BioScript™ | Highly Sensitive Ultra-Stable Reverse Transcriptase

The conversion of RNA to cDNA (reverse transcription) is an important technique in molecular biology for a wide variety of assays including: viral detection, gene-expression analysis, and cloning of RNA transcripts from different biological sources. A successful reverse transcription reaction is dependent on many factors, including the reverse transcriptase used in the reaction. The enzyme should be able to generate high quality full-length cDNA from different types of templates and under various conditions. Commercially available reverse transcriptases include the Moloney Murine Leukaemia Virus (MMLV) reverse transcriptase and the Avian Myeloblastosis Virus (AMV) reverse transcriptase. These retroviral enzymes exhibit RNA-dependent DNA polymerase activity and RNA-degrading RNase H activity. Many variations of these enzymes are currently available on the market, including the wild type MMLV and AMV, which display full RNase H activity, and variations of MMLV which either lack the RNase H domain or contain its modified version, which greatly reduces its basal activity.

BioScript™ is a Moloney Murine Leukaemia Virus (MMLV) Reverse Transcriptase, which possesses low RNase H activity. This results in higher efficiency reverse transcription, as the RNA is less prone to degradation. This application note examines and compares the performance of BioScript with other commercially available reverse transcriptases.

BioScript™ PRODUCES HIGH YIELD OF cDNA, WHICH SHOWS EXCELLENT PERFORMANCE IN REAL-TIME RT-PCR EXPERIMENTS

Reverse transcription polymerase chain reaction (RT-PCR) is the most sensitive technique for mRNA detection and quantification currently available. Good quality cDNA is essential to this technique when high sensitivity is desired. In order to demonstrate the quality of cDNA synthesized by BioScript™, both end-point and real-time RT-PCR assays were performed.

Comparative yield experiments were carried out to determine the variation in yield of cDNA reverse transcribed using BioScript and two commercially available MMLV enzymes from competitors N and P. Total RNA was purified from NIH3T3 fibroblast cells using TRIsure™ (Cat No. BIO-38032). The quality of RNA was determined using an Agilent BioAnalyzer and the concentration measured by spectrophotometry on a Beckman Coulter DU®530. Identical starting amounts (0.5µg) of total RNA was reverse transcribed using 200 Units of each MMLV enzyme. 0.25µg of Oligo $(dT)_{18}$ was used for priming. The reactions were incubated at 42°C for 1 hour followed by 75°C for 15 minutes to inactivate the enzyme. To measure the yield of cDNA, quantitative PCR using SYBR® Green I chemistry was carried out using primers against the moderately to highly expressed genes. All steps were carried out in triplicate, giving 9 data points for each enzyme. This data has been used for the quantification with a modified delta-delta Ct method. It is important to note that all PCR reactions were assumed to have an efficiency of 100% (actual efficiencies calculated between 98-100%, data not shown), which leads to a doubling in PCR product for every cycle. Therefore a shift in 1 Ct corresponds to a two-fold difference in yield. The yields of all genes have been normalized to the yield obtained using BioScript.

Figure 1A demonstrates the differences in Ct value and Figure 1B shows the comparative yields of cDNA obtained between BioScript and reverse transcriptases from competitors P and N, respectively. cDNA obtained using BioScript showed the highest yield of amplification products as compared to the cDNA obtained using competitor enzymes.

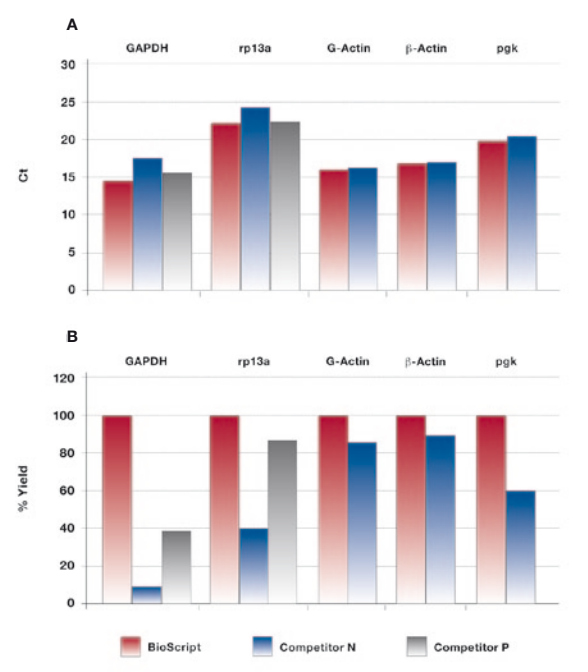


Fig. 1 Relative quantification of five different cDNA transcripts synthesized with BioScript and two competing enzymes. Figure 1A summarizes the differences in Ct values observed in qPCR reactions with mouse cDNA synthesized with BioScript, and enzymes from competitors N and P. Figure 1B show the calculated relative yields normalized to the yield obtained with BioScript cDNA.

To examine the effect of the high activity of BioScript in end-point RT-PCR, decreasing amounts of total HeLa cell RNA (20ng to 50pg) were reverse transcribed at 42°C for 1 hour with Oligo (dT) primer. One quarter of each reaction was used for amplification of an 860bp β-actin fragment. The reactions were analyzed on a 1% agarose gel (Fig. 2). PCR products were detected from as little as 50pg of template RNA, demonstrating that BioScript produces high quality cDNA even from very low amounts of templates.

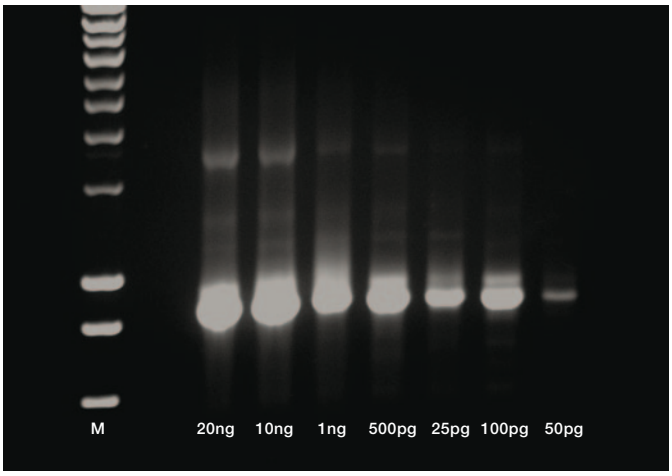
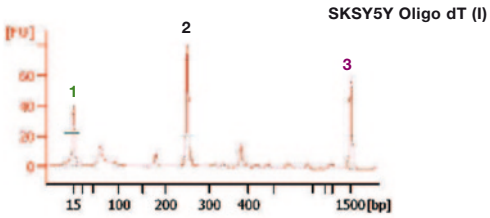


Fig. 2 Amplification of human β-actin from cDNA reverse transcribed from decreasing amounts of cDNA. cDNA was synthesized using BioScript from 20ng, 10ng, 1ng, 500pg, 250pg, 100pg and 50pg RNA extracted from HeLa cells. An 860bp β-actin fragment was amplified from the cDNA and detected by agarose gel electrophoresis. Marker is Hyperladder™ I (Cat No. BIO-33025).

BioScript SHOWS EXCELLENT PERFORMANCE WITH GENE-SPECIFIC PRIMERS, OLIGO $(dT)_{18}$ AS WELL AS RANDOM HEXAMERS, AND SYNTHESIZES cDNA WITH MORE SPLICE VARIANTS

In order to show the universal and superior performance of BioScript, cDNA was synthesized with gene-specific primers, Oligo $(dT)_{18}$ primer and random hexamers respectively by BioScript and a competitor's enzyme in three parallel RT-PCR experiments. Total RNA isolated from human SK-SY-5Y neuroblastoma cells was used as template, and the quality was tested by using the RNA 6000 Nano LabChip kit and the 2100 BioAnalyzer. Reverse transcription was carried out for 60min at 42°C after an initial 5min denaturation step at 70°C for all reactions and a 10min annealing step at 25°C for random hexamer primers only. One tenth of the first reaction was used in the second step PCR: cDNAs synthesized with Oligo $(dT)_{18}$ or gene specific primers were amplified with specific primers for the NTRK1 gene, cDNA synthesized with random hexamers was amplified with primers for the house keeping gene GAPDH. Amplified products were analyzed by using the DNA 1000 kit and the 2100 BioAnalyzer from Agilent. Several splice variants of NTRK1 amplification products, including 181bp, 250bp and 317bp variants, were detected in capillary electrophoresis. The cDNA synthesized with gene-specific primers using BioScript showed all three variants, whereas the cDNA synthesized by the enzyme from a competitor showed only the 250bp variant (Fig. 3). Similarly, two splice variants were detected in PCR products from cDNA synthesized by BioScript with Oligo $(dT)_{18}$, the 65bp variant and the 251bp form, but only the latter one was synthesized by the competing reverse transcriptase. The results were supported by the quantifications of the BioAnalyzer (Fig. 4). The 65bp, 181bp, 250bp and 317bp NTRK1 splice variants are accurately quantified in PCR reactions with cDNA synthesized by BioScript. PCR products from the corresponding cDNA synthesized by the competing enzyme show only the 251bp isoform at quantitative level. These findings demonstrate that BioScript synthesizes cDNA with higher sensitivity than the competing enzyme.

Competitor



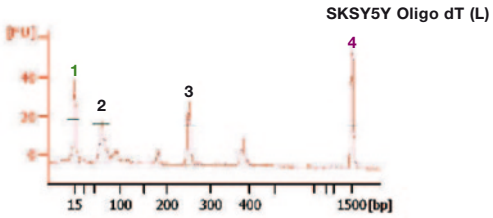
Overall results for sample 3: **SKSY5Y Oligo dT (l)**

Number of peaks found: 1

Peak table for sample 3: **SKSY5Y Oligo dT (l)**

Peak	Size (bp)	Conc. (ng/µl)	Molarity (nmol/l)	Observations
1	15	4,20	424,2	Lower Marker
2	250	4,59	27,8	
3	1500	2,10	2,1	Upper Marker

BioScript



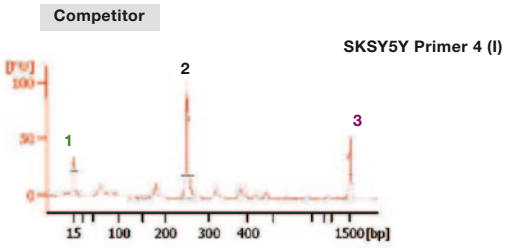
Overall results for sample 4: **SKSY5Y Oligo dT (L)**

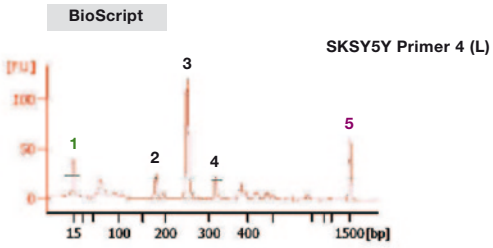
Number of peaks found: 2

Peak table for sample 4: **SKSY5Y Oligo dT (L)**

Peak	Size (bp)	Conc. (ng/µl)	Molarity (nmol/l)	Observations
1	15	4,20	424,2	Lower Marker
2	65	3,70	86,1	
3	251	1,82	11,0	
4	1500	2,10	2,1	Upper Marker

Fig. 3 Electropherograms of PCR products from cDNA synthesized with Oligo dT. cDNA was synthesized with Oligo dT. The left graph shows PCR products from cDNA synthesized by the competing reverse transcriptase, and the right graph shows PCR products from cDNA synthesized by BioScript. Data kindly provided by Dr. S. D'Amelio, Italy.





Overall results for sample 1: **SKSY5Y Primer 4 (I)**

Number of peaks found: 1

Peak table for sample 1: **SKSY5Y Primer 4 (I)**

Peak	Size (bp)	Conc. (ng/µl)	Molarity (nmol/l)	Observations
1	15	4,20	424,2	Lower Marker
2	251	6,76	40,8	
3	1500	2,10	2,1	Upper Marker

Overall results for sample 2: **SKSY5Y Primer 4 (L)**

Number of peaks found: 3

Peak table for sample 2: **SKSY5Y Primer 4 (L)**

Peak	Size (bp)	Conc. (ng/µl)	Molarity (nmol/l)	Observations
1	15	4,20	424,2	Lower Marker
2	181	1,40	11,8	
3	250	6,36	38,5	
4	317	1,11	5,3	
5	1500	2,10	2,1	Upper Marker

Fig. 4 Electropherograms of PCR products from cDNA synthesized with gene-specific primers. cDNA was synthesized with gene-specific primers (NTRK1 target gene). The left graph shows PCR products from cDNA synthesized by the competing reverse transcriptase, and the right graph shows PCR products from cDNA synthesized by BioScript. Data kindly provided by Dr. S. D'Amelio, Italy.

BioScript SHOWS SUPERIOR PERFORMANCE IN REVERSE TRANSCRIPTION OF LONG TEMPLATES

For many applications full-length cDNA of long templates is required. To test the performance of BioScript for the synthesis of long transcripts, comparative experiments were carried out using BioScript and a comparable MMLV enzyme from competitor P. Total RNA was isolated from mouse NIH3T3 RNA with TRIsure (Cat No. BIO-38032). Reverse transcription was carried out at 42°C for 1 hour and analyzed by quantitative PCR using SYBR® Green I chemistry. Primers for comparative length analysis by qPCR were 1Kb, 3Kb, 6Kb and 9Kb upstream of the poly(A) tail of giantin and 1Kb, 7Kb and 12Kb upstream of the poly(A) tail of dynein (Fig. 5). Yields were calculated as described above.

The highest yields for giantin were achieved for the fragment 3Kb upstream and lowest, as expected, for the 9Kb fragment upstream of the poly(A) tail. Independent of the amplicon position, the cDNA produced with BioScript contained higher amounts of long transcripts than the cDNA synthesized with the competitor's enzyme. Similar results were obtained for dynein (Fig. 5). The highest yield was achieved for fragments 1Kb upstream of the poly(A) tail amplified from cDNA synthesized by BioScript. cDNA yields for the other fragments were comparable for both enzymes.

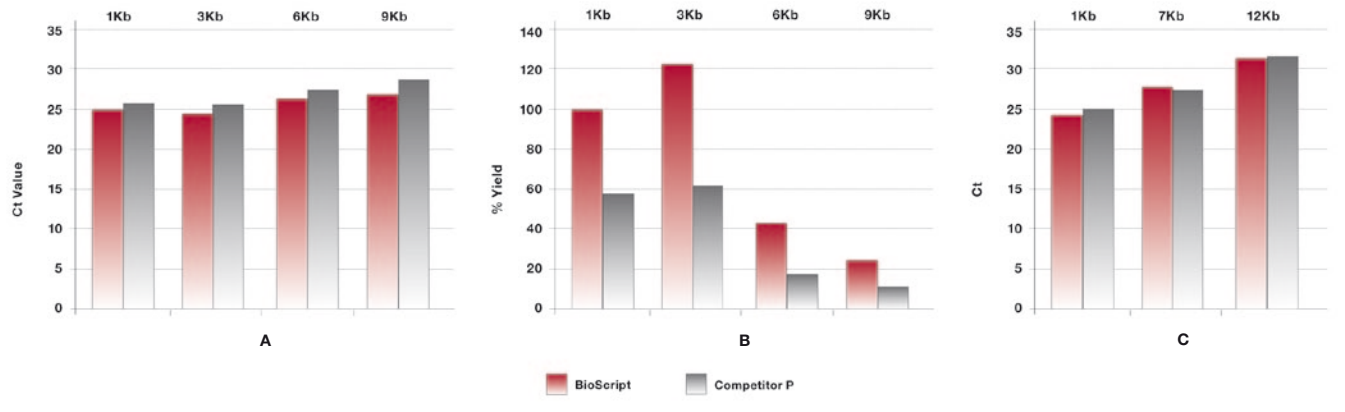


Fig. 5 Quantification of giantin and dynein transcripts of increasing length synthesized with two different reverse transcriptases. Figure 5A shows the differences in Ct for the amplification of giantin fragments 1Kb, 3Kb, 6Kb and 9Kb upstream of the poly(A) tail. Figure 5B shows the relative quantifications of the PCR products. Yield achieved with BioScript cDNA for amplification of the giantin transcript 1Kb upstream of the poly(A) tail is assumed as 100%. Figure 5C summarizes the Ct differences for the amplification of dynein fragments 1Kb, 7Kb and 12Kb upstream of the poly(A) tail.

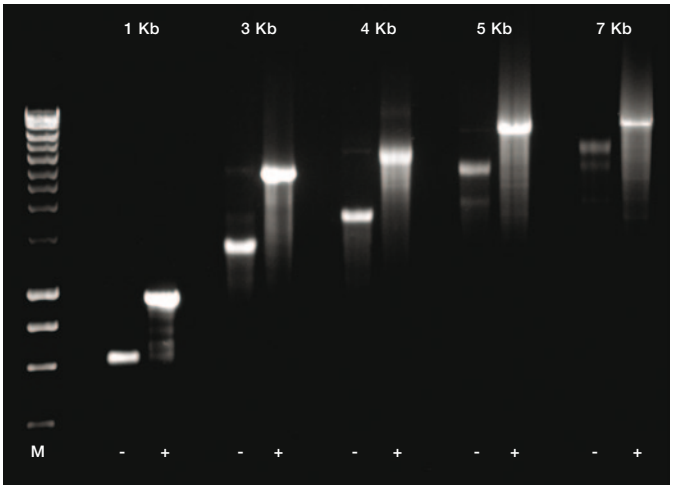


Fig. 6 cDNA of increasing length synthesized with BioScript reverse transcriptase. Transcripts of 1Kb, 3Kb, 4Kb, 5Kb and 7Kb length were reverse transcribed with BioScript at 42°C. One fifth of each reaction (+) and the respective no enzyme controls (-) were analyzed on a 1% agarose gel. Marker is Hyperladder™ I (Cat No. BIO-33025).

The excellent performance of BioScript for reverse transcription of templates of increasing size becomes apparent by analysis of the cDNA products by gel electrophoresis. Transcripts from 1Kb to 7Kb in length were reverse transcribed by BioScript for 1 hour at 42°C. Reactions were stopped by addition of Loading Buffer Blue and one fifth of each reaction was analyzed on a 1% agarose gel (Fig. 6). Highest yields were obtained for cDNAs up to 5Kb. The cDNA yield with the 7Kb long template is lower. The quality, in all cases, is excellent, as evidenced by minimal smearing and the absence of degraded or truncated products.

BioScript SYNTHESIZES HIGH YIELD OF cDNA OVER A WIDE RANGE OF TEMPERATURES

Many RNA transcripts form stable secondary structures at lower temperatures, making them less suitable as templates for RT-PCR at those temperatures. Therefore, it is important that a reverse transcriptase is not only active at 37°C-42°C but also at higher temperatures without a loss of activity.

To demonstrate the activity of BioScript over a wide range of temperatures, total RNA purified from NIH3T3 fibroblast cells using TRIsure, was reverse transcribed at temperatures ranging from 37°C - 55°C for 1 hour. The variation in yield of cDNA reverse transcribed using BioScript was compared against cDNA yield from a MMLV reverse transcriptase from Competitor Q using identical starting amounts of template. The quality of the RNA template was determined using an Agilent BioAnalyzer. To comparatively measure the yield of cDNA, quantitative PCR was carried out using primers against γ-actin and succinate dehydrogenase complex, subunit A (ssdha). The amplicons lie approximately 1-2Kb upstream from the poly(A) tails of each transcript.

Figure 7 shows the differences in Ct values for cDNA synthesized with BioScript and with the reverse transcriptase of competitor Q at 37°C and 55°C respectively. Variations in Ct values are minor at 37°C but become significant at 55°C. The Ct values for BioScript cDNA at 55°C are lower for both transcripts analyzed than the Ct values for the competing enzyme. This also means that, assuming the efficiency of the PCR is 100%, BioScript produces higher yield of cDNA than the competing enzyme.

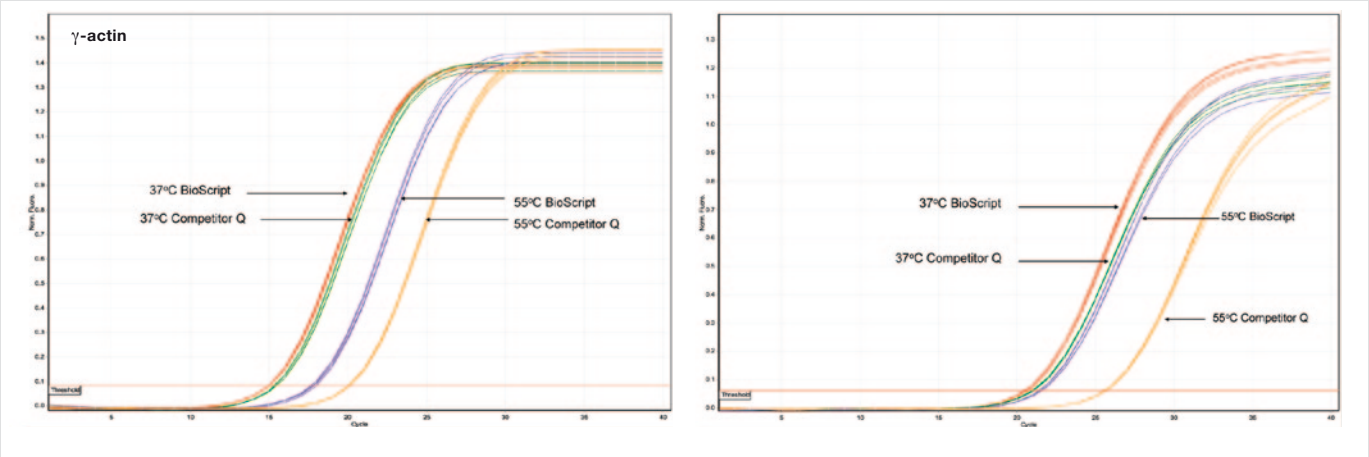


Fig. 7 BioScript exhibits lower Ct values for the amplification of γ-actin and ssdha at 37°C and 55°C. γ-actin (left panel) and ssdha (right panel) were amplified from cDNA reverse transcribed with BioScript and competitor Q's MMLV at 37°C and 55°C respectively. While the Ct values for cDNAs amplified at 37°C are relatively close together, the Ct values for 55°C are significantly lower for cDNA synthesized using BioScript, indicating higher thermostability of BioScript.

Figure 8 summarizes the data for γ-actin and ssdha amplified from cDNA synthesized at increasing temperatures with BioScript and the reverse transcriptase of competitor Q respectively. Yields for ssdha were higher at 37°C, 50°C and 55°C for BioScript cDNA. For γ-actin, BioScript showed higher cDNA yields for all temperatures tested.

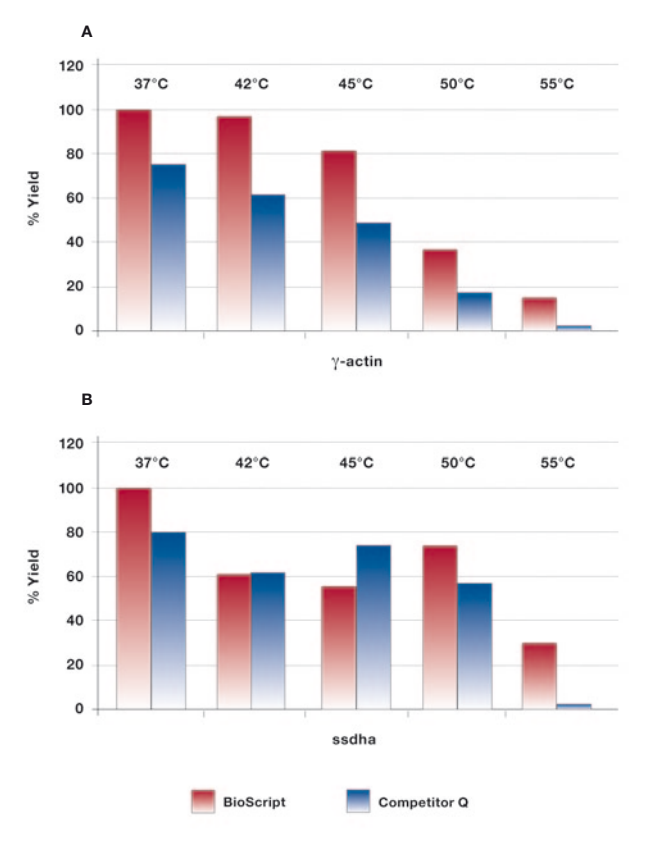


Fig. 8 Quantification of γ-actin and ssdha in cDNA synthesized at increasing temperatures. γ-actin (Figure 8A) and ssdha (Figure 8B) were amplified from cDNA reverse transcribed with BioScript and the reverse transcriptase of competitor Q. Yields were normalized to the yield at 37°C with BioScript cDNA.

To demonstrate the impact of synthesis at different temperatures on the amount and quality of the cDNA, a 1Kb lambda transcript was reverse transcribed with BioScript and a reverse transcriptase from competitor P respectively at increasing temperatures (Fig. 9). BioScript produced high cDNA yields of very good quality at all temperatures tested. The competing enzyme produced significantly less cDNA and the quality was noticeably poorer when analyzed by gel electrophoresis.

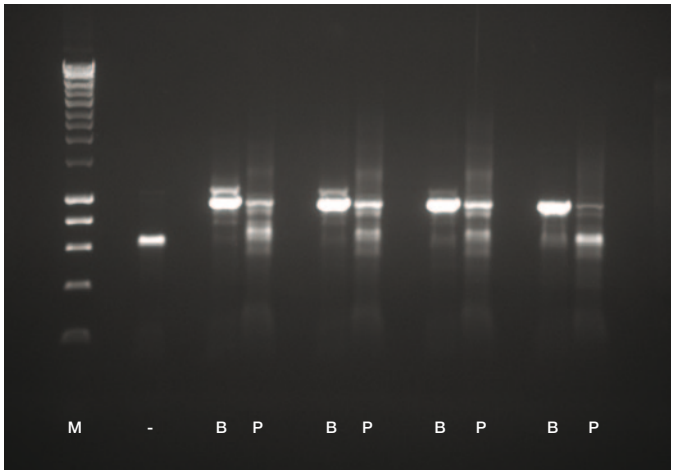


Fig. 9 Amplification of a 1Kb lambda transcript from cDNA synthesized at increasing temperatures. cDNA synthesized using BioScript and a reverse transcriptase from competitor P. Reactions were incubated at 37°C, 42°C, 45°C and 48°C for 1 hour. One fifth of each transcription was run on the gel. As control a reaction was incubated at 37°C without enzyme (-). Marker is HyperLadder™ I (Cat No. BIO-33025).

To demonstrate the temperature stability of BioScript, the enzyme was subjected to incubation at different temperatures for one week. It was then used in RT-PCR to generate a 500bp fragment of lambda DNA (Fig. 10). In all cases, no loss of activity was detected, demonstrating the stability of BioScript.

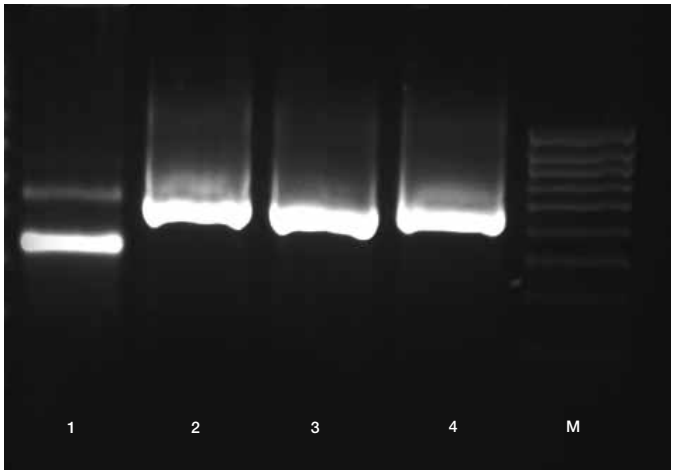


Fig. 10 Stability test of BioScript reverse transcriptase. A 500bp transcript from lambda DNA was reversed transcribed using BioScript which had been subjected to one week incubation at -20°C (lane 2), 4°C (lane 3) or room temperature (lane 4). Lane 1 is control reaction without enzyme. Marker is HyperLadder™ IV (Cat No. BIO-33029).

SUMMARY

BioScript reverse transcriptase is suitable for first-strand cDNA synthesis, cDNA library construction and the production of templates for RT-PCR analysis of gene expression. BioScript can be used with total RNA, mRNA and *in-vitro* transcribed RNA and shows excellent performance with gene-specific primers, Oligo (dT) as well as random hexamers. It is highly sensitive, even for low amounts of RNA, delivers high yields and is superior to leading competitor enzymes in the production of full-length cDNA, especially for long templates. BioScript is robust, easy to use and works well under a wide range of temperatures. To conclude, BioScript is an all-purpose reverse transcriptase with excellent features and is superior to many other MMLV reverse transcriptases available commercially.

Please visit www.bioline.com/bioscript to request a sample of BioScript.

United Kingdom
Bioline Ltd
16 The Edge Business Centre
Humber Road
London NW2 6EW
United Kingdom
Tel: +44 (0)20 8830 5300
Fax: +44 (0)20 8452 2822

Germany
Bioline GmbH
Im Biotechnologiepark TGZ 2
D-14943 Luckenwalde
Tel: +49 (0)3371 681 229
Fax: +49 (0)3371 681 244

Australia
Bioline (Aust) Pty Ltd
PO Box 122
Alexandria NSW 1435
Australia
Tel: +61 (0)2 9209 4180
Fax: +61 (0)2 9209 4763

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