Atlas® cDNA Expression Arrays User Manual

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I. Introduction

The human genome project's large-scale sequencing efforts have generated partial sequence data for thousands of genes (Aaronson et al., 1996; Hillier et al., 1996). Although many of these genes have been assigned to functional classes, the roles they play in various biological processes have yet to be elucidated. An important step toward understanding these roles is defining gene expression profiles—i.e., comparing patterns of expression in different tissues and developmental stages, in normal and disease states, or in distinct in vitro conditions. This can be accomplished using RT-PCR, RNase protection assays, or Northern blot analysis, but these methods focus on only a few genes at a time. A more promising approach for analyzing multiple genes simultaneously is the hybridization of entire cDNA populations to nucleic acid arrays—a method adopted for high-throughput analysis of gene expression. This technology has a wide range of applications, including investigating normal biological and disease processes, profiling differential gene expression, and discovering potential therapeutic and diagnostic drug targets.

Nucleic acid arrays and their applications have been described previously (Chalifour et al., 1994; DeRisi et al., 1996; DeRisi et al., 1997; de Saizieu et al., 1998; Heller et al., 1997; Lockhart et al., 1996; Milosavljevic et al., 1996; Nguyen et al., 1995; Piétu et al., 1996; Schena, 1996; Schena et al., 1996; Shalon et al., 1996; Zhao et al., 1995). In general, these studies have attempted to include as many cDNAs or oligonucleotides as possible in a single array. Because these arrays are difficult and expensive to produce, and analysis requires expensive fluorescence detection equipment, they are not easily accessible to the average researcher.

Atlas® cDNA Expression Arrays include hundreds of cDNAs spotted on positively charged nylon membranes. Plasmid and bacteriophage DNAs are included as negative controls to confirm hybridization specificity, along with several housekeeping cDNAs as positive controls for normalizing mRNA abundance. For a complete list of the cDNAs and controls on each array, as well as the GenBank accession numbers, please see the Array Gene List provided. This list is also available at our web site, **www.clontech.com**.

Unlike other nucleic acid arrays, Atlas Arrays are carefully designed to include genes that are of great interest to researchers in many fields. Moreover, these genes have been reported to play key roles in many different biological processes. On the larger arrays, the cDNAs are arrayed into functional classes that represent many current areas of research, as indicated in the Array Gene List accompanying each array. Because the genes included on the arrays are so meticulously selected, Atlas experiments generate highly informative results. A single hybridization experiment generates an expression profile for hundreds of genes at once.

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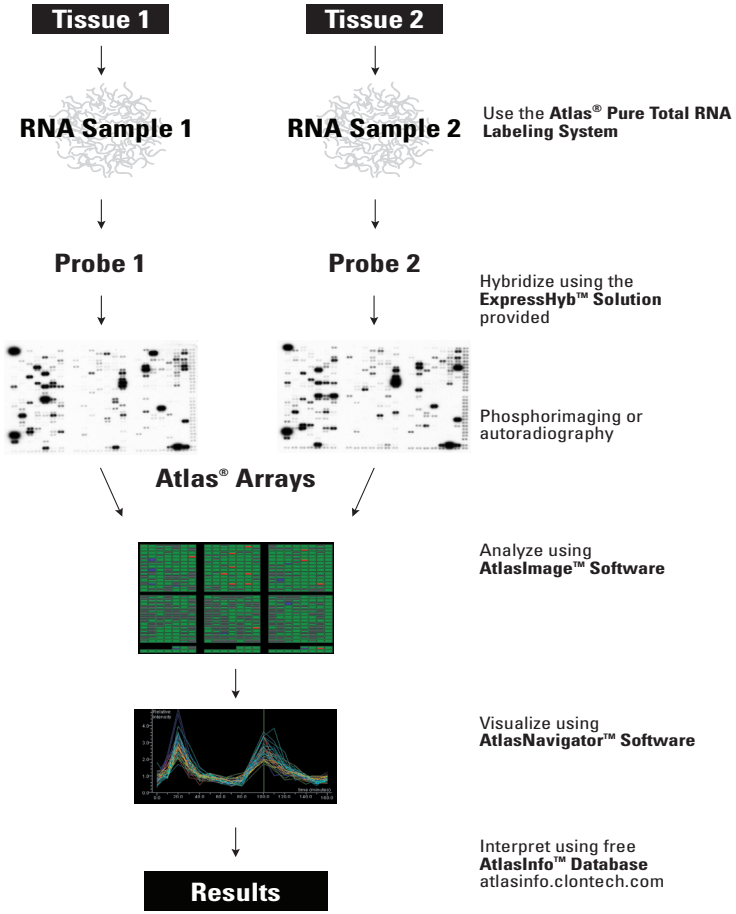

Figure 1. Broad-scale expression profiling with Atlas® Arrays. Side-by-side hybridizations with cDNA probes prepared from two different RNA populations allow the simultaneous comparison of the expression levels of all the cDNAs on the array. Examples of RNA populations that can be compared by this method include normal and diseased tissues, two different normal tissues, or cells in differing in vitro conditions (e.g., growth factor-stimulated vs. unstimulated cells).

Overview of the Atlas® procedure

The Atlas procedure is outlined in Figure 1. Identical membranes are included with each purchase, so the expression profiles of different mRNA populations can be compared side-by-side. The first step is to synthesize probe mixtures by reverse transcribing each RNA population using the cDNA Synthesis (CDS) Primer Mix included and [α-^{32}P] or [α-^{33}P]dATP. The CDS Primer Mix is a mixture of primers

I. Introduction continued

specific for each different type of Atlas Array—**they are not interchangeable**. These Primer Mixes ensure that cDNAs are only synthesized for the genes on your particular array. Each radioactively labeled probe mix is then hybridized to separate Atlas Arrays. After a high-stringency wash, the hybridization pattern can be analyzed by autoradiography and/or quantified by phosphorimaging. The relative expression levels of a given cDNA from two different RNA sources can be assessed by comparing the signal obtained with a probe from one RNA source to that obtained with a probe from another source. Each membrane can be reused at least three times.

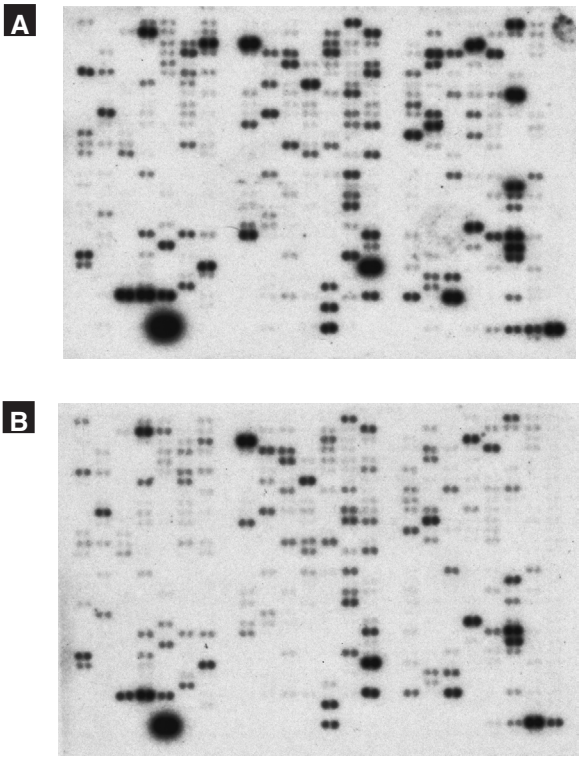

Figure 2. Gene expression profiling using poly A$^+$ or total RNA. ^{32}P-labeled cDNA probes were prepared from 1 µg of poly A$^+$ RNA (Panel A) or 2 µg of total RNA (Panel B) from Bcl-2 knockout mice. The probes were hybridized to separate Atlas Mouse cDNA Expression Array (Cat. No. 634539) membranes. Results were analyzed by autoradiography. The hybridization patterns are remarkably similar, although they are not identical. We have confirmed that differentially expressed genes can be reproducibly detected using either total or poly A$^+$ RNA to compare two different tissues (data not shown). The arrays shown here include duplicate spots of each cDNA. On other Atlas Arrays such as the 1.2 series, the cDNAs are printed in single spots.

I. Introduction continued

Because the Atlas technology uses a mixture of gene-specific primers for probe synthesis (patent pending), the hybridization probes are significantly less complex than probes generated using oligo(dT) or random primers. Reducing probe complexity results in an approximately 10-fold increase in sensitivity, with a concomitant reduction in nonspecific background. Because of this high sensitivity, even total cellular RNA can be used as starting material to synthesize probes—a clear advantage when only small amounts of tissues or cells are available (Figure 2).

The quality of RNA is the single most important factor for generating high-sensitivity hybridization probes. For best results, use the Atlas® Pure Total RNA Labeling System (Cat. No. 634562) for RNA purification and probe synthesis. Other commercially available kits for RNA isolation are not compatible with the Atlas technology because they result in a high level of nonspecific background. When used in conjunction with components supplied with your Atlas Arrays, Atlas Pure allows you to isolate pure total RNA from cells or tissues and synthesize probes directly from the total RNA. If you have already isolated total or poly A^+ RNA and it is not possible for you to make another preparation with the Atlas Pure System, you can use your samples to synthesize probes by following instructions in this User Manual.

Sensitivity, background, and linear range of detection

The cDNAs immobilized on each Atlas Array have been specially prepared to minimize the problem of nonspecific hybridization. Each cDNA fragment is 200 to 600 bp and has been amplified from a region of the mRNA that lacks the poly-A tail, repetitive elements, or other highly homologous sequences. When the array is hybridized with a ^{32}P- or ^{33}P-labeled cDNA probe, the background level is sufficiently low to permit detection of an mRNA that is present at only about 10–20 copies per cell—an abundance level of approximately 0.0025%. Because each cDNA immobilized on the arrays is species specific, probes from different species cannot be used for hybridization.

Figure 3 demonstrates that individual mRNAs can be detected at low levels within a cDNA probe mixture. For this experiment, RT-PCR was first used to confirm that six RNAs were expressed at very low levels (<1 molecule/cell) in human placenta. Then, each of the six corresponding cDNAs was cloned downstream of a T7 promoter, and the RNAs were synthesized with T7 RNA polymerase. Known amounts of each test RNA were "spiked" into placenta poly A^+ RNA at the indicated percentages, and ^{32}P-labeled cDNA probes were generated from each sample, including the control (placenta RNA only). The probes were then hybridized separately to seven array membranes. For each individual RNA, the difference between the signal from the spiked cDNA probes and that from the unspiked control cDNA probe clearly demonstrates the specificity of the hybridization signal. Furthermore, the control cDNA probe generated a background of less than 0.002%. The linear correlation between each hybridization signal and the abundance of each RNA suggests that detection is linear for RNAs present at

I. Introduction continued

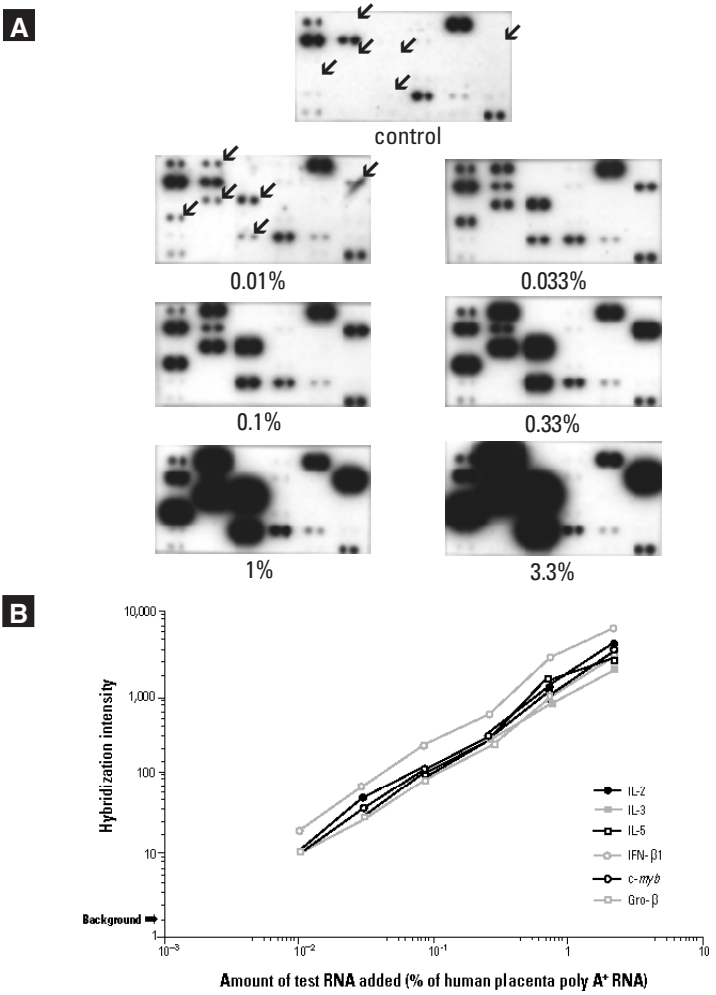

Figure 3. Hybridization signal is linearly related to target concentration. RT-PCR was first used to confirm that the six test RNAs are expressed at very low levels (<1 molecule/cell) in human placenta. Then, each of the six corresponding cDNAs was cloned downstream of a T7 promoter, and the RNAs were synthesized with T7 RNA polymerase. Known amounts of each test RNA were spiked into placenta poly A^+ RNA at the indicated percentages, and ^{32}P-labeled cDNA probes were generated from each sample, including the control (placenta RNA only). The probes were then hybridized separately to seven array membranes. **Panel A.** Blots were exposed to x-ray film overnight. The percentage of each test RNA in the cDNA probe template is indicated beneath each blot. The arrows on the control and 0.01% blots show the locations of the six cDNAs that hybridized to the probes containing the test cDNAs. Note the consistency in all seven blots of the spots that hybridized to the control and all six test probes. **Panel B.** Blots were exposed to a phosphorimaging screen overnight and the hybridization signal was plotted against the amount of test RNA in the probe template. The arrow indicates the background level of hybridization for the control cDNA probes. The Atlas Arrays shown here include duplicate spots of each cDNA. On other Atlas Arrays such as the 1.2 series, the cDNAs are printed in single spots.

I. Introduction continued

levels of 0.01–3% of the total population (Figure 3). Other experiments indicate that Atlas Arrays can be used reliably to quantify expression of RNAs represented at levels as low as 0.005% of the total population.

As seen in Figure 3, RNAs present at equal concentrations in the probe template can give signals that differ by several-fold. Thus, hybridization data are semiquantitative—i.e., the intensity of the hybridization signal allows you to only approximate the abundance level of an individual cDNA using the cDNA probe (within a 2- to 5-fold range). An approximate estimate of the abundance level of a target cDNA in an RNA population can be made by comparing its signal to the signals obtained with housekeeping genes of known abundance, which are included as positive controls on each Atlas Array. Because the level of mRNA expression may vary up to 10,000-fold for different genes, it is important to expose the array to x-ray film for varying lengths of time before you draw any conclusions about the presence or absence of a transcript in a given RNA sample. For the most accurate estimate of relative transcript abundance, a phosphorimager can be used to directly quantify the hybridization signal.

Some genes are expressed at very low levels. Detection of rare transcripts using Atlas Arrays depends on the quality of RNA samples, as well as successful synthesis and labeling of a cDNA probe with high specific activity, and requires low levels of nonspecific hybridization. The labeling reagents provided with the arrays have been optimized for generating the most efficient and sensitive cDNA probes for hybridization. ExpressHyb™ Hybridization Solution, a low-viscosity hybridization solution that significantly enhances the sensitivity of detection and reduces background (Yang & Kain, 1995), is also provided. For the best possible results from an Atlas experiment, it is important to use the reagents provided instead of substituting others.

^{32}P vs. ^{33}P detection

With Atlas Arrays, you have a choice of using ^{32}P or ^{33}P in the labeling reaction. The more appropriate method depends on the nature of your experiment. For general purposes, we recommend using ^{32}P because this isotope provides greater sensitivity in the procedure. High sensitivity will be especially important if you are interested in any non-abundant mRNAs. On the other hand, ^{33}P offers the advantage of higher-resolution signal, meaning that the signal produced by a spot on the array will be more closely confined to the spot's center, preventing signal "bleed" to neighboring spots. High signal bleed can complicate the interpretation of results for nearby genes. The ^{33}P method is particularly useful if highly abundant transcripts are of interest or you plan to quantitatively analyze your results by phosphorimaging. However, ^{33}P detection is generally only one-fourth as sensitive as ^{32}P detection. See Figure 8 (Section VIII) for a comparison of images generated using these two methods.

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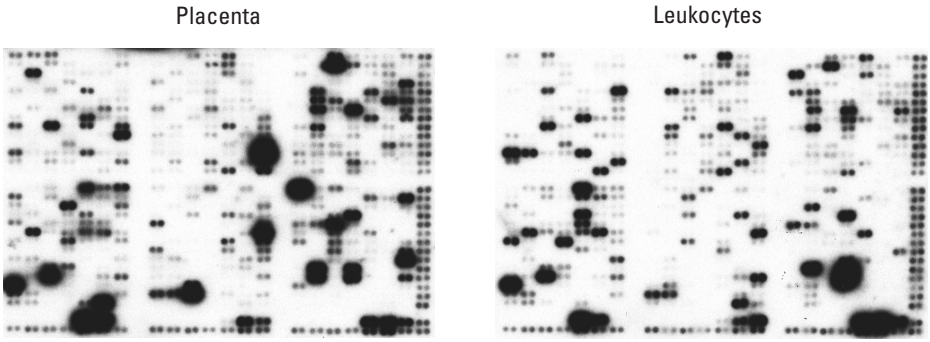

Figure 4. Differential gene expression in human placenta and leukocytes. Poly A^+ RNA was isolated from the indicated tissue. ^{32}P-labeled cDNA probes were generated from each poly A^+ RNA sample and hybridized to the Atlas Human Array (Cat. No. 634511) according to this User Manual. Membranes were exposed to Kodak BioMax MS x-ray film with a BioMax MS intensifying screen at –70°C for one day. The arrays shown here include duplicate spots of each cDNA. On other Atlas Arrays such as the 1.2 series, the cDNAs are printed in single spots.

Normalization of transcript abundance

"Housekeeping genes" produce the mRNAs necessary for a cell's basal metabolism. In contrast to the cDNAs included on Atlas Arrays, which are under tight transcriptional control, the levels of housekeeping gene transcripts remain relatively constant in different tissues, cells, developmental stages, and diseases (Adams et al., 1993; Adams et al., 1995; Liew et al., 1994). The term "normalization" refers to using the expression levels of one or more housekeeping genes as standards for measuring the expression levels of other genes. Normalization allows you to make adjustments to your hybridization signals if you inadvertently used different amounts of RNA (e.g., different concentrations or purity of your RNA samples) for generating cDNA probes. In addition, normalization is important for experiments which compare unrelated cells or tissues—situations in which the expression levels of individual housekeeping genes may vary.

Several studies have demonstrated that the most thorough way to normalize mRNA expression levels is to use a set of housekeeping genes as hybridization standards (Adams et al., 1993; Adams et al., 1995; Liew et al., 1994; Spanakis & Brouty-Boyé, 1994; Spanakis, 1993). This allows you to compensate for tissue- or cell-specific variation in the expression level of a single housekeeping gene.

Multiple housekeeping cDNAs are spotted on each Atlas Array for use as positive controls. While these genes all perform different functions and are expressed in cells at different levels relative to one another, all exhibit relatively constant expression levels in different tissues, cells, developmental stages, and diseases. When comparing the relative expression levels of a cDNA in two different RNA populations, you should normalize the hybridization signals to the signals obtained from one or more of the housekeeping gene control cDNAs. In hybridization

I. Introduction continued

TABLE I. RNA REQUIREMENTS FOR ATLAS® LABELING METHODS

Method	Total RNA	Poly A+ RNA
Atlas® SMART™ Probe Amplification Kit	50 ng	N/A
Atlas® Pure Total RNA Labeling System	10–50 µg	N/A
Atlas® SpotLight™ Labeling Kit	50 µg	2 µg
Conventional direct labeling (Section VI)	2–5 µg	1 µg

experiments where only a few genes exhibit changes in expression, you can use other genes for normalization. The list of housekeeping genes included on your Atlas Array can be found on the enclosed Array Gene List.

Although housekeeping genes are expressed at similar levels in a variety of cell types, some variation is seen when comparing divergent tissues. Figure 4 shows an experiment in which Atlas Human Array (Cat. No. 634511) membranes were hybridized with cDNA probes from human placenta and leukocytes. These patterns of hybridization are highly reproducible. Notice the variation in expression levels of individual housekeeping genes (second row from bottom). This experiment probably represents the most complicated case, in which RNA from divergent tissues is being compared. For more information on housekeeping genes and normalization, see Interpretation of Results (Section VIII).

Corroboration of results by RT-PCR

Because of sequence-dependent hybridization characteristics and variations inherent in any hybridization reaction, Atlas data and any other array data should be considered semiquantitative. We strongly recommend that the results of your array experiment be corroborated by RT-PCR.

Other products in the complete Atlas® system

As shown in Figure 1, the Atlas line of products provides a complete system for not only gene expression profiling but also for the analysis and interpretation of data. The following supplemental tools make Atlas analysis accessible to more types of samples and applications than any other array system:

- **Atlas® SMART™ Probe Amplification Kit (Cat. No. 634607)**

  For applications in which the amount of starting material is extremely limiting, the Atlas SMART Kit provides the best solution. When used in conjunction with the SMART™ PCR cDNA Synthesis Kit, Atlas SMART can be used to synthesize highly sensitive cDNA probes from as little as 50 ng of total RNA—the amount obtained from about 1,000 cells or 0.1 mg of tissue (Table I). Please note that amplified probes should not be directly compared to non-amplified probes. For more information, download the Atlas SMART Probe Amplification Kit User Manual (PT3503-1) from **www.clontech.com**.

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- **SpotLight™ Chemiluminescent Detection**

  Used together, the Atlas® SpotLight™ Labeling Kit (Cat. No. 634803) and the SpotLight™ Chemiluminescent Hybridization & Detection Kit (Cat. No. 634802) provide an excellent nonradioactive alternative to Atlas Array analysis. This chemiluminescent system provides comparable results to traditional radioactive detection after exposures that are just a few minutes in length. See the User Manuals at our website to find out more.

- **Atlas® Glass Microarrays**

  Atlas Glass Microarrays provide all the features and benefits of the membrane-based Atlas technology in the glass format. These arrays are an ideal solution for high-throughput expression profiling studies. See Section XI for a listing of products in the Atlas Glass family. For more information and to download the Atlas Glass Microarrays User Manual (PT3453-1), visit our website and use the Atlas Expression Arrays Products Quick Link.

- **Atlas® Plastic Microarrays**

  Atlas Plastic Microarrays combine the best properties of membrane-based and glass-based arrays. These arrays promote lower background and accurate analysis in an easy to use plastic format. See Section XI for a listing of products in the Atlas Plastic family. For more information and to download the Atlas Plastic Microarrays User Manual (PT3591-1), visit our website.

- **AtlasImage™ 2.7 Software** (Cat. No. 634650)

  To simplify the analysis of your results, use AtlasImage. Starting with either a phosphorimager scan or a scanned autoradiogram, the AtlasImage user can quantitate gene expression, compare gene expression profiles, and generate detailed reports in a straightforward, intuitive manner. AtlasImage was designed entirely by Clontech employees and includes a carefully chosen set of options for customizing analyses and generating reports that provides both versatility and simplicity. Version 2.01 or higher is required for use with Atlas cDNA Expression Arrays. Download a demo version of AtlasImage from our website.

I. Introduction continued

- **AtlasInfo™ Bioinformatics Database**

  Once you have identified interesting genes in an Atlas analysis, the free AtlasInfo Database can be used as a simple first step in learning more about them. For each Atlas Array gene, we include: alternative names, known functions, related diseases, journal citations, homologous genes, chromosomal locations, and links to related databases. Visit AtlasInfo at our website.

- **Additional products for analyzing differential gene expression**

  For investigating differential gene expression, Atlas Arrays provide a complementary approach to methods such as Clontech PCR-Select™ cDNA Subtraction (Cat. No. 637401; Diatchenko et al., 1996; Gurskaya et al., 1996; October 1995 Clontechniques)). Whereas Atlas Arrays allow you to survey the expression patterns of hundreds of **known** genes, these techniques are excellent choices for identifying **novel** differentially expressed genes.

II. List of Components

Note: The components included in the Atlas® Human Array Trial Kit are different from those listed below. Please refer to the enclosed Product Analysis Certificate for a precise listing.

Store Box 3 at room temperature.
Store Control Poly A^+ RNA at –70°C.
Store Atlas Arrays at –20°C, sealed in a plastic bag away from light.
Store all other reagents at –20°C.

Box 1:

- **Atlas® cDNA Expression Array membranes**
- **CDS Primer Mix**
  Note: Each different type of Atlas Array comes with a unique CDS Primer Mix. These Primer Mixes **are not** interchangeable.
- **Blank Nylon Membrane**
- **Orientation Grid**

Box 2:

- **10X dNTP Mix** (for [α-^{32}P] or [α-^{33}P]dATP label)
  (5 mM each dCTP, dGTP, dTTP)
- **5X Reaction Buffer**
  (250 mM Tris-HCl [pH 8.3], 375 mM KCl, 15 mM $MgCl_2$)
- **MMLV Reverse Transcriptase**
- **DTT** (100 mM)
- **10X Termination Mix** (0.1 M EDTA [pH 8.0], 1 mg/ml glycogen)
- **Control Poly A^+ RNA** (0.5 µg/µl)
- **C_0t-1 DNA** (1 mg/ml)
- **Deionized H_2O**

Box 3:

- **ExpressHyb™ Hybridization Solution**
- **Atlas® Nucleospin® Extraction Kit:**
  – NucleoSpin Extraction Spin Columns
  – 2-ml Collection Tubes
  – Buffer NT2
  – Buffer NT3 (Add 95% ethanol before use as specified on the label.)
  – Buffer NE

III. Additional Materials Required

The following reagents are required but not supplied:

For isolation of total RNA and synthesis of probes:

- Atlas® Pure Total RNA Labeling System (Cat. No. 634562)

  Notes:

  – The Atlas Pure Total RNA Labeling System is required for high-quality results. The System contains materials required for total RNA isolation and DNase I treatment as well as reagents for magnetic bead affinity enrichment of poly A^+ RNA and synthesis of cDNA probes.

  – The Atlas Pure System uses a phenol-based method for RNA purification. If you prefer a phenol-free method, you may use the spin column format in our NucleoSpin® RNA II Kit (Cat. Nos. 635990, 635991). For best results, use NucleoSpin® RNA II in conjunction with the Atlas Pure Total RNA Labeling System for probe synthesis. Alternatively, you may purify poly A^+ RNA and proceed with the probe synthesis procedure in Section VI of this User Manual. Please note that NucleoSpin RNA II is a different technology than the Nucleospin Extraction Columns included with this kit for probe purification.

For DNase treatment of RNA if you are not using the Atlas® Pure Total RNA Labeling System or an Atlas® Trial Kit:

- **RNase-free DNase I**
  - Epicentre #D9910K (1 unit/µl)

  or
  - Boehringer-Mannheim #776-785 (10 units/µl); dilute to 1 unit/µl in 1X DNase I Buffer prior to use.
- **10X DNase I Buffer**

  400 mM Tris-HCl (pH 7.5)
  100 mM NaCl
  60 mM $MgCl_2$
- **Phenol:chloroform:isoamyl alcohol**
  (25:24:1; equilibrated with 0.1 M sodium citrate (pH 4.5), 1 mM EDTA)
- **95% Ethanol**
- **2 M NaOAc** (pH 4.5)
- For DNase treatment of **Total** RNA, prepare an additional 100 µl of **10X Termination Mix** per sample:

  0.1 M EDTA (pH 8.0)
  1 mg/ml glycogen (Sigma #G1508 or Boehringer-Mannheim #901-393)

For cDNA probe synthesis:

- **[α-^{32}P]dATP** (10 µCi/µl; 3,000 Ci/mmol; Amersham #PB10204)

  OR
- **[α-^{33}P]dATP** (10 µCi/µl; >2,500 Ci/mmol; Amersham #BF1001)

  Note: See the Introduction for guidelines on choosing ^{32}P or ^{33}P. **Do not use Amersham's Redivue or any other dye-containing isotope.**

III. Additional Materials Required continued

For hybridization and washing:

- **Sheared salmon testes DNA** (10 mg/ml; Sigma #D7656)
- **10X denaturing solution**

  1 M NaOH
  10 mM EDTA

- **2X neutralizing solution** (1 M NaH_2PO_4 [pH 7.0])

  27.6 g $NaH_2PO_4 \cdot H_2O$

  Add 190 ml of H_2O. Adjust pH to 7.0 with 10 N NaOH if necessary. Add H_2O to 200 ml. Store at room temperature.

- **20X SSC**

  175.3 g NaCl
  88.2 g $Na_3Citrate \cdot 2H_2O$

  Add 900 ml of H_2O. Adjust pH to 7.0 with 1 M HCl if necessary. Add H_2O to 1 L. Store at room temperature.

- **20% SDS**

  200 g SDS

  Add H_2O to 1 L. Heat to 65°C to dissolve. Store at room temperature.

- **Wash Solution 1**

  2X SSC
  1% SDS

  Store at room temperature.

- **Wash Solution 2**

  0.1X SSC
  0.5% SDS

  Store at room temperature.

- **X-ray film or a phosphorimager**
  We recommend Kodak BioMax MS film (Kodak #118 8077, with the corresponding BioMax MS intensifying screen). Alternatively, use a phosphorimager.

IV. Overview & General Considerations

PLEASE READ ENTIRE PROTOCOL BEFORE BEGINNING.

Use of Supplemental Atlas® Kits

Several of the supplemental Atlas Kits (**Atlas® Pure, Atlas® SMART™ & Spot-Light™**) described in the Introduction are supplied with their own protocols for RNA isolation, probe labeling, and/or probe hybridization. Table II indicates what protocols are used with each of the different supplemental kits. Please be sure to use the correct protocol for each step in the analysis. Figure 5 shows a general flow chart for users of the Atlas Pure Total RNA Labeling System or the conventional Atlas procedure.

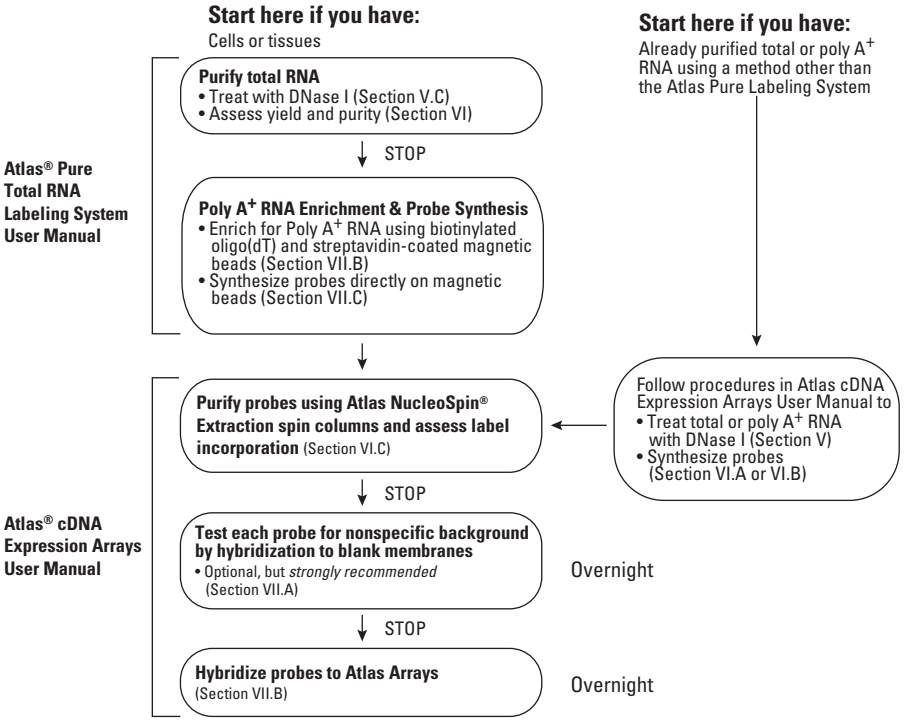


Figure 5. Experimental overview for using the Atlas® Pure System and conventional Atlas® procedure. You have the option of pausing at any of the indicated points as well as a few other steps as described in the protocol. See Table II for the sources of other supplemental Atlas kit protocols.

IV. Overview & General Considerations continued

TABLE II. PROTOCOL SOURCES FOR SUPPLEMENTAL ATLAS® KITS

Supplemental Kit	RNA Isolation	Probe Synthesis	Probe Hybridization
Atlas® SMART™ Probe Amplification Kit	Use preferred*	Atlas SMART	Atlas SMART
Atlas® Pure Total RNA Labeling System	Atlas Pure	Atlas Pure	Atlas Arrays
SpotLight™ Chemiluminescent System	Use preferred*	SpotLight Label	SpotLight Hyb
Conventional procedure	Atlas Arrays	Atlas Arrays	Atlas Arrays

Atlas SMART = Atlas® SMART™ Probe Amplification Kit User Manual (PT3503-1)
Atlas Pure = Atlas® Pure Total RNA Labeling System User Manual (PT3231-1)
SpotLight Label = Atlas® SpotLight™ Labeling Kit User Manual (PT3517-1)
SpotLight Hyb = SpotLight™ Chemiluminescent Hybridization & Detection Kit User Manual (PT3518-1)
Atlas Arrays = Atlas® cDNA Expression Arrays User Manual (PT3140-1; this document)

*See Section V of this User Manual for guidelines. If desired, RNA isolation reagents and protocol supplied with the Atlas Pure Total RNA Labeling System may be used.

RNA Preparation

- **The quality of the RNA used to make probes is the most important factor influencing the sensitivity and reproducibility of the hybridization pattern.** A poor-quality RNA preparation leads to high background on the membrane and/or an inaccurate hybridization pattern. These problems are typically caused by residual RNase and genomic DNA contamination. For best results, please use the Atlas Pure Total RNA Labeling System (Cat. No. 634562) for purifying total RNA. This System uses a phenol:chloroform-based method to produce high yields of quality total RNA that is virtually free of genomic DNA, nucleases, and other impurities, and is designed specifically for use with Atlas Arrays. If you prefer a non-phenol-based method, you may use the NucleoSpin RNA II Kit (Cat. Nos. 635990, 635991) for RNA purification. This NucleoSpin kit includes a complete protocol optimized for use with Atlas. Please note that the columns and reagents included with the NucleoSpin RNA II Kit are different than the Nucleospin Extraction Kit components included with this array. Also note that other commercially available kits for RNA isolation are not compatible with Atlas technology because they result in a high level of nonspecific background and nonreproducible results.
- If it is not possible for you to use the Atlas Pure Total RNA Labeling System or the NucleoSpin RNA II Kit to purify your RNA, we recommend that you use poly A^+ RNA for probe synthesis. In general, Atlas probes synthesized from poly A^+ RNA are more sensitive than total RNA probes. However, total RNA can be used without a significant decrease in the ratio of signal to background.

IV. Overview & General Considerations continued

- Contamination by genomic DNA is particularly troublesome. Therefore, **we strongly recommend that all RNA samples be treated with RNase-free DNase I** prior to being used for probe synthesis. DNase I and other reagents for performing this step are also provided in both the Atlas Pure Total RNA Labeling System and the NucleoSpin RNA II Kit. Although DNase I treatment of total RNA is preferable, treatment can be performed on poly A^+ RNA if necessary. Protocols for both methods are provided in Section V. Following these protocols is especially important if you are not using the Atlas Pure System.
- When using Atlas Arrays to compare mRNA populations, **you must use the same method to isolate total or poly A^+ RNA from each sample**. For successful and accurate detection of differentially expressed cDNAs, it is essential that all RNA samples used for analysis be of the same quality. Otherwise, you will be unable to determine whether differences in the pattern of hybridization are due to differential gene expression or differences in RNA quality. For example, if one RNA sample is significantly more degraded than the other, the differences in hybridization patterns may actually reflect differences in individual RNA stability rather than differences in expression. Furthermore, you should not compare total RNA samples to poly A^+ RNA samples. Also, we recommend that you compare Atlas Arrays that came from the same lot (see the bag containing your arrays for the lot number).

Probe Preparation

The Atlas Pure Total RNA Labeling System includes a protocol for probe synthesis that should be followed by users of that product. Atlas Pure users will resume the procedure in this User Manual at the NucleoSpin Extraction column probe purification section (see below and Section VI.C).

- Please see the Introduction for guidelines on using ^{32}P vs. ^{33}P in probe labeling.
- Use the reagents provided for preparing radioactively labeled cDNA probes; **do not** substitute other reagents.
- To confirm that the probe preparation protocol works in your hands, we recommend that you perform a parallel labeling with the Control Poly A^+ RNA (or Control Total RNA for Atlas Pure users). If necessary, the Control Poly A^+ probe can also be used for troubleshooting hybridization; however, because the control probe generates a very strong hybridization signal, we suggest that you do **not** hybridize it to the Atlas Array unless you have problems with your experiment. The Control **Total** RNA probe (Atlas Pure users) should not be used as a hybridization control because it contains a mixture of RNA from several different species. See the Troubleshooting Guide for more information.

IV. Overview & General Considerations continued

- Atlas Arrays should be used with radioactive detection methods, as described in this User Manual. Follow your institution's guidelines for working with radioactivity and disposing of radioactive materials.
- Radioactively labeled probes should have high specific activity. This is especially critical for detecting low-abundance mRNAs. Use only fresh (<one week old) ^{32}P- or (<two weeks old) ^{33}P-labeled nucleotide of high specific activity (typically 3,000 Ci/mmol). To compare RNA populations, both probes should have the same specific activity.
- To remove unincorporated ^{32}P- or ^{33}P-labeled nucleotides from labeled cDNA probes, be sure to use the NucleoSpin Extraction columns provided.

Hybridization

- Prior to your first experiment, test each of your probes by hybridization to a blank membrane as described in Section VII.A. Alternatively, you can perform a test hybridization to the membranes included with an Atlas® Array Trial Kit.
- Use the ExpressHyb™ Hybridization Solution shipped with each array; do **not** substitute your own recipe. This procedure has been optimized to generate a strong hybridization signal with low background.
- ExpressHyb may be stored at room temperature. However, when the temperature drops below 25°C, a precipitate may form. (This often occurs during shipping.) Before use, always warm the solution at 68°C for 30–60 min, stirring thoroughly to completely dissolve any precipitate. Avoid foaming by placing a stir-bar into the bottle and stirring slowly on a magnetic plate.
- Continuous agitation of the array is necessary during all prehybridization, hybridization, and washing steps. For best results, we recommend that you use a hybridization incubator with rotating bottles. If using an adjustable-speed hybridization incubator, rotate bottles continuously at 5–7 rpm during prehybridization and hybridization and at 12–15 rpm for washes. Alternatively, prehybridization, hybridization, and washing steps may be performed in a sealed plastic bag or small plastic container with an air-tight lid, placed in a shaking incubator.

Handling Atlas® Arrays

- The printed surface is facing up when the notched corner of the array is located on the upper right hand side (Figure 6).
- Always use forceps to handle the array membranes and grip the membranes by the edges only. Never touch the areas where the cDNAs are immobilized—even if you are wearing gloves.
- Never allow the array membranes to dry, even slightly. After your final wash, shake off excess solution with forceps (do not blot-dry), and immediately wrap the membrane completely with plastic wrap. When stripping, unwrap the membrane and immediately place it in the stripping solution; avoid prolonged exposure of the membrane to air.

IV. Overview & General Considerations continued

- Each Atlas Array may be stripped and reprobed at least 3 times; however, the strength of the hybridization signal may decrease at each reprobing.
- For effective stripping of a cDNA probe from the array membranes, it is crucial that the membrane be stored at –20°C when not in use. Keep the membrane at room temperature for as briefly as possible. After you have finished exposing the membrane to x-ray film, immediately strip the membrane, wrap it in plastic, and put it in a –20°C freezer—instead of leaving it on your laboratory bench.

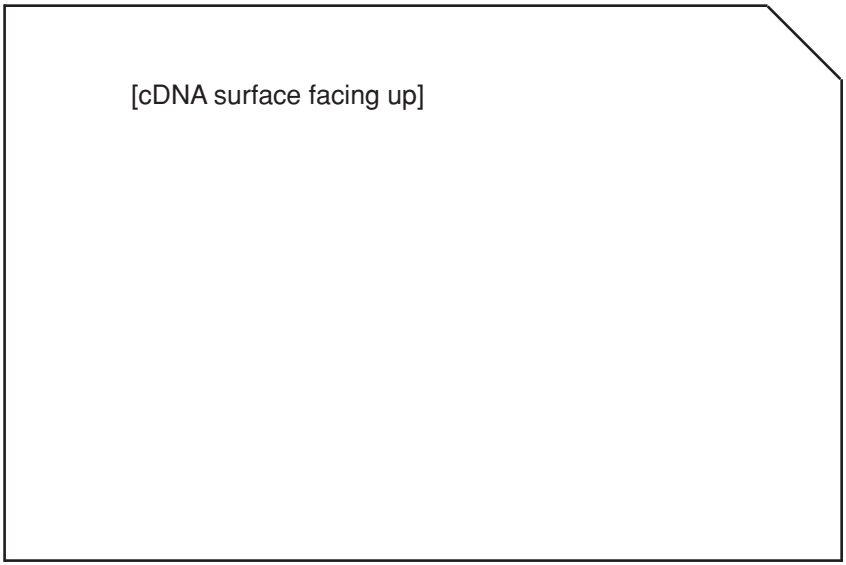


Figure 6. Membrane orientation of Atlas® Nylon Arrays. The upper right hand corner of the array membrane has been cut off. Position this notch at the upper right hand corner of the Orientation Grid to ensure that the printed surface is facing up and that the array is correctly aligned.

V. Preparation of RNA

For isolation of total RNA, we strongly recommend that you use the Atlas Pure Total RNA Labeling System (Cat. No. 634562). **If you are using the Atlas® Pure System or an Atlas® Trial Kit, skip Section V and follow the procedure in the Atlas® Pure User Manual.** Otherwise, follow the guidelines below for RNA purification and the appropriate protocol in Section V.B (total RNA) or Section V.C (poly A^+ RNA) for DNase treatment. The removal of DNA contaminants from your RNA is a critical factor in obtaining good results.

A. Recommended Purification Methods

- Poly A^+ RNA

  Different methods and kits for purification of poly A^+ RNA can yield varying results. In general, you should first isolate total RNA using two or three rounds of guanidinium thiocyanate/acid phenol:chloroform extraction. Then purify poly A^+ RNA using one or two rounds of oligo(dT)-cellulose or oligo(dT) latex bead chromatography. Using this procedure, rRNA contamination is usually <20–30% and genomic DNA impurities are usually <0.01%. However, the level of contamination may vary two- to five-fold, depending on the procedure used for RNA isolation. We do not recommend that you purify total RNA using silica membrane-based methods.

- Total RNA

  Most commercially available kits for RNA isolation, including many spin-column formats, are not compatible with the Atlas technology because they result in a high level of nonspecific background.

B. DNase Treatment of Total RNA

DNase I treatment can be performed on either total or poly A^+ RNA. However, because the quantity of poly A^+ RNA is often more limiting, we recommend performing DNase I treatment on total RNA whenever possible. The following protocol for DNase I treatment of 0.5 mg of total RNA prior to purification of poly A^+ RNA can be integrated into most standard RNA purification protocols. If you are starting with more or less than 0.5 mg, adjust all volumes proportionally.

We recommend that you divide your total RNA samples prior to DNase treatment and only perform the DNase treatment described below on a portion. This is a safeguard, since significant degradation can occur during the incubation with DNase I if samples still contain impurities at this point. In our experience, prior to performing this procedure, some RNase-rich human tissues (for example, pancreas, liver, leukocytes, and cerebellum) must be phenol:chloroform extracted twice, then precipitated and resuspended in RNase-free H_2O in order to completely remove RNases.

V. Preparation of RNA continued

Note: For DNase treatment of total RNA, you will need to prepare an additional 100 µl of 10X Termination Mix per sample. This is prepared as:

0.1 M	EDTA (pH 8.0)
1 mg/ml	glycogen

1. Combine the following reagents in a 1.5-ml microcentrifuge tube for each sample (you may scale up or down accordingly):

500µl	Total RNA (1 mg/ml)
100µl	10X DNase I Buffer
50µl	DNase I (**1 unit/µl**)*
350 µl	Deionized H_2O
1.0ml	**Total Volume**

* If using 10units/µl DNase I, dilute 10-fold in 1X DNase I Buffer prior to use.

Mix well by pipetting up and down several times.

2. Incubate the reactions at 37°C for 30 min.
3. Add 100 µl of 10X Termination Mix. Mix well by pipetting up and down.
4. Split each reaction into two 1.5-ml microcentrifuge tubes (550 µl per tube).
5. Add 550 µl of phenol:chloroform:isoamyl alcohol (25:24:1; pH 4.5) to each tube and vortex thoroughly.
6. Spin in a microcentrifuge at 14,000 rpm for 10 min at 4°C to separate phases.
7. Carefully transfer the top aqueous layer to a new 1.5-ml microcentrifuge tube. Avoid pipetting any material from the interface or lower phase.
8. Add 550 µl of chloroform to the aqueous layer and vortex thoroughly.
9. Spin in a microcentrifuge at 14,000 rpm for 10 min at 4°C to separate phases.
10. Being careful not to pipet any chloroform, remove the top aqueous layer and place in a 2.0-ml microcentrifuge tube.
11. Add 1/10 volume (50 µl) of 2 M NaOAc (pH 4.5) and 2.5 volumes (1.5 ml) of 95% ethanol.
12. Vortex the mixture thoroughly; incubate on ice for 10 min.
13. Spin in a microcentrifuge at 14,000 rpm for 15 min at 4°C.
14. Carefully remove the supernatant.
15. Gently overlay the pellet with 500 µl of 80% ethanol.
16. Spin in a microcentrifuge at 14,000 rpm for 5 min at 4°C.
17. Carefully remove the supernatant and any traces of ethanol.
18. Air dry the pellet for approximately 10 min or until pellet is dry.

V. Preparation of RNA continued

19. Dissolve the precipitate in 250 µl of RNase-free H_2O and mix well for 1–2 min. After checking yield and purity as described in Section V.D, proceed with oligo(dT) purification of poly A^+ RNA.
    Alternatively, store your dissolved RNA at –70°C.

C. DNase Treatment of Poly A^+ RNA

If you did not treat your total RNA with DNase I, or if you suspect that your poly A^+ RNA may still be contaminated with genomic DNA, treat your poly A^+ RNA with DNase I using the following protocol. This protocol is written for treatment of 2 µg of poly A^+ RNA, but it can be used for up to 10 µg by increasing the volumes of components proportionally. Depending on the volume/concentration of your poly A^+ RNA, you may not need to add deionized H_2O in steps 1 or 3.

1. For each RNA sample, combine the following reagents in a 0.5-ml microcentrifuge tube:

   2.0µl Poly A^+ RNA (1 mg/ml)
   1.0µl 10X DNase I Buffer
   0.2µl DNase I **(1 unit/µl)***
   6.8 µl Deionized H_2O
   10.0 µl Total Volume

   * If using 10 units/µl DNase I, dilute 10-fold in 1X DNase I Buffer prior to use.

   Mix well by pipetting up and down several times.
2. Incubate the reactions at 37°C for 30 min in an air incubator.
3. Add 1 µl of 10X Termination Mix and 20 µl of deionized H_2O to the tube.
4. Add 30 µl of phenol:chloroform:isoamyl alcohol (25:24:1; pH 4.5) and vortex thoroughly.
5. Spin the tube in a microcentrifuge at 14,000 rpm for 10 min at 4°C to separate phases.
6. Carefully transfer the top aqueous layer to a new 0.5-ml microcentrifuge tube. Avoid pipetting any material from the interface or lower phase.
7. Add 30 µl of chloroform:isoamyl alcohol (24:1) to the aqueous layer and vortex thoroughly.
8. Spin the tube in a microcentrifuge at 14,000 rpm for 10 min at 4°C to separate phases.
9. Being careful not to pipet any chloroform, remove the top aqueous layer and place in a new 0.5-ml microcentrifuge tube.
10. Add 1/10 volume (3 µl) of 2 M NaOAc (pH 4.5) and 2.5 volumes (80 µl) of 95% ethanol.
11. Vortex the mixture thoroughly; incubate on ice for 10 min.

V. Preparation of RNA continued

12. Spin in a microcentrifuge at 14,000 rpm for 15 min at 4°C.
13. Carefully remove the supernatant.
14. Gently overlay the pellet with 100 µl of 80% ethanol.
15. Spin in a microcentrifuge at 14,000 rpm for 5 min at 4°C.
16. Carefully remove the supernatant and any traces of ethanol.
17. Air dry the pellet for approximately 10 min or until pellet is dry.
18. Dissolve the precipitate in 2 µl of deionized H_2O; mix thoroughly. Use this RNA for generating your ^{32}P-labeled cDNA probe. You can store your dissolved RNA at –70°C.

D. Analyzing RNA by Gel Electrophoresis

Before embarking on your experiment, you should always check the quality of your total or poly A^+ RNA by electrophoresing 0.5–2 µg on a denaturing formaldehyde/agarose/EtBr gel. For comparison, you can run 1–2 µg of the Control Poly A^+ RNA supplied with the Atlas Array. Mammalian poly A^+ RNA samples should produce smears from 0.5–12 kb, with weak ribosomal RNA (rRNA) bands at approximately 4.5 and 1.9 kb, representing 28S and 18S rRNAs, respectively. For some gland tissues (e.g., pancreas, stomach, thyroid gland) or tissues that overproduce several proteins (e.g., skeletal muscle), the size distribution may be smaller, containing several additional bands that correspond to the most abundant mRNAs.

If you are using total RNA, you should observe the two bright 28S and 18S rRNA bands at approximately 4.5 and 1.9 kb, respectively. The ratio of intensities of these bands should be 1.5–2.5 : 1.

When comparing expression profiles of two RNA populations, make sure the RNA samples are of similar quality—i.e., confirm that one is not significantly more degraded than the other.

E. Testing for DNA Contamination by PCR

A simple test for genomic DNA contamination is to use your poly A^+ or total RNA directly as a template in a PCR reaction with primers for any well-characterized gene (e.g., actin or G3PDH). Select primers that will amplify a genomic DNA fragment <1 kb. Be careful that the primers are not separated by a long intron. If this reaction produces bands that are visible on an agarose/EtBr gel, your RNA almost certainly contains genomic DNA. As a positive control, use different concentrations of genomic DNA as a template for PCR. This control will allow you to determine the approximate percentage of DNA impurities in your RNA sample. For a successful experiment, your RNA should contain <0.001% genomic DNA or produce no visible PCR product after 35 cycles.

VI. Preparation of cDNA Probes

If you are using the Atlas Pure Total RNA Labeling System or an Atlas Trial Kit, follow the probe synthesis procedure included in the Atlas Pure User Manual. Then return to this section and proceed with Column Chromatography in Section VI.C below. If you are using the Atlas SMART Probe Amplification Kit or the Atlas SpotLight Labeling Kit, follow the protocols supplied with those kits.

If you are not using one of these other products, continue with the appropriate procedure below to prepare probes from **poly A$^+$ RNA** (Section A) or **total RNA** (Section B).

Note: The quality of your RNA is the single most important factor in achieving a successful analysis with Atlas Arrays. If you have not followed the guidelines for RNA preparation in Section V, be sure to do so before proceeding.

A. Probe Synthesis from Poly A$^+$ RNA

The 10-µl reaction described below converts 1 µg of poly A$^+$ RNA into ^{32}P- or ^{33}P-labeled first-strand cDNA. When labeling your experimental cDNA, you should perform a parallel labeling reaction with the Control Poly A$^+$ RNA that is provided. This will verify that the cDNA synthesis system works in your hands and will allow you to estimate the quality and concentration of your experimental cDNA samples.

Note: Always keep MMLV RT on ice and return the tube to the freezer promptly after use.

1. Prepare a Master Mix for all labeling reactions plus one extra reaction to ensure that you have sufficient volume. Combine the following reagents in a 0.5-ml microcentrifuge tube **at room temperature**:

	per rxn	4 rxns
5X Reaction Buffer	2 µl	8 µl
10X dNTP Mix (for dATP label)	1 µl	4 µl
[α-^{32}P]dATP (3,000 Ci/mmol, 10 µCi/µl)	3.5 µl	14 µl
-or-		
[α-^{33}P]dATP (>2,500 Ci/mmol, 10 µCi/µl)		
DTT (100 mM)	0.5 µl	2 µl
Total volume	**7 µl**	**28 µl**

2. Preheat a PCR thermal cycler to 70°C.
3. For each experimental poly A$^+$ RNA and the Control Poly A$^+$ RNA, combine the following in a labeled 0.5-ml PCR tube:

poly A$^+$ RNA sample	1 µg (1–2 µl)
CDS Primer Mix	1 µl*

* If you have observed weak signals in previous hybridizations, use 2 µl CDS Primer Mix, but do not exceed 3 µl total volume.

To each tube, add deionized H_2O to a final volume of 3 µl (if necessary).

4. Mix well by pipetting and spin tubes briefly in a microcentrifuge.
5. Incubate tubes in preheated PCR thermal cycler at 70°C for 2 min.

VI. Preparation of cDNA Probes continued

6. Reduce the temperature of the thermal cycler to 50°C (or 48°C if you are using an unregulated heating block or water bath) and incubate tubes for 2 min. During this incubation, add 1 µl MMLV Reverse Transcriptase per reaction to the Master Mix (add 4 µl MMLV RT for the 4-reaction Master Mix). Mix by pipetting, and keep the Master Mix at room temperature.
7. After completion of the 2-min incubation at 50°C, add 8 µl of Master Mix to each reaction tube.

   Note: Do not remove the RNA samples from the thermal cycler for longer than is necessary to add the Master Mix.
8. Mix the contents of the tubes by pipetting and immediately return them to the thermal cycler.
9. Incubate tubes in the PCR thermal cycler at 50°C (or 48°C) for 25 min.
10. Stop the reaction by adding 1 µl of 10X Termination Mix.
11. Proceed with the column chromatography in Section C below. If necessary, you can store your probe on ice or at 4°C for a few hours.

B. Probe Synthesis from Total RNA

The 10-µl reaction described below converts 2–5 µg of total RNA into ^{32}P- or ^{33}P-labeled first-strand cDNA. Such a small amount of total RNA is adequate because of the sensitivity provided by the gene-specific CDS Primer Mix. Starting with less than 2 µg could decrease the sensitivity of your probe, while starting with more than 5 µg will be too concentrated in a 10-µl reaction volume. If necessary, the reaction volume can be increased to 20 µl to accommodate a larger volume of RNA. In this case, double all reaction components **except** use 5 µl of [α-^{32}P] or [α-^{33}P]dATP and 1 µl of CDS Primer Mix per reaction. When labeling your experimental RNA, you should perform a parallel labeling reaction with the Control Poly A$^+$ RNA that is provided. This will verify that the cDNA synthesis system works in your hands and will help you estimate the quality of your experimental cDNA samples.

Note: Always keep MMLV RT on ice and return the tube to the freezer promptly after use.

1. Prepare a Master Mix for all labeling reactions plus one extra reaction to ensure that you have sufficient volume. Combine the following reagents in a 0.5-ml microcentrifuge tube **at room temperature**:

	per rxn	4 rxns
5X Reaction Buffer	2 µl	8 µl
10X dNTP Mix (for dATP label)	1 µl	4 µl
[α-^{32}P]dATP (3,000 Ci/mmol, 10 µCi/µl)	3.5 µl	14 µl
-or-		
[α-^{33}P]dATP (>2,500 Ci/mmol, 10 µCi/µl)		
DTT (100 mM)	0.5 µl	2 µl
Total volume	**7 µl**	**28 µl**

VI. Preparation of cDNA Probes continued

2. Preheat a PCR thermal cycler to 70°C.
3. For each reaction, combine the following in a labeled 0.5-ml PCR tube:

For experimental RNA samples	For Control Poly A+ RNA
1–2 µl* RNA (2–5 µg)	1 µl RNA
1 µl† CDS Primer Mix	1 µl CDS Primer Mix

* Or up to 5 µl if the reaction volume is doubled.

† If you have observed weak signals in previous hybridizations, use 2 µl CDS Primer Mix, but do not exceed 3 µl total volume (6 µl if the reaction volume is doubled).

To each tube, add deionized H_2O to a final volume of 3 µl (if necessary).

4. Mix well by pipetting and spin tubes briefly in a microcentrifuge.
5. Incubate tubes in a preheated PCR thermal cycler at 70°C for 2 min.
6. Reduce the temperature of the thermal cycler to 50°C (or 48°C if you are using an unregulated heating block or water bath) and incubate tubes for 2 min. During this incubation, add 1 µl MMLV Reverse Transcriptase per reaction to the Master Mix (add 4 µl MMLV RT for the 4-reaction Master Mix). Mix by pipetting, and keep the Master Mix at room temperature.
7. After completion of the 2-min incubation at 50°C, add 8 µl of Master Mix to each reaction tube.

   Note: Do not remove the RNA samples from the thermal cycler for longer than is necessary to add the Master Mix.
8. Mix the contents of the tubes by pipetting and immediately return them to the thermal cycler.
9. Incubate tubes in the PCR thermal cycler at 50°C (or 48°C) for 25 min.
10. Stop the reaction by adding 1 µl of 10X Termination Mix.
11. Proceed with the column chromatography in Section C below. If necessary, you can store your probe on ice or at 4°C for a few hours.

C. Column Chromatography

To purify the labeled cDNA from unincorporated ^{32}P- or ^{33}P-labeled nucleotides and small (<0.1 kb) cDNA fragments, follow this procedure for each reaction tube. Before use, be sure to add 95% ethanol directly to Buffer NT3 as specified on the bottle label.

1. Dilute probe synthesis reactions to 200 µl total volume with Buffer NT2; mix well by pipetting. If you are continuing from Section VI.A or VI.B, add 190 µl Buffer NT2. If you are continuing from the Atlas Pure Total RNA Labeling System protocol, separate beads and pipet the supernatant (~20 µl) into 180 µl Buffer NT2.

VI. Preparation of cDNA Probes continued

2. Place a NucleoSpin Extraction Spin Column into a 2-ml Collection Tube, and pipet the sample into the column. Centrifuge at 14,000 rpm for 1 min. Discard Collection Tube and flowthrough into the appropriate container for radioactive waste.
3. Insert the NucleoSpin column into a fresh 2-ml Collection Tube. Add 400 µl Buffer NT3 to the column. Centrifuge at 14,000 rpm for 1 min. Discard Collection Tube and flowthrough.
4. Repeat Step 3 twice.
5. Transfer the NucleoSpin column to a clean 1.5-ml microcentrifuge tube. Add 100 µl Buffer NE, and allow column to soak for 2 min.
6. Centrifuge at 14,000 rpm for 1 min to elute purified probe.
7. Check the radioactivity of the probe by scintillation counting:
   a. Add 2 µl of each purified probe to 5 ml of scintillation fluid in separate scintillation-counter vials.
   b. Count ^{32}P- or ^{33}P-labeled samples on the ^{32}P channel, and calculate the total number of counts in each sample. (Multiply counts by a dilution factor of 50.)

   Probes synthesized using the procedure in this User Manual should have a total of **5–20 x 10^6 cpm** for probes made from poly A^+ RNA or **2–10 x 10^6 cpm** for probes made from total RNA. Probes made using the Atlas Pure Total RNA Labeling System typically have an activity of **1–10 x 10^6 cpm**. Store probes at –20°C.

   Discard flowthrough fractions, columns, and elution tubes in the appropriate container for radioactive waste.

VII. Hybridization Procedure

A. Test Hybridization to Blank Membrane

Before hybridizing labeled cDNA probes to Atlas Arrays, you should check the quality of each probe by hybridizing it to a control (blank) nylon membrane (supplied). This will allow you to estimate the level of nonspecific background resulting from impurities in your RNA samples. If you lack sufficient RNA for hybridization to a blank membrane, test for genomic DNA contamination by PCR (Section V.E). It is critical that your RNA samples be free of genomic DNA impurities. For the test hybridization, follow the procedure described below in Section B, except in Step B.4, use only 1/5 of your total pool of probe. Adjust each of the other volumes in that step proportionately. For performing the test hybridization with additional probes, you can cut the blank membrane into separate pieces if necessary. If you observe a high level of background on the control membrane, you should treat your total RNA sample(s) again with DNase I (Section V.B) and remake your probe(s).

B. Hybridizing cDNA Probes to the Atlas® Array

Notes: Because probes made from the Control RNA generate strong hybridization signals, we suggest that you do not hybridize control probes to Atlas Arrays unless you have problems with your experiment. See the Troubleshooting Guide for more information.

1. Prepare a solution of ExpressHyb and sheared salmon testes DNA:
   a. Prewarm 5 ml of ExpressHyb at 68°C.
   b. Heat 0.5 mg of the sheared salmon testes DNA at 95–100°C for 5 min, then chill quickly on ice.
   c. Mix heat-denatured sheared salmon testes DNA with prewarmed ExpressHyb. Keep at 68°C until use.
2. Fill a hybridization bottle with deionized H_2O. Wet the array by placing it in a dish of deionized H_2O, and then place the membrane into the bottle. Pour off all the water from the bottle; the membrane should adhere to the inside walls of the container without creating air pockets. Add 5 ml of the solution prepared in Step 1. Ensure that the solution is evenly distributed over the membrane. Perform this step quickly to prevent the array membrane from drying.
3. Prehybridize for 30 min with continuous agitation at 68°C.

   Notes:
   - Do not remove the array from the container during the prehybridization, hybridization, or washing steps.
   - If performing the hybridization in roller bottles, rotate at 5–7 rpm during prehybri-dization and hybridization steps.

VII. Hybridization Procedure continued

4. To prepare your probe for hybridization, add 5 µl C_0t-1 DNA to your entire pool of labeled probe and incubate in a boiling (95–100°C) water bath for 2 min. Then incubate probe on ice for 2 min.
   Note: If you are performing the test hybridization to the blank membrane, use 1/5 volume for each of the components in these steps.

   [Optional] If you are not using Atlas Pure or an Atlas Trial Kit, or if you prefer an alkaline denaturing procedure, you may use the following steps instead:

   a. Mix together:

Labeled probe (entire pool; 0.5–20 x 10^6 cpm)	~100 µl
10X denaturing solution (1M NaOH, 10 mM EDTA)	~11 µl*
Total Volume	**~111 µl**

   *or 1/10 Total Volume

   b. Incubate at 68°C for 20 min.

   c. Add the following to your denatured probe:

C_0t-1 DNA	5 µl
2X neutralizing solution (1 M NaH_2PO_4, pH 7.0)	~115 µl†
Total Volume	**~230 µl**

   †or 1/2 Total Volume

   d. Continue incubating at 68°C for 10 min.

5. Being careful to avoid pouring the concentrated probe directly on the surface of the membrane, add the mixture prepared in Step 4 directly to your array and prehybridization solution. Make sure that the two solutions are mixed together.
6. Hybridize overnight with continuous agitation at 68°C. Be sure that all regions of the membrane are in contact with the hybridization solution at all times. If necessary, add an extra 2–3 ml of prewarmed ExpressHyb.
7. The next day, prewarm Wash Solution 1 (2X SSC, 1% SDS) and Wash Solution 2 (0.1X SSC, 0.5% SDS) at 68°C.
8. Carefully remove the hybridization solution and discard in an appropriate radioactive waste container. Replace with 200 ml of prewarmed Wash Solution 1. Wash the array for 30 min with continuous agitation at 68°C. Repeat this step three more times.
   Note: If using roller bottles, fill to 80% capacity and rotate at 12–15 rpm during all wash steps.
9. Perform one 30-min wash in 200 ml of prewarmed Wash Solution 2 with continuous agitation at 68°C.
10. Perform one final 5-min wash in 200 ml of 2X SSC with agitation at room temperature.

VII. Hybridization Procedure continued

11. Using forceps, remove the array from the container and shake off excess Wash Solution. Do not blot dry or allow the membrane to dry. **If necessary, place the array in H_2O while preparing an adequate sized piece of plastic wrap. If the membrane dries even partially, subsequent removal of the probe (stripping) from the Atlas® Array will be difficult.**
12. Immediately wrap the damp membrane in plastic wrap. If possible, seal the edges of the wrapped array using a heat sealer to prevent the array from drying.
13. Mount the plastic-wrapped array on Whatman paper (3 MM Chr). Expose the array to x-ray film at –70°C with an intensifying screen. When setting up your exposure, make sure you are aware of the orientation of the array by noting the position of the orientation notch at the upper right-hand corner of the membrane. Also, be sure to try several exposures for varying lengths of time (e.g., 3–6 hr, overnight, and 3 days). Alternatively, use a phosphorimager. When exposing the array to a phosphorimaging screen at room temperature, be sure to seal the array membrane in plastic to prevent drying.

C. Stripping cDNA Probes from the Atlas® Array

To re-use the array after exposure to x-ray film or phosphorimaging, you may remove the cDNA probe by stripping.

Note: Perform all steps in a fume hood with appropriate radiation protection.

1. In a 2-L beaker, heat 500 ml of 0.5% SDS solution to boiling.
2. Remove the plastic wrap from the array and immediately place the membrane into the boiling solution. Avoid prolonged exposure of the membrane to air.
3. Continue to boil for 5–10 min.
4. Remove the solution from heat and allow to cool for 10 min.
5. Rinse the array in Wash Solution 1 (2X SSC, 1% SDS).
6. Remove the array from the solution and immediately wrap the damp membrane in plastic wrap. Check the efficiency of stripping with a Geiger hand counter and by exposure to x-ray film. If radioactivity can still be detected, repeat the stripping procedure (steps 1–5).
7. Place the array into a hybridization container and proceed with the next hybridization experiment. Alternatively, the array can be sealed and stored in plastic wrap at –20°C until needed. Do not allow the membrane to dry, even partially.

VIII. Interpretation of Results

A. Sensitivity of Detection and Background Level

After hybridization and washing, we recommend that you perform a "trial run" exposure of your array membranes to x-ray film or a phosphorimaging screen. This will allow you to assess the sensitivity and quality of your hybridization pattern so that you can determine the optimal exposure time for your experiment. For x-ray film, expose the membranes to Kodak BioMax MS film (with the corresponding BioMax MS intensifying screen) at –70°C overnight. In our experience, other x-ray films are 2- to 5-fold less sensitive than BioMax MS film. If available, a phosphorimager affords approximately the same sensitivity as BioMax MS film, and allows you to quantify hybridization signals.

Exposure Time

Figure 7 shows an Atlas Human Array that was hybridized to a ^{32}P probe prepared from the Control Poly A$^+$ RNA (from human placenta). The upper panel shows an overnight exposure to BioMax MS x-ray film at –70°C. As long as your RNA is of high quality, the signals corresponding to medium- to high-abundance mRNAs (0.05–0.5% of poly A$^+$ RNA) can be easily detected after several hours or an overnight exposure. If you have treated your RNA samples with DNase I as recommended, the genomic DNA spots should not appear until after 3–6 days of exposure to x-ray film. Do not be concerned if you do not observe a signal for the genomic DNA spots; this indicates that your samples are free of genomic DNA.

Usually, an overnight exposure is not sufficient to reveal hybridization signals from rare- to medium-abundance mRNAs, especially when using ^{33}P-labeled probes. In our experience, a 3- to 7-day exposure will reveal hybridization signals for about 20–70% of the arrayed cDNAs. The lower panel of Figure 7 shows a 3-day exposure of an Atlas Array to BioMax MS x-ray film at –70°C. The exact number of hybridization signals depends on the complexity of the experimental RNA sample, and may differ by several-fold. The practical limit for sensitivity is the level of background generated by nonspecific hybridization of the probe to the membrane. Longer exposure times (>7 days) are useful only if the background level is low. Overexposure is not an issue if using a phosphorimager.

Orienting Your X-ray Film

Use the Orientation Grid provided to identify the positions of your hybridization signals. The dark spots for the housekeeping genes are good guidelines for precisely aligning your array to the grid. The gene locations are diagrammed in the enclosed Array Gene List. If the orientation notch on the upper right-hand corner of the array image is visible, verify that it is positioned at the upper right-hand corner of the drawing on the transparency. If you encounter difficulty in aligning the Orientation Grid to your array, see the Troubleshooting Guide (Section IX.E)

VIII. Interpretation of Results continued

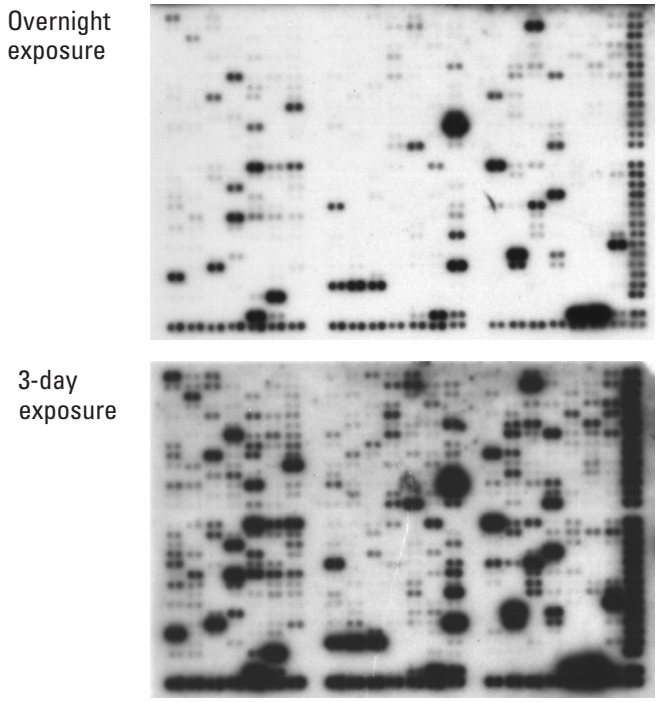


Figure 7. Sample overnight and 3-day exposures using the Atlas® Human cDNA Expression Array and the Human Control Poly A+ RNA. The Control Poly A+ RNA (from human placenta) was used to make a ^{32}P-labeled cDNA probe, which was hybridized to the membrane according to this User Manual. The membrane was exposed to Kodak BioMax MS x-ray film with a BioMax MS intensifying screen at –70°C for the indicated amount of time. Please note that due to the strong hybridization signal generated with the control probe, we recommend that you only perform the control hybridization experiment if necessary for troubleshooting purposes. The exposure time required to obtain comparable results will depend on the specific activity of your hybridization probe. The intensity of hybridization signals for the control genomic DNA spots can vary with different RNA samples.

The pattern generated by hybridization to the cDNAs arrayed in the various regions should be unique for each RNA sample (see Figure 4 in the Introduction). Some samples may produce signals that are similar or even higher in intensity than the abundant housekeeping genes. After an overnight exposure with ^{32}P-labeled probes, you should observe signals for the most abundant housekeeping genes, including ubiquitin, phospholipase A2, α-tubulin, β-actin, and G3PDH. These genes are expressed at about 0.1–0.5% abundance in mammalian tissues or cells and can be used as universal positive controls. Please note that the ratio of intensities of signals for different housekeeping genes may differ 2- to 5-fold for different tissues

VIII. Interpretation of Results continued

or cells. If you cannot see signals generated by the positive control genes after 1–3 days of exposure, please refer to the Troubleshooting Guide.

Another important parameter is the level of nonspecific hybridization, or background. After overnight exposure, you generally will not see hybridization with blank regions of the membrane or with the negative DNA controls (arrayed in region G). In addition, depending on the source of your RNA, only 5–20% of arrayed cDNAs should reveal hybridization signals after overnight exposure. If you observe hybridization for the negative controls and/or for nearly all arrayed cDNAs, please refer to the Troubleshooting Guide for recommendations on reducing nonspecific hybridization.

B. Normalization of Hybridization Signals

To analyze differential gene expression using Atlas Arrays, it is important to identify one or more housekeeping genes that generate equally intense hybridization signals for both samples being compared. This housekeeping gene or genes can then serve as a positive control. In cells or tissues that are closely related—i.e., where only a few genes change their expression levels—expression of housekeeping genes generally remains constant. However, as seen in Figure 4 (Introduction), the expression levels of individual housekeeping genes may be variable, depending on your experimental system. Figure 4 probably represents the most complicated case, in which RNA from different human tissues are being compared. Note the variation in expression levels of the nine housekeeping genes on the array.

The best approach for comparing hybridization signals for different samples is to equalize the intensity of the hybridization signals by adjusting exposure times. If one array is uniformly darker than the other, adjust the exposure time of one array until the signals for the housekeeping genes are approximately the same on both arrays. The most common reason for different hybridization intensities is the quality of RNA samples used to prepare the hybridization probes. For more details about RNA quality, please refer to the Troubleshooting Guide.

When normalizing the results of closely related cells or tissues, you can expect that the ratio of intensities of the hybridization signals for most of the nine housekeeping control cDNAs will be approximately equal. In this case, usually only a few genes will exhibit different levels of expression. Choose one or two of the least variable housekeeping control cDNAs to normalize the results from both membranes being compared. On the other hand, when comparing unrelated tissues or cells, as in Figure 4, it is often easiest to use the signals of cDNAs surrounding a target cDNA for normalization. The advantage of this approach is that the cDNAs being compared are in close proximity on the filter. For use as standards, choose only those cDNAs whose expression levels remain constant under your experimental conditions. Alternatively, you can use the average intensity of all nine

VIII. Interpretation of Results continued

housekeeping genes to normalize the results from both membranes. In our experience, these are the most convenient and accurate approaches to normalization.

C. Comparison of Results Using ^{32}P- and ^{33}P-labeled Probes

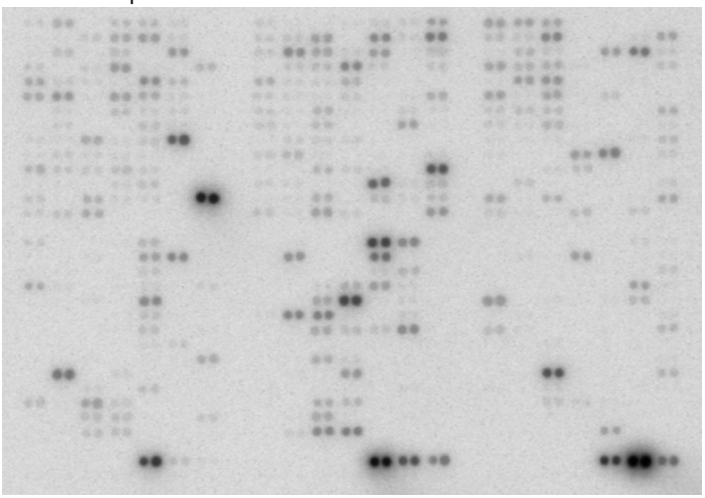
As discussed in the Introduction, both ^{32}P- and ^{33}P-labeling methods are compatible with Atlas Arrays. Figure 8 shows arrays that were hybridized to either a ^{32}P- or ^{33}P-labeled probe. Compared to ^{32}P, the spatial resolution and quality of the images is improved with ^{33}P. These characteristics tend to facilitate image analysis and signal quantification. However, also note that ^{33}P signals are approximately four times less intense, decreasing assay sensitivity.

D. Corroboration of Results by RT-PCR

Because of sequence-dependent hybridization characteristics and variations inherent in any hybridization reaction, Atlas data and any other array data should be considered semiquantitative. We strongly recommend that you corroborate the results of your experiment using RT-PCR.

VIII. Interpretation of Results continued

^{32}P-labeled probe



^{33}P-labeled probe

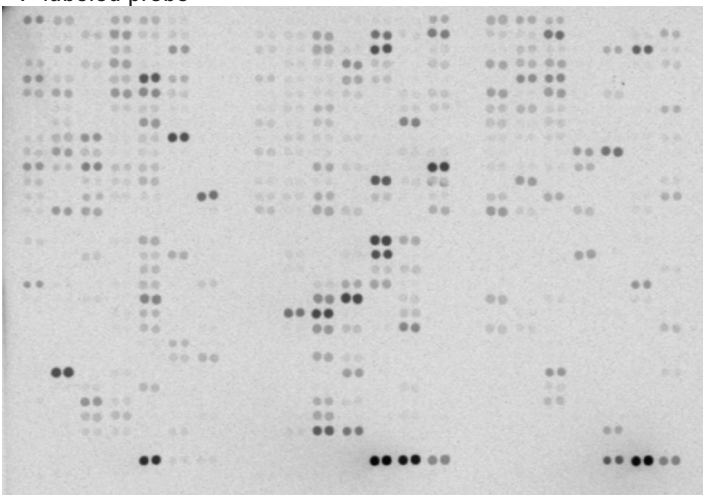


Figure 8. Comparison of ^{32}P- and ^{33}P-labeled probes using Atlas® Human Cancer Arrays. Radiolabeled cDNA was prepared from poly A$^+$ RNA from the Jurkat human T-cell leukemia cell line. The cDNA was hybridized to the membranes according to the protocol. Phosphorimaging screens were exposed overnight. ^{32}P-labeled probes: 1 x 10^6 cpm. ^{33}P-labeled probes: 4 x 10^6 cpm.

IX. Troubleshooting Guide

Users seldom encounter problems with Atlas Arrays when following the procedures described in this User Manual. These procedures may seem arduous, but they should be followed closely. Not only do they improve the odds of success, but they also make troubleshooting much easier. For best results, use the labeling reagents and ExpressHyb Hybridization Solution included with these arrays. Do **not** substitute your own reagents.

If you do encounter problems, consult the following troubleshooting guide.

A. RNA Quality

As discussed in Sections IV and V, the quality of the RNA used to make your probe is the single most important factor determining the success or failure of Atlas experiments. The following guidelines may be helpful for troubleshooting problems with your experimental RNA samples. If you suspect that your RNA is not of sufficient quality, we strongly recommend that you use the Atlas Pure Total RNA Labeling System (Cat. No. 634562) if you are not already doing so. Otherwise, prepare fresh RNA according to the guidelines described in Sections IV and V.

1. Genomic DNA Contamination

   If your background is high, genomic DNA contamination is the most likely cause. There are several steps you can take to test for genomic DNA contamination and to correct the problem.

   a. If you have not already done so, perform a test hybridization with your probe and the blank membrane as described in Section VII. A.

   b. If you have not already done so, test your RNA for genomic DNA contamination by PCR as described in Section V.E.

   c. If you have not already done so, treat your RNA with DNase I as described in Sections V.B and V.C, then prepare fresh probe.

2. Additional Troubleshooting of RNA Quality

   a. The Control poly A^+ RNA (Total RNA for Atlas Pure users) is a good positive control for preparing your hybridization probe. Compare the incorporation of ^{32}P or ^{33}P for cDNA made from the Control RNA and your experimental RNA samples. Incorporation using the Control RNA should be 2–20 x 10^6 cpm. If you obtain this level of incorporation with the Control Poly A^+ RNA, but cannot incorporate at least 1 x 10^6 cpm into cDNA made from your experimental RNA samples, your experimental RNA samples are probably of poor quality or the concentrations of your samples are significantly lower than you expected. On the other hand, if the Control RNA fails to yield good incorporation, you should troubleshoot the labeling procedure or check the quality of the isotope used for labeling. See Section IX.C for more details.

IX. Troubleshooting Guide continued

b. If you are not using the Atlas Pure Total RNA Labeling System, be sure that you are using the optimum amount of poly A^+ RNA to prepare your hybridization probe. We typically use 1 µg of poly A^+ RNA or 2–5 µg of total RNA (measured from the A_{260} of a concentrated stock) for each labeling reaction.

c. If you are using Atlas Pure, be sure to work through the enrichment/probe synthesis steps quickly, without pausing. Additionally, to help reduce any chance of RNA degradation, you may add 100 units of Ambion's ANTI-RNase (#2692) after adding magnetic beads to your sample.

d. If, on a denaturing formaldehyde/agarose/EtBr gel, your experimental poly A^+ RNA appears as a smear that is no larger than 2 kb, the RNA may be degraded. If this is the case, we suggest you prepare fresh RNA after checking your purification reagents for RNase or other impurities. If problems persist, you may need to find another source of tissue/cells.

e. Impurities in RNA samples can inhibit reverse transcriptase. In this case, you may need to perform additional steps to purify your total RNA starting material. Try treating your total RNA twice with phenol: chloroform and once with chloroform, followed by precipitation with 1/10 volume of 2 M NaOAc (pH 4.5) and 2.5 volumes of ethanol. This will help ensure the removal of any protein and other impurities that may not have been removed effectively during initial RNA purification. You can perform additional RNA purification methods such as CsCl centrifugation or gel filtration if necessary. Such procedures should be optimized for each particular tissue/cell type separately.

B. High Nonspecific Background (with or without hybridization signals)

The negative controls immobilized on the Atlas Array should not generate strong hybridization signals. Moreover, in a typical experiment with this array, only 20–70% of the cDNAs give hybridization signals (depending on the length of exposure time to x-ray film). If the signals for the negative controls are comparable to the signals for the cDNAs, or if more than 80% of the cDNAs give strong signals, you should reduce the background.

If the background is high, use the following guidelines to troubleshoot your experiment. First, strip the probe from the array as described in Section VII.C. Monitor the stripping process with a Geiger counter; cpm should be reduced to almost background levels. If stripping does not substantially lower the signal level, successful stripping may not be possible. You will have to wait for the signal to decay to re-use the array. Once the signal level is reduced, repeat the hybridization, making sure that you adhere to the protocol and take note of the guidelines listed below.

IX. Troubleshooting Guide continued

Several factors may account for high levels of nonspecific background:

1. RNA used to make the hybridization probe is of poor quality

   Refer to Section A above.

2. Unincorporated [α-^{32}P] or [α-^{33}P]dATP is not fully removed from the probe

   If for some reason you did not purify your cDNA probe, use the NucleoSpin Extraction Spin Columns provided with the Atlas Array and follow the procedure described in Section VI.C. Do not use another purification method.

3. Probe is too old

   Use your cDNA probe as soon as possible after preparation because radioactive decay results in probe fragmentation. Small radioactive decay products can contribute to high background.

4. Composition of hybridization mix is incorrect

   Use the ExpressHyb Hybridization Solution provided with the array and follow the procedure described in Section VII.B. Do **not** substitute another hybridization solution.

5. ExpressHyb solution has precipitated

   When the temperature drops below 25°C, a precipitate may form in the hybridization solution. This often occurs during shipping. Before using, always warm the solution at 68°C for 30–60 min, stirring thoroughly to dissolve any precipitate. Avoid foaming by placing a stir-bar into the bottle and stirring slowly on a magnetic plate.

6. Alternative reagents substituted for making hybridization probe

   The optimal size of the cDNA fragments in your probe is 200–800 nucleotides. The labeling reagents provided with the Atlas Array have been optimized for this size range. Using other reagents or labeling kits may increase background and reduce sensitivity. Do **not** substitute other reagents.

7. Hybridization probe contains repetitive sequence(s)

   Be sure to include C_ot-1 DNA in your hybridization mixture.

C. Hybridization Signals Absent or Very Weak

Each cDNA sample on the membrane is confined to a dot containing 10 ng of a PCR-amplified fragment. Using the reagents provided, you can generate labeled cDNA probes that should enable you to detect expression of mRNAs present at 0.005–0.01% of poly A$^+$ RNA—a sensitivity corresponding to approximately 10–20 target transcripts per cell. Some mRNAs are expressed at very low levels—sometimes less than this sensitivity limit—even in the tissues or cells from which the cDNA probe was originally derived. To detect rare messages, always expose x-ray film at –70°C with an intensifying

IX. Troubleshooting Guide continued

screen and use fresh solutions for developing the film. We recommend Kodak BioMax MS film (Kodak #118 8077, with the corresponding BioMax MS intensifying screen). Using a phosphorimager allows you to further increase sensitivity by two-fold.

If you use the conditions for labeling and hybridization described in this User Manual, an overnight exposure of an Atlas Array to BioMax MS x-ray film at –70°C should reveal signals for the control housekeeping cDNAs for ubiquitin and G3PDH; these mRNAs correspond to an abundance level of about 0.1–0.2%. Likewise, a 5–6 day exposure should reveal signals for the control genomic DNA. (See Section VIII for more details.) Sensitivity can be further increased by lengthening exposure times (up to two weeks) or reducing nonspecific background (see Section VIII.A). This is especially important for rare mRNAs, where the hybridization signal may be comparable with the level of nonspecific background.

If you still do not see a hybridization signal for the control housekeeping genes and genomic DNA after 5–6 days of x-ray film exposure, use the following guidelines to troubleshoot your experiment:

1. RNA used to make the hybridization probe is of poor quality

   Refer to Section A above.

2. Your MMLV RT may be degraded

   This can happen if MMLV RT is not kept on ice at all times, or it is not returned to the freezer promptly after use.

3. Hybridization probe has low specific activity
   - If the ^{32}P-dATP you used for labeling was older than one week or your ^{33}P-dATP was older than two weeks, make a new probe with fresh nucleotide. Some lots of dATP may inhibit incorporation. If you obtain $<0.5 \times 10^6$ cpm incorporation for the Control RNA, procure a new lot of dATP and repeat the labeling procedure.
   - Try using 2 µl CDS Primer Mix to make probes in SectionsVI.A or VI.B instead of 1 µl.
   - If you are using the Atlas Pure Total RNA Labeling System, you can increase the amount of RNA used for labeling to as much as 50 µg. Increasing the amount of RNA can dramatically improve results in some cases.

4. Array has been stripped too many times

   If you are reprobing the array and fail to see a hybridization signal, you should confirm that the cDNA was not removed from the membrane during stripping. Using the Control Poly A$^+$ RNA included with this array, make a positive control probe by following the procedure in Section VI of **this** User Manual and hybridize the probe to the array. Because the control probe generates a strong hybridization signal, we recommend

IX. Troubleshooting Guide continued

that you only perform this control hybridization if you are having problems with your experiment. Furthermore, we suggest that you only use 1/10 of the total volume of positive control probe in the hybridization.

D. Unable to Strip and Reprobe

If you observe high background when reprobing the array, the membrane may not have been stripped completely or may have been allowed to dry. If a membrane is allowed to dry even partially, subsequent removal of the probe will be very challenging. To prevent drying after your final wash, shake off excess solution with forceps (do not blot dry) and immediately wrap the membrane in plastic wrap or seal it in a polyethylene bag. When reprobing, unwrap the array, immediately place it in stripping solution, and follow the rest of the protocol provided for removing probes (Section VII.C). If the membrane has not partially dried, consult the paragraph discussing high background in Section B above.

E. Difficulty Orienting or Aligning the Orientation Grid

When exposing your array to x-ray film or a phosphorimager screen, make sure that you note the orientation of the membrane. The upper right-hand corner of the array membrane has been cut off to make this easier. When analyzing your results using the Orientation Grid, verify that this orientation notch is positioned at the upper right-hand corner of the drawing on the transparency (Section IV, Figure 6). Also, use the dark housekeeping gene spots as guidelines to precisely align your array to the grid. The gene locations diagrammed in the enclosed Array Gene List will prove helpful.

Occasionally, you may have difficulty precisely aligning all of the array spots with the boxes on the Orientation Grid. This sometimes results from membrane warping during the experimental steps. In this case, you should confine your analysis to one region of the array at a time, and then adjust the Orientation Grid as necessary when continuing to the next area.

F. No Signal Produced by Genomic DNA Spots

If you have treated your RNA samples with DNase I as recommended, the genomic DNA spots should not appear until 5–6 days after exposure to x-ray film. Do not be concerned if you do not observe a signal for the genomic DNA spots; this indicates that your samples are free of genomic DNA.

G. Low Specific Activity in Probe Fractions

Scintillation counting provides an approximate measure of the radioactivity of your samples. The counts per minute (cpm) returned by this method can vary a great deal from counter to counter. If your cpm counts are significantly lower than the recommended level, this may be a sign that your scintillation counter is operating at a low efficiency. In order to accurately assess the activity of your probe, we recommend that you convert your numbers from

IX. Troubleshooting Guide continued

cpm counts to disintegrations per minute (dpm) counts which precisely represent radioactivity. To make this conversion, you must first obtain the ^{32}P channel efficiency of your scintillation counter. Typically this information is determined from calibrations of the instrument and is available at your institution's radiation safety office. The total dpm for each fraction is calculated as:

$$dpm = \frac{cpm}{efficiency}$$

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XI. Related Products

For a complete listing of all Clontech products, please visit **www.clontech.com**

Products	Cat. No.
Atlas® Nylon Membrane Macroarray Products	
• Human Array Trial Kit	634563
• Pure Total RNA Labeling System	634562
• NucleoSpin® RNA II Kit	635990 635991
• SMART™ Probe Amplification Kit	634607
• SpotLight™ Labeling Kit	634803
• SpotLight™ Chemiluminescent Hybridization & Detection Kit	634802
• ExpressHyb™ Hybridization Solution	636831 636832
• NucleoSpin® Extraction Kit	635961
Atlas® Glass Microarray Products	
• Glass Microarrays	many
• Glass Fluorescent Labeling Kit	634709
• Glass Total RNA Isolation Kit	634708
• Glass Hybridization Chamber	634706
Atlas® Plastic Microarray Products	
• Plastic Microarrays	many
• Plastic Printing Kit	634806
• Plastic Array Hybridization Box	634810
• PlasticHyb™ Hybridization Solution	634807
Atlas® Software Products	
• AtlasImage™ 2.7 Software	634650
• Nylon RT-PCR Primer Sequences CD-ROM	634564

XI. Related Products continued

Products	Cat. No.
Other Products for Gene Cloning and Expression Analysis	
• Mouse Multiple Tissue Expression (MTE™) Array	636827
• Multiple Tissue Northern (MTN®) Blots	many
• Clontech PCR-Select™ cDNA Subtraction Kit	637401
• Poly A^+ and Total RNAs	many
• Marathon-Ready cDNAs	many
• QUICK-Clone™ cDNAs	many
• Multiple Tissue cDNA (MTC™) Panels	many
• RT-PCR Amplimer Sets	many