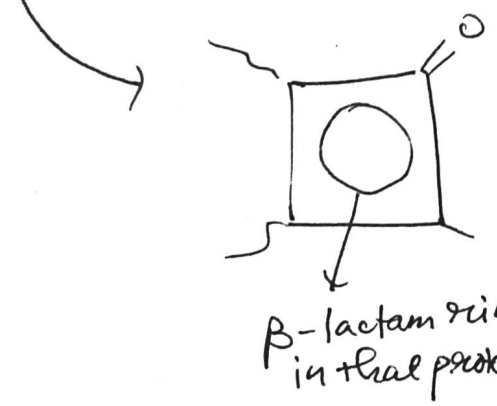
Biolab → Transformation. 1st Feb 2024

The marker that we are using amp – ampicillin.
It is a PET plasmid.

~~Above~~ Now we add ~~to~~ 1 mL LB then incubate at 37°C for 50 min. Why?
It produces the product that the plasmid codes for during this time, gaining resistance.
How does penicillin work?
It prevents cell wall biosynthesis. How does it do that? The protein that links sugar and amino acids. ~~binds to penicillin~~ to produce peptidoglycan binds to penicillin.
Ampicillin works akin to this.

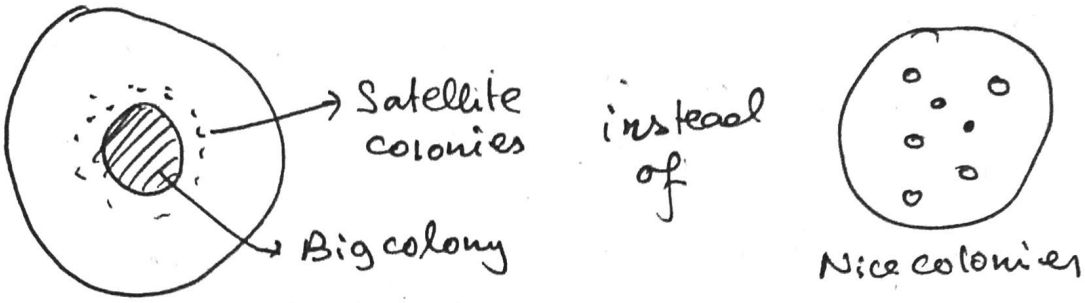


In the plasmid, There is ORF that codes for β-lactamase, which cuts the ring and makes it not bind to ampicillin
We add 5×10^8 cells in competent phase, 25 ng of plasmid. (5KB)
1 bp is 600 Dalton.
(I am not going to do calculation)
~~5Kb → 3 × 10^6 Dalton.~~

$\Rightarrow 5 \times 10^9$ Plasmids.

This is in excess. compared to number of of cells.

If we come late to see petridish, we will see,



Because other non-transformed bacteria are alive just unable to grow. when time passes, the gens leak out and allow satellites to grow.

We use 100 μg/mL ampicillin as it is above lethal dose.