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pCP20 transformation to remove Kanamycin cassette

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Behavioural Genomics



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Protocol status: Working

We use this protocol and it's working

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Abstract

Transformation with pCP20 to remove the Kanamycin cassette from Keio *E. coli* BW215113 single gene deletion mutants - Cassandra Backes (Host-Microbe Co-Metabolism Laboratory, MRC-LMS)



Materials

Making TSS broth(for final volume 10 mL): Make fresh!

- 10% (w/v) Polyethylene Glycol (PEG) 3350* – on media shelf; add 1 g
- LB broth – add 9 mL
- 50 mM Mg²⁺ (MgSO₄ or MgCl₂) – use 1M stock of MgSO₄ used for NGM; add 500 uL
- Sterilise-filter into new tube
- 5% (v/v) DMSO –chemical metal chest; add 500 uL

Making TSS enhanced buffer (for final volume of 10 mL):

- Add 8.2 mL of dH₂O to 30 mL tube
 - 100mM KCl – (to prepare 1M stock: 1.491 g in 20 mL of H₂O); add 1 mL
 - 30mM CaCl₂- use 1M stock for NGM; add 0.3 mL
 - 50mM MgSO₄ - use 1M stock for NGM; add 0.5 mL
 - Vortex well and filter-sterilise into 2 mL tubes
 - Keep in fridge
- *There are other molecular weights of PEG, so look at label carefully and choose one with 3350 on it!



- 1 Make buffer and broth the day before and keep them in the fridge
- 2 Day before: Grow O/N cultures of the strains of interest. Can also prepare buffers (see materials).
- 3 Dilute overnight culture in LB broth at an OD = 0,2 and grow until OD_{595nm} = 0.5 – 0.8 (mid-log phase).
UNTIL NOW, KEEP CULTURES ON ICE
- 4 Spin down culture(s): transfer first to 15 mL falcon, spin 4500 RPM, 10 min, 4°C
- 5 In the meantime, label 2 mL tubes with strain names and add 80uL **TSS buffer** and 1 - 5 uL of pDNA (50-75ng). vortex and keep on ice for 10 min.
- 6 Remove supernatant from 14 mL tubes and resuspend pellet in 1mL **TSS broth**(cold). Resuspend gently and keep on ice.
- 7 Then transfer cells to a 2 mL tube.
- 8 Add 200uL of bacterial culture in **TSS broth** to the 2 mL tubes containing pDNA in **TSS buffer**, Mix gently by pipetting.
- 9 Incubate for 20 min on ice.
- 10 Add 1mL LB.
- 11 Shake at 30°C for 1 hour 700 rpm.
- 12 Spin down (4500 RPM, 5 min), resuspend pellet in 300uL LB.
- 13 Spread 50 and 150ul on a plate and incubate at **30C** in Chloramphenicol plates!



- 14 Keep remaining transformation mix at 4°C.
- 15 Check plates next day for single colonies, and confirm by PCR/Sequencing.