cDNA Library Construction

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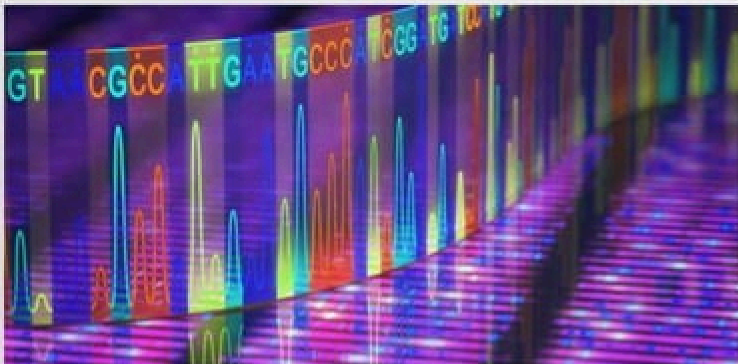
The complete genome sequences of a number of organisms, including mammals, have recently become available because of rapid advances in DNA sequencing technology. Nevertheless, the analysis of transcripts still plays a crucial role in bridging the gap between the genome and the proteome, particularly in mammals. This is mainly because so far we cannot accurately predict the structures of transcripts derived from a particular gene from the genomic information alone. Therefore, as a method for the analysis of transcripts, cDNA library construction is crucial, even in the post-genome sequencing era.



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An Overview of cDNA Library

Complementary DNA (cDNA) is produced from a fully transcribed messenger ribonucleic acid (mRNA) that contains only the expressed genes of an organism. Clones of such DNA copies of mRNAs are called cDNA clones. A cDNA library is a combination of cloned cDNA fragments constituting some portion of the transcriptome of an organism which are inserted into many host cells.

The mRNA is spliced before translation into protein in eukaryotic cells. The DNA synthesized from the spliced mRNA doesn't have non-coding regions or introns of the gene. Therefore, the protein under expression can be sequenced from the DNA which is the key advantage of cDNA cloning over genomic DNA cloning.

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The Workflow of cDNA Library Construction

Creation of a cDNA library starts with mRNA instead of DNA. Messenger RNA carries encoded information from DNA to ribosomes for translation into protein. To create a cDNA library, these mRNA molecules are treated with the enzyme reverse transcriptase, which is used to make a DNA copy of an mRNA (i.e., cDNA). A cDNA library represents a sampling of the transcribed genes, but a genomic library includes untranscribed regions.

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The Workflow of cDNA Library Construction

1. Isolation of mRNA

First of all, it involves the isolation of total mRNA from a cell type or tissue of interest. It may be desirable to remove highly abundant tRNAs and rRNAs which might otherwise constitute the majority of the final library to the detriment of the detection of low abundance RNAs. We routinely remove tRNAs and other small RNAs <200 nt using a Kit from Creative Biogene and remove rRNAs using magnetic bead-based depletion kits. The 3′ ends of eukaryotic mRNA are composed of a string of 50 -250 adenylate residues (poly A Tail) which makes the separation easy from the much more prevalent rRNAs and tRNAs in a cell extract through a column containing oligo-dTs tagged onto its matrix.

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2. Synthesis of the first strand of cDNA

mRNA being single-stranded cannot be cloned and is not a substrate for DNA ligase. It is first converted into DNA prior to insertion into a suitable vector.

1) A short oligo (dT) primer is annealed to the Poly (A) tail on the mRNA.
2) Reverse transcriptase extends the 3´ -end of the primer by mRNA molecule as a template producing a cDNA: mRNA hybrid.
3) The mRNA from the cDNA: mRNA hybrid can be removed by alkaline hydrolysis or RNase H to give a single stranded (ss)-cDNA molecule.

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3. The second strand of cDNA generation

The ss-cDNA is converted into double stranded (ds) cDNA by either RTase or *E. coli* DNA polymerase. (It is essential to use only the minimal number of amplification cycles needed to obtain sufficient material for sequencing to avoid over-amplification of the libraries, which is a major source of bias in the results.)

4. Incorporation of cDNA into a vector

The ds-cDNA can be trimmed with S1 nuclease to obtain blunt–ended ds-cDNA molecule followed by addition of terminal transferase to tail the cDNA with C's and ligation into a vector. Because the blunt-end ligation is inefficient, short restriction-site linkers are first ligated to both ends.

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5. Cloning of cDNAs

cDNAs are commonly cloned in phage insertion vectors. Bacteriophage vectors possess the following advantageous over plasmid vectors:

- Are more desirable when a large number of recombinants are required for cloning low-abundant mRNAs as recombinant phages are produced by *in vitro* packaging.
- Can easily handle and store large numbers of phage clones, as compared to the bacterial colonies carrying plasmids.

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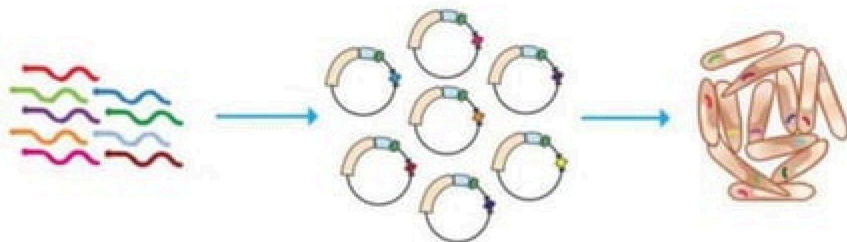
cDNA Library Construction Services

Creative Biogene's advanced technologies and highly experienced staffs are available to provide you a range of cDNA libraries construction service, including standard cDNA library, subtractive cDNA library, normalized cDNA library, full length cDNA library, yeast two-hybrid cDNA library and SSH cDNA library. Creative Biogene's goal is to provide you with the most affordable and high-quality cDNA libraries construction service to ensure your satisfaction in a timely and professional manner.



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A subtractive cDNA library is a collection of cDNA clones which is rare and likely to be poorly expressed. Subtractive cDNA libraries are produced using a proprietary technique which relies on the removal of dsDNA formed by hybridization between a control and test sample, thus eliminating cDNAs of similar abundance and retaining the transcripts which are differentially expressed or variable in sequence.

Subtractive cDNA library

Normalized cDNA libraries are produced using a special normalization procedure which can enhance the gene discovery rate of a cDNA library and facilitate the identification and analysis of rare transcripts. This procedure is imperative for transcriptome sequencing, and useful in other applications, such as functional screening, construction of specific RNA libraries, and transcript end sequence profiling.

Normalized cDNA library

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Yeast two-hybrid cDNA library is a powerful molecular biology tool in studying the protein interactions, to find the domain that plays a key role in protein-protein interaction or to discover a new protein interaction with target proteins. The establishment of the yeast two-hybrid library is based on the well understanding of the regulation of the transcription initiation process of eukaryotic cells.

Yeast two-hybrid cDNA library

Suppression Subtractive Hybridization (SSH) is an efficient tool to selectively amplify target cDNA fragments (differentially expressed) and simultaneously suppress non-target DNA amplification. Using a small quantity of either total RNA or poly(A)+ mRNA from each of two populations, the SSH procedure can simultaneously subtract and partially normalize the abundance of target cDNA in the subtracted population.

SSH cDNA Library

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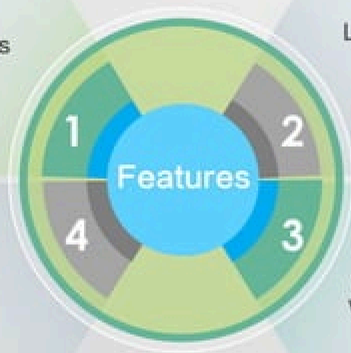
Large numbers of primary clones

Low vector background and high percentage of recombinants



Competitive prices and fast turnaround time

The cDNAs are cloned directionally into our standard vector or your vector of choice

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