**1L of NGM**

(NOTE: even if you are making several liters, just use this same protocol in parallel with several bottles)

1. Add ~500mL ddH20 and a stir bar to a 1L bottle and start a magnetic stirrer.

2. Add each of the following while stirring for ~5 minutes (Note: agar will not dissolve):

 3g NaCl

 17g Agar

 2.5g Peptone

3. Add ddH2O up to 1L.

4. Prepare materials for autoclave sterilization: Wrap pouring tubes (2) in foil and add a small piece of autoclave tape. Fill two 1L bottles with water to autoclave (needed for flushing the pouring tube and pump before and after pouring plates).

5. Add water to a Nalgene autoclave tray to about 1 inch deep. Place bottles in tray and place in autoclave. Place wrapped tubes in autoclave outside of tray. Autoclave for 20 minutes on the **liquid** cycle.

6. While autoclave is running, set up plates near the Pour Boy: make stacks 10 plates high - you will need about 100 plates (10 stacks) per liter.

7. When autoclave is done, cool the bottle of NGM to 55-60C by placing it into a water bath. Don’t let it cool below 55C because it will begin to solidify.

8. Once cooled to 55-60C, use good sterile technique to add the following reagents to the bottle while stirring:

 1ml of 5mg/ml Cholesterol

 1ml of 1M CaCl2

 1ml of 1M MgSO4

 25ml of KP Buffer (pH 6.0)

9. Flush the PourBoy system with 500ml hot sterile water (that you autoclaved with the NGM) and keep the other bottle of autoclaved sterile water on a hot plate for use after pouring.

10. Use PourBoy to pour plates with 10ml per 60x15mm plate. Each liter should be enough for almost 100 plates.

11. Immediately after pouring the last plate, flush the PourBoy with hot sterile autoclaved water that you have kept hot on a stir plate while pouring.

12. Take the tubes off the PourBoy and hang them up to dry. Clean the bottles. Leave the plates on the bench to solidify overnight.

13. The next day, seed plates with a small drop of OP50 bacteria and let the plates sit at room temp on the bench until dry - ~2 days.

**NGM Plates for RNAi (with Ampicillin and IPTG)**

Follow the same procedure for regular NGM plates but add two additional reagents once the autoclaved bottle cools to 55-60C:

 1ml of 100mg/ml Ampicillin

 1ml of 1M IPTG

**Cholesterol (5mg/ml)**

Simply add 0.5 grams of Cholesterol to 100ml of 95% Ethanol (do not autoclave or filter).

**KP Buffer (pH 6.0)**

1. Add about 800ml of ddH2O to a graduated cylinder containing a stir bar. Place on a magnetic stirrer.

2. Add 98.4g KH2PO4 and 48.2g K2HPO4 while the solution is stirring.

3. Add ddH2O up to 1L. Continuing stirring until the solution is clear.

4. Do **not** autoclave. Autoclaving causes it to crash out of solution. Filter sterilize with vacuum pressure.