Author: Ylenia Longo
created: 06.08.2018 15:02
updated: 06.08.2018 15:21
Entry 1/22: Part improvement and Part Submission Level 1
In Project: Part submission
With tags: part submission, level 1, part improvement

The iGEM headquarters requires each team to submit at least one part, which represents an improvement from a previous iGEM submitted part to the iGEM community, as well as the submission of some parts related to the own project.

For this purpose, a restriction of the iGEM backbone pSCB1C3 and the part to be inserted is done. As a template, the protocol suggested from iGEM (with some changes) is used (http://parts.igem.org/Help:Protocols/Linearized_Plasmid_Backbones)

Enzyme Master Mix

-5µl NEB Buffer 3.1

-1µl EcoRI -HF

-1µl PstI-HF

water up tp 25µl

RESTRICTION: Then: 4µl Plasmid pSCB1C3 and 4µl Enzyme Master Mix/ and 3µl of desired plasmid and 4µl Enzyme Master Mix are mixed together

The samples are restricted at 37°C for 30 min and then inactivated up to 80°C for 20 min.

LIGATION:

-2µl of Plasmid backbone pSCB1C3

- equimolar amounts of digested part to be inserted (<3µl)

- 1µl T4 DNA ligase buffer

- 0.5µl T4 DNA ligase

- up to 10µl H20

Ligate at 16°C for 30min and heat inactivate at 80°C for 20min.

After this step 1-2µl are supposed to be transformed into competent cells.

In this case p2iGEM0333-336 are constructed.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
Entry 2/22: Part improvement
In Project: Part submission
No tags associated

created: 13.08.2018 13:07
updated: 13.08.2018 13:09

The part improvement testrestriction was negatve and has been repeated:

For this purpose, a restriction of the iGEM backbone pSCB1C3 and the part to be inserted is done. As a template, the protocol suggested from iGEM (with some changes) is used (http://parts.igem.org/Help:Protocols/Linearized_Plasmid_Backbones)

Enzyme Master Mix

-5µl NEB Buffer 3.1

-1µl EcoRI -HF

-1µl PstI-HF

water up tp 25µl

RESTRICTION: Then: 4µl Plasmid pSCB1C3 and 4µl Enzyme Master Mix/ and 2µl of desired plasmid and 4µl Enzyme Master Mix are mixed together

The samples are restricted at 37°C for 30 min and then inactivated up to 80°C for 10 min.

LIGATION:

-2µl of Plasmid backbone pSCB1C3

- equimolar amounts of digested part to be inserted (<3µl)

- 1µl T4 DNA ligase buffer

- 0.5µl T4 DNA ligase

- up to 10µl H20

Ligate at 16°C for 30min and heat inactivate at 80°C for 10min.

After this step 1-2µl are supposed to be transformed into competent cells.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo Entry 3/22: Part improvement In Project: Part submission With tags: part improvement	created: 14.08.2018 15:34 updated: 14.08.2018 15:35

Inoculation of colonies for part improvement in LB Cam and incubation at 37°C at 220rpm overnight.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
Entry 4/22: Part improvement, miniprep and testrestriction
In Project: Part submission
With tags: part improvement

created: 15.08.2018 10:55
updated: 15.08.2018 11:03

Miniprep of p2iGEM0336 according to the Promega pure yield miniprep kit:

- 600µl culture+ 100 µl Lysis buffer --> mix +350 µl Neutralisation buffer (cold) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 900 µl) to column
- centrifuge 30 sec max rpm --> discard supernatant
- add 200µl column wash
- centrifuge 30 sec max rpm --> discard supernatant
- For elution use a new tube
- 30 µl Millipore water on column (at 37°C)
- Let incubate for 1h Min at RT
- Centrifuge for 30 Sek. max

The plasmids are then testrestricted according to the following protocol:

1.5µl CutSmart

1.5µl Plasmid

0.5µl EcoRI

0.5µl PstI

up to 15µl H20

The samples are incubated at 37°C for 2hours.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
Entry 5/22: Sequencing
In Project: Part submission
With tags: sequencing, part improvement, part submission

created: 21.08.2018 16:01
updated: 21.08.2018 16:02

Sequencing of the part submission parts was performed using the pSCB1C3 forward and reverse primer.

The following protocol was used:

500ng Plasmid

2.5µl Primer

up to 10µl H20

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
Entry 6/22: Sequencing part sumbission
In Project: Part submission
With tags: sequencing

created: 22.08.2018 14:42
updated: 22.08.2018 14:43

Sequencing of the part submission plasmids resulted in positive inserts in the iGEM backbone.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Katharina Polzen
Entry 7/22: Part improvement
In Project: Part submission
With tags: iGEM part

created: 31.08.2018 11:27
updated: 31.08.2018 11:29

The iGEM headquarters requires each team to submit at least one part, which represents an improvement from a previous iGEM submitted part to the iGEM community, as well as the submission of some parts related to the own project.

For this purpose, a restriction of the iGEM backbone pSCB1C3 and the part to be inserted is done. As a template, the protocol suggested from iGEM (with some changes) is used (http://parts.igem.org/Help:Protocols/Linearized_Plasmid_Backbones)

Enzyme Master Mix

-5µl NEB Buffer 3.1

-1µl EcoRI -HF

-1µl PstI-HF

water up tp 25µl

RESTRICTION: Then: 4µl Plasmid pSCB1C3 and 4µl Enzyme Master Mix/ and 3µl of desired plasmid and 4µl Enzyme Master Mix are mixed together

The samples are restricted at 37°C for 30 min and then inactivated up to 80°C for 20 min.

LIGATION:

-2µl of Plasmid backbone pSCB1C3

- equimolar amounts of digested part to be inserted (<3µl)

- 1µl T4 DNA ligase buffer

- 0.5µl T4 DNA ligase

- up to 10µl H20

Ligate at 16°C for 30min and heat inactivate at 80°C for 20min.

After this step 1-2µl are supposed to be transformed into competent cells.

Made with: p2iGEM0262-0270; p2iGEM0296, p2iGEM0314, p2iGEM0275, p2iGEM0276, p2iGEM0283, p2iGEM0284

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Susanne Vollmer

created: 05.09.2018 18:15
updated: 06.09.2018 17:24

Entry 8/22: restriction ligation cloning of atzD, trzC, guaD, ptxDopt into the pSCB1C3
In Project: Part submission
With tags: restriction, ligation, cloning

restriction and ligation cloning of trzC, guaD, atzD ptxDopt into the iGEM backbone pSCB1C3 with the following protocol:

Restriction:

Mastermix:

- 5 µl NEB 3.1 Buffer
- 1 µl EcoRI-HF
- 1 µl PstI-HF

add to 4 µl of the backbone (pSCB1C3) 4 µl of the mastermix, add to 3 µl of the desired plasmid (p2iGEM0373, p2iGEM0309, p2iGEM0294, p2iGEM0295) 4 µl Mastermix

incubation at 37°C for 30 min

Ligation:

Mastermix:

- 2 µl Backbone
- 3 µl of the desired plasmid
- 1 µl T4 ligase buffer
- 0,5 µl T4 ligase
- 3,5 µl milli Q

Cycler program:

- 16°C for 8h
- 80°C for 20 min
- 12°C HOLD

must be restarted cause the cycler rune only for 8 minutes, bevor restart add T4 ligase again.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Susanne Vollmer
Entry 9/22: Transformation of E.coli T 10 with p2iGEM0379-p2iGEM0382
In Project: Part submission
With tags: Transformation

created: 06.09.2018 16:37
updated: 06.09.2018 17:43

transformation of E.coli Top 10 with p2iGEM0379-p2iGEM0382 with the following protocoll:

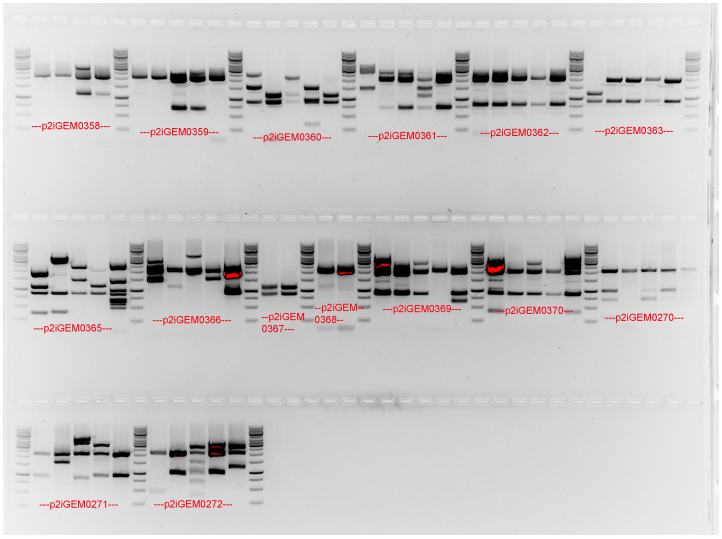
- thraw competent cells (*Top 10 and DH5a*) 5-10 min on ice
- add 2 µl of the Plasmid DNA on the competent cells
- flick the tube 3-4 times. Do not vortex
- incubate on ice for 30 min
- heatshock for 45 sec on 42°C
- place on ice for 5 min
- pipette 300 µl LB without Antibiothica (sterile)
- place at 37°C for 60 min, shake 300 rpm
- centrifuge with 6000 rpm for 2 min, decate 180 µl supernatant (sterile!), resuspend the rest (120 µl)
- plate 120 µl on LB can
- incubate at 37°C over night

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Katharina Polzen
Entry 10/22: Test restriction evaluation
In Project: Part submission
With tags: iGEM part

created: 07.09.2018 09:20
updated: 07.09.2018 09:24

Gel_2018-09-06_13hr_43minKPBack.jpg



Test restriction for the iGEM Backbones:

p2iGEM0358: nothing right

p2iGEM0359: #3 and #4 right

p2iGEM0360: #2 and #5 right

p2iGEM0361: #5 right

p2iGEM0362: All right

p2iGEM0363: #1 right

p2iGEM0365: #4 right

p2iGEM0366: nothing right

p2iGEM0367: #1 and #2 right

p2iGEM0368: nothing right

p2iGEM0369: #2 right

p2iGEM0370: #2 and #4 right

p2iGEM0371: #1 and #5 right

p2iGEM0372: #5 right

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
Entry 11/22: Part submission/Level 1 Cidar Quorum sensing testrestriction
In Project: Part submission
With tags: part, part submission

created: 10.09.2018 11:04
updated: 10.09.2018 11:05

Enzyme Master Mix

-5µl NEB Buffer 3.1

-1µl EcoRI -HF

-1µl PstI-HF

water up tp 25µl

RESTRICTION: Then: 4µl Plasmid pSCB1C3 and 4µl Enzyme Master Mix/ and 3µl of desired plasmid and 4µl Enzyme Master Mix are mixed together

The samples are restricted at 37°C for 30 min and then inactivated up to 80°C for 20 min.

LIGATION:

-2µl of Plasmid backbone pSCB1C3

- equimolar amounts of digested part to be inserted (<3µl)

- 1µl T4 DNA ligase buffer

- 0.5µl T4 DNA ligase

- up to 10µl H20

Ligate at 16°C for 30min and heat inactivate at 80°C for 20min.

After this step 1-2µl are supposed to be transformed into competent cells.

In this case p2iGEM0358,364,366,368,385,386,387,403,409,411,413,431,432 and 433 are created.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Susanne Vollmer
Entry 12/22: restriction liagation cloning and transformation
In Project: Part submission
With tags: restriction, ligation, cloning, Transformation

created: 10.09.2018 11:27
updated: 12.09.2018 21:00

restriction Ligation cloning of p2iGEM0294, p2iGEM0295, p2iGEM0309, p2iGEM0373.2, p2iGEM0332, p2iGEM0256, p2iGEM0257, p2iGEM0258 into the iGEM Backbone pSCB1C3 with the following protocol:

Enzyme Master Mix for p2iGEM0294, p2iGEM0295, p2iGEM0309, p2iGEM0373.2

-5µl NEB Buffer 3.1

-1µl EcoRI -HF

-1µl PstI-HF

water up tp 25µl

Enzyme Master Mix for p2iGEM0332, p2iGEM0256, p2iGEM0257, p2iGEM0258

-5µl NEB Buffer 3.1

-1µl EcoRI -HF

-1µl PstI-HF

water up tp 25µl

RESTRICTION: Then: 6µl Plasmid pSCB1C3 and 6µl Enzyme Master Mix/ and 3µl of desired plasmid and 4µl Enzyme Master Mix are mixed together

The samples are restricted at 37°C for 1 h and then inactivated up to 80°C for 5min.

LIGATION:

-2µl of Plasmid backbone pSCB1C3

- digested part 3µl

- 1µl T4 DNA ligase buffer

- 0.5µl T4 DNA ligase

- 4,5 µl milli Q

Ligate at 16°C 2 h 20 min and transform directly 4 µl into E.coli T10

inactivate the rest at 80°C for 5 min

transformation of E.coli T10 with the iGEM Backbone cloning (p2iGEM0388,p2iGEM0389, p2iGEM0390, p2iGEM0391, p2iGEM0321, p2iGEM0322, p2iGEM0323, p2iGEM0324) and p2iGEM0373,2 with the following protocol:

- thraw competent cells (*Top 10*) 5-10 min on ice
- add 4 µl of the Plasmid DNA on the competent cells
- flick the tube 3-4 times. Do not vortex
- incubate on ice for 30 min
- heatshock for environ 45 sec on 42°C
- place on ice for 5 min
- pipette 300 µl LB without Antibiothica (sterile)
- place at 37°C for 60 min, shake 300 rpm
- centrifuge with 6000 rpm for 2 min, decate 200 µl supernatant (sterile!), resuspend the rest (100 µl)
- plate 100 µl on LB can-> expect p2iGEM0373,2 add all the culture to 3ml LB and 30 µl ampicilline
- incubate at 37°C over night

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
Entry 13/22: Transformation part submission
In Project: Part submission
With tags: transformation

created: 11.09.2018 11:16
updated: 11.09.2018 11:18

p2iGEM0358,364,366,368,385,386,387,403,409,411,413,431,432 and 433 are transformed into competent E.coli Top 10 cells according to the following protocol:

- thraw competent cells (*Top 10*) 5-10 min on ice
- add 4 µl of the Plasmid DNA on the competent cells
- flick the tube 3-4 times. Do not vortex
- incubate on ice for 30 min
- heatshock for environ 45 sec on 42°C
- place on ice for 5 min
- pipette 300 µl LB without Antibiothica (sterile)
- place at 37°C for 60 min, shake 300 rpm
- centrifuge with 6000 rpm for 2 min, decate 200 µl supernatant (sterile!), resuspend the rest (100 µl)
- plate 100 µl on LB Cam
- incubate at 37°C over night

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
Entry 14/22: Inoculation of colonies on Lb Cam
In Project: Part submission
With tags: inoculation, part submission

created: 12.09.2018 11:36
updated: 12.09.2018 11:37

Grown colonies are incoulated in liquid LB medium (+Cam) and grown overnight at 37°C at 220rpm.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Susanne Vollmer
Entry 15/22: Miniprep of p2iGEM0379-p2iGEM0382, and p2iGEM0288-p2iGEM0392
In Project: Part submission
With tags: miniprep

created: 12.09.2018 20:58
updated: 12.09.2018 21:12

Miniprep of the inocultated colonies (inoculation a day before, each Colony in 3 ml LB chloramphenicol, incubation over night at 37° C) of p2iGEM0379, p2iGEM0380, p2iGEM0381, p2iGEM0382, p2iGEM0388, p2iGEM03789, p2iGEM039, p2iGEM0379 with the follwing protocol:

Kit used: Promega PureYield^{TM} Plasmid Miniprep System

- centrifuged for 5min at max rpm (in the culture tubes), most of the supernatant was discarded and the pellet in the remaining (600 µl) resuspended.
- remaining culture was put into an 1,5 ml Eppi
- 100 µl Lysisbuffer was added and mixed well
- 350 µl neutralisation buffer (cold) was added and mixed
- centrifuge at 3 min max rpm
- Addition of supernatant (800 µl) to column
- centrifuge 30 Sek. max rpm --> discard supernatant
- add 200µl Endotoxin removal wash
- 30 sex max rpm
- add 400µl column wash
- 30 sec max rpm
- insert collumn into new tube and add 30µl of Elution buffer
- incubate for 45 min at roomtemperature
- 30 sec max rpm

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
Entry 16/22: Miniprep part submission
In Project: Part submission
No tags associated

created: 13.09.2018 10:49
updated: 13.09.2018 10:50

The 60 inoculated colonies are miniprepped according to the Miniprep Pure Yield Protocol of Promga.

- 600µl culture + 100 µl Lysisbuffer --> mix + 350 µl neutralisation buffer (4°C) --> mix
- centrifuge at 3 min max rpm
- Add supernatant (~ 800 µl) to column
- centrifuge 30 sec. max rpm --> discard supernatant
- add 200µl endotoxin removal wash
- centrifuge 30 sec. max rpm
- add 300µl column wash
- 30 sec max rpm
- For elution use new tube+ 30 µl 37°C warm milli Q water and centrifuge down for 1 min.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Susanne Vollmer
created: 13.09.2018 10:51
updated: 13.09.2018 18:48

Entry 17/22: Testrestricion, gel electrophoresis and sequencing of the iGEM Backbone cloning with p2iGEM0379-p2iGEM0382 and p2iGEM0389-p2iGEM0391
In Project: Part submission
With tags: testrestriction, gel electrophoresis, sequencing

Testrestriction to test if the iGEMbackbone cloning worked, with the follwing protocol:

Mastermix:

- 1 µl cutsmart
- 1 µl template Plasmid
- 0,2 µl NotI-HF
- 7,8 µl milli Q

incubation at 37°C for 2h

no inaktivation, just add loading dye an load on gel

1 % agarose gel

90 V 95 min

in each pocket: 10 µl (all of the restriction) and 2 µl loading dye

1kb ladder

gel picture below:

expected bands:

pSCB1C3,iGEM backbone (negative): 2046 bp, 24 bp

pSCB1C3_atzD (p2iGEM0382): 2046 bp, 1145 bp

pSCB1C3_Dur1.2p2 (p2iGEM0391): 3011 bp, 2364 bp

pSCB1C3_trzE (p2iGEM0389): 2364 bp, 938 bp

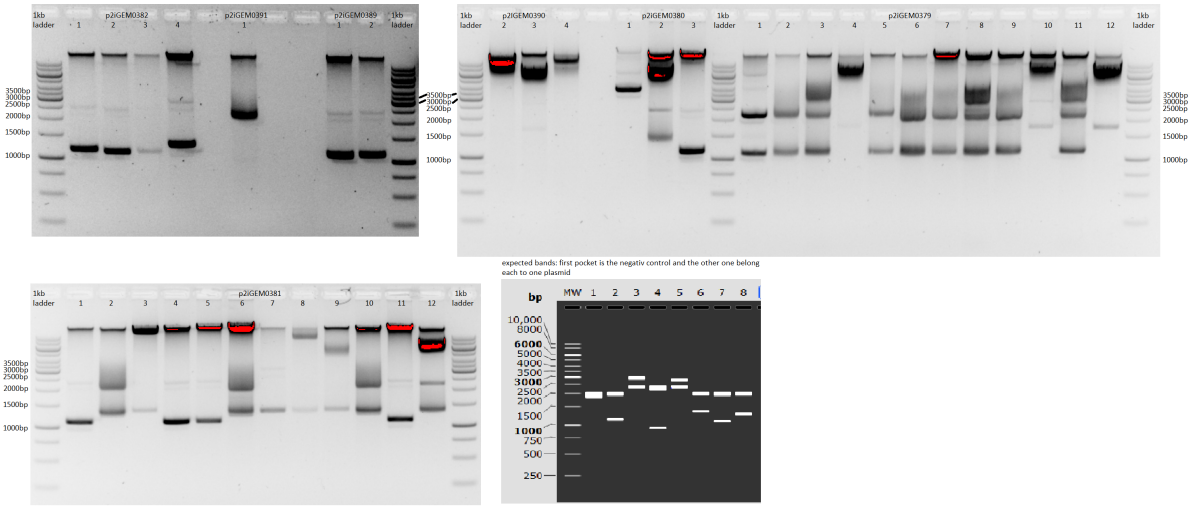
pSCB1C3_Dur1.2p1 (p2iGEM0390): 2874 bp, 2364 bp

pSCB1C3_guaD (p2iGEM0380): 2046 bp, 1376 bp

pSCB1C3_ptxDopt (p2iGEM0379): 2046 bp, 1089 bp

pSCB1C3_trzC (p2iGEM0381): 2046 bp, 1292 bp

Gel_2018-09-13_14hr_19min_testrestriction_notI_iGEMbackbones_SV_90V_1kb_95_min_bearbeitet.png



sequencing of possibly positiv plasmids of the iGEMbackbone cloning, with the following protocol:

in each tube 400-500 ng of DNA, 2,5 µl primer and add milli Q up to 10 µl, put a Barcode on the tubes, spin short, collect them in a bag and then bring to the GATC box

Barcode	Template	Primer	content of plasmid
69BC13	p2iGEM0382, 2	O_iGEM18_0092	6,25 µl
69BC14	p2iGEM0382, 2	O_iGEM18_0093	6,25 µl
69BC15	p2iGEM0389, 2	O_iGEM18_0092	7,5 µl
69BC16	p2iGEM0389, 2	O_iGEM18_0093	7,5 µl
69BC17	p2iGEM0380, 5	O_iGEM18_0092	1,2 µl
69BC18	p2iGEM0380, 5	O_iGEM18_0093	1,2 µl
69BC19	p2iGEM0380, 6	O_iGEM18_0092	1,85 µl
69BC20	p2iGEM0380, 6	O_iGEM18_0093	1,85 µl
69BC21	p2iGEM0379, 5	O_iGEM18_0092	3,82 µl
69BC22	p2iGEM0379, 5	O_iGEM18_0093	3,82 µl
69BC23	p2iGEM0381, 2	O_iGEM18_0092	5,62 µl
69BC24	p2iGEM0381, 2	O_iGEM18_0093	5,62 µl

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
created: 13.09.2018 16:14
updated: 13.09.2018 17:16
Entry 18/22: Testrestriction part submission
In Project: Part submission
With tags: test, restriction

Plasmids are testrestricted according to the following protocol:

0.3µl EcoRi

0.3µl PstI

1µl CuTsmart

1µl Plasmid

up to 10µl H20

The samples are incubated at 37°C overnight

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
Entry 19/22: Gel testrestriction part submission
In Project: Part submission
With tags: part submisiion, testrestriction

created: 14.09.2018 13:56
updated: 14.09.2018 15:38

The samples restricted the previous day are loaded on a 1% agarose gel

the following bands are expected:

p2iGEM0385: 2029bp and 1028bp

p2iGEM0368: 2029bp and 885bp

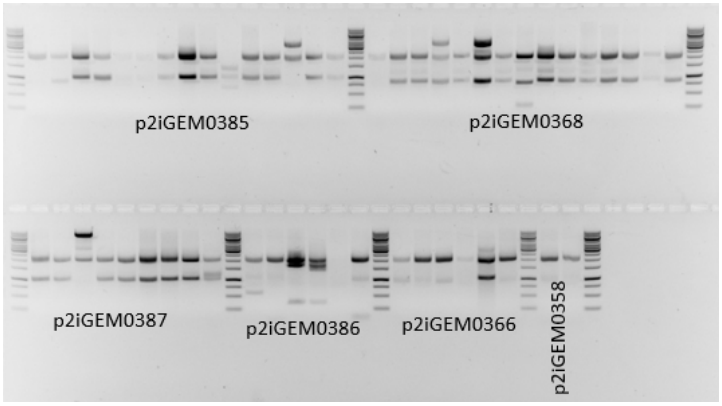
p2iGEM0387: 2029bp and 1075bp

p2iGEM0386: 2029bp and 604bp

p2iGEM0366: 2029bp and 547bp

p2iGEM0358: 2029bp and 682bp

Part_Improvement.PNG



Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
Entry 20/22: Level 2 plasmids QS
In Project: Part submission
With tags: QS, level2

created: 24.09.2018 11:10
updated: 24.09.2018 11:13

The level 2 plasmids are cloned into the iGEM backbone according to the following protocol:

Enzyme Master Mix

-5µl NEB Buffer 3.1

-1µl EcoRI -HF

-1µl PstI-HF

water up tp 25µl

RESTRICTION: Then: 4µl Plasmid pSCB1C3 and 4µl Enzyme Master Mix/ and 3µl of desired plasmid and 4µl Enzyme Master Mix are mixed together

The samples are restricted at 37°C for 30 min and then inactivated up to 80°C for 20 min.

LIGATION:

-2µl of Plasmid backbone pSCB1C3

- equimolar amounts of digested part to be inserted (<3µl)

- 1µl T4 DNA ligase buffer

- 0.5µl T4 DNA ligase

- up to 10µl H20

Ligate at 16°C for 30min and heat inactivate at 80°C for 20min.

After this step 1-2µl are supposed to be transformed into competent cells.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
Entry 21/22: Inoculation of colonies
In Project: Part submission
With tags: colonies

created: 25.09.2018 16:13
updated: 25.09.2018 16:14

Inoculation of colonies in 3mL LB Cam and incubation at 37°C at 220 rpm.

Date:	Signed and understood by:
Date:	Witnessed and understood by:

Author: Ylenia Longo
Entry 22/22: Testrestriction Lvl2
In Project: Part submission
With tags: testrestriction

created: 27.09.2018 11:05
updated: 27.09.2018 15:48

A testrestriction is performed according to the following protocol:

0.3µl EcoRi

0.3µl PstI

1µl CutSmart

7.6µl H20

The samples are incubated at 37°C for an hour.

The following bands are expected:

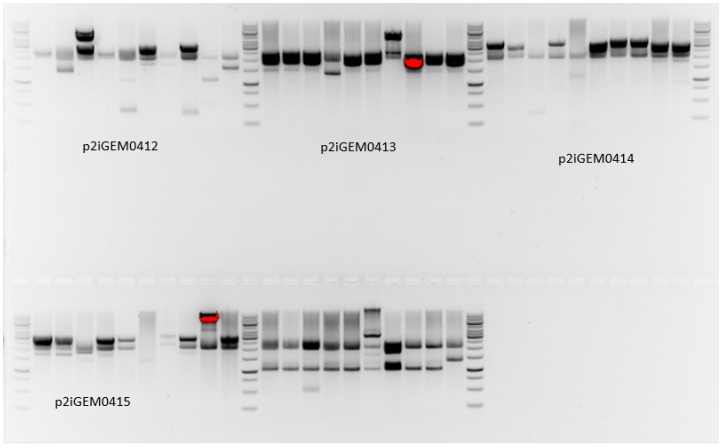
p2iGEM0412: 2233bp and 2029bp

p2iGEM0413: 2029bp and 1863bp

p2iGEM0414: 3155bp and 2029bp

p2iGEM0415: 2677bp and 2029bp

sniüpp.PNG



Date:	Signed and understood by:
Date:	Witnessed and understood by: