



Application

Oligo dT verification of FastGene® Scriptase II cDNA 5x ReadyMix OdT

Category

Reverse transcriptase

Product name

FastGene® Scriptase II cDNA 5x ReadyMix OdT
(Master Mix type/random hexamer + oligo dT primer included) Cat.No.: LS65

Manufacturer

Nippon Genetics Europe GmbH

The following data was kindly provided by Dr. Chie Umatani, Bioinformatics Laboratory, Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Japan

Overview

The conventional product (FastGene® Scriptase II cDNA 5x ReadyMix, LS64) was a master mix (premix) type reverse transcriptase reagent containing only "random hexamers" as reverse transcription primers.

This product tended to have a lower reverse transcription efficiency when compared to the product of Company T containing "random hexamer" and "oligo dT primer".

Therefore, we have newly prepared FastGene® Scriptase II cDNA 5x ReadyMix OdT (LS65), which is the conventional product (LS64) with "oligo dT primer" added.

When the effect of oligo dT Primer was verified in this application note, it was found that the reverse transcription efficiency was improved, and even when compared with the product of T company, the reverse transcription efficiency was comparable.

This result also means that the time-saving protocol used in conventional products can be applied.

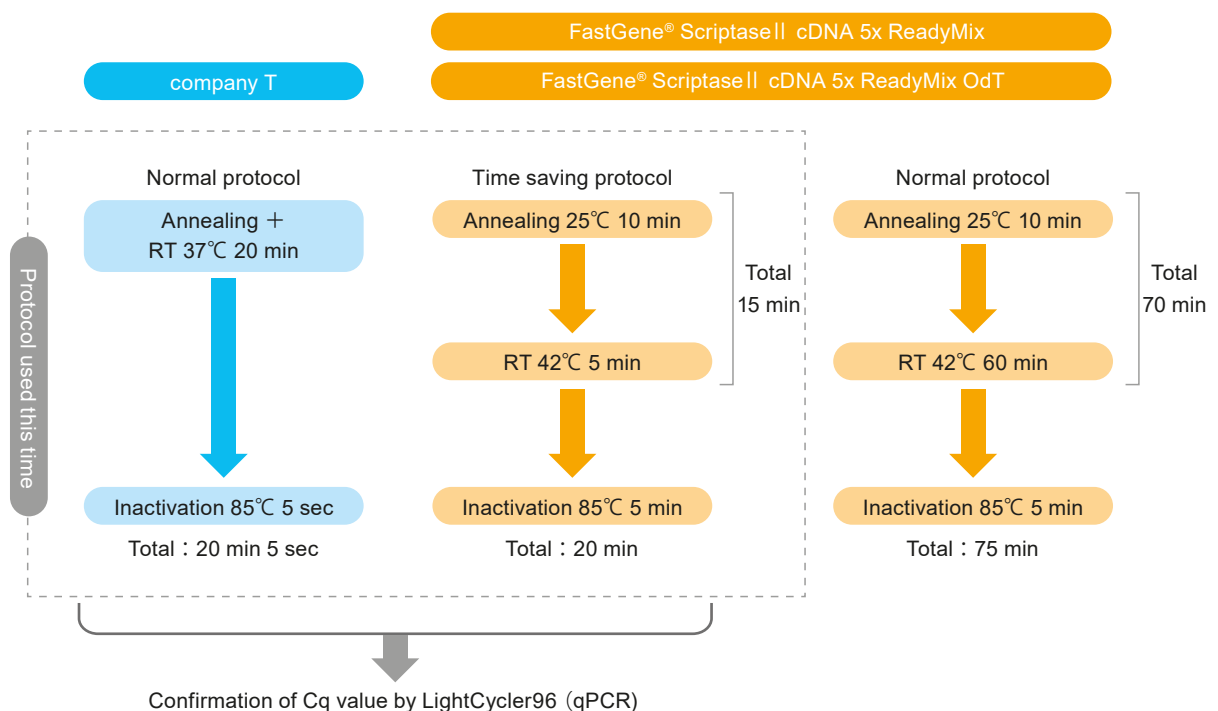
[Supplement]

In this application, we will compare our product with reverse transcriptase reagent of Company T under similar conditions, so please refer to Technical Note 2017 <EU1>. Reverse transcription was performed using the described time-saving protocol to perform comparative verification of reverse transcription efficiency.

Experimental method

Total RNA was extracted from the whole brain of medaka (*Oryzias*).

Cq value was obtained by performing reverse transcription and qPCR once, using 250 ng of total RNA





Results

【Comparison between company T and the conventional product (random hexamer only) (LS64)】

qPCR results: Cq value comparison (n=1)

company T	FastGene® (LS64 : Random hexamer only)
19.47	21.65

Gene: rps13
PCR product: 93 nt
PCR primer target region (region from 62 to 154 nt, counting from 5')

【Comparison between Company T and this product (including random hexamer and oligo dT primer) (LS65)】

qPCR results: Cq value comparison (#1 to #3 are medaka individuals)

Template	company T	FastGene®
#1	19.35	18.82
#2	19.36	19.09
#3	19.51	19.04
Ave	19.41	18.98

Gene: rps13
PCR product: 93 nt
PCR primer target region (region from 62 to 154 nt, counting from 5')

Reverse transcription was rapidly performed by adding the oligo dT primer.

● Conclusion

This product (LS65), which added the "oligo dT primer" to the conventional product, showed improved performance compared to the reverse transfer efficiency of the conventional product (LS64).

Also, in the reverse transcription reaction using this product, the reverse transcription reaction time was shortened from the usual 60 minutes to 5 minutes ["Time saving protocol"].



Thus, it was found that the reverse transcription efficiency is equal to or higher than that of the T company kit and can be achieved in a time comparable to that of the T company kit.



This product, which added the "oligo dT primer" to the conventional product, started up as early as 2 cycles and was able to obtain better reverse transcription efficiency.

Customer's comment

FastGene® Reverse Transcription Kit List

Reverse transcriptase specifications comparison table	FastGene® Scriptase Basic		FastGene® Scriptase II				
							
	Reverse transcriptase	cDNA synthesis kit	Reverse transcriptase	cDNA synthesis kit	Premix type		
Cat.No	LS52	LS62	LS53	LS63	LS64	LS65	
RT reaction temperature	42°C~50°C		42°C~50°C		42°C		
Time required	60 min		50 min (TN available for reduced time)		(5 min~) 60 min		
RNase H activity	Yes		Has reduced activity				
Maximum RNA addition	Up to 5 µg/20 µL		Up to 1 µg/20 µL		Up to 1 µg/20 µL		
Kit contents	Reverse transcriptase	○	○	○	○	●	●
	Buffer	○	○	○	○	●	●
	dNTP mixture	○	○	○	○	●	●
	Sterile water	○	○	○	○	×	×
	DTT	×	×	○	○	×	×
	RNase Inhibitor	×	○	×	○	●	●
	Oligo dT primer	×	○	×	○	×	●
	Random hexamer primer	×	○	×	○	●	●
Auxiliary Protein	×	×	×	×	●	●	
Remark	Reverse transcription primer can be selected according to the application				Suitable for qPCR		

○ : It is included in the kit as a separate tube and can be adjusted in any amount.

× : Not included in the kit.

● : Included in 5x FastGene® Scriptase II ReadyMix as premix type.