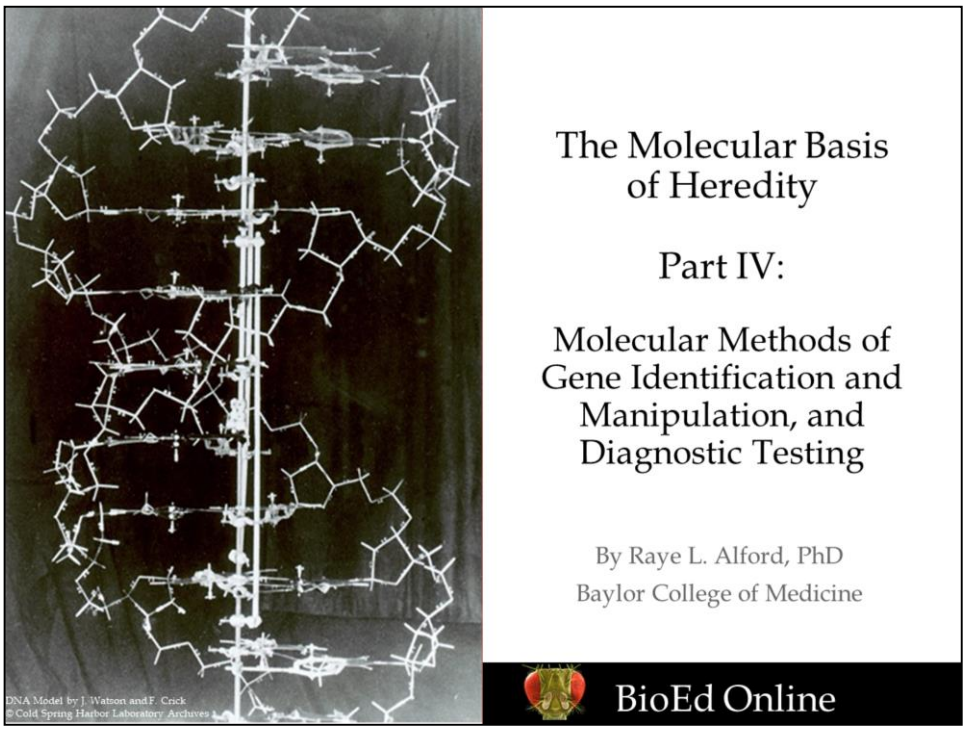

Molecular Methods of Gene Identification and Manipulation, and Diagnostic Testing

In this slide set, we will explore some of the more common molecular genetic laboratory methods for the identification of disease genes, the manipulation of genetic material, and the molecular genetic diagnosis of heritable disorders. The methods to be discussed include linkage analysis, PCR, cloning, restriction analysis, allele specific oligonucleotide hybridization and DNA sequencing.

Illustration:

The image on this slide is a photograph of the model of the DNA molecule that was built by Drs. James Watson and Francis Crick in 1953. Drs. Watson and Crick used this model to depict their proposed structure for the DNA double helix. The proposed structure was derived from X-ray diffraction data produced by Drs. Maurice Wilkins and Rosalind Franklin. The model was constructed from metal scraps obtained from a machine shop.

Drs. Watson and Crick published their proposed DNA structure in the journal Nature on April 2, 1953 (Volume 171, page 737). Drs. Watson, Crick and Wilkins were awarded the Nobel Prize in Physiology or Medicine in 1962 for their work. Dr. Franklin died before 1962. Since Nobel Prizes are only awarded to living individuals, she could not be honored.

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Image Reference:

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Gene Discovery

Gene identification is an important step in understanding the molecular basis of any heritable disorder. Identification of the gene(s) associated with a disorder opens the door for understanding the molecular components and biochemical pathways involved in the disorder, enables experimentation into improved diagnostic testing methodologies, provides targets for drug development, and suggests new avenues for therapeutic research, including gene therapy, stem cell therapy, and enzyme replacement therapy. The laboratory mouse and the fruit fly are vital tools for the study of human disorders and the preclinical evaluation of novel therapeutic approaches.

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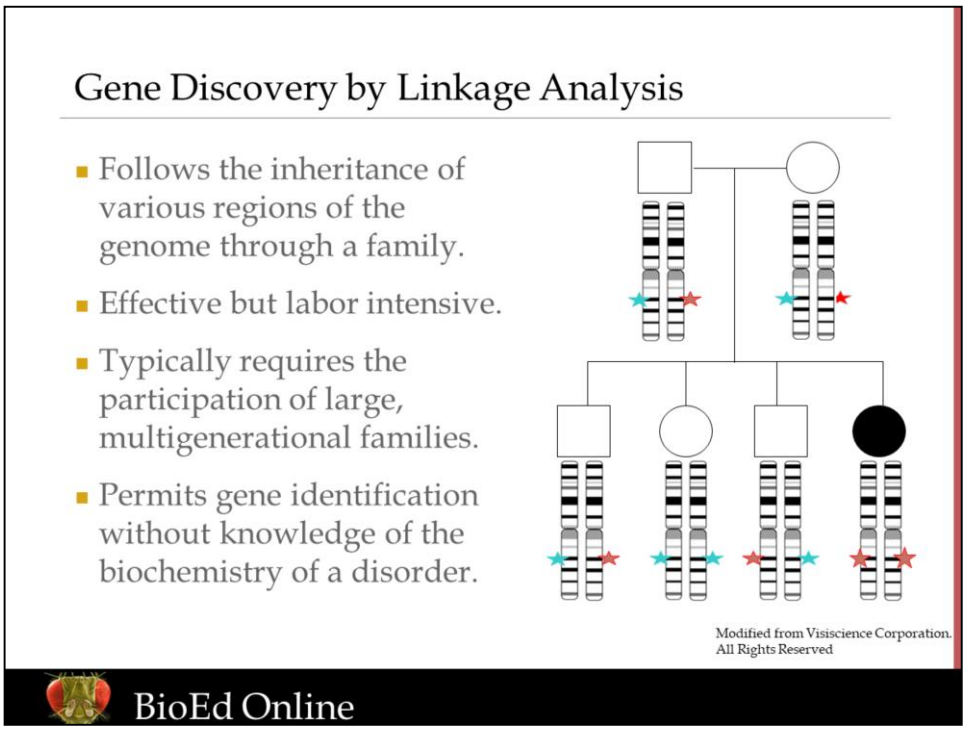

Gene Discovery by Linkage Analysis

Many genes have been discovered by an approach called linkage analysis. Linkage analysis relies on Mendelian inheritance of chromosomes (and genes). In linkage analysis, polymorphic genetic markers (sites of DNA sequence variations) spread throughout the genome are used to track the transmission of various genomic regions through a family. With linkage analysis, all members of a family are genotyped for the polymorphic markers. The genotype of each family member at each marker is then compared to the genotypes of other family members to determine which markers are present in the family members affected by the disorder. Since linkage analysis does not detect mutations in genes directly, but rather traces a potential disease allele through a family because of a tight association (genetic linkage) between the potential disease gene and the marker, further investigation is required once a genomic region of interest is identified. The goal of the investigation is to identify the gene and mutations associated with the disorder. Since linkage analysis is not a direct mutation detection method, its level of sensitivity relies on how many different forms of the markers there are, the density of the markers throughout the genome, and the genetic distance between the marker and the disease gene. Highly polymorphic markers, and lots of them, improve the success rate of linkage analysis.

Linkage analyses must be performed with data sets based on large, multigenerational families. This is because one needs many individuals, and typically more than one affected individual, within a family to identify the genomic regions associated with a disorder with statistical significance. However, large families segregating genetic disorders are not always easy to find, especially in highly mobile societies like the United States. In some cases, a several small families can be used instead, but this approach requires that the disease is caused by the same gene in all the families. For example, if the disorder is caused by mutations in the same gene in every individual affected by the disorder, then the results of linkage analysis of many small families can be added together because everyone affected by the disorder should share the same disease gene. One example of such a disorder is cystic fibrosis (CF), in which all individuals affected by the disorder have mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). On the other hand, if a disorder is caused by mutations in more than one gene, linkage analysis will be more successful if large,

multigenerational families are used because two different families might have the disease because of mutations in different genes. In these cases, the linkage data would appear contradictory and would not be additive. One example of a highly heterogeneous genetic disorder is nonsyndromic hearing loss. More than 100 genes have been mapped so far for hereditary deafness.

Genetic heterogeneity has complicated the search for the genetic factors associated with a number of common complex disorders such as schizophrenia and autism. In these disorders, multiple genetic factors are likely to be involved and each may have only a small effect on susceptibility to develop the disorder. As such, the disorder in any two families may be associated with different sets of genes. Sorting out the genetic factors involved is quite complicated and requires extensive additional investigations.

As a result of the Human Genome Project, researchers now have a collection of polymorphic genetic markers that provide excellent coverage of the genome. These markers are powerful tools for mapping disease genes. Linkage analysis strategies are precise, accurate and effective, but labor intensive. However, while they often require data from large families, they do not require an investigator to know anything about the underlying biochemical mechanisms involved in the disorder.

In the simplified diagram on this slide, a polymorphism associated with an autosomal recessive disorder is indicated by the red star. Only the affected individual has inherited two copies of the disease-associated polymorphism. From this finding, researchers would explore the genetic region surrounding the marker to identify genetic alterations unique to the disease-associated form of the marker.

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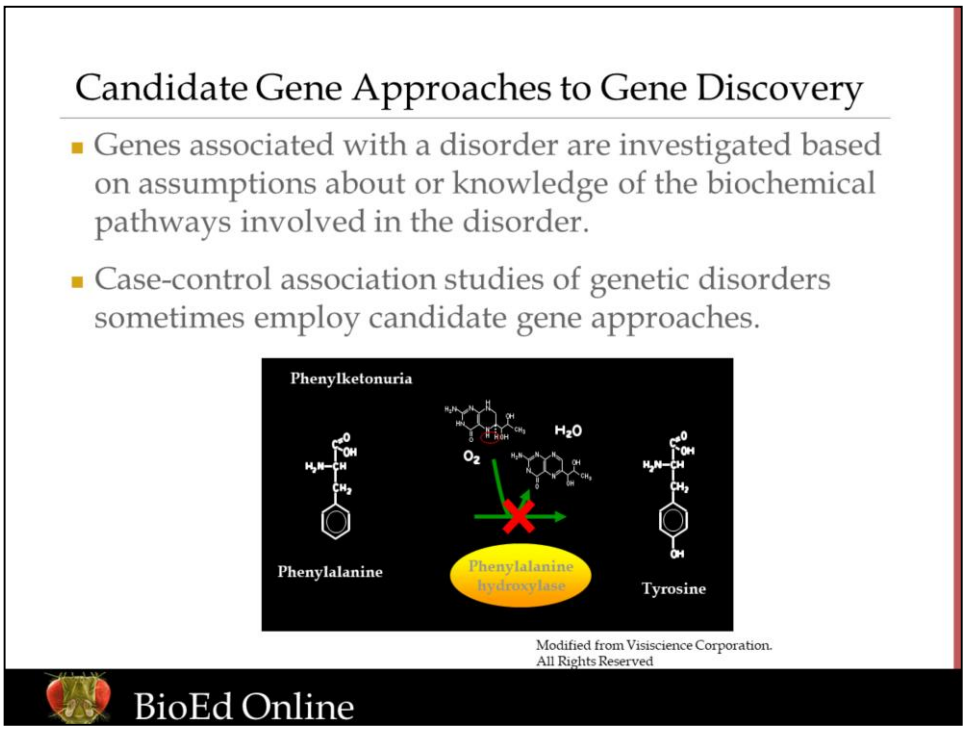

Candidate Gene Approaches to Gene Discovery

Another approach to discovering genes associated with disease is candidate gene approach, which is based on assumptions about or knowledge of the biochemistry of a disorder. For example, if the primary symptom of a particular disorder is activation of inflammatory pathways in a particular tissue, genes that encode proteins involved in inflammatory processes might be reasonable candidates to investigate for disease-associated genes. In this case, one might limit the selection of polymorphic markers to be analyzed to those that lie within genes involved in inflammation, as opposed to selecting a group of markers distributed throughout the genome. This approach has been used successfully to discover a number of disease genes. Its advantage is that case-control groups can be studied, eliminating the need to collect data from large, multigenerational families. Its disadvantage is that one must make certain assumptions about the pathophysiology of the disorder. If those assumptions are incorrect, or if the case and control groups are not carefully selected, gene discovery may not be successful.

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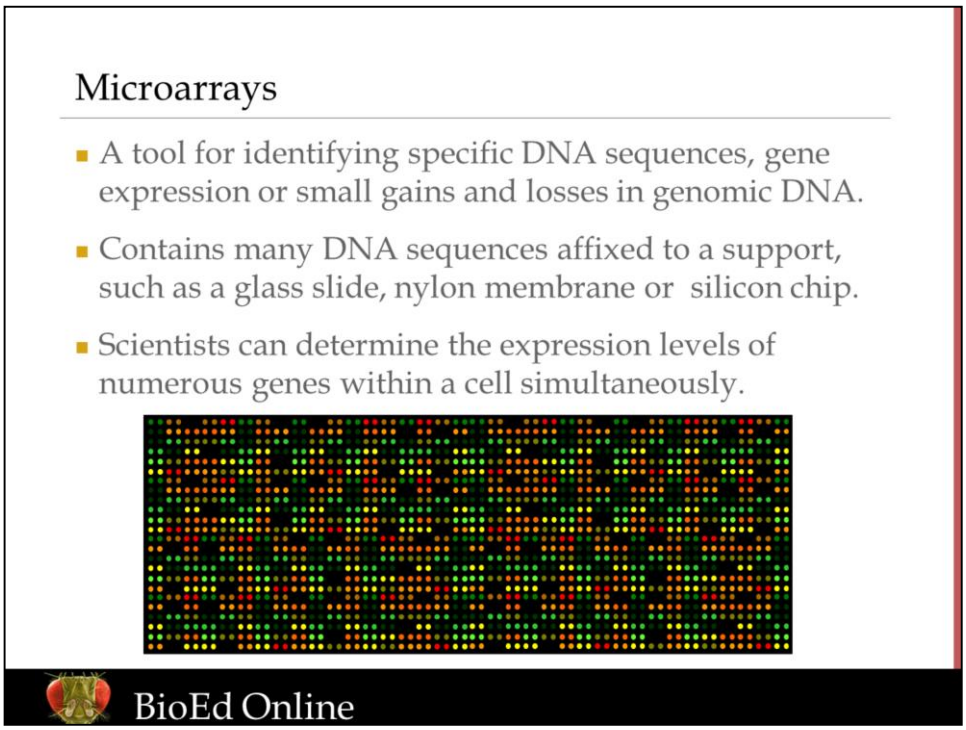

Microarrays

For the microarray image shown on this slide, let us assume that each spot on the array contains the DNA of a different form of a polymorphic marker. For every form or variation of the marker, there is a separate spot on the array. DNA from the test specimen is applied to the array and allowed to hybridize (bind specifically) to the DNA on the microarray. This hybridization is based on the complementarity of the DNA double helix. Under the proper conditions, the DNA from the specimen will find its complementary counterpart on the array and hybridize (bind) to it. The DNA from the specimen is labeled with a fluorescent tag so that it can be seen with a color camera. In this way, the specimen can be genotyped for each marker represented on the array, based on the pattern of spots to which it hybridizes. For example, if a dimorphic marker (marker with two forms– A and B) is used, test samples can be genotyped as AA, BB or AB based on the pattern of fluorescence on the array. Using this technology, the entire genome of a specimen can be investigated for a large number of markers simultaneously, significantly reducing the labor, time and cost involved in the analysis. Imagine that for a particular marker a preponderance of the B allele is found in the case group but not in the control group: one might suspect that there is a susceptibility gene somewhere in the region of that particular marker. The genomic region surrounding the marker could then be further investigated to attempt to identify disease-associated genes and mutations.

Microarrays also can be used for other purposes as well, such as investigating gene expression in cells. In this application of microarray technology, DNA sequences representing all (or many) of the genes in the genome are immobilized on the array. Fluorescently labeled probes derived from the RNA of a particular cell type are applied to the array to see which genes are being expressed in the cells (and which are not). The spots corresponding to expressed genes will light up because a fluorescently labeled specimen hybridizes there. Spots corresponding to unexpressed genes will remain unlabelled. Under the proper experimental conditions, quantitation of the amount of gene expression also may be possible. This is a useful approach for comparing gene expression between normal and disease tissue. Any differences found could indicate candidate genes associated with susceptibility to or progression of the disease.

To interpret the microarray shown in this slide in a slightly different way, let us now assume that this is a gene expression microarray and that there is a spot on the microarray bearing DNA from every gene in the human genome. Let us also assume that control cDNA, cDNA derived from normal tissue has been tagged with GREEN fluorescent dye and hybridized to the target DNA on the microarray, and that sample cDNA, cDNA derived from diseased tissue is tagged with RED fluorescent dye and hybridized to the target DNA on the microarray. The spots that show green suggest that there is no expression of that particular gene in the disease tissue. The spots that show red suggest that there is no expression of that particular gene in normal tissue. The spots that show yellow would suggest that there is expression of that particular gene in both normal and disease tissue. The spots that show no fluorescence suggest that there is no expression of that particular gene in either normal or disease tissue. Discovery of the differences in gene expression between normal and disease tissue allows researchers to focus their studies on the genes whose expression differs between the normal and disease state and accelerate their understanding of the biochemical pathways that might be activated, or inactivated, in association with a particular disease.

Another use of microarray technology is to search for small gains or losses in genomic DNA in patients with particular disorders. For this application, DNA representing a large percentage of the DNA in the human genome is ordered on an array. A specimen from a patient with a disorder of unknown etiology is labeled with one fluorescent dye and mixed with equal quantities of control DNA from a person without the disorder that has been labeled with a different fluorescent dye. These two DNA samples are then allowed to hybridize to the DNA on the array. By analyzing the fluorescence bound to each of the spots on the array, scientists can determine if portions of the genome are missing from the patient DNA, or occur in more copies than usual in the patient DNA. In this way, researchers can identify regions of the genome where losses or gains of genetic information might be associated with the symptoms in the patient. This is called comparative genomic hybridization.

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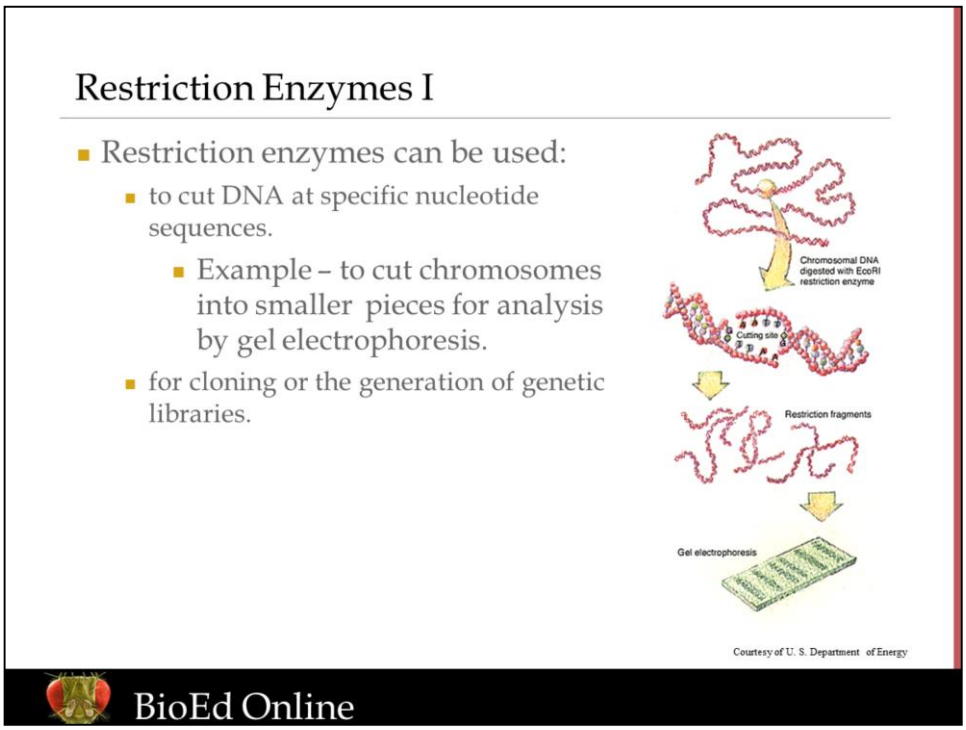

Restriction Enzymes I

Restriction enzymes are enzymes produced by bacteria that recognize specific DNA sequences and cut the DNA strand at those sequences. Restriction enzymes can be used to cut DNA for a variety of purposes.

Restriction enzymes can be used to digest the genomic (chromosomal) DNA of an organism and generate a set of DNA fragments of manageable size. For example, digested DNA can be run on electrophoresis gels for evaluation of banding patterns. Variations in the banding patterns (pattern of restriction digestion) of certain genes between individuals affected by a genetic disease and those not affected may indicate the presence of an underlying molecular genetic alteration associated with the disease. Restriction analysis can also be used to detect polymorphic sites that, while not associated with disease, still interrupt the ability of a restriction enzyme to cut a particular segment of DNA. Restriction enzyme digestion of polymorphic sites and evaluation of the banding patterns that resulted were the basis for early DNA fingerprinting methods. Today, it is more common to find forensic DNA technology using length variations in repetitive elements to identify and distinguish among individuals because repetitive sequences can be more polymorphic (have more forms) than restriction fragments (gain or loss of restriction digestion) and have greater power to distinguish among individuals.

In the creation of DNA libraries, the DNA of an organism is digested (cut into fragments) with a restriction enzyme and cloned into vectors (bacteriophage, plasmid, cosmid) for further evaluation in the laboratory. Vectors are small, independently replicating DNA molecules carried by viruses or bacteria that can be manipulated in the laboratory to carry, and copy,

genes of interest to researchers. Libraries can also be constructed using RNA as the starting material to synthesize complementary DNA molecules. In this case, a library called a cDNA library is created. Because cDNA libraries are based on RNA (transcribed genes) rather than genomic (total cellular) DNA, they are enriched for expressed (transcribed) genes whereas genomic DNA libraries contain all (or most of) the genes from an organism regardless of whether they were expressed (transcribed). Separate cDNA libraries from various individual cell types can be investigated to discover differences in gene expression between different types of tissues.

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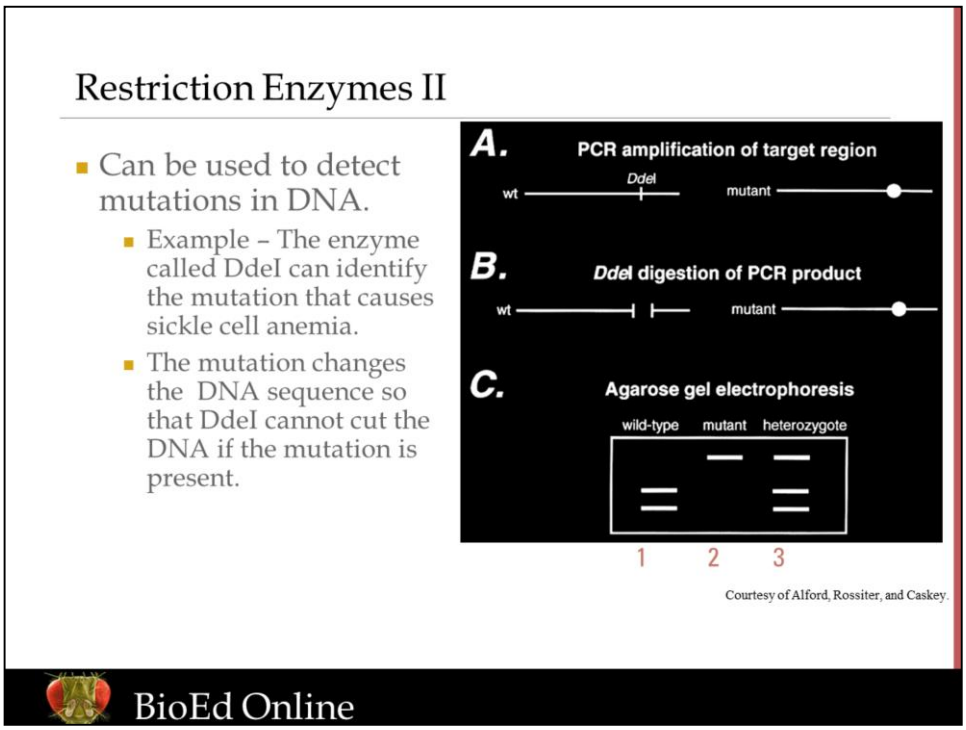

Restriction Enzymes II

Because of the specificity of their DNA sequence recognition, restriction enzymes can also be used to detect mutations associated with genetic diseases. In the image shown in this slide, the restriction enzyme DdeI is used to identify the mutation in the beta-globin gene that is associated with sickle cell disease in a fragment of DNA amplified from patients by polymerase chain reaction (PCR). In this case, the mutation associated with sickle cell disease changes the DNA sequence of the beta-globin gene in such a way that it is no longer recognized by DdeI (Panel A). As a result, normal, non-sickle, beta-globin genes are digested by DdeI while sickle cell-associated alleles are not (Panel B).

When DNA from patients is amplified by PCR and the PCR products are digested with DdeI and run on agarose gel electrophoresis, a person with two normal beta-globin genes will demonstrate a two band pattern, indicating that the beta-globin DNA was cut by DdeI (Panel C, Row 1). A person with two copies of the sickle cell-associated gene will show only an uncut band, indicating that neither of the beta-globin genes was cut by DdeI (Panel C, Row 2). A carrier for sickle cell disease will demonstrate both cut and uncut bands (Panel C, Row 3), indicating that he or she has one normal gene and one sickle cell-associated gene.

Understanding the molecular genetic basis of a disorder and developing a DNA-based methodology for mutation detection enable precise DNA-based diagnosis of the disorder, permitting identification of affected and unaffected individuals, and carriers of recessive diseases. In this example, the individual in row 2 of Panel C is affected by sickle cell disease, while the individual in row 3 is a carrier for sickle cell disease.

References:

Alford, R., Rossiter, B., & Caskey, C. (1994). DNA Diagnosis in Monogenic Diseases. *International Journal of Technology Assessment in Health Care, 10*(4), 628-643.
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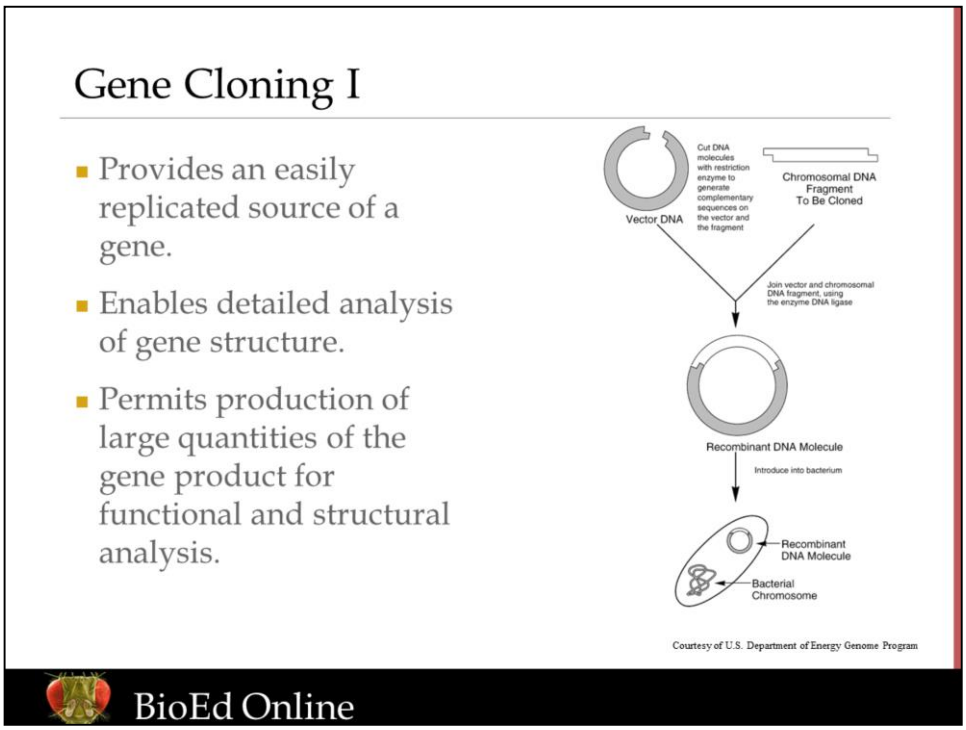

Gene Cloning I

Genes isolated from genomic DNA by restriction digestion can be cloned into vectors (bacteriophage, plasmid, cosmid) for further evaluation in the laboratory. Vectors are small, independently replicating DNA molecules carried by viruses or bacteria that can be manipulated in the laboratory to carry, and copy, genes of interest to researchers. In the cloning process, target DNA is cut by a restriction enzyme. The vector DNA is cut by the same enzyme. The target and vector DNA are mixed together and DNA ligase is added, to join the ends of the target DNA to the ends of the vector DNA. After ligation, the vector is once again a circle but now it carries a piece of the target DNA inserted into the cloning site. The recombinant vector is then transferred into cells in a process called transfection. Once inside the cells, the vector and its cloned gene can be cultured to create millions of copies of the target DNA. Plasmids that replicate in bacterial cells are an efficient mechanism by which genes can be replicated in vitro, but they are limited in that the target DNA cloned into the cloning site can only be of a certain size. Fragments too large are not able to be propagated by the bacterial cells efficiently. Other vectors such as cosmids and phages that replicate in either bacteria or in eukaryotic cells can accommodate larger pieces of cloned DNA.

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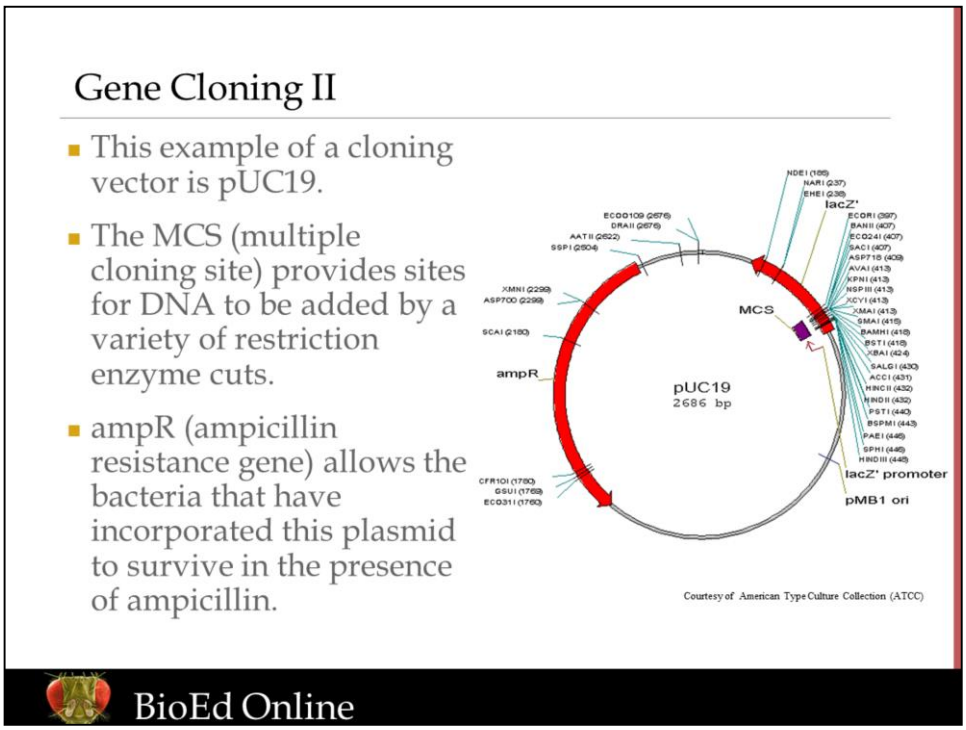

Gene Cloning II

Genes isolated from genomic DNA by restriction digestion can be cloned into vectors for further analysis. Cloning vectors come in a number of varieties, including phage, plasmid, cosmid, and others. This slide shows a map of the bacterial plasmid vector called pUC19. On the right is the **m**ultiple **c**loning **s**ite (purple box labeled MCS). This site contains a number of different restriction enzyme digestion sites, allowing DNA cut by a variety of enzymes to be cloned into this vector. Once inside the bacterial cells, the plasmid and its cloned gene can be cultured to create millions of copies of the target DNA. Plasmids are efficient ways to replicate genes in vitro, but they are limited in that the target DNA cloned into the MCS can only be of a certain size. Fragments too large are not able to be propagated by the bacterial cells efficiently. Other vectors, such as cosmids and phages, that replicate in either bacterial or eukaryotic cells can accommodate larger pieces of cloned DNA.

On the left side of the plasmid drawing is an ampicillin resistance gene (ampR). This gene allows any bacteria harboring this plasmid to survive treatment with the antibiotic ampicillin. In this way, cells that have taken up the recircularized plasmid, called transfected, survive in cultures containing ampicillin, while those that do not carry the plasmid die. Therefore, only bacteria carrying the plasmid are grown, enriching the culture for the cloned target DNA. Naturally occurring plasmids, not genetically engineered ones, are the basis for much of the antibiotic resistance seen in bacterial diseases today.

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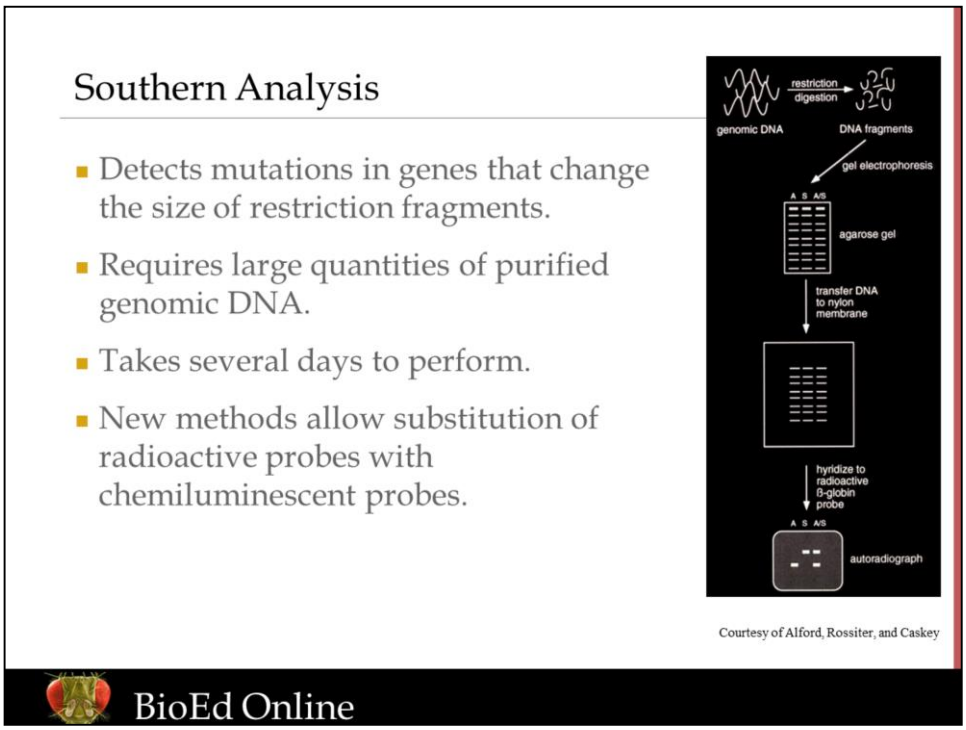

Southern Analysis

Southern analysis permits the detection of mutations and polymorphisms in genes because changes in the sequence of genes result in changes in restriction patterns. In Southern analysis, genomic DNA is isolated from a patient's cells and digested with an enzyme. The resulting DNA fragments are run on an agarose gel for separation according to size. To stabilize the DNA in its electrophoretic separation pattern, the DNA is hybridized (affixed) to a nylon membrane. A labeled DNA probe (short sequence of DNA tagged isotopically or chemically) specific for the gene of interest is mixed with the membrane under conditions that allow the probe to find and bind to (hybridize with) its complementary DNA on the membrane, resulting in a characteristic banding pattern for the normal gene upon isotopic or chemical signal detection. This approach can be used to analyze a particular nucleotide change if the change occurs at the site of action by a restriction enzyme and alters the ability of the enzyme to cut the DNA. This approach also can detect alterations in genes that disrupt the regular banding pattern because of deletions or other rearrangements in the gene that span restriction sites. Southern analysis also can be effective in identifying changes in the size of repetitive elements, such as triplet repeats that are associated with disease. In many cases, the changes in size of the gene associated with expansion of the repeat sequence is large enough to be seen on Southern analysis as a change in the size of the band associated with the gene. In cases of smaller repeat expansions a different type of gel substance, called polyacrylamide, is used because it is better able to separate smaller sized DNA fragments.

In the image on the slide, genomic DNA is digested with a restriction enzyme and separated by gel electrophoresis according to fragment size. The DNA is then transferred from the gel to a nylon membrane and hybridized with a radioactively labeled probe unique to the sequence of interest. In this case, the probe is complementary to the beta-globin gene and is designed to

detect the mutation associated with sickle cell disease, which interrupts a restriction enzyme site. The sickle cell-associated genes remain undigested by the enzyme while the normal gene sequence is cut by the enzyme into a smaller piece of DNA. In the lane labeled A, an individual with two normal copies of the beta-globin gene was analyzed. The size of the fragment seen on the autoradiograph is that of the digested DNA, indicating two normal alleles and no sickle cell-associated allele. In the column labeled S, an individual with sickle cell disease and two copies of the sickle cell gene was analyzed. The size of the fragment seen is larger than the fragment in column A, indicating that the restriction enzyme failed to cut the DNA due to the presence of two copies of the sickle cell-associated mutation. In the column labeled AS, an individual with one copy of the normal gene and one copy of the sickle cell-associated gene was analyzed. Two bands are seen: one for the digested smaller allele on the bottom; and, one for the uncut sickle cell-associated allele on the top. This individual is a carrier for sickle cell disease.

References:

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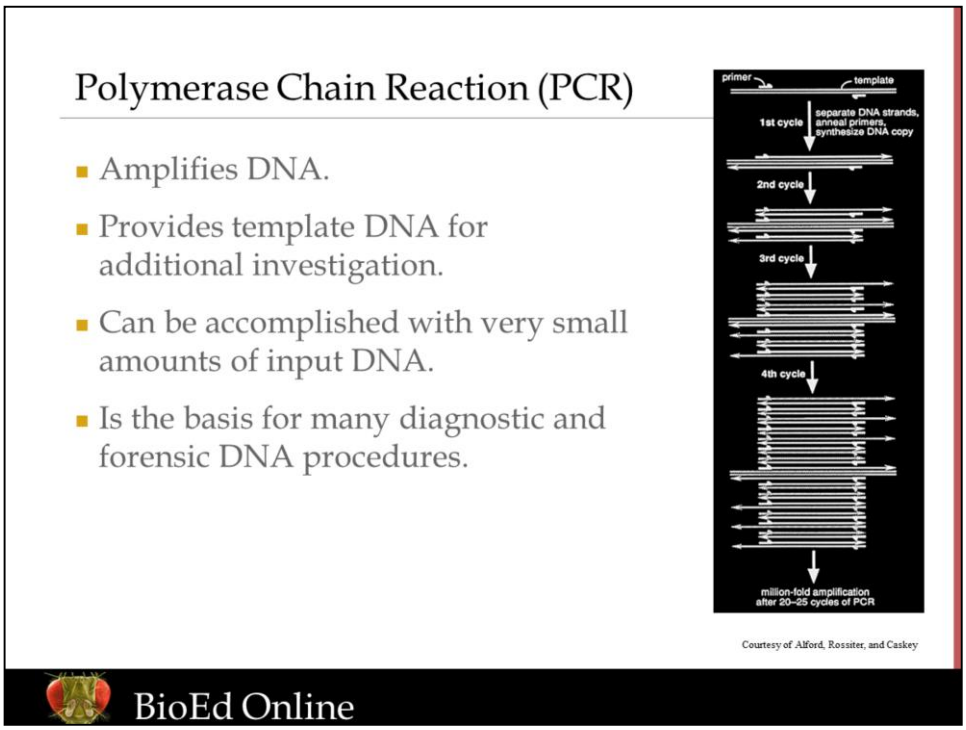

Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) was invented in 1985 by Kary Mullis, PhD. Dr. Mullis received the Nobel Prize in Chemistry in 1993 for his work.

PCR is the enzymatic amplification of DNA, creating millions of copies of a segment of DNA from only a small amount of starting material. To begin, DNA primers are manufactured that are complementary to a portion of the target DNA sequence. The primers and the target DNA are mixed and denatured (separated into single strands) by high temperature. The mixture is then cooled, allowing the primers to hybridize (bind) to the target DNA. After hybridization, a special DNA polymerase enzyme that is resistant to heat inactivation synthesizes new DNA strands from the primers, using the target DNA as a template. The process is repeated for 30-50 cycles of heat denaturation, primer binding, and DNA synthesis. After many cycles of PCR, millions of copies of the target DNA are created where only a few existed before. PCR is also fast. Most PCR reactions take only a few hours to perform in the laboratory.

DNA fragments generated by PCR can be subjected to a variety of different analyses, depending on the type of mutation or polymorphism sought. For example, gel-based methods that separate fragments based on size are ideal for detecting small expansions or deletions such as occur in repetitive elements, such as trinucleotide repeats, while DNA sequencing will detect changes, such as single nucleotide substitutions, in the sequence of a gene.

PCR has several advantages over methods like Southern analysis. First, PCR uses very small amounts of DNA as starting material, while Southern analysis and other methods require large amounts of input DNA. PCR also can use degraded DNA as starting material because even a degraded DNA sample (DNA that is broken into pieces by decay) is likely to have pieces still large enough to be bound by the primers and amplified while Southern analysis tyipcally examines much larger pieces of DNA. These properties make PCR useful for forensic DNA analyses where evidentiary DNA is frequently scarce and/or compromised by environmental or other exposures.

In the early days of PCR, the methodology could only amplify DNA fragments a few hundred to two or three thousand base pairs in length. Recent improvements in PCR technology allow today's scientists to amplify DNA fragments that are up to several thousand base pairs in length, but even today PCR cannot amplify very long target DNA sequences.

References:

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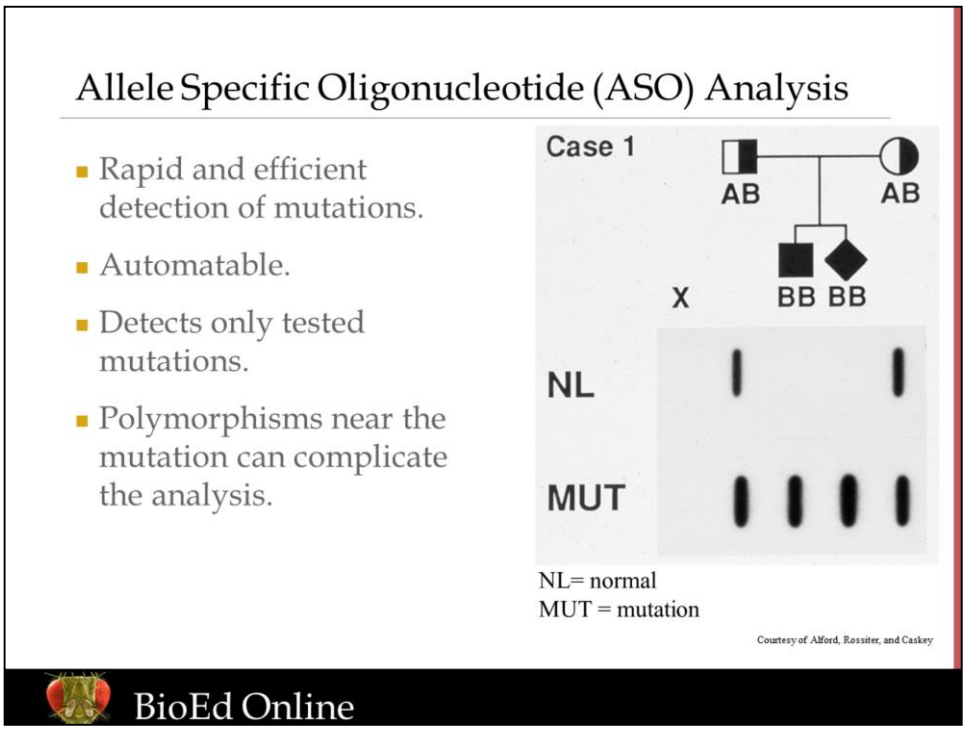


Allele Specific Oligonucleotide (ASO) Analysis

ASO (allele specific oligonucleotide) hybridization begins with PCR. Once amplified, the target DNA is hybridized (affixed) to a nylon membrane or another solid substrate in spots (without prior electrophoresis). Oligonucleotide probes specific for normal and mutant sequences are radioactively, chemiluminescently, or fluorescently labeled and hybridized to the substrate-bound target DNA. Competitive binding between mutant and normal probes permits probe binding dependent upon the sequence of the target DNA.

ASO is very effective and reliable and is used for many DNA tests where only one or a few mutations cause the disease. The development of 96- and 384- tray formats permit automation and the simultaneous analysis of many specimens, making ASO useful for high throughput diagnostic testing. ASO effectively identifies sought after mutations, but unlike sequencing, cannot typically detect mutations not directly tested. Another common problem with ASO is with polymorphic sequences that occur near the sequence variation that is being sought. If these polymorphisms interfere with probe binding, false positive or false negative results can be obtained. In these cases, special consideration must be given to probe design to avoid errors in interpretation of ASO test results.

The image on the slide shows the ASO analysis of a mutation in the CFTR gene associated with cystic fibrosis (CF) in a family with one affected child and a fetus of unknown genotype. In this example, the parents are both carriers of a CF-associated allele. On ASO analysis, they show a signal with both the normal and mutant probes, consistent with their carrier status. The child, who is affected by CF, shows an ASO signal only for the mutant probe, indicating inheritance of two copies of the CF allele, one from each parent. The fetus also has inherited a CF-associated allele from each parent and does not show a signal from the normal probe. This fetus is predicted to be affected with CF. The column labeled X represents the negative control.

There are a number of variations on allele specific methods, all based on the same principle: primers and probes only bind to complementary DNA sequences. This feature of DNA

hybridization is exploited to identify normal and mutant alleles in patient specimens.

References:

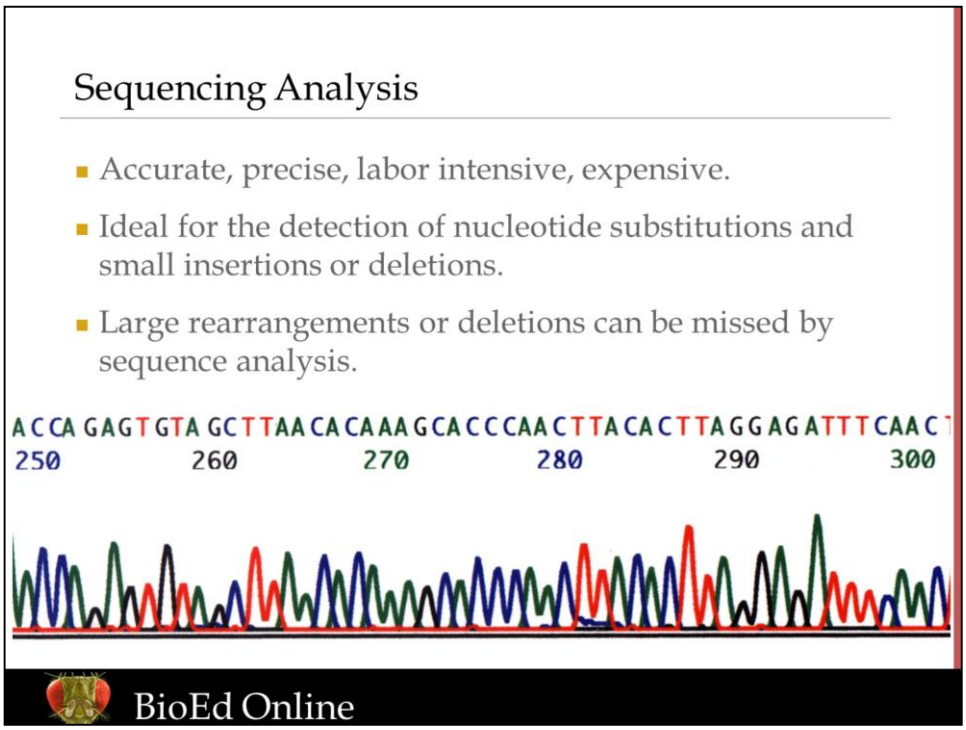
Alford, R., Rossiter, B., & Caskey, C. (1994). DNA Diagnosis in Monogenic Diseases. *International Journal of Technology Assessment in Health Care, 10*(4), 628-643.

Cystic Fibrosis Foundation. Retrieved 3-10-2005 from http://www.cff.org/home/

Nussbaum, R. L., McInnes, R. R., & Willard, H. F. (2004). *Thompson & Thompson: Genetics in Medicine* (6th ed.). Saunders, an imprint of Elsevier.

Image Reference:

Alford, R., Rossiter, B., & Caskey, C. (1994). DNA Diagnosis in Monogenic Diseases. *International Journal of Technology Assessment in Health Care, 10*(4), 628-643.



Sequencing Analysis

DNA sequencing is the method of choice for the diagnostic detection of nucleotide substitutions or small insertions or deletions in genes. For DNA sequencing, short segments of DNA are amplified by PCR and subjected to DNA sequencing. Most DNA sequencing today uses a modified PCR reaction, called cycle sequencing, in which dideoxynucleotides (ddNTPs) are added to the PCR reaction mixture. Because ddNTPs lack a 3' OH group, they cannot be extended by the addition of another nucleotide, and DNA synthesis during PCR stops after the incorporation of a ddNTP into a segment of DNA. Fluorescent tagging of the each of the ddNTPs (ddATP, ddCTP, ddGTP, and ddTTP) with a different colored tag allows colorimetric detection of the last base in each nucleotide chain. Size separation by gel electrophoresis allows the colors to be read step by step until the entire sequence of a DNA fragment is deciphered.

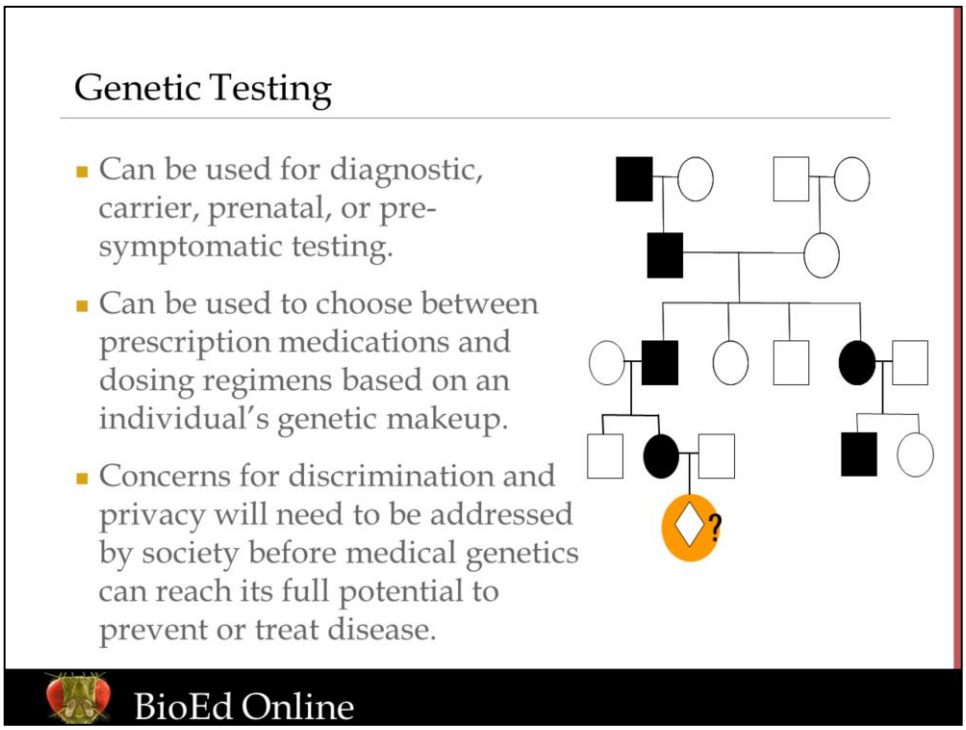
Although the method is powerful for detection of point mutations and small insertions or deletions, any alteration that compromises the ability of the PCR primers to amplify a gene can result in false negative results. For example, deletion of one of the copies of a gene in a cell will result in PCR only from the remaining copy. If the remaining gene is of normal sequence, the sequence will be interpreted as normal. Unless the likelihood of the deletion occurring is taken into account, a false negative diagnosis may result. However, if the testing laboratory is aware of the possibility of deletion and applies an additional methodology to detect the deletion, the accuracy of the diagnostic analysis can be improved.

Even with recent advances in technology, DNA sequencing is still labor intensive and expensive, however unlike some analytic methods, such as ASO, that can detect only sought after mutations, sequencing will detect any alteration in the nucleotide sequence of the amplified fragment, provided that the alteration does not interfere with the PCR reaction itself. The detection of unexpected or previously unreported changes in the nucleotide sequence of a gene is common with diagnostic sequencing. The detection of these sequence changes of unknown pathological significance complicates interpretation of the test results and may fail to resolve the clinical diagnosis clearly in some cases.

The slide shows an electropherogram generated by automated fluorescent DNA sequencing of a segment of DNA using an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequence of the DNA fragment is read from left to right. This output sequence is compared to the known normal sequence of this fragment of DNA for the detection of any alterations. This particular sequence is a portion of the mitochondrial 12S rRNA gene and is identical to the normal sequence for this region in humans.

Reference:
Nussbaum, R. L., McInnes, R. R., & Willard, H. F. (2004). *Thompson & Thompson: Genetics in Medicine* (6th ed.). Saunders, an imprint of Elsevier.

Image Reference:
Alford, R. (2006). Laboratory image.



Genetic Testing for Inherited Disorders

Genetic testing has many applications. In the case of individuals showing signs or symptoms of a disorder of known genetic etiology, the gene associated with the disorder can be tested for diagnostic purposes. DNA testing also can identify unaffected carriers of recessive diseases so that risk analysis may be performed and parents can understand their risks for having a child affected by a particular genetic disorder. For example, cystic fibrosis (CF) is common in Caucasians. Carrier testing of Caucasian couples planning to have children can provide couples with information about their risks for having a child with CF. In contrast to disorders that affect individuals early in life, some genetic diseases do not begin to affect individuals until later in life. Knowing the genetic basis for such disorders provides an opportunity for presymptomatic testing of individuals at risk for the disorder. In cases where medical or lifestyle interventions can prevent or delay onset of the disease, or reduce the severity of symptoms, presymptomatic genetic testing might be of interest to at-risk individuals.

Finally, genetic testing is being used more frequently in the prescription of medications because researchers are beginning to understand the genetic basis for variable responses to medications and are applying this knowledge to the personalization of drug therapy. In the future, an individual might be tested for various genes that can predict that person's response to a drug before the drug is prescribed. Those for whom the typical dose of the medication is predicted to have little or no effect, or those likely to experience an adverse reaction to the drug, can be offered a customized dose of the medication or an alternative medication, if one is available.

The adequacy of laws protecting the confidentiality of personal genetic information and prohibiting genetic discrimination in insurance and employment need to be evaluated and updated to keep pace with advances in genetic technologies so that the full potential of genetic medicine can be realized.

In this slide, a family segregating an autosomal dominant disorder is illustrated. The fetus, circled in orange, is at 50% risk of inheriting the gene responsible for the disorder. Genetic

testing, if available, could provide information about whether the fetus has inherited the gene associated with the disorder.

References:

Cystic Fibrosis Foundation. Retrieved 3-10-2005 from http://www.cff.org/home/
Nussbaum, R. L., McInnes, R. R., & Willard, H. F. (2004). *Thompson & Thompson: Genetics in Medicine* (6th ed.). Saunders, an imprint of Elsevier.

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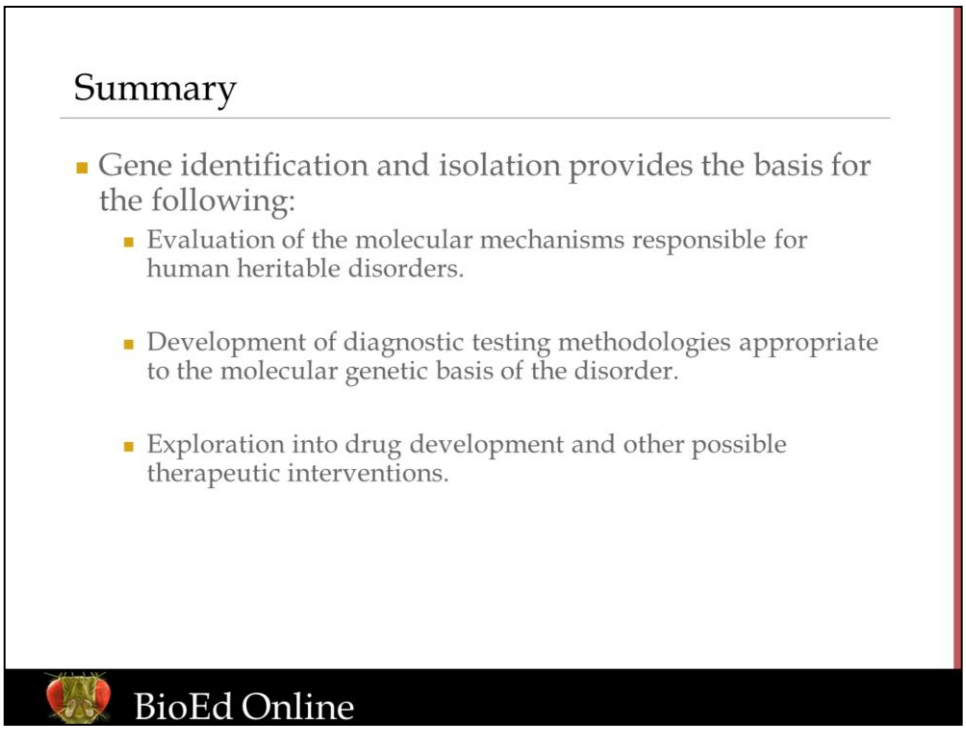

Summary

Gene identification enables investigation into the causes of genetic disorders, the development of diagnostic tests for genetic disorders, and the exploration of new therapies.

A variety of genetic testing technologies are available, but not every technology is appropriate for every disorder. For example, while a karyotype is very effective for detecting Down syndrome (a chromosomal aneuploidy), it will not diagnose cystic fibrosis (CF, a single gene disorder). Thus, the selection of the appropriate testing strategy for the detection of a particular genetic disorder must be based on an understanding of the molecular basis of the disorder.

A variety of treatment approaches have the potential to impact the course of genetic diseases. For example, enzyme replacement therapies hold promise for patients affected by loss of function mutations in certain enzymes. But this approach will not work for the treatment of muscular dystrophy that is caused by abnormalities in the architecture of muscle cells. Therefore, the investigation into treatments for genetic disorders requires an understanding of the biochemical mechanisms of each disease.

The completion of the Human Genome Project has given scientists new tools with which to identify and understand the genetic factors that contribute to disease, but much work remains to be done. Over the next few decades, the discoveries of genetic factors that influence human health and disease, and patient responses to medications, are expected to revolutionize the practice of medicine. Education is crucial if genetic medicine is to fulfill its potential. We need well-prepared teachers to train students who will become the next generation of researchers, doctors, lawmakers and patients.

References:

The National Human Genome Research Institute. Retrieved 3-10-2005 from http://www.genome.gov/
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