**A: cscB CRISPR Knockouts**

**A: PCR**

samples:

label primer1 primer2 template product

A1 cscB1 pTargRev pTargetF pTarget-cscB1/ipcr

A2 cscB2 pTargRev pTargetF pTarget-cscB2/ipcr

A3 cscB3 pTargRev pTargetF pTarget-cscB3/ipcr

source:

label location note

pTargetF templates/A1 plasmid already diluted 25x

cscB1 oligos1/F1

cscB2 oligos1/G1

cscB3 oligos1/H1

pTargRev oligos1/A1

destination: thermocycler1A

program: Q5/Q5-4K

note:

When complete, save the 10 uM stock of pTargRev in oligos1/B1

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.

**A: Zymo**

source: thermocycler1A

samples:

reaction label elution\_volume destination product

A1 A1p 50 uL boxA/A1 pTarget-cscB1/pcrpdt

A2 A2p 50 uL boxA/A2 pTarget-cscB2/pcrpdt

A3 A3p 50 uL boxA/A3 pTarget-cscB3/pcrpdt

**A: Digest**

reaction:

33.5 uL ddH2O

10 uL DNA

5 uL NEBBuffer2

1 uL SpeI

0.5 uL DpnI

samples:

label dna source product

A1 A1p boxA/A1 pTarget-cscB1/spedig

A2 A2p boxA/A2 pTarget-cscB2/spedig

A3 A3p boxA/A3 pTarget-cscB3/spedig

destination: thermocycler1A

program: main/SPE1

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.

**A: Zymo2**

source: thermocycler1A

samples:

reaction label elution\_volume destination product

A1 A1d 10 uL boxA/B1 pTarget-cscB1/dig

A2 A2d 10 uL boxA/B2 pTarget-cscB2/dig

A3 A3d 10 uL boxA/B3 pTarget-cscB3/dig

**A: Ligate**

reaction:

7.5 uL ddH2O

1 uL T4 DNA Ligase Buffer

1 uL DNA

0.5 uL T4 DNA Ligase

samples:

label digest source product

A1 A1d boxA/B1 pTarget-cscB1/lig

A2 A2d boxA/B2 pTarget-cscB2/lig

A3 A3d boxA/B3 pTarget-cscB3/lig

destination: thermocycler1A

program: main/LIGATE

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.

**A: Transform**

source: thermocycler1A

samples:

label product strain antibiotic incubate

A1 pTarget-cscB1 Mach1 Spec 37°C

A2 pTarget-cscB2 Mach1 Spec 37°C

A3 pTarget-cscB3 Mach1 Spec 37°C

rescue\_required: yes

note:

Because the antibiotic is spectinomycin, there needs to be a rescue step. After the transformation completes, transfer the cells to a 1.5 mL eppendorf tube (labeled on lid with label) and 200 uL of 2YT medium. Tape the tubes to the inside of the shaker. Another experimentalist will perform plating.

**A: Plate**

samples:

label product strain antibiotic incubate

A1 pTarget-cscB1 Mach1 Spec 37°C

A2 pTarget-cscB2 Mach1 Spec 37°C

A3 pTarget-cscB3 Mach1 Spec 37°C

**A: Pick**

samples:

source product strain antibiotic incubate number labels

A1 pTarget-cscB1 Mach1 Spec 37°C 2 A1A, A1B

A2 pTarget-cscB2 Mach1 Spec 37°C 2 A2A, A2B

A3 pTarget-cscB3 Mach1 Spec 37°C 2 A3A, A3B

**A: Miniprep**

samples:

culture label location

A1A pTarget-cscB1-A boxA/C1

A1B pTarget-cscB1-B boxA/D1

A2A pTarget-cscB2-A boxA/C2

A2B pTarget-cscB2-B boxA/D2

A3A pTarget-cscB3-A boxA/C3

A3B pTarget-cscB3-B boxA/D3

note:

Write the sample like A1A on the top of the eppendorf, put the full name (the label) on the side of the tube

**A,B,C: Sequencing**

sources:

label location product

A1A boxA/C1 pTarget-cscB1-A

A2A boxA/C2 pTarget-cscB2-A

A3A boxA/C3 pTarget-cscB3-A

B1A boxB/C1 pTarget-cscR1-A

B2A boxB/C2 pTarget-cscR2-A

B3A boxB/C3 pTarget-cscR3-A

C1A boxC/C1 pTarget-tyrB1-A

C2A boxC/C2 pTarget-tyrB2-A

C3A boxC/C3 pTarget-tyrB3-A

L4440 oligos1/J1

Instructions:

* Resuspend the oligo L4440 to the appropriate volume for a 100 uM stock
* In an eppendorf, Prepare a 2.66 uM dilute stock of L4440 as:
  + 487 uL ddH2O
  + 13.3 uL of 100 uM oligo
* 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)
* Label the tops of the tubes with the “label”, ie “A3A”
* When done, save the L4440 stock at:
  + oligos1/A5
* Take the sequencing reactions and order form to:
  + 237 Stanley Hall (second floor cold room)

A,C Transform pCas

Transform 1 uL of pCas miniprep into 50 uL aliquot of Mach1 competent cells

A,C: Pick pCas/Mach1 Sydney Ghoreishi Wednesday, 3/13/19 3pm

* Label two 15mL snap-cap tubes as pCas/Mach1-A and pCas/Mach1-B
* Add 4mL each of 2YT+Kan medium into each tube
* Inoculate each with a colony of pCas/Mach1
* Take to Stanley 3rd floor 30°C room and place in rotary shaker

In practice, also streaked the two clones on LB+K plates at 30 and 42 to test for ts phenotype and confirm wasn’t contamination.

A,C: pCas induction growth James Bartlett,Eugene Pang Thursday, 3/14/19 9:30am

* Label two 15mL snap-cap tubes as pCas+Ara and pCas+null
* Add 4mL each of 2YT+Kan medium into each tube
* Add 4uL of 20% Arabinose to the pCas+Ara tube
* Add 50uL of Mach1/pCas cells into each tube
* Take to Stanley 3rd floor 30°C room and place in rotary shaker

A,C: pCas cotransformation Yun Yu Thursday, 3/14/19 3:30pm

* Transfer 1 mL pCas+Ara and pCas+null into labeled eppendorf tubes
* Chill in ice water bath until cold
* Spin 30 seconds, discard supernatant, return to ice bath
* Add 100 uL of TSS solution, pipette and stir gently to resuspend, return to ice
* Use these cells according to the normal transformation protocol

label location plasmid cells antibiotics temperature

C2A+ boxC/C2 pTarget-tyrB2-A pCas+Ara kan/spec 30°C

C3A- boxC/C3 pTarget-tyrB3-A pCas+null kan/spec 30°C

C2A+ boxC/C2 pTarget-tyrB2-A pCas+Ara kan/spec 30°C

C3A- boxC/C3 pTarget-tyrB3-A pCas+null kan/spec 30°C

A,C: IPTG outgrowth Tuesday, 3/19/19 9:30am James Bartlett

Begin repeat of thread with pTarg1

**Ab: cscB CRISPR Knockouts**

**Ab: PCR**

samples:

label primer1 primer2 template product

A1 cscB1 pTargRev pTarg1 pTarg1-cscB1/ipcr

A2 cscB2 pTargRev pTarg1 pTarg1-cscB2/ipcr

A3 cscB3 pTargRev pTarg1 pTarg1-cscB3/ipcr

source:

label location note

pTarg1 boxK/C1 Needs dilution 25x, labeled “KA” in the box

cscB1 oligos1/F1 Needs dilution to 10uM

cscB2 oligos1/G1 Needs dilution to 10uM

cscB3 oligos1/H1 Needs dilution to 10uM

pTargRev oligos1/B1 Already diluted to 10uM

destination: thermocycler1A

program: Q5/Q5-4K

note:

When complete, save the cscB\* diluted stocks in boxA E1, E2, E3 respectively

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.

**Ab: Zymo**

source: thermocycler1A

samples:

reaction label elution\_volume destination product

A1 Ab1p 50 uL boxA/A5 pTarg1-cscB1/pcrpdt

A2 Ab2p 50 uL boxA/A6 pTarg1-cscB2/pcrpdt

A3 Ab3p 50 uL boxA/A7 pTarg1-cscB3/pcrpdt

**Ab: Digest**

reaction:

33.5 uL ddH2O

10 uL DNA

5 uL NEBBuffer2

1 uL SpeI

0.5 uL DpnI

samples:

label dna source product

A1 Ab1p boxA/A5 pTarg1-cscB1/spedig

A2 Ab2p boxA/A6 pTarg1-cscB2/spedig

A3 Ab3p boxA/A7 pTarg1-cscB3/spedig

destination: thermocycler1A

program: main/SPE1

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.

**Ab: Zymo2**

source: thermocycler1A

samples:

reaction label elution\_volume destination product

A1 Ab1d 10 uL boxA/B5 pTarg1-cscB1/dig

A2 Ab2d 10 uL boxA/B6 pTarg1-cscB2/dig

A3 Ab3d 10 uL boxA/B7 pTarg1-cscB3/dig

**Ab: Ligate**

reaction:

7.5 uL ddH2O

1 uL T4 DNA Ligase Buffer

1 uL DNA

0.5 uL T4 DNA Ligase

samples:

label digest source product

A1 Ab1d boxA/B5 pTarg1-cscB1/lig

A2 Ab2d boxA/B6 pTarg1-cscB2/lig

A3 Ab3d boxA/B7 pTarg1-cscB3/lig

destination: thermocycler1A

program: main/LIGATE

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.

**Ab: Transform**

source: thermocycler1A

samples:

label product strain antibiotic incubate

A1 pTarg1-cscB1 Mach1 Amp 37°C

A2 pTarg1-cscB2 Mach1 Amp 37°C

A3 pTarg1-cscB3 Mach1 Amp 37°C

rescue\_required: no

**Ab: Pick**

samples:

source product strain antibiotic incubate number labels

A1 pTarg1-cscB1 Mach1 Amp 37°C 2 A1A, A1B

A2 pTarg1-cscB2 Mach1 Amp 37°C 2 A2A, A2B

A3 pTarg1-cscB3 Mach1 Amp 37°C 2 A3A, A3B

**Ab: Miniprep**

samples:

culture label location

Ab1A pTarg1-cscB1-A boxA/C5

Ab1B pTarg1-cscB1-B boxA/D5

Ab2A pTarg1-cscB2-A boxA/C6

Ab2B pTarg1-cscB2-B boxA/D6

Ab3A pTarg1-cscB3-A boxA/C7

Ab3B pTarg1-cscB3-B boxA/D7

note:

Write the sample like Ab1A on the top of the eppendorf, put the full name (the label) on the side of the tube

**Ab: Sequencing**

sources:

label location product

Ab1A boxA/C1 pTarget-cscB1-A

Ab2A boxA/C2 pTarget-cscB2-A

Ab3A boxA/C3 pTarget-cscB3-A

L4440 oligos1/A5

Instructions:

* Resuspend the oligo L4440 to the appropriate volume for a 100 uM stock
* 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)
* Label the tops of the tubes with the “label”, ie “Ab2A”
* Take the sequencing reactions and order form to:
  + 237 Stanley Hall (second floor cold room)