Strength measurement

Obey local laws and regulations. Strictly follow stanard experiment procedures.

Plasmid construction

Restriction Digest
Materials

Your two Part Samples, A and B: Miniprepped 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
dH20

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 dH20
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 dH20
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 dH20
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.

Transfomation

Thaw a tube of NEB Express Competent E. coli cells on ice until the last ice crystals disappear. Mix gently and carefully pipette 50 l of

cells into a transformation tube on ice.
Add 1-5 l 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 42 for exactly 60 seconds. Do not mix
Place on ice for 5 minutes. Do not mix.
Pipette 950 l of room temperature SOC into the mixture.
Place at 37 for 60 minutes. Shake vigorously (250 rpm) or rotate.
Warm selection plates to 37.
Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC.
Spread 50-100l of each dilution onto a selection plate and incubate overnight at 37. Alternatively, incubate at 30 for 24-36 hours or at 25 for 48 hours.

Fluorescence measurement

Day 1:transform Escherichia coli DH5 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 37C 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 37C and 220 rpm.
Take 100 L (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 Fl measurement)
Record data in your notebook

System construction and conformation

Obey local laws and regulations. Strictly follow stanard experiment procedures.

Plasmid construction

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Materials

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NEB Buffer 2
BSA
dH20

Digest

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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 dH20
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 dH20
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 dH20
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
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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 tubs 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 polysterene 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 its 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 tubecentrifuge at 6000x rcf at 4 C for 10 mins. best use 18-slot centrifuge by Todd's bench, 6000x rcf converts to8800 rpm.
5. Pipette supernatant into the corresponding cryotube (DON'T throw the pellet away!!!) and place in -20C 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.
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

All the vectors are constructed by standard biobricks and backbones.

BREVIBACTERIUM MCBRELLNERI ATCC 49030 - pSB1AK3

TACTAGTAGCGGCCGCTGCAGTCCGGCAAAAAAACGGGCAAGGTGTCACCACCCTGCCCTTTTTCTTTAAAACCGAAA
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TEPIDANAEROBACTER ACETATOXYDANS - pSB1AK3

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