BEST BASIC PART

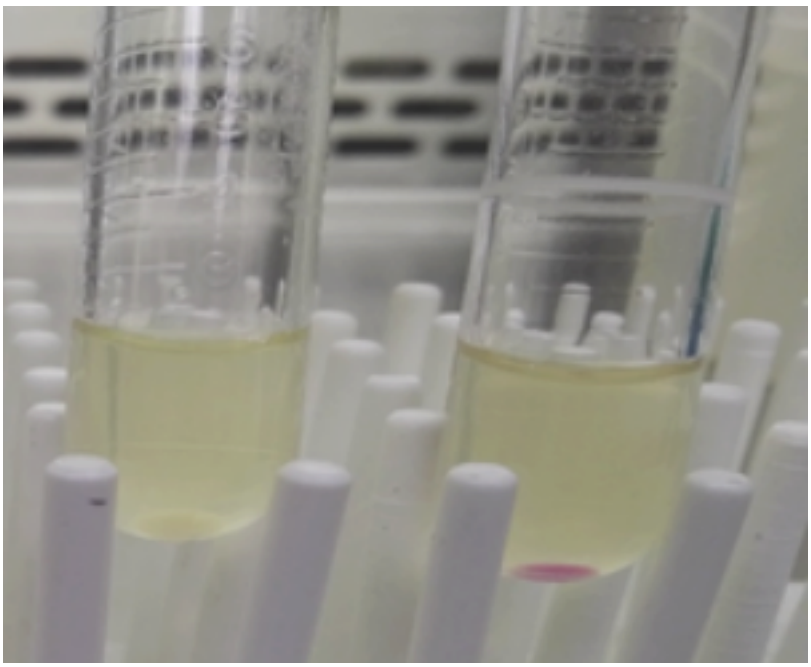
BBa_K2491030

Broad Host Range Vector with Kanamycin resistance (Plasmid Backbone)

We are proud to present our best basic part: a designed plasmid with a RK2 origin of replication to generate a shuttle vector as a broad host range vector (i.e. can replicate in E.coli and other microorganisms).

See our improvements page for more information about this part!

Clone verification: The following primers annealing in the TRFA region of the RK2 backbone vector and the kanamycin region were used to verify the construct by amplification and sequencing of the backbone: TRFA_F: 5'-CCGACGGATGTTCGACTATTT-3', TRFA_R: 5'-AGTAAAGCGCTGGCTGAA-3', KM_F: 5'-AAAGCCGTTTCTGTAATGAAGG-3', and KM_R: 5'-CCTGTTGAACAAGTCTGGAAAG-3'. The data showed that the 2 regions were present. In addition, cultures containing the RK2 backbone harboring the RFP gene [biobrick part BBa_K2491030 containing biobrick part BBa_J04450] showed a red pellet (right vial) compared to a control (left vial) without the RFP gene.



BEST COMPOSITE PART

BBa_K2491027

We describe our best composite part, the P1P2 construct, which provide a platform to efficiently degrade phenanthrene, a toxic polycyclic aromatic hydrocarbon (PAH) present in crude oil spills.

The biodegradation of hydrocarbons is accomplished by a multiple step process under the control of complex sets of 10-15 genes typically dispersed in the genome and plasmids, encoding various classes of enzymes, mainly oxygenase, hydrogenase, and carboxylase. Additional genes are also involved in the regulation of expression of the pathway (activator), genes involved in secreting surfactants to allow the bacteria to mix well with crude oil, and genes involved in the transport of PAH inside the microorganism where the degradation takes place.

To be able to degrade as many aromatic components as possible, our unique approach is to converge the pathways and employ gene augmentation. This approach is possible because there are some intermediates that are common between pathways.
To that end, we cloned the catabolic genes of phenanthrene upstream of the common intermediates and introduced them into a bacteria that would already have the ability to metabolize the intermediates downstream, containing those genes. The degradation maps of phenanthrene can converge with many other PAHs at 2 points: salicylate or phthalate. Therefore, we would introduce the upstream genes of phenanthrene into a strain that is degrading other PAH via the salicylate degradation pathway.

This composite part was created by gene synthesis of selected genes from the phenanthrene pathway from *Burkholderia* sp. RP007. Two separate blocks were synthesized [phnF-phnE-phnC-phnD] and [phnAc-phnAd-phnB]. Then, each block was cloned under the control of three promoters of various strengths from the Anderson series (BBa_J23100, BBa_J23101, and BBa_J23110) and inserted into a high copy plasmid (pSB1C3). Each fragment was then assembled together on vector pSB1C3.

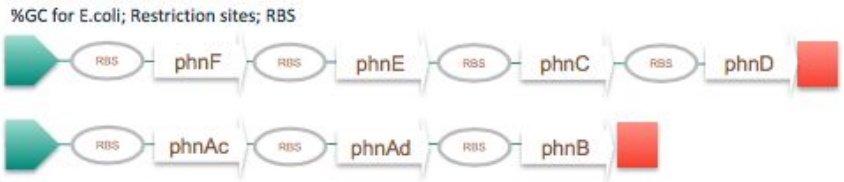


Figure 1. Map of 2 synthetic blocks of 4227 bp and 3174 bp responsible for phenanthrene catabolic pathway. The promoter was either an inducible T7-modified promoter or one of 3 constitutive promoters from the Anderson series. The 2 fragments were cloned separately on a pUC plasmid and then assembled together on a single high copy number plasmid. RBS: Ribosome Binding Site; Blue arrows: promoter; Orange squares: terminator.

Genes	Function	AA	MW (kDa)
phnF	Aldehyde dehydrogenase	562	62.2
phnE	Hydratase-aldolase	360	41.7
phnC	Extradiol dioxygenase	497	52.6
phnD	Isomerase	330	36.5
phnAc	ISPα (large) subunit	275	30.0
phnAd	ISPβ (small) subunit	196	21.9
phnB	Dihydrodiol dehydrogenase	272	28.4

Table 1. Genes of the Upper Catabolic Pathway of Phenanthrene from *Burkholderia* sp. RP007

The construct degraded effectively phenanthrene and crude oil as assessed in biotransformation experiments. Biodegradation experiments consisted of comparing the growth in minimal medium with phenanthrene or crude oil of recombinant E.coli containing the catabolic pathway vs. recombinant E.coli containing an empty vector. Our characterization data are reported on our results page.

Key advantages:

- The design with 2 polycistronic blocks rather than one provide maximum flexibility for subsequent genetic manipulations. If one block would have been toxic, it could have been cloned onto a low copy plasmid.
- By having 2 promoters, this design provides a high expression level to all genes thus minimizing accumulation of toxic intermediates.

- Genes involved in degradation pathway are not always arranged in discrete operons but are frequently dispersed throughout the genome. Our construct has the synthetic genes responsible for phenanthrene degradation [phnF, phnE, phnC, phnD, phnAc, phnAd, and phnB], all clustered in one island.
- This degradation pathway leads to salicylic acid, a compound that is not toxic to bacterial cells and that is a point of convergence between PAH catabolic pathways.
- This set of synthetic genes was designed to be used for eventual gene augmentation considerations.

FOR FURTHER CHARACTERIZATION OF PART PLEASE CHECK BBa_K2491027

PARTS COLLECTION

Composite Part Name	Type	Nickname	Clone Description	Designer
BBa_K2491000	Coding	100_F1	Composite part consisting of the upper fluorene catabolic pathway synthetic ORFs [flnB (1,1a-dihydroxy-1-hydro-9-fluorenone dehydrogenase), dbfA1 (angular dioxygenase large subunit), and dbfA2 (angular dioxygenase small subunit)] under the control of the constitutive promoter BBa_J23100 and RBS BBa_B0034 and carries the terminator BBa_B0015.	Philippe Hansen-Estruch
BBa_K2491001	Coding	100_F2	100_F2 flnE, flnD1, ORF16, and flnC fluorene ORFs Composite part consisting of the upper fluorene catabolic pathway synthetic ORFs [flnE (meta cleavage compound hydrolase), flnD1 (extradiol dioxygenase large subunit), ORF16 (extradiol dioxygenase small subunit and ferredoxin fusion protein), and flnC (short-chain dehydrogenase/reductase] under the control of the constitutive promoter BBa_J23100 and RBS BBa_B0034 and carries the terminator BBa_B0015.	Philippe Hansen-Estruch
BBa_K2491002	Coding	101_F1	Composite part consisting of the upper fluorene catabolic pathway synthetic ORFs [flnB (1,1a-dihydroxy-1-hydro-9-fluorenone dehydrogenase), dbfA1 (angular dioxygenase large subunit), and dbfA2 (angular dioxygenase small subunit)] under the control of the constitutive promoter BBa_J23101 and RBS BBa_B0034 and carries the terminator BBa_B0015.	Philippe Hansen-Estruch
BBa_K2491003	Coding	101_F2	Composite part consisting of the upper fluorene catabolic pathway synthetic ORFs [flnE (meta cleavage compound hydrolase), flnD1 (extradiol dioxygenase large subunit), ORF16 (extradiol dioxygenase small subunit and ferredoxin fusion protein), and flnC (short-chain dehydrogenase/reductase] under the control of the constitutive promoter BBa_J23101 and RBS BBa_B0034 and carries the terminator BBa_B0015.	Philippe Hansen-Estruch
BBa_K2491004	Coding	110_F1	Composite part consisting of the upper fluorene catabolic pathway synthetic ORFs [flnB (1,1a-dihydroxy-1-hydro-9-fluorenone dehydrogenase), dbfA1 (angular dioxygenase large subunit), and dbfA2 (angular dioxygenase small subunit)] under the control of the constitutive promoter BBa_J23110 and RBS BBa_B0034 and carries the terminator BBa_B0015.	Philippe Hansen-Estruch

BBa_K2491005	Coding	110_F2	Composite part consisting of the upper fluorene catabolic pathway synthetic ORFs [flnE (meta cleavage compound hydrolase), flnD1 (extradiol dioxygenase large subunit), ORF16 (extradiol dioxygenase small subunit and ferredoxin fusion protein), and flnC (short-chain dehydrogenase/reductase] under the control of the constitutive promoter BBa_J23110 and RBS BBa_B0034 and carries the terminator BBa_B0015.	Philippe Hansen-Estruch
BBa_K2491007	Coding	100_P1	Composite part consisting of the upper phenanthrene catabolic pathway synthetic ORFs [phnF (Aldehyde dehydrogenase) phnE (Hydratase-aldolase), phnC (Extradiol dioxygenase), and phnD (Isomerase)] under the control of the constitutive promoter BBa_J23100 and RBS BBa_B0034 and carries a terminator BBa_B0015.	Philippe Hansen-Estruch
BBa_K2491008	Coding	100_P2	Composite part consisting of the upper phenanthrene catabolic pathway synthetic ORFs [phnAc (ISPα (large) subunit), phnAd (ISPβ (small) subunit), and phnB (Dihydrodiol dehydrogenase)] under the control of the constitutive promoter BBa_J23100 and RBS BBa_B0034 and carries a terminator BBa_B0015.	Philippe Hansen-Estruch
BBa_K2491009	Coding	101_P1	Composite part consisting of the upper phenanthrene catabolic pathway synthetic ORFs [phnF (Aldehyde dehydrogenase) phnE (Hydratase-aldolase), phnC (Extradiol dioxygenase), and phnD (Isomerase)] under the control of the constitutive promoter BBa_J23101 and RBS BBa_B0034 and carries a terminator BBa_B0015.	Philippe Hansen-Estruch
BBa_K2491010	Coding	101_P2	Composite part consisting of the upper phenanthrene catabolic pathway synthetic ORFs [phnAc (ISPα (large) subunit), phnAd (ISPβ (small) subunit), and phnB (Dihydrodiol dehydrogenase)] under the control of the constitutive promoter BBa_J23101 and RBS BBa_B0034 and carries a terminator BBa_B0015.	Philippe Hansen-Estruch
BBa_K2491011	Coding	110_P1	Composite part consisting of the upper phenanthrene catabolic pathway synthetic ORFs [phnF (Aldehyde dehydrogenase) phnE (Hydratase-aldolase), phnC (Extradiol dioxygenase), and phnD (Isomerase)] under the control of the constitutive promoter BBa_J23110 and RBS BBa_B0034 and carries a terminator BBa_B0015.	Philippe Hansen-Estruch
BBa_K2491012	Coding	110_P2	Composite part consisting of the upper phenanthrene catabolic pathway synthetic ORFs [phnAc (ISPα (large) subunit), phnAd (ISPβ (small) subunit), and phnB (Dihydrodiol dehydrogenase)] under the control of the constitutive promoter BBa_J23110 and RBS BBa_B0034 and carries a terminator BBa_B0015.	Philippe Hansen-Estruch
BBa_K2491013	Project/ CCA-48	100_F1 -F2	CCA-30 [Fluorene insert 1_Ter_BBa_B0015 XbaI/PstI + Promoter BBa_J23100_BBa_B0034 EcoRI/SpeI] as EcoRI/SpeI CCA-36 [Fluorene insert 2_Ter_BBa_B0015 XbaI/PstI + Promoter BBa_J23100_BBa_B0034 EcoRI/SpeI] as XbaI/PstI Vector pSB3T5 as EcoRI/PstI	Philippe Hansen-Estruch
BBa_K2491025	Project/ CCA-51	101_F1 -F2	CCA-30 [Fluorene insert 1_Ter_BBa_B0015 XbaI/PstI + Promoter BBa_J23101_BBa_B0034 EcoRI/SpeI] as EcoRI/SpeI CCA-38 [Fluorene insert 2_Ter_BBa_B0015 XbaI/PstI + Promoter BBa_J23101_BBa_B0034 EcoRI/SpeI] as XbaI/PstI Vector pSB3T5 as EcoRI/PstI	Philippe Hansen-Estruch

BBa_K2491026	Project/ CCA-54	110_F1 -F2	CCA-30 [Fluorene insert 1_Ter_BBa_B0015 XbaI/PstI + Promoter BBa_J23110_BBa_B0034 EcoRI/SpeI] as EcoRI/SpeI CCA-40 [Fluorene insert 2_Ter_BBa_B0015 XbaI/PstI + Promoter BBa_J23110_BBa_B0034 EcoRI/SpeI] as XbaI/PstI Vector pSB3T5 as EcoRI/PstI	Philippe Hansen-Estruch
BBa_K2491027 BEST COMPOSITE	Project/ CCA-57	100_P1 -P2	CCA-23 [Promoter BBa_J23100 /RBS_BBa_B0034 EcoRI/SpeI + Phenanthrene insert 1_Ter_BBa_B0015 XbaI/PstI] as SpeI/PstI CCA-42 [Phenanthrene insert 2_Ter_BBa_B0015 XbaI/PstI + Promoter BBa_J23100_BBa_B0034 EcoRI/SpeI] as XbaI/PstI	Philippe Hansen-Estruch
BBa_K2491028	Project/ CCA-60	101_P1 -P2	CCA-26 [Promoter BBa_J23101 /RBS_BBa_B0034 EcoRI/SpeI + Phenanthrene insert 1_Ter_BBa_B0015 XbaI/PstI] as SpeI/PstI CCA-44 [Phenanthrene insert 2_Ter_BBa_B0015 XbaI/PstI + Promoter BBa_J23101_BBa_B0034 EcoRI/SpeI] as XbaI/PstI	Philippe Hansen-Estruch
BBa_K2491029	Project/ CCA-64	110_P1 -P2	CCA-29 [Promoter BBa_J23110 /RBS_BBa_B0034 EcoRI/SpeI + Phenanthrene insert 1_Ter_BBa_B0015 XbaI/PstI] as SpeI/PstI CCA-46 [Phenanthrene insert 2_Ter_BBa_B0015 XbaI/PstI + Promoter BBa_J23110_BBa_B0034 EcoRI/SpeI] as XbaI/PstI	Philippe Hansen-Estruch

Basic Part Name	Type.	Clone Description	Resistance gene	Designer	Vector Backbone Improvement
BBa_K2491030 *BEST BASIC*	Plasmid Backbone	Broad Host Range Vector	Kanamycin	Philippe Hansen-Estruch	pSB3K3