

Technically Speaking

RiboClone® cDNA Synthesis Systems and Related Cloning Products

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Promega offers a variety of cDNA synthesis and cloning options including two different reverse transcriptases and four different primers or primer-adaptors as well as five cDNA cloning vectors and their corresponding bacterial host strains. These components are available in a number of configurations as RiboClone® Systems. This article is designed to help you choose the specific components that best fit your cDNA synthesis and cloning needs.

Q: What are some experimental uses for cDNA libraries?

A cDNA library can be used to clone an RNA of interest for use in gene structure or expression studies. One strategy used is to make a cDNA library from mRNA extracted from appropriate tissue or cells. The cDNA clone of interest is then identified within the population of cDNA clones by screening with a nucleic acid probe consisting of a previously cloned cDNA fragment, genomic DNA, or a synthetic oligonucleotide specifying a particular RNA sequence (1).

A cDNA expression library can be used to identify the DNA sequence that encodes a polypeptide or protein of interest. Certain lambda vectors (such as lambda-gt11) facilitate the expression of the polypeptide encoded by its DNA insert and can be screened with an antibody raised against the protein of interest.

Q: How do I choose the correct cDNA synthesis system for my application?

Promega has two different reverse transcriptases, AMV(Avian Myeloblastosis Virus) and M-MLV(H-) (Moloney Murine Leukemia Virus), each available in combination with four different primers or primer-adaptors to fit a variety of needs.

Random Primers are used when the mRNA of interest is not polyadenylated (such as bacterial mRNA) or for cloning the 5' or internal regions of an RNA molecule. **Oligo(dT) Primers** are used with poly(A)⁺ RNA templates.

Both of these primers yield cDNA which is cloned in a random orientation. cDNA produced in this manner is usually modified by the addition of linkers or adaptors (such as the RiboClone *EcoR* I Adaptor Ligation System or RiboClone *EcoR* I Linker Ligation System) for cloning into a single, specific restriction site.

Directional or orientation specific cDNA cloning can be accomplished using the ***Not* I or *Xba* I Primer-Adaptors**. These consist of oligo(dT) primers adjacent to a unique restriction site (*Not* I

or *Xba* I). *EcoR* I linkers or adaptors are added to the resulting cDNA and then it is digested with *Not* I or *Xba* I to yield a molecule with *EcoR* I on one terminus and *Not* I or *Xba* I on the other.

Directional cloning is valuable in two major applications: *In vitro* transcription of directionally-cloned inserts in vectors LambdaGEM®*-2 and LambdaGEM-4 can generate either sense or antisense RNA probes that represents all of the sequences in the library. For expression libraries, directional cloning can increase by a factor of two the likelihood of expressing the insert as the correct polypeptide.

*U.S. Pat. No. 4,766,072 has been issued to Promega Corporation for transcription vectors having two different bacteriophage RNA polymerase promoter sequences separated by a series of unique restriction sites into which foreign DNA can be inserted.

Q: What is the difference between the two reverse transcriptases?

AMV Reverse Transcriptase is isolated directly from the blood of infected chickens. This native enzyme contains endogenous RNase H activity which can lead to shortened cDNAs; typically cDNAs in the size range of 1-3kb are produced. AMV Reverse Transcriptase is more processive than M-MLV Reverse Transcriptase and is the best choice for GC-rich templates or templates containing significant secondary structure (2).

Promega's M-MLV (H-) Reverse Transcriptase is a recombinant molecule which lacks RNase H activity. cDNAs as large as 7.5 kb can be synthesized with this reverse transcriptase if the template lacks significant secondary structure or GC-rich regions. M-MLV Reverse Transcriptase is less processive than AMV Reverse Transcriptase and so ten-fold more enzyme is required per reaction.

Q: How do I choose a cloning vector?

Promega offers a variety of lambda cloning and expression vectors for directional or nondirectional cloning. Many of the vectors are available as predigested, dephosphorylated arms which give a low background of nonrecombinants.

Lambda gt10 is a cloning vector with a unique *EcoR* I site for nondirectional cloning of cDNAs produced using oligo(dT) or random primers followed by modification with RiboClone *EcoR* I Adaptor or Linker Systems. This vector can accept DNA inserts of up to 7.6 kb. Lambda gt10 is available both uncut and precut as *EcoR* I Arms.

LambdaGEM-2 was made by inserting the multiple cloning region and SP6 and T7 promoters of plasmid pGEM®-1 into lambda-gt10. This multiple cloning region contains a unique *EcoR* I site and so can be used for nondirectional cloning. This vector also contains an *Xba* I site which can be used in combination with the *EcoR* I site for orientation specific cloning of cDNAs produced with the *Xba* I Primer-Adaptor System. This cloning vector can accept inserts up to 7.1 kb. It is available uncut or as either *EcoR*I Arms or *EcoR* I-*Xba* I Arms.

LambdaGEM-4 is similar to LambdaGEM-2 except the entire pGEM-1 plasmid (which contains the ampicillin resistance gene) has been inserted into lambda-gt10. The cloning capacity of this vector is 4.7kb. *Spe* I digestion and religation of this vector yields recombinant pGEM-1 plasmids containing cDNA inserts. LambdaGEM-4 is available uncut or as *EcoR* I-*Xba* I Arms.

Lambda gt11 is a cloning and expression vector which contains a unique *EcoR* I site for cloning cDNAs synthesized using oligo(dT) or random primers followed by the addition of *EcoR* I linkers or adaptors. The *EcoR* I insertion site is located within the *lacZ* gene upstream from the beta-galactosidase translation termination codon. Recombinant phage can be recognized by their ability to form colorless plaques when plated on *lac*- hosts in the presence of X-gal; parental plaques are blue. DNA inserts up to 7.2 kb can be cloned into lambda-gt11. This vector is available uncut or as *EcoR* I Arms.

Lambda gt11 *Sfi*-*Not* Vector is derived from lambda-gt11 with the addition of *Not* I and *Sfi* I sites in close proximity to the *EcoR* I site. This modification allows directional cloning of cDNA synthesized with the *Not* I Primer Adaptor and modified with *EcoR* I linkers or adaptors. Lambda gt11 *Sfi*-*Not* Vector is available uncut or as *EcoR* I-*Not* I Arms.

Q:How do I package my lambda clones?

Promega's Packagene® System is a phage-infected *E. coli* extract which supplies, in a single tube, the mixture of proteins and precursors required for encapsidating lambda DNA. Packagene Extracts are available in combination with cDNA cloning vector arms or can be purchased separately.

Q: What bacterial strain do I use to grow my clones?

Promega has a variety of bacterial strains each suited to a particular vector or application.

LE 392 is a permissive host allowing growth of both parental and recombinant phage. It is recommended as the primary host for amplification of recombinant phage and for screening a cDNA library with nucleic acid probes. This host is compatible with all Promega Lambda cDNA Cloning Vectors for amplification or screening with nucleic acid probes.

C600 and C600*hfl* are the bacterial hosts of choice when using lambda-gt10. C600 is a permissive host and is the preferred host to titer and propagate lambda-gt10. C600*hfl* carries a lysogeny mutation which causes production of the parental phage to be suppressed 50- to 100-fold. Morphologic selection of phage is possible with C600*hfl* because the parental lambda-gt10 phage are lysogenic and their plaques appear turbid, while the recombinant phage are lytic and their plaques appear clear. C600*hfl* should be maintained in the presence of 15µg/ml tetracycline due to the presence of a transposon (Tn10) encoding for tetracycline resistance.

Y1090 is used for screening expression cDNA libraries such as those produced with lambda-gt11 or lambda-gt11 *Sfi*-*Not* Vectors. This strain allows a lytic infection due to the *supF* mutation which represses the normally defective lysis of lambda-gt11 and lambda-gt11 *Sfi*-*Not* recombinants. This enables antibody screening of plaque lifts at a high density with low signal to noise ratios. Y1090 also contains the mutation delta-*lon* which decreases protease activity and may increase the stability of recombinant fusion proteins in this strain. Cells of this bacterial strain contain a plasmid, pMC9, which carries the *lac* repressor and also ampicillin and tetracycline resistance. Expression of the fusion protein is repressed and can be induced by the addition of isopropyl beta-D-thiogalactopyranoside (IPTG). This is especially useful if the fusion protein is toxic to the host cell. Plaque formation can be initiated without expression of the fusion protein and then after a large number of cells have been infected, gene expression can be turned on by the addition of IPTG (1). Y1090 allows color selection of recombinant (clear) or parental (blue) plaques of lambda-gt11 or lambda-gt11 *Sfi*-*Not* in the presence of IPTG and X-Gal. This strain

also carries resistance to tetracycline (transposon Tn10). To maintain pMC9, it is necessary to initially streak this strain on LB plates supplemented with 100µg/ml ampicillin and 15µg/ml tetracycline.

Y1089 is the strain of choice to express large quantities of beta-galactosidase fusion proteins from expression vectors such as lambda-gt11 or lambda-gt11 *Sfi-Not*. This strain is similar to Y1090 in that it also contains pMC9 (*lac* repression and antibiotic resistance), is tetracycline resistant (Tn10), carries the delta-*lon* mutation and allows for color selection. Unlike Y1090, strain Y1089 carries the *hflA150* mutation which enhances the frequency of phage lysogeny. To produce preparative amounts of recombinant fusion protein, Y1089 is lysogenized with the lambda-gt11 or lambda-gt11 *Sfi-Not* clone of interest and grown to a high cell density. Production of the fusion protein is induced by the addition of IPTG to the medium and the cells are harvested and lysed to release the protein (1).

Q: What Promega products can help me screen an expression library?

The ProtoBlot® Immunoscreening System enables the detection of rabbit or mouse primary antibodies used to screen for lambda-gt11 or lambda-gt11 *Sfi-Not* recombinants expressing beta-galactosidase fusion proteins. ProtoSorb® *lacZ* Immunoaffinity Adsorbent is designed to purify beta-galactosidase fusion proteins. The column matrix consists of a purified mouse anti-beta-galactosidase monoclonal antibody coupled to cross-linked agarose beads.

References

1. Huynh, T. *et al.* (1985) In *DNA Cloning Techniques: A Practical Approach*, Vol 1, Glover, D.M., ed., ILR Press, Oxford, 49.
2. Kimmel, A.R. and Berger, S.L. (1987) *Meth. Enzymol.* **152**, 307.

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