Last modification: 18 Feb., 2011

Ciona Electroporation protocol:

Prep steps:

1. For small volume electroporations, EtOH precipitate DNA and resuspend directly in mannitol. For normal "800 ul" volumes, DNA stock should be about 5 ug/ul. Use up to 100 ug per 800ul electroporation.
2. Turn on plate chiller
3. Put 4 glass bowls (one with an 80 filter) with once-filtered sea water in 18C tank and let them reach temperature
4. Put gelatin-coated plates on cold plate:
    a. 1 – 35x10mm plate (dechorionation plate)
    b. 4 – 60x15mm plates with 10mL twice-filtered SW (wash plates)
    c. x – 60x15mm plate with 10mL twice-filtered SW, 10uL 1000x pen/strep, 2uL 0.5M EDTA (overnight plates)
5. Set cuvets on cold plate
6. Prepare 100mg of sodium thioglycolate in 10mL of twice-filtered SW (thioglycolic acid is aliquoted in -20C freezer; after using, wrap with parafilm and return to -20C)
7. Add 500uL of 0.77M mannitol to your DNA (250uL for ½ electroporation + 150uL eggs)
    a. Need ~25 ug DNA per 800 ul electroporation, less than this and you get variable expression. You can scale electroporation volumes down to 100 ul, just maintain all ratios of reagents.

Electroporation:

NOTE: time between fertilization to electroporation must be ~20 minutes or less

1. Fertilize eggs for 2 minutes (induce fertilization by swirling eggs and sperm using scissors or pipette tip). During the 2 minutes:
    a. Thaw protease in 4C (protease is in -80C bottom drawer)
    b. Add 28uL of 10M NaOH to thioglycolate/SW solution (to achieve proper pH)
    c. Add ~3mL of thioglycolate/SW solution to dechorionation plate
2. Filter sperm through rinse bowls 3X using 80 filter
3. Concentrate embryos in filter and add to dechorionation plate using Pasteur pipets (continue using Pasteur pipets for all remaining transfers)
4. Remove as much thioglycolate solution from dechorionation plate without disturbing the embryos, and add most of the remaining 5mL thioglycolate solution (leave some solution in tube for next step).
5. Add whatever is left in thioglycolate tube to protease solution, mix gently, then add the mixture to the dechorionation plate.
6. Periodically pipet embryos for the next 5-7 minutes
    a. Embryos will go through dechorionation, changing color from brown (fertilized) -> yellow (chorion) -> brown (dechorionated)
7. Once dechorionated, concentrate embryos into middle of dish by swirling
8. Transfer embryos to successive wash plates 4X using Pasteur pipets
9. Concentrate embryos in last wash plate by swirling
10. Add correct volume of embryos to DNA in microcentrifuge tube.
11. Transfer embryos + DNA mixture to cuvet
12. Electroporate
13. Disperse embryos in appropriate overnight dish.