# User Guide

YOUSEQ

# NGS Library Quantification kit

## (For Illumina Specific libraries)

qPCR quantification of NGS libraries

96, 384 & 960 reactions

Version 3.3

# Kit contents

| | Cap colour | Size | | |
|---|---|---|---|---|
| | | 96 rxns | 384 rxns | 960 rxns |
| Library specific primers* |  | 0.5 mL | 2 mL | (3x) 1.7 mL |
| YouSeq qPCR MasterMix |  | 1 mL | (3x) 1.4 mL | (6x) 1.75 mL |
| Standards 1-4* |  | (4x) 0.1 mL | (12x) 0.1 mL | (20x) 0.1 mL |
| YouSeq dilution buffer |  | 50 mL | (2x) 100 mL | (4x) 125 mL |

* Arrived lyophilised, require resuspension (Instructions below)

## Other items to be supplied by the user

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

# Resuspension step

Resuspend the kit contents with the correct reagents as per this table:

| | Reagent | Volume |
|---|---|---|
| **Library Specific Primers** | YouSeq Dilution Buffer | 550 µl |
| **Standards Template 1-4** | YouSeq Dilution Buffer | 100 µl |

# qPCR plate set up

1. Each reaction requires the mixture below, to be loaded into each well
    i. Recommendation – Make a bulk mix and dispense this into all appropriate wells
    ii. e.g. 10 reactions (plus 1 rxn overage):
        1. 110 µL YouSeq qPCR MasterMix
        2. 55 µL Library specific primers
    iii. Remember an additional 4 wells are required for the provided standards.

10 µL YouSeq qPCR MasterMix
5 µL Library specific primers

## Library Dilutions and Loading

2. Dilute each library by **1:10,000 or 1:100,000** factor in provided dilution buffer. The dilution will depend on the library preparation used. If first attempt, run both dilutions and see which most appropriate.

3. Use **1 µL** of Library Product to dilute

    i. **We'd recommend:**
        1. 1 µL library product plus 99 µL dilution buffer (tube 2)
        2. 1 µL tube 2 plus 99 µL dilution buffer (**1:10,000 dil**)
        3. 10 µL tube 3 plus 90 µL dilution buffer (**1:100,000 dil**)

4. Then add the following amount of the final dilution to the appropriate well(s)

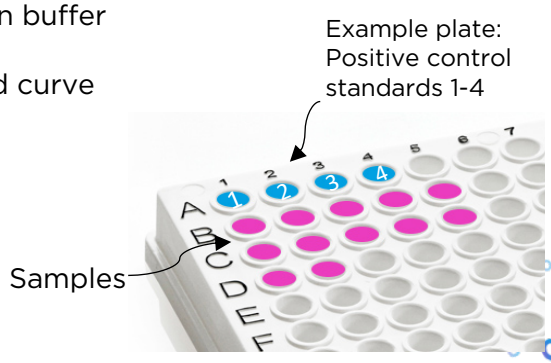
5 µL Diluted Library

## Positive control standards

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

6. Load 4 wells to create your positive control standard curve

5 µL standard 1, 2, 3, or 4

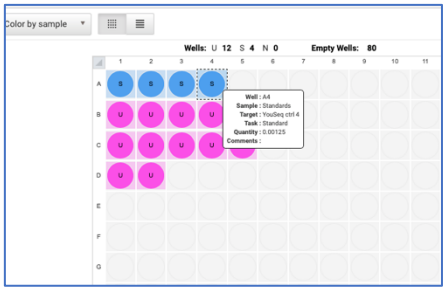


7. Seal the plate/tubes and load into your qPCR instrument.

# Standard curve set up

8. 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 |



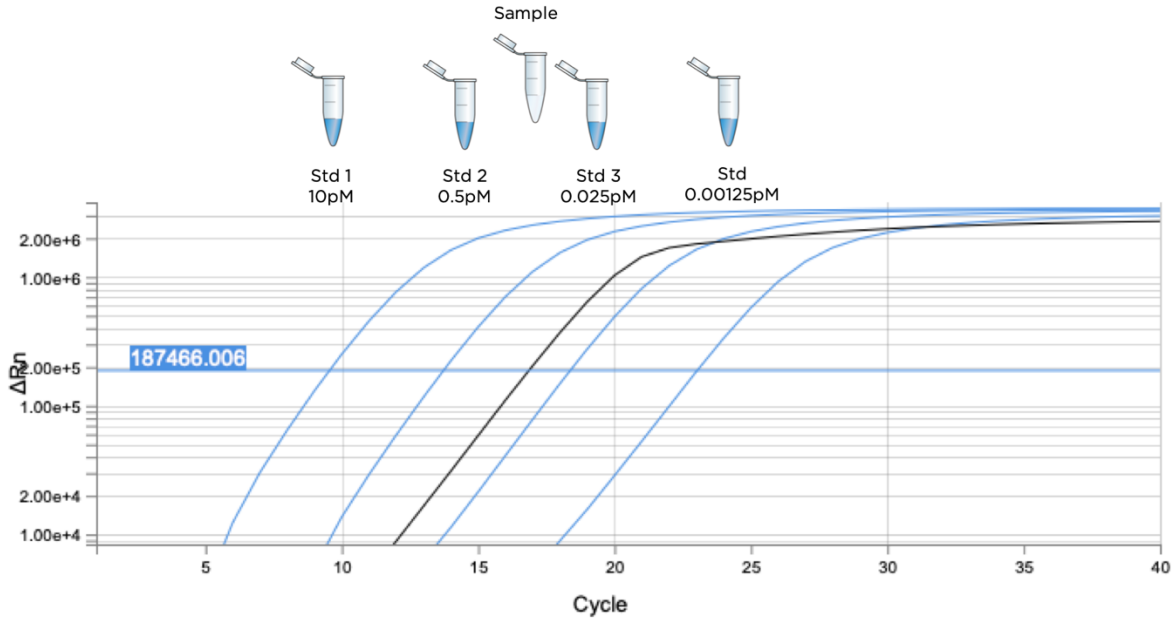
## qPCR protocol set up

9. Run the following PCR protocol:

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

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

# Data interpretation




## 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**
- Standard one should have a Cq value within 9.2 +/- 1 Cq value

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 1:10,000 before loading and it recorded a calculated concentration of 0.6 pM. With average 370 bp fragment length.

- Therefore, adjusted conc = 0.6 x (252 / 370) x 10,000 =

  4086.4 (pM) or 4.0864 nM