

## LESSON ONE: LOOKING THROUGH A FATHER'S EYES

**KEY QUESTION(S):** What is Pompe disease? What is the fundamental biology of the disease?

**OVERALL TIME ESTIMATE:**

- Advanced Preparation: 60 minutes (30 minutes to assemble section packets; 30 minutes background reading)
- Student Procedure: 45-60 minutes

**LEARNING STYLES:** Visual and auditory

**VOCABULARY:**

**Pompe disease:** Pompe disease is an inherited disorder caused by the buildup of a complex sugar called glycogen in the body's cells. The accumulation of glycogen in certain organs and tissues, especially muscles, impairs their ability to function normally. There are three types of Pompe disease, which differ in severity and the age at which they appear. These types are known as classic infantile-onset, non-classic infantile-onset, and late-onset.

**Lysosome:** Lysosomes are cellular organelles that contain acidic digestive enzymes to break down waste materials and cellular debris.

**Enzyme:** Enzymes are proteins that catalyze (i.e., increase the rates of) chemical reactions. Almost all chemical reactions in a biological cell need enzymes in order to occur at rates sufficient for life.

**DNA:** Deoxyribonucleic acid is a nucleic acid containing the genetic instructions used in the development and functioning of all known living organisms.

**LESSON SUMMARY:** A first person story is presented to the students to hook their interest in the disease. Using a jigsaw approach, students will learn about the fundamentals of Pompe disease and share information during a whole class discussion. This activity sets the stage for further investigation of Pompe disease specifically, but more generally the steps of translational research. This lesson also challenges students to think in reverse. We often know the symptoms and pathology of diseases and perform a differential diagnosis to determine which disease fits the symptoms, then proceeding with treatment accordingly. In this lesson, students will understand the basis of the disease, know what cellular structures are affected, deduce the symptoms and propose treatment.

**STUDENT LEARNING OBJECTIVES:**

The student will be able to...

1. Describe an enzyme.
2. Explain the role of an enzyme in the human body.
3. Define gene and understand how a gene relates to a protein
4. Explain where a lysosome is located and its role in the function of a cell
5. Explain the genetics of Pompe disease
6. Associate disease pathology with symptoms
7. Suggest treatment methods for Pompe disease
8. Create a model (e.g., flow chart, concept map) of the cause and effect relationship of Pompe disease

**STANDARDS:**

SC.912.L.14.6  
SC.912.L.14.19  
SC.912.L.14.20  
SC.912.L.16.2  
SC.912.L.16.3

SC.912.L.18.1  
SC.912.L.18.2  
SC.912.L.18.4  
SC.912.L.18.11  
SC.912.N.1.6

**MATERIALS:**

- 1 copy of *Teacher Pages: Jigsaw – Enzymes - Lysosomes*
- 1 copy of *Teacher Pages: Section Name Cards*
- Envelopes or clips (for keeping information pages together)
- 1 copy of *Student Worksheet: Looking Through a Father’s Eyes* per student

**BACKGROUND INFORMATION:** Teachers are encouraged to read the student information (four sections: enzymes, molecular biology, genetics, and lysosome) prior to the activity. This activity specifically focuses on the fundamentals of Pompe disease to help students understand what it is so they can think further about how it might be diagnosed and treated.

**ADVANCE PREPARATION:**

1. Make section cards: *Teacher Pages: Section Name Cards*, laminate if desired, and cut into individual cards.
2. Make information section packets: *Copy Teacher Pages: Jigsaw – Enzymes - Lysosomes*. Laminate if desired. Cut into smaller reading sections by cutting between paragraphs. Place all slips for a section in an envelope or clip together.
3. Make 1 copy of *Student Worksheet: Looking Through a Father’s Eyes* for each student.
4. Make overhead or prepare paper copies of JAMA overview of basic science research <http://jama.ama-assn.org/content/287/13/1754.full.pdf>
5. If having a student read Calum’s story, make an extra copy for the student to read from.

*Implementation note: For students and classrooms unaccustomed to jigsaws, it can seem a bit confusing and chaotic. Have patience. Collaborative learning experiences are a valuable part of scientific discovery.*

**PROCEDURE AND DISCUSSION QUESTIONS WITH TIME ESTIMATES:**

1. (2 minutes) Read aloud or ask a student to read Calum’s story aloud.
2. (5 minutes) Ask the students to share their thoughts on the story. Record their comments/questions on the board or other medium that can be referenced throughout the unit (i.e., flip chart paper, transparency, Smartboard, etc.) Students may note the year 1993 and suggest that surely there is a cure or at least a treatment now. This is also a good time to assess prior knowledge (particularly related to the movie Extraordinary Measures).
3. (2 minutes) Tell the students they are going to have a chance to learn more about Pompe disease over the course of the next several classes. Their first task is to learn the fundamentals of the disease (the basic science).
4. (5 minutes) Project the JAMA overview of basic science research. Allow students to read and summarize the main points. <http://jama.ama-assn.org/content/287/13/1754.full.pdf>

*Implementation note: Optional stopping point and/or inclusion of an enzyme activity.*

5. (5 minutes) Have students assemble into groups of 4. This is their home group, so encourage them to remember the members of their home group. For a class with extra students, have them join to make groups of 5 rather than have a group without a member which would put extra burden on the smaller group to read more.
- Once settled, give each member a card with one of the following section names: Enzymes, Molecular Biology, Genetics, or Lysosome. *See Teacher Pages: Section Name Cards.*

Group	Member A	Member B	Member C	Member D
1	Enzymes	Molecular Biology	Genetics	Lysosome
2	Enzymes	Molecular Biology	Genetics	Lysosome
3	Enzymes	Molecular Biology	Genetics	Lysosome
4	Enzymes	Molecular Biology	Genetics	Lysosome
5	Enzymes	Molecular Biology	Genetics	Lysosome
6	Enzymes	Molecular Biology	Genetics	Lysosome
7	Enzymes	Molecular Biology	Genetics	Lysosome
8	Enzymes	Molecular Biology	Genetics	Lysosome

6. Ask students to regroup according to their section name, forming four large groups.

*\*You can allow students to stay in these large groups, or divide them in half yielding 2 Enzyme sections, 2 Molecular Basics sections, 2 Genetics sections, and 2 Lysosome sections. This option requires producing two copies of each section information packet.*

Section Name	Group	Member #
Enzymes	Group 1-8	Member A
Molecular Biology	Group 1-8	Member B
Genetics	Group 1-8	Member C
Lysosome	Group 1-8	Member D

7. Distribute information packets to each section.
8. Distribute *Student Worksheet: Looking Through a Father's Eyes* to each student.
9. (10 minutes) Have students remove and equally distribute the information slips from the envelope, read their slip(s), and share with the other members of their section. Encourage students to take notes and summarize their section to share back in their home group. Move around the groups to ensure understanding.

10. Ask students to put their information slips back in the envelope or clip. Have one member return the envelope to the front of the room while the groups redistribute back to their home group.

Group	Member A	Member B	Member C	Member D
1	Enzymes	Molecular Biology	Genetics	Lysosome
2	Enzymes	Molecular Biology	Genetics	Lysosome
3	Enzymes	Molecular Biology	Genetics	Lysosome
4	Enzymes	Molecular Biology	Genetics	Lysosome
5	Enzymes	Molecular Biology	Genetics	Lysosome
6	Enzymes	Molecular Biology	Genetics	Lysosome
7	Enzymes	Molecular Biology	Genetics	Lysosome
8	Enzymes	Molecular Biology	Genetics	Lysosome

11. (10 minutes) Once back in their home groups, the students should each take a turn sharing what they learned about Pompe disease in their section group. Again, move around the groups to ensure understanding. They can use the *Student Worksheet: Looking Through a Father's Eyes* to guide their discussion.
12. (10-15 minutes) Drawing from the student comments/questions from the beginning and using the *Student Worksheet: Looking Through a Father's Eyes*, call on groups to answer the questions on the worksheet aloud. Use the *Teacher Answer Key* to check for understanding. Encourage other groups to share their interpretations as well. Clarify uncertain concepts. Students may need to be guided to answer questions 5 – 7 on the worksheet or these may be left for students to complete on their own. See the *Teacher Answer Key* for suggested questions to pose.
13. Conclude by telling the students they should now understand the cause of the disease and have suggested symptoms and treatment. This is the beginning of the basic science of translational research, the process of producing a therapeutic product. Next they will look closer at the history and future of Pompe disease.

#### ASSESSMENT SUGGESTIONS:

- Student worksheet can be checked for completion.

#### EXTENSIONS:

ACTIVITIES: An enzyme lab to demonstrate enzyme activity-

- Peroxide activity: [http://www.biologycorner.com/worksheets/enzyme\\_lab.html](http://www.biologycorner.com/worksheets/enzyme_lab.html)
- Pineapple juice gelatin lab: [http://mdk12.org/instruction/curriculum/hsa/biology/enzyme\\_activity/teachers\\_guide\\_engagement2.html](http://mdk12.org/instruction/curriculum/hsa/biology/enzyme_activity/teachers_guide_engagement2.html)

#### RESOURCES/REFERENCES:

- Background information: Online Mendelian Inheritance of Man <http://omim.org/entry/232300>

- Background information: <http://www.amda-pompe.org/index.php/main/about/amd/>
- Background information: <http://www.pompe.com/en/>
- Background information and informational materials: <http://www.pompe.org.uk/>
- Calum's story: <http://pompestory.blogspot.com/2009/04/calum.html>
- Additional patient stories: <http://www.amda-pompe.org/index.php/main/patients/>
- Section information: A Guide for Families by Kevin O'Donnell, 1994  
[http://www.pompe.org.uk/images/stories/guide\\_for\\_families.pdf](http://www.pompe.org.uk/images/stories/guide_for_families.pdf)
- Rare Disease Day, February 29, 2012: <http://www.rarediseaseday.org/>

## CALUM'S STORY

Written by Kevin O'Donnell

[http://pompestory.blogspot.com/2009\\_04\\_01\\_archive.html](http://pompestory.blogspot.com/2009_04_01_archive.html)

It was Friday, May 28, 1993 and one of the happiest days of my life. I was driving across the border from England into Scotland, to a new home in Edinburgh - the city I had always wanted to live in - and to my dream job, running my own molecular biology laboratory. Best of all, I had my family in the car with me - my wife Elaine and our two-month old baby son, Calum. Life was sweet. Yet within a few short months that sweetness turned to dust in my mouth.

Everything was fine for a couple of months. I busied myself with settling in to my new job, while Elaine and Calum went house-hunting. Then Calum seemed to be getting a lot of colds and sniffles, which took longer and longer to go away. It never occurred to us for one moment that something could be seriously wrong. Even when, at six months old, Calum was hospitalized with pneumonia we didn't think it was anything other than a temporary blip. In hindsight we were incredibly naive - but what does anyone know with their first child?

An x-ray showed an enlarged heart and, even then, we didn't think anything other than "So he shouldn't play rough sports? Guess he'll just have to be geeky like his dad!" Unknown to us though, alarm bells were beginning to ring at the hospital and blood tests were taken "Just routine, nothing to be concerned about..." And we weren't concerned. We found a house (next to a park - and a school - perfect!) and started fixing it up prior to moving in.

Then we got the news that brought our world crashing down. On a follow-up hospital visit we were told that the blood tests showed Calum had something called Pompe disease which was a type of glycogen storage disease. It was untreatable and fatal; children with this disease did not usually live beyond their first birthday.

We just couldn't believe it. We thought that there must have been some terrible mistake. Our beautiful child, the light of our lives, going to die? How could such a thing be possible? And whoever heard of an untreatable disease in this day and age - those doctors really needed to keep up with what was happening in the world of medicine!

For the next two weeks we frantically found out everything we could about Pompe disease and what little information there was available was not good. Everything confirmed what the hospital had told us; there was no hope. None. While we were still in shock, Calum declined quite rapidly and he went back into the hospital. He died at the Hospital in Edinburgh, on November 18, 1993, just two weeks after we were given the diagnosis.

## TEACHER PAGES: JIGSAW - ENZYMES

Pompe disease is caused by a defective enzyme, acid alpha-glucosidase (pronounced "AL-fa glue-CO-sih-days" and often abbreviated GAA). But what is an enzyme and why are they so important? In your body, right now, thousands of different chemical reactions are taking place. The chemical reactions that take place in the body are controlled by enzymes. Of direct interest to us, are the reactions concerning sugar.

Small units of sugar, glucose, are being bound together to make glycogen to use for energy later. In turn, when energy is needed, glycogen is broken down into small sugar molecules. Yet, if you take a lump of sugar and place it on your kitchen table you will wait a very long time before it turns into either energy or glycogen. You need a *catalyst*, something which will help the sugar to change into something else. In the body, **enzymes** are the catalysts which make reactions like this happen.

(images of glycogen and glucose)

<http://i585.photobucket.com/albums/ss297/stcasey34/glucose-metabolism.jpg?t=1276562215>

<http://www.balancingcenter.com/images/glucose-glycogen%20.jpg>

There are enzymes for all sorts of purposes: one enzyme may catalyze the formation of glycogen and another may break it down. Each one of the thousands of reactions which make up a human being is catalyzed by an enzyme. This is under strict control, otherwise there would be chaos. The control is done by the body being divided into compartments (of which we see more later) and by the activity of the enzymes being regulated by...other enzymes. You will gather from this that enzymes determine what we are and that living things are extremely complicated.

So, having explained what they do, what are enzymes? Enzymes are proteins, made up of a chain of building blocks called amino acids. There are twenty different amino acids and an almost infinite number of ways in which they can be arranged to make different proteins. Not all proteins are enzymes - some proteins are part of the body's structure. For example, muscle is largely protein, as are fingernails and hair. The function of a protein is determined by which amino acids make it up and how they are joined together. This is because although proteins are made up of a single long chain of amino acids, they don't exist as a long piece of string.

If a protein was big enough to see with the naked eye, it would look more like a tangled piece of string. This is because certain amino acids cause kinks in the chain, causing it to double back on itself or go at an angle. Also, some amino acids will form bonds with others they find themselves close to due to the kinks and bends in the chain. So, although it might look like a tangled piece of string, the shape of proteins is not random. Every time the body makes a copy of a particular protein it has exactly the same shape. This is important.

Remember that enzymes are in fact very tiny. They are not much bigger than the chemicals that they are joining or breaking apart. It is this specific 3-dimensional (3D) shape of enzymes that determines how they interact with the chemicals that make up the body. For example, an enzyme with one shape may hold two sugar molecules together, while another enzyme may break them apart. This is a simplified statement, but that is essentially how enzymes work. If an enzyme for some reason has a wrong amino acid in the chain, then its shape will be different and it may not work. That is what happens in metabolic diseases such as Pompe disease.

The next question is: how is this sequence of amino acids determined? And why does the wrong sequence sometimes get put together? The answer is in our genes.

## TEACHER PAGES: JIGSAW - MOLECULAR BIOLOGY

**DNA** (deoxyribonucleic acid) is the chemical in which all of our genetic information is stored. A complete copy of our DNA is in every cell in our body (except mature red blood cells which lack a nucleus). Like proteins, it is made up a series of building blocks. In the case of DNA however, there are only four building blocks called nucleotides: adenine, thymine, cytosine and guanine. We will refer to them as A, T, C and G. These are the letters in which the genetic code is written. These letters join together to make extremely long stretches of DNA called chromosomes. The letters are not joined together at random. They make up a series of 'words'. Each 'word' is made up of three letters, and signifies (or 'codes for') a specific amino acid. Each amino acid has at least one three letter 'word' coding for it, some have more than one. This is the genetic code.

Some 'words' (or **codons** as they are called) do not code for amino acids but are punctuation marks. They tell the machinery of the body which reads the genetic code to either start or stop reading. The 'words' in between a start and stop signal code for amino acids which, when joined together, will make up a single protein. This unit of information is called a **gene**. This is the way in which the information in DNA is used to make each protein and therefore all living things.

When cells grow, they divide into two, to make new cells. When this happens, the entire genome (three billion letters of DNA) must be copied (replicated). In each human, there are around 25,000 genes, each coding for thousands of nucleotides and hundreds of amino acids. Occasionally mistakes are made and one nucleotide letter is substituted for another. When a gene which contains a wrong letter is read, the wrong amino acid is placed in the protein. (An enzyme is a protein.) This can give the protein a different 3D shape.

With Pompe disease, we are concerned with the structure and function of the GAA protein which is an important enzyme. A misfolded protein may result in reduced function and performance of the enzyme. This is particularly damaging if the new 'word' formed with the wrong letter is the one which says 'stop reading', in which case a shortened enzyme is formed which will have no function at all. Also very damaging is when an extra base is inserted into the DNA sequence or one is deleted without being replaced. This means that from that point on, all the three letter 'words' will be wrong. For example, take AAT TTA GGC. If an extra C is inserted at the beginning, this will give CAA TTT AGG C. This is a completely different series of words giving a completely different, and possibly functionless, enzyme.

Changes in the DNA sequence are called mutations. Most go undetected; however, some are harmful. The DNA change may result in an enzyme produced which is not as good as the original one. This disrupts the normal function of the cell and body systems and can lead to severe complications. In a single cell, mutations usually don't matter very much and would not even be noticed. The problems come when the cell in which the mutated DNA is present is a germ cell (sperm or egg) which goes to make up a new person. This means that every cell of that person has a faulty copy of DNA. Even then, this is not a disaster since DNA exists in the form of chromosomes. We each have two complete sets of chromosomes, one from each parent. So, if one set has a faulty gene, the copy of the gene from the other parent can be used instead.

This is a great system, so good in fact that each of us has several faulty genes which are undetectable because we function perfectly well with the remaining functional copy. If we each have, say, 5 faulty genes out of 25,000, then the chances of meeting someone with the corresponding faulty genes are very small. That is why diseases like Pompe are so rare.

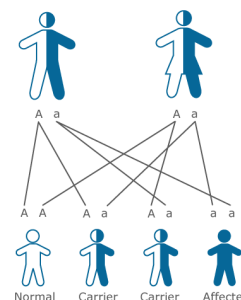


## TEACHER PAGES: JIGSAW - GENETICS

For parents of children with Pompe disease, each parent has one good gene and one faulty one (for the alpha-glucosidase enzyme GAA). They each pass on one gene to their children through random assortment. In the case of Pompe disease, both parents have passed on the faulty gene, meaning that the child has no copy of the gene to make the functioning enzyme, leading to all the Pompe symptoms.

Pompe disease is an example of an autosomal recessive disorder. In order for symptoms of Pompe disease to present, an affected individual must have inherited a mutation from each parent. Pompe disease is quite rare; only 1 in 40,000 people are affected. Therefore, most individuals in the population do not have a mutation for Pompe, and their genotype is defined as AA. These individuals are referred to as **homozygous dominant**: they have the same genes on each chromosome and that gene corresponds to the dominant phenotype. In the example of Pompe disease, AA individuals have two normal functioning GAA genes.

**Homozygous** individuals have two copies of the same gene. **Heterozygous** individuals have different copies of the gene. In a normal, non-affected individual, if a faulty GAA gene is present, it is masked by a functioning, normal gene (dominant). Let's define this person's genotype as Aa. A person with this genotype is considered a carrier since he or she carries a copy of the defective gene. So, unless symptoms are present, we assume a non-affected individual to be either AA (homozygous dominant) or Aa (heterozygous). Most heterozygous individuals don't know they carry the mutant gene until a family member presents with the disease. In order for symptoms to be present in a recessive disorder, the affected individual must have two faulty copies of the gene. In this case, two bad copies of the GAA gene. The genotype for this individual is written aa (**homozygous recessive**).



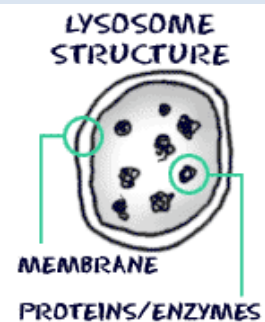
So, if both parents are carriers, what is the likelihood of their children having Pompe disease? Using a Punnet square illustrates the possible genetic combinations. Remember, **A** indicates a normal gene for GAA; **a** indicates the mutated GAA gene. The parents are both carriers with the genotype Aa. We put one parent on the top of the square; the other parent on the left side. The possible offspring combinations are denoted within the 4 squares: 1 in 4 (25%) possibility that the child will be affected (aa); 2 in 4 (50%) that the child will be a carrier (Aa); 1 in 4 (25%) that the child will have only normal genes (AA). Since this is an autosomal disease, the mutation can be passed by either mother or father and all children, regardless of gender, have an equal chance of inheriting mutations.

	<b>A</b>	<b>a</b>
<b>A</b>	<b>AA</b>	<b>Aa</b>
<b>a</b>	<b>Aa</b>	<b>aa</b>

## TEACHER PAGES: JIGSAW - LYSOSOMES

The body is divided into tiny cells, each containing an entire copy of the DNA for the whole body. Not all of it is used in each cell. Liver cells read only the bits about liver cells, muscle cells only the bits about muscle cells and so on. That's why there are different types of cells in the body. Cells themselves are banded together to form organs. Just as the body is divided into organs, each with its own specific function, the cells themselves are divided into compartments called organelles.

There are a number of different organelles in cells. Energy is produced in organelles called mitochondria; DNA, in the form of chromosomes, is kept in the nucleus; but our interest is in an organelle called the lysosome. Lysosomes are sacks (vesicles) of enzymes that have pinched off of the Golgi apparatus. Lysosomes are found in varying amounts in varying shapes but always consist of a central space filled with enzymes completely enclosed by a membrane.



[http://www.biology4kids.com/files/cell\\_lysome.html](http://www.biology4kids.com/files/cell_lysome.html)

Lysosomes act as tiny vacuum cleaners, tidying up the cell and ingesting anything that there is too much of such as complex sugars and proteins. The substances they ingest are then broken down and released into the cell as smaller units for re-use. The breaking down is done by enzymes found only in the lysosomes. The environment inside the lysosomes is more acidic than that of the surrounding cell cytoplasm, so only acidic enzymes can function inside the lysosome.

Enzymes are very sensitive to acidity and so enzymes which work inside the lysosome would not work outside and vice-versa. That is why Pompe disease does not have the same symptoms of low sugar levels that some of the other glycogen storage diseases produce - glycogen continues to be broken down normally in the cells outside the lysosomes and blood sugar levels are maintained. It is the excess glycogen that is taken into the lysosome that creates a problem.

The reason that Pompe disease produces such severe symptoms is twofold. Firstly, the missing enzyme is one of the lysosomal enzymes. For this reason, Pompe disease is referred to as a lysosomal storage disease. There are around 50 known lysosomal storage diseases. In each case there is a common problem: the lysosomes take up a substance that they do not possess the enzyme to break down. This means that the lysosomes grow larger and larger until they disrupt cell function, and therefore, the function of the organ or tissue that the cells make up.

This leads us to the second reason that Pompe disease is so severe, even amongst lysosomal storage diseases. As glycogen is the energy storage compound for the body, there is a lot of it in muscle. This means that in Pompe disease, where there is no enzyme to break down the glycogen in the lysosomes, the lysosomes in the heart and skeletal muscle quickly accumulate large deposits of glycogen. As the lysosomes increase in size, the muscle cells are displaced. Additionally, when the lysosome bursts due to the large size, the acidic contents are released, degrading the surrounding tissue. The muscles are therefore progressively weakened.

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## STUDENT WORKSHEET: LOOKING THROUGH A FATHER'S EYES

Name: \_\_\_\_\_

Date: \_\_\_\_\_

Home group members: \_\_\_\_\_

### A. Guiding Questions

Directions: Use the guiding questions 1-4 below as you share information about Pompe disease in your home group. Answer as thoroughly as possible. Include illustrations as needed.

1. What is an enzyme and how does it work?
  
  
  
  
  
  
  
  
  
  
2. Discuss the molecular biology from DNA → protein. What is a mutation and how does it affect the protein?
  
  
  
  
  
  
  
  
  
  
3. Is Pompe disease an inherited disorder? If so, describe how it is inherited and the probability.
  
  
  
  
  
  
  
  
  
  
4. What is a lysosome? What is its function?

## **B. Reflective Questions**

Directions: Now that you have a shared understanding of the basic science of Pompe disease, answer the questions below 5-7 on your own.

5. What role does each of the above (enzyme, molecular biology, genetics, lysosome) play in Pompe disease?
6. Considering #5, what symptoms might you expect to see in an individual with Pompe disease?
7. How would you treat the disease? Think beyond simply “medicine” but what type of medicine? How would you administer it?

TEACHER PAGES: SECTION NAME CARDS

Copy and cut. Distribute one card per student. (Laminate for repeated use.)

Lysosome	Lysosome
Lysosome	Lysosome
Lysosome	Lysosome
Lysosome	Lysosome

Enzyme	Enzyme
Enzyme	Enzyme
Enzyme	Enzyme
Enzyme	Enzyme

Teacher Page: Section Cards, second page

Copy and cut. Distribute one card per student. (Laminate for repeated use.)

Molecular Biology	Molecular Biology
Molecular Biology	Molecular Biology
Molecular Biology	Molecular Biology
Molecular Biology	Molecular Biology

Genetics	Genetics
Genetics	Genetics
Genetics	Genetics
Genetics	Genetics

