Stem Cell Biology
The Adventures of Cryptic Ron Stem Cell

Overview:

This collection of activities is designed to introduce students to the basic biology of stem cells and highlight two current types of stem cell research; the use of stem cells to study genetic disorders and induced pluripotent stem cells.

The instructions given are meant to allow teachers to replicate the experimental procedures and case study method used at the Life Sciences Learning Center. We have also created a video/DVD that can be used to supplement the lesson. Starring graduate students, post-doctoral fellows, faculty, and a group of Life Sciences Learning Center Summer Science Academy students and incorporating many of the animations used in the slideshow, this lighthearted rendition of the Adventures of Cryptic Ron is meant to both amuse, engage, and educate.

The video blends skits with animations, and is meant to be paused by the teacher whenever he/she wants to digress, discuss, or expand on the topics presented in the video. Sections labeled "In The Lab" are meant to be paused to allow students to do the laboratory activities (set up for those activities are included in this guide). Sections labeled "What do you know, What do you want to know" are meant to be paused to initiate discussion on students' prior knowledge as well as whatever questions they may have at the time on the story, the topic, or general questions about content. Sections labeled "What do you know NOW" follow results of the lab activity, where students use the results of the experiments to advance their knowledge of stem cells, as well as the story.

If you don't want to use the video, a .swf slideshow file can be downloaded to explain key points in each part.

If you have any questions on the set up or any aspect of this lesson, please email us at LSLC_media@urmc.rochester.edu. Our media team is always online, and happy to assist you and take any suggestions you might have to make our programs better.

Summary of Activities:

Activity title (Estimated time required)	**Students will…**	**Purpose**
Plant Tissue Culture	• Use their knowledge about the three characteristics of stem cells to determine how to identify and isolate stem cells from a plant (Cauliflower) • Use sterile technique to cut out parts of a cauliflower that they think contain stem cells and place them in culture media for further observation.	Apply sterile technique to cell culture Apply knowledge of stem cells to design an experiment.
DNA Fingerprinting: Paternity testing for Cryptic Ron	• Students load an agarose gel, or use paper DNA to determine if Cryptic Ron is related to the donor father and mother	Introduction to gel electrophoresis and heredity alleged
Using Growth Factors to differentiate Embryonic and Adult Stem Cells	• Learn about the differences in adult and embryonic stem cells and use this knowledge to determine if CrypticRon is an embryonic stem cell. • Practice laboratory skills in combining "growth factors" in different ratios and testing their combinations on "embryonic" and "adult stem cells."	Model how chemical factors and cell history influence the ability of a cell to differentiate.
Microarray to find out what genes are expressed in embryonic versus adult stem cells	• Learn about how gene expression (what genes are turned on and off) determines the ability of a cell to differentiate • Learn about how microarrays are used to determine differences in gene expression	Introduction to microarray technology and model how differences in gene expression can determine the fate of a cell.
Induced Pluripotent Stem Cells…or how to make a multipotent cell, pluripotent	• Learn about how gene transfer can be used to turn genes "on" in a cell to change its fate • Practice laboratory skills in a mock gene transfer exercise. Apply the knowledge of how growth factors can be used to differentiate cells to testing whether or not their experiment was successful.	Illustrate how gene transfer can be used to reprogram differentiated cells

Correlation with New York State Learning Standards:

Standard 1

1.1c Science provides knowledge, but values are also essential in making effective and ethical decisions about the application of scientific knowledge.

1.2a Inquiry involves asking questions and locating, interpreting, and processing information from a variety of sources.

1.3b All scientific explanations are tentative and subject to change or improvement. Each new bit of evidence can create more questions than it answers.

3.1a Interpretation of data leads to the development of additional hypotheses, the formulation of generalizations, or explanations of natural phenomena.

Standard 4

1.2f Cells have particular structures that perform specific jobs. These structures perform the actual work of the cell.

2.1k The many body cells in an individual can be very different from one another, even though they are all descended from a single cell and thus have essentially the same genetic instructions. This is because different parts of these instructions are used in different types of cells, and are influenced by the cell's environment and past history.

5.2 Biological research generates knowledge used to design ways of diagnosing, preventing, treating, controlling, or curing diseases.

7a Societies must decide on proposals which involve the introduction of new technologies. Individuals need to make decisions which will assess risks, costs, benefits, and trade-offs.

Laboratory Skills

- Organizes data through the use of data tables and graphs
- States an appropriate hypothesis
- Identifies the control group and/or controlled variables
- Collects, organizes, and analyzes data
- Analyzes results from observations/expressed data
- Formulates an appropriate conclusion or generalization from the results of an experiment
- Recognizes assumptions and limitations of an experiment

Part 1: Plant Tissue Culture

Summary:

Students are introduced to the mysterious CrypticRon, who is allegedly an embryonic stem cell breaking federal law because he's using federal monies to culture himself.

Objectives:

- Students will learn basic information about stem cell law (what is the law restricting the use and study of embryonic stem cells) and biology (What are the three characteristics of all stem cells).
- Students will apply their knowledge to an experiment in which they have to isolate a plant stem cell.

Preparing for class:

- Each student will need one copy of the Stem Cell Biology Handout
- Prepare plant tissue culture media and materials for each lab group
    1. 1 plate MS media
    2. 1 pair of sterile scissors (sterilized in rubbing alcohol)
    3. 1 pair of sterile forceps (sterilized in rubbing alcohol)
    4. 1 "Sterile working surface" (a clean sheet of aluminum foil – the sterile side is the one facing the inside of the roll)
    5. 1 Sterilized piece of cauliflower

Sterilizing Cauliflower Pieces

1. Cut out one piece of cauliflower (include cauliflower curd and some of the stem) per student
2. Place all the pieces into the beaker with 10% bleach. Make sure all the pieces are submerged in the 10% bleach solution (you may need to place a weight on top to submerge them)
3. Soak the cauliflower for 10 minutes
4. Pour off the cauliflower (use your gloved hands to hold the cauliflower pieces in the beaker)
5. Pour in 500mL cooled sterilized water (stir the pieces with the sterile forceps)
6. Pour off water
7. Repeat steps 5-6 two more times
8. Cover the beaker with foil (face the "Sterile" side of the foil towards the inside of the cauliflower, i.e., the side that is wrapped on the inside of the roll) and store in the fridge for up to one week.

MS media plates

1. Autoclave 370mL water with 16g Murashige and Skoog powdered media (ordered from Sigma Aldrich, cat # M 9274) on liquid cycle
2. Allow to cool so that you can comfortably handle the bottle (approximately 50 degrees)
3. Add 0.75mL PPM (http://www.ppm4plant-tc.com/) using a STERILE TRANSFER PIPETTE (*Optional)
4. Swirl the media to mix
5. Pour about 30mL media into a deep Petri dish (100x20mm dish), allow to solidify
6. Seal the plates with parafilm. Store the containers UPRIGHT at ROOM TEMPERATURE for no more than one week

Part 2: DNA fingerprinting

Summary:

Students have to determine if CrypticRon is related to two parents who created IVF embryos in 2002 (A year after the federal law was passed that restricted the use of federal funds to create stem cell lines!).

Objectives:

- Students will learn about gel electrophoresis and how it can be used in paternity testing

Preparing for class (Using paper gels):

- Each student will need one copy of the paper gel and a pair of scissors and tape or glue.

Preparing for class (Agarose gel electrophoresis):

- Each lab group will need a 1% agarose gel (1g agarose in 100mL TAE or TE buffer). To save on gels you can pre-pour agarose gels, then cut them into small gels (With at least 3 wells to each small gel). Students can load their gels at their desks (place each gel onto a plastic tray, such as a weigh boat), and then carefully place them in an electrophoresis tank (more than one small gel can go into a tank)
- Each lab group will need access to:
    1. An electrophoresis tank and power supply
    2. A micropipettor that can deliver 10 microliters. (A 10 microliter fixed volume pipettor works great!)
    3. Clean pipette tips
- Each lab group will need the following "DNA" bands (Each lab group will load 10 microliters of each of these DNA bands
    1. Cryptic Ron
    2. Ladder

"Ladder":
- 0.1g Xylene Cyanole
- 0.1g Bromophenol Blue
- 30mL glycerol
- 70mL water

"Cryptic Ron DNA":
- 0.1g Xylene Cyanole
- 30mL glycerol
- 70mL water

Part 3: Growth Factors and Differentiation

Summary:

CrypticRon claims that he is an adult stem cell, not an embryonic stem cell, and therefore the federal laws do not apply to him! Students have to determine whether or not CrypticRon is telling the truth by comparing the ability of CrypticRon cells to differentiate to the ability of embryonic stem cells to differentiate

Objectives:

- Students will learn that adult stem cells are multipotent (limited potential to differentiate into different cells) and embryonic stem cells are pluripotent (full potential to differentiate into different cells)
- Students will learn that the application of growth factors to cells in the laboratory can be used to force cells to differentiate

Preparing for class

- Each lab group will need the following:

- Each lab group will need the following:
    1. Disposable transfer pipettes (minimum 7)
    2. Growth Factor 1 (At least 1mL)
    3. Growth Factor 2 (At least 1mL)
    4. Clear 12 well strips (can also use 12 well plates or, if students are careful, a sheet of saran wrap placed on white paper
    5. CrypticRon Stem Cells (At least 1mL)
    6. Embryonic Stem Cells (At least 1mL)
    7. At least 3 empty microtubes
    8. Gloves

"Growth Factor 1":
- pH 2 buffer

"Growth Factor 2":
- pH 10 buffer

"CrypticRon Stem Cells":
- 70% Ethanol, mixed with yellow and green food coloring to match the color of the "Embryonic Stem Cells"

"Embryonic Stem Cells":
- A 50:50 dilution of 2% Methyl Red and 2% thymolphthalein
    1. Make 2% Methyl Red by mixing 2g of Methyl Red powder with 70mL 100% ethanol. When Methyl Red is dissolved, add 30mL water to make up 100mL 2% Methyl Red solution
    2. Make 2%mg/mL thymolphthalein by mixing 2g of thymolphthalein powder with 70mL 100% ethanol. When thymolphthalein is dissolved, add 30mL water to make up 100mL 2% thymolphthalein solution

Notes for this part:

- You can either allow students to come up with their own ratios or lead them to a good set of ratios of growth factors. Ones that definitely work are:
    1. 100% Growth Factor 1
    2. 50% Growth Factor 1, 50% Growth Factor 2
    3. 50% Growth Factor 2

  The students are using a common technique used in labs to come up with a titration of the two growth factors to see what their combined effects are on cells. It's typical to start with 100% of one growth factor, and end with 100% of another growth factor, and then use a graded series of combinations in between. If you had a lot of time, you might do this:
    1. 100% Growth Factor 1
    2. 75% Growth Factor 1, 25% Growth Factor 2
    3. 50% Growth Factor 1, 50% Growth Factor 2
    4. 25% Growth Factor 1, 75% Growth Factor 2
    5. 50% Growth Factor 2

  But the set of three combinations shown above will cause the embryonic stem cells to differentiate into the three different kinds of cell types.

  If you want them to test out different combinations of growth factors on their own, it's important to point out that the first thing they ought to do is figure out if the combinations really do cause the embryonic stem cells to differentiate into all three cell types. Once they've established that, then they know what combinations they should use to test the CrypticRon cells. If the CrypticRon cells do not react in the same way as the embryonic stem cells...it definitely suggests that they are not embryonic stem cells.

  Based on what the CrypticRon stem cells are capable of differentiating into, students should be able to make a guess about what type of adult stem cell he is. Since he can only differentiate into blood cells...it suggests that he's a blood stem cell.

  This set of mock experiments is based on real stem cell studies in which growth factors are used to induce stem cells to differentiate.

  ***Make sure the students do not throw away their growth factor combinations! They'll use them again in part 5. If you won't be doing part 5 for awhile, it's OK to throw them away, but the important thing is for them to remember how they used them to test whether or not CrypticRon was an embryonic stem cell. In part 5, they'll be trying to make CrypticRon cells becoming pluripotent by adding engineered genes. Once they've done that, they'll need to use their knowledge of what combinations of growth factors can make embryonic stem cells differentiate into the different cell types to determine whether or not their experiment to make CrypticRon pluripotent worked!*

Part 4: Microarrays – what the difference between embryonic and adult stem cells anyhow?

Summary:

CrypticRon's true story comes out – he is a blood stem cell taken from a child who died of an unknown neurodegenerative disorder. He hopes to be able to figure out why she died...but how? Students are introduced to the work of Dr. Christoph Proschel, a University of Rochester Medical Center researcher whose innovative study on neural stem cells in a patient with Vanishing White Matter disease revealed the cause of that disease, with hopes for a possible cure. CrypticRon is hopeful...but there are no neural stem cells from his patient to collect anymore. How does a blood stem cell become a neural stem cell? Students have to figure out that if they can make an adult stem cell revert back to an embryonic stem cell...maybe they can turn a blood stem cell into a neural stem cell. To do this, they need to determine what genes are differentially expressed between embryonic and adult stem cells

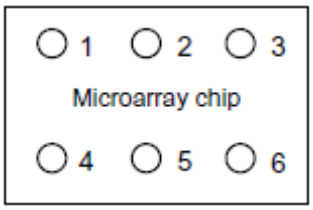
Objectives:

- Students will learn that differences in gene expression cause cells to take on different fates and functions. An embryonic stem cell and an adult stem cell may have the same DNA, but because of differences in gene expression, one is pluripotent and one is multipotent.
- Students will learn that microarray technology can be used to determine differences in gene expression.

Preparing for class

**Although this section has a lot of tubes to set up...once you've made a class set of tubes, if the students are careful about not cross contaminating things, 1mL of each solution will last for many classes, since each lab group uses only a tiny amount of each*

- Each lab group will need the following:
    1. A "microarray chip." The following image can be printed in sets on chromatography paper, or any absorbent paper. Normal computer paper can also be used, but Whatman 3MM chromatography paper (15-20cm) fits nicely onto a standard laser printer. You can also simply have students draw this image onto a piece of filter or chromatography paper using a pencil.



    2. Minimum 8 Q-tips
    3. 1mL tubes of each gene 1, 2, 3, 4, 5, 6
        - "1: C-myc" = 70% ethanol
        - "2: Nanog" = 70% ethanol
        - "3: HSF" = 70% ethanol
        - "4: Ribosomal Pr" = 2% phenolphthalein
        - "5: Myosin" = 70% ethanol
        - "6: Cyclin D" = 2% phenolpthalein
    4. 1mL tube of "Cryptic Ron (Adult Stem Cell) RNA" = Water
    5. 1mL tube of "Developing Solution" = 0.5% NaOH (0.5g NaOH in 100mL water)

Part 5: Genetic Engineering, Growth Factors and Differentiation

Summary:

Based on Part 4, students should now be aware that embryonic stem cells express two genes that adult stem cells do it. To turn CrypticRon into a pluripotent embryonic stem cell, perhaps all they need to do is to turn on those two genes in Cryptic Ron! To do this, they learn about how genes can be engineered to always be expressed, and inserted into cells.

Objectives:

- Students will learn that that genes can be engineered to always be expressed, and inserted into cells to change their fate and function.
- Students will apply their knowledge of how to test for pluripotent cells (From part 3) to testing engineered cells.

Preparing for class

- Each lab group will need the following:
    1. Growth Factor 1 (Same as part 3)
    2. Growth Factor 2 (Same as part 3)
    3. At least 3 microtubes

    **Or, students can use the growth factor combinations they made in part 3

    4. Cryptic Ron Stem Cells (same as part 3)
    5. 1mL "Engineered Nanog" (See recipe below)
    6. 1mL "Engineered C-myc" (See recipe below)
    7. Disposable transfer pipettes (4 if using pre-made combinations, 6 if making fresh combinations)
    8. Clear 12 well strips (can also use 12 well plates or, if students are careful, a sheet of saran wrap placed on white paper
    9. Gloves

"Engineered Nanog" and "Engineered C-myc":

- A 50:50 dilution of 2% Methyl Red and 2% thymolphthalein
    1. Make 2% Methyl Red by mixing 2g of Methyl Red powder with 70mL 100% ethanol. When Methyl Red is dissolved, add 30mL water to make up 100mL 2% Methyl Red solution
    2. Make 2%mg/mL thymolphthalein by mixing 2g of thymolphthalein powder with 70mL 100% ethanol. When thymolphthalein is dissolved, add 30mL water to make up 100mL 2% thymolphthalein solution

Part 6: The real research

Summary:

This section is here to point out that the work that they did has basis in real life. Vanishing White Matter disease is a fatal disease that affects children, and Dr. Proschel's study on stem cells went a long way to understanding how to treat this disease.

What they did to "insert" two genes and turn an adult stem cell into a pluripotent stem cell also has basis in real research. "Induced Pluripotent Stem Cells" or "IPSC" came to the forefront of stem cells research in 2008, with the discovery that human skin cells could be induced to be like embryonic stem cells by turning on four genes, two of which were Nanog and C-myc. While the research is an exciting step towards being able to harness the power of pluripotent cells without the controversy involved in embryonic stem cell research, it's just a step, and much more research needs to be done.