




Jul 22, 2022

Functionality test (Borax Buffer)

In 1 collection

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 Nadine Mowoh

ABSTRACT

This protocol describes the comparative analysis of visual and band patterns after electrophoresis with the BenBio Borax buffer 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 100 Volts and visualize on a Blue light transilluminator or UV transilluminator.

The Borax buffer has an advantage of allowing the gels to be run at very high voltages (100 V and 150 V) without the buffer heating up and affecting the gel and also faster reading of gel results within 10 to 15 minutes.

Contrary to the Borax buffer, TBE buffer gels are run at 80 V to 90 V to avoid heating up the buffer and it takes up to 20 to 30 minutes to read gel results.

PROTOCOL CITATION

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COLLECTIONS ⓘ

**Beneficial Bio: Quality control tests**

KEYWORDS

Functionality of Rapid Borax buffer, Functionality of Borax electrophoresis buffer

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[Beneficial Bio: Quality control tests](#)

GUIDELINES

This protocol describes the steps in preparing and testing the functionality of our formulation of Borax buffer

MATERIALS TEXT

Equipment

Micropipette

Microwave

Gel casting tray

Well comb

UV transilluminator / Blue light transilluminator

Voltage source (Electrophoresis unit)

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)

DNA template PCR amplicon (lambda 0.5 and 1kb or other)

Commercial 1x TBE buffer ([Recipe here](#)), prepared from a 10x TBE stock to be used as "Standard"

Borax buffer to be prepared from a 10x Borax sachet

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 as they are known mutagen and UV radiations are harmful to the skin. To minimize risks Blue light transilluminator can be used in place of 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

5m

1 Preparing PCR amplicons

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 follows:

- 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 below.



Figure 1

Any DNA template of choice can be used but we adopt the 0.5 and 1kb Lambda DNA. 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.

2



5m

Pipetting

Assemble the reaction mix into 20 ul volume in 0.2 mL PCR tubes in the order shown in table 1 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)

Table 1

- 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	

Table 2

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

Functionality test of Borax buffer

40m

4 Preparing 1x Borax buffer

5m

Our lab's standard DNA gel buffer has become 10 g of Borax into 1 L of water which gives a 10x stock solution. Small gels run in 10-15 minutes at 100 Volts and 200 Volts.

To make a 1x Borax buffer - 1 L

- Open up a sachet of 10x Borax buffer (containing 10 g buffer powder) and pour into a 2 L capacity beaker.
- Measure 1 L of water using a measuring cylinder into the beaker and stir until 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.
- 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 Borax buffer is stored at room temperature and is stable for up to 1 year.

5 Preparing 1.5 % Borax agarose gel

3m

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. 0.5 g 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 % Borax agarose gel for electrophoresis 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 Borax 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

30m

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 Borax buffer to cover the gel (the amount of running buffer to use will depend on the size of the electrophoresis unit).
4. Pipette 3 µL of 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 2*).
5. Pipette 1 part of the 6x DNA loading dye and 5 parts of the DNA amplicon, mix and load into the wells (depending on the number of amplicons) (*Figure 3*).
6. 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 100 to 200 Volts for 15 minutes run).



Figure 2

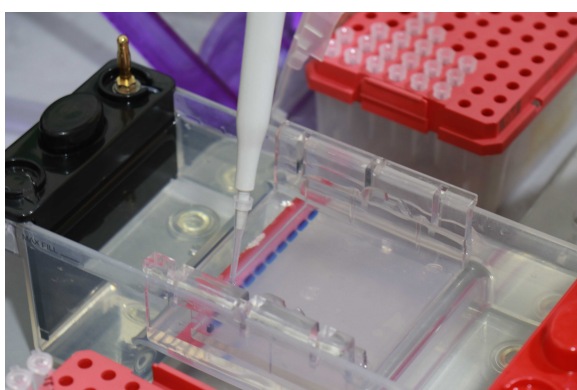


Figure 3

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.

- Repeat the main steps 4 to 6 to prepare another gel that would be used as control gel, this time using 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 Borax buffer with TBE buffer to show that the Borax buffer does not**



negatively affect the migration of DNA nor hinder visualization of the DNA bands on the gel.