Lab Protocols

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Introduction

Part I

Synthetic Biology

P1 Transduction

Strains: Donor *E. coli* (eg. Keio knockout), Recipient *E. coli* (eg. K12), P1 phage stock (high titer)

Reagents: NZCYM media, 1M Sodium Citrate, 10mM CaCl2, antibiotic stocks, P1 salts solution (LB with 10mM CaCl2 and 5mM MgSO4), LB plates

Time required: 2-3 days (excluding day 0)

A. Donor lysate preparation

Day 0

1. Inoculate a 3ml overnight culture of donor strain in LB supplemented with antibiotics (eg. Kan for keio collection donors).

Day 0

2. Inoculate a 3ml overnight culture of recipient strain in LB. (For part B)

Day 1

3. Dilute saturated donor culture 1:100 into 3mL NZCYM supplemented with 10Mm CaCl2 and antibiotic if required (eg. Kan for keio collection donors). Incubate at 37C and start checking for cells after 40 minutes.
    - Make an additional such dilution to visually check for phage clearing later. No P1 phage will be added to this control culture.
    - Start a 42C water bath for incubation after addition of P1 phage.

Day 1

4. After around 45-60 minutes, if the culture has swirls when held up the light and shaken, add 50uL of the high-titer P1 phage stock to the culture. Incubate at 42C for 1 hour.

Day 1

5. After 60 minutes, check if the culture with P1 phage has started to clear (compared to control). If so, move the culture to 37C for 2 hours.

Day 1

6. Transfer lysed culture to a 15mL conical centrifuge tube and add 200uL $CHCl_3$. Vortex the tube vigorously to lyse any remaining cells and incubate at 37C for another 5 minutes.

    - At this point, start fast-cooling the centrifuge to 4C.

Day 1

7. After 5 minutes, centrifuge the 15 mL conical tubes at 9200g for 5 mins (or at max speed for 10 minutes).

    - Collect syringes and 0.45um filters for filtering the lysate.
    - Label new tubes that will be used to store the lysate.

Day 1

8. After centrifugation, carefully transfer the supernatant to the syringe and filter it through the 0.45um filter into its final tube. This P1:Donor lysate can now be stored at 4C.

    - Discard the chloroform contaminated tubes in biohazard bins.

B. Recipient transduction

Day 1 or 2

1. Pellet 1mL of saturated recipient culture for 2 minutes at maximum speed.

Day 1 or 2

2. Resuspend the cells in 500uL P1 salts solution. Transfer 100uL of these cells to 3 tubes (100uL each).

    - One of these tubes is a no-phage control and gets no P1:Donor lysate.
    - Add 10uL P1:Donor lysate to one of the other tubes.
    - Add 100uL P1:Donor lysate to the final tube.

Day 1 or 2

3. Incubate the tubes at 37C without shaking (desk top heater w/ water in the wells) for 30 minutes to allow the phage to adsorb to the cells.

Day 1 or 2

4. After 30 minutes, add 1mL of LB and 200uL of 1M Sodium Citrate to each tube and incubate for 1 hour with shaking.

- Spread plain LB plates with 5mM Sodium Citrate (125uL of 1M for a 25uL plate) and any antibiotics required for selection of the transduced strain. (eg. Kan for keio collection donors).

Day 1 or 2

5. After 1 hour, spin down the cells, discard the supernatant, and resuspend in 100uL LB media. Spread, the cells on LB plates containing 5mM Sodium Citrate and antibiotic. Incubate the plates at 37C

Day 2 or 3

6. Check the plates the next day, no-phage plates should have no colonies. Restreak colonies from the plates made with P1:Donor lysate transduced cultures onto selective plates. You can directly inoculate these colonies (after restreaking) for step 2 of FLP recombination.

FLP Recombination

Strains: Transformant strain

Reagents: TSS buffer, pcp20 plasmid, LB plates, antibiotics

Time required: 5 days (excluding day 0)

A. pcp20 Transformation

Day 0

1. Inoculate a 3ml overnight culture of transformant strain in LB supplemented with antibiotics (eg. Kan for keio collection transduced strains).

Day 0

2. Dilute the overnight culture 1:100 in 3mL LB supplemented with antibiotics and incubate at 37C for 2-3 hours.

Day 1

3. After 2-3 hours, once the culture is translucent but not opaque, swirl the culture in ice water bath for 5-10 minutes to stop cell growth.

Day 1

4. Centrifuge the culture in two 1.5mL centrifuge tubes at maximum speed for 5 minutes to get a cell pellet. Resuspend the cell pellet in 100uL TSS buffer.
    - One of these tubes can be used as a control and will get no plasmid DNA.

Day 1

5. Add 1uL pcp20 plasmid DNA to the tube and incubate on ice for 30 minutes.
    - Set a desk-top heater to 42C for the heat-shock step.

Day 1

6. Heat shock the cells twice by doing two 45 second heat exposures with a two minute gap on ice (45 sec shock → 120 second on ice → 45 sec shock). After these heat-shocks, let the tubes incubate on ice for 2 minutes and add 500uL SOC to each. Incubate the cells at 30C for 1 hour.
    - Make LB + Carbenicillin plates for plating transformants.

Day 1

7. Plate 100uL of the culture on LB + Carbenicillin plates. Grow the plates overnight at 30C.

B. Recombination, curing, and screening

Day 2

1. After checking for the absence of colonies on the control, inoculate 3 transformed colonies in 3mL LB and grow at 42C overnight for curing of the plasmid.

Day 3

2. Dilute the overnight culture by a factor of 106 (3x dilution of 10uL in 990uL LB) and plate 125uL of this dilution on LB plates. Incubate the plates at 30C.

Day 4

3. Pick three colonies from each plate (total 9 colonies) and restreak them on LB+Kan → LB+Carb → LB plates (in that order) followed by resuspension in 10uL nuclease free water in a PCR tube. Incubate the LB and LB+Kan plate at 37C and the LB+Carb plate at 30C overnight.
    - The colonies in nuclease free water can be used directly for a Colony PCR to check for deletion of the Kanamycin casette.

Day 5

4. The next day, isolate colonies that grew on LB but not on LB+Kan and LB+Carb plates. These are the recombined clones. Verify deletions with PCR for binding sites flanking the gene of interest.

TSS Transformation

Strains: Transformant strain

Reagents: TSS buffer, plasmid, LB plates, antibiotics

Time required: 4-5 hours (excluding Day 0)

A. pcp20 Transformation

Day 0

1. Inoculate a 3ml overnight culture of transformant strain in LB supplemented with any antibiotics required for plasmid/insert maintenance.

Day 1

2. Dilute the overnight culture 1:100 in 3mL LB supplemented with antibiotics and incubate at 37C for 1-2 hours.

Day 1

3. After 1-2 hours, once the culture is translucent but not opaque, swirl the culture in ice water bath for 5-10 minutes to stop cell growth.

Day 1

4. Centrifuge the culture in two 1.5mL centrifuge tubes at maximum speed for 5 minutes to get a cell pellet. Resuspend the cell pellet in 100uL TSS buffer.
    - One of these tubes can be used as a control and will get no plasmid DNA.

Day 1

5. Add 1uL plasmid DNA to the tube and incubate on ice for 30 minutes.
    - Set a desk-top heater to 42C for the heat-shock step.

Day 1

6. Heat shock the cells twice by doing two 45 second heat exposures with a two minute gap on ice (45 sec shock → 120 second on ice → 45 sec shock). After these heat-shocks, let the tubes incubate on ice for 2 minutes and add 500uL SOC to each. Incubate the cells at 37C (or another temperature required for plasmid stability) for 45 minutes.
    - During this time, make selection plates for plating the transformants (with previous and new antibiotics)

Day 1

7. Plate 100uL of the culture on selective plates. Grow the plates overnight at 37C (or alternative temperature for plasmid).

Colony PCR

Strains: Plate with any colonies to be tested (Plate of interest)

Reagents: Appropriate primers for verification, restreak plate with appropriate antibiotic

Time required: <1 day.

1. Create/use LB Agar plates that contain the appropriate antibiotic that was used for selection of the colonies that are to be verified.
2. Divide the plate area into any number of subsections for restreaking the colonies. (I usually divide a plate into 8 sectors).
3. For 10uL reactions and N colonies, create a batch PCR mix that is 10 times (N+1) uL in volume. For example, for 8 samples, I will create a PCR master mix that is $10 \times (8+1) = 90$ uL in volume. This master mix will contain,
    - Water
    - Forward Primer
    - Reverse Primer, and
    - Polymerase w/ Buffer (i.e., PCR Master Mix)
4. Decide how much water, primers, and polymerase MM goes into this batch mix using the appropriate enzyme documentation and based on the total volume calculated above. For colony PCR, I suggest using a non-high fidelity enzyme like Promega GoTaq Green (but NEB Q5 can be used if the first try with GoTaq fails.)
5. Mix the batch using vortexing and spin it down. Divide the batch mix into individual PCR tubes, with 10ul volume per tube. Label one of them the negative control (-) and the rest as C1 to CN, where N is the number of colonies you wish to PCR.
6. Now, number N colonies on your plate of interest with a marker (from 1 to N). Number the subdivisions on the new plate the same way.
7. For each colony, pick it using a sterile pipette tip from the plate of interest and streak it in the correct section on the new plate. Then dip the same pipette tip into the prepared PCR reaction tube with the correct colony number. The negative control tube (-) gets no colonies.

8. Perform the PCR reactions with the appropriate settings for the polymerase and run the samples on a 1% Agarose gel for checking the results.

Part II

Microbiology

3-Color Infection Assay

This protocol is used to check for phage *E. coli* receptor usage using three one-receptor strains containing only the LamB, OmpF, or OmpC receptor each. The phage are plated on a plate containing 1:1:1 ratio of the three strains, each containing a different fluoroscent reporter, and then imaged using a fluoroscent microscope setup.

Strains:

- 3-color strains
  - *E. coli* ΔLamB ΔOmpF w/ mClover3 (eOC)
  - *E. coli* ΔOmpF ΔOmpC w/ mTurquoise2 (eLB)
  - *E. coli* ΔOmpC ΔLamB w/ mScarlet-I (eOF)

Reagents: LB Media, 0.3% NZCYM Agar, 1000x Kanamycin stock, phage sample.

Time required: 2 days (excluding day 0)

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Part III

Recipes

TSS Buffer

Based on the Bennett lab TSS protocol

Pre-sterilized components

Makes **50mL** TSS Buffer

Component	Stock Concentration	Sterilization method	Amount
LB Media	1x	Autoclaved	42.5mL
PEG-3350	1g/mL	Filter-sterilized	5mL
MgCl2	1M	Autoclaved	1mL
DMSO	100%	–	2.5mL

Store at 4°C.

Post-sterilized components

Makes **50mL** TSS Buffer

Component	Stock Concentration	Amount
PEG-3350	pure	5g
MgCl2	1M	1mL
DMSO	100%	2.5mL
LB media	1x	make up to 50mL (~46.5mL)

Filter sterilize and store at 4°C.