NIPPON Genetics

Customers Product Feedback

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

Manufacturer: NIPPON Genetics EUROPE

Application: Examination of RNA purification using mouse breast carcinoma-derived FM3A cells (confirmed by PCR and electrophoresis)

The following data was published due to the kindness of Mr. Koichiro Kashiwaba, Genomic Biology Laboratory (Department of Biological Science and Technology, Tokyo University of Science Department of Biological Engineering), Japan.

Overview

In recent years, analysis of all genetic information (genome) has been completed in all major species and the nucleotide sequence of all genes has been clarified. Our laboratory utilizes this genome information to elucidate the mechanism of gene expression in humans and mice and conducts research as the foundation for treating diseases. We analyse gene expression patterns in tissues and cells by microarray and RNA-seq, and conduct both basic and application-related research. Since RNA is unstable, it is important to be able to purify it particularly stably when used for experiments.

For this reason we examined wether FastGene® RNA Premium Kit can be used equally for RNA purification as compared with conventional kit (product of Q company).

Method

- RNA purification conditions
  - Sample type: FM3A cells derived from mouse breast cancer
  - Sample volume: 4×10^6 cells
  - DNase I treatment: FastGene treated sample with DNase I after elution
    Company Q treated sample with DNase I on column
  - RNA elution buffer volume: 30µl
- Reverse transcription conditions
  - Input RNA amount: 1 µg (per 20 µl reaction volume)
  - Reverse transcriptase: ReverTra (TOYOBO)

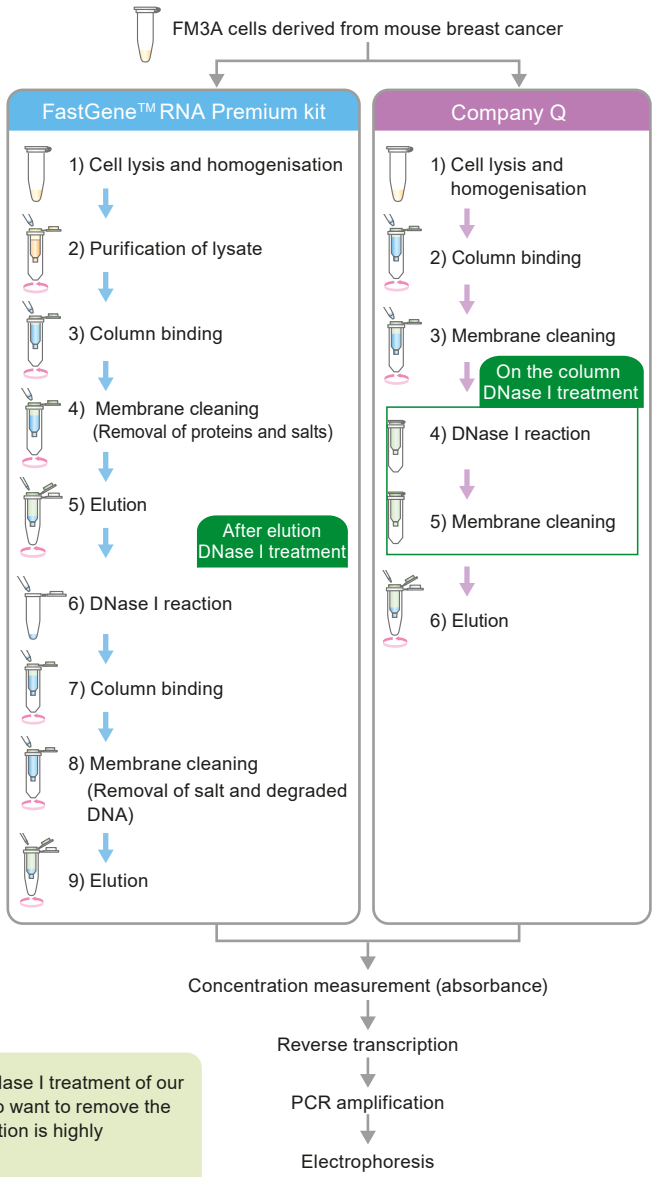
Reaction compostion	+ RT	-RT
total RNA	5 µg	5 µg
D.W.	12-X µl	13-X µl
oligo dT20 Primer (10µM)	1 µl	1 µl
5×Buffer	4 µl	4 µl
10 mM dNTPs	2 µl	2 µl
ReverTra Ace (100 U/µl)	1 µl	0 µl
Total volume	20 µl	20 µl

Reaction program:

Annealing 30 °C • 10 min
↓
Enzyme reaction 42 °C • 30 min
↓
Denaturation 99 °C • 5 min

- PCR conditions
  - Input cDNA amount: 1 µl (reaction solution under reverse transcription conditions)
  - PCR reagent: KOD - Plus - Neo (TOYOBO)
  - PCR instrument: T 100 (Bio-Rad)
- Electrophoresis conditions
  - 1 % TAE agarose gel, 100 V, 30 min
  - Nucleic acid stain reagent: ethidium bromide (after dyeing)
  - Illuminator: UV (ATTO AE-6932 GXES print graph)
  - For the RNA run loading buffer containing formamide was used.

Workflow



We carried out the recommended protocol for DNase I treatment of our FastGene® RNA Premium Kit. For customers who want to remove the gDNA thoroughly the DNase I treatment after elution is highly recommended.
gDNA removal efficiency:
DNase I teatment on column < DNase I treatment after elution.
For more details read our technical note TN 2017 <2>.

Results

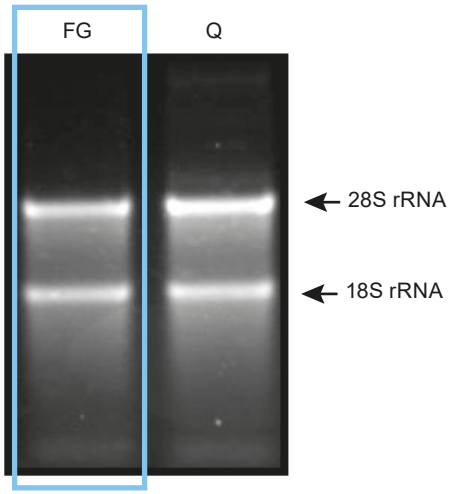
Yield and purity measured by NanoDrop

	260/280	260/230	Yield (µg)
FG	2.07	2.23	33.0
Q	2.09	1.65	34.2

FG: FastGene® RNA Premium Kit
Q: Q company RNA Extraction Kit (with DNase I treatment)

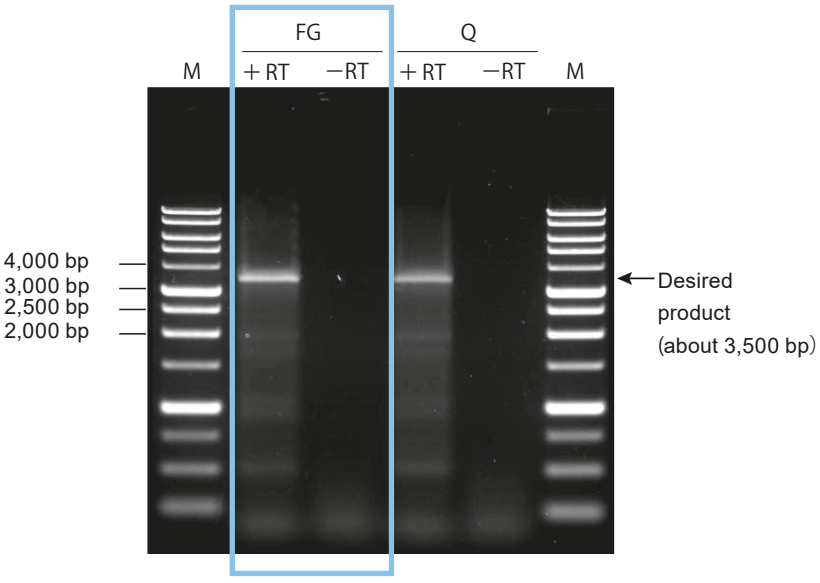
Both yield and purity were the same as those using Q company' s kit.

Electrophoresis reslut A (purified RNA)



500 ng of extracted RNA was analyzed on a 1 % non-denaturing agarose gel. Clear bands of 28S rRNA and 18S rRNA were obtained by using both FastGene® RNA Premium Kit and Q RNA Extraction Kit.

Electrophoresis result B (amplification of gene A using cDNA)



Using the extracted RNA, cDNA was synthesized with oligo dT20 primer and reverse transcriptase ReverTra Ace (TOYOBO). From the obtained cDNA, the full length of gene A (about 3,500 bp) was amplified using PCR enzyme KOD-Plus-Neo (TOYOBO) and cloning into pUC19 vector was attempted.
When 5 µl of the PCR product were electrophoresed on a 1 % agarose gel, a band was confirmed at the expected position when using RT for both the FastGene® RNA Premium Kit and the Q company RNA extraction kit. The successful insertion of this DNA fragment into pUC19 vector was confirmed by analysis of the nucleotid sequence.

Customer's comments

We used the FastGene® RNA Premium Kit for the isolation of gene A (about 3,500 bp), which is involved in cell division of mouse cultivated cells. An evaluation comparison with the kit of company Q showed that the yield and purity of the isolated total RNA are very similar. We synthesized cDNA from the obtained RNA and attempted amplification of gene A by PCR. At the expected position a band was detected. After purification of this fragment and it' s insertion into the vector by using restriction enzymes, gene A could be isolated without problems. Furthermore our laboratory has for other qRT-PCR experiments the same results as using the Q company' s kit. The FastGene® RNA Premium Kit allows a reproducible purification of stable high-purity RNA with a high yield and is moreover relatively inexpensive, so we strongly recommend it to those who seek a good price-performance ratio.

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