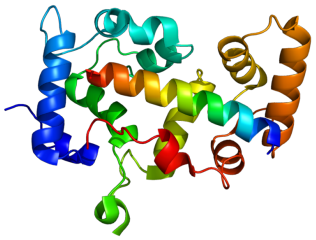
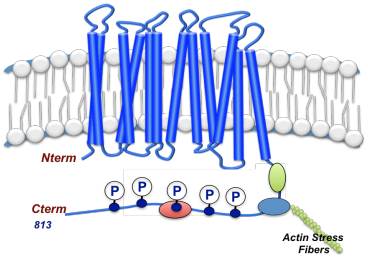
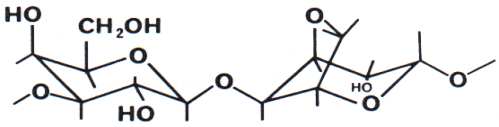


Agarose Gel Protocol

See long version for background

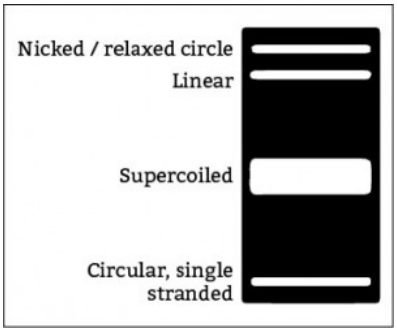


DNA gels are used to separate fragments of DNA and RNA. Unlike most protein separations which use acrylamide polymers, use agarose in a submerged horizontal orientation, and at time called horizontal gel electrophoresis. This handout will cover the details of agarose gels, the theory of separation by agarose gel electrophoresis and tips for conducting successful gel electrophoresis. Agarose is a seaweed extract (red algae agar) and is a long polymer of D and L galactose and derivatives in a linear polymer bonded by two different glycosidic bonds.



Repeating pattern of agarose

For most plasmids and restriction digests a 0.8% to 1.2% gel will work just fine. 1-10 ng of a single, double stranded DNA band should be appropriate.



A simple approach (but not fully correct) to describe the migration is the negative charge of the phosphate backbone will attract the nucleotide to the positive electrode, the size and shape of the DNA will impact its mobility through the gel pores. As created by *E. coli,* plasmid DNA is double stranded and tightly wound. Supercoiled DNA, while having the same mass will take a much smaller volume of space than relaxed DNA and thus run as if it were a smaller size. The two larger, slower migrating bands, are the result if one of the strands of a supercoiled plasmid were cut and the plasmid unwound and relaxed. It is still a circular plasmid but as a fully relaxed nucleotide will have a retarded run through the agarose gel pores and migrate at a higher more expected molecular weight. If both strands are cut by mechanical or enzymatic means, the DNA will no longer be circular and will run at its "true" molecular mass as a linear piece of DNA.

Additionally, if there is RNA contamination, it will run as a wide band at a much smaller molecular mass than plasmid or genomic DNA.

Molecular biology agarose**:** This is a general-purpose agarose that has a high exclusion limit. This type of agarose has high gel strength and is easy to handle at low percentages making it ideal for preparative gels and recovery of DNA. Analytical separation is >1,000 base pairs (bp).

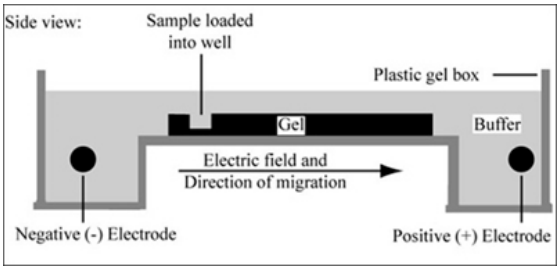
Low-melt agarose**:** The main use of low-melt agarose is for preparative electrophoresis. It is ideal for in-gel applications such as ligation, PCR, restriction enzyme digestion, transformation, and sequencing. This agarose has a gelation temperature of 26°C and high resolving capacity, >1,000 kb.

Buffers - There are a few basic buffers for running DNA gels. ***TAE, TBE, TPE and Borate buffers*** (not the same as a TBE buffer). Each has a different use.

Each gel and buffer should match the correct use. TAE is best used if recovering DNA from gel slice, while TBE is better for smaller (<1kB) DNA strands. See figure and table for details.

TAE Buffer	TBE Buffer	TPE Buffer	Na Borate Buffer
• Use when DNA is to be recovered • Use for large > 12kB DNA • Low ionic strength • Low buffering capacity - may need to recirculate for extended runs	• Used for <1kB DNA - provides tighter bands with higher % gels • Decreased DNA mobility • High ionic strength • High buffering capacity • Not best buffer if recovering DNA after run	• High buffering capacity • Will work for recovering DNA after run • Good for long runs • Used for analysis of single-stranded DNA • Will interfere with phosphate-sensitive reactions of recovered DNA	• Used for high voltages providing faster runs • Limited resolution • Best for quick analytical gels of purified DNA or restriction digests
50X TAE Stock • 242.0 g Tris Base • 57.1 ml Glacial Acetic Acid • 18.61g $Na_2EDTA{\cdot}2H_2O$ • QS to 1.0 liter with water - do not adjust pH, but check... *note: can use stock EDTA to make buffer, will need to check and adjust pH depending on stock (100 ml of 0.5 M EDTA at ph 8.0)* 1X=40 mM Tris pH 7.6-8.0, 20 mM acetic acid, 1 mM EDTA	**10X TBE Stock** • 108.0 g Tris Base • 55.0g boric acid • 40 ml 0.5M EDTA (pH 8.0) • QS to 1.0 liter with water 1X=89 mM Tris pH 8.3, 89 mM boric acid, 2 mM EDTA	**10X TPE Stock** • 108.0 g Tris Base • 15.5 ml 85% Phosphoric acid • 7.44 g $Na_2EDTA{\cdot}2H_2O$ • QS to 1.0 liter with water 1X=89 mM Tris pH 8.3, 89 mM boric acid, 2 mM EDTA	**1X Na Borate (SB)** • Prepare 1M boric acid (6.1 g/100 ml water). • Carefully add 1.0 ml of 10 M NaOH to 500 ml water with stirring • Adjust pH of NaOH solution to pH 8.5 using a 1M Boric Acid slurry in a dropwise fashion. *note: Boric acid may not go into solution easily, slightly warm and/or use as a well mixed slurry. Biotechniques 36:214-216 2004*

OTHER IMPORTANT FACTORS:



Buffer Depth and Depletion: For any buffer the depth of buffer over the gel should range from 3 to 5 mm.

Preparing Gels: Choose a flask that is 2-4 times to volume of the solution

- Mass the correct amount of agarose (0.8% gel = 0.8g of agarose in 100 ml 1X buffer)
- Sprinkle in the agarose powder while solution is rapidly stirred
- Cover with plastic wrap and puncture hole for ventilation
- Heat flask on high until bubbles appear.
- Remove beaker and GENTLY swirl the beaker to resuspend any settled powder and gel pieces. BE CAREFUL!!! A microwaved solution can become overheated and when agitated can bump molten hot agar onto your hand. Wear SAFETY GLOVES AND EYE PROTECTION.
- Reheat the flask until the solution comes to a boil. It may be safe to use short time periods depending on the microwave. Hold the boil for about one minute.
- Gently swirl the flask (USING SAFTEY OVEN MITS/GLOVES) to mix the agarose solution
- *Cool solution to 60°C (about 5 min) before adding stain and casting*.

SYBR Safe DNA Stain [Safe Alternative]

SYBR Safe is a cyan based, non-mutagenic dye (no observed in an acute oral toxicity study in rats) form of SYBR Green dye. The dye absorbs in the blue range, fluoresces only when complexes with DNA and then emits in the green (lambda max 520 nm). The dye is purchased as a highly concentrated stock (often 10,000X). Add to cooling agarose gel before pouring into a casting stand.

- For 50 ml of gel, 5 ul of 10,000X will be enough for most minigels

Casting the Gel:

- Measure the required volume for a gel (should be 3-4 mM thick). Thick gels will cause problems during electrophoresis. Smaller DNA fragments will be lost or fuzzy. Thick gels also show much higher background staining. See figure.
- Level and assemble the tray (tape or damns at the end of the tray as appropriate).
- Allow a small 0.5-1.0 mm space between bottom of comb and tray.
- Pour gel and allow to cool at room temperature for 30 min.
- Slowly wiggle and remove the comb

Running the Gel:

- Place the tray into the gel unit and cover with 3-5 mm buffer. Ensure loose fragments are flushed out of loading wells
- Load samples. Slowly, keeping positive pressure on the pipetter, dispense DNA into the gel.
  - 1-10 ng per lane of purified DNA band or 50-100 ng of a sample with many bands
  - 1 KB DNA LADDER – Mix 10 µl of Stock (0.01 µg/µl 1KB ladder) with 2 µl of 5 or 6X DNA Sample Buffer
    - Prepare 0.01µg/µl stock by mixing 5 ul of 1 µg/µl ladder + 360 µl of 10mM Tris-Cl pH 8.5 (you can use leftover EB buffer from the Qiagen kits!).

- Run the gel at either 100 or 125 constant volts for 45-90 min (keep an eye on the dye front, for standard plasmid gels with digests let the dye travel 2/3 or more to the end of the gel before stopping)

Visualization: Depending on stain, follow manufacturer's instructions. If visualizing directly on UV tansluminator, wear protective eyewear or best, face shield to protect from UV damage to eyes and sunburn.

- 1-10 ng of a single, double stranded DNA band should be appropriate. Less is possible with some stains. Do not exceed 100 ng of DNA per band. 1-5 ng per band for ethidium bromide and 3-10 ng per band will give reasonable results.
  - This is not total DNA - account for all potential bands!
- Loading the smallest volume will give the best results
- The recommended voltage [actually *electrical field strength*] is 4–10 V/cm ([Total voltage divided by the] distance between anode and cathode, not the length of the gel) in the gel electrophoresis unit. If the voltage is too low, then the mobility is reduced and band broadening will occur due to diffusion. If the voltage is too high, the band resolution is reduced, mainly because of gel overheating.
- Typical runs in mini-gel format are 50-100 volts.
- Run until bromophenol blue dye is 2/3 of the way through the gel.