How-To-Do-It

Biotechnology for Non-Biology Majors

An Activity Using a Commercial Biotechnology Laboratory

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The area of biotechnology is quickly overwhelming the general public as the mainstream media are replete with news of daily discoveries involving the DNA and genes of different organisms. Unfortunately, many of those in the general population lack the background knowledge to understand the scientific basis behind much of the technology involved in these discoveries and in fact there is a large amount of anxiety associated with this lack of knowledge (Grace 1997). This apparent lack of knowledge in the area of biotechnology could be addressed by improved education at the high school, college and university levels. Another survey conducted by Zeller (1994) resulted in the author proposing that high schools double the amount of time spent on genetics and biotechnology and also increase the number of laboratory activities involving biotechnology.

Although these recommendations address the problem at the high school level, they certainly can be applied to those students that are attending postsecondary educational institutions. The challenge at this level is to engage the students with activities that will stimulate their interest in the area of biotechnology, while at the same time not overwhelming them with the science behind the process. A simultaneous challenge is to insure that the activity is not too costly in terms of necessary equipment and supplies. We have developed an inexpensive activity in partnership with a local biotechnology company that confronts these challenges.

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The activity is included in the biology course titled Biology in a Human Context and is offered at the University of Cincinnati. This course (five credits/quarter) was developed with partial National Science Foundation funding and involves the collaboration of instructors from five different colleges within the University of Cincinnati. The course involves a three-quarter sequence with respective themes that involve the human body, human genetics and reproduction, and humans and their environment. The course is tailored for nonscience majors and fulfills the natural science requirement for the Arts and Sciences at the University of Cincinnati. The goal of the course is to produce biologically literate students by exploring the major concepts in biology using humans as the primary focus. Presently, the course is taught simultaneously at four of the five colleges using a variety of multimedia and pedagogic methods.

This activity is utilized in the middle term of the Biology in a Human Context sequence that stresses human reproduction and genetics. The biotechnology company that collaborates in this activity is *Genetica® DNA Laboratories, Inc.* located in Cincinnati, Ohio. Although Genetica® provides other DNA-related services, it primarily performs paternity testing for a national client base. The laboratory is within reasonable driving distance from all the colleges and offers public education to interested groups.

Background Provided Prior to Activity

Basic properties of DNA and its central role in genetics have already been covered in the course. This activity is one example of the many uses to which we can put our understanding of DNA to work. During the first four weeks of the course, the structure and function of DNA, meiosis and mitosis, the concepts of Mendelian genetics, and the basic theory and techniques of gel electrophoresis are explored. Several steps are taken to assure that students understand these topics, including a weekly three-hour practicum where a maximum group size is approximately 25 students and where more class time can be devoted to each topic than is common practice in a traditional nonmajors biology class. Additionally, the use of multimedia, including the concurrent development of a CD-ROM, has increased the learning of our students. Even though the instruction of these topics remains idiosyncratic to each of the four college instructors, by the end of the first four weeks of classes our students recognize and apply the basic terminology of reproductive genetics and DNA.

Objectives of this Activity

The objectives of this activity are for students to be able to:

1. Describe how a commercial laboratory uses DNA data for determining the biological father in cases of disputed paternity.
2. Describe the operation of equipment as well as the procedures involved in carrying out such analyses, and the importance of certifying labs.
3. Analyze whether an alleged father (AF) is or is not the biological father (with 99.9% accuracy) in a specific example calculating appropriate probabilities.
4. Explain at least one important use of DNA technology in modern society.

5. Evaluate some of the ethical, legal and social issues accompanying this technology.

When addressing objectives in our activities, we all adhere to a pedagogical model that includes the Five-Es Learning Cycle: Engagement, Exploration, Explanation, Elaboration and Evaluation. The remainder of this paper will describe this activity in reference to this approach to learning.

Engagement

Engagement is meant to capture the students' attention, stimulate their thinking and help them access prior knowledge. In this activity, engagement involves demonstrating to the students how their recently acquired knowledge of DNA can be used in an area of significant concern to our society—identifying the biological father of a child, particularly in cases of out-of-wedlock births. Out-of-wedlock births are a significant social issue in the United States. For example, in 1997 there were more than 1.2 million out-of-wedlock births in the United States (Center for Disease Control and Prevention 1997), which accounted for over 32% of all births that year.

Exploration

Exploration gives students time to investigate, plan and organize the collected information. With regards to this activity, we discuss why parentage testing is important and under what circumstances it is used. For example, we indicate that reliable parentage testing is possible when one biological parent, mother or father, is unavailable (or deceased). When the mother is deceased, samples from the child and alleged father (AF) can still be sufficient for determination of paternity. If appropriate genetic factors are not in common between the child and the AF, the AF is excluded. If genetic factors are in common, more testing can compensate for the absence of the mother. The testing of maternal grandparents may be useful to improve the calculations, but generally are not needed. When the AF is deceased or unavailable, samples from the child's paternal grandparents can be submitted. In these cases, the laboratory performs a grandparentage test, which determines whether the alleged paternal grandparents are excluded. The probability that they could transmit the genes necessary for their son to be the biological father is calculated. Other relatives such as full siblings or known children of the AF can also be used. It is also possible to test the remains of the AF. A common example is obtaining DNA from a toxicology specimen collected from an alleged father who dies violently (e.g. in an automobile accident).

Case studies are needed to introduce the technology to the students so that they are not overwhelmed during the Explanation phase. For example, the last junta that occurred in Argentina produced many orphans whose parents had been killed by the government, and the relatives wanted their children back. Of course, records had been destroyed to protect the guilty. Thus, identification of the children had to be accomplished using samples obtained from the grandparents.

A specific example of how parentage in this situation might be assigned is demonstrated using Figure 1. In this situation we explain that each of the bands within a lane can be considered alleles, the VNTRs will be explained more fully later, but for now we will concentrate on just the matching of the grandparent alleles with the two children in question. In this case, we can exclude Child 1 as coming from those grandparents. Child 2 matches for all loci, which only means that these four individuals could have been the grandparents, not that they are the grandparents.

Explanation: Genetica® Field Trip

Through reflective activities the students are provided a fuller understanding of how paternity analyses are carried out. Our reflective activity is the field trip to Genetica®. The total visit lasts approximately one-and-a-half hours and includes an introductory slide show, interactive tour of the facility, and analysis of sample paternity cases to calculate the probability of paternity. The 20- to 30-minute presentation describes the value of paternity testing, reorients students to DNA and its structure, reviews the procedures used and outlines how the test results help determine paternity cases by using an example that consists of a mother, child and AF. The most difficult concept for the student to understand is the idea of the VNTRs and the frequencies in which these VNTRs occur in certain identifiable groups of humans. This problem is quickly overcome with examples provided by the presenter.

On some human chromosomes, a short sequence of DNA has been repeated a number of times. In any particular chromosome the repeat number may vary from 1 to 30 repeats. Since these repeat regions are usually bounded by specific restriction enzyme sites, it is possible to cut out the segment of the chromosome containing this variable number of tandem repeats

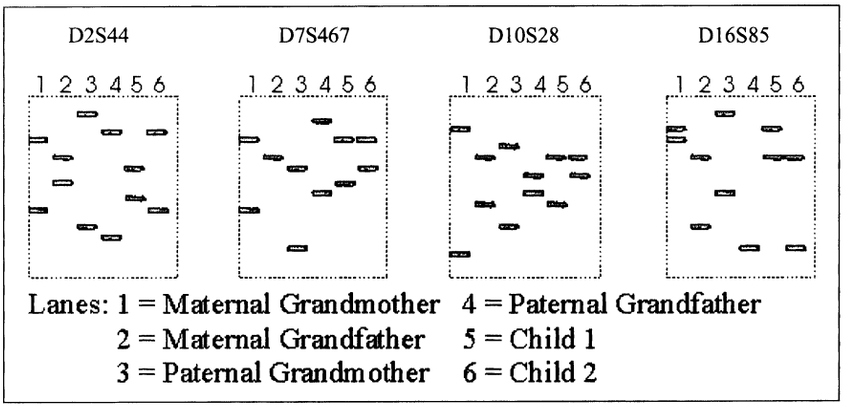

Figure 1. Four VNTR (variable number of tandem repeats) loci. Each of the four loci is on different chromosomes, and these have assorted independently. Each band represents a region of that locus which contains several short sequences of DNA that are repeated several times (i.e. VNTR). These regions are usually bounded by specific restriction sites and can be "cut" out and identified using the Southern blot procedure. Results of the Southern blots for each locus are given above. The samples are from all grandparents and from the two children under question. In the case above, all the bands of the children (Lanes 5 and 6) should match up with at least one of the bands of the maternal (Lanes 1 and 2) and paternal (Lanes 3 and 4) grandparents for each locus. Child 1 matches, however child 2 does not match up in loci D2S44 and D7S467 and only matches with the maternal grandparents in the remaining loci.

or VNTRs, run the total DNA on a gel, and identify the VNTRs by hybridization with a DNA probe. A DNA probe is special piece of DNA that is developed to target specific segments of DNA. In Figure 2, the top represents the chromosomes of the two parental individuals of the pedigree below. The father has one chromosome with four repeated sequences and the other with six repeated sequences. The mother has one chromosome with five repeated sequences and the other with three repeated sequences. At the bottom of the figure is a pedigree of the mating between the two individuals and their four children. The DNA of each of the individuals has been analyzed for the VNTR repeat number and the gels are shown below each other by the VNTRs at this one genetic locus. If several VNTR loci were used, the uniqueness of each individual would become even more distinct.

After the presentation, students are divided into two smaller groups of approximately 10 to 12 individuals. One group goes on a tour of facility with the lab director while the other group performs an actual paternity test led by the course instructor. Each activity lasts approximately 30 minutes and then the groups switch so that each is able to take the tour and complete a paternity test. Provided the background information and presentation are given prior to these activities, this amount of time appears to be sufficient as it still allows for questions and comments in each activity.

The tour provides a step-by-step walk through of the paternity-testing procedure starting with sample collection (blood or buccal swab), DNA extraction, and ending with a photograph of the finished gel showing VNTR sites of the mother, child and AF. Students are able to view the following; the DNA pellet extracted from white blood cells or buccal cells of the cheek, the electrophoresis apparatus and Southern blot procedure, separation of the VNTRs through electrophoresis, DNA probing, DNA transfer to nylon membrane, and the final photographic plate. At each point, procedure and methods are explained in understandable terms to the student, and technicians are present to demonstrate techniques. It should be stated here that if all the preceding steps were actually observed in their entirety, the trip would last several hours. What the students are actually observing are the discrete steps that are involved and the end product of those steps.

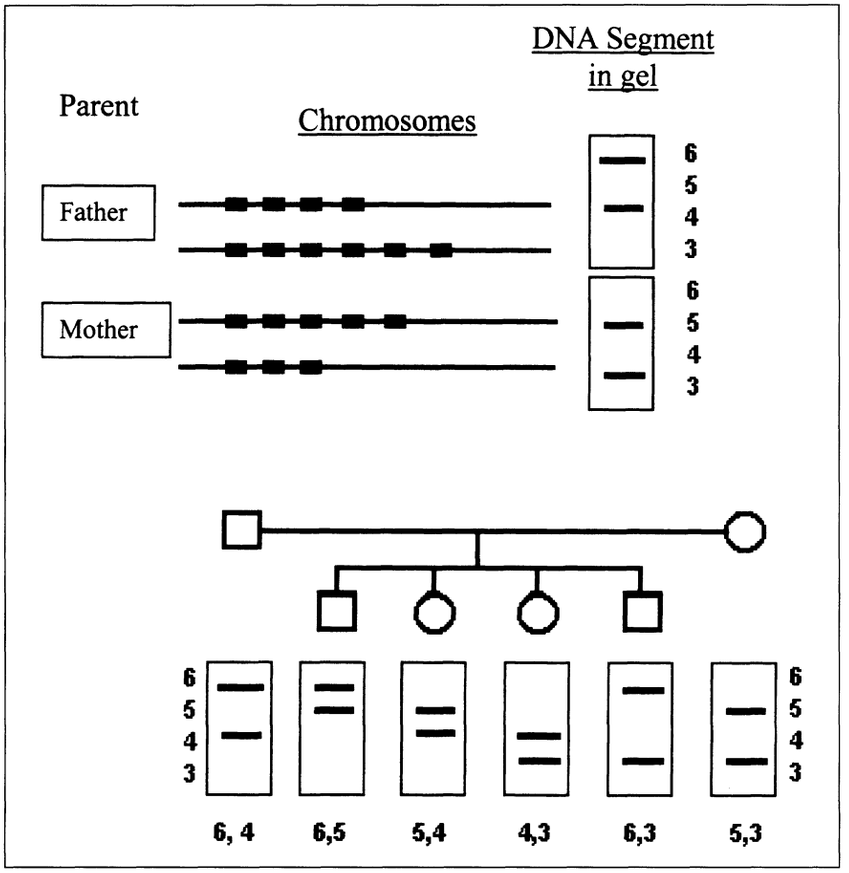

Figure 2. A representation of VNTR sites of both parents and how those sites would be represented in hypothetical offspring. This describes how each offspring shares at least one allele (VNTR site) with each parent even though the distribution of those sites among siblings may be entirely different.

In the conference room, those students not on the tour go through the calculations involved in the DNA Parentage Test. An example used, shown in Table 1, is handed to the students as an actual but anonymous case. Each of the four sites in Table 1 represents a specific VNTR located on a different chromosome. It is critical to the analysis that each VNTR segregate independently during meiosis and it is important to the students to know that alleles shared by the child and parents follow simple Mendelian genetic rules. The numbers under the Mother, Child and AF refer to the size (kilobases) of the VNTR, and are considered alleles since the child will inherit one from each parent. Those with two different numbers are heterozygous; those with only one are homozygous for that particular VNTR at the specific site being analyzed.

Students first examine the alleles of the child and AF at each site and determine if shared alleles exist. The shared alleles of the child and AF in Table 1 are 2.1, 5.4, 6.6 and 2.6 at the respective four sites. Two things are reinforced at this point. First, for the AF to be the biological father, he must share one VNTR (allele) at each site studied with the child (the child obviously shares a VNTR at each with the mother). If the AF does not share one of the VNTRs, further testing is done. If the AF does not share two or more VNTRs, Genetica® labs concludes he cannot be the biological father, and thus he is exonerated.

Second, even though the child and AF share alleles at all four sites, it does not necessarily mean the AF is the biological father. This emphasizes the importance of calculating a Paternity Index (PI) when the AF is not excluded (exonerated) from being the biological father. The PI calculates how many times an AF is more likely to be the biological father compared to picking a male at random from a population of males in the particular race in question. It is critical to the calculation that the frequencies of each VNTR at each site be known. Genetica® either purchases these frequency data sets from other companies who have derived them empirically through sampling populations within several racial classifications or have developed

Table 1. An actual case used at Genetica® labs to demonstrate paternity analysis.

Chromosome Site	Mother's Alleles	Child's Alleles	AF's Alleles
D2S44			
A	1.1	2.1	2.1
B	2.3	2.3	
D7S467			
A	2.4	2.4	2.5
B	4.2	5.4	5.4
D10S28			
A	2.5	2.5	3.1
B	5.0	6.6	6.6
D16S85			
A	1.1	2.6	2.6
B	3.0	3.0	4.2

Table 2. Abridged table of VNTR frequencies for four independently segregating sites in North American Caucasians. The frequency refers to how often that particular allele occurs in a particular population.

Chromosome Site	Allele	Frequency	Allele	Frequency	Allele	Frequency
D2S44	1.1	0.1	3.1	0.05	5.1	0.05
	1.2	0.05	3.2	0.1	5.2	0.04
	2.1	0.4	4.1	0.02	6.1	0.2
	2.3	0.06	4.3	0.3	6.3	0.02
D7S467	1.4	0.02	3.4	0.02	5.4	0.01
	1.5	0.03	3.5	0.05	5.5	0.1
	2.4	0.3	4.4	0.06	6.4	0.02
	2.5	0.1	4.5	0.1	6.5	0.03
D10S28	1.6	0.02	3.6	0.4	5.6	0.06
	1.7	0.05	3.7	0.03	5.7	0.07
	2.6	0.2	4.6	0.2	6.6	0.1
	2.7	0.07	4.7	0.05	6.7	0.05
D16S85	1.8	0.04	3.8	0.06	5.8	0.05
	2.6	0.2	4.8	0.04	6.8	0.06
	2.9	0.05	4.9	0.07	6.9	0.1

and validated their own. In the early 1990s the validity of the PIs was questioned by population geneticists who correctly understood that VNTR frequencies varied among racial groups. Most of their concerns were satisfied by intensive development of reliable frequency data for individual race categories.

Calculating PIs proceeds as follows. The PI is a calculation that takes into account the probability of the father sharing a specific allele with the child and the frequency of the shared allele in the population. Thus PI = X/Y, where X is the probability of the father sharing a specific allele and Y is the frequency of the shared allele in the population. Using the first chromosome site in Table 1 (D2S44), the AF has only one VNTR, 2.1, so he is homozygous for this particular "allele." Therefore he must share that allele with the child if he was the father, so X = 1 in this instance. The rest of the sites (D7S467, D10S28, D16S85) have two alleles associated with the AF. In these cases the probability of sharing one of those alleles with the child is 50%, based upon independent assortment of chromosomes in meiosis I. Therefore, X = 0.5 for calculating PIs for these sites. Table 2 presents the frequencies of the VNTRs for each of the four sites being used. Using this table, allele 2.1 at site D2S44 has a frequency of .4, so the PI for this site = 1/.4 = 2.5. The remaining PIs for the three remaining sites are as follows:

D7S467, PI = .5/.01 = 50, (Allele 5.4 has a frequency of .01)

D10S28, PI = .5/.1 = 5, (Allele 6.6 has a frequency of .1)

D16S85, PI = .5/.2 = 2.5, (Allele 2.6 has a frequency of .2)

An obvious question asked by the students is why calculate all four PIs? Genetica® labs assures for all cases that either 99.9% or higher probability of paternity (PP) will be obtained if the tested AF is included as being the father of the child or a 100% exclusion if the tested individual is truly not the biological father of the child. Additionally, if the tested AF is included after testing, Genetica® labs guarantees that 99.9% of the male population will also be excluded as a possible father. The PP is calculated by converting a PI into a percentage or 1/(1+(PI)). We expect a percentage of less than 100% because no alleles at any of the chromosome sites have frequencies greater than 1.0. Using the first calculated PI of 2.5, the PP obtained is 1/(1+(1/2.5)) = 71.4%. This percentage falls short of the 99.9% PP that Genetica® guarantees if the AF is included after testing. Therefore, additional genetic sites are analyzed by Genetica® to gain better resolution as to whether the tested individual is the biological father of the child or not. Thus a combined paternity index (CPI) is calculated. The CPI is the product of the calculated individual PIs. In most cases, four chromosome sites and their PIs appear to be sufficient. However, depending on frequency of alleles in the individuals being tested, it may be necessary to look at more than just four chromosome sites in order to reach that PP or 99.9%. In the present example the CPI = 2.5*50*5*2.5 = 1562.5. Now if the PP is calculated with the CPI, PP = 1/(1+(1/1562.5))*100 = 99.94. Thus, the PP is greater than 99.9%, so it meets the minimum standards set by Genetica® labs and we can be statistically assured the AF is the biological father with a 99.94% probability. Another way of viewing the PP is that it is over 1000 times more likely (1562 to be exact) that the AF is the biological father compared to a male chosen at random from the racial population in question.

Evaluation

The final part of the learning, Evaluation, involves generating instruments that assess the understanding gained from the field experience. For this, we use two writing assignments and specific exam questions. The two written assignments are constructed as "Biodiaries" in our course. Biodiaries are written works developed by each student.

In the first Biodiary, students are instructed to write a page to a significant person in their life describing the procedure used for paternity testing. The students are informed not to use many technical terms, but explain what the purpose of paternity testing is, how it is carried out, its accuracy, and the value it provides to society. The students are to specifically include the importance of variation among individuals, and population frequencies of the various alleles, and why these two points are important. Additionally, the student writes a page describing other uses of DNA technology that have been previously discussed in class. This includes DNA fingerprinting, DNA cloning, gene therapy and genetic engineering. The description is to include the value of these different technologies and their own sense of how valuable they are or will be to society.

The second Biodiary has the student performing calculations and analysis for two to three unknown paternity cases that are constructed by one of the authors. Students are expected to show their calculations and conclude whether the AF was or was not the biological father with 99.9% accuracy.

Although not evaluative, a third written assignment is asked of the students. They are to write a half-page summary of their own feelings about this activity, indicating how worthwhile they thought it was, the level of difficulty they had in understanding the procedures and calculations, and how much the trip contributed to their understanding of DNA technology and its uses. Representative responses are below:

- "...interesting and helped my knowledge of genetic testing."
- "...useful...very related to class study...gained clearer understanding of DNA testing"
- "...loved it...before the visit I did not understand DNA at all...now I'm really interested"
- "...very worthwhile...learned a lot more than reading text...useful teaching alternative; adds excitement."

Exam questions generated from this activity include:

- Explain the reason why four different chromosome sites are used to calculate the combined paternity index.
- How does whether the AF is heterozygous or homozygous at a particular VNTR site effect calculation of their PI?
- Explain why the race of the AF is important in determining paternity.
- Sample PI and CPI calculations and conclusions.

An additional activity we have discussed includes constructing a mock courtroom scenario using students as different courtroom participants (judge(s), jury, plaintiff, defendant and lawyers). It has been demonstrated that the courtroom scenario is successful in other biology classes addressing DNA technology (Rubenstein et al. 1996). The debate style format of the courtroom scenario provides a good arena for discussing the ethical, legal and social issues (ELSI) of this technology. The ELSI issue is important since recently Lindell and Milczarek (1997) stated that most undergraduate biology courses do not examine the social implications of existing technologies.

What To Do in the Absence of a Genetic Laboratory?

An obvious problem for many is what to do if a commercial laboratory is not available. We are fortunate here in Cincinnati to have this partnership with Genetica® so that students can experience firsthand the power of biotechnology. In lieu of a laboratory, there are several commercial and interactive multimedia modules that adequately describe the procedures involved, e.g. *Case It*, published by BioQuest Curriculum Consortium. Most DNA testing laboratories are also willing to provide anonymous examples for educational purposes and provide educators with the appropriate data to complete those examples. Finally, the authors would be happy to provide further information for anyone interested in incorporating such an activity into his/her curriculum (contact the senior author, Frank.Wray@uc.edu).

Conclusion

Activities that incorporate active learning, such as the one outlined here, have more far-reaching learning potential than other more traditional laboratory activities. The excitement produced by this activity was evident in ways that cannot be quantified. For example, in the weeks following the activity, several students spontaneously brought news articles into class that pertained to genetic testing. The articles produced stimulating discussions and the sense of students' applying what they learned was excitingly evident! With this type of evidence, it is our firm belief that the knowledge gained by such an activity reaches far beyond the classroom; it reaches into the students' everyday lives. As instructors, application of learning is often a driving goal.

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References

Bergland, M. *Case It*. BioQuest Curriculum Consortium.

Centers for Disease Control and Prevention. (1997). Out-of-wedlock births. *National Vital Statistics Reports, 47*(18), 1–2.

Grace, E. (1997). *Biotechnology Unzipped: Promises and Realities*. Washington, DC: National Academy Press.

Lindell, T.J. & Milczarek, G.J. (1997). Ethical, legal, and social issues in the undergraduate biology curriculum. *Journal of College Science Teaching, 26* (Mar/Apr), 345–9.

Rubenstein, E.C., Anderson, C.J. & Hall, W.B. (1996). Drama drives DNA fingerprinting lab exercise. *Journal of College Science Teaching, 26* (Nov), 103–6.

Zeller, M. F. (1994). Biotechnology in the high school curriculum: the future is here! *The American Biology Teacher, 56*(9), 460–464.