IP RNA

- **Preparation of the beads**

  - 50µL Magnetic Prot A beads per condition (Dynabeads® Protein A, Invitrogen)
  - Wash 2 x with PBS-DEPC
  - Block with yeast tRNA 50µg in 500µL PBS-DEPC 1h at room temperature rotating
  - Wash 2 x with PBS-DEPC
  - Block with protein lysate 2h at RT rotating (Tissue: 3x sc in 3mL Lysisbuffer, 500µL per beads)
  - Wash 3 x with PBS-DEPC
  - Add 500µL PBS-DEPC with Antibodies
    - 10µg Cpeb 1 Santa Cruz (50µL)
    - 10µg Tsc2 as isotope (50µL)

    Over night 4°C rotating

- **IP Lysis Buffer**

100mM	KCl
5mM	MgCl2
10mM	HEPES
0,5%	NP-40
1mM	DTT
2,5µL/mL	RNasin (fresh)
50x	Roche (fresh)
2mM	Vanadyl ribonucleosides (fresh)

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- **Couple Beads to the Antibodies**

    - Equilibrate 1M DMP 2HCl to room temperature, heat sodium borate to solve it.
    - Wash 1x with PBS-DEPC
    - Wash 2x with 0,2M sodium borate pH9,0
    - Resuspend in 400µL 0,2M sodium borate + 8µL DMP
    - 30´ RT rotating
    - Wash 2x 5´ with 0,05MGlycin 5´RT
    - Wash 2x with PBS-DEPC
    - Resuspend in PBS + 0,1% azid
    - Store at 4°C

- **Start the IP**

mice

- Perfuse the mice with HBSS
- 2,5cm around the lesion side
- Resuspend each sc in 500µL Lysis Buffer +++
- Centrifuge, pool & divide for all the conditions
- Take away 200µL for Input RNA Isolation and a sample for the Input Protein sample
- Add the lysate to the coupled beads!
- ON 4°C rotating

cells

- Scratch the cells in PBS-DEPC
- Resuspend in Lysis buffer, 500-1000µL
- Centrifuge, BCA Protein quantification
- Take away something for the western blot input (25µg)
- Add the lysate to the coupled beads! (the same amount for each reaction, 500-1000µg if possible)
- ON 4°C rotating

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- **After the incubation**

  - Take away the supernatant for WB depleted
  - Wash 6x with IP Lysis Buffer
  - Wash 1x with Lysis Buffer + 1M Urea
  - Add 200µL Prot K Buffer (200mM TRis-HCl pH 7,5, 100mM NaCL, 10mM EDTA, 0,5%SDS) + 7µL Proteinase K (10mg/mL)
  - 30´ 50°C shaking
  - Take the supernatant for further steps
  - + 1 Vol. Phenol-chloroform-isoamylalc
  - Vortex 60´´, centrifuge 5´ 13.000rpm RT
  - Upper phase into a new tube
  - + 1Vol Chloroform
  - Vortex and centrifuge
  - Upper phase (around 100µL) +

3M Sodium acetate pH 5,5	1/10 Vol
Pellet paint	1µL
Glycogen	1µL
EtoH 100%	2,5 Vol

-80°C ON

- **Precipitation and DNase Digestion (don´t forget the input sample here, digest 5µg)**

- 13.000rpm 30´ 4°C
- Washes: 80% EtoH
  100% EtoH
  Let it dry 5´
  Resuspend in 85µL DEPC H2O
- + 10µL RQ1 DNase Buffer
- + 5 RQ1 DNase (Promega)
- 30´ 37°C
- + 1µL DNase Stop Solution
- 10´ 65°C

3M Sodium acetate pH 5,5	1/10 Vol
EtoH 100%	2,5 Vol

-80°C ON or 2h

- **Precipitation**

- 13.000rpm 30´ 4°C
- Washes: 80% EtoH
  100% EtoH
  Let it dry 5´
  Resuspend in 13µL DEPC-H2O

- **Concentrations**

- Photometer, µg/µL and 260/280
- Put the whole outcome of the IP into the cDNA Synthesis (Superscript 3, Invitrogen) (12µL)

- **cDNA Synthesis**

RNA	12µL
Annealing Buffer	2µL
Oligo dT Primer	2µL
H2O	

5´ 65°C
On ice at leat 1´

Reaction 1	16µL
2x Reaction Buffer	20µL
Enzyme Mix	4µL

50´ 50°C
5´ 85°C
∞ 4°C

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- **Gene specific PCR**

Either you take the same volume (e.g. 1µL) for each sample or you determine the concentration by photometer and put 500ng into the reaction

Primers:

ma_ip_**prkacb**_#2_for	ACGCCGCGCTCTAGGGTCTG
ma_ip_**prkacb**_#2_rev	CTTTCACGCTCTCCACTTCGCTGC
ma_ip_**cpeb3**_#2_for	CAGAGCCAGCTGCGCAAACCA
ma_ip_**cpeb3**_#2_rev	CTGCCGAAGGACGGCGACAG
ma_ip_**creb**_#1_for	CCAGCAGAGTGGAGATGCTGCTG
ma_ip_**creb**_#1_rev	GCTGGCATGGATACCTGGGCT
ma_ip_**ywhaz**_#2_for	CGACTGGAAAGGCAGGGCGTC
ma_ip_**ywhaz**_#2_rev	GATCTGGCTGCTCACAGGCTACA
Foxp1_IP_18.08.10_for	CGGATCAGCCATCCAGAACGGGT
Foxp1_IP_18.08.10_rev	GGTGCCTCTCCGTTGGACCG
CPEB1_fw1	CCCATTGGGTCTGGTCGTGTGAC
CPEB1_rev1	GGGGCTATGGTGGCGTAGGC

mGAPDH_Eli!

cDNA	0,5µL - 1µL
Puffer Mango Taq 5x	5µL
dNTP´s	1µL
MgCl2	1,6µL
Primer for 1:10	1µL
Primer reverse 1:10	1µL
Mango Taq	0,5µL
H2O	Add 25µL

95°C	5´		
95°C	30´´	|	
x°C	1´	|	?x
72°C	1´	|	
72°C	10´		
4°C	∞		

- **12% PAA-Gels and Silverstaining**