pCP20 transformation to remove Kanamycin cassette

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

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Behavioural Genomics

Saul Moore

DISCLAIMER

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)

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MATERIALS TEXT

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+ ($MgSO_4$ or $MgCl_2$) – 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 $CaCl_2$- 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!

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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 OD595nm = 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.

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11 Shake at 30^{o}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^{o}C.

15 Check plates next day for single colonies, and confirm by PCR/Sequencing.

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