# Integration via Irradiation of C. elegans

As described by Erik Andersen, written by Renée Brielmann

Procedure:

*It is advisable to integrate at least 2-3 independent extachromosomal lines per construct since some arrays integrate more readily than others. The transmission frequency for these strains should be 25-35%.*

1. **Synchronize** transgenic worms by either egg lay or bleaching.
    a. Egg Lay: Pick 10 gravid adult transgenic worms onto each of four 6cm seeded plates (40 worms total). Let the worms lay eggs for 6 hours and then remove the adult worms.
    b. Bleaching: Use a 20% alkaline hypochlorite solution to bleach gravid worms for several minutes or until worms begin to dissolve. Wash eggs at least three times with M9 to remove residual bleach/NaOH. Let eggs hatch overnight in M9 with gentle rocking and then distribute the liquid onto 6cm seeded plates.
2. **Incubate** worms at 20°C for approximately 48 hours until they reach the late L4 stage. Make sure there are at least 250 array positive worms total.
    Note: When your transgenic line is developmentally delayed it will take longer than 48 hours to reach the late L4 stage. As a result, the transgenic worms may be overgrown by the non-transgenic worms, causing the plates to starve before they are ready.
3. **UV irradiate:** Put your plate with at least 250 positive worms in the Stratalinker UV Crosslinker 2400 and irradiate for 300 microjoules/cm2 × 100.
4. **After irradiation**: Pick five array-positive P0s from the irradiated plates to each new 6cm plate. Pick around 50 plates.
5. Wait 4-5 days, **chunk** to one plate per P0 plate.
    Note: Make sure the size of the chunk is big enough to get plenty of transgenic worms – this will depend on the transmission rate but a 1x1cm chunk is fine in most cases.
6. Wait 3-5 days, **chunk** each plate to a new seeded plate.
7. Wait 3-5 days, **chunk** each plate to a new seeded plate.
8. The next day, **single** 3-5 array-positive worms from each 6cm plate onto a 3cm plate (150 – 300 worms total, one worm per 3cm plate)
9. **Score** those plates for 100% transmission.
10. Since gamma irradiation can cause background mutations, it is wise to **outcross** the recovered integrated strains by mating with wild type males several times.
11. **Sequence** for the integrated gene using appropriate primers.

***Materials needed:***

- 200x 6cm plates seeded with OP50 (for picking after irradiation and consecutive chunking).
- Approx. 300 small plates seeded with OP50 to single out at the end.