NIPPON Genetics EUROPE

Application

Comparative study of RT-reaction by using RNA extracted from mouse lymph node

Product: FastGene® Scriptase II cDNA Kit（LS63）

Manufacturer: NIPPON Genetics EUROPE GmbH

The following data was kindly provided by Ms. Haruko Hayasaka, Department of Life Sciences, Faculty of Science and Engineering, Kinki University, Japan.

Overview

Our laboratory has cloned the full-length cDNA by reverse transcription from mouse lymph node-derived RNA. In the current kit (T company), the signal of the target gene (390 bp) could not be detected, because short size cDNA is synthesized preferentially. By using the FastGene® Scriptase II cDNA Kit (LS63), the signal of the target gene was detected and the result was improved.

Experimental method

Sample: Lymph node (inguinal, mesenteric) harvested from one mouse

Qiagen: Purify total RNA with RNeasy Mini Kit

Harvest all of lymp node (muscle, mesenterium) from mouse
Sample quantity: 23 mg

↓

FastGene® Scriptase II cDNA Kit（LS63）

Input amount of RNA: 1µg

sample RNA	8	µL
oligo dT primer	1	µL
random hexamer	1	µL
dNTP	2	µL
H_2O	0.5	µL

↓ 65℃ 5min

Adding components

5x FastGene® Scriptase II buffer	4	µL
0.1 M DTT	2	µL
RNase Inhibitor	0.5	µL

↓ 42℃ 2min

Add 1 µL of FastGene® Scriptase II to RNA suspension on ice

↓ 42℃ 50min

↓ Incubate at 72℃ for 15 minutes

Storage at 4℃

FastGene® Scriptase II
（Cat No. LS63）

- cDNA can be synthesized from a small amount of RNA
- Obtain longer cDNA due to low RNase H activity

Beside this, it can be used in various applications like RT-qPCR and NGS.
MasterMix (5x) are also available for qPCR (Cat No. LS64).
→ containing all necessary components.

↓

PCR with KAPA2G Robust

● PCR program

Initial denaturation	98℃	2min	
Denaturation	95℃	15sec	35 cycles
Annealing	57℃	30sec	
Extension	68℃	2min	

KAPA2G Robust（Cat No.KK5004）

- Resistant to PCR inhibitors and less susceptible to the refining condition of the template.
- Low effect of freeze/thaw by unique enzyme stabilizer

Also with dye that is convenient for electrophoresis（Cat No.KK5706）

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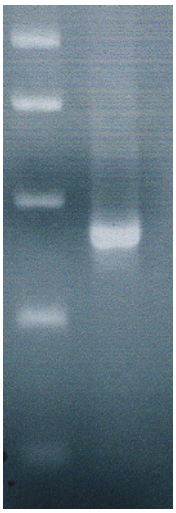
Electrophoresis

● Electrophoresis conditions

Electrophoresis unit	: Mupid-exU（ADV EXU-1 ）
Nucleic acid staining reagent	: RedSafe（INB-21141 ）
Electrophoresis buffer	: TBE buffer
Agarose gel concentration	: 2 %
Voltage and migration time	: 100 V / 30 min

Result



← 390bp
A clear signal of the target gene was confirmed

Reference data

← 390bp
When used other company RT enzyme + oligo dT Primer, the signal was weak
※Simultaneous comparison was not done

Customer's comment

As a result of RT-PCR using lymph node-derived RNA, we were able to detect the expression of the target gene (a gene which PCR amplification was unstable in reverse transcription using other reverse transcription reagents).
In addition, it was possible to confirm the band of the full length cDNA by gel electrophoresis.
I think this kit is a good product.

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