Name: _____ TOC#

CSI in Biotechnology

Part 1: Gel Electrophoresis Webquest

Gel Electrophoresis Background

In any species genome, there is also a lot of "junk DNA." This does not perform any known function and, therefore, is free to vary at random. Intraspecific analysis of DNA (sometimes called "DNA fingerprinting") provides an accurate means of comparison between individuals of the same species. It is used extensively in the field of forensic science.

Scientists identify differences in DNA sequences by measuring the length and number of fragments created by digestion with restriction enzymes. A technique called gel electrophoresis is used to separate fragments according to length. DNA fragments, cut with specific restriction enzymes, are placed on one end of the specially prepared block of agarose called a gel. The gel is like a sponge with small holes in it. AN electric current is applied across the agarose which causes the strands to migrate through the gel. (Since DNA molecules are negatively charged, they migrate towards the positively charged electrode.) The larger fragments move more slowly and are found nearer the point of origin, while the smaller fragments are found further from the origin (closer to the positive electrode). Scientists then use a special stain to make the DNA fragments visible as bands. By counting the number of bands the researchers can tell how many fragments exist. By observing the distance each fragment has migrated, they determine how big each fragment is.

Gel Electrophoresis

Go to the follow website: http://learn.genetics.utah.edu/content/labs/gel/

Click through the online activity, and read the information to answer the questions below. These questions go in order of the activity.

1. By what factor does gel electrophoresis sort DNA?

2. What other types of molecules can gel electrophoresis be used to sort?

3. The gel is the _____ that _____ DNA strands. It's like a sponge made of Jello with many small _____ in it.

4. Where do we put DNA in the gel?

5. ANALYSIS: What do you think you must do to the DNA before you place it in the gel?

6. By adding an _____, we can make the DNA move.

7. Is the DNA at the positive or negative side of the gel? Which way will the DNA move?

8. Which strands will move the farthest, why?

9. We can't see a single strand of stained DNA, but we can see _____.

10. What causes the bands in the gel?

In the following questions, follow along completing the steps of running a gel, and answer these questions.

11. Make the gel in step 1. What are the ingredients of agarose gel?

12. What does the liquid buffer do?

13. As the gel cools, _____ will form in it.

14. What is the comb for?

15. Why do we use the DNA Size Standard?

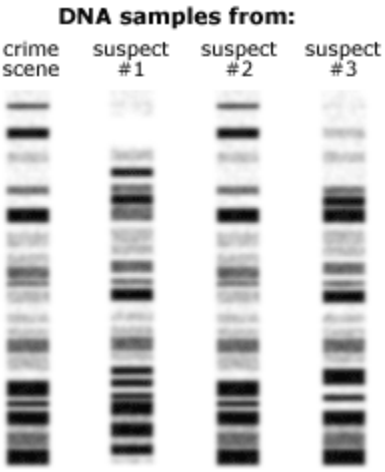
16. In the last step, the stain we use on the DNA is a chemical that _____ to DNA and shows up under _____ light.

17. Although we can't see _____ DNA strands, we can see large groups of stained DNA strands. These groups will show up as bands in the gel.

18. How many base pairs long was each of the three bands of DNA that showed up?

19. ANALYSIS: explain, in detail, why we use gel electrophoresis.

Part 2: Using DNA in Crime



1. What is the picture to the right showing?

2. Who else should the investigators compare the DNA to?

3. Which suspect should be arrested based on this information?

4. What do the different bands represent?

Read the following article titled "Innocent or Guilty?"

- Highlight any information you find interesting or shocking.

When you are finished, answer the reflection/opinion question.

Innocent or Guilty?

Advances in biotechnology such as the polymerase chain reaction and DNA sequencing are making it possible for scientists and doctors to make huge strides in treating diseases. However they have also had an amazing impact on the ability of the police to both identify criminals – and to prove that people are innocent of crimes. The first ever murder conviction which was brought about as a result of DNA evidence also cleared a man of rape and murder using the same techniques. It is an incredible story….

In 1983 15 year old Lynda Mann was raped and murdered in Narborough in Leicestershire. A semen sample was taken from her body but at that point scientists could only tell the blood group and certain enzyme types from the evidence. Three years later another 15 year old girl, Dawn Ashworth was also found raped and murdered in the same area. All the evidence pointed to it being the work of the same man. The semen samples showed that the blood groups matched.

The police had a suspect – a local man who eventually confessed to Dawn's murder but denied having anything to do with Lynda's death. The policemen in charge of the case – Chief Constable Michael Hirst and

Chief Superintendent David Baker were convinced that if he had committed one crime, he had committed both. They contacted Dr Alec Jeffreys and his team at Leicester University. They had published work showing how new DNA analysis techniques – what we now call DNA fingerprinting – could be used to help solve crimes.

When Alec Jeffreys ran a comparison between the semen from both rapes, and the blood of the suspect, he found that the crimes had certainly been carried out by the same man – but it wasn't the man who had confessed!

5. Should there be a national or international DNA database of all our DNA profiles to help police identify criminals from the clues – blood, skin, saliva, semen, faeces – that they leave behind at almost every crime scene?

Read the following article about "familial searching."

- Highlight any information you find interesting or shocking.

When you are finished, answer the reflection/opinion questions

Familial Searching

Familial searching is an additional search of a law enforcement DNA database conducted after a routine search has been completed and no profile matches are identified during the process. Unlike a routine database search which may spontaneously yield partial match profiles, familial searching is a deliberate search of a DNA database conducted for the intended purpose of potentially identifying close biological relatives to the unknown forensic profile obtained from crime scene evidence. Familial searching is based on the concept that first-order relatives, such as siblings or parent/child relationships, will have more genetic data in common than unrelated individuals. Practically speaking, familial searching would only be performed if the comparison of the forensic DNA profile with the known offender/arrestee DNA profiles has not identified any matches to any of the offenders/arrestees.

Familial searching is often confused with what occurs when a partial match results from the routine search of the DNA database. A partial match is the spontaneous product of a regular database search where a candidate offender profile is identified as not being identical to the forensic profile but because of a similarity in the number of alleles shared between the two profiles, the offender may be a close biological relative of the source of the forensic profile.

While familial searching is now being performed in several jurisdictions in the United States, the United Kingdom (UK) has the most experience conducting familial searching of their National DNA Database. Since 2003, the UK has conducted approximately 200 familial searches resulting in investigative information used to help solve approximately 40 serious crimes (as of May 2011). The UK has developed detailed protocols for familial searches that include an approval process, considerations for prioritization, research of family history, and training of law enforcement officers. One of the key components responsible for the effectiveness of the

UK's system is that the search is not based upon genetics alone. Age, and more importantly, geographic location, are combined with the genetic data to produce a ranked list of potential relatives of the unknown forensic profile.

In considering whether familial searching should be implemented in your jurisdiction, it is important to recognize that a relative must already be in the database in order for the search to identify them as a potential relative of the forensic profile. It should be noted that even if a relative is in the database, it is possible that the relative may not be included in the ranked list produced by the familial search. For example, California's validation of their familial searching protocol showed that approximately 93% of fathers and 61% of full siblings were identified by their familial search procedure using the CODIS 13 core loci in searching a database of approximately one million DNA profiles (96% of fathers and 72 % of full siblings were identified using 15 loci). However, regardless of whether or not a relative is in the database, a familial search will always generate a ranked list of potential candidates for evaluation.

Is familial searching currently conducted at the national level? No, familial searching is not currently performed at the National DNA Index System.

What States perform familial searching? As of June 2011, California, Colorado, Texas and Virginia perform familial searching.

6. What is "familial searching"?

7. Currently, our state uses familial searching to identify possible suspects in certain cases. Do you agree with our state using this method? Why or why not?

Part 3: DNA Double Take Article

Directions: Read the article at this station. Answer the following questions after reading the article.

DNA Double Take

By CARL ZIMMER

From biology class to "C.S.I.," we are told again and again that our genome is at the heart of our identity. Read the sequences in the chromosomes of a single cell, and learn everything about a person's genetic information — or, as 23andme, a prominent genetic testing company, says on its Web site, "The more you know about your DNA, the more you know about yourself."

But scientists are discovering that — to a surprising degree — we contain genetic multitudes. Not long ago, researchers had thought it was rare for the cells in a single healthy person to differ genetically in a significant way. But scientists are finding that it's quite common for an individual to have multiple genomes. Some people, for example, have groups of cells with mutations that are not found in the rest of the body. Some have genomes that came from other people.

"There have been whispers in the matrix about this for years, even decades, but only in a very hypothetical sense," said Alexander Urban, a geneticist at Stanford University. Even three years ago, suggesting that there was widespread

genetic variation in a single body would have been met with skepticism, he said. “You would have just run against the wall.”

But a series of recent papers by Dr. Urban and others has demonstrated that those whispers were not just hypothetical. The variation in the genomes found in a single person is too large to be ignored. “We now know it’s there,” Dr. Urban said. “Now we’re mapping this new continent.”

Dr. James R. Lupski, a leading expert on the human genome at Baylor College of Medicine, wrote in a recent review in the journal Science that the existence of multiple genomes in an individual could have a tremendous impact on the practice of medicine. “It’s changed the way I think,” he said in an interview.

Scientists are finding links from multiple genomes to certain rare diseases, and now they’re beginning to investigate genetic variations to shed light on more common disorders.

Science’s changing view is also raising questions about how forensic scientists should use DNA evidence to identify people. It’s also posing challenges for genetic counselors, who can’t assume that the genetic information from one cell can tell them about the DNA throughout a person’s body.

Human Blueprint

When an egg and sperm combine their DNA, the genome they produce contains all the necessary information for building a new human. As the egg divides to form an embryo, it produces new copies of that original genome.

For decades, geneticists have explored how an embryo can use the instructions in a single genome to develop muscles, nerves and the many other parts of the human body. They also use sequencing to understand genetic variations that can raise the risk of certain diseases. Genetic counselors can look at the results of genetic screenings to help patients and their families cope with these diseases — altering their diet, for example, if they lack a gene for a crucial enzyme.

The cost of sequencing an entire genome has fallen so drastically in the past 20 years — now a few thousand dollars, down from an estimated $3 billion for the public-private partnership that sequenced the first human genome — that doctors are beginning to sequence the entire genomes of some patients. (Sequencing can be done in as little as 50 hours.) And they’re identifying links between mutations and diseases that have never been seen before.

Yet all these powerful tests are based on the assumption that, inside our body, a genome is a genome is a genome. Scientists believed that they could look at the genome from cells taken in a cheek swab and be able to learn about the genomes of cells in the brain or the liver or anywhere else in the body.

In the mid-1900s, scientists began to get clues that this was not always true. In 1953, for example, a British woman donated a pint of blood. It turned out that some of her blood was Type O and some was Type A. The scientists who studied her concluded that she had acquired some of her blood from her twin brother in the womb, including his genomes in his blood cells.

Chimerism, as such conditions came to be known, seemed for many years to be a rarity. But “it can be commoner than we realized,” said Dr. Linda Randolph, a pediatrician at Children’s Hospital in Los Angeles who is an author of a review of chimerism published in The American Journal of Medical Genetics in July.

Twins can end up with a mixed supply of blood when they get nutrients in the womb through the same set of blood vessels. In other cases, two fertilized eggs may fuse together. These so-called embryonic chimeras may go through life blissfully unaware of their origins.

One woman discovered she was a chimera as late as age 52. In need of a kidney transplant, she was tested so that she might find a match. The results indicated that she was not the mother of two of her three biological children. It turned out that she had originated from two genomes. One genome gave rise to her blood and some of her eggs; other eggs carried a separate genome.

Women can also gain genomes from their children. After a baby is born, it may leave some fetal cells behind in its mother’s body, where they can travel to different organs and be absorbed into those tissues. “It’s pretty likely that any woman who has been pregnant is a chimera,” Dr. Randolph said.

Everywhere You Look

As scientists begin to search for chimeras systematically — rather than waiting for them to turn up in puzzling medical tests — they’re finding them in a remarkably high fraction of people. In 2012, Canadian scientists performed autopsies on the brains of 59 women. They found neurons with Y chromosomes in 63 percent of them. The neurons likely developed from cells originating in their sons.

In The International Journal of Cancer in August, Eugen Dhimolea of the Dana-Farber Cancer Institute in Boston and colleagues reported that male cells can also infiltrate breast tissue. When they looked for Y chromosomes in samples of breast tissue, they found it in 56 percent of the women they investigated.

A century ago, geneticists discovered one way in which people might acquire new genomes. They were studying "mosaic animals," rare creatures with oddly-colored patches of fur. The animals didn't inherit the genes for these patches from their parents. Instead, while embryos, they acquired a mutation in a skin cell that divided to produce a colored patch.

Mosaicism, as this condition came to be known, was difficult to study in humans before the age of DNA sequencing. Scientists could only discover instances in which the mutations and the effects were big.

In 1960, researchers found that a form of leukemia is a result of mosaicism. A blood cell spontaneously mutates as it divides, moving a big chunk of one chromosome to another.

Later studies added support to the idea that cancer is a result of mutations in specific cells. But scientists had little idea of how common cases of mosaicism were beyond cancer.

"We didn't have the technology to systematically think about them," said Dr. Christopher Walsh, a geneticist at Children's Hospital in Boston who recently published a review on mosaicism and disease in Science. "Now we're in the midst of a revolution."

Benign Differences

The latest findings make it clear that mosaicism is quite common — even in healthy cells.

Dr. Urban and his colleagues, for example, investigated mutations in cells called fibroblasts, which are found in connective tissue. They searched in particular for cases in which a segment of DNA was accidentally duplicated or deleted. As they reported last year, 30 percent of the fibroblasts carried at least one such mutation.

Michael Snyder of Stanford University and his colleagues searched for mosaicism by performing autopsies on six people who had died of causes other than cancer. In five of the six people they autopsied, the scientists reported last October, they found cells in different organs with stretches of DNA that had accidentally been duplicated or deleted.

Now that scientists are beginning to appreciate how common chimerism and mosaicism are, they're investigating the effects of these conditions on our health. "That's still open really, because these are still early days," Dr. Urban said.

Nevertheless, said Dr. Walsh, "it's safe to say that a large proportion of those mutations will be benign." Recent studies on chimeras suggest that these extra genomes can even be beneficial. Chimeric cells from fetuses appear to seek out damaged tissue and help heal it, for example.

But scientists are also starting to find cases in which mutations in specific cells help give rise to diseases other than cancer. Dr. Walsh, for example, studies a childhood disorder of the brain called hemimegalencephaly, in which one side of the brain grows larger than the other, leading to devastating seizures.

"The kids have no chance for a normal life without desperate surgery to take out half of their brain," he said.

Dr. Walsh has studied the genomes of neurons removed during those surgeries. He and his colleagues discovered that some neurons in the overgrown hemisphere have mutations to one gene. Two other teams of scientists have identified mutations on other genes, all of which help to control the growth of neurons. "We can get our hands on the mechanism of the disease," said Dr. Walsh.

Other researchers are now investigating whether mosaicism is a factor in more common diseases, like schizophrenia. "This will play itself out over the next 5 or 10 years," said Dr. Urban, who with his colleagues is studying it.

Moving Cautiously

Medical researchers aren't the only scientists interested in our multitudes of personal genomes. So are forensic scientists. When they attempt to identify criminals or murder victims by matching DNA, they want to avoid being misled by the variety of genomes inside a single person.

Last year, for example, forensic scientists at the Washington State Patrol Crime Laboratory Division described how a saliva sample and a sperm sample from the same suspect in a sexual assault case didn't match.

Bone marrow transplants can also confound forensic scientists. Researchers at Innsbruck Medical University in Austria took cheek swabs from 77 people who had received transplants up to nine years earlier. In 74 percent of the samples, they found a mix of genomes — both their own and those from the marrow donors, the scientists reported this year. The transplanted stem cells hadn't just replaced blood cells, but had also become cells lining the cheek.

While the risk of confusion is real, it is manageable, experts said. "This should not be much of a concern for forensics," said Manfred Kayser, a professor of Forensic Molecular Biology at Erasmus University in Rotterdam. In the cases where mosaicism or chimerism causes confusion, forensic scientists can clear it up by other means. In the Austrian study, for example, the scientists found no marrow donor genomes in the hair of the recipients.

For genetic counselors helping clients make sense of DNA tests, our many genomes pose more serious challenges. A DNA test that uses blood cells may miss disease-causing mutations in the cells of other organs. "We can't

tell you what else is going on," said Nancy B. Spinner, a geneticist at the University of Pennsylvania, who published a review about the implications of mosaicism for genetic counseling in the May issue of Nature Reviews Genetics.

That may change as scientists develop more powerful ways to investigate our different genomes and learn more about their links to diseases. "It's not tomorrow that you're going to walk into your doctor's office and they're going to think this way," said Dr. Lupski. "It's going to take time."

8. What can genome sequencing be used for?

9. What is chimerism? Give an example.

10. What are some different ways that people can acquire different genomes?

11. What is mosaicism? Give an example.

12. Give an example of how chimeras are helpful.

13. Give an example of how chimeras are harmful.

14. What are the implications for humans having different genomes in their body in terms of testing, such as blood tests?

15. What are the implications that could arise with this new information in terms of using DNA in the justice system?