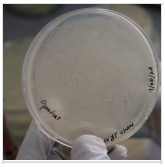

Aug 13, 2021

Plate Protein Expression on Autoinduction media

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dx.doi.org/10.17504/protocols.io.bv4pn8vn

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ABSTRACT

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.

DOI

dx.doi.org/10.17504/protocols.io.bv4pn8vn

PROTOCOL CITATION

Stephane Fadanka, Chiara Gandini 2021. Plate Protein Expression on Autoinduction media. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bv4pn8vn

KEYWORDS

Plate, Expression, Protein, Auto-intduction

CREATED

Jun 25, 2021

LAST MODIFIED

Aug 13, 2021

PROTOCOL INTEGER ID

51055

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 37C **37 °C** .

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:
4x YT (0.5L in 1L bottle) liquid
6g Na2HPO4
3gKH2PO4
20g Tryptone
5g Yeast Extract
5gNaCl

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

4 Prepare 50% glycerol, 10% glucose and 5% lactose solutions 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 Enough distilled water to dilute and reconstitute media after sterilisation (**1 L**)

5 Sterilize all solutions by autoclaving.

6 After reconstituting the media, add an appropriate antibiotic 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).*

30m

7 Allow the plates to solidify for about **00:30:00**

8 After Autoclaving, reconstitute autoinduction medium

Reconstitution:

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8.1
- 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.
- Bring to 1L with sterile water and carefully shake the flask to mix the solution

Preparation of auto-induction media (2X YT agar)

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

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

Collection of Cell Biomass

11
- After incubation, period, check cell growth and collect Biomass from each plate using 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** .

Functionality Testing of Expressed Protein

12 Successful expression and functionality of the produced enzyme were evaluated the next day by SDS PAGE and PCR. 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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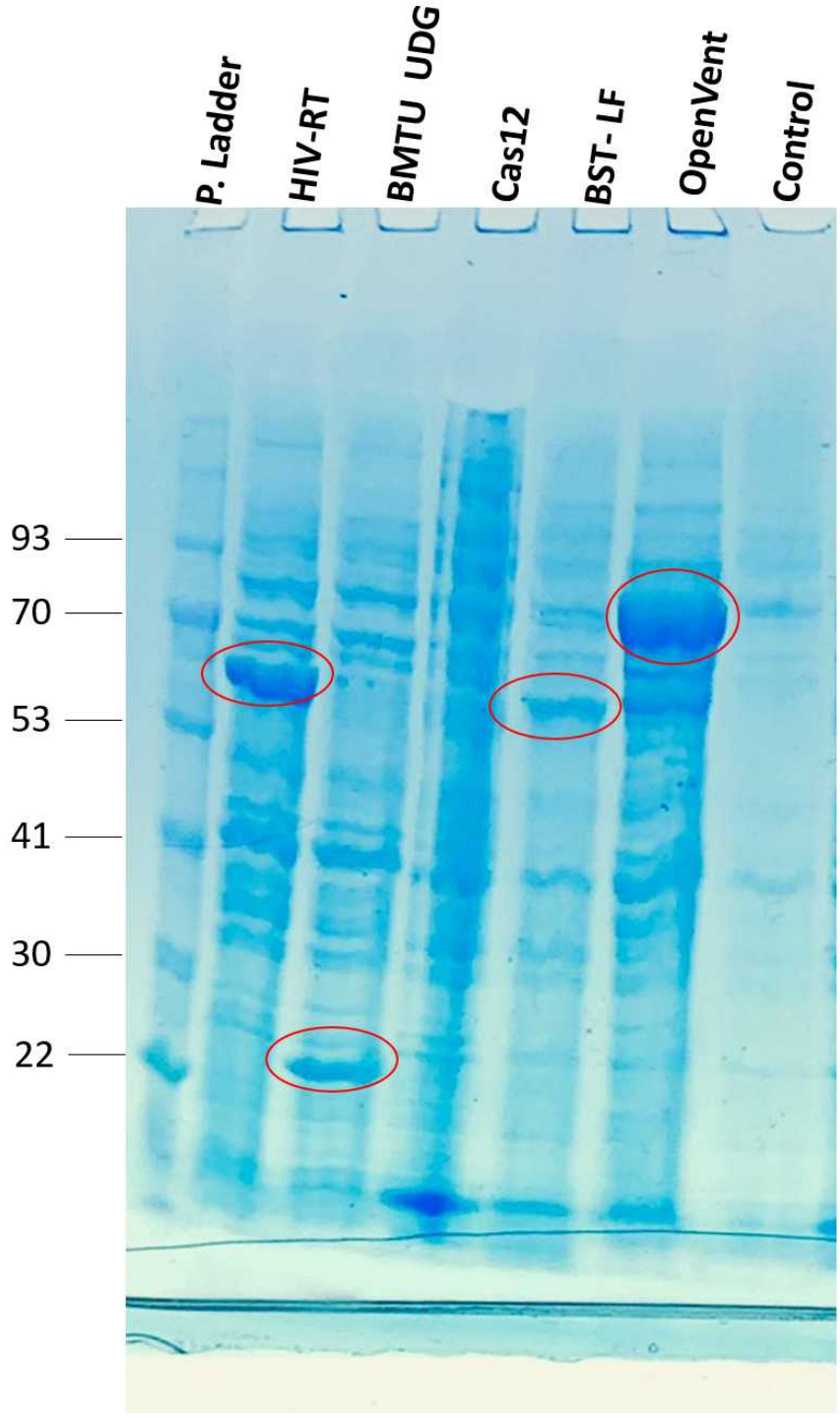

SDS PAGE gel after enzymes expression using plate auto-induction medium.

12.1 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:

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.

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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

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.

12.2 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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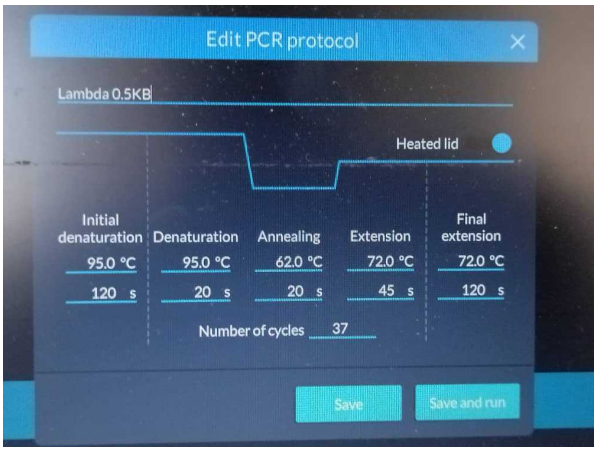
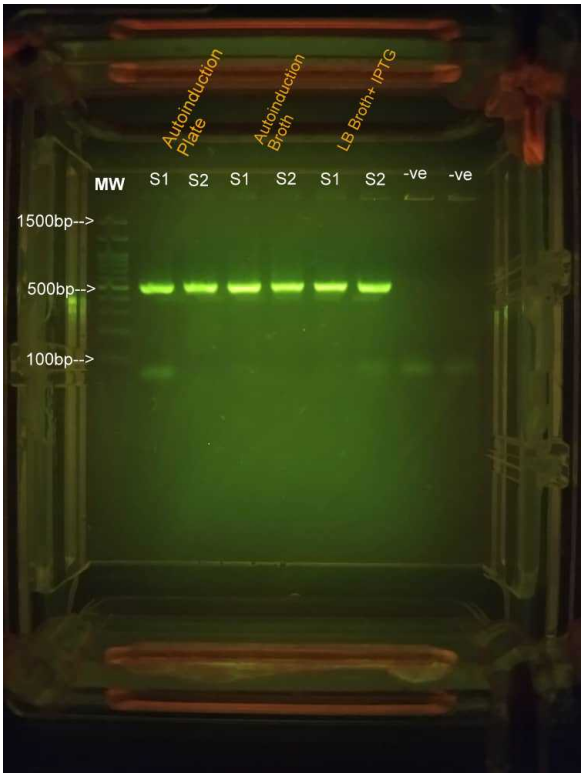


PCR parameter (MiniPCR mini 16)

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.



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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