Small scale protein expression from T7 promoters in BL21star(DE3) cells

Aim:
- Express proteins preceded by a T7 promoter

Timeframe:

- Starter cultures: 14h
- 50 mL expansion: 4-5h
- Expression: 4-24h (dependant on conditions)
- Cell harvesting: 10min

Materials:
- Agar plate containing BL21star(DE3) cells transformed with plasmid of interest
- LB media
- Antibiotic stocks
- 50 mL falcons (sterile)
- 250mL flasks (sterile / autoclaved) - stoppered with foam and covered with foil

Procedure:

At all times ensure correct aseptic techniques.

Day 1: Prepare starter cultures - This step can be skipped if you already have a starter culture of your cells.
1. Add 5-10 mL of LB to 50 mL falcon tubes.
2. Add antibiotic to working concentrations(dependant on antibiotic resistance of theplasmid).
3. Inoculate these cultures by picking colonies of BL21star(DE3) cells from an agar plate.
4. Incubate overnight at 37ºC, 200 rpm shaking.

Day 2:
1. Add 50ml of sterile LB to a sterile 250 mL flask supplemented with the appropriate antibiotic.
2. Measure the OD600 of the overnight culture against a standard curve.
3. Using the OD600 measurement inoculate the 50mL culture to a final OD600 of approximately 0.1.
4. Incubate the 50 mL culture at 37 °C, 200 rpm, until the OD600 reaches 0.6-0.8 (approx 4 hours).
5. Once the OD600 has reacher 0.6-0.8 induce expression of your protein with IPTG to a final concentration of 400-4000 uM - some optimisation may be necessary to find the ideal amount.

6. Incubate the culture to allow for expression. This can be done at a variety of temperatures. 25°C , overnight , shaking at 200 rpm worked well for us.

Day 2 or 3 - depending on expression time:

1. Harvest the cells by centrifugation at (5000 rpm) for 10 min at 4°C to pellet the cells.
2. Cells can then be frozen until use or one can proceed directly to cell lysis and proteinn purification.