cDNA Synthesis with the Transcriptor First Strand cDNA Synthesis Kit from Formalin-Fixed Paraffin-Embedded Tissue (FFPET) Samples

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Partial degradation of RNA is a common problem when working with FFPET samples. Semi-quantitative RT-PCR assays amplifying the 5´- and 3´-end sequences of the housekeeping gene β-actin present a solution: calculating the ratio of the 3´- to 5´-amplicon allows assessment of RNA integrity. An important step for reproducible quantification is the reverse transcription reaction. The applicability of the new Transcriptor First Strand cDNA Synthesis Kit to partially degraded FFPET RNA was evaluated. The new kit is compared with the widely used First Strand cDNA Synthesis Kit for RT-PCR (AMV). Under modified reaction conditions (temperature, time) the kit reversely transcribes the FFPET samples with high efficiency.

Introduction

Gene expression analysis at the transcription level is used for molecular differentiation of solid tumors in research studies and may have the potential to guide future treatment decisions. Retrospective studies using microarray technology on long-term archived tissues might be valuable for the detection of new prognostic markers. The poor integrity of RNA derived from formalin-fixed paraffin-embedded tissue (FFPET) samples, however, might cause a bias in the quantification of different transcripts.

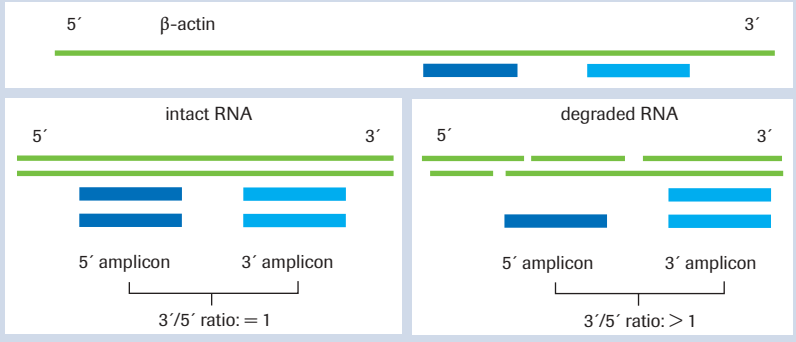

Figure 1: RT-PCR assay to determine RNA integrity. Intact RNA transcripts exhibit a ratio of one, while an increased ratio is the result of RNA degradation.

To investigate whether formalin-fixed tissue can be used for microarray analysis, we estimated RNA degradation levels by performing a semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) assay. This assay is based on quantitative amplification of 5´- and 3´-end sequences of the housekeeping gene β-actin. Calculating the ratio of 3´- to 5´-amplicons allows assessment of RNA integrity, since intact RNA transcripts exhibit a ratio of one while degraded RNA increases the ratio (Figure 1). In this study, reverse transcription of partially degraded RNA with the new Transcriptor First Strand cDNA Synthesis Kit for RT-PCR was assessed.

Materials and Methods

Total RNA was extracted from 5-µm FFPET sections of xenografts and archived research samples using the High Pure RNA Paraffin Kit. Using anchored-oligo$(dT)_{18}$ primers and the Transcriptor First Strand cDNA Synthesis Kit according to the pack inserts, 200 ng total RNA was reverse transcribed. The reaction conditions were either as described in the pack insert: 30 minutes at 55°C, or in a modified reaction: 10 minutes at 25°C, 60 minutes at 42°C, 5 minutes at 99 °C (the last 5-minute step to inactivate the enzyme).

Real-time PCR was performed using the LightCycler® Instrument and the LightCycler® FastStart DNA Master SYBR Green I Kit together with 2 µl cDNA as a template. For amplification and detection of 3´ and 5´ β-actin amplicons, two pairs of primers were designed against the 1761-base human β-actin sequence at positions 1385 and 1650 (Arcturus, Mountain View, USA).

PCR conditions were: 10 minutes pre-incubation step at 95°C; 50 cycles of 0 seconds at 95°C, 5 seconds at 62°C and 10 seconds at 72°C, ramp time 20°C/sec. The 3´/5´

Table 1: CPs and 3´/5´ ratios of different RNA samples after cDNA synthesis with Transcriptor First Strand cDNA Synthesis Kit using the following incubation conditions: 30 minutes at 55°C or 10 minutes at 25°C, 60 minutes 42°C, 5 minutes 99°C.

Reaction conditions	30 minutes at 55°C		10 minutes at 25°C, 60 minutes 42°C, 5 minutes 99°C	
Sample	**CP**	**3´/5´ Ratio**	**CP**	**3´/5´ Ratio**
Calibrator RNA HBAC 3´	16.49	1.00	16.49	1.00
Calibrator RNA HBAC 5´	16.29		16.33	
Xenograft RNA HBAC 3´	18.79	12.79	17.61	7.79
Xenograft RNA HBAC 5´	21.97		20.78	
RNA_1 HBAC 3´	20.15	65.28	20.10	14.23
RNA_1 HBAC 5´	25.88		24.08	
RNA_2 HBAC 3´	19.52	45.54	19.53	11.17
RNA_2 HBAC 5´	24.74		23.22	
RNA_3 HBAC 3´	20.71	165.63	20.56	25.46
RNA_3 HBAC 5´	27.77		25.16	
RNA_4 HBAC 3´	20.23	30.17	19.18	14.53
RNA_4 HBAC 5´	24.83		23.25	

ratio was determined in relation to cDNA transcribed from an intact RNA (calibrator) using the LightCycler® Relative Quantification Software.

Results and Discussion

The degradation status of RNA isolated from FFPET xenografts and archived research samples was analyzed by calculating the 3´/5´ ratio of β-actin amplicons after cDNA synthesis using the Transcriptor First Strand cDNA Synthesis Kit.

The 3´/5´ ratios of cDNA synthesized with the Transcriptor First Strand cDNA Synthesis Kit for 30 minutes at 55°C (according to the package insert) were significantly higher than the ratios of cDNA synthesized in the modified reaction (Table 1). At the same time, a cDNA from a calibrator RNA reverse transcribed under both reaction temperatures showed identical 3´/5´ ratios with both conditions. The partially degraded FFPET RNA seems to be additionally degraded when Transcriptor Reverse Transcriptase is used at elevated reaction temperatures (55°C). On the other hand, with intact sample material (such as the calibrator RNA) an elevated reaction temperature has no influence on cDNA synthesis.

As a result, the RT incubation conditions were modified. A change in the temperature profile from 30 minutes at 55°C to 10 minutes at 25°C, 60 minutes at 42°C, 5 minutes at 99°C ensures a high efficiency of cDNA synthesis of FFPET RNA using the new Transcriptor First Strand cDNA Synthesis Kit (Table 1). The 3´/5´ ratios of cDNA reversely transcribed with this new kit were comparable to those with our old standard procedure using the First Strand cDNA Synthesis Kit for RT-PCR (AMV; data not shown). Under modified reaction conditions, the new Transcriptor First Strand cDNA Synthesis Kit gave excellent results when working with partially degraded RNA. ■

Order INFO

Product	Pack Size	Cat. No.
Transcriptor First Strand cDNA Synthesis Kit	1 kit (50 reactions)	04 379 012 001
Transcriptor Reverse Transcriptase	250 U (25 reactions)	03 531 317 001
	500 U (50 reactions)	03 531 295 001
	2000 U (200 reactions)	03 531 287 001
Protector RNase Inhibitor	2000 U	03 335 399 001
	10000 U	03 335 402 001