

Culturing Transformed Bacteria

1. Make and autoclave LB broth, wait for it to cool, so that it does not scald your hand when you touch the side of the flask.
2. Once pleasantly warm, add kanamycin and pipette 3 mLs into each glass culture tube.
3. Fill PCR tubes (1 tube/colony) with 40ul of LB broth
4. Fill a cap of one of the tubes with 10ul of autoclaved water.
5. Pick a colony with a 10ul pipette tip, and then resuspend it in the water in the cap by pipetting up and down.
 - a. Bacteria are easier to resuspend in water than LB, that's why we first resuspend it in water.
6. Repeat with all colonies. Spin down after each strip of 8 is done.
7. When all picked colonies are in the PCR tubes, add 25ul from each into the glass culture tubes. Grown up in the shaker under the water bath. The start/stop button controls the shaking mechanism.
 - a. If your samples don't grow, use the second 25ul in the PCR tube to try a second time.
8. If you are not planning on immediately doing the miniprep, you can spin down the bacteria in eppy tubes, drain off the LB, and then resuspend in some EB buffer.
 - a. The first buffer you use in the miniprep is kept in the fridge at +4 degrees C.