**L: pTarg2 Assembly**

**L: PCR**

protocol: phusion

31.5 uL ddH2O

10 uL 5x Phusion Buffer

5 uL 2mM dNTPs

1 uL primer1 (10 uM)

1 uL primer2 (10 uM)

1 uL template

0.5 uL phusion

samples:

label primer1 primer2 template product

L1 targAf targAr p20N5 pTarg2/pcrA

L2 targBf targBr pTargetF pTarg2/pcrB

source:

label location note

p20N5 templates/C1 plasmid already diluted 25x

pTargetF templates/A1 plasmid already diluted 25x

targAf oligos1/E7

targAr oligos1/F7

targBf oligos1/G7

targBr oligos1/H7

destination: thermocycler1B

program: main/PHU1

note:

You will need to make 10 uM dilutions from lyophilized oligos. When done, store the 100 uM blue-capped stocks in the oligos1 box as listed for location; store the 10 uM oligo stocks in boxL as follows:

targAf boxL/A1

targAr boxL/A2

targBf boxL/A3

targBr boxL/A4

note:

Never let enzymes warm up! Only take the enzyme cooler out of the freezer when you are actively using it, and only take the tubes out of it when actively dispensing.

**L: Gel and DpnI**

source: thermocycler1B

samples:

reaction size product

L1 1200 pTarg2/pcrA

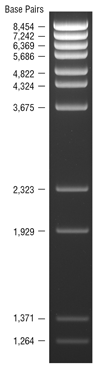
L2 1172 pTarg2/pcrB

protocol:

* In new PCR tubes, combine, mix and quick spin 6 uL of “1x Load” and 2 uL of PCR product
* Run a gel with the full volume of the mixture in each well, and an additional well with 5 uL of BstEII ladder
* Add 0.5 uL DpnI to each PCR reaction, mix, quick spin, run thermocycler
* Take an image of the gel and email to jcanderson@berkeley.edu

destination: thermocycler1B

program: main/SPE1



**L: Zymo**

source: thermocycler1B

samples:

reaction label elution\_volume destination product

L1 L1p 50 uL boxL/B1 pTarg2/pcrA

L2 L2p 50 uL boxL/B2 pTarg2/pcrB

**L: Assemble**

DNA Mix:

5 uL L1p

5 uL L2p

reaction:

4 uL ddH2O

1 uL DNA Mix

5 uL 2X Gibson Mix

source

dna location

L1p boxL/B1

L2p boxL/B2

samples

label fragments product

L L1p,L2p pTarg2

destination: thermocycler1B

program: main/GIB2

note:

Never let enzymes warm up! Only take the enzyme cooler out of the freezer when you are actively using it, and only take the tubes out of it when actively dispensing.

**L: Transform**

source: thermocycler1B

samples:

label product strain antibiotic incubate

L pTarg2 Mach1 Amp 37°C

rescue\_required: no

**L: Wrap**

samples:

source product strain antibiotic incubate

L pTarg2 Mach1 Amp 37°C

* Wrap the plate in parafilm
* Store it in the minifridge

**L: Pick**

samples:

source product strain antibiotic incubate number labels

L pTarg2 Mach1 Amp 37°C 2 LA, LB

**L: Miniprep**

samples:

culture label location

LA pTarg2-A boxL/C1

LB pTarg2-B boxL/C2

note:

Write pTarg2-# on the top of the Eppendorf

**L: Sequencing**

sources:

G00101 oligos1/B5

samples

label location plasmid oligo

LA boxL/C1 pTarg2-A G00101

Instructions:

* For each plasmid listed, mix the following sequencing reactions in an eppendorf tube:
  + 6 uL ddH2O
  + 4 uL miniprep DNA (undiluted)
  + 3 uL oligo (2.66 uM)
* Clearly label the tops of the tubes with the “label”, ie “LA”
* Take the sequencing reactions and order form to:
  + 237 Stanley Hall (second floor cold room)