Aug 03, 2022

Injection of algae into Ambystomid (salamander) embryo

John Burns[1], Hui Yang[2], Joost S Mansour[3]

[1]Bigelow Laboratory for Ocean Sciences; [2]University of Arizona, Molecular and Cellular Biology; [3]University of Arizona

dx.doi.org/10.17504/protocols.io.5jyl89y17v2w/v1

Symbiosis Model Systems
Tech. support email: **adam.jones@moore.org**

Joost Mansour
University of Arizona

DISCLAIMER

ABSTRACT

This protocol describes the steps taken to artificially introduce algal cells into salamander tissues by microinjection for the study of salamander-alga endosymbiosis. Microinjection allows experimental manipulation of the separate partners before the establishment of symbiosis. For instance, algae can be grown with a heavy nitrogen (^{15}N) or carbon (^{13}C) label prior to injections to trace nutrient transfer.

DOI

dx.doi.org/10.17504/protocols.io.5jyl89y17v2w/v1

Citation: John Burns, Hui Yang, Joost S Mansour Injection of algae intoÃÂ Ambystomid (salamander) embryo https://dx.doi.org/10.17504/protocols.io.5jyl89y17v2w/v1

PROTOCOL CITATION

John Burns, Hui Yang, Joost S Mansour 2022. Injection of algae into Ambystomid (salamander) embryo. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl89y17v2w/v1

FUNDERS ACKNOWLEDGEMENT

Moore Foundation - Solar Salamanders? Algal Invasion of a Vertebrate
Grant ID: GBMF5604

KEYWORDS

micro injection, alga, symbiosis, salamander, artificial, manipulation

CREATED

Jul 26, 2022

LAST MODIFIED

Aug 03, 2022

PROTOCOL INTEGER ID

67676

GUIDELINES

Embryos (*A. maculatum*) for injection should be between stages 26-31. Injections of younger embryos (~stage 19) ended with the death of all embryos. Injection of embryos at stage 33+ can be problematic because as the embryos flex, they can tear the injection site, preventing the wound from healing and causing eventual death. Injection of embryos at stages 26-30 has good survival: 95% survival at 24 hours, >50% after 5 days

MATERIALS TEXT

- Salamander eggs
- Dense *Oophila* algal cultures in a growth state (cultures should have mostly motile algae) --> 180,000 cells per mL or more.

- Small steel strainer
- 70% ethanol in a squeeze bottle
- Milli-Q water in a squeeze bottle
- Sterile 20% Holtfreter's solution (https://en.wikipedia.org/wiki/Holtfreter%27s_solution)
- Binocular dissecting microscope
- Tweezers (or needles to control and limit the eggs movements)
- Microinjection apparatus, such as a 3-axis micromanipulator with a nanofil syringe attached (https://www.wpiinc.com/var-3167-sub-microliter-injection-system).
- 35 gauge steel needle (beveled) for nanofil syringe (https://www.wpiinc.com/var-3184-nanofil-needles).
- Optional: motorized injection controller.
- Disposable Plastic Base Molds (e.g., https://www.fishersci.com/shop/products/fisher-scientific-tissue-path-disposable-base-molds-5/p-91758 or https://www.fishersci.com/shop/products/flat-embedding-mold-double-end/50930509)
- 24 well plate
- Petri dishes
- Plastic transfer pipette, cut such that the opening is large enough to hold one egg.

DISCLAIMER:

BEFORE STARTING

Salamander egg clutches need to be collected as close to laying as possible (i.e. very young). Collected egg clutches can be cleaned following the same steps as the first section of this protocol. Once washed the egg clutches should be incubated in the dark to prevent algal growth. This way there should be no algae other than the ones injected during this protocol. Leave the egg clutches to incubate and grow to the desired stage.

Prepare the embryos

Load whole eggs containing embryos into a strainer.

Citation: John Burns, Hui Yang, Joost S Mansour Injection of algae intoÃÂ Ambystomid (salamander) embryo https://dx.doi.org/10.17504/protocols.io.5jyl89y17v2w/v1

1

1.1

Whole eggs will be washed with 70% ethanol prior to decapsulating the embryos to remove external microorganisms. The ethanol wash would kill embryos, so the eggs must be intact at this point. For *A. mexacanum* eggs, individual jellied eggs can be used. For *A. maculatum* eggs, intact eggs need to be removed from the egg mass before being placed in the strainer.

2

Wash eggs with 70% ethanol for ~20 seconds.

Citation: John Burns, Hui Yang, Joost S Mansour Injection of algae intoÃÂ Ambystomid (salamander) embryo https://dx.doi.org/10.17504/protocols.io.5jyl89y17v2w/v1

2.1

Squirt 70% ethanol over eggs in strainer while rotating strainer around to make sure to thoroughly wash all eggs.

3 Wash eggs with Milli-Q water for ~20 seconds.

Milli-Q water is used to wash off the ethanol

3.1

*wash steps can be repeated if desired.

4 Following the water rinse, transfer eggs to a 100 mm petri dish containing sterile 20% Holtfreter's solution.

Citation: John Burns, Hui Yang, Joost S Mansour Injection of algae intoÃÂ Ambystomid (salamander) embryo https://dx.doi.org/10.17504/protocols.io.5jyl89y17v2w/v1

Embryos do not need to be decapsulated and are better of left in the egg.

5

Embryos are now ready for injection.

Prepare the algae

6 Pour 40 mL of algal culture into a 50 mL falcon tube.

7 8m

To pellet the algae centrifuge at **300 x g, 00:08:00**

8 Remove supernatant, and place tube upside-down on a kimwipe to remove excess liquid.

9 Add 20 mL sterile 20% Holtfreter's solution to the algal pellet and resuspend it.

Citation: John Burns, Hui Yang, Joost S Mansour Injection of algae intoÃÂ Ambystomid (salamander) embryo https://dx.doi.org/10.17504/protocols.io.5jyl89y17v2w/v1

10 8m

Repeat centrifuge at **300 x g, 00:08:00**

11 Remove supernatant, remove excess liquid as before (**go to step #8**)

The centrifugation and wash steps have the effects of:
(a) concentrating the algae
(b) removing the high-nutrient growth medium
(c) removing many contaminating bacteria as they do not pellet well at 300xg.

12 Resuspend the pellet in 400 uL of 20% Holtfreter's solution.
This is the 100x concentrated algal solution that will be used for injections.

Depending on the algal density this resuspension can be fone into a larger volume

13

Fix a sample of the concentrated algal culture in 0.1% glutaraldehyde for cell counts. This can be used to verify the concentration of the culture finally used.

Or another preferred fixing method for cell counting

Inject the embryos with algae

14 Prepare an individual embryo in a well in plastic mold (e.g., **Fisherbrand™ Disposable Base Molds** or any kind of well that will hold the egg snug).
Eggs are typically kept and injected underwater (20% Holtfreter's)

15 Place the embryo in its mold/well on the dissecting microscope.

Citation: John Burns, Hui Yang, Joost S Mansour Injection of algae intoÃÂ Ambystomid (salamander) embryo https://dx.doi.org/10.17504/protocols.io.5jyl89y17v2w/v1

16 Prepare the syringe by backfilling the syringe with the concentrated algal solution. Close the syringe by adding the needle.

17 Prep the needle by evacuating air, and push out some solution to ensure that algae are coming out during injections.

18 ⚠

Carefully penetrate the egg and then break into the embryo's skin surface with the needle (beveled). It is not necessary to push the needle deep into the embryo.

Indeed it is very disadvantageous for the needle to pierce through the other side of the embryo.

Be careful during egg and embryo penetration to NOT hit the needle onto other surfaces and bent or break it.

19 Inject algae.

For stage 30 embryos, a good quantity of algal concentrate for injection seems to be about 300 nanoliters (0.3 uL).

20 Carefully withdraw the needle straight back.

21 Transfer the embryo to an individual well in a 24 well plate containing 1.5 mL 20% Holtfreter's solution.

21.1 Repeat until all embryos have been injected (**go to step #18**).

Citation: John Burns, Hui Yang, Joost S Mansour Injection of algae intoÃÂ Ambystomid (salamander) embryo https://dx.doi.org/10.17504/protocols.io.5jyl89y17v2w/v1

Video of algal injection. Example of algal injection into an early (21-23) stage *A. maculatum* embryo. Later stages such as 25-28 are recommended.

22 Place the 24-well plate containing the injected embryos in an incubator with a 12/12 light-dark cycle.

Most embryos should survive and heal after a few (3-5) days.

Citation: John Burns, Hui Yang, Joost S Mansour Injection of algae intoÃÂ Ambystomid (salamander) embryo https://dx.doi.org/10.17504/protocols.io.5jyl89y17v2w/v1