

Our main interest is to reproduce complex patterns using a bacterial model. We used *Escherichia coli* because is widely used in molecular biology and there is a lot of information about it. We used bacteria that produce red and green fluorescent proteins to monitor their behaviour.

We transformed *E.coli* JM109 with the following biobricks:

BBa\_I13521: Ptet + mRFP, swithed off by tetracycline

BBa\_I13522: ptet GFP

BBa\_J04430: GFP coding device switched on by IPTG

BBa\_J04450: RFP coding device switched on by IPTG

Cultures were made in Petri dishes with LB agar with 100 µg of ampicilin and were incubated overnight at 37°C.

Antibiotic	Transformant bacteria.
NA	Cell of the strain JM109 without transformation
Amp	Cell of the strain JM109 without transformation
Amp	<a href="#">BBa_I13521</a> RFP (25µl) + <a href="#">BBa_J04430</a> GFP (25µl)
Amp	<a href="#">BBa_I13522</a> GFP (25µl) + <a href="#">BBa_J04450</a> RFP (25µl)
Amp	<a href="#">BBa_I13521</a> RFP (50µl)
Amp	<a href="#">BBa_J04430</a> GFP (50µl)
Amp	<a href="#">BBa_I13522</a> GFP (50µl)

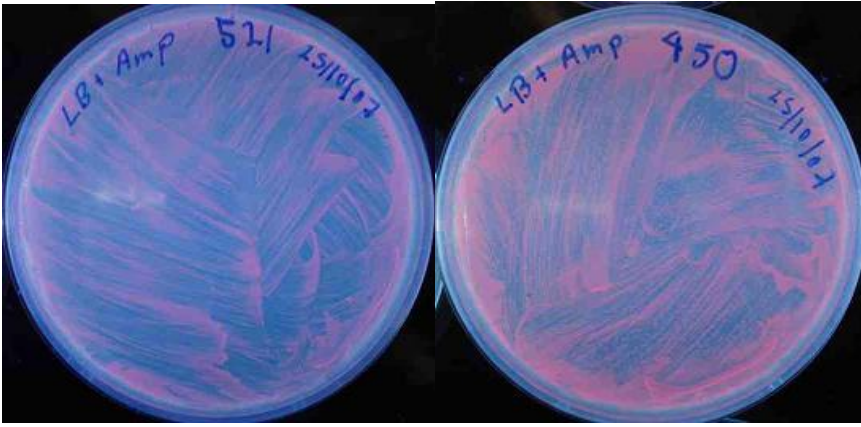
In order to prove that bacteria form complex patterns we did the following:

We tested the bacteria in small Petri dishes on semisolid Agar. We platted 5µl of an overnight culture (10 ml LB + 100 µg Amp/ml + glycerol stock).

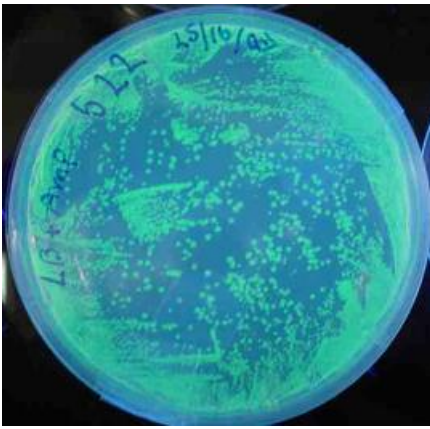
To 5 µl of culture we added 45 µl of LB medium to dilute bacteria and platted 50 µl.

First we platted individual clones to use as positive controls:

Clones	
521 RFP	5 µl culture
450 RFP	5 µl culture
522 GFP	5 µl culture

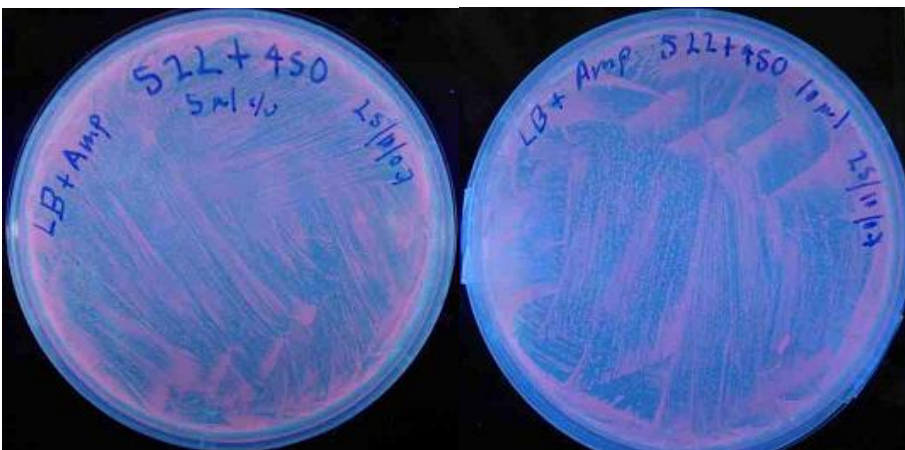


Too many colonies for clones 521 and 450



Individual clones were visualized in clone 522 that produces GFP

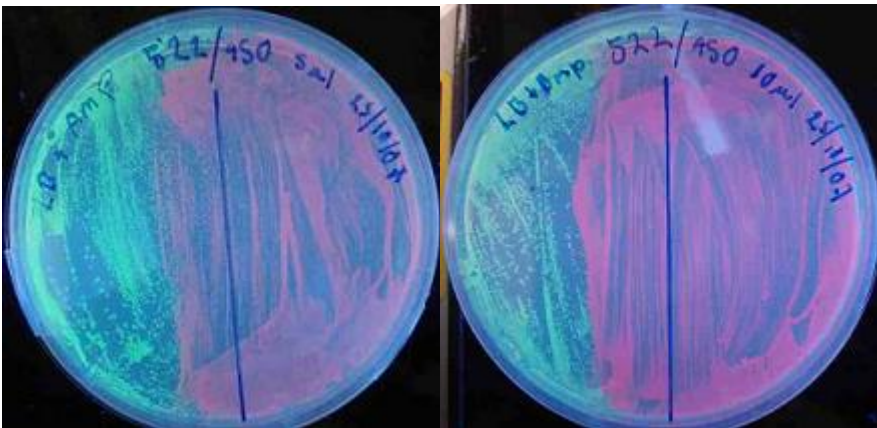
Then we mixed bacteria that produce GFP and RFP. We used 5  $\mu$ l of each culture and observed the following:



522 + 450  
5  $\mu$ l of each

522 + 450  
10  $\mu$ l of each

For this part we mixed the clones and plated them together and separately as showed below:



Note: Small green colonies were observed.

Conclusions: due to the great amount of bacteria that produces RFP we weren't able to clearly see any pattern.

We'll have to repeat the experiment with less amount bacteria.

## TRANSFORMATION

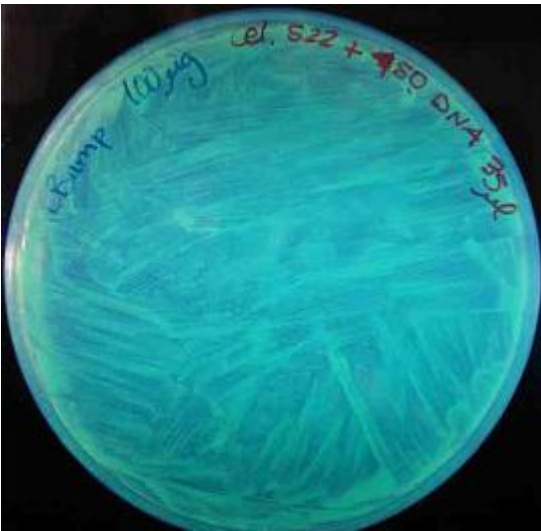
We want to insert biobrick BBa J04450 that codes for RFP into previously transformed bacteria with biobrick BBa I13522 that produces GFP.

We prepared competent cells according to the previously described protocol and used aprox. 35 ng of purified plasmid from biopart BBa J04450.

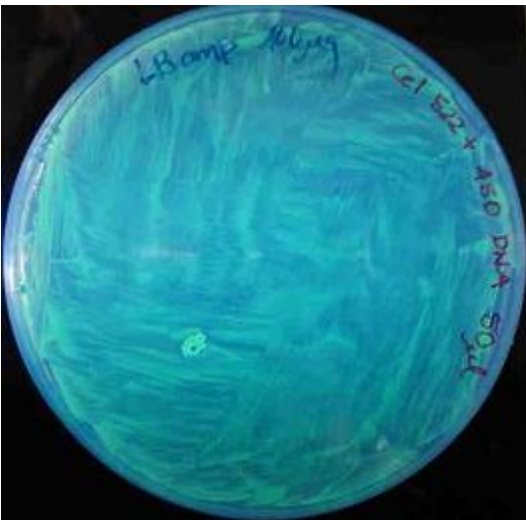
We obtained the following:



Competent cells that produce GFP without DNA



Competent cells that produce GFP + 450 DNA



Competent cells + 450 DNA

Conclusion: we didn't obtain transformants.