

## *C. elegans* Osmotic Avoidance Assay

### **Reagents Needed:**

unseeded NGM plates  
4M NaCl/Trypan blue solution

### **Procedure:**

1. Draw circle 2cm in diameter on the cover of a 6cm NGM plate.
2. Place the cover with the circle on it under an unseeded NGM plate
3. Transfer approximately 50mL of the NaCl/Trypan blue solution onto the NGM plate using the 2cm diameter circle as a guide.
4. Allow the ring to be absorbed into the NGM. The trypan blue will allow you to see the salt ring once it has been absorbed. It should take no longer than 5 minutes for the ring to dry.
5. Once the ring is dry, pick 20-30 worms onto center of the plate and ring you just pipetted. Try not to transfer any OP50 onto the unseeded plate, as it could interfere with your experiment.
6. Once the worms have been transferred to the plate, start a timer and score the number of worms that cross the ring at whatever times you desire. N2 worms generally will not cross the ring at all, while *osm* mutants, for example, will cross it without hesitation.
7. The osmolarity of the ring decreases constantly throughout the experiment.  
*Do not take data for more than 10 minutes after the ring has been absorbed, because by this time the osmolarity of the ring will have decreased significantly. It will no longer repel N2 worms once a certain osmolarity is reached, and the plate will be useless.*

### **Recipes:**

#### 4M NaCl/Trypan Blue Solution (110mL)

- Add 2.34g NaCl to 10mL Trypan blue.
- Allow to dissolve.
- Add to 100mL 4M NaCl
- Mix well.

### **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.