

Lifespan Assay

Reagents needed:

Lifespan assay using loss-of-function mutants

- **6-cm NGM/FUDR plates** - standard NGM recipe + 50µg/ml flurodeoxyuridine (FUDR - inhibits growth of progeny) seeded with a 50µl spot of OP50

Lifespan assay using RNAi

- **6cm NGM/FUDR RNAi plates** - standard NGM recipe + 50µg/ml FUDR + 1mM IPTG + 25µg/ml carbenicillin) seeded with a 50µl spot of appropriate RNAi bacteria
- **6cm NGM RNAi plates** - standard NGM recipe + 1mM IPTG + 25µg/ml carbenicillin) seeded with a 50µl spot of appropriate RNAi bacteria. NO FUDR!!

Controls

- *daf-2(e1370)* or *daf-2(RNAi)* - long lifespan
- *daf-16(mgDf50)* or *daf-16(RNAi)* - short lifespan
- Empty vector
- RNAi control - *unc-22(RNAi)* - causes a “twitcher” phenotype.

Protocol

1. Per genotype / RNAi strain, you will need 1 -FUDR plates and at least 8 + FUDR plates.
- 2a. **Loss-of-function alleles**
 - Seed hypochlorite isolated eggs onto a standard NGM OP50 for 2 days at 20°.
 - On the 2nd or 3rd day, pick 50-75 L4s to NGM + FUDR plates
- 2b. **RNAi knockdown**
 - Seed hypochlorite isolated eggs onto a standard NGM RNAi plate for 2 days at 20°.
 - On the 2nd or 3rd day, pick 50-75 L4s to NGM + FUDR + IPTG/CARB plates
3. Score daily for live, dead, and ‘censored’ worms.
 - “Dead” = no visible movement, even in response to light touch with a pick.
 - “Censored” - Animals that crawl up the sides of plates and die or expire from a “bag of worms” (progeny hatching inside mother). Data from censored events will be included up to the time of censoring
4. Repeat daily until all animals are dead
5. Record survival data using GraphPad prism and plot survival curves in Origin

Notes

1. Do **NOT** put animals on FUDR plates until they are L4 or older. The FUDR will stop their growth.
2. **WHAT DOES DEAD MEAN??** To determine this, GENTLY touch the animal on the tail and look for movement. If no movement is noted, touch the mid-body

and look again. Finally, if no movement is noted, gently touch the head and look. The worms don't have to dance around; they just need to show some type of response. Worms that don't respond are dead.

3. **What is a censored animal?** Animals that crawl off the plate, desiccate on the side of the plate, extrude internal organs, die from internally hatched progeny ('bag-of-worms' phenotype), or that are simply lost are censored.

Lifespan assay worksheet

Date: _____

Mean Survival (+/- SE) _____

Genotype: _____

Experimenter: _____

Median Survival _____

Temperature: _____

Day	Live	Dead	Censored
1			
2			
3			
4			
5			
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