Teacher Guide: Let's Clone a Mouse, Mouse, Mouse . . .

ACTIVITY OVERVIEW

Abstract:
Somatic Cell Nuclear Transfer (SCNT) is a cloning method that involves transferring a nucleus from a somatic cell of the individual to be cloned to an enucleated egg. This activity simulates, step-by-step, the Honolulu technique for SCNT used by researchers at the University of Hawaii. It allows students be the scientist and carry out SCNT via paper illustrations.

Module:
Cloning in Focus

Key Concepts:
Somatic cell nuclear transfer, cloning, somatic cell, enucleated cell, morula, artificial embryo twinning

Materials:
Student handouts, scissors, tape, crayons/colored pencils/markers

Prior Knowledge Needed:
At least a basic knowledge of cell structure and the function of the nucleus and cytoplasm. The nucleus contains the information (DNA, in the form of genes on chromosomes) related to inheritance of traits. The cytoplasm contains the machinery (organelles, proteins, etc.) for the cell to carry out its functions. Artificial embryo twinning is a technique in which a very early embryo is manually separated into individual cells, each of which develops into a "twin".

Appropriate For:
Ages: 12 - 16
USA grades: 7 - 12

Prep Time:
30 minutes (copying and review time)

Class Time:
45 - 70 minutes

Activity Overview Web Address:
http://gslc.genetics.utah.edu/teachers/tindex/overview.cfm?id=howclone

Other activities in the ***Cloning in Focus*** module can be found at:

http://gslc.genetics.utah.edu/teachers/tindex/

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I. PEDAGOGY

A. Learning Objectives

- Students will be able to explain, step by step, the Honolulu method of cloning.
- Students will be able to differentiate somatic cell nuclear transfer from artificial embryo twinning.
- Students will exercise hand-eye coordination.

B. Background Information

Clones are produced naturally when the cells in a developing zygote become separated after the two-cell stage. The two cells (or two groups of cells) develop into identical twins.

In artificial embryo twinning, a very early embryo is separated into individual cells in a Petri dish in a laboratory. Each cell is allowed to divide and develop on its own and then implanted into a surrogate mother. The resulting "offspring" are genetically identical (clones) since they all came from the same zygote.

Somatic cell nuclear transfer is used to create a clone from an adult organism. DNA from a somatic cell taken from the adult is injected into an unfertilized, enucleated egg and then implanted into the uterus of a surrogate mother. The resulting "offspring" is a clone of the adult DNA donor.

It is important for students to understand the difference between artificial embryo twinning and somatic cell nuclear transfer. Both techniques produce clones. However, artificial embryo twinning does not preserve the genotype of the adult "parent" while somatic cell nuclear transfer does. In artificial embryo twinning, recombination between each parent's chromosomes occurs during formation of the egg and sperm that are united to form the egg, which is then split to create twins. No recombination occurs in somatic cell nuclear transfer since the nucleus of a somatic cell (which has a diploid number of chromosomes) is used to create the initial "egg". Artificial embryo twinning is a form of sexual reproduction while somatic cell nuclear transfer is a form of asexual reproduction.

For additional information see *What is cloning?*, available on the Genetic Science Learning Center website at http://gslc.genetics.utah.edu/units/cloning/whatiscloning/

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C. Teaching Strategies

1. Timeline

- Day before activity:
  - Make photocopies of the student handouts on white paper
  - Gather scissors, tape and crayons/colored pencils/markers
- Day of activity:
  - Discuss cloning and the difference between artificial embryo twinning and somatic cell nuclear transfer
  - Have students carry out the simulated cloning activity
  - Discuss the questions at the end of the activity

2. Classroom Implementation

- Begin by discussing the science of cloning, differentiating between artificial embryo twinning and somatic cell nuclear transfer. You may want to use the information and animations in *What is Cloning* (http://gslc.genetics.utah.edu/units/cloning/whatiscloning/)

- To carry out the simulated cloning activity:
  - Divide students into groups of three.
  - Have each student in a group choose an adult mouse.
  - Follow the instructions in the student handout to carry out the cloning procedure.
  - See the Annotated Procedure (pages 6-9) for more detailed instructions and additional Background Information relevant to each step. You may choose to share this information with your students, if appropriate or in response to their questions.

- When students have completed the activity, assess their understanding using the questions at the end of the activity (see Assessment Suggestions, below).

3. Adaptations

- Lead students through the steps of the procedure using the Click and Clone interactive animation (http://gslc.genetics.utah.edu/units/cloning/clickandclone/). View a step and then have students carry it out with their paper materials.
- Enlarge the illustrations for ease of handling.
- 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.

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4. Assessment Suggestions

- Use the questions at the end of the student handout for assessing students' understanding of the cloning process (Answer Key on pages 10-11). Suggestions for using these questions include:
  - Have students discuss the questions within their small groups and then as a class.
  - Discuss the questions as a class.
  - Have students individually write answers to the questions and then discuss them as a class.
- You may also use the following additional questions to assess student understanding:
  - Identify the steps in somatic cell nuclear transfer in order.
  - Compare and contrast genetic inheritance in artificial embryo twinning and somatic cell nuclear transfer.

II. ADDITIONAL RESOURCES

A. Activity Resources - linked from the online Activity Overview:

http://gslc.genetics.utah.edu/teachers/tindex/overview.cfm?id=howclone

- Article: Leutwyler K. (July 27, 1998) Send in the clones. *Scientific American*. Describes the successful cloning of mice using the Honolulu technique. Includes photos of the mice, the donor nucleus, and the scientists.
- News release: First Reproducible Cloning of Mammals from Adult Cells Reported in July 23 Issue of the Journal *Nature*. (July 22, 1998)
  News release from the University of Hawaii describing the successful cloning of mice. Includes a description of the Honolulu technique and background information on cloning.

B. Other Resources

- Paper: Wakayama T, Perry ACF, Zuccotti M, Johnson KR, and Yanagimachi R. (1998) Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* 394: 369-374.
  This is the original paper from the University of Hawaii reporting successful cloning of mice using the somatic cell nuclear transfer technique.
- Paper: Wilmut I, Beaujean N, de Sousa PA, Dinnyes A, King TJ, Paterson LA, Wells DN, and Young LE. (2002) Somatic cell nuclear transfer. *Nature* 419:583-586.

An update on progress to date in cloning mammals using the somatic cell nuclear transfer technique. Includes a discussion of challenges and cellular factors that influence the outcome of cloning.

III. MATERIALS

A. Detailed Materials List

- Student handouts (S-1 to S-5)
- Scissors - 1/student or group
- Crayons/colored pencils/markers (blue, green, yellow, brown, and black; white - optional) - 1 set/group
- Tape - 1 dispenser/group, or several groups

IV. STANDARDS

A. U.S. National Science Education Standards

Grades 5-8:

- Content Standard C: Life Science - Reproduction and Heredity; individuals receive genetic information from their parents that specifies their inherited traits

Grades 9-12:

- Content Standard C: Life Science - The Cell; cell structure
- Content Standard C: Life Science - The Molecular Basis of Heredity; the instructions for specifying an organism's characteristics are carried in DNA; the transmission of genetic information [in sexual reproduction] occurs through the union of egg and sperm

B. AAAS Benchmarks for Science Literacy

Grades 6-8:

- The Living Environment: Heredity - in sexual reproduction, a specialized cell from the female merges with a specialized cell from the male; the genetic information from each parent is copied in each cell as the organism develops
- The Human Organism: Human Development - following fertilization, cell division produces a small cluster of cells that differentiate to form the embryo

Grades 9-12:

- The Living Environment: Heredity - the sorting and recombination of genes in sexual reproduction results in variation between parents and offspring; the information passed from parents to offspring is coded in the DNA molecule
- The Living Environment: Cells - cells have specialized parts

C. Utah Core Curriculum

Intended Learning Outcomes for the Utah Secondary Core Curriculum in Science:

Students will:

4. Demonstrate Awareness of the Social and Historical Aspects of Science
    b. Understand how technological advances have influenced the progress of science, and how science has influenced developments in technology.

Biology (9-12):

- Standard 2: Students will understand the classification and function of cells.
  Objective 1: Investigate structure and function of cells.
  - Sketch and describe cells and components.
- Standard 4: Students will evaluate the significance and impact of genetic alteration on living organisms.
  Objective 3: Research and analyze perspectives on issues related to genetic technologies.
  - Evaluate applications of genetic technologies (e.g., genetic engineering, DNA analysis, virology).

Biology: Human Biology (9-12)

- Standard 2: Students will understand the classification and function of cells.
  Objective 1: Investigate structure and function of cells.
  - Sketch and describe cells and components.
- Standard 3: Students will analyze how genetic information is passed from one cell to another.
  Objective 3: Describe the significance and impact of genetic alteration on living things.
  - Describe applications of genetic technologies (e.g., human genome project, gene therapy, DNA analysis)

V. CREDITS

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Louisa Stark, Genetic Science Learning Center
Harmony Starr, Genetic Science Learning Center (illustrations)

Funding:

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A. Annotated Procedure

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

- Divide students into groups of three.
- Have each student in a group choose an adult mouse.
- Have students cut apart the figures on the cut-out page, leaving white areas around each figure for ease in coloring.

1. Color the cells as follows:
- **Cumulus Cell Nucleus and Cumulus Cell, blue**
- **Egg Cell Nucleus and Egg Cell, blue**
- **Morula (the ball of cells), green**

Have each student color the cells in their mouse (the student with the Surrogate Mother colors the morula).

2. Color the three large female mice as follows:
- **Somatic Cell Donor, brown**
- **Egg Cell Donor, black**
- **Surrogate Mother, white**
- **Don't color the Mouse Pup just yet**

Have each student color their mouse (the white, Surrogate Mother mouse does not need to be colored if it is on white paper).

Background Information: The colors of the mice correlate with the phenotypes used in the Honolulu cloning experiment.

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

The mice can now be cut out more carefully.

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

   Direct students to focus on what is happening in this and subsequent steps by using questions such as the following: Why did you remove the somatic cell first? Why don't you remove the somatic nucleus right away? What do you think would make this technique more effective?

   Teaching Tip: Some students may find it difficult to cut out the cells. Use this as an opportunity to point out that scientists often work in less than desirable circumstances. Nuclei are removed from cells using microscopes and related tools. Dexterity is a beneficial skill to have as a scientist.

   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.

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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 (Sr^{2+}), which stimulates similar cellular processes when an egg is fertilized.

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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.

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B. Answer Key

Questions from Student Handout (S-3)

1. What does the Nucleus contain? What role do its contents play in the cell?
The Nucleus contains DNA, which is structured in the form of genes on chromosomes. Genes provide the information for the cell to carry out its functions.

2. What is the purpose of the Somatic Cell Donor?
Provides the DNA, which contains the information for creating the new individual.

3. What is a Somatic Cell?
Any cell other than a sex cell (egg or sperm).

4. What is the purpose of the Egg Cell Donor?
Provides the egg, which contains the machinery (organelles, proteins, etc. in the cytoplasm) to create the embryo.

5. What is the major difference between a somatic cell and an egg cell?
The DNA (in the form of chromosomes) in a somatic cell is diploid, while the DNA in sex cells is haploid.

6. What is the purpose of the Surrogate Mother?
Provides the womb where development of the morula takes place. The morula cannot be placed in the womb of the Egg Cell Donor because the Donor was treated with hormones that caused it to ovulate. The Somatic Cell Donor is not used as the mother so that it is clear that the DNA in the cloned individual came from the cloning process.

7. What color did you color the Mouse Pup? Why?
The Mouse Pup was colored brown. It inherited its brown color from the Somatic Cell Donor, which provided the nucleus (and thus the DNA) for the egg from which the Pup originated.

8. What gender (male or female) will the Mouse Pup be? Why?
The Mouse Pup will be female, the same as the Somatic Cell Donor. Both sex chromosomes are present in the DNA in the nucleus from the somatic cell, since it is diploid.
Note: Not all clones are female like Dolly the sheep and the first mice. In 1999 the University of Hawaii researchers cloned a male mouse that was derived from the nuclei of cells taken from the tail tips of adult males.

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9. How is this method of cloning different from artificial embryo twinning?
Artificial embryo twinning involves a fertilized egg that is separated after the first mitotic division. Somatic Cell Nuclear Transfer involves taking the nucleus from an individual and placing it into an enucleated egg.

Additional Questions for Discussion (listed in Assessment Suggestions)

Identify the steps in somatic cell nuclear transfer in order.

Remove cumulus cells from the somatic cell donor.
Collect egg cells from the egg cell donor.
Enucleate the egg cells.
Remove the nuclei from the cumulus cells.
Inject a nucleus into each enucleated egg cell.
Let the cells rest so that the nuclei can adjust to the egg cells.
Chemically stimulate the egg cells to divide.
After the egg cells divide to the morula stage, implant them in the womb of a surrogate mother(s).

Compare and contrast genetic inheritance in artificial embryo twinning and somatic cell nuclear transfer.

Both techniques produce clones. However, artificial embryo twinning does not preserve the genotype of the adult "parent" while somatic cell nuclear transfer does. In artificial embryo twinning, recombination between each parent's chromosomes occurs during formation of the egg and sperm that are united to form the egg, which is then split to create twins. No recombination occurs in somatic cell nuclear transfer since the nucleus of a somatic cell (which has a diploid number of chromosomes) is used to create the initial "egg". Artificial embryo twinning is a form of sexual reproduction while somatic cell nuclear transfer is a form of asexual reproduction.

Name_______________________________ Date___________

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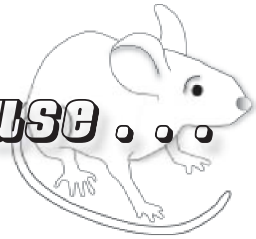

Activity Instructions - Page 1

FIRST, A LITTLE HISTORY:
In 1996, Dolly the sheep became the first mammal to be cloned by transferring the nucleus from an adult somatic cell into an enucleated egg cell. To confirm that Dolly was truly a clone, researchers at the University of Hawaii used a similar process in 1998 and ultimately cloned 50 mice. In this activity you will simulate their steps of somatic cell nuclear transfer to produce an exact clone, or genetic copy, of a mouse.

MATERIALS:
- Lab Benchtop illustration with 4 Petri Dishes
- Mice Cut-outs page
- Crayons, colored pencils or markers
- Scissors
- Tape

INSTRUCTIONS:

1. Color the cells as follows:
   - Cumulus Cell Nucleus and Cumulus Cell, blue
   - Egg Cell Nucleus and Egg Cell, blue
   - Morula (the ball of cells), green

2. Color the three large female mice as follows:
   - Somatic Cell Donor, brown
   - Egg Cell Donor, black
   - Surrogate Mother, white
   - Don't color the Mouse Pup just yet

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.

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

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

Genetic Science Learning Center

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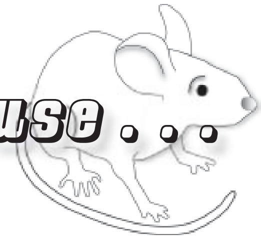

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Activity Instructions - Page 2

INSTRUCTIONS - Continued:

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

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

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

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.

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).

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.

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

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

16. Clean your lab station and answer the Activity Questions.

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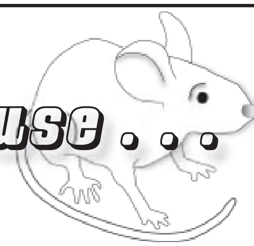


Activity Instructions - Page 1

1. What does the Nucleus contain? What role do its contents play in the cell?

2. What is the purpose of the Somatic Cell Donor?

3. What is a Somatic Cell?

4. What is the purpose of the Egg Cell Donor?

5. What is the major difference between a somatic cell and an egg cell?

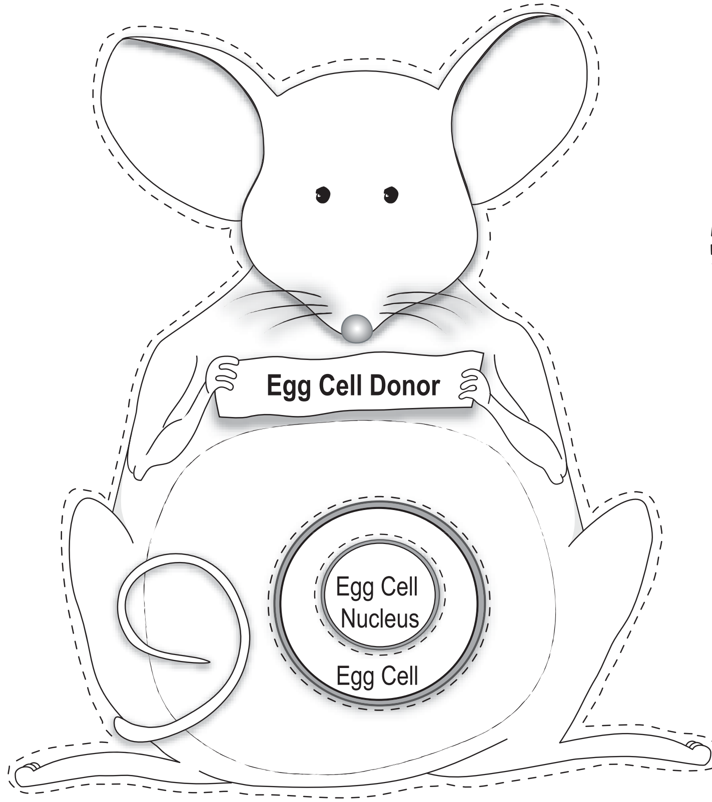
6. What is the purpose of the Surrogate Mother?

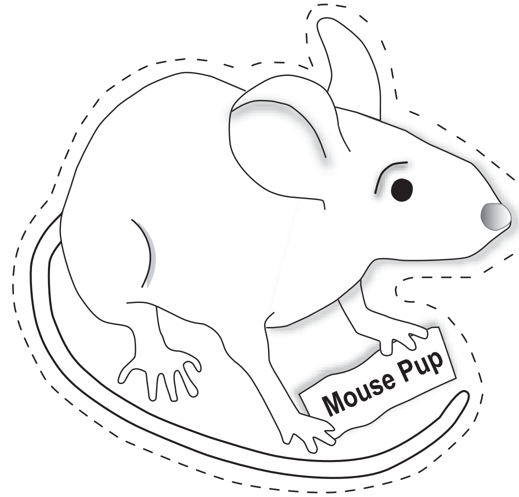
7. What color did you color the Mouse Pup? Why?

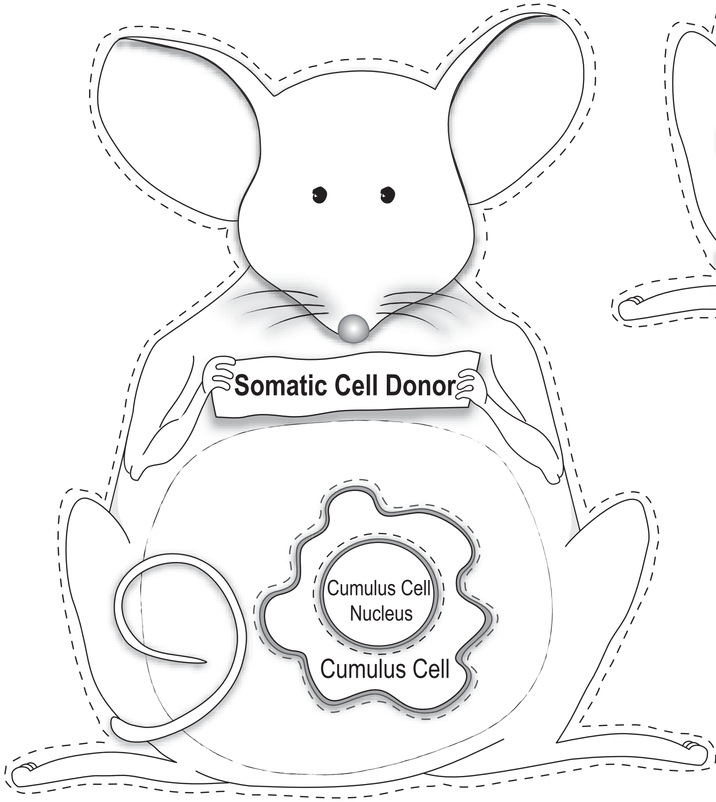
8. What gender (male or female) will the Mouse Pup be? Why?

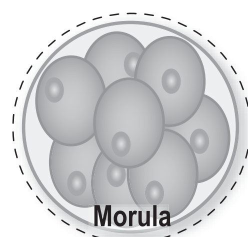
9. How is this method of cloning different from artificial embryo twinning?

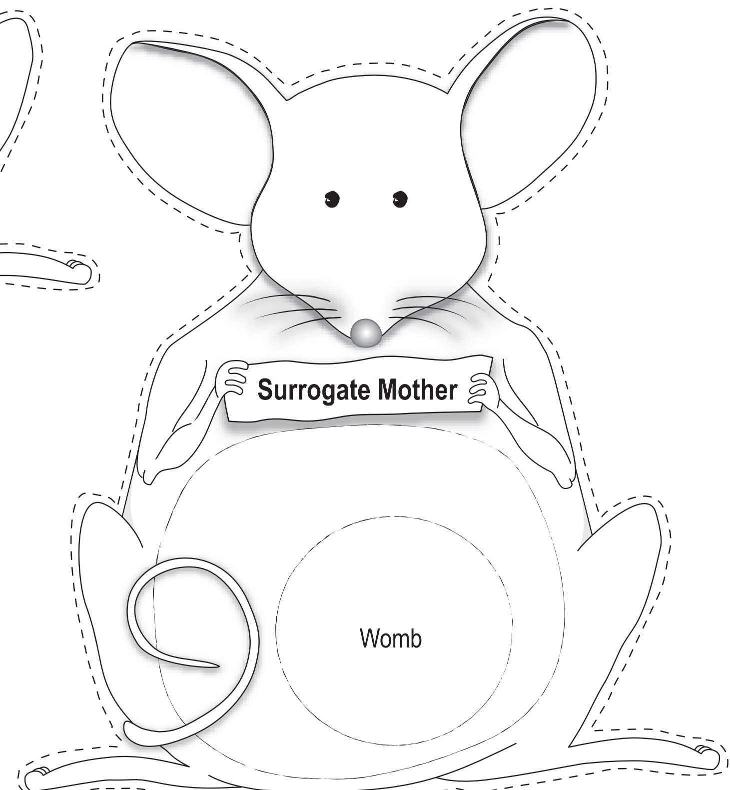
Mice Cut-outs











Lab Benchtop