

# **Growing RNAi bacteria**

## **Reagents Needed:**

- LB agar plates + 25ug/ml carbenicillin + 12.5ug/ml tetracycline
- LB media
- Sterile filtered 25mg/ml carbenicillin
- NGM plates containing 1mM IPTG and 25ug/ml carbenicillin

## **Protocol**

1. Streak HT115 bacteria containing RNAi insert to LB plates containing 25 ug / ml carbenicillin and 12.5 ug / ml tetracycline. Incubate plate at 37 degrees overnight.
2. Pick single colonies and inoculate to 5 ml of LB + 25ug / ml carbenicillin in a 15 ml culture tube
3. Shake overnight at 37 degrees for 12-18h (no longer than 24h)
  - a. If you are just spotting a couple of plates, you can inoculate ~200 ul of culture in the morning and spot it to plates in the afternoon (~8h of growth).
4. Spot culture to RNAi NGM plates (NGM + 1mM IPTG + 25ug/ml carbenicillin)
  - a. 12 wells – 40ul / well
  - b. 24 wells – 25ul / well
  - c. 6cm plates – 200 ul
  - d. 10cm plates – 500ul
5. Incubate at room temperature overnight, preferably in the dark
6. Place worms on plates or store plates at 4 degrees until use (good for up to 1 month)