Pull-down of RNA with GFP-tagged immobilized proteins

1. Purification of GFP-tagged proteins:

Materials:

- Lysis buffer
  - AM300 (AM100 containing 300 mM KCl)
  - 0.1%NP40
  - 1x Roche Complete
  - 0.5 mM PMSF
- GFP-Trap (Chromotec)

Protocol:

- Resuspend cell pellet in lysis buffer (1 ml for a 10 cm dish of HEK293T cells, 2 ml for a 15 cm dish)
- Homogenize cells using a glass homogenizer (1 ml size, 30 strokes).
- Incubate cell lysate for 1h on a turning wheel at 4 °C
- Centrifuge at 13,000 rpm for 45', 4°C
- Transfer supernatant to a new tube (keep an aliquot of 40-80 µl as input control → store at -20 °C)
- Add 3 µl of packed GFP-Trap beads for 1 ml of lysate. Mix GFP-Trap with Protein A/G Agarose Beads (6-7 µl of packed beads) to increase bead volume and facilitate efficient recovery of beads during washing steps
- Incubate for 3-4h on a turning wheel at 4 °C
- Remove supernatant and wash beads 3x with 0.5 ml of lysis buffer and 1x with 1 ml of lysis buffer without Roche Complete and PMSF
  - Washing steps are done by centrifuging the beads for 5' at 7.200 rpm at 4 °C followed by resuspension of beads in 0.and 5' on a turning wheel
- Remove supernatant, add 2 volumes of buffer AM100 (20µl if you used a total of 10 µl of packed beads)
- Subject 10% of the bead slurry for SDS-PAGE to estimate amount of bound protein
- Store beads at 4°C

Critical points

- Don't incubate the beads with the lysate overnight! You will get a lot of unspecific binding!
- Store immobilized proteins at 4°C, never at -20°C!
- Wash beads before adding to the lysate

2. Pull-down assay

Materials:

- Reaction buffer
  - AM100 (100 mM KCl; 20 mM Tris-HCl, pH 8.0; 5 mM $MgCl_2$; 0.2 mM EDTA, pH 8.0; 10% glycerol)

- 0.1% NP40
- 10 mM ß-MeOH
- 1x Roche Complete
- 0.5 mM PMSF

- In vitro synthesized transcripts (prepare radioactive or non-radioactive by in vitro transcription)

Protocol

- Set up the following reaction in a 0.5 ml DNA LoBind tube:
  - Appr. 1µg of protein bound to 1-2 µl of packed beads
  - Add 1-2 pmol of in vitro synthesized transcript
  - add reaction buffer to 200 µl
- incubate for 1 hour on a turning wheel at RT and pellet beads
- Wash beads 5x with reaction buffer
  - add 200 µl of buffer, invert tube 10x, centrifuge for 5 min, 7.200 rpm, 4°C, remove supernatant

- *If transcript was radiolabelled*
  - Monitor bead-bound radioactive transcripts by scintillation counting and/or by electrophoresis on agarose gels and PhosphorImaging

- *If transcript was not radiolabelled*
  - Isolate bead-bound RNA with TRIzol/TRI Reagent
  - Resuspend RNA in 10µl of H_2O, use 4µl for qRT-PCR