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Application

RNA isolation of Neuro 2a cells (mouse neuroblastoma) Analysis of purified RNA by Real Time PCR (qPCR)

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

Manufacturer Nippon Genetics Europe

The following data was published due to the kindness of the Department of Peripheral Nervous System Research, National Institute of Neuroscience, National Center of Neurology and Psyciatry, Japan

Method

Mouse derived neuroblastom Neuro2a cells were divided in 6 parts (per tube 1 Mio cells). Three tubes were used for purification by the Qiagen RNeasy kit and the other three tubes were used with the FastGene® RNA Basic Kit.

1 μg of RNA was reverse transcribed in a 20 μl reaction system using ReverTra Ace enzyme of TOYOBO. The reverse transcription reaction sample was diluted 20-fold with MQ (Millipore) water and 1 μl was subjected to qPCR. For the PCR reaction the THUNDERBIRD SYBR qPCR Mix from TOYOBO Co., Ltd. was used. Actin and Gene A were used as target genes. All experiments were performed using the 7300 Real-Time PCR System from Applied Biosytems.

Workflow

Cultured cells Neuro2a

Per tube
1.0×10^6 cells

Condition

1. RNA purification
Sample type: Neuro2a cells (mouse derived neuroblastoma)
Sample amount: 1.0×10^6 cells / prep
RNA purification kit: ①Company Q Kit ②FastGene® RNA Basic Kit (each n=3)
DNase I treatment: Neither ① nor ②
RNA elution volume: 30 μl for both kits

RNA purification ① Q company kit

RNA purification ② FastGene® RNA Basic Kit

・n=3
・DNase I not performed
・30μl elution

2. RT reaction
Input RNA amount: 1 μg (per 20 μl per reaction)
Reverse transcriptase: ReverTra Ace （TOYOBO）

Reaction set up:

total RNA	1μg
Random Primers (25 pmoles/μl)	1μl
5×Buffer	4μl
10 mM dNTPs	2μl
ReverTra AceR (100units/μl)	1μl
（Total Volume	20μl）

Set up:

Annealing   30℃・10min
↓
Enzyme reaction 42℃・30min
↓
Denaturation 99℃・5min

・RNA sample 1 μg/ 20 μl reaction

RT reaction

・20-fold dilution cDNA template 1 μl/ 20 μl reaction
・n=3
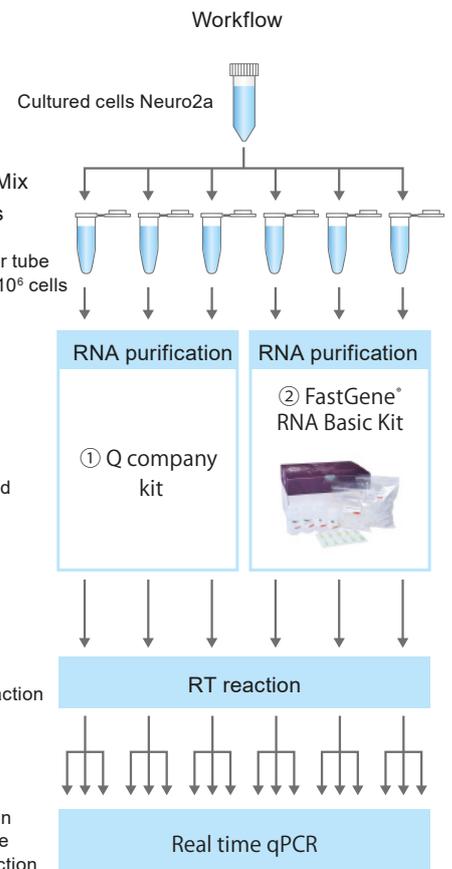
Real time qPCR

3. Real time PCR (qPCR)
cDNA volume: 1 μl of reverse transcription reaction solution diluted 20-fold
                 (20 μl per 1 reaction)
qPCR Reagent: THUNDERBIRD SYBR qPCR Mix （TOYOBO）
qPCR Instrument: Applied Biosystems 7300 Real-Time PCR System

Result

Yield and purity

RNA sample		yield		purity	
		(μg)	Mean (μg)	A260/280	A260/230
Company Q	1	17.6625		2.05	2.31
	2	18.4535	18.7547	2.05	2.47
	3	20.1480		2.04	2.50
FastGene®	1	18.9745		2.08	2.33
	2	21.4340	19.8202	2.06	2.49
	3	19.0520		2.07	2.42

NanoDrop
(Thermo Scientific™ Ltd)

There is just a little difference between both kits in regard to yield and purity.

qPCR result: actin

Sample		Ct	Mean	Mean Ct	StdDev Ct	P value
company Q	1	16.1606	16.1369	16.3551	0.29728	0.11140
		16.2475				
		16.0027				
	2	16.2128	16.2347			
		16.3102				
		16.1810				
	3	16.7193	16.6937			
		16.5803				
		16.7815				
FastGene®	1	15.9163	15.8943	15.8970	0.05795	
		15.8641				
		15.9024				
	2	15.9028	15.9563			
		15.8711				
		16.0950				
	3	15.9139	15.8405			
		15.8597				
		15.7479				

qPCR result 2: Gene A

Sample		Ct	Mean	Mean Ct	StdDev Ct	P value
company Q	1	21.7528	21.7298	21.9913	0.34464	0.17405
		21.5308				
		21.9058				
	2	22.3860	22.3819			
		22.5230				
		22.2366				
	3	21.5057	21.8623			
		22.2444				
		21.8369				
FastGene®	1	21.4406	21.4551	21.5940	0.13235	
		21.5040				
		21.4208				
	2	21.2879	21.6081			
		21.8181				
		21.7182				
	3	21.9397	21.7187			
		21.5256				
		21.6908				

In comparison with the company Q kit, the result of qPCR show little difference (P> 0.05).

We have used Q products so far, but the FastGene® RNA Basic Kit from Nippon Genetics is very convenient to use and yield and purity are even a little bit better.

Customers comment