THE INSTITUTE FOR GENOMIC RESEARCH *Standard Operating Procedure*			
TITLE: **POLY A+ RNA ISOLATION FROM TOTAL RNA BY DYNABEADS® OLIGO (dT)$_{25}$**			PAGE: 1 of 5
SOP #: M014	REVISION LEVEL: 0		EFFECTIVE DATE:
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1. PURPOSE

This protocol describes the isolation of poly A^{+} RNA from total RNA suspended in RNAse-free water using Dynabeads® Oligo (dT)$_{25}$.

2. SCOPE

The procedure is used by the Human, Mouse, and Arabidopsis Microarray projects under the supervision of John Quackenbush within the Mammalian Genomics Department.

3. MATERIALS

3.1 Dynabeads® Oligo (dT)$_{25}$ (Dynal Biotech; Cat# 610.05)
3.2 EDTA (Sigma; Cat# E2628-2)
3.3 Lithium Chloride (Sigma; Cat# L7026)
3.4 Tris-HCl pH 7.5 (Sigma; Cat# 93372)
3.5 DEPC-Treated Water (Ambion; Cat#9920)
3.6 Dry Bath Incubator and 1.7 mL Heating Block (Fisher Scientific; Cat# 11718 and 11781-9)
3.7 Magnetic Particle Concentrator (MPC), 1.7 mL (Dynal Biotech; Cat# 120.20)
3.8 Microcentrifuge (Eppendorf; Model# 5415D)
3.9 Pipette Tips, 20 µL
3.10 Pipette Tips, 200 µL
3.11 Tubes, 1.7 mL
3.12 Vortex

4. REAGENT AND EQUIPMENT PREPARATION

4.1 Binding Buffer

4.1.1 Prepare 50 mL of binding buffer with the following final concentrations: 20 mM Tris-HCl at pH 7.5, 1 M LiCl, and 2 mM EDTA. Use RNAse-free water.

4.1.2 Bring to room temperature prior to use. Store the remaining portion at 4° C.

4.2 Wash Buffer

4.2.1 Prepare 50 mL of wash buffer with the following final concentrations: 10 mM Tris-HCl at pH 7.5, 0.15 M LiCl, and 1 mM EDTA. Use RNAse-free water.

4.2.2 Bring to room temperature prior to use. Store the remaining portion at 4° C.

4.3 Elution Buffer

4.3.1 Prepare 15 mL of elution buffer with the following final concentration: 10 mM Tris-HCl at pH 7.5. Use RNAse-free water.

4.3.2 Keep buffer on ice just prior and during use. Store the remaining portion at 4° C.

4.4 Dry Bath Incubators

4.4.1 Adjust one dry bath incubator to 70° C.

4.4.2 Adjust another dry bath incubator to 85° C.

5. PROCEDURE

5.1. Bead Preparation

Note: According to the manufacturer, "Do not leave the Dynabeads® Oligo $(dT)_{25}$ unsuspended for a long period of time, as drying of the Dynabeads® Oligo $(dT)_{25}$ may lower their [binding] capacity."

5.1.1 Vortex Dynabeads® to resuspend. Be sure that the suspension is a uniform brown color.

5.1.2 Transfer 200 µL of bead suspension per 100 µg of total RNA into a 1.7 mL tube.

5.1.3 Place the tube in the Magnetic Particle Concentrator (MPC) for 1 minute. Check for good separation of beads and solution.

5.1.4 Remove as much of the storage buffer as possible from the tube by pipette without disturbing the pellet. Discard storage buffer.

5.1.5 Remove tube from MPC and completely resuspend the beads in 200 µL of binding buffer. Mix by pipetting.

5.1.6 Place tube in MPC for 1 minute. Remove and discard binding buffer.

5.1.7 Remove tube from MPC and add binding buffer in a volume equal to that of the total RNA sample being extracted (i.e. add 250 µL of binding buffer for a 250 µL sample of total RNA).

5.2 poly A^+ RNA Isolation

5.2.1 Incubate total RNA at 70° C for 5 minutes, and then add total RNA to bead-binding buffer suspension from step 5.1.7. Mix well by pipetting.

5.2.2 Leave at room temperature for 10 minutes to allow the poly A^+ RNA to hybridize to the beads.

5.2.3 Place tube in the MPC for 2 minutes.

5.2.4 Transfer buffer-sample mixture to a sterile tube, store at room temperature, and set aside for later re-extraction of any remaining poly A^+ RNA.

5.2.5 Remove tube with beads from MPC. Resuspend beads in 200 µL of wash buffer. Mix by pipetting.

5.2.6 Place tube in MPC for 1 minute and discard wash buffer without disturbing the pellet.

5.2.7 Repeat wash (steps 5.2.5 and 5.2.6).

5.2.8 Resuspend beads in at least 20 µL of elution buffer per 100 µg of total RNA.

5.2.9 Incubate tube at 85° C for 2 minutes.

Note: The following two steps must be performed as quickly as possible to prevent RNA degradation and maintain Dynabead® Oligo $(dT)_{25}$ integrity. (Please refer to note in 5.1.)

5.2.10 Immediately place tube in MPC and quickly transfer the elution to a sterile tube without disturbing the pellet. The elution is the poly A^+ RNA. Place poly A^+ RNA on dry ice.

5.2.11 Immediately resuspend beads in 200 µL of binding buffer and mix.

5.3 Bead Preparation for Re-Extraction

Note: Additional extractions may be performed to isolate any remaining poly A^+ RNA from the sample. This may be desirable if total RNA concentration is relatively high. Usually only 1 round of re-extraction is necessary to isolate the majority of the poly A^+ RNA from the sample. Follow steps 5.3.1 to 5.3.4 to prepare beads for each round of re-extraction.

Note: The beads may be used for up to 5 rounds of extraction (of the same sample). New beads should be used for different samples to prevent cross contamination.

Note: Subsequent elutions may be combined with the first-round elution or kept separately.

5.3.1 Place tube into MPC for 1 minute.

5.3.2 Remove and discard buffer without disturbing pellet.

5.3.3 Remove tube from MPC and resuspend beads in the buffer-sample solution from step 5.2.4.

5.3.4 Proceed with the protocol from steps 5.2.2 until 5.2.11 for each round of re-extraction.

5.4 Clean-Up

5.4.1 Discard buffer-sample solution and beads.

5.4.2 Use spectrophotometer to measure concentration (260nm) and ratio (260nm vs. 280nm) of poly A^+ RNA. Good quality poly A^+ RNA have ratios between 1.9 and 2.1. Typical yields range between 0.5% and 3% of total RNA yield.

5.4.3 Store poly A^+ RNA at -80° C as soon as possible.