**K: pTarg1 Assembly**

**K: 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

K1 yyBla-F yyBla-R p20N5 pTarg1/fragA

K2 yyEI-F yyEI-R pTargetF pTarg1/fragB

source:

label location note

p20N5 templates/C1 plasmid already diluted 25x

pTargetF templates/A1 plasmid already diluted 25x

yyBla-F oligos1/A7

yyBla-R oligos1/B7

yyEI-F oligos1/C7

yyEI-R oligos1/D7

destination: thermocycler1A

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 boxK as follows:

yyBla-F boxK/A1

yyBla-R boxK/A2

yyEI-F boxK/A3

yyEI-R boxK/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.

**K: Gel and DpnI**

source: thermocycler1A

samples:

reaction size product

K1 1183 pTarg1/fragA

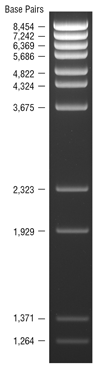
K2 1174 pTarg1/fragB

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: thermocycler1A

program: main/SPE1



**K: Zymo**

source: thermocycler1A

samples:

reaction label elution\_volume destination product

K1 K1p 50 uL boxK/B1 pTarg1/fragA

K2 K2p 50 uL boxK/B2 pTarg1/fragB

**K: Assemble**

DNA Mix:

5 uL K1p

5 uL K2p

reaction:

7 uL ddH2O

1 uL T4 DNA ligase buffer

1 uL DNA Mix

0.5 uL T4 DNA ligase

0.5 uL BsaI

source

dna location

K1p boxK/B1

K2p boxK/B2

samples

label fragments product

K K1p,K2p pTarg1

destination: thermocycler1A

program: main/GG1

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.

**K: Transform**

source: thermocycler1A

samples:

label product strain antibiotic incubate

K pTarg1 Mach1 Amp 37°C

rescue\_required: no

**K: Wrap**

samples:

source product strain antibiotic incubate

K pTarg1 Mach1 Amp 37°C

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

**K: Pick**

samples:

source product strain antibiotic incubate number labels

K pTarg1 Mach1 Amp 37°C 2 KA, KB

**K: Miniprep**

samples:

culture label location

KA pTarg1-A boxK/C1

KB pTarg1-B boxK/C2

note:

Write pTarg1-# on the top of the Eppendorf

**K: Sequencing**

sources:

G00101 oligos1/B5

samples

label location plasmid oligo

KA boxK/C1 pTarg1-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 “KA”
* Take the sequencing reactions and order form to:
  + 237 Stanley Hall (second floor cold room)