RNA extraction from low number of cells using FastGene® RNA Premium Kit

Product	FastGene® RNA Premium Kit (FG-81050, FG-81250)
Distributor	NIPPON Genetics EUROPE GmbH

The following data were kindly provided by a researcher at Kyoto University.

Purpose

This study evaluates a new protocol for extracting RNA from as few as 1000 cultured cells using the FastGene® RNA Premium Kit. The objective is to assess RNA yield and quality in comparison to a competitor kit from Company Q, ensuring that even low-input samples achieve reliable results for downstream applications.

Summary

Extracting high-quality RNA from a limited number of cells is essential for various molecular biology applications, yet it presents significant challenges. The FastGene® RNA Premium Kit is optimized for standard and large input protocols, but its performance with low-input samples required further investigation.

This study evaluates a new protocol for RNA extraction from as few as 1000 cultured cells using the FastGene® RNA Premium Kit. RNA yield and quality were assessed in comparison to a competitor kit from Company Q, the market leader in RNA extraction.

The results demonstrated that the **FastGene® RNA Premium Kit provides equivalent or superior Ct values and yields, highlighting its suitability as a reliable solution for low-input RNA extraction.**

Materials

- Mouse embryonic fibroblast (MEF) ~ 1000 cells
- RNeasy Micro kit (company Q)
- FastGene® RNA Premium Kit
- Chloroform
- TRIzol
- Vortex
- Centrifuge
- 70% EtOH
- Qubit™ 4 Fluorometer
- RNA HS Assay Kit
- FastGene® ScriptaseII Ready Mix (LS64)
- THUNDERBIRD SYBR qPCR Mix (TOYOBO)
- ABI StepOnePlus (Thermo)
- Primers

Experimental procedure

RNA purification

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 (total volume 400 µL).
3. Spin down at RT, max 30 s.

Protocol Company Q kit, n = 3

4. Discard flow through, reset the column, add 700 µL of RW1 and spin down at RT for max 30 s.
5. Discard flow through, reset the column, add 500 µL of RPE and spin down at RT for max 30 s.
6. Discard flow through, reset the column, add 500 µL of 80% EtOH and spin down at RT for max 30 s.
7. Place the column in a new collection tube and spin down at RT for 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 for max 1 min and collect flow through.

Protocol FastGene® RNA Premium Kit (simplified protocol), n = 3

4. Discard flow through, reset column, add 600 µL RW1 and spin down at RT for max 30 s.
5. Place the column in a new collection tube, add 700 µL of RW2 and spin down at RT for max 30 s.
6. Place the column in a new collection tube and spin down t RT for 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 min.
8. Centrifuge at RT for max 1 min and collect flow through.

The RNA purification workflows for both the FastGene® RNA Premium Kit and Company Q are depicted in figure 1.

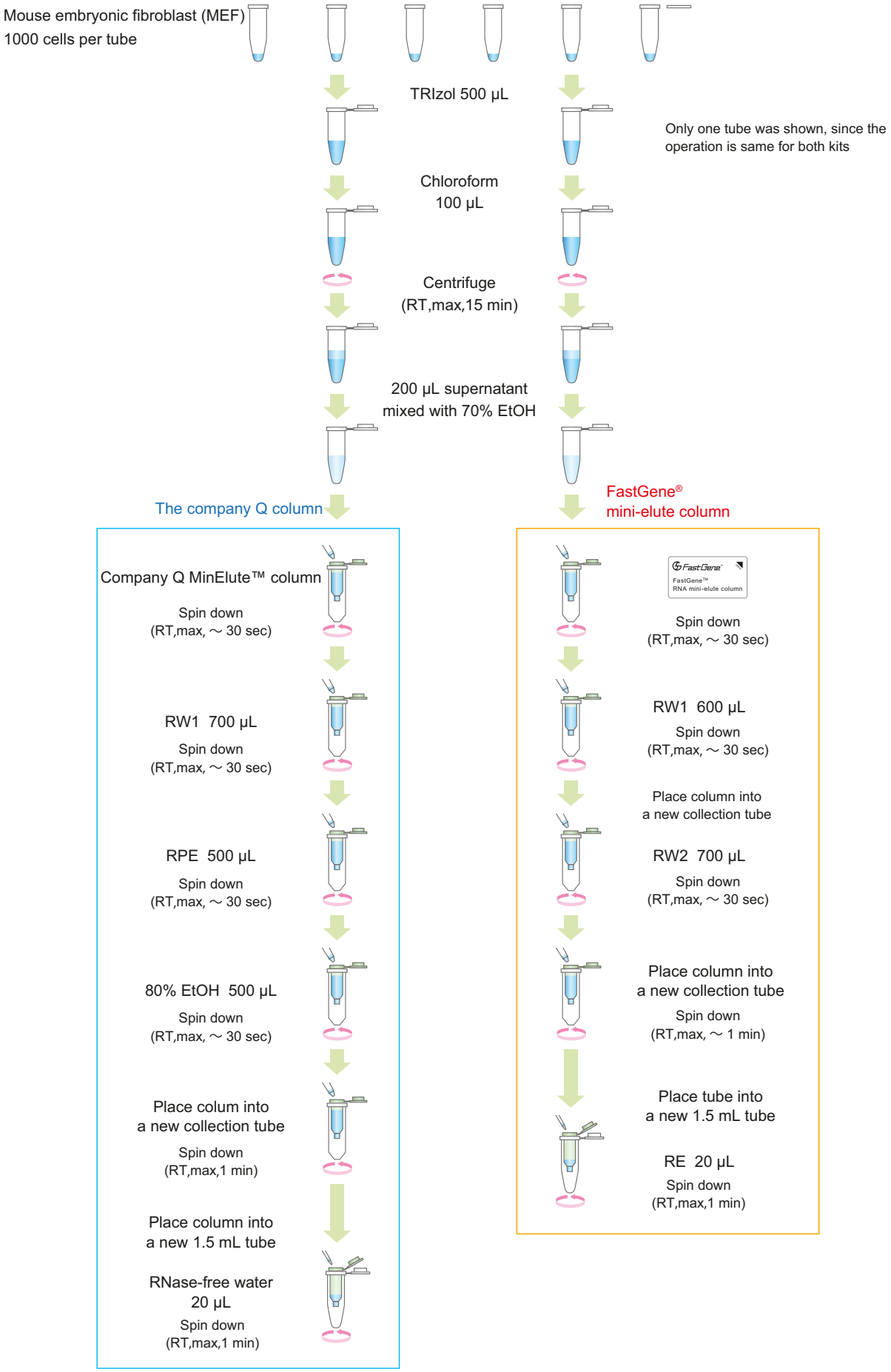

Figure 1: Workflow of RNA purification using Company Q Kit and FastGene® RNA Premium Kit.

RNA yield measurement

1. RNA concentration was measured using the Qubit™ 4 Fluorometer with the RNA HS Assay Kit.
2. For measurement, 10 µL of the Company Q sample and 3 µL of the FastGene® sample were used.

Reverse transcription

1. The reaction was carried out using FastGene® ScriptaseII Ready Mix (LS64).
2. A total of 1.5 µL of FastGene® Scriptase II Ready Mix (LS64) was added to 6 µL of the RNA sample (figure 2, top panel), followed by the following thermal cycling conditions:
   - 25°C for 10 minutes
   - 42°C for 60 minutes
   - 85°C for 5 minutes
   - 4°C hold.

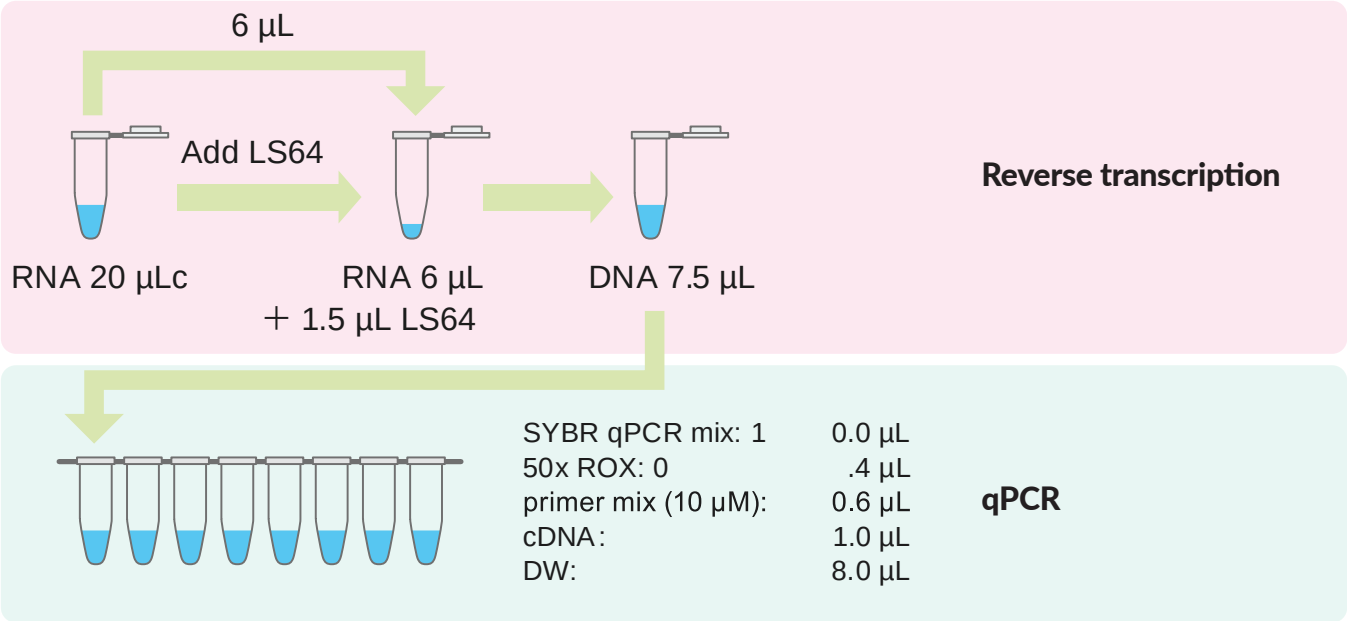

Figure 2: Workflow of reverse transcription and real-time PCR.
Reverse transcription was performed using FastGene® Scriptase II Ready Mix, followed by real-time qPCR analysis.

Real-time qPCR

1. The analysis was performed on target genes with high expression (high) and medium expression (medium).
2. The cycle conditions were followed according to the TOYOBO Thunderbird protocol.

Results

I. RNA yield extracted from a low number of cells

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		

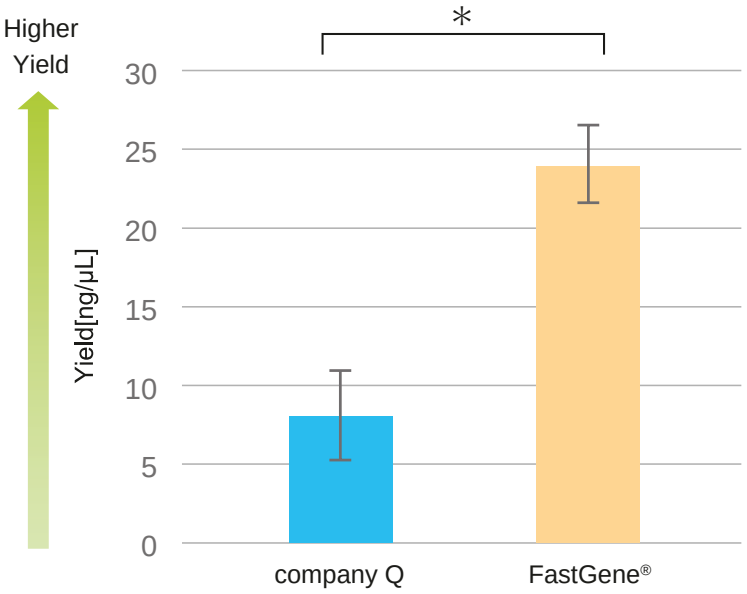

Figure 3: RNA yield measurements from the Company Q Kit and FastGene® RNA Premium Kit were performed using the Qubit™ 4 Fluorometer.

Extraction of RNA from only **1000 cells** using the **FastGene® RNA Premium Kit resulted in yields three times higher than those obtained with Company Q**. Statistical analysis using a t-test revealed a significant difference ($P < 0.05$), highlighting the superior efficiency of the FastGene® RNA Premium Kit even with low cell numbers.

II. Validation of RNA yield extracted from a low number of cells

Target gene with high expression level

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			

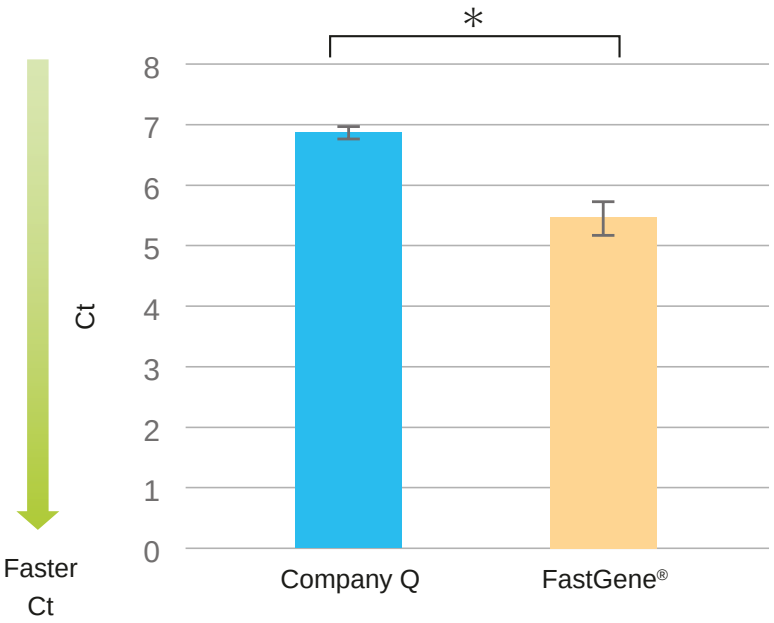

Figure 4: Comparison of Ct values for a high-expression gene target between Company Q and FastGene® RNA Premium Kit using RNA extracted from 1000 cells.

For **high-expression** gene, the **FastGene® RNA Premium Kit produced significantly lower Ct values** compared to Company Q, indicating a **higher RNA yield and more efficient amplification**. This result was statistically significant ($P < 0.05$), demonstrating that the FastGene® RNA Premium Kit outperforms Company Q in RNA extraction from low-input samples for high-expression targets.

Target gene with medium expression level

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			

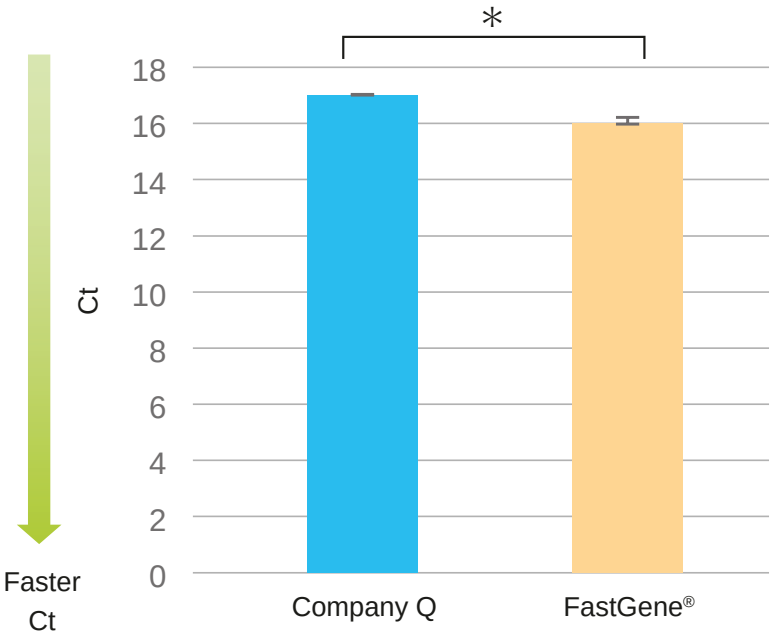

Figure 5:Comparison of Ct values for a medium-expression gene target between Company Q and FastGene® RNA Premium Kit using RNA extracted from 1000 cells.

Similarly, for **medium-expression gene**, the **FastGene® RNA Premium Kit effectively extracted and amplified RNA from low sample volumes**, showing reliable performance even with moderate gene expression. The difference in Ct values was statistically significant ($P < 0.05$), supporting the FastGene® RNA Premium Kit's capability to handle a variety of gene expression levels efficiently.

Conclusion

The results of this study demonstrate that the **FastGene® RNA Premium Kit** offers superior **RNA extraction efficiency and amplification performance**, even from as few as 1000 cells. The kit produced **yields three times higher** than those obtained with Company Q, with statistically significant differences ($P < 0.05$). For **both high-expression and medium-expression genes**, the **FastGene® RNA Premium Kit consistently delivered lower Ct values** compared to Company Q, indicating more efficient RNA extraction and reliable amplification across a range of gene expression levels. These findings underscore the **effectiveness of the FastGene® RNA Premium Kit for RNA extraction from low-input samples**, making it a highly efficient solution for various research applications.

Customer comment

In this experiment, we tested a new protocol with the goal of achieving cost efficiency. The samples used contained very low cell numbers and minimal protein and DNA contamination, which is why the full protocol, including DNase I digestion, was not necessary. As a result, the modified protocol reduced the procedure time without compromising RNA yield or qPCR performance.

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