

LIFESPAN ASSAY

Materials and Reagents:

Plates: standard NGM plates with 5' flurodeoxyuridine (FUDR, to inhibit progeny growth)

Make normal NGM media. Dissolve the contents of one vial of FUDR in dH₂O (3mL) and filter sterilize. Add to medium at the same time as Ca, Mg, etc. after autoclaving. Final concentration is 5mg/mL.

Procedure:

- 1) Isolate synchronized embryos by bleaching adults on NORMAL NGM PLATES (no FUDR, otherwise the animals will not grow up). Incubate plates for 2 days at the desired temperature.
- 2) On the third day, pick adult animals onto seeded NGM/FUDR plates.
- 3) Score daily or every other day for live/dead worms. Animals are dead if the pharynx does not pump and they do not respond to prodding with a pick. Place a plate lid with a grid drawn on it underneath the plate that you are scoring to keep track of your position and avoid missing or double-counting worms.
- 4) Repeat until all animals are dead.

Tips/Troubleshooting

- 1) Temperature: lifespan assays are typically carried out at 20°C or 25°C. Mean adult lifespan at 20 is ~ 23-24 days. At 25, the average is ~14 days, so 25 is quicker, but be sure that any mutants you might be using are viable and behave normally at 25.
- 2) Plates: Any size plate can be used, however, 3cm work best in my experience because the worms are concentrated in a smaller area and are, therefore, easier to find. Up to 50-60 animals can be grown on a 3cm plate without exhausting food too quickly. For longer experiments, worms may need to be transferred to new plates every 7-10 days. If you transfer animals from one condition, you MUST transfer animals from all of the other plates in the experiment to be consistent.
- 3) Is that worm dead?: Seems simple, but is not always, especially in older worms. Because smacking the worm to see whether it moves likely has a negative impact on lifespan, be gentle. Also, try touching the agar right next to the worm first, as this will often get the animal to move without having to touch it. Failing that, touch the tail or mid-section of the worm. If that doesn't work, then go to the

head. Touching the very tip of the head will sometimes result in contraction of the nose in an animal that otherwise looks very dead. Remember, the worm doesn't have to get up and do a jig, it just has to respond.

References

1) Genetics 141: 1399-1406 1995

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