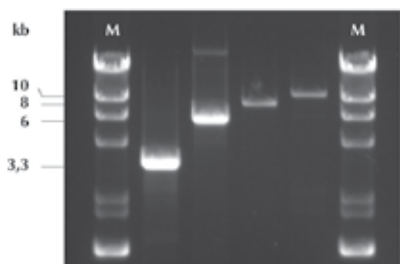


DNA Amplification

Reagents - RT-PCR kits

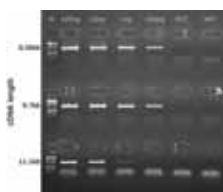


Robustness of different RT-PCR systems. A typical RT-PCR experiment for amplifying short cDNA fragments (192 to 1113bp). Finnzymes' RT-PCR system was compared to the RT-PCR systems from two major suppliers. Total RNA from human skeletal muscle was transcribed with random priming according to each supplier's recommendations. Amplification of cDNA was performed with a high-fidelity DNA polymerase recommended by the suppliers. Due to the various sizes of amplicons, a PCR protocol with the lowest annealing temperature and the longest extension time defined by amplicons was used. Robust Phusion RT-PCR kit produced all 11 amplicons with highest yields. With competitors' RT-PCR systems, less amplicons with varying yields were transcribed and amplified. M is a molecular size marker.



Phusion RT-PCR Kit is able to amplify long RT-PCR fragments. 3.3kb, 6kb, 8kb and 10kb fragments of dystrophin gene were reverse transcribed and amplified with Phusion RT-PCR kit. Oligo (dT) was used for priming. Starting template was human skeletal muscle total RNA (50ng). M is a molecular size marker.

*Polymerase Chain Reaction (PCR) is a process covered by patents owned by Hoffman-La Roche



Verso exhibits high affinity for mRNA templates giving long and high-yield cDNA providing an accurate cDNA pool for PCR.

Following reverse transcription using the Verso cDNA synthesis kit, PCR primers pairs were used to amplify short fragments 0.56kb, 9.7kb and 11.1kb from the mRNA/poly(A) junction. The successful amplification of all 3 amplicons, even from very low input concentrations, demonstrates that Verso RT has a high affinity for RNA and can reverse transcribe long RNA templates. NTC – no template control; NEC – no enzyme control.

RT Enhancer degrades genomic DNA, eliminating the need for separate DNase treatment.

The RT Enhancer, which is included in all Verso kits, is a unique enzyme that degrades contaminating genomic DNA at the start of the RT step. It is equally as effective as DNase I treatment but more convenient as it eliminates the need for a separate DNase I incubation prior to RT-PCR. Figure below demonstrates equal DNA degradation with RT enhancer and DNase I treatment.

Genomic DNA treated with:

1. No treatment
 2. RT enhancer
 3. No treatment
 4. DNase treatment
- M = DNA marker

*Polymerase Chain Reaction (PCR) is a process covered by patents owned by Hoffman-La Roche

Phusion® RT-PCR* kit

Thermo
SCIENTIFIC

NEW

87

- Broad range of RT-PCR products with high yields
- Short and simple cDNA synthesis and PCR* protocols
- cDNA amplification with 52x Taq fidelity
- Complete 2-step kit for RT-PCR

The kit contains the full set of reagents required for performing cDNA synthesis and PCR in two steps. In the first step, a variety of RNA templates and all priming options may be used for efficient synthesis of cDNA with M-MuLV reverse transcriptase. In the second step, Phusion® hot start II high fidelity DNA polymerase is used for cDNA amplification. This polymerase is extremely accurate and robust, and the hot start modification guarantees additional specificity in PCR. The superior features of Phusion hot start II DNA polymerase enable accurate cDNA amplification with high yields and short cycling times. Phusion RT-PCR Kit is an ideal choice when producing cDNA for cloning and gene expression studies.

Kit components

	FZF-546S	FZF-546L
RT enzyme mix (M-MuLV RNase H- RT + RNase inhibitor)	1 x 40µL	1 x 200µL
10x RT buffer (Includes 50mM MgCl ₂)	1 x 1.5mL	1 x 1.5mL
Oligo(dT) ₁₈ primer	1 x 20µL	1 x 100µL
Random primers (hexamers)	1 x 20µL	1 x 100µL
10mM dNTP mix	1 x 40µL	2 x 100µL
Phusion hot start II DNA polymerase	1 x 10µL	1 x 50µL
5x Phusion HF buffer	1 x 1.5mL	1 x 1.5mL
Control RNA with carrier	1 x 20µL	1 x 20µL
Control primer mix	1 x 20µL	1 x 20µL

Catalogue No	Alt. No	Description
FZF-546S	F-546S	20 reactions (cDNA synthesis in 20µL, PCR in 50µL)
FZF-546L	F-546L	100 reactions (cDNA synthesis in 20µL, PCR in 50µL)

RT-PCR* kits, Thermo Scientific ABgene Verso™

Thermo
SCIENTIFIC

LF

- High yields of full length cDNA
- Easy to use format
- Variety of kit options for 1 and 2 step RT-PCR* reactions for optimum flexibility and sensitivity

The Thermo Scientific ABgene Verso™ RT-PCR* system combines a reverse transcription enzyme, priming options and optimised buffers to generate high yield and full length cDNA. Several kit options are available from cDNA synthesis to ReddyMix™ formulations that reduce post PCR* handling steps. An RNase inhibitor is included with the Verso™ RT enzyme. This mix of enzymes significantly reduces the amount of RNase contamination while eliminating the extra steps of adding an inhibitor to the reaction. The ReddyMix™ buffer system for loading of PCR* products directly onto an agarose gel also saves time. Thermo-Start™ DNA polymerase kits are available to provide maximum specificity to PCR* reactions.

cDNA kits

Catalogue No	Alt. No	Description	Reactions per pack
MBK-170-010T	AB-1453/A	Verso™ cDNA kit	40 x 20µL
MBK-170-020Q	AB-1453/B	Verso™ cDNA kit	100 x 20µL
PCR-750-010G	AB-1454/A	Verso™ 1 step RT-PCR master mix with ThermoPrime Taq DNA polymerase	80 x 25µL
PCR-750-020D	AB-1454/B	Verso™ 1 step RT-PCR master mix with ThermoPrime Taq DNA polymerase	400 x 25µL
PCR-755-010W	AB-1454/LD/A	Verso™ 1 step kit ReddyMix master mix with ThermoPrime Taq DNA polymerase	80 x 25µL
PCR-755-020T	AB-1454/LD/B	Verso™ 1 step kit ReddyMix master mix with ThermoPrime Taq DNA polymerase	400 x 25µL
PCR-760-010W	AB-1456/A	Verso™ RNA control kit	40 x 50µL
PCR-765-010P	AB-1455/A	Verso™ 1 step RT-PCR master mix with Thermo-Start™ Taq DNA polymerase	80 x 25µL
PCR-765-020M	AB-1455/B	Verso™ 1 step RT-PCR master mix with Thermo-Start™ Taq DNA polymerase	400 x 25µL

