NIPPON Genetics EUROPE

Customer product feedback

Product name:	FastGene® RNA Premium Kit (FG-81050, FG-81250)
Manufacturer name:	NIPPON Genetics EUROPE
Application:	Quantitative PCR using RNA extracted from mouse brain tissue (cerebral cortex) (Real-time qPCR and digital PCR)

The following data was published by the courtsey of Univeristy of Tsukuba, International Institute for Integrated Sleep Medicine Research Organization Yanagisawa/ Futo Laboratory Tomoyuki Fujiyama, Japan

Background

Sleep is an universal recognized behavior in animals, but neuroscientific mechanisms controlling this are still unknown. Analysis of mechanisms controlling sleep/wake behavior and analysis of neural circuit functions requires elucidation of molecular mechanisms by basic research, and research using various animal species including mice is currently under way. In our laboratory, we succeeded in establishing a dreamless mouse family, which uses forward genetics to search for novel genes resposible for the molecular mechanism of sleep/wakefulness and markedly shortens REM sleeping time. Furthermore, we found that the causative gene is *Nalcn* encoding nonselective cation channel NALCN (Funato, Fujiyama *et al*., Nature 2016).

In this application note, samples were prepared to examine the expression level of Nalcn gene mRNA in *Nalcn* gene-modified mouse brain tissue with FLAG-HA tag sequence inserted at the N terminus using the CRISPR/Cas system. From the adult mouse cerebral cortex cells, mRNA was extracted using this kit, cDNA was synthesized by reverse transcription, and the expression level of *Nalcn* gene was analyszed by real time qPCR (qPCR) and Droplet digital PCR (ddPCR). In ths application note, I will show examples of using FastGene products in the flow of general sample preparation up to the PCR experiment.

Experimental conditions

In this study, RNA was extracted from mouse brain tissue (cerebral cortex) of three genetically modified mouse strains ($Nalcn^{FLAG\text{-}HA/+}$) (mouse A,B,C) in which the FLAG-HA tag sequence was inserted into the N terminal coding region of the *Nalcn* gene. The difference between A, B and C is that the first parental mouse used to establish the lineage of mouse strains created by CRISPR is different.

For reproducibility, expression quantitative analysis using GAPDH as a reference gene was performed on Nalcn gene target regions 1 and 2 by real time qPCR and Droplet digital PCR with different methods.

1. RNA purification

RNA purification kit	competitor Q kit	FastGene® RNA Premium Kit
Sample	Mouse brain tissue (cerebral cortex) > 20 mg for each kit	
Lysis buffer volume	1,000µL	600µL*
DNase I treatment	Not implemented	Implemented as recommended
RNA elution buffer volume	50µL	

* recommended amount according to the large input

2. Reverse transcription reaction

Input RNA amount : 0.2µg

Reverse transcriptase : PrimeScript RT PCR Kit (#RR014A) (Takara)

<Composition/ reaction program>

Total RNA	0.2	µg
Random 6mers (20µM)	0.5	µL
Oligo dT Primer (2.5µM)	1	µL
dNTPs (10mM each)	1	µL
RNase Free dH2O	up to 10.5	
(Total Volume	10.5	µL)

⬇

Annealing 65℃・5min

⬇

5×Buffer	4	µL
RNase Inhibitor	0.5	µL
PrimeScript RTase	0.5	µL
RNase Free dH2O	up to 20	
(Total Volume	20	µL)

⬇

Enyzmatic reaction 42℃・30min

⬇

Denaturation 95℃・5min

3. Real time qPCR

Input cDNA quantity : 1µL reverse transcription solution (undiluted)

qPCR reagent : SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) (#RR820A) (Takara)

qPCR device : ViiA™ 7 real-time PCR system (Thermo Fisher Scientific)

4. Digital PCR

Input cDNA quantity : 1µL reverse transcription solution (undiluted)

ddPCR reagent : ddPCR™ EvaGreen Supermix (#1864034) (Bio-Rad)

dPCR device : QX200™ Droplet Digital™ PCR system (Bio-Rad)

Result

Result 1. Yield (ng/µL) of purified RNA from mouse brain tissue (cerebral cortex)

Sample		company Q RNA extraktion kit
mouse A	1	273.0
	2	305.5
	3	259.5
mouse B	1	560.9
	2	218.7
	3	340.5
average		326.6
SEM		49.9

Sample		FastGene RNA extraktion kit
mouse C	1	259.2
	2	171.6
	3	101.0
Wildtype	1	259.9
	2	332.2
	3	99.4
average		203.9
SEM		38.8

Student's t test gives a p value of 0.08, but because of usage only 6 mice for each kit, the results were defined as n.s.
Both kits are comparable in RNA extraction, but the first observation was that FG had a little lower in yield than company Q. This was considered to be a difference caused by different samples and not because of a DNase treatment or not. The purity was satisfactory.

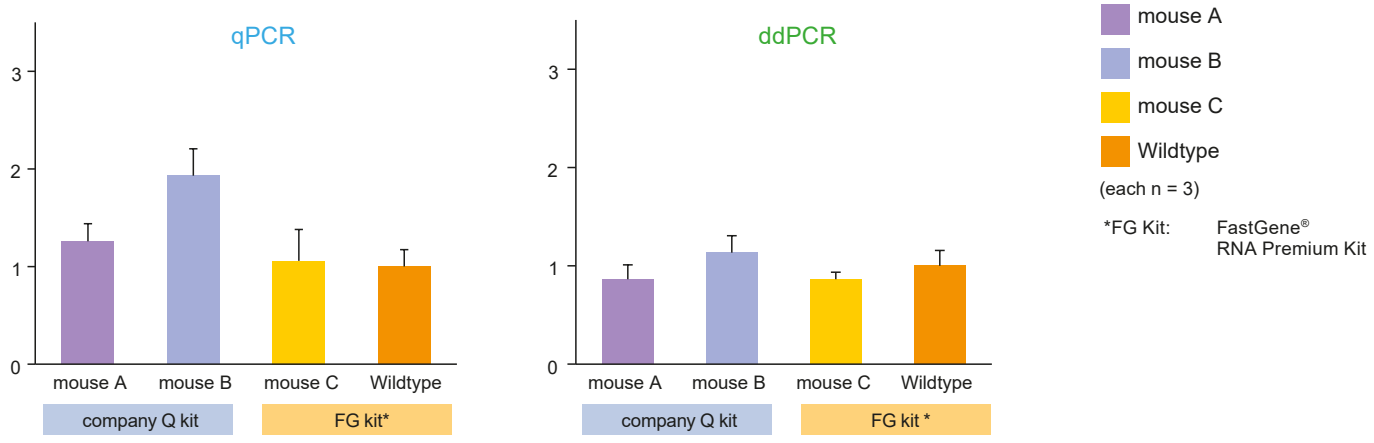
Supplement) About mouse A, B, C

It is a gentically modified mouse strain in which a FLAG-HA tag sequence is inserted into the N-terminal coding region of the *Nalcn* gene.
The difference between A, B, and C is that using different parental mice while establishing a lineage of mouse strains by CRISPR.

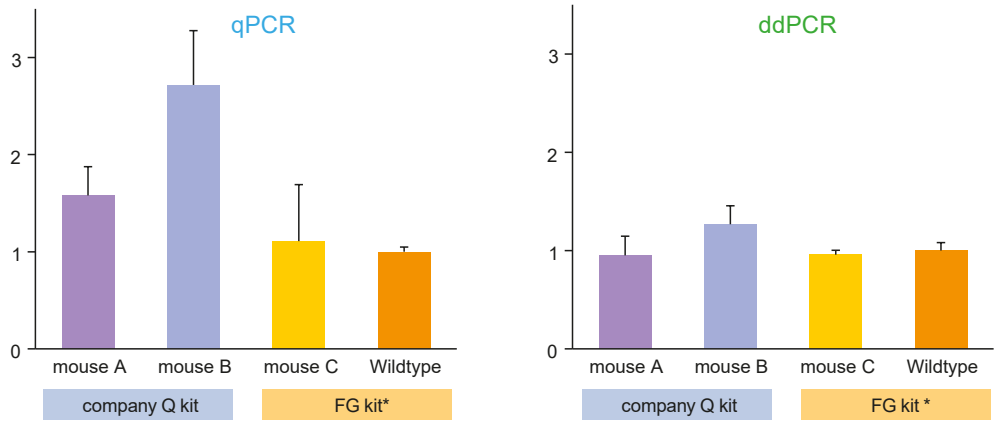
Result 2. Determination by real-time PCR and digital PCR

Real time qPCR and Droplet digital PCR was performed using RNA extracted from mouse brain tissue (cerebral cortex) of Wildtype and Nalcn gene modified mouse (*Nalcn*$^{FLAG\text{-}HA\,/\,+}$) of the same genotype. 3 mice per line were used (mouse A to C).
Specifically, quantitative analysis was performed on *Nalcn* gene traget regions 1 and 2 using GAPDH as a reference gene.
Since mutation was not added in the vicinity of the promoter region related to expression control of Nalcn gene in all three lines of mouse (A to C) this time, it was expected that the expression level of the target gene region was almost the same as wild type.
As a result, it was confirmed that in wildtype and mouse C, in which the RNA was extracted with FastGene® RNA Premium kit, the target gene regions 1 and 2 had almost equivalent expression levels as expected.

Nalcn gene target region 1



Nalcn gene traget region 2



<Customer's comments>

We performed qPCR and ddPCR experiments using RNA extracted with FastGene® RNA purification trial kit.
It came to the conclusion that the product performance was not only equivalent to what we used before, but also actually it was even better than before.
I am also satsified that the degree of purification of the extracted RNA is much higher than that obtained by the conventional methods.
Originally, NALCN channel is a very difficult protein to detect *in vivo* brain tissue. Therefore, we designed genetically modified mice by CRISPR with the aim of detecting NALCN with higher accuracy using tag antibody against brain tissue expressing tagged NALCN protein. As a result, it was possible to confirm highly specific signal by western blot using brain tissue lysate.
Based on the results of this PCR experiment, it is shown that there is no change in mRNA expression level compared with wild type, and it is expected that the expression level of NALCN protein itself will probably not be affected.
In future, I would like to analyze the molecular characteristics of NALCN channel in more detail using genetically modified mice expressing tagged protein.

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