Technical Data Sheet

Very fast reverse transcription reactions

- Purpose: Investigate the ability to perform very quick reverse transcription
- Methods: Reverse transcription; Endpoint PCR; quantitative PCR;
- Products: FastGene® Scriptase II cDNA Synthesis Kit (LS 63, NIPPON Genetics EUROPE)

Abstract

FastGene® Scriptase II is an engineered reverse transcriptase enzyme, able to deliver highest quality cDNA from low amounts 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 minimal time possible of a reverse transcription. The process was reduced down to 5 minutes using different concentration of starting material. The resulting cDNA was used in endpoint PCR experiments as well as in qPCR experiments.

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

Materials

- FastGene®Scriptase II cDNA Synthesis Kit
- RNA - Universal Human Reference RNA
  Concentration: 5 ng, 0.5 ng, 0.05 ng
- Primers:
  - TUBB (1006bp) - Endpoint PCR
  - GAPDH (138 bp) - qPCR
  - YWHAZ (249 bp) - qPCR

FastGene® Scriptase II cDNA Synthesis Kit

Experimental Procedure - RT

Reverse Transcription

FastGene®

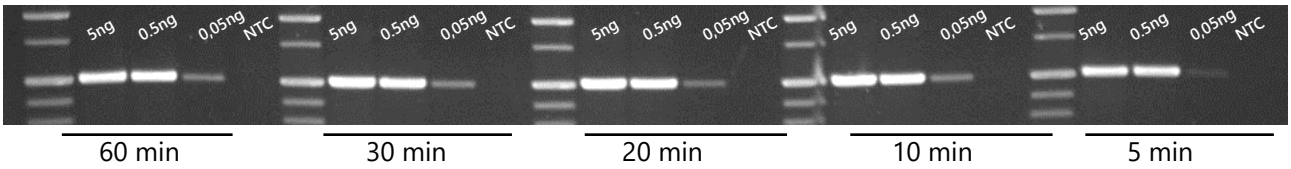
Component	Volume / µl
2 mM dNTP	2
80 µM Oligo dT	1
RNA*	5
Sterile H_2O	up to 10 µl
10 min incubation at 65 °C	
5 x Reverse Transcriptase Buffer	4
FastGene® Scriptase II	1
0.1 M DTT	1
RNase Inhibitor	0,5
Sterile Water	Up to 20 µl
5, 10, 20, 30, 60 min at 42 °C	
3 min at 95°C	

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

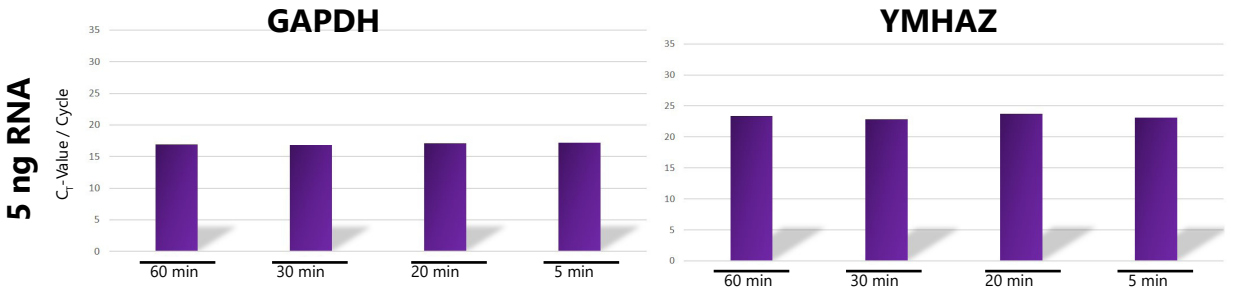
Endpoint PCR and qPCR were performed according to the manufacturer´s instructions

Results

1 Endpoint PCR

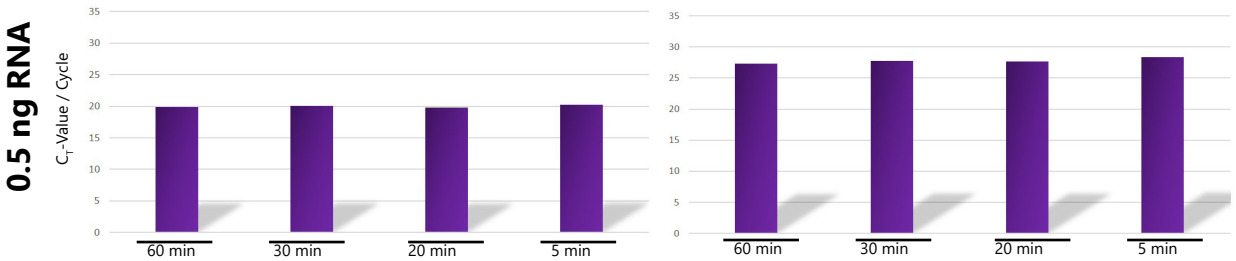


2 quantitative PCR



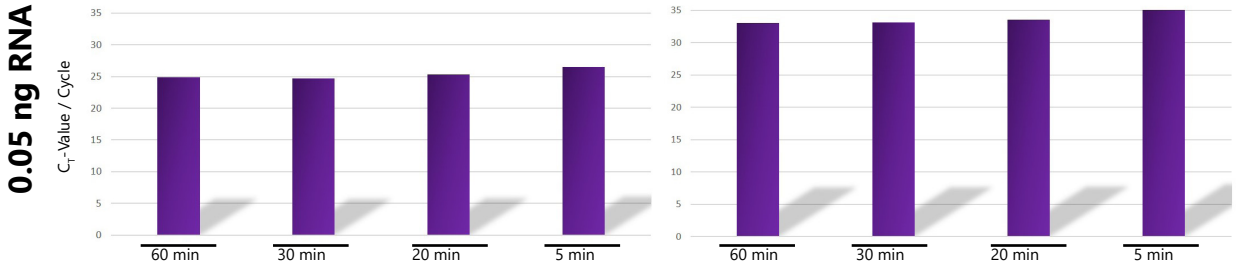
Gene / Average C_T	GAPDH
Manufacturer	**FastGene®**
RNA - 5 ng	17.0 ± 0.2

Gene / Average C_T	YMHAZ
Manufacturer	**FastGene®**
RNA - 5 ng	23.2 ± 0.4



Gene / Average C_T	GAPDH
Manufacturer	**FastGene®**
RNA - 0.5 ng	20.0 ± 0.3

Gene / Average C_T	YMHAZ
Manufacturer	**FastGene®**
RNA - 0.5 ng	27.8 ± 0.4



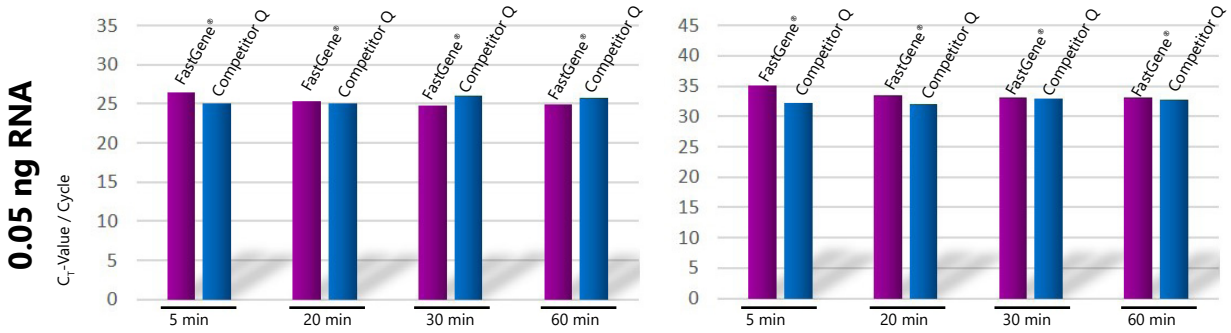
Gene / Average C_T	GAPDH
Manufacturer	**FastGene®**
RNA - 0.05 ng	25.3 ± 0.8

Gene / Average C_T	YMHAZ
Manufacturer	**FastGene®**
RNA - 0.05 ng	33.7 ± 0.9

Conclusion:

Different concentrations of RNA were used to perform a reverse transcription for the denominated times, followed by a PCR to amplify a 1006bp product (TUBB) or by two qPCR assays. All RNA concentrations produced a PCR product in endpoint as well as qPCR experiments, even with a 5 minutes RT, and a negative control without template (NTC) did not produce a product. Different concentrations of RNA were used to perform a reverse transcription with the labelled times, followed by two qPCR-assays aiming GAPDH (high expression) and YMHAZ (low expression. No difference was noted between cDNA produced in 5 min to 60 min, even at 0.05 ng starting material. All standard deviation were below ±1 cycle.

Results (continued)



Gene / Average C_T	GAPDH
Manufacturer	**FastGene®**
RNA - 0.05 ng	25.3 ± 0.8

Gene / Average C_T	YMHAZ
Manufacturer	**FastGene®**
RNA - 0.05 ng	33.7 ± 0.9

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) and YMHAZ (low expression. No difference was noted between cDNA produced in 5 min to 60 min, even at 0.05 ng starting material. All standard deviation were below ±1 cycle.

Conclusion

FastGene® Scriptase II is able to produce cDNA in 5 minutes. Result 1: For large products, the band of 0.05 ng RNA after 5 minutes was slightly weaker. Hence, for products of 1000 bp a 10-min RT step is recommended at low volumes. Result 2: No difference in C_T-values larger than ±1 cycle were detected (see next page for results).

FastGene® Scriptase II can therefore be recommended for very short RT-reactions.