GST Pull-down

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Plate 293T cells (Day 1):

*1.5 million cells per well in a 12-well plate

*Culture medium: DMEM+10%FBS+0.2% Primocin; 1 ml per well in a 12-well plate

- Prewarm the trypsin and medium in 37 ℃
- Add 1 ml of medium/well in a 12-well plate
- Add 3 ml of medium/well in a 6-well plate (Put 4 ml if you want to passage every 3 days)
- Save the old medium in a 15 ml tube
- Add 1 ml (for 6-well plate) of trypsin to a well of 293T cells
- Incubate in 37 ℃ incubator for 2 min
- Stop the reaction by adding 3 ml of old medium to the well
- Resuspend the cell gently (using p1000 tip)
- Count the cells using hemacytometer
- Plate 1.5 million cells/well in a 12-well plate

Hydrate agarose glutathione beads (Day 1):

- Take out the agarose glutathione beads in -20 ℃, let it warm up in room temperature
- Add some glutathione beads powder into a 1.5 ml tube
- Add PBS. (Add enough amount to hydrate the beads, no specific volume required.)

PEI Transfection (co-expression) (Day2, no more than 24h later than plating):

- 100 ul DMEM (No antibiotics) (For 1 ug DNA in total)
- 500 ng Plasmid A
- 500 ng Plasmid B
- 50 ng pEGFP-N3 (optional, the purpose is to test transfection efficiency)
- 3 ug PEI
- Mix it gently and incubate at room temperature for 30 min
- Add to the well(s).

Check the cell condition (Day 3 and Day 4):

- Take photos at bright field and GFP channel of a well. (If all the wells are of similar condition, just take one well for record.)
- Need to add or change medium on Day 3.

Pull-down (Day 4):

- Prepare the lysing buffer:
  - 1% triton, 5 mM EDTA, 5mM EGTA in PBS
  - Store in 4 ℃ , put on ice when using it.

- Pull-down:
  - Aliquot 50 ul of glutathione beads (mixture, 50% beads and 50% PBS) to new 1.5 ml tubes (Cut the tip when transferring the beads)
  - Take the plate out and put on ice for 10 min (Dampen the activity of protease in the cells)
  - Discard the medium in all the wells and put 250 ul of lysing buffer to each well.
  - Put on ice for 10 min to fully lyse the cells (Guo didn't do this step)
  - Collect the liquid in 1.5 ml tubes
  - Sonicate 10s/tube. (Short-time sonication doesn't require ice bath)
  - Centrifuge in the cold room at maximum speed for 20 min
  - Save 20 ul of the cell lysates as inputs, put in -20 ℃
  - Transfer the supernatant to the tubes containing glutathione beads
  - Rotate at the cold room (beside the cell culture room) overnight.

Pull-down (Day 5):

- 1000 rpm, 5 min in the cold room
- Discard the supernatant and add 1 ml of lysing buffer
- Rotate for 10 min and spin down (1000 rpm, 5 min, in the cold room)
- Wash in total 3 times (rotate for 5 min in the rest 2 washes)
- Remove all the supernatant after wash and spin down
- Add 25 ul 2x SDS loading buffer to all the inputs
- Add 25 ul 1x SDS loading buffer to all the pull-down samples (should have 25 ul of beads in the tube)
- Heat all samples in 55 ℃ (I usually do 95 ℃ , 5 mins)
- Samples are ready for western blot.