NIPPON Genetics EUROPE

Application

Contamination of DNA in purified RNA; Comparative evaluation of RNA extraction kit, by analyzing the lowest level of contamination

Product: FastGene® RNA Premium Kit（FG-81050, FG-81250）

Manufacturer: NIPPON Genetics EUROPE

The follwoing data is kindly provided by Mr. Tetsuro Ariyoshi, RIKEN Center for Biosystems Dynamics Research, Laboratory of Cell Polarity Regulation, Japan. Thank you for your kind publication.

Background

Quantitative analysis of expression of RNA is carried out, but since we are conducting experiments in which the detection of genomic DNA can not be avoided in the design of qPCR primer, we should suppress DNA contamination in the purified RNA as much as possible. It is necessary to analyse RNA extraction kits for this applicability.
In this case, FastGene® RNA Premium Kit which adopts “DNase I treatment in eluted high-purity RNA solution” and can expect high DNA removal efficency as a standard protocol, is compared to a commerially available RNA extraction kit with “DNase I treatment on-column” as the standard protocol, and the comparative evaluation was conducted.
As an evaluation method, we examined “whether amplification by residual genomic DNA with qPCR is observed” under the reaction condition “without addition of reverse transcripase (without RT enzyme)” .

Method

RNA is extracted using two kinds of RNA extraction kits, “when reverse transcriptase treatment was performed” and “when reverse transcription reaction was not performed” . qPCR was performed respectively, and the amplification curves were compared.

1. Type and amount of initial sample (per Prep)
   Animal cells（HEK293T 5×10^5 cells）

2. Comparison RNA extraction kit
   FastGene® RNA Premium Kit（FG-81050） ：DNase I treatment in eluted high purity RNA solution
   Company P RNA extraction kit ：DNase I treatment on-column

3. Final elution buffer volume during RNA extraction
   30 µL

4. Reverse Transcription and qPCR reaction reagents (one step RT-qPCR master mix)
   TaKaRa One Step TB Green PrimeScript PLUS RT-PCR Kit（RR096A）

5. Input amount of RNA
   Total RNA 60 ng

6. Composition of Reverse Transcription reaction and program

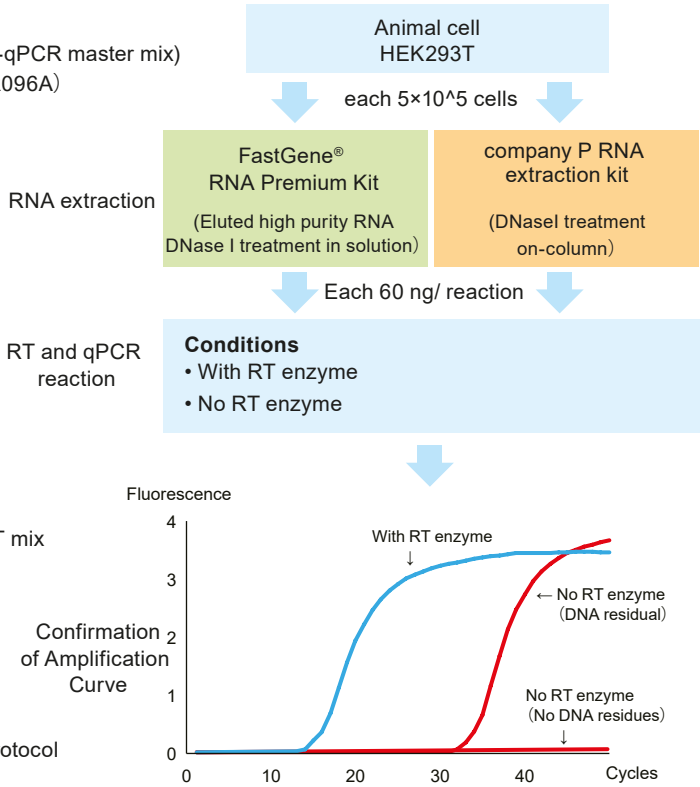
<One Step RT-qPCR reaction Composition (With RT enzyme)>

2×RT-qPCR Buffer	3.75 µL
qPCR Enzyme mix	0.45 µL
(Note) RT mix	0.15 µL
Target specific Primer	0.15 µL（Final 0.5 µM）
total RNA	3.00 µL（60 ng）
Total	7.5 µL

(Note) Under the condition of “without RT enzyme” ,
0.15 µL RNase free dH_2O was added instead of 0.15 µL RT mix

<Reaction program>
42℃ 5 min
95℃ 10 sec
95℃ 10 sec ┐
60℃ 30 sec ┘ ×50 cycle
＊Melting curve analysis as recommended by the instrument´s protocol

7. qPCR device used
   LightCycler96 (Roche)

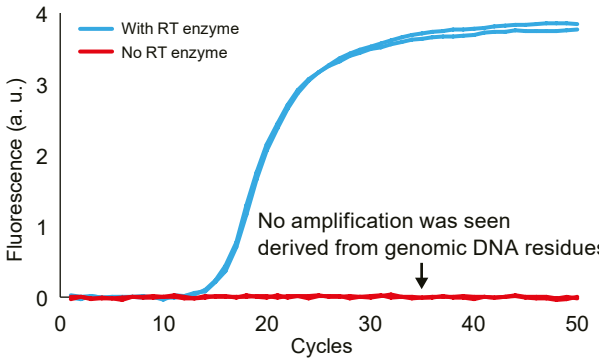


It is suggested that genomic DNA may remain in the RNA sample, when amplification was observed under the condition of “no RT enzyme” .

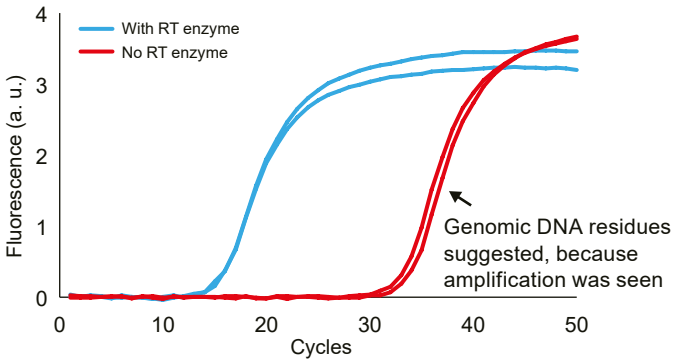
Result

Comparison of amplification curves in qPCR (primer: PGK1)

FastGene® RNA Premium Kit
(DNase I treatment in eluted high purity RNA solution)



company P RNA extraction kit
(DNase I treatment on-column)



Signals derived from contamination of genomic DNA are hardly detected from purified RNA using FastGene® RNA Premium Kit.

Customer`s comment

Quantitative analysis of expression of RNA is carried out, but since we are conducting experiments in which the detection of genomic DNA can not be avoided in the design of qPCR primer. I was looking for an extraction kit in which DNA contamination in purified RNA can be suppressed as much as possible.
In purified RNA using FastGene® RNA Premium Kit, almost no signal derived from contamination of DNA was detected, and the amount of expressed RNA could be more accurately quantified compared with purified RNA using competitor`s products. In experiments where it is necessary to minimize DNA contamination as much as possible, such as when primers with junctions can not be designed, the FastGene® RNA Premium Kit is the product of choice.

Binsfelder Straße 77,
52351 Düren, Germany

www.nippongenetics.eu
TEL +49 2421 554960 FAX +49 2421 55496 11 info@nippongenetics.eu