

TITLE: CRISPR cloning - 85nt scaffold bicistronic backbone

SOP Number: D-DEZ -PRO-012

Revision Number: 0

Effective Date: 07 Apr 2015

- a. pDNA 8-10 µg
- b. NEB 10X Buffer 2.1 4 µL
- c. BbsI (5U/ul) 2 µL (10 U)
- d. H₂O to 40 µL

2. Run on a 2% agarose gel and gel purify linearized product (elute in 30 µL).

** Note: Can set up multiple digests so as to obtain stock of linearized backbone for future cloning reactions

F. ANNEAL gRNA TARGET OLIGOS

1. In 0.2ml PCR tubes set up following:

- gRNA_{top} 8.5 µL
- gRNA_{bot} 8.5 µL

2. Anneal in PCR machine using ANNEAL program

G. PHOPHORYLATE ANNEALED OLIGOS

1. Phosphorylate Oligos:

- a. Annealed oligos 17 µL
- b. T4 DNA ligase buffer 2 µL
- c. T4 PNK 1 µL

2. Incubate at 37 deg for 1 hr

3. Dilute phosphorylated and annealed oligos 1:100 in molecular grade water.

H. LIGATE gRNA TARGET INTO BICISTRONIC BACKBONE

1. Set up 1:7 vector:insert ligation reactions for each insert.

2. In addition, set up a vector-only control reaction.

3. Experimental reaction(s):

- a. Linearized backbone 1 µL

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- b. Annealed/phosphorylated oligos 7 μ L
 - c. T4 DNA ligase buffer 2 μ L
 - d. T4 DNA ligase 1 μ L
 - e. H₂O 9 μ L
4. Control reaction:
- a. Linearized backbone 1 μ L
 - b. T4 DNA ligase buffer 2 μ L
 - c. T4 DNA ligase 1 μ L
 - d. H₂O 16 μ L
5. Incubate at 16 deg overnight*

* Alternatively, can incubate at room temperature for 2-3 hours before proceeding with transformation.

I. TRANSFORMATION

1. Transform 12 μ l of each ligation reaction into Stbl3 OneShot cells
2. Incubate on ice 5-20 min
3. Heat shock at 42°C for 45 sec
4. Incubate on ice 1-2 min
5. Add 250 μ L SOC
6. Outgrow at 37°C while shaking 45 min – 1hr
7. Plate 150 μ L outgrowth onto LB + Amp plate

J. COLONY ANALYSIS

1. Verify targets have been cloned successfully by culturing and mini prepping 5-10 colonies from each plate (except the vector-only controls) using Machery-Nagel NucleoSpin Plasmid mini kit.
2. Sequence each clone with LKO 1 5 sequencing primer

*** make a glycerol stock from positive culture ***

K. HISTORY

The University of Iowa Dezii Translational Vision Research Group

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Effective Date	Revision	Change
07 Apr 2015	0	PM-18 Rev. #3