Polysome sample processing

This document describes processing of fractionated polysome samples.

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Reagents and materials

- 1M HEPES pH 7.3 (Thermo Scientific, CAT#BP299)
- Dithiothreitol (Bio-Rad, CAT#1610611)
- 20% SDS (Thermo Scientific, CAT#BP1311)
- RNase A (10mg/mL, Thermo Scientific, CAT#EN0531)
- Ethanol, absolute (whatever vendor sells this to your lab)
- SP3 beads (Thermo Scientific, CAT#65152105050250, CAT#45152105050250) (see **Note 1**)
- Trypsin/rLysC mix (Promega, CAT#V5073)
- 1.5mL or 2.0mL Safe-Lock tubes (Fisher Scientific, CAT#05-402-25 or CAT#05-402-7)
- Thermomixer capable of holding 1.5mL or 2.0mL tubes
- Benchtop centrifuge (capable of holding 1.5mL or 2mL microfuge tubes)
- Magnetic rack for 1.5mL or 2.0mL tubes (I like this rack: Promega, CAT#Z5342)

Solution recipes

- 100mM HEPES, pH 7.3 (100uL of 1M stock combined with 900uL of water)
- 0.2M of dithiothreitol (DTT) - 15mg in 500uL of 1M HEPES, pH 7.3, prepare fresh and keep on ice
- 4X Lysis buffer (need 100uL per sample, recipe for 1mL)
  - 400mM HEPES pH 7.3 (400uL of 1M stock)
  - 4% (v/v) SDS (200uL of 20% stock)
  - 40mM DTT (200uL of 0.2M stock)
  - water to 1mL (200uL of clean water)
- 80% (v/v) ethanol (prepare fresh)

Protocol

1. Adjust the volume of the polysome samples to 100uL in a fresh 2mL tube using 100mM HEPES pH 7.3 and add 1uL of RNase A per sample.
2. Add 25uL of the 4X Lysis buffer and pipette mix. Incubate at +65C for 30-minutes in a Thermomixer at 1,000rpm.

3. During this incubation, prepare the SP3 beads stock (see **Note 2**):
    1. Vortex the bead stock to re-suspend the material.
    2. Take 500uL of each of the bead stocks and combine in a fresh 2mL tube.
    3. Place on a magnetic rack and wait for beads to settle. Discard the supernatant.
    4. Reconstitute the beads in 1mL of water with pipetting and place back on the magnetic rack. After the beads settle, discard the supernatant.
    5. Repeat the above rinse one additional time for a total of 2 rinses.
    6. Reconstitute the beads in 500uL of water. The bead stock can be stored at +4C indefinitely.
4. Add 10uL of SP3 beads to the sample and pipette mix.
5. Add 150uL of ethanol to the sample and gently shake the tube with your hand to mix the phases. Do not vortex.
6. Place in a shaking incubator at room temperature for 5-minutes.
7. While this is incubating, prepare your digestion solution by combining 0.5ug of trypsin/rLysC in 100uL of 100mM HEPES, pH 7.3 per sample and keep on ice (see **Note 3**). The original enzyme stock should be 0.1ug/uL.
8. Centrifuge the tube at 250g for 30-seconds to bring any liquid down from the top of the tube.
9. Place the tube on a magnetic rack and wait for the beads to settle. Discard the supernatant.
10. Remove the tube from the rack and reconstitute the beads in 800uL of 80% ethanol. Gently rinse the beads by pipetting.
11. Place the tube on a magnetic rack and wait for the beads to settle. Discard the supernatant.
12. Repeat **Steps 9 - 11** two additional times for a total of 3 rinses.
13. Place the tube back on the magnetic rack an wait for the beads to settle.
14. Discard the supernatant taking care to remove any residual ethanol remaining. It is not necessary to air dry the tubes.
15. Now digest your samples into peptides:
    1. Add 100uL of the digestion solution to the sample and pipette mix.
    2. Incubate at +37C in a shaking incubator or ThermoMixer overnight (around 14-hours).
    3. After digestion, gently shake the tube to reconstitute the beads.
    4. Centrifuge the tube at 20,000g for 2-minutes.
    5. Place on the magnetic rack and wait for the beads to settle.
    6. Transfer the supernatant to a fresh 1.5mL tube.
    7. The peptide sample can be stored at -80C indefinitely.

Notes

Note 1 - Other beads can be substituted here, such as BangsLabs Magnefy beads (CAT#MFY0001), or other carboxylate modified particles. Other companies,

such as MagReSyn also offer HILIC beads which are also compatible.

Note 2 - The bead stock from the vendor is 50mg/mL. We recommend using the beads at a 10:1 (ug of beads : ug of protein) ratio. This is far in excess of what is necessary, but will improve rinsing and elution efficiency by reducing the amount of 'clumping' that happens during binding.

Note 3 - I generally aim to have a trypsin:protein, ug:ug ratio of around 1:100, depending on the volume. For a larger volume I would likely scale this down to a 1:25 ratio. But these are personal preferences and digestion should be efficient at the values you would typically use in your lab.