PicoGreen protocol for 1 plate of samples, standards

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- standards made up in triplicate
- calibration plate with triplicate standards
- 96-well sample plate

Preparation

- label 1x TE, PicoGreen (PG) centrifuge tubes
- label optical plates. Gen5 protocols are set for Costar 96 black opaque 3915
- have on hand 1 full box each of P10, P200 tips + 4 columns P200
- turn on plate reader in advance of use

1. prepare 1x TE buffer (10 mM Tris-HCl, 1 mM EDTA, ph 7.5) from the supplied 20x

purpose	-	expansion	-	with overage
96 samples	@ 99 ul ea	9 500		
120 vol. dilute PG	@ 100 ul ea	12 000	@ 1.09	13 100
standards	separate protocol	3 930		
total	-	25 430	@ 1.09	**27 700**

- 26 315 ul H2O
- 1 385 ul 20X TE

2. aliquot off 13 034.5 ul 1x TE for PG solution
3. drop 99 ul of 1x TE in the sample plate wells

4. drop in 100 ul standard curve per well on calibration plate

·	1	2	3
A	1x TE	1x TE	1x TE
B	64	64	64 ng / ml
C	128	128	128
D	256	256	256
E	512	512	512
F	640	640	640
G	800	800	800
H	1000	1000	1000

5. homogenize dna on thermomixer 1 min. 400 rpm, 4C.
6. drop tips for negative controls, both boxes
7. spin down microplate
8. add 1 ul to each sample well

missed aliquots: catch with a P10

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

9. prepare a 200-fold dilution of PG in 1x TE

- Keep reagent in foil while thawing and in diluted state. Vortex well, spin down.
- For 13 100 ul pg solution:
  - 13 034.5 ul 1x TE
  - 65.5 ul PG

10. Add 100 ul diluted PG to every well. Aspirate 10X. New tips each time, both plates.
11. Cover optical plates with old Costar plates or foil. Incubate at RT for 5 min.
12. Read the plates.