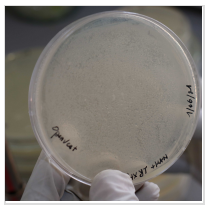

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Mar 09, 2022

Plate Protein Expression on Autoinduction media V.2

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Open Bioeconomy Lab

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The current protocol describes the preparation and use of 2X YT autoinduction medium for recombinant protein expression on Petri dishes. This protocol allows for reproducible and time effective expression experiments to be undertaken with minimal user intervention as compared to standard procedures using IPTG.

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More details on media preparation, List of required material and reagents, Video illustration on how to prepare the media (BioReach Cambridge).

Plate, Expression, Protein, Auto-intduction

protocol ,

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Reagents:

- Na2HPO4
- KH2PO4
- Tryptone
- Yeast Extract
- NaCl
- Distilled H2O
- Agar (Bacteriological grade)
- Glycerol
- Glucose
- Lactose
- LB broth supplemented with appropriate antibiotic
- Desired bacteria strain

Equipment:

- 3x250 ml Duran Bottles
- 1L Duran Bottle
- Petri Dishes
- 1.5 ml Eppendorf tubes
- Glas slides
- Autoclave or pressure cooker
- Beakers
- Measuring cylinders
- Electronic scales
- Magnetic stirrer
- Weighing boats
- Laboratory spatula
- Cell spreaders (Glass or plastic)
- 37 C Incubator
- Bunsen Burner

Preparation of the Overnight Pre-inoculum

1 Grow culture **10 mL** of desired bacteria strain in LB broth supplemented with the appropriate antibiotic, grow overnight at **37 °C** in an incubator.

Preparation of Auto-induction media 30m

2 Prepare the needed amount of 4xYT autoinduction agar medium

- (*It is recommended to prepare and use Auto induction media the same day, make sure to prepare just the amount needed for the experiment) e.g 20ml of Autoinduction in 9cm Petri plates).*

2.1 Composition of 4x YT autoinduction media for 1L final volume :

6g - Na2HPO4

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3g - KH2PO4
20g - Tryptone
5g - Yeast Extract
5g - NaCl
15g - Agar

3 Weigh and dissolve all powders and salts (including Agar) in **0.5 L** of distilled water and transfer to a **1 L** Duran bottle.

4 Prepare 50% glycerol, 10% glucose and 5% lactose stock solutions to be used for reconstitution of the autoinduction media after sterilisation:

4.1 50% (vol/vol) glycerol
- - Measure 50ml of glycerol in a 250ml bottle
- - Add 50 ml of distilled water and mix by shaking.

4.2 10% (weight/volume) glucose
- - Weight 10g of glucose
- - Dissolve in 100ml of water

4.3 5% lactose
- - Weight 10g of lactose
- - Dissolve in 200ml of water

4.4 Include enough distilled water (**500 mL**) to reconstitute autoinduction medium to 1 L after sterilisation.

5 Sterilize all solutions by autoclaving.
- (*Also sterilise microscope slides, as well as Petri Dishes and Cell spreaders if made of glass).*

6
- After Autoclaving, add 50% glycerol, 10% glucose and 5% lactose to the 4xYT to reconstitute the autoinduction medium

Reconstitution of Autoinduction media 30m

7
- Add into the 1L bottle containing 0.5L of 4xYT: **12 mL** of 50% glycerol **5 mL** of 10% glucose **40 mL** of 5% lactose.

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- *Sterile pipette tips and containers (such as 50ml Falcon tubes) can be used to accurately measure the exact volumes of the various solutions to reconstitute the medium.*

- Bring to 1L with sterile water and carefully shake the flask to mix the solution.

Preparation of auto-induction media (4xYT agar)
1. Gather all reagents needed; **2.** Mix all ingredients in 1L Duran bottle; **3.** Autoclave and Reconstitue 4xYT media

8 After reconstituting the culture medium, add an appropriate amount of desired antibiotic 50ug/ml Kanamycin and pour **20 mL** of reconstituted media in 9cm Petri dishes.

- *(Carefully determine the number of plates needed and prepare the volume of media to prepare accordingly make sure to prepare 3 plates per culture and to include replicates for the control as well).*

9 Allow the plates to solidify for about **00:30:00** 30m

10 Inoculate the plates with **0.2 mL** of prepared overnight culture using the cell spreading method.

- ***(See Video for illustration - protocol 1: Autoinduction scale up - by BioReach Cambridge).***

11 Incubate the plate overnight at **37 °C** .

Collection of Cell Biomass

12
- After incubation, period, check cell growth and collect Biomass from each plate using a sterile microscope slide, a scalpel blade or any other available utensil enabling to scrap the surface of the plate without carrying the gel.

- Cell Biomass can be collected and stored in a suitable container (1.5ml Eppendorf tubes; 15-50ml Falcon tubes.. etc.) and stored at **-20 °C** or **-80 °C** .
- ***(See Video for illustration - protocol 1: Autoinduction scale up - by BioReach***

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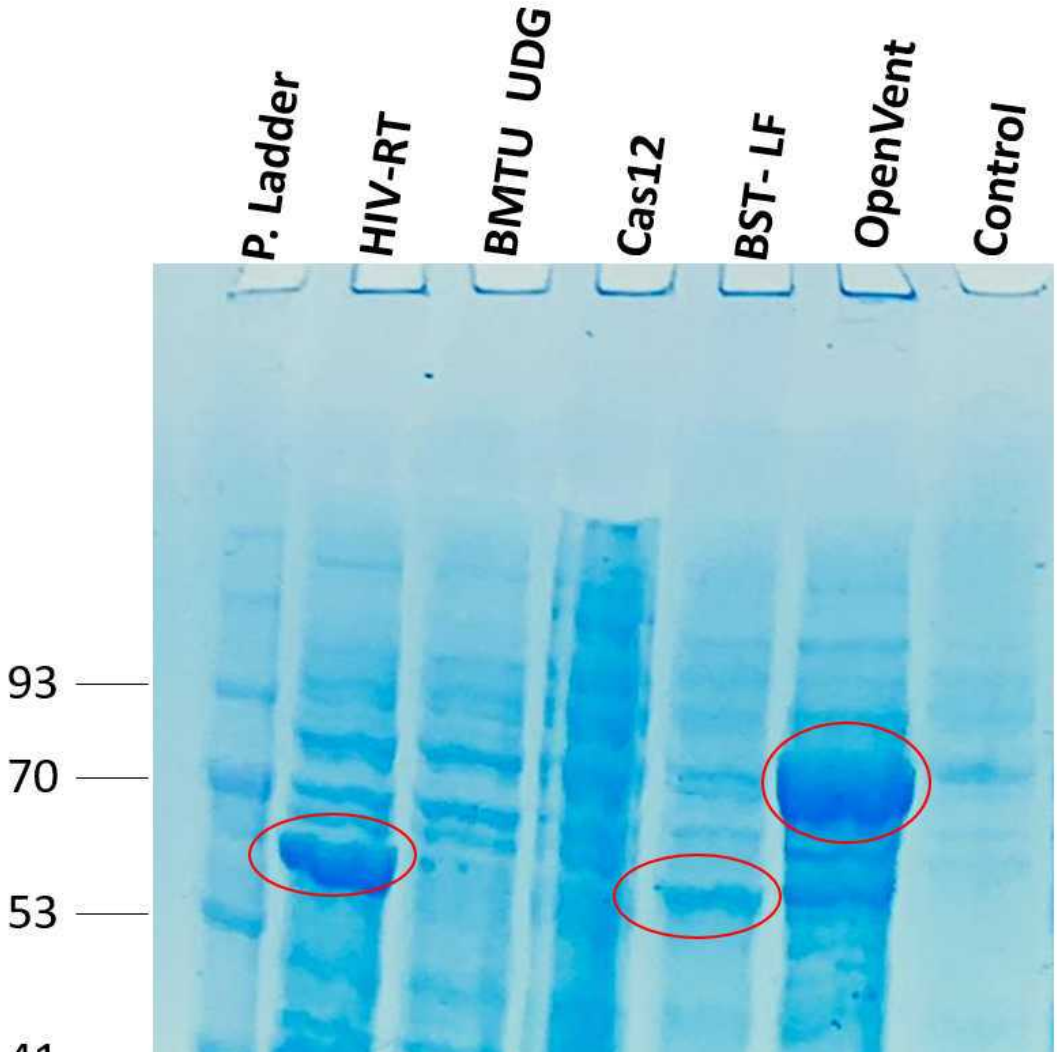
Cambridge).

Checking for successful protein expression

13 Successful expression of the produced enzyme was evaluated the next day by SDS PAGE. use a small fraction of stored cell pellet to run an SDS PAGE and check if the protein were successfully expressed:
following results were obtained using NuPage Pre-cast gel, MOPs sample Buffer and 2X SDS sample preparation buffer.

- Various enzymes were expressed using prepared auto-induction media and successful enzyme expression was confirmed by the presence of bands at the expected size on the gel.



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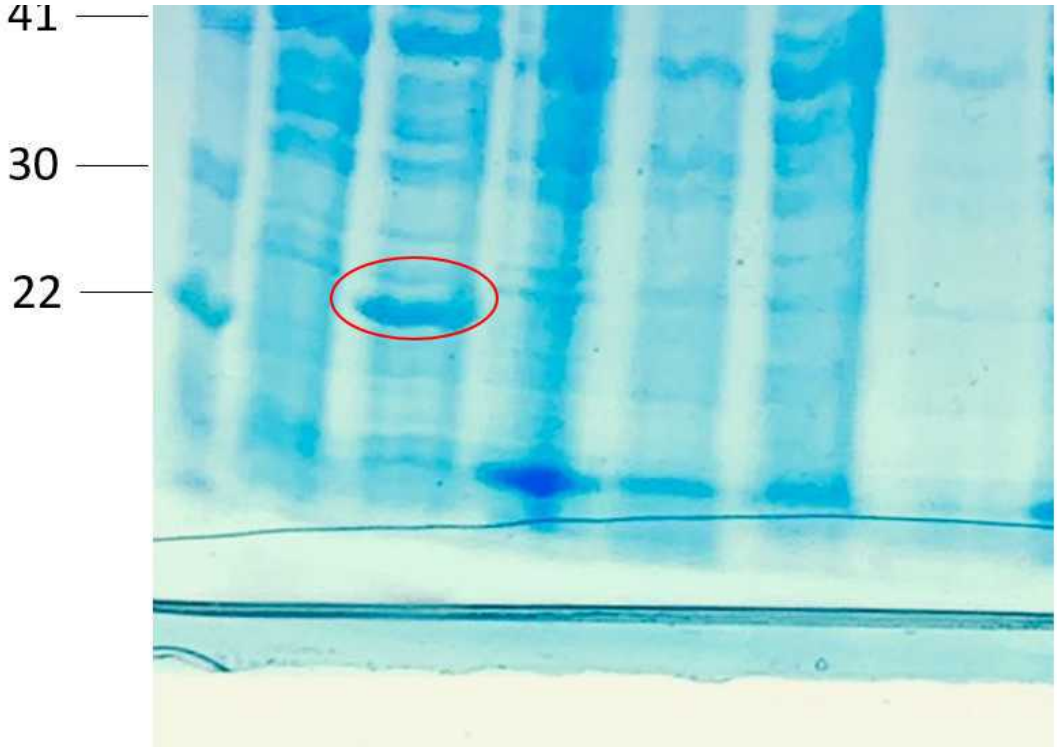

SDS PAGE gel after enzymes expression using plate auto-induction medium.

13.1

Preparation of Cellular reagents from Plate Auto-induction media

1. Transfert a small lump of cell biomass into a fresh tube and freeze the rest at -20C.

2. Resuspend the cells into 1.2ml of Cold PBS and follow the original protocol for cellular reagents preparation from **step6.2.9** (Protocol here).

3. Dilute resuspended cell pellet into cold PBS to obtain a suspension of A600 between 6.5 and 8

Measure A600 of a neat, 1:10 or 1:100 dilution. Multiply the value to get the actual final A600 number.

You might *have to dilute several* times *before you get the right OD.*

4. Calculate the volume of your final cell suspension to aliquot in each PCR tube

(that would contain 2 x 10^8 cells), using the equation:

Volume to Aliquot **= 200/final A600 of cell suspension.**

e.g. if your final A600 is 6.5, then

- volume containing 2x10^8 cells = 200/6.5
- volume containing 2x10^8 cells = 31µl

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5. Aliquot either single reaction or 10X reactions worth of cellular reagents into 8-tube strips
of 0.2 ml PCR tubes e.g. using the example above, 3.1 µl (1x reaction) or 31 µl (10x);

6. Label tubes with reagent, date and operator

7. Incubate the tubes at 60C for 10min in a thermocycler or Heat block (heat treatment)
to make sure that produced cellular reagents are free from any living bacteria.

8. Place the tube strips with aliquoted cellular reagents carefully in a container 1/2 filled with desiccant,
leave tubes opened (using vacuum Tupperware is ideal).

9. Place the container overnight in a 37 °C static incubator.

10. After 18-24hrs check to see if the cellular reagents are completely dry.
Note: Leaving the reagents longer at 37 °C should not hurt their efficacy.

11. Once dry, close the lids and place them in a small bag at +4°C with a small amount of desiccant.

Functionality testing

13.2 The functionality of produced enzymes was assessed for OpenVent DNA Polymerase by carrying out a PCR reaction using cellular reagents preparations produced with an in-house enzyme:

A PCR reaction was carried out using prepared cellular reagents for OpenVent DNA Polymerases using Lambda genome template 0.5kb.

PCR conditions:
T annealing **62**C, extension 72C for **45s**, 37 cycles, **25ul** total volume using 1ul of OpenVent, template:lambda genome 1ul of a 1:10 dilution (50ng/ul concentration of template), primers to amplify 0.5 kb.

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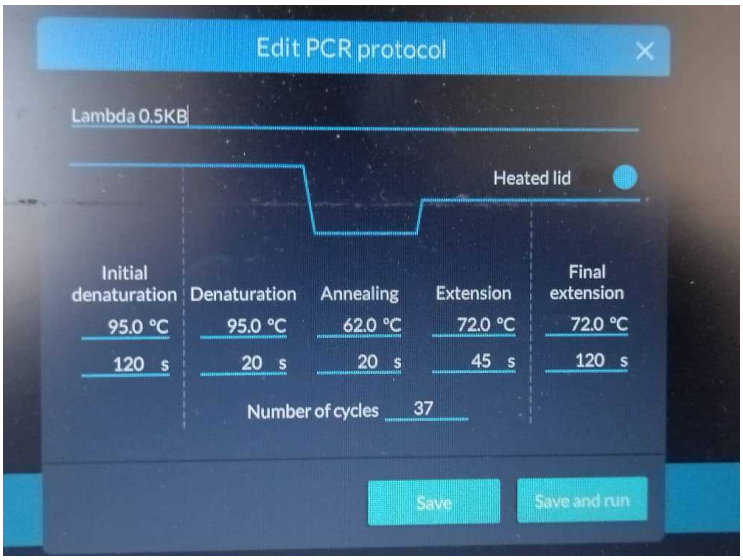

PCR parameter (MiniPCR mini 16)

PCR Results were visualised via Agarose gel electrophoresis on 1.5% agarose gel using TBE buffer system, with 9ul of each amplicon loaded;

Bands of expected sizes were spotted after running the gel.

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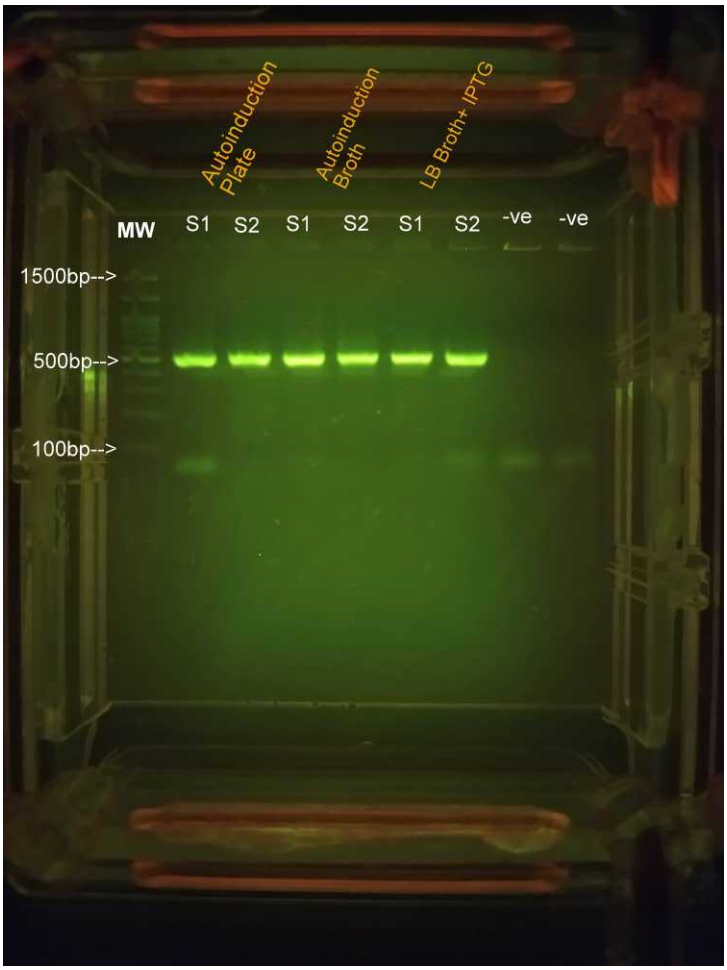

Functionality testing of in-house OpenVent DNA polymerase prepared using auto induction media on Plate; 1.5% Agarose gel, 9ul of each PCR template on TBE Buffer system: MW= Molecular weight marker; S= single PCR reaction (2 replicates; -ve= Negative control.

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https://dx.doi.org/10.17504/protocols.io.b5qrq5v6