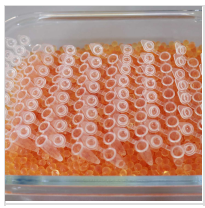

Version 3

Jul 28, 2022

Functionality test (OpenVent polymerase, PCR Master Mixes) V.3

In 1 collection

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Low-cost, high-quality ...

Nadine Mowoh

ABSTRACT

After production, we typically subject our products to a batch of quality control assays to ascertain their functionality, efficacy and ability to meet their intended purpose.

Strict quality control is necessary when producing or using enzymes (whether purified or unpurified) and other molecular biology reagents.

Quality control for functionality involves incubating the enzymes or PCR master mix in a PCR reaction mixtures and testing for their ability to amplify a specific region of a DNA template and the results recorded in terms of the presence or absence of amplification by bands on an electrophoresis agarose gel.

The Quality control tests are done at specific regular intervals and results documented for follow up. Incase any product fails the quality control test, actions are taken to verify the cause and make sure it does not repeat.

More than one test may be carried out for a particular product and the quality manager must approve and sign the certificate of analysis (a document that reveals the different quality control tests that have been performed on a reagent) to show that the product passes all the quality control tests assigned for it before a product is released for use.
Quality control of a product is done in two stages - the first involves a batch of tests carried out within the facility where the reagents are produced (Internal quality control) and the second involves testing for functionality by an external facility (External quality control).

In this protocol we describe how to carry out Functionality test of the following:

- OpenVent Enzyme
- PCR Master Mix - All formulations (wet and dry).

PROTOCOL CITATION

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COLLECTIONS

Beneficial Bio: Quality control tests

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PARENT PROTOCOLS

Part of collection
Beneficial Bio: Quality control tests

GUIDELINES

Before carrying out quality control on products research should be carried out on the possible assays that apply for that particular product and availability of resources.

MATERIALS TEXT

The materials required for a quality control assays would depend on the products to be tested (whether Enzyme or PCR Master mix).

Reagents

- DNA polymerase of interest (either cellular reagent or purified enzyme or PCR master mixes)
- Control DNA polymerase (known to work)
- Forward Primer (20 pMol)
- Reverse Primer(20 pMol)
- PCR grade water
- Enzyme reaction buffer
- dNTP Mix (if necessary)
- Agarose (electrophoresis grade)
- TBE running buffer

Materials/Equipment

- Ice
- PCR machine (mini PCR-blueGel)
- Pipettes (P-10, P-20 and P-200)
- Sterile Pipette tips (10µl and 20µl)
- Bowl
- Sterile 0.2ml PCR tubes
- Microwave
- Gel casting tray
- Well comb
- UV transilluminator/blue light transilluminator
- Voltage source (Electrophoresis unit)
- Electrophoretic gel tank and components (blueGel)
- Waste container
- weighing balance

SAFETY WARNINGS

- Wear protective clothing and all recommended Laboratory PPE to avoid contaminating the reagents as this may affect their functionality.
- PPE may also protect you from accidental spills or splashes that may be dangerous to the eye or skin.
- Take special care when handling the EtBr gel stain and the UV transilluminator

BEFORE STARTING

Clean and disinfect all work surfaces with a 1:10 dilution of bleach followed by 70% alcohol.

Functionality test - PCR amplification 37m

1 DNA polymerase Enzyme and PCR Master mix

In this section we describe the procedure for testing enzymes (DNA polymerases - OpenVent) and PCR Master Mix for functionality.

- We adopt **20 µL** PCR reaction in our lab, **25 µL** and/or **50 µL** reactions can be made but do not forget to calculate the corresponding amount of PCR components to add.

Preparing reagents

1. Thaw all reagents on ice in a bowl
2. Label reaction tubes (0.2 mL PCR tubes) according to the number of samples, and include controls in each run (negative and positive controls) as needed.

Polymerase enzyme type:

1. If using a 10x cellular reagent enzyme, rehydrate the enzyme with 30 µL of sterile PCR grade water, flick tube and keep on ice.
2. If using a pre-purified polymerase or Master mix, remove from the freezer and keep on ice as shown in figure 1 below.

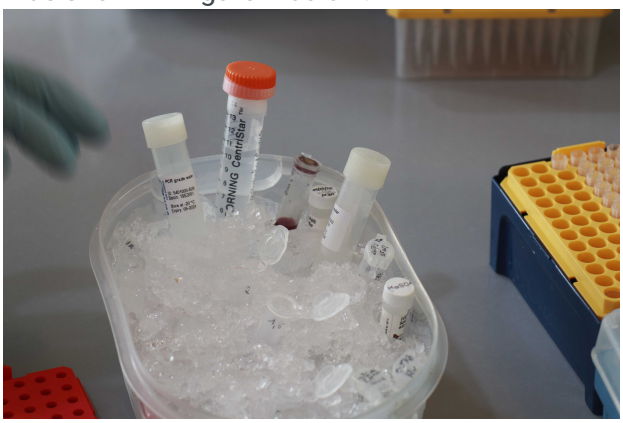

Figure 1

2

Pipetting

- In each PCR tube, pipette and combine the following PCR reaction components in the order shown in table 1 below *(work on ice while pipetting)*.

A	B	C	D	E
PCR component	**Test sample- 1x PCR Master Mix (uL)**	**Test sample- DNA polymerase enzyme (uL)**	**Negative control (for OpenVent Enzyme) (uL)**	**Positive control (for OpenVent Enzyme) (uL)**
PCR water	Variable to 20	Variable to 20	Variable to 20	Variable to 20
dNTP(10mM)	/	0.4	0.4	0.4
PCR buffer(10x)	/	2	2	2
Forward primer	1	1	1	1
Reverse primer	1	1	1	1
DNA template	1	0.5-1	/	0.5-1
Test enzyme	17	1	1	/
Control Enzyme (Commercial DNA polymerase)	/	/	/	1

Table 1

- Hold the tubes up and gently flick the tubes to mix the components and place in a thermocycler.
- Input the cycling parameters as indicated in the table below and run.

1. The negative control is done by pipetting all PCR components without the DNA template to check that there is no contamination in the reagents and also to detect false positives.
2. The positive control is done by pipetting all PCR components with a commercial DNA polymerase enzyme as a standard to confirm DNA amplification and detect false negatives.
3. The control parameters in this table are for OpenVent enzyme. For the PCR master mix, we use the same principle with the appropriate amount of reagents corresponding to the Master mix.

3

Thermocycling:

A	B	C	D
Phase	**Temperature (°C)**	**Duration (sec)**	**Number of cycles**
Initial Denaturation	95	120	
Denature	95	30	
Anneal	50-65 (depending on primers)	20	30 to 35
Extend	72	Variable based on primers	
Final Extension	72	120	

Table 2

The running time will depend on the size of the DNA template (usually we use do 35 to 40 cycles for 0.5kb Lambda-50ng/ul).

- At the end of the thermocycling process, run agarose gel electrophoresis to check for amplification of the DNA template and establish functionality of the enzyme and PCR master mix.

4 Preparing Agarose gel for Electrophoresis

18m

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).

Casting the gel

- 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.
- After the gel is solidified, remove the comb and place the casting tray into the gel box or electrophoresis unit.
- 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).

5 Loading and running the gel wells

17m

The PCR Master mixes and DNA ladder are loaded directly without mixing with loading dye since they already contain the tracking dye and components to help them sink in the well

- Pipette 3 µL of DNA ladder (the size of the ladder would depend on size of the DNA template) into the first well.
- Pipette 1 part of the DNA loading dye and 5 parts the negative control carefully to avoid bubbles and load into the second well.
- Pipetting 1 part of the DNA loading dye and 5 parts of DNA amplicons or test samples, mix and load in the subsequent wells. *(Do same for the positive control)*.
- 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 Volts for 15 minutes run).

6

2m

Visualization of gel and Interpretation of gel results

1. The amplification results are visualized by transferring the gel into a UV transilluminator or blue light transilluminators.
2. With the help of the DNA ladder, determine if the amplification product is of the expected size and the bands are sharp and distinct by comparing with the DNA polymerase enzyme and PCR Master Mix standards.

- If bands are seen at the positive control and test amplicon lanes, it signifies a successful amplification and might mean the test reagents are working as expected.
- If bands are seen on the positive control but no bands are present on the test amplicon lanes, it may signify that the test reagents are not functioning as expected.
- If bands are seen at the negative control, it may suggest contamination of the reaction

components.

- If no bands are seen at the positive control, but present on the test amplicon lane, it may suggests failed amplification which could be caused by many reasons one of them being pipetting error.

The results obtained will determine if the reagent needs further testing or follow up or would be declared "good to use".