**INTEGRATION BY GAMMA IRRADIATION**

**Materials:**

Worm bleach for small scale synchronization:
- 2 ml bleach
- 2 ml H2O
- 1 pellet NaOH

Solution will keep for approximately 2 weeks.

**Procedure:**

1. Synchronize transgenic worms in order to have 100-150 animals in the late L4 stage. Animals can be bleach synchronized by putting 15 animals in a drop of worm bleach, for 4 plates.
2. Pick 150 transgenic animals to be irradiated with 4000 rads of gamma rays (at 400 rads/min irradiate for 10 min). Or, skip this step and irradiate the entire plate as described in step 3 and pick out late L4s afterwards.
3. Pick animals off irradiated plates – 5 animals/large plate (9cm)- for 20-30 plates. Let animals starve at room temperature for 11 days.
4. Chunk large, starved plates to medium plates (6cm) and let sit for 1-2 days.
5. Pick clones – 1 animal/small plate (3cm). 5-15 small plates per 1 large plate depending on how difficult the integration is expected to be.
6. Wait 2-3 days then check progeny for integration (be sure there are NO non-transgenic animals on the plate to be sure the line is integrated).
7. Backcross newly integrated line with appropriate ‘wild-type’ line to ensure newly integrated lines carry the single, desired mutation.

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