



## SUBMARINE ELECTROPHORESIS

### Steps of Submarine Electrophoresis:

- Prepare the gel and or fit the precast gel in the tank.
- Add the buffer in the tank.
- Run electrophoresis.
- Stain the gel.

### Gel Casting:

1. Following your protocol (kind and size of the gel) calculate the concentration of agarose, the needed amount of buffer, the kind of casting platform and the well forming comb.
2. Add a definite amount of agarose to a definite volume of electrophoresis buffer in a glass bottle, close the bottle airtight, and put it in 90 degree water bath for 20 mins.
3. Put the melted agarose solution in a 55<sup>0</sup>C water bath to cool [and add 1µl from Ethidium bromide stock solution per 10 ml agarose solution and mix it].
4. Prepare the gel casting platform. To prevent leakage hot agarose solution can be applied with a micropipette to the joints and edges of the platform. Insert the well forming comb, pour the agarose solution on it on a horizontal position, and let it solidify [about 20 mins].
5. Gently remove the comb.
6. Fill the tank with buffer until the buffer is 1-2 mm deep over the gel [the volume depends on the kind of the tank].

Cat No	Lot	Tank Length/width (inside) in mm	Length/width/mm of UV Transparent Basis(and gel)	Volume of running buffer in ml
SES 01	SET01	163X59	83X59	123
SES 02	SET01	139X83	59X83	170
SES 03	SET01	150X90	70X90	185
SES 04	SET01	170X70	90X70	147

7. Carefully pipette each sample (5-10µl) into a well in the gel.
8. Control: Load one well with the prepared known ladder. The samples and ladder should be on the same buffer (salt concentration affects migration rate).
9. Connect the tank to the power supply [wells at the negative end (black)], turn on the power and run electrophoresis until the dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized. [ DNA and RNA are negatively charged] Bromphenol blue runs with a DNA fragment of about 200-400 bp.  
**CAUTION: DO NOT REMOVE THE COVER OF THE TANK WITHOUT DISCONNECTING THE POWER LEADS. ELECTRICAL SHOCK MAY RESULT!**

10. If the gel was not stained with EBr during the run, stain the gel in a EBr solution [0.5µg/ml] until the DNA has taken up the dye and is visible (about 5-10 mins) under UV light.
11. Staining: If the gel was not stained with EBr during electrophoresis carefully empty the buffer from the tank and add in it EBr solution [ 0.5µg/ml H<sub>2</sub>O] until the gel is submarine, and let it for 5-10 mins at room temperature.
12. Distaining: Replace the EBr solution 2 times with in H<sub>2</sub>O for 5 mins. To avoid the background fluorescence caused by unbound EBr soak the gel for 5 minutes in 0.01M MgCl<sub>2</sub>.
13. Photography: Place the gel on the transilluminator (excitation 302nmUV/B1: emission 590nm, orange – red) and follow the instruction of the transilluminator. **Warning:** The transilluminator can damage the eyes!

## How much DNA should be loaded?

The big question. You may be preparing an analytical gel to just look at your DNA. Alternatively, you may be preparing a preparative gel to separate a DNA fragment before cutting it out of the gel for further treatment. Either way you want to be able to see the DNA bands under UV light in an Ethidium bromide-stained gel. Typically, a band is easily visible if it contains about 20ng of DNA.

Too much DNA loaded onto a gel is a bad thing. The band appears to run fast (implying that it is smaller than it really is) and in extreme cases can mess up the electrical field for the other bands, making them appear the wrong size also.

Too little DNA is only a problem in that you will not be able to see the smallest bands because they are too faint.

**MW ladders:** Unknown DNA samples are typically run on the same gel with a “ladder”. That is a sample of DNA where the sizes of the bands are known.

## STAINING OF THE DNA FRAGMENTS

After electrophoresis the visualization of the fragments in the gel can be done by staining with Ethidium bromide.

### ETHIDIUM BROMIDE (EBr)

**(It is powerful mutagen and therefore it must be handled with gloves!)**

EBr is an aromatic cation. It binds to double stranded DNA by inserting into the stacked bases. It can be used to detect both single- and double- stranded nucleic acids. The presence of EBr in the gel saves time but the migration will be showered.

Minimum amount of DNA detectable by EBr on a 3-5 mm thick gel and a 5 mm wide lane is 1ng.

**Stock Solution: 10mgEBr/ml H<sub>2</sub>O (1%).** Store it in refrigerator. Protect it from light. Add 1 µl of EBr stock solution in 20ml agarose by 55 °C.

**EBr –Staining solution:** Immerse the gel in EBr solution (10µl of 1% EBr stock solution per 100 H<sub>2</sub>O) for 10-15 mins.

Photograph the gel under the UV light.

10ng DNA in a single, sharp band can be visualized with EBr, while carrying capacity of an agarose gel is about 1 µg per band: Thus 10 ng to 20 µgDNA can be loaded.

### **Visualization of nucleic acids:**

After migration, the bands should be visualized by adding a dye.

Ethidium bromide or silver nitrate are commonly used dyes. Ethidium bromide is a very commonly used fluorescent dye to detect nucleic acids in agarose gels. Ethidium bromide can be added directly into the gel before pouring or it can be added to the migration buffer after migration. It fluoresces under UV light (254 nm) and allows the visualization of the bands on the gel. Pictures can be taken for subsequent analysis.

Silver nitrate can also be used since it presents a high affinity for nucleic acids. Even though it is less dangerous to use, it is not as commonly used as Ethidium bromide: the gels stained should be unstained before reading and the background is higher than with Ethidium bromide.

### **The rate of migration is affected by a number of factors such as:**

- The concentration of agarose.
- The conformation of DNA [super helical, nicked, and linear]. Each form runs differently, the super helical the fastest and the linear form the slowest.
- The presence of Ethidium bromide (EtBr) in the gel causes DNA to run slower due to EtBr's ability to intercalate and uncoil DNA.
- The voltage is also a factor in migration and can only be so high before a decrease in resolution (~5-8 V/cm).

## **BUFFERS**

### **1. LOADING BUFFER (10X concentrated):**

<b>Na<sub>2</sub>EDTA</b>	<b>20mM</b>
<b>Glycerol</b>	<b>50% v/v</b>
<b>Bromphenol blue</b>	<b>0.05% w/v</b>
<b>Store at -20 or 4 °C</b>	
<b>Use: Add 1:10</b>	

### **2. LOADING BUFFER (6X concentrated):**

<b>Bromphenol blue</b>	<b>0.25%</b>
<b>Xylene Cyanol FF</b>	<b>0.25%</b>
<b>Ficoll type 4000</b>	<b>15%</b>
<b>Na<sub>2</sub>EDTA</b>	<b>120mM</b>

### **3. RUNNING BUFFERS**

#### **3A) TAE 50X concentrated:**

<b>H<sub>2</sub>O</b>	<b>800ml</b>	<b>In 1 Liter H<sub>2</sub>O, pH 8.0 ( adjust with NaOH)</b>
<b>Tris Base</b>	<b>242g</b>	
<b>Na<sub>2</sub>EDTA .2H<sub>2</sub>O</b>	<b>37.2 ( or 100ml 0.5M)</b>	
<b>Glacial Acetic Acid</b>	<b>57.1ml</b>	
<b>Store at 10-25 °C</b>		
To use dilute it 1:50. TAE provides optimal resolution of fragments >4kb in length. It is better for cloning work. Use it by electrophoresis of supercoiled DNA fragments. Because of its lower buffering capacity it should be circulated by running of electrophoresis.		

#### **3B) TBE 10X concentrated:**

<b>Tris Base</b>	<b>108g</b>	<b>In 1 liter H<sub>2</sub>O, pH 8.0 (adjust with NaOH)</b>
<b>Boric acid</b>	<b>55 g</b>	
<b>Na<sub>2</sub>EDTA</b>	<b>8.3g ( or 40 ml of 0.5M )</b>	
<b>Store at 10-25 °C</b>		
TBE provides optimal resolution of 0.1-3 kb fragments. The TBE has both a higher buffering capacity and lower conductivity than TAE .Use it by electrophoresis with Volts >150. It generates less heat than TAE and does not allow a significant pH drift.		

#### **3C) TTE 20X concentrated:**

<b>Tris Base</b>	<b>216g</b>	<b>In 1 liter</b>
<b>Taurine</b>	<b>72g</b>	
<b>Na<sub>2</sub>EDTA</b>	<b>4g</b>	

## Notes:

1. Migration of DNA is retarded and band distortion can occur when too much buffer covers the gel.
2. Storage of DNA in Agarose after electrophoresis for long time can be done in 70% methanol.
3. **Electrophoresis Conditions:** Volts should not be more than 5 volts/cm (refers to the distance between electrodes of the electrophoresis tank). 20-80 mA.
4. Temperature: Not more than 30 °C. Cooling of the system results better results.
5. Electrophoresis of GEL without EBr preferable
6. UV-Light: It can damage the DNA.
7. The transilluminator can damage the eyes!

## IMPORTANT NOTES:

### You must:

- **Be technical trained personnel to work with this system.**
- **Follow thoroughly the instructions.**
- **Isolate the electrophoresis tank from the power supply before removing the cover of the tank.**
- **Do not exceed the maximum operating voltage.**
- **Always wear gloves to avoid contact with Ethidium bromide (mutagen).**
- **Always wear safety goggles when using UV light source.**
- **Use only distilled water to clean the tank (not alcohols etc).**
- **Do not use voltage greater than 5 Volts/cm (refers to the distance between the electrodes of the tank).**
- **Run electrophoresis in temperature less than 30°C. Use the cooling box or other system to cool the gel and the running buffer.**

## References:

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