**O: Ortholog Swap Test**

**O: Dilutions**

For each plasmid, dispense 1 uL into 25 uL of water in an Eppendorf tube:

label source destination

pim3-A boxG/H5 boxO/A10

pimar1 donner1/B1 boxO/A1

pimar2 donner1/C1 boxO/A2

pimar3 donner1/D1 boxO/A3

pimar4 donner1/E1 boxO/A4

pimar5 donner1/F1 boxO/A5

pimar6 donner1/G1 boxO/A6

pimar7 donner1/H1 boxO/A7

pimar8 donner1/I1 boxO/A8

**O: PCR1**

>Resuspend all oligos to 100 uM:

source:

label location

orfF1 oligos1/H8

orfF2 oligos1/I8

orfR1 oligos1/G9

orfR2 oligos1/H9

nbackR0 oligos1/I9

backF2 oligos1/J9

>Prepare 10 uM dilutions, store dilution tubes at destination when complete:

label destination

orfF1 boxO/B1

nbackR0 boxO/B2

>Set up standard Q5 reaction:

samples:

label primer1 primer2 template product

O9 orfF1 nbackR0 pim3 b0

source:

label location note

pim3 boxO/A10 plasmid already diluted 25x

destination: thermocycler2A

program: Q5/Q5-8K

**O: PCR2**

mastermix:

134 uL ddH2O

42.5 uL 5x Phusion Buffer

21.25 uL 2mM dNTPs

4.25 uL primer1 (10 uM)

4.25 uL primer2 (10 uM)

2.125 uL phusion

reaction:

24.5 uL mastermix

0.5 uL template

samples:

label primer1 primer2 template product

O1 norfF0 orfR0 pimar1 p01

O2 norfF0 orfR0 pimar2 p02

O3 norfF0 orfR0 pimar3 p03

O4 norfF0 orfR0 pimar4 p04

O5 norfF0 orfR0 pimar5 p05

O6 norfF0 orfR0 pimar6 p06

O7 norfF0 orfR0 pimar7 p07

O8 norfF0 orfR0 pimar8 p08

destination: thermocycler2B

program: main/phu1

**O: Gel and DpnI**

source: thermocycler2A,2B

samples:

reaction size product

O1 1286 p01

O2 1035 p02

O3 1037 p03

O4 1076 p04

O5 1278 p05

O6 989 p06

O7 1027 p07

O8 1269 p08

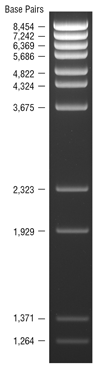
O9 6943 b0

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: thermocycler2A

program: main/SPE1



**O: Gel and DpnI**

source: thermocycler1A

samples:

reaction size product

O1 1286 p01

O2 1035 p02

O3 1037 p03

O4 1076 p04

O5 1278 p05

O6 989 p06

O7 1027 p07

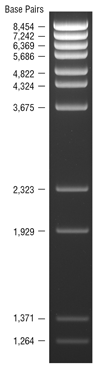
O8 1269 p08

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
* After confirming bands, add 0.5 uL DpnI to PCR reactions O1, O3, and O7, mix, quick spin, run thermocycler
* Take an image of the gel and email to jcanderson@berkeley.edu

destination: thermocycler2A

program: main/SPE1



**O: Zymo**

source: thermocycler2A

samples:

reaction label elution\_volume destination product

O9 O9 50 uL boxO/C9 b0

Redesign due to failed PCRs

**Ob: Ortholog Swap Test, redesigned**

**Ob: 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

Ob orfF0b orfR0 pim7 O7b

Oc orfF0c orfR0 pim7 O7c

Od orfF0d orfR0 pim7 O7d

source:

label location note

pim7 boxJ/A4 Needs dilution 25x

orfR0 oligos1/J8 Dilute

orfF0b oligos2/F1 Resuspend and dilute

orfF0c oligos2/G1 Resuspend and dilute

orfF0d oligos2/H1 Resuspend and dilute

destination: thermocycler2A

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 as follows:

orfF0b boxO/C1

orfF0c boxO/D1

orfF0d boxO/E1

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.

**Ob: Gel and DpnI**

source: thermocycler2A

samples:

reaction size product

Ob 1025 O7b

Oc 1039 O7c

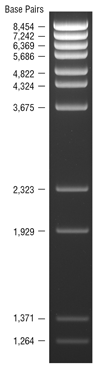
Od 1044 O7d

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: thermocycler2A

program: main/SPE1



**Ob: Zymo**

source: thermocycler2A

samples:

reaction label elution\_volume destination product

Ob Ob 50 uL boxO/C2 O7b

Oc Oc 50 uL boxO/D2 O7c

Od Od 50 uL boxO/E2 O7d

**Ob: DpnI Treatment**

Set up the following reaction for each sample in PCR tubes, mix well, run the thermocycler:

Reaction:

44 uL PCR product

5 uL NEB Buffer 2

1 uL DpnI

samples:

reaction label destination product

Ob Ob boxO/C2 O7b

Oc Oc boxO/D2 O7c

Od Od boxO/E2 O7d

destination: thermocycler2A

program: main/SPE1

**Ob: Zymo2**

source: thermocycler2A

samples:

reaction label elution\_volume destination product

Ob Ob 50 uL boxO/C2 O7b

Oc Oc 50 uL boxO/D2 O7c

Od Od 50 uL boxO/E2 O7d

Note: if there are already similarly-labeled tubes at the boxO locations, throw the old samples away and replace with your zymo cleanups**Ob: Assemble**

reaction:

4 uL ddH2O

0.5 uL frag1

0.5 uL frag2

5 uL 2X Gibson Mix

Source:

dna location

Ob boxO/C2

Oc boxO/D2

Od boxO/E2

O9 boxO/C9

Samples:

label fragments product

Ob Ob,O9 pim9b

Oc Oc,O9 pim9c

Od Od,O9 pim9d

destination: thermocycler2A

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.

**Ob: Transform**

source: thermocycler2A

samples:

label product strain antibiotic incubate

Ob pim9b Mach1 Amp 37°C

Oc pim9c Mach1 Amp 37°C

Od pim9d Mach1 Amp 37°C

rescue\_required: no

**O: Pick**

samples:

label product strain antibiotic incubate number labels

Ob pim9b Mach1 Amp 37°C 2 ObA,ObB

Oc pim9c Mach1 Amp 37°C 2 OcA,OcB

Od pim9d Mach1 Amp 37°C 2 OdA,OdB

**O: Miniprep**

samples:

culture label location

ObA pim9b-A boxO/C3

ObB pim9b-B boxO/C4

OcA pim9c-A boxO/D3

OcB pim9c-B boxO/D4

OdA pim9d-A boxO/E3

OdB pim9d-B boxO/E4

note:

Write “ObA” label on the top of the Eppendorf, and pim##-# on the side

**O: Sequencing**

sources:

ca998 oligos1/C5

jbagSeq oligos1/I5

samples

label location plasmid oligo

ObAf boxO/C3 pim9b-A jbagSeq

OcAf boxO/D3 pim9c-A jbagSeq

OdAf boxO/E3 pim9d-A jbagSeq

ObAr boxO/C3 pim9b-A ca998

OcAr boxO/D3 pim9c-A ca998

OdAr boxO/E3 pim9d-A ca998

Instructions:

* For each plasmid listed, mix the following sequencing reactions in an eppendorf tube:
  + 2 uL ddH2O
  + 8 uL miniprep DNA (undiluted)
  + 3 uL oligo (2.66 uM)
* Label the tops of the tubes with the “label”, ie “OcAr”
* Take the sequencing reactions and order form to:
  + 237 Stanley Hall (second floor cold room)