attempt to contribute to the ongoing gene discovery efforts, we have further optimized our original procedure for construction of normalized directionally cloned cDNA libraries[1] and we have successfully applied it to generate a number of human cDNA libraries from a variety of adult and fetal tissues [2]. To date we have constructed libraries from infant brain, fetal brain, adult brain, fetal liver-spleen, full-term and 8-9 week placentae, adult breast, retina, ovary tumor, melanocytes, parathyroid tumor, senescent fibroblasts, pineal glands, multiple sclerosis plaques, testis, B cells, fetal heart, fetal lung, 8-9 week fetuses and pregnant uterus. Several additional libraries are currently in preparation. All libraries have been contributed to the IMAGE consortium, and they are being widely used for sequencing and mapping.

However, given the large scale nature of the ongoing sequencing efforts and the fact that a significant fraction of the human genes has been identified already, the discovery of novel cDNAs is becoming increasingly more challenging. In an effort to expedite this process further, in collaboration with Greg Lennon (LLNL) we have developed and applied subtractive hybridization strategies to eliminate pools of sequenced cDNAs from libraries yet to be surveyed. Briefly, single-stranded DNA obtained from pools of arrayed and sequence I.M.A.G.E. clones are used as templates for PCR amplification of cDNA inserts with flanking T7 and T3 primers. PCR amplification products are then used as drivers in hybridizations with normalized libraries in the form of single-stranded circles. The remaining single-stranded circles (subtracted library) are purified by hydroxyapatite chromatography, converted to double-stranded circles and electroporated into bacteria. Preliminary characterization of a subtracted fetal liver-spleen library indicates that the procedure is effective to enhance the representation of novel cDNAs.

In an effort to enhance the representation of full-length cDNAs in our libraries, as we strive towards our final objective of generating full-length normalized cDNA libraries, we have adapted our normalization protocol to take advantage of the fact that it is now possible to produce single-stranded circles in vitro by sequentially digesting supercoiled plasmids with Gene II protein and Exonuclease III (Life Technologies). This has proven significant because it circumvents the biases introduced by differential growth of clones containing small and large cDNA inserts when single-strands are produced in vivo upon superinfection with a helper phage.

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References

[1] Soares, M.B., Bonaldo, M.F., Su, L., Lawton, L. & Efstratiadis, A. (1994). Construction and characterization of a normalized cDNA library. Proc. Natl. Acad. Sci. USA 91(20), 9228-9232.

[2] Bonaldo, M.F., Lennon, G. and Soares, M.B. (1996). Normalization and subtraction: Two approaches to facilitate gene discovery. Genome Research 6, 791-806.

Mapping in Man-Mouse Homology Regions

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Numerous studies have confirmed the notion that mouse and human chromosomes resemble each other closely within blocks of syntenic homology that vary widely in size, containing from just a few to several hundred related genes. Within the best-mapped of these homologous regions, the presence and location of specific genes can be accurately predicted in one species, based upon the mapping results obtained in the other. In addition, information regarding gene function derived from the analysis of human hereditary traits or mapped murine mutations, can also be extrapolated from one species to another. However, syntenic relationships are still not established for many human regions, and local rearrangements including apparent deletions, inversions, insertions, and transposition events, complicate most of the syntenically homologous regions that appear simple on the gross genetic level. Because of these complications, the power of prediction afforded in any homology region increases tremendously with the level of resolution and degree of internal consistency associated with a particular set of comparative mapping data. Our groups have been interested in further defining the borders of syntenic linkage groups in human and mouse, upon elucidating mechanisms behind evolutionary rearrangements that distinguish chromosomes of mammalian species, and upon devising means of exploiting the relationships between the two genomes for the discovery and analysis of new genes and other functional units in mouse and man.

One of the larger contiguous blocks of mouse-human genomic homology includes the proximal portion of mouse chromosome 7 (Mmu7). Detailed analysis of this large region of mouse-human homology have served as the initial focus of these collaborative studies. Our results have shown that gene content, order and spacing are remarkably well-conserved throughout the length of this approximately 23 cM/29 Mb region of mouse-human homology, except for six internal rearrangements of gene sequence in mouse relative to man. One of these differences involve a small segment of H19q13.4 genes whose murine counterparts have been transposed out of the large Mmu7/H19q conserved synteny region into a separate linkage group located on mouse chromosome 17. The six internal rearrangements, including two transpositions and four local