**Yeast RNA isolation from low cell number**

Work on ice and cool down benchtop centrifuge to 4C.

Spin cells in EtOH at maximum speed for 30s. Discard EtOH and wash with PBS. Wash again in PBS then remove all PBS.

On ice, re-suspend pellet in 50µl Lysis/Binding buffer (from mirVANA kit), transfer to 2ml bead beater tubes containing 50µl Zirconium beads

Lyse cells in bead beater in cold room, 5 cycles 30s on 6.5m/s, 30s ice

Add 250µl Lysis/Binding buffer, vortex to mix

Add 15µl miRNA Homogenate Additive, vortex, leave on ice for 10min

Add 300µl Acid Phenol:Chloroform (from Ambion, with the kit), vortex 30s

Centrifuge 5min top speed in the hood at room temperature, extract the upper phase into a 1.5ml LoBind tube

Add 2µl glycogen[[1]](#footnote-1) then 400µl room temperature 100% ethanol, vortex

Put at -20C for one hour

Spin 15min top speed in cold room

Remove supernatant

Add 1ml cold 70% ethanol, vortex, spin 30s at room temperature

Pour off ethanol

Spin 30s at room temperature

Remove residual ethanol with a pipette

Dry pellet for 5 minutes

Add 10µl water, vortex briefly to resuspend.

Quantify 0.2µl using PicoGreen RNA or Qubit.

1. vortex the glycogen stock – the glycogen can settle to the bottom of the tube… [↑](#footnote-ref-1)