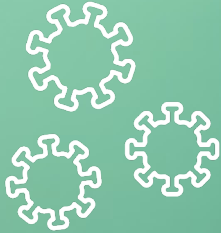


UltraScript® cDNA Synthesis Kit



- Unbiased cDNA synthesis for real-time PCR
- Thermostable reverse transcriptase
- 30 minute protocol

The UltraScript® cDNA Synthesis Kit uses the latest developments in reverse transcriptase technology and buffer chemistry to enhance cDNA synthesis speed, yield and representation. The reverse transcriptase, buffer system and combination of random hexamers with anchored oligo(dT) allow for unbiased, efficient and sensitive cDNA synthesis.

Features

- Unbiased representation of 5' and 3' mRNA ends
- Sensitive detection of low copy number transcripts
- High cDNA yields from as little as 4 pg total RNA
- Simple 2 tube system
- 5x buffer contains anchored oligo(dT), random hexamers, enhancers, dNTPs and MgCl₂
- 20x thermostable reverse transcriptase blended with RNase inhibitor
- Also available in a format with separate oligos for maximum setup flexibility

Applications

- cDNA synthesis for real-time PCR analysis
- Low copy number transcripts
- Viral RNA targets
- Efficient synthesis from total RNA or poly(A)+ RNA

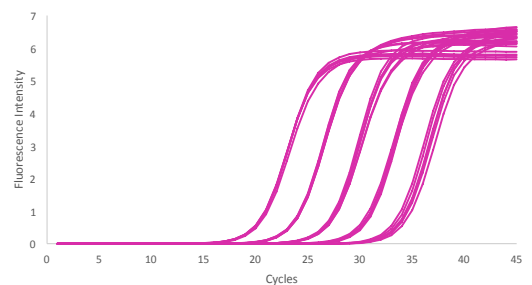


Figure 1. Broad reverse transcription dynamic range

UltraScript® cDNA Synthesis Kit was used for cDNA synthesis using a 10 fold serial dilution of mouse total RNA from 40 pg to 400 ng. qPCR was performed using qPCRBIO SyGreen Mix amplifying a 122 bp fragment of the mouse ACTG gene. Efficiency was measured at 96% across the range tested.

The results demonstrate that qPCRBIO cDNA Synthesis Kit efficiently reverse transcribes RNA across a broad dynamic range of substrate.



PCRBIOSYSTEMS
simplifying research

High quality cDNA synthesis for downstream qPCR analysis is essential for successful expression studies. Many factors affect cDNA synthesis including the reverse transcriptase, buffer systems, enhancers and priming strategy. The UltraScript® cDNA synthesis mix removes the need for user optimisation of these critical factors.

The modified MMLV reverse transcriptase (RTase) is both thermostable and extremely active. The enzyme is blended with RNase inhibitor preventing degradation of RNA by contaminating RNase. The RTase is not inhibited by ribosomal and transfer RNAs, total RNA is an ideal substrate. The 5x cDNA synthesis mix can be used with up to 0.4 µg total RNA.

The relative concentrations of random hexamers and anchored oligo(dT) have been optimised for the generation of cDNA for use in real-time PCR experiments. Alternatively, users can opt for the UltraScript® cDNA Synthesis Kit Separate Oligos to choose their own priming strategy.

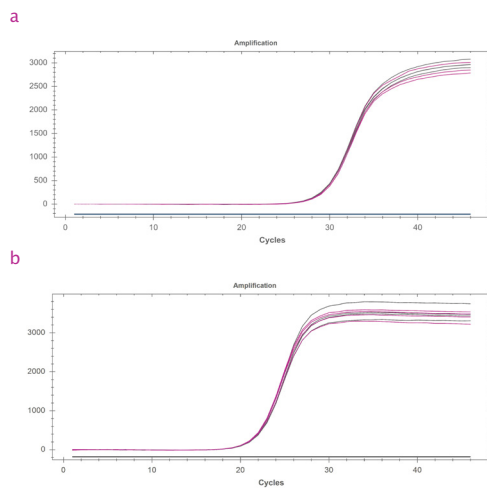


Figure 2. Unbiased representation of mRNA ends

a) UltraScript® cDNA Synthesis Kit was used to synthesise cDNA from mouse liver total RNA. 2 primer pairs were designed against the 5' (pink traces) and the 3' (grey traces) ends of the 4.2 kb mouse CANX transcript. qPCR BIO SyGreen® Mix was used for analysis. The primer pairs were 4 kb apart and did not show any reverse transcription bias, hence the amplification traces overlap.

b) 2 primer pairs against the 5' (pink) and 3' (grey) traces of RNS18 gene (1.8 kb). Again, no reverse transcription bias was evident.

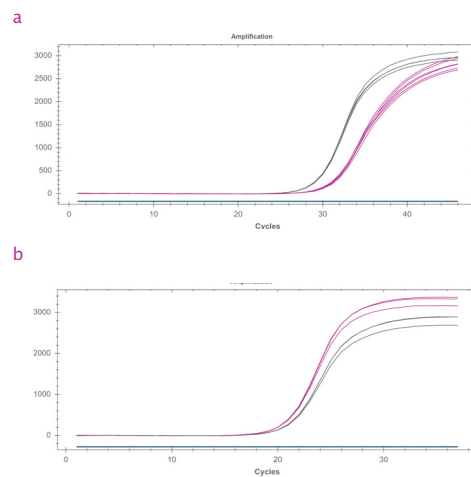


Figure 3. Thermostable enzyme for high GC%

a) UltraScript® cDNA Synthesis Kit was used to synthesise cDNA from mouse liver total RNA at 42 °C (pink) and also at 55 °C (grey). A primer pair was designed against GJB2, generating an 84% GC amplicon. qPCR BIO SyGreen Mix® was used for analysis. The higher temperature incubation generated more GC rich cDNA than the low temperature incubation.

b) A control amplicon of 55% GC from GAPDH was amplified from the 2 cDNAs described above. For this GC% no advantage of higher temperature incubation was achieved. The yield was slightly lower with the higher temperature.

Catalogue Number	Product Name	Pack Size	Presentation
PB30.11-02	UltraScript® cDNA Synthesis Kit	25 Reactions	[1 x 25 µL UltraScript] & [1 x 100 µL reaction mix]
PB30.11-10		100 Reactions	[1 x 100 µL UltraScript] & [4 x 100 µL reaction mix]
PB30.15-02	UltraScript® cDNA Synthesis Kit Separate Oligos	25 Reaction	[1 x 25 µL UltraScript] & [1 x 200 µL buffer] & [1 x 100 µL Anchored Oligo(dT)18] & [1 x 100 µL Random Hexamers]
PB30.15-10		100 Reactions	[1 x 100 µL UltraScript] & [2 x 200 µL buffer] & [1 x 100 µL Anchored Oligo(dT)18] & [1 x 100 µL Random Hexamers]
PB30.12-01	UltraScript® Reverse Transcriptase	10,000 units	[2 x 25 µL UltraScript, 200 units/µL] & [1 x 200 µL buffer]
PB30.12-04		40,000 units	[2 x 100 µL UltraScript, 200 units/µL] & [4 x 200 µL buffer]