Micro-Injection Protocol for femtoJet 4i https://online-shop.eppendorf.com/eshopdownload/downloadbykey/72717_186

TIMETABLE

Day 1	Plant beans or radish
Day 8	Place 2 adults of the recipient strain on 1 week old seedlings
Day 10	Remove adults from seedlings, keep offspring
Day 15	Microinjection: Transfer haemolymph from donor to 4-5 d old recipient. Place all injected aphids of the same clone and treatment on the same plant. Label it with "donor strain → recipient strain, date"
Day 20 (once nymphs appear)	1st transfer: Place surviving recipients on plants – one per plant. Label with "strain of donor → strain of recipient, A/B/C…"
Day 24	2nd transfer: Transfer surviving recipients onto new plants, keep the offspring from the 1st transfer
Day 27	3rd transfer: Transfer recipients onto new plants, keep the offspring from the 2snd transfer
Day 31	4rd transfer: Transfer recipients onto new plants, keep the offspring from the 3snd transfer
Day 34	If possible, transfer recipients a 5th time and keep the offspring from the 4th transfer.
Day 36	Place 3-4 youngest daughters ("F1") of the recipients singly on plants. Label them "strain of donor → strain of recipient, A1/A2/A3/B1…". Discard all older offspring of the recipient.
Day 40 (once nymphs appear)	Keep the F2 generation offspring and test the F1 adult for endosymbionts. Only keep the offspring of F1 aphids carrying the endosymbiont.
Day 47	Transfer four F2 adults to 1 week old plants
Day 51	After it has produced enough offspring, test the F2 adult for endosymbiont to ensure it is transferred faithfully from mother to daughter

PROTOCOL

1) Remove the flexible injection tube from the femtoJet before switching it on.
2) Switch on the femtoJet with the main power button. The main power button is hidden behind the engine, as on all fancy machines. 
3) Wait until the microinjection pump stops asking you to wait.
4) Set the pressures values. The optimal values are likely to depend on the aphid species, but the following setting seems to work well:
   - Compensation pressure: pc = 0hPa
   - Injection time (ti) = between 0.1s – 0.5 s, or set it such that it ejects as long as you press the pedal.
   - Injection pressure: pi = 60 – 300 hPa.
5) Reconnect the flexible injection tube to the femtoJet.
6) Prepare a needle: The new needle is still closed at the tip. It needs to be cut open with a blade. Make sure that the needle is still fine enough, so cut it more towards the tip and not too far back. The tip of the needle should be thinner than an aphid tibia. Place the needle into the holder:

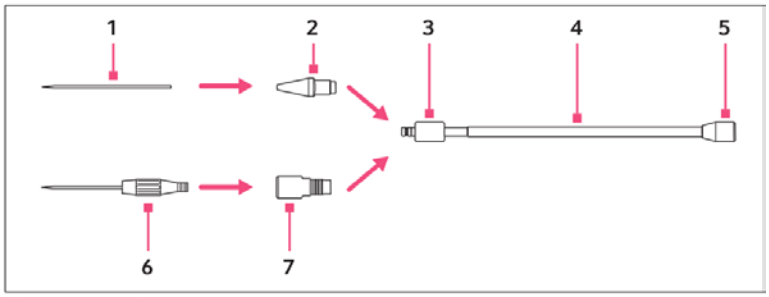
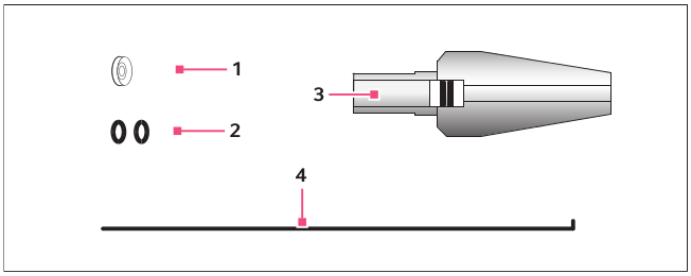


Fig. 3-9: Universal capillary holder

1 Capillary
2 Grip head
3 Knurled screw
4 Universal capillary holder
5 Connection for injection tube
6 Femtotips
7 Adapter for Femtotips

The holder is already assembled and we are not using parts 6 and 7.
- Completely loosen the knurled screw (3)
- Gently push the needle (1) into the transparent grip head (2), until the black rubber O-ring, go through them up to the stop (you will feel it).
- Slightly tighten knurled screw (3)



1 Sealing washer
2 O-rings
3 Grip head with sealing washers and o-rings
4 Removal tool

7) Prepare one eppendorf tube per strain of donors, recipients, and transfected.
8) Prepare the aphids:
   a. Donors (of hemolymph with bacteria): Prepare a microscope slide with double- faced adhesive tape. There is a black microscope slide dedicated to this in the transparent plastic tray dedicated to microinjection material. Choose large adult aphids of your desired clone as donors because they have a lot of hemolymph. Place them ventral side facing up on the double- faced adhesive tape. To make the operation for them a bit easier, you can benumb them in CO_2. But they are aphids, they are often naturally benumbed.
   b. Recipients: choose enough 2nd to 3rd instar larvae and store them in a labelled tube (min. 10 individuals, rather more). Put one recipient individual at a time on a microscope slide, ventral side facing up, but not on the adhesive tape! You need to keep them alive.
   c. In case you are injecting a compound, then you don't have any donor. A convenient way to load the compound into the capillary, is to use a 10µL pipette to load the compound by the back of the needle before setting it into the holder. Then, use the femtoJet with a pressure of approximately 600hPa to push the compound toward the tip of the needle.
9) Injection: stick the needle cautiously into one of the donor aphids. A hemolymph drop will ooze out of the aphid. Hold the needle into this drop. Capillary suction will fill the needle with hemolymph. Sting the recipient aphid immediately, otherwise the needle will clog up. The best place for stinging is close to the hind legs. Hold the needle rather steeply. Because recipients are not settled on the adhesive tape, while you sting them, the best position of the needle is as vertical as possible. Be cautious, put the needle only slightly into the aphid, just beneath the skin. Then press the pedal of the pump and inject the hemolymph into the aphid. Put it into a labelled eppendorf tube, until you have finished the injections.
10) Aphids are full, of sugar, and their hemolymph tend to obstruct the needle. To limit this, rinse the needle into a container with water between each injections, and check that the needle is still open by pressing the pedal in the water. When you can see bubbles the needle is still open. But even with this procedure, it will slowly obstruct. You will thus have to sometime change the needle.
11) Whatever the needle obstruction, for every donor clone, change the needle and the water in the container, and the container! Discard needles into the dedicated petri dish that is into the transparent plastic tray.
12) When the injections are done, clean the place and shut down the whole system:
    Remove the flexible injection tube from the femtoJet. Hold "standby" until the engine exhale the air pressure. Then switch off with the main button (hidden behind the engine).
13) Place all stung aphids of the same clone and treatment on the same bean plant. Label it with an explicit code such as | number of recipient ← number of donor + date of injection|.
14) Most of the nymphs will die. Once the survivors start to give birth, place them singly on plants, and add a letter to their label, to identify them individually. e.g.: A15-172 ← A15-27 10/11/2015 A
15) To be sure which aphids is the "mother" (= the stung aphid), place her on a new plant every 4 to 5 days. Keep the plants with her babies. Do that until the mother dies (this will take 2 – 3 weeks in total). Then, keep her last offspring (3-4 babies) and place them singly on plants. Label them as the mother, but add a number behind the letter. Discard the older offspring that have been laid before. Wait until these aphis start to give birth. As soon as this happens, keep enough of her offspring (grand-daughters of the stung aphid) and check daughters for the endosymbionts presence. If it is positive, keep the grand-daughters, otherwise discard them. To be sure that the endosymbionts is successfully transmitted from generation to generations: check again the great-grand daughters of the stung aphid, before you add them to the clone bank.

If you need to inject a counpound in the aphid (and not simply to transfer hemolymph), you might need to fill the needle with a big amount of counpound.
To do so, you can use the tube you use to manipulate aphids, but without any tip at the end:

Once the needle is set to the capilary holder, a trick is to disconnect the capilary holder from it's tube (position 5 on figure 3-9, 1st page). Then you can connect the conic part of your tube instead of the femtoJet injection tube. This will allow you to soak the needle into the counpond and to suck it up into the needle.