R.E- Enzymes → Type I, Type II, III, IV

- Type-I → I Enzymes have different subunits → Recognition, cleavage, modification units
  - → It requires energy → ATP molecule
  - → requires cofactor → S-adenosyl methionine
  - → Methylation at 1 base pair ← modification
  - → Recognition → 1000 bp away from the methylation
  - → This are not used more.

- Type-II → 2 ~~different~~ enzymes → Cleavage, modification
  - → recognize palindromic sequence
  - → They need require ATP, cofactor.
  - → Sequences are symmetrical.
  - → widely used in genetic engineering.

- Type-III → same as type-1, 2 subunits → recognition, modification (DD) → same sequence; Cleavage
  - → Cleavage is done at 24-26 bp away from recognition site.

• Expression vector
- not only carry the foreign DNA also expresses
- have Regulatory elements — Prom, Ter.
- Ribosome binding site
- Ori site, marker gene, multiple cloning sik

eg: PET.

- Vector. extrachromosomal, self relplicating, autonomous recognition site, restriction enzymes, Ori site, marker gene, multiple cloning / cleavage site, Transformation, mobilisation, selection, cloning vector, Expression vector, gene of interest, self regulatory elements, promoter, terminator, ribosomal binding site, DNA, mRNA, protein synthesis, plasmid, cosmid, BAC, YAC, PET, Ampicillin resistant, antibiotic Tetracycline, Streptomycine, Host cell, E.coli, Ligase, express, flurescent gene, green flurescent protein, isolation & purification, 10kb.

PBR322 Vector

- first vector developed by Boliver & Rodriguez → 1977
  - P — Plasmid
  - B — Boliver
  - R — Rodriguez.
- molecular weight → 2.83×10^6 Daltons
  - have ori site
  - marker gene → ampicillin, tetracycline (AD) → recognition site → PstI, SalI, EcoRI, HindIII, EcoRV
- first artificial plasmid. (vector)
- Origin of replication in Plasmid PBR322 is known as PMB1