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**Lab 5**

**Enzymes**

**What are the enzymes used in recombinant DNA technology?**

DNA manipulative enzymes can be grouped into four broad classes, depending on the type of reaction that they catalyse:

**1-Nucleases**

Enzymes that attack and degrade DNA from the end known as exonucleases

enzymes that cut DNA at internal site called endonucleases

**2- DNA ligase**

Enzymes that join or stick two duplexes together

**3-DNA polymerases**

Enzymes that synthesize DNA, but also have exonucleae function too.

**4-DNA Modifying Enzymes**, remove or add chemical groups.

**Nucleases**

We will principally focus on endonucleases that cut specific DNA sequence

within DNA molecules.

Endonucleases used for cloning DNA are known as restriction enzymes. They

discovered for first time in the early of 1950, when it was shown that some

strains of bacteria are immune to bacteriophage infection, Restriction occurs

because the bacterium produces an enzyme that degrades the phage DNA

before it has time to replicate and direct synthesis of new phage particles. The

bacterium’s own DNA is protected from attack because it carries additional

methyl groups that block the degradative enzyme action.

**How is restriction enzyme naming ?**

A restriction enzyme is simply named by three italic letters that identify the

host bacterium, and then a capital letter and roman number to identify the

strain or serotype and the order of identification e.g. BamHI denotes a restriction enzymes from *Bacillus amyloliquefaciens* Strain or serotype H and was first identified in this Bacillus *sp.*HindIII *Haemophilus influenza*

**Types of enzymes:-**

1. Type I enzymes cut DNA at random far from their recognition

sequences. They are multifunctional protein with both restriction and

methylase activities

2. Type II restriction endonucleases cut DNA at specific nucleotide

sequences. Restriction and methylase activities are separate.

3. Type III enzymes cleave outside of their recognition sequences. They

cut DNA about 20-30 base pairs after the recognition site.They are

multifunctional protein with both restriction and methylase activities

4. Type IV enzymes target methylated DNA.

**Restriction enzymes mechanism**

Restriction enzymes recognise the sequence, attach and cut through the DNA

by catalysing the hydrolysis of the bond between adjacent nucleotides. These

enzymes cut specific sequence called recognition site and its palindromic

sequence.



**Blunt ends and sticky ends**

Many restriction endonucleases make a simple double-stranded cut in the

middle of the recognition sequence, resulting in **a blunt end or flush end.**

Other restriction endonucleases, the two DNA strands are not cut at exactly

the same position. Instead the cleavage is staggered, usually by two or four

nucleotides, so that the resulting DNA fragments have short single-stranded

overhangs at each end. These are called sticky or cohesive ends, as base

pairing between them can stick the DNA molecule back together again. One important feature of sticky end enzymes is that restriction endonucleases with different recognition sequences may produce the same sticky ends.





**DNA ligase**

These enzymes catalyse the final step in creating recombinant DNA molecule.

The main role of DNA ligases in the cells is in DNA replication and repair.

However, it is now used to generate recombinant DNA molecules.

Main ligase used in cloning is that of the viral T4 DNA ligase.

**Application of restriction enzymes**

1- They are used to assist insertion of genes into plasmid vectors during

gene cloning and protein expression experiments.

2- Restriction enzymes can also be used to distinguish gene alleles by

specifically recognizing single base changes in DNA known as Single

Nucleotide Polymorphisms (SNPs)

3- Map DNA molecules and many other applications

**Procedure:**

A typical restriction digestion reaction:

Up to 1 μg DNA (5-10 μL)

1 μL of Restriction Enzyme\*

2 μL 10x Buffer (1x final concentration)

1 μL 20x BSA\*\* (if recommended)

x μL dH2O (to bring total volume to 20μL)

Mix gently by pipetting.

Incubate tube at appropriate temperature (usually 37 °C and some

enzymes require a different temp.) for 1-2 hours. **Always** follow the

manufacturer’s instructions.

After that you can check the digestion of your plasmid by running on

agarose gel alongside uncut plasmid (control).

\* Always add restriction enzyme last thing.