Extrachromosomal array integration protocol (Takao)

1. Generate a stable transgenic line (extrachromosomal array) by DNA injection. I've used *rol-6(d), lin-15, unc-119,* and *dpy-20* as the coinjection marker.

2. Irradiate with gamma ray or X-ray with 3500 ~ 4000 rads (either X-ray or gamma). If the calibration is questionable, worms irradiated with ~7000 rads will die off within 1 or 2 generations, so that can be used as a reference point.

3. Pick 2 irradiated adults per plate to 10cm plates. [I usually pick about 40 adults to 20 plates.]

4. Grow until starvation + 2 days. Usually about 11 days total.

5. Take one small chunk from each large plate and transfer each chunk to a small (5cm) plate. Approximately 100 ~ 500 transgenic larvae should be transferred per chunk.

6. Grow until starved or almost starved. (~ 4 days)

7. From each small plate, pick 8 transgene bearing animals, one worm per plate. Keep track of which worms came from the same original plate, so you can tell which integrants are independent. Pick young larvae if possible. [Typically about 160 plates]

8. ~ 4 days later, check for 100% transmission. Clone ~3 animals from each plate segregating only transgene

containing animals.

9. Confirm 100% transmission in all clones.

All plates are standard NG agar seeded with OP50.

	# large plates	individual worms picked	number of independent integrants	radiation	dose (rads)	coinjection marker
E	16	128	2	X-ray	~4000	*unc-119*
F	18	144	4	X-ray	~4000	*unc-119*
J	19	152	4	X-ray	~4000	*unc-119*
K	19	152	7	X-ray	~4000	*unc-119*
M	14	112	0	X-ray	~6000	*unc-119*
N			died out	X-ray	~8000	*unc-119*

O	8	64	1	X-ray	~6000	*unc-119*
P	12	96	2	X-ray	~4000	*unc-119*
Q	10	80	4	X-ray	~4000	*unc-119*
R	20	160	5	gamma-ray	~3500	*unc-119*
S	20	160	4	gamma-ray	~3500	*dpy-20*
T	19	152	9	gamma-ray	~3500	*unc-119*
V	16	128	4	gamma-ray	~3500	*unc-119*
X	12	96	3	gamma-ray	~3500	*unc-119*