

***Bacillus thuringiensis* toxin plate assay**
By Aroian lab, adapted by Erik Andersen February 7, 2009

1. Pour Bt toxin 6 cm. plates.
2. Inoculate one colony of *E. coli* containing the toxin expression plasmid and the control plasmid to separate 3 mL cultures of LB supplemented with ampicillin (75 $\mu\text{g}/\text{mL}$).
3. Grow overnight at 37°C.
4. Dilute culture 5 fold and grow for 1 hour at 37°C.
5. Add IPTG to final concentration of 50 μM , grow for 3 hours at 30°C (until O.D.600 of 2.0).
6. Mix toxin and control *E. coli* strains. For resistance mutants, the Aroian lab uses 100% Cry5B expressing *E. coli* or 2.5% Cry21A expressing *E. coli*. For scoring hypersensitive strains, use 10% Cry5B (with OP50). When scoring unknown strains, you should setup a dose-response assay first.
7. Spread 30 μL of mixed bacteria onto 6 cm Bt toxin plates.
8. Grow at 25°C overnight.
9. Setup assay by picking 10-20 L4 larvae to each plate.
10. Score animals 48 to 72 hours later at 20°C. Worms should look “sick” or have a reduced brood size.

Bt toxin plates, per 100 mL

0.3 g	NaCl
1.7 g	Bactoagar
0.5 g	Bactopeptone
0.1 g	yeast extract
97.3 mL	water

Autoclave, cool to 55°C, then add:

0.1 mL	cholesterol (5 mg/mL in ethanol)
0.1 mL	1 M CaCl_2
0.1 mL	1 M MgSO_4
2.5 mL	1 M KH_2PO_4 (pH 6)
0.1 mL	carbenicillin (50 mg/mL 1000X stock)
0.1 mL	IPTG (100 mM 1000X stock)