NIPPON Genetics EUROPE

Application

Comparative evaluation of reverse transcriptase products for expression analysis of target genes in cultured cells

Product: FastGene® Scriptase II ReadyMix (5X) (LS64)

Manufacturer: NIPPON Genetics EUROPE GmbH

The following data has been provided by the curtesy of Kyoto University customers.

Overview

The comparative study was conducted between FastGene® Scriptase ReadyMix (LS64) and a “two-step RT-qPCR” competitor product, which was currently used. This study was carried out for expression analysis of target genes in cultured cells.
Reverse transcription reaction conditions were evaluated using qPCR as a downstream application, using the current program as it is and obtained cDNA.

Method (reverse transcription reaction)

- RNA sample: RNA recovered from MEF cells (mouse fetal fibroblast), or the same RNA diluted 1:10000 in water
- Reverse transcription kit: ① FastGene® Scriptase II ReadyMix (includes random primer)
  ② Th cDNA synthesis kit (current product) ※Two-step RT- qPCR kit (including random primer)
- Reaction composition:

① FastGene® Scriptase II ReadyMix

Sample RNA	14 µL
FastGene® Scriptase II ReadyMix	4 µL
dH_2O	2 µL ※
total	20 µL

※In this study, dH_2O was added separately, because the same sample RNA solution was used for reaction composition of the current kit ②.

② Th cDNA synthsis kit (current product)

Sample RNA	14 µL
5 x reaction mix	4 µL
10 x enzyme mix	2 µL
total	20 µL

- Reaction conditions

25℃	10 min
42℃	60 min
85℃	5 min
4℃	hold

※ For both kits, the reaction conditions of Th’ s cDNA synthesis kit (current product) were used.

- Downstream application:
  qPCR using SYBR Green I master mix (reagent)
  Device used: StepOnePlus™(Thermo Fisher Scientific)

FastGene® Scriptase II Series

FastGene™

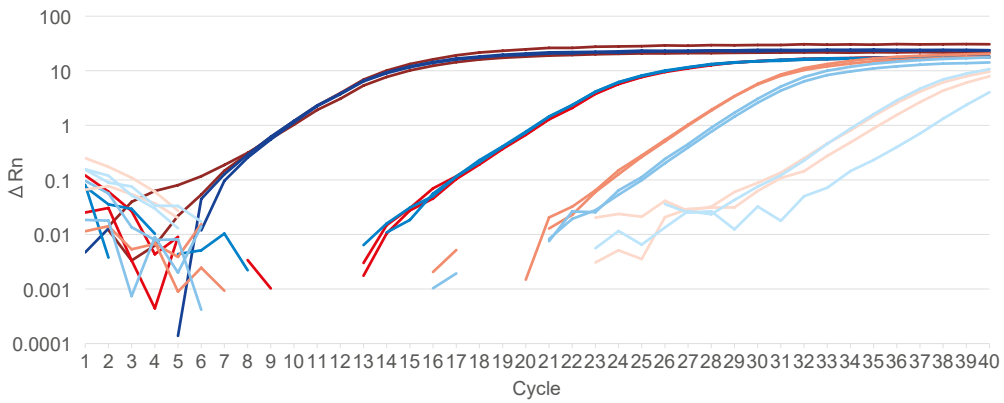
The FastGene® Scriptase II reverse transcriptase has a mutation in the reverse transcriptase MMuLV to “improve thermal stability” and “repress the RNase H activity” . This allows more complex applications such as long chain cDNA synthesis for cloning purpose, RT-qPCR, NGS etc.
You can choose from the following three types:

- FastGene® Scriptase II Reverse Transcriptase (LS53)
- FastGene® Scriptase II cDNA Synthsis kit (LS63),
- FastGene® Scriptase II ReadyMix (LS64) Note: For RT-qPCR

FastGene® Scriptase II ReadyMix (5X) (LS64)

This is a “revese transcription reaction ReadyMix including random primers” released for “quantification of gene expression by qPCR” .
In particular, ” quantitative expression of genes by qPCR” often requires processing multiple samples, since this product is a ReadyMix type, it can reduce the time and effort of multiple-sample dispensing operation.

Result



	Sample	Gene expression level	RT kit
	1	High	Scriptase II
	2		
	1		company Th kit
	2		
	1	Middle	Scriptase II
	2		
	1		company Th kit
	2		
	1	Low	Scriptase II
	2		
	1		company Th kit
	2		
	1	Middle 1:10000 dilution	Scriptase II
	2		
	1		company Th kit
	2		

Gene expression level	RT kit	Sample	Cт	Cт Mean	Cт SD	Tm1
High	Scriptase II	1	8.35	8.36	0.01	85.26
		2	8.37			85.26
	company Th kit	1	8.54	8.45	0.12	85.26
		2	8.37			85.26
Middle	Scriptase II	1	20.14	20.11	0.05	86.45
		2	20.07			86.45
	company Th kit	1	19.81	19.84	0.04	86.60
		2	19.86			86.45
Low	Scriptase II	1	28.38	28.40	0.03	83.49
		2	28.42			83.49
	company Th kit	1	29.90	29.75	0.21	83.49
		2	29.60			83.49
Middle 1:1000 dilution	Scriptase II	1	34.66	34.22	0.63	86.60
		2	33.78			86.45
	company Th kit	1	33.71	35.36	2.33	76.47
		2	37.01			80.79

- Description of target genes:
  High： High expression gene
  Middle： moderately expression gene
  Low： Low expression gene
- Each qPCR measured by duplicates

- Both kits used the reverse transcription reaction program of the current Th company kit. Reverse transcription was performed under the same conditions, and the results of qPCR using the cDNA were compared. As a result, almost identical amplification curves were obtained for both kits, so it was judged that the reverse transcription reaction was stably performed even when the FastGene® Scriptase II ReadyMix was used under the conditions of the current product.
- The reverse transcription of Middle (diluted solution) at a dilution of 1:10000 is expected to be detected about 13.3 cycles later than that of the undiluted solution, but it was clearly confirmed as an experimental result in FastGene® Scriptase II ReadyMix.

Customer's comment

The product was very easy to handle, as I did not use ReadyMix products until now.
In addition, the reverse transcription efficency of the low expression gene RNA was higher than that of the products conventionally used.

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