LARGE SCALE GST FUSION PROTEIN INDUCTION AND PURIFICATION
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1. Use a bacterial colony containing the pGEX-derived construct to inoculate 10ml LB/Amp and 1%glucose in a 50ml tube. Grow the culture @ 37°C, shaking overnight .
2. Use 1ml overnight culture to inoculate 200ml LB/Amp (with 1% glucose). Grow the culture @37°C, shaking to an OD600 of 1.0 (usually about 2 hours)
3. Add IPTG(isopropyl b-D-thiogalactopyranose) to induce expression of the tac promoter-driven fusion gene to a final concentration of 0.1mM. Grow the culture an additional 4-6 hours.
4. Chill the cells on ice for 5-10 min.(It's very important to keep the cells/lysates @4°C for the duration of the procedure)
5. Transfer the culture to two sterile 250ml centrifuge bottles. Spin down @4°C , 3800rpm X 20 min, discard the supernatant.
6. Resuspend the cell pellets in 25 ml ice cold PBS-PI and transfer the solution to a 50 ml tube.
7. Lyse the cells by sonication(power=10) 4-6 X 10 second bursts @4°C.
8. Add Triton X-100 to final concentration of 1% and tumble the solution @ 4°C, 30 min. Spin down@ 4°C ,3800rpm X 20 min. Remove the supernatant into a new 50 ml tube.
9. To purify the GST-fusion protein, add 500μl of 50% slurry of glutathione-agarose beads ,tumble@ 4°C, 30rpm X 30mi.
10. Spin down the beads @ 4°C, 3800rpm X 5min, discard the supernatant and resuspend the beads with 10 ml ice cold PBS-PI, wash the beads three times.
11. Recover purified GST-fusion protein by competitive elution with 5 time volumes elution buffer, tumble it @4°C, 30rpm X 20-30 min. Spin down and collect the supernatant. Keep it at -80°C.