

## **UV integration of extrachromosomal arrays**

### **Reagents needed**

- UV Cross linker (Stratalinker or similar, in the UPenn Microarray Core Facility, just ask for permission to use)
- Extrachromosomal array with <50% transmission frequency
- 60 x NGM 24 wells with OP50

### **Protocol**

1. Wash a well fed population of transgenic worms off a plate and rinse once with water
2. Place rinsed worms onto a clean NGM plate
3. Dry the plate in the hood for ~10 minutes
4. Take the plate to the UV cross linker
5. Set 'Energy' to '300' (30,000  $\mu\text{J} / \text{cm}^2$ )
6. Remove the lid from the worm plate and place it into the Crosslinker
7. Press 'Start'
8. Wait for the UV light to go off and for the machine to beep (takes ~10 seconds)
9. Pick ~50 L4 transgenic hermaphrodites back to an NGM plate with OP50
10. Let them recover at 20° for 24 hours
11. Pick the 50 P0s from the original plate to 2 new 6cm OP50 plates (25 P0s / plate) - keep the original plate
12. After 24 hours, check the original plate. If the UV mutagenesis was successful, there should be many unhatched eggs on the original plate.
13. Pick 480 transgenic F1s (20 x 24 wells - 1 animal / well)
14. After 3-5 days, pick 2 F2s from each F1 to a 24 well (1 animal / well)
15. Examine the F2s after 3-5 days for lines that exhibit 100% transgenic animals - these are candidate integrants.
16. Freeze this strain ASAP
17. Outcross integrants to N2 males at least 3X and then reisolate homozygous lines

### **Notes**

1. This screen can be performed at any temperature, and strains can be moved between temps to accommodate your schedule.
2. To prevent silencing, freeze stocks of both non-outcrossed and outcrossed integrants as soon as possible