**Project proposal**

**Aim :**

Who between starving *E. coli* and satiated ones go faster to a food area ?

**Protocol:**

Material:

* We use two strain of *E.coli* one RFP and GFP.
* normal LB media
* min poor LB media
* 30 petri dishes
* counting chambers
* pipettes (15mL, 30ml, 1mL)
* agar + water solution (0.3% agar)
* arduino
* stepper motor of this type (<https://www.adafruit.com/products/918>) <https://hackspark.fr/fr/17pm-k054-nema-17-stepper-motor.html>

16 Rue Alexandre Dumas, 75011 Paris ouvert de 10h30 a 13h puis 16h 19h

* camera ( go pro)
* plexiglas cercle of

<https://www.researchgate.net/post/Is_there_any_good_substitute_for_M9_minimal_medium_for_E_coli>

**Robot:**

* We print the draw for stabilize the plate with ‘gravure’ at 100% power and 10% Speed. It’s very long (more than 4 hours for half of the circle)
* this is the code that finally works :
* To attach the plate to the robot we have been help by the mechanic near cochin.
* The plexi plate was way too heavy (but more because of its large surface than its weight)
* we thought about make the go pro moving (but because of the quality is not a good alternative)
* a good camera is too heavy to turn
* we might thought of making an engrenage for make the plate moving as first expected.

**Media**:

* Prepare a poor liquid media: … % of nutrient on liquid LB
* Prepare a rich liquid LB media (... % of nutrient)

***E. coli* culture:**

* RFP rich media \*2
* RFP poor media \*2

if time :

* GFP rich media \*3
* GFP poor media \*3

The bacteria didn’t grow in poor media because of how the media was made or how the we insert the bacteria.

So we change our experiment to just compare RFP and GFP movement through the gradient.

**Counting and fluorescence:**

* kill bacteria with … to prevent them from moving (not do)
* insert 7 microliter in a counting chamber and deduce the number of E coli in 1 microliter.
* quantify fluorescence for a specific volume of bacteria. Make a photo and analyse it with imagej to link a number of bacteria to a surface of fluorescence
* repeat for each culture

We didn’t do that because we were studying the population/colonie scale.

Other wise when we tried we saw that there were really too much bacteria so we made a dilution by 100.

**Slide/Plate without nutrient gradient:**

* Take 1 L of distilled water.
* Filter the water with a syringe and a filter.
* Divide the 1 L of the filter water in two different sterile bottles ( 0,5 L in each)
* Then add in each bottle 1,5 g of Agar and put the bottle in the microwave for 5 minutes with thestopper a little open.
* Once the water boil, get out the bottle from the microwave and shake it for two minutes in order to dissolve completely the agar in the water.

**Slide/Plate with nutrient gradient:**

* Prepare media with only water and agar and a rich solid LB
* draw a line under the plate to delimit both side ( we didn’t do that )
* Insert some rich media on one side and solid and agar on the other ( we put first our rich media and tilt the plate to make a ‘triangle’ gradient than we put agar and water on the top.)
* 10 minutes for the LB to solidify
* 10 minutes for water and agar to solidify
* Let this for 24h ??? to let time to create gradient

**Purify media :**

* We first take 1mL in an ependorff and centrifugated it but we must do this for the all falcon in a huge centrifuger. So we centrifuge 1 minute at 9.5 rotation per second than take out the surnagent and put filtrate water instead, and repeat this two times before take 50 microlitre and put it in a plate. Then parafilm the plate.

**Controls**: \*3

* insert a known number/volume of bacteria from rich red (R+) and rich green (V+) media in the middle CLIC!!!! hurry up!!!!!
* measure fluorescence with fluorescence microscope: take a picture of the plate. (Just under the light with a filter and just take a picture)
* Analyse the picture with ImageJ: associate volume of bacteria to a surface on the plate + to a fluorescence
* Wait for ??? (30 micrometer per second) to let bacteria move
* Take a picture of the plate
* Analyse image: determine level of fluorescence of each culture and deduce the number of bacteria on the final area
* Repeat for : R-V-, R+V+ no nutrient nowhere , R-V- no nutrient

We take picture every 20 second for all night but under white light with a go pro, the quality is very bad we don’t know how to analyse them.

**Experiments : \*5**

* in the gradient plate put a define quantity of RFP rich and the same quantity of GFP poor than the inverse (RFP poor, GFP rich)
* measure with imagej the fluorescent at the beginning and after … minutes.

We just take a photo after 2 hours in the plate and after one night (14 hours) and a time laps that we can’t analyse because of the quality.

We did only RFP rich and GFP rich.

2 control avec gradient (12.5 mL agar+eau, 12,5 mL LB)

R+, V+ et R-, V-: 2\*5

2 control sans gradients (eau+agar)

2 expériences avec gradients (12.5 mL agar+eau, 12,5 mL LB)

1\*5

1\*5

20 voire 25 avec gradients

10 voire 15 eau+ agar

Cultures:

* une verte dans le pauvre
* une verte dans le riche
* une rouge dans le pauvre
* une rouge dans le riche

Counting: