Isolation of mRNA

Note; This technique is FYI as newer protocols have been greatly simplified.

Overview of the general techniques:

Guanidium Salt: Rapidly and completely kills and lyses cells (deactivates many enzymes that may degrade the cells.)

CeCl gradient is used to isolate fraction with RNA and then ppt w/ Et-OH.

Into columns with oligo Poly T (to catch poly A tails of mRNA)

Spin elutent, pellet, resuspend in an isotonic solution.

Technique in more detail.

- Guanadinium isothiocyanate (Oxygen inhibitor and proteinase inhibitors.) Kills cells on contact.
- Tissue was then homogenized using tissue homogenizer.
- Alternative technique would be to gently rupture the cells and to isolate the nuclei (intact)
- *Either way… speed is of the essence.*
- All glassware and appropriate chemicals were treated with DEPC. This chemical will destroy enzymes and when heated degrades to relatively non toxic CO2 AND ET-OH.
- With the knowledge that RNA has a greater buoyant density that DNA, samples were added to gradient centrifuge tube (w/ CeCl)
- RNA will pelletize and top fractions are removed (with appropriate rinsing)
- Resuspend pellet in water, acetic acid (Both w/ DEPC) and ppt with Et-OH (note no DEPC) .
- Note: Lots of others steps to purify FYI… Kits have greatly simplified the process.
- Assay purity with Abs 260/280 ratio: 260 is absorbed by RNA and 280 is absorbed by protein.
- Samples are run through column (with 5X the volume of the column.)
- Samples are heated and quenched on ice (to ensure SSRNA) and run through with loading buffer (contains lots of salt to encourage binding of mRNA to column.)
- Sample passed through twice to get as much product bound as possible.
- Washed twice and eluted with low salt (to disassociate mRNA from column.)
- Purity again assessed by abs 260/280 and fractions with greatest and purist mRNA are ppt and stored.
- Now mRNA can be used for creating of cDNA library.