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Efficient Preparation of Competent Cells and Recombinant Protein Expression in E. coli BL21 (DE3) Using IPTG Induction V.1

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Protocol status: Working
We use this protocol and it's working

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Abstract

Reproducible method for preparing TSS-competent *E. coli* BL21 (DE3) cells, transforming them with plasmids, inducing protein expression using IPTG and expression analysis by SDS-PAGE.

Competent Cells Preparation by TSS Method (DAY 1)

1 Selection of a healthy strain of BL21 (DE3) *E. coli* Culture from an Agar Plate

2 Inoculate the isolated colony and incubate
200 rpm, 37°C, 24:00:00 Overnight

Competent Cells Preparation by TSS Method (DAY 2) 40m

3 Prepare 50 mL of L.B Agar and autoclaved it at 121 °C for 15 Minutes
2 g of L.B Agar in 50 mL Water

4 Prepare 100 mL of L.B Broth
2.5 g in 100 mL of Water

5 Dilute the overnight culture in a 1:100 ratio
1 mL of overnight culture in 99 mL Autoclaved L.B Broth

6 Incubate at 180 rpm, 37°C Till it reaches 0.5 - 0.6 OD

7 Prepare the TSS Solution (10 mL Total Volume)
10 % of PEG for a 10 mL solution is 1 g
5 % of DMSO for a 10 mL solution is 500 µL
50 mM MgCl2 for a 10 mL solution is 47 mg
Add all the components in 8.45 g of Autoclaved L.B Broth to make total volume of 10 mL

8 After reaching 0.5 OD, cells should be arrested by incubating the culture on ice for 30m
00:30:00 (30 Minutes)

9 Cells were harvested by centrifuging at 4000 rpm, 4°C, 00:10:00 , (10 Minutes) 10m

10 Discard the supernatant. Suspend the cells by adding 1 mL of TSS Solution by tapping them to ice

11 After resuspension of cells, Aliquote 50 µL in each 1.5 mL Eppendorf tubes (Pre-Chilled)

Checking & Validation (DAY 2)

12 To check the experiment, add 50 µL of competent cells in 25 ml Agar Plates + Kanamycin Antibiotics
In this step, if we can't see any colonies, it is likely expected that competent cells are ready.

Transformation (DAY 3)

1d 1h 30m

13 Make 50 mL L.B Agar and pour it into the plates (25 mL each)
In the upcoming steps, this L.B Media will be used

14 Make 25 mL L.B Broth and store in 50 mL Falcon tube
In the upcoming steps, this L.B Media will be used

15 Thawed the cells by keeping the eppendorf containing competent cells at 0 °C on ice

16 Add 100 ng Plasmid to the Eppendorf
100ng= 0.1 ug

17 Incubate the Eppendorf for 15 Minutes on Ice

18 Heat-shock the Eppendorf containing competent cells 42 °C for 45 Seconds

19 Put Eppendorf on ice for 2-4 Minutes

20 Add 800 µL of L.B Broth in Eppendorf and incubate at 180 rpm, 37°C, 01:00:00 1h
This step is known as regeneration

21 After regeneration, harvest the cell by centrifugation 4000 rpm, 37°C, 00:30:00 30m

22 Discard the supernatant and add 200 µL of L.B Broth , resuspend it till you see a clear solution
(Don't resuspend the cells vigorously, it may damage transformant cells.)

23 Pour 100 µL of Transformant cells into an agar plate (25 mL plate) and add 10 µL Kanamycin

24 Incubate it overnight at 180 rpm, 37°C, 24:00:00 1d

25 Colonies are likely to appear if they have an uptake of plasmid

Induction & Expression Screening (DAY 4-5) 1d 20h 15m

26 Make 40 mL L.B Broth and aliquot 10 mL each in 15 mL Falcon

27 Pick a healthy, medium-sized, isolated colony from an agar plate and inoculate it in 10 mL of L.B Broth (Primary Culture) ; Incubate at 180 rpm, 37°C, 24:00:00 1d

28 Take 1% 0.1 µL of the Transformant cells from an overnight culture and inoculate them into 10 mL Falcon (All 4 of them). Incubate them at 180 rpm, 37°C Till it reaches 0.5 OD (Secondary culture)

29 Add 0.5 mM of IPTG into two Falcon tubes and 0.1 mM of IPTG into two Falcon tubes.

30 Incubate two Falcon tubes containing 0.1 & 0.5 mM concentration of IPTG at 25 °C 20h
16:00:00

Incubate two Falcon tubes containing 0.1 & 0.5 mM concentration of IPTG at 37 °C

04:00:00

31 Harvest all 4 Falcon Tubes at 4000 rpm, 37°C, 00:15:00 15m

(At their respective timing)

32 Run SDS-PAGE and check the protein expression