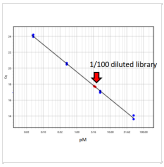

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Illumina TruSeq Library quantification with qPCR probe method

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

A homemade solution for quantification of Illumina library.

Because the method uses a quenched-probe (not intercalator), it is expected to be relatively stable for the difference of size distribution of libraries.

Currently, we confirmed this protocol with a probe for TruSeq type adapter.

PROTOCOL CITATION

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MATERIALS

NAME	CATALOG #	VENDOR
UltraPure™ Salmon Sperm DNA Solution	15632011	Thermo Fisher
PhiX Control v3	FC-110-3001	Illumina, Inc.
PrimeTime® Gene Expression Master Mix	1055770	Integrated DNA Technologies

ABSTRACT

A homemade solution for quantification of Illumina library.

Because the method uses a quenched-probe (not intercalator), it is expected to be relatively stable for the difference of size distribution of libraries.

Currently, we confirmed this protocol with a probe for TruSeq type adapter.

Preparing calibrators and primer/probe

1 Prepare low-TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) containing 5 ng/µl salmon sperm DNA.

1 M Tris-HCl, pH8.0	100 µl
0.5 M EDTA	10 µl

UltraPure™DNA Solution 10 mg/mL	5 µl
milli-Q water	9889 µl
Total	**10 mL**

Store in -20 °C.

2 Prepare dilution series of PhiX control with low-TE buffer containing 5 ng/µl salmon sperm DNA in 8-strip PCR tubes. These are used as calibrators.

50 pM	100 µl
5 pM	100 µl
0.5 pM	100 µl
0.05 pM	100 µl

Store in -20 °C.

3 Prepare 20x primers & probe mix solution as below.

Probe	5 µM
P5 primer	5 µM
P7 primer	5 µM

Store in -20 °C.

Double-quenched probe (IDT): /56-FAM/ACACTCTTT/ZEN/CCCTACACGACGCTCTTC/3IABkFQ/

P5 primer: 5'-AATGATACGGCGACCACCGA-3'
P7 primer: 5'-CAAGCAGAAGACGGCATACGA-3’

4 Prepare the following qPCR master mix per reactions for the number of your samples (+ calibrators).

Prime Time PCR master mix	10 µl
20x primers & probe mix	1 µl
H2O	8 µl

Distribute above to each well.

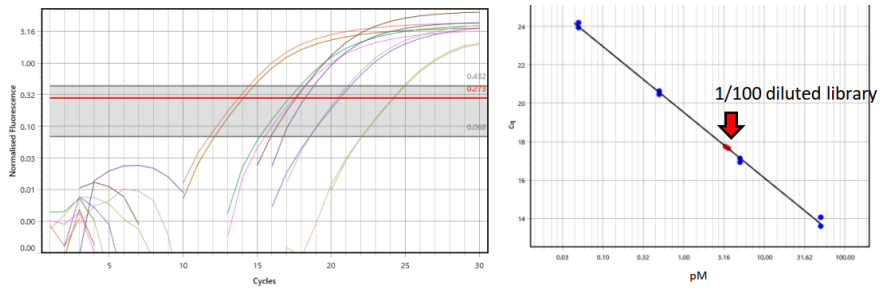
Add 1 µl of template (diluted library or calibrator) to each well.

5 Conduct PCR with the following condition.

95 °C for 2 min
30 cycles of
  97 °C for 5s
  62 °C for 10s
  68 °C for 15s (photo with a filter appropriate for SYBR-green or FAM)

Result

6



An example of expected result

Calculate the original concentration of your library.