Transformation of Agrobacterium

Modified Nov 2013 by David Neece

Preparation of Competent Cells

1. Inoculate 200 ml of LB media with 1 ml of an overnight culture of the chosen strain of *Agrobacterium*. Incubate at 28ºC with vigorous agitation. Start the culture in the late afternoon to be harvested the following morning.

2. Grow the cells to log phase (OD_{550} 0.5-0.8).

3. Pellet the cells in a benchtop centrifuge at 5000 x g for 10 minutes at room temperature. (*Agrobacterium* takes longer to pellet than *E. coli*).

4. Wash the pellet with sterile 1X TE.

5. Resuspend the cells in 0.1X the original volume of LB, and aliquot 250µl fractions in microcentrifuge tubes.

6. Snap-freeze in liquid nitrogen and store at -70ºC.

Transformation

7. Thaw competent *Agrobacterium* on ice (use 250 µl per transformation reaction), and add DNA (up to 10 µl, 100-1000ng) and flick tube gently to mix.

8. Keep the mixture on ice for 5 minutes, and then transfer to liquid nitrogen for 5 minutes.

9. Incubate the mixture for an additional 5 minutes in a 37ºC water bath.

10. Transfer 250 µl of cells to a 15 ml Corning tube containing 1 ml of LB and shake at 28ºC for 2 hours.

11. Collect the cells by spinning 2 min at 5000 rpm, and resuspend cells in 100-200 µl LB. Spread them on two LB agar plates containing the appropriate antibiotic.

12. Incubate the cells for 2 days at 26-28ºC for colonies to form.

Adapted from: Höfgen R., Willmitzer L. (1988) Storage of competent cells for Agrobacterium transformation. Nucleic Acids Res. 16:9877