

preparation of large scale GST-fusions.

- inoculate 2 liters with o/n cultures
 - a GST control
 - b GST T Koppin
 - c GST R6.
- 6 liters total LB broth
amp 100 µg/ml
- 37°C for 6 hours
- Add IPTG = 1 mM final conc.
- shake 37°C for 4 hours.
- harvest cells at 5000 rpm 5 min each. 4°C
- resuspend in 15 ml cold STE with 100 µg/ml lysozyme
1 mM PMSF.
- incubate 15 min on ice.
- Adjust to 5 mM DTT: Add 75 µl 1 M DTT stock vortex.
- Add Sarkosyl up to 1.5%
- use 2-7 ml of 10% stock in STE buffer.
- Sonicate for 1 minute or longer.
- Centrifuge 10,000 rpm 5 min. 4°C.
- Adjust to 2% Triton X-100: Add 4.5 ml 10% stock in STE
- Vortex.
- For 10 ml supernatant, add 50 µl of 50% bead slurry
- bind for 1 hour at 4°C
- Wash beads 3X in NETN
- Elute using 2 ml total 20 mM glutathione.
- Resuspend remaining pellet from 50 ml culture in 5 ml
 - load 10 µl
 - load 20 µl Soap before beads added.