NGS Library Quantification Kit for Ion torrent

Item No.: N665925

Storage condition: -20℃, 12 months, if need to be used frequently, can be stored in 2-8℃, try to avoid repeated freezing and thawing.

Product content

Component	N665925-1ml	N665925-5ml
2×SYBR qPCR Master Mix 1	1ml	5ml
qPCR Primer Mix 1	100μl	500μl
DNA Standard I	100μl	500μl
DNA Standard II	100μl	500μl
DNA Standard III	100μl	500μl
DNA Standard IV	100μl	500μl
DNA Standard V	100μl	500μl
50 x High ROX	40μl	200μl

Product Introduction

This product is a real-time fluorescence quantitative PCR of the products after NGS library construction using a dye method (SYBR Green I).
(qPCR). The kit provides the reaction mixes, DNA primer mixtures, standards and sample dilutions required for the qPCR process, making the reagent system complete and easy to use. The fluorescent dye SYBR Green I contained in the reaction mixture can bind to all double-stranded DNA; the GoldStar Taq DNA Polymerase used is a chemically modified new high-efficiency hot-start polymerase, and the activation of the enzyme needs to be incubated at 95℃ for 10 minutes. the product has high specificity, high amplification efficiency, and is able to quickly and accurately quantify the concentration of the constructed libraries. The product is highly specific and efficient in amplification, and can quickly and accurately quantify the concentration of the constructed library.
ROX dye is used to correct the fluorescence signal error generated between wells of a quantitative PCR instrument, and is generally used in Real Time PCR amplifiers from ABI, Stratagene, and other companies. The excitation optics vary from instrument to instrument, so the concentration of ROX dye must be matched to 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 requiring 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, and others.
Instruments requiring High ROX calibration: ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One/Step One Plus, etc.
Note: High Rox and Low Rox are formulated as described in Use 2.

Scope of application

This product is designed for absolute quantification of the concentration of Ion torrent platform second generation sequencing libraries. The end of the library contains Ion torrent P5 and P7 microarray binding sequences, the length of which does not exceed 1kb, and the concentration is not less than 0.005pM

can be used to perform quantitative experiments with this product. The qPCR Primer Mix provided in the kit contains the following two primer sequences:
Primer 1:5'-CCA TCT CAT CCC TGC GTG TC - 3' Primer 2: 5'-CCT CTC TAT GGG CAG TCG GTG AT-3'
The primer sequence can be used in advance to confirm whether the library can be amplified by that primer pair.

Usage

1. Amplification template preparation
The library samples to be detected were diluted with TE (10 mM Tris-Cl, pH 8.0, 1 mM EDTA), and the concentration after dilution was as close as possible to the range of 0.05-50 pM. 4° C on ice was set aside.
2. qPCR reaction system preparation
The desired cryopreservation reagent is pre-melted completely and mixed by inverting several times before preparation, then centrifuged briefly and set aside.
The base reaction system for 20 μl was as follows:

reagents	20μl reaction system
2×SYBR qPCR Master Mix	10μl
qPCR Primer Mix 1	0.8μl
Template	4 μl
ddH_2O	5.2μl

Description: High Rox model: add 1 μl High Rox per 50 μl of reaction system; Low Rox model: 1 μl High Rox per 500 μl of reaction system.
Prepare a sufficient amount of reaction system mixture according to the need, mix well and add to the reaction wells in a volume of 16 μl per well, add the same volume of TE to the blank control, and then add the prepared standards and diluted samples to the corresponding reaction wells in a volume of 4 μl/well. It is recommended to use 20 μl reaction system, if you need to carry out a smaller system reaction, the system components can be reduced in equal proportion.
3. qPCR reaction program

Steps	Temperature	Time	Cycles
Pre denaturation	95° C	10min	1
denaturation	95° C	10sec	40
Annealing/Extension	60° C	30sec	
Dissolution curve analysis	65-95° C		

(1) Please use 60-64℃ as a reference for setting range of annealing temperature, and increase the annealing temperature when non-specific reaction occurs.
2) If the average length of the library is greater than 700bp, the annealing/extension time should be increased appropriately.

data analysis

1. Standard curve production

The standard curve was plotted using Ct values in the valid range. The standard curve correlation coefficient R2 should not be less than 0.99 and the slope should lie between -3.1 and -3.6. If the standard curve parameters are not reasonable, it is recommended to repeat the experiment.

DNA Standard name	DNA Standard concentration
DNA Standard I	50pM
DNA Standard II	5pM
DNA Standard III	0.5pM
DNA Standard IV	0.05pM
DNA Standard V	0.005pM

2. Library concentration calculation

The difference in Ct between the three replicate wells of the experiment should be no more than 0.2, otherwise the invalid data should be deleted or the experiment should be repeated. Do not use the Ct outside the valid Ct range of the standard curve to calculate the concentration of the diluted libraries. Please refer to the data processing Excel of this product for the specific library concentration calculation method.

matters needing attention

1. Before testing, these instructions should be read in detail. It should be operated by personnel with professional experience or qualified by training.
2. For use, please mix gently by turning up and down, avoid foaming as much as possible, and use it after centrifugation for a short period of time.
3. Avoid repeated freezing and thawing of the product, repeated freezing and thawing may degrade the performance of the product.
4. When preparing the reaction solution, please use new or non-contaminated tips and centrifuge tubes to prevent contamination as much as possible.