**Diagnosing Niemann Pick Disease**

**STUDENT HANDOUT-Modified from SANFORD PROMISE LAB**

**Overview**

Niemann-Pick disease is a rare inherited neurological condition involving lipid metabolism, which is the breakdown, transport, and use of fats and cholesterol in the body. In people with this condition, abnormal lipid metabolism causes harmful amounts of lipids to accumulate in the spleen, liver, lungs, bone marrow, and brain. Due to the accumulation of lipids, the disease is progressive and life-threatening.

Niemann-Pick disease type C usually appears in mid-to-late childhood, although infant and adult onsets are possible. Signs of Niemann-Pick disease type C include severe liver disease, breathing difficulties, developmental delay, seizures, poor muscle tone (dystonia), lack of coordination, problems with feeding, an inability to move the eyes vertically, and enlargement of the spleen or liver. People with this disorder can survive into adulthood. The rarity of Niemann-Pick type C and the heterogeneous nature of symptoms mean that it is often misdiagnosed or goes undetected. Niemann-Pick disease type C is further subdivided into types C1 and C2, each caused by a different gene mutation.

**Objective**

Your summer job is as intern in a genetics lab at a Mount Blueberry Children’s hospital. A doctor comes to your team and says that he has a family in which he suspects three cousins of all have Niemann-Pick type C disease. Testing to confirm NP-C typically includes biochemical testing of fibroblasts from skin biopsy, histological analyses of cells in bone marrow, MRI imaging to detect cerebral atrophy, and genetic testing.   
The family would like to confirm that:  
 1) the children indeed have Niemann-Pick type C  
 2) what are the risks of future children in the family developing the disease.

The doctor has collected DNA from the following patients:

Grandpa Jones

Grandma Jones

Terry Jones (son)

Lisa Jones (wife of Terry)

Becky Jones (daughter of Terry and Lisa – died @ 17 with disease related symptoms)

Bobby Jones (son of Terry and Lisa – thought to have the same problems as sister Becky)

Rita Robinson (daughter)

Rick Robinson (husband of Rita)

Phil Robinson (son of Rita and Rick)

Paul Jones (son)

Gina Jones (ex-wife of Paul)

Ryan Jones (non-identical twin son of Gina and Paul – show symptoms of disease)

Raelyn Jones (non-identical twin daughter of Gina and Paul)

Your job is the following:

1. Set up a PCR & DNA Electrophoresis screen with each of the patient samples to determine who is a carrier for the disease
2. generate a pedigree map for the family showing affected vs carrier patients

**Part 1 – Polymerase Chain Reaction (PCR)**

Polymerase chain reaction is a common method used to amplify specific regions of DNA for further analysis. There is an exponential increase in the number of DNA copies synthesized when performing PCR. For example, if you start with 1 molecule of DNA, there will be 2 identical copies after one cycle; 4 copies after two cycles; 8 copies after three cycles and so on.

There are several important steps in the PCR process:



Step 1: **Denature** the double-stranded DNA into single strands.

Step 2: **Anneal** the primers to a specific region of DNA.

Step 3: **Extend** by synthesizing new DNA using the enzyme DNA polymerase which uses the original strand as a template for nucleotide placement.

You will utilize PCR to amplify the region of DNA associated with the NPC1 gene on the long arm of chromosome 18 – which causes Niemann-Pick disease, type C1.

Protocol:

1. Centrifuge DNA sample at 12000rpm for 1 minute.
2. Transfer 5uL of DNA sample to 0.2mL PCR tube.
3. Add 20uL of PCR reaction mix to DNA sample in 0.2mL PCR tube and pipet up and down to mix.
4. Label tube and place into the thermocycler and run PCR cycle:

95˚C for 2 minutes

95˚C for 30 seconds

60˚C for 30 seconds 20 cycles

72˚C for 1 minute

72˚C for 5 minutes

1. PCR cycle will take just under 1 hour to complete.

**Part 3 – DNA Electrophoresis**

Now that you have performed a PCR reaction to amplify *NPC1*, we need to analyze these samples using DNA electrophoresis to separate DNA based on the physical properties: size.

Protocol:

1. Assemble DNA gel electrophoresis equipment.
2. Make a 1% Agarose Gel by:
   1. Adding 0.7g agarose powder to flask containing 70mL TAE buffer and swirl to mix.
   2. Microwave for 1 minute, swirl solution and incubate for additional 45 seconds. Solution will be very hot and be careful to avoid overflow during heating.
   3. Add 3uL of GelRed to agarose solution and swirl to mix.
   4. Pour solution into casting tray and cool at room temperature for 20 minutes.
3. Turn gel 90 degrees so wells are on negative side of gel box.
4. Submerge gel in TAE buffer and remove comb.
5. Load the gel with 10uL of DNA Ladder in one well and 15uL of PCR sample in the remaining wells. Be sure to hold sample in micropipette directly above well in TAE buffer. Pipet slowly to make sure sample enters well and to avoid any sample loss.



1. Attach lid and run gel at 100 volts for approximately 45 minutes.
2. Check box for bubbles on both sides; after ~5 minutes, ensure color is traveling in correct direction.
3. Remove casting try and gel and view on UV box.

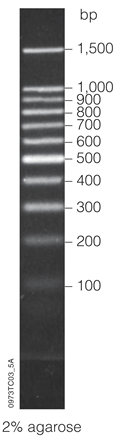


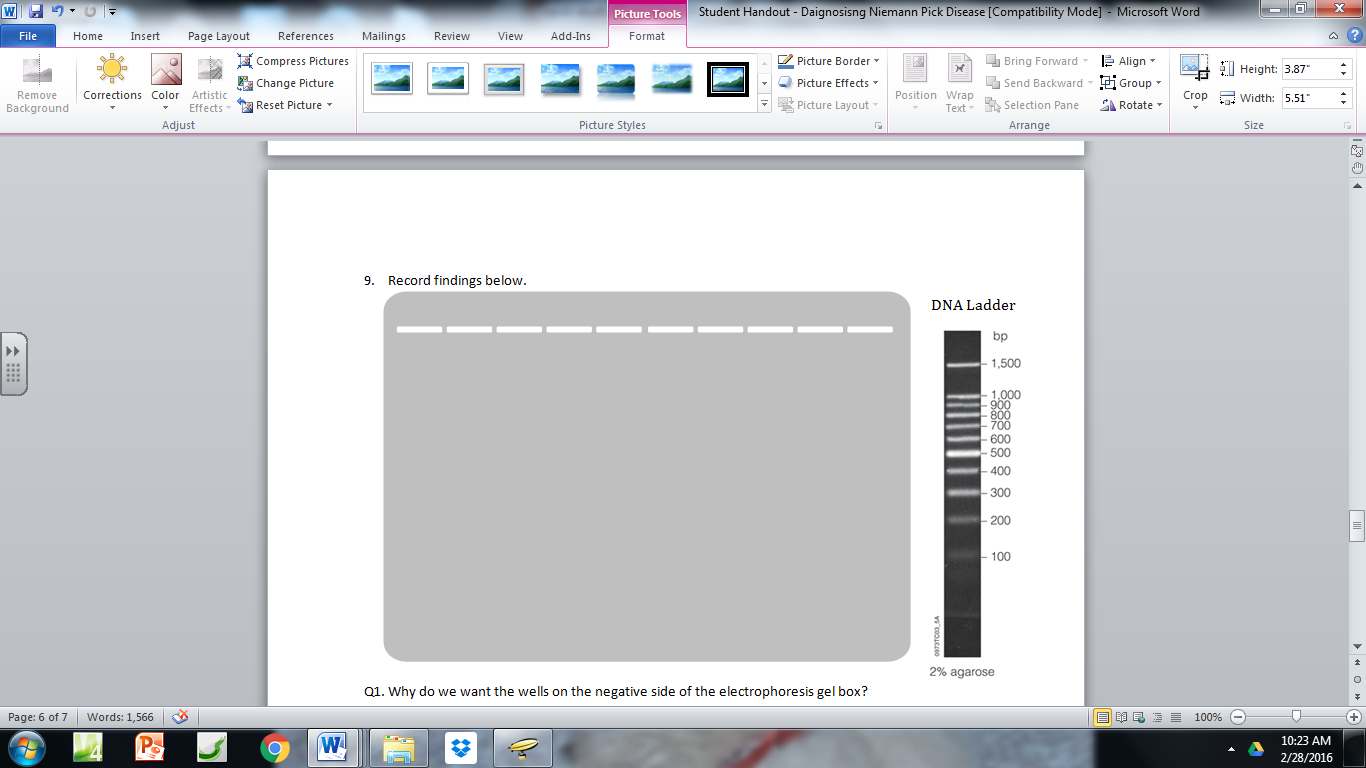
GelRed in the agarose gel binds to DNA and gives off visible light (that we can see) when excited by UV light so that we can distinguish DNA on the gel.

Record findings below.

**JONES FAMILY**

DNA Ladder





**Part 2 – Generating a Family History**

**How is Niemann Pick Inherited?**

This condition is inherited in an autosomal recessive pattern, which means both copies of the gene in each cell have mutations. The parents of an individual with an autosomal recessive condition each carry one copy of the mutated gene, but they typically do not show signs and symptoms of the condition.

**What is the genetic cause of Niemann Pick type C?**

Mutations in either the NPC1 or NPC2 gene cause Niemann-Pick disease type C. The NPC1 gene provides instructions for producing a protein that is involved in the movement of cholesterol and lipids within cells. A deficiency of this protein leads to the abnormal storage of lipids within cells as seen in people with Niemann-Pick disease type C1. The NPC2 gene provides instructions to produce a protein that binds and transports cholesterol. Reduced or absent levels of this protein lead to the abnormal accumulation of lipids and cholesterol in the cells as seen in people with Niemann-Pick disease type C2. The exact functions of the NPC1 and NPC2 proteins are not fully understood.

**CREATE A PEDIGREE SHOWING THE JONES FAMILY showing family relationships and the results of your DNA ANALYSIS**

Questions:

PCR  
  
1. What components are in the PCR sample?

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2. How is a specific region of DNA targeted for amplification during PCR?

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3. Why is Taq DNA polymerase used instead of human DNA polymerase in the PCR mix?

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3. What occurs during the following steps in PCR:

Denaturation \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Primer annealing \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Extension \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

GEL ELECTROPHORESIS

4. Why do we want the wells on the negative side of the electrophoresis gel box?

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5. Instead of running the gel with a “ladder”, you ran lanes containing Unaffected control (UC), affected control (AC) and carrier control (CC) DNA. EXPLAIN WHY A LADDER WASN’T USED? (Don’t say they were controls)

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