or BACs. Hence, it facilitates retrofitting existing PAC/BAC clones (made with the new vectors) with desirable sequences without affecting the inserts. The new vector(s) are being applied to generate second generation libraries for human (female donor), mouse and rat.

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Development of Affinity Technology for Isolating Individual Human Chromosomes by Third-Strand Binding

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Prior to the onset of this grant, solution conditions had been developed for binding a 17-residue third strand oligodeoxyribonucleotide probe to a specific human chromosome (HC) 17 multicopy alpha satellite target sequence cloned into DNA vectors of varying size up to 50 kb. Binding was shown to be both highly efficient and specific. Moreover, initial experiments with fluorescent-labeled third strands and human lymphocyte metaphase spreads and interphase nuclei proved similarly successful. During the current research period, the technology for such third strand-based cytogenetic examination, i.e., Triplex *In Situ* Hybridization or TISH, of such spreads was perfected, so that it is now a highly reproducible method. Comparison of spreads of different individuals by TISH and FISH analysis has provided a new basis for detecting alpha satellite DNA polymorphisms, the basis of which requires further investigation.

This year work also commenced on the development of comparable probes specific for alpha satellite sequences in HC-X, 11, and 16. The work with HC-X has reached the stage where we are ready to test the probe for TISH-based cytogenetic analysis. Solution studies of the interaction of the probes designed for HC-11 and HC-16 alpha satellite targets are following the well-established path we employed for HC-17 and HC-X. With the expectation of success in these cases during the coming year, the way should be clear for the development and application of comparable probes for alpha satellite sequences of any other human chromosomes that may be of interest, and possibly of other eukaryotic species.

Meanwhile, we have begun to turn our attention to two other goals, one being the exploitation of our probes for the isolation of individual human chromosomes by affinity purification, as we originally proposed. The other goal is to exploit our probes as aids in flow sorting human chromosomes, a direction of work we expect to pursue in collaboration with the Los Alamos National Laboratory, just as soon as they indicate a readiness to do so. Finally, we have begun to evaluate the possibility of using third-strand binding fluorescent probes for detection of single copy genes by means of photon counting, a goal which we plan to undertake with our colleague Robert Austin of our Physics Department.

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Chromosome Region-Specific Libraries for Human Genome Analysis

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The objective of this project is to construct and characterize chromosome region-specific libraries as resources for genome analysis. We have used our chromosome microdissection and MboI linker-adaptor technique (PNAS 88, 1844, 1991) to construct region-specific libraries for human chromosome 2 and other chromosomes. The libraries have been critically evaluated for high quality, including insert size, proportion of unique vs repetitive sequence microclones, percentage of microclones derived from dissected region, etc.

We have constructed and characterized 11 region-specific libraries for the entire human chromosome 2 (the second largest human chromosome with 243 Mb of DNA), including 4 libraries for the short arm and 6 libraries for the long arm, plus a library for the centromere region. The libraries are large, containing hundreds of thousands of microclones in plasmid vector pUC19, with a mean insert size of 200 bp. About 40-60% of the microclones contain unique sequences, and between 70-90% of the microclones were derived from the dissected region. In addition, we have isolated and characterized many unique sequence microclones from each library that can be readily sequenced as STSs, or used in isolating other clones with large inserts (like YAC, BAC, PAC, P1 or cosmid) for contig assembly. These libraries have been used successfully for high resolution physical mapping and for positional cloning of disease-related genes assigned to these regions, e.g. the cloning of the gene for hereditary nonpolypsis colorectal cancer (Cell 75, 1215, 1993).

For each library, we have established a plasmid sub-library containing at least 20,000 independent microclones. These sub-libraries have been deposited to ATCC for permanent maintenance and general distribution. The ATCC Repository numbers for these libraries are: #87188 for 2P1 library