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Removal of FRT flanked antibiotic resistance gene

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ABSTRACT

Protocol: Removal of antibiotic resistance marker with FRT sites using pCP20 plasmids

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ABSTRACT

Protocol: Removal of antibiotic resistance marker with FRT sites using pCP20 plasmids

Protocol based on Datsenko 2000

- 1 Transform with pCP20 following transformation protocol. **Rescue at 30 °C** and spread cells on Amp plates.
- 2 **Incubate overnight at 30 °C** .(While cells are growing at 30 °C the flippase should be in action and should flip out the resistance marker at FRT sites.

- 2.1 Optional additional step: Streak colonies on Amp, **incubate at 30 °C** overnight. Will allow for a

longer period of flippase action. (This helps if perform protocol first time without it and get low efficiency of flipping out of antibiotic marker)

- 3 Patch multiple colonies (~25) onto LB only, LB + Ab with frt sites (eg. Cm or Kan), and LB+Amp and **incubate at 42 °C overnight**. (pCP20 is a temperature-sensitive plasmid and this will help lose the pCP plasmid.)
 - 3.1 Take a colony and serially patch onto the plates, starting with LB only, in the order mentioned above. **Incubate overnight** at the appropriate temperature.
 - 3.2 Original protocol says to patch on LB last to ensure adequate cells are transferred onto antibiotic plates, but BB suggests LB first.

4 Interpretation of Results

- 4.1 No growth on any antibiotic plate – successful removal of marker and nopCPin cells
- 4.2 Growth on Amp plates – pCP20 not lost from cells
 - Perform second isolate streak and incubation at **42 °C overnight** and repeat steps 3 to 4.
- 4.3 Growth on Ab (to be flipped out) plates – failed removal (see optional step 2a for possible troubleshooting)