Gene cloning (the construction of a cdna library...)

Science, Chemistry

The Construction of a cDNA Library from an Extract of Human Brain Cells 909 words The Construction of a cDNALibrary from an Extract of Human Brain Cells A complementary DNA (cDNA) library refers to a blend of reverse transcribed mRNA pieces inserted into a group of host cells. cDNA is made from wholly transcribed mRNA found in the nucleus of cells and therefore contains only the expressed genes of an organism. Establishment of cDNA libraries is important in disease research, genome construction and research for drugs and alternative medicine. By purchasing an already constructed cDNA library, research scientists avoid the process of collecting mRNA from human tissue, hence avoiding tissue contamination, enzyme manipulation and complicated purification processes that usually accompany the process. One also gets to enjoy working with the best quality of starter cDNA and stand a better chance of getting more accurate results. An array of human cDNA libraries can be made from diverse tissues such as kidney breast, heart, spleen and brain (Adams et al, 1991). The process of construction of a cDNA library from brain cells consists of; isolation and collection of mRNA from brain cells, generation of cDNA by the enzyme reverse transcriptase (RT), incorporating the cDNA into a vector (plasmids), and isolation of the plasmids and purification of the DNA. This process is discussed into detail below. First is the Isolation and Collection of mRNA from Brain Cells. The mRNA is purified from the rest of the RNAs extracted from brain cells. Here, several methods for purifying RNA such as column purification and trizol extraction and can be used. The former technique is achieved by using oligomeric dT nucleotide coated resins that ensure only the mRNA having the poly-A tail will bind, leaving the RNAs. The mRNA is further purified using a

buffer to separate the mRNA strands from oligo-dT, some heat is required during the process (Freshney, 2006). The second stage involves generation of cDNA by the Enzyme Reverse Transcriptase (RT). After purification of mRNA, creation of a complementary cDNA strand requires that oligo-dT is tagged as a complementary primer that binds to the poly-A tail hence providing a free 3'-OH end which will be extended by reverse transcriptase (RT). When this completes, the mRNA is detached using an RNAse enzyme, this process leaves a single stranded cDNA. Once the initial cDNA strand has been generated it is necessary to produce a second strand of DNA. A convenient method of doing this involves exposing the DNA/RNA mixture to a combination of DNA polymerase and RNAase-H. RNAase-H produces single-stranded nicks in the RNA, while DNA polymerase then uses these single-stranded nicks to start the synthesis of second strand cDNA (Sim et al, 1999). The third stage involves incorporating the cDNA into a vector. A vector refers to a self-replicating organism usually designed to replicate the DNA material created. They are majorly derivatives of viruses such as plasmids and retroviruses and must include some mechanism of reproduction. The vector can be prepared by treating it with the same nuclease, or one that produces the same restriction site, to produce a single-stranded region complementary to that in the cDNA. Mixing the cDNA of interest and the vector a medium with enzyme ligase allows proper incorporation of the cDNA into the vector. An obstacle in this procedure is that the vector itself will tend to form a vector without any cDNA insert. Treating the vector with enzyme phosphatase minimizes this problem and also prevents unwanted side reactions. The DNA created is therefore

inserted into plasmids, and further the plasmids into bacteria for growth. Once a sizeable population size of the clones is achieved, the plasmids are isolated and the DNA purified. The DNA is then sequenced to compile the cDNA library (Sutcliffe et al, 1982). Methods of screening cDNA Libraries The following are three possible ways which may be used to screen a cDNA library for a gene whose protein product has been previously isolated. Screening by Databases In cases where partial protein sequence or partial cDNA sequence data is available, performing a query on a data base often result in identifying candidate clones with genes whose protein product has been previously isolated. The rapid buildup of sequence information and genetic data has enabled scientists to sidestep the long procedures required to isolate cDNAs since databases include the sequence of entire genomes (Sim et al, 1999). PCR-based Screens Regions of homology, known to be conserved within a family, can be identified once two or more members of a family have been isolated. With this knowledge, PCR primers can be designed and used to amplify reverse transcriptase products of mRNAs in an appropriate tissue. The molecular weight of known members of the family can be predicted and new mRNAs may give rise to products. These products in turn can be used to screen cDNA libraries. a single region of conserved structure is sufficient to isolate novel genes using this strategy. Homology Screening This screening method works on the basis that once a unique gene has been isolated from a species, it becomes easy to use a hybridization approach to isolate cDNAs from another species. In the same manner, additional family members of the same species can easily be identified based on similar genetic make up (Sutcliffe et al, 1982). Conclusion

The construction of a cDNA library is an important approach that benefits disease research, genome construction and research for drugs and alternative medicine. This is because it saves time and improves chances of getting more accurate results and must therefore be encouraged.

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