Application

RNA extraction from medaka brain - Comparison of RNA yield and DNA contamination

Product: FastGene® RNA Basic Kit（FG-80050, FG-80250）

Manufacturer: NIPPON Genetics EUROPE

The following data was kindly provided by Mr. Shinji Kanda, Mr. Daichi Kayo, Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Japan

Background

Analysis of RNA is carried out in various manner such as quantitative PCR. In recent years silica colum based extraction is increasing. However, since DNA and RNA have similar properties, it is difficult to separate DNA from RNA and DNA contamination is a serious problem, especially in quantitative PCR. For that reason, we have taken measures such as removal by DNase I and utilization of a genomic DNA removal column.

As a result of examining the RNA yield and DNA contamination amount of the commercially available RNA extraction kit, we found that FastGene® RNA Basic Kit has extremly low DNA contamination without any special operation.
Therefore, we thought that quantitative RT-PCR with sufficient precision can be performed with this kit alone even if the “control without reverse transcription” experiment was performed in real time quantitative PCR afterwards.
(If amplification is observed under “control without reverse transcription” , it is suggested that genomic DNA may remain in the RNA sample).
About the above, we will introduce the experimental results including comparison with an other company`s products.

Method

RNA extraction

1. Sample preparation
   Ten brains of Himedaka were excised, added to 3.5 mL of lysis buffer of each kit and homogenized using a hand micro homogenizer (physcotron NS-310E Microtech - Nition).
   It was stored in a deep freezer until RNA extraction treatment was performed.

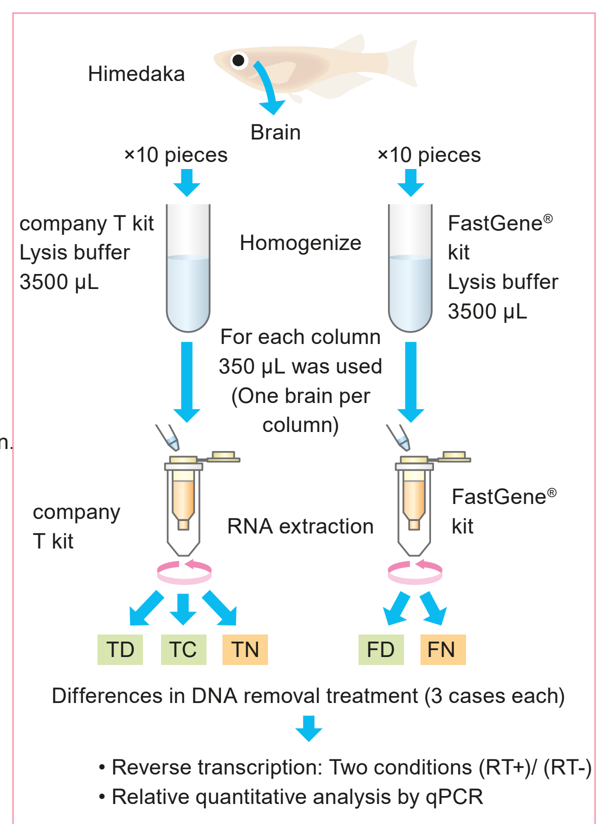
2. RNA extraction
   Use kit: (1) company T RNA extraction kit (2) FastGene® RNA Basic Kit
   Dispense 350 μL of sample homogenized in Lysis buffer
   (one brain per column), RNA was extracted under each condition.

3. DNA removal treatment
   It was conducted under the following five conditions. Three columns were used for each condition.

		company T kit	FastGene® kit
With DNA removal	DNase treatment*1	TD	FD
	Column removal*2	TC	—
No DNA removal*3		TN	FN

∗1: Performing DNase treatment
After RNA binding column was treated, before washing the membrane.
T company DNase was used and treated on membrane at RT for 15 min.
∗2: Performing genomic DNA removal column treatment.
Performed according to recommended protocol of T company`s kit.
∗3: DNA removal treatment has not been performed.
Both steps *1, *2 above were skipped.

4. Elution
   Eluted with 50 μL of elution buffer included in each kit.



Evaluation of extracted RNA samples was carried out as followed:

1. Measurement by Qubit
   RNA yield was measured using Qubit.
   Also, the amount of remaining DNA was examined by measuring the yield.

2. Relative determination by qPCR
   Reverse transcription of RNA was performed (40 ng) in a 5 μL reaction volume system using TaKaRa, PrimeScript RT Master Mix.
   Relative expression of ribosomal protein 13 (rps 13) gene was quantified using complete cDNA as a template.
   In addition to the presence of reverse transcription (RT+), qPCR was also performed without RT (RT-) and the extent to which genomic contamination affects quantification was also verified.

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Result

Yield (Qubit)

company T kit : TD、TC、TN
FastGene® RNA Basic kit : FD、FN

RNA yield

RNA (ng/μL)	TD	FD	TC	TN	FN
	With DNA removal treatment			No DNA removal treatment	
Column 1	67.4	20.4	40.2	54.8	41.6
Column 2	70.6	35.0	43.4	55.6	39.8
Column 3	59.0	17.3	41.8	53.8	55.8

Residual DNA amount

DNA (ng/μL)	TD	FD	TC	TN	FN
Column 1	1.100	0.450	0.370	17.0	0.614
Column 2	0.848	0.480	0.258	14.4	0.500
Column 3	1.050	0.466	0.374	17.1	0.552

Approximately any necessary amount of RNA was obtained.
The variation of FastGene® kit with DNase I treatment (FD) is presumed to be caused by what was done on the on-column.
In company T kit without DNA removal treatment (TN), DNA remained.
On the other hand, in FastGene® Basic kit, DNA contamination was very low even without DNase I treatment (FN).

qPCR

company T kit : TD、TC、TN
FastGene® RNA Basic kit : FD、FN

Cq value（rps13）

RT+	TD	FD	TC	TN	FN
	With DNA removal treatment			No DNA removal treatment	
column 1	18.23	17.79	18.33	17.18	17.87
column 2	17.80	17.69	18.34	17.34	18.17
column 3	18.82	17.79	18.74	17.20	17.84

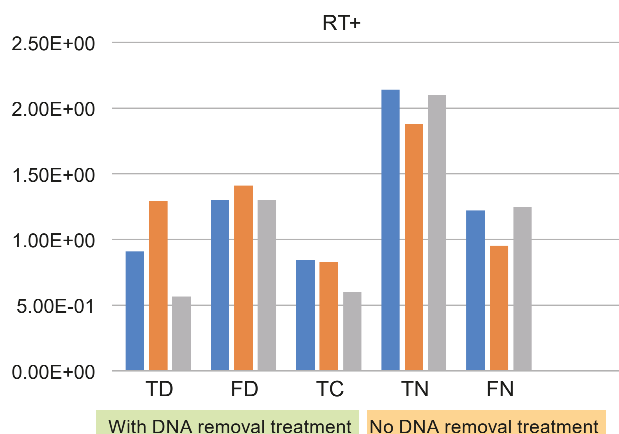
RT-	TD	FD	TC	TN	FN	NTC
column 1	30.79	30.83	29.97	26.47	30.41	32.74
column 2	30.95	28.90	30.08	26.67	30.75	
column 3	30.44	30.83	30.21	26.50	30.47	

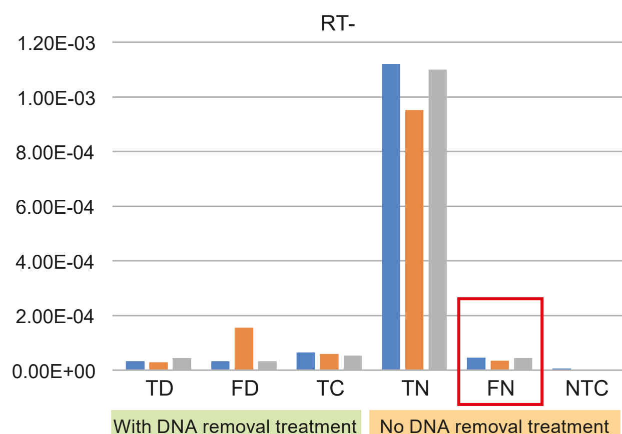
Relative expression of rps13

company T kit : TD、TC、TN
FastGene® RNA Basic kit : FD、FN

RT+	TD	FD	TC	TN	FN
	With DNA removal treatment			No DNA removal treatment	
column 1	9.09E-01	1.30E+00	8.41E-01	2.14E+00	1.22E+00
column 2	1.29E+00	1.41E+00	8.31E-01	1.88E+00	9.53E-01
column 3	5.64E-01	1.30E+00	5.99E-01	2.10E+00	1.25E+00

RT-	TD	FD	TC	TN	FN	NTC
column 1	3.35E-05	3.24E-05	6.48E-05	1.12E-03	4.56E-05	6.81E-06
column 2	2.94E-05	1.55E-04	5.96E-05	9.52E-04	3.45E-05	
column 3	4.44E-05	3.23E-05	5.36E-05	1.10E-03	4.34E-05	





In the absence of reverse transcription (RT-), residual DNA was suggested in company T kit without DNA removal treatment (TN).
On the other hand, the omitted DNA removal treatment (FN) of the FastGene® kit suggested that DNA contamination was extremely small as with each condition (TD, FD, TC) with DNA removal treatment.

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The yield of RNA extraction for one medaka adult fish brain did not differ greatly in any of the kits.
By using DNase I treatment or the accompanying genomic DNA column, RNA isolated by competitor`s kit was specific which excluded the influence of genome and was able to processed.
With FastGene`s column it was found that even without this treatment the genomic contamination was as low as the level done by other company`s genomic DNA removal column treatment and the RNA yield was sufficient. The results of quantitative PCR are also stable.
We also believe that being relatively inexpensive is a major advantage of FastGene® RNA Basic Kit.

Customer`s comment

Comment from Nippon Genetics Co., Ltd.

In the commercially available RNA extraction kit, the degree of "residual genomic DNA" to the extracted RNA sample depends on the type of starting materials, the amount of sample and other factors.
Generally, as the number of cells contained in the starting materials increases, the possibility of genomic DNA residual increases since the amount of genomic DNA correlates with the number of cells.

This time, thanks to Mr. Daichi Kayo and Mr. Shinji Kanda, "With the FastGene® RNA Basic Kit there is little DNA contamination without special DNA removal treatment, and in the downstream qPCR results there where hardly any effect" . In this case, as shown in the data in this document, the residual genomic DNA was checked by a method that can specifically measure DNA called "control without reverse transcription" . To check the influence of residual genomic DNA, qPCR was carried out in parallel.

If residual genomic DNA is observed or if it is necessary to avoid the risk of residual genomic DNA in downstream application, we recommend the use of FastGene® RNA Premium Kit containing DNase I treatment mentioned in the protocol.

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