XYLENE_INTERACTION_WITH ECOS

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Question: Will ECOS create AmilCP in the presence of xylene? If so to what extent?

Hypothesis: If the concentration of xylene is great enough, we will get blue colonies/pellets. We predict the dark colonies possibly had issues during ligation and therefore will not properly react to the xylene. Or no color difference will be detectable if they are already producing AmilCP. The light colonies, should produce AmilCP if enough xylene is present.

Materials: xylene, plates containing ECOS ligated to AmilCP, broth containing ECOS ligated to AmilCP, alginate beads, containing ECOS ligated to AmilCP, micro pipettes, incubator shaker table, recording materials.

Variables: Manipulated- concentration of xylene and mode of delivery (plates vs Lb vs beads)
Responding- Amount of AmilCP produced
Controlled- time left to grow, cell cultures used, antibiotics used, amount of broth/growth allowed.

Procedure: 1) Ensure you have adequate amounts of ECOS. Spread plates with 200 microliter of LB broth. Make four plates from each tube. 16 plates in total. Ensure to label properly. Aliquot LB into four tubes per tube (9 mL of straight LB and 1 mL LB with ECOS). 16 tubes in total. Let grow for 3-5 hours in incubator and shaker table respectively. Create beads with LB containing ECOS

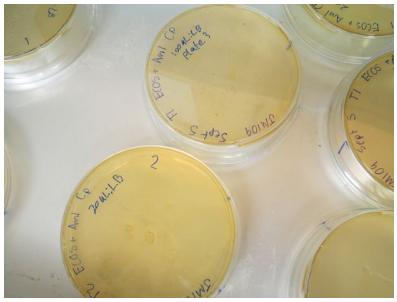
2) Add different concentrations of Xylene to plates, tubes and beads.

3) Let grow, periodically checking results.

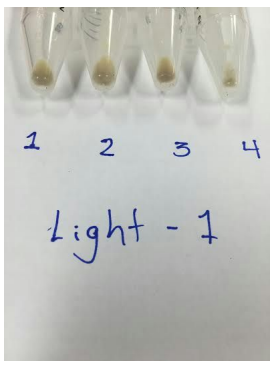
4) Record results

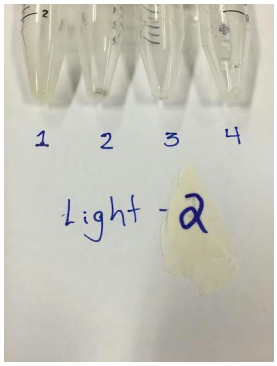
Results Trial One:

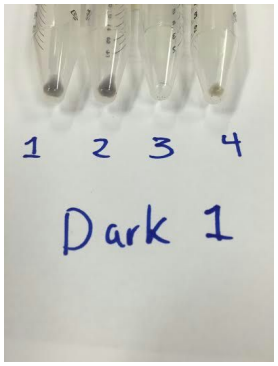
Plates- Trial One	Light 1	Light 2	Dark 1	Dark 2
1-control	Overgrowth	Overgrowth	Overgrowth	Overgrowth
2-10 microliter	Over Growth	Overgrowth	Overgrowth	Overgrowth
3-100 microliter	Overgrowth	Overgrowth	Overgrowth	Overgrowth
4-250 microliter	Overgrowth	Overgrowth	Overgrowth	Overgrowth

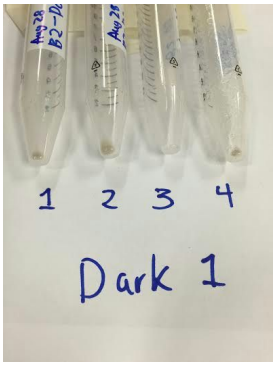


Tubes- Trial One	Light 1	Light 2	Dark 1	Dark 2
1-control	Dark cells around edge. Light in centre	Light cells	Very dark	Dark
2-1 microliter	Dark cells around edge	Small amount of dark cells	Very dark	Dark
3-25 microliter	Dark cells all over.	No growth	No growth	No growth
4-500 microliter	Some dark cells	No growth	Dark	Dark









Analysis: We did have some positive results with this lab. It seems that the dark colonies are simply producing amilCP constantly as our negative controls had dark growth as well. We are therefore focusing on our light colonies. Light two had light cells in our negative control and dark cells in the one tube that grew which was good. We were producing cells when xylene was present in 100ppm. In light one, it seemed there may have been an error with our lab work as there was some dark cells around the very edges of the cells when they were

spun down. We had better growth in light one although it seems our production of AmilCP was regulated more by the level of cell growth than by the concentration of xylene present.

Conclusion: Our hypothesis was correct in that we saw amilCP production when there was xylene present. This was encouraging although a colour difference was difficult to detect. We will need to run more trials to come to a proper conclusion concerning the optimum point in their growth and to eliminate variables such as level of growth and human error in colour differentiation.

Errors: Negative control in light one seemed to produce some xylene. This may have been a mistake in putting xylene in the wrong tube or even just leaving the lid open when xylene was open in the room. Cells also grew overnight and then were left in the incubator with xylene for several more hours. This may have affected growth outcome.

Results Trial Two:

Tubes- Trial Two	Light 1.1	Light 1.2	Light 2.1	Light 2.2
1-control	Light Cell Growth	Light cells	Light Cells	Light Cells
2- .5 microliter	Dark Tinge when compared with control.	Dark tinge	Dark in centre	Dark tinge
3- 10 microliter	Dark Cells in Centre	Semi dark cells	Dark tinge with compared with control	Dark Cells
4- 50 microliter	Dark cells	Dark Cells	Dark Cells	Dark Cells




Analysis: During this trial we used only light colonies and chose different concentrations of xylene to add to our 10mL of LB. We found that this time, leaving them in the incubator for shorter periods of time, we had much more uniform growth. Our output therefore did not have any correlation with the amount of cell growth. We did see very positive results. We had the darkest cells in the 50 uL tube and no dark cells in the negative control. We also were able to detect 50ppm which was quite positive news.

Conclusion: We have successfully ligated AmilCP into the backbone of JM109 with ECOS. We are seeing production of AmilCP in the presence of xylene and in correlation to the concentration of xylene.

Errors: Our plates once again did not grown so we were unable to see results in that capacity. The color change was also not a drastic one and it is difficult to see the change without a comparison to the negative control. We need a computer program to pick up the exact wavelength if we are going to be able to create a scale of any sort to use in the field.