

## Rip Seq

B060

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## **mRNA CoIP for miRNA targetome identification (RIP-Seq) using protein G sepharose**

### **Day 1:**

#### Lyse of cells:

- Harvest cells by centr. @ 1000rpm / 10min/ 4°C
- Wash cells once with ice cold PBS
- Lyse cells with 100µl of ice cold polysome lysis buffer for 5min inverting on 4°C
- Store lysate @ -80°C over night to enhance lysis

### **Day 2 (approx. 5.5 hours):**

#### Prepare beads:

- Take 50µl of Protein G Sepharose (GE healthcare), pulse in a centrifuge to remove solution
- Wash Sepharose 2x with 500µl NT2 buffer
- Add 500µl NT2, add 10µg of Ago2 Antibody (11A9) / same with isotype control Antibodies in a separate tube
- Incubate 1 hour @ 4°C rotating
- Wash Sepharose 2x with 500µl NT2 buffer

#### IP:

- thaw cell lysate on ice
- Centr. 15000g/ 4°C / 15 min
- Add 850µl of ice cold NT2 buffer to each lysate and add 5µl RNaseOUT + 2µl vandyli ribonucleoside complex + 10µl of DTT (100mM) + 10µl of EDTA + 10µl Protease inhibitor
- Immediately remove 100µl for total lysate fraction
- Incubation 1 hour @ 4°C rotating
  
- Spin down the mix and store supernatant @ -80°C for later analysis on Western blot
- Wash Beads 5x with 1000µl of ice cold NT2 buffer
- resuspend beads in 50µl Glycin (1M, pH 2,3) and incubate @ RT for 15min
- Transfer eluate into a new tube and add 50µl Tris-HCL (1M, pH 8) immediately
- Add 3µl of Proteinase K and incubate 10min @ 55°C
- Cool mix down and add 350µl of TRIZOL LS, vortex
- Add 100µl of Chloroform and vortex
- Transfer mix to a Phase lock tube and spin 5min @ 13000rpm and RT
- Transfer upper phase (usually 250µl) into a new Tube
- Add 1.5x Vol. EtOH 100% (ice cold), mix and isolate RNA using the miRNeasy Kit according to the manufacturers protocol (one column for all parallel IPs)
- Test RNA on an Agilent Bioanalyzer Pico Chip

### **Day 3 and 4**

- Illumina TruSeq RNA library preparation according to the manufacturers instructions

## Buffers

Prepare all buffers and Stocks in RNase free water

### Polysome lysis buffer 5ml:

4,7 ml	RNase free H <sub>2</sub> O	
50µl	1M HEPES (pH 7.0)	4,766 g for 20ml Stock
500µl	1M KCl	1,4912 g for 20ml Stock
25µl	1M MgCl <sub>2</sub>	
25µl	NP-40	

### **@ the time of use add:**

		<u>for 500µl</u>
50µl	1M DTT	5µl
12,5µl	100U/ml RNaseOUT	1,25µl
200µl	Protease inhibitor cocktail	20µl
10µl	200mM Vanadyl ribonucleoside complex	1µl

### NT2 buffer 50ml:

40 ml	RNase free H <sub>2</sub> O	
2,5 ml	1M Tris (pH 7,4)	6,057g for 50ml Stock
7,5 ml	1M NaCl	2,922g for 50ml Stock
50µl	1M MgCl <sub>2</sub>	
25µl	NP-40 (=Igepal)	