Removal of antibiotic cassette with pCP20

JEUDI 05/08/2021

Day 1: Transform with pCP20

- Make competent cells of the strain

See protocol scaled down to a fifth (10ml of culture)

- Transform with pCP20
  - Thaw the competent cells from glycerol stock
  - Add 2 to 5uL of plasmid to 100uL of competent cells
  - Incubate for 20min on ice
  - Heat shock at 42°C for exactly 45sec
  - Add 950uL of LB to the tube
  - Incubate during one hour at 37°C with shaking

Allows for the antibiotic resistance to be expressed before selection

- Plate 200uL of the culture on an LB + amp plate
  - 20% at original concentration + the rest spinned down and concentrated

200uL directly and the rest centrifuged before resuspension in 200uL

- Grow overnight at 30°C

Cells grow slow at 30°C, do not be surprised if there are no colonies after 18 hours.

Day 2: Induce recombination

- Pick a single colony from the LB + amp plate
- Inoculate into 5mL plain LB
- Grow overnight at 43°C to induce FLP recombinase expression an select for loss of pCP20

Day 3: Plate to get single candidate recombinants

- Make a 10^6 dilution of the overnight culture via 3 serial dilutions.

50 µl into 4.5 ml each time

- Plate 50 µl of this dilution on LB. This should yield a couple hundred colonies.
- Grow overnight at 30°C to prevent partial loss of plasmid from colonies founded by cells that did not lose plasmid.

Day 4: Screen for genomic recombination and plasmid loss

- Patch six individual colonies from this plate onto LB + Kan, LB + Amp, and LB plates.

*Do the patching by picking a colony and then streaking a small spot on each plate **in this order**. Be sure you patch on LB last. Failure to grow can sometimes occur because no cells were transferred to the later plates. This order ensures that, if you get the desired growth on the last plate and no growth on the LB + Kan and LB + Amp plates, it was not due to "running out" of cells in the later patches.*

- Grow overnight at 37°C for LB and LB + Kan and grow at 30°C for LB + Amp plates.
- NOTE: To save a day, you can inoculate several colonies into 5mL LB at the same time that you patch them (*after streaking on plain LB, use the same loop to inoculate the overnight culture*).

You will proceed with the liquid LB cultures that correspond to colonies that were Kan and Amp sensitive. If you do this, be sure to only use colonies that have growth on the LB-only plate, in addition to no growth on the LB-Kan and LB-Amp plates.

Expected results: Most if not all patches should be completely Kanamycin and Ampicillin sensitive.

Day 5: Grow up successful recombinants

- Inoculate 5 ml LB from patches on LB plates that score as sensitive to both antibiotics.
- Grow overnight at 37°C, shaking at 120 rpm.
- Colony PCR for the absence of kanamycin cassette

Day 6: Archive clones

- Make glycerol stocks of the successful clones