iGEM2017 – Microbiology – BMB – SDU

Project type: iGEM2017	**Creation date:**
Project title: Biobrick assembly	**Written by:** FN & JB
Sub project: Cellulose secretion	**Performed by:** FN, FP, MA & JB

1. SOPs in use

iGEM2017_SOP01_v02_FN_LA_plates_antibiotic
iGEM2017_SOP02_v02_FN_ONC_E.coli
iGEM2017_SOP03_v02_EG_Gel_purification
iGEM2017_SOP04_v02_FN_Colony_PCR_with_MyTaq
iGEM2017_SOP05_v02_EG_Plasmid_MiniPrep
iGEM2017_SOP06_v02_SJ_TSB_transformation
iGEM2017_SOP07_v02_SJ_Fast_digest
iGEM2017_SOP09_v02_JB_Ligation
iGEM2017_SOP11_v02_JB_Bacterial freezing stock
iGEM2017_SOP12_v02_EG_Making_LB_and_LA_media
iGEM2017_SOP13_v02_EG_Agarose_gel_DNA
iGEM2017_SOP14_v02_EG_Table_Autoclave

2. Purpose

Create a cellulase the secretion system BBa_K2449026, along with other biobricks.

3. Overview

Cex

Day	SOPs	Persons	Experiments
17.06.14	SOP07	FN	Fast digest Cex med x+s
17.06.15	SOP03 SOP09	FN	Gel purification of Cex concentraion = 5 ng/µl ligering with backbone and cex
17.06.16	SOP06	FN	Transformaiton of the ligation.
17.06.17	SOP04	FN & JB	Coloni PCR of Cex
17.06.18	SOP05	FN & JB	Miniprep
17.06.19		JB	Sequencing

HlyB-HlyD

Day	SOPs	Persons	Experiments
17.06.16	SOP06	FN	Transformation of 6I kit plate 4 2017, biobrick Bba_K1166002
17.06.17	SOP04 SOP02	FN & JB	Coloni PCR, ONC
17.06.18	SOP05	FN & JB	Miniprep
17.06.19			Getting primer for getteing HlyB and hlyD out of the brick
17.07.13	SOP10 SOP03	JB	Phusion PCR, to get HlyB and hlyD out of the brick. Gelpurification
17.07.14	SOP07 SOP09	FP	Fast Digest + ligation of HlyB and HlyD
17.07.15	SOP06	FP	Transformation to Top10
17.07.16	SOP04 SOP02	FP	Coloni PCR, ONC
17.07.18	SOP05 SOP11	JB	Miniprep Freezstock

CenA

Day	SOPs	Persons	Experiments
17.07.27	SOP07 SOP03	JB & FN	Fast digest, Gel purification, Ligation, Transformation

	SOP09 SOP06		
17.07.28	SOP04 SOP02	JB & FN	Coloni PCR, ONC
17.07.29	SOP05 SOP11	JB & FN	Miniprep, Freezestock

Combination of bricks with PenI promoter (BBa_R0074)

Day	SOPs	Persons	Experiments
17.10.03	SOP07 SOP03 SOP09	JB	Fast digest, gel purification and ligation
17.10.04	SOP06	MA	Transformation
17.10.05	SOP04 SOP02	JB	Coloni PCR and ONC
17.10.06	SOP11 SOP05 SOP07 SOP03 SOP09 SOP06	JB & MA	Freeze stock, miniprep, fast digest, gel purification and ligation
17.10.07	SOP04 SOP02	MA	Coloni PCR and ONC
17.10.08	SOP11 SOP05	JB	Freeze stock and miniprep
17.10.18	SOP07 SOP03 SOP09 SOP06	FN	Fast digest, ligation and transformation
17.10.19	SOP11 SOP05	JB & FN	Coloni PCR and ONC
17.10.20	SOP11 SOP05 SOP07 SOP03 SOP09 SOP06	JB & FN	Freeze stock Miniprep, fast digest, gel purification, ligation and transformation
17.10.21	SOP11 SOP05	JB	Freeze stock and miniprep

4. Experiment history

Cex

Day	SOPs	Persons	Experiments
17.06.14	SOP07	FN	Fast digest: Fast digest of Cex with X + S and pSB1C3 backbone with X + S.
17.06.15	SOP03 SOP09	FN	Gel purification: After separation on gel the plasmids was purified. Ligation: Ligation of pSB1C3 backbone and the cutted Cex.
17.06.16	SOP06	FN	Transformation: Transformation of the ligations to the E.Coli strain Top10
17.06.17	SOP04 SOP02	FN & JB	Coloni PCR: Coloni PCR of the transformations from the previous day. ONC: ONC of the good looking colonies.
17.06.18	SOP05	FN & JB	Miniprep: Miniprep of the ONC from the previous day
17.07.13	SOP19	JB	Sequencing: The part was sent to sequencing and it was confirmed to be the correct part. We also got confirmed that the brick was heading the correct way after the dangerous X + S cut.

HlyB-HlyD

Day	SOPs	Persons	Experiments
17.06.16	SOP06	FN	Transformation: BBa_K1166002 got transformed into the E. Coli strain Top10.
17.06.17	SOP04 SOP02	FN & JB	Coloni PCR: Coloni PCR of the transformations from the previous day. ONC: ONC of the good looking colonies.
17.06.18	SOP05	FN & JB	Miniprep: Miniprep was performed on ONC from the previous day.
17.06.19			We ordered primers for cutting HlyB and HlyD out of the biobrick.
17.XX.XX			We received primers for cutting HlyB and HlyD out of the biobrick
17.07.13	SOP10 SOP03	JB	Phusion PCR: Phusion PCR was used to get the HlyB and HlyD part out of BBa_K1166002.
17.07.14	SOP07 SOP09	FP	Fast Digest: Fast Digest of the HlyB and HlyD part with X + P. Ligation: Ligation of the cutted HlyB and HlyD part and a pSB1C3 backbone.

17.07.15	SOP06	FP	Transformation: The ligations from the previous day was transformed into the E.Coli strain Top10
17.07.16	SOP04 SOP02	FP	Coloni PCR: Coloni PCR of the transformations from the previous day. ONC: ONC of the good looking colonies.
17.07.18	SOP05 SOP11	JB	Miniprep: Miniprep was performed on ONC from the previous day. Freeze stock: Freeze stock was made of the ONC.

CenA

Day	SOPs	Persons	Experiments
17.07.27	SOP07 SOP03 SOP09 SOP06	JB & FN	Fast digest:The synthesized CenA part was cut with E + P. Gel purification: After separation on gel the plasmids was purified. Ligation: The cutted CenA was ligated together with a pSB1C3 backbone. Transformation: The ligations was transformed into the E.Coli strain Top10.
17.07.28	SOP04 SOP02	JB & FN	Coloni PCR: Coloni PCR was performed on the transformations from the previous day to confirm the ligation had worked, and some of the correct looking colonies was used for ONC
17.07.29	SOP05 SOP11	JB & FN	Miniprep: Miniprep was performed on ONC from the previous day. Freeze stock: Freeze stock was made of the ONC.

Combination of bricks with PenI promoters

Day	SOPs	Persons	Experiments
17.10.03	SOP07 SOP03 SOP09	JB	Fast digest: BBa_K2449013 and BBa_K2449014 was cut with x+p and the BBa_R0074 was cut s+p. Ligation: Ligations of BBa_K2449016 and BBa_K2449017 was made.
17.10.04	SOP06	MA	Transformation of BBa_K2449016 and BBa_K2449017 into Top10.
17.10.05	SOP04 SOP02	JB	Coloni PCR was performed on the transformations from the previous day to confirm the ligation had worked, and some of the correct looking colonies was used for ONC
17.10.06	SOP11	JB & MA	Freeze stock: was made of BBa_K2449016 and

	SOP05 SOP07 SOP03 SOP09 SOP06		BBa_K2449017 Miniprep: Miniprep was performed on BBa_K2449016 and BBa_K2449017. Fast digest: BBa_K2449016 was cut with s+p and e+s, BBa_K2449014 was cut with x+p and BBa_B0015 was cut with e+x Ligation: Ligations of BBa_K2449027 and BBa_K2449024 was made. Transformation: The ligations was transformed into the Top10.
17.10.07	SOP04 SOP02	MA	Coloni PCR was performed on the transformations from the previous day to confirm the ligation had worked, and some of the correct looking colonies was used for ONC
17.10.08	SOP11 SOP05	JB	Freeze stock: was made of BBa_K2449027 and BBa_K2449024 Miniprep: Miniprep was performed on BBa_K2449027 and BBa_K2449024
17.10.18	SOP07 SOP03 SOP09 SOP06	FN	Fast digest: BBa_K2449024 was cut with s+p and BBa_K2449015 was cut with x+p Ligation: Ligation of BBa_K2449025 was made Transformation: BBa_K2449025 was transformed into Top10.
17.10.19	SOP11 SOP05	JB & FN	Coloni PCR was performed on the transformations from the previous day to confirm the ligation had worked, and some of the correct looking colonies was used for ONC
17.10.20	SOP11 SOP05 SOP07 SOP03 SOP09 SOP06	JB & FN	Freeze stock: was made of BBa_K2449025 Miniprep: Miniprep was performed on BBa_K2449025 Fast digest: BBa_K2449025 was cut with x+p and BBa_K2449014 was cut with s+p Ligation: Ligation of BBa_K2449026 was made Transformation: The BBa_K2449026 was transformed into the Top10.
17.10.21	SOP11 SOP05	JB	Freeze stock: was made of BBa_K2449026 Miniprep: Miniprep was performed on BBa_K2449026

5. Results and conclusions

Following biobricks has been produced in this assembly:

BBa_K2449013

BBa_K2449014

BBa_K2449015

BBa_K2449016

BBa_K2449017

BBa_K2449024

BBa_K2449025

BBa_K2449026

BBa_K2449027

6. Appendices