**1. Digest watermelon backbone**

(Add enzyme last)

|  |  |
| --- | --- |
|  | Amount |
| Backbone | 20 μg=20ul |
| CutSmart | 11 |
|  Sbf1 | 4 |
| water | 75 |
| **Total** | 110 |
|  |  |

Incubate O/N at 37 degrees

2. Gel purify

Add 9 uL water to each well of a 1% E-Gel

Distribute digest reaction 10 uL per well

Run gel

Run 3 E-gels with 10 lanes of digestion reaction

Every half a E-gel (5 lanes) goes to 1 epi-tube

 -Extract using using gel purification protocol (no need to do PCR cleanup)

-increase volume to 100ul

 -SPRI Clean 0.7X

-Elute in 40 uL (expect 50-200 ng/uL I got 125ng/ul)

**-** Qbit measurements

-Run on a gel- you should not see the second lower!

2. **Prepare lineage barcode insert**

1. **one cycle PCR**

Do a 1:1 molar ratio 500ng of each primer

|  |  |  |
| --- | --- | --- |
| # | Primer  | seq |
| 1 | Barcode\_F  | GGCTGGCAACTAGAAGGCACAGTCGAGGCTGATCAGCGGGTTTCCTGCAGGGTTTGTC |
| 2 | Barcode\_R (IDT seq format) |  CTGTACAAGTAAAttataaCCTGCAGGATGCGTCTTC(N1:50000050) (N2:00505000)(N1)(N2) (N1)(N2)(N1) (N2)(N1)(N2) (N1)(N2)(N1) (N2)(N1)(N2) (N1)(N2)(N1) (N2)(N1)(N2) (N1)(N2)(N1) (N2)(N1)(N2) (N1)(N2) GAAGACAAACCCTGCAGGAAACC |

-I used neb Q5 protocol for a 50ul reaction.

Single cycle PCR:

*98C, 30s (initial denaturation)*

*98C, 10s (denaturation)*

*60C, 10s (annealing)*

*72C, 10s (extension)*

*72C, 2 minutes (final extension)*

*4C (hold)*

-run on a gel (no need to do PCR cleanup)

-SPRI 2.5X

**-** Qbit measurements

**3. Gibson assembly**

**-total backbone 100ng**

**-ratio of insert to backbome: 5X**

* 4 hour incubation at 50 degrees

-SPRI and eluted in 15ul (control well gave 10ng/ul in nanodrop)

**4. Electroporation**

a. Electroporate entire volume into Lucigen Endura Competent Cells (25ul).

b. Estimate the number of transformed colonies:

by plating a serial dilution of transformation mixture as described in Wang, Lander, and Sabatini, 2016. Cold Spring Harb Protoc; doi:10.1101/pdb.prot090803.

c. Expand the cells in LB with carbenicillin for 16 hours at 30°C (~5pm-9:00am).

**5. maxi prep library**

1. spin down cultures and Purify pool with Qiagen EndoFree Maxi Kit
2. pick a handful of single colonies from the LB diversity plates mini-prep and send to Sanger sequencing
3. Proceed with miseq sequencing to confirm library diversity