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Working

Creating a more isogenic strain of worms starting from parental strain

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ABSTRACT

This protocol describes how to create a more isogenic strain starting from a parental strain.

MATERIALS

NAME	CATALOG #	VENDOR
15 ml sterile falcon tubes and rack		
Sterile conditions (e.g. laminar flow or a flame)		
cryopreservation tube	View	
2x freezing buffer	View	
bleaching solution for C. elegans	View	

STEPS MATERIALS

NAME	CATALOG #	VENDOR
bleaching solution for C. elegans	View	
cryovial	View	

SAFETY WARNINGS

A Bunsen burner will be used to work in sterile conditions.

1 Pick a single worm into a new fresh agar NGM plate. Let it move away from the point of deposition. Pick it again into a new fresh plate. This procedure of first picking into a plate and then into another makes sure that no other worms or eggs are co-picked with the single worm of interest.

bench-top dissecting light microscope

fillin ?

Bunsen burner

? ?

2 Let the single worm populate the whole plate. This will likely take a few days.

3 When the plate is full of worms, chunk the agar into two or more plates. This strain is now called differently, for example starting with SX, the code given to the Miska lab. Alternatively, you can repeat steps 1 and 2 to increase homozygosity.

4 Freeze one of the plates. Always freeze vials. White plug in one of the 5 coloured caps. New strain from single worm is called SX.... . SX is the Miska lab designation in the world. If you are not working in the Miska lab, your new strain name will start with a different lab code.

5 Once a plate is full of gravid adults, bleach it and synchronise overnight.

6 Take the arrested L1s and pour them onto new plates. Grow them until they just start laying eggs.

7 Bleach a plate of synchronised gravid adults that have just started laying eggs. This will maximise the number of L1s (who are the stage that best survives cryopreservation and with a high potential of giving birth to progeny). Synchronise overnight in 2.5 ml M9.

bleaching solution for C. elegans
View

8

cryovial
View

Check that you have many thrashing L1s. Add 2.5 ml freezing buffer 2x and aliquot 1 ml into each of 5 cryopreservation tubes. Transfer the vials into a polystyrene box. Tape the polystyrene box shut. Put the polystyrene box in the -80 C freezer. The box allows the temperature decrease to be slower. The day after, put test tube in tap water for 5 min. Pour on plate and check that worms are alive after a few hours. Put other tubes in inbox (4 tubes) and email lab manager. New strain SX.... is ready. One tube will be put in liquid nitrogen for long term storage. Three tubes will be put in the -80 C freezer for mid term storage.