

Exp1

Plasmid construction

Restriction Digest Materials Your two Part Samples, A and B: Miniprep DNA (in BioBrick RFC[10] plasmid backbones) Linearized Plasmid Backbone (with a different resistance to the plasmid backbones containing your part samples) EcoRI, XbaI, SpeI, PstI, DpnI NEB Buffer 2 BSA dH2O Digest Enzyme Master Mix for Plasmid Backbone (25ul total, for 5 rxns) 5 ul NEB Buffer 2 0.5 ul BSA 0.5 ul EcoRI-HF 0.5 ul PstI 0.5 ul DpnI (Used to digest any template DNA from production) 18 ul dH2O Enzyme Master Mix for Part A (25ul total, for 5 rxns) 5 ul NEB Buffer 2 0.5 ul BSA 0.5 ul EcoRI-HF 0.5 ul SpeI 18.5 ul dH2O Enzyme Master Mix for Part B (25ul total, for 5 rxns) 5 ul NEB Buffer 2 0.5 ul BSA 0.5 ul XbaI 0.5 ul PstI 18.5 ul dH2O Digest Plasmid Backbone Add 4 ul linearized plasmid backbone (25ng/ul for 100ng total) Add 4 ul of Enzyme Master Mix Digest Part A Add 4 ul Part A (25ng/ul for 100ng total) Add 4 ul of Enzyme Master Mix Digest Part B Add 4 ul Part B (25ng/ul for 100ng total) Add 4 ul of Enzyme Master Mix Digest all three reactions at 37C/30 min, heat kill 80C/20 min Ligation Add 2ul of digested Plasmid Backbone (25 ng) Add equimolar amount of Part A (EcoRI-HF SpeI digested) fragment (< 3 ul) Add equimolar amount of Part B (XbaI PstI digested fragment) (< 3 ul) Add 1 ul T4 DNA ligase buffer. Note: Do not use quick ligase Add 0.5 ul T4 DNA ligase Add water to 10 ul Ligate 16C/30 min, heat kill 80C/20 min Transform with 1-2 ul of product Note: For linearized plasmid backbones provided by iGEM HQ, a plasmid backbone with an insert of BBa_J04450 was used as template. As a result any red colonies that appear during your ligation may be due to the template as a background. Digesting with Dpn1 before use should reduce this occurrence.

Transformation

Thaw a tube of NEB Express Competent E. coli cells on ice until the last ice crystals disappear. Mix gently and carefully pipette 50 SIL of cells into a transformation tube on ice. Add 1-5 SIL containing 1 pg-100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA. Do not vortex. Place the mixture on ice for 30 minutes. Do not mix. Heat shock at exactly 42IAC for exactly 60 seconds. Do not mix Place on ice for 5 minutes. Do not mix. Pipette 950 SIL of room temperature SOC into the mixture. Place at 37IAC for 60 minutes. Shake vigorously (250 rpm) or rotate. Warm selection plates to 37IAC. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC. Spread 50-100 SIL of each dilution onto a selection plate and incubate overnight at 37IAC. Alternatively, incubate at 30IAC for 24-36 hours or at 25IAC for 48 hours.

Coculture

This takes an hour to an hour and a half. Do not rush. All samples with cells or media separate, pipette them up and down Two of the flasks are in Brian's shaker and the rest are in the normal one. 1. Pipette 0.5 ml out of each flask into 2 ml centrifuge tubes. When there is a lot of filter paper gunk, use the cut pipette tips. Do this twice for each flask. Shake the flasks well immediately before pipetting and pipette up and down when drawing your sample. Look at the tip and make sure there are no air bubbles or filter paper you will get an inaccurate measurement later. When there is a lot of filter paper gunk, use the cut pipette tips. Close the lids of the centrifuge tubes fully. Close the lids on the flasks but make sure the little top thing is off. Immediately put the flasks back into the shaker. 2. Vortex the centrifuge tubes and then immediately pipette (Do not let them sit between.) the 0.5ml of liquid through the mesh on the caps of cytometer tubes (5 mL Falcon polystyrene round bottom tube with cell-strainer cap). Alternative method(replaces steps 2 - 4): use scissors and cut off the lids of the labeled centrifuge tubes (save the lids, don't bother labeling the lids because they were cut off before any liquid was in any centrifuge tube, so they can go on any centrifuge tube). Take the mesh caps off the cytometer tubes and cap them on the lidless centrifuge tubes (throw away capless cytometer tubes). Now pipette the .5ml of liquid through the mesh caps directly into the centrifuge tubes. When done pipetting, throw out mesh caps and re-cap the centrifuge tubes with the saved, cut-off lids. Now centrifuge with the 18-slot centrifuge 10 min at 8800 rpm. Make sure to close the centrifuge tube before vortexing. Press tip of pipette pretty firmly down on the mesh and pipette up and down in quick short movements. Be careful of stuff splashing out though and watch for bubbles. The goal is to get all of the fluid on the inside of the tube, if any ends up on top of the mesh, aspirate it up and try again. If it's not going through, pipette up and down and try twisting the tube or try a different spot on the mesh. 3. After you pipette through the mesh, hand centrifuge the liquid to the bottom, a lot tends to stick to the sides. 4. Pipette up and down right before decanting the filtered contents of each cytometer tube into another centrifuge tube, centrifuge at 6000x rcf at 4 C for 10 mins. best use 18-slot centrifuge by Todd's bench, 6000x rcf converts to 8800 rpm. 5. Pipette supernatant into the corresponding cryotube (DON'T throw the pellet away!!!) and place in -20 C iGEM freezer TIP: Hold the tip against the side of the tube to minimize any instability in your hands, then get the tip within around a millimeter of the pellet and aspirate slowly. 6. Resuspend pellet from centrifuge tubes in 1ml PBS. Remember to pipette up and down. 7. Pipette up and down while transferring 1ml into a cryotube. 8. Add 1ml 50% glycerol to each cryotube from step 7, vortex FOR 1 MINUTE to thoroughly mix, and place in iGEM box in the -80 degree freezer (It is in the stack of boxes where the rest of our boxes always are, however it is 2nd from the bottom). If box 1 is full then put in box 2, filling from top left corner; make sure lid is lined up with box using the black corner markings. Wear latex gloves; don't mess with freezer bare-handed. DO NOT OPEN THE -80 UNLESS IT IS BELOW -75C SERIOUSLY. Minimize time in the -80. Again, DO NOT OPEN THE -80 UNLESS IT IS BELOW -75 C. 9. Mark down on the table below that you have completed this measurement. Make sure to replenish any materials that ran out so that the next group doesn't have to go looking. Also leave out things like test tube racks.

Concentration measurement

1. Ethyl acetate extracts from bacterial cultures were subjected to HPLC-MS analyses 2. which was performed with an Alliance chromatographic module coupled to a 2996 photo- diode array detector and a ZQ4000 mass spectrometer (Waters, Mi- cromass, Milford, MA) 3. A Symmetry C18 column (2.1*150 mm, Waters) was used with acetonitrile and formic acid (1%) in water as solvents 4. Elution started at 10% acetonitrile for 4 min, followed by a linear gradient up to 88% at 30 min, and a final isocratic hold at 100% for 5 min, at a flow rate of 0.25 mL/min 5. Mass analysis was done by electrospray ionization in the positive mode, with a capillary voltage of 3 kV and cone voltages of 20, 60 and 100 V 6. Compounds were identified based on their HPLC elution time, UV- visible absorption characteristics and mass spectra, compared to previously published data.

DNA Sequences

Some DNA

Exp2

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Fluorescence measurement

Day 1:transform Escherichia coli DH5SA or TOP10 with plasmid Day 2: Pick 2 colonies from each of plate and inoculate it on 5-10 mL LB medium + Chloramphenicol. Grow the cells overnight (16-18 hours) at 37IAC and 220 rpm. Day 3: Cell growth, sampling, and assay Note the differences, depending on whether you are making your readings in a plate reader or 1 ml cuvettes: Set your instrument to read OD600 Measure OD600 of the overnight cultures Record data in your notebook Dilute the cultures to a target OD600 of 0.02 in 10ml 0.5x TB medium + Chloramphenicol in 50 mL falcon tube (if using cuvettes, you can use 100 ml in a 500ml shake flask). Incubate the cultures at 37IAC and 220 rpm. Take 100 SIL (1% of total volume) samples of the cultures at 0, 1, 2, 3, 4, 5, and 6 hours of incubation(if using cuvettes, remove 1 ml from 100 ml culture). Place samples on ice. At the end of sampling point you need to measure your samples (OD and FI measurement) Record data in your notebook

DNA Sequences

Some DNA