Phallusia mammillata electroporation

Based on S. Darras and C. Hebras protocols

Material needed:

- 1.2M D-mannitol solution
- Nuclease-free water
- Dissecting scope
- FSW and FSW + BSA 0.1%
- GF-coated Petri dishes (Ø 10cm and 20 cm)
- Glass Pasteur pipettes
- Eppendorf tubes*[1]
- Low-binding 1.5 ml microtubes
- BTX ECM830 square electroporator
- 4 mm electroporation cuvettes (Gene Pulser®/MicroPulser™ Electroporation Cuvettes, 0.4 cm gap)

A. DNA preparation:

1. Put D-mannitol*[2] aliquot (1.2M, stored at -20°C) at 70°C on hotplate to defrost and to solubilize it.
2. Mix your DNA (diluted in nuclease-free water) with D-mannitol 1.2M :
   - 50µg DNA diluted in 125 µl of nuclease-free water
   - 125 µl of mannitol 1.2

3. Let it cool down to 18°C *[3]

B. Eggs preparation:

1. Collect eggs and sperm from *P. mammillata*, and dechorionate eggs (see *Protocol for Harvesting Ascidian gametes, Protocol for preparation of Ascidian dechorionated eggs*)
2. Fertilize the eggs: when fertilized (eggs are deformed), start the timer
3. Wash the embryos 3x in FSW to clean up the sperm
4. Transfer the embryos to a Ø10 cm GF-coated petri dish with FSW+BSA 0.1% (0.5g BSA in 500ml FSW)
5. Go to electroporation room
6. Once there, wash once and then fill up the electroporation cuvettes with FSW+BSA 0.1% until electroporation

Notes :

*1. All eppendorf tubes are pre-autoclaved

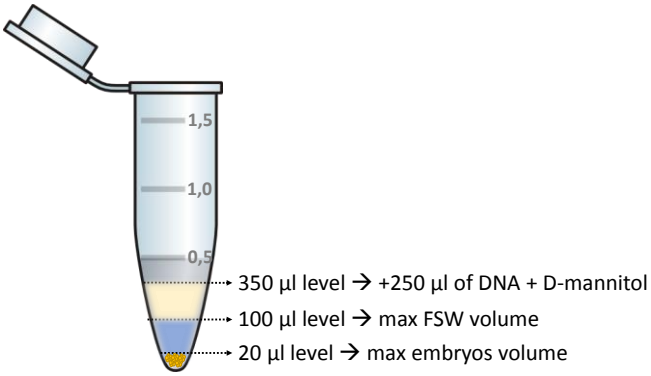
*2. Mannitol is used to maintain the osmotic balance between the environment and the egg

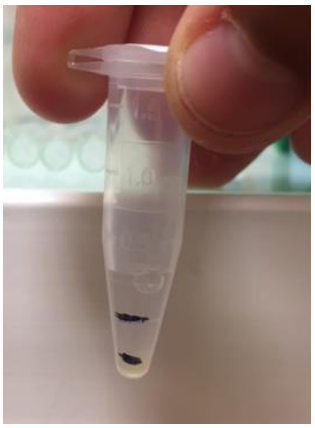
*3. If the temperature outside the labs is higher than 18°C (e.g. in summer), please carry the embryos in a closed box with ice to avoid heat shock! 18°C temperature should be maintained during the all procedure.

C. Electroporation between 45 and 50 minutes post fertilization*[4] (mpf):

1. Wash the FSW+BSA from the electroporation cuvettes
2. With glass Pasteur pipette*[5], carefully*[6] transfer 1 ml of embryos to low-binding Eppendorf (1.5ml)

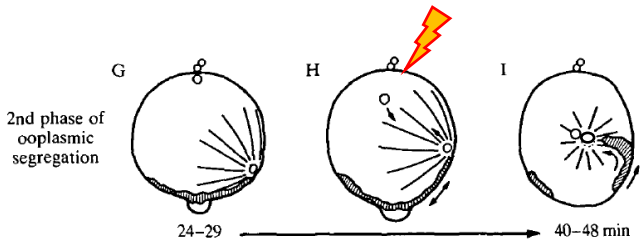
3. Leave the eggs to settle to obtain about 20µl pellet of concentrated embryos*[7]
4. Remove excess water
5. Fill with FSW+BSA up to 100µl level
6. Add the 250µl DNA+Mannitol and mix gently





Notes :

*4.The pulse should be done towards the end of the first interphase (but before entry into first mitosis): at ~45/50min post-fertilization (18°C).Just before electric shock, check carefully the development in the dissecting scope, to be sure about electroporation time

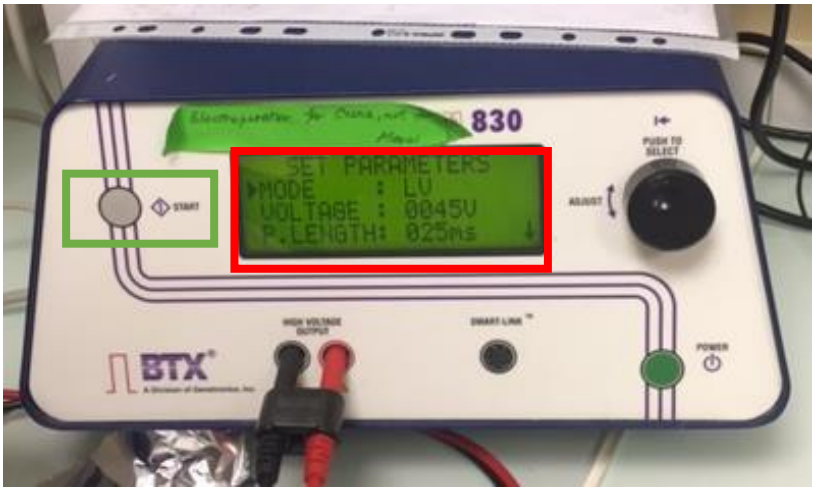


*5. For each DNA to eletroporate, CHANGE the glass Pasteur pipette to avoid contamination!
*6. DO NOT MAKE BUBBLES when pipetting the embryos, or you will kill them all! Proceed very carefully!
*7. Respect the embryo quantity (20 µl), too many embryos will not increase electroporation efficiency

7. Then transfer the embryos+DNA mix to the electroporation cuvette*[8]

8. Keep a GF-coated petri dish with **non-electroporated embryos** to follow development and compare electroporation side-effects

9. Place the cuvette in the electroporator cuvette holder and apply the electric pulse: **45V, 25ms (Voltage /P length)** by pressing on **START** (see electroporation video)



10. Fill the cuvette with FSW+BSA*[9]
11. Transfer the embryos into Ø20 cm GF-coated petri dish.
12. Incubate the embryos at 18°C. Do not touch before 16-cell stage.
13. Leave the embryos to develop until gastrula stage in electroporation room (~6hpf).
14. At gastrula stage Wash 2x embryos from BSA → just transfer them into Ø20 cm GF-coated petri dish with FSW)

Notes:

*8. Each electroporation cuvette can be used for **3-4 times** maximum. Each cuvette is used for a single DNA. Between 2 uses, to wash electroporation cuvette with water and EtOH 70%.

*9 If more than one electroporation is about to be done, do it and fill up the cuvette with FSW+BSA, and proceed for the next electroporations; only at the end you will transfer the embryos to GF-coated dish, otherwise the 2-cell stage arrives and electroporation can no longer be performed!