard.
- ☐ For **LightCycler,** you need **50 µL** total per sample or standard.

☐ 05 Prepare the working solution – **QuantiFluor Dye 1:400 in 1× TE** buffer.
e.g. To measure 10 samples and 5 standards on LightCycler, you will need 15×50=750 µL total, meaning you need 2 µL of the QF Dye, 40 µL of TE and 758 µL of water.

☐ 06 Mix the working solution properly. Vortex and quick spin.

☐ 07 Combine the working solution and samples/standards.

- ☐ For **Qubit,** you need **198 µL** of working solution and **2 µL** of sample/standard. You must use the optical grade 0.5 mL tubes.
- ☐ For **LightCycler,** you need **48 µL** working solution and **2 µL** of sample/standard. You must use white plates or white strips and optical grade foils or caps.

☐ 08 Vortex the samples. Do a quick spin.

☐ 09 Incubate at RT for **3 minutes**. Should be kept in dark.

☐ 10 Measure the samples and standards. Write down the relative fluorescence.

☐ 11 Construct the calibration curve in Excel and calculate the concentration of samples.