(region 2p23-p25, comprising 25 Mb); #87189 for 2P2 library (2p21-p23, 28 Mb); #87103 for 2P3 library (2p14-p16, 22 Mb); #87104 for 2P4 library (2p11-p13, 28 Mb); #77419 for 2Q1 library (2q35-q37, 28 Mb); #87308 for 2Q2 library (2q33-q35, 24 Mb); #87309 for 2Q3 library (2q31-q32, 26 Mb); #87310 for 2Q4 library (2q23-q24, 19 Mb); #87409 for 2Q5 library (2q21-q22, 23 Mb); #87410 for 2Q6 lbrary (2q11-q14, 31 Mb); and #87411 for 2CEN library (2p11.1-q11.1, 4 Mb). Details of these libraries have been described: Hum. Genet. 93, 557, 1994 (for 2P1 library); Cytogenet. Cell Genet. 68, 17, 1995 (for 2P2 library); Somat. Cell Mol. Genet. 20, 353, 1994 (for 2P3 library); Somat. Cell Mol. Genet. 20, 133, 1994 (for 2P4 library); Genomics 14, 769, 1992 (for 2Q1 library; Somat. Cell Mol. Genet. 21, 335, 1995 (for 2Q2, 2Q3 & 2Q4 libraries); Somat. Cell Mol. Genet. 22, 57, 1996 (for 2Q5, 2Q6 & 2CEN libraries).

Region-specific libraries and short insert microclones for chromosome 2 are particularly useful resources for its eventual sequencing because this chromosome is less exploited and detailed mapping information is lacking. We have also constructed 3 region-specific libraries for the entire chromosome 18 using similar methodologies, including 18P library (18p11.32-p11.1, 22 Mb); 18Q1 library (18q11.1-q12.3, 25 Mb); and 18Q2 library (18q21.1-q23, 34 Mb). Details of these libraries have been described (Somat. Cell Mol. Genet. 22, 191-199, 1996).

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*Identification and Mapping of DNA-Binding Proteins Along Genomic DNA by DNA-Protein Crosslinking

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In 1995-1996 we continued to map and identify nonhistone proteins binding at loci along the yeast chromosome. Using DNA-protein crosslinking in vivo, we detected two polypeptides that probably correspond to core subunits of yeast RNA-polymerase II in the coding region of the transketolase gene (TKL2). Several nonhistone proteins were detected that bind to the upstream region of TKL2 and to an intergenic spacer between calmodulin (CMD1) and mannosyl transferase (ALG1) genes. The apparent molecular weight of these proteins was estimated. We also developed a new method to synthesize strand-specific probes.

Using DNA-protein crosslinking in vitro, we found the amino acid residues of the Lac-repressor that interacts with DNA. Only Lys-33 crosslinks with the Lac-operator in the specific complex.

In addition to Lys-33, the N-terminal end of the protein also crosslinks in a nonspecific complex. Our results demonstrate that, in the presence of an inducer, the repressor's N-termini crosslink to the operator's outermost nucleotides. We suggest that binding of an inducer changes the orientation of the DNA-binding domain of the Lac repressor to the opposite of that found for the specific complex.

We plan to use a new method to increase resolution and thus identify amino acids and nucleotides that participate in DNA-protein recognition. The mechanisms of transcription regulation of some yeast genes will thus be further elucidated. Our approaches are based on DNA-protein crosslinking. Detailed analysis will be done for specific and nonspecific complexes, in the presence and absence of inducers. This will allow us to make some conclusions about possible conformational rearrangements in DNA-protein complexes during gene activation at the protein's DNA-binding domains.

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A PAC/BAC Data Resource for Sequencing Complex Regions of the Human Genome: A 2-Year Pilot Study

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While the complete sequencing the human genome at 99.99% accuracy is an immediate goal of the Human Genome Project, a serious technical deficiency remains the ability to rapidly and efficiently construct sequence ready maps as sequencing templates. This is particularly problematic in regions with unusual genome structure. An understanding of these troublesome regions prior to genome-wide sequencing will provide quality assurance as well as reliable sequencing strategies in these regions.