Part 1: Stem Cell Biology

Specialized: When referring to a cell, these cells have specific structures that allow them to carry out specific functions and tasks.

Culture: when referring to cells, it means to let cells grow and divide in a dish or tube.

Cell line: A population of cells grown in culture that is made from, and therefore genetically identical to, a single common ancestor cell.

It's a dark and stormy night, and Agent X and Agent Z are creeping up on an abandoned warehouse, preparing for a midnight raid.

Agent X: What's in here anyhow? This is creepy…

Agent Z: Shhh! We've heard rumors that a rogue stem cell has started to culture himself.

Agent X: Not CrypticRon Stem Cell!?

Agent Z: That's right…we've suspected that Ron was an embryonic stem cell for awhile. He has the three characteristics of stem cells.

- He's not **specialized**.
- He gives rise to cells that go on to become specialized cells.
- When he does divide, he makes an exact copy of himself so that he can keep doing this for long periods of time.

Agent X: Crazy…but is this a problem? Researchers **culture** stem cells all the time.

Agent Z: True, but Ron has been taking federal money for a few years now. In 2001, a law was passed saying that federal grant money could not be used to create new embryonic stem **cell lines**.

Agent X: And CrypticRon has only been around since 2003…

Agent Z: That's right. This means he's illegal if he's taking federal money. Let's go!

Agents X and Z rush into the warehouse, where they are faced with a massive group of cells staring at them in confusion.

Agent X: Which one of you is CrypticRon Stem Cell!!??

Cell: You're too late!! CrypticRon took off! You'll never find him! He's hiding in this head of cauliflower, disguised as a plant cell! Just try and find him amongst all these cells!*

Agent Z: Oh we'll find him…he can't hide his stemness from us...

**In real life, human cells can not turn into plant cells, or vice versa. But CrypticRon is another story…*

EXPERIMENTAL PROCEDURE: How will you find CrypticRon (a stem cell) in a plant?

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Part 2: Stem Cell Biology

Agent X: Give it up CrypticRon…we've got you now.

Ron: *(defiant)* Got what? You got nothin' on me!

Agent Z: Oh yeah? We'll just see about THAT! I've got the DNA from the two adults that your **in-vitro fertilized embryo** was made from and we'll match you to them. They made their embryos in 2002; a full year after the federal law was passed!

Agent X: We'll just take a sample of your DNA then…

Ron: Are you crazy? I'm a single cell; if you take my DNA I'm dead!

Agent X: Oh. Right. Never mind. We'll take DNA from one of your **progenitor cells**. You can always make more, right, stem cell guy?

In-vitro fertilized embryo (IVF embryo): An embryo produced by mixing sperm and an egg in a laboratory dish.

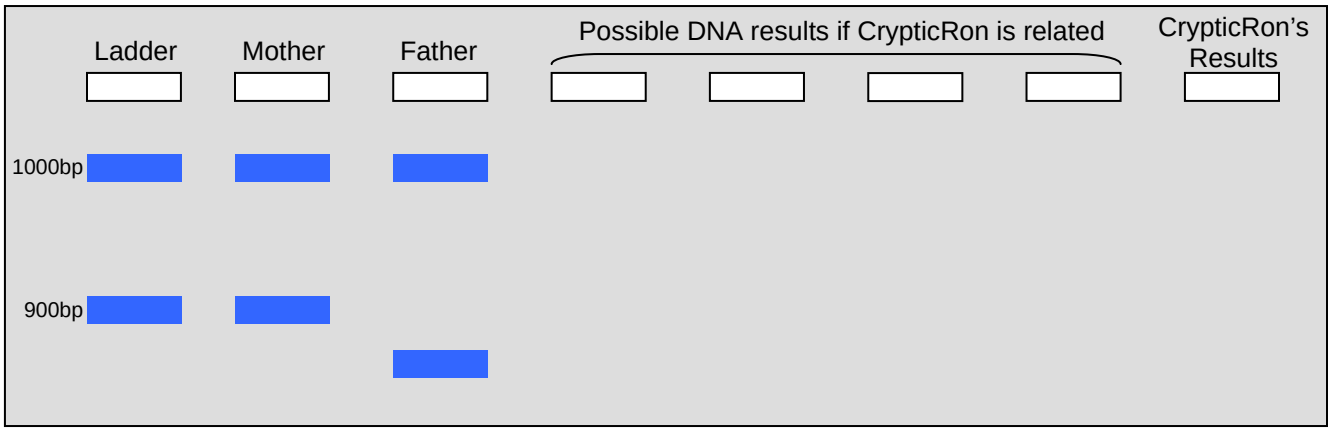
Progenitor Cells: Partly specialized cells, these are daughter cells of a stem cell. Progenitor cells divide to give rise to more and more specialized cells.

LABORATORY OBSERVATIONS:

Humans have 2 copies of every chromosome. One from the father and one from the mother. Shown below are the DNA fingerprints of CrypticRon's alleged donor father and mother. The DNA fingerprint shows the two copies of a gene from each person being tested. This gene can come in different sizes, which gives each person a unique pattern of DNA when it is run on an agarose gel.

If CrypticRon is related to the donor father and mother shown below, what might his DNA gel results look like?

On your gel – load 10 microliters of Ladder into one well, and 10 microliters of CrypticRon DNA into another. Run your gel at 120V for about 10 minutes, then make your observations...



Conclusions:_____

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Part 3: Stem Cell Biology

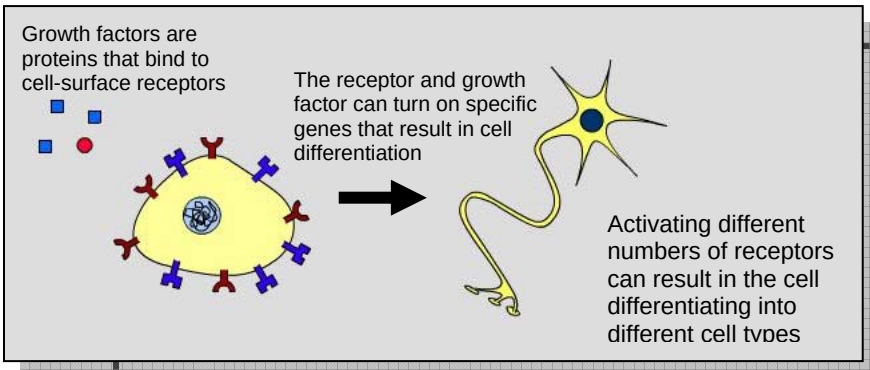
Agent Z: This is it Ron, read it and weep…you're a match.

Ron: No, wait! I'm not an embryonic stem cell!!

Agent X: Yeah right…you exhibit all the qualities of a stem cell, and the two donors who made your IVF embryo don't have any kids.

Ron: *(angry)* Amateur! They DID have a child…but she died of a genetic disease before she turned 3. You see that DNA? I have two copies of a mutation. I'm an adult stem cell that was taken from the child after she died.

Agent X: *(shocked)* ADULT stem cell? What's that?



You are given:

- Growth factor 1
- Growth factor 2
- An embryonic stem cell line
- CrypticRon's stem cell line
- Empty microfuge tubes

Adding different combinations of Growth Factors 1 and 2 will result in differentiation into different cell types, depending on the potential of the cell to differentiate.

Yellow: Red and white blood cells
Red: Neurons and Glial (support) cells of the brain
Green: Muscle cells

How will you determine if CrypticRon is an embryonic or adult stem cell?

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EXPERIMENTAL PROCEDURE

Combinations of Growth Factors (you can do up to 4 different combinations)

_____mL GF-1 _____mL GF-2	_____mL GF-1 _____mL GF-2	_____mL GF-1 _____mL GF-2	_____mL GF-1 _____mL GF-2

Mix together 1 drop of cells and 1 drop of growth factors in the clear 12 well-strips and record your observations.

LABORATORY OBSERVATIONS:

Conclusions_____

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Part 4: Stem Cell Biology

White matter – whitish nerve tissue of the brain and spinal cord, consisting mostly of nerve fibers coated in an insulating layer of myelin

Expression – When referring to a gene, expression refers to whether a gene is on (transcribed to RNA) or off (not transcribed to RNA)

Agent Z: OK CrypticRon…you're right. You're an adult stem cell. But why are you culturing yourself? You've got a genetic defect. What are you trying to do? Make more genetically defective cells?

Ron: Actually yes. But I want to study them. I think my kid died because something was wrong with her brain cells…but what?

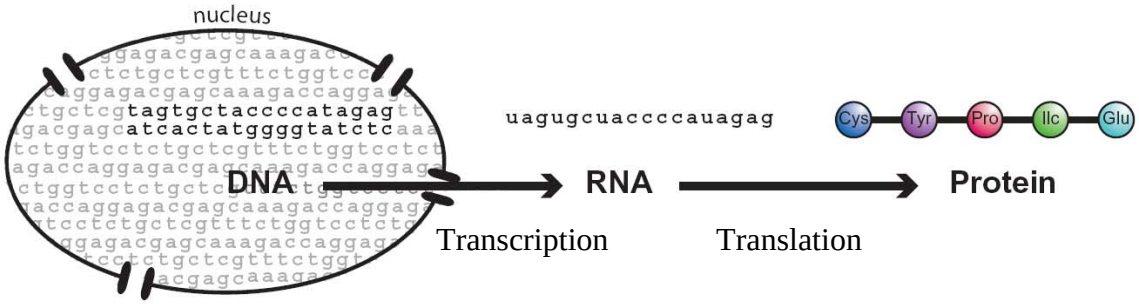
Agent X: I thought you might have that question, so I brought Dr. Chris Proschel (*Prosh-EL*) from the University of Rochester Medical Center. He studied a patient who had a similar problem

Dr. Proschel: Our patient had something called Vanishing White Matter disease. I'm not sure if that's the same thing your child had, but in this case, all the cells of the **white matter**, cells called "oligodendrocytes" (*Awl-i-go-DEN-dro-sites*) slowly disappeared.

Ron: Were those cells defective?

Dr. Proschel: That's what we all thought we would find, but when we isolated and studied the neural stem cells, what we found is that the patient's neural stem cells could make normal oligodendrocytes, but could not differentiate into astrocytes (*AS-tro-site*). Astrocytes are a type of support cell in the brain.

Ron: If only I could have been a neural stem cell…I could do the same type of study. How could that be done?



There is always double stranded DNA in the nucleus. Some of the sections of DNA code for genes. The genes can be transcribed into RNA whenever the gene product (the protein) is needed in the cell.

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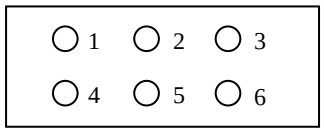
PROTOCOL: Setting up and using a microarray:

1) Place one microarray chip into a disposable plastic tray

2) Apply some of each **DNA solution** onto the correct spot using a Q-tip.



Dip a clean Q-tip into each DNA solution (key shown below). Touch the Q-tip to the chip to apply some of the DNA solution onto the correct spot on the chip. (DNA 1 should go in spot 1, etc)

3) After all six DNA solutions have been applied, apply the **Cryptic Ron (Adult Stem Cell) RNA** onto each spot using a Q-tip. Dip the Q-tip in the RNA solution ONCE, and apply to all six spots.

4) Wait for 20 seconds

5) Dip a clean Q-tip into the **developing solution** and gently touch each of the circles. Record your observations in the space below. If the circle turns purple, it means that that gene is being transcribed and translated.

LABORATORY OBSERVATIONS

Complete the microarray for CrypticRon, and compare it to the Embryonic Stem Cell microarray shown below:

1. Use a Q-tip to spot each of the genes onto the appropriate spot (for example, "gene 1: C-myc," should be spotted onto the spot labeled "1."
2. Allow the spots to dry (About 30 seconds)
3. Apply "CrypticRon (Adult Stem Cell) RNA" to each of the spots using another Q-tip. Dip the Q-tip into the "CrypticRon RNA" and dab a bit on each spot. There's no need to change Q-tips…just use one Q-tip for all the spots
4. Apply "Developing Solution" to each spot. Dip the Q-tip into the "Developing solution" and dab a bit on each spot. If the there's CrypticRon RNA that matches the genes, the RNA will have stuck, and will be turn purple when the "Developing solution" is applied.

Gene 1: c-myc (transcription factor)

Gene 2: nanog (transcription factor)

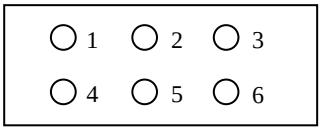
Gene 3: Heat shock factor (stress enzyme)

Gene 4: Ribosomal protein

Gene 5: Myosin (muscle protein)

Gene 6: Cyclin D (Cell cycle gene

CrypticRon Stem Cell (adult) gene expression



Conclusions:_____

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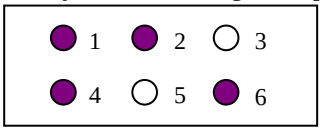
Embryonic stem cell gene expression (data filled in)



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Part 5: Stem Cell Biology

Transcription Factors
In humans, most genes are not transcribed unless a transcription factor (TF) is present. The presence or absence of a transcription factor can turn a gene or set of genes on or off.

Agent X: We found two genes that are actively expressed in the embryonic stem cells and are not expressed in you.

Ron: Maybe if I could turn on those genes…I might behave like an embryonic stem cell!

Agent Z: Wait a minute here…you only looked at 8 genes! AND, how can just two proteins change a multipotent cell into a pluripotent cell?

Dr. Proschel: Those proteins are **transcription factors** – they are responsible for turning genes on and off. By putting in just one transcription factor, you could be turning on or off many other genes down the road. And look! One of the genes is *c-myc*, a transcription factor that's known to be important in keeping cells undifferentiated.

Agent X: It's worth a try. We can add these genes to see if they make CrypticRon pluripotent.

EXPERIMENTAL PROCEDURE:

Mix Blood Stem Cells (BSC) with genes

1. Label a clean tube "BSC + genes"
2. add 4 drops Blood Stem Cells, 2 drops _____

Test the cells in each tube with the growth factors as you did in part 3 to see if the blood stem cells can differentiate into more different types of cells

OBSERVATIONS

Conclusions_____

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Important brain finding results from boy's rare, fatal disease

On May 7, 2002, 12-year-old Nathan Van Vleck of Pittsford died after a nearly lifelong fight with an exceedingly rare inherited disease known as vanishing white matter (VWM) disease. As Nathan's illness progressed, the family discussed how it might help other families and patients coping with VWM, and the family decided to allow the study of some of Nathan's brain cells for research purposes. Immediately upon his death in the hospital, a team of neuropathologists and neurobiologists worked through the night to isolate some of Nathan's brain cells, which were then grown and studied in the laboratory.

Chris Proschel, research assistant professor in the Department of Biomedical Genetics, led the laboratory study of Nathan's cells. Since doctors know that the disease affects myelin, the fatty material that insulates nerves and allows them to send their signals crisply, Proschel's team expected to find defects in or a shortage of oligodendrocytes, the brain cells that produce myelin.

The team found no such thing. The oligodendrocytes looked normal and were present in healthy numbers – but the team did observe a dearth of astrocytes, and those that were present did not appear healthy. "Normally, astrocytes are much easier to grow than fragile neurons or oligodendrocytes," says Proschel. "So the last thing you'd expect is fewer astrocytes."

"This is another step towards closing the gap between the origin of the disease and how it is manifested in children," Proschel says. "Chances are that someday, we'll have to try to replace either the defective gene or the defective cells. That will mean either gene or cell therapy. In both cases, you must deliver the payload to the right target. This paper shows that if you target only the oligodendrocytes, you might miss the target."

Adapted from http://www.eurekalert.org/pub_releases/2005-04/uorm-ibf042505.php

Feb 2008

The researchers genetically altered human skin cells using four regulator genes, according to findings published online in the Feb. 11 edition of the journal *Proceedings of the National Academy of the Sciences.*

The result produced cells called induced pluripotent stem cells, or iPS cells, that are almost identical to human embryonic stem cells in function and biological structure. The reprogrammed cells also expressed the same genes and could be coaxed into giving rise to the same cell types as human embryonic stem cells, the researchers said.

"Our reprogrammed human skin cells were virtually indistinguishable from human embryonic stem cells," lead author Kathrin Plath, an assistant professor of biological chemistry and a researcher with the Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, said in a prepared statement. "Our findings are an important step towards manipulating differentiated human cells to generate an unlimited supply of patient specific pluripotent stem cells. We are very excited about the potential implications."

"Reprogramming normal human cells into cells with identical properties to those in embryonic stem cells…may have important therapeutic ramifications and provide us with another valuable method to develop human stem cell lines," study first author William Lowry, an assistant professor of molecular, cell and developmental biology, said in a prepared statement. "It is important to remember that our research does not eliminate the need for embryo-based human embryonic stem cell research, but rather provides another avenue of worthwhile investigation."

Adapted from http://www.forbes.com/forbeslife/health/feeds/hscout/2008/02/11/hscout612519.html

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