Mapping Chromosome 5 *Deborah Grady*

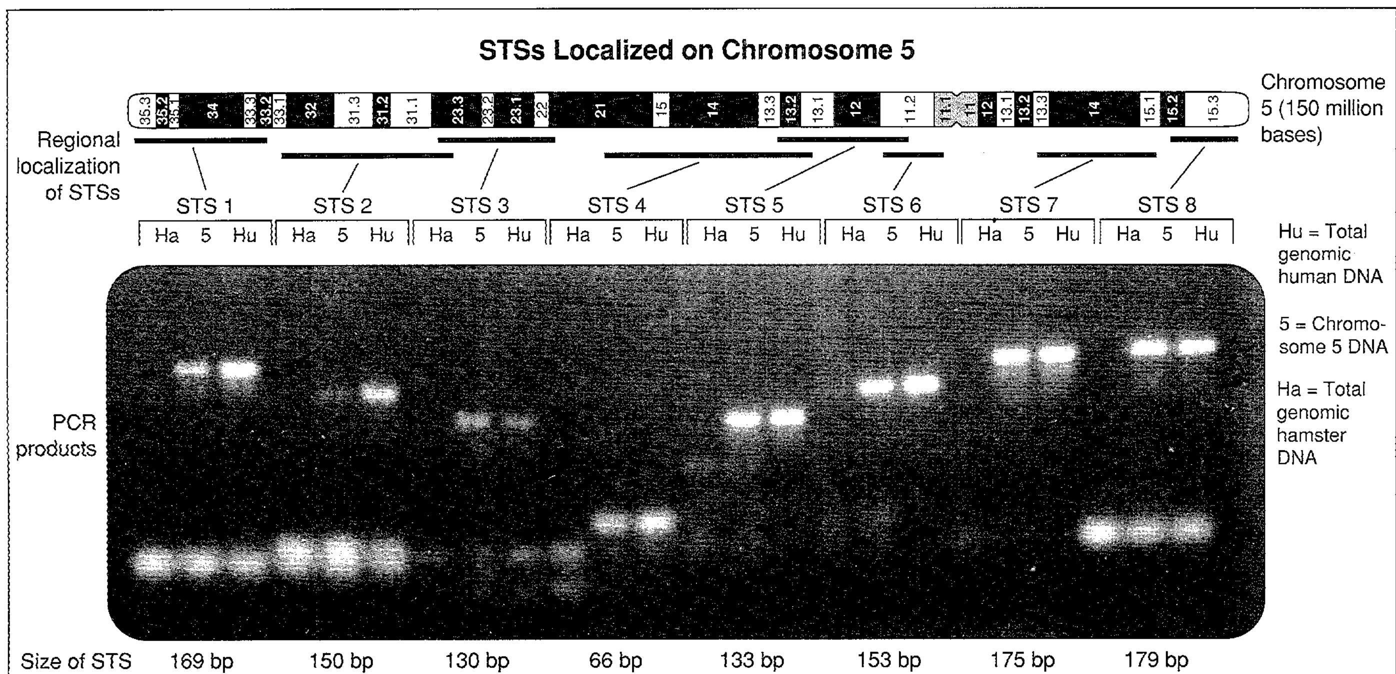
Constructing physical maps of complex genomes relies on the ability to isolate DNA segments for detailed analysis and to position those segments along the genome by identifying physical landmarks within them. The chromosome-16 physical map, now nearing completion, is a high-resolution map of DNA segments that have been isolated through cloning in cosmid and YAC vectors. The cloned fragments have been assembled into contigs and positioned along the chromosome based on detailed information about the positions of restriction sites, repetitive sequences, and the unique physical landmarks called STSs, or sequence-tagged sites. The chromosome-16 contig map provides information at a resolution of about 10,000 base pairs and will prove useful in studying chromosomal structure and organization.

In view of the need to complete physical maps of other chromosomes both rapidly and efficiently, we are adopting a different approach in mapping a second chromosome, chromosome 5. The goal is to construct a lower-resolution map consisting of (1) a series of STSs spaced evenly across the chromosome; and (2) YAC contigs assembled and ordered along the chromosome on the basis of their STS content. The project is being carried out in collaboration with John Wasmuth of the University of California at Irvine.

Our starting strategy utilizes the Los Alamos technologies for constructing chromosome-specific libraries to rapidly build a map covering 60 percent of the chromosome. The first step is to create a "framework" map of STS markers spaced at intervals of 0.5 to 1 million bases along chromosome 5. Given the statistics associated with generating STS markers at random and the fact that chromosome 5 is 194 million bases long, we will have to generate at least 400 STS markers to produce an STS map with a resolution of 1 million base pairs. We are developing the STS markers from a chromosome 5-specific library of M13 clones constructed at Los Alamos specifically for this purpose. Generating an STS involves sequencing a short cloned fragment of genomic DNA and identifying unique primer pairs from that sequence, which, when used in the polymerase chain reaction (PCR), will amplify a unique site in the genome. (See "The Polymerase Chain Reaction and Sequence-tagged Sites.")

Wasmuth is localizing the position of each STS to one of the intervals along human chromosome 5 defined by a panel of 30 hamster/human hybrid cells each containing various portions of chromosome 5. This localization is accomplished by determining through PCR screening which hybrid cells contain the STS and which do not. This method allows regional localization at a resolution of between 5 and 10 million base pairs. Plans are being made to refine the localization to a resolution of 200,000 base pairs using radiation-hybrid mapping. This mapping technique is analogous to genetic-linkage mapping in that distances are measured by how often two markers on the same chromosome become separated from one another. In linkage studies the separation is due to crossing over during meiosis, and the frequency of crossing over, the so-called genetic distance, is not necessarily proportional to the physical distance. In radiation-hybrid mapping the separation occurs through radiation-induced chromosome breakage, and the frequency of the radiation-induced breakage between two markers is linearly proportional to the physical distance separating the markers. Moreover, the technique is readily applied to any unique markers, in particular, to STSs.

Once generated and regionally localized on the chromosome, each STS will be "anchored," or located, on a non-chimeric YAC clone from a chromosome 5-specific YAC library, which has been constructed at Los Alamos. The cloning technique used to construct non-chimeric clones from flow-sorted chromosomes is discussed in "Libraries from Flow-sorted Chromosomes."



The non-chimeric YACs, localized along chromosome 5 by their STS content, will provide a solid base on which to build YAC contigs covering the chromosome. At Los Alamos, we will concentrate on mapping the short arm of chromosome 5 (52 million base pairs). Special emphasis will be placed on the region of chromosome 5 involved in the Cri du chat syndrome, one of the most common terminal-deletion syndromes in humans.

The figure (above) illustrates our early work on STS generation and regional localization. The upper portion shows the regional localization along chromosome 5 of eight STSs generated from our chromosome 5-specific M13 library. The regional localization (indicated with bars) will be reduced to intervals of 5 to 10 million bases once all available hybrid cells are screened for the presence of each STS.

The photograph in the lower portion of the figure shows the results of testing for the existence and uniqueness of each STS. The three gel lanes for each STS show the PCR products generated from total-genomic human DNA (right lane), chromosome-5 DNA (middle lane), and total-genomic hamster DNA (left lane) using the primer pairs that operationally define each STS. The PCR products from the three reactions were separated in parallel in a 3 percent agarose gel and stained with ethidium bromide to visualize the DNA. In all cases a single PCR amplification product of the same size resulted from the total-genomic human DNA and the chromosome-5 DNA. The hamster DNA served as a control to ensure that a positive signal from the chromosome-5 DNA did not represent a spurious signal arising from hamster DNA. In all cases, the hamster DNA yielded no PCR product. The test also shows that human/hamster hybrid cells can be screened for an STS without concern that false positive signals will arise from the hamster DNA in the hybrid cell. The PCR results demonstrate the existence of each STS as a unique landmark on chromosome 5 and the specificity of the PCR protocol defining each STS. The size of each STS is given at the bottom of the figure. ■