Reagents and Procedures for Performing RNAi in *C. elegans*

Growing colonies of RNAi bacteria clones from frozen glycerol stocks

We use rectangular single-well plates (Fisher Scientific: 12-565-285) for stamping out glycerol stocks from 96-well RNAi library plates using a 96-pin applicator (Boekel Scientific: 140500), but standard petri dishes can be used if glycerol stocks will be streaked out manually one at a time.

RNAi bacteria growth plate preparation

Ampicillin (Sigma: A9518)
Stored as powder at 4˚C
Prepare 100 mg/ml stock
1 g ampicillin in 10 ml sterile H_2O, pass through syringe filter
Aliquot 500 µl/tube and store at -20˚C
Tetracycline (Sigma: 87218)
Stored as powder at 4˚C
Prepare 1.5 mg/ml in 100% ethanol
Aliquot 500 µl/tube and store at -20˚C
NaCl (Sigma: S9888)
Tryptone (Fisher Scientific: DF0123-17-3)
Yeast extract (Fisher Scientific: DF0127-17-9)
Agar (Fisher Scientific: DF0140-15-4)

1. For 250 mL (prepared in 500 mL flask):
   2.5 g NaCl
   2.5 g Tryptone
   1.25 g Yeast extract
   3.75 g Agar
   dH20 to 250 mL
2. Autoclave on standard 30 min. liquid cycle (note: autoclaved LB-agar can be stored and later melted in microwave when needed)
3. When cool enough to comfortably hold, add:
   a. 125 µL of ampicillin (100 ug/ml stock)
   b. 2.5 mL of tetracycline (1.5 mg/ml stock)
4. Dispense ~24 mL into each rectangular single-well plate
5. Leave uncovered under Bunsen burner for 20-30 min until solidified then store at 4˚C for at least 24 hours before use

Transferring RNAi bacteria from glycerol stocks to RNAi bacteria growth plates

Glycerol stock plate(s) (Horizon Discovery: RCE1181)
96-pin applicator (Boekel Scientific: 140500) or sterile pipette tips if transferring one clone at a time
*dH_2O**
*10% bleach**
*200 proof ethanol**
Bunsen burner
Dry ice
RNAi bacteria growth plate (see above)
Aluminum foil sealing tape (Fisher Scientific: 12-565-398)
15 ml conical tube or lab marker

**not required if using disposable sterile pipette tips instead of 96-pin applicator*

1. Remove glycerol stock RNAi plate from -80˚C freezer and immediately put on dry ice for duration of time spent outside freezer – **do not let the plate thaw**
2. Position RNAi bacteria growth plate underneath lilt Bunsen burner
3. Carefully peel back existing foil from frozen glycerol stock plate – avoid potential cross contamination from any ice crystals
4. Transfer RNAi bacteria from glycerol stock plate to RNAi growth plate. If using a 96-pin applicator follow step 5, if using sterile pipette tips, follow step 6 instead
5. 96-pin applicator:
    a. Submerge tips of 96-pin applicator in container with 200 proof ethanol
    b. Thoroughly sterilize pins in Bunsen burner – allow 15 sec to cool
    c. Place the applicator pins into a 96-well glycerol stock plate and carefully move them in a circular motion to scrape some of the glycerol stock onto the pins
    d. Align the applicator with the with the RNAi growth plate to ensure faithful replication of the 96-well format
    e. Gently place the 96-pin applicator on surface of flat plate and carefully move the pins along the agar surface producing small circular spots – do not puncture the agar surface
    f. Cover the flat plate
    g. Submerge the tips of the applicator pins in 10% bleach for 15 sec
    h. Wash off bleach from applicator pins by submerging tips in dH_2O
    i. Rinse the applicator pins in ethanol, sterilize using the Bunsen burner, and air dry
6. Sterile pipette tip:
    a. Gently scrape the surface of the glycerol stock well using a sterile pipette tip – anything visible on the tip should be sufficient for growth on the new plate
    b. Gently touch the pipette tip to the desired area of the new RNAi bacteria growth plate and make a small circle - do not puncture the agar surface
    c. Dispose of the pipette tip and repeat for any additional bacterial clones, making sure each spot on the new RNAi bacteria growth plate is labeled accordingly
    d. Cover the RNAi growth plate
7. Thoroughly seal foil sealing tape over the 96-well glycerol stock plate by using a conical tube or lab marker to press around the perimeter, along columns and rows between wells, and gently over surface of the plate
8. Set new RNAi bacteria growth plates upside down (lid on bottom) in 37˚C incubator to grow overnight

Growing Cultures of RNAi Clones for Spotting on RNAi NGM Knockdown Plates

96-pin applicator (Boekel Scientific: 140500) or sterile pipette tips if transferring one clone at a time
*dH_2O**
*10% bleach**
*200 proof ethanol**
Bunsen burner
RNAi bacteria growth plate with overnight colonies
Sterile 2,000 µl 96-well assay block (Fisher Scientific: 07-200-700)
LB medium with 50-100 µg/ml ampicillin (LB Amp)
    Ampicillin (Sigma: A9518)
    Stored as powder at 4˚C
    Prepare 100 mg/ml stock
    1 g ampicillin in 10 ml sterile H_2O, pass through syringe filter
    Aliquot 500 µl/tube and store at -20˚C
P1000 and tips
Breathe Easy sealing membrane (Fisher Scientific: 50-550-304)

**not required if using disposable sterile pipette tips instead of 96-pin applicator*

1. Fill all needed wells of assay block with 1 ml LB Amp
2. Transfer RNAi bacteria from RNAi growth plate to liquid LB Amp. If using a 96-pin applicator follow step 3, if using sterile pipette tips, follow step 4 instead
3. 96-pin applicator
    a. Submerge tips of 96-pin applicator in container with 200 proof ethanol
    b. Thoroughly sterilize pins in Bunsen burner – allow 15 sec to cool
    c. Carefully place the applicator pins onto the RNAi bacteria growth plate to pick up a small amount of each colony onto the tip of each pin
    d. Dip the 96-pin applicator pins into the LB Amp in the assay block wells and swirl gently
    e. Submerge the tips of the applicator pins in 10% bleach for 15 sec
    f. Wash off bleach from applicator pins by submerging tips in dH_2O
    g. Rinse the applicator pins in ethanol, sterilize using the Bunsen burner, and air dry
4. Sterile pipette tip:
    a. Gently scrape a small amount of the desired colony from the RNAi growth plate using a sterile pipette tip – anything visible on the tip should be sufficient for growth in the liquid culture
    b. Swirl the pipette tip in the LB Amp assay well
    c. Dispose of the pipette tip and repeat for any additional bacterial clones
5. Cover the RNAi growth plate
6. Seal assay block with Breathe Easy sealing membrane
    a. Remove backing and place on top of assay block.
    b. Seal by pressing around the perimeter, along columns and rows between wells, and gently over surface of the wells of the assay block using a 15 ml conical tube or lab marker
    c. Remove top sheet of the Breathe Easy membrane
7. Incubate assay block overnight in 37˚C incubator (right side up)

Preparing RNAi Knockdown Plates with Carbenicillin and IPTG

Carbenicillin (Sigma: C1389)
Stored as powder at 4˚C
Prepare 25 mg/ml stock
0.25 g ampicillin in 10 ml sterile H_2O, pass through syringe filter
Aliquot 500 µl/tube and store at -20˚C
Isopropyl β-D-1-thiogalactopyranoside (IPTG) (Sigma: I6758)
Stored as powder at 4˚C
Prepare 1M stock in sterile H_2O, pass through syringe filter
Aliquot 500 µl/tube and store at -20˚C
**note: IPTG is light sensitive
NaCl (Sigma: S9888)
Peptone (Fisher Scientific: DF0118-17-0)
Agar (Fisher Scientific: DF0140-15-4)
$MgSO_4$ 1M
55.49 g in 500 ml distilled H_2O, autoclave and prepare 50 ml aliquots
$CaCl_2$ 1M
60.183 g in 500 ml distilled H_2O, autoclave and prepare 50 ml aliquots
KH_2PO_4 pH 6 1M
dissolve 136.8 g KH_2PO_4 in 800 ml distilled H_2O
Add 17.9 g KOH pellets, stir until dissolved
Add distilled H_2O to 1 L
Divide into 2 x 500 ml aliquots, each in 1 L bottle, autoclave
Cholesterol 5 mg/ml
Dissolve in 95% ethanol, store at 4˚C
60 mm Petri dishes (Tritech Research: T3308)

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