Month of Practicing:

2016.10-2016.11

We practiced the microinjection skills for MiniMos injection. We injected GFP markers in N2(wild type) *C. elegans.*

2016.11-2016.12

We practiced the microinjection skills for MiniMos injection. We injected GFP markers in N2(wild type) *C. elegans.*

2017.01-2017.02

We practiced the microinjection skills for MiniMos injection. We injected mcherry markers in N2(wild type) *C. elegans.*

2017.02-2017.03

We practiced the microinjection skills for MiniMos injection. We mcherry markers in N2(wild type) *C. elegans.*

After practiced the injection skills, we finally got the worms with GFP and RFP, so we have mastered the injection skills.

Month of Experiments:

Having mastered the injection skills, as well as getting the plasmids, we began our experiments using MiniMos transformation method. After many times' attempts during the February to May, we decided our final plans and do our experiments in these months.

The primary MiniMos system we used:

Chrimson system:

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

CochR system:

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

2017.04.01-2017.04.07: the unc-119 *C. elegans* were injected the chrimson system, we injected about 50 worms. No F1 have insertions.

2017.04.08-2017.04.15: the unc-119 *C. elegans* were injected the chrimson system, we totally injected about 60 worms. No F1 have insertions.

2017.04.16-2017.04.23: the unc-119 *C. elegans* were injected the chrimson system, we totally injected about 50 worms. The results was we obtained 3 F1 worms with free moving and RFP, but after 2 days, all F2 lost the fluorescence, meaning that the vectors were not successfully transformed into the chromosomes.

2017.05.01-2017.05.07: the unc-119 *C. elegans* were injected the chrimson system, we injected about 40 worms. No F1 have insertions.

2017.05.08-2017.05.15: the unc-119 *C. elegans* were injected the chrimson system, we totally injected about 65worms. No F1 have insertions.

2017.05.16-2017.05.23: the unc-119 *C. elegans* were injected the chrimson system, we totally injected about 60 worms. No F1 have insertions.

2017.05.24-2017.05.31: the unc-119 *C. elegans* were injected the chrimson system, we totally injected about 55 worms. We obtained 5 F1 worms with free moving and RFP, but after 2 days, all F2 lost the fluorescence, meaning that the vectors were not successfully transformed into the chromosomes.

2017.06.01-2017.06.07: the unc-119 *C. elegans* were injected the chrimson system, we injected about 50 worms. No F1 have insertions.

2017.06.08-2017.06.15: the unc-119 *C. elegans* were injected the chrimson system, we totally injected about 50 worms. No F1 have insertions.

2017.06.16-2017.06.23: the unc-119 *C. elegans* were injected the chrimson system, we totally injected about 50 worms. The results was that we got 5 free moving worms with RFP and GFP fluroescence, but in F2 and F3 generation, all the offsprings lost their fluroescence.

2017.06.24-2017.06.30: the unc-119 *C. elegans* were injected the chrimson system, we totally injected about 55 worms. But no results.

2017.07.07: we went to HKBU for guidance and assistance on microinjection. We microinjected 40 worms, 20 used Chrimson system and 20 used CochR system. The results showed that in F1, each system had more than 6 worms successfully expressed the plasmid. We obtained the unc-rescued worms with fluorescence.

2017.07.17: 10 days after microinjection, we used heatshock to check the insertion. However, after heatshock(34℃, 4h), all worms injected with Chrimson system died. For worms with CochR system, one plate had more than 30 alive worms, and they all had GFP.

2017.07.24: After heatshock, we singled out the alive worms with CochR system to see whether they obtained the stable phenotype. To check the normal expressions of plasmids, we observed the proteins under the confocal microscope. And the result was the same as the model in the database. In other words, we successfully got the insertion in the worms!
Later on, we did the inverse PCR and the mapping results showed that, the inserted gene is on the first chromosome of *C. elegans*. (This was done in August.)

2017.08.01: we went to HKBU to microinject the Chrimson system. We injected 20 worms.
After 12 days, we did the heatshock and got about 10 worms expressed plasmids without arrays. On 08.21, we obtained worms with stable inheritance.

The final 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

2017.09: We went to the HKBU to microinject worms with Chrimson system. Meanwhile, we also microinject Chrimson system in worms expressed CochR system (This was obtained in August). We also did the mating experiment to combine the two phenotype in one strain.
Results:
1) We successfully got worms expressed mix system (Chrimson + CochR).
2) We got worms expressed two proteins in F2.

Protocol

Protocol A: the synchronization of the C.elegans