

Picogreen quantification

Kit component: 20X TE Buffer, Quant-IT PicoGreen assay and lambda standard (100ngr/ul)

Batch of 72 genotypes, use std curve with 8 points and ~8 blanks (use one of the blanks a std with 0 ngr/ul of DNA).

1. Prepare 20ml 1X TE Buffer using the 20X concentrated TE. Label and keep separate from other TE buffers. You need ~20ml for each batch of 72. Prepare 100ml by mixing 5ml 20X in 95 ml H₂O. Divide in 2 falcon of 50ml.
2. Prepare lambda DNA solutions (1:2 serial dilution) using the standard DNA provided by the kit and the 1X TE diluted in step 1*. Prepare std in excess in strip tubes, so you can use the multichannel pipette later.
3. Dilute the PicoGreen 1:200 in 1X TE for having a total volume of 18ml (90ul PicoGreen and 17910 ul 1X TE). You will need 17.6ml for the the plate. Prepare in falcon tube and cover with aluminium foil.
4. Aliquote 200ul of solution in each well for std, samples and blanks. If the solution is not enough (i.e. error in pipetting) reduce the number of blanks.
5. Plate 3ul of STD and sample in the solution. Mix well or incubate 5 min RT
6. Read in plate reader at ~485nm excitation and ~525 nm emission. Calculate sample concentration based on standard curve.

* Standard concentration

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|---|------------|
| A | 100ng/ul |
| B | 50 ng/ul |
| C | 25 ng/ul |
| D | 12.5 ng/ul |
| E | 6.25 ng/ul |
| F | 0 ng/ul |
| G | blank |