Gel electrophoresis Standard Operating Procedure

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A step by step guide for the standard protocol for gel electrophoresis as performed in the Biology department using gel box FB-SB-710.

**Gel electrophoresis**

Standard Operating Procedure

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# Preparing TAE and TBE Buffer for electrophoresis systems

These buffers are used because they both have a basic pH which gives the phosphate group of the DNA a net negative charge allowing migration of the DNA toward the positive anode.

**Buffer TAE or TBE** TAE is best used when DNA is to be recovered from the gel and for electrophoresis of large (>20kb) fragments. TBE is best used for smaller (<1kb) fragments due to high ionic strength and high buffering capacity. Also it reacts with agarose making smaller pores and tighter matrix.

## TAE-Tris acetate w/EDTA (40mM Tris base, 40mM acetic acid, 1mM EDTA)

**50x Stock solution, pH ~8.5**

242g Tris base

57.1ml glacial acetic acid

18.61g Na2EDTA-2H2O (MW 372.24)

Distilled/RO water to 1 liter final volume

## TBE-Tris borate with EDTA (89mM Tris base, 89mM boric acid, 2mM EDTA)

**10x Stock solution**

108g Tris base

55g boric acid

7.44g Na2EDTA-2H2O (MW 372.24) (Or 40ml of 0.5M EDTA, pH 8.0)

Distilled/RO water to 1 liter final volume

# Making a GEL

**Please note** an increased % in agarose gives better separation of small fragments and bands that are close in size.

|  |  |
| --- | --- |
| Agarose % (w/v) | Approximate range of separated DNA fragments (kb) |
| 0.3 | 60 to 5 |
| 0.5 | 30 to 1 |
| 0.7 | 12 to 0.8 |
| 1.0 | 10 to 0.5 |
| 1.2 | 7 to 0.3 |
| 1.5 | 4 to 0.2 |
| 2.0 | 3 to 0.1 |
| 3.0 | <0.1 |

Mobility range of DNA in different percentage agarose gels. *Fisher Scientific Horizontal Electrophoresis systems Installation and operation Manual.* 217754 Rev. 2. 2012.

### For making a 1% gel

1. Dissolve 0.4g of agarose in 40ml of electrophoresis buffer (TBE or TAE) by heating in a microwave.

**! CAUTION HOT USE GLOVES WHEN REMOVING!**

1. After heating and fully dissolving the agarose add 4uL of SYBR SAFE directly into the liquid via pipette, mix by gentle swirling.
2. Use this mixture to cast a gel
3. Place the gel tray into the gel box so that the gasket (Ends with rubber strip) forms a seal against the walls of the gel box make sure to press the gel tray all the way down so that the gel box and gel tray are level.
4. After the gel mix has cooled to 60C (Higher temps will damage and warp the gel box) pour the mix into the gel tray. Upon pouring the gel mix immediately insert the gel comb with the desired number of teeth/wells.
5. Allow the gel to solidify completely. Then lift the gel tray out of the gel box turn it 90O and replace it into the gel box with the comb closest to the cathode.
6. Pour running buffer into the gel box to fill the chamber and completely submerge the gel (300ml).
7. Carefully remove the comb using a light tapping motion to avoid damage to the wells.

# Loading samples

Please note the maximum volume for a gel of this size using a B1A-10 comb is 13.5uL

For other combs use the calculation below to determine well volume and x 0.75 of this value for loading volume. Please note well height will remain **6mm** unless volume of gel cast is increased from 40ml.

(Well height (mm)-1.5) x (Tooth width x Comb thickness)

1. Pipette 10uL of your sample into a clean tube.
2. Add 2uL of loading dye (Bromophenol blue) (Blue dye) for visual tracking.
3. Mix by low vortex and spin on mini-fuge to ensure all sample is at the bottom of the well.
4. Plan out your gel before pipetting and create a reference chart/diagram to ensure correct samples are put into the correct wells. Also that you have a negative and positive control in addition to a hyperladder on one or both ends of the gel (First and last well).
5. Carefully pipette all of sample into the correct well on your gel using 10xL pipette tips (Or any compatible extended length tips)
6. Add 10uL of the hyperladder for reference/comparison in at least one or if possible both ends of your gel (First and last wells)

# Connecting and starting power supply

1. Carefully place the lid on the top of the gel box, ensure it is completely pressed down and level.

**! MAKE SURE RED WIRES CONNECT TO RED PORT ON BOX AND BLACK WIRES TO BLACK PORT!**

1. Connect the wires to the correct color terminals on the power supply.
2. Plug in the power supply
3. Set voltage to 150V
4. Ensure bubbles start to form on both sides of the gel box where the buffer reservoirs are.
5. After 45 mins the gel should be complete check that samples have migrated by looking through the lid.
6. Turn off power supply.
7. Disconnect power supply from outlet.

# Visualizing the gel

1. Ensure power supply is turned off.
2. Remove lid from gel box.
3. Wait approx. 10mins for gel to cool.
4. Carefully remove gel.
5. Place gel onto a blue light transilluminator (Optimal due to maxima excitation wavelength).
6. Place orange filter over to protect eyes.
7. Turn on transilluminator, bands should be visible in addition to a clear hyperladder
8. Take picture via gel doc system on other device (Cell phone) as soon as possible to avoid degradation of fluorescence.
9. Annotate gel picture to transcribe your reference chart for sample in each well including negative and positive control (See below).



 **HL 1 2 3 N 4 5 6 P HL**

 HL 1 2 3 N 4 5 6 P HL