In vitro RNA-protein binding assay

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Ilse Hofmann i.hofmann@dkfz-heidelberg.de

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The following samples are required for the assay:

- Transcribe target RNA in vitro according to the RiboMAX Large Scale RNA production system (Promega Kit)
- Synthesize protein under study in vitro according to the TnT® Coupled Reticulocyte Lysate System (Promega Kit)

Procedure:

- Use 25 µl Dynabeads each (Novex), besides IP-sample (beads+ antibody+ protein+ RNA), two additional negative controls are recommended (control 1: beads +RNA-to control if RNA binds unspecific to the beads; control 2: beads + antibody +RNA, control if antibody binds RNA). As positive control we use PABPC1 binding to poly(A)-RNA. Any RNA may be labeled using the enzyme (Poly(A) polymerase, Ambion)
- Set up two samples in parallel: one sample is needed to control specific loading with in vitro synthesized protein by western blotting, the other sample is used to detect RNA bound to the protein under study.
- Wash beads 3x with IP-buffer.
- Add antibody (we use 2 µg) to the beads.
- Incubate for 2 hour at 4°C on a rotating wheel.
- Wash Beads 3x with 1 mL IP-buffer.
- Add in vitro synthesized protein (amount depends on the protein control amount on WB, we use 6 µl out of 50 µl TNT sample of in vitro translation kit) in 400 µl IP-Buffer to the beads and incubate for 1 hour at 4°C on a rotating wheel.
- Wash beads 3x with 1 mL IP- Buffer, for last wash step use IP-buffer substituted with RNAse inhibitor
- Add approx. 1 µg of in vitro transcribed RNA to 400 µl IP-buffer substituted with RNAse inhibitor, mix the solution with the beads specifically loaded with the in vitro synthesized protein.
- Incubate ON, at 4°C, on a rotating wheel.
- Remove an aliquot from the suspension (approx. 200 µl), mix with 1 mL TRIZOL (→ RNA isolation: reference sample)
- Wash the beads 3x with 1 mL IP-buffer substituted with RNAse inhibitor
- Transfer the beads to a new tube to reduce background of unspecific bound RNA.
- Wash beads 3x with 1 mL IP-buffer substituted with RNAse inhibitor.
- Remove IP-buffer and add 1 mL TRIZOL to the beads (→ RNA isolation: IP-sample).
- Detect binding of RNA to protein by RT-PCR using primers specific for the target gene. For cDNA synthesis use 2 µg RNA form the reference sample and total RNA isolated from the IP-sample or the two negative controls (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems)

IP-buffer: 140 mM NaCL, 5 mM EDTA, 20 mM Hepes (pH 7.5), 1% Nonidet-P40

RNAse Inhibitor: RNasin RNA Plus Inhibitor (0.5 µl/1 mL IP-Buffer)

TRIZOL (Trizol reagent, Ambion)