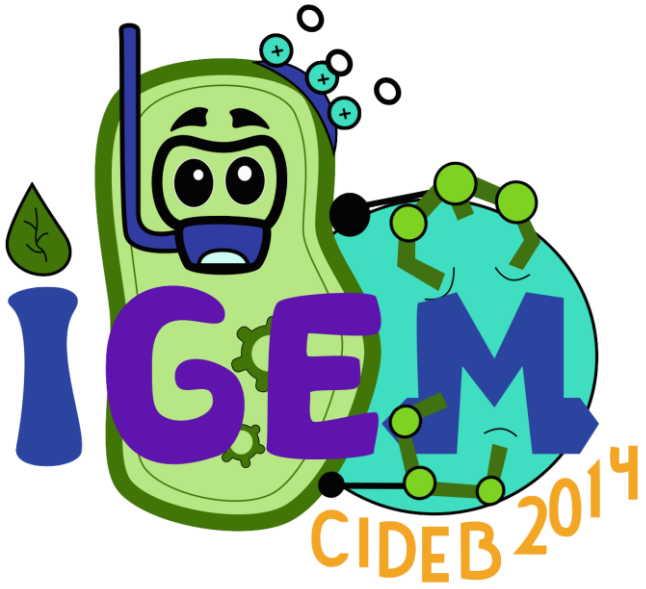
# Laboratory Protocols

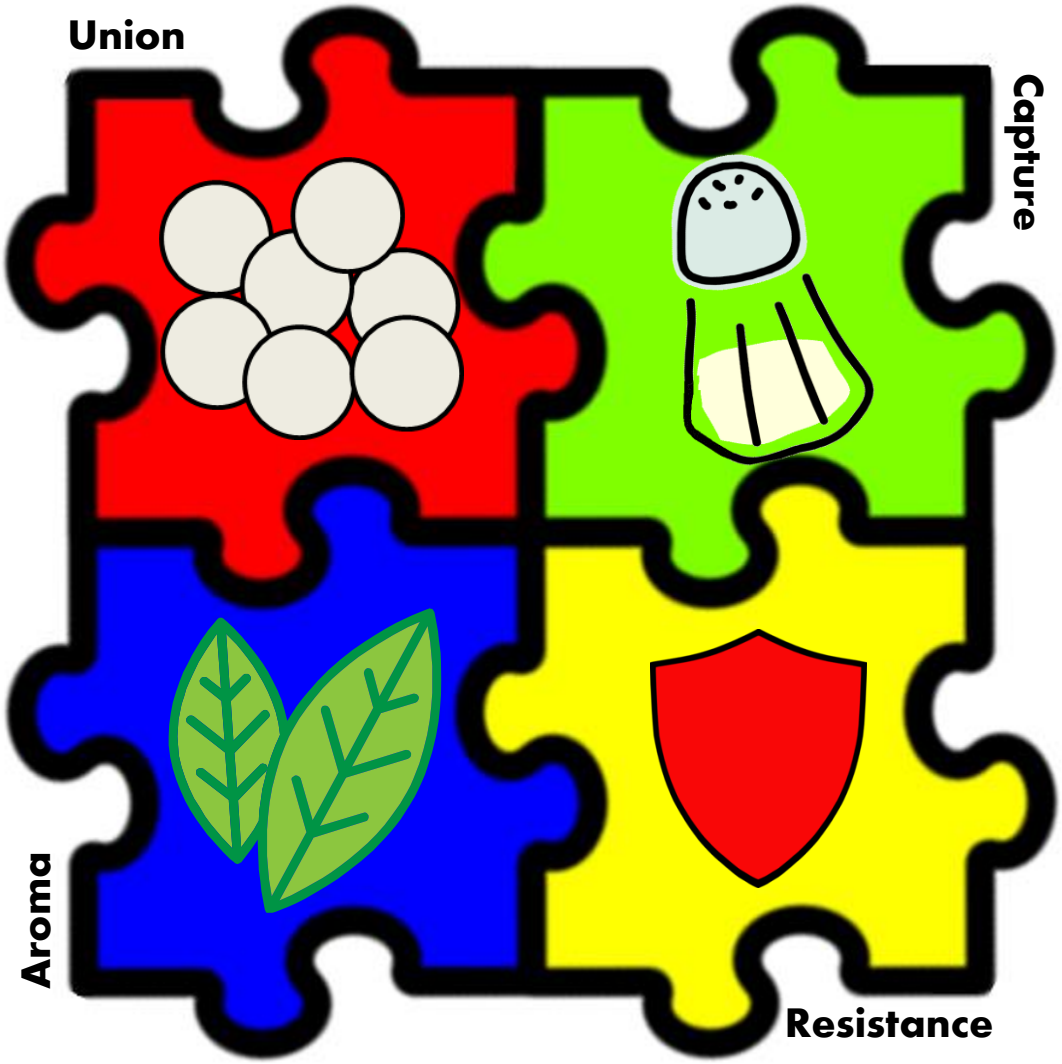
## Project's construction plan

iGEM CIDEB 2014



# E. CARU project

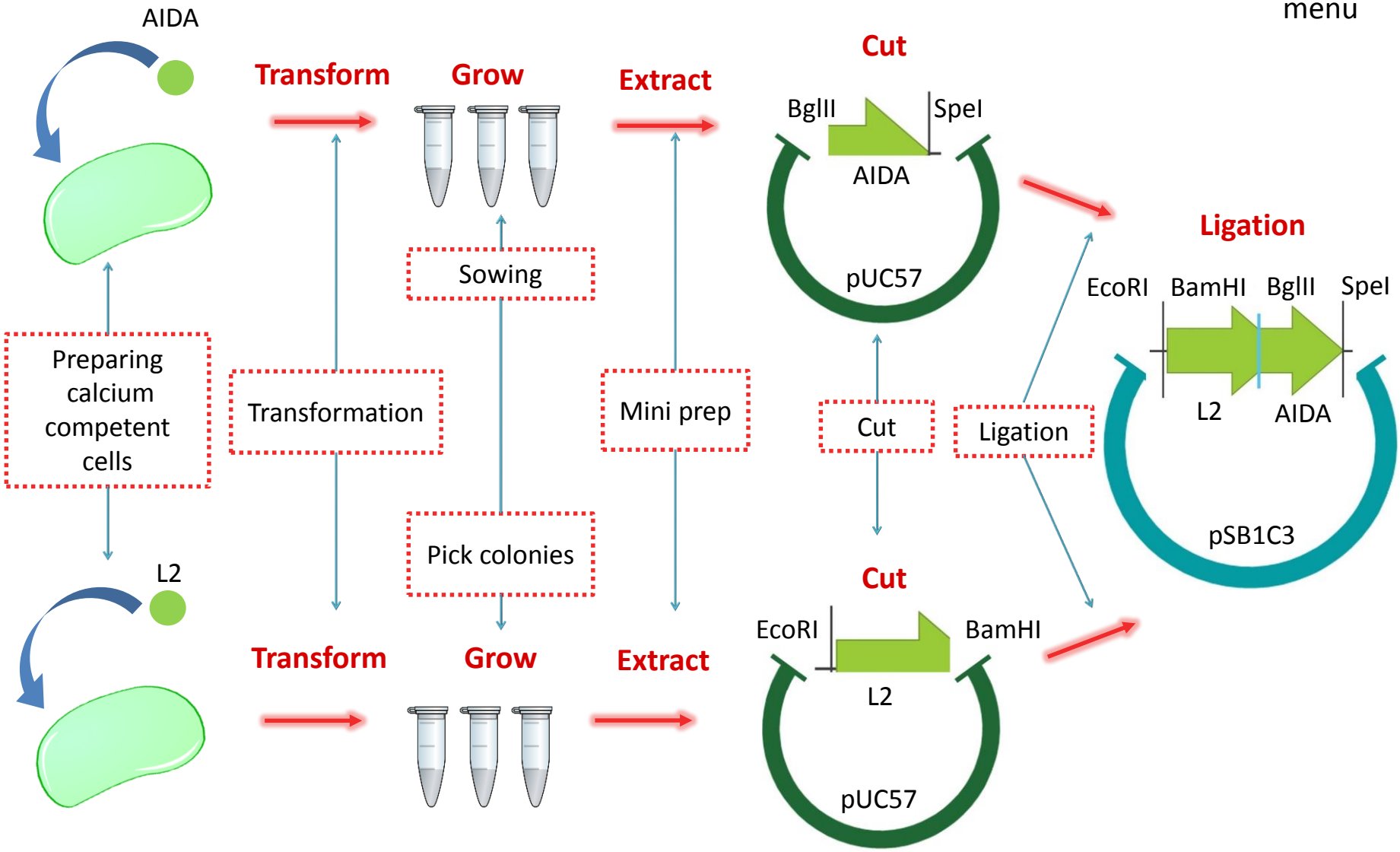
Click on each module



Click on each

# Union module

Return to menu



Click on each

Return to menu

# Capture module

Preparing calcium competent cells

NhaS

Transformation

Transform

Sowing

Pick colonies

Grow

Mini prep

Extract

Cut

Cut

EcoRI

PstI

NhaS RFP

pUC57

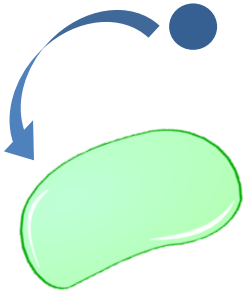
Ligation

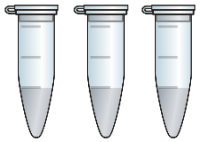
Ligation

pSB1C3

NhaS RFP

pSB1C3

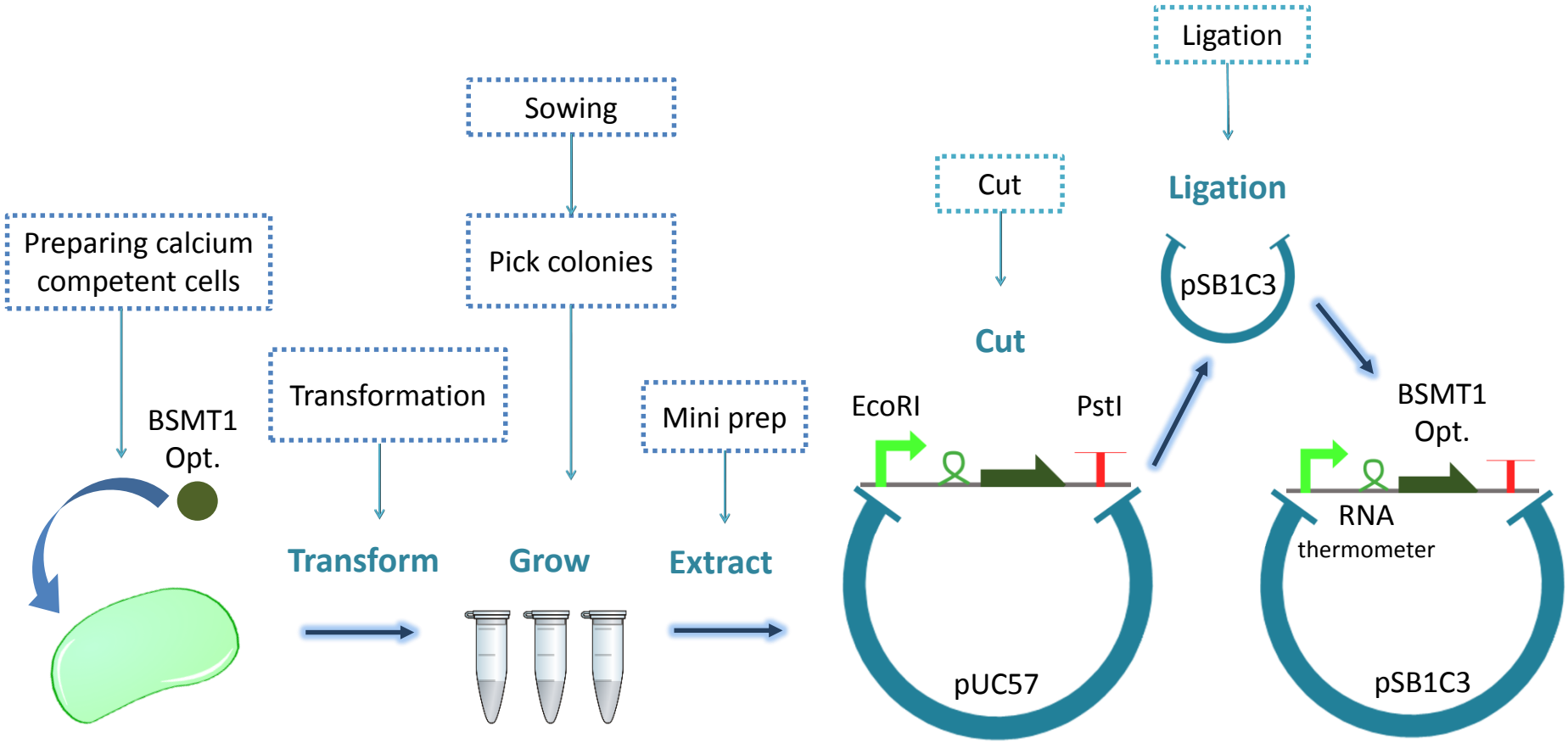




Click on each

Return to menu

# Aroma module



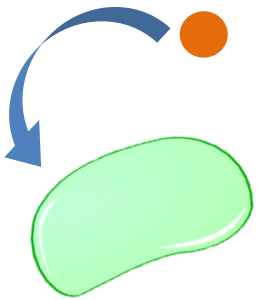
Click on each

Return to menu

# Resistance module

Preparing calcium competent cells

IRRE



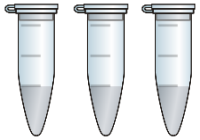
Transformation

Transform

Sowing

Pick colonies

Grow

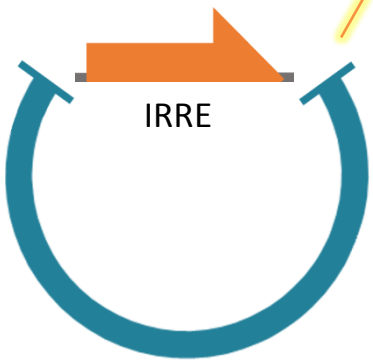


Mini prep

Extract

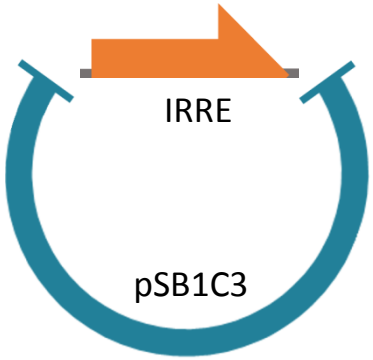
Ligation

Cut




Ligation


Cut

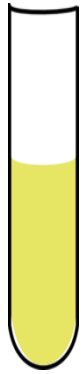


# Calcium Competent Cells Preparation

1

Inoculate a DH5-α cell colony in 3 mL of Luria Bertani (LB) broth with no antibiotic, incubate all night at 37°C with constant stirring (shaker).

Inoculate DH5- α

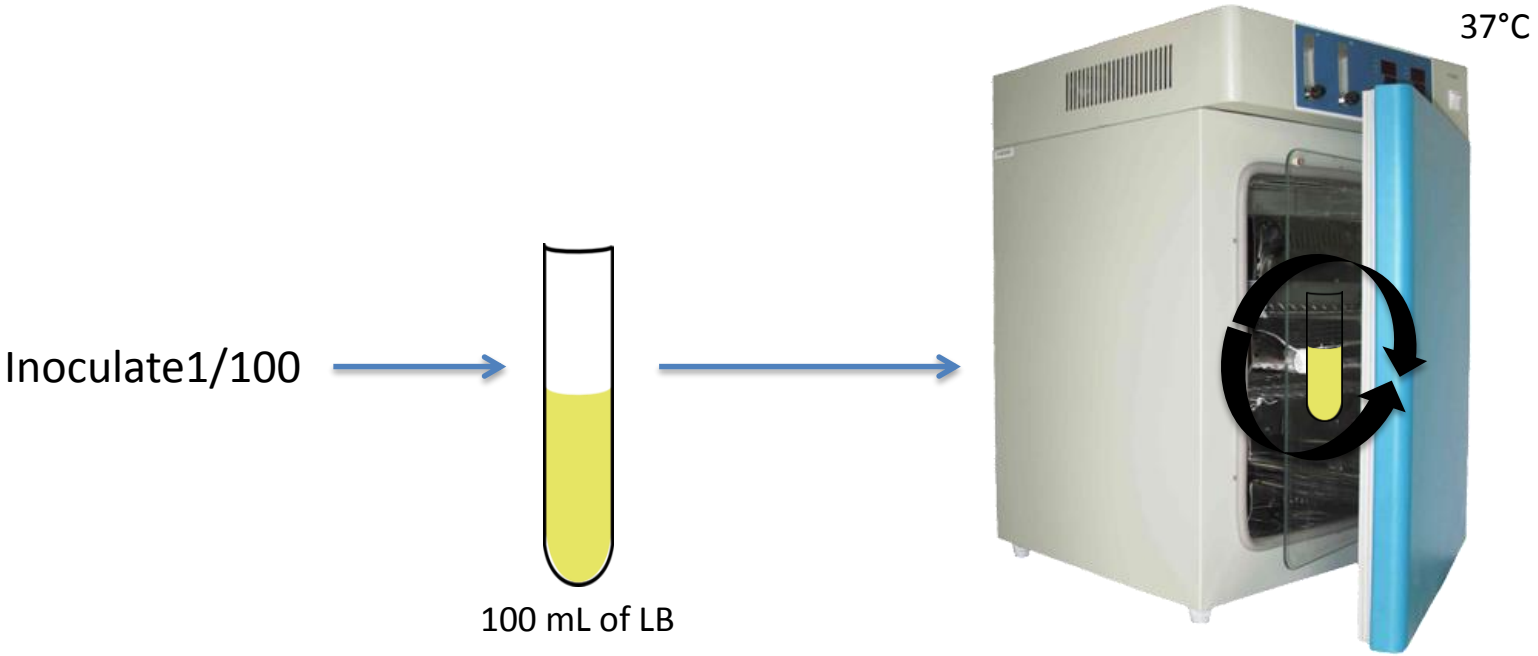


3 mL of LB

37°C

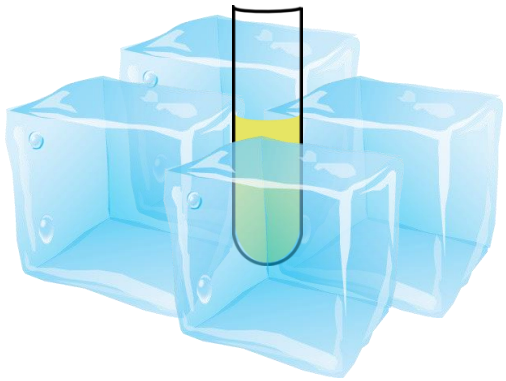
2

Inoculate 1/100 from the volume of these cells to 100 mL of LB broth, incubate at 37°C with constant stirring up to a $DO_{500}$ 0.375 (~$5x10^8$ cel/mL).



3

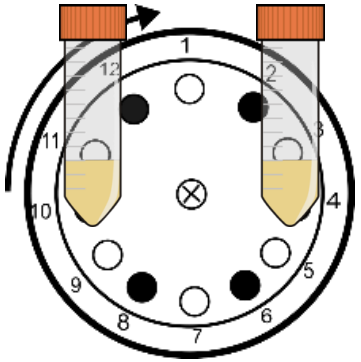
Cool the culture on ice for 5 minutes.



4

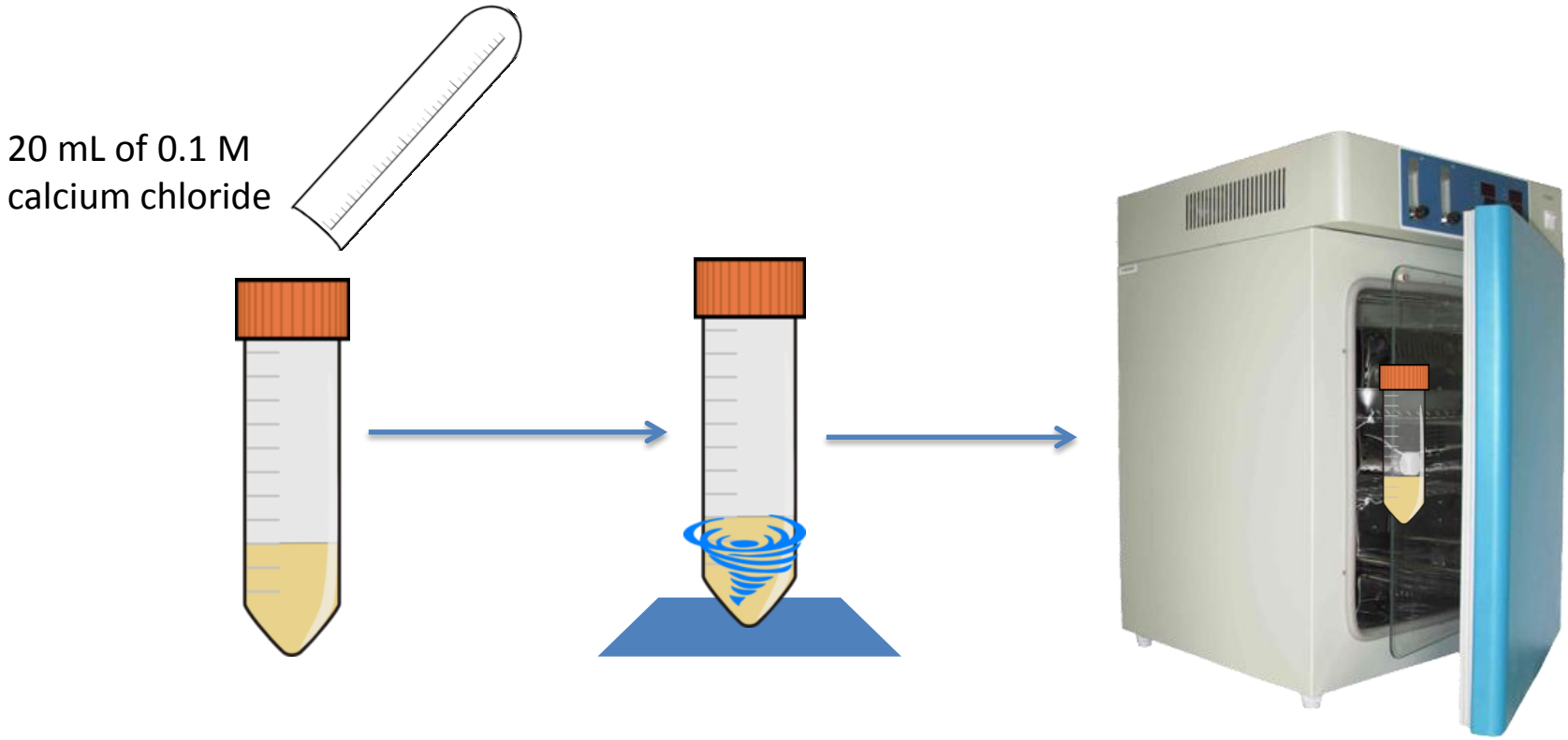
Spin-dry for 8 minutes at 1,700 x g 4°C in 2 Falcon tubes of 50 mL each.

1700 x g 4°C



5

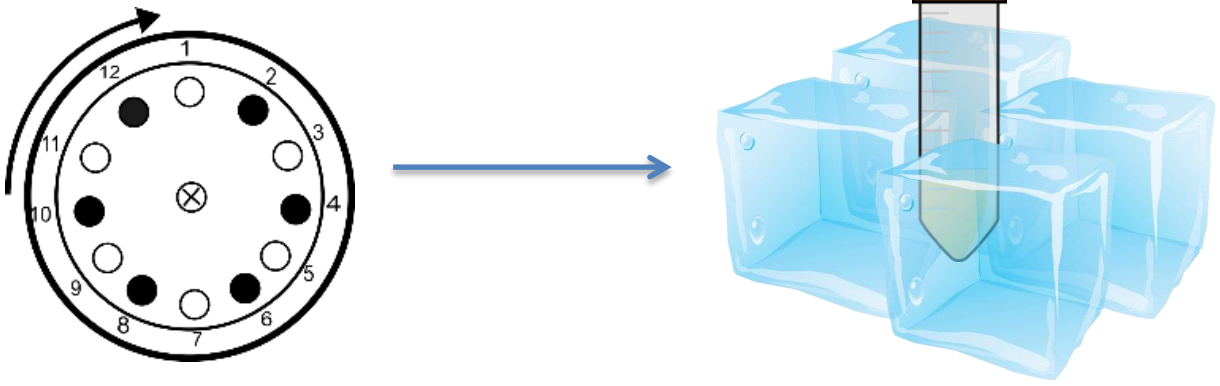
Resuspend by vortexing the pellet in 20 mL of 0.1 M calcium chloride cooled in ice and incubate at 4 ° C for 30 min.



6

Spin-dry 8 minutes at 1,700 x g 4°C and introduce in ice.

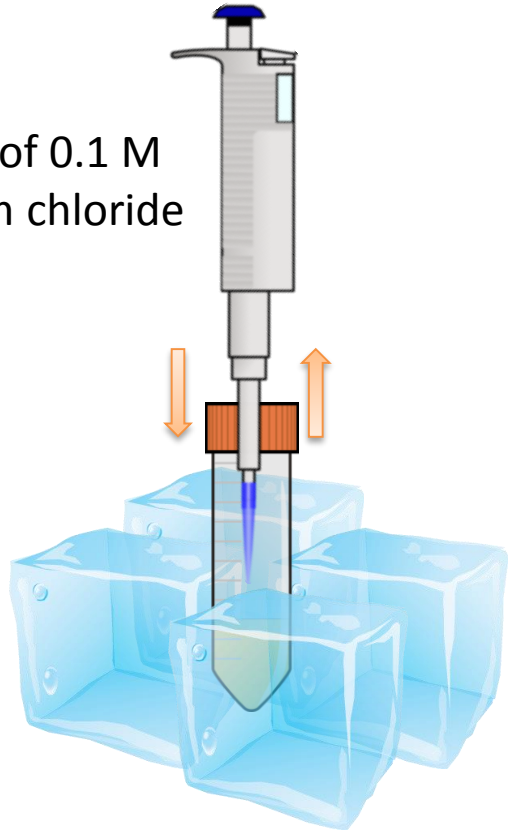
1,700 x g 4°C



7

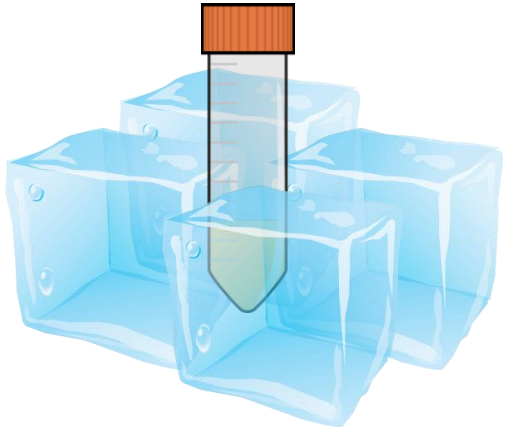
Re-suspend the pellet using a micropipette with 4 mL of 0.1 M calcium chloride cooled in ice. (Inside the ice).

4 mL of 0.1 M calcium chloride



8

Store on ice for one week until use.



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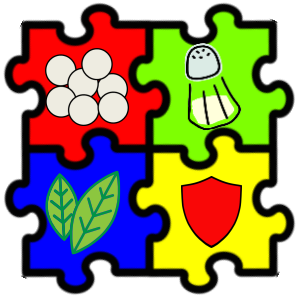
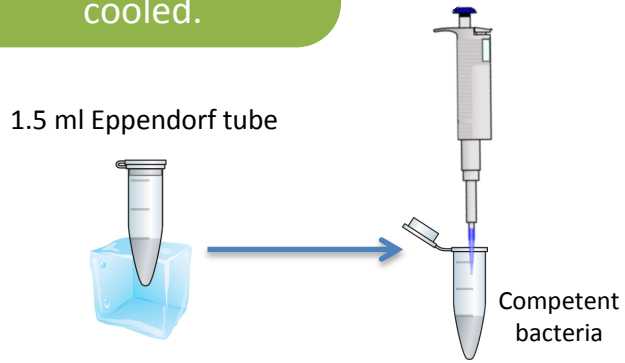


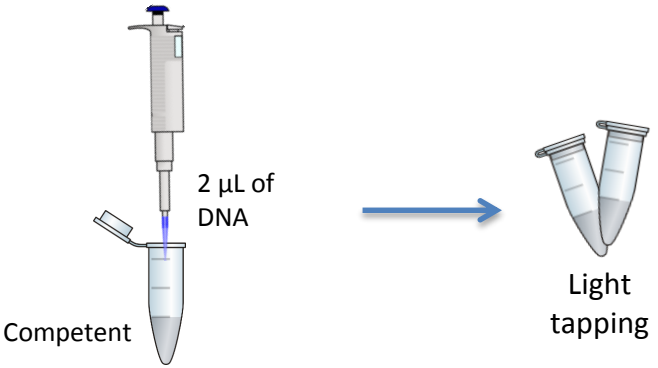
*It is important to keep all material at 4°C.

# Transformation

1. Add 50µL of competent bacteria to an Eppendorf tube previously cooled.

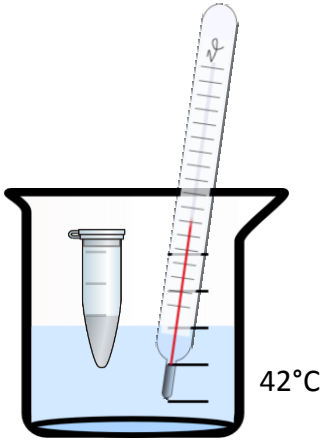



2. Add 2 µL of DNA and mix giving light tapping.



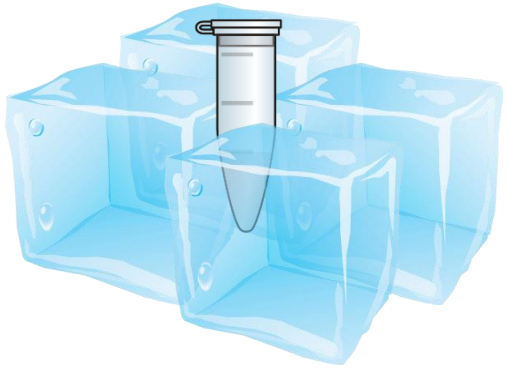

3. Let it rest in ice for 20-30 minutes.

4. Give thermal shock by immersing the Eppendorf tubes in a beaker with water at a temperature of 42 ° C for 1 minute.



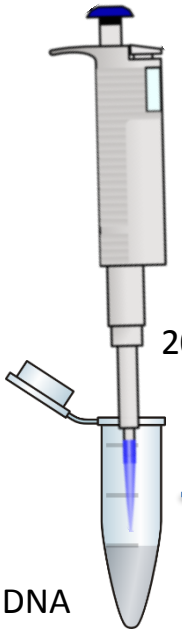
5

Let rest in ice for 2 minutes.



6

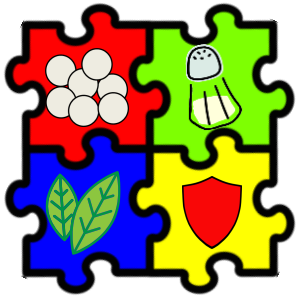
Add 200 µL of LB broth and incubate at 37°C during 20-30 minutes.



200 µL of LB

Competent + DNA

37°C



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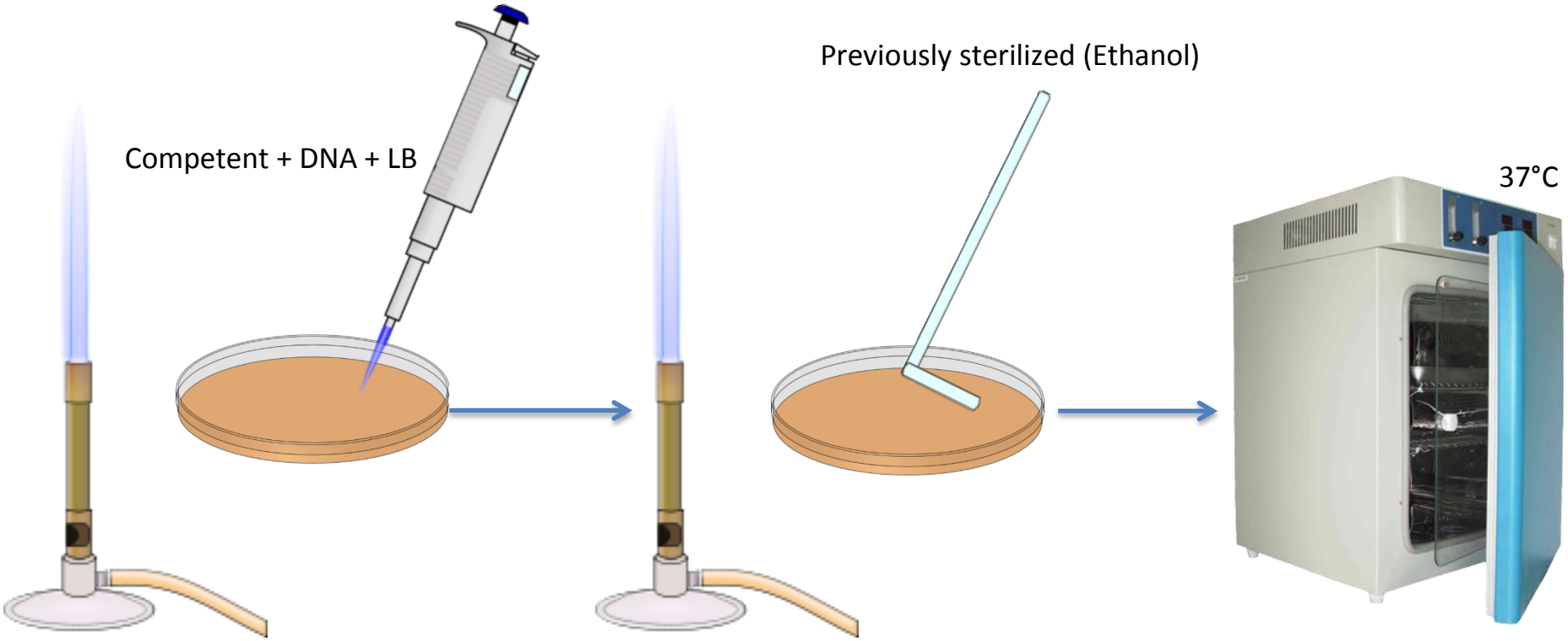
# Sowing

1 Plating on LB boxes with their respective antibiotic and incubate at 37 ° C overnight.

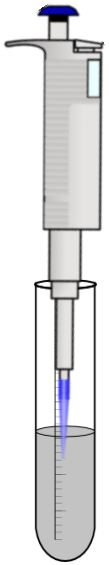
Click on the module you were viewing



# Pick colonies

1

To a test tube containing culture broth add the appropriate antibiotic.

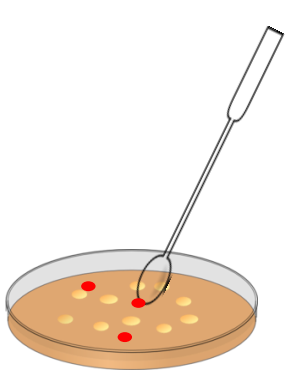


Appropiate antibiotic

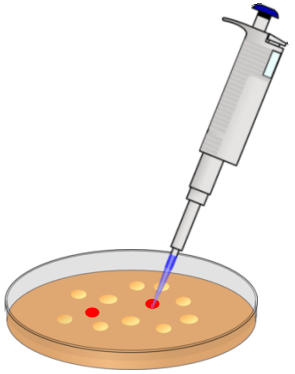
Culture broth

2

Chop a red colony with a previously sterilized bacteriological loop or chop with a micro pipette tip.

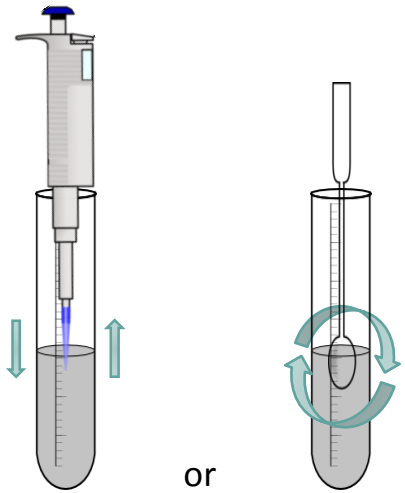


or



3

Stir in small circles in the culture (if taken with handle) or up and down a few times growing (if micro pipette was used).

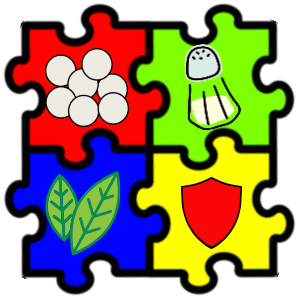


or

4

Incubate at 37°C with vigorous stirring and leave it for 16-18 hours.

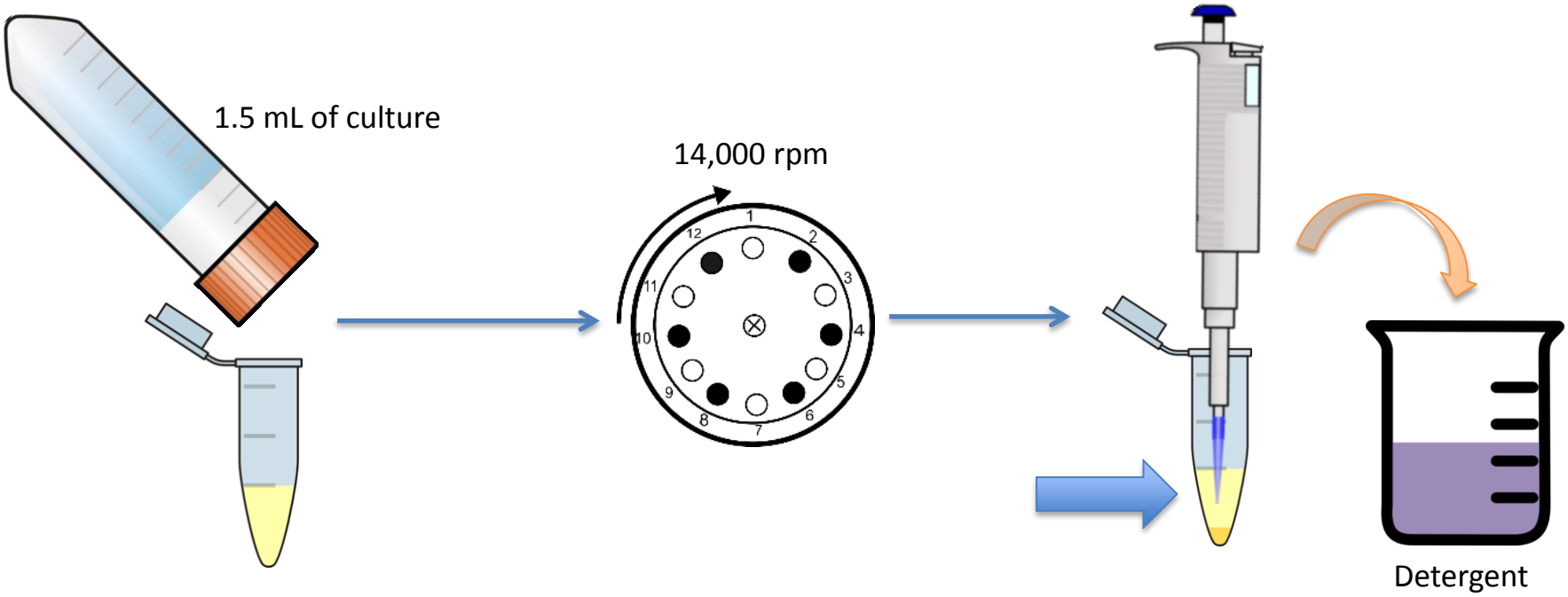

Click on the module you were viewing



# Mini preparation of plasmidic DNA

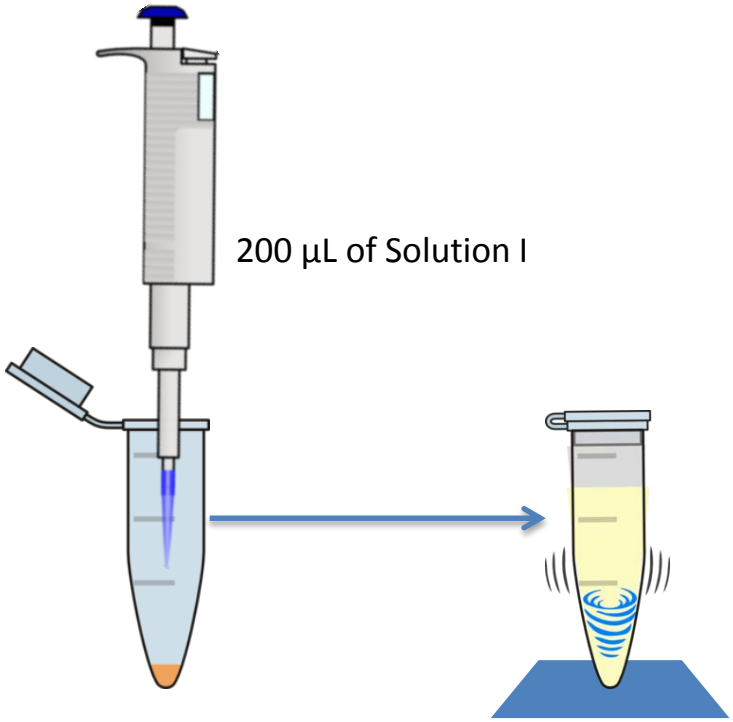
1

Add 1.5 mL of culture to an Eppendorf tube, spin-dry at 14,000 rpm for 30 seconds and put the supernatant in a container with 0.1% chlorine or liquid soap.



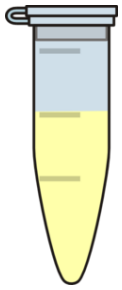
2

Add 200 uL of Solution I and mix giving vortex until the pellet is completely dissolved. (You can use a micro pipette if it is difficult to dissolve)



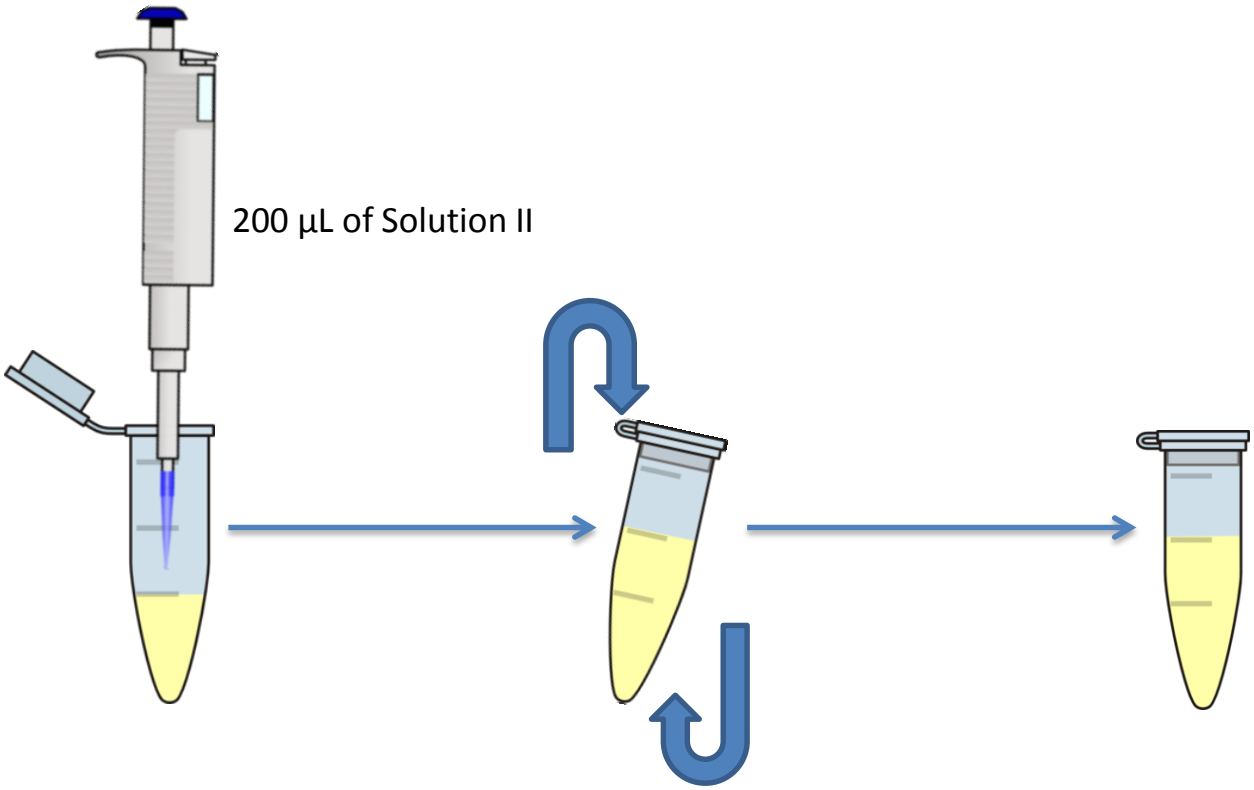

3

Leave at room temperature for 10-20 minutes.



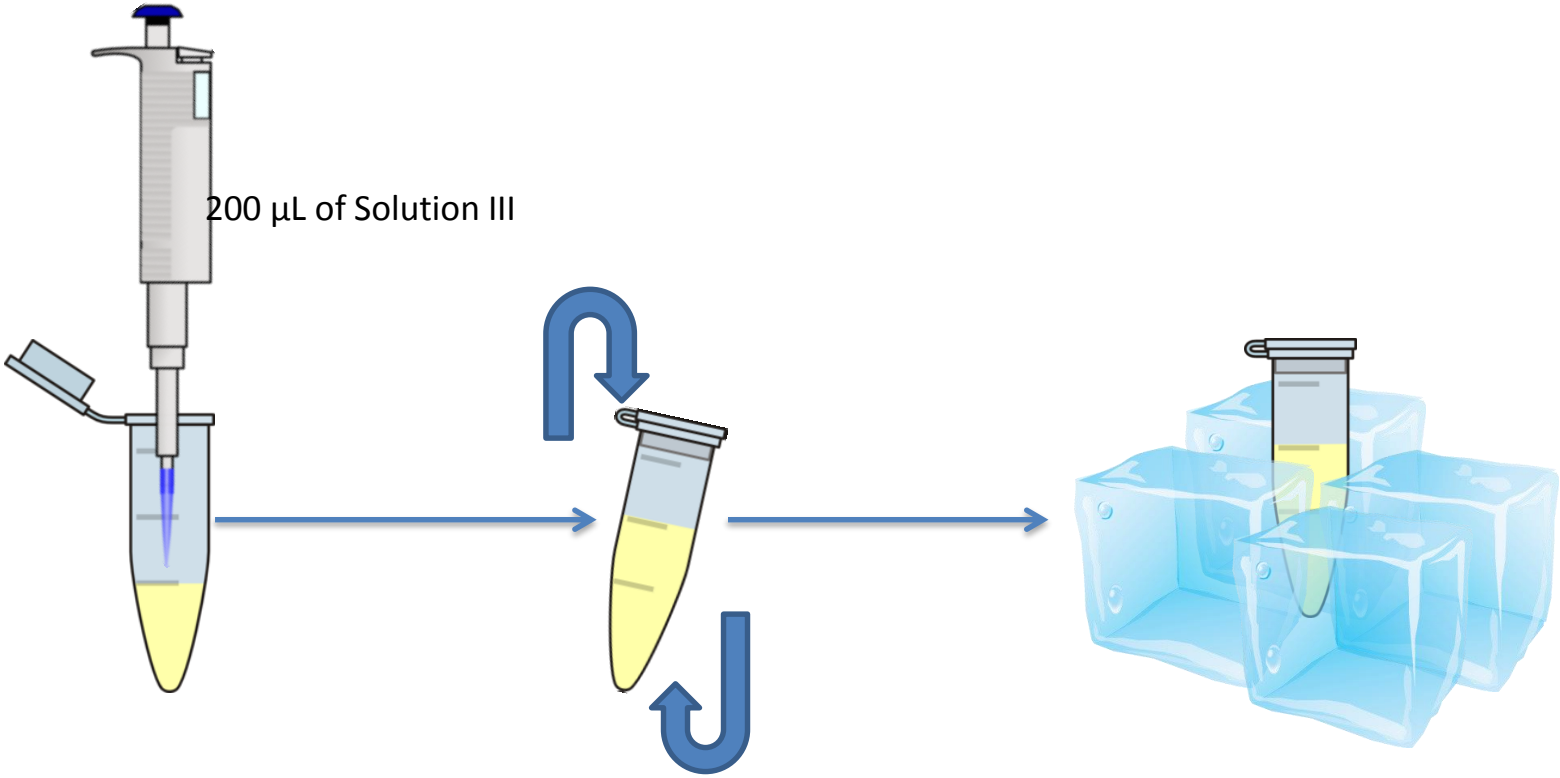
4

Add 200 uL of Solution II and mix by inversion. Leave at room temperature for 5-10 minutes.



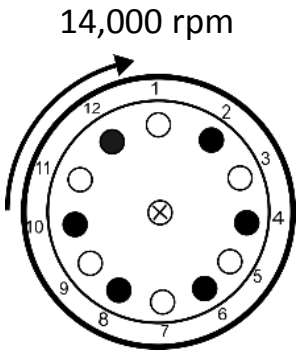
5

Add 200 uL of Solution III and mix by inversion. Leave on ice for 10 minutes.



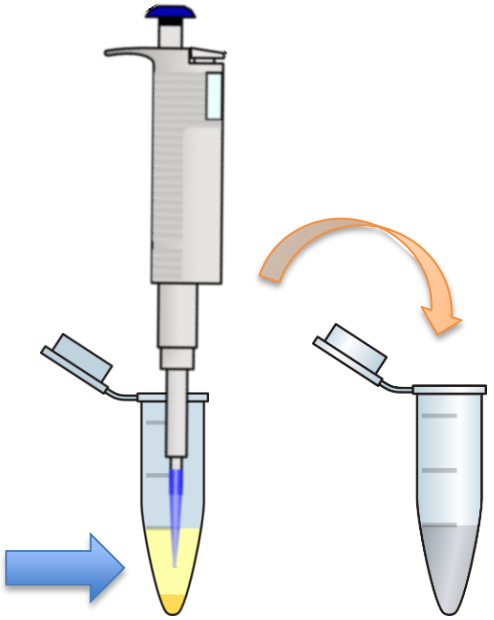
6

Spin-dry at 14,000 rpm for 5 minutes.




7

Pass the supernatant to a new Eppendorf tube containing 1 mL of 100% ethanol using a tip of a micro pipette being careful not to touch any precipitate.



8

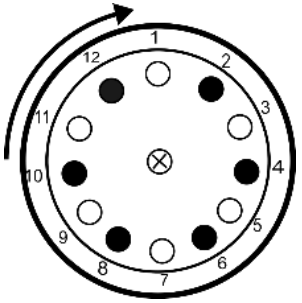
Incubate at -20°C for 10 minutes. (From 10 minutes to 2 hours).

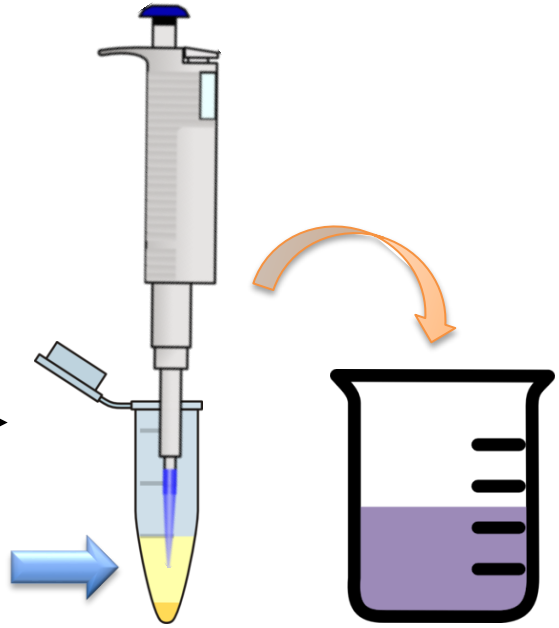
-20°C

9

Spin-dry at 14,000 rpm for 10 minutes and throw the supernatant away.

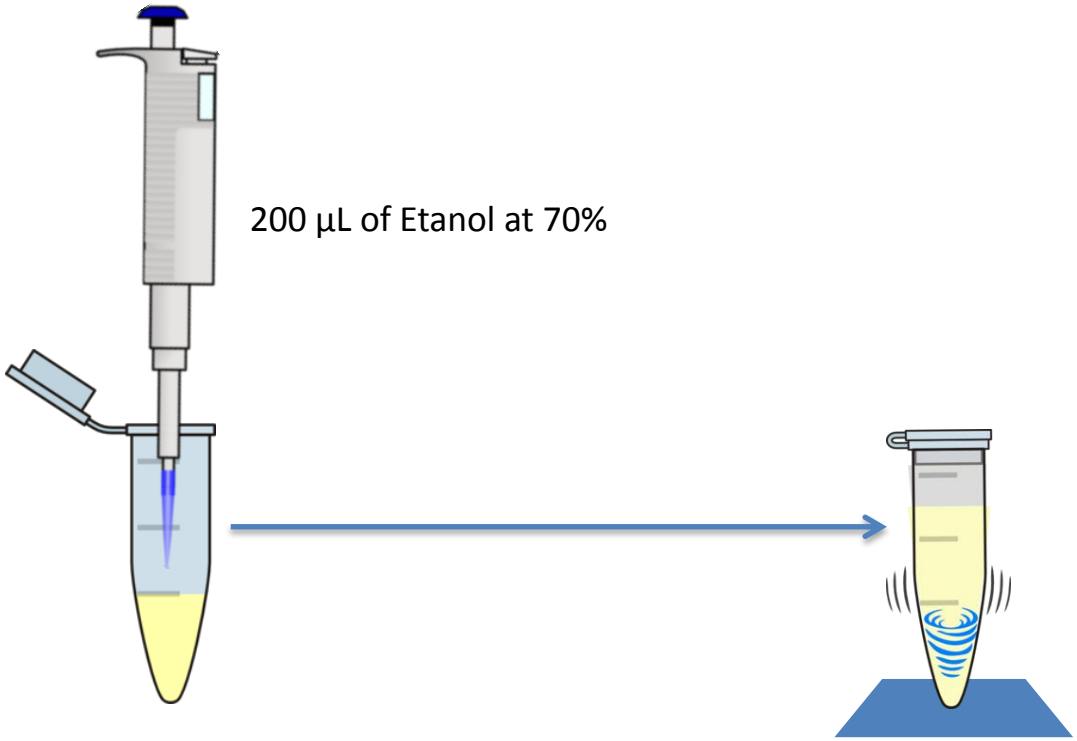
14,000 rpm



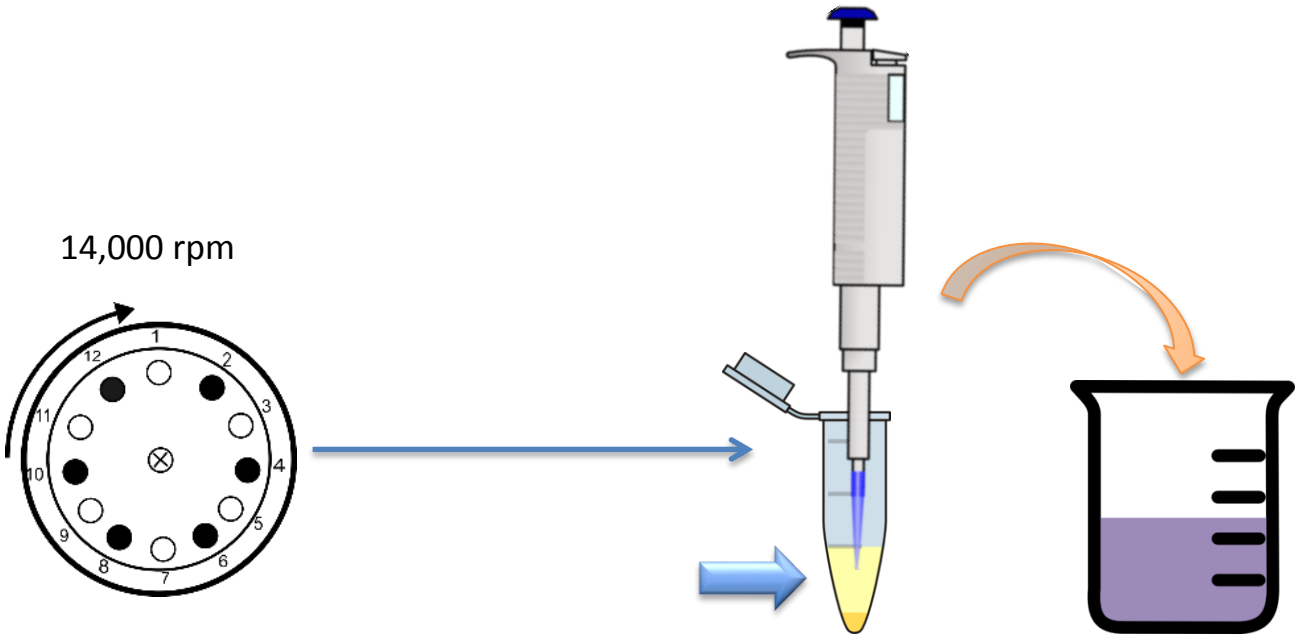
10

Add 200 µL of Etanol at 70% and vortex a few seconds.



11

Spin-dry at 14,000 rpm for 5 minutes and remove the supernatant with a micro pipette.



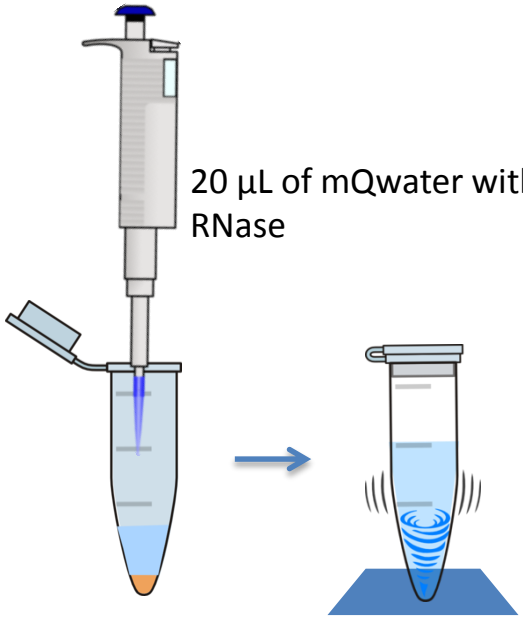
12

Dry the pellet at 37 °C for 5 minutes in the incubator.

37°C

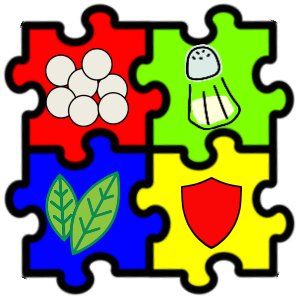
13

Add 20 uL of mQ water with RNase (10ng/µL) and resuspend by vortexing.



20 µL of mQwater with RNase

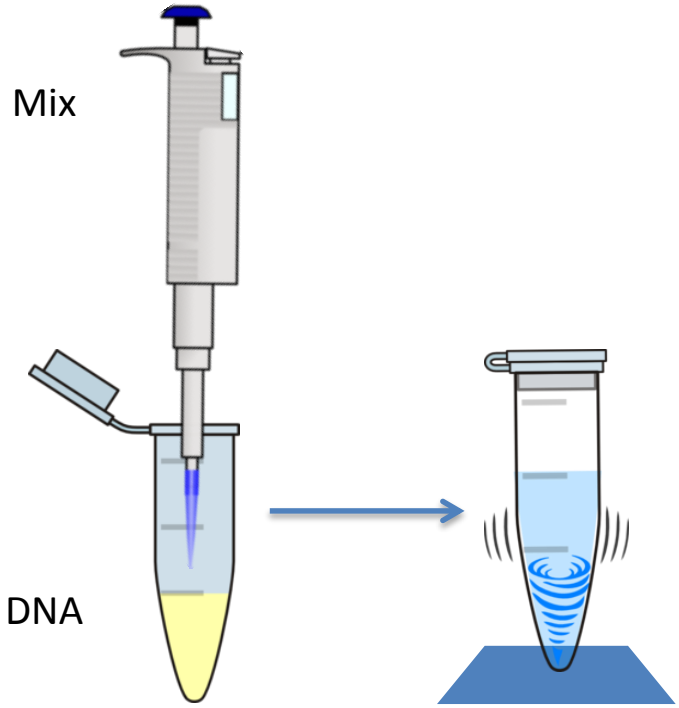
Click on the module you were viewing



# Cut

1

Make the mix with mQ water, buffer and the desired enzimes. Add it uniformly to the DNA and vortex.

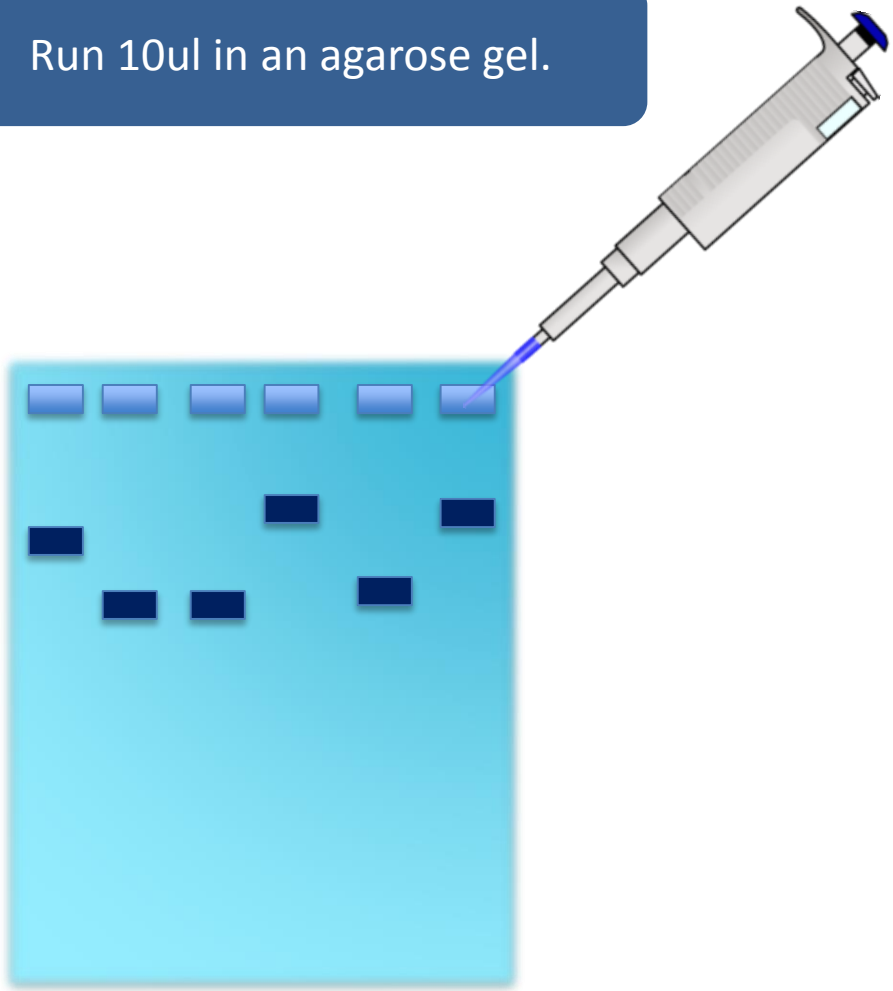



2

Incubate at 37° C for 1 or 2 hours.

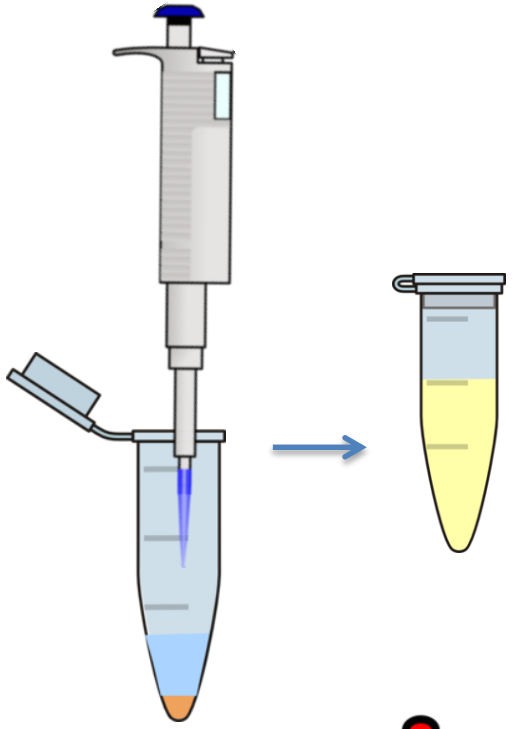
3

Run 10ul in an agarose gel.



4

Save 10ul for ligation.



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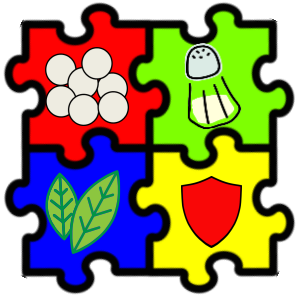
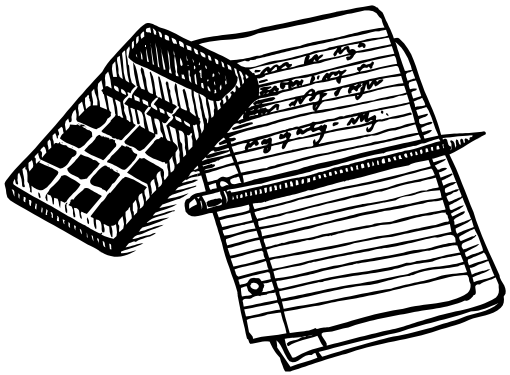


# Ligation

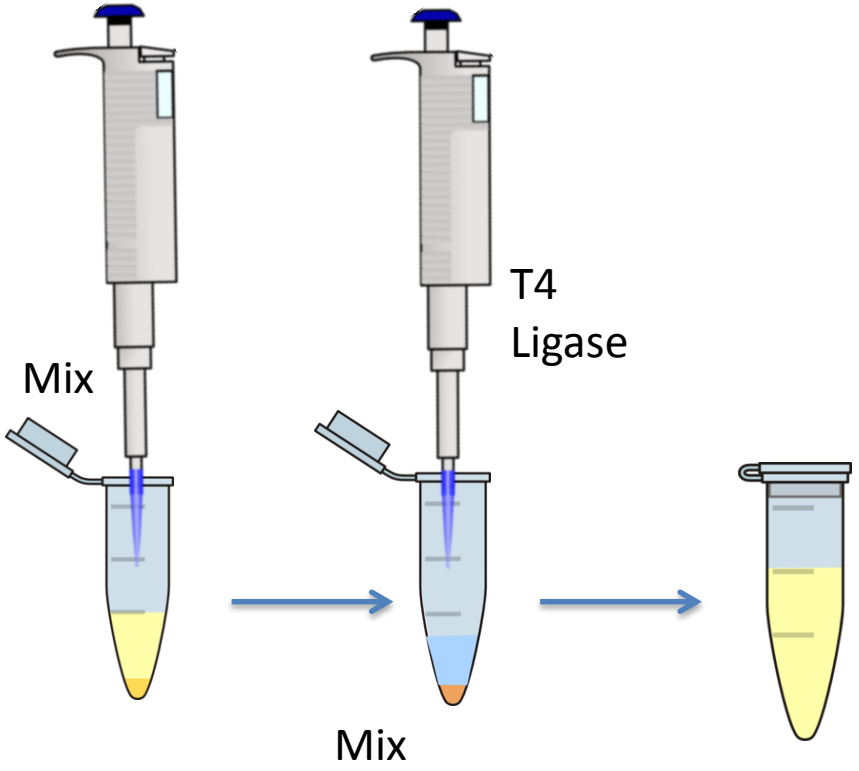
1

Calculate using the ligation calculator the quantities for preparing the ligation mix with final volume of 20ul.



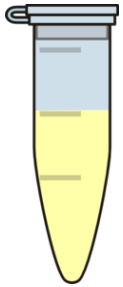
2

Prepare the mix with mQ water, ligation buffer, vector and fragment. Add ligase T4.



3

Incubate overnight at room temperature (25° C).



25° C

Click on each module