



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 4.0

For Research Use Only



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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) 0.1 mL	(16x) 0.1 mL	(40x) 0.1 mL
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.

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

qPCR REACTION SET UP

Set up the reaction on ice. Follow the table below for reaction mix creation.

- i. N = reaction quantity of samples PLUS 4x Standards PLUS 1x No Template Control (NTC)
- ii. Overage is applied in the calculation ($N + 4 + 1$)

Each reaction requires the mixture below to be loaded into each well. It is recommended to make a bulk mix and dispense this into all appropriate wells. Below is an example of bulk mix with 10 reactions (plus 1 rxn overage).

Component	Per Well	Volume Required	
		$N+1 \times \text{rxn}$	e.g. 10 rxns
YouSeq qPCR MasterMix	10 μL	$N+1 \times 10 \mu\text{L}$	160 μl
Library specific primers	1 μL	$N+1 \times 1 \mu\text{L}$	16 μl
Water	4	$N+1 \times 4 \mu\text{L}$	64 μL
Total Volume	15 μL	$N+1 \times 15 \mu\text{L}$	240 μL

LIBRARY DILUTIONS

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

1. Add 99 μl of dilution buffer into 2 tubes and label them 2 and 3
2. Add 90 μl of dilution buffer into 1 tube and label it 4
3. Pipette 1 μl of library product 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 1 μl of tube 3 into tube 4
8. Mix by pipetting up and down 5 times

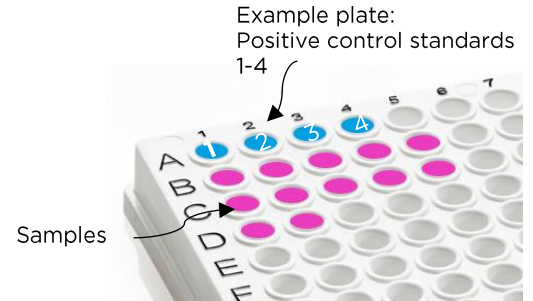
Tube No.	Dilution factor
3	1:10,000
4	1 : 100,000

Pipette 5 μl of diluted library product – Tube 4 - to the designated wells.

Please note: On the first time of using each library, dilutions from tube 3 and tube 4 can be used in separate wells to ensure the most appropriate concentration is included.

POSITIVE CONTROL

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

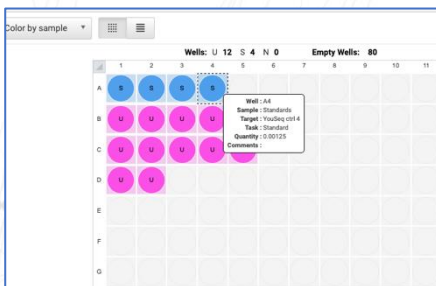


Seal the plate carefully and spin it briefly in a plate centrifuge to ensure that all the reagents are at the bottom of the well. Load the plate into the qPCR instrument.

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



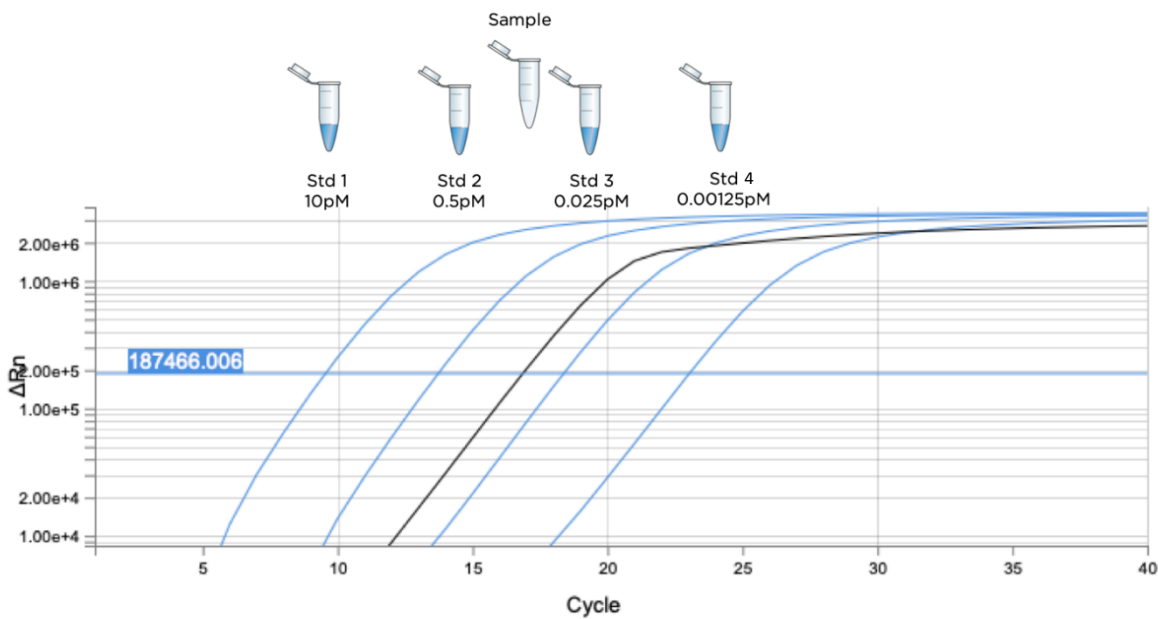
qPCR AMPLIFICATION PROTOCOL

Run the following PCR Protocol:

	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



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 standard

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 1:10,000 before qPCR and returned a calculated concentration of 0.6 pM. with average 370 bp fragment length.

Adjusted concentration = $0.6 \times (252 / 370) \times 10,000$

Adjusted concentration = 4086.4 pM or 4.0864 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

For customer support, please contact us:

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