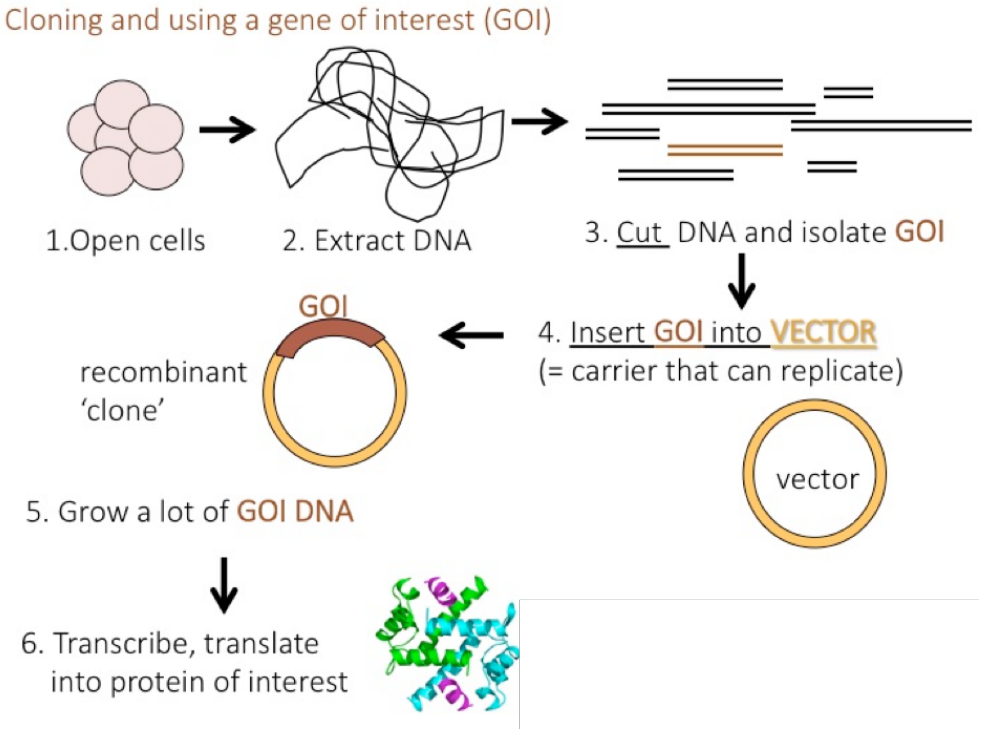
Vectors and ligation enzymes – Reference Summary

Cloning

Cloning is the process of isolating a gene of interest (GOI) and expressing it in an organism.

A **vector** is any carrier of DNA that can deliver that DNA, *i.e.* the GOI, to the cell and be replicated. One common example of a vector is a **plasmid**, which is a piece of small, circular DNA naturally present in bacteria.

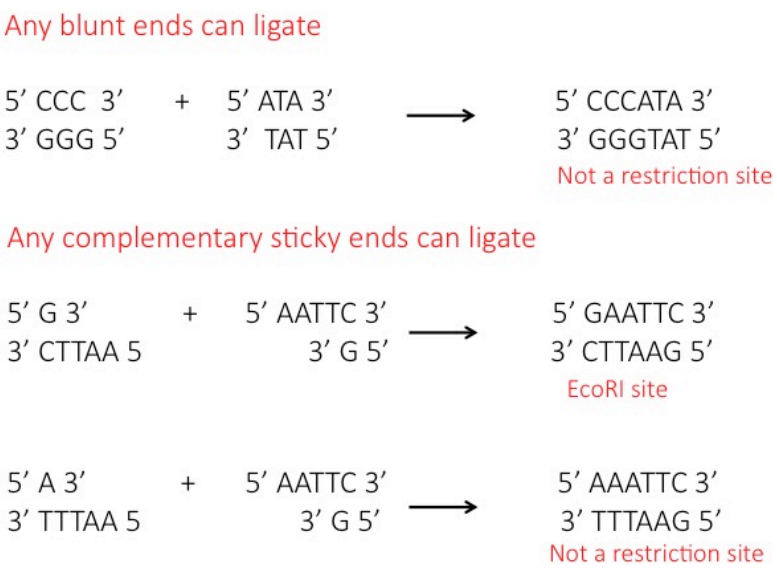
The cloning process is depicted in detail below.



Restriction Endonucleases: Compatible Ends

Restriction endonucleases (Res, see Lesson 17) are used to cut out and isolate the GOI (step 3 in the diagram above) and to cut the vector so that the GOI can be integrated into the vector (step 4 in the diagram above).
In order for the GOI to integrate into the vector, the two need to be cut in a way that gives them compatible ends. In this way, they can match up and ligate into a new circular plasmid without any breaks. There are specific enzymes that carry out recombinant DNA ligation to give a recombinant plasmid. These are called **DNA ligases**.

The different types of compatible ends are detailed below.



Note that the GOI and the vector do not need to be cut with the same enzyme to be compatible. However, if possible use the same RE to both cut out your GOI and the target restriction site on your vector.

In the case of blunt ends, all blunt ends can match with other blunt ends. In the case of sticky ends, only the overhanging base pairs need to match up.
However, ligating DNAs from two different restriction sites may prevent the ligation point from serving as a restriction site in the future because you alter the sequence on the other half of the restriction site (in the case of blunt ends) or around the overhanging base pairs (in the case of sticky ends).