UBC iGEM 2015 Screening Notebook

May 4, 2015

1. Created stocks of fosmid pools obtained from the Hallam Lab
    - Goal:
        - We wanted to screen fosmid libraries from the Hallam Lab for imidacloprid-degradation enzymes, and incorporate the respective genes into our imidacloprid-degrading bee-gut bacteria (gilliamella or snodgrassella)
        - We hoped to create stocks of fosmids with environmental DNA from soil and other sources, as collected by the Hallam Lab
    - Method:
        - Prepared approximately 80 ml of LB+10% glycerol media, and added 80 microliters of arabinose, leucine, and 12.5 M chloramphenicol (dissolved in ethanol).
        - In four 96-well plates, 100 microliters of this prepared media was added to each well.
        - 100 microliters from each sample of Hallam lab fosmid pools were pipetted into each well – which corresponded to a particular fosmid pool
        - The plates were left under 37°C for about 3-4 hours and then transferred to a -80°C freezer for storage until use.
        - In total, 4 plates were made, with each plate corresponding to glycerol stocks of fosmid pooled samples belonging to different metagenomic libraries. Plates 1-3 samples were collected from soil environments, whereas plate 4 samples were collected from various other sources

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INITIAL SCREENING ASSAY

May 27, 2015

1. Determined how toxic acetonitrile was to E. coli (Epi-300)
    - Goal:
        - We wanted to determine whether acetonitrile was toxic to E. coli at some concentration, since imidacloprid was ordered suspended in acetonitrile solution
    - Method:
        - Prepared 6 ml of minimal media, and added 6 microliters of arabinose, leucine and 12.5 M chloramphenicol
        - In a 96-well plate, different concentrations of acetonitrile solution were dissolved in minimal media: (0%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 5%, 10%)

    - A total of 200 microliters of the prepared media (minimal media + acetonitrile) were added to each well - with three replicates made for each corresponding concentration
    - 5 microliters of E. coli cells (from Epi-300 glycerol stocks) were then added to each well with the above components
    - The plate was incubated on a shaker at 37°C

2. Made minimal media with glucose as the sole carbon source
    - 5x M9 Salts; 1 M $MgSO_4$; 1 M $CaCl_2$; 20% Glucose; MilliQ (Distilled) Water
    - Combined the above and filter-sterilized

May 28, 2015

1. Results from May 27 Experiment
    - Wells with 0-2% acetonitrile turned cloudy white, indicating that biomass grew
    - Wells with 5% and 10% acetonitrile stayed colorless, meaning no growth

2. Determined how toxic imidacloprid suspended in acetonitrile solution was to E. coli (Epi-300)
    - Goal:
        - We wanted to determine whether imidacloprid was toxic to E. coli at some concentration, in order to determine the direction of our screening assay (whether we were going to screen with imidacloprid as the sole carbon source, or with imidacloprid at its toxic concentration)
    - Method:
        - Prepared 5 ml of minimal media, and added 5 microliters of arabinose, leucine and 12.5 M chloramphenicol
        - In a 96-well plate, different concentrations of imidacloprid suspended in acetonitrile were dissolved in minimal media (0%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 2.5%) - assuming there was 100 nanograms of imidacloprid per microliter of acetonitrile
        - A total of 200 microliters (of minimal media + imidacloprid dissolved in acetonitrile) were added to each well
        - 5 microliters of E. coli cells (from Epi-300 glycerol stocks) were then added to each well containing the above components - three replicates were made for each corresponding concentration
        - The plate was incubated on a shaker at 37°C

May 29, 2015

1. Results from May 28 2015 Experiment
    - All wells turned cloudy white, indicating that biomass grew at all the tested imidacloprid (suspended in acetonitrile) concentrations

June 1, 2015

1. Determined how toxic imidacloprid solution was to E. coli (Epi-300)
    - Goal:
        - We wanted to determine whether imidacloprid was toxic to E. coli at a concentration beyond that tested on 05.29.2015. It was not possible to test higher concentrations of imidacloprid, due to acetonitrile toxicity beyond a concentration of 2.5%.
- Method:
    - Prepared 3 ml of minimal media, and added 3 microliters of arabinose, leucine and 12.5 M chloramphenicol
    - A stock solution of Imidacloprid at (5 mg/1 ml) was made in an eppendorf tube, by suspending 5 mg of imidacloprid in 1 ml of minimal media
    - In a 96-well plate, this stock solution was diluted by 20x, 100x, 200x, 500x, 1000x, 2000x
    - A total of 200 microliters (of prepared minimal media + diluted imidacloprid solution) were added to each well
    - 5 microliters of E. coli cells (from Epi-300 glycerol stocks) were then added to each well containing the above components - with duplicates made for each corresponding concentration
    - The plate was incubated on a shaker at 37°C

June 2, 2015

1. Results from June 1 Experiment
    - All wells turned cloudy white, indicating that biomass grew at all tested imidacloprid concentrations
        - We concluded that Imidacloprid is not significantly toxic to E. coli
    - Decided to perform a screening assay with imidacloprid as a sole carbon source and to use a 1:1000 dilution of 5mg/1ml imidacloprid in minimal media due to imidacloprid's relatively low solubility. Growth of biomass was also decided as the phenotype to measure the expression of potential imidacloprid-degrading enzymes, as we are making the assumption that overwhelming growth in imidacloprid-present conditions would indicate the ability of cells to degrade imidacloprid and use its degradation-products for metabolic processes.

2. Screening of plates 1 and 2 for imidacloprid-degrading enzymes
    - Method:
        - Prepared 50 ml of minimal media, and added 50 microliters of arabinose, leucine, 12.5 M chloramphenicol, and imidacloprid stock solution (5 mg/ml)
        - In two new 96-well plates, a total of 200 microliters of the prepared media were added to each well
        - 5 microliters of fosmids from each well of plates 1 and 2 (prepared on May 4, 2015) were added to each corresponding well on the two new plates

- Film was added on top of the new plates to prevent drying, and were incubated on a shaker at 37°C
- Plates 1 and 2 glycerol stocks containing fosmid samples were returned to the -80°C freezer for storage

June 3, 2015

1. Results from June 2 Experiment
    - None of the wells turned significantly cloudy white, indicating that no biomass grew
        - The plates were put back on a shaker at 37°C for one more day, to determine if there would be any further growth after an extended incubation period

June 5, 2015

1. Results from further incubation on June 3
    - Still, none of the wells turned significantly cloudy white, indicating that no biomass grew
        - These plates were disposed of in the biohazardous waste bin

2. Screening of plates 3 and 4 for imidacloprid-degrading enzymes
    - Same procedure as what was done for plates 1 and 2

June 6, 2015

1. Results from June 5 Experiment
    - Several of the wells in plates 3 and 4 turned cloudy white, indicating that biomass grew
        - Decided to passage biomass from all wells into new 96-well plates

2. Passaged cells obtained from plates 3 and 4 (made on June 5) into new 96-well plates
    - Goal:
        - Passaging cells was done in order to extend the lifespan of the culture as well as to increase the number of cells present. Passaging also ensured that residual growth was eliminated and thus prevented false positive results.
    - Method:
        - Prepared 50 ml of minimal media, and added 50 microliters of arabinose, leucine, 12.5 M chloramphenicol, and imidacloprid stock solution (5 mg/ml)
        - In two new 96-well plates, a total of 200 microliters of the prepared media were added to each well
        - 5 microliters of cells from each well of plates 3 and 4 were added to each corresponding well on the two new plates

- Film was added on top of the plates to prevent drying, and were incubated on a shaker at 37°C
- Plates 3 and 4 containing initial cultures were put in a 4°C room for storage

June 8, 2015

1. Results from June 6 Experiment
    - After visual inspection, clumps of cloudy white cells were found settled at the bottom of wells A1, A2, and D2 of plate 4, indicating that biomass grew only in these wells
        - SCR005_2, SCR005_1, FOS62_28 were designated as potential hits
        - All other wells were clear, indicating misses

2. The corresponding potential hits in plate 4 were cultured in test tubes
    - Goal:
        - Potential hits were cultured in test tubes in order to facilitate more growth of biomass. A higher concentration of imidacloprid solution was also used in order to more rigorously induce and select for potential imidacloprid-degrading enzymes.
    - Method:
        - Prepared 135 ml of minimal media, and added 135 microliters of arabinose, leucine, and 12.5 M chloramphenicol.
        - 10 ml of the prepared media was added into each of 12 test tubes
        - Each test tube was labelled with the names of the wells corresponding to potential hits as well as a "+" (indicating the addition of imidacloprid solution) or a "-" (indicating the addition of only minimal media containing glucose, but no imidacloprid); duplicates of each were made
        - 50 microliters of imidacloprid was added into 6 of the test tubes labelled "+", whereas 50 microliters of minimal media (with only glucose) was added into 6 of the test tubes labelled "-"
        - 10 microliters of fosmids from the respective wells on plate 4 (prepared on May 4) was added to the corresponding test tubes
        - The test tubes were capped and incubated on a shaker at 37°C
        - Plate 4 glycerol stocks containing fosmid samples was returned to the -80°C freezer for storage

3. Re-screening of plates 1 and 2 for imidacloprid-degrading enzymes
    - Screening was repeated for plates 1 and 2 due to the possibility that the initial experiment may not have been accurate.
    - See rationale and protocol for this experiment under June 2, 2015 notes

June 9, 2015

1. Results from June 8 experiments

- The “- imidacloprid” test tubes containing cultures of potential hits from plate 4, showed just as much, if not more biomass than the corresponding “+ imidacloprid” test tubes
    - Decided to return the test tubes for incubation on a shaker at 37°C for an additional 24 hours in order to see if more growth would ensue
- Several of the wells in plates 1 and 2 turned cloudy white, indicating that biomass grew
    - Decided to passage biomass from all wells into new 96-well plates

2. Passaged cells obtained from plates 1 and 2 (made on June 8) into new 96-well plates
    - Same procedure as that for plates 3 and 4

June 10, 2015

1. Results from June 9 Experiments
    - After visual inspection, clumps of cloudy white cells were found settled at the bottom of wells D11, E2, E12, and G12 of plate 1, as well as A1, B9, C9, C10, D9, E6, E9, E12, and G10 of plate 2; indicating that biomass grew only in these wells
        - NA002_1, NA003_2, NA004_4, NB001_21, NR001_1, NR001_21, NR001_33, NR001_34, NR001_45, NR001_54, NR001_57, NR001_60, and CA231_4 were designated as potential hits
        - All other wells were clear, indicating misses
    - The test tube cultures (prepared on June 8) corresponding to potential hits from plate 4 still showed the same result
        - Decided to return the test tubes for incubation on a shaker at 37°C for an additional 24 hours in order to see if more growth would ensue since it is possible that imidacloprid degradation for these cultures is not immediate

2. The corresponding potential hits in plates 1 and 2 were cultured in test tubes
    - Same procedure as that for plate 4 potential hits

June 11, 2015

1. Results from June 10 Experiments
    - The “- imidacloprid” test tubes containing cultures of potential hits from plates 1 and 2, showed just as much, if not more biomass than the corresponding “+ imidacloprid” test tubes
        - Decided to return the test tubes for incubation on a shaker at 37°C for an additional 24 hours in order to see if more growth would ensue
    - The test tube cultures (prepared on June 8) corresponding to potential hits from plate 4 still showed the same result
        - Decided to return the test tubes for incubation on a shaker at 37°C for an additional week, and check on them daily, in order to see if more growth

would ensue since it is possible that imidacloprid degradation for these cultures is not immediate

2. Screening of pooled metagenomic libraries from plate 1-4 for imidacloprid-degrading enzymes
    - Goal:
        - Because there were no significant hits from cultures of individual fosmid pools, we decided to combine all said individual fosmid pools that belonged to the same metagenomic library together - resulting in a total of 9 fosmid library pools for plate 1. It is possible that imidacloprid-degradation is the result of cooperation between different bacteria in the environment, so pooling fosmid pools belonging to the same library together would yield a closer representation of this phenomenon.
    - Method:
        - 29 cryogenic tubes were labelled with the library names belonging to the plates 1-4 glycerol stocks (made on May 4)
        - 5 microliters of each fosmid pool belonging to a particular library were combined in the respective cryogenic tube
        - Prepared 95 ml of minimal media, and added 95 microliters of arabinose, leucine, 12.5 M chloramphenicol
        - 5 ml of the prepared media was added into each of 58 test tubes
        - Each test tube was labelled with the names of the libraries on plate 1-4 as well as a “+” (indicating the addition of imidacloprid solution) or a “-” (indicating the addition of only minimal media containing glucose, but no imidacloprid)
        - 25 microliters of imidacloprid was added into 29 of the test tubes labelled “+”, whereas 25 microliters of minimal media (with only glucose) was added into 29 of the test tubes labelled “-”
        - 5 microliters of fosmids from the respective cryogenic tubes was added to the corresponding test tubes
        - The test tubes were capped and incubated on a shaker at 37°C
        - The 9 cryogenic tubes of glycerol stocks containing pooled fosmid library samples were put in the -80°C freezer for storage

June 12, 2015

1. Results from June 11 Experiments
    - The test tube cultures corresponding to potential hits from plate 4 still showed the same result
    - The test tube cultures corresponding to potential hits from plate 1 and 2 also still showed the same result
        - Decided to return the test tubes for incubation on a shaker at 37°C for an additional week, and check on them daily, in order to see if more growth would ensue since it is possible that imidacloprid degradation for these cultures is not immediate

- The “- imidacloprid” test tubes containing cultures of pooled fosmid library samples from plate 1, showed just as much, if not more biomass than the corresponding “+ imidacloprid” test tubes
    - Decided to return the test tubes for incubation on a shaker at 37°C for an additional 24 hours in order to see if more growth would ensue

June 15, 2015

1. Results from all previous experiments
    - “+” test tubes corresponding to fosmid pooled libraries: SCR006, NR001, and CG23A showed slightly more biomass than their “-“ test tube counterparts
    - The rest of the test tube cultures corresponding to potential hits had little to no change
    - It was decided that a new screening assay would be adopted in order to more rigorously screen for imidacloprid-degradation enzymes, as such all previous plates and test tubes were disposed of

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NEW SCREENING ASSAY

1. Experimentation was shifted towards high-throughput screening methods
    - All screening was done in either 96-well or 384-well plates
    - Degradation genes for imidacloprid were screened for using two protocols: where imidacloprid was used either as the sole carbon source or as the sole nitrogen source for fosmid growth
    - Significantly more imidacloprid was used – 1.5 mg/ml imidacloprid solution was added into each well
    - To determine the extent of cell growth, we measured optical density (OD) at an absorbance of 600 nm using a plate reader
    - From June 22 - 29, screening was done with imidacloprid as sole carbon source
    - From July 1 - onwards, screening was done with imidacloprid as sole nitrogen source

June 22, 2015

1. Screening of plates 1 - 4 for imidacloprid-degrading enzymes
    - Method:
        - Prepared 35 ml of minimal media, by adding 7 mL of 5x M_9 salts, 70 microliters of 1 M $MgSO_4$, 3.5 microliters of 1 M $CaCl_2$, 1.75 mg of imidacloprid, and 1.4 mg of glucose, distilled milliQ water (to 35 mL mark),

35 microliters each of arabinose, 12.5 M chloramphenicol and leucine together, mixing this solution using a vortex, and then filter-sterilizing it using a syringe and acro-disk
    - In one new 384-well plate, a total of 80 microliters of the prepared media were added into each well using a multi-channel pipette
    - 2 microliters of fosmids from each well of plates 1 – 4 (prepared on May 4, 2015) were correspondingly added to the 384-well plate in the following fashion:

	1	2
A	Fosmid from Plate 1	Fosmid from Plate 2
B	Fosmid from Plate 3	Fosmid from Plate 4

    - Film was added on top of the new plate to prevent drying, and were incubated on a shaker at 37°C
    - Plates 1-4 glycerol stocks containing fosmid samples were returned to the -80°C freezer for storage

June 23, 2015

1. Results from June 22 Experiment
    - Several of the wells turned cloudy white, indicating that biomass grew
    - Decided to passage biomass from all wells into a new 384-well plates

2. Passaged cells obtained from 384-well plate (made on June 22) into a new 384-well plate
    - Method:
        - Prepared 35 ml of minimal media, by adding 7 mL of 5x M_9 salts, 70 microliters of 1 M $MgSO_4$, 3.5 microliters of 1 M $CaCl_2$, 50 mg of imidacloprid, distilled milliQ water (to 35 mL mark), 350 microliters of DMSO, 35 microliters each of arabinose, 12.5 M chloramphenicol and leucine together, mixing this solution using a vortex, and then filter-sterilizing it using a syringe and acro-disk
        - In one new 384-well plate, a total of 80 microliters of the prepared media were added into each well using a multi-channel pipette

- Fosmids from each well of the plate prepared on June 22 were added to each corresponding well on the new 384-well plate using a QPIX Robot
- Film was added on top of the new plate to prevent drying, and were incubated on a shaker at 37°C
- The old 384-well plate was placed in a 4°C room for storage

June 24, 2015

1. Results from June 23 Experiment
    - None of the wells on the passaged 384-well plate turned cloudy white, meaning that no biomass grew
    - Decided to re-passage the 384-well plate prepared on June 22 on yet a new 384-well plate, and dispose the one prepared on June 23

2. Re-passaged cells obtained from 384-well plate (made on June 22) into a new 384-well plate
    - Method:
        - Prepared 35 ml of minimal media, by adding 7 mL of 5x M_9 salts, 70 microliters of 1 M $MgSO_4$, 3.5 microliters of 1 M $CaCl_2$, 50 mg of imidacloprid, distilled milliQ water (to 35 mL mark), 350 microliters of DMSO, 35 microliters each of arabinose, 12.5 M chloramphenicol and leucine together in a 50 mL Falcon tube, mixing this solution using a vortex, and then filter-sterilizing it using a syringe and acro-disk
        - In one new 384-well plate, a total of 80 microliters of the prepared media were added into each well using a multi-channel pipette
        - 2 microliters of fosmids from each well of the plate prepared on June 22 were added to each corresponding well on the new 384-well plate using a a multi-channel pipette
        - OD at 600 nm absorbance was measured using a plate-reader in order to determine a baseline OD at Day 0
        - Film was added on top of the new plate to prevent drying, and were incubated on a shaker at 37°C

3. Screening of pooled libraries for imidacloprid-degrading enzymes
    - Method:
        - Prepared 20 ml of minimal media, by adding 20 mL of (5x M_9 salts + 1 M $MgSO_4$ + 1 M $CaCl_2$), 28.4 mg of imidacloprid, distilled milliQ water (to 20 mL mark), 200 microliters of DMSO, 20 microliters each of arabinose, 12.5 M chloramphenicol and leucine together in a 50 mL Falcon tube, mixing this solution using a vortex, and then filter-sterilizing it using a syringe and acro-disk

- In one new 96-well plate, a total of 200 microliters of the prepared media were added into each well using a multi-channel pipette
- 2 microliters of pooled fosmid libarires from each cryogenic tube prepared on June 11 were added to 3 wells of the new plates (to represent 3 replicates) in the following fashion:

	1	2	3	4	5	6	7	8	9	10	11	12
A		NR001			NO002			NA001			NA002	
B		NA003			NA004			NB001			CB001	
C		SCR003			NR001			CO002			CO003	
D		CA231			CA233			CO004			CA232	
E		CB002			CB003			CB004			CB005	
F		CB006			SCR00			SCR002			SCR004	
G		SCR005			SCR00			FOS62			CG23A	
H		Beaver										

- OD at 600 nm absorbance was measured using a plate-reader in order to determine a baseline OD at Day 0
- Film was added on top of the new plate to prevent drying, and were incubated on a shaker at 37°C

June 25, 2015

1. Results from June 24 Experiment
    - Wells corresponding to NR001, CB004, SCR004, and FOS62 showed increased growth when OD was measured after 1 day
        - These pooled libraries were designated as potential hits.

June 26, 2015

1. Determining whether DMSO had any effect on cell growth of pooled libraries designated as potential hits, and whether increasing imidacloprid concentration would affect cell growth
    - Method
        - Prepared 10 ml of minimal media, by adding 10 mL of (5x M_9 salts + 1 M $MgSO_4$ + 1 M $CaCl_2$), distilled milliQ water (up to 10 mL mark), 10 microliters each of arabinose, 12.5 M chloramphenicol and leucine together in a 50 mL Falcon tube, mixing this solution using a vortex, and then filter-sterilizing it using a syringe and acro-disk

- Prepared 4 (1.7 ml) Eppendorf tubes each labelled with experimental conditions, and added the corresponding components:
    1. 3 mg imidacloprid/2 mL minimal media + 1% DMSO
    2. 6 mg imidacloprid/2 mL minimal media + 1% DMSO
    3. 9 mg imidacloprid/2 ml minimal media + 1% DMSO
    4. 3 mg imidacloprid/2ml minimal media (no DMSO added)
- In one new 96-well plate, a total of 200 microliters of the prepared solutions in the Eppendorf tubes were added into each well using a multi-channel pipette in the following fashion:

	1	2	3	4	5	6	7	8	9	10	11	12
NR001		3 mg/2mL imidacloprid + 2 microliters DMSO			6 mg/2mL imidacloprid + 2 microliters DMSO			9 mg/2mL imidacloprid + 2 microliters DMSO			3 mg/2mL imidacloprid + no DMSO	
CB004												
SCR004												
Fos62												

- OD at 600 nm absorbance was measured using a plate-reader in order to determine a baseline OD at Day 0
- Film was added on top of the new plate to prevent drying, and were incubated on a shaker at 37°C

June 27, 2015

1. Results from June 26 Experiment

- Only wells corresponding to the "no DMSO" condition of NR001, showed more growth than the corresponding "+ 1% DMSO" condition for the same pooled library when OD was measured after 1 day
    - NR001 individual fosmid pools were subject to further experimentation to determine whether cells were growing due to the presence of DMSO and that the acquired result is not a false positive

June 28, 2015

1. Determining whether DMSO had any effect on cell growth of NR001 individual fosmid pools

- Method
    - Prepared 12.5 ml of minimal media, by adding 12.5 mL of (5x M_9 salts + 1 M $MgSO_4$ + 1 M $CaCl_2$), distilled milliQ water (up to 12.5 mL mark), 12.5 microliters each of arabinose, 12.5 M chloramphenicol and leucine together in a 50 mL Falcon tube, mixing this solution using a vortex, and then filter-sterilizing it using a syringe and acro-disk
    - Prepared 2 (1.7 ml) Eppendorf tubes each labelled with experimental conditions, and added the corresponding components:
        1. 3 mg imidacloprid/2 mL minimal media + 1% DMSO
        2. 3 mg imidacloprid/2ml minimal media (no DMSO added)
    - In one new 384-well plate, a total of 200 microliters of the prepared solutions in the Eppendorf tubes were added into each wells using a multi-channel pipette in the following fashion:

	1	2
A	NR001_ 1 "+"	NR001_ 1 "-
B	NR001_ 1 "+"	NR001_ 1 "-"

        1. One quadrant of wells corresponds to one individual fosmid pool belonging to NR001
        2. "+" indicates that 1% DMSO was added
        3. "-" indicates that no DMSO was added
    - OD at 600 nm absorbance was measured using a plate-reader in order to determine a baseline OD at Day 0
    - Film was added on top of the new plate to prevent drying, and were incubated on a shaker at 37°C

June 29, 2015

1. Results from June 28 Experiment
    - None of the wells corresponding to the "no DMSO" condition of NR001, showed more growth than the corresponding "+ 1% DMSO" condition for the same pooled library when OD was measured after 1 day
        - All individual fosmid pools belonging to NR001 were misses

July 2015

- Falcon-tubed some libraries in order to grow more promising cultures
- Passaged pools, 0.1% imidacloprid, 1 microlitre bacteria
- The following fosmid pools were designated as potential hits

NB001	Nr003	NO00_1	NO00_2	NA00_2,3,4	NA001	CO004	CO002+3	CBOO1+2	CBOO4+5	CA23	SRC001+2	SCR003+5

July 22, 2015

- Passaged NO00_1 and NA001 in a 96-well plate + 0.1% imida, + 0.5 microlitres bacteria

July 24, 2015

- Prepared 8.3 mL total of minimal media with 8.3 microlitres each of CM, arab, leucine and 4 mg imidacloprid
- Passaged CO004 pooled library with 0.1% imidacloprid in a 96-well plate along with 0.5 microlitres bacteria

July 27, 2015

- Checked the plates and there was no significant growth

July 29, 2015

- Plate read 2 plates: NA001 and NO001 plates show promise
    - Although these could be false positives, with only ~0.45 OD difference
- Repassaging NA001_2, NA001_4, NA001_6, NA001_7, NA001_10, NA001_14 (as these all show an OD greater than 2.5)
- CA004 pooled library is a no go at all.

August 1, 2015

- Re-passaged two promising hits from earlier trials

August 6, 2015

- The NO001 and SCR pools in 2ml falcons tubes don't have any growth when using imidacloprid as the sole nitrogen source

- 16ml of imidacloprid was added into 8 ml of minimal media for the positive condition as well as 2 duplicates of each in the negative condition, and then 0.5 microliters of bacteria was re-inoculated

August 10, 2015

- SCR001_24 and NO001 pooled library both grew in all three triplicates, clearly indicating that some other component was being degraded.