CONSTRUCT DESIGN

Phalate

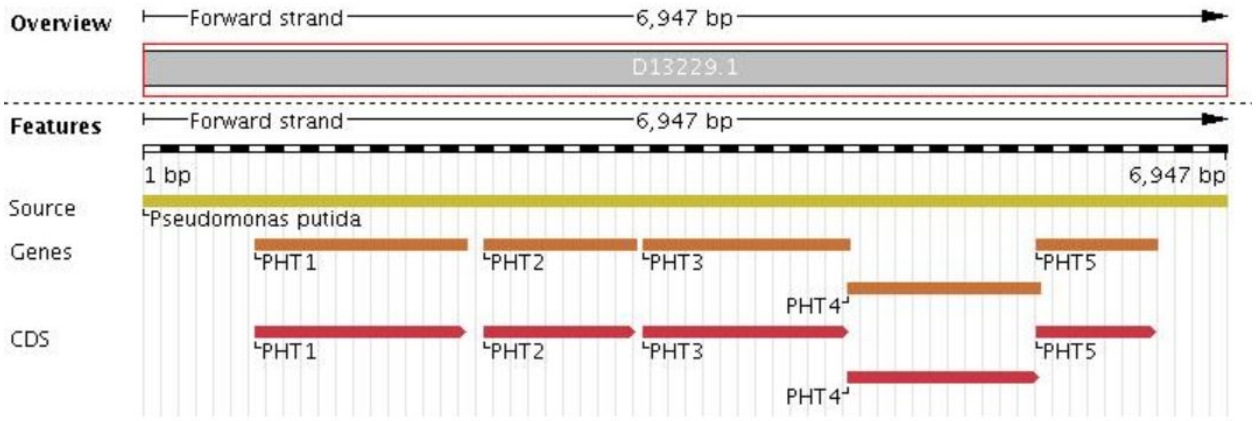
Purpose/Goals:
Identify genomic data and identify genes contributing to the breakdown of phthalate degradation.
Design synthetic genes and implement them into *E. coli*.

Rationale:
Phthalate (benzene-1,2-dicarboxylate) is another common intermediary, along with salicylate in degradation pathways of many polycyclic aromatic hydrocarbons. PAHs are identified as a priority pollutant by the EPA due to their toxicity to organisms and their relative increase in abundance due to human activity which are mainly derived from oil-related activities and combustion.
Although, many non-photosynthetic bacteria can degrade Phthalate; Very little is known about the specific enzymes involved in the breaking down of Phthalate. Also, many of the non-photosynthetic bacteria that can degrade PAHs are harmful to humans.

Design:
The catabolic pathway was synthesized as one operon with the codon-optimized for expression in *E.coli*. The source of the genes was from Pseudomonas putida. Since the operon consists of only 5 genes,the catabolic pathway was kept as one and was added a promoter(>BBa_J23100_RBS) and the BBa_K731721 T7 terminator sequence for several reasons:
(i) To facilitate the synthesis of the genes (cost-effective and in a timely manner) by submitting short sequences when added in conjunction to e.coli with other operons whose final product is phthalate;
(ii) To ensure a good level of expression of the polycistronic genes;
(iii) To determine if there were orientations of the two polycistronic operons that may be more favorable for expression, in other words, to optimize the gene order;
(iv) To minimize toxicity issues that may arise when the full pathway is synthesized with all the genes;
(v) To identify which, if any, fragment would present a toxic or metabolic burden to E.coli; and
(vi) To give a certain level of modularity and make it more flexible for others to use in additional applications

The synthetic sequences were designed according to IGEM requirement removing restriction sites that are restricted to prefix and suffix sequences. The codon was optimized for expression in E.coli with percent of GC around 50%.

Codon Map:



PHT 1:
Phthalate transporter
This protein is involved in the pathway phthalate degradation, which is part of Xenobiotic degradation.Currently no research points to a specific functionality of this protein

PHT2:
Phthalate 4,5-dioxygenase oxygenase reductase subunit
This protein is involved in step 1 of the sub pathway that synthesizes 3,4-dihydroxybenzoate from phthalate.

PHT3:
Phthalate 4,5-dioxygenase oxygenase subunit
This protein is involved in step 1 of the sub pathway that synthesizes 3,4-dihydroxybenzoate from phthalate.

PHT4:
Putative 4,5-dihydroxy phthalate dehydrogenase
This protein is involved in step 2 of the sub pathway that synthesizes 3,4-dihydroxybenzoate from phthalate.

PHT5:
4,5-dihydroxyphthalate decarboxylase
This protein is involved in step 3 of the sub pathway that synthesizes 3,4-dihydroxybenzoate from phthalate.

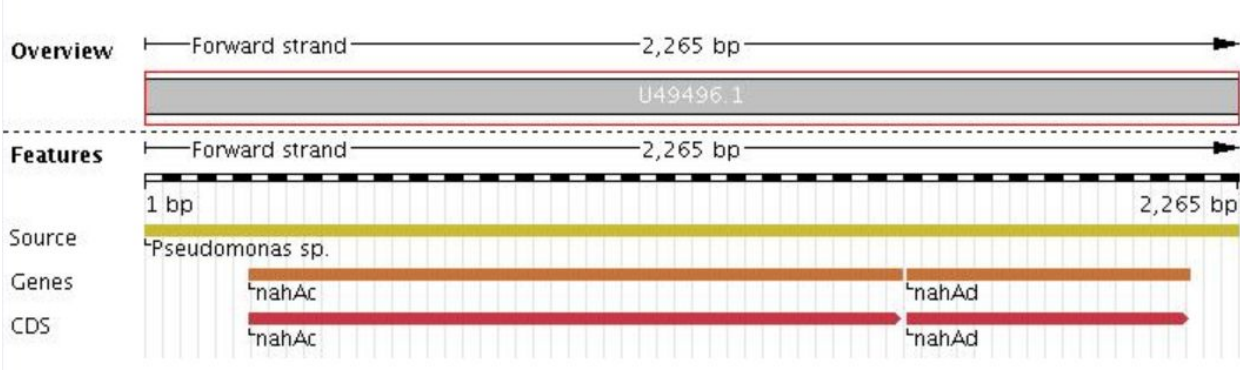
Salicylate

Purpose/Goals:
Identify genomic data and identify genes contributing to the breakdown of salicylate.
Design synthetic genes and implement them into E. coli.

Rationale:
Salicylate (2-hydroxybenzoic acid) is a common intermediary in degradation pathways of many polycyclic aromatic hydrocarbons. PAHs are identified as a priority pollutant by the EPA due to their toxicity to organisms and their relative increase in abundance due to human activity which are mainly derived from oil-related activities and combustion.. We assumed that salicylate is metabolizable by *E.coli* but it was not the case, halting our progress in developing a catabolic pathway for Naphthalene degradation. This our main reason for developing a catabolic pathway for Salicylate.
Although, many non-photosynthetic bacteria can degrade Salicylate; Very little is known about the specific enzymes involved in the breaking down of Salicylate Also, many of the non-photosynthetic bacteria that can degrade PAHs are harmful to humans.

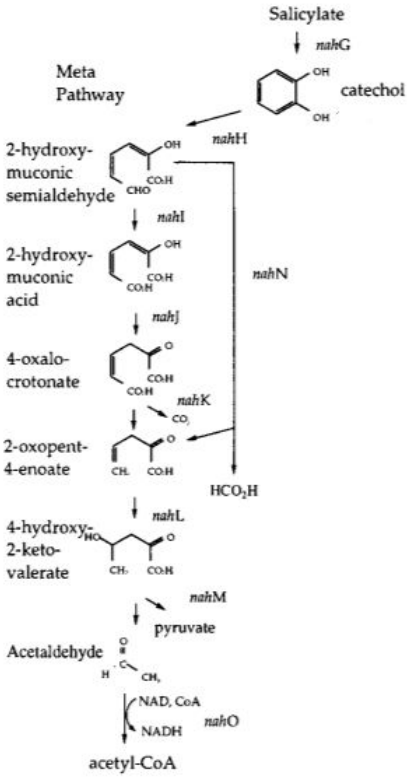
Design:
The catabolic pathway was synthesized as one operon with the codon-optimized for expression in *E.coli*. The source of the genes was from Pseudomonas sp. strain NCIB 9816-4 . Since the operon consists of only 2 genes,the catabolic pathway was kept as one and was added a promoter(>BBa_J23100_RBS) and the BBa_K731721 T7 terminator sequence for several reasons:
(i) To facilitate the synthesis of the genes (cost-effective and in a timely manner) by submitting short sequences when added in conjunction to *E.coli* with other operons whose final product is salicylate;
(ii) To ensure a good level of expression of the polycistronic genes;
(iii) To determine if there were orientations of the two polycistronic operons that may be more favorable for expression, in other words, to optimize the gene order;
(iv) To minimize toxicity issues that may arise when the full pathway is synthesized with all the genes;
(v) To identify which, if any, fragment would present a toxic or metabolic burden to *E.coli*; and
(vi) To give a certain level of modularity and make it more flexible for others to use in additional applications
The synthetic sequences were designed according to IGEM requirement removing restriction sites that are restricted to prefix and suffix sequences. The codon was optimized for expression in *E.coli* with percent of GC around 50%.

Codon Map:



nahAc:
Component of the naphthalene dioxygenase (NDO) multicomponent enzyme system which catalyzes the incorporation of both atoms of molecular oxygen into naphthalene to form cis-(1R,2S)-dihydroxy-1,2-dihydronaphthalene. The alpha subunit has a catalytic role in the holoenzyme. Also able to catalyze the cis-dihydroxylation of biphenyl and phenanthrene
nahAd:
The beta subunit seems to have a structural role in the holoenzyme.

Degradation Pathway:



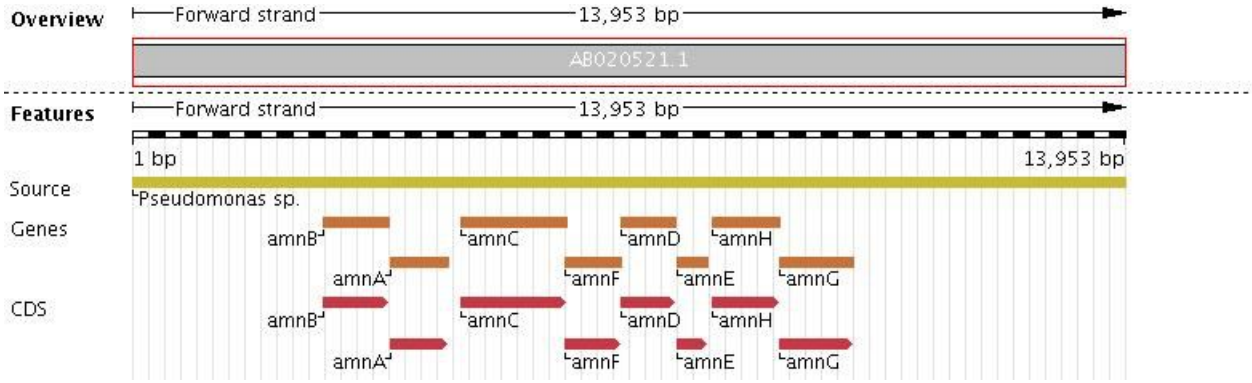
Chrysene

Purpose/Goals:
Identify genomic data and identify genes contributing to the breakdown of chrysene (1,2-benzphenanthrene). Design synthetic genes and implement them into *E. coli*.

Rationale:
Chrysene is a common intermediary in degradation pathways of many polycyclic aromatic hydrocarbons. PAHs are identified as a priority pollutant by the EPA due to their toxicity to organisms and their relative increase in abundance due to human activity which are mainly derived from oil-related activities and combustion. Although, many non-photosynthetic bacteria can degrade chrysene; very little is known about the specific enzymes involved in the breaking down of salicylate. Also, many of the non-photosynthetic bacteria that can degrade PAHs are harmful to humans.

Design:
The catabolic pathway was synthesized as one operon with the codon-optimized for expression in E.coli. The source of the genes was from *Pseudomonas fluorescens*. Since the operon consists of only 2 genes,the catabolic pathway was kept as one and was added a promoter(>BBa_J23100_RBS) and the BBa_K731721 T7 terminator sequence for several reasons:
(i) To facilitate the synthesis of the genes (cost-effective and in a timely manner) by submitting short sequences when added in conjunction to *E.coli* with other operons whose final product is salicylate;
(ii) To ensure a good level of expression of the polycistronic genes;
(iii) To determine if there were orientations of the two polycistronic operons that may be more favorable for expression, in other words, to optimize the gene order;
(iv) To minimize toxicity issues that may arise when the full pathway is synthesized with all the genes;
(v) To identify which, if any, fragment would present a toxic or metabolic burden to *E.coli*; and
(vi) To give a certain level of modularity and make it more flexible for others to use in additional applications
The synthetic sequences were designed according to IGEM requirement removing restriction sites that are restricted to prefix and suffix sequences. The codon was optimized for expression in *E.coli* with percent of GC around 50%.

Codon Map:



- **amnA** catalyzes the ring fission of 2-aminophenol to produce 2-aminomuconic 6-semialdehyde.
- **amnB** catalyzes the ring fission of 2-aminophenol to produce 2-aminomuconic 6-semialdehyde. AmnB seems to be the catalytic subunit of the complex. The enzyme is also active toward 2-amino-p-cresol, 6-amino-m-cresol, 2-amino-m-cresol, 2-amino-4,5-dimethylphenol, 2-amino-4-chlorophenol, and catechol
- **amnC** is involved in the modified meta-cleavage pathway for 2-aminophenol catabolism. The enzyme is also active toward 2-hydroxymuconic 6-semialdehyde, acetaldehyde, propionaldehyde, and butyraldehyde.
- **amnD** is a meta-cleavage pathway for the 2-aminophenol catabolism. Only active toward 2-amino muconic acid.
- **amnE** is involved in the modified meta-cleavage pathway for the 2-aminophenol catabolism.
- **amnF** is involved in the modified meta-cleavage pathway for the 2-aminophenol catabolism. This protein is in *E. coli* so it can intrinsically degrade this compound.
- **amnG** catalyzes the retro-aldol cleavage of 4-hydroxy-2-oxopentanoate to pyruvate and acetaldehyde. It is involved in the meta-cleavage pathway for the degradation of 2-aminophenol. This protein is in *E. coli* so it can intrinsically degrade this compound.
- amnH catalyzes the conversion of acetaldehyde to acetyl-CoA, using NAD+ and coenzyme A. Is the final enzyme in the meta-cleavage pathway for the degradation of 2-aminophenol. This protein is in *E. coli* so it can intrinsically degrade this compound.

Degradation Pathway: