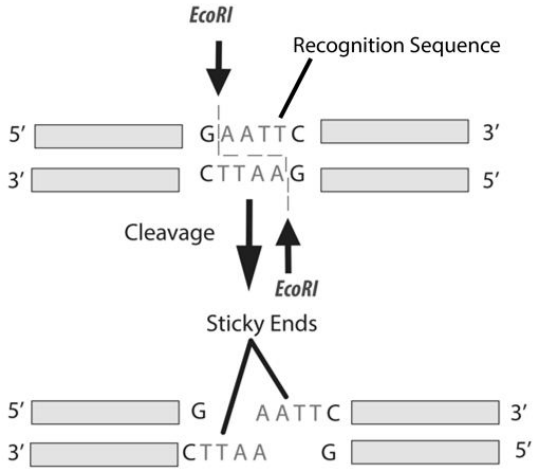
Restriction Digestion

Aim: Restriction digestion of plasmid DNA with *EcoRI* restriction enzyme.

Principle: Restriction enzymes, also referred to as restriction endonucleases, are enzymes which recognize short, specific (often palindromic) DNA sequences. They cleave double-stranded DNA (dsDNA) at specific sites within or adjacent to their recognition sequences. Most restriction enzymes (REs) will not cut DNA that is methylated on one or both strands of their recognition site, although some require substrate methylation. Each restriction enzyme has specific requirements to achieve optimal activity. Ideal storage and assay conditions favour the most activity and highest fidelity in a particular enzyme's function. Conditions such as temperature, pH, enzyme cofactor(s), salt composition and ionic strength affect enzyme activity and stability. One popular recombinant DNA tool is the EcoRI endonuclease, which cleaves DNA at GAATTC and it is isolated from strains of *E. Coli.*



Materials requirement:

1. Plasmid DNA
2. Restriction enzymes
3. Agarose and Buffers for Electrophoresis
4. Sterile nuclease free water, sterile tips.

Procedure:

Restriction digestion of Plasmid DNA

1. Make the reaction mix as follow:

Component	**Vol. (Micro litre)**
Restriction Enzyme , EcoRI (10,000 U/ml)	1
10X Digestion Buffer	2
Plasmid DNA	5
Molecular biology grade water	12
Total Volume of Reaction	20

2. After mixing the above components, give a short spin.
3. Incubate at 37 ^{O}C water bath for 1.5 hr to 2 hr.
4. Analyze the digested sample by agarose gel electrophoresis. (Load DNA ladder , undigested and digested sample)

Result and observation: