Gel electrophoresis

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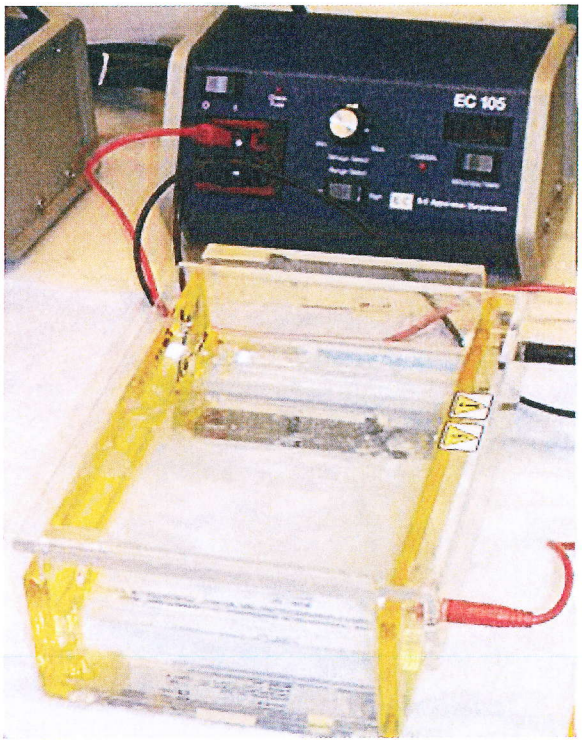
Gel electrophoresis is a technique used for the separation of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or protein molecules using an electric field applied to a gel matrix.[1] DNA Gel electrophoresis is usually performed for analytical purposes, often after amplification of DNA via PCR, but may be used as a preparative technique prior to use of other methods such as mass spectrometry, RFLP, PCR, cloning, DNA sequencing, or Southern blotting for further characterization.

Gel electrophoresis



Gel electrophoresis apparatus - An agarose gel is placed in this buffer-filled box and electrical field is applied via the power supply to the rear. The negative terminal is at the far end (black wire), so DNA migrates toward the camera.

Classification Electrophoresis

Other Techniques

Related
- Capillary electrophoresis
- SDS-PAGE
- Two-dimensional gel electrophoresis
- Temperature gradient gel electrophoresis

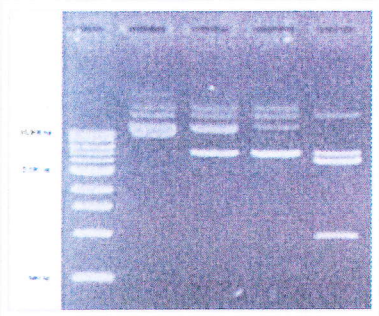
Separation

The term "gel" in this instance refers to the matrix used to contain, then separate the target molecules. In most cases, the gel is a crosslinked polymer whose composition and porosity is chosen based on the specific weight and composition of the target to be analyzed. When separating proteins or small nucleic acids (DNA, RNA, or oligonucleotides) the gel is usually composed of different concentrations of acrylamide and a cross-linker, producing different sized mesh networks of polyacrylamide. When separating larger nucleic acids (greater than a few hundred bases), the preferred matrix is purified agarose. In both cases, the gel forms a solid, yet porous matrix. Acrylamide, in contrast to polyacrylamide, is a neurotoxin and must be handled using appropriate safety precautions to avoid poisoning. Agarose is composed of long unbranched chains of uncharged carbohydrate without cross links resulting in a gel with large pores allowing for the separation of macromolecules and macromolecular complexes.

"Electrophoresis" refers to the electromotive force (EMF) that is used to move the molecules through the gel matrix. By placing the molecules in wells in the gel and applying an electric field, the molecules will move through the matrix at different rates, determined largely by their mass when the charge to mass

ratio (Z) of all species is uniform, toward the anode if negatively charged or toward the cathode if positively charged.[2]

Visualization



I Agarose gel prepared for DNA analysis - The first lane contains a *DNA ladder* for sizing, and the other four lanes show variously-sized DNA fragments that are present in some but not all of the samples.

After the electrophoresis is complete, the molecules in the gel can be stained to make them visible. Ethidium bromide, silver, or coomassie blue dye may be used for this process. Other methods may also be used to visualize the separation of the mixture's components on the gel. If the analyte molecules fluoresce under ultraviolet light, a photograph can be taken of the gel under ultraviolet lighting conditions. If the molecules to be separated contain radioactivity added for visibility, an autoradiogram can be recorded of the gel.

If several mixtures have initially been injected next to each other, they will run parallel in individual lanes. Depending on the number of different molecules, each lane shows separation of the components from the original mixture as one or more distinct bands, one band per component. Incomplete separation of the components can lead to overlapping bands, or to indistinguishable smears representing multiple unresolved components.

Bands in different lanes that end up at the same distance from the top contain molecules that passed through the gel with the same speed, which usually means they are approximately the same size. There are molecular weight size markers available that contain a mixture of molecules of known sizes. If such a marker was run on one lane in the gel parallel to the unknown samples, the bands observed can be compared to those of the unknown in order to determine their size. The distance a band travels is approximately inversely proportional to the logarithm of the size of the molecule.

Applications

Gel electrophoresis is used in forensics, molecular biology, genetics, microbiology and biochemistry. The results can be analyzed quantitatively by visualizing the gel with UV light and a gel imaging device. The image is recorded with a computer operated camera, and the intensity of the band or spot of interest is measured and compared against standard or markers loaded on the same gel. The measurement and analysis are mostly done with specialized software.

Depending on the type of analysis being performed, other techniques are often implemented in conjunction with the results of gel electrophoresis, providing a wide range of field-specific applications.

Nucleic acids

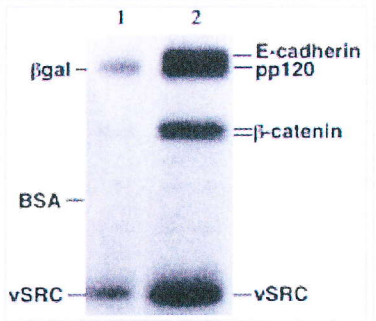
In the case of nucleic acids, the direction of migration, from negative to positive electrodes, is due to the naturally-occurring negative charge carried by their sugar-phosphate backbone.[3]

Double-stranded DNA fragments naturally behave as long rods, so their migration through the gel is relative to their size or, for cyclic fragments, their radius of gyration. Single-stranded DNA or RNA tend to fold up into molecules with complex shapes and migrate through the gel in a complicated manner based on their tertiary structure. Therefore, agents that disrupt the hydrogen bonds, such as sodium hydroxide or formamide, are used to denature the nucleic acids and cause them to behave as long rods again.[4]

Gel electrophoresis of large DNA or RNA is usually done by agarose gel electrophoresis. See the "Chain termination method" page for an example of a polyacrylamide DNA sequencing gel. Characterization through ligand interaction of nucleic acids or fragments may be performed by mobility shift affinity electrophoresis.

Proteins

Proteins, unlike nucleic acids, can have varying charges and complex shapes, therefore they may not migrate into the polyacryl amide gel at similar rates, or at all, when placing a negative to positive EMF on the sample. Proteins therefore, are usually denatured in the presence of a detergent such as sodium dodecyl sulfate/sodium dodecyl phosphate (SDS/SDP) that coats the proteins with a negative charge.[1] Generally, the amount of SDS bound is relative to the size of the protein (usually 1.4g SDS per gram of protein), so that the resulting denatured proteins have an overall negative charge, and all the proteins have a similar charge to mass ratio. Since denatured proteins act like long rods instead of having a complex tertiary shape, the rate at which the resulting SDS coated proteins migrate in the gel is relative only to its size and not its charge or shape.[1]



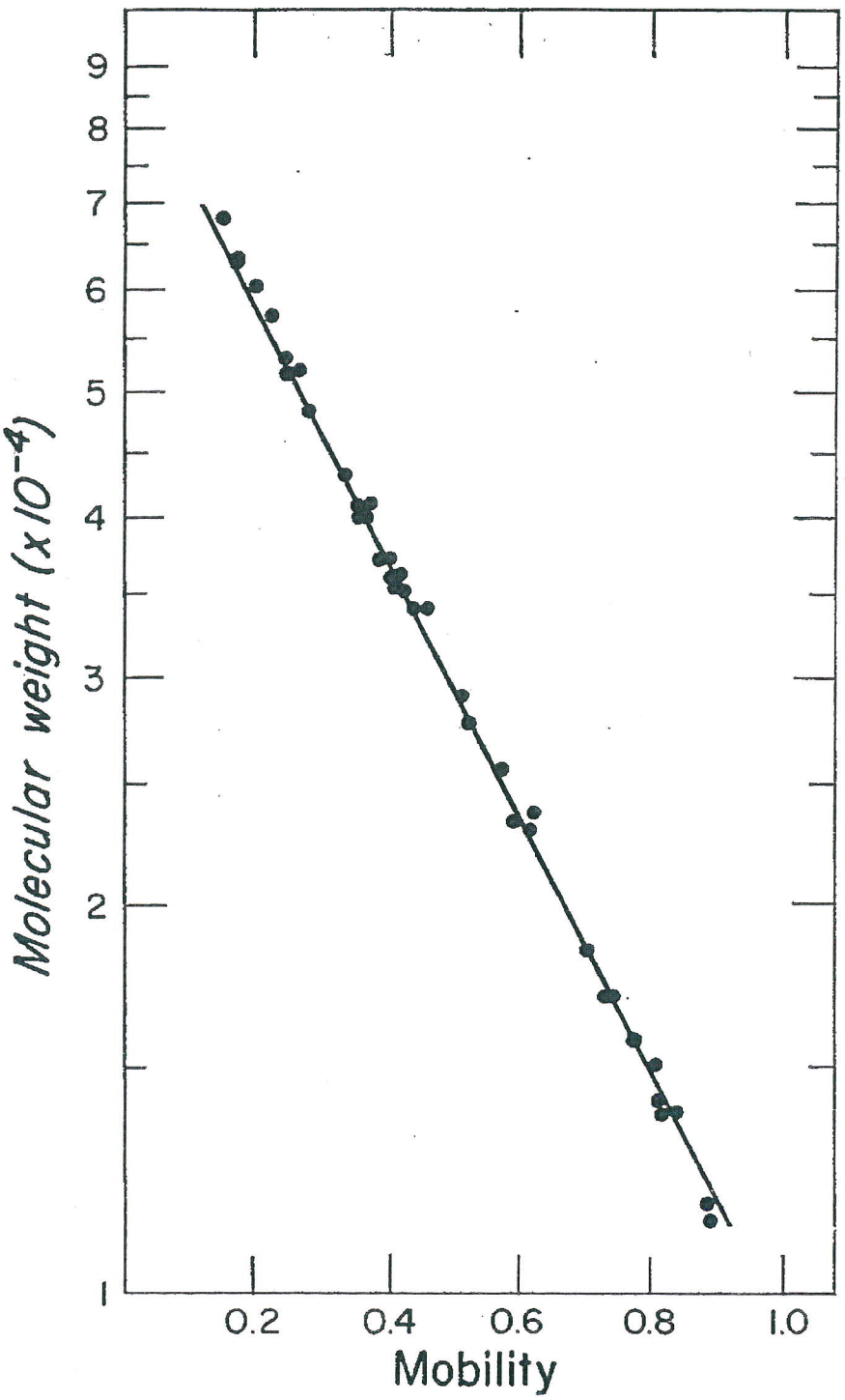
SDS-PAGE autoradiography - The indicated proteins are present in different concentrations in the two samples.

Proteins are usually analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), by native gel electrophoresis, by quantitative preparative native continuous polyacrylamide gel electrophoresis (QPNC-PAGE), or by 2-D electrophoresis.

Characterization through ligand interaction may be performed by electroblotting or by affinity electrophoresis in agarose or by capillary electrophoresis as for estimation of binding constants and determination of structural features like glycan content through lectin binding.

History

- 1930s - first reports of the use of sucrose for gel electrophoresis
- 1955 - introduction of starch gels, mediocre separation
- 1959 - introduction of acrylamide gels (Raymond and Weintraub); accurate control of parameters such as pore size and stability
- 1964 - disc gel electrophoresis (Ornstein and Davis)
- 1969 - introduction of denaturing agents especially SDS separation of protein subunit (Weber and Osborn)[5]



Comparison of the molecular weights of 37 different polypeptide chains in the molecular weight range from 11,000 to 70,000 with their electrophoretic mobilities on gels with the normal amount of cross-linker.

SDS-PAGE (PolyAcrylamide Gel Electrophoresis)

The purpose of this method is to separate proteins according to their size, and no other physical feature. In order to understand how this works, we have to understand the two halves of the name: **SDS** and **PAGE**.

SDS

Since we are trying to separate many different protein molecules of a variety of shapes and sizes, we first want to get them to be linear so that the proteins no longer have any secondary, tertiary or quaternary structure (i.e. we want them to have the same linear shape). Consider two proteins that are each 500 amino acids long but one is shaped like a closed umbrella whle the other one looks like an open umbrella. If you tried to run down the street with both of these molecules under your arms, which one would be more likely to slow you down, even though they weigh exactly the same? This analogy helps point out that not only the mass but also the shape of an object will detrmine how well it can move through and environment. So we need a way to convert all proteins to the same shape - we use SDS.

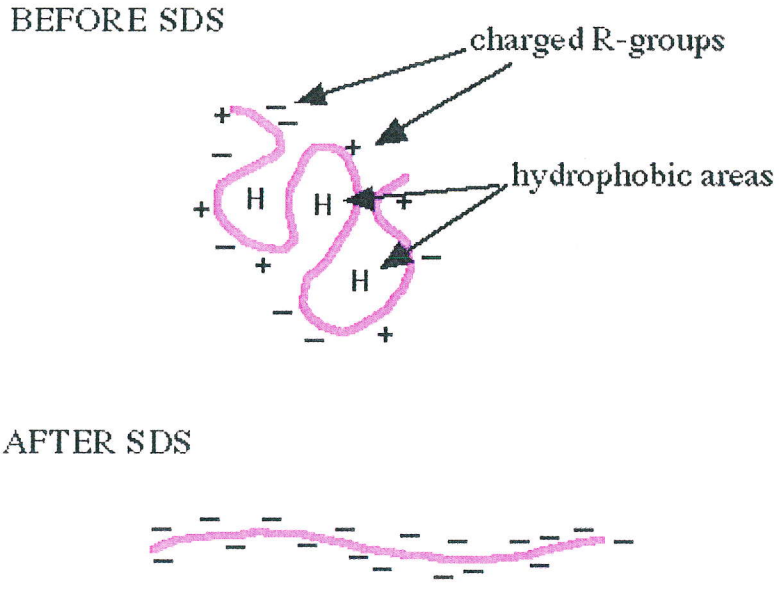


Figure 1. This cartoon depicts what happens to a protein (pink line) when it is incubated with the denaturing detergent SDS. The top portion of the figure shows a protein with negative and positive charges due to the charged R-groups of the particular amino acids in the protein. The large H represents hydrophobic domains where nonpolar R-groups have collected in an attept to get away from the polar water that surrounds the protein. The bottom portion shows that SDS can break up hydrophobic areas and coat proteins with many negative charges which overwhelms any positive charge in the protein due to positively charged R-groups. The resulting protein has been denatured by SDS (reduced to its primary structure) and as a result has been lenearized.

SDS (Sodium dodecyl Sulfate) is a detergent (soap) that can dissolve hydrophobic molecules but also has a negative charge (sulf**ATE**) attached to it. Therefore, if a cell is incubated with SDS, the membranes will be dissolved and the proteins will be soluablized by the detergent, plus all the proteins will be covered with many negative charges. So a protein that started out like the one shown in the top part of figure 1 will be converted into the one shown in the bottom part of figure 1. The end result has two important features: 1) all proteins contain only primary structure and 2) all proteins have a large negative charge which means they will all migrate towards the positve pole when placed in an electric field. Now we are ready to focus on the second half - PAGE.

PAGE

If the proteins are denatured and put into an electric field, they will all move towards the positive pole at the same rate, with no separation by size. So we need to put the proteins into an environment that will allow different sized proteins to move at different rates. The environment of choice is polyacrylamide, which is a polymer of acrylamide monomers. When this polymer is formed, it turns into a gel and we will use electricity to pull the proteins through the gel so the entire process is called polyacrylamide gel electrophoresis (**PAGE**). A polyacrylamide gel is not solid but is made of a laberynth of tunnels through a meshwork of fibers (figure 2).

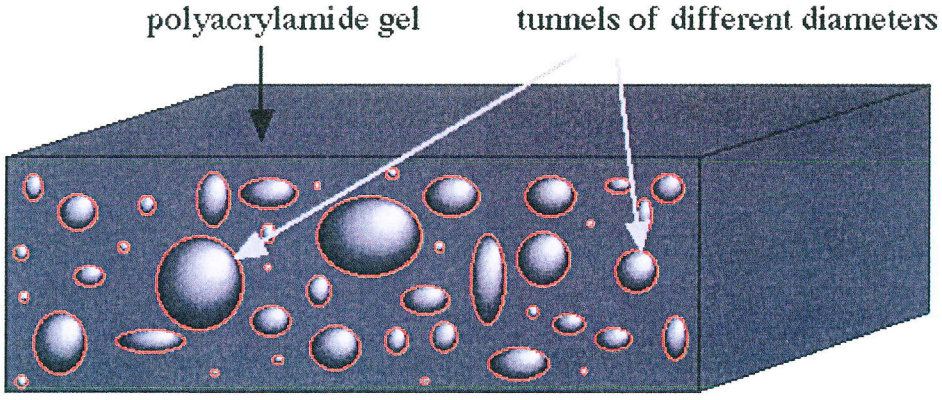


Figure 2. This cartoon shows a slab of polyacrylamide (dark gray) with tunnels (different sized red rings with shading to depict depth) exposed on the edge. Notice that there are many different sizes of tunnels scattered randomly throughout the gel.

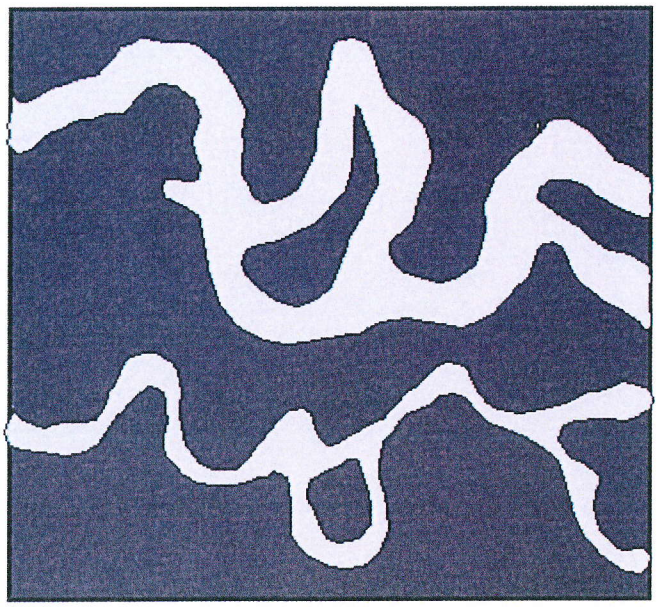


Figure 3. This is a top view of two selected tunnels (only two are shown for clarity of the diagram). These tunnels extend all the way through the gel, but they meander through the geland do not go in straight lines. Notice the difference in diameter of the two tunnels.

Now we are ready to apply the mixture of denatured proteins to the gel and turn on the current (figure 4). If all the proteins enter the gel at the same time and have the same force pulling them towards the other end, which ones will be able to move through the gel faster? Think of the gel as a tiny forrest with many branches and twigs througout the forrest but they form tunnels of different sizes. If we let children and adults run through this forrest at the same time, who will be able to get through faster? The children of course. Why? Because of their small size, they are more easily able to move through the forrest. Likewize, small molecules can manuver through the polyacrylamide forrest faster than big molecules.

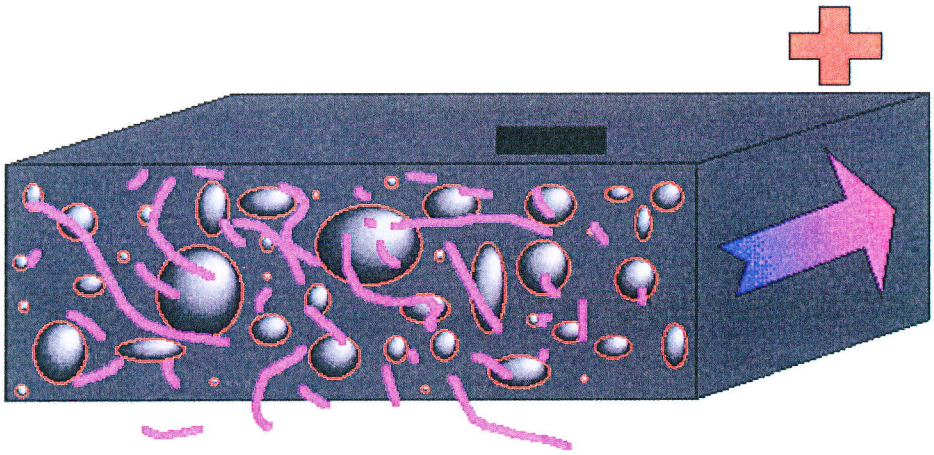


Figure 4. Cartoon showing a mixutre of denatured proteins (pink lines of differen lengths) begining their journey through a polyacrylamide gel (gray slab with tunnels). An electric filed is established with the positive pole (red plus) at the far end and the negative pole (black minus) at the closer end.

Since all the proteins have strong negative charges, they will all move in the direction the arrow is pointing (run to red).

You have to remember that when we work with proteins, we work with many copies of each kind of protein. As a result, the collection of proteins of any given size tend to move through the gel at the same rate, even if they do not take exactly the same tunnels to get through. Back to our analogy of the forrest... If we were in a hot air ballon above the forrest and watched 100 children, 100 teenagers, and 100 large adults running through the forrest, we would see collection (or band) of children moving quickly, a band of teenagers moving slower, and a third band made of adults plodding their way through the forrest. Likewize, proteins tend to move through a gel in bunches, or bands, since there are so many copies of each protein and they are al the same size. When running an SDS-PAGE, we never let the proteins electrophorese (run) so long that they actually reach the other side of the gel. We turn off the current and then stain the proteins (normally they are colorless and thus invisible) and see how far they moved through the gel. Figure shows a cartoon gel and figre 6 shows a one real. Notice that the actual bands are equal in size, but the proteins within each band are of different sizes.

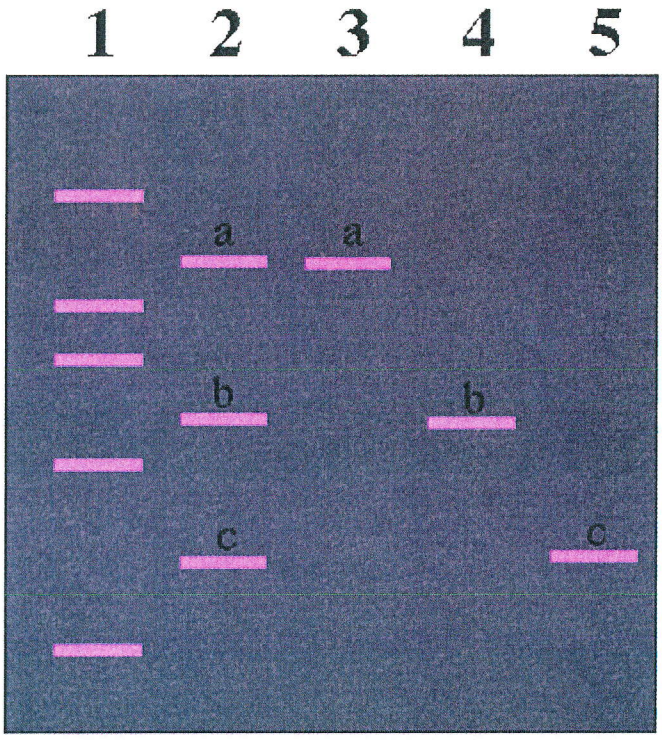


Figure 5. This shows a top view of an SDS PAGE after the current has been on for a while (positive pole at the bottom) and then turned off. The gel (gray box) has five numbered lanes where five different samples of proteins (many copies of each kind of protein) were applied to the gel. (Lane 1, molecular weight standards of known sizes; Lane 2, a mixture of three proteins of different sizes with a being the biggest and c being the smallest protein; Lane 3, protein a by itself; Lane 4, protein b by itself; Lane 5 protein c by itself.) Notice that each group of the three proteins migrated the same distance in the gel whether they were with other proteins (lane 2) or not (lanes 3-5). The molecular weight standards are used to measure the relative sizes of the unknow proteins (a, b, and c).

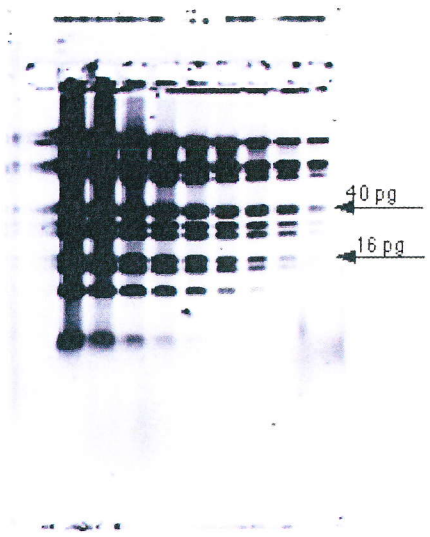


Figure 6. This photo shows a variety of different proteins being separated on a gel. This particular image is showing a serial dilution of the same protein sample to indicate how little protein is needed (16 picograms = $16 \cdot 10^{-12}$ grams) in order to be detected.
This image was taken from a home page operated by Hitachi Software (http://www.hitachi-soft.com /hitsoft/gs/fmbio/feb.htm)

There is a caveot to this method that you must always keep in mind. SDS-PAGE separates proteins based on their primary structure of size but not amino acid sequence. Therefore, if we had many copies of two different proteins that were both 500 amino acids long, they would travel together through the gel in a mixed band. As a result, we would not be able to use SDS-PAGE to separate these two proteins from each other.