Integration protocol for *lin-15+* containing arrays into a *lin-15(lf)* background

*Adapted from Mariol et al., 2013*

1. Prepare culture plates:
   1. Prepare about 500 small NGM plates
   2. Seed each plate with OP50
2. Evaluate the transmission rate of the transgenic line to be integrated:
   1. Determine the percentage of muv vs. non-muv progeny for each strain that is being considered for integration
3. Choose a transgenic line for transgene integration:
   1. Select the transgenic line with the highest transmission rate as possible (ideally ≥80%).
4. Obtain a population of transgenic animals synchronized at the L4 larval stage for integration:
   1. Pick 30 non-muv adults onto five culture plates (six animals by plate)
   2. Let the worms lay eggs for 4-5 hrs at 20 °C.
   3. Eliminate the adults from the plates after checking for the presence of a few dozen eggs per plate – wait longer to remove the adults if not enough eggs have been laid.
   4. Culture the animals in a worm incubator at 20 °C until the progeny reaches the L4 larval stage (~48 hrs at 20 °C).
5. UV Irradiation and Recovery of Transgenic Worms
   1. Pick 100 fluorescent transgenic L4 animals onto separate culture plates (15-20 animals by plate).
   2. Place the plates, with the lids removed, upside-down in the UV gel transilluminator in the BMS Core room. Irradiate the plates for 15 seconds with the time starting when the UV light has come on and stopped flickering (as seen through the small window in the door of the transilluminator).
6. Worm recovery after irradiation:
   1. Place plates with irradiated worms overnight at 15 °C for recovery.
   2. Check for the number animals that are alive - a survival rate of around 80-90% suggests efficient irradiation.
   3. Grow the irradiated animals at 20 °C until the progeny has reached adulthood
7. Selection of F1 animals:
   1. Pick single 150-200 non-muv F1 animals onto separate culture plates. For highly transmitting lines (≥80%), 100 F1 animals are enough.
   2. Keep these F1 plates at 20 °C until the progeny reaches adulthood
   3. Discard all F1 plates exhibiting either i) no progeny, indicating that the F1 animal was sterile or died, or ii) no or only few non-muv F2 animals, indicating that the F1 animal did not transmit the transgene at the expected rate.
8. Selection of F2 animals:
   1. Pick non-muv F2 animals from each selected F1 plate. If a fluorescent transgene is being integrated (ex. mCherry or gfp), you should pick F2 worms with a high level of fluorescence as this could indicate that these animals are homozygous for the integrated array.

*\*Note: when picking F2 animals it is critical not to carry along eggs or larvae in order to avoid false negatives in the next step.*

* 1. Grow F2 animals at 20 °C.

1. Isolation and validation of integrated transgenic strains:
   1. Screen F2 plates for 100% non-muv F3 worms. The screen is quite rapid as the presence of a single muv worm indicates that the plate should be thrown away.
   2. Pick four non-muv F3 animals from selected plates to confirm the 100% inheritance of the transgene.
   3. If possible keep several independent integrated transgenic strains, i.e. strains recovered from different F1 animals.
2. Outcross integrated strain
   1. Cross integrated L4’s with wild-type males
   2. Find an F2 isolate that is homozygous for the integration (usually done by finding a plate with 100% fluorescent progeny)
   3. Repeat steps 10.1 and 10.2 two more times

Selectable genetic markers for nematode transgenesis

Rosina Giordano-Santini & Denis Dupuy

Genome Regulation and Evolution, Inserm U869, Université de Bordeaux, Institut Européen de

Chimie et Biologie (IECB), 2, rue Robert Escarpit, 33607 Pessac, France.

**C. elegans transgenesis: how to?**

Stable transformation of C. elegans was first achieved by microinjection of the DNA of interest into the syncytial gonad of the hermaphrodite [3, 28]. Exogenous DNA (plasmid or PCR product) is directly introduced into the cytoplasm of the germline. Then, as individual oocytes separate from the syncytium, it is incorporated into fertilized eggs. The exogenous DNA undergoes intermolecular ligation leading to the formation of tandem repeats of about 80 to 300 plasmid copies, also referred to as multicopy extrachromosomal arrays [3, 28]. These arrays are semi-stable, thus displaying non-Mendelian segregation and leading to mosaic animals. An array that is transmitted to the F2 generation is generally inherited by the following generations with a characteristic transmission rate ranging from 10-90% [28] (Figure 1).