BioBrick Formation Step-by-Step Overview:

(protocol compiled by Bethany Bruno, Liz Kelly, & Elyse McMillen, last updated 9/24/13)

As a multistep, complex process, each aspect of biobrick formation takes approximately two to three days. The general protocol involves the following steps:

Thoroughly read all protocol before beginning! Happy labbing, and good luck :)

1. Restriction Enzyme Digestion

Materials: (per 1-1.5 mL microcentrifuge reaction tube)

- 2 uL 10X Corresponding Buffer
- 2 uL 10X Cutsmart BSA
- 0.5 uL Enzyme #1 (& 0.5 uL enzyme #2 if double digestion)
- __ uL DNA (either insert, vector, or plasmid)
- __ uL DI water

Preparation:

Enzyme Selection: Before digestion, select desired enzymes (EcoRI, XbaI, SpeI, or PstI). For BioBrick formation, the XbaI/SpeI scarring pattern is generally used; should you be inserting a DNA sequence into a linearized backbone, however, use EcoRI and PstI. After BioBrick formation, confirmatory digests may also be conducted with EcoRI and PstI. In accordance with iGEM policy, ensure that EcoRI, XbaI, SpeI, and PstI do not cut within the insert nor vector sequence (disregarding standard cut sites).

Reaction Volume Determination: To determine the correct reaction volumes of DNA and deionized water, the insert and vector DNA concentrations must be known beforehand; these may be found via Nanodrop. For BioBrick formation, use 500 ng of insert DNA (if insert is one kilobase or less) and 200-300 ng of vector DNA. This provides sufficient DNA proportions for ligation post sea-plaque gel electrophoresis. For confirmatory digests and agarose gels, use 400 ng plasmid DNA. Add water so that the final reaction volume is 20uL

	Insert	Vector	
BioBrick Formation	500 ng of DNA	250 ng of DNA	
Confirmatory Digest	--	--	400 ng of DNA

NOTE: Insert DNA and vector DNA are digested separately in different reactions.

Materials Location: Disregarding water, all materials may be found in the -20 degree freezer and should be be kept on ice throughout the procedure. Ensure, however, that frozen materials have fully defrosted before use. Hold and rub the tubes with fingers if they remain frozen at time of use. Enzymes should not be warmed; they should only be removed from the -20 degree freezer immediately prior to addition to reaction mixture.

Dilution Warning: Before beginning, ensure that the BSA and buffer are both 10X. If only 100X is available, make a 10X dilution by adding 1 uL of 100X to 9 uL of DI water for 10 uL total volume; equivalent proportions may be used if larger stock is desired.

Procedure:

1. Label one 1-1.5 uL microcentrifuge tube per digestion reaction, following proper labeling protocol.
2. Using a p20 pipette, add appropriate volume of deionized water to each reaction tube.
3. Using a p2 pipette, add 2 uL 10X Cutsmart buffer to each reaction tube.

Note: Prevent air bubble formation by carefully pipetting additions directly into the reaction mixture while pushing to the first pipette stop. Pipet up and down to mix the solution; the pipette tip may also be used to stir the solution. Remove the tip from the mixture, and hover barely above the mixture while pipetting to the second stop.

4. Using a p2 pipette, add 2 uL 10X BSA to each reaction tube.
5. Using either p2 or p20 pipette, add appropriate volume of DNA to each reaction tube.
6. Using p2 pipette, add 0.5 uL enzyme to each reaction tube.
7. Place reaction tubes into 37 degrees Celsius incubator. One hour is generally used for all digestion reactions; remove reaction tubes from the incubator for use in sea-plaque gel after allotted time-span. If gel electrophoresis is to be conducted immediately after digestion, prepare sea-plaque agarose gel during the digestion.

Note: For convenience, digestion products may be left on lab bench temporarily after one hour incubation at 37 degrees Celsius. In general, do not wait more than a few hours before loading the products for gel electrophoresis.

2. Gel Preparation

Agarose Gel Preparation

Safety Considerations

Ethidium Bromide (EtBr) is carcinogenic. Avoid skin contact and do not inhale. Prepare gel within hood specifically used for EtBr work, and dispose of all waste which contacted EtBr within EtBr labeled water container (gloves, pipette tips, gel tape, etc).

Materials

- Agarose
- 1X TAE
- Ethidium bromide (EtBr)
- Gel mold
- Tape
- Comb (either 6 or 10 well)
- Weigh boat
- Scoopula
- Fine scale
- 50-100 mL graduated cylinder
- 250 mL erlenmeyer flask, specifically designated for EtBr
- Microwave
- Saran wrap

Preparation

Expected Gel: Plan how samples will be run, drawing an expected gel image. Use comb with the correct number of wells in accordance to the expected gel. Ladders should be loaded in the middle of the gel to provide clear, distinct bands.

Procedure

1. Carefully tape the open edges of the gel mold such that the seal prevents molten gel from leaking out of the mold.
2. Select appropriate comb according to expected gel and place in the notches towards one end of the gel mold.
3. Weigh 0.4 g of agarose into a weigh boat using a fine scale. Pour agarose from weigh boat into 250 mL flask specifically for EtBr.
4. Measure 50 mL of 1x TAE in graduated cylinder. Pour into agarose-containing 250 mL erlenmeyer flask.
5. Place flask in microwave for 30 second intervals until the agarose is completely dissolved.

Note: Do NOT allow the mixture to bubble or boil. If this does occur, stop the microwave and let the mixture cool by swirling. Be careful with handling as the flask can become quite hot; use hot pads and hold the flask at arms length to avoid burns.

6. Let the mixture cool for approximately 5 minutes. Wait until the flask is cool enough that it can tolerably be held for 10 second without hot pads.
7. Under the hood, add 1.0 uL of EtBr to the gel and swirl until the EtBr is no longer visible.

Note: EtBr is carcinogenic. Be very careful using this chemical, using gloves and a hood to avoid inhaling fumes. Preferably, use a designated p2 pipette and p2 tip box for EtBr use.

8. Pour the mixture into the gel mold and let cool until solid within the hood (approximately 20 minutes).
9. Dispose of all gloves and paper towels in EtBr designated hazardous waste container.

SeaPlaque Agarose Gel Preparation

Description

8% SeaPlaque agarose gels are used strictly for BioBrick formation gel extractions of digested products to be used in ligation reactions. They are extremely fragile and thus require special precautions not taken with regular agarose gels.

Safety Considerations

Ethidium Bromide (EtBr) is carcinogenic. Avoid skin contact and do not inhale. Prepare gel within hood specifically used for EtBr work, and dispose of all waste which contacted EtBr within EtBr labeled water container (gloves, pipette tips, gel tape, etc).

Materials

- SeaPlaque agarose
- 1X TAE
- Ethidium bromide (EtBr)
- Gel mold
- Tape
- Comb (either 6 or 10 well)
- Weigh boat
- Scoopula
- Fine scale
- 50-100 mL graduated cylinder
- 250 mL erlenmeyer flask, specifically designated for EtBr contamination
- Microwave
- Saran wrap

Preparation

Expected Gel: Plan how samples will be run, drawing an expected gel image. Use comb with the correct number of wells in accordance to the expected gel. Ladders should be loaded in the middle of the gel to provide clear, distinct bands.

Procedure

1. Carefully tape open edges of the gel mold such that the seal prevents molten gel from

later leaking out of the mold.

2. Select appropriate comb according to expected gel and place in the notches to one end of the gel mold.
3. Weigh 0.4 mg of SeaPlaque agarose into a weigh boat using a fine scale. Pour agarose from weigh boat into 250 mL flask labelled for EtBr.
4. Measure 50 mL of 1x TAE in graduated cylinder. Pour into agarose-containing 250 mL erlenmeyer flask.
5. Place flask in microwave for 30 second intervals until the agarose is completely dissolved.

Note: Do NOT allow the mixture to bubble or boil. If this does occur, stop the microwave and let the mixture cool by swirling. Be careful with handling as the flask can become quite hot; use hot pads and hold the flask at arms length to avoid burns.

6. Let the mixture cool for approximately 5 minutes. In general, wait until the flask is cool enough that it can tolerably be held for 10 second without hot pads.
7. Under the hood, add 1.0 uL of EtBr to the gel and swirl until the red EtBr is no longer visible.

Note: EtBr is carcinogenic. Be very careful using this chemical, using gloves and a hood to avoid inhaling fumes. Preferably, use a designated p2 pipette and p2 tip box for EtBr use.

8. Pour the mixture into the gel mold and let cool for 10 minutes within the hood.
9. Wrap gel mold in saran wrap and place in a 4 degree fridge for 1 hour.
10. Dispose of all gloves and paper towels in EtBr designated hazardous waste container.

3. Gel Electrophoresis

Description

This protocol is for the running of a SeaPlaque agarose gel for extraction of digested products. Techniques may be applied to other gels.

Materials

- SeaPlaque agarose gel
- 1X TAE
- Parafilm
- PCR tube tray
- Gel electrophoresis box
- Voltmeter
- 6x loading dye
- Distilled water
- p2 pipette
- p2 tips
- p200 pipette
- p200 tips

Preparation

Calculations: Calculating the proper amount of dye and product as well as dye and ladder is an integral part of the gel process. There needs to be enough DNA in the well to be extracted. When using 6x dye, create mixtures that dilute the final concentration to 1x dye. For 20uL of digested products add 4uL of 6x dye. Ladder concentrations may vary and water can be used to make up the difference. Add 200-300 nanograms of large(1KB) DNA and 500 nanograms for smaller DNA.

Note: Running controls with undigested DNA is recommended

Set up: Fill the gel electrophoresis box with fresh 1X TAE. If there is old TAE in the box, empty and dispose of the liquid properly. Remove the gel (still in the gel mold) from the 4 degree fridge and gently remove the saran wrap. Remove the tape from the sides of the mold. Be sure not to apply pressure to the gel itself and do not let it slide off the mold.

Procedure

Part I: Prepare the Gel

1. Place the gel mold with gel into a gel box already filled with TAE. Make sure the TAE covers the whole gel.
    a. Be sure that the wells of the gel are on the same side as the negatively charged current.
2. CAREFULLY, without jostling the comb back and forth, pull the comb straight up and out of the gel.

Note: This step is very fickle. Be extremely careful and go slowly. The gel is fragile and will not stay on the mold as you pull it up.

3. Using a p200 pipet set to 50uL, flush each well wit h 1x TAE. This clears out any extra

pieces of agarose that may have fallen into the well while pulling out the comb.

Note: The gel moves a lot. Be sure not to push the gel off the mold

4. Using a p2 set to 0.5uL, add a drop of 6x dye to the bottom of each well. This will identify if any wells have holes in the bottom. Leaky wells will lead to poor results.
    a. If there are leaky wells, do not use that well.

Part II: Prepare the Products

5. Place a square of parafilm down onto a PCR tube tray.
6. Press down on the parafilm, creating little wells for the products to be mixed.
7. Aliquot correct proportions of the dye.
8. Aliquot correct proportions of the distilled water(optional) and pipet up and down to properly mix.
9. Add ALL the digested products (20uL) to the aliquoted dye. Again, pipet up and down to thoroughly mix the products and the dye.
10. Transfer ALL of the mixture in the parafilm well into the agarose gel
    a. Be sure to pipet slowly. If pipetted too fast, products will be expelled from the well and run into other wells.
11. DO NOT FORGET THE LADDER. This allows analysis of the gel results. Without the ladder, the gel is inconclusive.

Part III: Running the Gel

12. Place the lid of the gel box on to the correct electrodes ie. black with black and red with red.
13. Turn on the voltmeter to 60 volts. DO NOT increase the voltage more than this. SeaPlaque is a low melting point agarose and more voltage will lead to poor results.

Note: For non-SeaPlaque gels, increase the voltage to 90 volts.

14. If the electrodes are connected correctly, there will be bubbles rising in the liquid TAE in the gel box.
15. Let the DNA run for an hour
    a. Check on the gel every 10 minutes for any signs of gel degradation.
    b. Gel may be run for longer than an hour if desired. Be sure the check the gel to ensure quality.
16. When the gel has run for an hour (or desired length of time), turn off the voltmeter.

4. Gel Extraction

Description
This protocol is for the extraction of DNA bands from a SeaPlaque gel.

Materials
- Seaplaque gel
- Gel imager
- Transilluminator
- Clean razor blades
- Lab coat
- Face shield
- 0.5mL Eppendorf tubes (autoclaved)

Preparations
Gel extraction is an important step in BioBrick Formation. Make sure to plan out what the gel should look like and which bands to cut. This will be helpful when it comes to cutting the bands.

Procedure
1. Remove gel CAREFULLY from the electrophoresis box. Hold the mold next to the wells and the end of the gel so that it does not slide off the mold. Keep this hold until using the transilluminator or imager.
2. Carefully place the gel WITH THE MOLD on/in the imager and take a good quality picture. This will be important for later ligations.

Note: The gel is too fragile to remove from the mold before placing it on the transilluminator
Note: Try to limit the amount of time the gel spends in UV light. The UV light can cause thymine dimers of the DNA

3. Carefully take the gel off the imager and place on the transilluminator WITH OUT THE MOLD.
4. Put on long sleeved lab coat and the face shield. The transilluminator uses UV light and that will burn your skin and eyes if exposed for too long.
5. Turn on the transilluminator to the lowest possible setting to avoid dimers .
6. Cut a path to the first band of interest for easier access.
7. Using a new side of the blade for each band, make four cuts in a square around each band.

Note: If experienced user, try and cut the excess gel off. It should be clear where the DNA is because it glows in the UV light.

8. Using the edge of the blade, plop the gel chunk on to the top of the gel and push into a labeled 0.5mL Eppendorf tube.
9. In between each cut turn off the transilluminator to prevent dimers.
10. If more bands, return to step 5 and repeat.
11. When complete, turn off the transilluminator, wipe off excess gel and liquid, and place ALL materials into the EtBr waste bucket.

5. Ligation Reaction

Description

This protocol is for ligating two digested products, a vector and an insert, that have been extracted from a SeaPlaque agarose gel.

Materials

- Extracted agarose chunks of digested DNA
- 2 waterbaths
- Eppendorf tubes (sterilized)
- 2uL 10X Ligase Buffer
- 0.75uL T4 Ligase
- ___uL Deionized water
- Ice bucket
- ice

Preparations

Material set up: Set 2 waterbaths: one to 65 degrees Celsius and another to 37 degrees Celsius. Cut the tip off of the p20 tips to allow for better uptake of the thicker agarose solution. Fill ice bucket with ice in order to keep the T4 ligase enzyme cold.

Calculations: Calculating the ratio of insert to vector is very important to the ligation process. Using a 3:1 insert to vector ratio is recommended. To calculate how many uL to pipet into the reaction, compare the base pair lengths of both fragments as well as the band brightness from the gel.
Example: If the insert is 700bp and the vector is 2100bp and the bands are equal in brightness, then there is 3X the amount of DNA in the insert band.

Procedure

1. Place the 1.5mL Eppendorf tube with the agarose chunk of digested DNA into the 65 degree water bath for 10 minutes but NO LONGER.

Note: Agarose can be placed in a 37 degree water bath to remain molten after the 10 minutes has elapsed.

2. Add appropriate amount of deionized water to new ligation reaction tube
3. Retrieve 10X Ligase buffer from -20 degree freezer. Add 2uL of 10X Ligation buffer to the same tube

Note: Ensure the Ligation Buffer has melted completely and has a fishy scent, otherwise retrieve fresh buffer.

4. Using the previously cut tips, SLOWLY pipet the molten agarose for both vector and insert into the same tube for the ligation reaction.

Note: The amount of vector and insert should be calculated beforehand.

5. Retrieve T4 ligase from the -20 degree freezer and place on ice immediately
6. Add 0.75uL of T4 ligase to the reaction tube.

Note:DO NOT forget the ligation control ie. just the vector without the insert.

7. Let sit on bench overnight at room temperature.

8. Return all materials to their respective storage conditions.

6. Transformation and Plating

Description

This protocol is for transforming plasmid into competent cells and then growing these transformed cells on LB media + antibiotics solid plates.

Materials

- Competent cells
- Plasmid of interest
- Liquid LB media(autoclaved)
- Solid LB media + Antibiotics plates
- Glass pasteur pipets
- Ice bucket
- Ice
- 70% EtOH
- Bunsen burner
- striker

Preparations

*Materials:*Competent cells can be premade in lab or ordered from a company. For this protocol, 50 uL of competent cells need to be aliquoted into an Eppendorf tube(sterilized).Be sure to fill ice bucket with ice before hand as the competent cells need to stay chilled. Retrieve enough tubes of competent cells to transform all ligation products, ligation control and transformation control. Controls are just as important as the products themselves.

Media Preparation: Premade media and plates are paramount. Make sure these are available within reach before starting this protocol. Correct concentrations of antibiotics is also key to the survival and selection of the bacteria of interest.
Set a water bath to 42 degree C

Transformation

1. Clean workspace with 70% EtOH
2. Light bunsen burner with striker
3. Add 5uL of ligation product to 50uL of competent cells.

Note: Because of the agarose chunks used in the ligation, the ligation mixture may be thick or sticky. Be sure to pipet up and down SLOWLY to accurately obtain the desired amount of ligation products.

4. Gently flick the cells 6 times to properly mix the cells and the ligation products.
    a. Be sure not to displace the cells all over the tube.

Note:Without proper mixture, the majority of the cells will not be able to adopt the plasmid.

5. Incubate on ice for up to 5 minutes (NO LONGER). Then heat shock the samples at 42 C for 1 minute using one of the water baths. Place the samples back on ice for 1 minute, then place them in a tube rack (at room temperature). The bacterial cells do not tolerate the agarose well so keeping them on ice for too long can lead to disastrous results.
6. Add 1 mL of LB liquid to each sample and place in shaker at 37 C for 2 hours.

Plating

1. Remove LB+antibiotic plates from 4 degree C and bring to room temperature.
2. After 2 hours, remove the transformation samples from the shaker.
3. Pipet 200uL of transformant onto a room temperature LB+antibiotic plate.
4. Use a glass pipet as a spreader and spread the pipetted transformants around the plate. One may also use glass beads.

Note: To make a glass pipet spreader, turn on a bunsen burner and melt the glass creating two right angles: one at the middle of the pipet and end. Glass beads are also an option.

7. Culturing

Description

This protocol is for growing a single colony from a plate in liquid LB for various purposes.

Materials

- Liquid LB media (autoclaved)
- Antibiotics (filter sterilized)
- Bacteria on solid LB + Antibiotic plates
- Culture tubes
- Inoculation loop
- 70% EtOH
- Bunsen burner
- Striker

Procedure

1. Clean the workspace with 70% EtOH
2. Light the bunsen burner with the striker to keep work area sterile.
3. Aliquot 7mL of LB into a culture tube
4. Add appropriate antibiotics to the correct concentration. Make sure the antibiotic is fully melted and mixed well.
5. Sterilize the inoculation loop with 70% EtOH and heating it in the flame. Repeat process three times.

Note: if using individually packaged plastic loops DO NOT FLAME
Note: if using pipette tips instead of loops, make sure the tips are autoclaved

6. Let the inoculation loop cool. A hot inoculation loop can shock and kill the bacteria
7. Gently scoop up an individual colony with the inoculation loop. The bacteria should be a visible lump on the loop.
    a. if using a pipette tip, the bacteria should be a visible clump at the end of the tip.

Note: Avoid taking large clumps of LB with the colony

8. Swirl the inoculation loop in the culture tube. Clump should not be visible on the loop any more.
    a. if using a pipette tip, drop the tip into the tube and leave there.
9. Place cap on the culture tube
10. Place on a shaker in an incubator overnight at a slant.

Note:The shaker should be set so that the LB is not frothing but there is aeration.

11. Turn off the bunsen burner
12. Parafilm the plate and store in 4 degree fridge for later reference
13. Clean workspace with 70% EtOH
14. Several hours after finishing this protocol split the culture into 2 culture tubes so that the final volume in each tube is 3.5mL. This is important for miniprep volumes.

8. Miniprep Protocol

Description

This protocol is designed to purify 50 uL plasmid DNA from 7 mL overnight E. coli culture in LB

media.

Materials

- RNase A solution
- Buffer P1
- Buffer P2
- Buffer N3
- Buffer PB
- Buffer PE
- Buffer EB
- Lyse Blue
- Ethanol (96%-100%)

Preparations

1. Cultures need to be extremely turbid prior to miniprep. A timeframe of 18-22 hours after culturing. Do not wait longer than 22 hours as the cells begin to die at a high rate.
2. Make sure Buffer P1 had LyseBlue and RNase A added. Retrieve from 4 degree fridge.
3. Make sure Buffer PE had ethanol added.
4. Check Buffers P2 and N3 for any salt precipitation and redissolve at 37 degrees Celsius if necessary
5. Put Buffer EB in the incubator before you start. This will be used for the last step
6. Set up two waste containers (one for tips, one for liquid waste)

Procedure

1. Start with a total of 7mL of culture from each colony, separated into two 15mL Falcon tubes with 3.5mL each. Remove any pipet tips in the tubes. For each colony, use a P1000 set at 750uL to put two pipets of culture in a labeled 1.5 uL microcentrifuge tube. Repeat two more times with two other microcentrifuge tubes. At this point, you should have 3 full microcentrifuge tubes from one colony, and about 1.5mL of liquid left in the Falcon tube. Set aside this extra liquid and MAKE SURE to use for a well-labeled glycerol stock before you discard the tubes. Centrifuge down the 3 microcentrifuge tubes from each colony at 8,000 rpm for 3 minutes at room temperature. Ensure the tubes are balanced and set up the same way in the centrifuge, with the points down.
2. Remove the tubes and QUICKLY so that the pellet stays on the side, draw out the supernatant. This can be done best with two people because one person can take out the majority of the liquid with a P1000 and another can follow to get ALL of the remaining liquid out without touching the pellet.
3. Resuspend pelleted bacterial cells in 250 ul Buffer P1 total. Since there are 3 tubes, split into 80 ul for the first tube, 80 ul for the second tube, and 90 ul for the third tube. Do this by picking up the Buffer P1 and shooting it repeatedly at the pellet by pipetting up and down. When all three tubes have been suspended, combine the liquid from the three tubes all into the first tube. Discard the two empty tubes.
4. Add 250 ul Buffer P2 and mix thoroughly by inverting the tube 4-6 times. Make sure to

complete step 5 (next step) within 5 minutes of adding the Buffer P2 (wait 4 minutes after adding P2, then do step 5 within the next 60 seconds).

Note: If using LyseBlue reagent, solution turns blue

5. Add 350 ul Buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times.

Note: If using LyseBlue reagent, solution turns colorless

6. Centrifuge for 10 min at 13,000 rpm.
7. Apply the supernatant (the liquid that does NOT stick to the bottom of the tube) from step 6 to the QIAprep spin column (tube with small inner tube inside) by pipetting. Make sure to not disturb the extended pellet.
8. Centrifuge for 1 min at 13,000 rpm. Discard the flow-through (whatever falls into the bottom larger tube) by removing the top section of the column and replacing when the liquid is discarded.
9. Wash the QIAprep spin column by adding 500ul Buffer PB directly to the white filter area of the spin column. Centrifuge for 1 min at 13,000 rpm and discard the flow-through.
10. Wash QIAprep spin column by adding 750ul Buffer PE directly to the white filter area of the spin column. Centrifuge for 1 min at 13,000 rpm and discard the flow-through.
11. Centrifuge for an additional 1 min to remove residual wash buffer.
12. To elute DNA, place the QIAprep column (referring to only the top inner tube) in a clean, labeled, 1.5 ml microcentrifuge tube (this is the new outer tube). Remove the Buffer EB from the incubator. Add 25ul Buffer EB directly to the VERY center of the white filter each QIAprep spin column. Let stand for 5 min and then centrifuge for 1 min. Repeat the process, adding 25 uL more to the filter, let stand 5 min, then centrifuge 1 min. Remove the tubes and discard the QIAprep column. The liquid left in the bottom of the microcentrifuge tube is your miniprepped product.

9. Glycerol Stock Protocol

Description

Every Miniprep should have an accompanying glycerol stock. Make sure there is enough material grown up for both the miniprep AND the glycerol stock. One should not be shirked off for the other. Both are important.

Note: exact number used in this protocol are examples. A proper ratio of 50% glycerol to 50% bacteria will suffice.

Materials

- 80-100% Glycerol (autoclaved)
- 1.5-2.0mL Eppendorf tubes (autoclaved)
- Bacterial cultures in LB media
- 70% EtOH
- Bunsen burner
- Striker

Preparations

Glycerol is very thick and sticky. Place in microwave, cap still on the bottle to keep sterile, and microwave at full power for 5-10 seconds. Glycerol heats up very quickly.

Procedure

1. Clean the workspace with 70% EtOH
2. Light the bunsen burner with the striker to keep work area sterile.
3. Add 500uL of warm glycerol to a 1.5 or 2.0mL Eppendorf tube.

Note: Be sure that the glycerol is not too hot as bacteria will not survive in a scalding hot environment.

4. Add 500uL of bacteria in suspension to the same tube.

Note:Make sure that the bacteria is well suspended. Pipette up and down a few times to ensure there is indeed bacteria going into the stock.

5. Invert the tube until the contents are well mixed.
6. Turn off the bunsen burner
7. Label well with date and store in -80 freezer.
8. Clean workspace with 70% EtOH

10. Nanodrop

Description

This protocol is to determine the concentration of DNA within any sample of DNA including miniprep products.

Materials

- Solution buffer (EB if from a miniprep)
- Kimwipes

- Samples
- Nanodrop

Preparation

Access: Be sure to have proper access to a Nanodrop. This instrument is expensive, but useful

Procedure

1. Turn on the computer connected to the Nanodrop
2. Open the Nanodrop program associated with the Nanodrop model
3. Select Nucleic Acid
4. Before finding the concentration of the DNA sample, the machine needs a blank. Use the buffer that the DNA sample is in ie. EB buffer or water.
5. Lift arm of the Nanodrop and place 1uL of buffer onto the Nanodrop
6. Set arm back down gently and select Blank
7. Lift arm, wipe off excess liquid iwth Kimwipe
8. Place 1uL of DNA sample onto the Nanodrop
9. Set arm back down gently and select Measure
10. Sample concentration appears as ng/uL
11. Important ratios to note: 260/280, this should be around 1.9

11. Growing up a Glycerol Stock Protocol

Description

This protocol is for when previous transformants need to be regrown up for multiple purposes such as minipreps or verification.

Materials

- liquid LB media (autoclave)
- Culture tubes

- Inoculation loop
- Antibiotics
- Glycerol stocks from -80
- Ice bucket
- Ice
- 70% EtOH
- Bunsen burner
- striker

Preparations

Fill a bucket with ice for the glycerol stock to stay cold in. The glycerol stock should not defrost or melt.

Procedure

1. Remove glycerol stock from the -80 degree freezer and place on ice.
2. Clean the workspace with 70% EtOH
3. Light the bunsen burner with a striker to keep the workspace sterile
4. Sterilize the inoculation loop with 70% EtOH and heating it in the flame. Repeat process three times.

Note: if using individually packaged plastic loops DO NOT FLAME

Note: if using pipette tips instead of loops, make sure the tips are autoclaved

5. Let the inoculation loop cool. A hot inoculation loop can shock and kill the frozen bacteria
6. Open the glycerol stock tube and remove a chunk of the stock.
7. Add removed chunk to no more than 3 mL of LB + antibiotic in a culture tube.
8. Place in an incubator/shaker overnight at a slant.

Note: The shaker should be set so that the LB is not frothing but there is aeration.

9. Turn off the bunsen burner
10. Return the glycerol stock to the -80 degree freezer
11. Clean workspace with EtOH