A. Preparation of Dynabeads

1. Resuspend the Dynabeads thoroughly before use (vortex).
2. Transfer Dynabeads needed for all samples (using 20 µl Dynabeads per mRNA isolation) from the stock tube suspension, to an RNase-free microcentrifuge tube.
3. Place the tube on a Dynal magnet.
4. After 30 seconds (or when the suspension is clear) remove the supernatant.
5. Remove the tube from the magnet and pre-wash Dynabeads by resuspending in Lysis/Binding Buffer to the original volume by pipetting.
6. Place the tube on the magnet and remove supernatant.
7. Remove the tube from the magnet and resuspend the beads in Lysis/binding buffer to the original volume. Aliquot 20 µl suspension to each sample tube.

Note: Do not allow the Dynabeads to dry, as this may lower their capacity.

Note: Bring all buffers, except the 10 mM Tris-HCl, to room temperature prior to use.

B. Preparation of lysate from tissue

1. Grind frozen tissue sample (up to 5 mg depending on the tissue type) in a microcentrifuge tube, using a manual tissue grinder. Work quickly.
2. Add 100 µl Lysis/Binding Buffer and thaw sample while continuing to grind until complete lysis is obtained (approx. 1-2 min). A rapid lysis in the Lysis/Binding Buffer is critical to obtain undegraded mRNA. If the raw extract is noticeably viscous a shearing step might be beneficial.
3. Spin the lysate for 30-60 seconds in a microcentrifuge to remove debris.

C. mRNA isolation for PCR amplification

4. The 100 µl lysate is combined with the 20 µl prewashed Dynabeads Oligo(dT)25.
5. Mix by pipetting up and down a few times.
6. Place the tube on a sample mixer or roller for 5 min at RT to allow the mRNA to anneal to the Dynabeads with continuous rotation.
7. Place the sample tube on the magnet and discard the supernatant.
8. Remove the sample tube from the magnet and resuspend the Dynabeads-mRNA complex in 100 µl Washing Buffer A by careful pipetting.
9. Place the sample tube on the magnet and discard the supernatant.
10. Repeat steps 8 - 9 once.
11. Resuspend the Dynabeads-mRNA complex in 100 µl Washing Buffer B.
12. Transfer the suspension to a new tube.
13. Place the new sample tube on the magnet and discard the supernatant.
14. Resuspend the Dynabeads-mRNA complex in 100 µl Washing Buffer B.
15. Place the sample tube on the magnet and discard the supernatant.
16. Remove the sample tube from the magnet and add 10-20 µl 10 mM Tris-HCl and incubate at 65-80°C for 2 minutes.
17. Place the tube on the magnet and immediately transfer the supernatant to a new microcentrifuge tube.