User Guide

YOUSEQ

NGS Library Quantification kit

qPCR quantification of NGS libraries

96 reactions

Version 2.0

Kit contents

Library QC/quant kit

	Cap colour	Size
Library specific primer		1.5 mL
YouSeq qPCR MasterMix		2 x 1.5 mL
Standards		4 x 0.1 mL*
YouSeq dilution buffer		5 mL

* Arrived lyophilised, require resuspension

Other items to be supplied by the user

- qPCR instrument
- Plasticware suitable for your PCR machine
- Pipettes and general laboratory equipment

qPCR plate set up

Load each well of your 96-well PCR plate with the following:

10 µL YouSeq qPCR Master Mix
5 µL Library specific primers

Library Dilutions and Loading

Dilute each library by 1:100,000 factor in provided dilution buffer

- Use 1 µL of Library Product to dilute
- We'd recommend:
  - 1 µL library product plus 99 µL dilution buffer (tube 2)
  - 1 µL tube 2 plus 99 µL dilution buffer (tube 3)
  - 10 µL tube 3 plus 90 µL dilution buffer (tube 4)
- Then add the following amount of the final dilution to the appropriate well(s)

5 µL Diluted Library

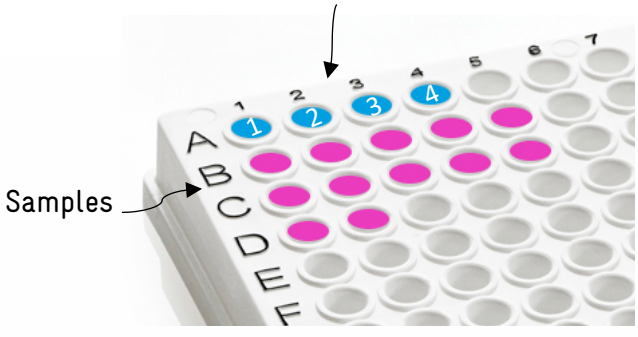
Positive control standards

Resuspend all 4 of the standards in **100 µL** of dilution buffer

Load 4 wells to create your positive control standard curve

5 µL standard 1, 2, 3, or 4
10 µL YouSeq qPCR Master Mix
5 µL Library specific primers

Example plate:
Positive control standards 1-4

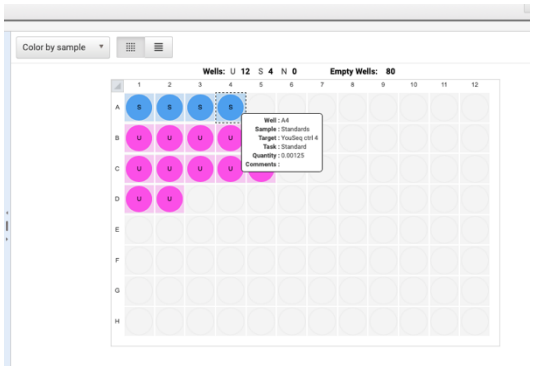


Seal the plate and load in to your qPCR instrument.

Standard curve set up in qPCR instrumentation software

Your qPCR instrument will have a function to programme a 'standard curve'. This is simply a matter of inputting the concentration of the 4 positive control standards supplied in the kit.

Standard no.	Concentration
1	10 pM
2	0.5 pM
3	0.025 pM
4	0.00125 pM



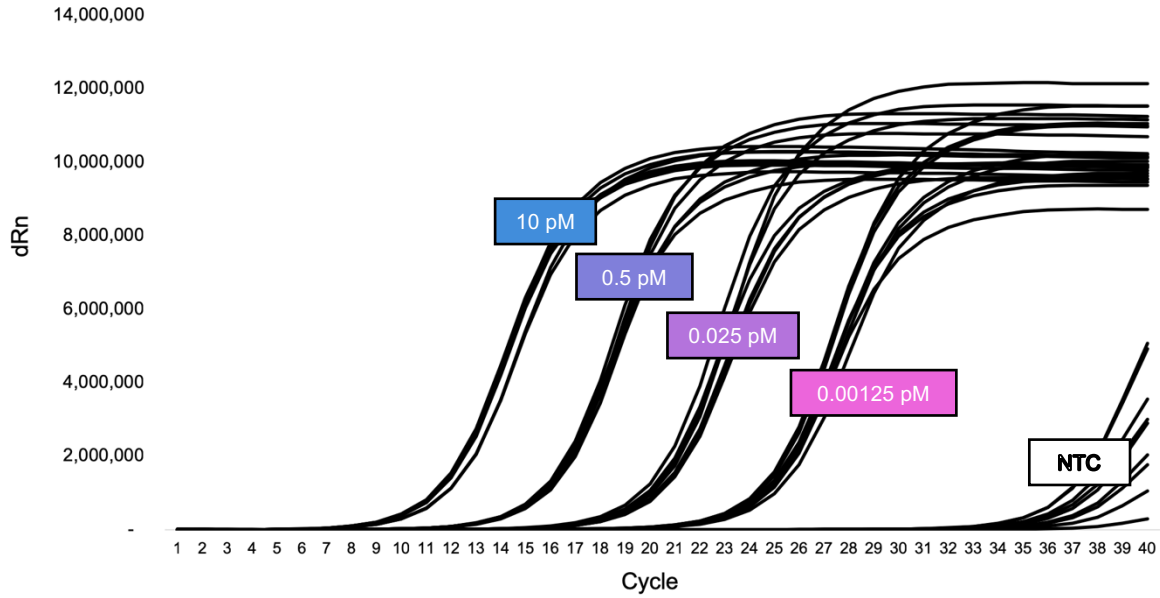
qPCR protocol set up

Run the following PCR protocol:

	Temp	Time
40 cycles	95°C	3 minutes
	95°C	10 seconds
	60°C*	60 seconds
	55-95°C	Melt Curve

*Make sure to collect fluorogenic data through the FAM/SYBR (Green) channel

Data interpretation



Example amplification plots for the standards 1-4

Pass Criteria:

The criteria need to be met to ensure the run was performed optimally and quantification values are as true as possible.

- PCR efficiency – 90 – 110% efficient

If this isn't met, the run has failed and would interpret an incorrect quantification. We'd recommend repeating this run.

Quantification of libraries

Your qPCR instrument software will automatically compare the Cq values obtained from your samples to those from the positive control standards in the kit. This calculation will deliver a 'calculated concentration' in pM of each of your diluted NGS Libraries.

To determine your adjusted concentration for your non-diluted library, follow the formula below for each library:

$$\text{Adjusted Concentration} = \text{Calculated Concentration} \times \frac{252}{\text{Average Fragment Length}} \times \text{Dilution Factor}$$

e.g. Library was diluted 1:100,000 before loading and it recorded a calculated concentration of 6 pM. With average 370 bp fragment length.

- Therefore, adjusted conc = 0.6 x (252 / 370) x 100,000 =

  40,864 (pM) or 40.864 nM