

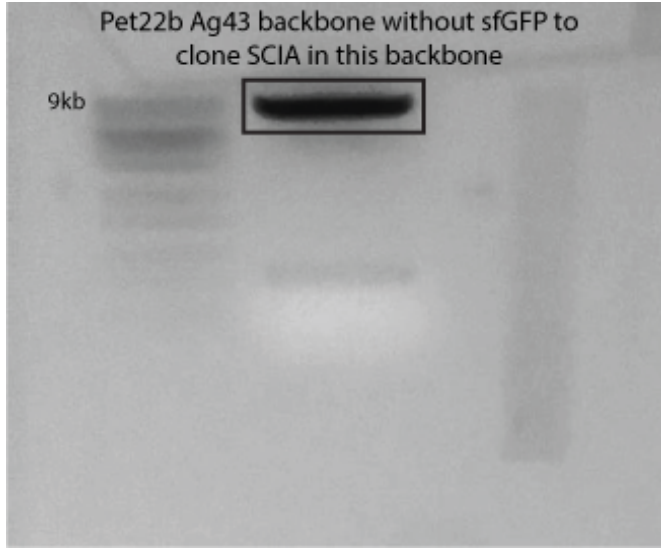
Sample ID	Corresponding IDT Construct
G1	1996 patent design with SCI57 a-b chains
G2	1996 patent design with wild type a-b chains
G3	Rajpal 15 design with SCI57 a-b chains
G4	Rajpal 15 design with wild type a-b chains
G5	Rajpal 27 design with SCI57 a-b chains
G6	Rajpal 27 design with wild type a-b chains
G7	SCI57 with its own linker a-b chains
G8	SCI 57 with wild type a-b chains

8 IDT constructs were diluted to 10 ng/ μ l with HyPure Nuclease-free water and stored in -20°C .

Restriction digestion to pet22b-pelB-sfGFP-Ag43-AmpR in order to remove sfGFP and clone IDT constructs:

- \approx 1200 ng DNA
- 2.5 μ l CutSmart
- 0.5 μ l BamHI
- 0.5 μ l AflII
- 18 μ l ddH₂O

Gel electrophoresis:



Gel extraction was done and nanodrop analysis was performed:

Conc. (ng/ μ l)	260/280	280/230
20.6	2.89	0.02

Gibson assembly was done:

- a. IDT G1 (insert) + Pet22b (backbone) (1:5)
- b. Reaction:
 - i. 1 μ l insert
 - ii. 2.5 μ l backbone
 - iii. 15 μ l Gibson Mix
 - iv. 1 μ l ddH₂O

Transformation of Gibson Product to PRO DH5 α was done, and the selected colony was inoculated.

Colony PCR was done to G1.



Gibson assembly was done to remaining 7 IDT constructs with the constructs and pET22b backbone:

1 μ l insert
2.5 μ l bb
7.5 μ l mix
6.5 μ l ddH₂O
insert: 380 bp
backbone: 8 kbp

IDT Gibson products were transformed into PRO DH5 α cells.

All IDT Gibson products have all grown.

Miniprep was done to IDT G1 and pET22b-pelB-sfGFP-Ag43.

Sample	Conc. (ng/ μ l)	260/280	260/230
IDT G1	114.0	1.95	1.75
pET22b	202.2	2.03	1.42

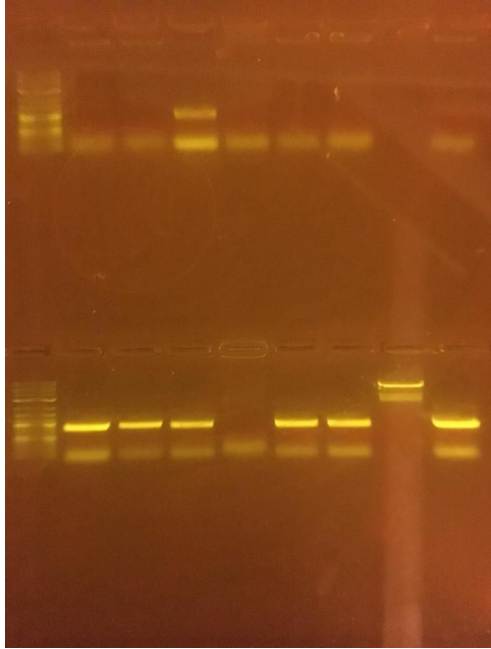
IDT-G1 was prepared for sequencing.

Sequencing result was successful.

Colony PCR was done on IDT G2, G3, G4, G5, G6, G7, G8:

PREA 34 & PGEMP 3 primers were used.

Annealing was set to 65°C



IDT cells were inoculated and cell stocks were taken, also miniprep was done:

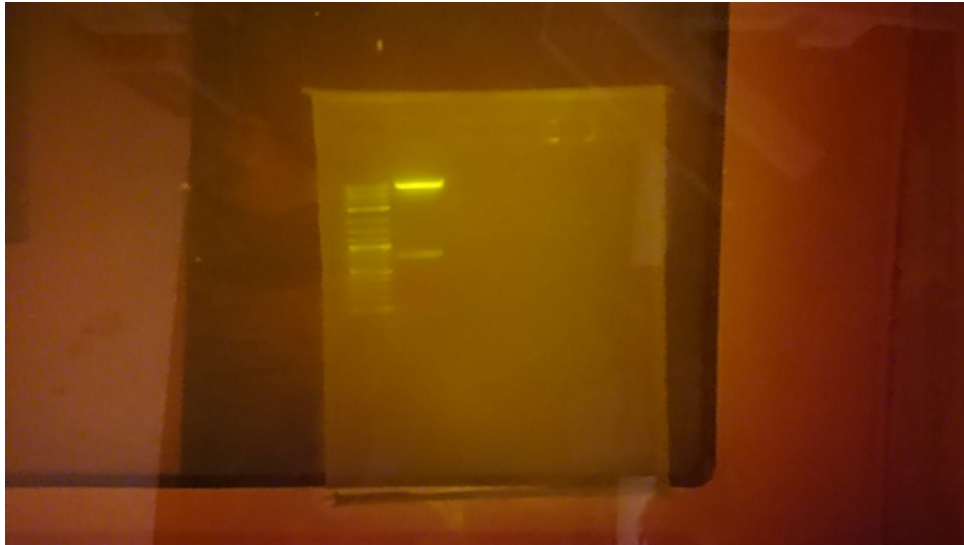
Sample	Conc. (ng/μl)	260/280	260/230
G2	164.1	2.05	1.77
G3	101.9	1.98	1.80
G4	229.6	2.07	2.11
G5	203.8	2.04	1.92
G6	1.8	1.43	0.13
G7	158.0	1.99	1.94
G8	120.7	1.98	1.92

Restriction Digestion to pET22b bb to remove sfGFP part:

- 2.5 μl CutSmart
- 0.5 μl BamHI
- 0.5 μl AflII
- 18 μl ddH₂O

Sample	Conc. (ng/ μ l)	260/280	260/230
pET22b bb	23.2	2.62	0.03

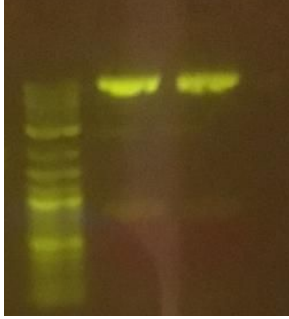
1. Gibson on IDT product
 - a. 2.3 μ l backbone
 - b. 2 μ l insert
 - c. 1.8 μ l ddH₂O



Miniprep to two pET22b duplicates

Sample	Conc. (ng/ μ l)	260/280	260/230
pET22b 1	234.4	1.98	2.25
pET22b 2	272.1	2.03	2.23

Digestion on pET22b plasmid with BamHI & AflIII



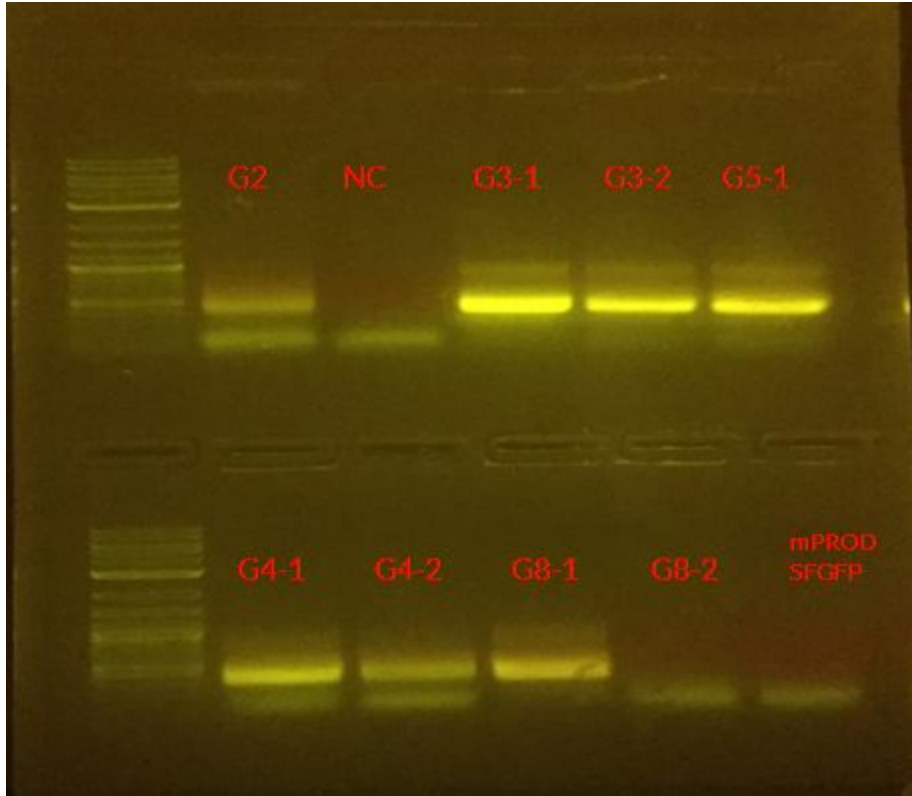
Gel extraction on digested pET22b (backbone)

Sample	Conc. (ng/ μ l)	260/280	260/230
pET22b digest	42.1	1.90	0.24

Gibson Assembly:

15 μ l mix

G2, G3, G4, G5, G8 IDT products were grown on plate; G6 & G7 were not grown
G2, G3, G4, G5, G8 were verified with colony PCR.



Verified colonies were inoculated in LB overnight.

G2-1, G3-1, G5-1, G4-1, G8-2

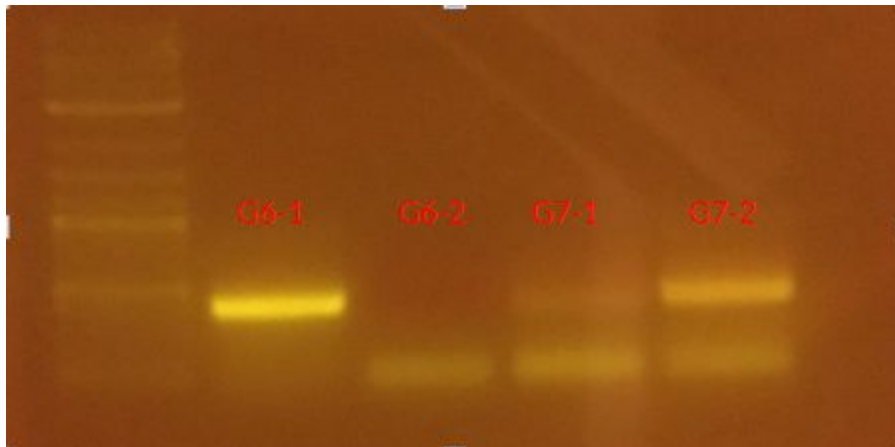
Gibson to IDT G6 and IDT G7 constructs was done. Transformed to PRO DH5 α competent cells.

Cell stocks were taken from PRO DH5 α cells containing IDT G2, G3, G4, G5 and G8. Miniprep was done.

Nanodrop Analysis:

Sample	Conc. (ng/ μ l)	260/280	260/230
IDT G2	300.1	2.04	2.22
IDT G3	177.4	2.01	2.23
IDT G4	355.1	2.07	2.26
IDT G5	126.8	2.00	1.93
IDT G8	271.3	2.04	2.26

IDT G6 and IDT G7 constructs were verified by colony PCR.

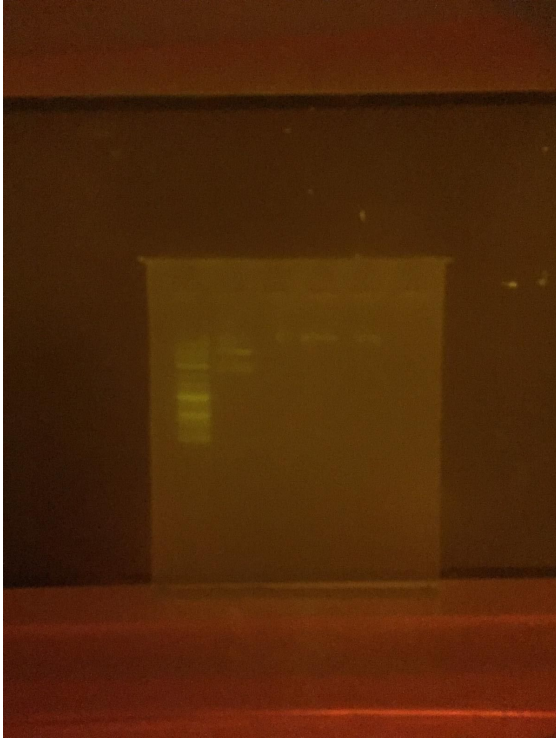


RESULT: G6 col1 and G7 col2 were verified.
Verified colonies for G6 and G7 were inoculated.

G5 & G6 sequencing was failed. Therefore:

1. Cell inoculation
 - a. DH5 α PRO cells with IDT-G5
 - b. DH5 α PRO cells with IDT-G6
2. Miniprep
 - a. DH5 α PRO cells with IDT-G5
 - b. DH5 α PRO cells with IDT-G6

Sample	Conc. (ng/ μ l)	260/280	260/230
IDT-G5	247.4	2.05	2.15
IDT-G6	204.6	2.04	2.21



gel order: ladder-digest-IDT G5-IDT G6
(IDT G5 and G6 were run on the gel for verification)

Gibson assembly to pet22b and IDT G5 & G6 was repeated. Transformation was done.

All IDT constructs were verified by sequencing.

All IDT constructs (except G1) were transformed into BL21 cells.

Cell inoculation

- a. Chosen from the plates
 - i. IDT - G2 - BL21
 - ii. IDT - G3 - BL21
 - iii. IDT - G4 - BL21
 - iv. IDT - G5 - BL21
 - v. IDT - G6 - BL21
 - vi. IDT - G7 - BL21
 - vii. IDT - G8 - BL21

Cell stock were prepared from BL21- IDT cells.