iGEM2017 – Microbiology – BMB – SDU

Project type: iGEM2017	**Creation date:** 2017.07.21
Project title: Biobrick assembly	**Written by:** EG, SJ
Sub project: Dormancy System Analysis	**Performed by:** EG, SJ, SM

1. SOPs in use

iGEM2017_SOP03_v03_EG_Gel_purification
iGEM2017_SOP04_v02_FN_Colony_PCR_with_MyTaq
iGEM2017_SOP05_v03_EG_Plasmid_MiniPrep
iGEM2017_SOP06_v02_SJ_TSB_transformation
iGEM2017_SOP07_v03_SJ_Fast_digest
iGEM2017_SOP09_v03_JB_Ligation
iGEM2017_SOP10_v02_JB_Phusion_PCR
iGEM2017_SOP13_v02_EG_Agarose_gel_DNA

2. Purpose

To analyse the dormancy system and its components. Choice of vectors and verification of cloning was particularly assessed.

3. Overview

3.a Low or high copy plasmid requirement for LacI hybrid promoter

Date (YY.MM.DD)	SOPs	Persons	Experiments
17.07.24	SOP06	EG, SJ	Transformation
17.07.25	SOP06	EG, SJ	Transformation
17.07.26	SOP05	EG, SJ	Miniprep
17.07.26	-	EG, SJ	Expression and strength of LacI-regulated hybrid promoter
17.07.26	SOP07	EG, SJ	Fast digest
17.07.26	SOP13	EG, SJ	Gel electrophoresis
17.07.27	SOP03	EG, SJ	Gel purification
17.07.27	SOP09	EG, SJ	Ligation
17.07.27	SOP06	EG, SJ	Transformation
17.07.29	SOP04	EG, SJ	Colony PCR
17.07.29	SOP13	EG, SJ	Gel electrophoresis
17.07.29	SOP05	EG, SJ	Miniprep
17.07.29	SOP06	EG, SJ	Transformation
17.07.30	SOP04	EG, SM	Colony PCR
17.07.30	SOP13	EG, SM	Gel electrophoresis
17.08.05	SOP06	EG, SJ	Transformation
17.08.06	SOP04	EG, SJ	Colony PCR
17.08.06	SOP13	EG, SJ	Gel electrophoresis
17.08.31	SOP07	EG, SJ	Fast digest
17.08.31	SOP13	EG, SJ	Gel electrophoresis
17.08.31	SOP03	EG, SJ	Gel purification
17.08.31	SOP09	EG, SJ	Ligation
17.09.01	SOP06	EG, SJ	Transformation
17.09.16	SOP06	EG, SJ	Transformation
17.09.19	SOP05	EG, SJ	Miniprep
17.09.19	SOP07	EG, SJ	Fast digest
17.09.19	SOP13	EG, SJ	Gel electrophoresis
17.09.19	SOP03	EG, SJ	Gel purification
17.09.19	SOP09	EG, SJ	Ligation
17.09.21	SOP06	EG, SJ	Transformation
17.09.22	SOP04	EG, SJ	Colony PCR
17.09.22	SOP13	EG, SJ	Gel electrophoresis
17.09.23	SOP07	EG, SJ	Fast digest
17.09.23	SOP13	EG, SJ	Gel electrophoresis
17.09.23	SOP03	EG, SJ	Gel purification
17.09.23	SOP09	EG, SJ	Ligation

3.b Low or high copy plasmid requirement for LacI promoter

Date (YY.MM.DD)	SOPs	Persons	Experiments
17.09.27	SOP07	EG, SJ	Fast digest
17.09.27	SOP13	EG, SJ	Gel electrophoresis
17.09.27	SOP03	EG, SJ	Gel purification
17.09.30	SOP07	EG, SJ	Fast digest
17.09.30	SOP13	EG, SJ	Gel electrophoresis
17.09.30	SOP03	EG, SJ	Gel purification
17.09.30	SOP09	EG, SJ	Ligation
17.09.30	SOP06	EG, SJ	Transformation
17.10.01	SOP04	EG, SJ	Colony PCR
17.10.01	SOP13	EG, SJ	Gel electrophoresis
17.10.02	SOP09	EG	Ligation
17.10.02	SOP06	EG, SJ	Transformation
17.10.03	SOP04	EG, SJ	Colony PCR
17.10.03	SOP13	EG, SJ	Gel electroporesis
17.10.05	SOP05	EG	Miniprep
17.10.05	SOP07	EG	Fast digest
17.10.05	SOP13	EG	Gel electrophoresis
17.10.07	SOP06	EG, SJ	Transformation
17.10.08	SOP04	EG, SJ	Colony PCR

3.c Low or high copy plasmid requirement for AraC promoter

Date (YY.MM.DD)	SOPs	Persons	Experiments
17.10.10	SOP06	SJ	Transformation
17.10.11	SOP05	SJ	Miniprep
17.10.12	SOP07	EG	Fast digest
17.10.12	SOP13	EG	Gel electrophoresis
17.10.12	SOP03	EG	Gel purification
17.10.14	SOP07	EG	Fast digest
17.10.14	SOP13	EG	Gel electrophoresis
17.10.14	SOP03	EG	Gel purification
17.10.14	SOP09	EG	Ligation
17.10.15	SOP06	EG, SJ	Transformation
17.10.16	SOP04	EG, SJ	Colony PCR
17.10.16	SOP13	EG, SJ	Gel electrophoresis
17.10.17	SOP05	EG, SJ	Miniprep
17.10.17	SOP07	EG, SJ	Fast digest

17.10.17	SOP13	EG, SJ	Gel electrophoresis
17.10.17	SOP03	EG, SJ	Gel purification
17.10.17	SOP09	EG, SJ	Ligation
17.10.18	SOP06	EG, SJ	Transformation
17.10.19	SOP04	EG, SJ	Colony PCR
17.10.19	SOP13	EG, SJ	Gel electrophoresis
17.10.20	SOP05	EG, SJ	Miniprep
17.10.20	SOP07	EG, SJ	Fast digest
17.10.20	SOP13	EG, SJ	Gel electrophoresis
17.10.20	SOP06	EG, SJ	Transformation
17.10.21	SOP04	EG, SJ	Colony PCR
17.10.21	SOP13	EG, SJ	Gel electrophoresis
17.10.23	-	EG, SJ	Fluorescence microscopy
17.10.24	-	EG, SJ	Fluorescence microscopy
17.10.30	-	EG, SJ	Flow cytometry

3.d PCR and sequencing of chromosomal insert

Date (YY.MM.DD)	SOPs	Persons	Experiments
17.07.30	SOP10	EG, SM	Phusion PCR
17.07.30	SOP13	EG, SM	Gel electrophoresis
17.07.31	SOP04	SJ	Colony PCR
17.07.31	SOP13	SJ	Gel electrophoresis
17.08.06	SOP10	EG, SJ	Phusion PCR
17.08.06	SOP13	EG, SJ	Gel electrophoresis
17.08.06	SOP10	EG, SJ	Phusion PCR
17.08.06	SOP13	EG, SJ	Gel electrophoresis
17.08.12	SOP10	EG, SJ	Phusion PCR
17.08.12	SOP04	EG	Colony PCR
17.08.12	SOP13	EG, SJ	Gel electrophoresis
17.08.12	SOP03	EG, SJ	Gel purification
17.08.13	SOP04	EG, SJ	Colony PCR
17.08.13	SOP13	EG, SJ	Gel electrophoresis

3.e BioBrick containing RelE controlled by the OmpR promoter

Date (YY.MM.DD)	SOPs	Persons	Experiments
17.10.19	SOP10	EG, SJ	Phusion PCR
17.10.20	SOP07	EG, SJ	Fast digest
17.10.20	SOP13	EG, SJ	Gel electrophoresis

17.10.20	SOP03	EG, SJ	Gel purification
17.10.20	SOP09	EG, SJ	Ligation
17.10.21	SOP06	EG, SJ	Transformation
17.10.22	SOP04	EG, SJ	Colony PCR
17.10.22	SOP13	EG, SJ	Gel electrophoresis
17.10.22	SOP09	EG, SJ	Ligation
17.10.22	SOP06	EG, SJ	Transformation
17.10.23	SOP06	EG, SJ	Transformation
17.10.20	SOP07	EG, SJ	Fast digest
17.10.20	SOP13	EG, SJ	Gel electrophoresis
17.10.20	SOP03	EG, SJ	Gel purification
17.10.23	SOP09	EG, SJ	Ligation

4. Materials required

Materials in use

Name	Components (Concentrations)	Manufacturer	Other specifications	Safety considerations

5. Experiment history

5.a Low or high copy plasmid requirement for LacI hybrid promoter

Date (YY.MM.DD)	SOPs	Alterations to SOPs and remarks to experiments
17.07.24	SOP06	Transformation pSB4A5 into TOP10. 10 µL water added to the kit, 5 µL used. 45 min phenotypical expression at 300 rpm.
17.07.25	SOP06	Transformation 10 µL B65 inserted into KG22 ΔEnvZ strain 45 min phenotypical expression at 300 rpm.
17.07.26	SOP05	Miniprep pSB4A5 Very red ONC and pellet.

		Red turned to yellow at addition of lysis solution.
17.07.26	SOP07	Fast digest pSB4A5 digested with EcoRI and PstI and treated with AP. 30 min digestion time.
17.07.26	SOP13	Gel electrophoresis 1 % agarose gel, small-thick comb. 2.5 µL ladder, 2.5 µL loading dye.
17.07.27	SOP07	Fast digest LacI-regulated hybrid promoter with GFP digested with EcoRI and PstI. 30 min digestion time.
17.07.27	SOP13	Gel electrophoresis 1 % agarose gel, small-thick comb. 2.5 µL ladder, 2.5 µL loading dye.
17.07.27	SOP03	Gel purification 60 °C melting temperature
17.07.27	SOP09	Ligation 1:0, 1:1, 1:2, and 1:4 ligations were made.
17.07.27	SOP06	Transformation pSB4A5 plasmid with LacI regulated hybrid promoter controlling GFP into KG22 ΔEnvZ (OD_{600}=0.9) and MG1655 (OD_{600}=0.8) NB: All four MG1655 plates were overgrown with 50/50 red and white colonies. The KG22 ΔEnvZ 1:0 plate was overgrown with a thin film of red bacteria. The 1:1, 1:2, and 1:4 plates likewise, but they also had distinct white colonies dispersed just like normal transformants.
17.07.29	SOP04	Colony PCR 22 of the KG22 ΔEnvZ transformants were restreaked. Of those, 12 turned green. These were used for colony PCR. 25 s elongation. 3 min extra elongation.
17.07.29	SOP13	Gel electrophoresis 1 % agarose gel, small-thick comb. 2.5 µL loading dye, 2.5 µL DNA ladder mix. All samples were loaded in the following order: Ladder, 1.1, 1.2, 1.3, 1.4, 2.1, 2.2, 3.1, 3.2, 3.3, 4.1, 4.2, 4.3 Result: 3.1 was the best
17.07.29	SOP05	Miniprep $LacI^P$-GFP on low copy plasmid from colony 3.1.
17.07.29	SOP06	Transformation $LacI^P$-GFP on low copy plasmid into MG1655 (OD_{600}=0.5) 45 min phenotypical expression at 300 rpm.
17.07.30	SOP04	Colony PCR Two colonies were restreaked and used for colony PCR. 15 s elongation 2.5 min extra elongation.
17.07.30	SOP13	Gel electrophoresis

		1 % agarose gel, small-thick comb. 2.5 µL loading dye, 2.5 µL DNA ladder mix. All sample loaded in the following order: Ladder, (RelB1, RelB2, RelB4,) GFP1, GFP2, (other…)
17.08.05	SOP06	Transformation $LacI^P$-GFP on high copy plasmid (pSB1A2) into MG1655 OD_{600}=0.44 5 µL plasmid (B65) added. 45 min phenotypical expression
17.08.06	SOP04	Colony PCR The plate contained yellow/green and white colonies. One of each was restreaked and used. M1 = white, M2 = yellow/green. 15 s elongation, 2.5 min extra elongation.
17.08.06	SOP13	Gel electrophoresis 1% agarose gel, small-thick comb, 2.5 µL DNA ladder mix, 2.5 µL 6x loading dye. Loading order: Ladder, M1, M2, (other…) Both had correct bands at 1100 bp. The yellow/green colony was used further on.
17.08.31	SOP07	Fast digest $LacI^P$-GFP digested with EcoRI and PstI 45 min digestion time.
17.08.31	SOP13	Gel electrophoresis 1 % agarose gel, broad-thick comb, 2.5 µL 6x loading dye, 6 µL 1 kb ladder. Loading order: Ladder, (other x5), $LacI^P$-GFP 75V, 40 min
17.08.31	SOP03	Gel purification 60 °C melting temperature
17.08.31	SOP09	Ligation $LacI^P$-GFP with low copy backbone. 1:0, 1:1, 1:2, and 1:4 ligations were made.
17.09.01	SOP06	Transformation MG1655 OD_{600}=0.7 $LacI^P$-GFP on low copy plasmid. All ligation product used. 1 hr phenotypical expression at 300 rpm.
17.09.16	SOP06	Transformation. 5 µL pSB4K5 from 2016 kit plate 4 well 6H in TOP10. OD_{600}=0.5 45 min phenotypical expression at 300 rpm. Plated on 30 ɣ KAN plate.
17.09.19	SOP05	Miniprep 5 mL ONC of pSB4K5 from a red colony.
17.09.19	SOP07	Fast digest pSB4K5 digested with EcoRI + PstI for 30 min, heat inactivated

		for 5 min at 80 °C. Treated with AP for 2 hr. LacI^{P}-GFP digested with EcoRI + PstI for 30 min.
17.09.19	SOP13	Gel electrophoresis 1% agarose gel, thick comb, 2.5 µL 6x loading dye, 6 µL 1 kb ladder Loading order: Ladder, pSB4K5, LacI^{P}-GFP 75 V, 40 min
17.09.19	SOP03	Gel purification 60 °C melting temperature
17.09.19	SOP09	Ligation LacI^{P}-GFP into pSB4K5 1:0, 1:1, 1:2, and 1:4 ligations made and placed at 16 °C O.N.
17.09.21	SOP06	Transformation All ligation product LacI^{P}-GFP into pSB4K5 into MG1655. OD_{600}=0.9.
17.09.22	SOP04	Colony PCR Colonies used: #1 red from 1:0, #2 white from 1:0,#3-8 from 1:1, #9-13 from 1:2, #14-16 from 1:4 15 s elongation, 2.5 min extra elongation.
17.09.22	SOP13	Gel electrophoresis 1% agarose gel, small-thick comb, 2.5 µL DNA ladder mix, 2.5 µL 6x loading dye. Loading order: Ladder, #1-14 Ladder, #15-16
17.09.23	SOP07	Fast digest pSB4K5 digested with EcoRI and PstI for 45 min, heat inactivated at 80 °C for 5 min, treated with AP for 2 hr LacI^{P}-GFP digested with EcoRI and PstI for 45 min
17.09.23	SOP13	Gel electrophoresis 1 % agarose gel, small-thick, 2.5 µL 6x loading dye, 2.5 µL DNA ladder mix. Loading order: Ladder, pSB4K5, LacI^{P}-GFP 75V, 40 min
17.09.23	SOP03	Gel purification 60 °C melting temperature
17.09.23	SOP09	Ligation LacI^{P}-GFP with pSB4K5 1:0, 1:1, 1:2, and 1:4 ligations were made and placed at 16 °C O.N.

5.b Low or high copy plasmid requirement for LacI promoter

Date	SOPs	Alterations to SOPs and remarks to experiments

(YY.MM.DD)		
17.09.27	SOP07	Fast digest pSB4K5 digested with EcoRI and PstI for 45 minutes. Heat inactivated at 80 °C for 5 minutes. Fast AP added for two hours.
17.09.27	SOP13	Gel electrophoresis 1% agarose gel, small-thick comb, 2.5 µL 6x loading dye, 2.5 µL DNA ladder mix 75 V, 40 min.
17.09.27	SOP03	Gel purification 60 °C melting temperature
17.09.30	SOP07	Fast digest LacI promoter + GFP digested with EcoRI and PstI for 45 min.
17.09.30	SOP13	Gel electrophoresis 1% agarose gel, small-thick comb, 2.5 µL 6x loading dye, 2.5 µL DNA ladder mix 75 V, 40 min.
17.09.30	SOP03	Gel purification 60 °C melting temperature
17.09.30	SOP09	Ligation $LacI^P$-GFP with pSB4K5 1:0, 1:1, 1:2, and 1:4 ligations made and placed at RT for 2 hrs.
17.09.30	SOP06	Transformation All ligation product into competent KG22.
17.10.01	SOP04	Colony PCR No colonies had appeared on the 1:0 and 1:1 plates. 8 colonies from $LacI^P$-GFP on low copy backbone used. Colony 1 from 1:2 and colonies 2-8 from 1:4.
17.10.01	SOP13	Gel electrophoresis 1% agarose, small-broad comb, 2.5 µL 6x loading dye, 2.5 µL DNA ladder mix Loading order: Ladder , 1-8 $LacI^P$-GFP 80 V, 20 min. NB: we plated on wrong plates.
17.10.02	SOP09	Ligation $LacI^P$-GFP with pSB4K5 1:0, 1:1, 1:2, and 1:4 ligations made and placed at RT for 2 hrs
17.10.02	SOP06	Transformation All ligation product into competent KG22. Plated on 30 ɣ KAN plates.
17.10.03	SOP04	Colony PCR One red colony from the 1:0, one white colony from the 1:1, 7 green colonies from the XXX plates where used. 1 min elongation 5 min extra elongation
17.10.03	SOP13	Gel electrophoresis 1% agarose, small-broad comb, 2.5 µL 6x loading dye, 2.5 µL

		DNA ladder mix Loading order: Ladder, 1-8 $LacI^P$-GFP 75 V, 30 min.
17.10.05	SOP05	Miniprep 5 mL ONC from colony #2 and #3 used
17.10.05	SOP07	Fast digest 500 ng from plasmid #2 and #3 digested with EcoRI and PstI for 30 min.
17.10.05	SOP13	Gel electrophoresis 1% agarose gel, small-thick com, 2.5 µL 6x loading dye, 2.5 µL DNA ladder mix Loading order: Ladder, #2, #3 75 V, 30 min.
17.10.07	SOP06	Transformation Fresh KG22 strain. OD_{600}=0.75 Duplicates made with 2 µL and 5 µL $LacI^P$-GFP on pSB4K5 #3 respectively. 45 min phenotypical expression at 300 rpm. Plated on 30 ɣ KAN plates. NB: both plates were overgrown and green
17.10.08	SOP04	Colony PCR One green colony and the leftover 20 white colonies from the old KG22 plates. 1 min elongation 10 min extra elongation NB: None were correct

5.c Low or high copy plasmid requirement for AraC promoter

Date (YY.MM.DD)	**SOPs**	**Alterations to SOPs and remarks to experiments**
17.10.10	SOP06	Transformation pSB3K3 into TOP10.
17.10.11	SOP05	Miniprep For pSB3K3 ONC colony #2 was used. 10 mL of the ONC was used for the miniprep.
17.10.12	SOP07	Fast digest pSB3K3 digested with EcoRI and PstI for 2 hr. Heat inactivated at 80 °C for 5 min. Treated with AP for 2 hr.
17.10.12	SOP13	Gel electrophoresis pSB3K3. Made for us
17.10.12	SOP03	Gel purification pSB3K3 Made for us

17.10.14	SOP07	Fast digest $AraC^P$-YFP and -CFP digested with EcoRI and PstI for 45 min.
17.10.14	SOP13	Gel electrophoresis 1 % agarose gel, small-thick comb, 2.5 µL 6x loading dye, 2.5 µL ladder 60 V 50 min
17.10.14	SOP03	Gel purification 60 °C melting temperature
17.10.14	SOP09	Ligation $AraC^P$-YFP and -CFP with pSB3K3. 1:0, 1:1, 1:2, and 1:4 ligations were made and placed at 16 °C O.N.
17.10.15	SOP06	Transformation All ligation product into MG1655.
17.10.16	SOP04	Colony PCR
17.10.16	SOP13	Gel electrophoresis
17.10.17	SOP05	Miniprep From 5 mL ONC of $AraC^P$-YFP and pSB3K3 new from freezestock.
17.10.17	SOP07	Fast digest Two hours digest of $AraC^P$-YFP with EcoRI, PstI, and XhoI. Two hours digest of pSB3K3 with EcoRI, PstI, and fast AP.
17.10.17	SOP13	Gel electrophoresis 75 V for 45 minutes.
17.10.17	SOP03	Gel purification Melting temperature 60 °C.
17.10.17	SOP09	Ligation 1:0, 1:1, 1:2, 1:4, and 1:8 ligations of $AraC^P$-YFP with pSB3K3 made and placed at 16 °C O.N.
17.10.18	SOP06	Transformation The ligation product was split into two. One of the half was transformed into MG1655 from the iGEM stock and the other half was transformed into MG1655 from Thøgers stock. OD_{600}=0.8 for the iGEM and OD_{600}=0.9 for Thøger.
17.10.19	SOP04	Colony PCR 6 colonies from Thøgers plates and 6 colonies from the iGEM plates used. 1 min elongation 5 min extra elongation
17.10.19	SOP13	Gel electrophoresis 1% agarose gel, small-thin comb, 2.5 µL DNA ladder mix Loading order (upper row of gel): Ladder, colonies from Thøgers plate 1-6, colonies from iGEMs plate 1-6. 80 V, 25 min
17.10.20	SOP05	Miniprep

		5 mL ONC of $AraC^P$-YFP Thøger #5 on pSB3K3
17.10.20	SOP07	Fast digest $AraC^P$-YFP on pSB3K3 digested with EcoRI and PstI for 2 hr
17.10.20	SOP13	Gel electrophoresis 1% agarose gel, small-thick comb, 2.5 µL 6x loading dye, 2.5 µL DNA ladder mix 75 V, 45 min
17.10.20	SOP06	Transformation $AraC^P$-YFP on pSB1C3 into MG1655. 45 min phenotypical expression
17.10.21	SOP04	Colony PCR 8 colonies from the transformation plate used. VF2 and VR primers 45 s elongation 5 min extra elongation
17.10.21	SOP13	Gel electrophoresis 1% agarose gel, small-thin comb, 2.5 µL DNA ladder mix All 8 had bands at 2 kbp. #4 was chosen for ONC.
17.10.23	-	Fluorescence microscopy $AraC^P$-YFP on pSB3K3
17.10.24	-	Fluorescence microscopy $AraC^P$-YFP on pSB1C3
17.10.30	-	Flow cytometry

5.d PCR and sequencing of chromosomal insert

Date (YY.MM.DD)	**SOPs**	**Alterations to SOPs and remarks to experiments**
17.07.30	SOP10	Phusion PCR RFP insertion in both ΔEnvZ and ΔOmpR. The control plates were empty. One colony from each of the plates with 10 ng and 100 ng were restreaked and used for PCR. HF+DMSO+$MgCl_2$ buffer was used 10 s denaturation 30 s annealing at 53 °C 1.5 min elongation 10 min extra elongation 8 °C hold
17.07.30	SOP13	Gel electrophoresis RFP insertion in both ΔEnvZ and ΔOmpR. 1 % agarose gel, small-thick comb. 2.5 µL loading dye, 2.5 µL DNA ladder mix. All sample loaded in the following order: Ladder, (RelB1, RelB2, RelB4, GFP1, GFP2, LSS1, LSS2,

		LSS4, empty), ΔEnvZ10ng, ΔEnvZ100ng, ΔOmpR10ng, ΔOmpR100ng. All four bands were at 1 kbp. Since there were more transformants, this was repeated with new colonies.
17.07.31	SOP04	Colony PCR RFP insertion in both ΔEnvZ and ΔOmpR. 10 new colonies from the four transformant plates were restreaked and used for colony PCR. 1.5 min elongation 5 min extra elongation 8 °C hold
17.07.31	SOP13	Gel electrophoresis RFP insertion in both ΔEnvZ and ΔOmpR. 1 % agarose gel, small-thick comb. 2.5 µL loading dye, 2.5 µL DNA ladder mix. All sample loaded in the following order: Ladder, 1, 2, 3, 4, 5, 6, 7, 8, 10 (other...) Result: No. 2 and 10 (ΔEnvZ100ng) as well as 4 and 5 (ΔOmpR100ng) were successful at 3 kbp. These are used for phusion PCR.
17.08.06	SOP10	Phusion PCR RFP insertion in both ΔEnvZ and ΔOmpR. Buffer used (amounts per reaction): HF, 0.4 µL 50 mM $MgCl_2$, 0.2 µL DMSO 0.5 µL template from ONCs 10 s denaturation 30 s annealing at 53 °C 90 s elongation 10 min extra elongation Hold at 8 °C
17.08.06	SOP13	Gel electrophoresis RFP insertion in both ΔEnvZ and ΔOmpR. 1% agarose, small-thick comb, 2.5 µL DNA ladder mix, 2.5 µL 6x loading dye Loading order: Ladder, ΔEnvZ2, ΔEnvZ10, ΔOmpR5, ΔOmpR4 No ladder was visible, and the samples did not yield any distinct bands.
17.08.06	SOP10	Phusion PCR RFP insertion in both ΔEnvZ and ΔOmpR. Buffer used (amounts per reaction): HF, 0.4 µL 50 mM $MgCl_2$, 0.2 µL DMSO 1 µL template from ONCs 10 s denaturation 30 s annealing at 53 °C 90 s elongation 10 min extra elongation

		Hold at 8 °C
17.08.06	SOP13	Gel electrophoresis RFP insertion in both ΔEnvZ and ΔOmpR. 1% agarose, small-thick comb, 2.5 µL DNA ladder mix, 1 kb DNA ladder, 2.5 µL 6x loading dye Loading order: DNA ladder mix, 1 kb ladder, ΔEnvZ2, ΔEnvZ10, ΔOmpR4, ΔOmpR5 The ladders did not separate correctly, and no distinct bands were visible in the samples.
17.08.12	SOP10	Phusion PCR RFP insertion in both ΔEnvZ and ΔOmpR. Buffer used (amounts per reaction): HF, 0.4 µL 50 mM $MgCl_2$, 0.2 µL DMSO Template: for each of the four bacterial solutions, 1 µL ONC was diluted with 100 µL water. This was boiled for 10 min. Each PCR was made in dublicates with either 1 µL or 0.5 µL template. 10 s denaturation 30 s annealing at 53 °C 90 s elongation 10 min extra elongation Hold at 8 °C
17.08.12	SOP04	Colony PCR RelE insertion in ΔEnvZ. 40 colonies used. VF2 and VR primers used by mistake. 90 s elongation. 5 min extra elongation.
17.08.12	SOP13	Gel electrophoresis RFP insertion in both ΔEnvZ and ΔOmpR. 1 % agarose gel, small-thick comb. 2.5 µL DNA ladder mix, 2.5 µL 6x loading dye. Loading order: Ladder, ΔEnvZ2-1, ΔEnvZ10-1, ΔEnvZ2-0.5, ΔEnvZ10-0.5, ΔOmpR4-1, ΔOmpR5-1, ΔOmpR4-0.5, ΔOmpR5-0.5. Result: All had very strong bands at 1000 bp, but also distinct bands at around 2700 bp.
17.08.12	SOP03	Gel purification RFP insertion in both ΔEnvZ and ΔOmpR. The eight bands were cut out and pooled in the respective pairs. 60 °C melting temperature. NB: the concentration was to low.
17.08.12	SOP13	Gel electrophoresis RelE insertion in ΔEnvZ. 1 % agarose gel, small-thick comb. 2.5 µL DNA ladder mix, 2.5 µL 6x loading dye.

		Loading order: Gel 1: Ladder, 1-14 Gel 2: Ladder, 15-28 Gel 3: Ladder, 29-40 Result: There was no difference between the control and the rest, the electroporation will be repeated.
17.08.13	SOP04	Colony PCR RFP insertion in both ΔEnvZ and ΔOmpR. 40 µL from the ONCs were plated out. From these plates, 10 colonies were used. Ins-H8 F NarP R primers used 75 s elongation. 3 min extra elongation.
17.08.13	SOP13	Gel electrophoresis

5.e BioBrick containing RelE controlled by the OmpR promoter

Date (YY.MM.DD)	**SOPs**	**Alterations to SOPs and remarks to experiments**
17.10.19	SOP10	Phusion PCR OmpR-RelE ligation product template (see protocol 3) HF+$MgCl_2$ buffer VR + VF2 primers 30 s elongation 10 min extra elongation
17.10.20	SOP07	Fast digest OmpR promoter (B22) digested with EcoRI and PstI for 2 hr. pSB3K3 digested with EcoRI, PstI, and AP for 2 hr
17.10.20	SOP13	Gel electrophoresis 1% agarose gel, small-thick comb, 2.5 µL 6x loading dye, 2.5 µL DNA ladder mix 75V, 45 min
17.10.20	SOP03	Gel purification 60 °C melting temperature
17.10.20	SOP09	Ligation OmpR promoter on pSB3K3 1:0, 1:1, 1:2, 1:4, and 1:8 ligations were made and placed ON at 16 °C.
17.10.21	SOP06	Transformation Half of the ligation product into TOP10 45 min phenotypical expression at 300 rpm
17.10.22	SOP04	Colony PCR 8 colonies used VF2 VR primers 15 s elongation 2.5 min extra elongation

17.10.22	SOP13	Gel electrophoresis 2 % agarose gel, small-thin comb, 2.5 µL 100 bp ladder. 3 with intense band at >300 bp was chosen. 8 with weak band at ~400 bp was chosen.
17.10.22	SOP09	Ligation 1:8, 1:5, 1:30 ligations made Split up for 2 hr RT and ON. 16 °C
17.10.22	SOP06	Transformation RT ligation product used in TOP10 NB: no colonies appeared
17.10.23	SOP06	Transformation Rest of ligation product used
17.10.23	SOP07	Fast digest OmpR with EcoRI and PstI for 2 hr pSB3K3 with EcoRI and PstI and AP for 2 hr
17.10.23	SOP13	Gel electrophoresis
17.10.23	SOP03	Gel purification
17.10.23	SOP09	Ligation 1:0, 1:1, 1:2, and 1:4 ligations made
17.10.24	SOP04	Colony PCR Colonies taken from 1:8, 1:15, and 1:30 plates transformed with the “residue”-ligation product.
17.10.24	SOP13	Gel electrophoresis First 16 colonies with 1% agarose gel and 17-32 with 2 % agarose gel, both small-thin combs. Colonies taken from 1:8, 1:15, and 1:30 plates.
17.10.24	SOP6	Transformation Half of the ligation product into TOP10 (OD_{600}=0.7) 45 min phenotypical expression.

6. Sample specification

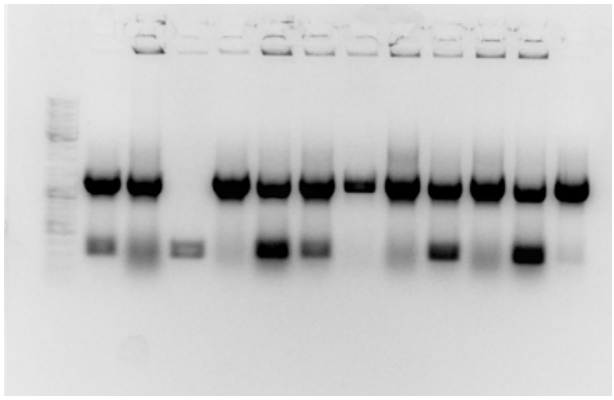
Sample name	Sample content	From	Used for / Saved where
W1			
w2			
w3			
w4			
w5			
w6			
w7			

7. Results and conclusions

7.a Low or high copy plasmid requirement for LacI hybrid promoter

High copy fluorescence microscopy: Test af pLac i top10 og kg22 -> fuldt udtryk i alle top10, KG22 >100 fuldt, 10 medium, 0 slukket. Alle var noisy, men mindre noise i KG. Konklusion: vi skærer LacI-GFP over i low copy og laver samme test.

Colony PCR with 12 KG22 ΔEnvZ transformants



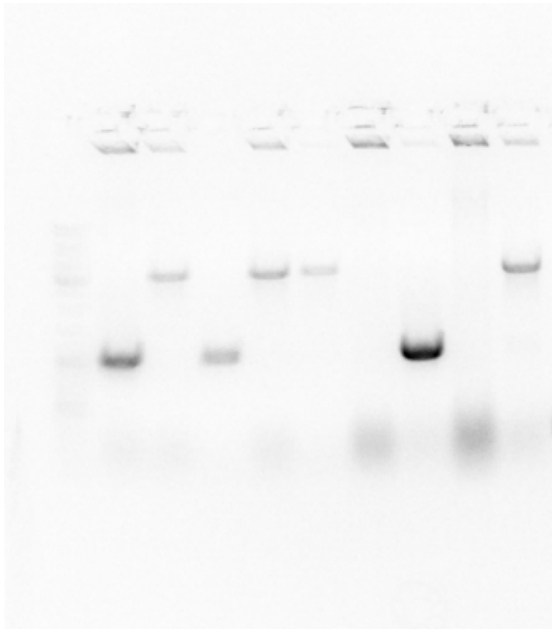
The insert should be 1230 bp, which is in accordance with the top row of bands. We chose no. 3.1 to work with further.

7.b Low or high copy plasmid requirement for LacI promoter

7.c Low or high copy plasmid requirement for AraC promoter

7.d PCR and sequencing of chromosomal insert

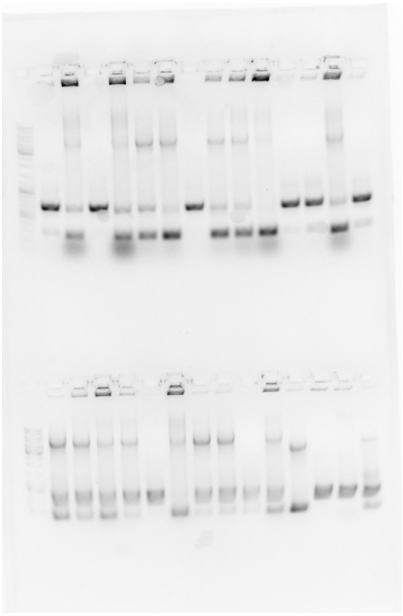
17.07.31

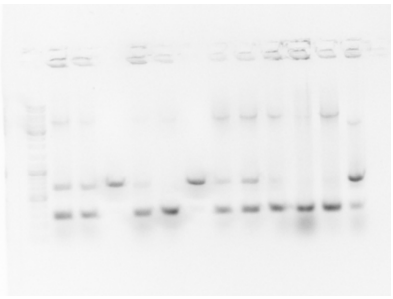


Ladder, 1, 2, 3, 4, 5, 6, 7, 8, 10

Result: No. 2 and 10 (ΔEnvZ100ng) as well as 4 and 5 (ΔOmpR100ng) were successful at 3 kbp. These are used for phusion PCR.

17.08.12





Gel 1: DNA ladder mix, 1-14
Gel 2: DNA ladder mix, 15-28
Gel 3: DNA ladder mix, 29-40

8. Appendices