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

by Erik Andersen (June 2010)

The end of this assay seems a little unfinished since there is a "How?" written in. Plus, should we be more explicit about DNA isolation, making libraries, etc.?

1. Streak out *Pseudomonas aeruginosa* (strain PA14) from frozen stock.(Day 1)

2. Prepare SKA plate medium and autoclave (Day 2)

	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. (Day 2)

3. Inoculate 100 ml LB with *Pseudomonas ariginosa* (strain PA14) from a freshly streaked plate and grow overnight at 37ºC for 24 hours. Because you need to grow the bacteria for 24 hours, you might want to inoculate earlier in the day. (Day 2)

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. (Day 3)

6. Remove the plates from 37ºC and keep at room temp for 24 hours. Use plates immediately. after the 24 hour room temperature incubation (Day 4)

7. Put 1000-2000 L4 hermaphrodites onto each SKA plate and put the plates at 25ºC. ***Do you want to include an instructions about generating that many L4s?*** (Day 5)

8. At 96 hours, collect the living worms. How? (Day 9)
4. Isolate DNA by lysis
5. Make libraries
6. Run sequence
7. Look for enrichment of genomic regions in neighborhood of *npr-1*.