Application Of Cloning Sites

- Let's say that we have a gene of interest, for example we need to administer a vaccine for Hepatitis B, for that we would need to clone antigen of Hepatitis B in order to amplify it so that it can be mass produced & can be administered to patients, so that patients can develop antibody against it.

How Does The Process Work?

1. We need a source from where we are obtaining the genetic material, in this case it is the Hepatitis B Virus (Alien DNA = DNA of the Source)
2. Vectors like bacteriophages, plasmids, retroviruses etc.. are used
    - why? if we directly introduce this DNA to a cell, 2 things will happen
        - It won't be able to take it & might degrade
        - speed of the process will be slow
    - we use weakened viruses as vectors & we put our gene of interest in these vectors
    - We need certain characteristics in these vectors
        1. Multiple sites of restriction enzymes (So that this plasmid (vector) can be identified by our restriction enzymes)
        2. This plasmid should not have multiple sites for the same enzyme, because then the restriction enzyme will cut the plasmid from multiple sites & our purpose won't work
        3. So, there should be sites for multiple restriction enzymes, but only 1 site for 1 restriction enzyme
3. We will use a restriction enzyme which can identify that particular gene in the source DNA & cut it, thus separating it from the source.
    - We use the same restriction enzyme to cut the vector as well, so that both these strands can be attached properly, complementary nucleotides are there.
4. Now we need to introduce the alien DNA in the vector, so that it can multiply, so how do we know that, the integration of alien DNA is completed, has it been successful or not?
5. Here comes the role of ori or selectable markers
    - There are now 3 scenarios possible :-
        1. Transformed Cell
            - Vector has entered this cell, whether the gene of interest has combined with the host DNA is not clear
            - i.e. the plasmid might not get integrated & join itself i.e. Non-Recombinant Cell

    - or the plasmid might get integrated & thus forming Recombinant Cell.
2. Recombinant Cell
    - Vector has entered this cell &
    - Gene of Interest has been combined with the Host DNA
3. Non-Transformed Cell
    - Vector has not entered this cell

Resistance Genes

- This plasmid has antibiotic resistance gene but the bacteria (host) does not have it, we can use this to our advantage.
    - Tetracycline Resistant Gene
        - The Gene of Interest we are adding to the plasmid is added to the "tetracycline resistant gene", & so, in recombinant cells, this gene gets impaired
        - So, bacteria is no more resistant to tetracycline, even if the plasmid gets integrated.
    - Ampicillin Resistant Gene
        - This is present in the plasmid

Results

- All the transformed cells will have ampicillin resistance
- All the non-transformed will not have ampicillin resistance
- This will differentiate transformed cells with the non-transformed cell
- Now, we will differentiate recombinant from non-recombinant cells
- Both will have ampicillin resistance gene because that is present in the plasmid
- But only the non-recombinant will have the resistance to tetracycline gene as the site for tetracycline is not impaired.