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RNAi Library screen V.1

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Protocol status: Working
We use this protocol and it's working

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Keywords: rnai knockdown library, rnai library screen protocol, phenotypic effect

Abstract

Protocol for screening a RNAi knockdown library in for behavioural and phenotypic effects in *C. elegans*

Materials

NGM agar plates
M9 Buffer
Tracking plates (UNIPLATE 650,96,PS,CLEAR:WHAT7701-1651)
Tracking plate lids (LID,POLYSTYRENE,CLEAR,UNIVERSAL PK100 : 512-1093)
96 well culture plates (Life technologies 268200)
Boekel pin replicator (140500 boekel)
Integra VIAFILL
Intrgra VIAFLO
Tips for VIAFLO (12.5 µl GRIPTIP, Sterile, LONG - 5 XYZ Racks of 384 Tips: 6404)

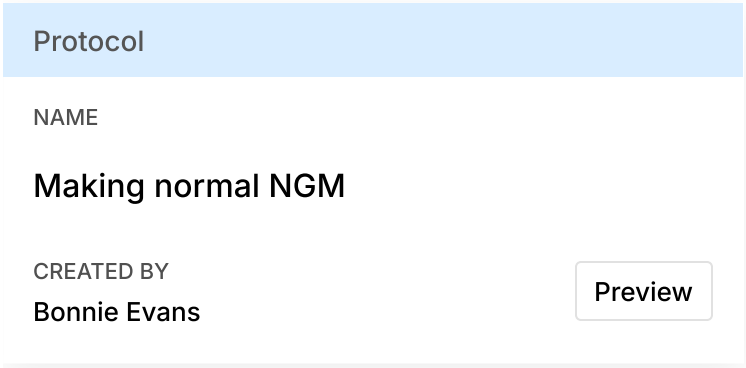
IPTG: 1M stock (dissolved in H20)
Nystatin: 5000x
Carbenicillin: 100 mg/ml (1000x) (dissolved in H20)
Tetracycline: 50mg/ml (5000x) (dissolved in 70% EtOH)
FUDR: 1g/10 ml (1000x) (dissolved in H20)

Troubleshooting

Perparing *C. elegans*

1 **Prepare in advance**
Prepare ten 90 mm NGM agar plates for each replicate of the screen for worm maintenance.

To prepare NGM follow the steps in the protocol for "Making normal NGM" and pour 35 ml per plate.



2 Seed each NGM agar plate with 1 ml of OP50 bacteria, and allow to dry.

3 **11 days before tracking**
Chunk worms onto ten seeded 90 mm NGM agar plates.

Prepare tracking plates

4 **11 days before tracking**
Prepare 75 tracking plates (WHAT7701-1651, PK100 : 512-1093) containing NGM agar, prepared as above, with the addition of 1 mM IPTG, 100 µg/ml carbenicillin, 10 µg/ml tetracycline, 1x nystatin (60 Units/ml).
Dispense 200 µl per well using the protocol "Dispensing agar into multiwell plates".

Protocol

NAME

Dispensing agar into multiwell plates

CREATED BY

Ida Barlow

Preview

4.1 Dry the tracking plates in a drying oven (with lids removed) until they lose 2.5 to 3.5% of their total weight.

Bleach synchronising *C. elegans*

5 **8 days before tracking**
Bleach synchronise worms from ten 90 mm plates according to the protocol "Bleach synchronisation of *C. elegans*".
Leave the eggs in M9 buffer in 15 ml faclon tubes on a rotator at 20 dgerees for two days.

Protocol

NAME

Bleach synchronisation of C. elegans

CREATED BY

Ida Barlow

Preview

Grow up RNAi library

6 We are using a version of the Ahringer RNAi library containing 6198 C. elegans genes with orthologues in humans.
This library is stored as frozen glycerol stocks in seventy three 96 well plates.

8 days before tracking
Grow up the RNAi library.

Citation

Hernando-Rodríguez, B., Erinjeri, A.P., Rodríguez-Palero, M.J. et al. (2018)
. Combined flow cytometry and high-throughput image analysis for the study of essential genes in Caenorhabditis elegans. .
BMC Biol .

https://doi.org/10.1186/s12915-018-0496-5 LINK

Citation

Kamath, R., Fraser, A., Dong, Y. et al. (2003)
. Systematic functional analysis of the Caenorhabditis elegans genome using RNAi.
Nature.

https://doi.org/10.1038/nature01278 LINK

7 Dispense LB broth (containing 100 µg/ml Carbenicillin, and 10 µg/ml Tetracycline), into seventy four 96 well plates (numbered 1-74) using the Integra VIAFILL.

8 Replicate all wells of the RNAi library into corresponsing plates of LB (with carbenicillin and tetrecycline).

8.1 Use a metal 96 pin replicator (Boekel) to transfer glycerol stock from stock plates to the LB culture plates.

To do this remove plates a few at a time from the -80 on dry ice.
Allow each plate around 1 minute to deforst slightly.
Firmly press the replicator into the stock plate, making sure to pick up bacteria from each well.
Transfer replicator to corresponding LB culture plate.

8.2 Between replicating each plate, the replicator is dipped in 100% ethanol and flame sterilised. This process is repeated twice to ensure full sterilisation.

One negative control plate is inncoluated after double flame sterilisation of the replicator.

Safety information

Take caution when flame sterilising the replicator.
Do not place flaming replicator back into the ethanol bath.
Work in a clear area - remove any flammable items, papers, tissue, card board boxes etc
Familiarise yourself with nearest safety blanket.

9 Place the LB plates containing the replicated library into large plastic boxes containing moistened paper towel, and incubate for 15 hours at 37 degrees.

10 Record OD600 values of 10 of the library plates using a plate reader (Tecan, Spark) to evaluate library growth.
Visually check the whole library and manually record any wells which have not grown.

Seeding tracking plates

11 **7 days before tracking**
Seed the tracking plates with the bacterial cultures using the Integra VIAFLO.

Using the Integra VIAFLO with GRIPTIPs mix each well of the library by pipetting 12.5 µl up and down 6 times, before transferring 10 µl from the library to the tracking plates twice.

Note

This double transfer is required to pipette a total of 20 µl of bacterial culture to each well of the tracking plates since the max volume of this VIAFLO is 12.5 µl.

12 Dry the tracking plates in a biosafety cabinet with lids removed for 3 hours.

13 Stack the tracking plates plates (upside down) in a sealed plastic box containing damp paper towel and incubate at 20 degrees.

Dispensing *C. elegans to tracking plates*

14 **6 days before tracking**
Dispense *C. elegans* onto the tracking plates using the protocol "Dispensing C. elegans to 96 well tracking plate using Integra VIAFILL".

We aimed to dispense an average of 4 worms per well, but will have some unavoidable variation between wells.

Note

This step should be carried out at the same time for each replicate, such that FUDR can be added 53 hours after dispensing worms.

For example: We dispensed worms at 4pm on Wednesday and add FUDR at 9 am on the following Friday.

Protocol

NAME

Dispensing C. elegans to 96 well tracking plate using Integra VIAFILL

CREATED BY

e.warren Warren

Preview

15 Leave plates to dry for 1 hour in a biosafety cabinet with lids removed.

16 Stack the tracking plates plates (upside down) in a sealed plastic box containing damp paper towel and incubate at 20 degrees.

Addition of FUDR

17 **4 days before tracking**,

53 hours after dispensing worms, add FUDR to each well of the tracking plates to prevent hatching of progeny.

DIspense 10 µl of 20X FUDR to each well using the Integra VIAFILL with the small 8 channel cassette.

18 Leave plates to dry for 1 hour in a biosafety cabinet with lids removed.

Note

Check that plates are visibly dry.

19 Stack the tracking plates plates (upside down) in a sealed plastic box containing damp paper towel and incubate at 20 degrees.

Tracking using Hydra rigs

20 **On the day of tracking**

Move tracking plates into the tracking room at 12 pm, one hour before tracking commences to aclimatise to temerature and humidity.

21 Record worm behaviour using the syngenta script (5 mins prestim; 6 mins bluelight with 60 sec OFF, [10sec ON, 90sec OFF] x 3 times; 5 mins postsim).

21.1 Run the following script
~/scripts$ python run_syngenta_experiment_v2.py -f 291122_rnaifullscreen_run1 – r 01 02 03 04 05

-f : date_file_ run number :
-r : specifies which rigs to start recording on

Note

Do not use blank spaces in file name
Include the run number for each run of tracking

22 Move all files to the NAS and then transfer files from the NAS to the group drive on the network for processing.

Citations

Step 6

Hernando-Rodríguez, B., Erinjeri, A.P., Rodríguez-Palero, M.J. et al.. Combined flow cytometry and high-throughput image analysis for the study of essential genes in Caenorhabditis elegans.
https://doi.org/10.1186/s12915-018-0496-5

Step 6

Kamath, R., Fraser, A., Dong, Y. et al.. Systematic functional analysis of the Caenorhabditis elegans genome using RNAi
https://doi.org/10.1038/nature01278