Procedure

This plasmid is quite hard to maintain and it is recomended that these growth conditions are followed.

1. Plasmid TH116. Full length human P50 dynamitin cloned into T7 between Bam and EcoR1.

   Transform freshly P50 into BL21.

2. Next day, grow up a culture from the plate at 20 until growing nicely.

   IMPORTANT: Do not over grow.

   Dilute into 1L LB amp in 5L flasks over night at 20C so that they will by about OD 0.4. next day. Assume 1 hour generation time.

   The keys here are

   1. to make sure that the cultures never go into stationary phase. Otherwise the secreted b latamase can cause loss of your plasmid.
   2. Plenty of aeration. We use 1L in a 5L flask.
   3. Induce at the same temperature you grow at. There is no point shifting down the temperature at induction.

3. Add IPTG to 100mM from 1M stock in water kept at -20C.
4. Induce for 4-5 hours.
5. Harvest by spinning in 1L bottles 5K 10'. Resuspend in 20mls PBS and spin 15K 15 mins in 2059 tubes. Pour off PBS and freeze LN2.
6. Cool down HB4
7. Thaw pellets at RT. When just thawed, add 1ml lysis buffer to each tube
8. Add:

   - 50mg/ml lysozyme (add as powder).
   - 1mM PMSF (-20C in Fedor's drawer 50ml conical).
   - 1/2000 LPC.
   - 0.1% BME.

   Keep on Ice

   The key to this step is to reuspend the bacteria in a small volume so that the sonnication is much more efficient. There is no point trying to sonnicate 50 mls. 1 ml of resuspension will sonnicate very quickly.

   Sonnicate in differentiation sonnicator, full power 1 times 30secs.

   Careful not to move tip to surface which induces bubbles.

9. Add lysis buffer to 10mls and mix.
10. Leave 10mins on ice
11. Spin 10K 1hour HB4.
12. recover sup through cheese cloth.
13. Purification. All steps at 4C

14. Preequilibrate Superose 12 overnight in
    - 50mM Kpipes pH 7.0
    - 100mM KCl
    - 1mM EGTA
    - 0.1% BME
    - 10% glycerol.
15. Take 5mls of sup and spin 50K 10mins TL100.
16. 5mls of supernatant was applied to a 1ml MonoQ equilibrated in
    - 50mM Bis Tris Propane pH 7.0,
    - 1mM EGTA
    - 0.1%bME (Important. Sensitive sulhydryl).
    - 20% glycerol (important. Protein is hydrophobic)
    - 100mM KCl
17. Flow rate 0.3 mls/min. Back pressure quite high due to glycerol. Fraction size 0.3 mls.

    Wash extensively in 10%B. Takes along time for UV to return to zero.

    elute with a 100-500mM KCl gradient. 10 column volumes.

    Then 1000mM KCl 5 column volumes.
18. P50 comes off at 330mM Kcl
19. Run a gel to check peak fractions
20. 0.5 ml of the Peak fractions were applied to a Superose 12 column in
    - 50mM Kpipes pH 7.0
    - 100mM KCl
    - 1mM EGTA
    - 0.1% BME
    - 10% glycerol
    - Run at 0.2 mls/ min. Max back pressure 2.4
    - Fraction size 0.5 mls. Came off at 8mls.

    Freeze in LN2 small aliquots
21. Purified fractions were collected and if nessesary concentrated by a salt bump from a MonoQ column on the SMART system.

Solutions

Lysis buffer

- PBS
- 1mM EGTA
- 1mM EDTA