iCLIP2 - Lysate preparation

Revised: 2020.3.9

0. Preparations

0.1 Pre-cool Bioruptor

0.2 Add protease inhibitors and RNAse inhibitors to lysis buffer (per 10 ml)

- ~~10 µl 1 M DTT (1 mM final) - only for GFP trap~~
- 1 tb cOmplete EDTA-free
- 25 µl 200 mM AEBSF (1:400, 0.5 mM final)
- 50 µl 200 mM RVC (1:200, 1 mM final)

1. UV cross-linking, lysate preparation

Frozen powdered tissue

1.1 On dry ice, pre-cool porcelain mortar and pestle + 6-well tissue plate + pipette tips. Pour LN2 into mortar, add tissue and grind up. Scrap powder into a 6-well plate.

1.2 Irradiate 4x with 150-250 mJ/cm2 at 254 nm while still on dry ice. Mix the powder with LN2-cooled spatula in between irradiations.

1.3 Move the plate to wet ice and lyse directly on the plate, then transfer to 1.5 ml tube.

1.4 Add 1:1000 RNasin/Ribolock. Incubate on ice for 10 min with occasional flicking.

1.5 Sonicate using a Bioruptor for 6 cycles of 30 s on/off at high intensity

1.6 Centrifuge lysate 10 min at 17,900 g at 4˚C, take supernatant. (Do not aspirate floating fat layer)

1.7 Clarify supernatant with 0.45 µm or 0.22 µm filter. Pool. (Co-star columns, 16,000 g, 5 min, 4˚C)

1.8 Measure lysate concentrations, and dilute all samples to 1-2 mg/ml.

1.9 Use 1 ml aliquot for IP. Snap-freeze the rest and store at -80˚C

Dissected brain tissue

1.10 Dissect 50 brains at a time in PBS. Transfer to a 6-well plate with 1 ml cold PBS supplemented with 0.2 mM AEBSF (1000x).

1.11 Irradiate 4x with 150 mJ/cm2 at 254 nm on ice. Mix the suspension in between irradiations.

1.12 Collect to Protein Lo-Bind tube. Remove as much s/n as possible. Snap-freeze.

1.13 For each 50 brains add 250 µl supplemented lysis buffer and homogenise (45 s each)

1.14 Pool the lysates and sonicate using a bioruptor for 6 cycles of 30 s on/off at high intensity

1.15 Centrifuge lysate 10 min at 17,900 g at 4˚C, take supernatant

1.16 Clarify the lysate using either 0.22 µm filter or spin column (16,000 g, 5 min, 4˚C)

1.17 Measure lysate concentrations

1.18 Snap-freeze and store at -80˚C