

Gamma Irradiation of *C. elegans*
(to integrate extrachromosomal transgenes)

Procedure:

It is advisable to integrate at least 2-3 independent extrachromosomal 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.

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. Irradiate the worm with 4000rads of gamma rays for 10 minutes.
4. Pick 5 irradiated L4 transgenic worms onto each of 20 10cm *enriched* NGM plates (100 worms total).
5. Incubate worms for 12-14 days at 20°C or until the worms are starved.

Note: After 10 days you can wrap the plates in Parafilm to avoid loss of moisture.
6. Chunk the worms onto 6cm plates and incubate at 20°C for 24-36 hours.
7. Single 20 worms from each plate onto 3cm plates (400 worms total).
8. Incubate at 20°C for 24-36 hours.
9. Identify plates where 100% of worms are transgenic.

Note: A small bacterial lawn can make it easier to screen for transgenic progeny. Try to avoid contamination as it makes it harder to screen.
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.