

Transcriptor First Strand cDNA Synthesis Kit: Efficient and Fast – a Comparison to Other Kits

Monika Jung^{1*}, Chuanliang Xu^{1,2}, Falk Ohl¹, Ann-Kathrin Mager¹, and Klaus Jung¹

¹Department of Urology, Charité-University Medicine Berlin, Germany

²Department of Urology, Shanghai Hospital, Second Military Medical University, Shanghai, China

*Corresponding author: monika.jung@charite.de



Monika Jung

We compared the efficiency of three reverse transcription kits for first-strand cDNA synthesis by quantifying HPRT, PBGD, and CD59 transcript fragments in cDNA samples of prostatic cell lines using the LightCycler® System. Oligo(dT), random, and mixed priming were used for transcription. The Transcriptor First Strand cDNA Synthesis Kit not only generated higher yields of cDNA independent of the priming method but was also the fastest in comparison to other commercially available kits. For longer mRNA sequences, the Transcriptor First Strand cDNA Synthesis Kit uses anchored oligo(dT) priming and with this kit, the complete 7.6-kb low-abundant CD59 mRNA was efficiently transcribed.

Introduction

A successful quantification of gene expression is influenced by two essential steps prior to RT-PCR: the isolation of an intact and pure total RNA or mRNA, followed by its efficient reverse transcription (RT) to generate a complete first-strand cDNA. Furthermore, the efficiency of the *in vitro* reverse transcription reaction is affected by the [1]:

- ➔ quality and quantity of the RNA
- ➔ GC content of the RNA, and its secondary or tertiary structures
- ➔ features of the reverse transcriptase (source and thermostability)
- ➔ efficiency of priming (depending on primer sequence and concentration)

For a variety of applications in molecular biology research, sensitive detection of low-abundant genes (e.g., in single cell studies, or for the determination of circulating tumor cells) is a permanent analytical challenge. Moreover, for estimating gene expression by relative quantification [2], genes with differences in RNA structure, property, and quantity have to be detected from the same cDNA sample in a two-step RT-PCR. Therefore, a powerful transcription kit which guarantees full-length cDNA synthesis of low-abundant and abundant mRNAs is necessary.

To study gene expression in microdissected cells from prostatic tissue, we looked for an efficient RT procedure. We compared the newly introduced Transcriptor First Strand cDNA Synthesis Kit with two other transcription kits using different priming strategies. The Transcriptor First Strand cDNA Synthesis Kit includes Transcriptor Reverse Transcriptase, an enzyme which achieves a broad dynamic detection range from very low to very high copy numbers of *in vitro* transcribed RNA [3].

The performance of three cDNA synthesis kits was compared using RNA samples from four human prostate cell lines. The transcription efficiency was estimated by the yield of cDNA as measured by real-time RT-PCR with the LightCycler® 1.5 Instrument. The expression of two house-keeping genes (hypoxanthine phosphoribosyl transferase [HPRT] and porphobilinogen deaminase [PBGD]) and the target gene protectin (CD59) was quantified and compared using the different kits. HPRT and PBGD as well as CD59 mRNAs belong to the class of low-abundant mRNAs. CD59 is a complement regulatory cell-surface protein which is crucial for tumor progression. It is discussed as a therapeutic target gene for different tumors [4].

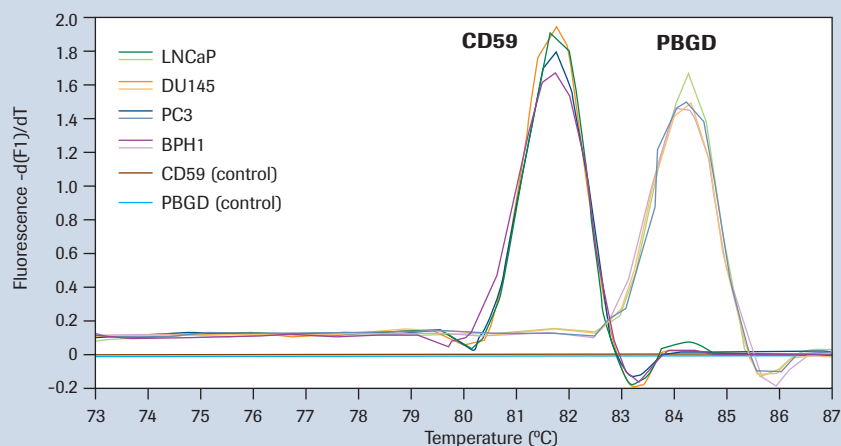


Figure 1: Melting-curve analysis. Melting peaks are displayed for PBGD and CD59 PCR products. The cDNAs from four prostatic cell lines were used. As a negative control for both CD59 and PBGD, no template was used.

Materials and Methods

Cell lines and cell culture

The three permanent human malignant prostatic cell lines LNCaP, DU 145, PC3 (American Type Culture Collection) and the benign prostatic cell line BPH-1 (German Collection of Microorganisms and Cell Cultures DSMZ) were grown in RPMI-1640 medium (Life Technology) as described elsewhere [5].

RNA isolation

Total RNA was isolated from cells immediately after harvesting and counting with a commercially available RNA isolation kit, including a DNase digestion step. Total RNA was isolated from 2-5 x 10⁶ cells of each cell line and quantified with a NanoDrop ND-1000 spectrophotometer. RNA integrity was checked on the Agilent 2100 Bioanalyzer.

cDNA synthesis

One microgram of RNA was reverse transcribed using the three first-strand cDNA synthesis kits. All RNA samples were denatured at 65°C for 10 minutes. For each sample, three priming methods were used: an oligo(dT) primer, a random primer, and a mix of both primers. cDNA synthesis was performed according to the package inserts without further optimization of any component or step. All kits included RNase inhibitors. For all cell lines and for each priming method, a control for genomic DNA contamination was carried out. Kit-specific differences were as follows:

- Kit from supplier A included a kit-specific recombinant heterodimeric reverse transcriptase expressed in *E. coli* with RNase H activity. Primers were not provided with the kit. We added 1 μM oligo(dT)₁₅, 10 μM random (dN)₆, and a mix of both primers. cDNA synthesis was performed at 37°C for 60 minutes.
- The First Strand cDNA Synthesis Kit for RT-PCR (AMV) included AMV reverse transcriptase, oligo(dT)₁₅ primer, and random (dN)₆ primer. Gelatin was not added. cDNA synthesis was performed at 25°C for 10 minutes for primer annealing, at 42°C for 60 minutes for the RT reaction and at 99°C for 5 minutes for transcriptase denaturation.
- The Transcriptor First Strand cDNA Synthesis Kit included a new recombinant reverse transcriptase expressed in *E. coli* with RNase H activity, random (dN)₆ primer, and a newly designed anchored oligo(dT)₁₈ primer. RNA denaturation was carried out in the presence of the primers. When random primer or mixed primers were used, a primer annealing step of 10 minutes at 25°C was performed before synthesis. All Transcriptor cDNA synthesis reactions were carried out for 30 minutes at 55°C, and at 85°C for 5 minutes for enzyme inactivation.

LightCycler® PCR

Real-time RT-PCRs of the two housekeeping genes were performed with different detection formats. *In vitro* transcribed HPRT mRNA was measured and quantified with the LightCycler® h-HPRT Housekeeping Gene Set and the LightCycler® FastStart DNA Master^{PLUS} HybProbe Kit. A total volume of 20 μl included 1 μl 1:4 diluted cDNA as template. Amplification was performed under the following conditions: a pre-incubation step of 10 minutes at 95°C, followed by 45 cycles of 15 seconds at 95°C, 55°C and 72°C (temperature ramp was 20°C/s).

The cDNA fragments of PBGD and CD59 were detected in a SYBR Green I quantification format. The same 1:4 diluted cDNA was used as described above (1 μl for PBGD quantification and 2 μl for CD59 quantification). For

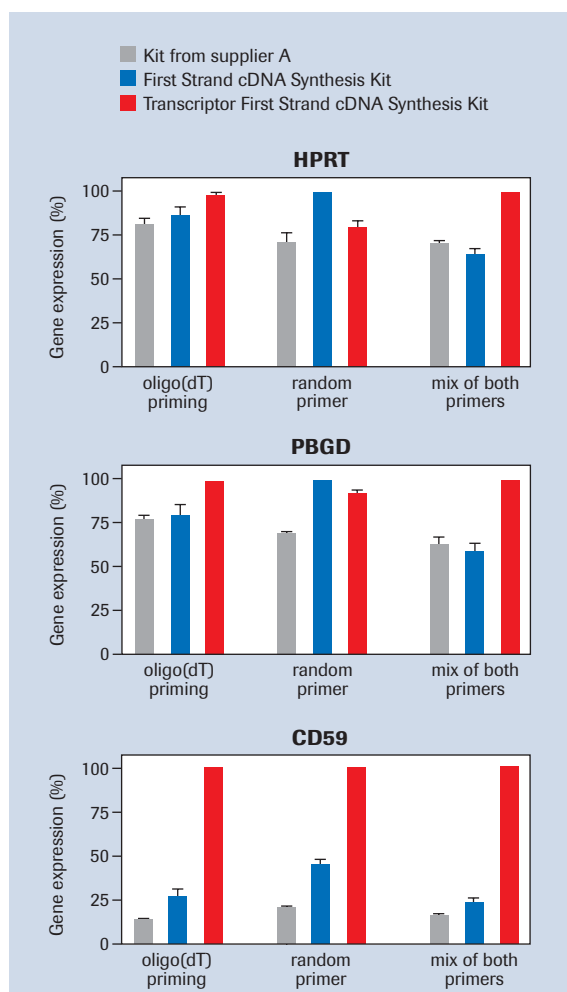
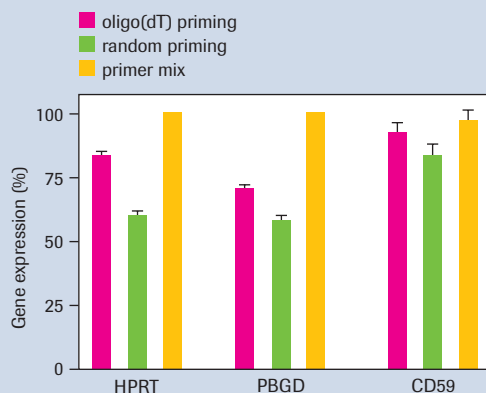


Figure 2: Comparison of reverse transcription kits.

The columns represent the mean ± standard deviation of gene expression levels of the four investigated prostatic cell lines. The highest expression levels at equal priming were set at 100%. The cDNA yield of the other syntheses was related to it and is shown for gene fragments of HPRT, PBGD, and CD59.

Figure 3: Influence of priming method on the cDNA yield using the Transcriptor First Strand cDNA Synthesis Kit.

The gene fragments of HPRT, PBGD and CD59 were quantified. The columns represent the mean \pm standard deviation of gene expression levels of four investigated prostatic cell lines. The highest expression level per gene was set at 100%.



amplification of PBGD the forward primer 5'-GCAACG-GCGGAAGAAAAC-3' and the reverse primer 5'-CCCT-GTGGTGGACATAGCA-3' were used. CD59 primers were located at the 5' end of the 7.6 kb mRNA (sequences as published [4]). The final concentration of each primer was 0.5 μ M. The amplified PBGD product was 156 base pairs (bp) long, with a specific melting point of about 84°C. The amplified CD59 product was 209 bp long, with a melting point of about 82°C (Figure 1). Amplifications were performed under the following conditions: 15 minutes pre-incubation followed by 45 cycles of 15 seconds at 95°C, 20 seconds at 59°C for PBGD and at 60°C for CD59, and each 20 seconds at 72°C (temperature ramp was 20°C/s).

cDNA quantification

Transcribed HPRT mRNA was quantified using RNA standards included in the LightCycler® h-HPRT Housekeeping Gene Set. In each PCR, pooled cDNA sample was used as a homologous standard for importing the HPRT standard curve. CD59 expression was quantified using the PBGD standard curve. The imported PBGD standard curve was generated with diluted cDNA from LNCaP cells. Data were analyzed with the LightCycler® Software, version 3.5. The runs were evaluated using the Second Derivative Maximum.

Results and Discussion

In comparison to two other RNA transcription kits, the new Transcriptor First Strand cDNA Synthesis Kit resulted

in the highest cDNA yield. The high yield was independent of the priming method and was confirmed for all three investigated gene fragments (Figure 2).

The observed differences in performance apparently depended on sequence and structure of the target RNA. For CD59, cDNA yields were 50%-90% higher using the Transcriptor First Strand cDNA Synthesis Kit. The significantly lower cDNA yield generated by the other two kits – particularly using oligo(dT) priming – clearly shows the advantage of using the Transcriptor Reverse Transcriptase in combination with the supplied anchored oligo(dT) primer for longer mRNA sequences. CD59 mRNA has a length of approximately 7.6 kb, which is about five times longer than the mRNA of both housekeeping genes HPRT and PBGD – this 7.6-kb CD59 mRNA was transcribed completely. With the Transcriptor Reverse Transcriptase full-length cDNA from RNA of up to 14 kb can be synthesized [3].

Figure 3 shows the influence of different RT priming methods using the Transcriptor First Strand cDNA Synthesis Kit. The highest amount of cDNA was obtained using the primer mix consisting of anchored oligo(dT) and random primer for all investigated gene fragments, followed by anchored oligo(dT) priming and random priming. No significant differences were detected between oligo(dT) priming and the primer mix for both HPRT and CD59. The priming-dependent efficiency of the RT reaction varies among different genes – this was also observed in other studies [1, 6].

In summary, the kit-specific Transcriptor Reverse Transcriptase and the anchored oligo(dT) primer improve the performance of cDNA synthesis rate of long RNA or RNA molecules with difficult structures. This is an important advantage of the Transcriptor First Strand cDNA Synthesis Kit, which helps to achieve a higher sensitivity when studying low-abundance genes.

We strongly recommend this cDNA synthesis kit with the shortest reaction time of the tested kits. We also recommend testing priming methods prior to new applications. The Transcriptor First Strand cDNA Synthesis Kit guarantees a fast, complete and high-yield synthesis of cDNA. ■



Product	Pack Size	Cat. No.
Transcriptor First Strand cDNA Synthesis Kit	1 kit	04 379 012 001
Transcriptor Reverse Transcriptase	1 kit (25 reactions)	03 531 317 001
	1 kit (50 reactions)	03 531 295 001
Protector RNase Inhibitor	1 kit	03 335 402 001
LightCycler® h-HPRT Housekeeping Gene Set	1 kit	03 251 891 001
LightCycler® FastStart DNA MasterPLUS HybProbe Probes	1 kit	03 515 575 001

References

1. Stahlberg A *et al.* (2004) *Clin Chem* 50:509–515
2. Technical Note No. LC 13/2001
3. *Biochemica* (2003) 4:17–19
4. Xu C *et al.* (2005) *Prostate* 62:224–232
5. Jung M *et al.* (2003) *Prostate* 55:89–98
6. Lekanne Deprez RH *et al.* (2002) *Anal Biochem* 307:63–69