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# Verifying Pluripotency in Acorn Worms

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**Abstract:**

This study aims to verify the pluripotency of acorn worm (Enteropneusta) cells using two different experimental methods. Acorn worms are marine invertebrates whose unique characteristics make them ideal subjects for this research. The first method involves transplanting stained tail tissue into the wound site of a healthy recipient to observe the integration and function of the labeled tissue during the regeneration process. The second method uses fate mapping technology, labeling specific cells with fluorescent dye in early embryos, and then transplanting these labeled cells into recipient embryos to track their migration, division, and differentiation during development. The results of both methods indicate that acorn worm cells can differentiate into various tissues and organs, confirming their pluripotency. These findings provide new insights into the developmental and regenerative mechanisms of deuterostomes.

**Keywords:** pluripotency, acorn worm, tail tissue transplantation, fate mapping, regenerative biology

## 1. Introduction

### 1.1 Acorn worms

Acorn worms (scientific name: Enteropneusta) are marine invertebrates belonging to the phylum Hemichordata, named for their acorn-shaped head. They have elongated bodies divided into three parts: the proboscis, collar, and trunk. Acorn worms inhabit the ocean floor and play a crucial ecological role by mixing sediments to promote nutrient cycling [1]. Their distinctive feature is the presence of microscopic ossicles composed of calcium carbonate, which vary in shape and composition among different species [2].

### 1.2 DiI dye

DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate) is a lipophilic fluorescent dye with an excitation wavelength of 549 nm and an emission wavelength of 565 nm [3]. It can embed into the lipid bilayer of cell membranes, emitting red fluorescence. It is widely used in neuroscience and cell biology research to label and trace cells and their structures, provide detailed depictions of neuron morphology, study cell-to-cell connections [4], and track the migration and integration of transplanted cells in vivo.

### 1.3 Fate mapping

Fate mapping is an important technique used in

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developmental biology. It involves precisely labeling specific cells or regions in an embryo with fluorescent dyes, genetic markers, or other labeling methods, and then tracking the division, movement, and differentiation paths of these labeled cells during embryonic development using microscopy or other imaging techniques. This allows researchers to determine in detail the specific tissues and organs these cells form in the adult organism [5,6-9].

### 1.4 Significance of Proving Pluripotency in Acorn Worms

Previous studies have not addressed the verification of pluripotency in hemichordates. This project aims to verify the pluripotency of acorn worm cells, which is crucial for understanding the developmental and regenerative mechanisms in deuterostomes. Acorn worms, as hemichordates, share significant evolutionary links with chordates, and studying their cellular pluripotency can reveal fundamental principles of cell differentiation and tissue formation. Through this experiment, we aim to understand how these cells divide, migrate, and differentiate during embryonic development and regeneration, ultimately forming specific tissues and organs. This research not only enriches our foundational knowledge of developmental biology but also opens new avenues for regenerative medicine.

## 2. Project Description

We objective of this project is to verify pluripotency in acorn worm cells using fate mapping techniques. The author uses two methods (Figure 1): Method one involves excising the tail tissue of the acorn worm, labelling it with a fluorescent dye (such as DiI), and then transplanting it into the wound site of a healthy recipient. The worm is then cultured and the integration and function of the labeled tissue are observed during the regeneration process. Method two involves injecting a fluorescent dye into specific cells of the acorn worm embryo at an early developmental stage to label them. These labeled tissues are then transplanted into corresponding regions of recipient embryos. The embryos are cultured and the division, movement, and differentiation paths of the labeled cells are tracked throughout development. Through these two methods, the final positions and functions of the labeled cells in the adult worm are studied to determine their pluripotency.

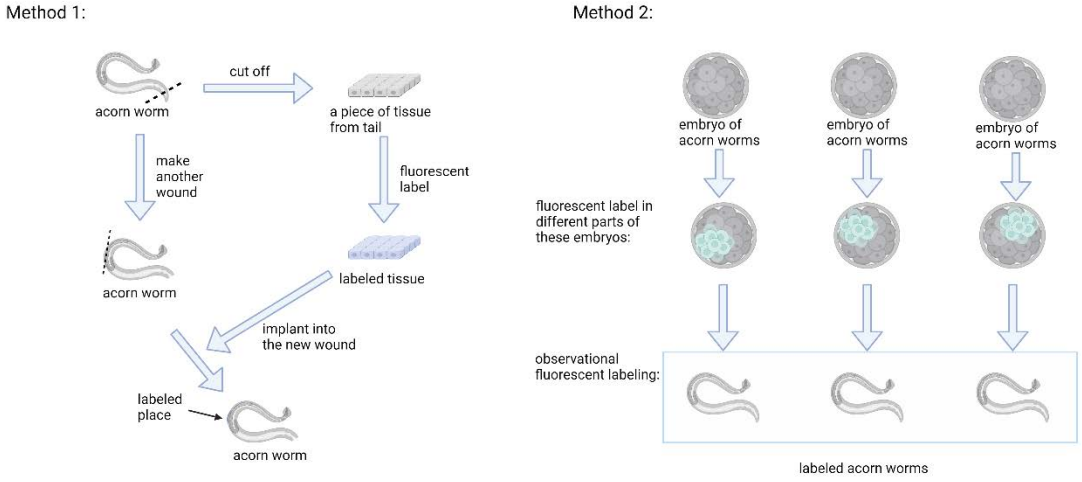


**Figure 1. Graphical Abstract**

## 3. Method

### 3.1 Method 1

#### 3.1.1 Excise the tail tissue of the acorn worm

First, anesthetize the acorn worm by placing it in a 7% magnesium chloride solution until it is fully relaxed [4]. Once anesthetized, fix the worm on the surgical platform, ensuring the tail is exposed for the procedure. Using fine scissors, excise the desired portion of the tail tissue and place it in a prepared DiI dye solution for several hours to ensure thorough permeation of the dye into the tissue [3]. After labeling, wash the tissue multiple times with sterile saline or PBS buffer to remove any excess dye.

#### 3.1.2 Transplant the excised tail tissue to the wound site

Next, select a healthy recipient acorn worm, anesthetize it again, and fix it on the surgical platform, exposing the pre-

determined transplant site. Create a small incision at the transplant site using fine scissors or a scalpel, ensuring the wound is large enough to accommodate the labeled tissue. Carefully transplant the labeled tail tissue into the recipient's wound using fine forceps, ensuring close contact between the transplant and recipient tissues to promote healing and integration. Suture or seal the wound with fine surgical stitches or tissue glue, ensuring the closure is secure but not overly tight to avoid excessive pressure on the recipient.

After the transplant, place the recipient acorn worm back into an appropriate culture environment, providing adequate oxygen and a suitable temperature. Regularly monitor the health status of the recipient and the integration of the transplanted tissue. Periodically examine the wound regeneration using a fluorescence microscope to observe the position and state of the labeled tissue. Record the fluorescence signals in the regenerating tissue and analyze the distribution and integration of the labeled tissue. Through multiple repeated experiments and observations at different time points, verify the integration and function of the transplanted tail tissue in the regeneration process.

### 3.2 Method 2

#### 3.2.1 Preparation and Injection of Fluorescent Dye

Select an appropriate fluorescent dye (DiI), collect acorn worm embryos at an early developmental stage (the 2-cell or 4-cell stage), [4] and use a microinjection setup equipped with fine needles and a micromanipulator to carefully inject the fluorescent dye into specific cells or regions of the embryo, ensuring minimal disruption to the embryo structure.

#### 3.2.2 Culturing and Monitoring Embryos

Place the injected embryos in a suitable culture medium, maintaining optimal temperature, pH, and salinity conditions specific to acorn worms, and regularly monitor the embryos under a fluorescence microscope to ensure the dye is retained in the labeled cells and to track their *development [8-9]*.

#### 3.2.3 Transplantation of Labeled Tissue

Select healthy, fluorescently labeled donor embryos and corresponding stage-matched recipient embryos, anesthetize the embryos using a suitable anesthetic solution to minimize movement, use fine dissection tools to carefully cut out the labeled tissue from the donor embryo, and transplant it into the corresponding region of the recipient embryo, ensuring proper alignment and minimal damage [6].

#### 3.2.4 Culturing and Observation Post-Transplantation

Place the transplanted embryos in an appropriate culture environment to facilitate healing and normal development, use a fluorescence microscope to track the integration and behavior of the labeled tissue within the recipient embryo, and document the developmental stages and the movement or differentiation of the labeled cells to understand their fate [8].

#### 3.2.5 Analysis of Adult Structures

Allow the transplanted embryos to grow into adults under ideal conditions, examine the adult worms at the appropriate developmental stages to determine the presence and location of fluorescently labeled cells, and analyze the data to correlate the positions of labeled cells in the embryo with their final locations in the adult worm, thereby mapping the cell fate.

## 4. Conclusion

We have experimentally verified the pluripotency of acorn worm cells, and the results show that the cells in acorn worms can differentiate into various tissues and organs under specific conditions. Two different methods were used to track the migration, division, and differentiation paths of labeled cells during regeneration and development.

The first method is tail tissue transplantation. By excising and staining the tail tissue of the acorn worm and then transplanting it into the wound site of a healthy recipient, we observed the integration and function of the labeled tissue during regeneration. The results showed that the transplanted tail tissue successfully integrated and participated in the regeneration process in the recipient, verifying its pluripotency.

The second method is fate mapping. In the early developmental stages of acorn worm embryos, specific cells were labeled with a fluorescent dye and then transplanted into corresponding recipient embryos. By observing the behavior of these labeled cells during development under a microscope, we found that these cells could migrate, divide, and differentiate into various tissues in the recipient, further confirming their pluripotency.

In summary, this study successfully verified the pluripotency of acorn worm cells through two different experimental methods, providing new evidence and perspectives for understanding the developmental and regenerative mechanisms in deuterostomes.

In the future, we can further optimize and expand experimental techniques to obtain more precise and detailed cell fate mapping data. Advanced molecular labeling and gene editing technologies, such as CRISPR/Cas9, can be introduced to achieve more refined cell labeling and tracking.

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Additionally, we aim to explore the pluripotency of cells at different developmental stages and cell types, providing a comprehensive understanding of the regenerative capabilities and developmental potential of acorn worms. By comparing fate mapping data across different species, we can investigate the conservation and diversity of pluripotency in evolution, offering new perspectives for evolutionary biology research.

The results of this experiment will have wide-ranging impacts in several fields:

1)Regenerative Medicine: Understanding the pluripotency and regenerative mechanisms of acorn worm cells will provide a theoretical foundation and practical guidance for developing new therapies for tissue repair and organ regeneration. This has significant application potential for treating injuries and degenerative diseases.

2)Evolutionary Biology Research: By comparing cell fate maps of different species, we can study the changes in pluripotency throughout evolution, revealing the mechanisms of development in complex life forms. This will help us understand developmental innovations and adaptation strategies in the animal kingdom.

3)Bioengineering: Utilizing pluripotent cells for bioengineering experiments can enable the in vitro cultivation of specific cell types or tissues, providing new models and tools for medical research and drug testing, thereby accelerating scientific research and drug development.

4)Ecology and Environmental Science: Studying the regenerative capabilities and adaptation mechanisms of acorn worms can aid in the protection and restoration of marine ecosystems. These research findings can provide scientific evidence for ecological restoration in response to environmental changes and pollution issues.

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