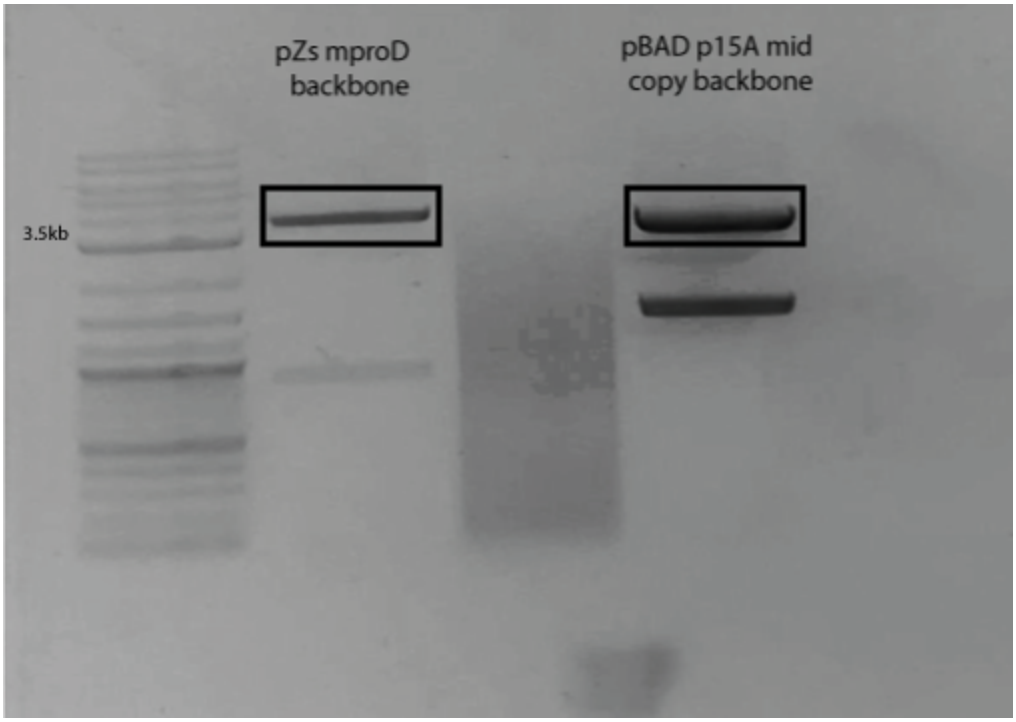
In order to construct low copy pelB-sfGFP-Ag43 construct,

pelB-sfGFP-Ag43-AmpR, to get pelB-sfGFP-Ag43,

pZs-mproD-UreR-KanR, to get low copy backbone,

were inoculated, miniprep was done and nanodrop analysis performed.

Sample	Conc. (ng/µl)	260/280	260/230
pelB-sfGFP-Ag43-AmpR	369.1	1.92	1.81
pZs-mproD-UreR-KanR	375.9	1.99	2.00



1) Ladder, 2) pZs-mproD-UreR-KanR, 3) pZe-pBAD-ALP-AmpR , 4) pZa-pBAD-ALP-Cmr

Gel extraction done to pZs-mproD-UreR-KanR

Sample	Conc. (ng/µl)	260/280	260/230
pZs-mproD-UreR-KanR	25.2	1.67	0.03

Restriction Enzyme Digestion:

pZs-mproD-UreR-KanR (KpnI&HindIII):

4.5 µl nuclease-free water
1 µl Cutsmart
4 µl DNA
0.25 µl KpnI
0.25 µl HindIII

The following Gibson primers were designed to clone pelB-sfGFP-Ag43 to low copy backbone:

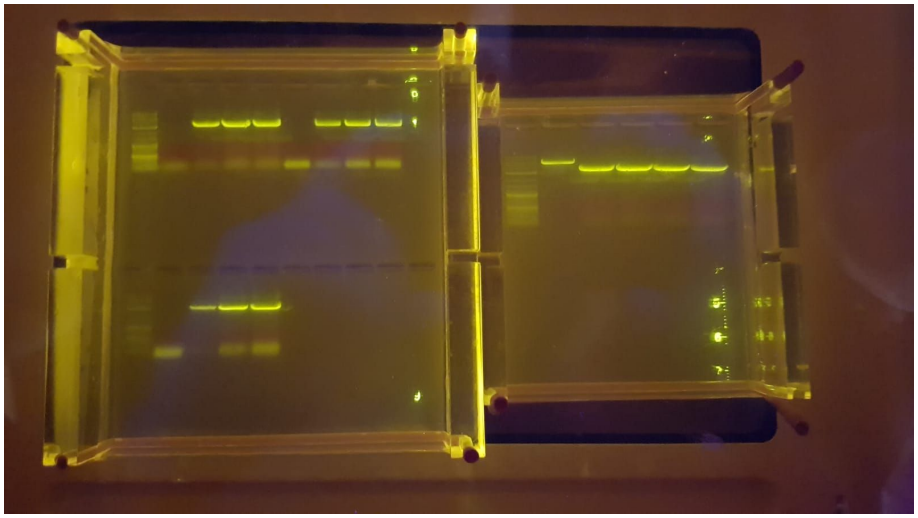
Construct	Name	Description	Base Pairs	Tm	Tm w/ Overhang	Anneal	100 µM stock- µl TE
Constitutive Active sfGFP display	iGEM2019-P1	Cons-Fwd-V1	51	65	78		468
	iGEM2019-P2	Cons-Rev-V1	59	65	82	65	468

For plasmid with Linker TEV + sfGFP

1. 5 µl P1, 5 µl P2 → 10 µl primers
2. Q5 High-Fidelity 2X Master mix → 50 µl
3. ddH2O → 40 µl

Total 100 µl to one tube.

- From 100 µl total mix without DNA → 25 µl was transferred to control tube.
- 0.3 µl plasmid DNA was added to 75 µl mix.
- 75 µl complete mix with DNA was split into 3 PCR tubes.



Gel extraction was done and nanodrop analysis was performed:

Sample	Conc. (ng/µl)	260/280	260/230
sfGFP	175.1	2.06	0.56

Gibson assembly was done:

pZS mproD + sfGFP
50 ng backbone (pZS mproD) + 46 ng insert (sfGFP)

=> 2 µl backbone + 0.26 µl insert + 0.24 µl ddH_2O + 7.5 µl Gibson Mix

Thermocycler, 50 °C, 1 hour

Transformation was not successful.

Gibson assembly was repeated:

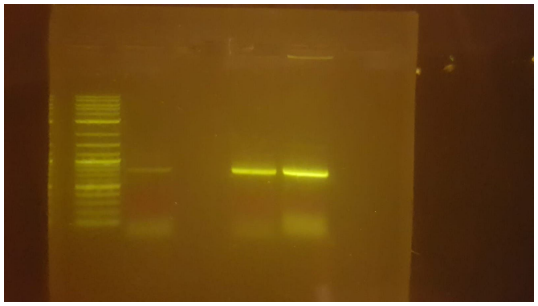
50 ng plasmid → 2 µl plasmid DNA
46 ng insert → 0.26 µl insert
→ 0.24 µl ddH_2O
→ 7.5 µl Gibson Mix
10 µl total mixture

Thermocycler, 50 °C, 1 hour

Following Gibson Assembly, sfGFP construct was transformed to PRO DH5α.

Colony PCR for sfGFP construct:

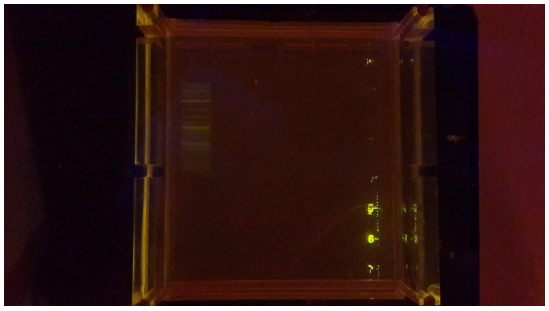
- 3 colonies were selected from the cells containing pZS-sfGFP-Kan.
- Same reaction and protocol were followed.
- sfGFP insert was verified.



Two different sfGFP colonies were chosen from agar plate and grown in 15 ml falcon overnight. When we checked the cells after overnight growing, none of them shined.

Restriction digestion was set up for verification:

2 µl sample
1 µl Cut Smart
0.25 µl KpnI
6.75 µl ddH_2O



sfGFP colonies were visualized under microscopy to see which colonies are shining.

Colony 3,7,8,10 and 11 were observed as shining a bit.

Cells taken from colonies observed as shining under microscopy were grown at 18 °C for three days.

Grown cells did not shine.
sfGFP Colony PCR For Verification From Colony 7 → Failed

Cells inoculation from sfGFP colony 3 & 11, and glycerol stocks were taken.

Miniprep for sfGFP Colony 3:

Conc. (ng/µl)	260/280	260/230
48.6	1.92	1.75

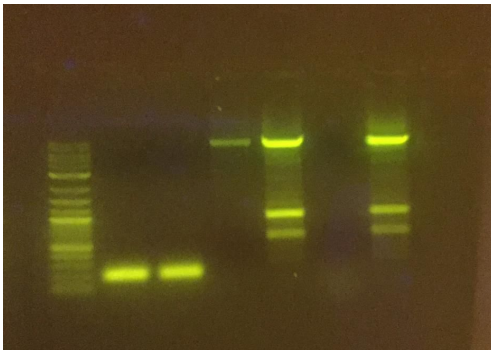
pZs-mproD-sfGFP was sent to sequencing.

PCR to proD

Primers used: P7, P8

PCR to pZS backbone

Primers used: P9, P10



Gel Order: ladder-proD-proD-pZS-pZS-pZS-pZS (Successful)

Gel to PCR products:

Sample	Conc. (ng/µl)	260/280	260/230
proD	29.4	1.93	0.08
pZS backbone	60.6	2.31	0.19

Gibson Assembly was done to extracted proD and pZS backbone

pZS backbone : 6900 bp

proD : 200 bp

6:1 reaction was performed

Transformation of Gibson products to PRO DH5***α***

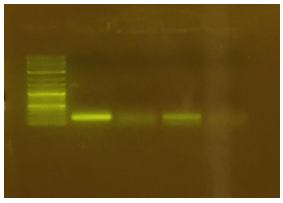
Positive control: pET22B backbone

-Also one negative control was made

There were no colonies. The experiments were repeated.
Gibson to proD and pZS backbone
Transformation of Gibson products to PRO DH5***α*** with two positive controls.

There were colonies. However, pZs proD colonies were not shining as desired.

Colony PCR was done to a pZS proD colony. Insert was verified.



5 colonies were chosen from pZS proD plate and inoculated and incubated overnight.

Colonies inoculated was checked under UV. Colony 2 and Colony 4 was observed as shining.

5 colonies grown overnight were centrifuged at 6000 rpm for 10 minutes. The pellet was resuspended with 1 ml 1X PBS. 400 µl of mixture was transferred to eppendorfs (2 replicas for each colony). One replica was heat released(60 ºC, 5 min) and the other was used as control group. Then cells were checked with transilluminator and M5 plate reader to check fluorescence intensity.

Cell stock was prepared from pZs proD col1, col2, col3, col4 and col 5
Heat Release Assay to pZs proD col1, col2, col3, col4 and col 5.

Miniprep to sfGFP proD col 2 & col 4:

Sample	Conc. (ng/µl)	260/280	260/230
sfGFP col 2	72.5	1.85	1.85
sfGFP col 4	55.9	1.91	1.62

pZs proD sfGFP col2 and col4 were transformed to BL21 Mar competents

pZs proD sfGFP transformed BL21 Marionette cells were inoculated.

Cell stocks were prepared from pZs proD sfGFP transformed BL21 Marionette cells that were inoculated.