07/20/16

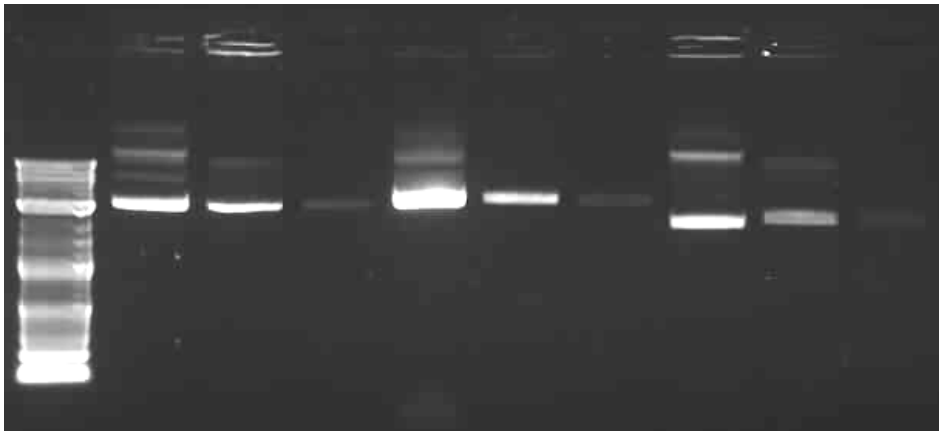
PCRs of pJS110 with oligos oEC11+oEC12 (product of 3000 pb) (no negative control…) to get amplified middle of dCas9.

07/21/16

PCRs of 113 + oEC7 + oEC14, 113 + oEC15 + oEC16, 113 + oEC17 + oEC8 to get amplified scFv.
PCR of 110 + oEC1 + oEC10 to get begining of dCas9
PCR of gDNA + oEC18 + oEC19 to get amplified pSET-16.

Single digest of 115 (156 ng/ul) with BbsI.
dna 25 ul
B (2.1) 3 ul
E 1 ul
dw 1 ul
Single digests of pSBIC3 with EcoR1 and Pst1 (at least 3 hours) :
dna 8 ul
B (2.1) 3 ul
E 1 ul
dw 18 ul

log 2	GFP uncut	GFP EcoRI	GFP PstI	pSBI C3 uncut	pSBI C3 EcoRI	pSBI C3 PstI	RBS uncut	RBS EcoRI	RBS PstI



Second digest of linearized pSBIC3 with Pst1 (overnight):
First digest : 29 ul
E : 1 ul

Gel to verify PCR products :
(no photo taken, oops)
=> Redo PCR of 17+8, 7+14, 15+16 and 18+19

Check PCR of 17+8, 7+14, 15+16, 18+19
Gel purification of PCR products and 115 digested with BbsI, pSBIC3 digested with EcoRI and PstI.

07/23/16

CIPage of linearized vectors 115 and non gel purified pSBIC3 :
Antartic Phosphatase : 0,5 ul
Buffer : 5 ul
Inactivation at 80 degrees for 30 minutes.

Anneal oligos upper&lower sg 1 to 6 :
oligos 0.5 mM (2ul)
kinase 1 ul
Buffer 2 ul
Put at 80 degrees for 15-20 minutes

Ligation of oligos + 115 :

	Vector alone	Vector + insert
115 guide ligase buffer dw		

Transformation of 20 ul of chemically competent bacteria with 1ul of each sgRNA

07/28/16

Redo sgRNAs cloning with 1.5mM (1ug of DNA) of oligos instead of 0.5 according to the NEB protocol.
First, oligos annealing
Typical Annealing Reaction

Primer	**1 µg = 10 ul**
10X T4 Ligase Buffer	**5 µl**
Nuclease-free Water	**To 50 µl**
Incubation	**85°C for 10 minutes, cool slowly (30-60 min.)**

Then phosphorylation of double stranded guides
Typical Annealing Reaction

Primer	**1 µg = 50 ul of the previous reaction**
10X T4 Ligase Buffer	**6 µl**
Nuclease-free Water	**To 60 µl**
Incubation	**85°C for 10 minutes, cool slowly (30-60 min.)**

Then ligate
Oligos 60
Vector (115) 2
B 7
E 1
Ligation overnight at 4 degrees.

Digest of pSB1C3 with Eco+Pst, Eco, Pst
DNA 25 (or 1ul)
B 3 ul
E 1 ul
dw 1 ul

07/29/16

Transformation of sgRNA 1, 2, 3, 4, 5, 6 and DHalpha without plasmid
DNA 10 ul (>100 ng)
Bacteria 25ul
At 4 degrees for 30 minutes, then 42 degrees for 30 sec and back to 4 degrees for 5 minutes. Resuspend in 3 ml (oops…...) of LB without antibiotic. Let it recover for 1 hour. Then try to spin at 1000 rpm for 5 minutes. Could not see any pellet. Then take 200 ul in the bottom of the tube and spread it on a Petri box with ampicillin. Put at 37 degrees.
In order to lose nothing, the remaining bacteria recieved ampicilling and put back in culture culture at 37 degrees.

Addgene order (DNMT3a3l) recieved. Bacteria resuspended in 4 ml of LB + kan.