

HARVESTING BACTERIAL COLONIES FROM AGAR PLATES

Adapted from BV's Cookbook, TCH 1/27/02

This protocol is useful for harvesting bacterial colonies from agar plates, as is done for screening bacterial libraries. This is an alternative to scraping bacterial colonies with a razor blade, which can tear out the agar, and is messy because bacteria stick to the blade. This method is also slightly faster per plate, which translates into much faster when doing multiple plates.

1. Plate bacteria on agar plates 16-24 hours prior to harvesting. If you leave the plates in the 37° incubator for greater than 24 hours, this procedure will not work well, as the colonies tend to really stick to the plate. In addition, the agar gets very dehydrated and will require more LB medium for harvesting (see below).
2. Add 3-4 ml of LB medium to each plate. If the plates are really dry, the medium will be absorbed and you will have to put more medium in the plate. It's best to use plates that are not too thick (i.e., don't have too much agar), as less medium will be absorbed.
I make my plates with 15-20 ml of agar/plate.
3. Take a plate and **LIGHTLY** tap it on the surface of the table repeatedly. You will see that 80-90% of the colonies come off the plate into the medium. The first few plates will require more tapping, the latter plates will require much less tapping, because the longer the colonies sit in LB, the easier they come off. With really sticky bugs, you can let the plates sit for several hours before tapping them. Next, swirl the plate GENTLY in a clockwise and then counterclockwise circular motion, WHILE the plate is on the table. This will also help remove more colonies.
4. Tilt the plate so that the medium pools to one end of the plate. Using a transfer pipette, aspirate the medium and squirt it on any colonies that remain stuck to the plate. Then transfer the medium to a tube.
5. Throw away the plate and transfer pipette, and repeat until all plates are done.