
A number of strategies can be used to reconstruct the original order of the DNA fragments in the genome. Many approaches make use of the ability of single strands of DNA and/or RNA to hybridize—to form double-stranded segments by hydrogen bonding between complementary bases. The extent of sequence homology between the two strands can be inferred from the length of the double-stranded segment. Fingerprinting uses restriction map data to determine which fragments have a specific sequence (fingerprint) in common and therefore overlap. Another approach uses linking clones as probes for hybridization to chromosomal DNA cut with the same restriction enzyme.

Restriction Enzymes: Microscopic Scalpels

Isolated from various bacteria, restriction enzymes recognize short DNA sequences and cut the DNA molecules at those specific sites. (A natural biological function of these enzymes is to protect bacteria by attacking viral and other foreign DNA.) Some restriction enzymes (rare-cutters) cut the DNA very infrequently, generating a small number of very large fragments (several thousand to a million bp). Most enzymes cut DNA more frequently, thus generating a large number of small fragments (less than a hundred to more than a thousand bp).

On average, restriction enzymes with

- 4-base recognition sites will yield pieces 256 bases long,
- 6-base recognition sites will yield pieces 4000 bases long, and
- 8-base recognition sites will yield pieces 64,000 bases long.

Since hundreds of different restriction enzymes have been characterized, DNA can be cut into many different small fragments.

Separating Chromosomes

Flow sorting

Pioneered at LANL, flow sorting employs flow cytometry to separate, according to size, chromosomes isolated from cells during cell division when they are condensed and stable. As the chromosomes flow singly past a laser beam, they are differentiated by analyzing the amount of DNA present, and individual chromosomes are directed to specific collection tubes.

Somatic cell hybridization

In somatic cell hybridization, human cells and rodent tumor cells are fused (hybridized); over time, after the chromosomes mix, human chromosomes are preferentially lost from the hybrid cell until only one or a few remain. Those individual hybrid cells are then propagated and maintained as cell lines containing specific human chromosomes. Improvements to this technique have generated a number of hybrid cell lines, each with a specific single human chromosome.