YOUSEQ

NGS LIBRARY QUANTIFICATION KIT USER GUIDE

CAT NO. YS-NGS-LQC-IL-96/384/960

96, 384 or 960 reactions (For Illumina Libraries)

VERSION 5.0

For Research Use Only

YOUSEQ

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INTENDED USE

The YouSeq NGS library quantification kit allows users to quantify their NGS libraries. Any libraries made with the Illumina P5/P7 adapter sequences can be quantified using this kit.

KIT CONTENTS

	Cap Colour	96 rxns Volume	384 rxns Volume	960 rxns Volume
NGS Library Quantification Kit primers		110 µl	(4x) 110 µl	(10x) 110 µl
YouSeq qPCR SYBR green MasterMix		1 mL	(4x) 1 mL	(10x) 1 mL
Standards 1-4*		(4x) 100 µl	(16x) 100 µl	(40x) 100 µl
YouSeq dilution buffer**		(1x) 50 mL	(4x) 50 mL	(10x) 50 mL
DNase/RNase free water		1.5 mL	(4x) 1.5 mL	(10x) 1.5 mL

* Supplied lyophilised and requires resuspension before use, see resuspension step below for instructions.
** Can be stored at 2-8C after thawing for subsequent use.

RESUSPENSION

Resuspend the designated kit contents with the correct reagents as per the table below. Spin or gently tap the vials to ensure all contents is at the bottom before opening.

After adding the resuspension reagent, pulse vortex each vial to ensure it is mixed well.

	Reagent	Volume to add
Standards Template 1-4	YouSeq dilution buffer	100 µl

MATERIALS REQUIRED BUT NOT PROVIDED

qPCR instrument

Pipettes, microcentrifuge tubes and general laboratory equipment

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qPCR REACTION SET UP

Set up the reaction on ice. Follow the table below to create a reaction mix.

i. N = 1x sample dilution PLUS 4x Standards PLUS 1x No Template Control (NTC) = 6

ii. Overage is applied in the calculation (N + 1) = 7

Each reaction requires the component volumes below to be loaded into each well. It is recommended to make a bulk mix and dispense this into all appropriate wells. Below is a calculation for the bulk mix required, including overage.

	Volume Required	
Component	Per Well	7 x rxn vol
YouSeq qPCR MasterMix	10 µL	70 µL
Library specific primers	1 µL	7 µL
DNase/RNase free water	4 µL	28 µL
Total Volume	15 µL	105 µL

LIBRARY DILUTIONS

Perform a serial dilution of your pooled library to create a library product at the optimum concentration.

1. Add 99 µl of dilution buffer into 2 tubes and label them ‘tube 2’ and ‘tube 3’.
2. Add 90 µl of dilution buffer into a tube and label it ‘tube 4’.
3. Pipette 1 µL of the purified library pool into tube 2
4. Mix by pipetting up and down 5 times
5. Change pipette tip and pipette 1 µL of tube 2 into tube 3
6. Mix by pipetting up and down 5 times
7. Change pipette tip and pipette 10 µL of tube 3 into tube 4
8. Mix by pipetting up and down 5 times

Tube No.	Dilution factor
4	1 : 100,000

9. Pipette 5 µL of tube 4 to the designated wells on your qPCR plate

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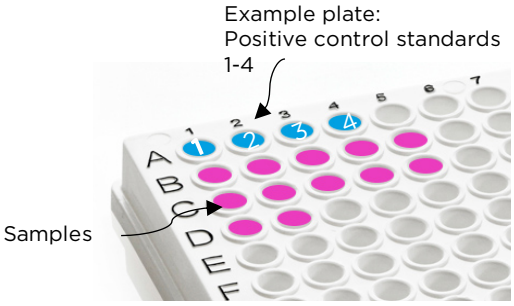
POSITIVE CONTROL

Pipette 5 µL of each positive control standard into your designated wells to create a positive control standard curve.

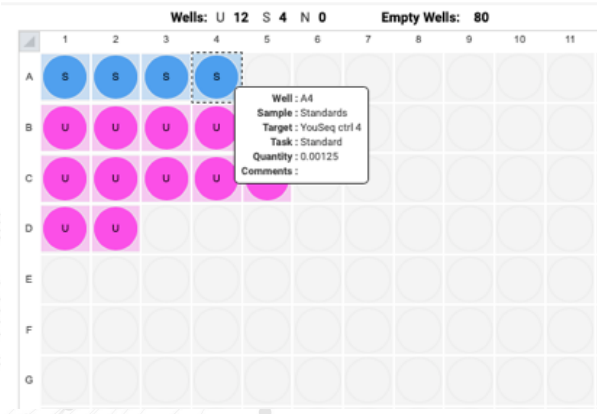


Seal the plate carefully and spin it briefly in a plate centrifuge to ensure that all the reagents are at the bottom of the well. Load the plate into the qPCR instrument.

STANDARD CURVE SET UP

Program a standard curve into the qPCR instrument software with the input concentrations as in the table below:

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



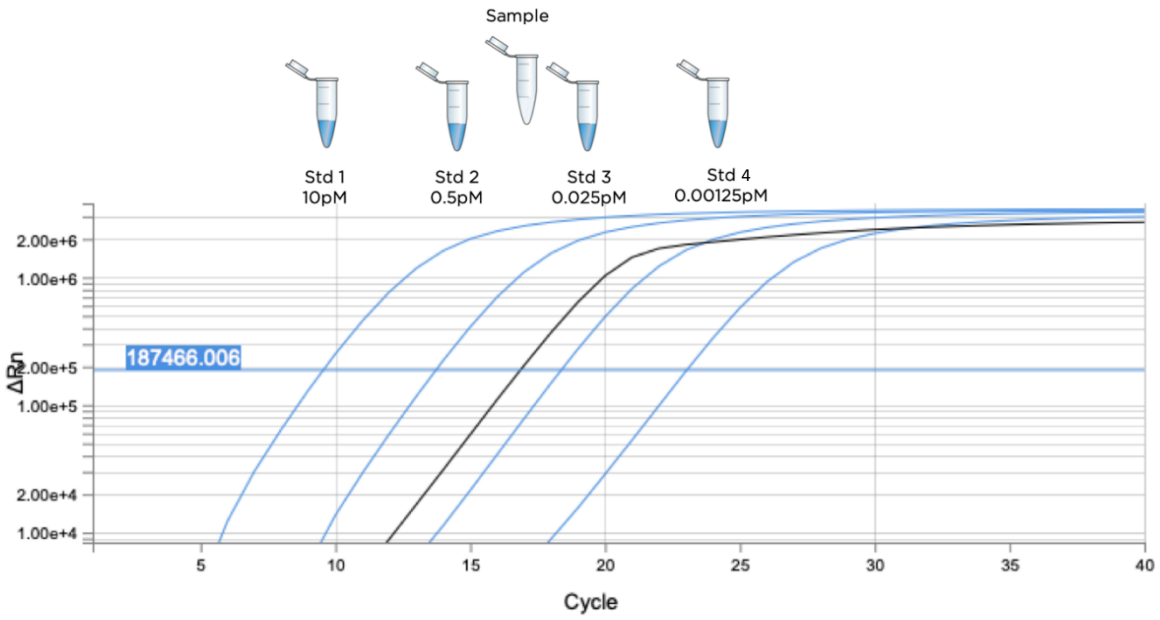
qPCR AMPLIFICATION PROTOCOL

Perform a PCR using the following cycling conditions:

	Temperature	Time
Hot Start	95°C	3 minutes
40 cycles	95°C	10 seconds
	60°C*	60 seconds
Melt curve	55-95°C*	

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

DATA INTERPRETATION



Your qPCR instrument software will precisely calculate the concentration of your library by comparing the Cq value from your library to the standard curve Cq values. Document this concentration carefully in your records.

qPCR DATA ANALYSIS

To ensure that the qPCR has performed sufficiently to provide accurate quantification values the following criteria must be assessed and met:

- PCR efficiency – 90 – 110% efficient
- Standard one should have a Cq value within 9.2 +/- 1 Cq value

If the criteria are not met, the run cannot be used for correct quantification and must be repeated.

Please note: the threshold should be manually set at 10% of the total RFU of the 10 pM standards

QUANTIFICATION OF LIBRARIES

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

To determine the library concentration for the non-diluted library, perform a calculation for each library using the formula below:

Worked Example:

Library was diluted 1:100,000 before qPCR and returned a calculated concentration of 0.6 pM. with average 370 bp fragment length.

Adjusted concentration = 0.6 x (252 / 370) x 100,000

Adjusted concentration = 40864 pM or 40.864 nM

PRODUCT SPECIFICATIONS

Storing your kit
Store at -20°C from arrival. The primers and MasterMix should be kept on ice while being used.

Laboratory Practices
To prevent contamination of the reactions and workspace, good molecular practice should be maintained at all times. Clean workspaces and equipment with DNA Away or 7% bleach solution prior to commencing the protocol.

Quality Control
In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of The ONE 16S NGS Library Preparation kit is tested against predetermined specifications to ensure consistent product quality.

Technical Assistance
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