NIPPON Genetics EUROPE

Application

Examination of RNA extraction method from a small number of cells (1000) using FastGene® RNA Premium kit

Product

FastGene® RNA Premium Kit（FG-81050, FG-81250）
FastGene® Scriptase II ready mix (LS64)

Manufacturer

Nippon Genetics Europe GmbH

The following data has been posted due to the kindness of Kyoto University customers.

Overview

FastGene® RNA Premium kit is recommended to extract RNA from 5×10^6 cultured cells using standard protocol and 1×10^7 for the large input protocol. In this Application Note, we investigated a new protocol for extracting RNA from a small amount of cells (1×10^3 cultured cells) using the FastGene® RNA Premium kit. We obtained yields and Ct values equivalent to or higher than a competitor kit from company Q.

Experimental conditions

1. RNA purification

Sample type ：Mouse embryonic fibroblast (MEF)
Sample amount ：Approximately 1000 cells
RNA purification kit ：① RNeasy Micro kit (company Q)　② FastGene® RNA Premium Kit
DNase I treatment ：①、② not implemented
RNA elution volume ：20 µL for both ①、②

RNA recovery method：

1. Add 100 µL of chloroform to TRIzol sample, vortex and then centrifuge at RT for 15 min
2. Mix 200 µL of the supernatant with 200 µl of 70% EtOH and transfer to company Q or FastGene® mini-elute column Volume (total 400 µL)
3. Spin down (RT, max、～30 s)

(For company Q kit, n = 3):

4. Discard flow through, reset the column, add 700 µL of RW1 and spin down (RT, max, ～30 s)
5. Discard flow through, reset the column, add 500 µL of RPE and spin down (RT, max, ～30 s)
6. Discard flow through, reset the column, add 500 µL of 80% EtOH and spin down (RT, max, ～30 s)
7. Place the column in a new collection tube and spin down (RT, max,1 min)
8. Place the column in a new 1.5 mL tube, add 20 µL RNase-free water on the silica membrane and incubate at RT for about 3 min
9. Centrifuge at RT, max, 1 min and collect flow through

（For FastGene® RNA Premium mini-elute column (simplified protocol), n = 3)

4. Discard flow through, reset column, add 600 µL RW1 and spin down (RT, max, ～30 s)
5. Place the column in a new collection tube, add 700 µL of RW2 and spin down (RT, max, ～30 s)
6. Place the column in a new collection tube and spin down (RT, max,1 min)
7. Place the column in a new 1.5 mL tube, add 20 µL of RE to the centre of the silica membrane and incubate at RT for about 3 minutes
8. Centrifuge at RT, max, 1 min and collect flow through

FastGene® RNA Premium Kit

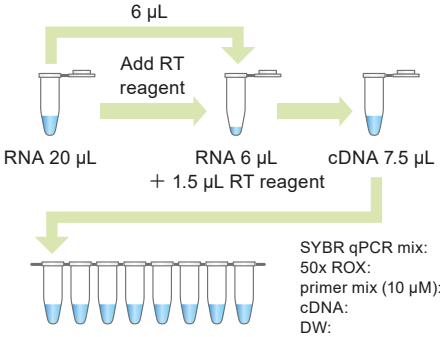
Cat.No.		
	FG-81006	6 rxn
	FG-81050	50 rxn
	FG-81250	250 rxn

RNA yield:

Using Qubit4, concentration was measured by RNA HS Assay Kit
For measurement, 10 µL was used for company Q sample and 3 µL for the FastGene® sample

2. Reverse transcription reaction

Performed using FastGene® ScriptaseII ready mix (LS64)
1.5 µL of reagent was added to 6 µL of RNA sample, following procedure was used
25℃ 10 min → 42℃ 60 min → 85℃ 5 min → 4℃ hold

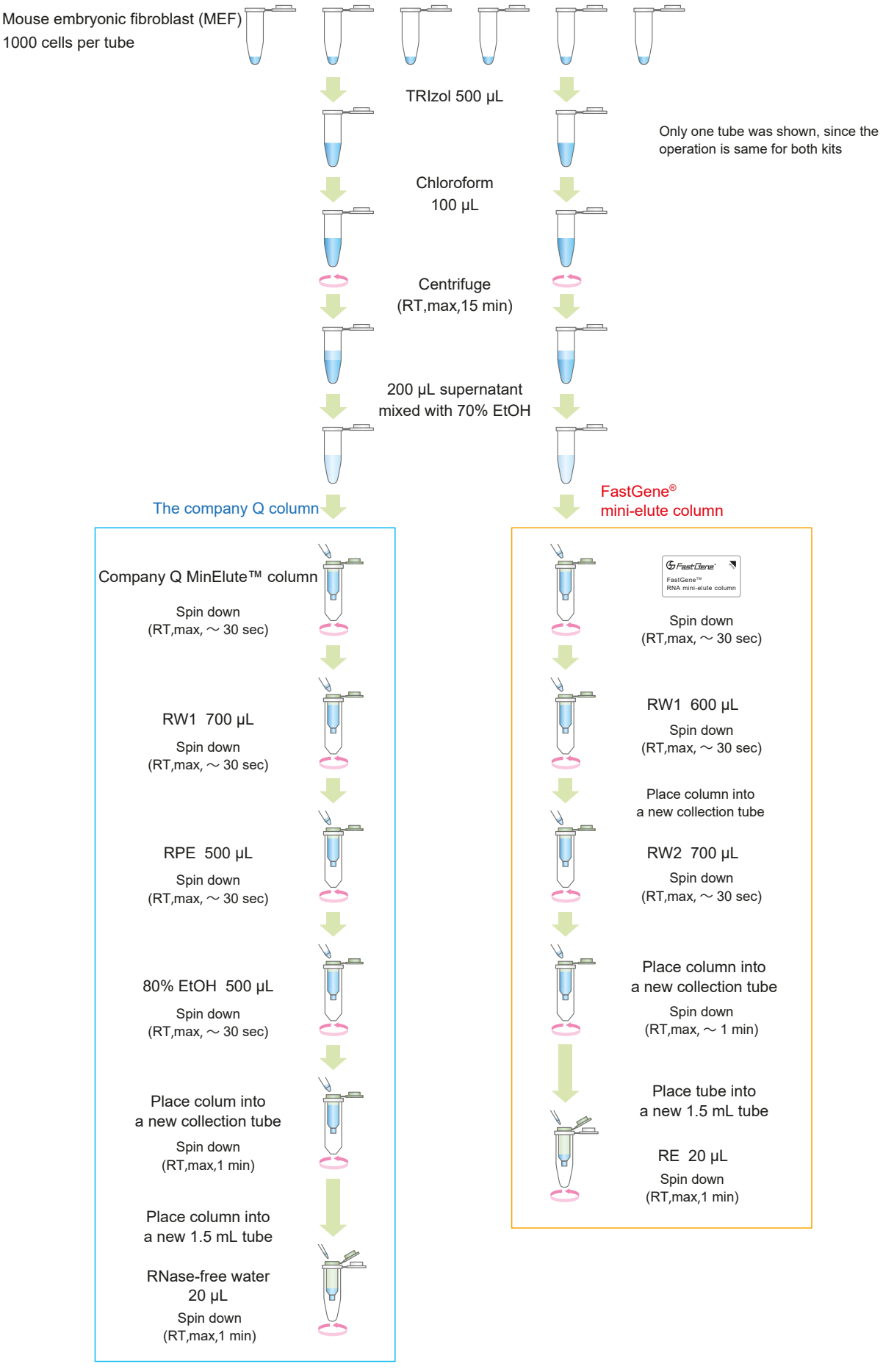


3. Real-time qPCR

Reagent: THUNDERBIRD SYBR qPCR Mix（TOYOBO）
Equipment: ABI StepOnePlus（Thermo）
(Analysis target genes: high expression (high) and medium expression (medium))
Cycle conditions: according to TOYOBO' s Thunderbird protocol

SYBR qPCR mix:	10.0 µL
50x ROX:	0.4 µL
primer mix (10 µM):	0.6 µL
cDNA:	1.0 µL
DW:	8.0 µL

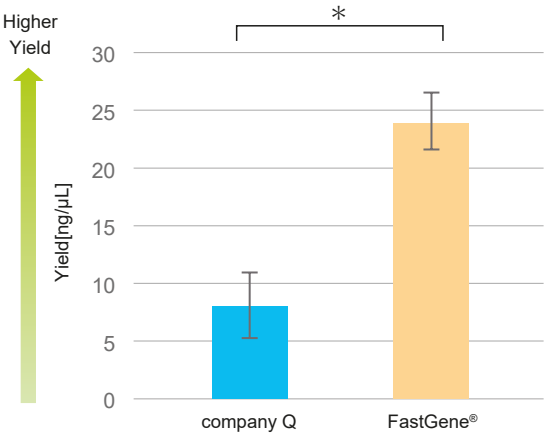
Study method workflow



Result

Yield measured by Qubit 4

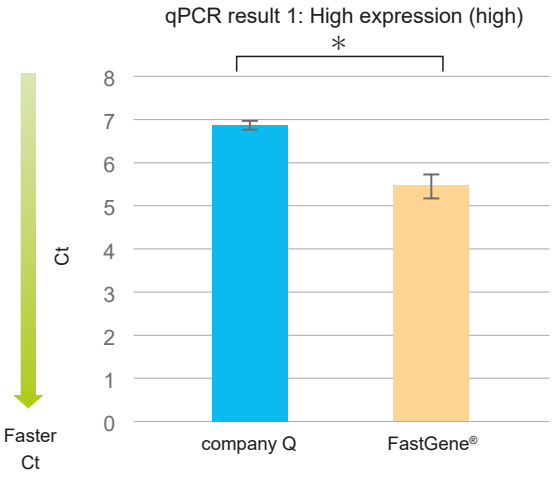
Used kit		Yield		
		Yield [ng/μL]	Mean [ng/μL]	SD
Company Q	1	5.06	8.03	3.16
	2	6.62		
	3	12.4		
FastGene®	1	25.3	23.90	2.42
	2	20.5		
	3	25.9		



Yield of FastGene® RNA Premium kit is 3 times higher as company Q [anlyzed with Qubit 4] (∗*t*-Test：P＜0.05)

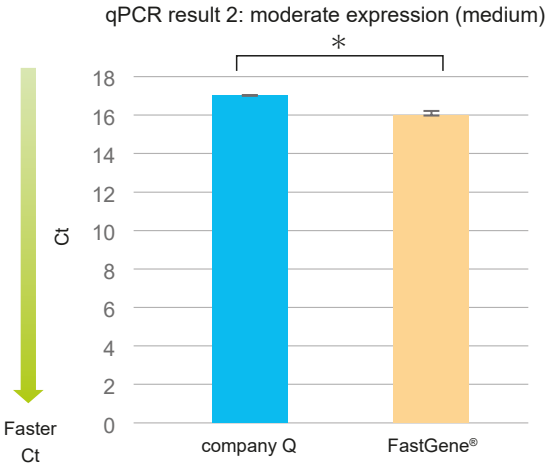
qPCR result 1: High expression level (high)

Used kit		Ct	Mean	Mean Ct	SD
Company Q	1	6.83	6.87	6.86	0.08
		6.92			
	2	6.74	6.78		
		6.83			
	3	6.98	6.93		
		6.87			
FastGene®	1	5.38	5.36	5.47	0.22
		5.34			
	2	5.78	5.77		
		5.77			
	3	5.32	5.27		
		5.23			



qPCR result 2: medium expression level (medium)

Used kit		Ct	Mean	Mean Ct	SD
company Q	1	17.15	17.12	17.03	0.09
		17.08			
	2	16.90	16.91		
		16.92			
	3	17.07	17.05		
		17.03			
FastGene®	1	15.90	15.87	16.00	0.14
		15.85			
	2	16.20	16.19		
		16.17			
	3	15.96	15.94		
		15.93			



Comparing Ct values by qPCR, FastGene® RNA Premium kit has lower Ct values compared to company Q for both high and medium expression level. (∗*t*-Test：P＜0.05)

Customer's comment

This time, we tried a new protocol in anticipation of cost advantages.
In this experiment we have used samples with very small cell number and tiny amount of protein and DNA contamination. Therefore, we did not perform the whole protocol including DNaseI digestion. Using this protocol we ended up in a shorter procedure time but no compramise in yield and qPCR performance.

Binsfelder Straße 77,
52351 Düren, Germany

www.nippongenetics.eu
TEL +49 2421 554960 FAX +49 2421 55496 1 info@nippongenetics.eu