C. elegans Osmotic Stress Resistance Assay

Reagents Needed:

M9 (common stock)
500mM NaCl NGM plates (unseeded)

Procedure:

1. Pour a thin layer of M9 onto an NGM plate containing *non-starved* worms. Gently swirl the plate to dislodge the worms.
2. With a glass pipet, collect the M9 and worms from the plate into microcentrifuge tubes.
3. Centrifuge the tubes at 2000 rpm for 1 minute. A pellet of worms will form.
4. With a glass pipet, place the pellet of worms into the center of a 500mM NaCl NGM plate. *Suck up as little liquid as possible*!
5. Use filter paper to remove any excess liquid from the NGM plate.
   It is important that you make sure all the excess liquid is gone, or the osmolarity of the plate will change.
6. Start a timer immediately after all the liquid is gone.
7. Score movement (# moving/total) as needed. Generally, movement is recorded at 3, 5, 7, 9, and 11 minutes. N2 worms should dehydrate and become paralyzed by 7 minutes after the start of the assay. Mutants resistant to osmotic stress will swim longer.

Recipes:

M9 (1L)
* Common lab stock in worm room.
5.8g $Na_2HPO_4 \cdot 7H_2O$
3.0g KH_2PO_4
5.0g NaCl
0.25g $MgSO_4 \cdot 7H_2O$
ddH_2O to 1L
• Filter (0.22µm) and bottle.

500mM NaCl NGM
Follow the standard NGM protocol, but instead of adding 3.0g/L of NaCl, add 29.22g/L of NaCl.
Mark plates with a green stripe to distinguish from normal NGM plates.

Reference:

This assay was developed in the Morimoto Laboratory.

Solomon A, Bandhakavi S, Jabbar S, Shah R, Beitel GJ, Morimoto RI. 2004. *Caenorhabditis elegans* OSR-1 Regulates Behavioral and Physiological Responses to Hyperosmotic Environments. Genetics, 167: 161-170.