Design of the IES plasmids

Date: 2020-04-17
Tags: *IES Plasmids DNA engineering*
Created by: Maarten Verhoeven

Summary:

This document describes the design and construction approach for the plasmid library that express the interspecific expression system that will be used in transformations of bacterial species from WWTPs. The plasmids will be assembled using Golden Gate Cloning and subsequent cloning into *E.coli*.. A Nanopore based sequencing pipeline will be setup to verify correct assembly. The budget for material (excluding general lab supplies) is estimated to be 24272 kr.

Introduction:

The interspecific expression system (IES) requires several DNA components to allow species independent expression of marker genes as well as the estrogenic pathway. In order to achieve this the system is build around the UBER (Universal Bacterial Expression Resource) system developed by Kushawa et al. To allow for flexibility when it come to the expressed pathway genes, markers and replication origins, the IES will contain a set of component the can be swapped to yield a large variety of plasmids. Ultimately the system will be used for two different research projects; 1. screen genetic accesability in WWTP cultures. 2. Recombinant bioaugmentation of microbes from WWTPs.

Approach:

The main bulk of the plasmids will be assembled from synthesised DNA pieces as well as DNA fragment PCR amplified from existing plasmid. Assembly of the fragments will be performed using Golden Gate Cloning (GGC). This technique uses Type II restriction nucleases that yield scarless joints between the DNA fragment that are assembled. The DNA is subsequently transformed into *E. coli*. Usually the restriction enzyme BsaI is used for GGC. However, since the UBER system contain a BsaI restriction site an alternative nuclease was chosen: BsmBI. GGC using this enzyme allows for correct assembly of all fragments except for IncP (which contains the respective restriction site). The assembly with this fragment will therefore be a lot less efficient and thus more colonies will need to be screened. The overal plasmid overview is shown below. Each of the swappable elements are listed in the figure.

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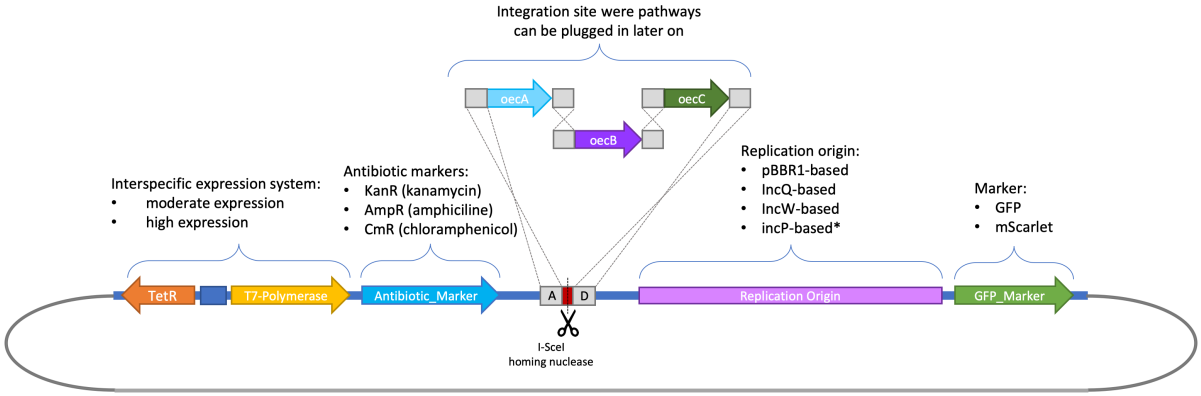

Overview of all components that combined can be used to make the plasmid library. The three oestrogen pathway genes can be added using a Gibson assembly step.

Besides the general components, the plasmid will additionally contain a plugin site with two homology regions and a homing nuclease site. Through Gibson assembly this plugin site can be used to incorporate the estrogenic degradation pathway when this is needed. In the table below all the DNA components with their corresponding source, length and ordering information is shown:

Plasmid components:	Features:	Length:	Origin:	Status:	Price:
IncP	Replication origin	2000bp	https://www.addgene.org/61263/	order as plasmid (Addgene)	$75
IncQ	Replication origin	5313bp	https://www.addgene.org/110602/	order as plasmid (Addgene)	$75
IncW	Replication origin	1900bp	https://www.addgene.org/109248/	order as plasmid (Addgene)	$75
pBBR1-based	Replication origin	1500bp	https://www.addgene.org/85168/	order as plasmid (Addgene)	$75
GFP	T7Prom_CDS_term	744bp	Kushawa et al.	Order as gBlock (IDT)	1240 kr.

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mScarlet	T7Prom_CDS_term	753bp	Kushawa et al.	Order as gBlock (IDT)	1240 kr.
oecA	T7Prom_CDS_term	1256bp	Chen et al.	as synthetic construct (GeneArt)	2932 kr.
oecB	T7Prom_CDS_term	1265bp	Chen et al.	as synthetic construct (GeneArt)	2950 kr.
oecC	T7Prom_CDS_term	1001bp	Chen et al.	as synthetic construct (GeneArt)	2417 kr.
Chloramphenicol	T7Prom_CDS_term	731bp	Addgene, (codon optimised for Burkholderia).	Order as gBlock (IDT)	1240 kr.
Ampiciline	T7Prom_CDS_term	932bp	Addgene	Order as gBlock (IDT)	1575 kr.
Kanamycin	T7Prom_CDS_term	889bp	Addgene	Order as gBlock (IDT)	1575 kr.
UBER_moderate expression	TetR, T7Polym	4456bp	https://www.addgene.org/71428/	order as plasmid (Addgene)	$75
UBER_high expression	TetR, T7Polym	4456bp	https://www.addgene.org/71431/	order as plasmid (Addgene)	$75

The IDT orders have an expected shipping time of 10 days. the GeneArt orders take about 20 days. for Addgene the delivery time are unknown but they indicate it might be longer due to the Corona Crisis. Besides the plasmids a set of primers have been designed to PCR amplify the required fragment for the donor plasmids, as well as for verification of correct assembly (see table below).

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Primer ID:	Sequence:	Purpose:	Price:
1_IncP_fw	CACCACACGTCTCGTACTAGCAGACAGTTTTATTGTTCATGA	Amplify fragment, Add BsmBI site	88 kr.
2_IncP_rv	CACCACACGTCTCGTGTGTCAGAATTGGTTAATTGGTTGTAACAC	Amplify fragment, Add BsmBI site	95 kr.
3_IncQ_fw	CACCACACGTCTCGTACTGTGCCACCTGTCATGACCAA	Amplify fragment, Add BsmBI site	80 kr.
4_IncQ_rv	CACCACACGTCTCGTGTGCCTGCCGTTGCTAGACATTG	Amplify fragment, Add BsmBI site	80 kr.
5_IncW_fw	CACCACACGTCTCGTACTCGCTGAGATAGGTGCCTCAC	Amplify fragment, Add BsmBI site	80 kr.
6_IncW_rv	CACCACACGTCTCGTGTGCTTCCTCGCTCACTGACTCG	Amplify fragment, Add BsmBI site	80 kr.
7_pBBR1_fw	CACCACACGTCTCGTACTCCCTCCCTTTTGGTGTCCAA	Amplify fragment, Add BsmBI site	80 kr.
8_pBBR1_rv	CACCACACGTCTCGTGTGCAGTTTGCTCAGGCTCTCCC	Amplify fragment, Add BsmBI site	80 kr.
9_pUBER_fw	CACCACACGTCTCGTTTGTACTCTTCCAAACGACGGCC	Amplify fragment, Add BsmBI site	80 kr.
10_pUBER_rv	CACCACACGTCTCGCCTGTGTGTGGAATTGTGAGCGGA	Amplify fragment, Add BsmBI site	80 kr.

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11_verify_IncP	TTGACAATTCTAGGGCGCGT	PCR amplify plasmid prior to sequencing	42 kr.
12_verify_IncQ	CTGGGCTGGACGGTAACC	PCR amplify plasmid prior to sequencing	38 kr.
13_verify_IncW	GCACAGCGCCGATTATCAAA	PCR amplify plasmid prior to sequencing	42 kr.
14_verify_pBBR1	CGGTCACGACTTTGCGAAG	PCR amplify plasmid prior to sequencing	40 kr.
15_verify_AmpR_fw	TCGCCGCATACACTATTCTCA	PCR amplify plasmid prior to sequencing	44 kr.
16_verify_AmprR_rv	TGATCCCCCATGTTGTGCAA	PCR amplify plasmid prior to sequencing	42 kr.
17_verify_CmR_fw	TGTTCCACGAACAGACGGAA	PCR amplify plasmid prior to sequencing	42 kr.
18_verify_CmR_rv	GTTTTCGATGAAGCCCTTCGG	PCR amplify plasmid prior to sequencing	44 kr.
19_verify_GFP_fw	ATCAGCAATGCCAGAAGGTT	PCR amplify plasmid prior to sequencing	42 kr.
20_verify_GFP_rv	CCATCTTCTTTGAAGTCAATACCTT	PCR amplify plasmid prior to sequencing	53 kr.
21_verify_KanR_fw	GAATATCCGGATAGCGGCGA	PCR amplify plasmid prior to sequencing	42 kr.

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22_verify_KanR_rv	CAAAATCGCTCGCATCCACC	PCR amplify plasmid prior to sequencing	42 kr.
23_verify_mScarlet_fw	AAGCTGAAGGTGACGAAGGG	PCR amplify plasmid prior to sequencing	42 kr.
24_verify_mScarlet_rv	CGCCGTCTTCGAAGTTCATC	PCR amplify plasmid prior to sequencing	42 kr.
25_verify_BSM_IncP_fw	TTCGCGTACTCCAACACCTG	verify correct assembly of IncP	42 kr.
26_verify_BSM_IncP_rv	CAGGCGGGTCAAATCAGGAA	verify correct assembly of IncP	42 kr.

Verification of correct assembly:

To validation and select for the correct plasmids, 4 to 8 colonies of each *E. coli* transformation will have to be analysed. Since normal colony PCR would be quite time consuming, it seems more efficient to use a sequencing-based approach. Currin et al. describe a Nanopore based pipeline that can be used to sequence a large set of plasmids. This method should be fairly straightforward to setup in our lab. It would allow for screening of 6x96 plates of constructs in 72h (according to the study).

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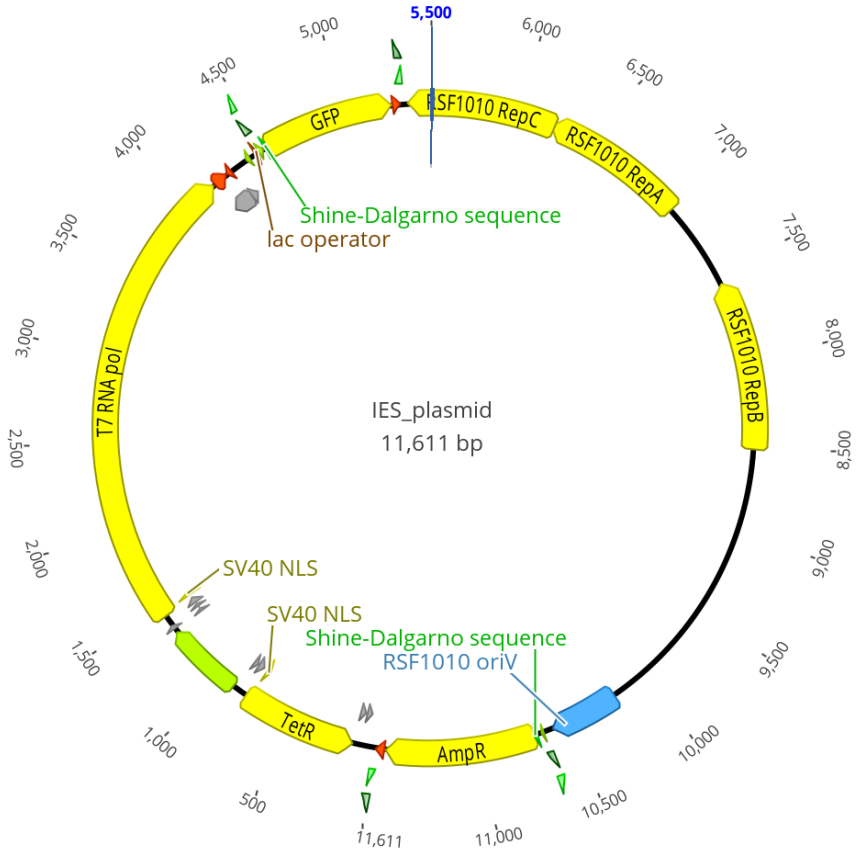

Overview for one of the in total 48 plasmids that will be constructed.

In the table below the overall list of kits that need to be ordered is show (excluding general lab supplies):

Name kit:	**Purpose**:	**Catalognr./Link:**	**Price:**
NEB® Golden Gate Assembly Kit (BsmBI-v2)	Assembly of fragments	E1602L	$400.00

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2X NEB 10-beta Competent *E. coli*	E. coli for cloning plasmids	C3019H	$231.00
I-SceI Homing nuclease	Cutting plugin site	R0694S	$71.00
NEBuilder® HiFi DNA Assembly Master Mix	Integrating estrogen pathway	E2621S	$159.00
DNA polymerase	PCR amplification	-	- (standard lab supply?)
NanoPore flowcell/Kit	Verify plasmid	-	- (standard lab supply?)

Budget overview:

Items:	**Price:**
Donor plasmids	3120 kr.
DNA fragments	15169 kr.
Additional Kits (excluding flow-cell, standard lab supply)	5983 kr.
Total:	24272 kr.

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Linked item

Workplan - WP_Contruction of interspecific expression system (IES)

Attached files

Screenshot.2020.04.20.at.11.48.21.png
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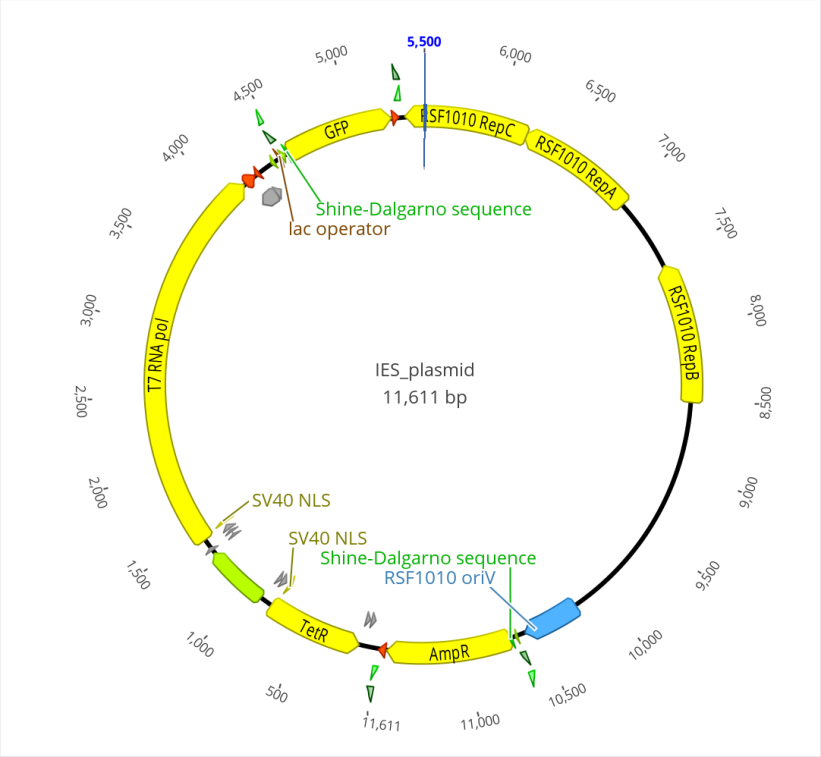

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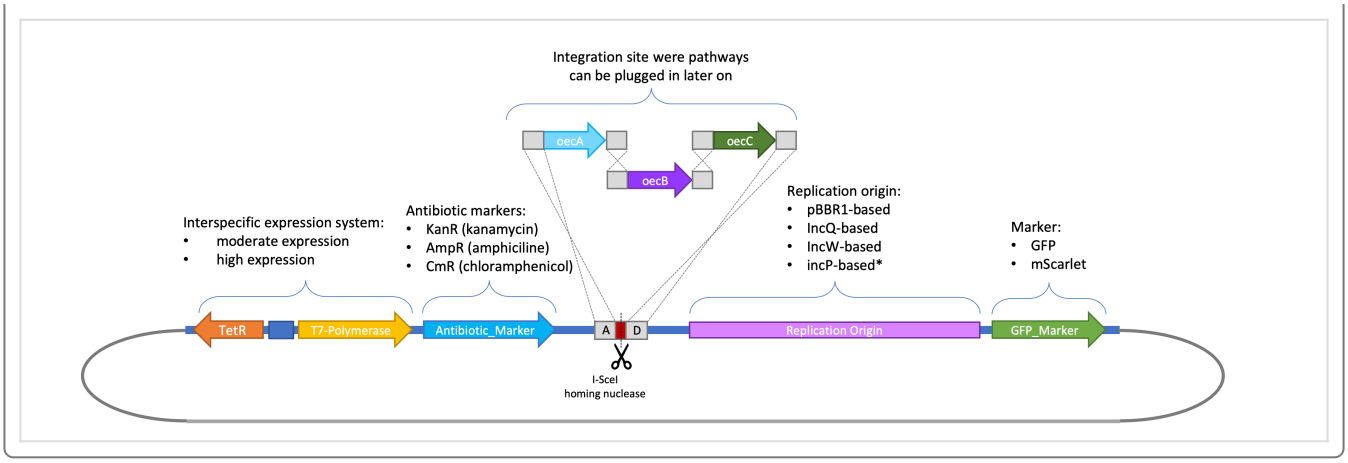

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