

Primer on Molecular Genetics

screened for additional polymorphisms, improving the genetic map of the region and further restricting the possible gene location. In addition, DNA fragments from the region can be used as probes to search for DNA sequences that are expressed (transcribed to RNA) or conserved among individuals. Most genes will have such sequences. Then individual gene candidates must be examined. For example, a gene responsible for liver disease is likely to be expressed in the liver and less likely in other tissues or organs. This type of evidence can further limit the search. Finally, a suspected gene may need to be sequenced in both healthy and affected individuals. A consistent pattern of DNA variation when these two samples are compared will show that the gene of interest has very likely been found. The ultimate proof is to correct the suspected DNA alteration in a cell and show that the cell's behavior reverts to normal.

Fig. 13. Cloning a Disease Gene by Chromosome Walking. After a marker is linked to within 1 cM of a disease gene, chromosome walking can be used to clone the disease gene itself. A probe is first constructed from a genomic fragment identified from a library as being the closest linked marker to the gene. A restriction fragment isolated from the end of the clone near the disease locus is used to reprobe the genomic library to find an overlapping clone. This process is repeated several times to walk across the chromosome and reach the flanking marker on the other side of the disease-gene locus. (Source: see Fig. 11.)

