In the majority of neurogenetic disorders, including Huntington's disease (HD), no specific biochemical defect has yet been identified as the fundamental cause of the clinical symptoms. Consequently, there is no straightforward approach for employing recombinant DNA techniques to directly clone a gene encoding the unknown defective protein or a regulatory DNA sequence. However, DNA methods, "Positional Cloning Method", can be used to facilitate and improve indirect methods of identifying the defect by first determining its chromosomal location, and then using this information to isolate the causative gene itself.

Huntington's disease (HD) is a progressive neurodegenerative disorder that displays autosomal dominant inheritance with high penetrance. HD is characterized by apparently normal cerebral development, followed by premature neuronal cell death, with the striatum most prominently affected. Clinical symptoms include progressive motor disorder (usually chorea), psychological manifestations and intellectual deterioration. Symptoms typically appear in the fourth or fifth decade of life, after the genes may have already been transmitted to progeny. There is no known therapy to cure this devastating disease, or even slow the inexorable course. Recent studies have defined a 2.5 Mbp region of chromosome 4p16.3 in where HD gene is likely to be located.

In order to identify the defective gene, we started to explore the strategy of identification of expressed sequences (genes) in this region using regional chromosome DNA clones. Here, we described a direct screening of the cDNA libraries using the human chromosome 4p16 region specific single copy DNA clones. We have newly developed a computer-controlled laser chromosome dissection and Single Unique Primer PCR (SUP-PCR) procedures to generate the regional chromosome DNA clones from a single human chromosome.

The generation of single copy DNA clones from a specific chromosome region valuable in screening various cDNA libraries for isolating expressed sequences from the region. For successful isolation of genes underlying genetic diseases, this approach is reliance on that cDNA clones isolated from relevant tissues can serve as candidate genes for an effective search for particular disease genes assigned to the specific chromosome region. We have taken this approach to attempt to isolate cDNA clones from HD region.
Laser chromosome dissection and SUP-PCR methods were applied to the distal half of the short arm of human chromosome 4 containing the Huntington's Disease locus (4p16.3) and resulted 4p16 DNA library consisting with approximately 1000-3000 non-overlapping sequences. Forty-five per cent of representative clones from the library identify single-copy DNA clones. After screening of the cDNA libraries from human striatum, caudate and fetal brain with one thousand single copy DNA clones (divided into twenty pools), 203 positive cDNA clones were isolated. Approximately sixty per cent of the positive clones were independent. So far, we have analyzed the positive cDNA clones, and identified 46 clones that were mapped on human chromosome 4. Three clones out of 46 cDNA clones were assigned as the candidate genes of Huntington's Disease. Both GeneBank and EMBL Data Bases established no significant similarities of the DNA sequences of these cDNAs to previously cloned primate genes, suggesting that these cDNAs encoded unknown proteins. The characterizations of these cDNAs are in progress. The isolation of regional chromosome specific cDNA clones described here appears to offer an additional powerful strategy to explore specific genes underlying neurogenetic disorders as well as inherited metabolic diseases.