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Preparing 1x PCR Master Mix V.2

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ABSTRACT

This protocol documents the production of BenBio 1X PCR Master Mix "Wet" and "Dry" formulations including the different colors of the Wet formulations (Rubis or oink and Saphir or blue). This method uses OpenVent cellular reagents. The PCR master mixes are then stored for subsequent PCR reactions.

Cellular reagents are defined as common molecular biology enzymes expressed in E.coli but not subsequently purified before use i.e. dried E.coli cells are used as the reagent (Bhadra et al (2018)).

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GUIDELINES

This protocol describes the steps in preparing and testing the functionality of 1X PCR master mix.

MATERIALS TEXT

Equipment
Thermocycler
Micropipette
0.2 uL PCR tubes
1.5 mL Eppendorf tubes
Microwave
Gel casting tray
Well comb
UV transilluminator
Voltage source (Electrophoresis unit)

Reagents
OpenVent DNA polymerase
PCR Primers
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
DNA loading dye (6x NEB)
DNA ladder (Bioline 1kb)
DNA gel stain (SYBR Safe or other Ethidium bromide, EtBr stain)
Trehalose
Azorubine
Bromophenol blue

SAFETY WARNINGS

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

BEFORE STARTING

- Ensure that the cellular reagent (OpenVent enzyme) to be used is available and has

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been pre-tested for functionality.
- Ensure that all the other components needed to prepare the PCR mix are available and free from contaminants..

Cellular reagents preparation

1

1d

Before making Master mixes, we typically start by producing the cellular reagents which will be used. The cellular reagents then under go a quality control tests to ensure the enzymes are functional and free from contaminating nucleases.

1. Prepare a fresh batch of cellular reagents (OpenVent) following BenBio protocol for plate protein expression on autoinduction media.

Functionality test

2

2h 30m

Remove the enzymes from storage, reconstitute and test for functionality as described in the BenBio protocol using the specific test that apply for cellular reagents (OpenVent enzyme).

Preparation of 1x PCR Master mix formulations

3 **Preparing the work surface and materials:**

5m

1. Clean the working surface first with 1:10 dilution of Bleach, then [M]**70 % (v/v)** Ethanol
2. Clean the micropipettes with [M]**70 % (v/v)** alcohol then with Lookout DNA erase solution; keep on a clean surface throughout manipulation.
3. Crush enough ice to fill the PCR box or a beaker bowl till it's 3/4 filled.
4. Remove all reagents needed from **-20 °C** and **4 °C** and thaw **On ice**
5. Prepare a sterile labelled **1.5 mL** microcentrifuge tubes or larger volume tubes depending on amount of PCR mix needed, and leave open **On ice** .

4 **Preparing reagent stocks**

[M]**20 Mass / % volume** **Trehalose (10 mL)**

- Weigh **2 g** of trehalose powder into a clean 50 mL capacity beaker.
- Measure 10 mL of distilled water and pour into the beaker to dissolve the powder.
- Filter the trehalose stock solution obtained through a 0.22 uM syringe filter in a sterile 15 mL eppendorf tube.
- Store at 4 °C for up to 12 months.

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[M]0.25 Mass / % volume Azorubine and [M]0.25 Mass / % volume Bromophenol blue (10mL)

- Use a weighing balance to accurately **0.025 g** of Bromophenol blue or Azorubine dye into a 15 mL centrifuge tube.
- From a 20 % trehalose stock, aliquot some volume into the Eppendorf tube to dissolve the dye powder, to make a 10 mL (dye) solution.
- Cork tightly the eppendorf tube and mix the content gently to homogenize (vortex can be used).
- Label the tube and either store it at room temperature (away from light) or 4 °C for up to 12 months.

5  10m

Pipetting components to make up the "Wet" mix

- Pipette the corresponding amount of PCR grade water to reconstitute the enzyme as was determined during preparation (*in this case 30 uL PCR water is used to reconstitute the enzyme)* and place on ice.
- Pipette all reagents in the tube following the order stated in the table below:

- We adopt 2 formulations of 1x PCR Master mix using 2 different dyes as trackers (Azorubine and Bromophenol blue), and have 2 states of the mix formulations as "Wet" and "Dry".
- Also a 2x concentration of the PCR master mix can be done to reduce the volume of water used for the preparation to ease drying and enhance shelf life of the PCR master mix formulation.

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A	B
Components	**Amount in µL for 1x concentration of PCR Master mix**
PCR grade H2O	8.8
dNTPs mix (25 mM)	0.6
10x Thermopol Buffer	2
MgSO4 (100mM)	0.6
Cellular reagents (Freshly prepared and heat treated)	3
20% (w/v) Trehalose stock solution	4.5
Bromophenol Blue 0.25% (w/v)Azorubine 0.25% (w/v)	0.5
	20

- Determine the total volume needed and make necessary calculations to adjust the amount of each component to be pipetted.
- Mix well by gentle agitations (avoid using vortex as the mixture will foam)

- At this point the Wet master mix formulation is ready for use after performing functionality tests and can be aliquoted into desired size screw cap tubes in the desired volume for long term storage (avoid frequent freezing and thawing).
- If the dry formulation is desired, continue with the steps described below.

Aliquoting to make a "Dry" mix

1. Carefully pipette **25 µL** of Mix formulation into each 0.2ml PCR tubes
2. Place PCR tubes (left Opened) in an Airtight container filled halfway with silica beads.
3. Place the container in an incubator and dry at **37 °C** **Overnight**
4. Store dried down tubes at **4 °C** in air-tied sachet or containers filled halfway with silica beads.

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Quality control - Functionality testing 2h 30m

6 2h

Check the functionality of the produced PCR Master Mix by running control PCR reactions.

We generally use the BenBio internal protocol which can be modified depending on the PCR master mix formulation and concentration being tested.

For functionality of **1x Wet mix formulation** we make a **20 µL** total volume PCR reaction as follows:

- Pipette **17 µL** of the mix into a **0.2 mL** PCR tube and add **1 µL** of each PCR primer and **1 µL** of DNA template and run the thermocycling process following the specific protocol for the DNA template and primers used.

For 1x Dry formulation:

- Rehydrate the dried mix tube with **17 µL** PCR water add **1 µL** of each PCR primer and **1 µL** of DNA template and run the thermocycling process following the specific protocol for the DNA template and primers used.

7 30m

Running Agarose gel and Visualization

After running the PCR reaction, the samples are visualized to check for amplification as follows:

- Prepare 1.5% agarose gel and run the gel electrophoresis to completion.
- Visualize to check for DNA bands which signify amplification to show that the enzyme or PCR master mix are functional (able to amplify a specific region of a test DNA template).

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