ENCOUNTER #1 : LETHAL FUSION

Description

During this experiment you will encapsulate 2 types of bacterial communities within hydrogel beads: natural (wild-type *E.coli*) bacteria, and genetically modified bacteria (*E.coli* carrying a plasmid).

Both encapsulations will be formed separately and evolved in different conditions, in accordance with their respective genotypes.

The conclusion of the experiment will be to introduce them in an environment that provokes the fusion of both encapsulated systems. We will observe how this encounter affected these ecosystems.

Act 1 : Encapsulation – bead formation

You need:

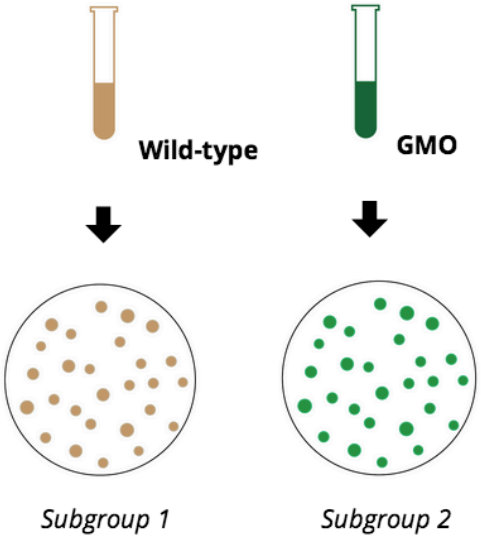
- Natural *E.coli* culture
- GMO *E.coli* culture

Note: GMO E.coli carries a plasmid allowing expression of Green Fluorescent Protein in presence of arabinose, and ampicillin resistance.

- Syringe
- 2% Alginate solution in Falcon tubes
- Calcium chloride (CaCl2) solution
- Beaker and magnetic stirrer
- Sterile conditions at all times

To do:

Divide your group in two subgroups. Each subgroup will separately generate a bacterial encapsulation using either natural cells or GMO cells.
The subgroups should both follow the subsequent steps for generating the alginate beads.
The difference in method (particular to each subgroup) transpires in step (II).



I. Label a beaker either **A** for wild type *E.coli* or **B** for GMO.

II. Add approximately 100mL of CaCl2 solution to one of the beakers. Install the beaker on the magnetic stirrer device and stir the solution with a magnet.

III. Add 200µL of bacterial culture (natural OR GMO) to a tube containing 20mL of alginate solution. Close the tube.

IV. Mix well by gently inverting the tube several times. Avoid forming bubbles.

V. Introduce the alginate mixture in a syringe. Gently add the mixture to the $CaCl_2$ solution, ONE DROP AT A TIME.

VI. Leave the formed beads to harden in the solution for 5-10 minutes.

VII. In the mean time: Label 3 Falcon tubes AND 3 Petri dishes with one of the following:

- Natural + GMO
- Natural
- GMO

Act II : Growth and evolution

You need:

- Encapsulated bacteria in $CaCl_2$ solution
- Plastic strainers
- Erlenmeyer containing **medium A**: OB growth medium (to grow natural cells)
- Erlenmeyer containing **medium B**: OB growth medium + Ampicillin + Arabinose (to grow GMO cells)
- Incubator

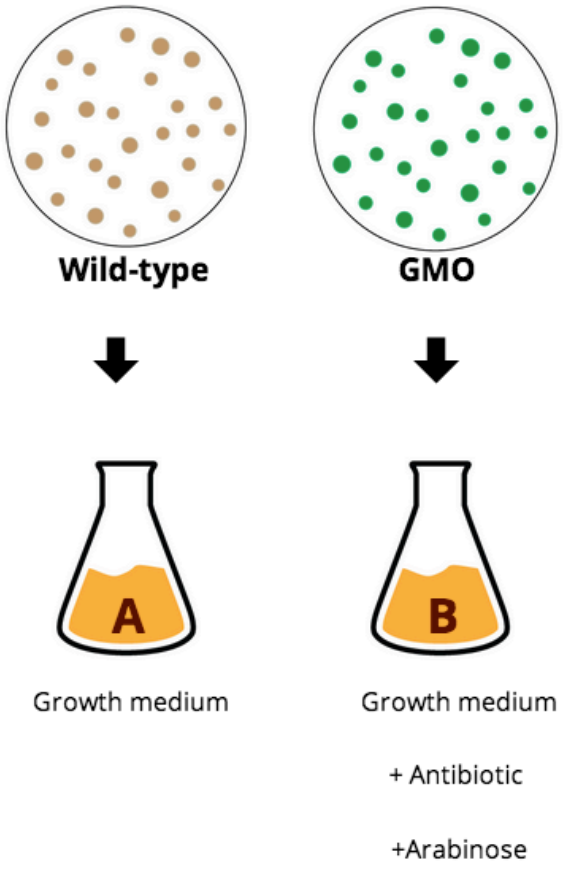
To do:

I. Separate the beads for the CaCl2 solution using the small plastic strainers

II. Place the beads in the corresponding growth medium according to the cells genotype (natural OR GMO).

- Natural cells = medium A
- GMO cells = medium B

III. Place the Erlenmeyer flasks in the shaking incubator at 37°C. Incubate for 35 minutes.



Act III : Encapsulations' encounter

You need:

- Plastic strainers
- Hydrophobic phase
- Falcon tubes
- Empty Petri Dishes
- Tissues

To do:

I. Add 25mL of hydrophobic phase to each Falcon tube previously labeled.

II. Separate the 'Natural' beads from the growth medium A. Decant the liquid as much as possible. Use a tissue to quickly remove remaining droplets.

III. Add half of this batch to the Falcon tube labeled 'Natural' and the other half to the 'Natural + GMO' tube.

IV. Repeat step III with the beads containing the GMO cells

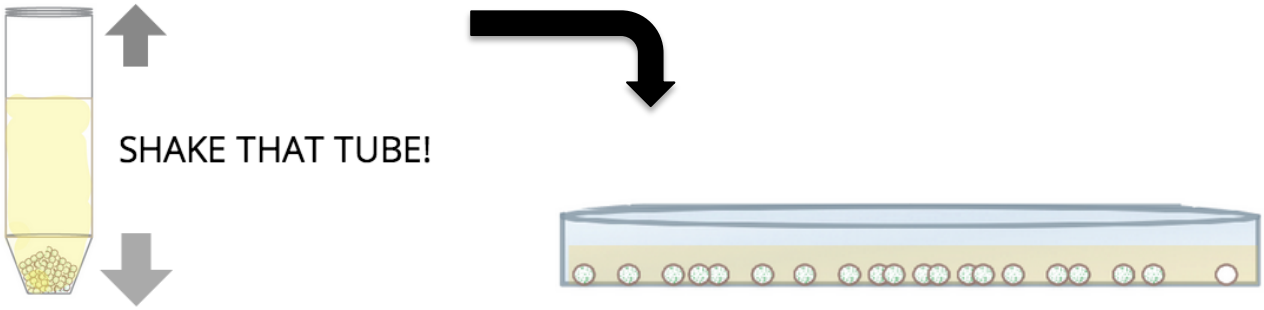
V. Add half of the batch to the Falcon tube labeled 'GMO' and the other half to the 'Natural + GMO' tube.

VI. Thoroughly mix the 3 Falcon tubes until obtaining a homogeneous emulsion.

VII. Put the mixture in the corresponding Petri dish

VIII. Make sure that most of the beads touch each other. You can move them in the plate to provoke contact with a sterile tip.

IX. Incubate overnight at 37°C.



Act IV : Conclusion

Observe the beads under the fluorescent microscope or by naked eye on top of a transilluminator.

ENCOUNTER #2 : LETHAL FUSION

Description

During this experiment you will encapsulate a bacterial community composed of genetically modified bacteria (E.coli carrying a plasmid) within hydrogel beads.

The identical bacterial encapsulations will be divided in two batches that will be evolved in different environments. Each environment will affect differently the phenotype of the immobilized bacteria.

The conclusion of the experiment will be to introduce them in an environment that provokes the fusion of the encapsulated systems initially cultivated separately. We will observe how this encounter affected these ecosystems.

Act 1 : Encapsulation – bead formation

You need:

- GMO *E.coli* culture
Note: GMO E.coli carries a plasmid allowing expression of Green Fluorescent Protein in presence of arabinose.

- 2% Alginate solution in Falcon tubes
- Calcium chloride
- Beaker and magnetic stirrer

To do:

I. Add approximately 200mL of $CaCl_2$ solution to one of the beakers. Install the beaker on the magnetic stirrer device and stir the solution with a magnet.

II. Add 200µL of bacterial culture to each tube containing 20mL of alginate solution. Close the tubes.

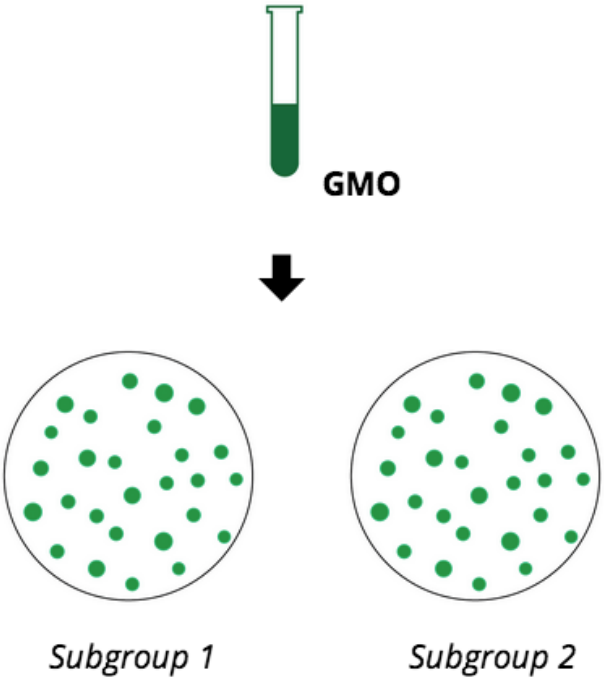
III. Mix well by gently inverting the tube several times. Avoid forming bubbles.

IV. Introduce the alginate mixtures in separate syringes. Gently add the mixture to the CaCl2 solution simultaneously, ONE DROP AT A TIME.

V. Leave the formed beads to harden in the solution for 5-10 minutes.

VI. In the mean time: Label 3 Falcon tubes AND 3 Petri dishes with one of the following:

(+) Arabinose
(-) Arabinose
(+/-) Arabinose



Act II : Growth and evolution

You need:

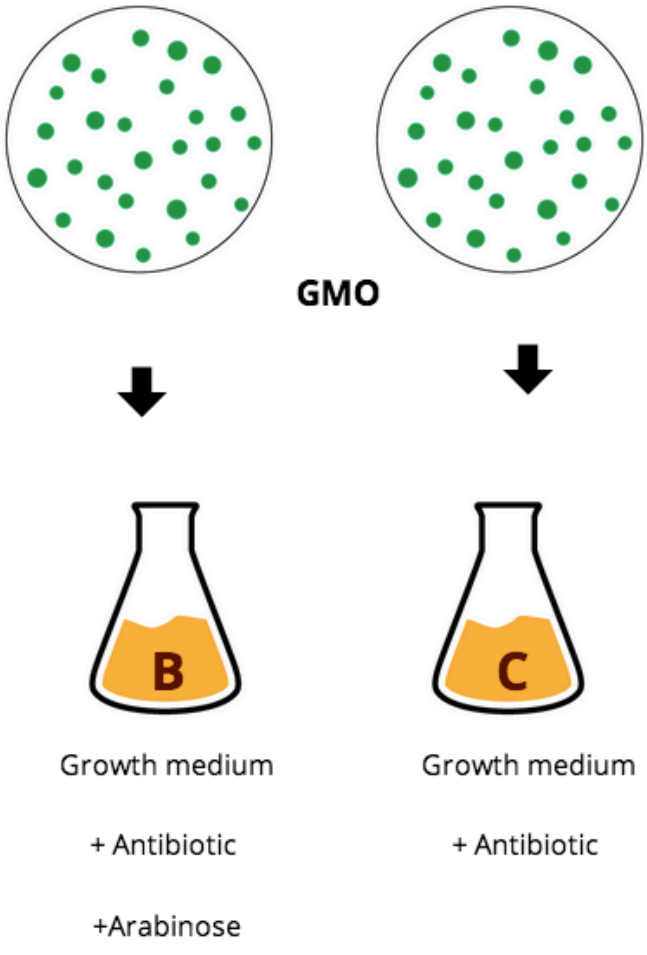
- Encapsulated bacteria in CaCl2 solution
- Plastic strainers
- Erlenmeyer containing **medium B**: OB growth medium + Ampicillin + Arabinose
- Erlenmeyer containing **medium C**: OB growth medium + Ampicillin
- Incubator

To do:

I. Separate the beads for the CaCl2 solution using the small plastic strainers

II. Place half of the beads in the flask containing medium B. Add the other half to medium C.

III. Place the Erlenmeyer flasks in the shaking incubator at 37°C. Incubate for 35 minutes.



Act III : Encapsulations' encounter

You need:

- Plastic strainers
- Hydrophobic phase
- Falcon tubes
- Empty Petri Dishes
- Tissues

To do:

I. Add 25mL of hydrophobic phase to each Falcon tube previously labeled.

II. Separate the beads from the growth medium B. Decant the liquid as much as possible. Use a tissue to quickly remove remaining droplets.

III. Add half of the batch to the Falcon tube labeled '(+) Arabinose' and the other half to the '(+/-) Arabinose' tube.

IV. Repeat step III with the beads incubated in medium C.

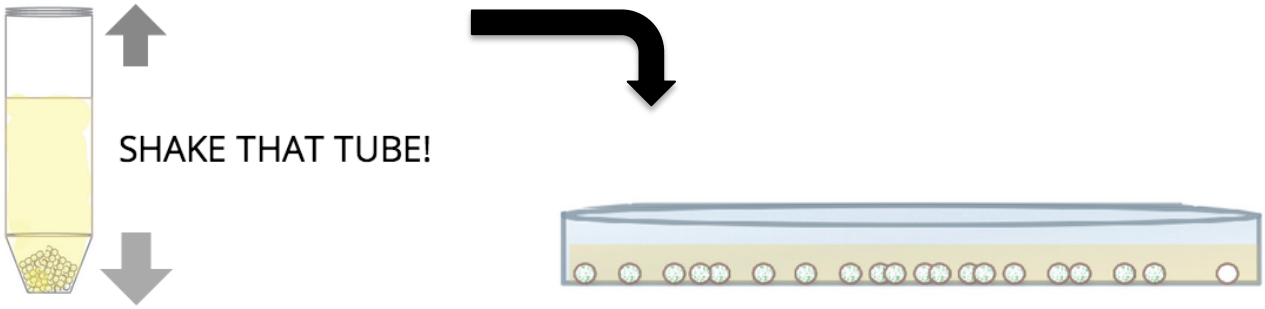
V. Add half of the batch to the Falcon tube labeled '(-) Arabinose' and the other half to the '(+/-) Arabinose' tube.

VI. Thoroughly mix the 3 Falcon tubes until obtaining a homogeneous emulsion.

VII. Put the mixture in the corresponding Petri dish

VIII. Make sure that most of the beads touch each other. You can move them in the plate to provoke contact with a sterile tip.

IX. Incubate overnight at 37°C.



Act IV : Conclusion

Observe the beads under the fluorescent microscope or by naked eye on top of a transilluminator.