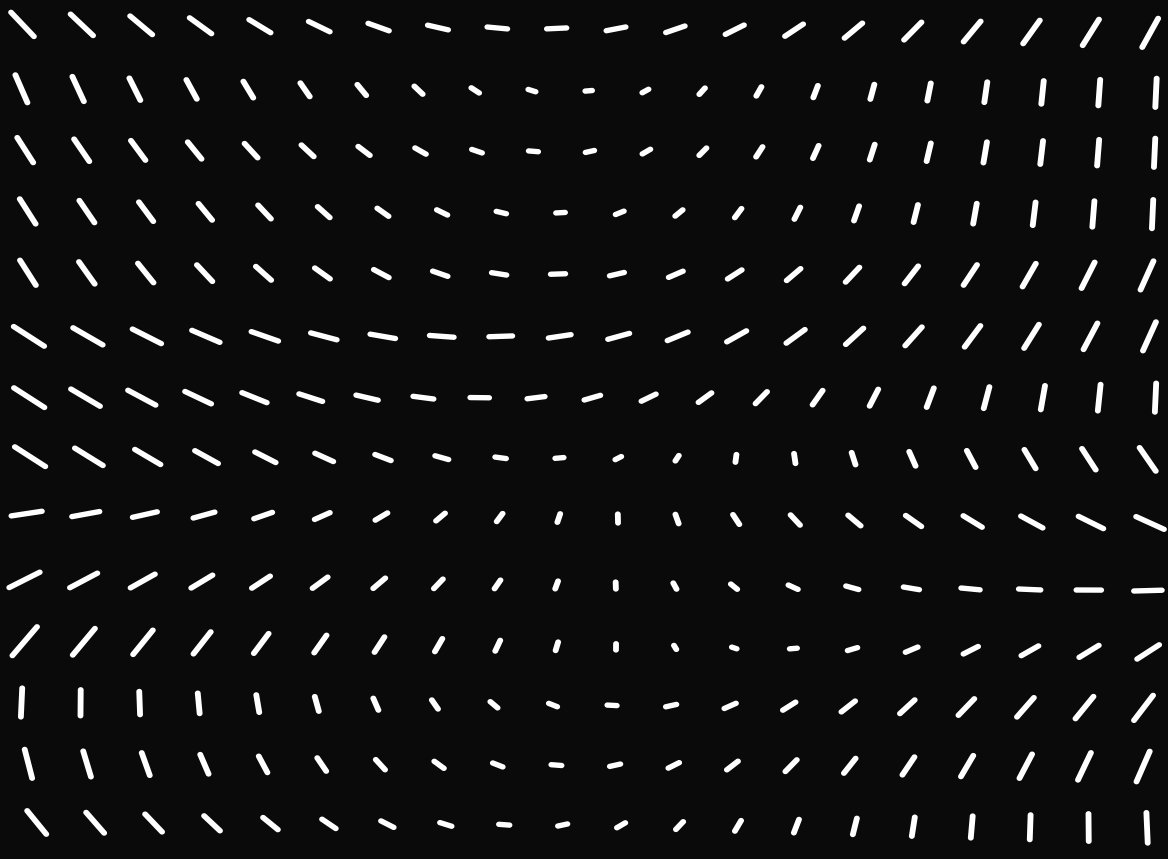
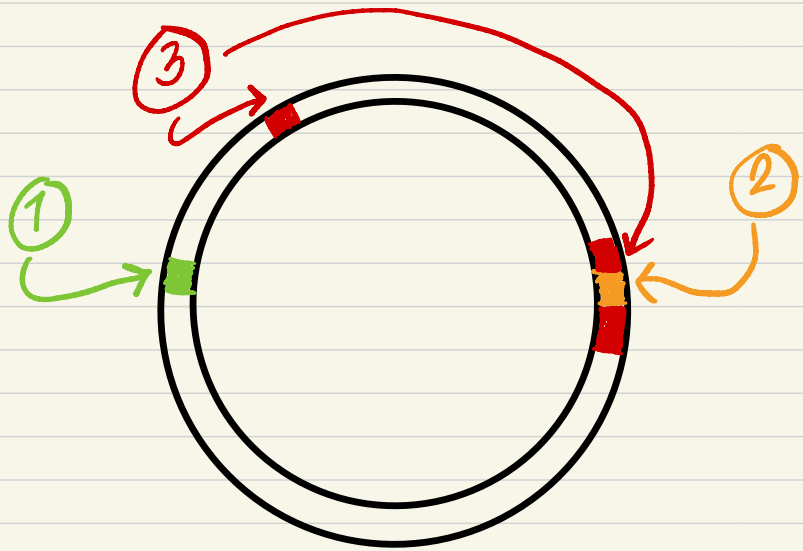
Pharmaceutical Biotechnology

- Zalib Muhammad Afnan



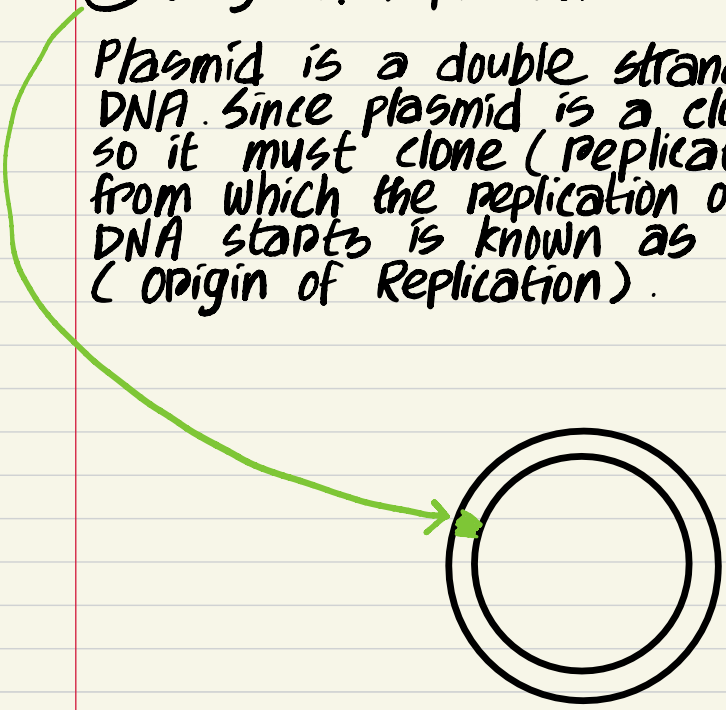
Plasmids:

A plasmid consists of three parts:



① Origin of Replication -

Plasmid is a double stranded circular DNA. Since plasmid is a cloning vector, so it must clone (replicate). The point from which the replication of plasmid DNA starts is known as ori (Origin of Replication).



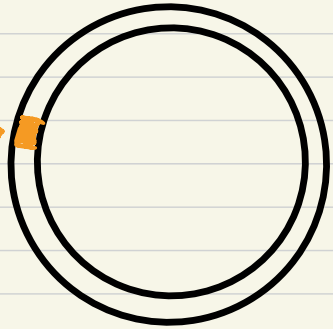
② Multiple Cloning site (also called polylinker)-

It is called polylinker because it is linking multiple restriction sites.

→ For definition, see L2.2

MCS contains recognition sequence for different restriction enzymes.
At the same time, MCS is able to produce any restriction enzyme.

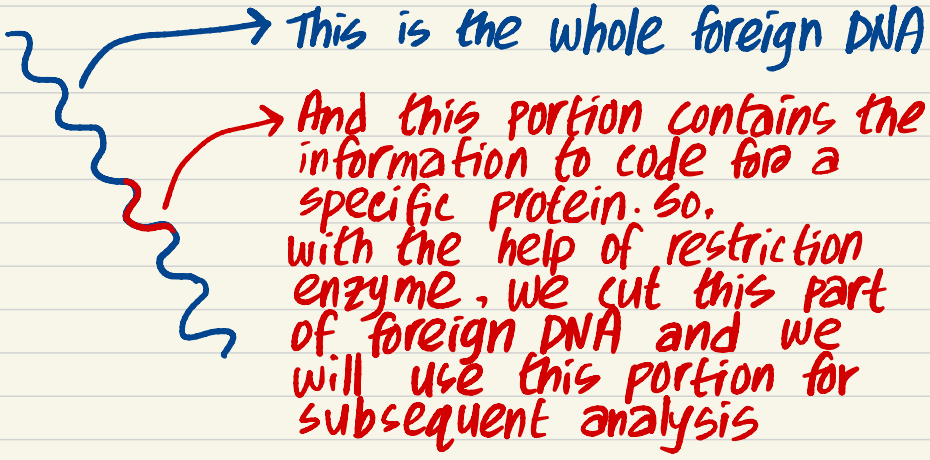
So, think about it -
MCS is producing restriction enzyme but since it also contains recognition sequence, hence, those restriction enzymes bind to the recognition sequence of MCS and cut the MCS (so that foreign DNA can be inserted here).



③ Two markers -

① lacZ gene -

Why do we need them ?
Consider the below scenario -



But now, there is a problem !

We have cut both foreign DNA & plasmid. But this foreign DNA & plasmid must join together. How can we ensure that both of them have joined together?

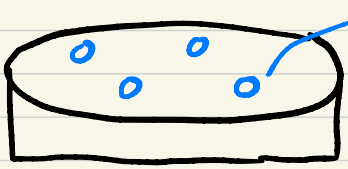
By using the lacZ gene!

lacZ gene expresses β-galactosidase enzyme.

lactose $\xrightarrow[\text{breaks}]{\beta\text{-galactosidase}}$ glucose + galactose

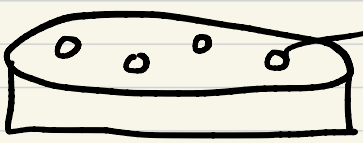
source of energy for bacteria

When bacteria (with intact lacZ gene) is incubated in X-gal media.
it will form blue colonies.
because intact lacZ gene can produce β-galactosidase enzyme which can hydrolyze X-gal and can form blue colonies.



Blue colonies!
So, X-gal hydrolyzed!
So, β-galactosidase present!
So, lacZ gene intact!
So, no foreign DNA in plasmid!

When bacteria (with cut lacZ gene) is incubated in X-gal media.
it will form white colonies.
because cut lacZ gene can not produce β-galactosidase enzyme. So. X-gal will not be hydrolyzed and we will not see blue colonies.
Rather, we will see white colonies.

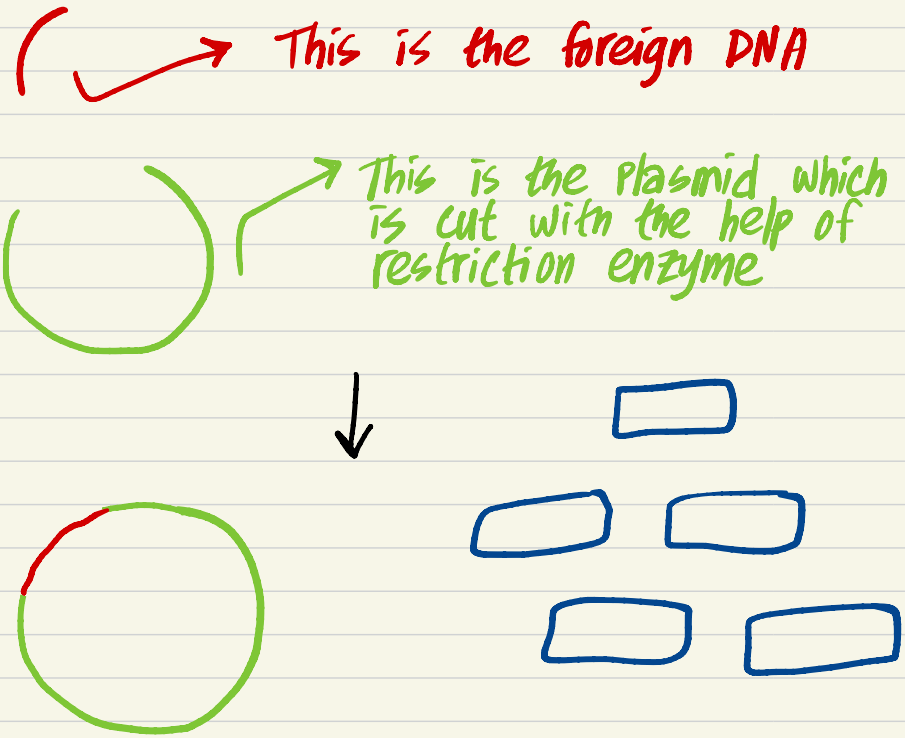


White colonies!
So. X-gal not hydrolyzed!
So. β-galactosidase absent!
So. lacZ gene cut!
So. foreign DNA in plasmid!

Hence,
the main reason for putting lacZ gene is to find out VISUALLY if the foreign DNA has been joined with the plasmid or not.

Remember.
Cutting foreign DNA/plasmid is one thing.
And ensuring that both of them have joined together is a complete different thing.
lacZ gene will ensure that foreign DNA has been successfully joined in plasmid.

(11) ampR gene -

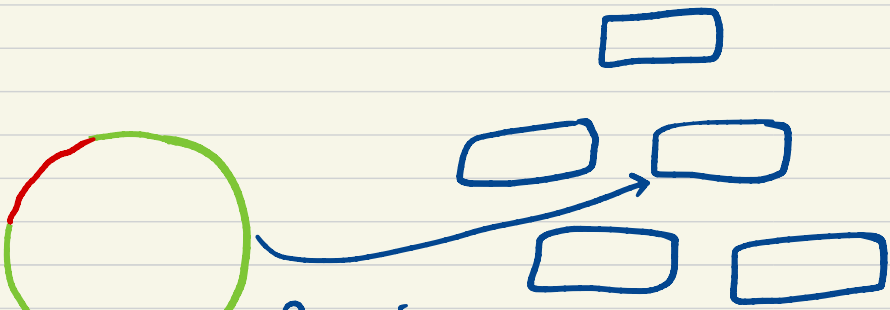


This is the plasmid carrying foreign DNA

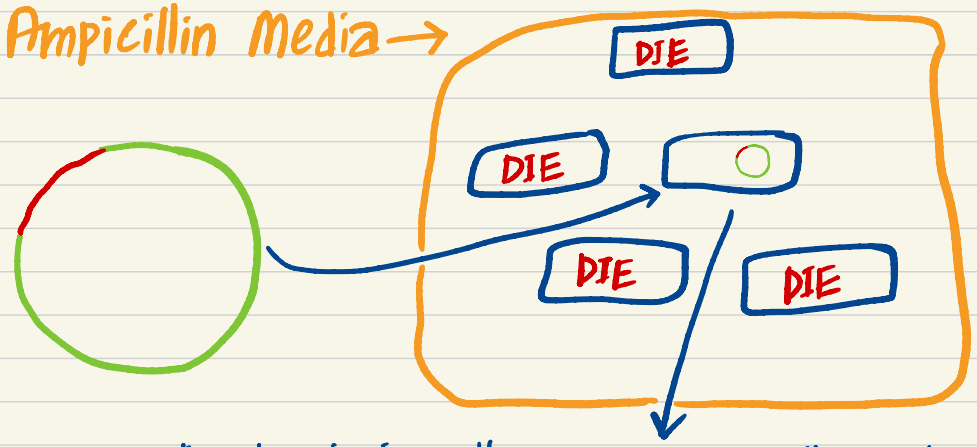
These are bacteria. Look carefully. What do you see? Is there any problem?

Yes, there is a problem!

There is only one plasmid and hundreds/ thousands/ millions/ even more bacteria present.
Only one bacteria will accept the plasmid. Others won't.
We must isolate that bacteria & culture only that bacteria.
After all, what's the point of culturing those bacteria which don't have the recombinant plasmid in it?
Hence, we must use a marker in our plasmid which is ampR gene.
This gene ensures the growth of bacteria in ampicillin media.



Imagine.
plasmid is entering in that bacteria.



This bacteria will grow in ampicillin media.

→ Because it contains ampR gene.

→ So, plasmid entered this bacteria.

→ So, we can isolate this bacteria & discard others.

That's why we need two markers -

(I) lac Z gene - To ensure that rDNA is present inside the vector

(II) ampR gene - To ensure that vector is present inside the host cell