Morimoto Lab RNAi Library

Making Glycerol Stocks

Original author unknown. Updated by Renee Brielmann 2019.

There are three forms of RNAi glycerol stocks:

1. 1 mL Cryovial tubes (for general use to pick individual RNAi clones)
2. Standard 96-well plates (for general use to replicate the whole plate)
3. Deep 96-well plates (for back-up only, or to copy to multiple plates)

I. 1 mL Cryovial Glycerol Stocks:

1. Grow the specific RNAi clone you want. This clone could be from someone's private stock, from the Ahringer RNAi Library, from another lab, or you could have made it.
    a. Using a pipette tip, scrape a very small amount (you only need 1 cell to grow the culture) of the source stock and put it in a glass culture tube containing 5mL of LB with 5µL of Ampicillin (100mg/mL).
    b. Grow overnight in the 37°C shaker.
2. Add 500µL of the overnight culture to a labeled Cryovial tube.
3. Add 500µL of 50% *sterilized* glycerol (final glycerol concentration of 25%). Pipette up and down a few times to make sure the culture and glycerol are fully mixed.
4. Store in -80°C freezer.
5. The remaining 4.5mL of the overnight culture can be used for sequencing. Use the QIAprep Spin Miniprep kit to extract and purify the DNA to send off for sequencing. The primers we use are the L4440F and L4440R.
    a. Once the sequence has been confirmed, the tube can go in the Morimoto Lab RNAi Library.

II. Deep 96-Well Plates:

1. Add 250µL of LB/Ampicillin (final Amp concentration: 100µg/mL) to each well of a *sterile* and labeled deep 96-well plate (250µL x 96 wells).
    a. You can use the multi-channel pipet for this.
2. Using a pipet tip, scrape a small amount off the top of the Cryovial glycerol stock and drop it in the corresponding well of the deep 96-well plate.
3. Repeat for all the RNAi clones.
    a. I usually leave the pipet tips in the wells until the end so it is easier to see which wells I have already inoculated.
4. Carefully remove the pipet tips making sure not to drip into any of the other wells.
5. Seal the plate with a foil lid making sure that each well is sealed from the wells around it (to prevent mixing via splashing).
6. Grow overnight in the 37°C shaker.
    a. Tape the deep 96-well plate to the bottom of the shaker to make sure it does not tip.

6. Add 250µL of 50% *sterilized* glycerol (final glycerol concentration of 25%). Pipette up and down a few times to make sure the culture and glycerol are fully mixed.
    a. You can use the multi-channel pipet for this.

III. Standard 96-Well Plates

1. Pipette 50µL of the bacteria/glycerol mixture from the deep 96-well into a labeled standard 96-well plate.
    a. You can use the multi-channel pipet for this.
2. Seal both the standard and deep plates with foil and store both at -80°C in the Morimoto Lab RNAi Library.
3. You can also copy from a standard 96-well plate to a new 96-well plate. Please see the protocol on the website.