Permission is hereby granted to teachers to photocopy any pages or figures in this laboratory kit for classroom use. Teachers may also make transparencies of pages in the Teacher’s guide. Requests or reprinting for photocopying for distribution outside of the typical classroom setting should be made to HudsonAlpha Educational Outreach via email at edoutreach@hudsonalpha.org.

This kit is intended for educational purposes only. It is not to be used for research or diagnostic purposes.

Special thanks go to Leah McRae, a life sciences teacher at Bob Jones High School in Madison, Alabama, for creating the initial version of the laboratory outline and instructor guide. The laboratory component was developed through the combined work of Dr. Bob Zahorchak and Mrs. Jennifer Carden, the 2008-09 HudsonAlpha Educator in Residence. Mrs. Kelly East developed the online supplement that accompanies this lab. Additional assistance was provided by Michelle Morris, April Reis, Kelly Hill and Griffin, Rebecca and Lisa Herod.

Thanks also to Steve Ricks, Robin Nelson and the outstanding Science in Motion Biology Instructors at the Alabama Math, Science, and Technology Initiative (part of the Alabama State Department of Education) for their cooperation in this endeavor.

Funding for the development of this module and its associated activities has been provided in part by the Educational Outreach Program at the HudsonAlpha Institute for Biotechnology, located in Huntsville, Alabama. The Institute, a unique partnership between scientific researchers and biotechnology companies, has a strong commitment to educating today’s youth about opportunities in biotechnology. For more information about the ongoing work at HudsonAlpha, please visit www.hudsonalpha.org.

This product was partially funded by a grant awarded under the Workforce Innovation in Regional Economic Development (WIRED) Initiative as implemented by the U.S. Department of Labor’s Employment & Training Administration. The information contained in this product was created by a grantee organization and does not necessarily reflect the official position of the U.S. Department of Labor. All references to non-governmental companies or organizations, their services, products or resources are offered for informational purposes and should not be construed as an endorsement by the Department of Labor. This product is copyrighted by the institution that created it and is intended for individual organizational, non-commercial use only.
Foreword

In the broadest sense, biotechnology is the use of biological processes, organisms or systems to develop products aimed to improve some aspect of life. Biotechnology at its roots is a very old science, stretching back 7,000 years to the creation of bread, cheese, wine, and vinegar (which all depend on harnessing and modifying some biological process). The field has expanded dramatically over the last quarter century, powered by our understanding of DNA, the recipe card inside the nucleus of our cells. This recipe card provides the instructions to make proteins and all the structures of the cell.

Biotechnology combines the disciplines of molecular biology, genetics, cell biology, biochemistry, and embryology, which in turn are linked to additional fields such as chemical engineering, information technology, and robotics. Over the next few years, biotechnology is poised to heavily impact several areas of society, including healthcare, the environment and agriculture.

Cancer diagnostics and treatment are on the frontline of this biotechnological revolution. Researchers and clinicians are identifying new methods to identify the genes implicated in the cancer pathway and are applying the knowledge of those genes to develop targeted therapies. This laboratory exercise looks at the genes linked to hereditary nonpolyposis colorectal cancer (HNPCC), and explores the connection between family history and testing for mutations in these genes. Students will construct a pedigree of a family impacted by HNPCC and identify at-risk individuals. Students will then analyze the results of a simulated genetic test to discern which family members have inherited the genetic mutations linked to HNPCC.

Additional student content including web-based exercises and activities are included in an online supplement that accompanies this activity - http://education.hudsonalpha.org/Kits/cancer.html. Career profiles of individuals who work in fields related to this activity, such as genetic counselors, are also included at this site.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVERVIEW</td>
<td>4</td>
</tr>
<tr>
<td>TIMEFRAME</td>
<td>6</td>
</tr>
<tr>
<td>LABORATORY SAFETY</td>
<td>7</td>
</tr>
<tr>
<td>TEACHER BACKGROUND</td>
<td>8</td>
</tr>
<tr>
<td>INSTRUCTOR PROTOCOL</td>
<td>17</td>
</tr>
<tr>
<td>STUDENT ACTIVITY ANSWERS</td>
<td>22</td>
</tr>
<tr>
<td>STUDENT HANDOUT</td>
<td>25</td>
</tr>
<tr>
<td>ACTIVITY PART ONE: PEDIGREE</td>
<td>25</td>
</tr>
<tr>
<td>ACTIVITY PART TWO: GENETIC TESTING</td>
<td>28</td>
</tr>
<tr>
<td>STUDENT DATA SHEET</td>
<td>30</td>
</tr>
<tr>
<td>HNPCC LAB CROSSWORD</td>
<td>32</td>
</tr>
</tbody>
</table>
HNPCC: Detecting Inherited forms of Cancer

Overview
This lab will provide the student with experience in drawing a family pedigree and interpreting the pedigree with respect to a specific form of inherited colon cancer. The students will then complete and analyze a simulated DNA-based diagnostic test to identify which members of a fictitious family have inherited the cancer-causing mutation. In the lab’s conclusion, students will take on the role of genetic counselor in order to discuss test results with one of the family members.

Please note: this kit is intended for educational purposes only. It is not to be used for research or diagnostic purposes.

Objectives

1. AHSGE Objectives
   a. IV – 1, IV – 2
2. Alabama Course of Study for Science Objectives:
   a. Biology: (1, 4, 6, 7, 8)
   b. Genetics: (3, 4, 5, 6, 7)

Learning Objectives
The students will:
1. Construct an accurate pedigree across multiple generations
2. Modify a pedigree using genetic testing data
3. Recommend genetic testing and lifestyle changes for individuals based on data obtained during the activity
4. Perform gel electrophoresis
5. Generalize genetic testing procedures and the consequences of the results to a variety of examples
6. Describe cancer as a multi-step process that includes the accumulation of multiple mutations
7. Explain the role of caretaker genes in the development of cancer
8. Role-play a genetic counselor communicating genetic testing results and the ethical dilemmas that arise from the results
9. Role-play a genetic testing laboratory technician obtaining and analyzing testing results

Suggested Unit Correlative
This lab could be completed after a study on the cell cycle and mitosis or after a module on genetic patterns of inheritance.
Materials

If the kit is to be stored for more than 2 weeks, it should be kept refrigerated. Otherwise, room temperature storage is acceptable. While students are setting up the reactions, all components can be maintained at room temperature – no reagents need to be kept on ice.

Included with this kit:
- Four (4) boxes of Student Sample box 1
  - Each contains: DNA Ladder, Normal Control, Tumor Biopsy, Heterozygous DNA
  - and 3 patient samples
- Four (4) boxes of Student Sample Box 2
  - Each contains: 6 additional patient samples
- Instructor manual
- Student manual

Required, but not included in this kit:
- Horizontal agarose gel electrophoresis system (for Alabama Science in Motion classrooms, the Lonza Flash Gel® is used)
- Micropipettes (2-20 µl) 1-2 per gel being run – a classroom of 32 students will use four gels and need 4-8 micropipettes
- Power supply for electrophoresis gels
- Tabletop microcentrifuge
- Tips for micropipettes

General laboratory safety instructions can be found on page 7 of this manual. A material safety data sheet and Safety Warnings/Conditions of Use agreement are found together with the packing list inside the kit.
Timeframe and Outline
Day One (Can be completed in 45-60 minutes)
I. What is Hereditary non-polyposis colorectal cancer?
   1. Description of hereditary non-polyposis colorectal cancer (HNPCC) – Tell the students that they will be working with a family affected with HNPCC. Using the information found in the Instructor section of this manual, explain the clinical symptoms of HNPCC, the inheritance pattern and the genetic cause – initiated by a mutation in mismatch repair genes followed by the acquisition of additional mutations as part of a multi-step model. Note: additional content and student activities can be found at the accompanying online supplement - http://education.hudsonalpha.org/Kits/cancer.htm.
   2. Have students research five additional facts on HNPCC as part of a homework assignment.

II. Constructing the HNPCC family pedigree
   1. Teach/Review how to construct a pedigree
   2. Discuss the role of a genetic counselor. A profile of this career can be found at http://education.hudsonalpha.org/Career_Profiles/Genetic_Counselor.html
   3. Students should imagine themselves in the role of a genetic counselor as they read the description of the family with colon cancer and create the family pedigree based on the information in the description. – This can be given as a homework assignment or begun in class and completed on Day Two.

Day Two (45-60 minutes depending on time to complete pedigree)
II. Pedigree information continued
   1. Have students finish pedigree construction if this was not a homework assignment.
   2. Review the completed pedigree with the students and identify individuals who are at risk of developing colon cancer based on the pedigree.
   3. Discuss genetic testing and issues for patients to consider before agreeing to be tested.
   4. Optional: Have students role-play in pairs. Have one act as a genetic counselor and the other as a member of the family being studied. Students can write a summary of their discussion, the patient’s decision and an explanation statement.

Day Three (Can be completed in 45 - 60 minutes)
III. Detection of HNPCC mutation using restriction digests and gel electrophoresis
   1. Discuss the use of restriction enzymes for the detection of the HNPCC mutation – for supporting information see the online supplement at http://education.hudsonalpha.org/Kits/cancer.htm.
   2. Discuss the career of a lab technician. (Coming soon to the HNPCC online supplement for this lab)
   3. Students should load and run the Flash Gel® electrophoresis system.
   4. Record test results.
   5. Have the students assume the role of the genetic counselor and add results to the pedigree using the Student Data Sheet questions as a guide.
      Optional: Have the students break into pairs as the genetic counselor and a member of the family. The genetic counselor should explain the test results and what they mean to the patient. The patient should ask questions they might have and discuss how these results make them feel. (Students could record this assignment for assessment purposes)
   6. Discuss the ethical issues surrounding genetic testing in this scenario and others.

If the classroom is scheduled on a block format (90 minute segments), the description of HNPCC and the overview and construction of the pedigree can be performed on Day One. The pedigree could be completed as a homework assignment if needed. On Day Two the students could review the pedigree, discuss the genetic test for HNPCC and run and analyze the electrophoresis gel.
Laboratory Safety

The best protection that you have while working in the laboratory is you. Following all safety guidelines and being aware of the potential for accidents can greatly minimize the possibility of an accident occurring.

1. Always wear eye protection when working with laboratory chemicals or biological materials. The major potential for damage to the eyes due to liquids is from splashes or vapors.

2. A laboratory coat/apron should be worn at all times when working with laboratory chemicals or biological materials.

3. Gloves should be worn at all times when working with chemicals or biological materials. Remove gloves before touching commonly used surfaces such as doorknobs and computer keyboards. Wash your hands after removing gloves frequently and before leaving the lab.

4. Dispose of all materials in the appropriate disposal receptacles. When in doubt always check with the instructor.

5. When utilizing a gel electrophoresis apparatus, follow all electrical safety precautions.

6. Never eat or drink in the lab.

7. Only closed toed shoes should be worn in the lab. Open-toed shoes or sandals are inappropriate for the lab.
Instructor Introduction & Background

Note to instructor: The student guide addresses these topics in much less detail. Additional content is provided here to support the instructor in discussion the concept of genetic modification with the class.

Additional student content including web-based exercises and activities can be found at the online supplement designed to accompany this laboratory exercise - http://education.hudsonalpha.org/Kits/cancer.htm.

What is a pedigree? A pedigree is a diagram showing family history and relationships in a concise, standardized format. It can be a very useful tool in medicine when tracking diseases through a patient’s family history. It can be used to highlight conditions or traits within a family that might have a genetic basis, make a diagnosis, determine patterns of inheritance and identify at-risk individuals. Basic pedigree notation is provided on the first page of the student handout. Pedigree notation varies somewhat, so if the basic symbols you have discussed with your students is different from what is printed, have the students make the appropriate changes on their handout.

What is genetic testing? Genetic testing is any type of medical test that identifies changes in chromosomes, genes or proteins. Genetic testing is often conducted to identify the presence of specific changes that may lead to disease or predispose an individual to a particular condition. Patients will usually talk with a genetic counselor to establish a family history and to learn more about the particular disease and genetic mutation for which they are being tested, as well as the implications of the outcome of the test. If the patient decides to go forward with the testing, a DNA sample would be obtained from the patient and sent to a specially certified laboratory for analysis.

In the lab, the patient’s DNA sample would be isolated and examined at the genetic region of interest. There are many ways to analyze these regions. In some cases the exact nucleotide sequence would be determined and compared to a standard sequence to check for differences.

When the results have been analyzed and confirmed by the testing laboratory, either the physician or genetic counselor will review the findings with the patient. This often includes a discussion of questions such as: What do my test results mean? What should I do now? What does this mean for my children? What is the probability I can pass this on to future children? Assuming a mutation is identified, other individuals in the family may have the option of speaking with a genetic counselor and considering personal testing for the mutation in question.

How are pedigrees and genetic testing related? Often a genetic family history is the first step in identifying individuals at risk for a genetic disorder who may benefit from a genetic test. Not every individual in the family will be a candidate for testing and not every candidate for testing will choose to be tested.

What is cancer? Cancer is a collection of diseases that are characterized by uncontrolled growth of cells and the subsequent spread to surrounding tissues. Although each cancer type is somewhat unique, there are several common features surrounding cancer. Nearly all types of cancer are genetic in nature – in other words, cancer is caused by changes in the genes that control cell growth and division. Some changes occur in genes, called proto-oncogenes, which are normally involved in stimulating cells to divide. The mutated form of these genes, called oncogenes, lead to overstimulation and increased cell division. This can be thought of as a gas pedal on a car being stuck in the “on” position. Another group of genes, known as tumor suppressors, produce proteins that normally block cell division unless the right conditions are present. If we continue our car analogy, mutations in tumor suppressors remove these blocks, similar to cutting the brake lines.
Although all cancers involve changes in the genes that control cell growth and division, only about 5% of cancer is strongly hereditary – caused primarily by mutations that are inherited from parent to child. Most cancers do not result from inherited mutations, but instead develop from an accumulation of DNA damage acquired during our lifetime. These cancers begin with a single “starting” cell that becomes genetically damaged at specific genes. The transformation from that initial cell into a tumor is a stepwise progression. When the cell divides, the resulting ‘daughter’ cells often acquire additional mutations in other oncogenes or tumor suppressors, becoming progressively more abnormal and ultimately invading surrounding tissues and/or spreading to other parts of the body (metastasis). The number of genetic mutations that are required to convert a genetically normal cell into a metastatic tumor varies with cancer type. These genetic changes may involve single “letter” substitutions, deletions, duplications or chromosomal rearrangements impacting vast sections of the genome.

In the case of colon cancer, the development of cancer takes a number of years. Abnormal cell growth in the colon leads to the appearance of polyps (also called adenomas). These are quite common and about 25% of humans have one or more by the age of 50. A fraction of these polyps acquire the necessary mutations to become cancerous.

For more information on cancer, the following educational websites may be of use:
http://www.hudsonalpha.org/pages/bio101-08fall.html
http://science.education.nih.gov/supplements/nih1/cancer/default.htm
www.insidecancer.org
www.cancerquest.org

What is HNPCC? This lab activity focuses on HNPCC (hereditary non-polyposis colorectal cancer), a type of colorectal cancer. HNPCC, also known as Lynch syndrome, can cause polyps in the colon. The average age of diagnosis is 44 years. HNPCC accounts for approximately 3% of people who have colon cancer, and in the U.S. there are about 160,000 new HNPCC cases each year.

HNPCC is sometimes confused with another type of colorectal cancer known as FAP (familial adenomatous polyposis). They can be distinguished on the basis of the number of colon polyps present. Individuals with FAP often contains hundreds or thousands of polyps along the entire expanse of the colon, many of which will ultimately become cancerous. In contrast HNPCC patients generally have only a few polyps, most likely observed on the right side of the colon.

HNPCC falls into that small frequency of cancer types discussed above with a strongly hereditary impact. Family pedigree studies have shown HNPCC is inherited in an autosomal dominant fashion, meaning that within a family affected by HNPCC, affected individuals are usually observed in every generation, men and women are equally likely to be affected and approximately 50% of the offspring of an affected parent will also have HNPCC.

There are four identified causative genes (MLH1, MSH2, MSH6 and PMS2), located on chromosomes 2, 3 and 7. These genes belong to a category known as “caretaker” genes – they scan the genome to identify and help repair specific types of DNA mutations (mismatches that result from errors in DNA replication or other damaging agents). There are two copies of each of these four genes, one inherited maternally and the other paternally. HNPCC generally occurs when both copies of a specific caretaker gene are mutated. Because one mutation is inherited, every cell in the body has only a single working copy of that gene. The rate of cell division in the wall of the colon is very high, as cells are continually shed into the digestive tract. This rapid cell division often leads to replication errors in the DNA. If the sole working copy of the caretaker gene also becomes mutated in a single cell, that cell lacks part of the damage detection and repair system. Unrepaired DNA damage accumulates in this cell and all the daughter cells that result from subsequent rounds of cell divisions. If the mutations occur in oncogenes or tumor suppressor genes, cancerous polyps will result.

As described above, while HNPCC (and other types of strongly hereditary cancers) are inherited in an autosomal dominant fashion, the cells that become cancerous have inactivated both copies of the mismatch repair gene, a finding that is
characteristic of a recessive disorder. One mutation is inherited, which predisposes the individual to cancer. Because the cells of the colon are dividing so rapidly, there is a high probability that any single colon cell will acquire mutation in the other copy of the mismatch repair gene. In this way, inheriting a single mutation leads to a very high probability of cancer development. This may be a confusing point for students as the pedigree analysis will show one type of inheritance pattern, but at the cellular level something slightly different is taking place.

Although mutations that impair any of the four DNA caretaker genes can lead to HNPCC, 90% of all detected mutations are in the MLH1 and MSH2 genes. Individuals with mutations in the HNPCC genes are also at increased risk for other cancers, including cancer of the endometrium, ovary, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain and skin. As described above, inheriting a mutation in one of these HNPCC genes does not guarantee the formation of cancer, since additional steps must occur along the cancer pathway. Incomplete penetrance, variable age of cancer development or early death may prevent cancer from occurring in an individual carrying the mutation. Still, clinical studies suggest that individuals who inherit one HNPCC mutation have an 80% lifetime risk of developing colorectal cancer, compared with a 5% risk for the general population.

**How are individuals with HNPCC identified?** Because the symptoms of colon cancer are typically not observed until very late in the cancer’s development, early detection is critical to improving patient survival. This begins by identifying individuals at risk for developing HNPCC based on family history. Colorectal cancers are fairly common and a large proportion of the population likely has someone in the extended family affected by this type of cancer. As noted above, HNPCC accounts for only about 5% of all colorectal cancers and is distinguished from other forms by a very specific set of family history requirements. The criteria for identifying potential HNPCC families (called the Amsterdam criteria) includes:

- at least three family members with a diagnosis of cancer associated with HNPCC
- one of the three family members must be a first-degree relative (parent, child or sibling) of the other two
- at least two successive generations in a family have been affected
- at least one relative must have been diagnosed with cancer before the age of 50

If a family meets these criteria, at risk individuals within the family will want to consider an aggressive screening approach to identify possible colon polyps early in their development. This includes various screening options such as colonoscopy, fecal blood screening and genetic testing. Current recommendations for HNPCC at-risk individuals include undergoing a colonoscopy every one to two years after reaching the age of 20, or 10 years before the earliest age of onset within the family, whichever is earlier. After the age of 40, colonoscopy should be performed annually.

Regardless of family history, if a colorectal tumor is identified in a patient, the cancerous tissue can be tested for the presence or absence of the proteins produced by the HNPCC caretaker genes. Alternately, a genetic test can be performed to search for mutations in the HNPCC genes themselves. This test can be utilized on both individuals diagnosed with colorectal cancer and their at-risk family members. A number of different testing approaches are used, including sequencing the entire coding region, sequencing only selected exons, analyzing the surrounding chromosomal regions and a targeted analysis of common mutation sites. The most comprehensive (yet also most time-consuming and expensive) approach is sequencing the full coding region of each gene. Several clinical genetics labs will sequence each of the four genes; other labs will only offer sequencing for the MLH1 and MSH2 genes, which account for 90% of all HNPCC cases.

Once an HNPCC mutation has been identified in an individual, it becomes easier to screen other family members for this mutation. Knowing the specific mutation present in a family allows for a more targeted genetic test, to examine only the DNA at the mutation site, rather than sequencing across several genes.

For the purposes of this laboratory activity, we will focus on a restriction enzyme digest of a targeted mutation site. This involves amplifying the DNA surrounding the mutation site with the polymerase chain reaction (PCR), followed by a digestion with a specific restriction enzyme. The genetic segment of interest is copied many millions of times using PCR to obtain a quantity of DNA that can be visualized by current techniques. These DNA copies are then exposed to a restriction enzyme - a protein that can be thought of as a type of molecular scissors. Restriction enzymes cut DNA only at specific
nucleotide sequences. In this laboratory example, the HNPCC mutation has created a new restriction enzyme recognition site. This provides a diagnostic flag – if the amplified DNA contains the mutation, it will be cut in two when mixed with the restriction enzyme. The non-mutated normal gene does not contain the specific cut site recognized by the restriction enzyme and the amplified fragment will remain uncut. Family members who have not inherited the mutation (and who’s amplified DNA remains as a single piece) can be easily distinguished from those who have inherited the mutation.

Remember that humans have two copies of most genes, one inherited from the mother and the other from the father. Individuals who inherit a single HNPCC mutation will have one normal copy and one mutated copy. When the genetic test is performed on these individuals, three DNA fragments of different lengths will be observed: one non-cut (normal) segment and two fragments from the mutated copy.

As HNPCC is a relatively rare cancer, nearly all affected individuals have inherited only one mutation. Although theoretically possible that two parents may each carry an HNPCC mutation in the same caretaker gene and pass the mutations along to a child, this is a very rare occurrence. If this were to happen, it is likely this child would develop multiple forms of cancer throughout the body early in life due to a lack of mismatch repair in every cell.

Overview of the Laboratory Activity
Students will assume the role of a genetic counselor to develop a family pedigree for a large family impacted by colorectal cancer. Using this information, students will identify who is at risk to develop this type of cancer and which individuals are candidates for genetic testing. For the second portion of the lab, students will be told that a member of the family has colorectal cancer has undergone genetic testing for the HNPCC genes. A single nucleotide change in the MSH2 gene has been identified. Consequently, members of the family are being offered targeted genetic testing to determine if they carry this specific mutation. The basic procedure for this genetic test is shown in figure 1. Students are given prepared (simulated) DNA, taken from a blood sample collected for each family member who agreed to be tested. To obtain a sufficient amount of DNA for visualization by gel electrophoresis the DNA samples have been amplified by PCR (polymerase chain reaction) for the region flanking the identified mutation (for a more detailed discussion with students regarding PCR, see “Optional Discussion Points with Students” below). Previous work has shown that the mutation is a single nucleotide change. Fortunately, the mutation creates a new cut site for the Apol restriction enzyme. If the mutation is present, mixing the amplified DNA fragment with the restriction enzyme will cleave it into two smaller pieces of differing size. If the mutation is not present, the amplified fragment will remain intact.

Gel electrophoresis is used to determine if the samples have been cut by the restriction enzyme, identifying individuals who have inherited the specific HNPCC mutation. Students will load the samples into the gel and analyze the results after separating the fragments by electrophoresis (figure 2). Family members with two normal copies of this gene will show only a single non-cut amplified DNA fragment (lane 2). Individuals who have inherited a copy of the mutation will present with three bands – one intact fragment that represents the normal copy and two smaller cleaved fragments derived from the mutated gene (lane 4).
Targeted HNPCC Genetic Test

normal MSH2 gene

...aagcaaatgctagattcagctagaga...
...ttcgtttacagtcaaatagtcgtct...

Apol
...aagcaaatgctagattcagctagaga...
...ttcgtttacagtcaaatagtcgtct...
No recognition sequence present

mutant MSH2 gene

...aagcaaatgctaAattcagctagaga...
...ttcgtttacagtcttaaatagtcgtct...

Apol
...aagcaaatgctaAattcagctagaga...
...ttcgtttacagtcttaaatagtcgtct...
Mutation creates enzyme recognition sequence

Amplify region around mutation by PCR

Add Apol restriction enzyme to amplified DNA

Separate bands by gel electrophoresis

DNA fragment not cut (1 band on gel)

...aagcaaatgctagattcagctagaga...
...ttcgtttacagtcaaatagtcgtct...

DNA fragment cut (2 smaller bands on gel)

...aagcaaatgcta Aattcagctagaga...
...ttcgtttacagtcttaaatagtcgtct...
Figure 2 – gel electrophoresis of HNPCC samples

Gel Analysis of HNPCC mutation

1  2  3  4

Uncut fragment from normal allele
Cut fragments from mutant allele

Lane 1 Standard ladder
Lane 2 DNA from blood sample of nonaffected individual
Lane 3 DNA from colorectal tumor sample
Lane 4 DNA from blood sample of affected individual
The student kit contains a number of non-patient samples to be loaded onto the gel (again refer to figure 2). These include:

1. A standard ladder composed of DNA fragments of known sizes chosen at 100 base pair intervals - a guide for estimating the size of the amplified DNA fragments (lane 1)
2. A sample representing this portion of the MSH2 gene from the blood sample of a normal, unaffected individual (lane 2)
3. A sample representing this portion of the MSH2 gene taken from a biopsy of a cancerous HNPCC polyp (lane 3)
4. A sample representing this portion of the MSH2 gene taken from the blood sample of an individual affected with HNPCC (lane 4)

Students should load these reference samples in the left-most four lanes of the gel and then begin loading their patient samples.

Notice the sample from the colorectal polyp (lane 3) contains only the smaller cut fragments. Remember that while an individual usually inherits only a single mutant copy of the HNPCC genes, the second copy of that gene must also be inactivated by an acquired mutation in any given cell for the cell to progress towards cancer. The cells obtained from the cancerous biopsy have mutated this second copy. The mutation may be the identical nucleotide change present in the inherited mutant copy. More likely, the entire normal copy of the gene has been deleted, a process that occurs frequently among cancers. Regardless of the mechanism for this particular family, the cancer cells contain no working copies of the MSH2 caretaker gene and only the cut fragments representing the mutated gene are observed on the gel.

Optional Discussion Points with Students:

Cancer Diagnosis and Treatment: Then and Now
Historically, the diagnosis of cancers has been based on 1) visual evaluation and staging of the cancer as determined microscopically by cell appearance and 2) spread to surrounding or distant tissues. Treatment decisions are often based upon this information. However, in many cases, individuals with similar appearing tumors will show markedly different responses to treatment. This suggests that differences at the molecular level may be responsible for the varying outcomes. Increasingly, oncologists are adding molecular analysis of a patient's cancer as part of the standard diagnosis. Such test may include:

Gene expression profiling measures the activity (or expression) of thousands of genes simultaneously within a cancer. This creates a global image of gene activity. Experiments of this kind are often able to sort patients into groups based on which genes are active and their level of activity. Often, the groups will show differences in response to a certain treatment or overall survival. If validated, these differences can be used to predict outcomes for new patients, helping physicians identify the most optimal treatment or course of action.
**Immunohistochemical testing** is another method to measure gene activity, but at a much narrower level, usually on the order of 10 genes or fewer at one time. This method uses specially labeled antibodies to detect the presence and level of specific proteins found within the cancer. For example, the estrogen, progesterone and HER2 growth factor are receptor-based proteins often identified immunohistochemically in breast cancer biopsies. Detecting whether each receptor is present and at what level is useful in determining which therapy will be most effective for treatment.

Understanding the molecular makeup of a cancer may lead to a specific pharmacologic therapy. Known as pharmacogenomics, this field deals with how a patient’s specific genetic variation (or in this case, the cancer’s specific variation) affects the response to certain drugs. For example, if the HER2 receptor described above is present at high levels on a breast cancer tumor, the anti-cancer drug Herceptin® is added to the patient’s treatment plan. Herceptin® binds the HER2 receptor and increases the efficacy of chemotherapy. In a similar manner, Gleevec® and Erbitux® may be prescribed for specific forms of chronic myeloid leukemia and colorectal cancer, respectively. Both medications prevent tumor cells from proliferating but each operates in a very pathway-specific process that is unique to a subset of each cancer type. This type of therapy based on molecular targets is slowly but surely gaining in success as additional genetic cancer pathways are identified.

**PCR – an overview of the Polymerase Chain Reaction**

As noted above, the Polymerase Chain Reaction functions essentially as a molecular copying machine. It is the laboratory version of DNA replication, focusing on a specific region of DNA to copy. PCR protocols include the following ingredients:

- **Template** - the DNA to be amplified
- **Primers** – Two short specific pieces of DNA whose sequence flanks the target sequence to be copied both in the forward direction and in the reverse direction
- **Nucleotides** – dATP (deoxyadenosine triphosphate), dCTP (deoxycytosine triphosphate), dGTP (deoxyguanosine triphosphate), dTTP (deoxythymidine triphosphate)
- **Magnesium chloride** - enzyme cofactor
- **Buffer** - maintains pH & contains salt
- **a DNA polymerase** – a thermophilic (heat loving) enzyme initially purified from organisms that thrive in hot places, such as hot springs or deep sea thermal vents

These chemical components are mixed together and placed in a thermal cycler - a machine designed to cycle the reaction through various temperature stages according to predetermined settings. The steps of PCR are graphically displayed in figure 3. At the beginning of each cycle, the temperature is increased to around 95°C, which causes the DNA strands to separate (denature). The temperature is then cooled to between 50-60°C, which allows the primers to bind (anneal) to the DNA on either side of the target region. The temperature is then increased to 72°C, activating the polymerase, adding the nucleotides to the ends of the primers and extending a newly replicated copy of the DNA strand. This cycle of denaturation, annealing and extension continues for 24-40 cycles. Each cycle doubles the number of copied fragments, leading to an exponential increase in target fragments. The PCR program run in this lab consists of 30 cycles, meaning that at the end of the last cycle over one million copies of the targeted sequence have been produced. This is a large enough quantity of DNA to be visualized using gel electrophoresis methods.
Figure 3 – The Polymerase Chain Reaction (PCR)

**Cycle One**

- Starting DNA Molecule
- Denatured DNA
- Primer 1
- Primer 2
- DNA Polymerase
- dNTPs
- Copied DNA

**Cycle Two**

- Copied DNA

**Cycle Three**

- Copied DNA

<table>
<thead>
<tr>
<th>Cycle</th>
<th># DNA molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td>10</td>
<td>1024</td>
</tr>
<tr>
<td>20</td>
<td>1048576</td>
</tr>
<tr>
<td>25</td>
<td>33554432</td>
</tr>
<tr>
<td>30</td>
<td>1073741824</td>
</tr>
</tbody>
</table>
Instructor Protocol

Prior to Day One
Students should have a basic understanding of cancer before beginning this laboratory activity. This understanding should include:

- Cancer is a disorder of abnormal cell growth
- The cell cycle is disturbed in cancer, leading to growth in the absence of proper cell signals
- Cancer is a multistep process that results from the stepwise accumulations of several mutations in proto-oncogenes and tumor suppressor genes
- Mutations in DNA can be both inherited and acquired
- Acquired DNA mutations can be caused by environmental factors (carcinogens)

Much of this information can be found at the National Institutes of Health (NIH) website about cancer: [http://science.education.nih.gov-supplements/nih1/cancer/default.htm](http://science.education.nih.gov-supplements/nih1/cancer/default.htm)

Make copies of the student activity guide for each student in the class. The activity guide begins on page 25 of this manual. Permission is hereby granted to teachers to reprint or photocopy in classroom quantities the pages or sheets needed for the students.

Day One - What is cancer?

1. Introduce hereditary non-polyposis colorectal cancer (HNPCC) – Tell the students that they will be working with a family affected with HNPCC. Using the information found in the Instructor section of this manual, explain the clinical symptoms of HNPCC, the inheritance pattern and the genetic cause – a mutation in mismatch repair genes.

   Note that additional content and student activities can be found at the accompanying online supplement - [http://education.hudsonalpha.org/Kits/cancer.html](http://education.hudsonalpha.org/Kits/cancer.html).

2. Have students research five additional facts on HNPCC for homework
3. Teach/Review how to construct a pedigree
4. Discuss the role of a genetic counselor. A profile of this career can be found at [http://education.hudsonalpha.org/Career_Profiles/Genetic_Counselor.html](http://education.hudsonalpha.org/Career_Profiles/Genetic_Counselor.html)
5. Students should imagine themselves in the role of a genetic counselor as they read the description of the family with colon cancer and create the family pedigree based on the information in the description. – This can be given as a homework assignment or begun in class and completed on Day Two.

Day Two: Pedigree Completion and Review

1. Have students finish pedigree construction if this was not a homework assignment.
2. Review the completed pedigree with the students and identify individuals who are at risk of developing colon cancer based on the pedigree.
3. Discuss genetic testing and issues for patients to consider before agreeing to be tested.
4. Optional: Have students role-play in pairs. Have one act as a genetic counselor and the other as a member of the family being studied. Students can write a summary of their discussion, the patient’s decision and an explanation statement.
Part One: Family Pedigree

Instructions: Use the information provided below to draw a family pedigree for a specific form of inherited colon cancer, HNPCC. Remember to use all the rules of pedigree construction as you complete this activity.

You are a genetic counselor whose job is to advise patients of the risk for an inherited disorder and discuss appropriate testing options and treatments. Your patient is Bob S who is 45 years old and in good health. His wife Jane is 42 and also in good health. Bob and Jane have a nineteen-year-old son Steven, and a twenty-one year old daughter, Claire. Bob explains that he has a sister Susan (currently age 50) who was diagnosed with colon cancer at age 42. His brother, Marshall, is 47 and last year was also diagnosed with colon cancer. Bob’s last sibling is a 38 year old sister named Sara. Bob’s parents both died young - his father Robert was killed in the war at age 35 and his mother Elizabeth was killed in a car accident at age 40. Elizabeth has a brother Stan who is 70 and was diagnosed at age 50 with colon cancer. Elizabeth also had three sisters and two brothers, all of whom are living. Bob’s maternal grandmother died at age 42 with a “female” cancer and his maternal grandfather died at age 85. Bob’s father Robert had three brothers who are deceased and three sisters, still living. Bob’s paternal grandparents both died in their 60’s, causes unknown. Bob’s wife Jane S. reports two brothers - one who has two sons and one with a daughter. Jane also has a sister who has three daughters. Jane’s parents are living – her mother Roberta is 67, and Herschel, her father is 70. Roberta has three living sisters, two living brothers, and a brother who died in his 70’s from a heart attack. Roberta’s parents (Jane’s maternal grandparents) are deceased, grandfather at age 65 from a heart attack and grandmother at age 75 with breast cancer. Herschel has two deceased sisters and a 78 year old brother named Warren who was diagnosed four years ago with colon cancer. Herschel’s parents are deceased, his father at age 80 and mother at age 75. Both Bob and Jane are African American. There is no other known cancer on either side of the family.

Prior to day three

Make copies of the Genetic Testing Student Activity handout.
Set out the following for each class of 32 students:

- 4 Flash Gel® docking stations with power supplies
- 2 micropipettes and a box of tips for each gel apparatus
- 4 boxes of samples 1-6 and 4 boxes of samples 7-12, both found in the HNPCC kit

This lab is designed for a group of 4 students to work from each sample box. Therefore, there will be 8 students per gel. (4 loading samples 1-6 and 4 additional students loading samples 7-12)

Before class begins, open the package containing the pre-cast gel. The DNA stain and buffer are already incorporated into the cassette. Set the cassette into the docking station. Each gel will hold up to 12 samples plus a lane for the DNA ladder.

You may make copies of the crossword puzzle in order to review the key terms in this lab. These items work as a great supplement for the students before, during (while the Flash Gels are being loaded), or after the lab.
Day Three: Preparing and Analyzing the Genetic Test Samples
Discuss the use of restriction enzymes for the detection of the HNPCC mutation – for additional information see the online supplement at http://education.hudsonalpha.org/Kits/Cancer.html

1. Students will transfer a portion of the patient samples into the gel and separate the products by electrophoresis (see student protocol below). Make sure the students know how to load the gels by demonstrating the process. Explain the role of the DNA marker as a molecular ruler, used to compare the sizes of the amplified bands.

Make sure that the gels are loaded in this order from left to right (6 µl per lane):

- Lane 1 – Ladder DNA fragments
- Lane 2 – Control DNA
- Lane 3 – Patient Tumor DNA
- Lane 4 – Heterozygote DNA
- Lane 5 – Stan, Bob’s Uncle (age 70) with colon cancer
- Lane 6 – Susan, Bob’s Sister (age 50) with colon cancer
- Lane 7 – Marshall, Bob’s Brother (age 47) who has a known HNPCC mutation
- Lane 8 – Sara, Bob’s Sister (age 38)
- Lane 9 – Bob
- Lane 10 – Warren, Jane’s Uncle (age 78) with colon cancer
- Lane 11 – Jane
- Lane 12 – Steven, Bob’s Son
- Lane 13 – Claire, Bob’s Daughter

2. After all wells have been loaded, connect the system to the power supply and turn on the power. Set the power supply to run at 275 volts and turn on the light on the gel box to observe the movement of the DNA fragments. Monitor the movement of the DNA bands carefully to ensure good separation (this should take 5-10 minutes). This system runs very quickly and the DNA can run off the end of the gel if not careful. Explain the reason that DNA moves from the negative electrode of the gel toward the positive pole is due to the negative charge present on DNA that results from the phosphate groups found in the DNA backbone. Also, explain the relationship between fragment size and rate of movement through the gel. (Smaller fragments move faster and farther while larger fragments move slower and stay closer to the wells)

3. Once the DNA fragments have been separated, the students should examine the gels in the docking stations with the UV light and record their results on their data sheet.

EXPECTED RESULTS
The results should show that Bob’s Uncle Stan, Bob’s Sister Susan with colon cancer (age 50), Bob’s Brother Marshall (age 47), Bob, and Bob’s daughter Claire all have inherited the HNPCC mutation. Bob’s sister Sara (age 38), Jane, Jane’s Uncle Warren, and Bob’s Son Steven do not have the HNPCC mutation.

4. Discussion should follow about the genetic testing results. The students should be able to complete the post lab questions. Have the students add results to the pedigree using the Student Data Sheet questions as a guide.

5. Discuss the ethical issues surrounding genetic testing in this scenario and others. If there is time, divide the students into pairs as the genetic counselor and a member of the family. The genetic counselor should explain the test results and what they mean to the patient. The patient should ask questions they might have and discuss how these results make them feel. (Students could record this assignment for assessment purposes)

These instructions are from the Student Guide, which begins on page 28.

Part Two: Genetic Testing
Student Procedure for Loading and Analyzing the HNPCC Gel

Based upon the previous information on Bob’s family, genetic testing was offered to Marshall, Bob’s brother who was recently diagnosed with cancer. This test examined the DNA sequence of all four genes known to be associated with HNPCC. A single nucleotide change was found in the MSH2 gene. Clinical studies have shown this mutation is associated with HNPCC. As a result, genetic testing has been recommended for those in the family who wish to participate.

You are the laboratory technician responsible for completing the genetic testing for these family members. You will be given a tube of prepared DNA, taken from a blood sample collected for each family member who agreed to be tested. The DNA has been amplified by PCR (polymerase chain reaction) to produce millions of copies of the specific region of DNA that contains the mutation identified in Marshall. There is enough DNA created by this test that it can be loaded onto an agarose gel and visualized by a technique known as gel electrophoresis.

Previous research has shown that the single nucleotide change found in Marshall’s DNA is recognized by a restriction enzyme, a type of protein that cuts DNA at specific sequences. If the mutation is present, mixing the amplified DNA fragment with the restriction enzyme will cut the DNA into two smaller pieces of differing size. If the mutation is not present, the restriction enzyme will not recognize the DNA sequence and the amplified fragment will remain intact.

As a lab technician, you will separate and visualize the DNA fragments from each family member by gel electrophoresis. Upon viewing the finished gel, you will analyze the fragments of DNA that represent the area around the possible mutation. You will record the results for each family member. These test results will then be returned to the patient’s doctor and a genetic counselor will be present to discuss them with each patient individually.

Important information for the lab technician:
Remember: If the mutation is present, the mutant fragment will have been cut into two smaller pieces. If no mutation exists, the single large uncut fragment will be found.
1. Use the DNA samples provided for the marker ladder, the control, the patient tumor, and various family members. Place the sample tubes in the microcentrifuge and spin for 15 seconds to make sure all the liquid is at the bottom of each tube.

2. Pipette 6 µl of each DNA sample into a well of the Flash Gel® cassette. Use the micropipettes and their tips to load the samples into the wells.

3. Make sure that the gels are loaded in the order shown below from left to right (6 µl per lane). Remember to change the tips for each sample loaded.

   Lane 1 – Ladder DNA fragments  
   Lane 2 – Control DNA  
   Lane 3 – Patient Tumor DNA  
   Lane 4 – Heterozygote DNA (one mutant copy of the gene and one normal copy)  
   Lane 5 – Stan, Bob’s Uncle (age 70) with colon cancer  
   Lane 6 – Susan, Bob’s Sister (age 50) with colon cancer  
   Lane 7 – Marshall, Bob’s Brother (age 47) who has a known HNPCC mutation  
   Lane 8 – Sara, Bob’s Sister (age 38)  
   Lane 9 – Bob  
   Lane 10 – Warren, Jane’s Uncle (age 78) with colon cancer  
   Lane 11 – Jane  
   Lane 12 – Steven, Bob’s Son  
   Lane 13 – Claire, Bob’s Daughter

4. After all the samples have been loaded the instructor will connect the electrophoresis equipment and run the gel (cassette) for 5-10 minutes. While the gel is running, observe the migration of the patient’s DNA bands.

5. After gel electrophoresis, determine the position of the DNA fragments by comparing them to the DNA ladder. Record this position of each fragment on your data sheet.

6. Use the results from the analysis to determine whether each family member has inherited the HNPCC mutation. Record this data on your sheet.

7. Answer the Student Data Sheet questions.

8. Using your answers as a guide:
   i. Pair with another student. One should take the role of a genetic counselor and the other should take the role of a patient - choose which one of the family members to represent. The genetic counselor should explain the test results and what they mean to the patient. The patient can ask questions and discuss how the test results make them feel. If there is time choose another patient and switch roles.
   ii. Discuss the ethical issues surrounding genetic testing in this scenario and other possible scenarios
Answers to Post-pedigree Questions:

1. Describe Bob’s risk for colon cancer? Describe Jane’s risk?

   Bob is at increased risk for early onset of colon cancer, possibly due to a single gene cancer disorder. Based on what appears to be an autosomal dominant mode of inheritance, it appears Bob may be at a 50% risk to develop colon cancer. Jane’s risk does not appear to be elevated based on a study of her family history. Although Jane has an uncle affected with colon cancer, his age of onset is relatively late (74) and is not consistent with the HNPCC pattern.

2. As Bob’s genetic counselor, what issues might you chose to discuss with Bob?

   Bob’s need to be screened for colon cancer based on his family history and the possibility of an inherited form of colon cancer being present in the family. This would also be the time to introduce the concept of genetic testing and which family members might be most appropriate to be tested.

3. Is there anyone in either family you’d like to find out more about?

   Pathology reports from Bob’s maternal grandmother – the “female cancer” could very likely be endometrial or ovarian, both of which are associated with HNPCC. Although medical records may not be available, it is possible that his mother or one of the aunts would know more about her diagnosis. Confirmation of this diagnosis would extend the cancer pattern back another generation and strengthen the evidence that this family is passing an HNPCC mutation across generations.

4. What is the relationship between cell cycle regulation and cancer? What makes a cancerous cell different from a normal cell?

   Cancer is a result of a loss of cell cycle control. Therefore the mutation or series of mutations that affect the DNA of a cancerous cell usually leads to a change in the regulation of the cell cycle and ultimately the rate in which a cell undergoes mitotic division. The cancerous cell has acquired many additional mutations that lead to uncontrolled cell division (as well as the ability to spread to other parts of the body – metastasis) whereas the normal cell still has control over the cell cycle.
Answers to Post-Lab Discussion Questions:

1. Does having the mutation mean that an individual will definitely develop cancer? Why or why not?
   *Having the mutation does not mean that someone will definitely develop cancer. In this case, it will take at least two consecutive mutations to fully inactivate the specific HNPCC tumor suppressor gene. Having one mutation means that the person is more likely to develop cancer than someone who does not have the mutation.*

2. What is the risk that Bob’s daughter Claire will pass the HNPCC mutation along to her children?
   *There is a 50% chance that each child will inherit the mutation from Bob’s daughter.*

3. Let’s assume that Bob did not want to know his mutation status and declined to be tested. Bob’s daughter Claire, however, very much wanted to be tested. Under these circumstances, a positive test on Claire will reveal Bob’s mutation status as well. Who has the right to determine if Bob’s daughter should be tested? Does Claire’s age make a difference? If she is tested and is found to be positive, how should she discuss the results with Bob?
   *This answer will vary because of the ethical dilemma posed in this situation. Who has the right to know and does the right of a parent trump that of a child?*
   
   *Note that if Bob’s daughter were a minor, standard testing practice does not test an individual under the age of 18 for HNPCC mutations. This is not an issue because of the age of Bob’s daughter, but it is a point for discussion. Many students may say that all children should be tested, but standard practice is to not test children for a genetic mutation that does not show symptoms until adulthood. To test these individuals as children, under the consent of the parent, takes away their right to not know their genetic information.*

4. Who outside the family (physicians, employers, insurance companies, teachers) has a right to know this genetic information?
   *This answer will vary and could lead to discussion of insurance companies and other organizations that might want to know the genetic results. This may also lead to a discussion of individual rights.*
HNPCC Lab Crossword

Across

1. The acronym that stands for familial adenomatous polyposis.
4. Intestinal growths that are usually benign, but are the first step in colon cancer.
7. An enzyme that cuts DNA at a specific site.
11. Term that indicates that a set of alleles for a gene are different.
12. Term that indicates that both alleles that code for a gene are the same.
13. Special screening in which a doctor uses a scope to examine the colon.
15. A trait that is expressed when combined with a recessive one.
16. Chart that shows an orderly presentation of an inherited trait in a family.

Down

2. Chromosome that is not a sex chromosome.
3. Disease in which cells grow continuously and form malignant tumors due to a change in their DNA.
5. Another name for HNPCC.
6. A change in DNA.
8. Process that separates DNA in a gel by using electricity.
9. Gene that contributes to the control of the cell cycle. When it is activated, a cell does not divide.
10. Middle structure that is a part of the large intestine. Functions to reabsorb water and electrolytes and form feces.
14. The acronym for human nonpolyposis colorectal cancer.
HNPCC: Detecting Inherited forms of Cancer
Student Activity

Overview: For this lab, assume you are a genetic counselor working in a clinic. It is your job to advise patients of their risk for inherited disorders based upon family history or clinical findings and to explain the nature of the disorder and risks to other family members. You will be working with an individual who has a family history of colon cancer that appears to be caused by an autosomal dominant disorder known as hereditary non-polyposis colorectal cancer, or HNPCC.

Later you will assume the role of a laboratory technician to complete and analyze a DNA-based diagnostic test identifying which members of this family have inherited the cancer-causing mutation.

What is a pedigree? A pedigree is a diagram showing family history and relationships in a concise, standardized format. It can be used to highlight conditions or traits within a family that might have a genetic basis. It can also help determine patterns of inheritance and identify at-risk individuals. Basic pedigree symbols are shown in the box at left.

What is genetic testing? Genetic testing is any type of medical test that identifies changes in chromosomes, genes or proteins. Genetic testing often identifies specific changes that may lead to or predispose an individual to a particular condition. If a patient decides to go forward with genetic testing, a DNA sample is obtained and sent to a specially certified laboratory to extract the patient’s DNA and examine the genes associated with the disease.

How are pedigrees and genetic testing related? Often obtaining a pedigree is the first step in identifying at risk individuals who may benefit from a genetic test. Not every individual in the family will be a candidate for testing and not every candidate for testing will choose to be tested.

What is cancer? Cancer is a collection of diseases characterized by uncontrolled cell growth and their spread to surrounding tissues. Nearly all types of cancer are genetic in nature – in other words, cancer is caused by changes in the genes that control cell growth and division. However, only about 5% of cancer is strongly hereditary – caused primarily by a mutation that is inherited from parent to child. Most cancers instead develop from an accumulation of DNA damage acquired during our lifetime. These cancers begin with a single “starting” cell that becomes genetically damaged. The transformation from that initial cell into a tumor is a stepwise progression. The number of genetic mutations that are required to convert a genetically normal cell into a metastatic tumor varies with cancer type.
What is HNPCC? This lab activity focuses on HNPCC (hereditary non-polyposis colorectal cancer), a type of colorectal cancer. The colon and rectum are parts of the body’s digestive system responsible for removing water and electrolytes from the digested food and forming and storing solid waste (feces) until it passes out of the body. The colon is the first 6 feet of the large intestine and the rectum is the last 8 to 10 inches. Cancer that begins in the colon is called colon cancer, and cancer that begins in the rectum is called rectal cancer. Cancers affecting either of these organs may also be called colorectal cancer.

HNPCC, also known as Lynch syndrome, results in polyps (abnormal growths) in the colon that ultimately may become cancerous. HNPCC accounts for approximately 3% of people who have colon cancer - about 160,000 new HNPCC cases each year in the U.S. HNPCC belongs in that rare group of cancer types primarily caused by inherited mutations. Family pedigree studies have shown HNPCC is inherited in an autosomal dominant fashion.

There are four identified genes (MLH1, MSH2, MSH6 and PMS2) that can cause HNPCC when mutated. These genes are known as “caretaker genes” – they scan the genome to identify and help repair DNA mutations. As with nearly all human genes, two copies of each are present - one inherited maternally and the other paternally. HNPCC occurs when both copies of a specific caretaker gene are mutated. Generally, one copy of the mutation is inherited from a parent, leaving every cell with only a single working copy. If a cell in the colon acquires a mutation in the remaining copy, the cell loses the ability to detect and repair DNA damage. Eventually, the buildup of this additional genetic damage leads to colorectal cancer.

Inheriting a mutation in one of these HNPCC genes does not guarantee the formation of cancer, as additional steps must occur along the cancer pathway. Still, clinical studies suggest that individuals who inherit one HNPCC mutation have an 80% lifetime risk of developing colorectal cancer.

How are individuals with HNPCC identified? Because the symptoms of colon cancer are typically not observed until late in the cancer’s development, early detection is critical to improving patient survival. This begins by identifying at risk individuals based on family history. Colorectal cancers are fairly common and a large proportion of the population is likely related to someone affected by colorectal cancer. HNPCC is distinguished from other forms of colon cancer by a very specific set of family history requirements.

If a family meets these criteria, at risk individuals are offered an aggressive screening approach to identify colon polyps early. This includes colonoscopy, fecal blood screening and genetic testing.

**Cancer Diagnosis and Treatment:** Historically, the diagnosis of cancers has been based on what the cancer cells look like under a microscope. Typical treatments for colorectal cancer include removal of all or a portion of the colon, followed by chemotherapy and/or radiation therapy.

Scientists have recently learned that they can identify the genes that are turned on in a cancer and monitor their activity in response to specific medication. This helps doctors see how well a patient’s cancer may respond to a particular drug or treatment. The amount of specific proteins found in certain types of cancer can also help doctors to know which therapy will be most effective for their patients.
Part One: Family Pedigree

Instructions: Use the information provided below to draw a pedigree for a family affected with HNPCC. Remember to use all the rules of pedigree construction as you complete this activity.

You are a genetic counselor whose job is to advise patients of the risk for an inherited disorder and discuss appropriate testing options and treatments. Your patient is Bob S who is 45 years old and in good health. His wife Jane, is 42 and also in good health. Bob and Jane have a nineteen-year-old son Steven, and a twenty-one year old daughter, Claire. Bob explains that he has a sister Susan (currently age 50) who was diagnosed with colon cancer at age 42. His brother, Marshall, is 47 and last year was also diagnosed with colon cancer. Bob’s last sibling is a 38 year old sister named Sara. Bob’s parents both died young - his father Robert was killed in the war at age 35 and his mother Elizabeth was killed in a car accident at age 40. Elizabeth has a brother Stan who is 70 and was diagnosed at age 50 with colon cancer. Elizabeth also had three sisters and two brothers, all of whom are living. Bob’s maternal grandmother died at age 42 with a “female” cancer and his maternal grandfather died at age 85. Bob’s father Robert had three brothers who are deceased and three sisters, still living. Bob’s paternal grandparents both died in their 60’s, causes unknown. Bob’s wife Jane S. reports two brothers - one who has two sons and one with a daughter. Jane also has a sister who has three daughters. Jane’s parents are living – her mother Roberta is 67, and Herschel, her father is 70. Roberta has three living sisters, two living brothers, and a brother who died in his 70’s from a heart attack. Roberta’s parents (Jane’s maternal grandparents) are deceased, grandfather at age 65 from a heart attack and grandmother at age 75 with breast cancer. Herschel has two deceased sisters and a 78 year old brother named Warren who was diagnosed four years ago with colon cancer. Herschel’s parents are deceased, his father at age 80 and mother at age 75. Both Bob and Jane are African American. There is no other known cancer on either side of the family.

Questions:
1. After constructing the pedigree, describe Bob’s risk for colon cancer. Describe Jane’s risk.

2. As Bob’s genetic counselor, what issues might you chose to discuss with Bob?

3. Is there anyone on either side of the family you’d like to find out more about to help support your views on a possible inheritance pattern for this cancer?

4. What is the relationship between cell cycle regulation and cancer? What makes a cancerous cell different from a normal cell?
Part Two: Genetic Testing

Based upon the information determined about the family, genetic testing was offered to Marshall, Bob’s brother who was recently diagnosed with cancer. This test examined the DNA sequence of all four genes known to be associated with HNPCC. A single nucleotide change was found in the MSH2 gene. Clinical studies have shown this mutation is associated with HNPCC. As a result, genetic testing has been recommended for those in the family who wish to participate.

You are the laboratory technician responsible for completing the genetic testing for these family members. You will be given a tube of prepared DNA, taken from a blood sample collected for each family member who agreed to be tested. The DNA has been amplified by PCR (polymerase chain reaction) to produce millions of copies of the specific region of DNA that contains the mutation identified in Marshall. There is enough DNA created by this test that it can be loaded onto an agarose gel and visualized by a technique known as gel electrophoresis.

Previous research has shown that the single nucleotide change found in Marshall’s DNA is recognized by a restriction enzyme, a type of protein that cuts DNA at specific sequences. If the mutation is present, mixing the amplified DNA fragment with the restriction enzyme will cut the DNA into two smaller pieces of differing size. If the mutation is not present, the restriction enzyme will not recognize the DNA sequence and the amplified fragment will remain intact.

As a lab technician, you will separate and visualize the DNA fragments from each family member by gel electrophoresis. Upon viewing the finished gel, you will analyze the fragments of DNA that represent the area around the possible mutation. You will record the results for each family member. These test results will then be returned to the patient’s doctor and a genetic counselor will be present to discuss them with each patient individually.

Important information for the lab technician:
Remember: If the mutation is present, the mutant fragment will have been cut into two smaller pieces. If no mutation exists, the single large uncut fragment will be found.
**Materials Needed Per Group**

- 2 sample boxes containing the DNA from various family members
  - 4 students – Sample Box 1
  - 4 students – Sample Box 2

- 2 Micropipettors and tips
- Flash Gel electrophoresis system
- Power Supply

**Procedure**

1. **Use the DNA samples provided for the marker ladder, the control, the patient tumor, and various family members.**
   Place the sample tubes in the microcentrifuge and spin for 15 seconds to make sure all the liquid is at the bottom of each tube.

2. **Pipette 6 µl of each DNA sample into a well of the Flash Gel® cassette.** Use the micropipettes and their tips to load the samples into the wells.

3. **Make sure that the gels are loaded in the order shown below from left to right (6 µl per lane).** Remember to change the tips for each sample loaded.
   - Lane 1 – Ladder DNA fragments
   - Lane 2 – Control DNA
   - Lane 3 – Patient Tumor DNA
   - Lane 4 – Heterozygote DNA
   - Lane 5 – Stan, Bob’s Uncle (age 70) with colon cancer
   - Lane 6 – Susan, Bob’s Sister (age 50) with colon cancer
   - Lane 7 – Marshall, Bob’s Brother (age 47) who has a known HNPCC mutation
   - Lane 8 – Sara, Bob’s Sister (age 38)
   - Lane 9 – Bob
   - Lane 10 – Warren, Jane’s Uncle (age 78) with colon cancer
   - Lane 11 – Jane
   - Lane 12 – Steven, Bob’s Son
   - Lane 13 – Claire, Bob’s Daughter

4. **After all the samples have been loaded the instructor will connect the electrophoresis equipment and run the gel (cassette) for 5-10 minutes.** While the gel is running, observe the migration of the patient’s DNA bands.

5. **After gel electrophoresis, determine the position of the DNA fragments by comparing them to the DNA ladder.** Record this position of each fragment on your data sheet.

6. **Use the results from the analysis to determine whether each family member has inherited the HNPCC mutation.** Record this data on your sheet.

7. **Answer the Student Data Sheet questions.**

8. **Using your answers as a guide:**
   - i. Pair with another student. One should take the role of a genetic counselor and the other should take the role of a patient - choose one of the family members to represent. The genetic counselor should explain the test results and what they mean to the patient. The patient can ask questions and discuss how the test results make them feel. If there is time choose another patient and switch roles.
   - ii. Discuss the ethical issues surrounding genetic testing in this scenario and other possible scenarios.
Detecting Inherited Forms of Cancer

Student Data Sheet

Results of Gel Electrophoresis:

Indicate whether each person was positive (+) or negative (−) for the HNPCC mutation:

Bob’s Uncle ____  Jane’s Uncle ___  Bob’s Sister (age 50) ___
Bob ___   Bob’s Sister (age 38) ___  Marshall (age 47) ___
Jane ___   Bob’s Son ___   Bob’s Daughter ___
Detecting Inherited Forms of Cancer

Student Discussion Questions

1. Does having the mutation mean that an individual will definitely develop cancer? Why or why not?

2. What is the risk that Bob’s daughter Claire will pass the HNPCC mutation along to her children?

3. Let's assume that Bob did not want to know his mutation status and declined to be tested. Bob’s daughter Claire, however, very much wanted to be tested. Under these circumstances, a positive test on Claire will reveal Bob’s mutation status as well. Who has the right to determine if Claire should be tested? Does Claire’s age make a difference? If she is tested and is found to be positive, how should she discuss the results with Bob?

4. Who outside of the family (physicians, employers, insurance companies, teachers) has a right to know this genetic information?
HNPCC Lab Crossword

Across
1. The acronym that stands for familial adenomatous polyposis.
4. Intestinal growths that are usually benign, but are the first step in colon cancer.
7. An enzyme that cuts DNA at a specific site.
11. Term that indicates that a set of alleles for a gene are different.
12. Term that indicates that both alleles that code for a gene are the same.
13. Special screening in which a doctor uses a scope to examine the colon.
15. A trait that is expressed when combined with a recessive one.
16. Chart that shows an orderly presentation of an inherited trait in a family.

Down
2. Chromosome that is not a sex chromosome.
3. Disease in which cells grow continuously and form malignant tumors due to a change in their DNA.
5. Another name for HNPCC.
6. A change in DNA.
8. Process that separates DNA in a gel by using electricity.
9. Gene that contributes to the control of the cell cycle. When it is activated, a cell does not divide.
10. Middle structure that is a part of the large intestine. Functions to reabsorb water and electrolytes and form feces.
14. The acronym for human nonpolyposis colorectal cancer.