Straight A's mRNA Isolation System

Product	Cat. #	Price
Straight A's mRNA Isolation System	69963-1	$250
Straight A's mRNA Isolation System plus Separation Stand	69962-1	$325

The Straight A's mRNA Isolation System provides sufficient components to isolate poly(A)$^+$ RNA from up to 2g animal tissue, 4g plant tissue, or 4×10^8 cultured animal cells.

Components:

- 100ml Lysis Buffer
- 3 x 50ml Wash Buffer
- 8ml Magnetight Oligo(dT) Particles, 10mg/ml
- 1.6ml 1M DTT
- 250µl Glycogen, 10mg/ml
- 1ml 3M sodium acetate, pH 5.2
- 2 x 1.5ml Nuclease-free Water
- Protocol

The Straight A's mRNA Isolation System plus Separation Stand contains all of the above components and includes one Magnetight Separation Stand.

Components available separately:

Product	Size	Cat. #	Price
Magnetight Separation Stand	1	69964-1	$85
Magnetight Oligo(dT) Particles	8ml	69593-1	$140
Straight A's Lysis Buffer	100ml	69960-1	$35
Straight A's Wash Buffer	3 x 50ml	69961-1	$50

Related Products	Page
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Ig-Prime® System	50
RNA Markers	54

Additional

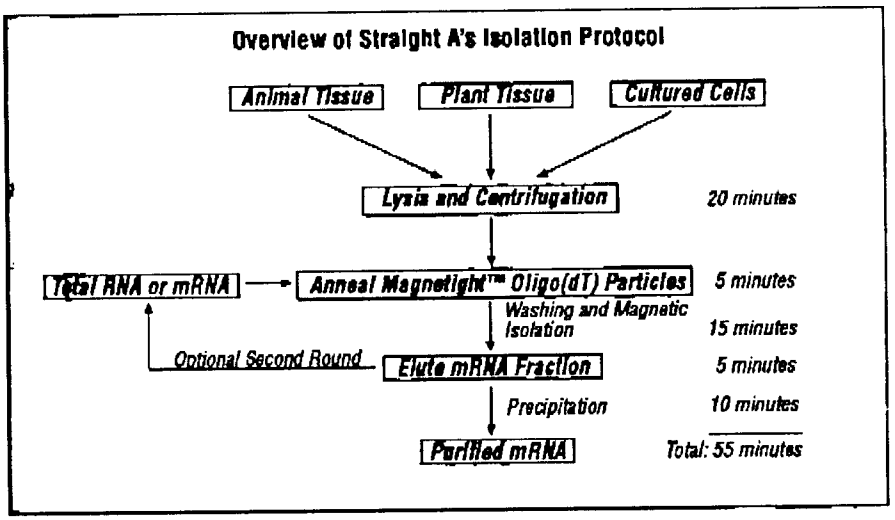
Protocol TB103
InNovatio No. 2

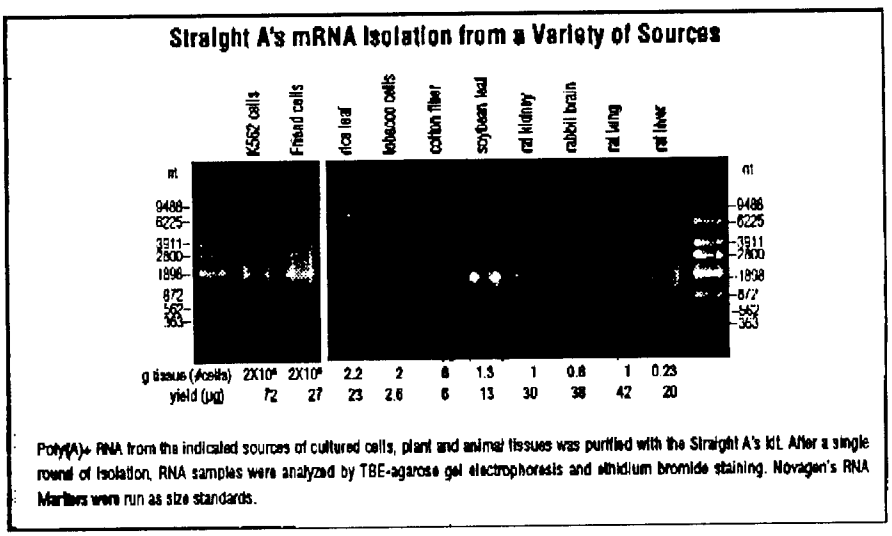
Post-it® Fax Note 7671 Date 12/13 # of pages ▶ 5
To Dr. Rausmag From Novagen
Co./Dept. Co. 1-800-207-0144
Phone # Phone #
Fax # 608-[illegible]-6969

The Straight A's™ mRNA Isolation System is designed for the rapid isolation of high quality poly(A)$^+$ RNA from various sources (1). The System provides a simple, rapid purification method using Magnetight™ Oligo(dT) Particles and a set of lysis and wash buffers suitable for both animal and plant tissues as well as cultured cells. The key component of the kit is the paramagnetic particles containing covalently attached oligo(dT)$_{25}$. The coated magnetite beads have been treated to minimize non-specific interactions and allow quantitative recovery of intact RNA. The diagram below shows the isolation protocol, including the time needed for each step. The entire procedure requires less than one hour and is applicable to fresh or frozen tissue. The Straight A's System components can be scaled to the amount of starting material available. Up to one gram of animal tissue (or plant tissue equivalent) per isolation and a total of two grams of animal tissue can be processed per kit. Since ribonuclease activity is effectively inhibited during tissue lysis, several samples can be processed in parallel without degradation of mRNA. As the flow diagram also indicates, the system can be applied to the selection of poly(A)$^+$ RNA from total RNA samples, or for further purification of mRNA isolated by other methods. Magnetight Oligo(dT) Particles can be reused several times to provide a very economical means of isolating high purity full length mRNA.

Reference

1. McCormick, M. and Hammer, B. (1994) *InNovations* 2, 8-11.





Poly(A)+ RNA from the indicated sources of cultured cells, plant and animal tissues was purified with the Straight A's kit. After a single round of isolation, RNA samples were analyzed by TBE-agarose gel electrophoresis and ethidium bromide staining. Novagen's RNA Markers were run as size standards.

Advantages

Speed

- Tissue directly to poly(A)⁺ RNA in less than 1 hour.

Purity

- Routinely > 75% poly(A)⁺ after one round, > 95% after two rounds (qualified for random primer cDNA synthesis).
- Intact mRNA ready for any application.

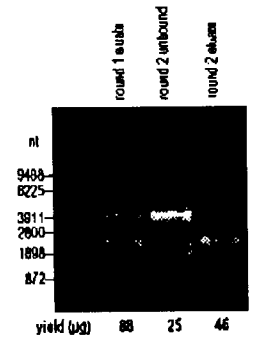
Simplicity

- No phenol or guanidine required.
- Minimal centrifugation; rapid magnetic separations (< 1 minute).
- Same system works with animal tissue, plant tissue, or cultured cells.
- Suitable for second round isolation and poly(A)⁺ selection from total RNA.
- Scales up to 1g animal tissue (2g plant) per isolation.

Economy

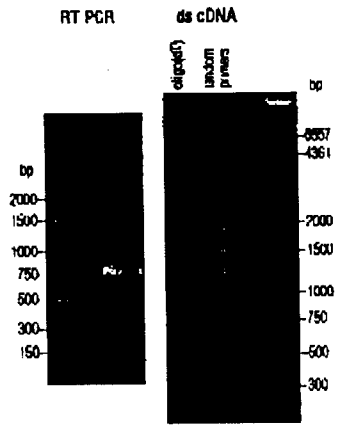
- Reusable Magnetight™ Oligo(dT) Particles
- Reasonably priced magnetic particles and separation stand.
- Individual components available separately.

Increased mRNA Purity after Two Rounds of Isolation



Rat liver mRNA was purified with the Straight A's kit according to the standard protocol. The indicated samples were analyzed by TBE-agarose gel electrophoresis and ethidium bromide staining. Novagen's RNA Markers were run as size standards.

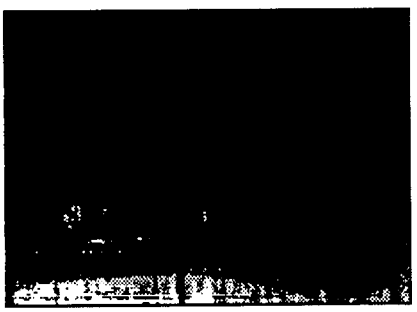
RT PCR and cDNA Synthesis with Straight A's mRNA



Poly(A)⁺ RNA purified from rabbit brain and rat liver was used RT PCR* and cDNA synthesis, respectively. First strand cDI was synthesized with MMLV reverse transcriptase usin defined primer (RT PCR), oligo(dT) and random primers indicated. For RT PCR, amplification was performed after t addition of the second primer under standard conditions, anc 5µl sample analyzed. For cDNA, second strand synthesis w performed and cDNA analyzed. Both gels were 1% TAE-agarc stained with ethidium bromide. Novagen's PCR Markers a λ *Hind* III markers were run as size standards.

* PCR is covered under patents owned by Hoffmann-La Roche

Magnetight Separation Stand



Stand with embedded magnet for isolation of Magnetight Oligo(dT) Particles. Versatile design accommodates several tube sizes: one 50ml or 15ml centrifuge tube; four 1.5ml microcentrifuge tubes.

ARTICLE

Straight A's™ mRNA Isolation System: Rapid, High-Quality Poly(A)+ RNA from Diverse Sources

Mark McCormick and Beth Hammer — Novagen, Inc.

In recent years, magnetic particle-based methods for batch-wise affinity purification of messenger RNA molecules have gained acceptance due to their convenience and speed. Initial systems were limited to separation of mRNA (poly(A)+ RNA) from contaminating RNA species, while more recent purification methods allow direct isolation of mRNA from a tissue or cell lysate in a single step without organic extractions(1,2). When compared to oligo(dT) cellulose and other traditional chromatography-based methods, magnetic particle systems represent fewer technical barriers for mRNA isolation. Some commercially available systems are, however, inconsistent in mRNA yield and purity and are often entirely unsuitable for some tissue sources, most notably plant tissue.

The Straight A's mRNA Isolation System provides a simple, rapid purification method using Novagen's Magnetight™ Oligo(dT) Particles and a set of lysis and wash buffers suitable for both animal and plant tissues as well as cultured cells. The key component of the kit is the paramagnetic particles, which are highly uniform beads containing covalently attached oligo(dT)$_{25}$. The coated magnetite beads have been treated to minimize non-specific interactions and allow quantitative recovery of intact RNA. Figure 1 shows a flow diagram of the isolation protocol, including the time needed for each step. The entire procedure requires less than one hour and is applicable to fresh or frozen tissue. The Straight A's System components can be scaled to the amount of starting material available. Up to one gram of animal tissue (or plant tissue equivalent) per isolation and a total of two grams of animal tissue can be processed per kit. Since ribonuclease activity is effectively inhibited during tissue lysis, several samples can be processed in parallel without degradation of mRNA. As the flow diagram also indicates, the system can be applied to the selection of poly(A)+ RNA from total RNA samples, or for further purification of mRNA isolated by other methods. Magnetight Oligo(dT) Particles can be reused several times to provide a very economical means of isolating high purity full length mRNA.

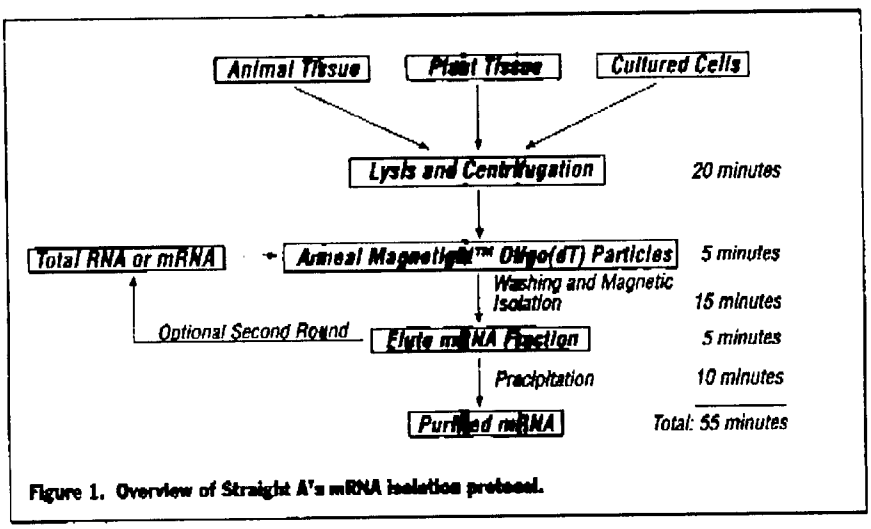


Figure 1. Overview of Straight A's mRNA isolation protocol.

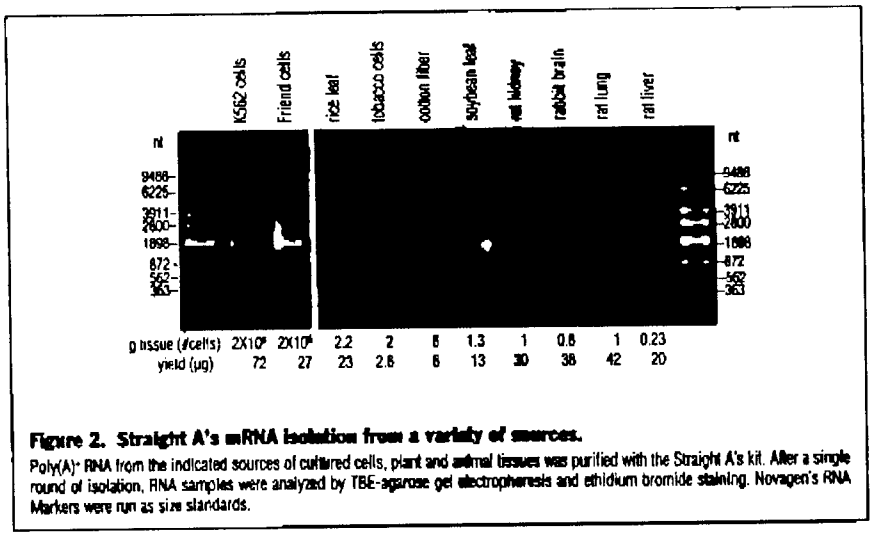


Figure 2. Straight A's mRNA Isolation from a variety of sources.

Poly(A)+ RNA from the indicated sources of cultured cells, plant and animal tissues was purified with the Straight A's kit. After a single round of isolation, RNA samples were analyzed by TBE-agarose gel electrophoresis and ethidium bromide staining. Novagen's RNA Markers were run as size standards.

mRNA Purification from a Variety of Sources

Commercial mRNA purification kits are generally compatible with one type of tissue and are rarely suitable for both plant and animal sources. Systems based on guanidine isothiocyanate are particularly poor for plant tissue samples and yields with these systems are usually very low. Furthermore, CuSCN-based methods can produce RNA pellets that are notoriously difficult to dissolve and can leave behind residual salts that may inhibit enzymic reactions. In contrast, the Straight A's System can isolate mRNA from a broad range of sources using the same protocol and reagents, without the disadvantages associated with CuSCN. Figure 2 shows gel analysis of RNA samples purified from plant and animal tissues and cultured cells. In all cases, a smear of RNA is seen corresponding to the size range expected for intact mRNA. Although rRNA bands of various intensities are also observed, the mRNA is typically greater than 75% pure after a single round of binding and elution from the Magnetight Oligo(dT) Particles. With most other commonly used methods, such as oligo(dT) cellulose chromatography, a single round of purification typically results in preparations containing 50% or more rRNA contaminants. The Straight A's System is particularly effective

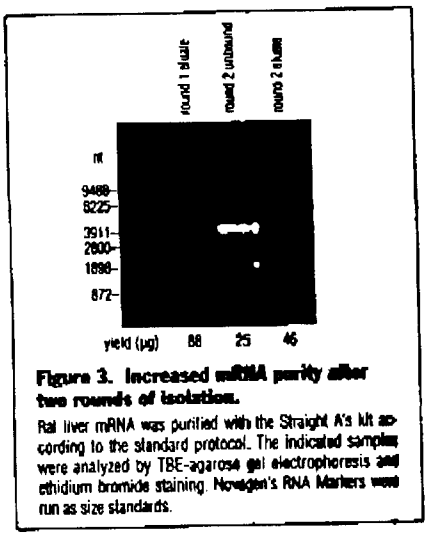


Figure 3. Increased mRNA purity after two rounds of isolation.

Rat liver mRNA was purified with the Straight A's kit according to the standard protocol. The indicated samples were analyzed by TBE-agarose gel electrophoresis and ethidium bromide staining. Novagen's RNA Markers were run as size standards.

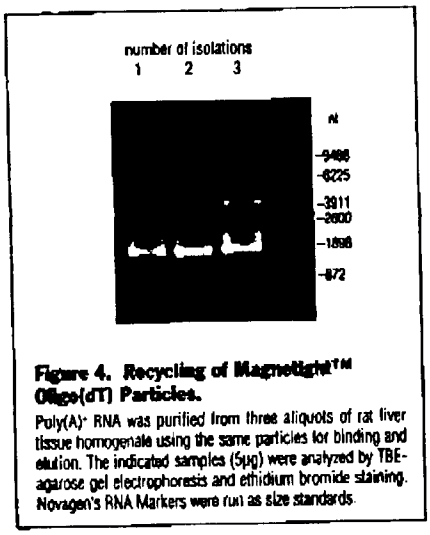


Figure 4. Recycling of Magnetight™ Oligo(dT) Particles.

Poly(A)+ RNA was purified from three aliquots of rat liver tissue homogenate using the same particles for binding and elution. The indicated samples (5µg) were analyzed by TBE-agarose gel electrophoresis and ethidium bromide staining. Novagen's RNA Markers were run as size standards.

in isolating mRNA from extremely low abundance sources such as cotton fiber, for which few effective methods exist. In addition, the protocol can be adjusted to the amount of starting material and can process up to 1 gram of animal tissue, 4 grams plant tissue or 2 x 108 cultured cells in a single isolation.

High Purity mRNA for Demanding Applications

Moderate levels of rRNA contamination will not interfere with a number of poly(A)+ RNA applications (*e.g.*, RT-PCR, Northern blotting, etc.), other than to cause an overestimation of mRNA yield based on UV absorbance. However, in some cases (*e.g.*, using random primers for cDNA synthesis) more complete removal of rRNA is desirable.

Figure 3 illustrates the additional purification possible with a second round of isolation using the Straight A's kit. Rat liver mRNA isolated from a single round of purification was subjected to a second round of binding, washing and elution from the Magnetight Oligo(dT) Particles, resulting in the specific removal of rRNA (seen in the unbound fraction from round 2). Material eluted from the second round of purification contained no visible rRNA bands by gel analysis. In fact, a prominent ~2.2kb mRNA band and several other abundant mRNA species were apparent in the absence of rRNA. The yields seen in the unbound and eluted fractions of the second round confirm that approximately 28% of the first round material was in fact rRNA, and that 81% of the sample was accounted for after the second round. The ability to rapidly perform a second round of purification with such high recovery is a unique feature of the Straight A's System.

Reusable Solid Phase

The stability of the oligo(dT) covalent linkage and the low non-specific binding properties of the Magnetight Oligo(dT) Particles make them ideally suited for repeated use. Figure 4 shows mRNA samples isolated from three successive purifications from rat liver lysate using the same sample of particles. There was no loss of particle binding capacity and no decrease in sample purity after three isolations. If desired, the particles can be treated with 0.1N NaOH between runs to hydrolyze RNA and minimize carryover. To make the Straight A's System more economical, the lysis and wash buffers are available separately to provide the maximum yield from system components.

Functional Performance of mRNA Samples

To test the quality and functionalit f
mRNA samples purified using the Str t
A's System, four common applications e
performed, including Northern blot ;
in vitro translation, RT PCR and cl A
synthesis.

Figure 5 shows a stained formaldeh -
agarose gel and corresponding Northern it
of several RNA samples. As also show n
Fig. 3, the stained gel shows that a se d
round of binding and elution remove e
residual 18s rRNA visible after the ir
round of purification from rat liver. .e
Northern blot shows strongly hybrid g
bands of the expected sizes of β-a n
mRNA (rat = 2000nt; human = 1765nt s-
tected with a homologous ^{32}P-labeled a ie
β-actin anti-sense RNA probe. In contra o
other isolation methods we have used ie
Straight A's system produced no evider of
mRNA degradation by this sensitive ot
analysis.

Figure 6 (p. 10) shows the *in vitro* t s-
lation products obtained with Red N t®
Lysate programmed with rat liver pol)*
RNA from a single round of isolation th
the Straight A's kit. The gel reveals a ge
number of protein bands covering a rar of
sizes consistent with those observed i ie
steady-state tissue (Coomassie stained el,
left panel). This analysis indicates tha ie
mRNA is free of translation inhibitor id
appears to consist of intact molecules.

Perhaps the widest use of poly(A)+ IA
is for cDNA synthesis, either for RT PC or

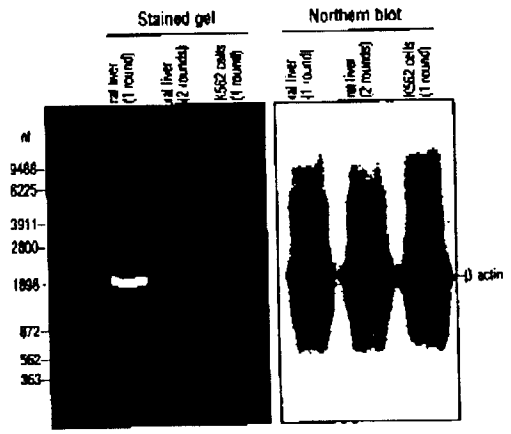


Figure 5. Northern blot of rat liver and K562 mRNA hybridized with actin probe.

Poly(A)+ RNA form the indicated sources was purified with the Straight A's kit. Duplicate samples (5µg) were run on a 1% for e-
hyde-agarose gel. Half of the gel was stained with SYBR Green II (Molecular Probes) and the other half blotted onto a nylon mer e.
The blot was hybridized with a ^{32}P-labeled mouse beta-actin anti-sense RNA probe synthesized with T7 RNA polymerase accor to
standard procedures and exposed to X-ray film for 2 hours. Novagen's RNA Markers were run as size standards.

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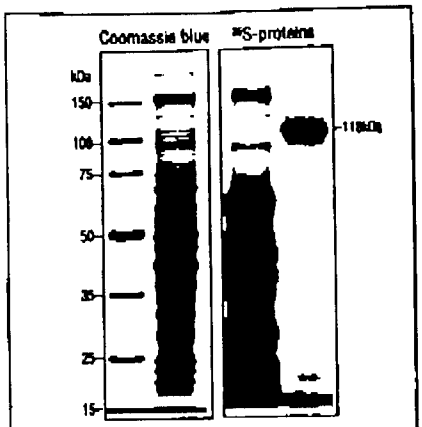

Figure 6. Translation of rat liver mRNA.
Poly(A)$^+$ RNA purified from rat liver (1 round) was translated in the presence of ^{35}S-methionine with Red Nova^{gen} Lysate. A 2µl sample of the reaction was analyzed by SDS-PAGE and fluorography, shown in right panel with 118kDa marker. The left panel shows a gel profile of total rat liver proteins stained with Coomassie blue. Perfect Protein^{TM} Markers were run as size standards.

cloning purposes. Figure 7 demonstrates the performance of mRNA isolated with the Straight A's System in both applications. The left gel shows RT PCR amplification of a 765 base rabbit brain mRNA segment with two specific primers. The gel on the right shows total double stranded cDNA synthesized from rat liver mRNA purified with two rounds of binding and elution from the Magnetight Oligo(dT) Particles. Oligo(dT) and random hexamer primers were used, and both produced a broad distribution of cDNAs extending beyond the 6.5kbp marker. Note the appearance of individual cDNA bands especially in the oligo(dT)-primed sample, which may represent abundant mRNAs in the population.

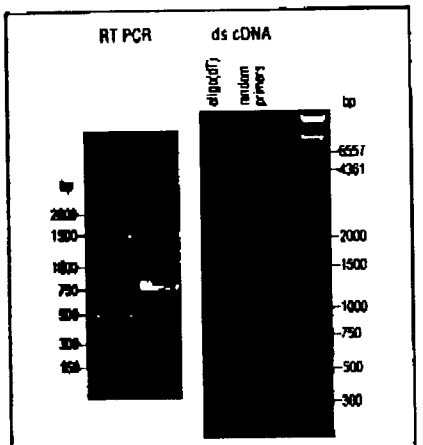

Figure 7. RT PCR and cDNA synthesis.
Poly(A)$^+$ RNA purified from rabbit brain and rat liver was used for RT PCR* and cDNA synthesis, respectively. First strand cDNA was synthesized with MMLV reverse transcriptase using a defined primer (RT PCR), oligo(dT) and random primers as indicated. For RT PCR, amplification was performed after the addition of the second primer under standard conditions, and a 5µl sample analyzed. For cDNA, second strand synthesis was performed and cDNA analyzed. Both gels were 1% TAE-agarose stained with ethidium bromide. Novagen's PCR Markers and lambda Hind III markers were run as size standards.

Summary

The Straight A's mRNA Isolation System offers a number of improvements over other methods, including greater ease of use, better reproducibility, and broader applicability. The following list describes its specific advantages and versatile applications. The Straight A's System:

- Isolates mRNA from plant tissue, animal tissue or cultured cells
- Produces highly intact RNA that can be used in the most stringent procedures
- Processes tissue to finished mRNA in less than 1 hour
- Can be used for large (1g) or small scale isolations
- Purifies mRNA from samples of total RNA
- Allows a second round of isolation for elimination of residual rRNA
- Permits recycling of magnetic particles
- Can be used with either fresh or frozen tissue
- Is compatible with several tissue homogenization methods, including Dounce and Polytron, with equivalent yield and purity (data not shown)
- Uses no GuSCN, β-mercaptoethanol or phenol
- Requires no high speed centrifugation, uses sterile disposable tubes only
- Allows efficient precipitation by using low elution volumes (5 minutes vs. overnight for other methods)
- Includes RNase-free glycogen and sodium acetate solutions for precipitation
- Is less expensive than other commercial kits

References

1. Jakobsen, K.S. Breivold, E. and Hornes, E. (1990) *Nucleic Acids Res.* **18**, 3669-3674.
2. Hornes, E. and Korsnes, L. (1990) *Genet. Anal. Techniques Appl.* 7, 145-150.

Acknowledgments

We thank Laura Crow (Agracetus), Michael Hanaway (University of Wisconsin Hospital & Clinics), Erika Meyer and William Fahl (McArdle Laboratory) for tissue and cell samples used for this work.

** The PCR process is covered by patents owned by Hoffmann-LaRoche.*