

In this pilot project proposal we are carrying out automated end-sequencing of approximately 40,000 PAC and BAC clones representing the entire human genome, as well as about 500 PAC clones localized to human chromosomes 11 and 15. The clones and resulting end-sequence data base will be utilized to 1) nucleate regions of interest for large scale sequencing concentrating on regions of chromosome 11 and 15, 2) correspond with regions mapped by other methods to confirm the mapping accuracy and 3) used to evaluate the use of random clone end sequence libraries. DNA sequencing is being carried out in an entirely automated fashion using a Beckman/Sagian robotic system, ABI 377 automated sequencers and automated sequence data processing, annotation and publication using a Hewlett Packard/Convex superparallel computer located at the UTSW genome center. FISH analysis of a sample of PAC clones has been carried out and defines the potential chimera rate in existing PAC libraries as less than 1.2%. This effort will be coordinated with efforts of other groups carrying out PAC and BAC library construction, PAC and BAC end-sequencing and FISH analysis to avoid duplication of effort and provide a comprehensive end-sequence library and data set for use by the international human genome sequencing effort.

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Automated DNA Sequencing by Parallel Primer Walking

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The development of efficient mapping approaches coupled with high throughput, automated DNA sequencing remains one of the key challenges of the Human Genome Project. Over the past few years, a number of strategies to expedite clone-by-clone DNA sequencing have been developed including efficient shotgun sequencing, sequencing of nested deletions, and transposon-mediated primer insertion. We have developed a novel sequencing strategy applicable to high throughput, large scale genomic analysis based upon DNA sequencing directly primed on of cosmid templates using custom-designed, automatically synthesized oligonucleotide primers. This approach of directed primer "walking" would allow the number of sequencing reactions and the efficiency of sequencing to be vastly improved over traditional shotgun sequencing.

Custom primer design has been carried out using software we developed for prediction of "walking" primers directly from the output of ABI377 automated DNA sequencers, and the output used to automatically program synthesis of the custom primers using 96 or 192 channel oligonucleotide synthesizers constructed at UTSW. Automated operation of the sequencing system is thus possible where results of each sequencing reaction is used to predict, synthesize, and carry out appropriate extension reactions for downstream "walking". A automated prototype system has been assembled where dye terminator DNA sequencing can be carried out from 96 cosmid templates simultaneously followed by prediction of oligonucleotide "walking" primers for extending the sequence of each fragment, and programming an attached 96-channel oligonucleotide synthesizer to initiate a second round of sequencing. Using a set of nested cosmids covering 800 kb at 5X redundancy, primer directed sequencing should allow completion of 800 kb of finished, high accuracy DNA sequence in 8 to 16 cycles. Furthermore, coupling of automated DNA sequencing instrumentation to DNA sequence analysis programs and multichannel oligonucleotide synthesizers will allow almost complete automation of sequencing process and the development of instrumentation for completely unattended DNA sequencing.

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***Parallel Triplex Formation as Possible Approach for Suppression of DNA-Viruses Reproduction**

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It is well known that homopurine or homopyrimidine single stranded oligonucleotides can bind to homopurine-homopyrimidine sequences of two-stranded DNA to form stable three-stranded helices. In such triplexes two identical strands have antiparallel orientation. We denote these triplexes as "antiparallel" or "classical" triplexes.

A particular interest of investigators to triplexes has arisen due to an elegant idea of using triplexes as sequence-specific tools for purposeful influence on DNA duplexes. Triplex forming oligonucleotides were shown to be potentially useful as regulators of gene expression and subsequently as therapeutical (antiviral) agents.

A significant limitation to the practical application of antiparallel triplex is the requirement for homopurine tracts in target DNA sequences. Numerous investigations slightly