MULTIPLE CHOICE™ cDNAs

Applications Guide

OriGene Technologies, Inc.

6 Taft Court, Suite 300
Rockville, MD 20850

Toll-free Phone: 888-267-4436
Phone: 301-340-3188
Fax: 301-340-9254
E-mail: custsupport@origene.com

http://www.origene.com

1. Package Contents and Storage Conditions

The following components are included:

-- First strand cDNAs derived from either human, rat or mouse tissues.

-- Control primers for β-Actin and Cyclophilin (for multiple choice™ cDNA kit only)

Store the Multiple Choice™ cDNA kit at -20°C.

2. Introduction

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. The Multiple Choice cDNA kit is set up 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.

Each step in the cDNA synthesis process is carefully monitored to ensure a quality product. Our first strand cDNA is synthesized from poly A+ RNA using an oligo(dT) primer and a reverse transcriptase which 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, cyclophilin. Small aliquots of these primers are included in the kit as controls.

3. Procedures

A. Primer Choice

Primer design is the most important part of the cDNA cloning process. However, there is no simple method for the choice of primers; rather there are a set of guidelines which are reported to aid in the amplification of specific cDNA products. Both unique and degenerate primers can be used with the first-strand cDNA provided. We typically design unique primers ranging in length from 17 to 25 nucleotides with a nearly equal A/T and G/C content. We avoid primer sequences which can form secondary structures which would inhibit the PCR reaction. Degenerate primers of a complexity of up to 128 combinations can be used with this kit. There are several commercially available computer programs for designing PCR primers.

B. PCR Conditions

We routinely use the following PCR components at the concentrations given below in 25 µl PCR reactions:

Stock Solution	Final Concentration
Multiple Choice cDNA (2-10 ng/µl)	2.5 µl
Unique 17mer Primers (6.5 µM)	0.65 µM
10X PCR Reaction Buffer	1 X
dNTPs (2 mM each)	0.2 mM each
AmpliTaq DNA Polymerase (5 units/µl)	0.75 units

The quantity of cDNA you need to amplify your gene of interest may vary. For mRNAs of rare abundance or for very large mRNAs, more cDNA is required in the reaction. It is important to identify the tissue which synthesizes the mRNA of interest in the highest abundance and use that cDNA as the template for your reaction. We use the Perkin-Elmer DNA Thermal Cycler (Model 9600). The standard cycling conditions we use are: 35 cycles of denaturation at 94 ^{o}C for 30 seconds, annealing at 55 ^{o}C for 1 minute, and extension at 72 ^{o}C for 1 minute. Final extension at 72 ^{o}C for 5 minutes. The number of amplification cycles needed to obtain sufficient material for other procedures will depend on the abundance and size of the target cDNA. It is best to use the minimum number of cycles possible, since Taq polymerase lacks proofreading activity. The error rate can be reduced by altering either the reaction pH and Mg^{2+} concentration (6).

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

C. Analysis of Reactions

1. Agarose Gel Electrophoresis

Results of the amplification are obtained by running 5-10 µl aliquots of the PCR reactions on 1.0% agarose gels. Please refer to the laboratory manual by Sambrook et al. (7) for agarose gel electrophoresis protocols.

D. Troubleshooting

1. No product is obtained with unique sequence primers: This result can occur for several reasons. The simplest explanation is that one of the reagents was omitted or is "old." Aliquots of PCR reagents, such as 10X buffer and dNTPs should be stored at -20^{o}C to prevent their degradation. This possibility can be controlled for by including a positive control, such as the primers included with the kit. It is also possible that the mRNA source you have chosen expressed the target gene at very low levels. We recommend that you try several of the Multiple Choice cDNAs in your initial screening.

2. No product is obtained with degenerate primers: It is important to determine whether the failure to obtain a PCR product is due to any of the reasons listed for unique sequence primers. you have eliminated all of the explanations listed above, there are several other likely causes for the lack of product with the degenerate primers.

a. The primers may contain too many mismatches. You can try reducing the annealing temperature or you can construct new primers.

b. If you are trying to amplify the 5' end of a very long mRNA, we recommend that you use cDNA which was primed with random hexamers rather than this cDNA which is oligo(dT) primed.

3. Too many PCR products: Try increasing the stringency of the PCR reaction by either increasing the annealing temperature or reducing the primer concentrations. Additionally tetramethylammonium chloride (TMAC), formamide and $MgCl_2$ have been reported to increase the specificity of PCR reactions using unique sequences. Alternatively alternate primers can be constructed or a "nested" PCR approach can be used.

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Multiple Choice™ Northern Blots
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