

mRNA Isolation

Stock 20X SSC

Need 1.5 ml 0.5X SSC = 37.5 ul 20X + 1.463 ml depc H₂O

Need 1.5 ml 0.1X SSC = 7.5 ul 20X + 1.493 ml depc H₂O

Resuspend large tube of beads

Remove 600 ul and place in 1.5 ml tube

Magnet and remove supernatant

Wash 3X with 0.5 ml of 0.5X SSC (0.3 ml per wash)

Resuspend in 0.1 ml of 0.5X SSC

Total RNA volume 500 ul

Heat at 65C for 10 min

Add 3 ul oligo dT probe

Add 13 ul 20X SSC

Incubate 10 min at room temp

Add RNA mix to beads

Capture and remove supernatant

Wash 4X with 0.1X SSC (0.3 ml each)

Add 100 ul depc H₂O

Capture beads

Supernatant = mRNA eluant

Repeat with 150 ul depc H₂O

Final Volume 250 ul mRNA

Precipitate mRNA

Add 1/10 volume 3M Na acetate

Add 1X volume isopropanol

Incubate -20C overnight

Centrifuge top speed 10 min at 4C

Decant

Wash with 80% etoh at -20C

Resuspend in depc H₂O (13 ul)