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Research Article

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Advances in Genetics Research

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Abstract

Genes are understandably crucial to physiology, morphology and biochemistry, but the idea of genes contributing to individual differences in behavior once seemed outrageous. Nevertheless, some scientists have aspired to understand the relationship between genes and behavior, and their research has become increasingly informative and productive over the past several decades. At the forefront of behavioral genetics research is the fruitfly Drosophila melanogaster, which has provided us with important insights into the molecular, cellular and evolutionary bases of behavior. By employing this development in their experiments with laboratory fruit flies, Gantz and Bier demonstrated that by arranging the standard components of this anti-viral defense system in a novel configuration, a mutation generated on one copy of a chromosome in fruit flies spreads automatically to the other chromosome. The end result, Bier says, is that both copies of a gene could be inactivated "in a single shot."

The two biologists call their new genetic method the "mutagenic chain reaction," or MCR. "MCR is remarkably active in all cells of the body with one result being that such mutations are transmitted to offspring via the germline with 95 percent efficiency. Thus, nearly all gametes of an MCR individual carry the mutation in contrast to a typical mutant carrier in which only half of the reproductive cells are mutant."

Bier says "there are several profound consequences of MCR. First, the ability to mutate both copies of a gene in a single generation should greatly accelerate genetic research in diverse species. For example, to generate mutations in two genes at once in an organism is typically time consuming, because it requires two generations, and involved, because it requires genetic testing to identify rare progeny carrying both mutations. Now, one should simply be able to cross individuals harboring two different MCR mutants to each other and all their direct progeny should be mutant for both genes."

Introduction

Modern humans and Neanderthals diverged from *H. heidelbergensis* or *H. erectus* is debated and that issue contributes to the stories on human ancestors published over the last couple of decades. Also, fossils of new species were discovered during the first decade of the 21st century, all of which coexisted with modern humans and Neanderthals. One is *Homo floresiensis*, which had a very small brain and another is *Homo sapiens denisova*; that's that Denisovians that have been so hot news cycles lately. Denisovian man is a popular topic for the public, because large segments of the modern human population possess more Denisovian sequences than Neanderthal sequences, but it also represents a scientific, technological milestone based on how it was discovered. First it was a tiny finger bone in Siberia in 2008 – the tip of the pinky– and more recently a Denisovian toe bone was found. From that pinky bone, researchers extracted DNA, separated out the DNA that was from soil bacteria and other contaminants, and accessed the DNA that had belonged to the ancient human, about 3 percent of the entire DNA sample. They sequenced it and compared it with sequences of modern humans and Neanderthals. That was possible because, the genetic database of Neanderthals has been growing substantially since 1997, when DNA from Neanderthal bone was first extracted and sequenced successfully. As for the result, sequence comparison showed that the pinky bone belonged to a human of an entirely different species. Thus, *H. sapiens denisova* became the first human species discovered by way of molecular genetics, rather than by comparative bone anatomy.

The two studies added to a growing awareness of human interspecies mixing tens of thousands of years ago. It's an idea that has complicated the older view that modern humans, *Homo sapiens* sapiens, completely replaced other human species by about 30,000 years ago, but the complexity does end with the fact that there was admixing and that also attention in the news. This month, for example, there was a big story, based on a Stanford University study, about how, despite interbreeding way back when, modern men lack Y chromosome genes from Neanderthals. This does not mean that Neanderthal men did not start paternal lines that persisted through modern human populations, but if they did then their Y chromosome genes eventually disappeared.

Going back one, two, and three years, there have been story after story about Neanderthals, Denisovians, ancient DNA, and early human species in general. So what's happening? Are we going through some kind of hominid fad, or are paleoanthropologists actually making discoveries with increasing speed? The details in the science literature suggests that it's the latter. They are progressing more rapidly than in the past. New data are coming in with increasing frequency and this has to do with advances in molecular genetics, especially in technology that's being applied to extracting and sequencing of ancient DNA.

Material & Methods

Gel electrophoresis separates DNA fragments by size in a solid support medium (an Agarose gel). DNA samples are pipetted into the sample wells, seen as dark slots at the top of the picture. Application of an electric current at the top (anodal, negative) end causes the negatively-charged DNA [remember it's an acid] to migrate (electrophorese) towards the bottom (cathodal, positive) end. The rate of migration is proportional to size: smaller fragments move more quickly, and wind up at the bottom of the gel. DNA is visualized by including in the gel an intercalating dye, ethidium bromide. DNA fragments take up the dye as they migrate through the gel. Illumination with ultraviolet light causes the intercalated dye to fluoresce with a pale pink colour. Note that the larger fragments fluoresce more intensely. Although each of the fragments of a single class of molecule are present in equimolarproportions, the smaller fragments include less mass of DNA, take up less dye, and therefore fluoresce less intensely. This is most evident in the lane at the extreme right, which shows a "*ladder*" set of DNA fragments of known size that can be used to estimate the sizes of the other unknown fragments.

A dye is added to the sample of DNA prior to electrophoresis to increase the viscosity of the sample which will prevent it from floating out of the wells and so that the migration of the sample through the gel can be seen.

A DNA marker (also known as a size standard or a DNA ladder) is loaded into the first well of the gel. The fragments in the marker are of a known length so can be used to help approximate the size of the fragments in the samples. The prepared DNA samples are then pipetted into the remaining wells of the gel.

When this is done the lid is placed on the electrophoresis tank making sure that the orientation of the gel and positive and negative electrodes is correct (we want the DNA to migrate across the gel to the positive end).

Separating the fragments

The electrical current is then turned on so that the negatively charged DNA moves through the gel towards the positive side of the gel. Shorter lengths of DNA move faster than longer lengths so move further in the time the current is run. The distance the DNA has migrated in the gel can be judged visually by monitoring the migration of the loading buffer dye.

The electrical current is left on long enough to ensure that the DNA fragments move far enough across the gel to separate them, but not so long that they run off the end of the gel.

Once the DNA has migrated far enough across the gel, the electrical current is switched off and the gel is removed from the electrophoresis tank. To visualize the DNA, the gel is stained with a fluorescent dye that binds to the DNA, and is placed on an ultraviolet transilluminator which will show up the stained DNA as bright bands. Alternatively the dye can be mixed with the gel before it is poured. If the gel has run correctly the banding pattern of the DNA marker/size standard will be visible.

It is then possible to judge the size of the DNA in your sample by imagining a horizontal line running across from the bands of the DNA marker. You can then estimate the size of the DNA in the sample by matching them against the closest band in the marker.

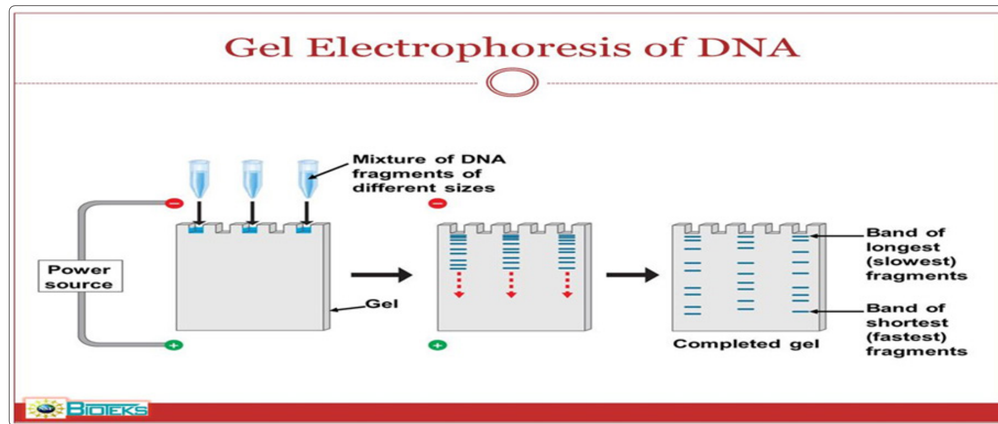
Agarose gel electrophoresis is the most effective way of separating DNA fragments of varying sizes ranging from 100 bp to 25 kb [1]. Agarose is isolated from the seaweed genera Gelidium and Gracilaria, and consists of repeated agarobiose (L- and D-galactose) subunits [2]. During gelation, Agarose polymers associate non-covalently and form a network of bundles whose pore sizes determine a gel's molecular sieving properties.

The use of Agarose gel electrophoresis revolutionized the separation of DNA. Prior to the adoption of Agarose gels, DNA was primarily separated using sucrose density gradient centrifugation, which only provided an approximation of size. To separate DNA using Agarose gel electrophoresis, the DNA is loaded into pre-cast wells in the gel and a current applied. The phosphate backbone of the DNA (and RNA) molecule is negatively charged, therefore when placed in an electric field, DNA fragments will migrate to the positively charged anode. Because DNA has a uniform mass/charge ratio, DNA molecules are separated by size within an Agarose gel in a pattern such that the distance traveled is inversely proportional to the log of its molecular weight [3]. The leading model for DNA movement through an Agarose gel is "biased reptation", whereby the leading edge moves forward and pulls the rest of the molecule along [4]. The rate of migration of a DNA molecule through a gel is determined by the following: 1) size of DNA molecule; 2) Agarose concentration; 3) DNA conformation 4) voltage applied, 5) presence of ethidium bromide, 6) type of Agarose and 7) electrophoresis buffer [5]. After separation, the DNA molecules can be visualized under uv light after staining with an appropriate dye. By following this protocol, students should be able to: Understand the mechanism by which DNA fragments are separated within a gel matrix Understand how conformation of the DNA molecule will determine its mobility through a gel matrix Identify an Agarose solution of appropriate concentration for their needs Prepare an Agarose gel for electrophoresis of DNA samples Set up the gel electrophoresis apparatus and power supply Select an appropriate voltage for the separation of DNA fragments Understand the mechanism by which ethidium bromide allows for the visualization of DNA bands Determine the sizes of separated DNA fragments.

Observing Separated DNA fragments

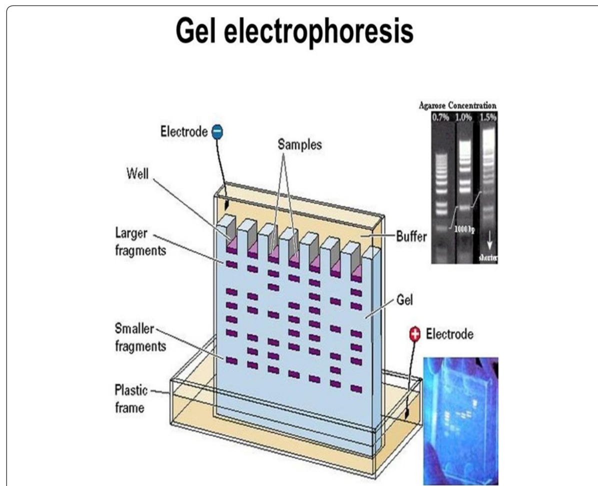
When electrophoresis has completed, turn off the power supply and remove the lid of the gel box. Remove gel from the gel box. Drain off excess buffer from the surface of the gel. Place the gel tray on paper towels to absorb any extra running buffer.

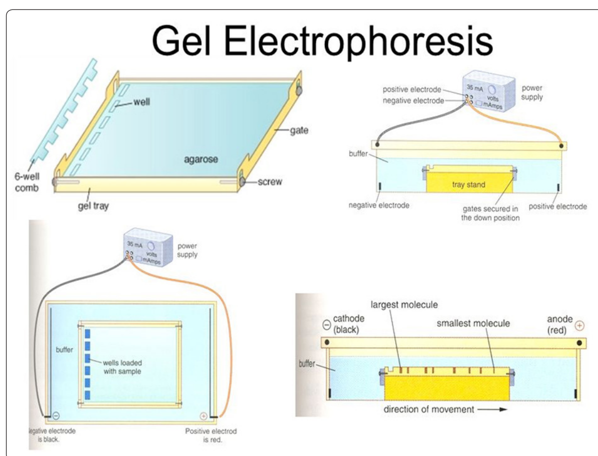
Visualising the results



Gel Electrophoresis of DNA

Remove the gel from the gel tray and expose the gel to uv light. This is most commonly done using a gel documentation system. DNA bands should show up as orange fluorescent bands. Take a picture of the gel. Properly dispose of the gel and running buffer per institution regulation





Gel Electrophoresis of DNA

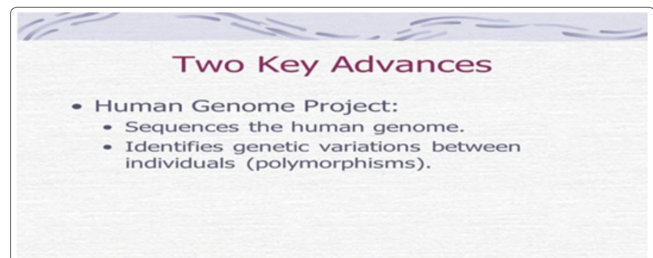
Representative Results

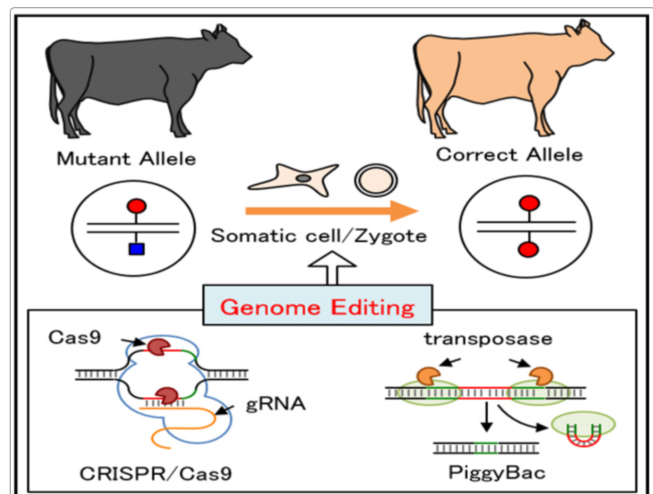
Represents a typical result after Agarose gel electrophoresis of PCR products. After separation, the resulting DNA fragments are visible as clearly defined bands. The DNA standard or ladder should be separated to a degree that allows for the useful determination of the sizes of sample bands. In the example shown, DNA fragments of 765 bp, 880 bp and 1022 bp are separated on a 1.5% Agarose gel along with a 2-log DNA ladder.

Genome Advance

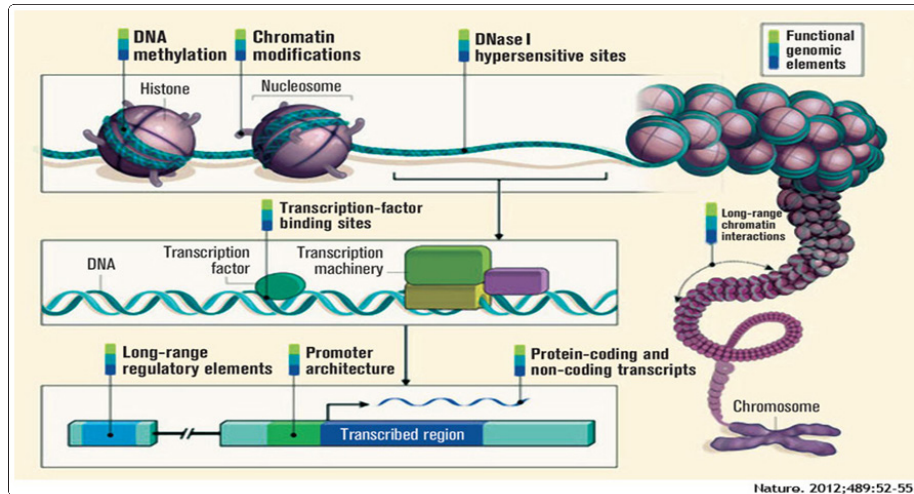
Genomics has become a fast-moving field, with findings pouring out of labs all over the world. Each month, the National Human Genome Research Institute will highlight what it considers the coolest genomic advances, broadly defined, of the previous month.

February's *Genome Advance of the Month* describes a leap forward in understanding the heritability and progression of schizophrenia in a study by researchers at Harvard Medical School and the Broad Institute. The study, published in the February 11, 2016 issue of *Nature*, signals the potential for research using large numbers of whole genome sequences, innovative biological methods and advanced software toolkits for analysis.





Your DNA forms thousands of loops, like those of a shoelace. Just as you learned to tie your shoes by forming separate “bunny ear” loops of string, your DNA forms many of these loops to create “genetic neighborhoods” within each bunny ear loop. These neighborhoods bring distant genes and specific gene control switches into close proximity. Genetic neighborhoods can be autonomous and remain separate from other neighborhoods. The December *Genome Advance of the Month* highlights a landmark study in *Nature* that describes what happens when two genetic neighborhoods merge in brain tumor cells.



Scientists are using an exciting gene editing tool called CRISPR/Cas9 to protect plants from harmful DNA viruses. The CRISPR/Cas9 system has previously been adapted for use in many organisms, and this latest iteration develops gene-editing for use in plants. The November *Genome Advance of the Month* describes how these scientists inserted the code for an ancient bacterial immune system into a plant’s genome to successfully strengthen the plant’s protection against viruses. Featured in *Genome Biology*, this research represents a promising method for cultivating plants’ resistance to harmful viruses.

September’s *Genome Advance of the Month* spotlights a Swiss study on women who are at increased risk for inherited breast cancer and the long term challenges they face in managing their care. It also identifies some of the challenges of providing care to at-risk individuals and highlights opportunities for improved models of care. The study is published in *Genetics in Medicine*.

Infertility - difficulty getting or staying pregnant - can come at a high financial and emotional cost, affecting about 6 million women and 4 million men in the United States. About half of these cases could be due to genetic factors. Now, new research techniques are yielding insight into the genetic roots of infertility. The August *Genome Advance of the Month* looks at a study in the *Proceedings of the National Academy of Sciences* that addresses the complex genetics of infertility using genetic sleuthing and cutting-edge, gene-editing technology.

Discussion

Agarose gel electrophoresis has proven to be an efficient and effective way of separating nucleic acids. Agarose’s high gel strength allows for the handling of low percentage gels for the separation of large DNA fragments. Molecular sieving is determined by the size of pores generated by the bundles of Agarose in the gel matrix [6]. In general, the higher the concentration of Agarose, the smaller the pore size. Traditional Agarose gels are most effective at the separation of DNA fragments between 100 bp and 25 kb. To separate DNA fragments larger than 25 kb, one will need to use pulse field gel electrophoresis, which involves the application of alternating current from two different directions [7]. In this way larger sized DNA fragments are separated by the speed at which they reorient themselves with the changes in current direction. DNA fragments smaller than 100 bp are more effectively separated using polyacrylamide gel electrophoresis. Unlike Agarose gels, the polyacrylamide gel matrix is formed through a free radical driven chemical reaction. These thinner gels are of higher concentration, are run vertically and have better resolution. In modern DNA sequencing capillary electrophoresis is used, whereby capillary tubes are filled with a gel matrix. The use of capillary tubes allows for the application of high voltages, thereby enabling the separation of DNA fragments (and the determination of DNA sequence) quickly.

Agarose can be modified to create low melting Agarose through hydroxyethylation. Low melting Agarose is generally used when the isolation of separated DNA fragments is desired. Hydroxyethylation reduces the packing density of the Agarose bundles, effectively reducing their pore size [8]. This means that a DNA fragment of the same size will take longer to move through a low melting Agarose gel as opposed to a standard Agarose gel. Because the bundles associate with one another through non-covalent interactions, it is possible to re-melt an Agarose gel after it has set [9].

EtBr is the most common reagent used to stain DNA in Agarose gels [10]. When exposed to uv light, electrons in the aromatic ring of the ethidium molecule are activated, which leads to the release of energy (light) as the electrons return to ground state. EtBr works by intercalating itself in the DNA molecule in a concentration dependent manner. This allows for an estimation of the amount of DNA in any particular DNA band based on its intensity. Because of its positive charge, the use of EtBr reduces the DNA migration rate by 15%. EtBr is a suspect mutagen and carcinogen, therefore one must exercise care when handling Agarose gels containing it. In addition, EtBr is considered a hazardous waste and must be disposed of appropriately. Alternative stains for DNA in Agarose gels include SYBR Gold, SYBR green, Crystal Violet and Methyl Blue. Of these, Methyl Blue and Crystal Violet do not require exposure of the gel to uv light for

visualization of DNA bands, thereby reducing the probability of mutation if recovery of the DNA fragment from the gel is desired. However, their sensitivities are lower than that of EtBr. SYBR gold and SYBR green are both highly sensitive, uv dependent dyes with lower toxicity than EtBr, but they are considerably more expensive.

Moreover, all of the alternative dyes either cannot be or do not work well when added directly to the gel, therefore the gel will have to be post stained after electrophoresis. Because of cost, ease of use, and sensitivity, EtBr still remains the dye of choice for many researchers. However, in certain situations, such as when hazardous waste disposal is difficult or when young students are performing an experiment, a less toxic dye may be preferred.

Loading dyes used in gel electrophoresis serve three major purposes. First they add density to the sample, allowing it to sink into the gel. Second, the dyes provide color and simplify the loading process. Finally, the dyes move at standard rates through the gel, allowing for the estimation of the distance that DNA fragments have migrated.

The exact sizes of separated DNA fragments can be determined by plotting the log of the molecular weight for the different bands of a DNA standard against the distance traveled by each band. The DNA standard contains a mixture of DNA fragments of pre-determined sizes that can be compared against the unknown DNA samples. It is important to note that different forms of DNA move through the gel at different rates. Supercoiled plasmid DNA, because of its compact conformation, moves through the gel fastest, followed by a linear DNA fragment of the same size, with the open circular form traveling the slowest [11-20].

In conclusion, since the adoption of Agarose gels in the 1970s for the separation of DNA, it has proven to be one of the most useful and versatile techniques in biological sciences research.

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