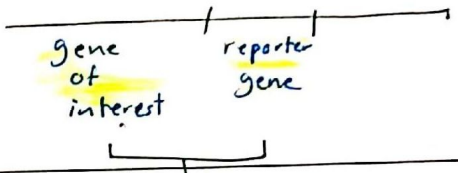
Reporter Gene Vector

- A gene that encodes a protein whose activity can be easily assayed in a cell in which it is not normally expressed
- These genes are linked to regulatory sequences whose function is being tested
- Changes in transcriptional activity from the regulatory sequences are detected by changes in the level of reporter gene expression



Shuttle Vectors

- Shuttle vectors can replicate in two different organisms, e.g. bacteria and yeast, or mammalian cells and bacteria.
- They have the appropriate origins of replication.
- Hence one can clone a gene in bacteria, maybe modify it or mutate it in bacteria, and test its function by introducing it into yeast or animal cells.

Cloning Strategy :-

- Strategy depends on the starting information and desired endpoint.
- Starting Information or Resources:
  - *Protein sequence*
  - *Positional cloning information*
  - *mRNA species / sequence*
  - *cDNA libraries*
  - *DNA sequence known or unknown*
  - *Genomic DNA libraries*
  - *PCR product*

* كل هاد مطلوب حتى أقدر أعمل recombinant DNA وأعمله expression داخل MO

Insertion of Genes Into Plasmids?

- To understand how genes are cloned, we need introduce three terms.
- Recombinant DNA- is mixed DNA
- Vector -it carries recombinant DNA into cells.
- Plasmids - are tiny circular pieces of DNA that are commonly found in bacteria.

Digestion of rDNA and Plasmid

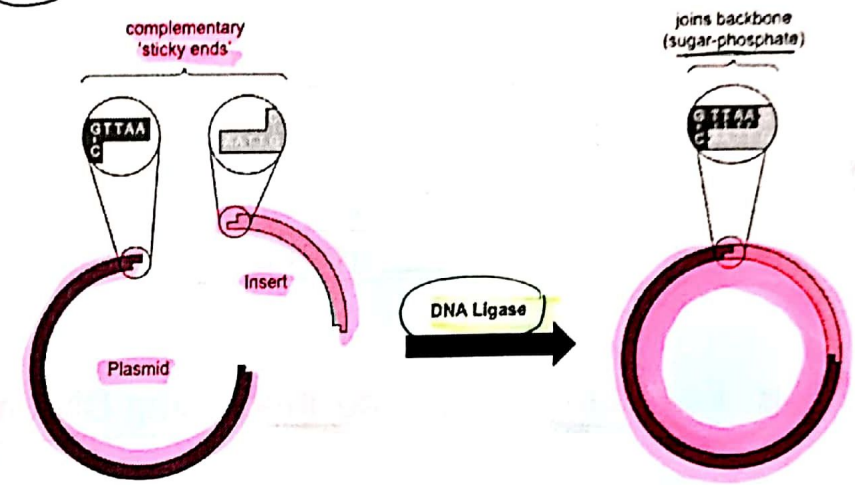
- both rDNA and Plasmid should be digested by the same enzymes
- Plasmid should be dephosphorylated by alkaline phosphatase to prevent self re-ligation
- Both digested plasmid and rDNA are run on the agarose gel and the bands are excised from the gel
- Each band will be purified using DNA purification kit

Alkaline Phosphatase

- Alkaline phosphatase removes 5' phosphate groups from DNA and RNA. It will also remove phosphates from nucleotides and proteins. These enzymes are most active at alkaline pH
- There are two primary uses for alkaline phosphatase in DNA manipulations:
  - *Removing 5' phosphates from plasmid and bacteriophage vectors that have been cut with a restriction enzyme. In subsequent ligation reactions, this treatment prevents self-ligation of the vector and thereby greatly facilitate ligation of other DNA fragments into the vector (e.g. subcloning).*
  - *Removing 5' phosphates from fragments of DNA prior to labeling with radioactive phosphate . Polynucleotide kinase is much more effective in phosphorylating DNA if the 5' phosphate has previously been removed*

Ligation of the rDNA to the plasmid

- Ligation id performed using DNA ligase catalyzes the formation of a phosphodiester bond between the 3'-OH at one end of a strand of DNA and th 5'-phosphate group of another.
- In animals and bacteriophage, ATP is used as the energy source for the ligatio while In bacteria, NAD^+ is used.



Factors affect ligation

- DNA concentration
- Ligase concentration
- Temperature
- Buffer composition

لازم يكون في تقارب بين تراكيزهم ...

optimum tem.

optimum pH