



Production of cellular reagents using IPTG

 In 1 collection

Aug 01, 2022

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ABSTRACT

This protocol documents the production of cellular reagents.

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)).

This protocol provides instructions for IPTG (T7 based) expression.

PROTOCOL CITATION

Shalo Minette, Jenny Molloy, Nadine Mowoh, Stephane Fadanka 2022.

Production of cellular reagents using IPTG. **protocols.io**

<https://protocols.io/view/production-of-cellular-reagents-using-iptg-cegjtbun>

COLLECTIONS

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KEYWORDS

Production of cellular reagents using IPTG, Protein expression in E.coli using IPTG, IPTG induction of protein expression in E.coli

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CREATED

Jul 28, 2022

LAST MODIFIED

Aug 01, 2022

PROTOCOL INTEGER ID

67819

PARENT PROTOCOLS

Part of collection

[Beneficial Bio: Internal protocols](#)

GUIDELINES

This protocol describes the use of IPTG in inducing protein expression in E.coli BL 21 DE3 strain.

MATERIALS TEXT

Equipment:

- Static Incubator
- Shaker incubator
- Centrifuge
- Micropipettes with sterile tips
- Sterile PCR tube (8-strip tubes)
- Sterile 1.5 mL Eppendorf tubes
- Tupperware or Glass jars
- Conical flask (50 mL and 250 mL)
- Duran bottles
- 10 µL wire loops
- Thermocycler or Water bath
- Spreader

Reagents:

- Silica gel beads desiccant
- PBS (Phosphate Buffered Saline) solution made using [this protocol](#)
- 100 mg/ml Kanamycin solution made using [this protocol](#)
- LB media and LB agar plate made up using [this protocol](#)

SAFETY WARNINGS

Wear protective clothing (gloves, lab coat, face masks)

BEFORE STARTING

Ensure all materials and reagents needed are available and all culture media prepared.

Preparation of overnight starter culture

1

To begin, we typically prepare an overnight starter culture from either a bacterial glycerol

sock or agar plate.

From glycerol stock

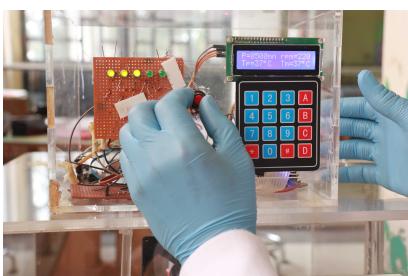
- Remove the glycerol stock from -20°C.
- Open the tube and use a sterile loop, sterile toothpick or sterile pipette tip to scrape some of the frozen bacteria off the top. *Do not let the glycerol stock thaw!*
- Streak onto LB agar plate which contains appropriate antibiotic, label with full details.
- Return glycerol stock to freezer.
- Incubate plate at **37 °C Overnight** in a static incubator
- Next day, check plate for growth of bacteria.
- Prepare 5 mL LB broth plus appropriate antibiotic as described in [this protocol](#).

e.g. 2.5µl 100 mg/mL Kanamycin stock in 5 mL LB broth, to give 50 µg/mL final kanamycin concentration.

- Inoculate prepared 5 mL LB Broth + Antibiotic in a 50 mL Falcon tube with 1 E.coli colony from the overnight the plate.
- Label and place the falcon tube in a shaking incubator at **37 °C Overnight**
- Store plate with colonies on at +4°C for up to 3 weeks.

From a pre-streaked plate

- Remove the plate from +4°C
- Use a wire loop to pick a colony from the plate
- Inoculate a prepared 5 mL LB Broth + Antibiotic in a 50 mL Falcon tube with 1 E.coli colony from the plate
- Label and place the falcon tube in shaking incubator at **37 °C Overnight**
- Store plate with colonies on at +4°C for up to 3 weeks.



Growth of main culture and preparation of cellular reagents

2 Growing main culture for IPTG-protein expression

- Prepare 50 mL LB broth plus appropriate antibiotic in a 100 mL flask
- Inoculate the 50 mL LB Broth + Antibiotic with **[M]0.5 % volume** of the starter culture
e.g. 250 µL of the overnight culture in 50 mL LB + Antibiotic
- Grow at **225 rpm, 37°C, 03:00:00** until $A_{600} = 0.5$
- Induce expression by adding **[M]0.5 millimolar (mM)** IPTG final concentration

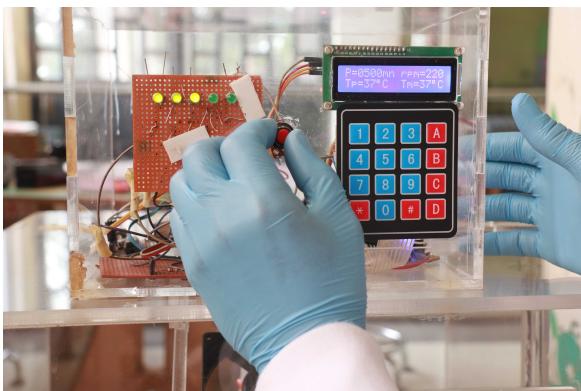
Calculate the amount of IPTG as follows:

$$\text{Amount of IPTG to add (\mu L)} = \frac{\text{concentration IPTG sought (mM)} \times \text{volume of culture (\mu L)}}{\text{Stock IPTG conc (mM)}}$$

e.g. 31.25 µL of [M]0.8 Molarity (M) stock to 50 mL

- Continue to incubate for **225 rpm, 37°C, 03:00:00**.
- Decant 1.5 mL (or desired volume depending on the tubes available) into centrifuge tubes or Eppendorf tubes.
- Centrifuge at **4000 rpm, 00:15:00**, pour off and discard the supernatant and keep the pellets at **4 °C**.
- Now make another set of tubes: This set is to be used for SDS PAGE to confirm successful protein expression.





After confirming successful protein expression

Wash cells

- Remove from the fridge the first set of tubes and resuspend cell pellet into 1.5 mL of cold PBS (vol of PBS = vol of harvested cell) – **First wash**

e.g. 1.5 mL harvested resuspend in 1.5 mL cold 1X PBS

- Centrifuge at **4000 rpm, 00:15:00**
- Perform **second wash**
- Resuspend pellet into 1.5 mL of cold 1X PBS

3 Aliquoting

- Measure A600 of a neat, 1:10 or 1:100 dilution. Multiply the value to get the actual final A600 number.
- Calculate the volume of your final cell suspension that would contain 2×10^8 cells, using the equation:

volume containing 2×10^8 cells = $200/\text{final A600}$ of cell suspension.

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

volume containing 2×10^8 cells = $200/6.5$

volume containing 2×10^8 cells = $31\mu\text{L}$

You can either aliquot $\sim 2 \times 10^7$ cells (enough for a single PCR reaction) or 2×10^8 cells (enough for 10 PCR reactions) into individual 0.2 mL PCR tubes in order to prepare dried Taq cellular reagents.

- 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, $3.1\mu\text{L}$ (1x reaction) or $31\mu\text{L}$ (10x).

4 Heating to kill the bacteria

- Transfer the 0.2 mL PCR tubes into a thermocycler
- Set a program for heating on the thermocycler at **60 °C** for **00:10:00** (leave some tubes unheated to serve as control and label the tubes accordingly).

Where a thermocycler is unavailable, a Water bath can be used by setting the water bath to **60 °C** and immersing the tubes in it for **00:10:00**

5 Drying

- Leave tube lids open
- Place the tube strips with pre- heated aliquoted cellular reagents carefully in a vacuum tupperware 1/2 filled with desiccant.
- place the lid of the tupperware on top and close gently making sure it closes properly on all the sides
- Use the vacuum pump to create a vacuum in the tupperware by pumping several times until the lid is visibly tough and sunken.
- Place the container static incubator **Overnight** at **37 °C**
- After 18-24 hours check to see if the cellular reagents are completely dry.
- Once dry, close the lids and place in a small bag at **4 °C** with a small amount of desiccant.

- We typically use this drying method to obtain cellular reagents that are stable for 3 to 6 months.
- In settings where a Lyophilizer is available, it can be used to obtain cellular reagents that can remain functional for longer periods.



6

Check the effectiveness of the heating step

- Reconstitute one of the pre-heated tubes with **30 µL** PCR water
- Mix by gently tapping onto the bottom of the tube and allow to stand for **00:05:00**
- Use a micropipette to aliquot **10 µL** inoculate on an LB agar plate supplemented with Kanamycin (100 mg/mL)
- Use a spreader to evenly spread the inoculum across the surface of the plate
- Repeat the above steps but this time reconstituting the control tube (tubes where cells were not pre-heated)
- Place both plates in a static incubator and incubated at **37 °C Overnight**

Check plates:

- The plate from pre-heated tubes should show no growth of bacteria confirming the complete killing of bacteria cells in cellular reagents making them safer to handle.
- The plate from non-heated tubes should show visible growth of bacteria confirming the need to preheat cellular reagents at 60c for 10 mins before drying and subsequent use.

Quality control

7 Proceed to carry out quality control of this batch of cellular reagents by testing the

 [functionality](#) and assessing them for [endonuclease activity](#).

After passing the quality control test, the cellular reagents can be used in a PCR amplification reaction.