Let's Clone a Mouse, Mouse, Mouse...

Abstract

A step-by-step, cut and paste simulation of cloning by somatic cell nuclear transfer.

Learning Objectives

- An organism can be cloned by transferring the nucleus from one of its somatic cells to an enucleated egg.

Estimated time

- Class time: 60 min.
- Prep time: 15 min.

Materials

- Copies of student handouts
- Scissors
- Tape
- crayons/colored pencils

Instructions

- Distribute materials to groups of three students. Each student will choose an adult mouse. Instruct them to cut apart the figures, leaving white areas around each for ease of coloring.
- Instruct them to follow the directions on the student sheet.
- For use in leading the activity, see the Annotated Procedure on the following pages for additional background information relevant to each step of the activity.

Adaptations

- Use real petri dishes instead of the paper ones on the "Lab Benchtop".
- Have students attempt to remove the nuclei while viewing the cells through a dissecting microscope.
- Have students use forceps to hold the cell while they are cutting out the nucleus.
- Use this activity in conjunction with the Click and Clone online activity on the Learn.Genetics website. Remove the instructions and have students use the cut-outs to create a poster that explains this cloning technique as an assessment.

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Annotated Procedure

Note: Text from the Student Activity Instructions is in **bold**.

1. **Color the cells as follows:**

2. **Color the three large female mice as follows:**

Background Information: The colors of the mice correlate with the phenotypes used in the actual cloning experiment at the University of Hawaii in 1998.

3. **Cut out the mice and the Morula, and spread them out in front of you.**

4. **From the brown mouse (the Somatic Cell Donor), cut out the Cumulus Cell and place it in Petri Dish #1.**

Background information: In theory, any somatic cell from a diploid individual, male or female, could be cloned. The University of Hawaii researchers tried nuclear transfer from cumulus cells, Sertoli cells, and neuronal cells in mice. Cumulus cells are found in a layer of cells that surround and nourish an egg cell in females; Sertoli cells are found in the seminiferous tubules in the male reproductive system; and neuronal cells are cells of the nervous system. Somatic nuclei injected into eggs are in the G0 or G1 stage of the cell cycle (resting and growth phases, respectively) so that the cycles of the two cells are compatible. In cloning Dolly the sheep, the cells were starved for 5 days to reduce them to G0. Although the three types of cells the University of Hawaii researchers tried are already in G0, only the nuclei from cumulus cells produced full term mouse pups. The cumulus cells are placed in Petri dish #1 to simulate the chemical bath they were stored in which helped separate the cells.

5. **From the black mouse (the Egg Cell Donor), cut out the Egg Cell and place it in Petri Dish #2.**

Background Information: In the University of Hawaii experiments, the egg cell donors were injected with hCG and eCG (human and equine Chorionic Gonadotropin) to stimulate ovulation and the eggs were collected about 13 hours later. The eggs are placed in Petri dish #2 to simulate the chemical bath that prepares the eggs to be enucleated.

6. **Cut out and discard the nucleus from the Egg Cell in Petri Dish #2. Do not remove any of the cytoplasm.**

Background Information: The cytoplasm of the egg cell is needed for embryonic development. Conserving as much of the cytoplasm as possible helps ensure the success of the procedure.

7. **Place the enucleated Egg Cell in Petri Dish #3.**

Background Information: Once the nucleus has been removed, the enucleated eggs are stored in a special solution (Petri Dish #3) until the new nucleus can be injected.

8. **Cut out the nucleus from the Cumulus Cell in Petri Dish #1, making sure that no cytoplasm is left surrounding the nucleus.**

Have students pay special attention to cut away any edges that could represent the cytoplasm of the somatic Cumulus Cell.

Background Information: Isolating the nucleus from the cytoplasm ensures that only the DNA from the somatic Cumulus Cell is placed into the enucleated egg.

9. Place the Cumulus Cell Nucleus into the enucleated Egg Cell in Petri Dish #3, and tape them together on the back.

Background Information: The process of removing the somatic cell nucleus and injecting it into the enucleated egg cell should take no more than 5 minutes.

10. Tape (on the back) the Egg Cell with the newly replaced nucleus onto Petri Dish #4 and let it rest for about 2 minutes. This waiting time represents the 1 to 6 hours that the new nucleus needs to successfully adjust to the Egg Cell.

Background Information: After nuclear transfer, the egg is placed in a chemical bath to prevent it from expulsing the final polar body that forms when an egg is fertilized. The eggs remain in the chemical bath 1-6 hours (simulated by the 2-minute wait). This time also allows the new nucleus and egg to adjust to each other.

11. The new Egg Cell needs to be chemically stimulated in order to divide and grow into an embryo. To represent this chemical activation, color Petri Dish #4, including the new Egg Cell, entirely with yellow (the yellow color over the new Egg Cell should hint at a green color).

Background Information: Before the new egg can be implanted into the surrogate mother, cell division must occur. A solution of chemicals is used to stimulate the egg to divide. The main activator in this solution is strontium (Sr2+), which stimulates similar cellular processes when an egg is fertilized.

12. After it is chemically stimulated, the new Egg Cell divides into a ball of cells called a Morula. Cover the new Egg Cell with the Morula (colored green).

13. After the new Egg Cell divides into a Morula, it is placed into the Womb of the Surrogate Mother mouse (colored white). Tape the Morula into the Womb of the Surrogate Mother.

Background Information: After the egg divides, it is placed either in the oviduct (2-8 cell stage) or uterus/womb (morula/blastocyst) of the Surrogate Mother mouse.

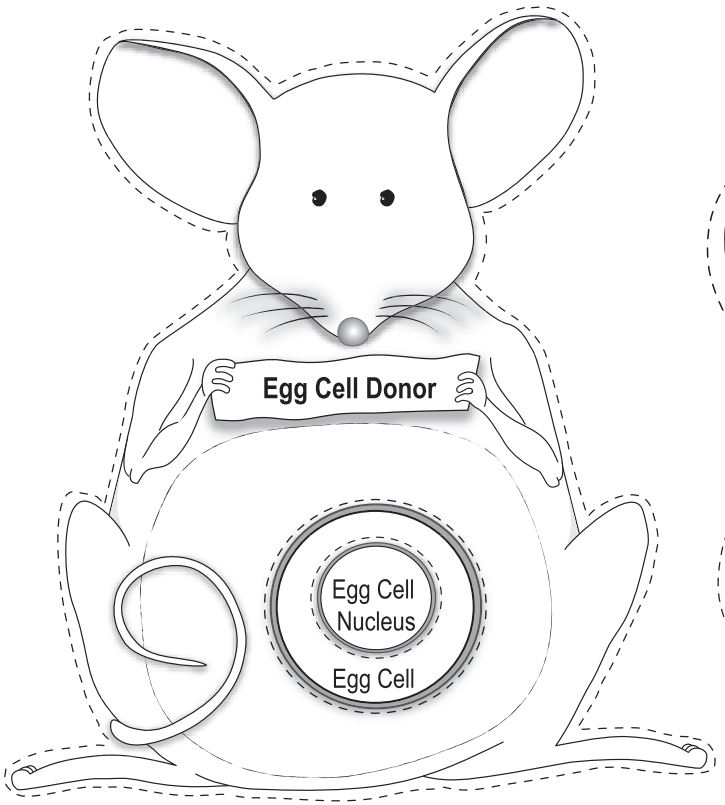
14. After about 19 days, the Surrogate Mother mouse will give birth to a new Mouse Pup.

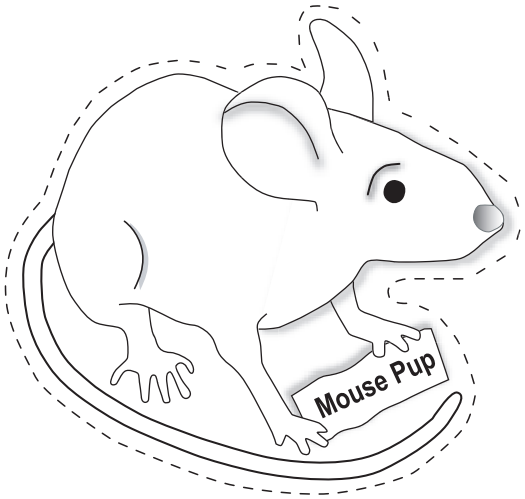
Background Information: After 18-19 days, the mouse pups cloned by the University of Hawaii researchers were delivered by caesarean section and were raised by lactating foster mothers.

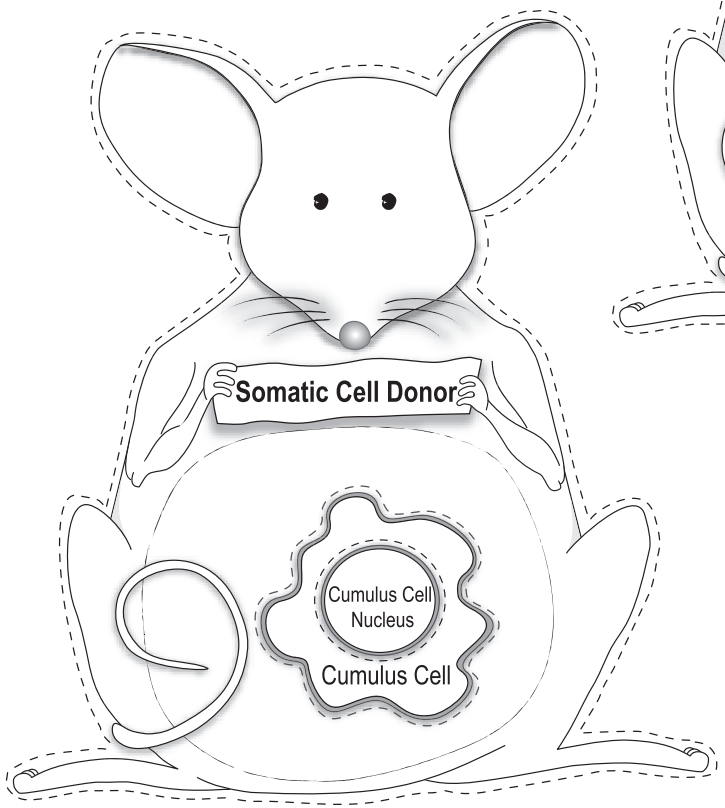
15. Which adult mouse will the Mouse Pup resemble? What color will it be? Color the newly delivered Mouse Pup this color.

- Have each group discuss and decide on the color of the new mouse pup.
- The Mouse Pup will resemble the Somatic Cell Donor, since this Donor provided the DNA for the cloned egg.
- The Mouse Pup will be brown (and female) like the Somatic Cell Donor.

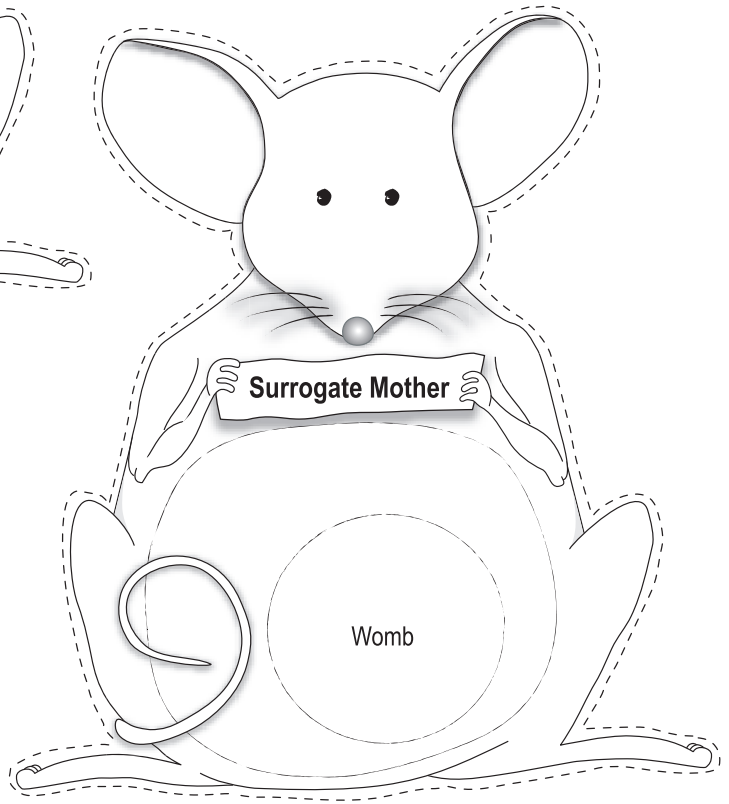
Cut-Outs


Egg Cell Donor
Egg Cell Nucleus
Egg Cell


Mouse Pup


Somatic Cell Donor
Cumulus Cell Nucleus
Cumulus Cell


Morula


Surrogate Mother
Womb