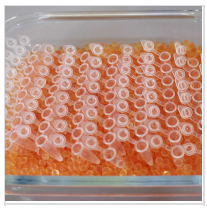

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Beneficial Bio Products: Quality control tests

Nadine Mowoh[1], Stephane Fadanka[1]

[1]Beneficial Bio, Mboalab

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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 involves incubating the enzymes or reagents in reconstituted recombination assays to eliminate the possibility of relevant protein or nucleic acid contaminants that may interfere with recombination assays and lead to misinterpretation of experimental data. Here batch by batch quality controls are done for each new product before it is released to the market.

The Quality control tests are done at specific regular intervals and results documented for follow up and action in case any product fails the quality control test. 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.
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).

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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 and the particular tests that apply for these products.

The Quality control (QC)tests that have been adopted for all of our products are the functionality and Nuclease activity, other specific additional tests may be done for the products as required.

Functionality test:

Reagents

- DNA polymerase of interest (either cellular reagent or purified enzyme or 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
- Electrophoretic gel tank and components(blueGel)

- Waste container

Nuclease activity:

Chemicals

- Agarose
- Enzyme of interest
- Enzyme specific buffer
- Nuclease free water
- Purified plasmid DNA
- DNAse I
- DNA loading dye/buffer
- TAE/TBE running buffer

Materials and Equipment

- 0.2ml PCR tubes
- 37°C water bath
- Horizontal gel tank including trays and power pack (as detailed in SOP016 Agarose gel electrophoresis)
- UV/blue light transilluminator
- Image recording device
- Agarose gel
- Agarose gel running buffer(TBE)
- DNase1-0.1g/ml (an endonuclease to act as positive control that digests double- and single-stranded DNA into oligo- and mono-nucleotides). 1mg/ml DNase1 can be used for optimal results. So up to 2µl can be used.

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.

BEFORE STARTING

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

Functionality tests

1

Pipetting:

After establishing a successful protein expression through SDS PAGE using the method described in this protocol, functionality test is performed and the procedure will depend on the product being tested. In this section we describe the procedure for testing Enzymes (DNA polymerases, OpenVent) and Master mixes enzyme.

- We adopt **20 µL** PCR reaction in our lab, **25 µL** and/or **50 µL** reactions are

can be done after calculating the corresponding amount of PCR reagents.

DNA polymerase and Master mix enzyme

1. Thaw all reagents on ice in a bowl
2. Label reaction tubes (PCR tubes) according to the number of samples, and including 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 30µlof 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.

In each PCR tube, pipette and combine the following reagent components following the order in the table below (work on ice while pipetting).

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

1. The negative control is done by pipetting all PCR components without DNA template to ensure there were no pipetting errors because no DNA amplification band will be seen after agarose gel electrophoresis.
2. The positive control is done by pipetting all PCR components with a commercial DNA polymerase enzyme as a standard to ensure thermocycling process was successful because the expected size of the amplified DNA would be seen on the gel.

2 Thermocycling:

1. Connect a Thermocycler and program the protocol to run following the thermocycling steps listed below. The exact annealing temperature will depend on the choice of DNA template and primers used.

Initial Denaturation	95	120 sec
Denature	95°C	30 secs
Anneal	50-65°C(depending on primers)	20 sec
Extend	72°C	Variable based on primers
Final Extension	72°C	120secs

- The time of run will depend on the size of the DNA template and number of cycles of amplification (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 template DNA.

3 Preparing Agarose gel for Electrophoresis

Follow this protocol to prepare and cast the gel.

4 Loading the gel wells and Visualisation of gel

Pipette 3 to 5µl of DNA ladder (size depending on size of the DNA template) unto the first well then pipette the negative control carefully to avoid bubbles and load into the second well.

Follow the step in loading by pipetting 1part of the DNA loading dye and 5 parts of amplicon or test sample, mix your amplicons with DNA loading dye and load in the subsequent wells (in the case of the master mixes, they are loaded directly as they already contain the tracking dye and components to help them sink in the well).

Allow the gel to run for 15-30mins at 48V on 0.8, .5% or 2% agarose gel in TBE buffer.

- The functionality test can be adopted to check the BenBio OpenVent PCR buffer in step 1 by including a positive control (10x Commercial PCR buffer).
- In step 3, the functionality of the BenBio TBE buffer and DNA gel stain can be checked by preparing the gel with a positive control (commercial Agarose gel electrophoresis buffer and DNA gel stain).
- In step 4, the functionality of BenBio DNA loading dye can be checked by loading the

PCR amplicons with a positive control (commercial DNA loading dye).

Visualization

The amplification results are visualized by transferring the gel into a UV transilluminator or blue gel transilluminator.

With the help of the DNA ladder, determine if the amplification product is of the expected size and the bands are sharp and distinct looking exactly like those from the DNA polymerase enzyme, PCR buffer, Agarose gel electrophoresis buffer, DNA gel stain and DNA loading dye standards.

Nuclease activity

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The Nuclease test performed will depend on the product whether it is the enzyme, buffer or stains.

For the enzymes, endonuclease test is carried out which involves:
Incubation of Reaction Buffer containing a minimum of 20 units of Enzyme (test enzyme/master mix) with 1 µg of pOBL1 or PUC19 plasmid DNA-50pg/ul for 4 hours at 37°C results determined by agarose gel electrophoresis 1.5% (w/v) agarose gel with TBE buffer system.
In our lab we use pOBL1 plasmid but any circular double stranded DNA can be used.

The incubation times may vary depending on the product being tested. We typically use 4 hours for Enzymes and 16 to 20 hours for other products(stains, buffers etc)

6 **Pipetting**
Pipette reaction mixtures in the order described below and incubate as indicated:

A	B	C	D	E
Component	**Test Sample (Enzyme and Master mix)**	**Negative control**	**Positive control**	**Test Sample (Stains and buffers)**
Enzyme specific buffer (10x)	2µl	2µl	2µl	2µl
Test Sample	5µg	N/a	N/a	5µg
Plasmid DNA	1µg	1µg	1µg	1µg
DNase1	N/a	N/a	2 units (0.5 to 2µl)	N/a
PCR water	Variable to 20µl	Variable to 20µl	Variable to 20µl	Variable to 20µl
Incubation time at 37c	4 hours	Same time as the test sample	Same time as the test sample	14-16 hours

Checking and interpreting results:
After incubation period the reaction is stopped, and an 1.5% agarose gel prepared, run and visualized as described in steps 3 and 4 above.

Any change in band intensity and migration position of the plasmid DNA bands compared to the negative control signals potential endonuclease activity. Linear DNA should run true to size(known size of plasmid used), supercoiled DNA should run faster and relaxed circle DNA should run slower(a single strand cut).

Visualization of low molecular weight DNA and low DNA concentration

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The confirmation of visualization tests are applicable for DNA gel stains, Electrophoresis buffers and DNA loading dye. The visualization tests could be modified slightly to suit a particular product.

The protocol described below describes the QC steps in confirming a products ability to allow visualization of low molecular weight DNA and low DNA concentration.

Follow the steps in preparing a 2% agarose gel as described in step 3 above and load by pipetting 1part of the DNA loading dye and 5 parts of amplicon or test sample . DNA templates or amplicon in this case is a 50 or 100bp DNA ladder therefore it can be loaded without loading dye needing 3-5ul of the ladder. Load directly into the gel well in replicates.
Run gel to finish and visualize the resulting band separation to confirm separation of the ladder into visible distinct bands (from the lowest to highest base pair bands) using a UV transilluminator or blue gel system.

To confirm a low DNA concentration detection/visualization property of the product:

Make 1:5 serial dilutions of the 100bp DNA ladder or any DNA amplicon and use the different dilutions as amplicons to load a gel well.
Allow the gel to run through and visualize to determine the lowest concentration detectable by the gel stain.

Physical property- Stability and pH

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The stability test is adopted to test Electrophoresis buffers by confirming the net weight of the dry powder to show it does not absorb moisture from the air after storage and the pH of the buffer solution upon dissolution of the buffer powder.

To confirm the weight of the powder buffer sachet:

1. Weigh the individual powder components that constitute the buffer (*the individual powders could be pre-dried if necessary in an incubator with silica gel beads at 37c*)
2. Put them together in a beaker and mix
3. Pour the powder mix in a zip lock sachet and store in an air tight container for 1 to 3 days.
4. After, open the sachets and pour the powder into a weighing boat and measure the weight of the powder
5. The weight should be approximately close to or equal to the weight of the powder 3 days before.

9 To confirm the pH of the buffer solution:

1. Pour the powder buffer into an appropriate size beaker and add the appropriate amount of distilled water as indicated on the powder sachet (e.g 1 sachet in 1L water).
2. Mix until a clear solution is obtained
3. Use a pH meter to check the pH of the solution which should be between 8.3 and 8.5.

Once a product has passed these tests they are suitable for use internally (in our lab)and externally (by other researchers).