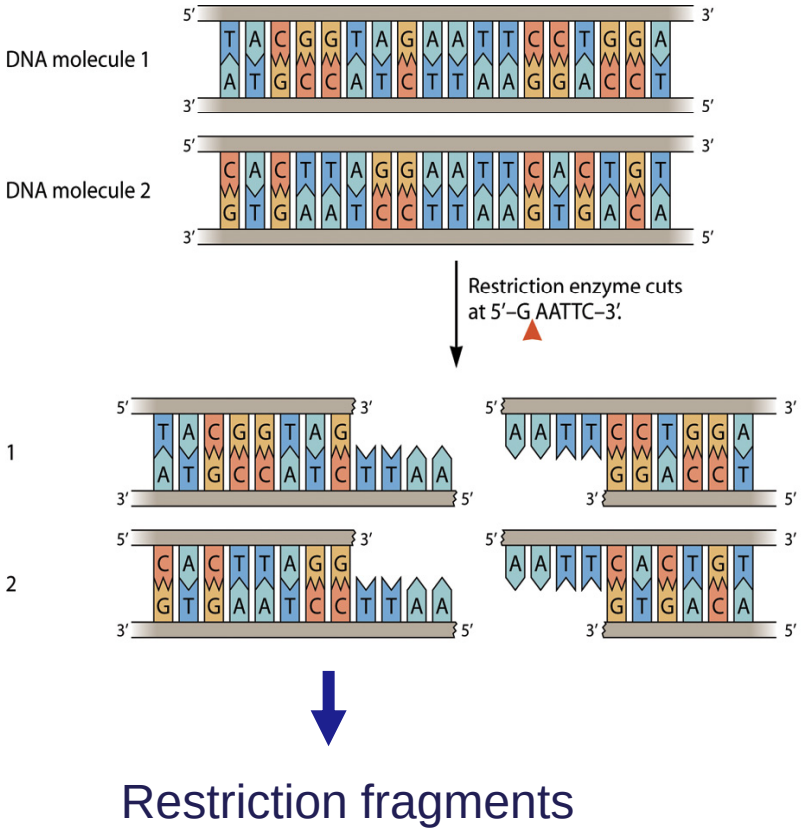
Chapter 15

The Biotechnology Toolbox

Cutting and Pasting DNA

- Cutting DNA
  - Restriction endonuclease or restriction enzymes
  - Cellular protection mechanism for infected foreign DNA
  - Recognition and cutting specific sites of DNA
    - Recognition sites are usually palindromic
      - e.g. 5’-GAATTC-3’



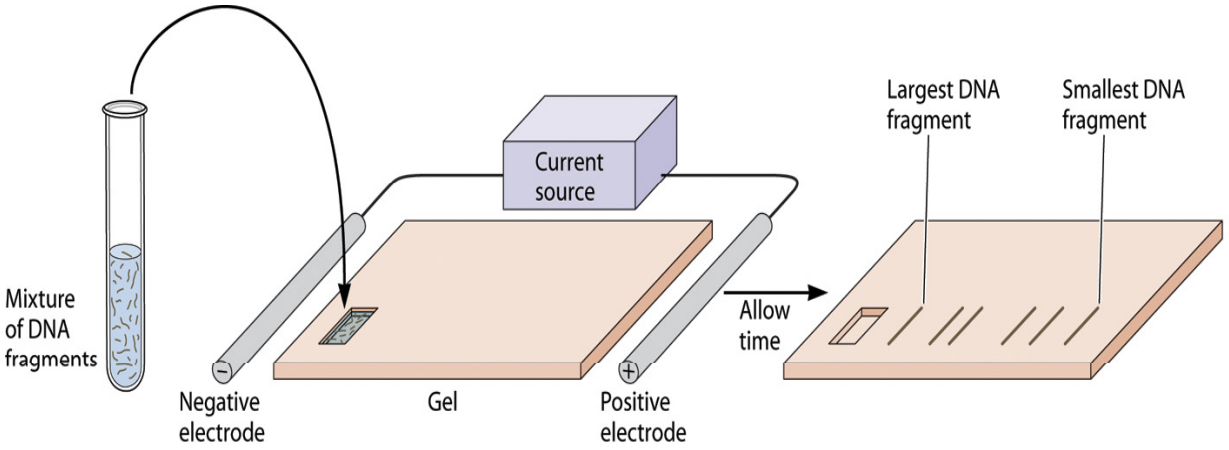
Recognition sites

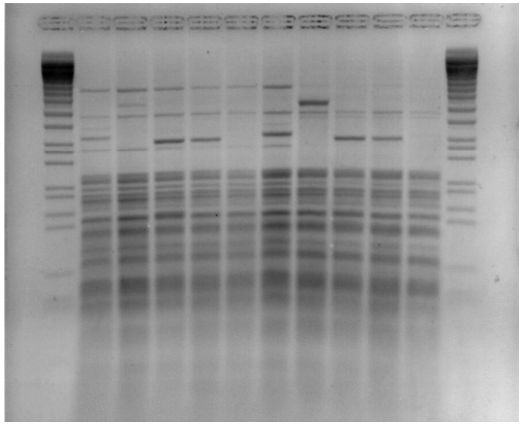
Table 4.1 Recognition sequences of some restriction endonucleases

Enzyme	Recognition site	Type of cut end
*Eco*RI	G↓A—A—T—T—C C—T—T—A—A↑G	5′-phosphate extension
*Bam*HI	G↓G—A—T—C—C C—C—T—A—G↑G	5′-phosphate extension
*Pst*I	C—T—G—C—A↓G G↑A—C—G—T—C	3′-hydroxyl extension
*Sau*3AI	↓G—A—T—C C—T—A—G↑	5′-phosphate extension
*Pvu*II	C—A—G↓C—T—G G—T—C↑G—A—C	Blunt end
*Hpa*I	G—T—T↓A—A—C C—A—A↑T—T—G	Blunt end
*Hae*III	G—G↓C—C C—C↑G—G	Blunt end
*Not*I	G↓C—G—G—C—C—G—C C—G—C—C—G—G—C↑G	5′-phosphate extension

Separating mixtures of DNA fragments

- Electrophoresis
  - Gels
    - Agarose : broad range of resolution
    - Polyacrylamide : high resolution for smaller DNA
  - Migration of DNA to the positive electrode under the electric current
  - Separation of DNA molecules by molecular weight and shape
    - $L = k\ 1/\log_{10}MW$ for linear DNA
  - Staining of DNA for visualization (Ethidium bromide, EtBr)





Pasting DNA

- DNA ligase
  - Joins DNA by forming new phosphodiester bond
- Recombinant DNA
  - DNA generated by joining DNA pieces from different sources