Yeast TCA Whole Cell Extracts

NOTE 1: This method is extremely efficient and probably more representative of the protein situation in the cell since it also extracts chromatin (based on the ability to see modified yeast histones). Also the TCA kills enzyme activity so you preserve labile modifications like phosphorylation or acetylation without the need for inhibitors. **NB.** <u>You can't IP from these extracts – they're only for westerns</u>.

1. Grow cells (5-10ml) to OD_{600} of ≈1.0. Collect by centrifugation (≈100µl cell pellet). Wash with 20% TCA and transfer to an ependorff. Aspirate TCA and freeze pellet –80ºC.

NOTE 2: All further steps performed on ice unless noted otherwise.

2. Thaw pellet on ice, resuspend in 250µl 20% TCA. Add 250µl glass beads. Chill ice 5'. Vortex max speed 3 x 1 min pulses, with 1 min on ice between.

3. Pierce bottom of ependorff with a red-hot 22G needle **(CAREFUL)** and place into another ependorff with lid cut off. Spin 6K, 2min, 4ºC. Beads should remain in top ependorff while bottom contains lysate and precipitate / debris.

4. Add 300µl 5% TCA to beads to wash. Spin 6K, 2min, 4ºC to collect with first spin. Discard top tube (keep lid to cap the bottom tube). Add 700µl 5% TCA (≈1.25ml final) and pipette to mix. Spin 14K, 10min, 4ºC.

5. Discard liquid and wash pellet with 750µl 100% EtOH (-20ºC). Remove liquid and resuspend pellet in 40µl 1M Tris Cl pH8.0. Add 80µl 2xSDS Loading bº. Boil 5 min 95ºC. Spin 14K, 5min, RTºC. Collect S/N to new ependorff tube and discard debris. Run 10-15µl on an SDS-PAGE mini gel.

Buffers:

<u>100% TCA</u> = 100g TCA + 45.4mls ddH_2O.
<u>2xSDS Loading bº</u>: 60mM Tris pH 6.8, 2% SDS, 10% Glycerol, 0.2% bromophenol blue, 100mM DTT.

Variant of protocol used in: Keogh *et al* (2006) *Genes Dev* **20:**660-5
Keogh *et al* (2006) *Nature* **439:**497-501

Whole cell extracts for Western Blotting
10ml cultures were grown in YPD medium to an OD_{600} of 1.0. Cells were collected by centrifugation and washed with 20% TCA. All purification steps were performed on ice with pre-chilled solutions. Cell pellets were resuspended in 250µl 20% TCA and subjected to glass bead lysis. The suspension minus the glass beads was collected, 1ml of 5% TCA was added, and precipitated proteins collected by centrifugation. Pellets were washed with 750µl 100% ethanol and proteins solubilized in 50µl 1M Tris pH8.0 / 100µl 2X SDS-PAGE loading buffer (60mM Tris pH 6.8, 2% SDS, 10% glycerol, 100mM DTT, 0.2% bromophenol blue). After 5 minutes at 95ºC, insoluble material was removed by centrifugation and the supernatant analyzed further.