AUGUST

WEEK 3

IPTG induction of BC2 cloned plasmid in BL21

16/08/21
17/08/21

Protocol:

- Primary inoculum: Take a 50ml falcon tube, add 10ml LB broth and 10ul Kan(50ng/ul). Select a colony from cloned BL21 cells and dip the tip in lb.
- Incubate for 12 hrs
- Secondary inoculum: (For one sample) Take 100ul of primary inoculum in 50ml falcon tube and add 10 ml LB and 10ul Kan.
- Keep it in incubation till OD reaches 0.6
- Take 10 ml of secondary inoculum and add IPTG (of required concentration)
- Keep it in incubation at req temperature and time. (for temp less than 34 incubation time is overnight and for temp greater than 34 incubation time is 5hr
- When the incubation period is over, pellet down the cells(centrifuge at 11,000 rpm 4℃ for 15min) and store it in -20℃

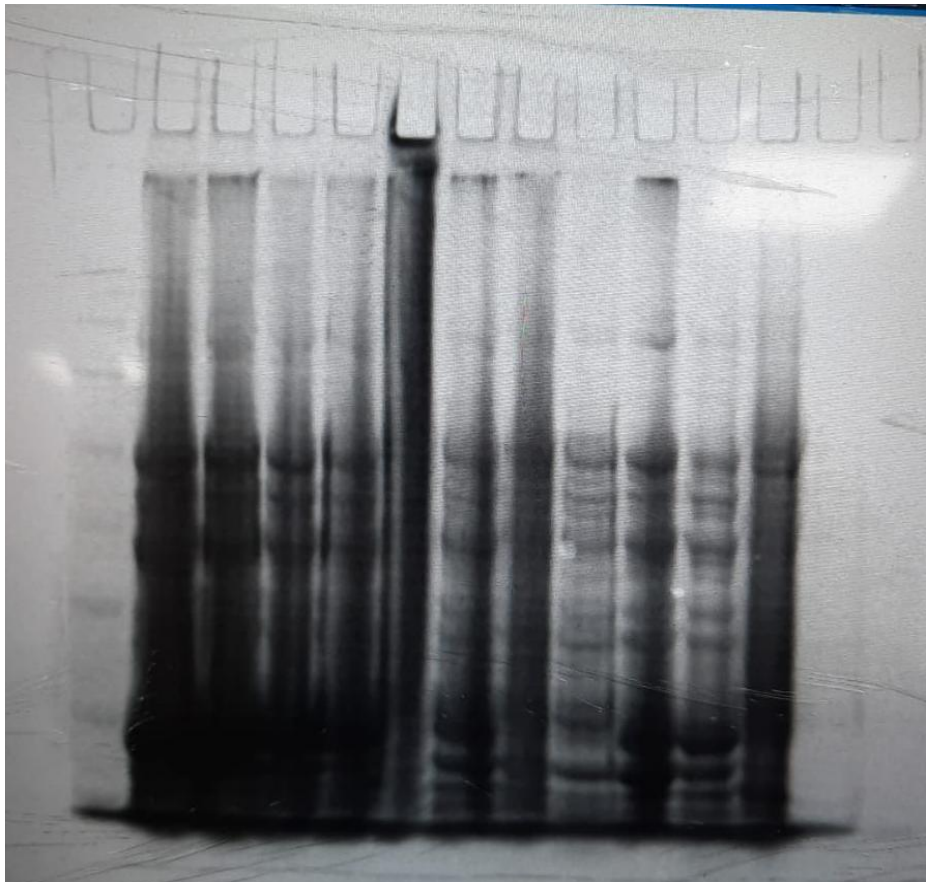
Total Samples taken:

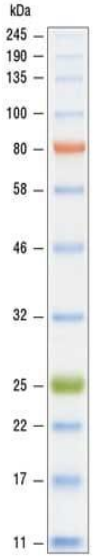
1. 35℃ 1mM IPTG
2. 35℃ 0.1mM IPTG
3. 37℃ 0.1mM IPTG
4. 37℃ 1mM IPTG
5. 30℃ 0.1mM IPTG
6. 30℃ 1mM IPTG
7. 26℃ 0.1mM IPTG
8. 26℃ 1mM IPTG
9. 16℃ 0.1mM IPTG
10. 16℃ 1mM IPTG
11. Uninduced primary inoculum
12. 26℃ 0.1mM Supernatant
13. 16℃ 1mM Supernatant

kDa

245
190
135
100
80
58
46

Req size- 43.262kDa





18/08/21

Aim: Check whether proteins are expressing in inclusion bodies or not

Protocol:

- Primary inoculum- 10 ml lb + kan(50ug/mL). Incubate for 12hr
- Secondary inoculum- 1050 ul primary inoculum 105 ml lb+ kan (50ug/mL). Incubate till 0D reaches 0.6. (preserve 1ml of secondary inoculum as a control)
- Add 1mM IPTG(200ul of 0.5M IPTG) and incubate at 35℃ for 5hr.
- Pellet it down. Take some pellet and add loading dye buffer.
- Take some pellet and add lysis buffer.
- Sonicate it and then centrifuge at 4C for 11000 rpm for 45 mins..
- Dissolve both pellet and supernatant in loading buffer separately.

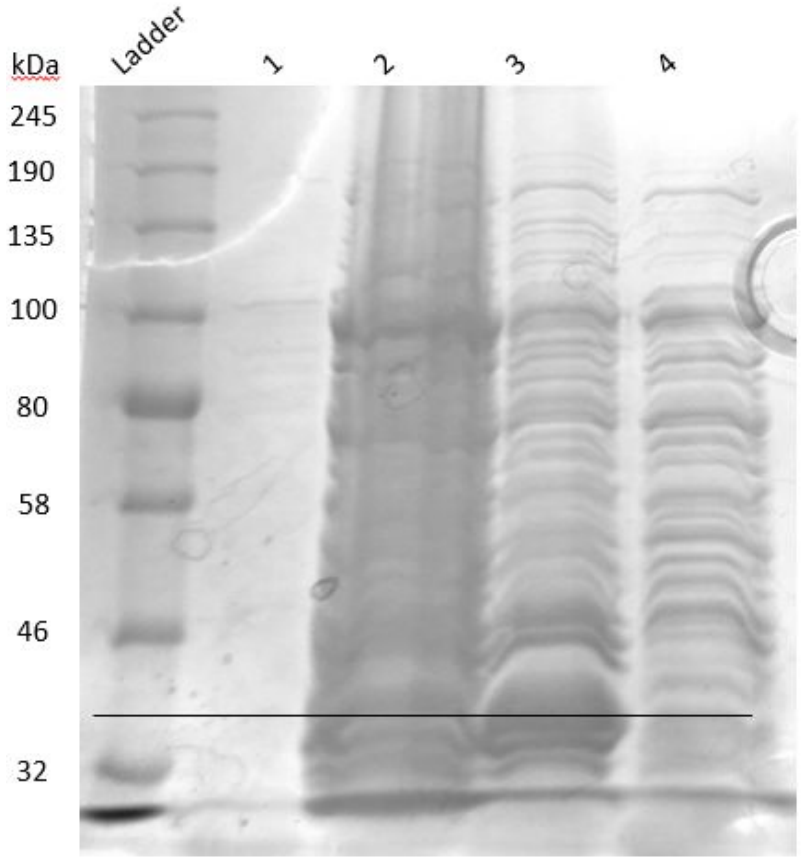
20-08-2021

Sonication

On time	30 secs
Off time	30 secs
Total cycles	15
Total time	15 mins

- Sonication carried out in pulse mode.
- Amplitude- 55%

SDS PAGE



Annotations:

Ladder- NEB Protein Standard ladder (11-245 kDa)
1: Uninduced Supernantent (Control)
2: Induced (pellet without lysis buffer)
3: Induced (pellet with lysis buffer)
4: Induced (supernatent with lysis buffer)

Result:: The proteins are forming in inclusion bodies. Try inducing at lower temperature 16C as inducing the protein at lower temp may cause the normal folding of proteins by giving it enough time to fold properly.