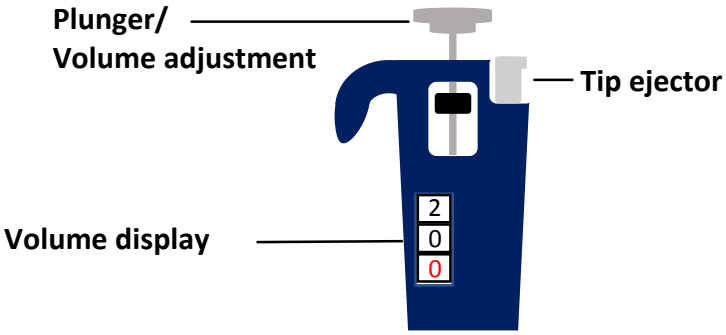
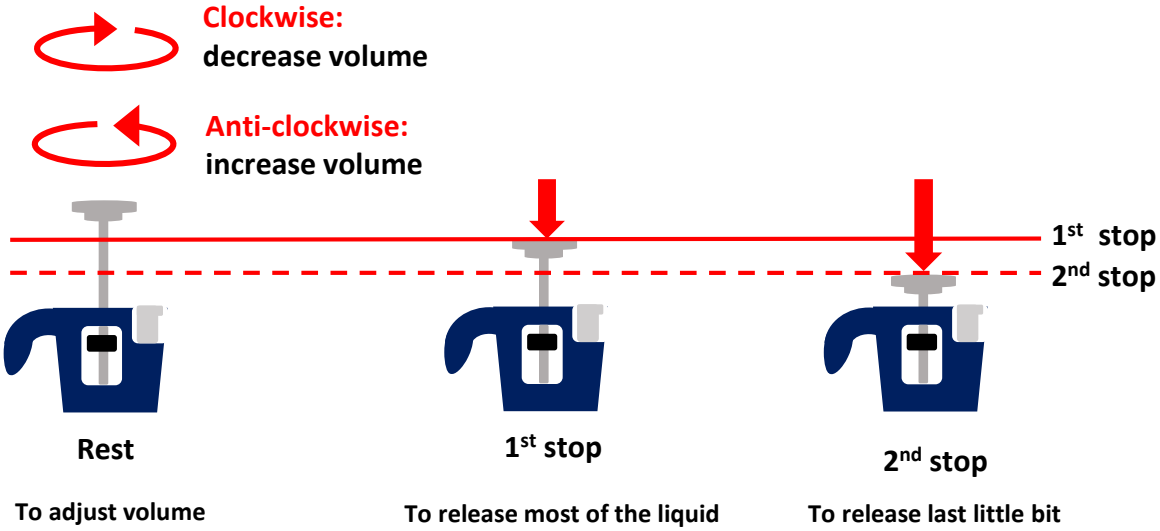
How to use a micropipette?

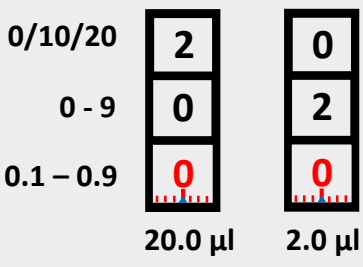




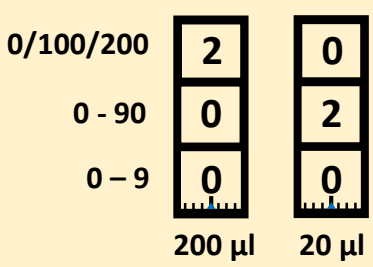
Do NOT over turn the plunger!

① Set Volume

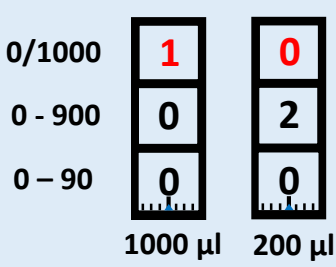
P 20 pipette = 2 – 20 µl



P 200 pipette = 20 – 200 µl



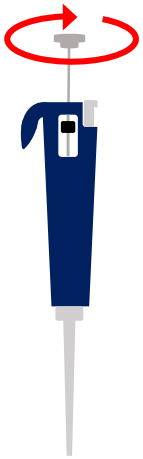
P 1000 pipette = 200 – 1000 µl



Never set volume above or below the limits!

② Step of pipetting liquid

a. Set volume



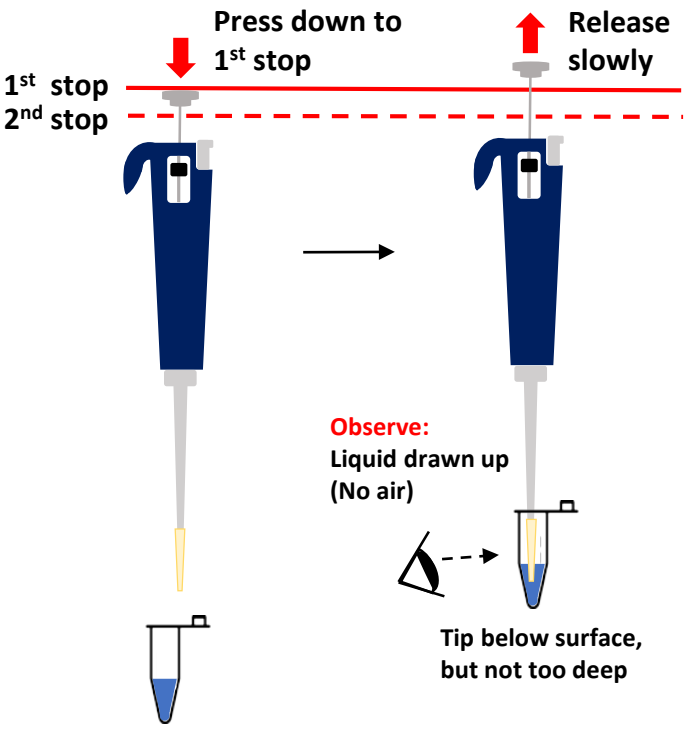
b. Insert pipette tip

Tip holder

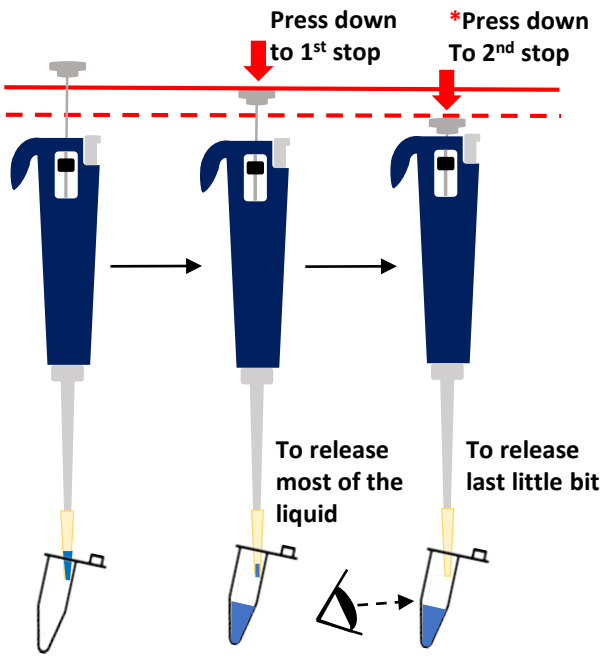
Never touch its tip with anything!

Push hard on the Pipette tip to make it tight

c. Suck up liquid

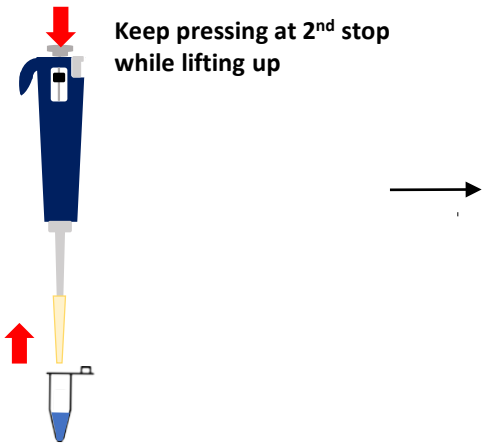


d. Release liquid



*When adding liquid in wells of the gel, press down to 1st stop only. Pressing to 2nd stop will eject air, making the liquid in the well spill out.

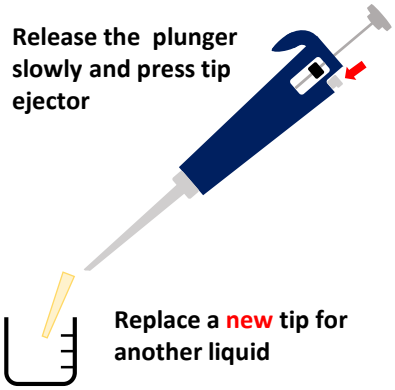
e. Lift up the tip



Keep pressing at 2nd stop while lifting up

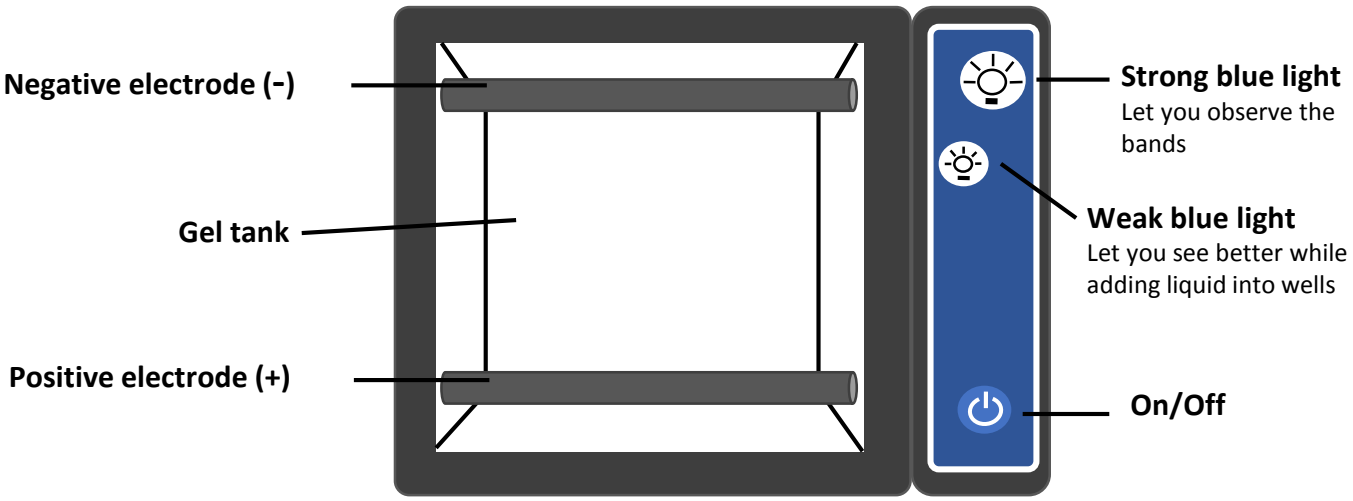
f. Eject the tip

Release the plunger slowly and press tip ejector



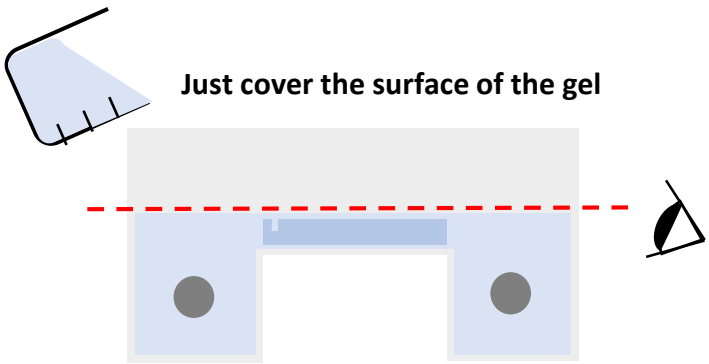
Replace a new tip for another liquid

How to do a gel electrophoresis?

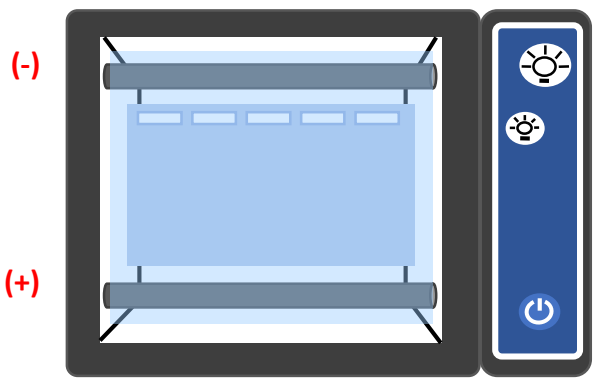


Gel System

a. Add buffer to the gel tank



b. Put the gel tank into the gel system



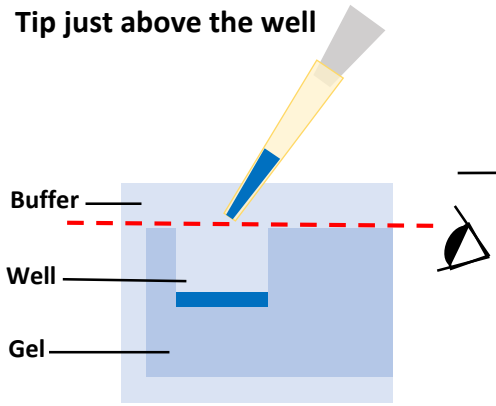
Make sure the wells are on the negative (-) side

How to do a gel electrophoresis?

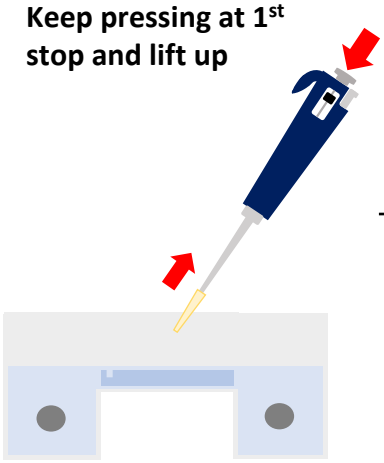
c. Add solution into the wells

Press to 1st Stop only

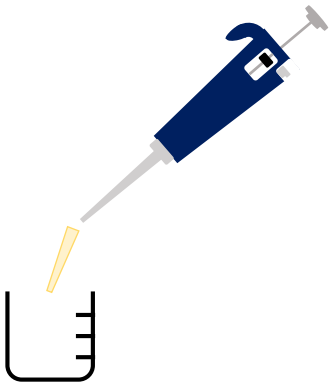
Tip just above the well



Keep pressing at 1st stop and lift up

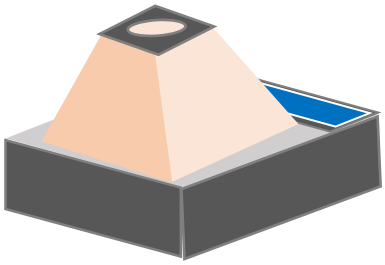


Add another solution with a new tip



Press weak blue light to make the wells easier to see

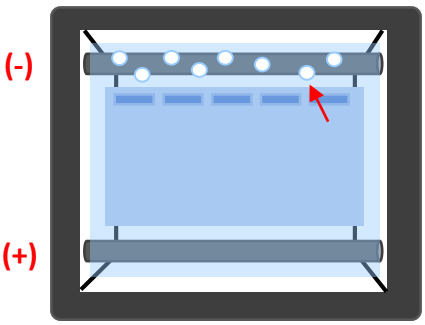
d. Turn on power



Place the photo hood on the gel system

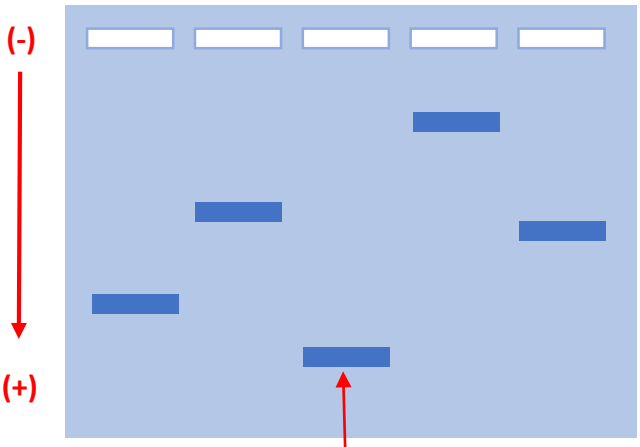
Press strong blue light to observe the bands

e. Start running the gel



Bubbles come out at negative (-) electrode

f. Observe bands appearing from (-) to (+) electrode



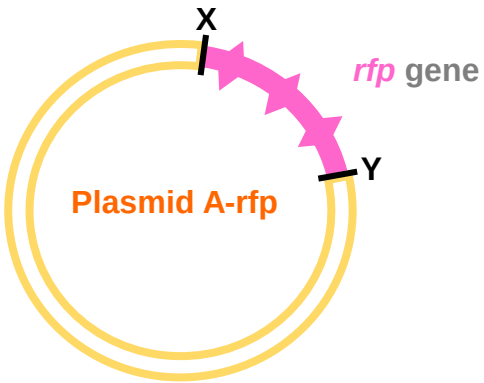
Larger (move slower)

Fragment Size

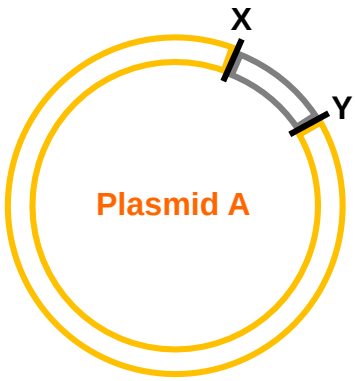
Smaller (move faster)

Bands show different sizes of DNA fragments

How to check if a plasmid has the *rfp* gene?



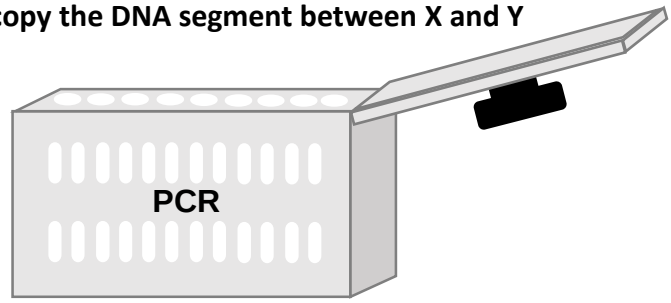
Plasmid with *rfp* gene



Plasmid without *rfp* gene

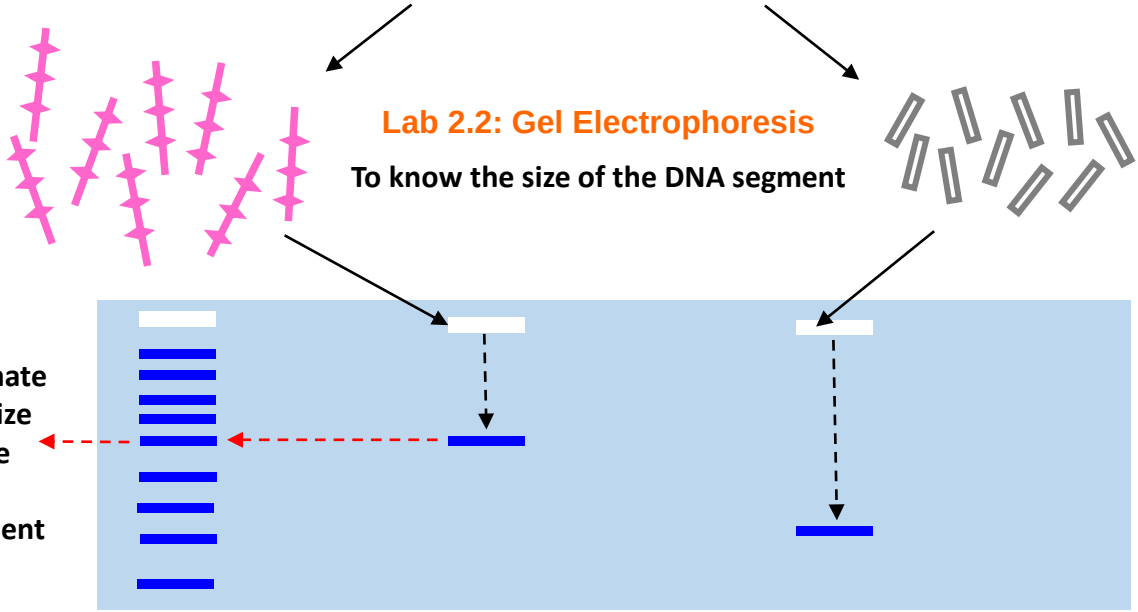
Lab 2.1: Polymerase Chain Reaction (PCR)

To copy the DNA segment between X and Y



Lab 2.2: Gel Electrophoresis

To know the size of the DNA segment



Estimate the size of the DNA segment

DNA Ladder

Many DNA segments in known size

Move slower

Larger DNA segment

Move faster

Smaller DNA segment

If the size is similar to the *rfp* gene, it is concluded that the *rfp* gene is in plasmid A

How to make a plasmid with the *rfp* gene?

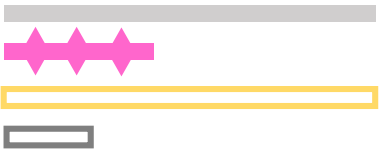
Source of *rfp* gene

Plasmid with DNA to express *rfp* gene
and make bacteria resistant to ampicillin.
So we know which bacteria have got the plasmid.

Lab 3.1: Restriction Digestion

To cut the plasmid at site X and Y

+ Restriction Enzymes



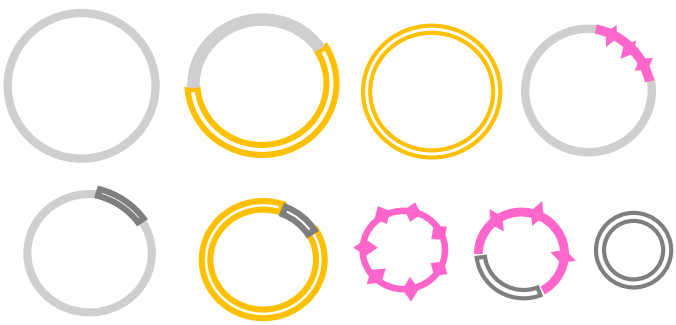
Lab 3.2: Ligation

To stick the *rfp* gene to plasmid A
between site X and Y

Mix the DNA fragments

+ DNA Ligase

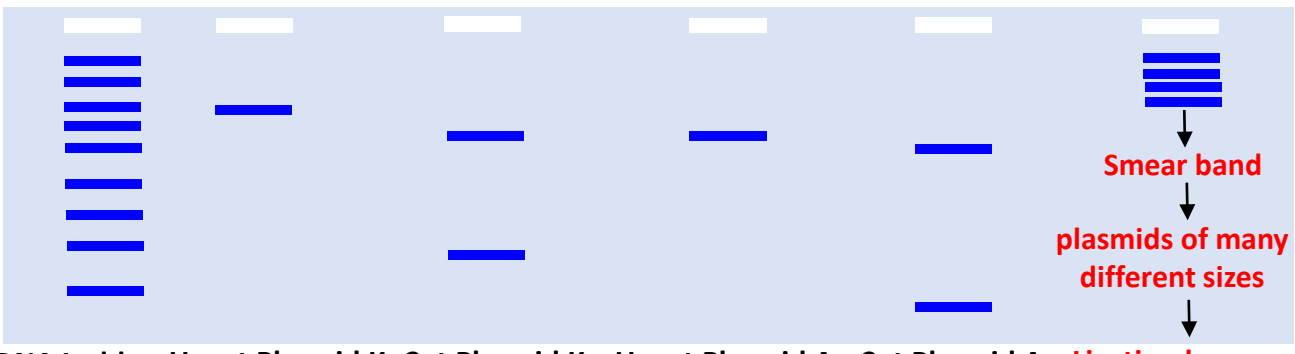
Target: Plasmid A with *rfp* gene



Other useless ligated plasmids

Lab 3.3: Gel Electrophoresis

To check if ligation occurs



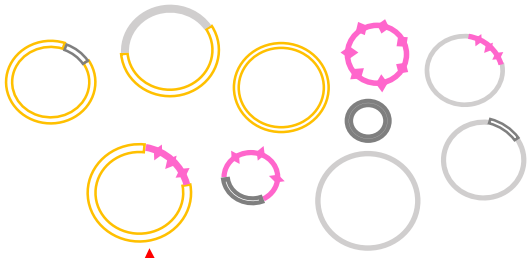
DNA Ladder | Uncut Plasmid K | Cut Plasmid K | Uncut Plasmid A | Cut Plasmid A | Ligation happens

Chapter 4 Creating Genetic Modified Bacteria

How to put the plasmid with *rfp* gene into a bacteria?

Plasmid A with *rfp* gene

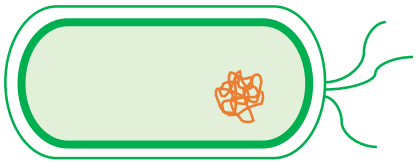
OR



Ligated plasmids
(include plasmid A with *rfp* gene)

Lab 4: Transformation

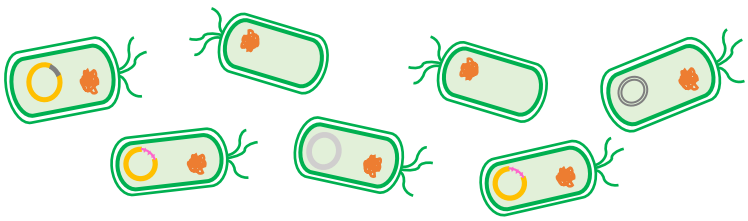
To put the plasmid A with *rfp* gene into bacteria



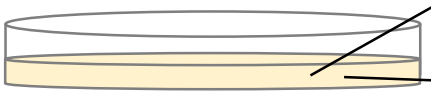
E. coli bacteria

Heat Shock

To open pores on the cell membrane so that plasmids can be taken into the bacteria.



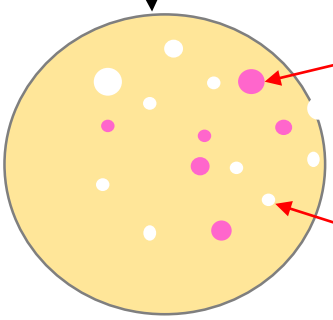
E. coli bacteria
(some take in plasmids, some do not)



Ampicillin
kill any bacteria without plasmid A that gives the bacteria resistance to ampicillin

Arabinose
Express the *rfp* gene and produce red fluorescent protein

Bacterial culture
grow individual bacteria into colonies



Red bacterial colony –
bacteria that have taken in plasmid A with *rfp gene*

White bacterial colony –
bacteria that have taken in plasmids with ampicillin resistance but no *rfp gene*