Clogmia Protocols:

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Goal of technique: dsRNA silencing in the fly Clogmia albipunctata.

Based on "Chironomus Riparius injection protocol" by Derek Athy and "Megaselia injection protocol" by Chun Wai Kwan and the tips from Jeff Klomp. Written and edited by Adi Bitansky 9.18.14.

Microinjections:

In order to get fresh eggs pull out ovaries right before injections. Put the ovaries in a glass dish filled with distilled water. The eggs are activated by the osmotic shock in the water and start to develop. The injections will be taken place within the first hour/ hour and a half after egg activation, after that the chorion is too hard to penetrate (happens approximately after 2 hours after egg activation). There is no chorion removing at this stage.

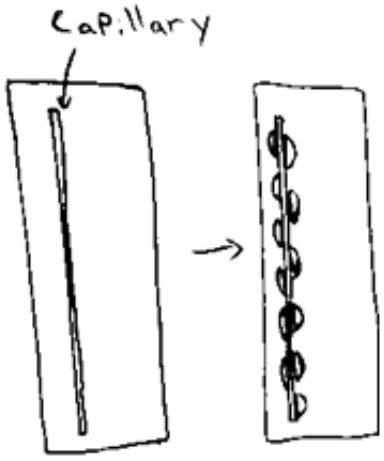
Needle pulling:

Needle pulling parameters: (using the machine P-87 FLAMING/BROWN MICROPIPETTE PULLER)

Cycle1: Heat: 570 pull: 115 velocity: 15 time: 250 pressure: 505
Cycle2: Heat: 595 pull: 130 velocity: 60 time: 250 pressure:505

Aligning:

The embryos were aligned in the injections room, with the temperature of 18◦C.
Use a clean slide for alignment (dry kimwipe will do).



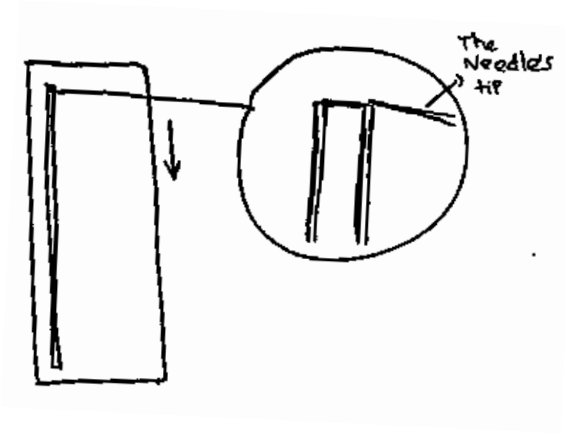
Afterwards, a capillary will be carefully placed on the slide, make sure one side is wider, that side will be for injected embryos, while the thinner side will be for alignment control embryos.
Use a pipette to transfer the embryos to the slide, try to transfer little water as possible. Align the embryos carefully on the slide by using a brush. The eggs are shaped like a bean, It is easier to inject the embryos when the flatter side in on the capillary. After aligning, if there is too much water you can remove them by using a kimwipe. If the embryos are still covered by a thin layer of water (you will see a thin black line surrounding them), Let the embryos dry in the room for 23min. (if the embryos look too dry, like a shrunken bean, you can try to decrease the drying time). If there is no water surrounding the embryos decrease drying time to 21-22 minutes.

**This part is crucial. Drying is important for injections, it prevents the cytoplasm from going up the needle and out of the embryo while injecting. Thus, it is better to dry the embryos as little as possible because drying affects the development of the embryos and might prevent them from developing through their germ band phase and hatch.
It is important to find the right balance.
After drying, cover the embryos with light oil, Halocarbon oil 27.

Breaking the needle's tip:

First, load the needle with substance, if you see bubbles in the needle tap it gently until there are no bubbles. Before injections the tip of the needle needs to be broken in order to let substance flow out.

You can try to break the tip in a few ways, you can gently push the needle against the capillary until the tip breaks and you can get substance to flow out, or (that's the one I used) focus on the capillary so you can see two lines on it, carefully place the needle on the top of the capillary, on the edge of the capillary (as shown in the picture). Make sure the needle is in not under or above the capillary but in the middle. Now, move down the slide (don't move the needle itself) and let the needle rub against the capillary until you can get some substance to come out, be gentle and careful. If nothing happens try again. Make sure you are on the right program and the right parameters on the injections machine before injecting.



Now you can finally inject the embryos. (!!!)

Injections:

While penetrating the membrane expect a little twitching. This twitching can tell you if you are touching the membrane and if you managed to penetrate through it.
If cytoplasm goes up your needle try to increase the balance pressure in the injection machine. If it doesn't help, it might mean that your embryos are not dry enough or that the hole in the tip of your needle it too big.
To prevent the needle from clogging, after every injected embryo, inject some substance out on the slide. If the needle gets clogged try using vent (looks like an arrow on the machine's screen ⟶) and empty the needle from whatever clogged it.
The size of the droplet: (the arrow is the needle) ⟶O
After injections, place the slide in a plastic box layered with a wet kimwipe. Make sure the embryos are in a moisture environment and in a room of 25◦C.

Fixation after injections:

When the embryos reach the wanted stage carefully move them away from the capillary by using a brush.
Collect the wanted embryos on one side of the slide.
Then, go to the fume hood.
Place the slide in a glass dish so the side with the wanted embryos is in it.
Pipette n-Heptane to wash the embryos away from the slide into the dish. Continue until the wanted embryos are all in the dish. (If the embryos stick to the slide use a brush and gently remove them from the slide)
Wash the embryos three times with n-heptane (in order to wash out the oil)
Wash three times with PBT

Chorion removal:

Remove PBT completely.
Add 10% bleach and wait 3:30-4 minutes, until you see at least one empty chorion (use a .
Wash 5 times with distilled water.

Fixing:*

After dechorionating, remove distilled water from dish (leave enough water that will allow you to collect the embryos with a pipette).
Collect the embryos with a pipette.
Add the embryos to a 1.5ml tube with a freshly made fixative 4% (950 µl substance) incubated with light rocking or on wheel (40 minutes).
Heptane and fixative are removed completely and 500 µl fresh n-heptane and 500 µl of methanol (in this order) are added to the embryos.
The tube is shaken vigorously for 30 seconds, most of the embryos sink to the bottom.
Note: if vitelline membrane needs to stay intact simply wash with methanol after fixation. If vitelline membrane continues to cause trouble consider temperature shocking during the first two methanol steps (add -80 MeOH and transfer with lid open to 60°C water bath for 60 seconds).
All solution is removed and the remaining embryos are washed three times with methanol.
Fixed embryos are stored at -20 °C.

* Based on:
Fixation of Blastoderm Stage Embryos for Whole Mount in situ Hybridization
Sean Ferguson, Schmidt-Ott lab protocol (Clogmia fixation)
Kosman, D et al. (2004) Science. 305 (5685):846. (Drosophila fixation)

Recipes:

Fixative 4%:

In 1.5 ml tube

500 µl l n-heptane

400 µl 1x PBS

50 µl 37% formaldehyd