

Extract mRNA for *Mnemiopsis* PartII

Lysis and m-RNA extraction with Dynabeads

Bead preparation

- mix the beads by shaking
- take 50 µl of resuspended beads per sample and transfer to a 1.5 ml tube (1 tube for each sample)
- place the tube on the magnetic stand, wait until liquid is clear
- remove liquid
- take tubes out of the stand, add equivalent amount of lysis-buffer and resuspend the beads
- place the tubes back on the magnetic stand wait until liquid is clear and remove liquid → beads are cleaned now
- take the beads off the magnet

Binding and washing

- thaw samples on ice
- add 1 µl of a 1:10 dilution of the spike-inns
- transfer the 300 µl lyses buffer with lysed embryos onto the aliquoted washed beads
- dissolve the bead pellet by pipetting up and down to mix well
- nutate it 5 min at RT
- place the beads on the magnet for 5 min
- remove liquid
- take the tubes off the magnet, add **600 µl washing buffer A**, resuspend the bead pellet by pipetting up and down
- place on the magnet for 2 min
- remove liquid
- take the tubes off the magnet and add another **600 µl washing buffer A**, resuspend the bead pellet by pipetting up and down
- place the tubes back on the magnet for 2 min
- remove liquid
- take the tubes off the magnet and add **300 µl washing buffer B**
- resuspend the bead pellet by pipetting up and down
- place the tubes on the magnet for 2 min
- remove liquid carefully
- take the tubes off the magnet and add **12.5 µl elution buffer** (Tris-HCl) onto the beads, make sure that all the beads are covered and all the beads are dissolved in the liquid
- place at 75 °C for 2 min
- place back on the magnet
- take the supernatant and transfer to a new tube
- store the tube on ice
- measure RNA concentration with the nanodrop or Bioanalyzer