

Gel Electrophoresis

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Electrophoresis is a technique for separating particles based on electrical charge, size, and shape. Particles to be separated are placed into an electrophoresis gel that is then subjected to a voltage, using electrodes of opposite charge placed at opposite ends of the gel. If particles to be separated have no charge, then electrophoresis cannot separate them. If they are charged, then they will move through the electrophoresis gel toward the electrode with opposing charge. Positive particles will move toward the negative electrode, and negative particles will move toward the positive electrode. Even particles of the same charge can be separated by electrophoresis, because particles of different size or different shape will move through the gel at different speeds. They will therefore end up at different places within the gel. In agarose gel electrophoresis, the gel is made using agarose, a polysaccharide derived from seaweed. The agarose is mixed with TAE to form a gel. TAE is a buffer (a substance that helps to maintain pH) that also conducts electricity. Wells in the gel serve to accept the samples, and dye is added to samples to allow the experimenter to know how far particles have traveled during a trial.

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Several factors affect how quickly or how far particles move through an electrophoresis gel:

- A higher concentration of agarose in the gel will make for a denser gel, which will slow all particles compared to a less dense gel.
- Small particles move through a gel faster than larger particles in the same gel.
- Shape affects the speed of particles because of drag. A particle with a compact shape has less drag than one that is more spread out, so the compact particle will move faster.
- The greater the voltage applied to the gel, the faster all particles in that gel will travel.
- The longer the voltage is applied to the gel, the farther all particles in that gel will travel.

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The agarose used to make electrophoresis gels is also a component of agar that is used in plates for growing bacteria and other cells.

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The particles moving through an electrophoresis gel form bands within lanes. The lanes correspond to the wells, and each band within a lane consists of many particles of the same charge, size, and shape that are therefore moving at the same speed. For most types of particles, the bands are not visible within the gel during the electrophoresis trial. Later treatment with dyes allows the bands to be seen either under normal room light or under ultraviolet light, depending on the dyes chosen for the experiment.

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Aside from separating particles, electrophoresis also provides a way of measuring the size of particles by comparing how far a particles of unknown size travels in relation to how far a particle of known size travels in the same gel. A mixture of particles (called a molecular mass ruler) of multiple known sizes is put into a well in the gel, and samples of particles of unknown size are put into other wells of the same gel. The particles in the molecular mass ruler form multiple bands (collectively called a ladder) in the ruler's lane, corresponding to the different known sizes. Bands for particles of unknown size can then be matched up against bands in the ladder to determine the unknown sizes.