MCLAB
Molecular Cloning Laboratories

Manual

Version 3.0

Product name: MCNext™ SYBR® Fast Library qPCR Quantification Kit

Cat #: IQPQ-UN, IQPQ-LR, IQPQ-RR

Introduction:

Accurate quantification of the NGS DNA library is critical to ensure efficient data generation and high quality reads. Quantitative Polymerase Chain Reaction (qPCR) is a highly sensitive and accurate approach for quantifying a NGS library and uses a minimal amount of material compared with other quantification methods. The qPCR quantification tracks library concentrations as a function of PCR cycle number in order to derive a quantitative estimate of the initial template concentration based on the known DNA concentration as a control or standard. Thus, the control template should be as similar as possible to the libraries for quantification, in terms of template size, GC content and library type. It is also very important that a constant quantity of the control template is available, as for each round of library quantification determines the cluster generation efficiency, in turn to adjust the loading amount of constructed libraries for the sequencing, even slight variation of the standard leads to fluctuation of cluster density, either compromise the data quality or waste the sequencing capacity.

Product Description:

The MCNext™ SYBR® Fast qPCR Library Quantification Kit provides a fast and reliable solution to determine the library concentration with a direct estimate cluster density. This direct measurement method significantly saves time and resource comparing with other qPCR library quantification kits on the market. This is achieved by providing a constructed library other than a single DNA fragment as the control standard with known cluster generation density on Illumina® sequencing platforms.

The MCNext™ SYBR® Fast qPCR Library Quantification Kit utilizes MCLAB's high performance fast PCR enzyme by a PhiX genome based Library Standards (six 10-fold dilutions) with an average size of 570 bp, providing quick and accurate quantification with 0.001 – 100 pM dynamic range in 60 min. Based on the quantification, the result could be directly converted to cluster numbers for loading volume reference. The MCNext™ SYBR® Fast qPCR Library Quantification Kit is a rapid solution for your library construction QC to add value to your workflow and increase confidence in your results. The proprietary PhiX Library Standards consist of a group of Illumina® sequencing adapter-ligated DNA fragments derived from the PhiX genome with well-characterized sequence and well-balanced GC content (50%). To use this library as a control for libraries constructed for sequencing on Illumina® sequencing platform is far superior to the single DNA fragment as control standard used by other qPCR quantification kits on the current market. It can be rapidly interpolated for the estimating cluster density and serves as a quantification baseline for sample libraries without the need to have another sequencing run for the cluster density measurement purpose.

It is an excellent control with measured cluster generation conversion parameter to examine the library quality and quantity, allowing you to quickly determine if an error is related to sample preparation before the high-cost sequencing run. Quantification is fast, direct and accurate by extrapolation against the standard curve generated using the six 10-fold Library Standards dilutions, followed by a simple cluster number conversion.

The MCNext™ SYBR® Fast qPCR Library Quantification Kit is available in 3 types (Regular ROX, Low ROX and Universal) based on the internal reference dye preferences of the PCR thermocyclers. The packages include ready-to-use 2X Master Mix, 10X Primer Mix and 6 pre-diluted PhiX Library Standards.

Features:

The MCNext™ SYBR® Fast qPCR Library Quantification Kit provides researchers with an accurate and sensitive method for quantifying NGS libraries.

- Direct quantify library cluster density before loading the samples
- Accurate library quantification using PhiX genome based Library Standards
- Consistent library quantification across a broad range of samples
- High performance fast PCR enzyme, high sensitivity and rapid detection, simplified steps
- Adaptable to high-throughput library quantification for larger barcoding applications
- Tailored for libraries made with Illumina® TrueSeq and Nextera workflows
- Convenient packages for all types real time PCR platforms

Kit Components:

MCNext™ SYBR® Fast qPCR Library Quantification Kit (Universal) (IQPQ-UN)

Component	Cat #	Volume
2x MCNext™ SYBR® Fast qPCR Master Mix (No ROX)	SFMM-UN	5mL
10x Primer Mix	IQPQ-PX	1 mL
PhiX Library Standard1-100pM	IQPQ-PHL1	200 µL
PhiX Library Standard2-10pM	IQPQ-PHL2	200 µL
PhiX Library Standard3-1pM	IQPQ-PHL3	200 µL
PhiX Library Standard4-0.1pM	IQPQ-PHL4	200 µL
PhiX Library Standard5-0.01pM	IQPQ-PHL5	200 µL
PhiX Library Standard6-0.001pM	IQPQ-PHL6	200 µL
Storage Buffer	IQPQ-DSB	3 mL
50x Low ROX	IQPQ-RXL	200 µL
50x High ROX	IQPQ-RXH	200 µL

MCNext™ SYBR® Fast qPCR Library Quantification Kit (Low ROX) (IQPQ-LR)

Component	Cat #	Volume
2x MCNext™ SYBR® Fast qPCR Master Mix (Low ROX)	SFMM-LR	5mL
10x Primer Mix	IQPQ-PX	1 mL
PhiX Library Standard1-100pM	IQPQ-PHL1	200 µL
PhiX Library Standard2-10pM	IQPQ-PHL2	200 µL
PhiX Library Standard3-1pM	IQPQ-PHL3	200 µL
PhiX Library Standard4-0.1pM	IQPQ-PHL4	200 µL
PhiX Library Standard5-0.01pM	IQPQ-PHL5	200 µL
PhiX Library Standard6-0.001pM	IQPQ-PHL6	200 µL
Storage Buffer	IQPQ-DSB	3 mL

MCNext™ SYBR® Fast qPCR Library Quantification Kit (Regular ROX) (IQPQ-RR)

Component	Cat #	Volume
2x MCNext™ SYBR® Fast qPCR Master Mix (Regular ROX)	SFMM-RR	5mL
10x Primer Mix	IQPQ-PX	1 mL
PhiX Library Standard1-100pM	IQPQ-PHL1	200 µL
PhiX Library Standard2-10pM	IQPQ-PHL2	200 µL
PhiX Library Standard3-1pM	IQPQ-PHL3	200 µL
PhiX Library Standard4-0.1pM	IQPQ-PHL4	200 µL
PhiX Library Standard5-0.01pM	IQPQ-PHL5	200 µL
PhiX Library Standard6-0.001pM	IQPQ-PHL6	200 µL
Storage Buffer	IQPQ-DSB	3 mL

Reference Dye	Compatible Instruments
No ROX	BioRad iCycler MiniOpticon, Opticon 2, Chromo 4, iQ5; Roche LightCycler 480; MJ Research DNA Engine Opticon 2, Chromo 4; Corbett Rotogene 3000, 6000
Low ROX	ABI 7500 qPCR Systems, ViiA 7, QuantStudio 12K Flex, Agilent Mx3000P Mx3005 and Mx4000
Regular ROX	ABI PRISM 7000, 7700, 7900HT, ABI 7300 qPCR Systems, GeneAmp 5700, StepOne, and the StepOnePlus

Storage Condition: -20°C

Protocol:

- Thaw the 6 pre-diluted PhiX Library Standard solutions, 2X master mix, and 10X Primer Mix on ice. Cover the 2X master mix with a piece of aluminium foil due to its light sensitivity.
- Low concentration DNA storage buffer included in the kit is recommended for the dilution of unknown library samples.
- Ensure that all the reagents (including 2X master mix, 6 DNA library standards and diluted unknown libraries) are thoroughly mixed prior to use.
- Vortex the dilution thoroughly followed by a brief spinning.

Step 1: Dilute Unknown Libraries

- Estimate the concentration of unknown library by OD_{260} reading. Dilute the libraries to approximately 10 nM in TE buffer.
- Add 998 µl of DNA storage buffer to 2 µl of the unknown library template to make a 500 fold dilution. This will give an approximate concentration of 20 pM.
- Vortex the dilution thoroughly followed by a brief spinning.
- Add 10 µl of the diluted library to 90 µl of DNA storage buffer to make a second 10-fold dilution.

Step 2: Prepare Reaction Mix

It is important to make a master mix to minimize pipetting errors. The method here makes enough master mix to fill a 96-well plate. Adjust volume accordingly for your samples.

- Prepare the reaction mix as follows:

Consumable	Reaction mix for one sample µl/well	Reaction mix for 110 samples µl/plate
2X MCNext™ SYBR® FAST qPCR Master Mix	10	1100
10X Primer Mix	2	220
Water	6	660
	18	1980

- Mix the reaction mix thoroughly and place it on ice until use.
- Vortex the 6 PhiX Library Standards and the unknown library dilutions thoroughly right before loading.
- For a total reaction volume of 20 µl, load each well 18 µl of reaction mix and 2 µl of standard DNA library or unknown diluted DNA library. Setting up each sample in triplicate is important for subsequent analysis.
- It is recommended to include a no template control in triplicate.
- Seal the plate tightly. No need to vortex the plate. Spin down the plate by brief centrifugation.

Step 3: Quantify by qPCR Cycling

- Place the plate in the qPCR thermocycler. Use the following thermal profile:

	Temperature	Time
Hot start/ denaturation	95°C	10 min
X 40 cycles	95°C	30 sec
	60°C	45 sec

- Shorter annealing/extenstion times for quantification could be used. But we recommend relatively long times to ensure all the templates in the library get amplified equally.

Steps 4: Analyze the Quantified libraries

- Ensure good amplifications for both the standard and unknown libraries.
- Generate a standard curve from the control template by plotting the Ct values against the log initial concentration.
- Ensure the efficiency of amplification of the control template is 90-110% (a slope of -3.58 to -3.10) and the R2 >0.9.
- Calculate the initial concentration of the unknown libraries based on the standard curve generated from the control template dilutions. Ensure that a size adjustment is calculated if there is difference in the average fragment size between the standard library (570 bp) and the unknown DNA libraries.

$$\text{Size adjusted conc. (pM)} = \text{Conc. calculated by qPCR (pM)} \times \frac{\text{Phix Library avg. fragment size}}{\text{Unknown library avg. fragment size}}$$