

First strand cDNA Synthesis using the NEB Protoscript kit

(Adapted from NEB protocol)

Overview

Reverse transcriptase is an enzyme capable of synthesizing a strand of DNA (the first strand) complementary to the mRNA template (cDNA). The cDNA produced in this reaction can be used in cloning procedures, or assays for measuring gene expression levels such as RT-PCR or qRT-PCR.

Oligo(dT) primers are a favorite choice for cDNA synthesis reactions because of their specificity for mRNA and because they allow many different targets to be studied from the same cDNA pool. An oligo-dT primer [d(T)₂₃VN] consists of a homogenous mixture of 23-mer thymidines that forces the primer to anneal to the beginning of the polyA tail of mRNAs.



A control reaction that includes the RNA sample and all the kit components except for the reverse transcriptase should be included in order to assess the impact of genomic DNA (gDNA) contamination in the samples. Sometimes primers will non-specifically bind to stretches of gDNA, resulting in the amplification of undesirable products in PCR reactions.

Kit components

- M-MuLV Enzyme Mix (10X)
- M-MuLV Reaction Mix (2X)
- Oligo d(T)₂₃VN* (50 μM)**
- Nuclease-free H₂O

*V = A, G or C; N = A, G, C or T

**Contains 1 mM dNTP

Procedure

Note: Intact RNA of high purity is essential for sensitive RT-PCR detection. RNA should have a minimum A₂₆₀/A₂₈₀ ratio of 1.7 or higher.

1. Before starting, check that the thermocycler has a program for the temperatures and times that are needed for the various steps. Enter a new program if none of the existing ones are suitable.

2. Thaw kit components and put on ice.

Text for [First Strand cDNA Synthesis Protocol \(E6300\)](#) removed due to copyright restrictions.
Please see: First Strand cDNA Synthesis Protocol (E6300), New England Biolabs.

Choice of primers for reverse transcription

Oligo d(T) priming is preferred for most applications because it ensures that all cDNA copies terminate at the 3' end of the mRNA and produces the longest contiguous cDNA. However, two other priming choices are possible:

- The Random Primer Mix is an optimized mix of the d(T)₂₃VN and random hexamer primers. It provides random priming sites covering the entire RNA templates including both mRNAs and non-polyadenylated RNAs (such as ribosomal RNAs). The Random Primer Mix yields shorter cDNAs on average and can be used for the detection of multiple short RT-PCR products. Random Primer Mix offers good performance in a wide range of RNA templates.
- When a gene-specific primer is used in a cDNA synthesis reaction, the cDNA product can be used only for amplification of the transcript for that gene. This priming method gives good results when the amount of RNA is limiting (below 10 ng) and only one particular cDNA is desired.

References

First strand cDNA synthesis protocol (E6300), New England BioLabs.
<https://www.neb.com/protocols/1/01/01/first-strand-cdna-synthesis-e6300>

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7.15 Experimental Molecular Genetics
Spring 2015

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