Neuroinformatics

Lab: Sample preparation for microarray data

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- **Lab: Sample preparation for microarray data**

How to prepare total RNA sample for microarray analysis?

1. Cell culture

2. RNA isolation

- Remove growth media from culture dish, wash cell with DEPC-PBS, and add 1 mL TRIzol Reagent
- lysis the cells directly in the culture dish by pipetting the cell up and down several times
- Incubate the homogenized sample for 5 m at RT to permit samples complete dissociation of the nucleoprotein complex
- Transfer the homogenized sample to new tube
- Add 0.2 mL of chloroform per 1 mL of TRIzol used for homogenization. Cap the tube securely
- Vortexing for 15 seconds
- Centrifuge at 13,000rpm for 15 minutes at 4°C.
- Remove the aqueous phase (500ul) of the sample by angling the tube at 45° and pipetting the solution out. (Avoid drawing any of the interphase or organic layer into the pipette when removing the aqueous phase.)
- Add 0.5 mL of 100% isopropanol to the aqueous phase
- Invert the tubes x 6 times
- Centrifuge at 13,000rpm for 10 minutes at 4°C
- Remove the supernatant from the tube, leaving only the RNA pellet.
- Wash the pellet, with 1 mL of 70% ethanol (-20°C)
- Invert the tubes x 3 times, then centrifuge the tube at 13,000rpm for 1 m at 4°C.
- Discard the supernatant
- Centrifuge the tube at 13,000rpm for 1 minutes at 4°C
- Discard remaining EtOH using pipette
- Air dry the RNA pellet for 7–10 minutes. (Do not allow the RNA to dry completely)
- Resuspend the RNA pellet in RNase-free water (20ul)-vortexing and tapping
- several times through a pipette tip.
- Proceed to downstream application, or store at –70°C
- RNA quantification

How to prepare total RNA sample for microarray analysis?

3. DNase I treatment

- Prepare the mixture as below in a 1.5ml tube
- 20 ~ 50ug RNA
- 5ul DNase I buffer (10X)
- 1ul DNase I (2U/ul)
- 0.5ul of RNase inhibitor (40U/ul)
- Fill up to 50ul (with D.W.)
- Incubate the mixture at RT for 10min
- Add 5 volume of buffer MS to 1 volume of sample and mix thoroughly
- Transfer the mixture to a micro spin column
- Centrifuge at >10,000 x g for 30s and discard the pass-through
- Apply 700ul of buffer RNW
- Centrifuge at >10,000 x g for 30s and discard the pass-through
- Centrifuge at >10,000 x g for an additional 1 min to remove residual wash buffer
- Transfer micro spin-column to a new 1.5ml tube
- Apply 20~50ul RNase-free D.W. to the center of the membrane in the micro spin-column.
- let it stand for 1min, and centrifuge at >10,000 x g for 1 min
- RNA quantification

- * QC criterion for microarray:
- The amount of total RNA
- Protein contamination (260/280 ratio)
- Organic contamination (260/230 ratio)
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- **4. Microarray experiment**