Removal of FRT flanked antibiotic resistance gene

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

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

PROTOCOL CITATION

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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)