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Functionality test (10x PCR buffer)

In 1 collection

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ABSTRACT

In this protocol we describe how to test the functionality of the BenBio 10x PCR buffer by showing that the buffer is able to provide a suitable condition for DNA polymerase to amplify a DNA template in a PCR reaction when compared to a commercial PCR buffer. We confirm the result by running agarose gel electrophoresis of the resulting DNA amplicon.

The BenBio 10x PCR buffer provides optimum PCR conditions for OpenVent in PCR amplification reactions.

PROTOCOL CITATION

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COLLECTIONS

Beneficial Bio: Quality control tests

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Beneficial Bio: Quality control tests

GUIDELINES

This protocol describes the steps in testing the functionality of our 10x PCR buffer formulation.

MATERIALS TEXT

Materials and Equipment

Thermocycler
Micropipette
Microwave
Gel casting tray
Well comb
UV transilluminator
Voltage source (Electrophoresis unit)
Ice
Ice bowl

Chemicals

10x PCR buffer (test buffer)
Commercial 10x PCR buffer (standard buffer)
10mM dNTP Mix
DNA Polymerase
Primers
Agarose (electrophoresis grade)
DNA template (lambda 0.5 and 1kb or other)
Commercial 1x TBE buffer (Recipe here)
DNA loading dye (6x NEB)
DNA ladder (Bioline 1kb)
DNA gel stain (SYBR Safe or other Ethidium bromide, EtBr stain)

SAFETY WARNINGS

EtBr is a mutagen so wear protective clothing when handling it. Also wear gloves and face shields to avoid contaminating the samples and reagents while pipetting.

BEFORE STARTING

- Make sure all PCR reaction components are available.
- Prepare an ice bowl because all pipetting will be done on ice.

Functionality test of 10x PCR buffer

1

Preparing PCR reagents and components

1. Thaw all reagents on ice in a bowl.
2. Label 2 reaction tubes (0.2 mL PCR tubes) "test buffer" and "control buffer" or more tubes depending on the number of replicates you choose to do.
3. Pipette the reaction components into the tubes as indicated in the table below *(do this while working on ice).*

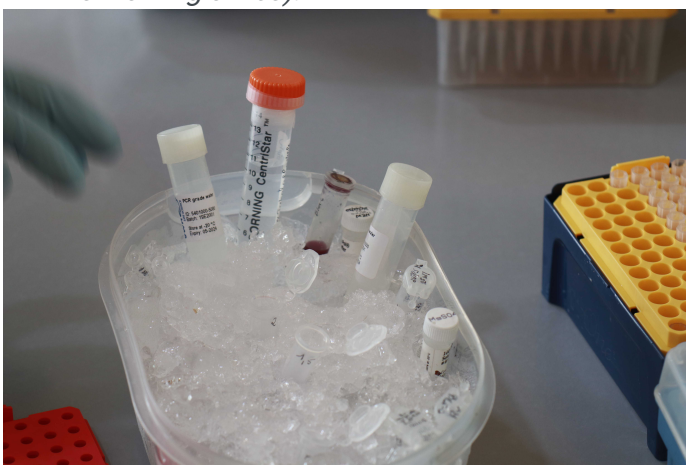

Figure 1

A	B	C
PCR Component	**Test Buffer tube (in uL)**	**Control buffer tube (in uL)**
PCR grade water	Variable up to 20 uL	Variable up to 20 uL
dNTP mix (10 mM)	0.4	0.4
Thermopol buffer (10x)	/	2
Test PCR buffer (10x)	2	/
Fw/Rv primers	1/1	1/1
DNA template	0.5	0.5
DNA polymerase	1	1

Table 1

2

Mixing

Hold the tubes up and gently flick the tubes to mix the components and place the tubes in a thermocycler.

3 

Thermocycling

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).*

A	B	C	D
Phase	**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 confirm the functionality of our PCR buffer by running a 1.5% agarose gel.

4 Performing Agarose gel electrophoresis

Prepare 10x and 1x TBE buffer

We use this internal protocol to prepare a 10x and subsequently a 1x TBE buffer which will be used in preparing the agarose gel and running buffer.

5 Preparing 1.5 % agarose gel for electrophoresis

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

1. Use a weighing balance to weigh out 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).
8. 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 as shown in Figure 2 below.

Figure 2

6 Casting and loading the Electrophoresis gel

1. After the gel is solidified, remove the comb and place the casting tray into the gel box or electrophoresis unit.
2. 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).
3. 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 3). (The DNA ladder is loaded without mixing with a loading dye because it contains a tracking dye already)*

4. Pipette 1 part of the 6x DNA loading dye and 5 parts of the DNA amplicon, mix and load into the next 4 wells (could load more or less wells depending on the number of amplicons) *(Figure 4).*
5. 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).
6. Run to finish (we adopt 80 to 100 Volts for 150 minutes run).

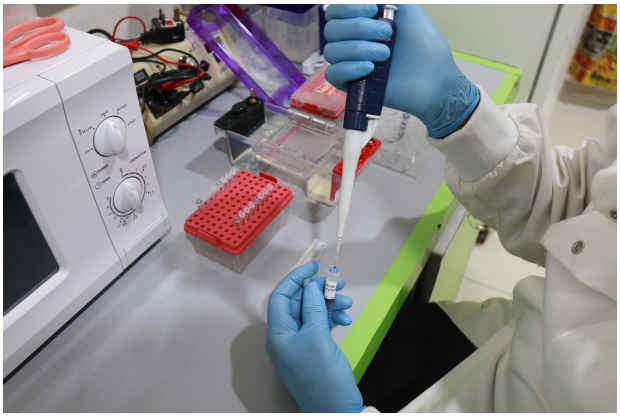

Figure 3

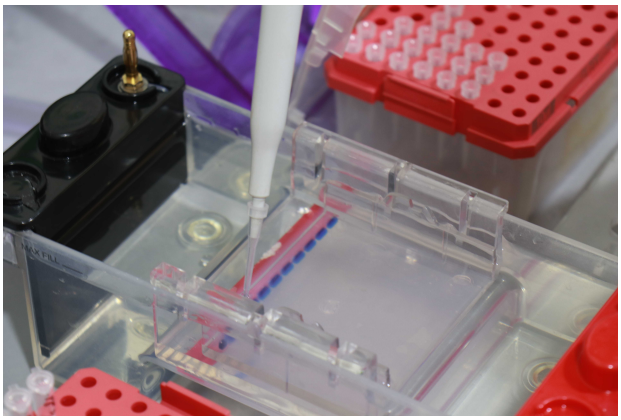

Figure 4

7

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.

After the run, compare the migration pattern and visibility of the DNA bands of the BenBio 10x PCR buffer with that of the commercial 10x PCR buffer to show that the BenBio 10x PCR buffer is able to provide a suitable PCR condition for the amplification of the specific DNA templates.