NIPPON Genetics EUROPE

Application

Comparative study of reverse transcriptase reaction using RNA extracted from zebrafish fertilized eggs

Product: FastGene® Scriptase Ⅱ（LS53）

Manufacturer: NIPPON Genetics EUROPE GmbH

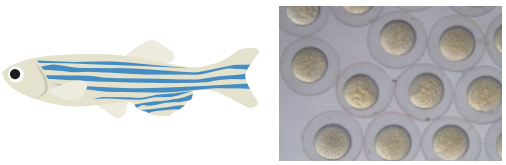
The following data was published through the courtesy of Tomoya Kotani' s of the Reproductive and Developmetal Biology, Faculty of Science, Hokkaido University, Japan

Introduction

Maternal factors stored in oocytes are extremely important for fertilization and development, but research is difficult due to the small expression rate of the transcription products.
Although transcripts that were attempted to be detected are thought to have important functions in development, it was predicted that the amount of expression is extremely small.
In addition to the study of detecting the transcripts by using FastGene® Scriptase II, we also confirmed that the resulting PCR products were without mutations, analyzed by cloning techniques after gel purification and reverse transcription.

Method

Initial sample: Zebrafish - Fertilized egg 50 pieces



RNA purification: TRIzol Reagent
(Thermo Fischer Scientific)

Reverse transcription reaction
(comparison of products)
- Conventional kit (competitor T)
- FastGene® Scriptase Ⅱ

PCR aparatus: GeneAtlas 485（ASTEC）

PCR enzyme: Expand High Fidelity PCR System
（Roche-Sigma）

PCR program

Predenature	94℃, 4 min	
↓		
Denature	94℃, 30 sec	35cycles
↓		
Annealing	55℃, 30 sec	
↓		
Extension	72℃, 60 sec./ kb	

Electrophoresis
- Electrophoresis apparatus: Mupid-2x
- Electrophoresis buffer: TAE
- Voltage: 100V
- Electrophoresis time: 15 min

FastGene® Scriptase Ⅱ

Input amount of total RNA 3 μg
＋
Mix 1 μL of oligo dT primer
＋
2 μL of dNTP Mixture was added

The amount of template RNA can be used up to the following amount
- Total RNA：1 ng-5 μg
- Messenger RNA（mRNA）：1 ng-0.25 μg
- Specific RNA：0.01 pg-0.5 μg

↓

Distilled water is added to a total amount of 12.5 μL

↓

Incubate at 65°C for 5 minutes, then cooled on ice

↓

Addition of components

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

↓

Incubate at 42°C for 2 minutes

↓

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

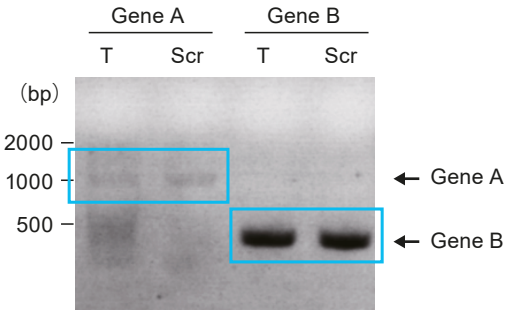
↓

Incubate at 42°C for 50 minutes

↓

Incubate at 70°C for 15 minutes to completely inactivate the enzyme

Result



T: conventional kit, competitor T
Scr：Scriptase II

Amplification size
Gene A (target amplification product): 1100 bp
Gene B (positive control): about 300 bp

Result

I was able to detect transcription products of very low expression levels with less background.

Customer comment

I was using an existing kit (competitor T), but I was looking for an inexpensive reverse transcriptase to replace it.
My laboratory uses several FastGene® products, because they are inexpensive, highly evaluated and of high quality.
After trying this product, I have seen that it is possible to detect transcripts with very small expression levels.
The FastGene® Scriptase II is not only inexpensive, but also proved to be a very good reverse transcriptase.
Therefore, I plan to use it in the future.

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