

Growing OP50 *E. coli*

1. Using sterile technique, inoculate Luria-Bertani (LB) media with a thawed vial of OP50.
2. Grow at 37°C in a shaker overnight.
3. Use immediately or aliquot into 50mL vials for storage at 4°C for a couple weeks.

NOTE: It is a good idea to make sure your OP50 is not contaminated by test seeding 5-10 plates and waiting a couple days to make sure nothing unwanted grows.

Seeding NGM Plates

1. Using sterile technique, pipette the desired amount of OP50 onto the dried NGM plates. The OP50 bacterial lawn should cover about 1/3 of the total plate surface area. As a general rule, you should use about 1 mL for 10 cm plates, 300 µL for 6 cm plates, and 100 µL for 3.5 cm plates. Try to keep the OP50 in the center of the plate (if the OP50 touches the side of the plate, there is greater risk of contamination).
2. Let the plates dry at room temperature. 6 and 3 cm plates can dry in about 24 hours and 10 cm plates take about 2 days to thoroughly dry. For stickier bacteria, let the plates dry for about 3 days at room temperature.
NOTE: During humid or rainy weather the OP50 generally takes longer to dry.
3. Store plates in the cold room (4°C) in a labeled box with a lid. They should be fine for several weeks.