1. For 500 mL (make in 1 L flask):
   1.5 g NaCl
   8.5 g Agar
   1.25 g Peptone
   dH20 to 500 mL
6. Autoclave on standard 30 min. liquid cycle (note: autoclaved media can be stored and later melted in microwave when needed)
2. Cool in 60˚C water bath
3. For each 500 ml, add:
   a. 0.5 ml of 1M $MgSO_4$
   b. 0.5 ml of 5 mg/mL cholesterol
   c. 12.5 ml of 1M KH_2PO_4 pH 6.0
4. Once cooled to 60˚C, for each 500 mL, add:
   a. 0.5 ml of 1M $CaCl_2$
   b. 0.5 ml of 25 mg/mL carbenicillin (25 µg/ml final)
   c. 2.5 ml of 1M IPTG (5 mM final)
5. Dispense 10 ml into 60 mm petri dishes
6. Cover and let solidify and dry for 24 hours before use
   **note: IPTG is light sensitive. Plates should be protected from light whenever possible

Seeding RNAi Knockdown Plates with RNAi Bacteria

Cultures of RNAi clones
RNAi knockdown plates
P1000 and tips

1. Label RNAi knockdown plates with appropriate identifying information for each clone of interest
2. Add 250 µl of RNAi bacteria culture to the center of each corresponding knockdown plate
3. Allow RNAi bacteria to dry for at least 2 days at room temperature before adding worms of desired developmental stage to the plates
   **note: IPTG in knockdown plates is light sensitive. Plates should be protected from light whenever possible

Performing One-Generation RNAi Knockdown in *C. elegans*

C. elegans strains of interest (ex: N2 for systemic, though not neuronal, RNAi or TU3401 or FJ1300 for neuronal RNAi)
Unseeded standard nematode growth medium (NGM) plates
Seeded RNAi knockdown plates
P200 pipette tip
Halocarbon oil (Sigma: H8898)
Glass coverslip (any)

To maximize consumption of RNAi bacteria, we recommend transferring worms to RNAi knockdown plates using halocarbon oil rather than standard *E. coli* food.

1. Using a P200 pipette tip (without the pipettor), add a small drop of halocarbon oil to a coverslip
2. Transfer L4 or gravid *C. elegans* from their plate to an unseeded standard NGM plate
3. Once worms have crawled away from any transferred *E. coli*, use a small amount of halocarbon oil on the end of the worm pick to transfer worms to the RNAi knockdown plate
4. Store plates wrapped in aluminum foil (IPTG in knockdown plates is light sensitive)
5. Assess offspring for RNAi phenotypes after desired incubation time