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$_2$HPO$_4$•7H$_2$O
3.0g KH$_2$PO$_4$
5.0g NaCl
0.25g MgSO$_4$•7H$_2$O
ddH$_2$O 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.