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NGS Library Quantitative Kit for Illumina

Product Number: PCK84

Shipping and Storage

-20 °C, 12 months. If frequently used, it can be stored at 2-8 °C to avoid repeated freeze-thaw as much as possible.

Components

Component	PCK84	PCK84
2×SYBR qPCR Master Mix	1 ml	5×1 ml
qPCR Primer Mix	100 µl	5×100 µl
DNA Standard 1	100 µl	5×100 µl
DNA Standard 2	100 µl	5×100 µl
DNA Standard 3	100 µl	5×100 µl
DNA Standard 4	100 µl	5×100 µl
DNA Standard 5	100 µl	5×100 µl
50×High ROX	40 µl	200 µl

Description

This product uses the dye method (SYBR Green I) to perform real-time fluorescence quantitative PCR (qPCR) on the products after NGS library construction. The reagent kit provides the reaction mixture, DNA primer mixture, standards, and complete reagent system required for the qPCR process, making the operation simple and convenient.This kit uses a chemically modified new highly efficient hot start polymerase, and the activation of the enzyme requires incubation at 95℃ for 10 minutes. This product has strong specificity and high amplification efficiency, and can quickly and accurately quantify the concentration of the constructed library. Suitable for fluorescence quantitative PCR instruments that do not require ROX as a calibration dye, such as Roche LightCyler 480, Roche LightCyler 96, Bio radioCyleriQ, iQ5, CFX96.

ROX dye is used to correct the fluorescence signal error generated between wells in quantitative PCR instruments, and is generally used in Real Time PCR amplification instruments from companies such as ABI and Stratagene. The excitation optical systems of different instruments vary, so the concentration of ROX dye must be matched with the corresponding fluorescence quantitative PCR instrument.

Instruments that do not require ROX calibration:Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, CFX96 etc.

Instruments that require Low ROX calibration:ABI Prism7500/7500 Fast, QuantStudio® 3 System, QuantStudio®5 System, QuantStudio®6 Flex System, QuantStudio®7 Flex System, ViiA 7 system, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000 etc.

Instruments that require High ROX calibration:ABI Prism 7000/7300/7700/7900, Eppendorf, ABI StepOne/Step One Plus etc.

Note:The preparation methods for High Rox and Low Rox are described in protocol Step 2.

Application

This product is designed for the Illumina platform second-generation sequencing library concentration absolute quantification. The end of the library contains Illumina P5 and P7 chip binding sequences, with a length of no more than 1 kb and a concentration of no less than 0.002 pM. This product can be used for quantitative experiments. The qPCR Primer Mix provided by the kit contains the following two primer sequences:

1. Primer 1: 5'-AAT GAT ACG GCG ACC ACC GA-3'
2. Primer 2: 5'-CAA GCA GAA GAC GGC ATA CGA-3'

It is possible to confirm in advance whether the library can be amplified by the primer pair through the primer sequence.

Protocol

1. Preparation of amplification template

Dilute the library sample to be tested with TE (10 mM Tris Cl, pH8.0, 1mM EDTA), and try to maintain a concentration between 0.01 and 20 pM after dilution. Place on ice at 4℃ for backup.

2. Preparation of qPCR reaction system

Before preparation, completely melt the required cryopreservation reagents and mix them multiple times, then centrifuge briefly for later use.

The basic reaction system for 20μl is as follows:

Reagent	20μlReaction system
2×SYBR qPCR Master Mix	10μl
qPCR Primer Mix	0.8μl
Template	4μl
ddH_2O	5.2μl

Note:High Rox model: 1μl High Rox is added to every 50μl reaction system; Low Rox model: Add 1μl High Rox to every 500μl reaction system.

Prepare a sufficient amount of reaction system mixture as needed, mix well, and add 16μl of each well to the reaction well. Add the same volume of TE to the blank control, and then add the prepared standard and diluted sample to the corresponding reaction well at a volume of 4μl/well. It is recommended to use a 20μl reaction system. If smaller system reactions are required, reduce the proportion of each component in the system.

3. qPCR reaction program

Step	Temperature	Time	Cycles
Pre denaturation	95℃	10 min	1
Denaturation	95℃	10 sec	40
Annealing/Extension	60℃	30 sec	
Dissolution curve analysis		65-95℃	

Note:1)The annealing temperature should be set at 60-64℃ as a reference range. When non-specific reactions occur, the annealing temperature can be increased.

2)If the average length of the library is greater than 700 bp, the annealing/extension time should be appropriately increased.

Data Analysis

1. Standard curve production

Draw a standard curve using Ct values within the valid range. The correlation coefficient R^2 of the standard curve should not be less than 0.99, and the slope should be between -3.1 and -3.6. If the parameters of the standard curve are unreasonable, it is recommended to repeat the experiment.

DNA Standard	DNA Standard Conc.
DNA Standard 1	20 pM
DNA Standard 2	2 pM
DNA Standard 3	0.2 pM
DNA Standard 4	0.02 pM
DNA Standard 5	0.002 pM

2. Library concentration calculation

The difference in Ct between the three composite wells in the experiment should not exceed 0.2, otherwise invalid data needs to be deleted or the experiment needs to be repeated. Do not use Ct outside the effective Ct range of the standard curve to calculate the concentration of the dilution library. Please refer to the data processing Excel of this product for the specific library concentration calculation method.

Note

1. Before the experiment, this instruction should be read in detail. The operation should be carried out by personnel with professional experience or qualified training.
2. Please gently mix it upside down to avoid foaming, and use after briefly centrifuging.
3. Avoid repeated freeze-thaw of this product, as repeated freeze-thaw may cause a decrease in product performance.
4. When preparing the reaction solution, please use a new or non polluting gun head and centrifuge tube to prevent contamination as much as possible.