Method for construction of normalized cDNA libraries

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Technology description

Summary

Problem or Unmet Need:
cDNA libraries are the main resource utilized for identification of genes. Finding the gene of interest, however, is usually a rather hard task. This is so because the frequency of the different cDNA clones in a conventional (non normalized) cDNA library vary over a wide range (typically over 1,000 fold). A few clones are present at high frequencies, whereas most genes are extremely under-represented. In a normalized cDNA library, on the other hand, all cDNAs are represented at comparable frequencies. Thus, the process of identification of new genes becomes not only much simpler but also more likely to be successful. Standard cDNA normalization protocols however utilize polymerase chain reactions (PCR) for amplification of cDNAs which favor short DNA fragments. The resulting normalized libraries lack longer cDNAs which contain considerable protein coding information.
Details of the Invention:
This technology represents a process for normalizing cDNA libraries from tissues of human or animal origin. It makes use of the preferential hybridization of prevalent cDNAs and the separation of single stranded from duplexed cDNAs to reduce the frequency of prevalent cDNAs. The range of frequencies of occurrence for all clones in the library has been shown to be reduced from 4 orders of magnitude to 1 order of magnitude following this normalization procedure. A number of tissue-specific normalized sub-libraries can also be generated by a series of subtractive hybridizations involving each of the individual normalized libraries, or combinations of them, and the cDNA catalogue.
Advantages

Application area

Normalized cDNA library construction for new gene identification
Generation of normalized cDNA libraries from various tissues, in various species, and at different stages of development

Advantages

Efficient and high yield reduction in prevalent clone frequency

Normalizes large sized cDNAs containing considerable protein coding information -- Important for new drug and drug target discovery
3’ non coding sequences are involved in the reassociation reaction eliminating the possibility of losing rare clones to cross hybridization with sequences of more prevalent members of the same gene family

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