# First Strand cDNA
for Human / Mouse / Rat

## APPLICATION GUIDE

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# PACKAGE CONTENTS AND STORAGE CONDITIONS

## Included Components

First strand cDNAs derived from either human, rat or mouse tissues
Control primers for β-Actin and
Control primers for Cyclophilin *(First Strand cDNA Set only)*

Store the First Strand cDNA kit at -20°C.

## Related Products

Rapid-Scan™ http://www.origene.com/geneexpression/rapid_scan/
Northern Blots http://www.origene.com/geneexpression/rna_blots/
cDNA libraries http://www.origene.com/reagents_tools/cdna_libraries/

# INTRODUCTION

## Overview

Amplification and cloning of cDNA by PCR* is an essential tool for today's molecular biologist. This technique can be used to clone members of a multi-gene family (1,2,3), homologues across species (4, 5) or previously identified genes. PCR amplification of cDNA and its subsequent subcloning can often save time and reduce cost by eliminating the need for the construction and screening of a cDNA library. OriGene's First Strand cDNA kit is designed for easy screening of a panel of tissues for the expression of your gene of interest. This cDNA panel is also ideal for the characterization of alternatively spliced mRNA, either within a single tissue or across the tissues of an organism.

## Description of First Strand cDNA

Each step in the process of First Strand cDNA synthesis is carefully monitored to ensure product quality. OriGene's First Strand cDNA is synthesized from poly $A^+$RNA using an oligo(dT) primer and a reverse transcriptase that favors the production of long products. Each panel of cDNAs is examined by PCR using standard conditions for the presence of some "house-keeping" cDNAs, such as β-actin and cyclophilin. Small aliquots of these primers are included in the kit as controls.

# METHODS

## Primer design

Primer design is a crucial factor in obtaining success in PCR. There are no simple rules; however, there is a general set of guidelines for primer design that are reported to aid in the amplification of specific product. Primers should be between 17 and 25 nucleotides in length, have about 50% G/C content and not form strong secondary structures. Avoid sequences that are susceptible to primer-dimer formation. There are several commercially available computer programs for designing PCR primers. Consult additional sources for strategies for designing degenerate primers.

* Polymerase Chain Reaction ("PCR") is covered by patents owned by Hoffmann-La Roche Inc. and F. Hoffmann-La Roche Ltd. ("Roche"), including, but not limited to, U.S. Pat. Nos. 4,683,202, 4,965,188, and 5,075,215, and their foreign counterparts. Purchase of any OriGene PCR-related products does not convey a license, expressly or by implication, to use the PCR process covered by these patents. Purchasers of these products must obtain a license to use the PCR process before performing PCR.

## PCR conditions

## Amplification of specific product

The quality of template cDNA required to amplify a given gene of interest can vary. For mRNAs of low abundance or for very large mRNAs, a higher amount of template cDNA is recommended. It is helpful to first identify a tissue that synthesizes the mRNA of interest at high levels and to use the cDNA from this tissue as the template for PCR.

First Strand cDNA is provided at a concentration of 2-10 ng/ul.

The following PCR protocol is suggested for amplification of a specific product using First-Strand cDNAs as templates.

| **Stock Solution** | **Volume** | **Final Concentration** |
|---|---|---|
| First Strand cDNA | 2.0 ul | --- |
| 10X PCR Buffer | 2.5 ul | 1x |
| Forward Primer (10 uM)+ | 1.5 ul | 0.6 uM |
| Reverse Primer (10 uM)+ | 1.5 ul | 0.6 uM |
| dNTPs (2 mM each) | 2.5 ul | 0.2 mM |
| Thermostable DNA Polymerase | --- | 0.5U |
| ddH2O | to 25 ul | |

+Gene-specific primers diluted to 10 pmol/ul

The following thermal cycling parameters are recommended:

| | |
|---|---|
| Denaturation | 94°C for 30 sec |
| Annealing | 55°C for 1 min |
| Extension | 72°C for 1 min (Cycles 35) |
| Final Extension | 72°C for 5 min |

The optimal number of cycles for amplification of specific PCR product will depend on the abundance and size of the target cDNA. It may be necessary to empirically determine this value. The annealing temperature should be 5°C lower than that of the $T_m$ of the primers. In general, use an extension time of 0.5 to 1 minute per kbp of expected product length.

Use caution with Taq DNA polymerase since it lacks proofreading activity and its error rate is influenced by pH and magnesium concentration (6).

## Amplification of control products

Following the same basic protocol as in (*i*) substitute your gene-specific primers with control primers (β-actin or cyclophilin). Use 2.5 ul each of Control Primer 1 and Control Primer 2 per 25 ul of reaction volumes.

The following table lists the expected size of the PCR products that are generated using the control primers:

| **Control primers** | **PCR product size** |
|---|---|
| Human β-Actin | 614 bp |
| Human cyclophilin | 417 bp |
| Mouse β-Actin | 575 bp |
| Mouse cyclophilin | 579 bp |
| Rat β-Actin | 575 bp |
| Rat cyclophilin | 495 bp |

## Analysis of Reactions

Separate an aliquot (10-20%) of the PCR reaction mix in an ethidium bromide/agarose gel in order to examine the product. Please refer to the laboratory manual by Sambrook et al. (7) for agarose gel electrophoresis protocols.

# TROUBLE-SHOOTING GUIDE

If no PCR product is obtained using gene-specific primers, repeat the reaction using control primers. Failure to generate expected products using control primers would suggest that the PCR reagents are at fault and that fresh components should be used in their place.

Should only the control PCRs generate expected products then it is likely that the target cDNA is in too low abundance or that the gene-specific primers are improper. Adjusting the annealing temperature of the reaction or choosing a new pair of primers may help improve the results. Additionally, a second pair of "nested" primers may be used in a secondary amplification of the target cDNA.

Multiple PCR products or smearing suggests that the chosen primers are inappropriate for amplification or that the cycling parameters are not optimal.

Detailed troubleshooting guides for PCR are widely available in the literature.

# APPENDIX

## References


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