Strategies for Sequencing Human Chromosome 16

FINAL REPORT

by

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This project funded for four years (02.92 to 01.96) was a renewal of a project funded for 2.5 years (07.89 to 01.92). This report covers the period 07.89 to 07.94. The original project was entitled "Correlation of physical and genetic maps of Human Chromosome 16". The aim over this period was to construct a cytogenetic-based physical map of chromosome 16, to enable integration of its physical and genetic maps. This was achieved by collaboration and isolation of new markers until each bin on the physical map contained a polymorphic marker on the linkage map. A further aim was to integrate all mapping data for this chromosome and to achieve contig closure over band q24.

Hybrid Cell Panel

The first aim was to construct a cytogenetic-based physical map with average bin sizes of 2 Mb by the isolation of naturally occurring rearranged chromosome 16s in mouse/human somatic cell hybrids.

The aim has been exceeded. The panel now consists of 88 hybrid cell lines representing 95 independently ascertained breakpoints on the chromosome. With the four fragile sites these divide the 85 Mb of euchromatin into 96 bins of average size 890 kb. There are four breakpoints in the 15 Mb of centromeric heterochromatin. Cloned DNA markers or STSs have been identified in 85 bins.

Subsets of the hybrid panel have been deposited in NIGMS cell repository and made available to our primary collaborators at LANL and numerous groups interested in specific regions of this chromosome and other chromosomes represented in the panel. The current state of the hybrid panel is shown in Fig. 1. This is the highest resolution cytogenetic-based physical map of any autosome.

Correlation of physical and genetic maps

During the initial stages of this project all available polymorphic markers typed on the CEPH panel of families were placed on the physical map. Gaps were targetted in the linkage map and closed by isolation and genotyping of additional PCR-based markers on these families. This resulted in the construction of a PCR-based linkage map which incorporated our 49 markers and 22 markers characterised by other laboratories, including those of the Weissenbach group. We coordinated the production of the CEPH consensus linkage map of this chromosome incorporating all PCR and non-PCR markers at this time. This linkage map has an average marker distance of 2.8 cM and the largest gap is 11.2 cM. This already exceeds the revised 5-year Research Goal of the US Human Genome Project to have a 2-5 cM genetic map completed by 1995. We have continued to integrate available microsatellite markers into the physical map and have now mapped many of the tri- and tetra-nucleotide markers.

Gene and Expressed Sequence Map

We have placed priority on the integration of genes and expressed sequences into the physical map of this chromosome. This has been achieved by mapping all available expressed sequences and cloned genes from published sources, databases and unpublished data obtained as a result of our numerous collaborations. The map now includes 83 genes,

150 expressed sequences, and 185 microsatellite markers and an updated version of this map was recently published (8).

Contig Construction

Development of an integrated mega-YAC, sorted chromosome 16 YAC and cosmid contig map of the chromosome by LANL with our collaboration has now been largely finalised and was published in the Nature Genome Directory (11).

We provided the somatic cell hybrid (CY18) from which chromosome 16 could be sorted to construct the cosmid library, the YACs were mapped by reagents which were first localised by us or LANL to our somatic cell hybrid panel and we provided some primers for STSs developed here to aid contig construction. We constructed the genetic maps for contig orientation and validation of the physical mapping of polymorphic markers.

The generation of sequence ready reagents, that is high resolution cosmid contigs for the region of 16q24, was initiated but could not be completed due to the cessation of funding.

Publications including work which was funded in full or in part by DOE contract DE-FG02-89ER60863

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