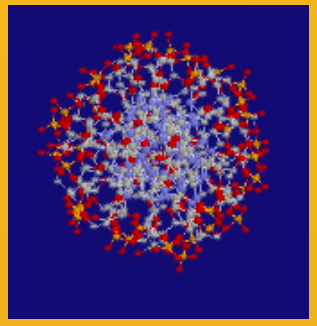
Gel Electrophoresis of DNA



What is Gel Electrophoresis?

- Electro = flow of electricity, phoresis, from the Greek = to carry across
- A gel is a colloid, a suspension of tiny particles in a medium, occurring in a solid form, like gelatin
- Gel electrophoresis refers to the separation of charged particles located in a gel when an electric current is applied
- Charged particles can include DNA, amino acids, peptides, etc

Why do gel electrophoresis?

- When DNA is cut by restriction enzymes, the result is a mix of pieces of DNA of different lengths
- It is useful to be able to separate the pieces - I.e. for recovering particular pieces of DNA, for forensic work or for sequencing

What is needed?

- Agarose - a polysaccharide made from seaweed. Agarose is dissolved in buffer and heated, then cools to a gelatinous solid with a network of crosslinked molecules
- Some gels are made with acrylamide if sharper bands are required

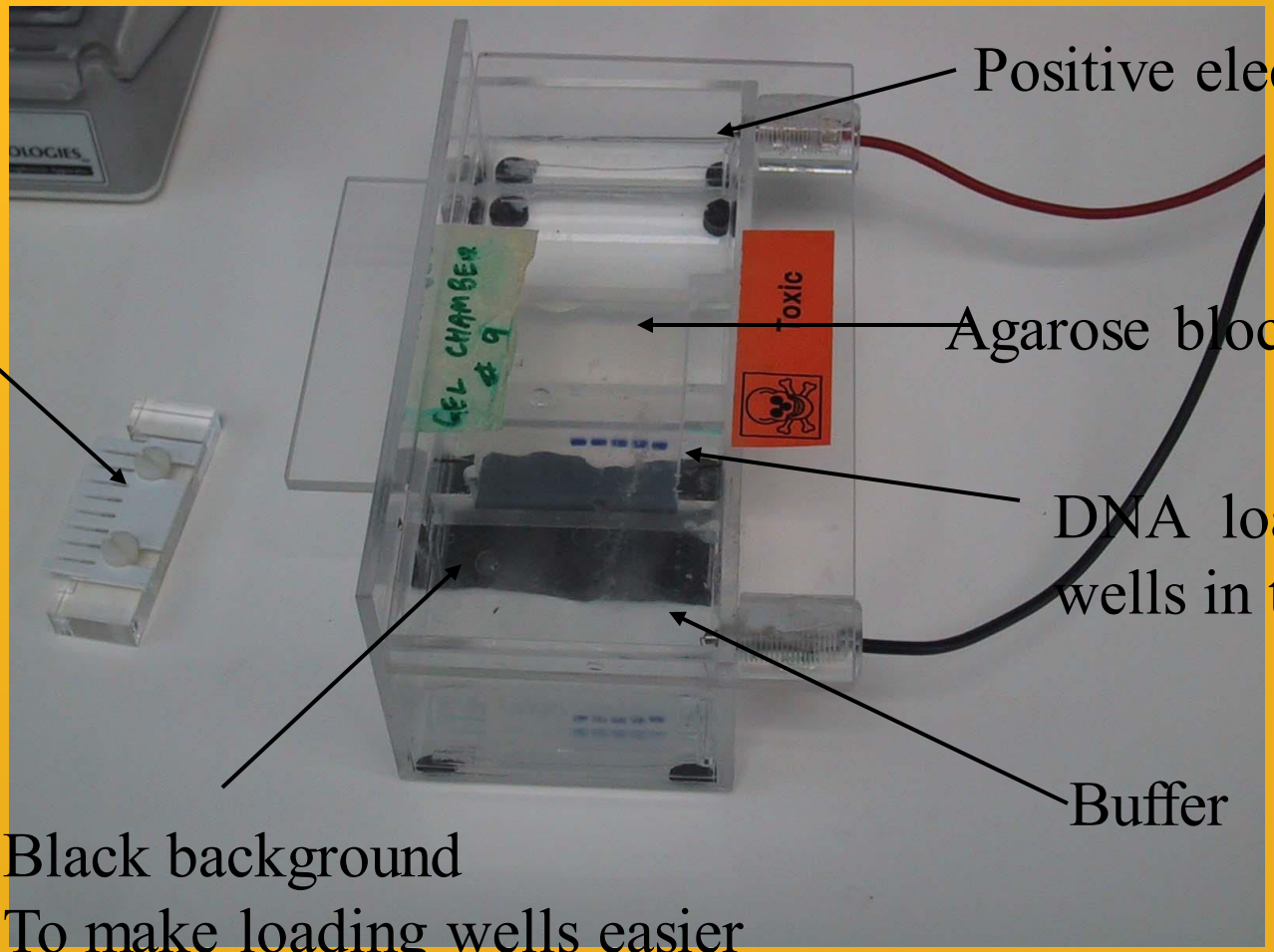
- Buffer - in this case TBE
- The buffer provides ions in solution to ensure electrical conductivity.
- Not only is the agarose dissolved in buffer, but the gel slab is submerged (submarine gel) in buffer after hardening

- Also needed are a power supply and a gel chamber
- Gel chambers come in a variety of models, from commercial through home-made, and a variety of sizes

How does it work?

- DNA is an organic acid, and is negatively charged *(remember, DNA for Negative)*
- When the DNA is exposed to an electrical field, the particles migrate toward the positive electrode
- Smaller pieces of DNA can travel further in a given time than larger pieces

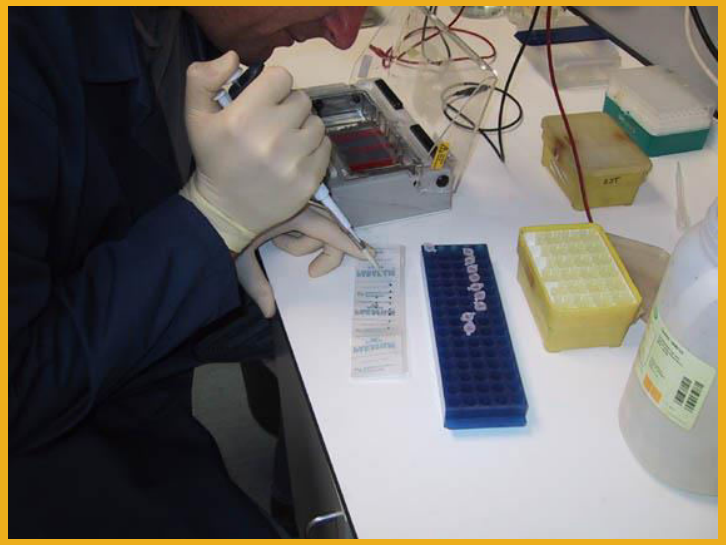
A gel being run



Steps in running a gel

- DNA is prepared by digestion with restriction enzymes
- Agarose is made to an appropriate thickness (the higher the % agarose, the slower the big fragments run) and 'melted' in the microwave
- The gel chamber is set up, the 'comb' is inserted
- The agarose may have a DNA 'dye' added (or it may be stained later). The agarose is poured onto the gel block and cooled

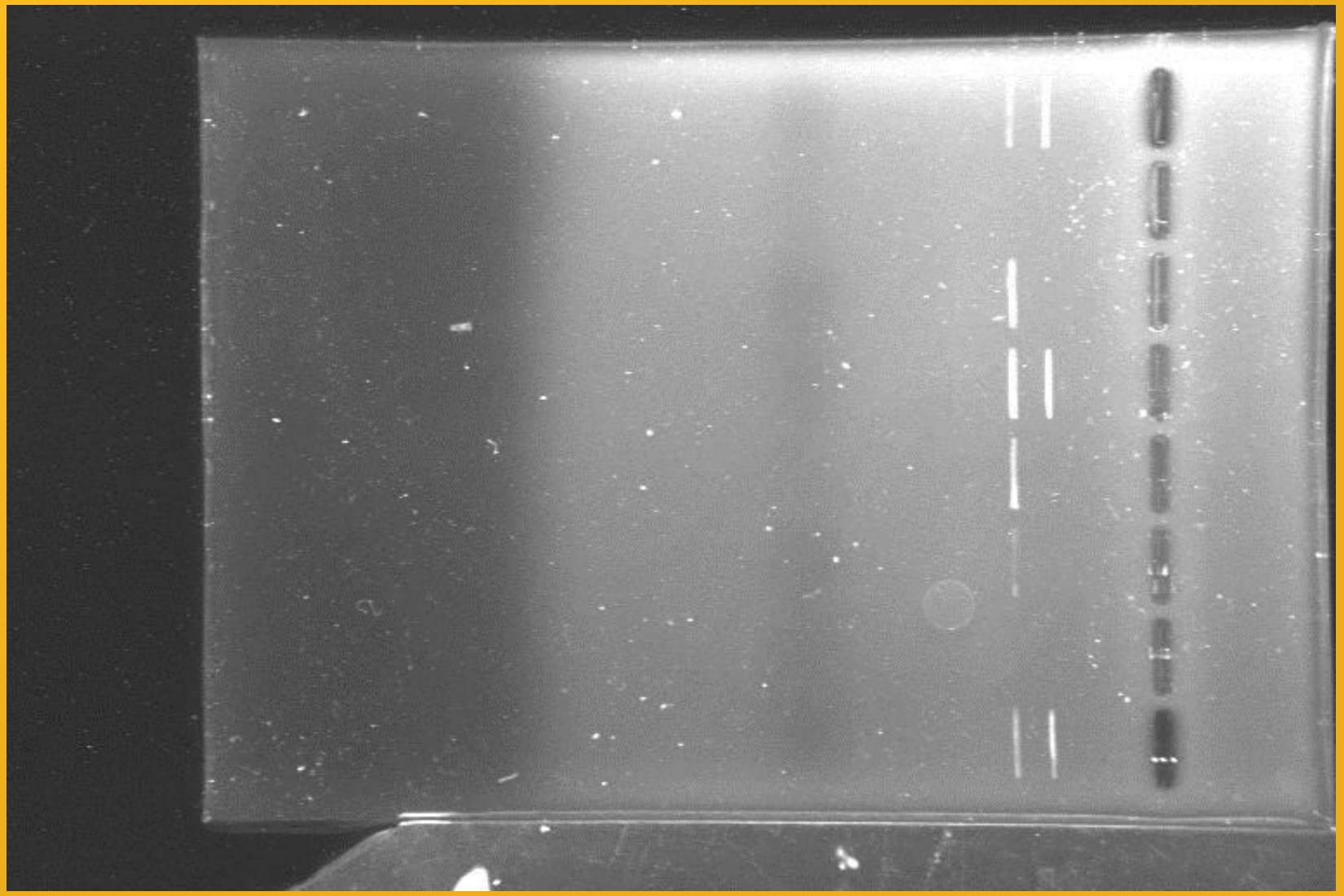
- The comb is removed, leaving little ‘wells’ and buffer is poured over the gel to cover it completely
- The DNA samples are mixed with a dense loading dye so they sink into their wells and can be seen



- The DNA samples are put in the wells with a micropipette.
- Micropipettes have disposable tips and can accurately measure 1/1,000,000 of a litre

Next?

- The power source is turned on and the gel is run. The time of the run depends upon the amount of current and % gel, and requires experimentation
- At the end of the run the gel is removed (it is actually quite stiff)
- The gel is then visualized - UV light causes the bands of DNA to fluoresce



A gel as seen under UV light - some samples had 2 fragments of DNA, while others had none or one

More……

- Many samples can be run on one gel- but it is important to keep track
- Most gels have one lane as a ‘DNA ladder’ - DNA fragments of known size are used for comparison