GST pull-down assay
Protocol adapted from Rexach’s laboratory (Reference below)

Sonicating bacterial cells:
* Buffers can also be used for yeast but yeast cell extracts must be dialysed.

1. **Sonication: (Sonicator Model: Misonix Sonicator 3000)**
   For each tube of induced cells (50 ml culture, O.D. 600 ~ 0.9-1.5):
   Resuspend cell pellet in a 15 ml conical tube with 10 ml of binding buffer adding the following protease inhibitors as below:
   - 20 ul PMSF (50 mg/ml)
   - 20 ul Leupeptin (1 mg/ml)
   - 20 ul Aprotinin (1 mg/ml)
   - 10 ul Pepstatin (1 mg/ml)
   - 20 ul DNAseI (3 mg/ml, stock)

   + Set **Power level to 5** for sonicking 10ml cell suspension
   - 20 sec ON, 25 sec OFF for a duration of 20 min.

   **NOTE:** Always keep samples on ice during sonication to prevent protein degradation due to heat generated from the sonicator tip.

2. **After sonication:**
   Transfer 1.5ml sonicated cell mix to microfuge tube and spin tubes at max speed (13.3K rpm) for 10 min at 4°C to pellet cell debris
   Transfer supernatant into a fresh tube.
   **Supernatant:**
   - Take 40 µl of the extract for immuno-blotting step + 10 µl 5xSLB (sample loading buffer)
   - Use 5ul to check protein concentration by Bradford assay.
   - Aliquot lysate containing 5mg protein into 1.5ml microfuge tube for future GST pull-down assay
   **Cell debris:**
   - Resuspend in 1.5 ml of Binding buffer which has been used.
   - Take 40 µl also for immuno-blotting step + 10 µl 5xSLB

**Binding GST-fusion protein to GS4B beads (from Amersham):**
1. **Prepare GS4B beads (50% slurry) prior binding GST-fusion protein**
   Put binding buffer on ice
   Transfer 133 µl of beads to a microfuge tube (make sure to mix the bead bottle well prior using)
   Spin down at 2000 rpm for 30 sec. at 4°C
   Wash beads 2x 1 ml of cold binding buffer to remove ethanol, re-spin and remove supernatant.
   Resuspend beads in 100 µl binding buffer to make a 50% slurry
2. **Binding GST-fusion protein to 50% slurry beads**
   Use 50ul of 50% slurry beads per 0.5 ml (~5mg) of protein extract (cell lysate)
   Add 1 µl of 10% Tween20 to the supernatant to make 0.1% (this will prevent glutathione beads from sticking to the tube wall when they are added to the cell extract)
   Incubate beads in cell lysate for 1 hour at 4°C on a nutator
   Spin down beads at 2000 rpm for 1 min & transfer lysate to a new tube or discard.

3. **Wash protein bound beads with binding buffer**
   - 1st and 2nd washes: 0.5 ml of cold binding buffer w/o ATP
   - 3rd and 4th washes: 0.5 ml of cold binding buffer with ATP
   - 5th and 6th washes: 0.5 ml of cold binding buffer w/o ATP
   After each wash, spin down beads at 2000 rpm for 30 sec. at 4°C

   **Binding Buffer:**
   20 mM HEPES pH6.8
   150 mM KOAc
   250 mM Sorbitol
   2 mM Mg(OAc)$_2$

   **For ATP** - Add 0.015 g of ATP per 25 ml of binding buffer to prevent any non-specific binding from bacterial cells

**Protein-Protein Interaction:**

1. **Incubating washed beads in Target protein extract (in binding buffer):**
   To each tube with washed beads bound by GST fusion proteins:
   Add 500 µl of target protein extract with ~5mg of Target protein in binding buffer
   Add 1 µl of 10% Tween20
   Incubate beads for 4-24 hours at 4°C on a nutator

2. **Collecting beads:**
   Spin down beads at 2000 rpm for 30 sec.
   Remove supernatant to a fresh tube or discard used protein extract
   **Optional:** Take 40 µl for western blot to see if target protein amount is reduced
   Wash beads six times (6x) as described above

3. **Elution:**
   Elute GST-bait protein-Target protein complex off beads with Elution buffer:
   Add 60 µl of elution buffer and incubate for 10 min at room temperature.
   Spin down beads, transfer supernatant to a fresh tube (keep this tube on ice)
   Repeat this elution step one more time ➔ Final volume is 120 µl
Determine protein concentration by O.D. \( \text{OD}_{280} \).
Do SDS-PAGE analysis by probing with anti-GST (Sigma Cat # G-1160) and anti-target protein.
Store Eluate in \(-80^\circ\text{C}\)

**5xSLB:**

- 10% w/v SDS
- 10 mM β-mercaptoethanol
- DTT
- 20% v/v Glycerol
- 0.2 M Tris-HCl, pH 6.8
- 0.05% w/v Bromophenol blue

**Elution Buffer:**

- 50 mM Tris, pH8.0
- 0.154g reduced glutathione
- Sterile filter

**Control:** Western blot analysis of GST pull-down samples, include at least one negative (-) control sample containing empty vector (GST only)

**References:**