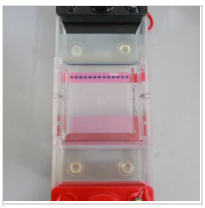

Jul 22, 2022

Functionality test (TBE electrophoresis buffer)

In 1 collection

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This protocol is published without a DOI.

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ABSTRACT

This protocol describes the comparative analysis of resolution of bands and band patterns after electrophoresis with the BenBio TBE buffer formulation as compared with a commercial TBE buffer. We use 0.5 kb and 1kb PCR amplicons loaded on 1.5% (w/v) agarose gel, run electrophoresis at 80V and visualized on a Blue light transilluminator or UV transilluminator.
Our formulation of TBE buffer is able to allow us obtain a higher resolution of smaller DNA fragments on agarose gels.

PROTOCOL CITATION

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COLLECTIONS

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GUIDELINES

This protocol describes the steps in testing the functionality of TBE buffer

MATERIALS TEXT

Equipment

Micropipettes
Microwave
Gel casting tray
Well comb
UV transilluminator/blue light system
Voltage source (Electrophoresis unit)

Materials and reagents

dNTP mix
10x PCR buffer
PCR grade water
PCR Primers
DNA template (0.5/1 kb Lambda DNA)
DNA polymerase enzyme
Agarose (electrophoresis grade)
Commercial 1x TBE buffer (Recipe here), prepared from a 10x TBE stock to be used as "standard"
1x TBE buffer "test buffer"
DNA loading dye (6x NEB)
DNA ladder (Bioline 1kb)
DNA gel stain (SYBR Safe or other Ethidium bromide, EtBr stain)

SAFETY WARNINGS

- Wear protective clothing like Lab coats, gloves and face masks during the process and avoid dust formation from the powders.
- Take special care when handling the EtBr gel stain and the UV transilluminator

BEFORE STARTING

Ensure the buffer sachet is not broken and powder remains free from moisture to enable the resulting buffer to stay longer and avoid compromising the gel results.

PCR amplification 1h 50m

1 In order to have the PCR amplicons to use for this experiment we typically amplify the 0.5 and/or 1kb regions of the Lambda DNA in a PCR reaction as follow: 5m

Preparing for PCR amplification of 0.5 and 1kb Lambda DNA

- Crush some ice and put in a clean bowl
- Remove and thaw all PCR reagents on ice (dNTP mix, 10x PCR buffers, primers, DNA template, DNA polymerase, PCR water)
- Label 3 or more 0.2 mL PCR tubes (E.g with the name of the DNA template and a negative control tube) and place them on ice as shown in figure 1.

In the negative control tube, all PCR reagents will be put except the DNA template (*replaced with PCR water*). This is to check whether there is any contamination within the reaction mix, and that there are no primer-dimers forming because no amplification is expected in the negative control tube as would be shown after agarose gel electrophoresis.

Figure 1

2  5m

- Assemble the reaction mix into 20 ul volume in 0.2 mL PCR tubes in the order shown in figure 2 below.

A	B	C
Component	**Amount in ul - in each 0.2 mL tube**	**Negative Control tube**
PCR water	variable up to 20ul	variable up to 20ul
Thermopol buffer 10x	2	2
dNTP Mix 10 mM	0.4	0.4
Fw/Rv primers	1/1	1/1
DNA template	0.5 (depending on the concentration)	/
Enzyme	2 units (1ul)	2 units (1ul)

Figure 2

- Hold the tubes up and gently mix by tapping tubes and place in a thermocycler.
- Input the cycling parameters as indicated in the table below and run. The amplification/running time will depend on the size of the DNA template.

3  1h 40m

Thermocycling

A	B	C	D
Phase cycle	**Temperature (°C)**	**Duration (sec)**	**Number of cycles**
Initial denaturation	95	120	
Denaturation	95	30	
Annealing	50 to 65 (depending on primers)	20	30 to 35
Extension	72	variable based on primers	
Final extension	72	120	

After amplification, we test the functionality of our TBE buffer by preparing a 1.5% agarose gel to run electrophoresis and visualize the gel.

Functionality test of TBE buffer 45m

4 5m

Preparing 1x TBE buffer from buffer powder sachet

Our lab's standard DNA gel buffer has become 1 sachet of 85.14 g TBE buffer powder into 0.5 L of distilled water which gives a 10x stock solution. Small gels run in 10-15 minutes at 80 Volts.

To make a 1x Buffer - 1 L

- Open up a sachet of 10x TBE buffer (containing 85.14 g of buffer powder) and pour into a 1 L capacity beaker.
- Measure 0.5 L of distilled water using a measuring cylinder into the beaker and stir until it the powder completely dissolves. *You may add a magnetic flea and use a magnetic stirrer to ease dissolution of the powder into a clear liquid which remains stable and clear for up to 1 year at room temperature.*
- Measure out 100ml of the 10x buffer into another 1 L capacity beaker
- Measure 900ml of distilled water and add into the beaker to make up the volume to 1 L. The 1x TBE buffer is stored at room temperature for subsequent use.

5 3m

Preparing 1.5 % TBE agarose gel

Agarose gels are commonly used in concentrations of 0.5% to 2% depending on the size of bands needed to be separated. Simply adjust the mass of agarose in a given volume to make gels of other agarose concentrations (e.g. 0.5g of agarose in 25 mL of TBE, TAE or Borax will make a 2% gel).

Make sure the buffer used in preparing the gel is the same buffer to be used as the gel running buffer, do not mix buffers and do not use water, as it might affect migration of the DNA samples.

Prepare a 1.5% TBE agarose gel as follows:

1. Use a weighing balance to weigh 0.375 g of agarose powder and pour into a 150 mL or any

appropriate size glass beaker.

2. Use a measuring cylinder to measure 25 mL of 1x TBE buffer and add to the agarose powder in the beaker (The volume of gel you will need to make will depend on the size of the casting tray.)
3. Swirl the beaker for about 5 seconds to dissolve the powder
4. Put the beaker into the Microwave and heat at medium high for 1 minute until it boils
5. Remove the molten agar from the Microwave and allow to cool for 30 seconds (about when you can comfortably keep your hand on the beaker).
6. Add EtBr gel stain to a final concentration of 0.5 µg/mL (usually about 2.5 µL to 3 µL of lab stock solution per 25 mL gel). EtBr binds to the DNA and allows you to visualize the DNA under ultraviolet (UV) light or Blue light.
7. Swirl the beaker gently to mix and distribute the DNA gel stain (swirl gently to avoid bubbles).

6 Casting and loading the Electrophoresis gel

35m

1. Pour the molten agar into the gel casting tray with the well combs in place. (Pour slowly to avoid bubbles which will disrupt the gel), allow to solidify for about 15 to 20 minutes.
2. After the gel is solidified, remove the comb and place the casting tray into the gel box or electrophoresis unit.
3. Fill the electrophoresis unit with 1x TBE buffer to cover the gel (the amount of running buffer to use will depend on the size of the electrophoresis unit).
4. Pipette 3 µLof DNA ladder (a collection of DNA fragments of known lengths that helps you determine the approximate length of a DNA fragment by running it on an agarose gel alongside the DNA amplicon), into the first well *(Figure 1).*
5. Pipette 1 part of the 6x DNA loading dye and 5 parts of the negative control, mix and load into the second well.
6. Pipette 1 part of the 6x DNA loading dye and 5 parts of the DNA amplicons, mix and load into the subsequent wells (depending on the number of amplicons) *(Figure 2).*
7. Connect the electrophoresis unit to a power or voltage source by connecting the electrodes from the power source to the electrophoresis unit (Black is negative, red is positive). The DNA is negatively charged and will run towards the positive electrode. Always Run to Red. Run to finish (we adopt 80 to 100 Volts for 20 minutes run).

Figure 1

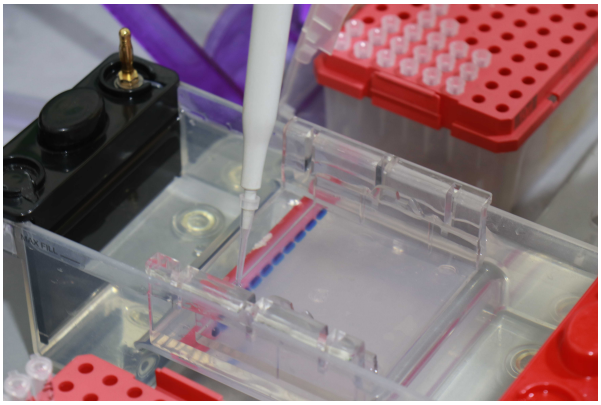

Figure 2

7 2m

Visualizing and Analyzing the gel

1. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box unto a gel visualization system.
2. Using any device that has UV light (UV transilluminator), visualize your DNA fragments. The fragments of DNA are usually referred to as ‘bands’ due to their appearance on the gel.
3. Using the DNA ladder in the first lane as a guide (the manufacturer's instruction will tell you the size of each band), you can infer the size of the DNA in your sample lanes.

Also check to make sure no band is present at the negative control lane.

- Repeat the main steps 4 to 6 to prepare another gel that would be used as control gel, this time using a commercial 1x TBE buffer.
- Remember to use the same buffer in preparing the gel as the running buffer so change the running buffer after the first gel run.
- The gel patterns and migration of the two gels will be compared.

- **After the run, compare the migration pattern of the DNA and visibility of the DNA bands of the BenBio TBE buffer with commercial TBE buffer to show that the BenBio TBE buffer does not negatively affect the migration of DNA nor hinder visualization of the DNA bands on the gel.**