Lab Notebook Entry 1

Date Performed

26-Apr

Procedure

Streak out starter plate of pTlp36-wasabi

Details

Purpose: AddGene ships us the plasmid in punctured agar, we need to plate these initial colonies. Materials: AddGene shipment, Ampicilin plate, burner, 70% ethanol, metal loop

Protocol

USE STERILE TECHNIQUE! Label the bottom of the plate you are using with your name, the date, and "AddGene pTlpA36-wasabi" Dip the loop in ethanol, flame the loop, let it cool, then place in the stab in the gel they sent us. Zig zag over a third of the plate, ending in a long line. Sterilize the loop again. Run the loop through the ending line perpendicularly, then zig zag over another third of the plate, again, end in a line. Do that one more time. Let the plate sit 5 mins, then put it upsidedown in the incubator at 37C.

Lab Notebook Entry 2

Date Performed

1-May

Procedure

Move plate of pTlpA36-wasabi to the incubator overnight

Details

Purpose: Get the cells growing again after being stored in the fridge over the weekend

Protocol

Move the plate into the incubator. Keep the plate updside down so the writing is facing up. Make sure the incubator is on. If it isnt, turn it on and make sure it is set to 37C.

Lab Notebook Entry 3

Date Performed

2-May

Procedure

Grow starter culture of pTlpA36-wasabi

Details

Purpose: AddGene ships us the plasmid already in a small amount of bacteria, we have to grow up those bacteria to have neough to use. Materials: LB media with ampicilin, plate with pTlpA36-wasabi colonies from previous step, 14mL snap cap plastic tube, stereological pipet

Protocol

USE STERILE TECHNIQUE! Do all of this in triplicate. In a 14mL falcon tube with a snap cap, use the serological pipet to add 5mL of LB with ampicilin. Then take a pipet tip and poke one of the colonies on the plate from the last step. Try to get only a single colony. eject the pipet tip into the 14mL tube. Label it "AddGene pTlpA36-wasabi" and your name and the date. Then put it on the shaking incubator at 37C, shaking ~200 rpm. Leave that overnight.

Lab Notebook Entry 4

Date Performed

5/3/17

Procedure

miniprep pTlpA36-wasabi

Details

Purpose: Get the pTlpA36 plasmid AddGene sent us out of the cells in DNA form. Miniprep kit , pTlpA36-wasabi starter cultures, eppindorf tubes

Protocol

Use the protocol for minipreps in the 2017 protocol file.

Lab Notebook Entry 5

Date Performed

3-May

Procedure

PCR of pTlpA36-wasabi

Details

Purpose: Make lots of our starter plasmid in a linear form. Eliminates LacI junk and adds Gibson assembly compatible ends. Materials: GET ICE FIRST. KEEP EVERYTHING COLD pTlpA36-wasabi plasmid , primers, PCR tubes, Q5 2x master mix, nuclease free water

Protocol

Use the protocol for PCR in the 2017 Protocol file. Use "switch_and_gibsonFOR" and "switch_and_gibsonREV" as primers. Use the following heat cycle program: 98C for 30secs, 30 cycles of [98C for 10secs, 72C for 3mins30secs], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 6

Date Performed

24-Apr

Procedure

PCR of t4 antiholin gene fragment

Details

Purpose: Make lots of our antiholin gene fragment Materials: GET ICE FIRST. KEEP EVERYTHING COLD t4 antilholin gene fragment, primers, PCR tubes, Q5 2x master mix, nuclease free water

Protocol

Use the protocol for PCR in the 2017 Protocol file. Use "T4-antiholinFOR" and "T4-antiholinREV" as primers. Use the following heat cycle program: 98C for 30secs, 30 cycles of [98C for 10secs, 63C for 30secs, 72C for 30secs], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 7

Date Performed

24-Apr

Procedure

PCR of sigma70 promoter gene fragment

Details

Purpose: Make lots of our sigma70 gene fragment Materials: GET ICE FIRST. KEEP EVERYTHING COLD t4 antilholin gene fragment, primers, PCR tubes, Q5 2x master mix, nuclease free water

Protocol

Use the protocol for PCR in the 2017 Protocol file. Use "sigma70 promoter FOR" and "sigma70 promoter REV" as primers. Use the following heat cycle program: 98C for 30secs, 30 cycles of [98C for 10secs, 65C for 30secs, 72C for 20secs], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 8

Date Performed

24-Apr

Procedure

PCR of T4 Endolysin and Holin gene fragment

Details

Purpose: Make lots of our endolysin/holin gene fragment Materials: GET ICE FIRST. KEEP EVERYTHING COLD t4 antilholin gene fragment, primers, PCR tubes, Q5 2x master mix, nuclease free water

Protocol

Use the protocol for PCR in the 2017 Protocol file. Use "holin/endolysin FOR" and "holin/endolysin REV" as primers. Use the following heat cycle program: 98C for 30secs, 30 cycles of [98C for 10secs, 62C for 30secs, 72C for 45secs], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 9

Date Performed

25-Apr

Procedure

Run holin/endolysin, antiholin, and sigma70, PCR products on gel

Details

Purpose: Check that our PCR products are the correct size. Materials: Agarose, 6X purple loading dye, ladder, TAE buffer, GelRed, pTlpA36-wasabi PCR product, sigma70 promoter PCR product, antiholin PCR product, endolysin/holin PCR product, empty PCR tube for each sample

Protocol

Use the protocol for gel electropheresis in the 2017 protocol file. Load each of the 4 PCR products in a seperate lane, plus the ladder, so you should have 5 lanes total.

Lab Notebook Entry 10

Date Performed

25-Apr

Procedure

2nd attempt: PCR of t4 antiholin gene fragment

Details

Purpose: Make lots of our antiholin gene fragment Materials: GET ICE FIRST. KEEP EVERYTHING COLD t4 antilholin gene fragment, primers, PCR tubes, Q5 2x master mix, nuclease free water

Protocol

Use the protocol for PCR in the 2017 Protocol file. Use "T4-antiholinFOR" and "T4-antiholinREV" as primers. Use the following heat cycle program: 98C for 30secs, 30 cycles of [98C for 10secs, 63C for 30secs, 72C for 30secs], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 11

Date Performed

5/3 after miniprep

Procedure

2nd attempt PCR of t4 holin/endolysin gene fragment using less template

Details

Purpose: Try again to make lots of our endolysin/holin gene fragment Materials: GET ICE FIRST. KEEP EVERYTHING COLD t4 endolysin/holin gene fragment, primers, PCR tubes, Q5 2x master mix, nuclease free water

Protocol

Use the protocol for PCR in the 2017 Protocol file EXCEPT USE ONLY 1uL OF THE GENE FRAGMENT DNA. Use "holin/endolysin FOR" and "holin/endolysin REV" as primers. Use the following heat cycle program: 98C for 30secs, 30 cycles of [98C for 10secs, 62C for 30secs, 72C for 45secs], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 12

Date Performed

4-May

Procedure

Run pTlpA36 PCR, 2nd attempt of holin/endolysin PCR and 2nd attmept of antiholin PCR product on gel

Details

Purpose: Check that our PCR products are the correct size. Materials: Agarose, 6X purple loading dye, ladder, 1X TAE buffer, GelRed, pTlpA36-wasabi PCR product, 2nd attempt antiholin PCR product, 2nd attempt endolysin/holin PCR product, empty PCR tube for each sample

Protocol

Use the protocol for gel electropheresis in the 2017 protocol file. Load each of the 4 PCR products in a seperate lane, plus the ladder, so you should have 5 lanes total.

Lab Notebook Entry 13

Date Performed

5/5/17

Procedure

3rd attempt PCR of Holin/endolysin with even less template

Details

Purpose: Try again to make lots of our endolysin/holin gene fragment Materials: GET ICE FIRST. KEEP EVERYTHING COLD t4 endolysin/holin gene fragment, primers, PCR tubes, Q5 2x master mix , nuclease free water

Protocol

Use the protocol for PCR in the 2017 Protocol file EXCEPT THIS TIME DILLUTE 2uL OF THE GENE FRAGMENT DNA IN 8uL OF PCR WATER IN A SEPERATE TUBE (label the tube and store in freezer box for future), THEN USE 1uL OF THAT AS TEMPLATE. Use "holin/endolysin FOR" and "holin/endolysin REV" as primers. Use the following heat cycle program: 98C for 30secs, 30 cycles of [98C for 10secs, 62C for 30secs, 72C for 45secs], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 14

Date Performed

5/5/17

Procedure

2nd attempt PCR of pTlpA36 with way less template

Details

Purpose: Our second attempt to make lots of our starter plasmid in a linear form. Eliminates LacI junk and adds Gibson assembly compatible ends. Materials: GET ICE FIRST. KEEP EVERYTHING COLD pTlpA36-wasabi plasmid, primers, PCR tubes, Q5 2x master mix, nuclease free water

Protocol

Use the protocol for PCR in the 2017 Protocol file, BUT THIS TIME DILLUTE 2uL OF THE TEMPLATE IN 18 uL OF PCR WATER IN A SEPERATE TUBE (label the tube and store in freezer box for future), THEN USE 1uL OF THAT AS TEMPLATE . Use "switch_and_gibsonFOR" and "switch_and_gibsonREV" as primers. Use the following heat cycle program: 98C for 30secs, 30 cycles of [98C for 10secs, 72C for 3mins30secs], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 15

Date Performed

5-May

Procedure

Run PCR products from 3rd attempt of holin/endolysin, 2nd attempt of pTlpA36, and 2nd attempt of antiholin on a gel

Details

Purpose: Check that our PCR products are the correct size. Materials: Agarose, 6X purple loading dye, ladder, 1X TAE buffer, GelRed, pTlpA36-wasabi PCR product, 2nd attempt antiholin PCR product, 2nd attempt endolysin/holin PCR product, empty PCR tube for each sample

Protocol

Use the UPDATED protocol for gel electropheresis in the 2017 protocol file. Load each of the PCR products in a seperate lane, plus the ladder. Last time the ladder came out fuzzy, Nick thinks it was because the agarose didnt dissolve all the way when you microwaved it. Be extra sure its all dissolved before you pour it into the mold.

Lab Notebook Entry 16

Date Performed

5-May

Procedure

Rerun PCR products from 3rd attempt of holin/endolysin, 2nd attempt of pTlpA36, and 2nd attempt of antiholin on a gel

Details

Purpose: Check that our PCR products are the correct size. Materials: Agarose, 6X purple loading dye, ladder, 1X TAE buffer, GelRed, pTlpA36-wasabi PCR product, 2nd attempt antiholin PCR product, 2nd attempt endolysin/holin PCR product, empty PCR tube for each sample

Protocol

Lab Notebook Entry 17

Date Performed

5/8/17

Procedure

4th attempt PCR of holin/endolysin (tweaking program)

Details

Purpose: Try again to make lots of our endolysin/holin gene fragment Materials: GET ICE FIRST. KEEP EVERYTHING COLD t4 endolysin/holin gene fragment, primers, PCR tubes, Q5 2x master mix, nuclease free water

Protocol

Use the protocol for PCR in the 2017 Protocol file EXCEPT THIS TIME DILLUTE 2uL OF THE GENE FRAGMENT DNA IN 8uL OF PCR WATER IN A SEPERATE TUBE (label the tube and store in freezer box for future), THEN USE 1uL OF THAT AS TEMPLATE. Use "holin/endolysin FOR" and "holin/endolysin REV" as primers. Use the following heat cycle program: 95C for 30secs, 30 cycles of [95C for 10secs, 62C for 30secs, 72C for 45secs], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 18

Date Performed

5/8/17

Procedure

3rd attempt PCR of pTlpA36 (tweaking program)

Details

Purpose: Our second attempt to make lots of our starter plasmid in a linear form. Eliminates LacI junk and adds Gibson assembly compatible ends. Materials: GET ICE FIRST. KEEP EVERYTHING COLD pTlpA36-wasabi plasmid, primers, PCR tubes, Q5 2x master mix, nuclease free water

Protocol

Use the protocol for PCR in the 2017 Protocol file, BUT THIS TIME DILLUTE 2uL OF THE TEMPLATE IN 18 uL OF PCR WATER IN A SEPERATE TUBE (label the tube and store in freezer box for future), THEN USE 1uL OF THAT AS TEMPLATE . Use "switch_and_gibsonFOR" and "switch_and_gibsonREV" as primers. Use the following heat cycle program: 95C for 30secs, 30 cycles of [95C for 10secs, 60C for 30secs 72C for 3mins], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 19

Date Performed

5/9/17

Procedure

Run a gel of 4th attempt of holin/endolysin PCR, 3rd attempt of pTlpA36 PCR, and 2nd attempt of antiholin

Details

Purpose: Check that our PCR products are the correct size. Materials: Agarose, 6X purple loading dye, ladder, 1X TAE buffer, GelRed, pTlpA36-wasabi PCR product, 2nd attempt antiholin PCR product, 2nd attempt endolysin/holin PCR product, empty PCR tube for each sample

Protocol

Use the protocol in the 2017 protocols file for the gel.

Lab Notebook Entry 20

Date Performed

5/10/17

Procedure

Run PCRs for Ptlp, Holin/Endolysin, and antiholin

Details

Purpose: to replenish our stock of PCR material and to confirm that our PCR process now works. We used diluted Ptlp from the first miniprep (2ul DNA : 8ul Water)

Protocol

Used protocols from most recent attempts of each PCR

Lab Notebook Entry 21

Date Performed

5/10/17

Procedure

Run Gel of leftover PCR from Monday

Details

Purpose to confirm if previous PCR worked. If so, we will perform a gel extraction.

Protocol

Used protocol in the 2017 file for the gel

Lab Notebook Entry 22

Date Performed

11-May

Procedure

Run a Gel ofthe PCRs run on 5/10

Details

Purpose: to confirm whether our PCR's have the right products

Protocol

Use the protocol in the 2017 protocols file for the gel.

Lab Notebook Entry 23

Date Performed

5/11/17

Procedure

PCR Purification of Holin/Endolysin and Antiholin

Details

Purpose: gets rid of excess primers and other junk from out pcr so we have nice clean DNA to work with

Protocol

Use the protocol that is in the PCR purification kit.

Lab Notebook Entry 24

Date Performed

5/11/17

Procedure

Gel Extraction of PtlpAs: includes purification

Details

Purpose: Extract DNA product from gels and purify DNA to have clean DNA to work with.

Protocol

Use the protocol that is in the Gel Extraction kit.

Lab Notebook Entry 25

Date Performed

12-May

Procedure

Do pcr cleanup kit on all pcr products we've made so far

Details

Purpose: gets rid of excess primers and other junk from out pcr so we have nice clean DNA to work with

Protocol

Use the protocol that is in the PCR purification kit.

Lab Notebook Entry 26

Date Performed

15-May

Procedure

Use nanodrop to find DNA concentration of holin/endolysin fragment and the pTlpA pcr fragment

Details

Purpose: we need to know the concentration of these two pcr products so that we know how much to use in our Gibson assembly

Protocol

Lab Notebook Entry 27

Date Performed

17-May

Procedure

Another pTlpA PCR

Details

Purpose: Our second attempt to make lots of our starter plasmid in a linear form. Eliminates LacI junk and adds Gibson assembly compatible ends. Materials: GET ICE FIRST. KEEP EVERYTHING COLD pTlpA36-wasabi plasmid, primers, PCR tubes, Q5 2x master mix , nuclease free water

Protocol

Use the protocol for PCR in the 2017 Protocol file, BUT THIS TIME DILLUTE 2uL OF THE TEMPLATE IN 18 uL OF PCR WATER IN A SEPERATE TUBE (label the tube and store in freezer box for future), THEN USE 1uL OF THAT AS TEMPLATE . Use "switch_and_gibsonFOR" and "switch_and_gibsonREV" as primers. Use the following heat cycle program: 95C for 30secs, 30 cycles of [95C for 10secs, 60C for 30secs 72C for 3mins], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 28

Date Performed

17-May

Procedure

Run new pTlpA PCR product on a gel, then do gel extraction

Details

Purpose: Try and get a higher concentration of pTlpA DNA than we did the first time.
Materials: Agarose, 6X purple loading dye, ladder, 1X TAE buffer, GelRed, pTlpA36 PCR product , empty PCR tube for each sample, Razor blade

Protocol

Use the protocol that is in the Gel Extraction kit. RUN THE WHOLE PCR PRODUCT ON THE GEL

Lab Notebook Entry 29

Date Performed

22-May

Procedure

Use nanodrop to find DNA concentration of the new pTlpA pcr fragment

Details

Purpose: we need to know the concentration of these two pcr products so that we know how much to use in our Gibson assembly

Protocol

Lab Notebook Entry 30

Date Performed

17-May

Procedure

Gibson assembly of isolated switch with gibson ends and T4 endolysin and holin

Details

Purpose: Combine the PCR product of the original plasmid with our holin/endolysin gene fragment. Materials: Gibson assembly kit, pTlpA36 PCR product, holin/endolysin PCR product, two empty PCR tubes, ddH20

Protocol

Please take extra care during this procedure, each gibson reaction costs us about $40 making it one of our more expensive procedures. Use the protocol for gibson assembly in the 2017 protocol file. Use 5uL of the 43.8ng/uL pTlpA36 PCR product (Sample D1) and 1uL of the holin/endolysin PCR product (H/E) in the reaction mixture.

Lab Notebook Entry 31

Date Performed

17-May

Procedure

transform new plasmid into high efficiency cells

Details

Purpose: Put our newly gibson assembeled plasmid into bacteria so they can make more of it for us. Materials: NEB High-efficiency chemically competent cells, SOC media, plate with ampicillin

Protocol

Use this protocol (https://www.neb.com/protocols/1/01/01/high-efficiency-transformation-protocol-c2987) and the LB-amp plates in the fridge

Lab Notebook Entry 32

Date Performed

Procedure

pick colonies and start 5ml culture of pThermolyse backbone

Details

Purpose: Grow up larger liquid cultures of the bacteria that contain our new pThermolyse plasmid so that we can miniprep to extract the DNA Materials: LB media with ampicilin, plate from gibson assembly with pThermolyse colonies from previous step, 14mL plastic "falcon" tubes with a snap cap, stereological pipet

Protocol

USE STERILE TECHNIQUE! Do all of this in triplicate. In a 14mL glass tube with a colored cap, use the serological pipet to add 5mL of LB with ampicilin. Then take a pipet tip (using a pipet, not your hands) and poke one of the colonies on the plate from the last step. Try to get only a single colony. eject the pipet tip into the 14mL tube. Label it "pThermolyse backbone" and your name and the date. Then put it on the shaking incubator at 37C, shaking ~200 rpm. Leave that overnight. DO NOT MISTAKE THE LARGE CENTRIFUGE FOR THE SHAKING INCUBATOR. INCUBATOR HAS A CLEAR PLASTIC LID AND DOES NOT HAVE A ROTOR INSIDE. IT SHAKES LIKE AN EARTHQUAKE, IT DOES NOT SPIN.

Lab Notebook Entry 33

Date Performed

5/19/17

Procedure

miniprep pThermolyse backbone

Details

Purpose: Get the pThermolyse backbone out of the cells in DNA form. Materials: NOTE: Our P1000 pipet doesnt work right now, so dont try using it, Miniprep kit (blue box on counter by the fridge), pThermolyse backbone starter cultures, eppindorf tubes

Protocol

Use the protocol for minipreps in the 2017 protocol file.

Lab Notebook Entry 34

Date Performed

23-May

Procedure

nanodrop gibson assembly product

Details

Purpose: determine concentration of gibson assembly product so that it can be sent for sequencing

Protocol

Lab Notebook Entry 35

Date Performed

24-May

Procedure

prep for sequencing and send

Details

Purpose: send to sequencing core

Protocol

Lab Notebook Entry 36

Date Performed

5/25/17

Procedure

transformation of pThermolyse backbone (we think...) into subcloning cells

Details

Purpose: get started making more of our backbone, so that if the sequencing shows that our gibson assembly was successfull we can procede and not have lost the two days it took to sequence. if it comes back with a bad sequence, we will toss this and start over. Materials: Subcloning chemically competent cells (KEEP ON ICE!), pThermolyse backbone miniprep products, Six LB-amp plates (if fewer than six, use what is there, and prioritize plating 500uL for each transformation before you plate 10uL, also let Aaron know that we are out), water bath (get it up to 42C when you're setting up), shaking incubator, SOC media, sterile disposabel spreader, foam floater for water bath

Protocol

Use protocol in 2017 file. You are going to be doing 3 transformations, one with each of the three DNA smaples we have from gibson assembly. Each of the transformations will be plated in two different concentrations on two paltes. Label the plates "pThermorlyse backbone #(1-3 depending on which smaple you used for that transformation)" and then with the amount you plated on that plate (10uL or 500uL). IF DENNIS SITLL HASNT PUT THE SHAKING INCUBATOR BACK: If he is there or there's someone in his office, ask for it back. If not: Initially heat the water bath to 42C. As soon as you put the cells back on ice after heat shocking them at 42C, turn the water bath down to 37C, and throw a good amount of ice in to help it cool quickly. You have 5 mins to get it to 37C or as close to 37C as you can get it. Wedge one of the foam floaters into one of the metal springs so that you can put the tube in the floater and it will shake a bit without risking it going under water. Turn on the shaking function of the water bath to 225rpm and shake for an hour in that instead of the shaking incubator.

Lab Notebook Entry 37

Date Performed

5/25/17

Procedure

Make more LB-amp?

Details

Purpose: See if we are running low, if yes, make more. Materials: LB base, flask, ampicilin

Protocol

use protocol in 2017 file to make 100 mL

Lab Notebook Entry 38

Date Performed

29-May

Procedure

PCR of pTlpA36-wasabi and of previous gel extracted pTlpA

Details

Purpose: We are going to try this part again. This time we will just do PCR cleanup instead of gel extraction since the gel extraction had really bad yeild. The product will have a mix of the two bands, but the "bad" bands hopefully wont be compatible with the other peice when we do gibson and will be filtered out by that step. Also trying to PCR the gel extracted product from last time since that is theoretically 'pure' pTlpA even if it has a bad concentration.

Protocol

Use the protocol for PCR in the 2017 Protocol file, BUT THIS TIME DILLUTE 2uL OF THE TEMPLATE IN 18 uL OF PCR WATER IN A SEPERATE TUBE (label the tube and store in freezer box for future), THEN USE 1uL OF THAT AS TEMPLATE . Use "switch_and_gibsonFOR" and "switch_and_gibsonREV" as primers. Use the following heat cycle program: 95C for 30secs, 30 cycles of [95C for 10secs, 60C for 30secs 72C for 3mins], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 39

Date Performed

30-May

Procedure

Run an analytical gel of PCR products from last step

Details

Purpose: Determine the products from our PCR

Protocol

Used 2017 Protocol file

Lab Notebook Entry 40

Date Performed

30-May

Procedure

2.0 PCR of pTlpA36-wasabi and of previous gel extracted pTlpA

Details

Purpose: See reasons above, but I screwed up and ruined our PCR product by adding the wrong buffer.

Protocol

Use the protocol for PCR in the 2017 Protocol file, BUT THIS TIME DILLUTE 2uL OF THE TEMPLATE IN 18 uL OF PCR WATER IN A SEPERATE TUBE (label the tube and store in freezer box for future), THEN USE 1uL OF THAT AS TEMPLATE . Use "switch_and_gibsonFOR" and "switch_and_gibsonREV" as primers. Use the following heat cycle program: 95C for 30secs, 30 cycles of [95C for 10secs, 60C for 30secs 72C for 3mins], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 41

Date Performed

31-May

Procedure

2.0 Run an analytical gel of PCR products from last step

Details

Purpose: See what we've got

Protocol

Used 2017 Protocol file

Lab Notebook Entry 42

Date Performed

31-May

Procedure

PCR cleanup of PCR products from last step

Details

Purpose: PCR cleanup to improve PCR products from last step

Protocol

Used protocol from qiagen kit box and used only 35uL of elution buffer (EB) to get higher concentration

Lab Notebook Entry 43

Date Performed

31-May

Procedure

Use nanodrop to find DNA concentration of the new pTlpA pcr fragment

Details

Purpose: we need to know the concentration of these two pcr products so that we know how much to use in our Gibson assembly

Protocol

Lab Notebook Entry 44

Date Performed

5/31/17

Procedure

Gibson assembly of isolated switch with gibson ends and T4 endolysin and holin

Details

Purpose: Combine the PCR product of the original plasmid with our holin/endolysin gene fragment. Materials: Gibson assembly kit, pTlpA36 PCR product, holin/endolysin PCR product, two empty PCR tubes, ddH20

Protocol

Please take extra care during this procedure, each gibson reaction costs us about $40 making it one of our more expensive procedures. Use the protocol for gibson assembly in the 2017 protocol file. Use 3uL of the pTlpA36 PCR product and 1uL of the holin/endolysin PCR product (H/E) in the reaction mixture.

Lab Notebook Entry 45

Date Performed

Procedure

transform new plasmid into high efficiency cells

Details

Purpose: Put our newly gibson assembeled plasmid into bacteria so they can make more of it for us. Materials: NEB High-efficiency chemically competent cells, SOC media, plate with ampicillin

Protocol

Use this protocol (https://www.neb.com/protocols/1/01/01/high-efficiency-transformation-protocol-c2987) and the LB-amp plates in the fridge and use 2uL of the gibson reaction

Lab Notebook Entry 46

Date Performed

1-Jun

Procedure

pick colonies and start 10ml culture of pThermolyse backbone

Details

Purpose: Grow up larger liquid cultures of the bacteria that contain our new pThermolyse plasmid so that we can miniprep to extract the DNA Materials: LB media with ampicilin, plate from gibson assembly with pThermolyse colonies from previous step, 14mL plastic tubes with a blue snap cap, stereological pipet

Protocol

USE STERILE TECHNIQUE! Do all of this in triplicate, assuming there are 3 or more colonies. In a 14mL plastic tube with a blue cap, use the serological pipet to add 10mL of LB with ampicilin. Then take a pipet tip (using a pipet, not your hands) and poke one of the colonies on the plate from the last step. Try to get only a single colony. eject the pipet tip into the 14mL tube. Label it "pThermolyse backbone" and your name and the date. Then put it in the hot water bath at 37C, shaking ~200 rpm. Leave that overnight.

Lab Notebook Entry 47

Date Performed

2-Jun

Procedure

miniprep pThermolyse backbone

Details

Purpose: Get the pThermolyse backbone out of the cells in DNA form. Materials: NOTE: Our P1000 pipet doesnt work right now, so dont try using it, Miniprep kit, pThermolyse backbone starter cultures, eppindorf tubes

Protocol

Do one miniprep for each of the starter cultures. Use the protocol for minipreps in the 2017 protocol file, EXCEPT: double the ammounts of buffers P1, P2, and N3 used. Also preheat the buffer EB by sticking it in the incubator for a few minutes before you use it.

Lab Notebook Entry 48

Date Performed

5-Jun

Procedure

nanodrop pThermolyse backbone (gibson assembly product)

Details

Purpose: determine concentration of gibson assembly product so that it can be sent for sequencing

Protocol

Lab Notebook Entry 49

Date Performed

6/5/17

Procedure

Pick new colonies and make new 8mL starter cultures of pThermolyse backbone

Details

Purpose: Grow up larger liquid cultures of the bacteria that contain our new pThermolyse plasmid so that we can miniprep to extract the DNA Materials: LB media with ampicilin, plate from gibson assembly with pThermolyse colonies from previous step, 14mL plastic tubes with a blue snap cap, stereological pipet

Protocol

USE STERILE TECHNIQUE! Do all of this in triplicate, assuming there are 3 or more colonies. In a 14mL plastic tube with a blue cap, use the serological pipet to add 10mL of LB with ampicilin. Then take a pipet tip (using a pipet, not your hands) and poke one of the colonies on the plate from the last step. Try to get only a single colony. eject the pipet tip into the 14mL tube. Label it "pThermolyse backbone" and your name and the date. Then put it in the hot water bath at 37C, shaking ~200 rpm. Leave that overnight.

Lab Notebook Entry 50

Date Performed

6/6/17

Procedure

Miniprep pThermolyse backbone

Details

Purpose: Get the pThermolyse backbone out of the cells in DNA form. Materials: NOTE: Our P1000 pipet doesnt work right now, so dont try using it, Miniprep kit, pThermolyse backbone starter cultures, eppindorf tubes

Protocol

Do one miniprep for each of the starter cultures. Use the protocol for minipreps in the 2017 protocol file, EXCEPT: double the ammounts of buffers P1, P2, and N3 used. Also preheat the buffer EB by sticking it in the incubator for a few minutes before you use it.

Lab Notebook Entry 51

Date Performed

Procedure

Nanodrop pThermolyse backbone (gibson assembly product)

Details

Purpose: determine concentration of gibson assembly product so that it can be sent for sequencing

Protocol

Lab Notebook Entry 52

Date Performed

5/25/17

Procedure

Make more LB-amp plates?

Details

Purpose: See if we are running low, if yes, make more. Materials: LB agar, flask, ampicilin , plates

Protocol

use 2017 protocol to make another sleeve of plates

Lab Notebook Entry 53

Date Performed

6/7/17

Procedure

Random PCR to check older Q5 master mix

Details

Purpose: Check if the older master mix works or if we need to buy more. Materials: GET ICE FIRST. KEEP EVERYTHING COLD pTlpA36-wasabi plasmid, primers, PCR tubes, Q5 2x master mix, nuclease free water

Protocol

Run two pcrs with each of the two old master mixes (4 PCRs Total). Use the protocol for PCR in the 2017 Protocol file, BUT THIS TIME DILLUTE 2uL OF THE TEMPLATE IN 18 uL OF PCR WATER IN A SEPERATE TUBE (label the tube and store in freezer box for future), THEN USE 1uL OF THAT AS TEMPLATE . Use "switch_and_gibsonFOR" and "switch_and_gibsonREV" as primers. Use the following heat cycle program: 95C for 30secs, 30 cycles of [95C for 10secs, 60C for 30secs 72C for 3mins], 72 for 2mins, 4C for infinity (just put in 99 hours or whatever the max is)

Lab Notebook Entry 54

Date Performed

7-Jun

Procedure

Pick new colonies and make new 5mL starter cultures of pThermolyse backbone

Details

Purpose: Grow up larger liquid cultures of the bacteria that contain our new pThermolyse plasmid so that we can miniprep to extract the DNA Materials: LB media with ampicilin, plate from gibson assembly with pThermolyse colonies from previous step, 14mL plastic tubes with a blue snap cap, stereological pipet

Protocol

USE STERILE TECHNIQUE! Do all of this in triplicate, assuming there are 3 or more colonies. In a 14mL plastic tube with a blue cap, use the serological pipet to add 5mL of LB with ampicilin. Then take a pipet tip (using a pipet, not your hands) and poke one of the colonies on the plate from the last step. Try to get only a single colony. eject the pipet tip into the 14mL tube. Label it "pThermolyse backbone" and your name and the date. Then put it in the hot water bath at 37C, shaking ~200 rpm. Leave that overnight.

Lab Notebook Entry 55

Date Performed

7-Jun

Procedure

XbaI digest to test pThermolyse

Details

Purpose: Cutting with XbaI will create a different band pattern depending on if our gibson assembly worked or not. Materials: pThermolyse backbone samples, XbaI (keep on ice when you take it out. restriction enzymes are super expensive and also very sensitive to heat), 10X Cut smart buffer, PCR water

Protocol

Do a digest for each of the 3 samples. Use the protocol in the 2017 protocols file. Here are the missing details: DNA amount-> You need to calculate this based on the concentration of each DNA sample, written on the side. You want to use 1ug of DNA, and the concentrations will be written in ng/uL so you will have to convery. Buffer-> XbaI uses cutsmart buffer. Heat cycler program-> 37C for 1 hour, 65C for 30 mins (heat inactivation), 4C for 99 hours (infinity)

Lab Notebook Entry 56

Date Performed

6/8/17

Procedure

miniprep pThermolyse backbone

Details

Purpose: Get the pThermolyse backbone out of the cells in DNA form. Materials: NOTE: Our P1000 pipet doesnt work right now, so dont try using it, Miniprep kit, pThermolyse backbone starter cultures, eppindorf tubes

Protocol

Do one miniprep for each of the starter cultures. Use the protocol for minipreps in the 2017 protocol file, EXCEPT: Preheat the buffer EB by sticking it in the incubator for a few minutes before you use it.

Lab Notebook Entry 57

Date Performed

6/8/17

Procedure

Run a gel to check Q5 master mix PCR

Details

Purpose: Analytical gel to see if the old master mixes work. Materials: Materials: Agarose, 6X purple loading dye, ladder, 1X TAE buffer, GelRed, pTlpA36 PCR product, empty PCR tube for each sample

Protocol

Use the protocol in the 2017 protocols file.

Lab Notebook Entry 58

Date Performed

6/8/17

Procedure

Gel of previous digest

Details

Purpose: Analytical gel to see if the old master mixes work. Materials: Materials: Agarose, 6X purple loading dye, ladder, 1X TAE buffer, GelRed, pTlpA36 PCR product, empty PCR tube for each sample

Protocol

Use the protocol in the 2017 protocols file.

Lab Notebook Entry 59

Date Performed

9-Jun

Procedure

Nanodrop backbone

Details

Purpose: Determine concentration of DNA in need to prep for sequencing.

Protocol

Lab Notebook Entry 60

Date Performed

12-Jun

Procedure

Prep for sequencing and send

Details

Purpose: Analyse DNA product to make sure experiment was complete.

Protocol

Lab Notebook Entry 61

Date Performed

10-Jun

Procedure

XbaI digest to test pThermolyse

Details

Purpose: Cutting with XbaI will create a different band pattern depending on if our gibson assembly worked or not. Materials: pThermolyse backbone samples, XbaI (keep on ice when you take it out. restriction enzymes are super expensive and also very sensitive to heat), 10X Cut smart buffer, PCR water

Protocol

Do a digest for each of the 3 samples. Use the protocol in the 2017 protocols file. Here are the missing details: DNA amount-> You need to calculate this based on the concentration of each DNA sample, written on the side. You want to use 1ug of DNA, and the concentrations will be written in ng/uL so you will have to convery. Buffer-> XbaI uses cutsmart buffer. Heat cycler program-> 37C for 1 hour, 65C for 30 mins (heat inactivation), 4C for 99 hours (infinity)

Lab Notebook Entry 62

Date Performed

10-Jun

Procedure

PstI digest to test pThermolyse

Details

Purpose: Cutting with PstI will create a different band pattern depending on if our gibson assembly worked or not. Materials: pThermolyse backbone samples, PstI (keep on ice when you take it out. restriction enzymes are super expensive and also very sensitive to heat), 10X NEB 3.1 buffer, PCR water

Protocol

Do a digest for each of the 3 samples. Use the protocol in the 2017 protocols file. Here are the missing details: DNA amount-> You need to calculate this based on the concentration of each DNA sample, written on the side. You want to use 1ug of DNA, and the concentrations will be written in ng/uL so you will have to convert. Buffer-> PstI uses NEB 3.1 buffer. Heat cycler program-> 37C for 1 hour, 80C for 30 mins (heat inactivation), 4C for 99 hours (infinity)

Lab Notebook Entry 63

Date Performed

11-Jun

Procedure

Run a gel of XbaI and PstI digests

Details

Purpose: See the results of the two restriction digests. Materials: Materials: Agarose, 6X purple loading dye, ladder, 1X TAE buffer, GelRed, restriction digests products, empty PCR tube for each sample

Protocol

Use the protocol in the 2017 protocols file.

Lab Notebook Entry 64

Date Performed

14-Jun

Procedure

colony pcr

Details

Purpose: Quickly screen all of the remaining gibson colonies

Protocol

Used Holin/Endolysin FOR and REV as primers

Lab Notebook Entry 65

Date Performed

15-Jun

Procedure

Run a gel of the colony pcr

Details

Purpose: See the results of the two restriction digests. Materials: Materials: Agarose, 6X purple loading dye, ladder, 1X TAE buffer, GelRed, restriction digests products, empty PCR tube for each sample

Protocol

Use the protocol in the 2017 protocols file. Run twice as much of each PCR product as the protocol says.

Lab Notebook Entry 66

Date Performed

20-Jun

Procedure

Run analytical gel of pTlpA on 0.8% gel

Details

Purpose: See the results of the two restriction digests. Materials: Materials: Agarose, 6X purple loading dye, ladder, 1X TAE buffer, GelRed, restriction digests products, empty PCR tube for each sample

Protocol

Use protocol in 2017 protocols file, standard analytical gel, .8% agarose

Lab Notebook Entry 67

Date Performed

Procedure

CONSTRUCT PLAN TWO (Sythesis+Assembly)

Details

Protocol

Lab Notebook Entry 68

Date Performed

15-Aug

Procedure

Digest part C with Hind3 and Nhe1

Details

Purpose: Cut the ends off of our synthesized part to make it compatible with the other parts for ligation.

Protocol

Lab Notebook Entry 69

Date Performed

15-Aug

Procedure

Digest of A and B with Bgl2

Details

Purpose: Cut the ends off of our synthesized part to make it compatible with the other parts for ligation.

Protocol

Lab Notebook Entry 70

Date Performed

15-Aug

Procedure

PCR Cleanup of A, B and C

Details

Purpose: Get rid of digest byproducts

Protocol

Lab Notebook Entry 71

Date Performed

15-Aug

Procedure

Digest of A with Nhe1

Details

Purpose: Cut the ends off of our synthesized part to make it compatible with the other parts for ligation.

Protocol

Lab Notebook Entry 72

Date Performed

15-Aug

Procedure

Digest of B with Hind3

Details

Purpose: Cut the ends off of our synthesized part to make it compatible with the other parts for ligation.

Protocol

Lab Notebook Entry 73

Date Performed

15-Aug

Procedure

PCR Cleanup of A and B

Details

Purpose: Get rid of digest byproducts

Protocol

Lab Notebook Entry 74

Date Performed

26-Aug

Procedure

Ligation of Parts

Details

Purpose: Connect our three synthesized parts together (A, B, and C)

Protocol

Ligation protocol: 10uL part A, 10uL part B, 10uL part C, 4uL T4 ligase buffer, 4uL PCR water, 2uL T4 Ligase. Add the ligase itself last and keep it in the freezer/on ice as much as possible. Pipet gently up and down a bunch to mix after it is all in the tube. Leave the reaction at room temp over the weekend with a sign saying not to touch it.

Lab Notebook Entry 75

Date Performed

28-Aug

Procedure

Transform into High Efficency Cells

Details

Purpose: Put our ligation back into cells to see if it worked and to make more of it if it did

Protocol

USE HIGH EFFICIENCY CELLS FROM GIBSON KIT

Lab Notebook Entry 76

Date Performed

29-Aug

Procedure

Make Starter Cultures

Details

Purpose: Make starter cultures of the plates that grew.

Protocol

Lab Notebook Entry 77

Date Performed

29-Aug

Procedure

Miniprep

Details

Purpose: Miniprep the starter cultures to extract DNA for further analysis.

Protocol

Lab Notebook Entry 78

Date Performed

29-Aug

Procedure

Make glycerol stocks

Details

Purpose: Make glycerol stocks from the starter cultures for experiment #2

Protocol

Lab Notebook Entry 79

Date Performed

3-Sep

Procedure

Redigest part C-JJ with Hind3 and Nhe1

Details

Purpose: Cut the ends off of our synthesized part to make it compatible with the other parts for ligation

Protocol

Lab Notebook Entry 80

Date Performed

3-Sep

Procedure

Digest of A and B with Bgl2

Details

Purpose: Cut the ends off of our synthesized part to make it compatible with the other parts for ligation

Protocol

Lab Notebook Entry 81

Date Performed

3-Sep

Procedure

PCR Cleanup of A, B and C-JJ

Details

Purpose: Get rid of digest byproducts

Protocol

Lab Notebook Entry 82

Date Performed

3-Sep

Procedure

Digest of A with Nhe1

Details

Purpose: Cut the ends off of our synthesized part to make it compatible with the other parts for ligation

Protocol

Lab Notebook Entry 83

Date Performed

3-Sep

Procedure

Digest of B with Hind3

Details

Purpose: Cut the ends off of our synthesized part to make it compatible with the other parts for ligation

Protocol

Lab Notebook Entry 84

Date Performed

4-Sep

Procedure

PCR Cleanup of A and B

Details

Purpose:Get rid of digest byproducts

Protocol

Lab Notebook Entry 85

Date Performed

4-Sep

Procedure

Ligation of Parts

Details

Connect our three synthesized parts together (A, B, and C)

Protocol

Ligation protocol: 10uL part A, 10uL part B, 10uL part C, 4uL T4 ligase buffer, 4uL PCR water, 2uL T4 Ligase. Add the ligase itself last and keep it in the freezer/on ice as much as possible. Pipet gently up and down a bunch to mix after it is all in the tube. Leave the reaction at room temp over the weekend with a sign saying not to touch it

Lab Notebook Entry 86

Date Performed

8-Sep

Procedure

Transform via High Efficiency Cells

Details

Put our ligation back into cells to see if it worked and to make more of it if it did

Protocol

Use high efficiency cells. Use whole ligation rxn into 50uL comp cells. Also transform 20uL pUC19 as a control.

Lab Notebook Entry 87

Date Performed

10-Sep

Procedure

Digest part C with Hind3 and Nhe1

Details

Cut the ends off of our synthesized part to make it compatible with the other parts for ligation

Protocol

Lab Notebook Entry 88

Date Performed

10-Sep

Procedure

Digest of A and B with Bgl2

Details

Cut the ends off of our synthesized part to make it compatible with the other parts for ligation

Protocol

Lab Notebook Entry 89

Date Performed

10-Sep

Procedure

PCR Cleanup of A and B

Details

Get rid of digest byproducts

Protocol

Lab Notebook Entry 90

Date Performed

10-Sep

Procedure

Digest of A with Nhe1

Details

Cut the ends off of our synthesized part to make it compatible with the other parts for ligation

Protocol

Lab Notebook Entry 91

Date Performed

10-Sep

Procedure

Digest of B with Hind3

Details

Cut the ends off of our synthesized part to make it compatible with the other parts for ligation

Protocol

Lab Notebook Entry 92

Date Performed

11-Sep

Procedure

PCR Cleanup of A, B and C

Details

Get rid of digest byproducts

Protocol

Lab Notebook Entry 93

Date Performed

11-Sep

Procedure

Ligation

Details

Connect our three synthesized parts together

Protocol

Ligation protocol: 10uL part A, 10uL part B, 10uL part C, 4uL T4 ligase buffer, 4uL PCR water, 2uL T4 Ligase. Add the ligase itself last and keep it in the freezer/on ice as much as possible. Pipet gently up and down a bunch to mix after it is all in the tube. Leave the reaction at room temp over the weekend with a sign saying not to touch it

Lab Notebook Entry 94

Date Performed

15-Sep

Procedure

transform

Details

Put our ligation back into cells to see if it worked and to make more of it if it did

Protocol

USE HIGH EFFICIENCY CELLS FROM GIBSON KIT

Lab Notebook Entry 95

Date Performed

16-Sep

Procedure

Make starter cultures

Details

Make large cultures to provide cells for our three experiments.

Protocol

Prewarm the LB to 37C in the incubator. Make 10mL cultures of 3 colonies from each of the 5 plates for a total of 15 cultures. Label them with a letter and then a number. 'P' for the pUC19 control, 'C' for the promoterless part C, and 'J' 'T' or 'L' for the three promoters, then 1, 2, and 3 for each type. So one tube will be P1, another P2, etc.

Lab Notebook Entry 96

Date Performed

19-Sep

Procedure

Make glycerol stocks

Details

Make glycerol stocks from the starter cultures for experiment #2.2

Protocol

Two stocks each from each of the 15 cultures. Use up to 5mL to make the stocks. We need the other 5mL for miniprep.

Lab Notebook Entry 97

Date Performed

19-Sep

Procedure

Miniprep

Details

Get DNA of our full constructs

Protocol

Miniprep all 15 cultures. Use up to 5mL for miniprep. We need the other 5mL for glycerol stocks.

Lab Notebook Entry 98

Date Performed

5-Oct

Procedure

Nanodrop

Details

Protocol

Lab Notebook Entry 99

Date Performed

5-Oct

Procedure

Sequence

Details

Protocol

Lab Notebook Entry 100

Date Performed

23-Sep

Procedure

Make starter cultures

Details

Make large cultures to provide cells for our three experiments.

Protocol

Prewarm the LB to 37C in the incubator. Make 5mL cultures of 3 colonies from each of the 5 plates for a total of 15 cultures. Label them with a letter and then a number. 'P' for the pUC19 control, 'C' for the promoterless part C, and 'J' 'T' or 'L' for the three promoters, then 1, 2, and 3 for each type. So one tube will be P1, another P2, etc.

Lab Notebook Entry 101

Date Performed

9-Oct

Procedure

Reminiprep

Details

Protocol

Lab Notebook Entry 102

Date Performed

11-Oct

Procedure

Nanodrop

Details

Protocol

Lab Notebook Entry 103

Date Performed

12-Oct

Procedure

Sequence

Details

Protocol

Lab Notebook Entry 104

Date Performed

Procedure

TESTING DIFFERENT PROMOTERS

Details

Protocol

Lab Notebook Entry 105

Date Performed

31-Aug

Procedure

Experiement #4: Can we regrow from plates stored at 4C

Details

Purpose: See if we can get the cells to grow again after being stored at 4C overnight

Protocol

Use Experiment #4 Protocol

Lab Notebook Entry 106

Date Performed

31-Aug

Procedure

Experiment #2: Can we regrow colonies from glycerol stocks

Details

Purpose: See if we can get the cells to grow again from a glycerol stock

Protocol

Use Experiment #2 Protocol

Lab Notebook Entry 107

Date Performed

1-Sep

Procedure

Experiment #1-A: shifting from 37C to 25C in liquid culture

Details

Purpose: See if the cells lyse as intended over different amounts of time at 25C

Protocol

Use Experiment #1 Protocol

Lab Notebook Entry 108

Date Performed

8-Sep

Procedure

Experiment #1-B: shifting from 37C to 25C in liquid culture

Details

Purpose: Get more data by repeating previous experiment

Protocol

Use Experiment #1 Protocol

Lab Notebook Entry 109

Date Performed

16-Sep

Procedure

Experiment #1-C: shifting from 37C to 25C in liquid culture

Details

Purpose: Get more data by repeating previous experiment

Protocol

Use Experiment #1 Protocol

Lab Notebook Entry 110

Date Performed

16-Sep

Procedure

Experiment #3: growing colonies on plates after time at 25C

Details

Purpose: can cells that have been at 25C for various amounts of time grow on plates

Protocol

Use Experiment #3 Protocol

Lab Notebook Entry 111

Date Performed

Procedure

SUBMISSION CLONING

Details

Protocol

Lab Notebook Entry 112

Date Performed

10-Sep

Procedure

Digest "T4 antiholin" with XbaI and SpeI

Details

Cut one of the parts we are submitting so it can be ligated into the backbone

Protocol

5uL template DNA, 15.5uL PCR H2O, 2.5uL buffer 2.1, 1uL XbaI, 1uL SpeI (25uL total volume). Program: 37C for 90mins, 80C for 20mins, 4C infinity

Lab Notebook Entry 113

Date Performed

10-Sep

Procedure

Digest "pSBB1C3" with XbaI and SpeI

Details

Cut the backbone so one of our parts can be ligated into it

Protocol

15uL template DNA, 46.5uL PCR H2O, 7.5uL buffer 2.1, 3uL XbaI, 3uL SpeI (split into three 25uL total volume rxns). Program: 37C for 90mins, 80C for 20mins, 4C infinity

Lab Notebook Entry 114

Date Performed

10-Sep

Procedure

Digest "holin sub" with XbaI and SpeI

Details

Cut one of the parts we are submitting so it can be ligated into the backbone

Protocol

5uL template DNA, 15.5uL PCR H2O, 2.5uL buffer 2.1, 1uL XbaI, 1uL SpeI (25uL total volume). Program: 37C for 90mins, 80C for 20mins, 4C infinity

Lab Notebook Entry 115

Date Performed

10-Sep

Procedure

Digest "endolysin sub" with XbaI and SpeI

Details

Cut one of the parts we are submitting so it can be ligated into the backbone

Protocol

5uL template DNA, 15.5uL PCR H2O, 2.5uL buffer 2.1, 1uL XbaI, 1uL SpeI (25uL total volume). Program: 37C for 90mins, 80C for 20mins, 4C infinity

Lab Notebook Entry 116

Date Performed

11-Sep

Procedure

Ligation of holin, endolysin, and antiholin into seperate backbones

Details

Ligate everything together

Protocol

Lab Notebook Entry 117

Date Performed

15-Sep

Procedure

Transformation

Details

Protocol

Lab Notebook Entry 118

Date Performed

18-Sep

Procedure

Starter cultures

Details

Make 5mL cultures of our synthesized cells.

Protocol

Lab Notebook Entry 119

Date Performed

19-Sep

Procedure

Miniprep

Details

Get our DNA back out

Protocol

Lab Notebook Entry 120

Date Performed

16-Sep

Procedure

Digest "TlpA36 sub" with XbaI and SpeI

Details

Cut one of the parts we are submitting so it can be ligated into the backbone

Protocol

5uL template DNA, 15.5uL PCR H2O, 2.5uL buffer 2.1, 1uL XbaI, 1uL SpeI (25uL total volume). Program: 37C for 90mins, 80C for 20mins, 4C infinity

Lab Notebook Entry 121

Date Performed

17-Sep

Procedure

Ligation of "tlpA36 sub" into backbone

Details

Ligate submission parts together for final product.

Protocol

Lab Notebook Entry 122

Date Performed

26-Sep

Procedure

Transformation

Details

Transform ligate into cells

Protocol

Lab Notebook Entry 123

Date Performed

27-Sep

Procedure

Starter cultures

Details

Make starter cultures of TlpA36 in submission vector

Protocol

5mL cultures, make 3

Lab Notebook Entry 124

Date Performed

28-Sep

Procedure

Miniprep

Details

Miniprep TlpA36 for further analysis of DNA.

Protocol

Lab Notebook Entry 125

Date Performed

9-Oct

Procedure

New starter cultures from plates

Details

Pick colonies and make new starter cultures of cells with holin, endolysin, and antiholin.

Protocol

Lab Notebook Entry 126

Date Performed

10-Oct

Procedure

Miniprep

Details

Miniprep of holin, endolysin, and antiholin (and our construct cell lines)

Protocol

Lab Notebook Entry 127

Date Performed

9-Oct

Procedure

Digest "TlpA36 sub" with XbaI and SpeI and "pSBB1C3" with XbaI and SpeI

Details

Cut one of the parts and backbone we are submitting so it can be ligated together.

Protocol

Lab Notebook Entry 128

Date Performed

10-Oct

Procedure

Ligate

Details

tlpa and backbone together

Protocol

Lab Notebook Entry 129

Date Performed

11-Oct

Procedure

Transform

Details

Transform ligate into cells

Protocol

Lab Notebook Entry 130

Date Performed

11-Oct

Procedure

Nanodrop

Details

Purpose: determine concentration of holin, endolysin, antiholin products so that it can be shipped

Protocol