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Lysis and transduction of E. coli with P1 phage - creation of double-knockout mutants

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Saul Moore: This protocol was carried out by Cassandra Backes of the Host-Microbe Co-Metabolism laboratory, MRC-LMS

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Behavioural Genomics


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DISCLAIMER

ABSTRACT

The creation of double-knockout mutants from single gene deletion mutants of the Keio Collection was performed by Cassandra Backes of the Host-Microbe Co-Metabolism laboratory, MRC-LMS.

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Lysis

1 Dilute an overnight culture of donor strain grown with selection for the marker to be transduced 1:100 in fresh LB supplemented with 5 mM CaCl2, and 0.2% (or 10 mM) D-Glucose. DO NOT ADD ANTIBIOTIC TO THIS CULTURE. Also prepare an extra tube (for no-phage control).
E. g. For each 5 mL culture add:
25 ul of 1 M CaCl2,
50 uL of 20%(or 1M) D-Glucose,

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50 uL O/N culture.

2 Incubate for 30 to 45 mins at 37C with shaking (220rpm) (or until you get cells to early log phase – tube should be slightly turbid, but noticeable growth)

3 Add P1 phage – 1 to 2 drops – using sterile/discardable Pasteur pipette to cultures (except to no-phage control tube)

4 Continue to incubate, with shaking. Monitor for 1–3 hr until the culture has lysed (you'll see cellular debris in the tube and the culture will have significantly lessened in its turbidity – compare with no-phage control tube).
a. Might take longer than 3 hours in some cases.
b. Sometimes the culture does not lessen in turbidity but if you can see some cellular debris when you shake
the tube then you can proceed to the next step.

5 Transfer lysed culture into 15 mL blue lid falcon tubes* and in the chemical fume hood add 125 uL of Chloroform and vortex for 1 min.
a. The chloroform ensures complete cell lysis and kills bacteria. It does not harm phage, but care should be
taken to avoid transferring the chloroform to the storage tubes during step 8, because storing of phage in
presence of chloroform can result in decreased viability of the stock overtime.
b. *Chloroform can dissolve the plastic on the 30 mL sterillin tubes – so make sure to transfer lysed culture to
15 mL falcon tubes first!

6 Spin down 10 min at 2000 RPM.

7 Pour supernatant into 5 mL syringe and sterile filter directly into 2x 2 mL tubes.
a. Avoid pouring the chloroform into syringe as it will be very hard to filter through otherwise.

8 Store at 4C (write date and donor strain genotype)

Transduction 1d

9 Overnight culture of the donor strain, carrying the mutation to be transduced, in LB + 5 mM CaCl2 at 37°C. 1d

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10 In a 15mL Falcon, add 50 uL of overnight culture + 5-10 uL phage P1, 20 min at 37°C WITHOUT shaking.
Negative control: tube without P1 phage

11 Add 5 mL LB CaCl2 5 mM and transfer to an Erlenmeyer flask at 37°C with stirring until OD_{600} ≈ 1
cell lysis and the stock is ready

12 Put everything back in a Falcon 15mL and add 500 uL chloroform
Store at 4°C. (CaCl2 concentration can be increased to 10 mM)

13 Overnight culture of recipient strain in LB

14 Dilute the overnight culture to 1/100th in 5 mL CaCl2
- At OD_{600} ≈ 1, take 5 Eppendorf tubes

1. Negative control tube – P1 lysate + 750 uL of recipient cells
2. 5 uL tube of P1 lysate + 750 uL of recipient cells
3. 10 uL tube of P1 lysate + 750 uL of recipient cells
4. 15 uL tube of P1 lysate + 750 uL of recipient cells
5. 20 uL tube of P1 lysate + 750 uL of recipient cells

Incubate for 20 min at 37°C WITHOUT stirring

15 Invert, centrifuge for 5 min at 5000 rpm and empty the supernatant

16 Resuspend the pellet in 1 mL of LB + 7.5 mM citrate and leave for 30 to 45 min at RT

17 Centrifuge again, resuspend in 100 µL and plate on LB + AB + 7.5 mM Citrate

18 The next day, restrict the clones on LB + citrate dishes to have isolated clones

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19 Verification of clones by PCR on colonies

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