

## **Agrobacterium freeze thaw transformation** (simplified from doi:10.1101/pdb.prot4666) – Rahul Patharkar

### Preparing competent cells

1. Inoculate 250 mL of LB with a fresh large colony or 100  $\mu$ L of a fresh liquid culture of your desired Agrobacterium strain (i.e. GV3101, EHA105,...). Grow overnight at 28°C, 300 rpm shaking.
2. Pellet the cells at 4500xg for 10 min.
3. Discard the supernatant.
4. Resuspend the pellet in 25 mL of fresh LB.
5. Aliquot 250  $\mu$ L cells in 1.5 mL tubes.
6. Snap freeze the tubes in liquid nitrogen and store at -80°C.

### Transforming Agrobacterium

1. Thaw frozen competent cells in your hands until they are almost completely thawed. Place on ice before the last sliver of ice melts.
2. Optionally, you can split the 250  $\mu$ L into two so that you have 125  $\mu$ L of cells per tube.
3. Add 1-5  $\mu$ L of standard miniprep binary plasmid (in my hands 50-100 ng DNA is sufficient to get plenty of colonies). Mix by gently flicking with your finger.
4. Freeze the tube in liquid nitrogen for 10 sec.
5. Place tubes in a 37°C water bath for 5 min.
6. Shake the tubes on their side at 28°C, 300 rpm shaking for 20-60 min.
7. Plate on a LB plate with appropriate antibiotic. Grow in a 28°C incubator for 2 days. You can expect 20-500 colonies for many commonly used binary vectors.

### Notes:

1. When freezing tubes in liquid nitrogen, it is important to put the tubes into liquid nitrogen vertically so that the cap portion of the tube does not initially get submerged in the liquid nitrogen. After the tube is frozen (10 sec.), the tubes can float horizontally in the liquid nitrogen. If the cap portion of the tube is submerged in liquid nitrogen during the first 10 sec. of freezing, a small amount of liquid nitrogen is often able to enter the tube. Tubes containing liquid nitrogen will often pop their cap off once they are removed from liquid nitrogen.

See a video version of this protocol here:

<https://rahulpatharkar.000webhostapp.com/2018/11/transforming-agrobacterium-by-the-freeze-thaw-method>