Microinjection Experiment Design:

1. miniMos system

1) Make miniMos vector with transgene chrimson and CoCHR (backbone is pCEH361).

2) Maintain healthy injection strain (TM4063).

Healthy worms are much easier to inject and result in higher insertion frequencies. Maintain unc-119 strains at 22° C.

3) Make injection mix.

The MiniMos system we used:

Chrimson system:

Chrimson plasmid	20ng/ul
Mos transponase	50ng/ul
Peel-1	10ng/ul
Mcherry	5ng/ul

CochR system:

CochR plasmid	20ng/ul
Mos transponase	50ng/ul
Peel-1	10ng/ul
GFP	5ng/ul

4) Inject worms and recover
   After injection, place in 22℃ incubator to recover for 4 hours. Then put plates in high temperature (30℃) incubator for 12hrs to enhance the rate of plasmid getting inside the embryos. Then put worms back to 22℃ incubator to culture.
5) Screen plates
   Observe F1 phenotype after 3 days. Select and transfer the free-moving worms expressed fluorescence of marks to new plates and incubate at 22℃.
6) Single and characterize insertion
   Wait until worms starve (about 6 days later), do the heatshock experiments(34℃, 4hrs). Take out plates and incubate at 22℃ for four hrs. Find the free-moving worms from plates. Single out and keep culturing.

(We used the miniMos vectors with Phsp:peel-1 in the backbone, so it can kill (most) array animals by a two hour heat-shock at 34° C in an air incubator. Array animals are dead approximately 4 hours after heat-shock.)