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## EXPRESSION

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# Human RNA Products That Facilitate Gene Expression Studies

**P**urification of quality RNA is an important first step in the study of RNA processing, gene expression, gene discovery, and gene isolation. Because RNA is easily degraded by mechanical shearing and by ribonuclease cleavage, it is difficult and time consuming to isolate intact RNA molecules. In addition, obtaining human tissues can be difficult. Here, we demonstrate the use of a variety of RNA blots and RNA samples from a range of human tissues including normal, tumor, and fetal tissues.

## METHODS

The GIBCO BRL® SEARCHLIGHTS™ and NORTHERN LIGHTS™ Blots were rehydrated with 5 ml of 4X SSC in a 50-ml conical tube. The SSC was discarded and the blots were prehybridized with 3 ml of hybridization buffer (48% deionized formamide, 4.8X SSPE, 5X Denhardt's solution, 0.1 mg/ml salmon sperm DNA, 10% dextran sulfate, and 0.2% SDS) in a hybridization incubator at 45°C for 2 to 4 h. The prehybridization solution was discarded and the blots hybridized at 45°C for 16 to 20 h in 3 ml hybridization buffer containing  $2 \times 10^6$  cpm of probe/ml. The blots were washed twice in 200 ml wash buffer 1 (2X SSC, 0.1% SDS) at room temperature for 5 min. The blots were washed once in 200 ml wash buffer 2 (0.25X SSC, 0.1% SDS) at 65°C for 30 min. The blots were air dried and exposed to Kodak® Biomax® film.

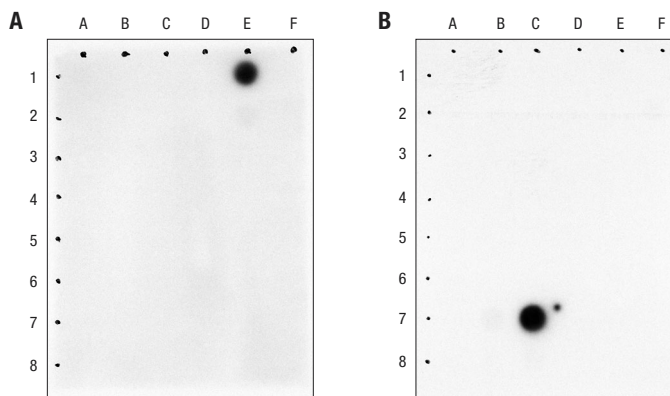
For reprobing, the blots were soaked in TE with 1% SDS at room temperature for 2 min, then boiled for 5 to 10 min, cooled to room temperature, and the strip buffer discarded. The blots were washed once with 4X SSC for 1 min, dried, and exposed to film overnight. If the background was high, the stripping was repeated.

## RESULTS AND DISCUSSION

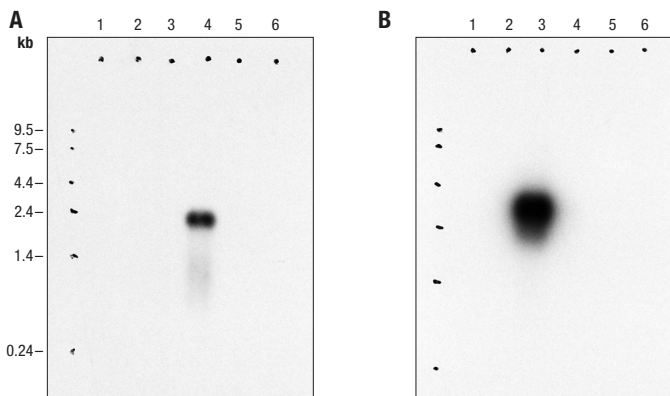
The SEARCHLIGHTS Multiple RNA Dot Blot was used to identify the tissue-specific expression of 2 moderately expressed (~1/10,000) genes (figure 1). As expected, the uromodulin (1) probe (a kidney-specific transcript) gave signal only with the kidney RNA dot. This blot was stripped and hybridized to a second probe for human serum albumin (2). Again as expected, signal was seen from the liver RNA dot. As a control, the dot blot was hybridized with a

labeled human 18S ribosomal DNA probe. The signal for each RNA dot was similar, demonstrating that equal quantities of RNA were in each dot (*data not shown*).

To further characterize the tissue-specific genes, the NORTHERN LIGHTS Blots containing human kidney and liver RNA were probed (figure 2). The uromodulin probe detected the full-length 2.3-kb transcript in the kidney mRNA. The full-length 2.6-kb serum albumin transcript was identified in the liver mRNA.



**FIGURE 1.** Tissue-specific gene detection with the SEARCHLIGHTS™ Human Multiple RNA Dot Blot. *Panel A.* The blot was hybridized to the <sup>32</sup>P-labeled uromodulin DNA probe and exposed without an intensifying screen at -80°C for 4 h. *Panel B.* The blot was stripped and hybridized to the <sup>32</sup>P-labeled serum albumin DNA probe and exposed with an intensifying screen at -80°C for 16 h. The blot has ink spots that were transferred manually to the film to mark the rows and columns.



**FIGURE 2.** Characterization of transcripts with NORTHERN LIGHTS™ Multiple mRNA Blots. *Panel A.* NORTHERN LIGHTS Blot III was hybridized to the <sup>32</sup>P-labeled human uromodulin probe and exposed without an intensifying screen at -80°C for 2 h. *Panel B.* NORTHERN LIGHTS Multiple mRNA Blot I was hybridized to the <sup>32</sup>P-labeled human serum albumin probe and exposed without an intensifying screen at -80°C for 2 h. The blots have ink spots on the left side corresponding to RNA molecular weights of 9.5, 7.5, 4.4, 2.4, 1.4, and 0.24 kb to facilitate size estimation. These dots were transferred manually to the film.

The NORTHERN LIGHTS Blots can be stripped and reprobed at least 3 times (figure 3). The first probe for  $\beta$ -actin was detected in all lanes (1.8-kb transcript). The second probe for transcription elongation factor 1  $\alpha$  (EF 1 $\alpha$ ) (5) was detected in every lane (2.1-kb transcript). The 2 MAP4 mRNA transcripts at 5.5 kb and 6.5 kb were detected.

After determining the tissue where the gene was expressed and the transcript size of the desired genes, further analysis is generally necessary; for example, full-length cDNA is isolated for functional genomic studies. High-quality total RNA and mRNA from the appropriate adult human tissues allow production of cDNA or RT-PCR products to isolate the full-length cDNA. The quality of GIBCO BRL RNA from human tissues is demonstrated by denaturing agarose gel electrophoresis (figure 4). The 28 S to 18 S ratios of the total RNAs were  $\geq 1.5:1$ . The  $A_{260/280}$  ratios were  $>1.8$ . Additionally, the first-strand cDNA yields for all 13 mRNA products were 15% to 30%. cDNA libraries have been constructed from several mRNAs and have been used successfully to identify rare genes (data not shown).

The ability to isolate large cDNAs from total RNA was demonstrated using RT-PCR to amplify an 8.5-kb fragment of adenomatous polyposis coli (APC), a moderately abundant gene (figure 5). Also, the 10-kb APC mRNA (7) transcript was detected in the NORTHERN LIGHTS Multiple mRNA Blot V (data not shown).

In summary, these results demonstrate that using a series of quality RNA products, tissue-specific genes can be identified and full-length transcripts can be isolated.

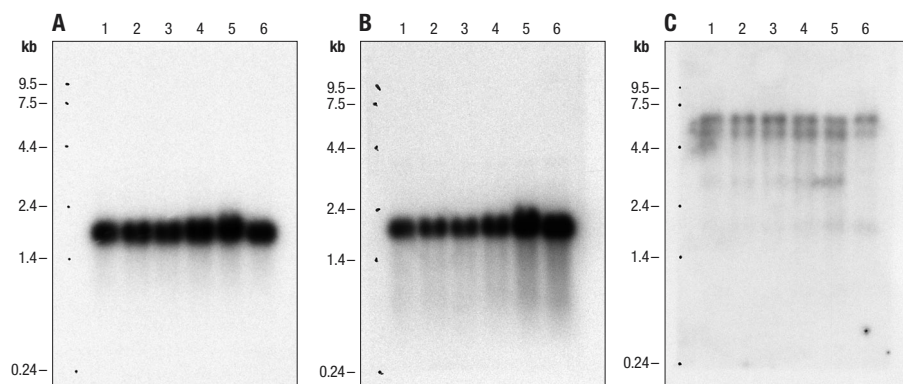
**ACKNOWLEDGEMENTS**

The authors thank Dr. Ray Hadley for providing the APC DNA and Dr. WuBo Li for the APC primers. **Focus**

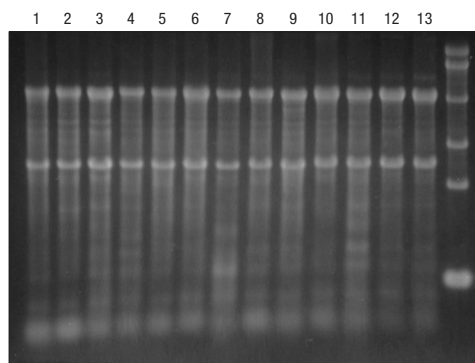
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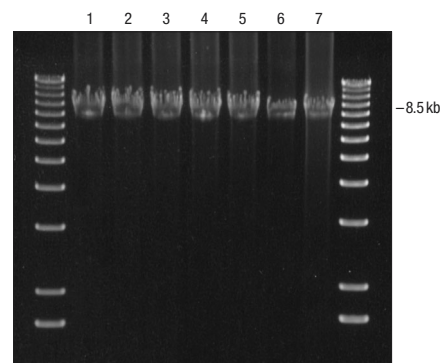
**Editor's Note:** The SEARCHLIGHTS Human Multiple RNA Dot Blot contains 46 total RNA samples from normal adult tissue immobilized on a positively charged nylon membrane. NORTHERN LIGHTS Multiple Human mRNA Blots are available for adult, fetal, and tumor tissues. For product information, see our on-line catalogue at [www.lifetech.com](http://www.lifetech.com).



**FIGURE 3. Reprobing blots.** Panel A. NORTHERN LIGHTS™ Multiple mRNA Blot V was hybridized with <sup>32</sup>P-labeled  $\beta$ -actin DNA and exposed without an intensifying screen at  $-80^{\circ}\text{C}$  for 4 h. Panel B. The blot was stripped, hybridized with <sup>32</sup>P-labeled human EF1 $\alpha$  (5) DNA, and exposed with an intensifying screen at  $-80^{\circ}\text{C}$  for 4 h. Panel C. The blot was stripped, hybridized a third time with <sup>32</sup>P-labeled human MAP4 (6) DNA, and exposed with an intensifying screen at  $-80^{\circ}\text{C}$  for 16 h.



**FIGURE 4. Denatured formaldehyde agarose gel of total RNA.** 2  $\mu\text{g}$  of each total RNA sample was electrophoresed on a denaturing formaldehyde/1.2% agarose gel. Human total RNA samples were from brain, heart, kidney, colon, liver, lung, placenta, fetal brain, fetal liver, fetal lung, breast tumor, colon tumor, and lung tumor, lanes 1–13, respectively.



**FIGURE 5. RT-PCR from human RNA.** cDNA was synthesized using THERMOSCRIPT™ RT from 25 ng human mRNA from brain, fetal brain, kidney, liver, and lung and 2.5  $\mu\text{g}$  of total RNA from lung, lanes 1–6, respectively, with a gene-specific primer (CAG ATG TCA CAA GGT AAG AC). One tenth of the cDNA was amplified with PLATINUM® Taq DNA Polymerase High Fidelity for 40 cycles of  $94^{\circ}\text{C}$  for 15 s,  $55^{\circ}\text{C}$  for 30 s, and  $68^{\circ}\text{C}$  for 10 min. Lane 7. Control PCR of the 8.5-kb APC fragment with 5 ng of APC DNA.

# Purification of Genomic DNA from Gram-Negative Bacteria

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**R**apid and efficient isolation of genomic DNA from bacteria has been hampered by the presence of a cell wall. Traditionally, either proteinase K (1) or lysozyme (2) has been used to break up the cell wall, followed by a phenol extraction and alcohol precipitation to remove proteins. RNA is removed by RNase treatment followed by a second phenol extraction and ethanol precipitation. This procedure requires at least 4 h from obtaining a cell pellet to dissolution of the genomic DNA pellet.

DNAZOL<sup>®</sup> Reagent is a reagent used to isolate genomic DNA from animal sources (3,4). The combination of high pH and guanidinium salts in DNAZOL Reagent lyses the animal cell and hydrolyzes the RNA in one step, resulting in fast genomic DNA isolation after an alcohol precipitation. DNAZOL Reagent has been used to isolate genomic DNA from bacteria, as well (5). However, significant physical manipulation was used to break up the bacterial cell wall, and genomic DNA isolation from bacteria was not as efficient. In this report, we describe a fast, efficient protocol for isolating high-quality genomic DNA from gram-negative bacteria using lysozyme in combination with DNAZOL Reagent.

## METHODS

Genomic DNA was isolated from *E. coli* (DH5 $\alpha$ <sup>™</sup> and DH10B<sup>™</sup> cells) and from *A. tumefaciens* (LBA4404). Saturated cultures (OD<sub>660</sub> > 1.1) were obtained by inoculating 4  $\mu$ l of a frozen glycerol stock culture into 4 ml of Luria Broth. Cultures were grown with shaking either for 16 h at 37°C (*E. coli*) or 40 h at 30°C (*A. tumefaciens*).

Genomic DNA was isolated from 1.5 ml of saturated culture using lysozyme/phenol/RNase A as previously described (2). Briefly, pelleted cells were resuspended in

Strain	Phenol/RNase A Method ( $\mu$ g)	Ratio (OD <sub>260</sub> /OD <sub>280</sub> )	DNAZOL <sup>®</sup> Reagent Method ( $\mu$ g)	Ratio (OD <sub>260</sub> /OD <sub>280</sub> )
DH5 $\alpha$ <sup>™</sup>	15	2.0	86	2.1
DH10B <sup>™</sup>	10	2.1	25	2.2
LBA4404	19	1.8	24	1.8

**TABLE 1. Comparison of methods for genomic DNA isolation.** Yields are calculated as the average of 2 experiments except for LBA4404 using phenol/RNase A, which is the result of 1 experiment.

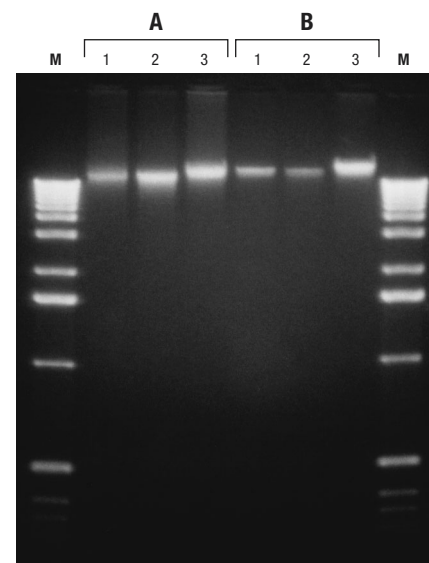
1 ml TES/sucrose/lysozyme [20 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0), 50 mM NaCl, 8% sucrose, 1 mg/ml lysozyme] by vortexing for 20 s. After a 5-min incubation at room temperature, 100  $\mu$ l of 10% SDS was added to stop the reaction. The mixture was extracted with an equal volume of phenol/chloroform/isoamyl alcohol and precipitated with 0.1 volumes of sodium acetate and 1 volume of isopropanol. After a 70% ethanol wash, the pellet was dissolved in 500  $\mu$ l TE by heating at 37°C. When the pellet was dissolved, 50 ng of RNase A was added and the mixture was incubated for 10 min at 37°C. The phenol extraction, isopropanol precipitation, and 70% ethanol wash were repeated, and the pellet was dissolved in 100  $\mu$ l TE.

A second procedure using lysozyme and DNAZOL Reagent was used to isolate genomic DNA. Cell pellets from 1.5-ml saturated cultures were resuspended in 300  $\mu$ l TES/sucrose/lysozyme by vortexing for 20 s. After a 15-min room-temperature incubation, 3 ml of DNAZOL Reagent was added, and the mixture was vortexed for 20 s. The DNAZOL Reagent–cell lysate mixture was centrifuged at 15,000  $\times$  *g* for 10 min at room temperature to remove any insoluble cell debris. The genomic DNA was precipitated from the supernatant by adding 0.5 volumes of 95% ethanol, inverting several times, and centrifuging for 2 min at 15,000  $\times$  *g* at room temperature. The pellet was washed twice with 3 ml 95% ethanol and dissolved in 100  $\mu$ l of 8 mM NaOH.

AFLP<sup>®</sup> analysis was performed with the GIBCO BRL<sup>®</sup> AFLP Analysis System for Microorganisms according to the manufacturer's directions using the selective primers *Eco*R I-A and *Mse* I-A. The radioactively labeled products were electrophoresed on a denaturing 6% polyacrylamide-urea gel and exposed to film for 16 h.

## RESULTS AND DISCUSSION

The amount of genomic DNA isolated was greater using the lysozyme and DNAZOL Reagent method than the phenol/RNase A method with similar OD<sub>260/280</sub> ratios (table 1). The procedure using DNAZOL Reagent required 1.5 h, compared with



**FIGURE 1. Agarose gel electrophoresis of bacterial genomic DNA.** Samples of genomic DNA prepared by phenol/RNase A (panel A), or DNAZOL<sup>®</sup> Reagent (panel B) were analyzed on a 1% agarose TBE gel containing ethidium bromide (0.5  $\mu$ g/ml). Strains used were DH5 $\alpha$ <sup>™</sup>, DH10B<sup>™</sup>, and LBA4404, lanes 1–3, respectively. M is  $\lambda$  DNA/*Hind* III fragments.

almost 4 h using the phenol method. These times do not include the overnight incubation at 37°C to ensure complete solubilization of the genomic DNA pellet in both procedures. The DNAZOL Reagent procedure without the addition of lysozyme yielded no detectable DNA. The genomic DNA was high molecular weight, with little smearing on an agarose gel (figure 1). The AFLP pattern of each strain was the same with both protocols (figure 2).

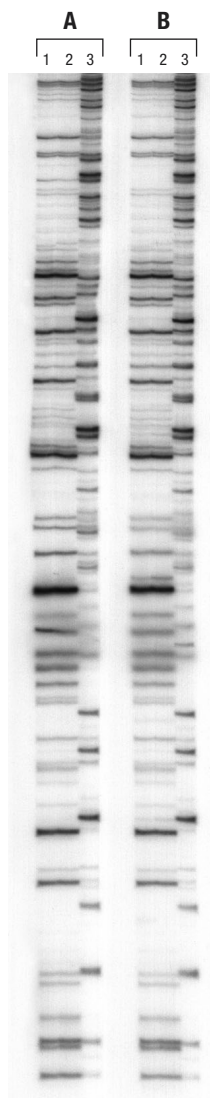
In summary, using lysozyme in combination with DNAZOL Reagent is a quick and efficient method for isolating genomic DNA from gram-negative bacteria. In addition, this protocol eliminates concerns involving the use of organics (such as phenol). The DNAZOL Reagent procedure can be scaled to a 1.5-ml microcentrifuge tube. Because it involves fewer manipulations, the lysozyme/DNAZOL Reagent procedure is more adaptable to high-throughput applications.

#### ACKNOWLEDGEMENTS

The author thanks Timothy Fawcett for helpful suggestions regarding lysozyme and Holly Anderson and Larry Mertz for useful discussions and critical reading of the manuscript. **FOCUS**

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**FIGURE 2.** AFLP<sup>®</sup> analysis of bacterial genomic DNA. Samples of genomic DNA prepared by phenol/ RNase A (panel A) or DNAZOL<sup>®</sup> Reagent (panel B) were analyzed using the selective primers *EcoR* I-A and *Mse* I-A. Strains used were DH5 $\alpha$ ™, DH10B™, and LBA4404, lanes 1–3, respectively.