NIPPON Genetics EUROPE

Technical Data

Very fast reverse transcription reactions

Product

FastGene® Scriptase II cDNA Synthesis kit (Cat.No. LS63)

Purpose

Consider a short-time protocol using FastGene® Scriptase II

Method

cDNA was used to analyze the reverse transcription reaction with 4 different incubation times. Afterwards, endpoint PCR and qPCR was carried out to confirm and evaluate the amplification by electrophoresis and rising Cq value.

Summary

FastGene® Scriptase II is an engineered reverse transcriptase, able to deliver highest quality cDNA from a small amount of RNA. Optimization of enzymatic design has led to one of the most reactive RT-enzyme on the market. This technical note shows the investigation of the minimum time possible of a reverse transcription. We were able to shorten time to 5 minutes with different concentrations of RNA. The resulting cDNA was used in endpoint PCR as well as in qPCR experiments.

Here we demonstrate the ability of FastGene® Scriptase II to reverse transcribe lowest amount of RNA in a very short incubation period.

Reagents

- FastGene® Scriptase II cDNA Synthesis kit (LS63)
- RNA：Universal Human Reference RNA (Agilent Technologies)
  Input RNA amount: 5 ng, 0.5 ng, 0.05 ng
- Primer:
  - TUBB（1006 bp）：Endpoint PCR
  - GAPDH（138 bp）：qPCR
  - YWHAZ（249 bp）：qPCR

FastGene® Scriptase II cDNA Synthesis kit

Experimental procedure

Reverse transcription reaction

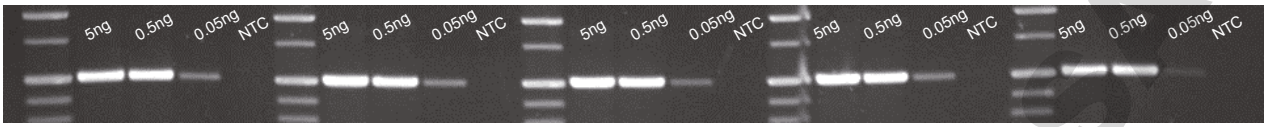
Components and Setup	Volume	
2 mM dNTP	2	µL
80 µM Oligo dT	1	µL
RNA*	5	µL
Sterile Water	up to 10	µL
10 min incubation at 65°C		
5 x Reverse transcription buffer	4	µL
FastGene® Scriptase II	1	µL
0.1 M DTT	1	µL
RNase Inhibitor	0.5	µL
Sterile Water	up to 20	µL
5, 10, 30, 60 min at 42°C		
3 min at 95°C		

* 50 ng, 5 ng, 0.5 ng of RNA were used in the RT-step.
10% of the RT reaction were used for PCR analysis

Endpoint PCR and qPCR were performed according to manufacturer' s instructions.

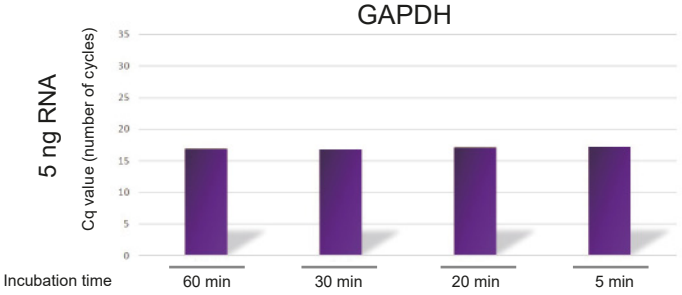
Result

PCR - electrophoresis result

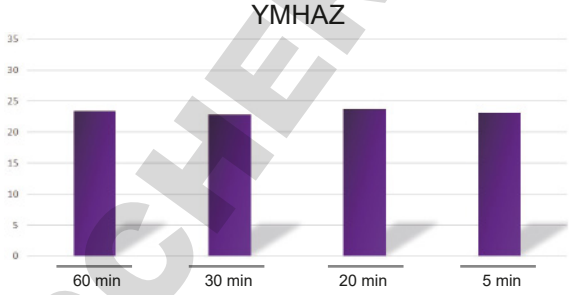


Incubation time: 60 min | 30 min | 20 min | 10 min | 5 min

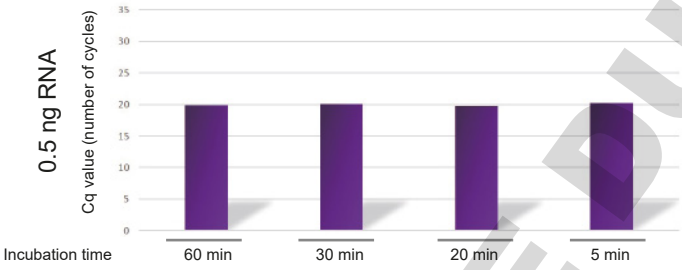
Quantitative PCR



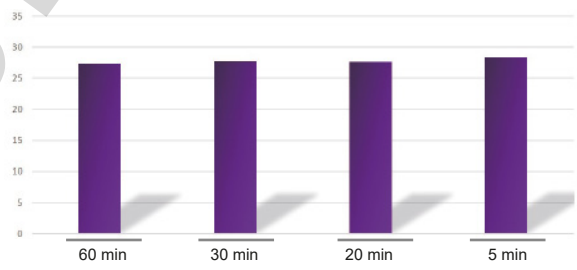
Average Cq value	GAPDH
Manufacturer	FastGene®
RNA - 5 ng	17.0 ± 0.2



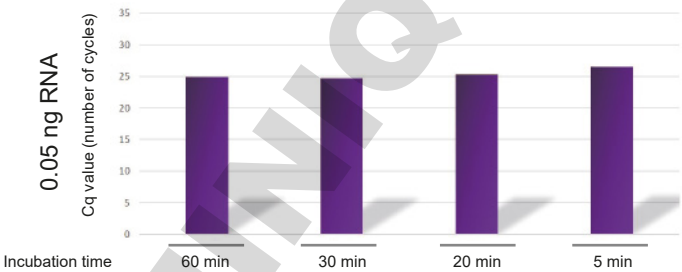
Average Cq value	YMHAZ
Manufacturer	FastGene®
RNA - 5 ng	23.2 ± 0.4



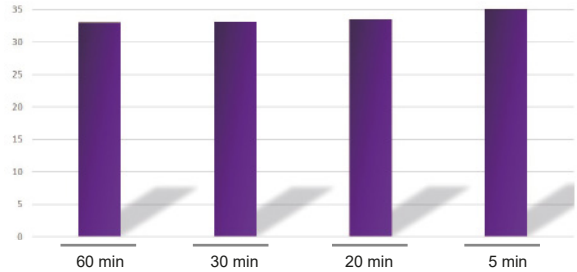
Average Cq value	GAPDH
Manufacturer	FastGene®
RNA - 0.5 ng	20.0 ± 0.3



Average Cq value	YMHAZ
Manufacturer	FastGene®
RNA - 0.5 ng	27.8 ± 0.4



Average Cq value	GAPDH
Manufacturer	FastGene®
RNA - 0.05 ng	25.3 ± 0.8

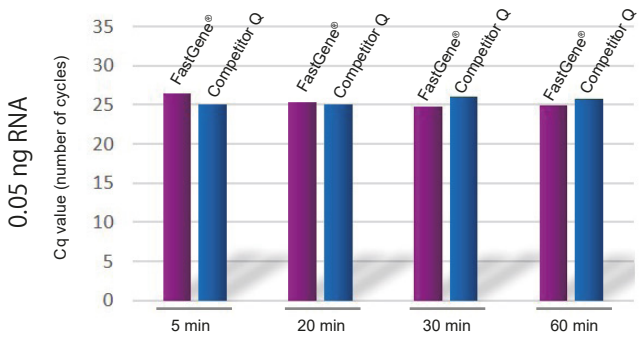


Average Cq value	YMHAZ
Manufacturer	FastGene®
RNA - 0.05 ng	33.7 ± 0.9

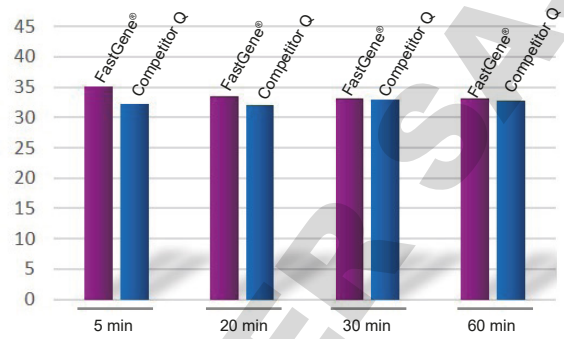
Summary

Different concentrations of RNA were used to perform a reverse transcription reaction for the specified time, followed by a PCR and two qPCRs to amplify a 1006 bp product (TUBB). All RNA concentrations produce a product even with a 5 min reverse transcription reaction. Negative controls without a template (NTC) did not produce PCR products. Reverse transcription was performed under the same conditions, followed by two qPCR-assays aiming GAPDH (high expression) and YMHAZ (low expression). No difference was noted between cDNA produced in 5 to 60 minutes, even at 0.05 ng starting material. All standard deviations were below ± 1 cycle.

Result



	Average Cq value	GAPDH
Manufacturer	FastGene®	Competitor Q
RNA - 0.05 ng	25.3 ± 0.8	25.5 ± 0.4



	Average Cq value	YMHAZ
Manufacturer	FastGene®	Competitor Q
RNA - 0.05 ng	33.7 ± 0.8	32.5 ± 0.4

2. Analysis using RT-qPCR

Different concentrations of RNA were used to perform a reverse transcription with the labelled times, followed by two qPCR-assays aiming GAPDH (high expression level) and YMHAZ (low expression level).
No difference was noted between cDNA produced in 5 to 60 minutes, even at 0.05 ng starting material. All standard deviations were below ± 1 cycle.

Conclusion

FastGene® Scriptase II was able to produce cDNA in 5 minutes.
Result 1: For large PCR products, the band of 0.05 ng RNA after 5 min was sligthly weaker. Hence for products of 1000 bp a 10-min RT step is recommended for low RNA amount.
Result 2: No difference in Cq value exceeding ± 1 cycle was detected.
FastGene® Scriptase II can therefore be recommended for short-term reverse transcripton reactions.

Binsfelder Straße 77,
52351 Düren, Germany

www.nippongenetics.eu
TEL +49 2421 554960 FAX +49 2421 55496 11 info@nippongenetics.eu