C. elegans Slow-killing assay (SKA) Large Plate Selection Protocol

by Erik Andersen June 22, 2010

Assay timing (each step is described below)

Day 1: Streak out PA14 from frozen stock.
Day 2: Pour SKA plates, inoculate PA14 to LB (at 2 PM)
Day 3: Spot PA14 to center of SKA plate, spread to cover 75% of plate, and put plates at 37ºC
Day 4: Take plates out of 37ºC (at 2 PM)
Day 5: Put 1000-2000 L4s on each SKA plate
Days 9: Collect worms alive after 100 hours on SKA plates

Plate preparation

1. Prepare SKA plate medium and autoclave

	250 mL	500 mL	1 L
NaCl	0.75 g	1.5 g	3.0 g
BactoAgar	4.25 g	8.5 g	17 g
Peptone	0.875 g	1.75 g	3.5 g
Sterile water	243.75 mL	487.5 mL	975 mL

Autoclave, allow to cool to 55 ºC and add the following

Cholesterol (5 mg/mL in EtOH)	0.25 mL	0.5 mL	1 mL
1 M $CaCl_2$	0.25 mL	0.5 mL	1 mL
1 M $MgSO_4$	0.25 mL	0.5 mL	1 mL
1 M KH_2PO_4 (pH 6)	6.25 mL	12.5 mL	25 mL
FUDR, filter sterile (100 mg/mL)	125 μL	250 μL	500 μL

2. Pour 25 mL per 10 cm plate. Flame tops of plates to remove bubbles, if needed.
3. Inoculate 100 ml LB with *Pseudomonas ariginosa* (strain PA14) from a freshly streaked plate.
4. Grow at 37ºC overnight for 24 hours.
5. Spot 800 μL of PA14 onto each plate, spread to cover 75% of plate, and put the plates at 37ºC for 24 hours in a closed box. Be careful to not scratch the tops of the plates.
6. Remove the plates from 37ºC and keep at room temp for 24 hours. Use plates immediately.

Slow-killing Assay Large Plate Selection

1. Put 1000-2000 L4 hermaphrodites onto each SKA plate.
2. Put the plates at 25ºC.
3. At 96 hours, collect the living worms. How?
4. Isolate DNA by lysis
5. Make libraries
6. Run sequence
7. Look for enrichment of genomic regions in neighborhood of *npr-1*.