

The Sanford PROMISE

Diagnosing Niemann Pick Disease

STUDENT HANDOUT



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 means 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 and 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 – shows symptoms of disease)

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

The mission of the Sanford PROMISE is to increase the community's understanding of science and their awareness about the benefits of research to our society.

SANFORD
RESEARCH

The Sanford PROMISE · 2301 E 60th Street North · Sioux Falls SD 57104 · P 605.312.6417 ·
SanfordOutreach@SanfordHealth.org · www.SanfordResearch.org/Education

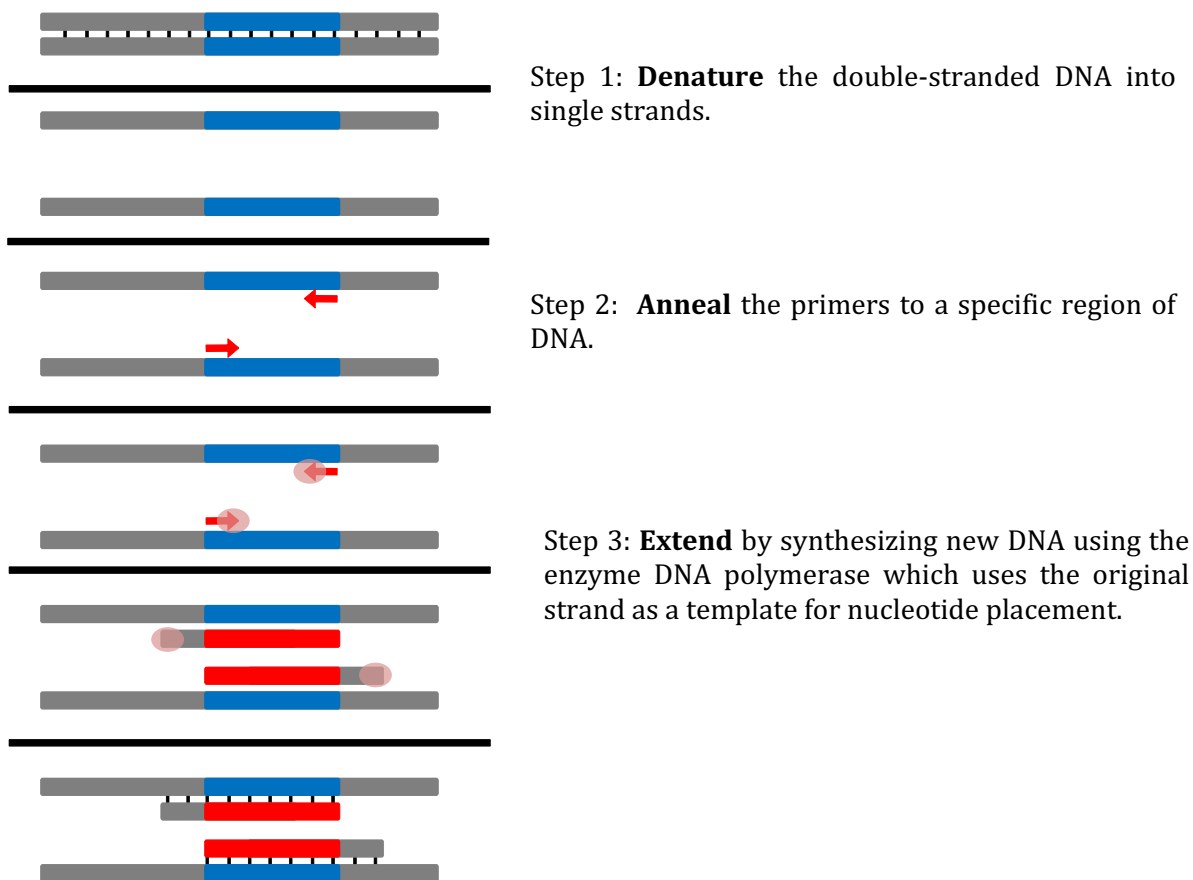
Your job is the following:

- 1) Set up a PCR & DNA Electrophoresis screen with each of the patient samples to determine who is a carrier for the disease and 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:



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	20 cycles
95°C for 30 seconds	
60°C for 30 seconds	
72°C for 1 minute	
72°C for 5 minutes	

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

Questions:

Q1. What components are in the PCR sample?

Q2. How is a specific region of DNA targeted for amplification during PCR?

Q3. What occurs during the following steps: denaturation, primer annealing, and extension?

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.

How do we track family history?

See Family History handout

Questions:

Q4. Based on the information provided to you from the doctor (ie., family relationships) generate a family history map of the Jones family (we will use our PCR results in determine affected/carriers)?

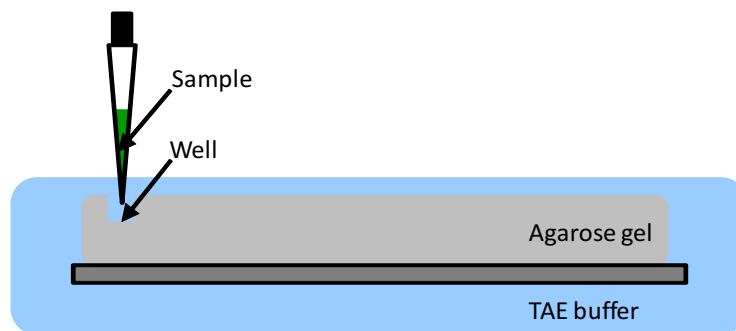
JONES FAMILY

Part 3 – DNA Electrophoresis

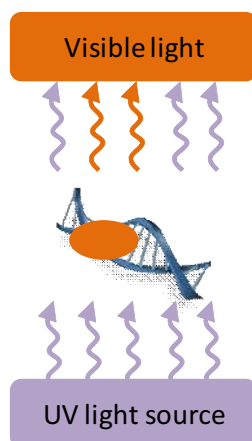
Now that you have performed a PCR reaction to amplify *NPC1*, we need to analyze these samples using DNA electrophoresis to separate DNA based on the physical properties: size.

Protocol:

1. Assemble DNA gel electrophoresis equipment.
2. Make a 1% Agarose Gel by:
 - a. Adding 0.7g agarose powder to flask containing 70mL TAE buffer and swirl to mix.
 - b. 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.
 - c. Add 3uL of GelRed to agarose solution and swirl to mix.
 - d. 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.



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

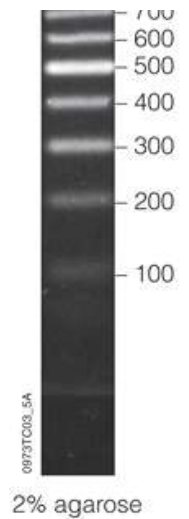


GelRed in the agarose gel binds to DNA and gives off visible light (that we can see) when excited by UV light so that we can distinguish DNA on the gel.

9. Record findings below.



DNA Ladder



Q1. Why do we want the wells on the negative side of the electrophoresis gel box?

Q2. What size DNA band(s) did you visualize in your PCR sample?

Q3. Was your sample positive or negative for the mutation? Support your conclusion with your data.

Q4. Return to your family history and determine which patients are affected vs. carriers? Fill them in on the family history.

Career Information

Clinical Geneticist

A physician that specializes in the field of genetics and how it applies to human health and disease. Services include diagnostics (screening), counseling, and management of birth defects and genetic diseases.

Clinical Geneticist - <http://medicine.jrank.org/pages/2049/Clinical-Geneticist.html>

Genetic Counselor

Provide information and support to families who have members with birth defects or genetic disorders and to families who may be at risk for a variety of inherited conditions. They identify families at risk, investigate the problem present in the family, interpret information about the disorder, analyze inheritance patterns and risks of recurrence, and review available options with the family.

Genetic Counselor –

http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genecounseling.shtml

Regional Highlights

Several regional companies perform work with relevance in the concept or biotechnology used in this activity:

Sanford Health (Sioux Falls, SD) - <http://www.sanfordhealth.org>

Sanford employs clinical geneticists and genetic counselors that work to diagnose, counsel, and manage patients with birth defects and genetic diseases.

Identity Genetics Inc. (Brookings, SD) - <http://identitygenetics.com/>

This company specializes in DNA biotechnology for family relationships (paternity testing) and forensic analysis of crime scene samples by PCR.

Glossary of Terms

Centrifuge – a piece of laboratory equipment that separates samples according to density through rotation.

DNA ladder – a set of known DNA sizes (in base pairs) used to determine the size of unknown DNA

DNA Polymerase – an enzyme that will synthesize dNTPs into DNA based on an existing DNA template

dNTP (deoxyribonucleic triphosphate) – the functional unit of DNA which may be either adenine (A), thymine (T), cytosine (C), or guanine (G)

GelRed – a DNA-binding dye that will give off visible light when excited by a UV light source so that we can visualize DNA on a gel

Gene – a DNA unit of heredity that encodes for an RNA or protein

Genome – an organism's entire genetic material, typically DNA

Genotype – the genetic makeup of an organism with specific reference to a particular gene

Heterozygous – two different genes are located at the same location on both chromosomes of an individual

Homozygous – two identical genes are located at the same location on both chromosomes of an individual

Lipid metabolism - the breakdown, transport, and use of fats and cholesterol in the body

Polymerase chain reaction (PCR) – a technique used to amplify specific regions of DNA

Primer – short piece of DNA used as a starting point for DNA synthesis

Thermocycler – a machine that is capable of quickly changing and holding temperatures that is typically used during PCR