

Activity 1: Before and After

Based on video content

20 minutes (10 minutes before and 10 minutes after the video)

Setup

Before watching the video, take a few minutes to consider your impressions of the field of genomics. In pairs, look at the list of some terms and topics that will be covered in the video. Discuss, or write a few brief sentences summarizing what comes to mind when you see the term.

After the video, go over the terms again as a group. For each one, discuss the group's impressions about the term before the video, and how they changed after viewing the video. As a group, try to come up with a brief definition or summary for each term.

Materials

- One copy of the List of Terms and Topics per two people (master copy provided)
- Tips and Suggested Answers

List of Terms and Topics

1. genome
2. Human Genome Project
3. open reading frame (ORF)
4. BLAST (basic local alignment search tool)
5. homology
6. SNPs (single nucleotide polymorphisms, “snips”)
7. DNA fingerprint
8. microarrays
9. cDNA (complementary DNA)
10. *Arabidopsis*
11. knockout organism
12. Type II diabetes

Note: For possible ways to define these terms, see the Tips and Suggested Answers that follow.

List of Terms and Topics, Suggested Definitions

1. genome
The entire collection of DNA found in an organism, including genes and additional sequences. The sequence varies slightly between individuals, but “genome” refers to the collective, common DNA of a species.
2. Human Genome Project
A collaborative effort to determine the sequence of the 3 billion nucleotides found in the entire collection of human DNA.
3. open reading frame (ORF)
A DNA sequence that has the potential to encode a protein. It has a series of amino acid codons without a stop and all in the same “reading frame” that is theoretically long enough to encode a protein.
4. BLAST (basic local alignment search tool)
A computer program that compares and aligns DNA sequences to find similarities. For example, it can look for genes that are common in two different species.
5. homology
Similarity between genes, DNA sequences, proteins, or other components of organisms, which suggests a common ancestral origin.
6. SNPs (single nucleotide polymorphisms, “snips”)
Places in the genome where single base differences are found when the DNA from individuals within the same species are compared.
7. DNA fingerprint
Variations in DNA base sequence that are characteristic of an individual.
8. microarrays
Glass chips dotted with tiny amounts of “probe” DNA. They can be used to detect which genes are expressed in an organism under different conditions by detecting the RNA from cells of the organism.
9. cDNA (complementary DNA)
DNA that is made from RNA by “reverse transcription.” It can be hybridized to a microarray of genes, to determine which RNAs are made by a cell under certain conditions.
10. *Arabidopsis*
A mustard plant that is used as a “model organism” for genetics.
11. knockout organism
An organism in which a gene has been deleted, or “knocked out” using genetic engineering techniques.
12. Type II diabetes
An inability to regulate blood glucose levels, because of defects in the regulatory system that makes and responds to insulin. This diabetes usually has an adult onset.

Activity 2: Making a Microarray

Based on video and online text content

40 minutes

Setup

Microarrays are small slides or chips of glass onto which microscopic dots of DNA are spotted. Each dot has DNA of a difference sequence. When the microarray of DNA dots is incubated with test DNA that has been fluorescently labeled, the test DNA hybridizes to specific spots, of known sequence, in the microarray. A fluorescence detector reveals where the test DNA has hybridized, so the microarray “probes” the test DNA to find out which sequences, and in what quantity, are in the test DNA.

Often, the test DNA is cDNA generated from the RNA of cells exposed to specific conditions. By comparing the RNA made by cells under the specific conditions to RNA made under standard conditions, we can see how cells respond to a drug or a new environmental condition.

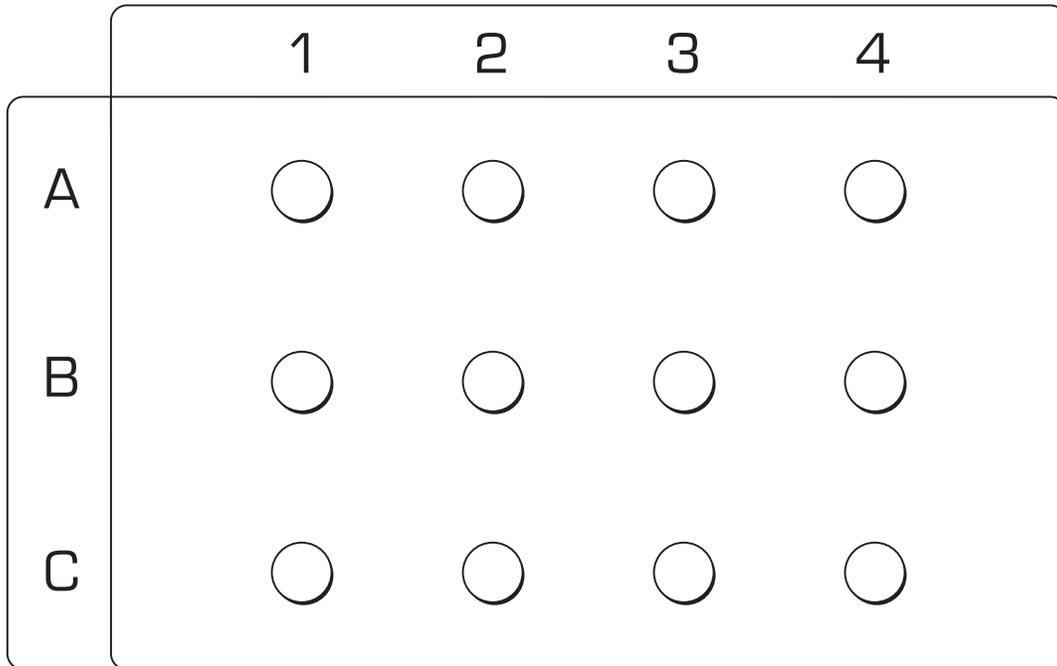
In this exercise, you will receive a diagram with a grid of dots that represents a microarray. You will also have a key to the gene sequences found at each dot on the grid, and an explanation of how the test DNA was made. Work in pairs to predict how each dot in the grid will hybridize to the test DNA. Then predict the appearance of each dot in the grid, and mark each dot according to your predictions. After everyone has marked the microarray, go over it as a group, and discuss any spots that are in dispute. See the Tips and Suggested Answers for explanations.

If necessary, review the microarray technique from the Genomics video or Genomics online text chapter, or look at Figure 5 from the text, before doing this exercise.

Materials

- One copy of the Microarray Grid Diagram per two people (master copy provided)
- One copy of the Key to the Genes on the Microarray per two people (master copy provided)
- Transparency of the Microarray Grid Diagram (or a sketch of the grid on a blackboard)
- Transparency of Aerobic and Anaerobic Pathways in Yeast (master copy provided)
- Transparency of Figure 5 from the Genomics online text chapter (master copy provided)
- One copy of the Genomics online text chapter per two people (available online at <http://www.learner.org/channel/courses/biology>)
- Optional: red, green, and yellow pens or red, green, and yellow sticky dots (one set per two people)
- Tips and Suggested Answers

Microarray Grid Diagram



On the microarray, a gene whose expression is *increased in aerobic growth* will appear **red**. A gene whose expression is *increased in anaerobic growth* will appear **green**. A gene that is *equally expressed in both conditions* will appear **yellow**.

For each microarray spot, show whether you would predict it to appear red, green, or yellow, by coloring it or labeling it R, G, or Y.

Key to the Genes on the Microarray

Background

This microarray will be used to see how the expression (transcription) of several yeast genes changes as the cells change their metabolism. First, a little background. Yeast cells can metabolize anaerobically or aerobically. Anaerobic metabolism (fermentation) uses the pathway of glycolysis to break down glucose to pyruvate. Pyruvate is converted to acetaldehyde, then ethanol, which is how the alcohol in wine or beer is produced. In aerobic metabolism, the glycolysis pathway enzymes are still active. However, pyruvate is not converted to ethanol. Instead, metabolic byproducts are sent to the TCA cycle (also called the citric acid, or Krebs cycle) and the electron transport chain. The TCA cycle and electron transport chain occur in mitochondria, and require oxygen.

How the Test DNA Was Made

The test DNA that is hybridized to the microarray contains two batches of labeled cDNAs made from yeast mRNAs. cDNAs from yeast growing *aerobically* are labeled with a *red* fluorescent dye; cDNAs from yeast growing *anaerobically* are labeled with a *green* dye.

On the microarray, *red* spots will show genes that are highly expressed in *aerobic* growth; *green* spots show genes that are highly expressed in *anaerobic* growth. If cDNA from a gene is equally abundant in both the green and red labeled cDNA batches, both will bind equally to a spot and it will appear *yellow*.

Key to Genes on the Microarray

Spot	Gene	Function of Gene Product
A1	PGK1	enzyme in glycolysis
A2	RPB8	RNA polymerase subunit, enzyme in transcription
A3	PDC1	enzyme that converts pyruvate to acetaldehyde
A4	ADH1	enzyme that converts acetaldehyde to ethanol
B1	DBP2	DNA polymerase subunit, enzyme in DNA replication
B2	COX1	cytochrome oxidase, part of the respiratory electron transport chain
B3	CIT1	enzyme in the TCA (citric acid, Krebs) cycle
B4	RPL10	ribosomal protein for translation in the cytoplasm
C1	MRP1	ribosomal protein for translation in mitochondria
C2	SIP4	activates transcription of genes required for aerobic metabolism
C3	HSP12	protects against oxidative (oxygen-generated) stress
C4	ACT1	actin protein for cell structure

Post-Activity Discussion

Once we know how genes increase or decrease in expression when yeast metabolism changes, how might this information be applied? Consider the fact that yeast is used in baking, brewing, and as a model organism for cell and genetic research. (See the Tips and Suggested Answers section for ideas.)

Source and Answers

The Tips and Suggested Answers has the information about which genes in this activity were expressed in aerobic and anaerobic conditions. The data come from microarrays described in the following article: DeRisi, J.L., Iyer, V.R., Brown, P.O. 1997. "Exploring the metabolic and genetic control of gene expression on a genomic scale." *Science* 278(5338):680–686.

Aerobic and Anaerobic Pathways in Yeast

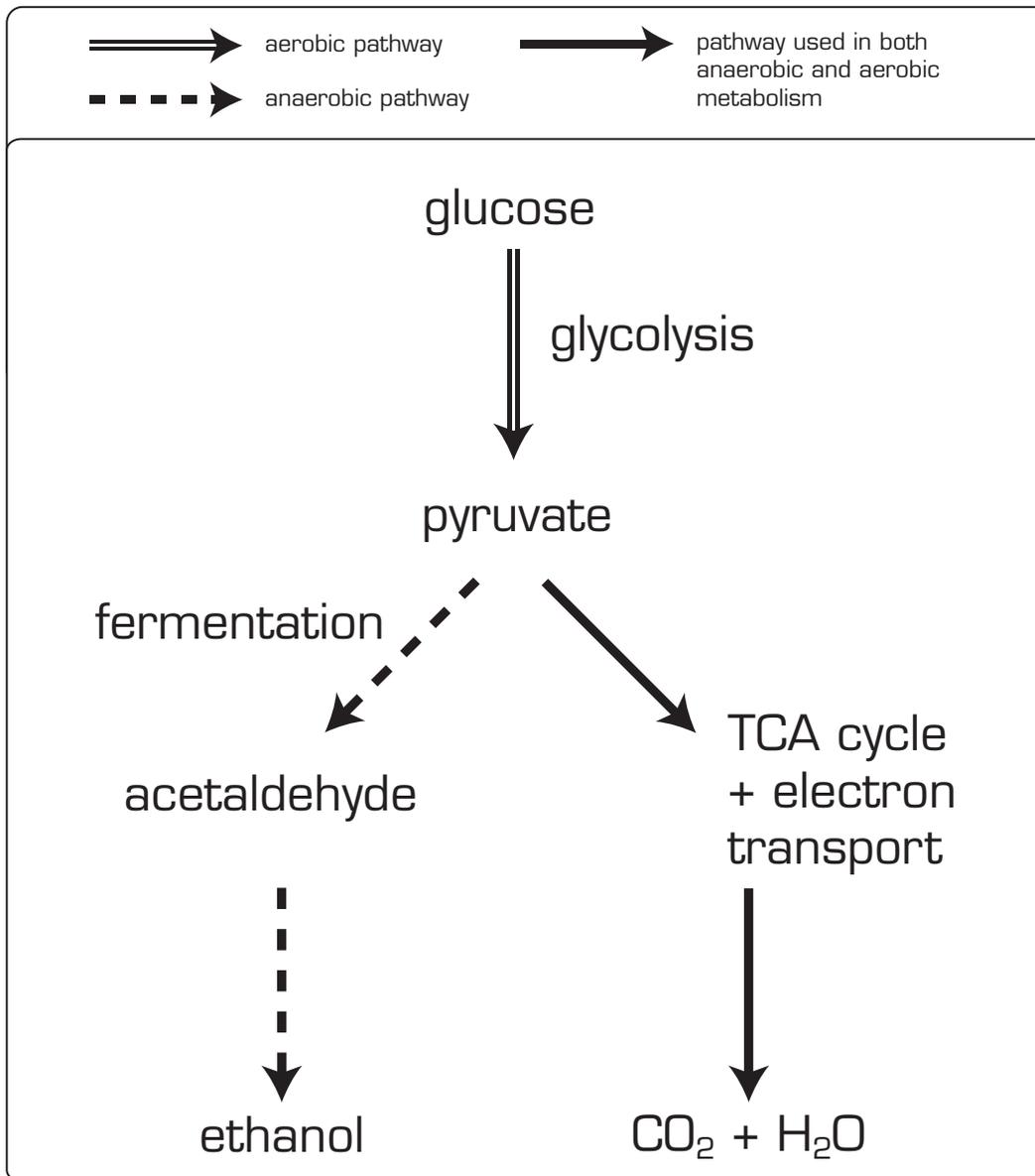
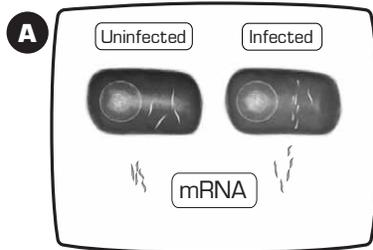
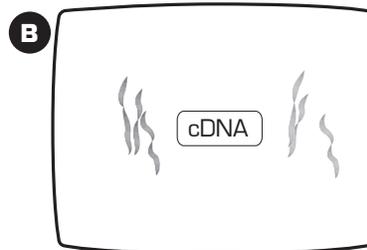


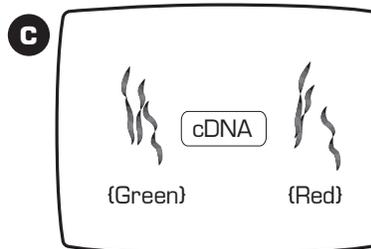
Figure 5 From the Online Text Chapter



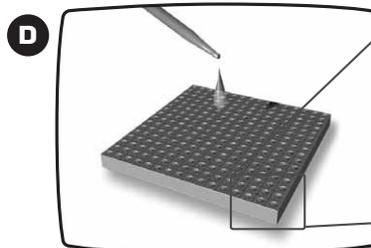
A) RNA is isolated from cells from two samples (in this illustration, infected and uninfected plant cells).



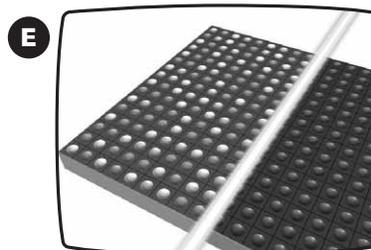
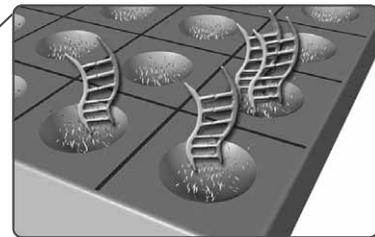
B) The mRNA from both samples is copied to a more stable form, called cDNA, using reverse transcriptase.



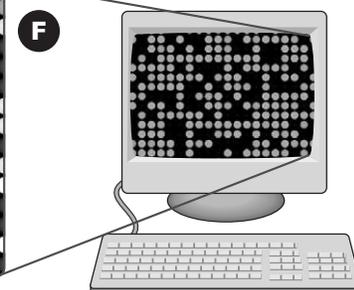
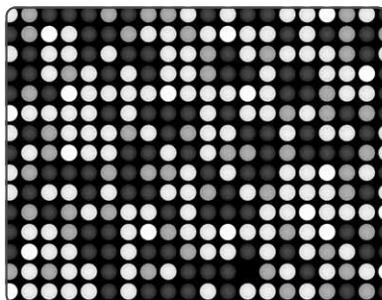
C) At the same time, the cDNA is labeled with fluorescent tags (a different color tag for each sample).



D) The tagged cDNA is placed on the microarray chip, where it binds to the corresponding DNA that makes up the genes that have been previously spotted on the chip.



E) The chip is placed in a laser scanner, which identifies the genes that hybridize to each sample (uninfected=green; infected=red; and both samples=yellow).



F) The data are displayed on a computer screen where expression of the individual genes can be identified.

Illustration — Bergmann Graphics

Making a Microarray

from DeRisi, J.L., Iyer, V.R., Brown, P.O. (1997). "Exploring the Metabolic and Genetic Control of Gene Expression on a Genomic Scale." *Science* Oct 24; 278(5338):680-686.

Microarray Answers

In general, we can divide the genes into three categories and use these to predict its expression in conditions of aerobic and anaerobic metabolism:

1. *Yellow* spots are predicted for genes that are expressed equally in aerobic and anaerobic conditions. These encode RNAs and proteins with "housekeeping" functions that occur in any conditions. Housekeeping genes include those for glycolysis, since many are active in both aerobic and anaerobic conditions.
PGK1, RBP8, DBP2, RPL10, ACT1
2. *Green* spots are seen for genes that encode enzymes that function in *anaerobic* growth (fermentation) and are more highly expressed under these conditions.
ADH1, PDC1
3. *Red* spots are seen for genes that encode enzymes that function in *aerobic* metabolism, mitochondrial function (where the TCA cycle and electron transport chain occur) or response to the increased oxidative damage that occurs during aerobic metabolism. The product of the *SIP4* gene is required for the transcriptional activation of other aerobic metabolism genes, so it also increases in aerobic metabolism.
COX1, CIT1, MRP1, SIP4, HSP12

In reality, this type of yeast normally grows anaerobically, so the expression level of several of the housekeeping genes like *RPL10* is higher during anaerobic growth. *ADH1* is expressed equally in aerobic and anaerobic conditions, indicating that the cell controls the protein, which is made constantly, rather than controlling the gene.

Post-Activity Discussion Question Answer

Knowing the gene expression changes that occur when yeast convert from anaerobic to aerobic growth, or v.v., might provide information that could be used to optimize brewing or baking applications. For example, strains of yeast that are specialized for brewing or baking could be subjected to the same microarray analysis, to find out if they are optimized for production of ethanol, or for anaerobic growth. New strains might be selected based on their gene expression "profile" determined by this experiment.

Since yeast cells are used as models for studying basic cellular processes in other organisms, learning how gene expression changes in yeast when metabolic conditions change might provide insights into what happens in the cells of other organisms under similar conditions. Of course, human cells do not ferment glucose to ethanol, but muscle cells ferment glucose to lactic acid under aerobic conditions.

Activity 3: “CSI, Crime Scene Investigation”

Based on video and online text content

45 minutes

Setup

This activity is a “whodunit” that uses DNA fingerprints as the identifying features of individuals. You are the Crime Scene Investigation team, investigating the death of a man in a household full of people.

The sequences used as identifying markers in DNA fingerprinting are STRs (short tandem repeats). These are repeated sequences that are found at a particular locus on a chromosome, but the number of copies of repeats varies in the population. Like the single nucleotide polymorphisms (SNPs) that are described in this unit’s video and text, STRs are inherited just like alleles of genes: one from each parent. Like SNPs, several STRs in a section of a chromosome make up a haplotype.

After reading the mystery story, use (fabricated) STR information to come up with possible solutions for the situation in the story.

Materials

- One copy of the Mystery Story per person (master copy provided)
- One Figure of DNA Fingerprints From the Inhabitants per person (master copy provided)
- One set of the Figures of DNA Fingerprint Evidence, cut into individual samples (master copy provided)
- Transparency of the Map of the Room (master copy provided)
- Tips and Suggested Answers

Mystery Story

A man is found dead of a head wound in the library of a large mansion. Near his head is a heavy, broken vase. The deceased is 20-year-old John, the chauffeur for the family who owned the mansion. John's parents, Jamison and Lilly, are the butler and maid of the mansion. The family who own the mansion are Sir Roderick and Lady Madeline, and their children Roberta (age 18) and Bertram (age 15). Also in the house is Roberta's fiancé Paul (age 21). The family has a cat named Spike and a dog named Spot.

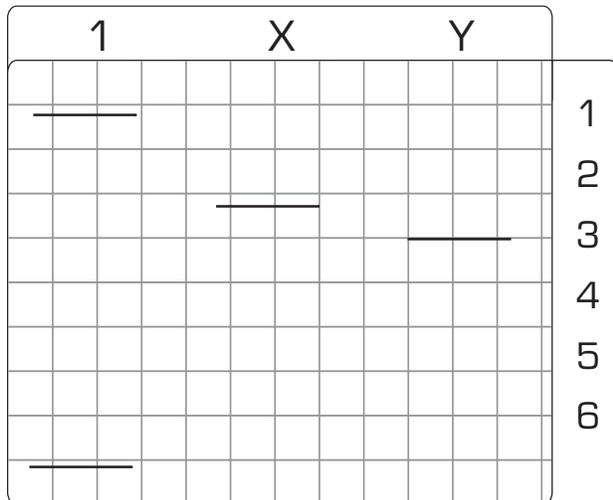
The window of the room was open. It was a dry, windy night and there were no footprints on the hard ground outside the window. A cat hair was found on the vase. Sir Roderick seems to think that a valuable stamp collection that was in the bookcase is missing, although his wife Madeline, daughter Roberta, and son Bertram claim they haven't seen the stamp collection for years, and thought he lost or sold it long ago.

Evidence samples have been taken from various parts of the room. In addition, cell samples were taken from each person in the household. DNA fingerprint analysis was performed on the evidence and the cell samples, and is now available for review by the CSI team. The following chromosomal regions were tested to generate the DNA fingerprints: a region on chromosome 1, a region on the X chromosome and for the males, a region on the Y chromosome. Note that the diagrams of DNA fingerprints supplied here are simplified, to save time. Normally, many more chromosomal loci are used in a DNA fingerprint investigation.

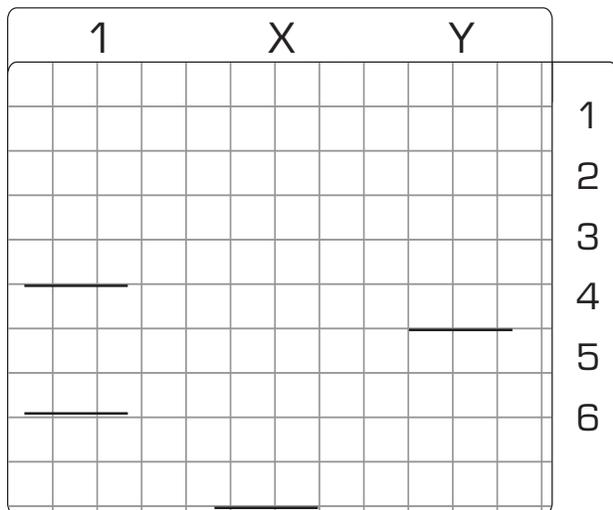
Each person will take at least one piece of DNA Fingerprint Evidence, along with a copy of the Figure of DNA Fingerprints From the Inhabitants. Use the Map of the Room and the Fingerprints From the Inhabitants to figure out who your evidence came from and where it was found. Come up with possible scenarios—accident or murder—that could have caused the death. If you suspect murder, was it an inside job or an intruder? What are the possible motives?

As a group, describe the scenarios you came up with to explain the evidence. See the Tips and Suggested Answers for possible answers.

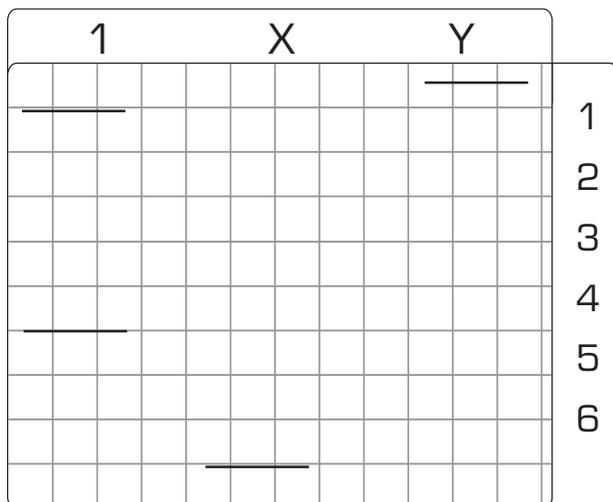
Figures of DNA Fingerprint Evidence



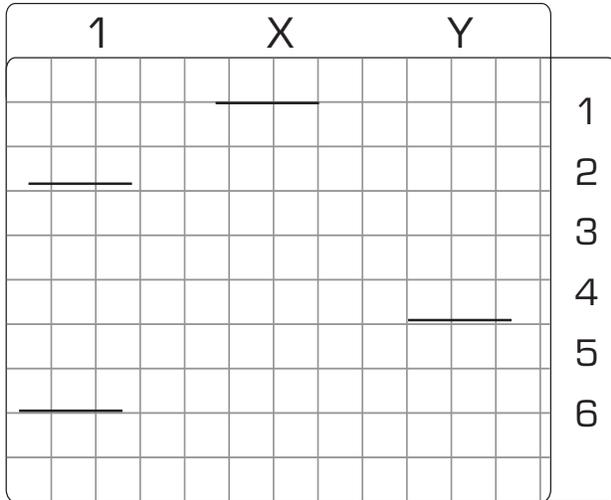
Evidence 1: From hair found on the floor (see map for location)
 (Grid lines and numbers along the side are for reference.)



Evidence 2: From skin cells found on the desk (see map for location)
 (Grid lines and numbers along the side are for reference.)

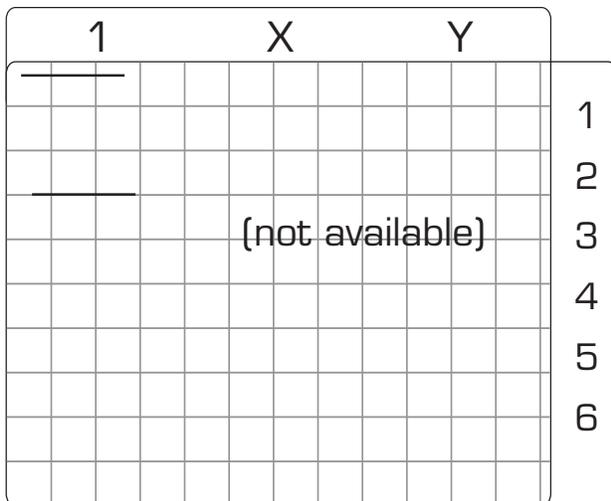


Evidence 3: From hair found on the floor (see map for location)
 (Grid lines and numbers along the side are for reference.)



Evidence 4: From blood found on the floor (see map for location)

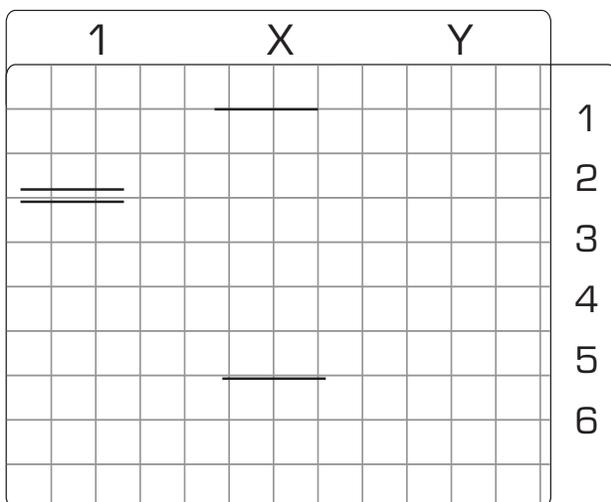
(Grid lines and numbers along the side are for reference.)



Evidence 5: Skin cells found on the vase (see map for location)

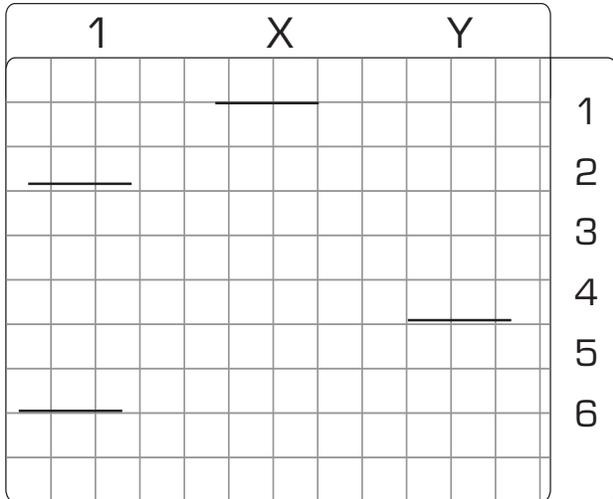
(A complete test of this sample was not available because of an error at the testing lab.)

(Grid lines and numbers along the side are for reference.)



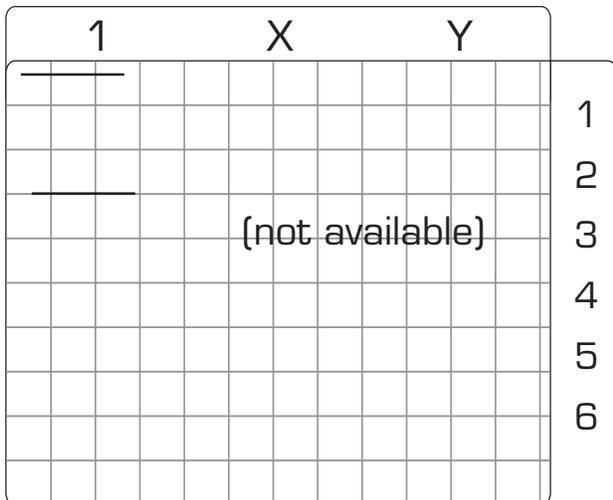
Evidence 6: Skin cells found on the vase (see map for location)

(Grid lines and numbers along the side are for reference.)



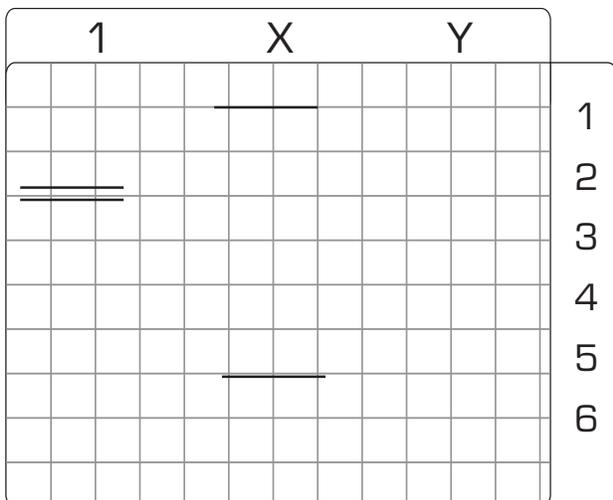
Evidence 7: Skin cells found on the door (see map for location)

(Grid lines and numbers along the side are for reference.)



Evidence 8: hair found on the floor (see map for location)

(Grid lines and numbers along the side are for reference.)



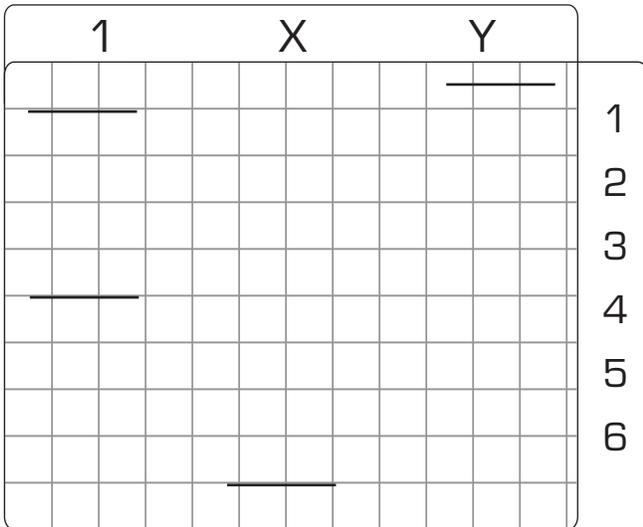
Evidence 9: Blood found on the floor (see map for location)

(Grid lines and numbers along the side are for reference.)

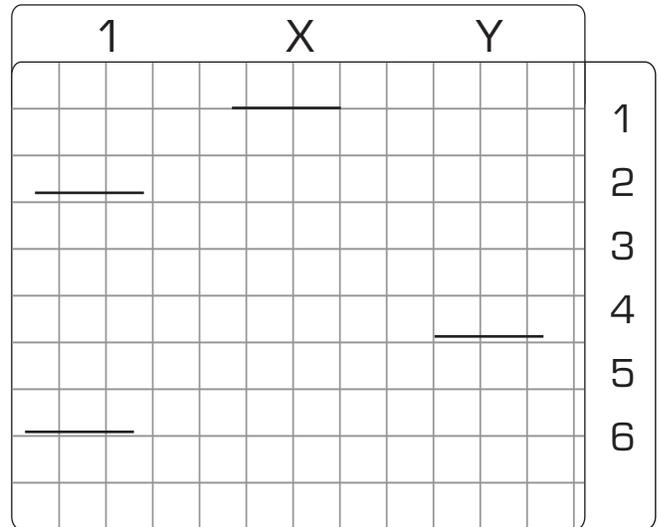
Figures of DNA Fingerprints From the Inhabitants

(Grid lines and numbers along the side are for reference.)

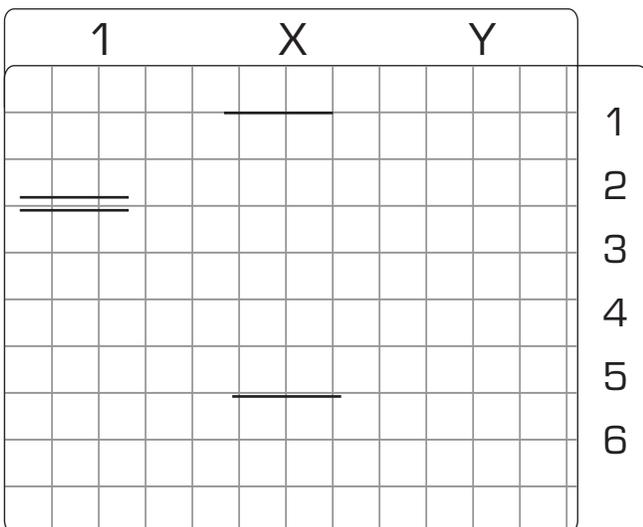
Butler (Jamison)



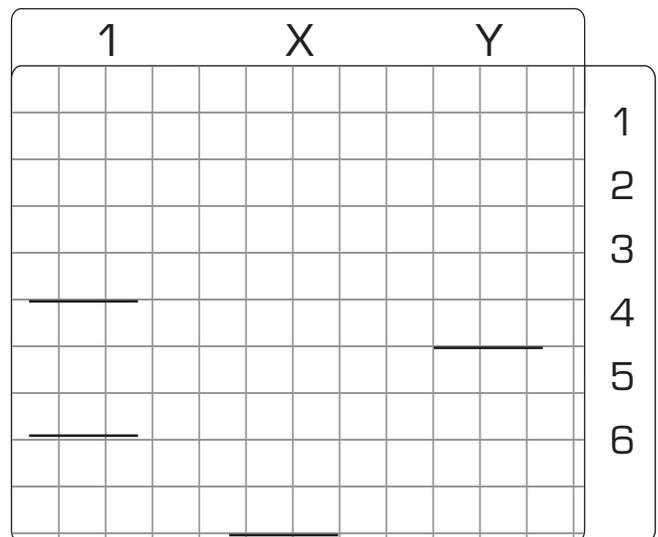
Chauffeur (the deceased, John)



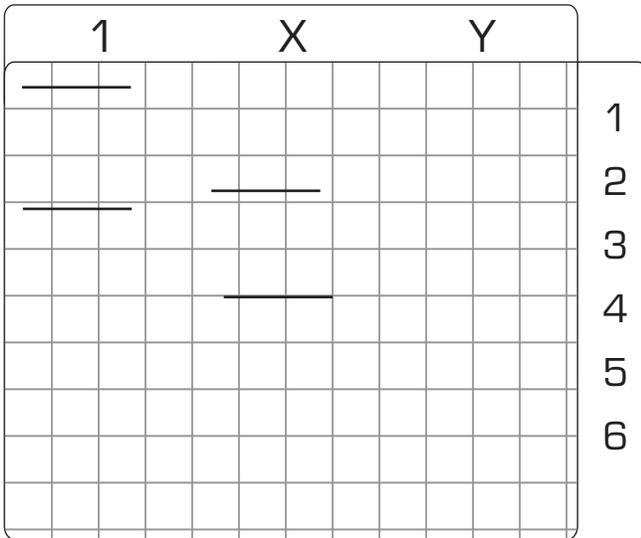
Maid (Lilly)



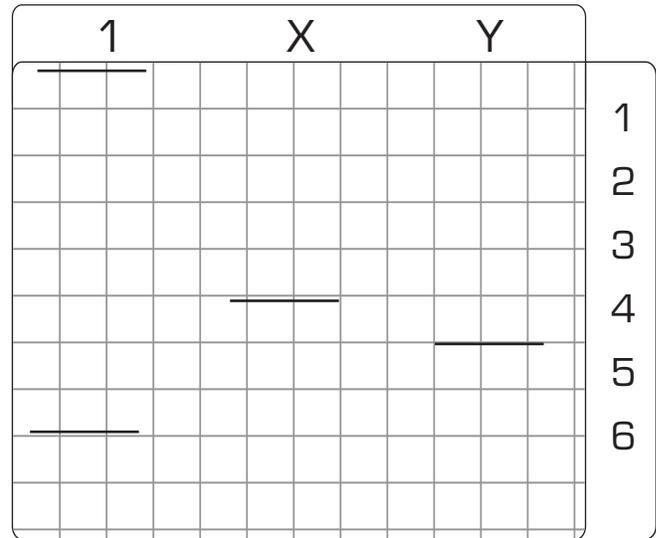
Owner of the mansion (Sir Roderick)



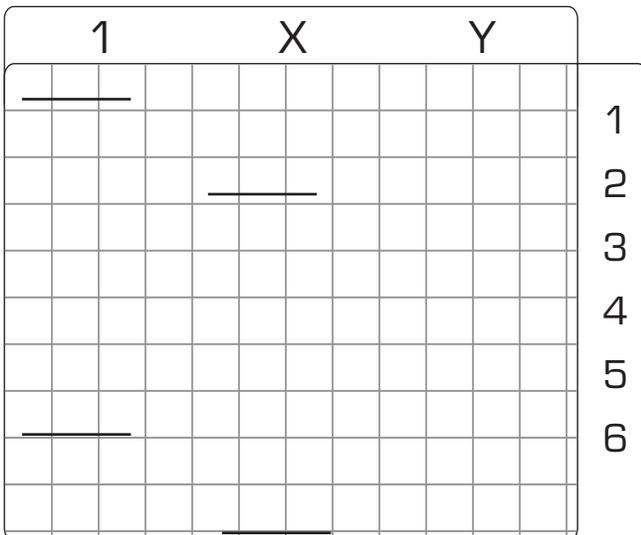
Owner of the mansion (Lady Madeline)



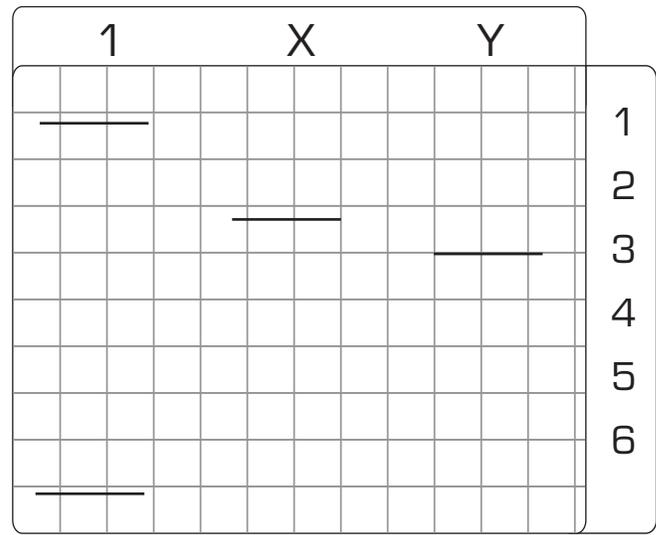
Son of Roderick and Madeline (Bertram)



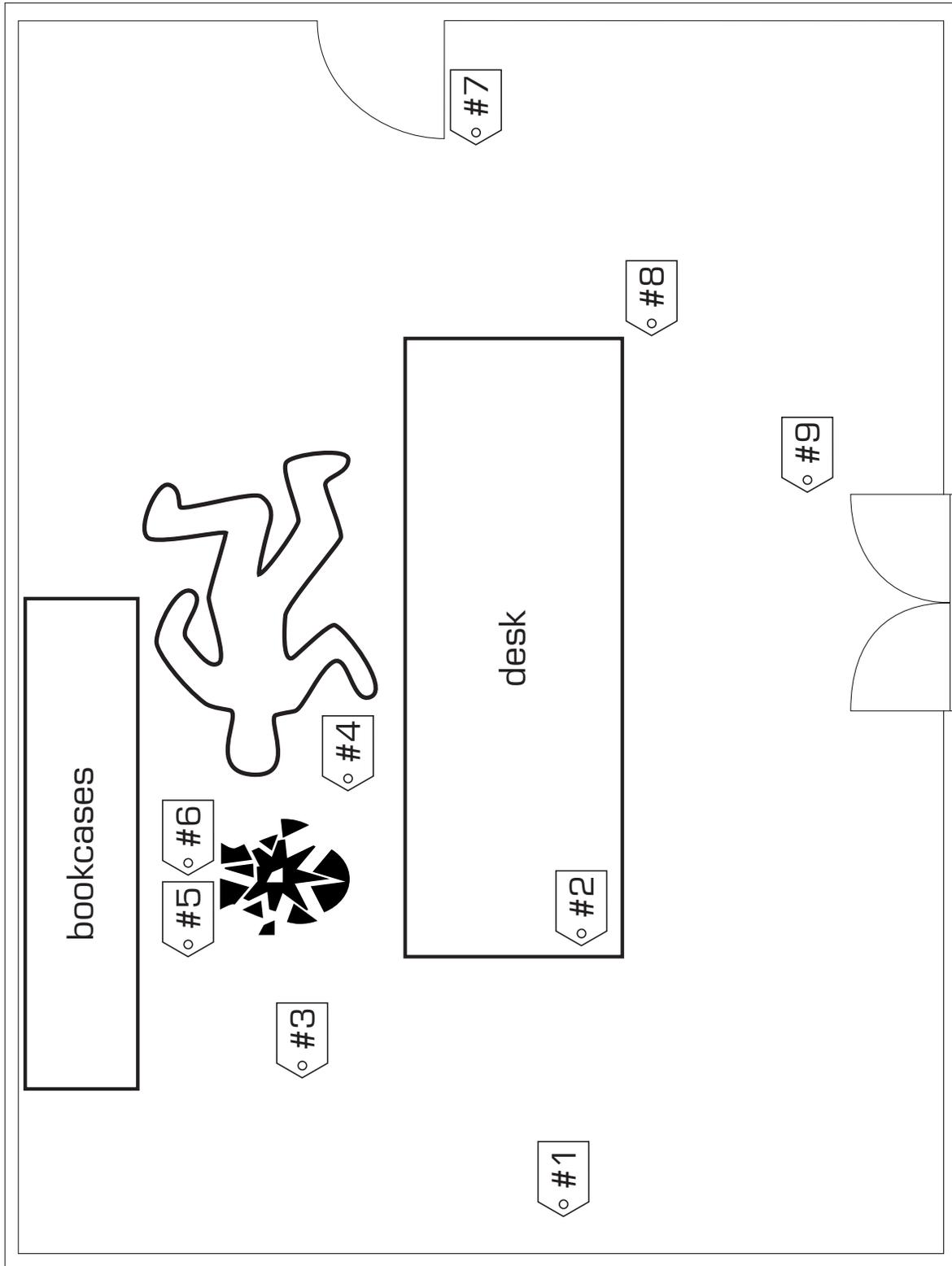
Daughter of Roderick and Madeline (Roberta)



Fiancé of Roberta (Paul)



Map of the Room



CSI, Crime Scene Investigation Possible Answers

1. The butler did it. His motivation? Paternity issues. Each person inherits one copy of each gene, SNP and STR from each parent. For autosomes (non-sex chromosomes like chromosome 1), everyone inherits 2, one from each parent. For the sex chromosomes and the genes and DNA fingerprint markers on them, males inherit an X from their mother and a Y from their father. Notice that the deceased, John, is supposed to be the child of the butler Jamison and the maid Lilly, but his DNA fingerprints suggest his father is Sir Roderick.
2. Sir Roderick did it, for the same reasons as the butler.
3. Lady Madeline did it. The partially damaged DNA fingerprint has some markers in common with her DNA fingerprint, although only for one of the loci tested here. Perhaps she recently discovered that her husband was the father of the chauffeur, and wanted to protect the inheritance of her own children.
4. Bertram did it, perhaps for the same reasons as Lady Madeline. His blood is on the floor near the window. Perhaps he cut himself on the broken vase and tried to escape out the window before the others came?
5. The maid did it. Lilly's skin cells are on the vase, although as the maid, she would be expected to pick it up and clean it from time to time.
6. The cat did it. The cat's hair is on the vase. It may have been up on the bookcases, perhaps chased by the dog, and dislodged the vase just as the victim walked below.
7. The wind did it. The window was open and it was a windy night. Perhaps the vase was in an unsteady position and blew over onto the victim.
8. An outsider did it, coming in and escaping through the open window, possibly stealing the stamp collection. (The partial DNA fingerprint might belong to the intruder.)

Activity 4: Quick Discussion

Based on video and online text content

15 minutes

Setup

Medicine is just one of the fields that is being revolutionized by the application of genomics techniques. Often, however, diagnostic applications precede applications that can cure or treat a condition. In pairs, spend a few minutes discussing each of the following questions.

Materials

- One copy of the Discussion Questions per two people
- Tips and Suggested Answers

Discussion Questions

1. Imagine it is possible to do a complete genomic analysis of an individual human. The analysis can determine which variants (alleles) of genes the person has, and the level at which the person's genes are expressed. Speculate about the kind of information a genomic analysis might reveal. What could one's genes, or their level of expression, predict about one's medical conditions, personality, or other personal characteristics? (See the Tips and Suggested Answers for possible answers.)
2. If individual genomic analysis tests were available, and they determined your risk of developing 100 diseases including Type II diabetes, different types of cancer, Alzheimer's, and susceptibility to viral and bacterial infections, would you have the test done? Why or why not?
3. If that type of data were available, who should have access to it? You and your physician? Your family members? Should insurance companies, the World Health Organization, the NIH, or the Center for Disease Control be able to access an anonymous, aggregate of the data, for statistical analysis?
4. What if genomics showed that a certain set of SNPs showed that some persons had an 80% chance of becoming alcoholic. Should we pass a law that would prevent them from buying alcohol?
5. Iceland automatically includes everyone's medical records in a database, unless a person requests to be left out. This database has found genes associated with several genetic disorders. Should we use a similar approach in this country?

Quick Discussion Answers

1. Imagine it is possible to do a complete genomic analysis of an individual human. The analysis can determine which variants (alleles) of genes the person has, and the level at which the person's genes are expressed. Speculate about the kind of information a genomic analysis might reveal. What could one's genes, or their level of expression, predict about one's medical conditions, personality, or other personal characteristics?

Possibilities include

- Information about a person's genes for insulin or the insulin receptor, or levels at which they are expressed, which might reveal information about susceptibility to diabetes.
- Information about a person's immune system genes or the level at which they are expressed might reveal information about their susceptibility to viral or bacterial infection, allergies, or risk of cancer.
- Knowing the level of expression of genes for enzymes that produce or release neurotransmitters or genes for neurotransmitter receptors might provide information about a person's mental health or personality.

Notes
