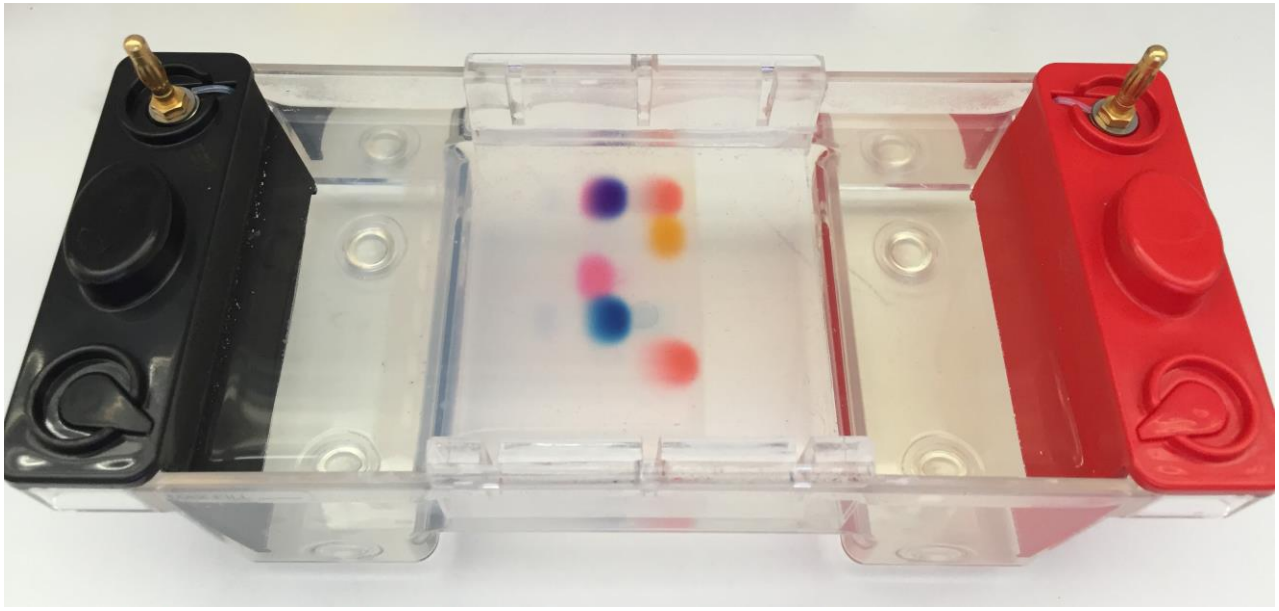
Instructional DNA Electrophoresis Kit

CSLDYEKIT1



Version 02062016

PACKING LIST:

- 6 x DYE samples premixed (130µl of each) in tubes labeled Sample A to F.
- CSL-AG5 Agarose powder
- 200ml Electrophoresis buffer as a 10X concentrate
- 10µl Mini Pipette
- Instructions
- MSDS for the various chemicals supplied

The packing lists should be referred to as soon as the kits are received to ensure that all components have been included. The components should be checked for damage when received.

Please contact your supplier if there are any problems or missing items.

The kit has various chemicals supplied in it so please ensure you wear the appropriate PPE at all time i.e eyes protection, gloves etc

Gel Electrophoresis

Gel electrophoresis is a separation technique used daily in research laboratories throughout the world. Using this method, the presence and size of nucleic acids and proteins in a sample can be determined.

Equipment required

Gel tank with trays, end dams and combs

(recommended for a class of 30 pupils TGT6XMINI six gel system. For smaller classes use a four (TGT4XMINI) or one gel system (TGT1XMINI)

Electrophoresis power supply

(recommended for running at up to 150 volts TPS150V and for better separation of the samples up to 250 Volts when using a nanoPAC300 and TGT6XMINI system only)

Other equipment required

Adjustable micropipettes and tips (or fixed volume pipettes)
Balance
Measuring cylinder
Vortex mixer
Microwave or hotplate stirrer
Large beaker or jug
DNA samples
Microcentrifuge tubes

Step by Step Instructions:-

Ensure the correct safety equipment is used when preparing and running the gels. These would include eye protection, gloves etc. Refer to MSDS sheets supplied for more information.

1. **Make the buffer.**
   Buffer is supplied as a 10X concentrate. Dilute by first establishing how much buffer is required for the tank volume and the gel volume by the particular gel system you are using and adding 1 part 10X buffer to 9 parts water (distilled or de-ionised is best but tap water is fine). For our recommended system TGT6XMINI dilute by measuring 200ml of buffer and adding to 1800ml of water. Stir.

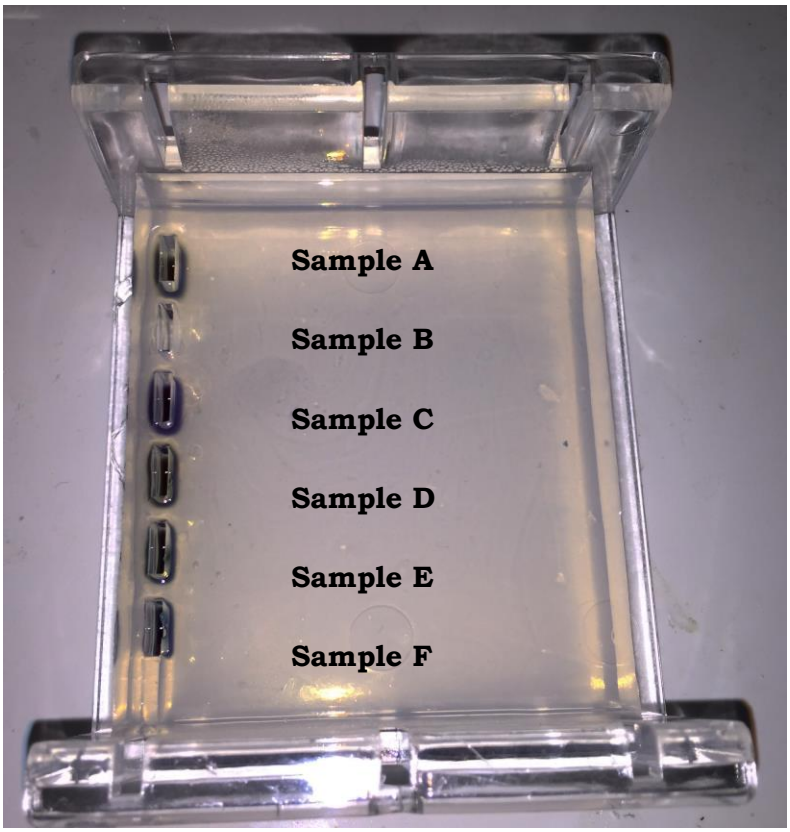
2. **Make the agarose gel.**
   Weigh out the required amount of agarose that will give a 1% gel for the gel volume you will be using so for a 100ml gel weigh out 1g of agarose. For our recommended TGT6XMINI weigh out 3g of agarose powder and mix with 300ml of diluted buffer Heat in microwave for 3 minutes or until the solution starts to bubble, stir and then follow by 30 second bursts until the agarose has completely dissolved. The solution will be completely clear. Set aside to cool. Alternatively, if you are dissolving the agarose using a heater stirrer or water bath, this should be set to 65 degrees centigrade and prepared beforehand.

3. **Pour gels.**
   Different gel systems have different methods for casting gels including traditional taping method, in tank casting and the use of casting systems. We recommend using the simple leak proof tray and end dams system of the Cleaver TGT systems. Instructions are below. For traditional tape casting please see page 8.

   Cleaver TGT systems Leak proof end dam casting:-
   Fit the end dams tightly to the gel trays. The best way of doing this is to place one dam on the bench and then push down firmly with the tray. Then repeat for the other end of the tray and dam. Insert the comb or combs depending on how many samples you wish to load. We recommend that each pupil loads one sample so there will need to be as many wells as required for the number of attendees. Each comb contains 6 wells and each gel tray in the TGT systems has at least two comb slots . Once the agarose has cooled to about 65°c pour it into the gel trays so that the base of the gel comb is submerged in the gel about 5mm – about 30 - 40ml of gel volume per tray used. Leave the gels to set. As they set the gels will turn cloudy. This will take about 20 minutes. Once set **very** carefully remove the comb(s) and end dams as the gel is easy to tear.

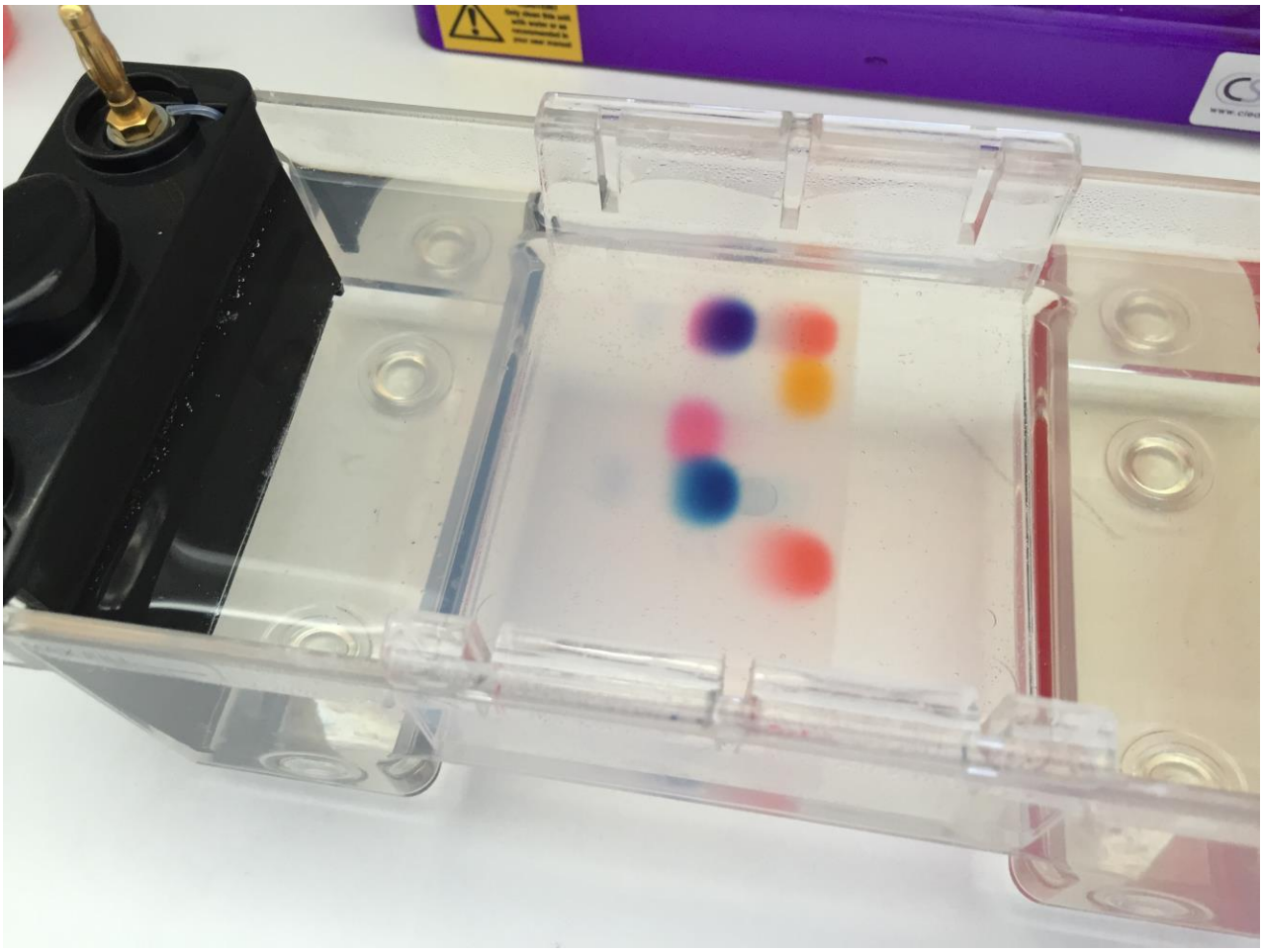
4. **Prepare samples.**
   You have six DYE samples provided in micro-centrifuge tubes. These are labelled A to F. Tube A contains a mixture of all the other dyes and acts as a marker to run alongside all the other samples (Dyes). It is advisable to shake the tube vigorously for 5 seconds then tap the tube on the bench for 5 seconds.

5. **Load the gels.**
   Use a micro pipette to load 10 µl of the contents of DYE sample A into the first well, DYE sample B into the second well etc, being careful to use a **fresh tip** each time so as not to contaminate the samples. Image below shows a gel which has been dry loaded. Please see suggested loading sequence.

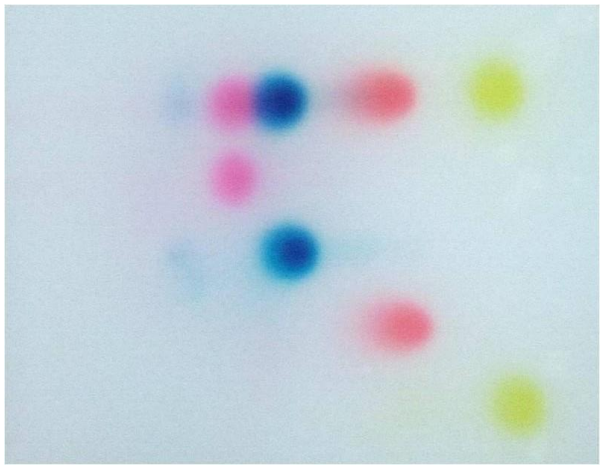


6. **Run the gels.**
   The gels trays can now be carefully transferred to the electrophoresis tank. The wells containing the samples need to be at the negative electrode (black electrode cassette) end of the tank.
   Carefully pour the diluted buffer into the tank reservoir being careful not to wash the

samples out from the wells.
Connect the leads by fitting the lid and set the power supply to 150v. Leave this to run for about 25 minutes. This will give a good separation of the bands. However, if you run the gel for 35mins or have a power supply with a higher capability and have limited time we would recommend setting to 250 Volts to give better separation of the sample bands. You can tell how far your sample has moved by viewing the dyes that are loaded with the DNA.



7. **Visualise the gels.**
   You will see the electrophoresis dyes separate out over time in their various colours. As mentioned previously Row A has a dye mixture containing all the dyes used so these separate out in a straight line. **See gel image on next page**



Using Traditional tape Gel Casting method:-

1. Autoclave or plastic backed general tape should be used. A length 5cm longer than the width of each end of the tray should be cut. One length should be placed over one end of the tray and stuck m1cm in from the tray edge. This should then be folded and the edges sealed securely. Repeat for the other end and place onto a level surface for gel pouring.
2. Place the comb(s) in the grooves. Each tray has more than one comb grove so that multiple combs can be used. Using multiple combs increases sample number available per gel but decreases run length and care must be taken to ensure that samples from the first wells do not migrate into the lanes of the second comb wells.
3. Pour in the agarose carefully so as not to generate bubbles. Any bubbles that do occur can be smoothed to the edge of the gel and dispersed using a pipette tip.
4. Allow the agarose to set, ensuring that the gel remains undisturbed.
5. Carefully remove the gel casting gates and comb and transfer the gel including tray to the main tank.

Teaching Tips

It is more usual in laboratories to wet load the gels in situ in the tanks through the buffer.
However, when dry loading the samples students are more easily able to see the wells and, if using a tank running multiple gels, it removes the bottleneck around the tank. Where time is a factor, provide the students with ready prepared gels, these can be made the day before and kept in the fridge. Buffer can be saved and used more than once.
A black plate put beneath the gel tray can be used to enhance visibility of the comb wells when loading the samples.

Additional safety controls and hints

Allow the agarose to cool before pouring as, if too hot, not only can it burn the skin if spilt but the gel tray could become warped. Practice pipetting technique using coloured water before pipetting the samples.

SAFETY PRECAUTION

WHEN USED CORRECTLY, ELECTROPHORESIS UNITS POSE NO HEALTH RISK.

HOWEVER, ELECTROPHORESIS UNITS CAN DELIVER DANGEROUS LEVELS OF ELECTRICITY AND ARE TO BE OPERATED ONLY BY QUALIFIED PERSONNEL FOLLOWING THE GUIDELINES LAID OUT IN THE EQUIPMENT INSTRUCTION MANUAL.