

PROTOCOL

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

Material

- two strain of E.coli one RFP and GFP.
- normal LB media
- min poor LB media
- 40 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 + phones)
- plexiglas cercle

[https://www.researchgate.net/post/Is there any good substitute for M9 minimal medium for E coli](https://www.researchgate.net/post/Is_there_any_good_substitute_for_M9_minimal_medium_for_E_coli)

Sample size → 40 plates

Biological replicates → 2

Technical replicates → 5

Controls → absence of gradient

Biological protocol

Prepare overnight RFP strain and GFP strain E.coli culture

→ prepare media water + 0.3% agar (jelly media)

Filter 1L of water thanks to 5 filters and one 50mL syringe, we should have put the all preparation water + agar at the AUTOCLAV the day before

- make two recipients of 500mL of filter water
- add 1.5 g of agar per recipients to have 0.3% concentration of agar at the end
- boil each recipient → make sure that recipients are half empty when boiling, if note it might exploded in the microwave
- 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.

Put 25mL of this media on 20 plates (non gradient plates)

→ prepare solid LB media gradient on 20 plates

Add 12.5mL of this media just coming out the AUTOCLAV on 20 plates

- to make a gradient place them a little bit tilt
- wait 15 minutes until it solidified

Add 12.5mL of water + 0.3% agar (jelly media) on those 20 plates with gradient of LB

→ prepare liquid LB media and media minimum M9

→ prepare bacteria growth on different media

- RFP strain → from the overnight culture

Take the overnight culture of RFP E.coli of 10mL

Make 2 serial dilutions until 10^{-6}

- pipette 100μL with P200 and place it on 900μL of LB (10^{-1})
- take 100μL of the previous dilution and put it on 900μL of LB (10^{-2})

- take 100µL of the previous dilution and put it on 900µL of LB
(10⁻³)
- take 100µL of the previous dilution and put it on 900µL of LB
(10⁻⁴)
- take 100µL of the previous dilution and put it on 10mL of LB (10⁻⁶)
- do it twice so you have 2 falcons of RFP well feed (R+1 and R+2)
- incubate at 37°C for one day to make them grow
 - pipette 100µL with P200 and place it on 900µL of M9
- take 100µL of the previous dilution and put it on 900µL of M9
(10⁻²)
- take 100µL of the previous dilution and put it on 900µL of M9
(10⁻³)
- take 100µL of the previous dilution and put it on 900µL of M9
(10⁻⁴)
- take 100µL of the previous dilution and put it on 10mL of M9 (10⁻⁶)
- do it twice so you have 2 falcons of RFP bad feed (R-1 and R-2)
- incubate at 37°C for one day to make them grow

- GFP strain - from the overnight culture

Take the overnight culture of GFP E.coli of 10mL

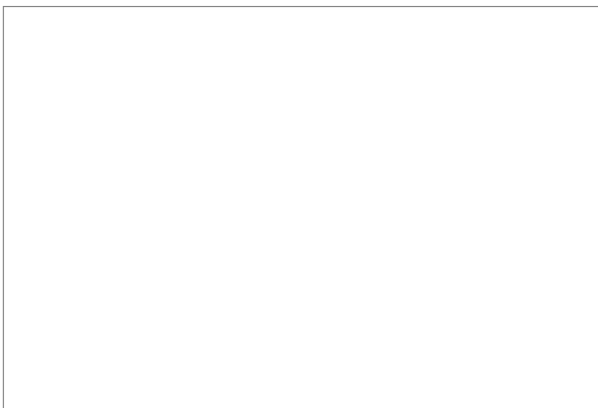
Make 2 serial dilutions until 10⁻⁶

- pipette 100µL with P200 and place it on 900µL of LB (10⁻¹)
- take 100µL of the previous dilution and put it on 900µL of LB
(10⁻²)
- take 100µL of the previous dilution and put it on 900µL of LB
(10⁻³)
- take 100µL of the previous dilution and put it on 900µL of LB
(10⁻⁴)
- take 100µL of the previous dilution and put it on 10mL of LB (10⁻⁶)
- do it twice so you have 2 falcons of GFP well feed (G+1 and G+2)
- incubate at 37°C for one day to make them grow
 - pipette 100µL with P200 and place it on 900µL of M9

- take 100µL of the previous dilution and put it on 900µL of M9 (10⁻²)
- take 100µL of the previous dilution and put it on 900µL of M9 (10⁻³)
- take 100µL of the previous dilution and put it on 900µL of M9 (10⁻⁴)
- take 100µL of the previous dilution and put it on 10mL of M9 (10⁻⁶)
- do it twice so you have 2 falcons of GFP bad feed (G-1 and G-2)
- incubate at 37°C for one day to make them grow

CAREFUL - don't pipette directly on the LB/M9 bottle to minimize contamination risks

- always manipulate under the hood



- The bacteria didn't grow in poor media because of how the media was made or how we insert the bacteria
- So we change our experiment to just compare RFP well feed and GFP well feed movement through the gradient

Which between RFP and GFP detect best nutrient gradient ?

→ counting bacteria and fluorescence

- 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 (colony) scale

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

→ purify R+ E.coli before putting them on 10 plates

- Take R+1 culture
- Put 1mL of this culture in 10 different eppendorfs
- Centrifuge them at 9.5 rcf - put a 1mL water eppendorf in it so that weight is equilibrate
- When centrifugate remove the liquid phase (950µL) and keep the concentrated bacteria at the bottom
- Add 950µL of filtered water
- Centrifuge again
- Repeat this process twice
- Then you have 10 bacteria's concentrated eppendorfs
- Put 5 of them on gradient plates on the less gradient side
- Put five of them on non gradient plates at one side of the plate (controls)
- Put parafilm around plates

- Take picture at $t=0$ under UV light to detect fluorescence
- Make a time lap with the go pro all night long

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.

- Take picture at $t=24h$ under UV light to detect new colonies
- purify G+ E.coli before puting them on 10 plates

- Take G+1 and G+2 culture of 10mL
- Centrifuge them with the big centrifugator at rpm (corresponding to 9.5

rcf) → put a 10mL water falcon in it so that weigh is

equilibrate

- Take off the liquid phase (10mL)
- Add 10mL of water
- Centrifuge again
- Repeat this process twice
- Last time don't add water
- Take loops and pick up colonies to put on plates
- Put 1 colony on 1 plate
- 5 gradient plates must have one colony and the less gradient side
- 5 non gradient plate must have one colony on one side of the plate
- Put parafilm around plates

→ take pictures under UV light each 1h to detect
fluorescent colonies