One-Step Reverse Transcription PCR (RT-PCR)

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- ***What is RT-PCR?***

Reverse transcription polymerase chain reaction (RT-PCR) is a variant of polymerase chain reaction (PCR). It is a sensitive method for the detection of mRNA expression levels. Traditionally RT-PCR involves two steps: the RT reaction and PCR amplification. RNA is first reverse transcribed into cDNA using a reverse transcriptase; the resulting cDNA is used as templates for subsequent PCR amplification using primers specific for one or more genes. RT-PCR can also be carried out as one-step RT-PCR in which all reaction components are mixed in one tube prior to starting the reactions. Although one-step RT-PCR offers simplicity and convenience and minimizes the possibility for contamination, the resulting cDNA cannot be repeatedly used as in two-step RT-PCR, sometimes abbreviated as RT-PCR.

- ***How does it work?***

One-step reverse transcription PCR is carried out using the Access RT-PCR System, (Promega, USA), to detect desired mRNA, using the specific designed primer sets. The total reaction mixture volume (25 µL) contained 5 µL (1µg) of total RNA, 1.5 mM MgSo4 (25 mM), 1 µM of each forward and reverse primer solutions (10 mM), 5 µL (1X) of *AMV/Tfl* 5X Reaction buffer, 0.2 mM dNTP mix (10 mM), 0.1 U/µl of 5 U/µl *AMV* Reverse transcriptase, and 0.1 U/µl of 5 U/µl *Tfl* DNA polymerase, topped up with DEPC treated water.

- ***How does it run?***

The thermal cycling profile is as follows: reverse transcription (45 °C for 45 minutes), inactivation of AMV reverse transcriptase (94 °C for 2 minutes), denaturation of RNA/cDNA primer, (94 °C for 2 minutes), denaturation (94 °C for 30 seconds), annealing (depends on the forward and reverse primers for 1 minute), extension (68 °C for 2 minute), final extension (68 °C for 7 minute), hold (4 °C) with the denaturation, annealing and extension steps repeated for 35 cycles. The PCR products were separated on a 1.0% agarose gel run at 120 V for 25 minutes. The PCR products will be analyzed under UV light after ethidium bromide staining (0.5 µg/ml).

- ***When RT-PCR fails, what is the Solution?***

Low yield or no amplification products may be a result of insufficient number of cycles, incorrectly programmed thermal cycler, improper reaction condition. Solution: reduce the annealing temperature or allow longer extension times.

Multiple or non-specific amplified products may be due to poor primer design, contamination by another target RNA/DNA, existence of multiple target sequence in the target RNA. Solution: Designing new sets of primers might help.

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BA: benzyladenine; PSI: photosystem I; WT: wild type

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A chapter in a book:

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Conference proceedings:

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