1996 Phase II

A Graphical Ad Hoc Query Interface Capable of Accessing Heterogeneous Public Genome Databases

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The interoperability of public genome databases is expected to be crucial in making the Human Genome Project a success. This project will develop software tools in which users in the genome community can learn or examine public genome database schemes in a relatively short time and can produce a correct Structured Query Language (SQL) expression easily. In Phase I, a concept system was constructed and the effectiveness of formulating ad hoc queries graphically was demonstrated. Phase II will focus on transforming the concept system into a product that is robust and portable. Two types of computer programs will be developed. One is a client program which is to be distributed to community users who intend to access public genomic databases and link them with local databases. The other is a server program and a suite of software tools designed to be used by those genome centers which intend to make their databases publicly accessible.

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Low-Cost Automated Preparation of Plasmid, Cosmid, and Yeast DNA

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MacConnell Research currently manufactures and sells a low cost automated bench-top instrument that can purify up to 24 samples of plasmid DNA simultaneously in one hour at a cost of $0.65 per sample and under $8000 for the instrument. The patented instrument uses a form of agarose gel electrophoresis to purify the plasmid DNA and electroelutes into approximately a 20 +l volume. The instrument has many advantages over other robotic and manual methods including the fact that is it two times faster, at least six times less expensive, much smaller in size, easier to operate, less cost per sample, and results in DNA pure enough for direct use in fluorescent automated sequencing. The instrument process begins with bacterial culture which is loaded directly into a disposable cassette in the machine.

In Phase II work we are developing an instrument which simultaneously purifies plasmid DNA from up to 192 (2 X 96) bacterial samples in 1.5 hours. Prototypes of this instrument thus far constructed have allowed the purification of 3–7 micrograms of high purity plasmid DNA per lane from 1.5 ml of bacterial culture. We have attempted to optimize all of the: instrument electrophoretic run parameters, lysis chemistry, lysis reagent delivery devices, reagent storage at room temperature, desalting processes and overall instrument mechanical and electronic control. Instrument prototypes have also been used to prepare cosmid or yeast DNA in quantities of 1–5 micrograms per cassette lane. Trials thus far have yielded plasmid DNA of sufficient purity for direct use in automated fluorescent and manual sequencing as well as other molecular biology protocols. We have studied the purity of the resulting DNA when directly sequenced on a Licor 4000 Long Reader and ABI 373A automated DNA sequencers. Results from the Licor 4000 instrument give routine read lengths of >850 base pairs with 98% accuracy while ABI 373A reads generally exceed 400 base pairs with similar accuracy.

The proposed 2 X 96-channel instrument will purify up to 1200 plasmid DNA preps per eight hour day. It will significantly reduce the cost and technician labor of high throughput plasmid DNA purification for automated sequencing and mapping.

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GRAIL-GenQuest: A Comprehensive Computational Framework for DNA Sequence Analysis

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Although DNA sequencing in the Human Genome Project is occurring fairly systematically, biotechnology companies have focused on sequencing regions thought to contain particular disease genes. The client-server DNA sequence analysis system GRAIL is the most accurate and widely used computer-based system for locating and characterizing genes in DNA sequences, but it is not accessible to many biotechnology environments. The GRAIL client software and graphical displays have been developed for high-end UNIX-based computer workstations. Such workstations are standard equipment in universities and large companies, but personal computers (PCs) and Macintosh computers are the prevalent technology within the biotechnology community. This Phase I project will design Macintosh- and Windows-based client graphical user interface prototypes for GRAIL.