

Protocol for 24-well AID-RNAi screen

Recipes:

LB Agar: 10 g Bactotryptone, 5 g yeast extract, 10 g NaCl, 15 g agar per liter. Autoclave, let it cool down to ~55°C before adding antibiotics.

NGM medium: 20 g agar, 2.5 g Bactopeptone, 3.0 g NaCl per liter. Autoclave, let it cool down to ~55°C before adding 1 ml cholesterol (5 mg/ml), 1 ml MgSO₄ (1 M), 1 ml CaCl₂ (1 M), 25 ml potassium phosphate buffer (1 M), antibiotics (ampicillin (100 µg/ml), tetracycline (12.5 µg/ml)) and 1 mM IPTG.

LB: 20 g LB per liter. Autoclave.

Screen outline:

Preparing NUNC plates:

1. Prepare LB agar containing ampicillin (100 µg/ml) and tetracycline (12.5 µg/ml).
2. Add 50 ml to each NUNC Omni Tray Plate (Thermo Scientific, 242811).

Replication of libraries:

3. Flame off twice before use with 100% ethanol and a Bunsen burner to sterilize.
4. Use Hedgehog (96 pin replicator) to replicate libraries (wells should thaw a little).
5. Put the Hedgehog replicator gently on the LB agar.
6. Flame off hedgehog twice before you replicate the next library
7. Incubate overnight at 37°C (95% - 100% of colonies should grow)
(Note: These plates can be stored up to 1 month at 4°C. You can regrow colonies if necessary by incubating the plates again at 37°C)

Preparing RNAi plates (On the same day as the first step of growing bacteria or earlier):

8. Fill 24-well plates with 1.5 ml NGM containing ampicillin (100 µg/ml), tetracycline (12.5 µg/ml) and 1 mM IPTG.
Note: You need four 24-well-plates per 96-well plate

Growing and seeding bacteria:

9. Fill wells of a 96-well plate with 100 µl of LB containing ampicillin (100 µg/ml), tetracycline (12.5 µg/ml).
10. Use hedgehog to inoculate bacteria. Be careful not to touch neighboring colonies. Move hedgehog replicator gently in the 96 wells
11. Flame off hedgehog twice, clean with brush and let dry briefly
12. Repeat for the next plate
13. Inoculate overnight in the 37°C on the shaker (200 RPM, fix plates with tape)
14. Next morning: Add 100 µl of LB Ampicillin (100 µg/ml), tetracycline (12.5 µg/ml) and 1.5 mM IPTG.
Note: Some liquid evaporates overnight. Antibiotics and IPTG concentration might therefore be slightly higher.
15. Incubate for 2 hours at 37°C.
16. Put 50 µl in every well of the 24-well plate. Label plates on the lid and at the bottom! Dry for 60-90 minutes in the sterile bench. Check if all wells are dry.

Seed LSD2096 eggs:

17. Wash off animals from 3-4 10 cm plates twice with M9 and discard.
18. Use a bent tip to scratch off eggs and collect in an Eppendorf tube.
19. Wash plates with 2 ml M9 (0.1% Tween) buffer and transfer to an Eppendorf tube.
20. Spin for 15 seconds at ~4000 rpm and wash 2-3 times until solution is not cloudy anymore with bacteria.
21. Pipette 30-50 eggs per well (it's okay if there is occasionally a hatched animal in a well).
22. Put at 20°C for 48 hours.

Apply auxin:

23. 48 hours later: Add 50 µl of 100 µM auxin (dilute auxin from 40 mM Stock in M9 buffer).
24. Shake plates und put into sterile hood until plates are dry.

Screen:

25. 24 hours later: Check for repression of GFP induction, note down other phenotypes.
26. Check again after 48 or 72 hours.

Verification:

27. Go back to the library (NUNC plates) and pick positive clones.
28. Grow bacteria in 5 ml LB with ampicillin (100 µg/ml), tetracycline (12.5 µg/ml) overnight in 50 ml falcon tube with lid loosely attached with tape. Add 20 ml of fresh LB with ampicillin (100 µg/ml), tetracycline (12.5 µg/ml) and grow for 5 hours. Spin down for 15 minutes at 3000 rpm. Discard liquid, add 5 ml of LB with 1.5 mM IPTG and dissolve pellet.
29. Seed RNAi plates (6cm) with 300 µl and make 25% glycerol stock (2 vials) for future use.
30. Repeat experiment on 6cm plates according to steps 17 – 26.
31. If positive: sequence clone.