



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 5 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

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

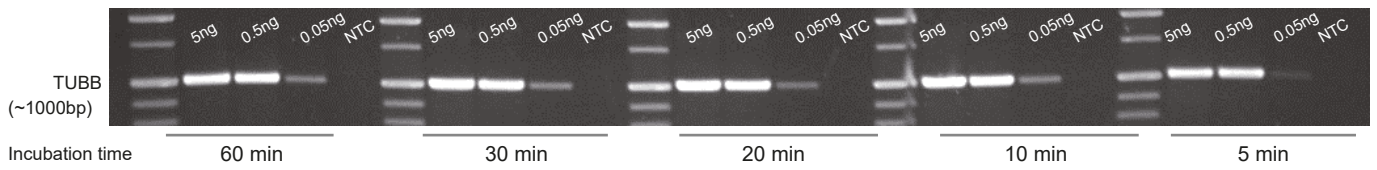


FastGene® Scriptase II cDNA Synthesis kit

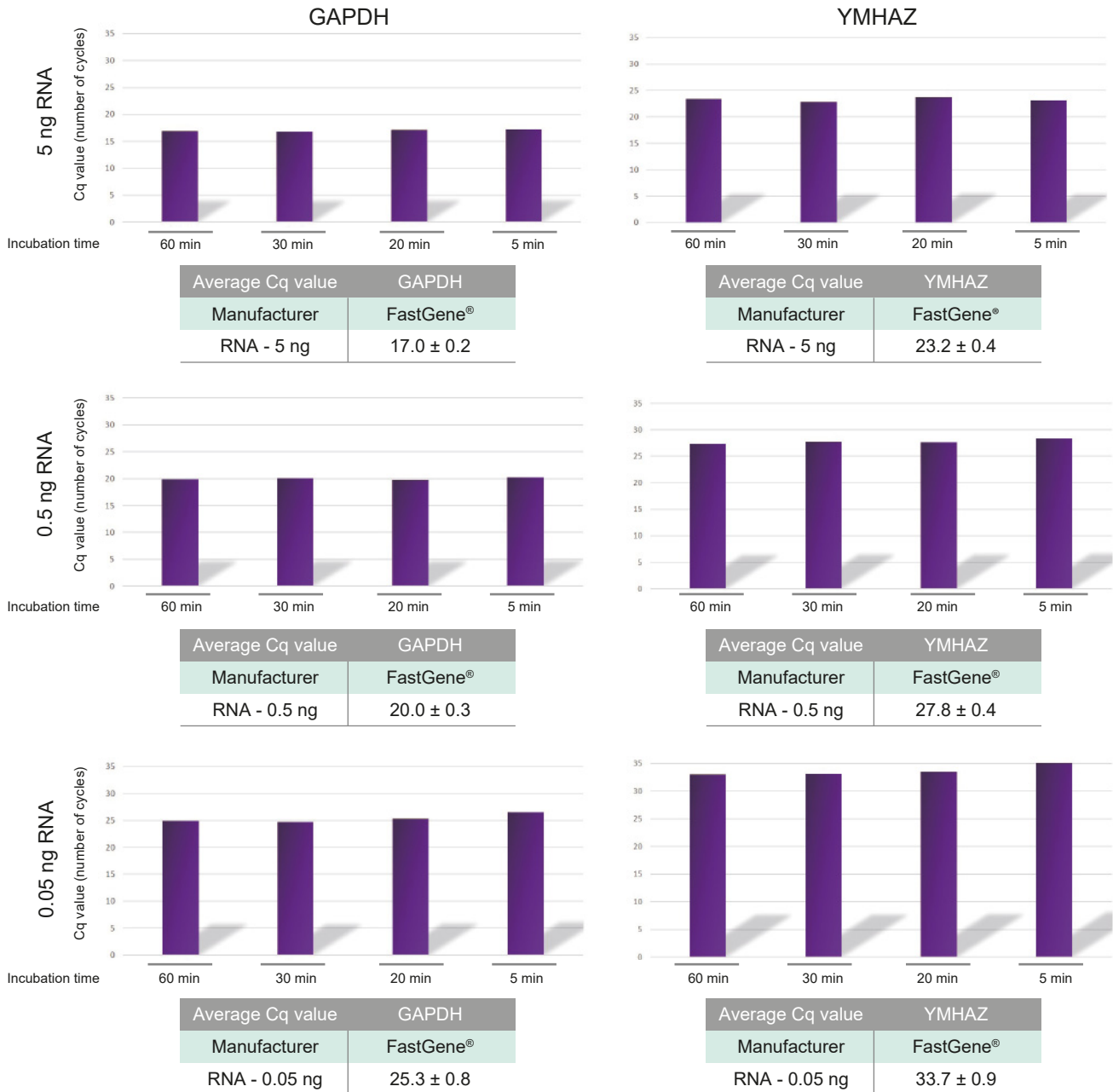
Endpoint PCR and qPCR were performed according to manufacturer's instructions.

Result

PCR - electrophoresis result



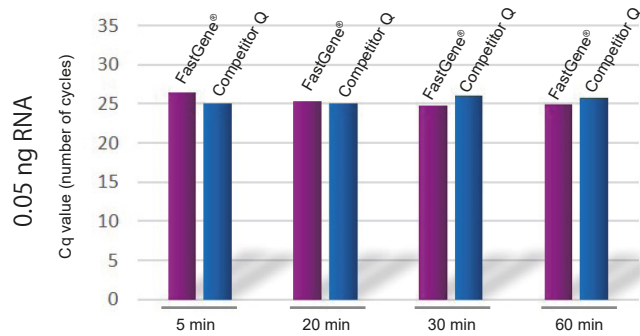
Quantitative PCR



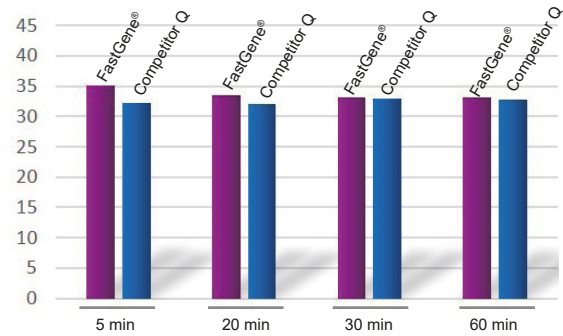
Summary

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 YMHZ (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	YMHAAZ
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 YMHAAZ (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 slightly 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 transcriptase reactions.