General Aim
To separate and identify DNA or RNA molecules by size, using an electric current.

Method
Electrophoresis of nucleic acids in agarose gels.

Learning Objectives (ILOs)
- Demonstrate proficiency with the protocol involved in agarose gel DNA electrophoresis.
- Identify the role of specific reagents and equipment in DNA electrophoresis.
- Prepare an agarose gel properly.
- Visualize and understand the precautions required during sample application in the gel.
- Practice basic laboratory techniques.
- Conclude downstream applications of DNA electrophoresis.

Theoretical Background/Context
- Gel Electrophoresis is a procedure used in molecular biology to separate and identify molecules (such as DNA and RNA) by size. The separation of these molecules is achieved by placing them in a gel made up of small pores and setting an electric field across the gel.
- The molecules will move based on their inherent electric charge (i.e., negatively charged molecules move away from the negative pole) and smaller molecules will move faster than larger molecules; thus, a size separation is achieved within the pool of molecules running through the gel. The gel works in a similar manner to a sieve separating particles by size; the electrophoresis works to move the particles, using their inherent electric charge, through the sieve.
- The concentration of gel = weight of agarose/volume of buffer (g/ml). For a standard agarose gel electrophoresis, 0.8% gel gives good separation of large 5–10kb DNA fragments, while 2% gel gives good resolution for small 0.2-1 Kb DNA fragments.
- During gelation, agarose polymers associate non-covalently and form a network whose pore sizes determine a gel’s molecular sieving properties. The phosphate in the backbone of DNA is negatively charged, therefore DNA fragments will migrate to the positively charged anode.
- DNA has a uniform mass/charge ratio, therefore 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.
- The rate of migration of a DNA molecule through a gel is determined by the following:
  1. Size of DNA band (the heavier, the slower)
  2. Agarose gel concentration (usually 0.8%).
  3. DNA conformation (linear/plasmid/etc.).
  4. Voltage
  5. Electrophoresis buffer.
  6. Ethidium bromide: EtBr is positively charged, thus; reduces the DNA migration rate by 15%. Other stains for DNA in agarose gels include SYBR Gold, SYBR green, Crystal Violet and Methyl Blue.
- UV light activates electrons in the aromatic ring of ethidium bromide releasing light as electrons return to ground state. EtBr intercalates itself in the DNA molecule in a concentration dependent manner. So higher intensity means higher amount of DNA.

Principle of Work
- Agarose is isolated from the seaweed genera Gelidium and Gracilaria. It’s mixed with a buffer and heated in a microwave, then left to cool down before pouring in the cast. A comb is added at a specific site to form the wells required for sample upload. Then gel is left to solidify.
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