USER GUIDE

ambion®
by life technologies™

*mir*Vana™ Probe & Marker

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life technologies™

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About This Guide

WARNING! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see the "Safety" appendix in this document.

IMPORTANT! Before using this product, read and understand the information the "Safety" appendix in this document.

Purpose

The *mirVana™ Probe & Marker User Guide* provides detailed procedures, reference information and troubleshooting for the kit.

User attention words

Five user attention words may appear in this document. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation or accurate chemistry kit use.

CAUTION! Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Except for IMPORTANTs, the safety alert words in user documentation appear with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instrument. See the “Safety” appendix for descriptions of the symbols.

1 *mir*Vana™ Probe & Marker

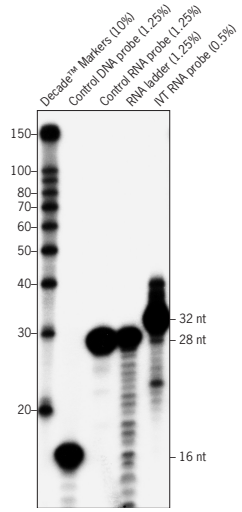
Introduction

Product Description and Background Information

Brief product description

Noncoding small RNAs such as transfer RNA (tRNA), ribosomal RNA (rRNA), small nucleolar RNA (snoRNA), and small nuclear RNA (snRNA) play critical roles in a variety of biological processes. In the past few years, two novel classes of small RNAs, microRNA (miRNA) and small interfering RNA (siRNA), have also emerged as powerful posttranscriptional regulators of gene expression (Carrington and Ambros 2003, Pasquinelli and Ruvkun 2002). Short probes are essential to detect small RNAs for analysis of their biogenesis and function. The *mir*Vana™ Probe & Marker Kit is designed for rapid 5' end labeling and clean-up of small RNA or DNA probes. The kit also contains reagents to prepare small radiolabeled RNA size markers (Decade™ Markers; 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, and 10 nt) and single-nucleotide RNA ladders (figure below). The purification procedure and components included in the kit are optimized for efficient recovery of small RNA probes, and can also be used to remove unincorporated nucleotides from radiolabeled probes prepared by in vitro transcription.

Figure 1 Probes and Markers Prepared with the *mir*Vana™ Probe & Marker Kit. Radiolabeled probes were prepared following the instructions in section "mirVana Probe & Marker Kit Procedure" on page 10. The 32 nt RNA probe was synthesized by in vitro transcription (IVT) using the *mir*Vana miRNA Probe Construction Kit. The Purification Cartridges and reagents were used to remove unincorporated nucleotides from all the labeled nucleic acids except the Control DNA. 1.25% of the purified Control RNA probe was subjected to alkaline hydrolysis as described in section "Alkaline Hydrolysis of RNA Probes" on page 22 to produce the RNA Ladder. The indicated percentage of each prep was resolved on a 15% denaturing polyacrylamide gel. The gel was exposed for 15 min.



5' end labeling

The *mir*Vana Probe & Marker Kit provides optimized reagents for the 5' end labeling of nucleic acids using bacteriophage T4 Polynucleotide Kinase (T4 PNK) and [γ-^{32}P]ATP (not included). T4 PNK catalyzes the transfer of the gamma phosphate from ATP to the 5'-hydroxyl of the nucleic acid molecule (thus only nucleic acids with a 5'-OH group can be labeled e.g., RNA or DNA oligonucleotides, PCR products. This phosphate transfer is commonly called a kinase or phosphorylation reaction.

IMPORTANT! DNA cut with most restriction enzymes, IVT RNAs, or small RNAs purified from biological samples have a 5'-PO_4 and, unless they are treated enzymatically to remove the phosphate first, are not substrates for T4 PNK.

Applications

Short radiolabeled probes prepared with the *mir*Vana Probe & Marker Kit are especially useful for the study of small RNA molecules such as rRNA, snoRNA, snRNA, siRNA or miRNA. DNA probes are typically used for Northern, Southern or primer extension experiments. RNA probes are also useful in hybridization reactions because RNA-RNA hybrids are more stable than RNA-DNA or DNA-DNA duplexes. In addition to standard nucleic acid detection procedures which rely on hybridization on a solid support, (Northern, Southern or dot blot), short antisense RNA probes can also be used for the sensitive detection of miRNA or siRNA by solution hybridization with the *mir*Vana miRNA Detection Kit. Used with the *mir*Vana miRNA Isolation Kit, designed for the rapid purification of representative total RNA population containing all of the small RNA species, the *mir*Vana miRNA Detection Kit and the *mir*Vana Probe & Marker Kit are an integrated system optimized for the study of the biogenesis and functions of siRNA and miRNA.

Short RNA probes are also useful for a variety of RNA structure/function experiments.

Radiolabeled RNA probes are also widely used to study protein-RNA interactions. The most common applications include bandshift assays in nondenaturing gels (EMSA), in vitro crosslinking with recombinant or native proteins, immunoprecipitation with antibodies specific for the protein of interest, and protein pull-down using recombinant tagged fusion proteins.

Control DNA Substrate

The Control DNA Substrate provided with the kit can be radiolabeled to generate an antisense DNA oligonucleotide probe specific for a highly conserved portion of the 5S rRNA. The probe sequence is 5'-TTAGCTTCCGAGATCA-3'. This probe was successfully used to detect 5S rRNA expression in several human, mouse, and rat tissues and cell lines by Northern blot (see example in Figure 2, and procedure in section "Northern Blot Analysis of Small RNA Molecules" on page 24). The 5S rRNA is a small RNA (120 nt) constitutively expressed at a high level in all cell types. Like other small RNAs, 5S rRNA is not efficiently recovered using RNA isolation procedures that are not specifically optimized for small RNA purification. Thus, 5S rRNA is an ideal loading control for experiments aimed at analyzing expression patterns of differentially expressed small RNAs such as miRNAs.

Control RNA Substrate

The Control RNA Substrate provided with the kit is an HPLC-purified RNA oligonucleotide that can be radiolabeled to generate an antisense RNA probe specific for miR-16 miRNA (Lagos-Quintana et al. 2001). This probe was successfully used to detect *mir-16* expression in several human, mouse, and rat tissues and cell lines by Northern blot (see example in Figure 2, and procedure in section "Northern Blot Analysis of Small RNA Molecules" on page 24). The Control RNA Substrate includes 6 nt at its 3' end that are not complementary to miR-16. This additional 6 nt sequence is cleavable by RNases A and T1 (see sequence below) making the Control RNA probe suitable for solution hybridization assays such as the *mir*-Vana miRNA Detection Kit (Figure 2). Cleavage of the noncomplementary 6 nt sequence results in a clear size difference between the full-length probe and the protected target-specific fragment after gel electrophoresis.

The target RNA sequence (miR-16, 22 nt) is:

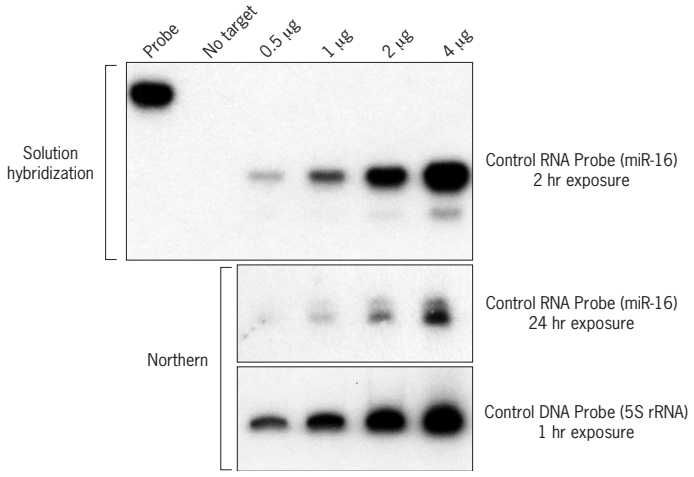
5'-UAGCAGCACGUAAAUAUUGGCG-3'

The Control RNA Substrate sequence (28 nt) is:

3'-GAGACCAUCGUCGUGCAUUUAUAACCGC-5'

Sequence not derived from the target RNA is underlined. This sequence will be removed from the protected probe sequence when used in the *mir*Vana miRNA Detection Kit.

Figure 2 Examples of Small RNA Detection with the Control Probes. ^{32}P-labeled Control probes were synthesized from the Control Substrates provided with the kit, and used to detect miR-16 miRNA or 5S rRNA in FirstChoice® Total RNA from mouse kidney. Solution hybridization assays (top panel) were performed with 5 x 10^4 cpm of Control RNA probe and the mirVana miRNA Detection Kit using the recommended procedure. Northern blots (bottom panels) were hybridized with 1 x 10^5 cpm of Control RNA or DNA probe per mL of hybridization solution following the procedure in section "Northern Blot Analysis of Small RNA Molecules" on page 24.



Reagents Provided with the Kit and Storage

Amount		Component	Storage
10	µL	Decade Marker RNA	below –70°C
40	µL	T4 Polynucleotide Kinase	–20°C
80	µL	10X Kinase Buffer	–20°C
10	µL	Control RNA Substrate	–20°C
10	µL	Control DNA Substrate	–20°C
400	µL	RNA Carrier Solution	–20°C
1	mL	Alkaline Hydrolysis Buffer	–20°C
1.4	mL	Gel Loading Buffer II	–20°C
1.4	mL	Elution Buffer	–20°C
28	mL	Binding/Washing Buffer Concentrate Add 15.4 mL 100% ethanol before use	4°C/room temp
200	µL	10X Cleavage Reagent	room temp
40		Purification Cartridge + Tubes	room temp
40		Elution Tube	room temp
1.75	mL	Nuclease-free Water	any temp†

† Store Nuclease-free Water at –20°C, 4°C or room temp.

*mir*Vana Probe & Marker Kit Procedure

Nucleic Acid Substrate Preparation and Planning

1. Suitable nucleic acid substrates for T4 PNK

   Use purified oligonucleotides if possible

   The *mir*Vana Probe & Marker Kit procedure was designed to accommodate gel-purified, HPLC-purified or simply desalted DNA and RNA oligonucleotides.

   Unpurified (desalted only) oligonucleotide preps may contain a significant amount of smaller-than-full-length products due to poor coupling efficiency during synthesis or subsequent degradation by nuclease contamination (to assess the quality of an oligonucleotide prep see section "Gel Analysis of Oligonucleotides and Labeling Products" on page 21). To obtain probes with the highest possible specific activity, we recommend using purified oligonucleotides when possible. This is especially important for demanding applications that require primarily full-length probes (e.g. primer extension with DNA probes or solution hybridization assays with RNA probe). Alternatively, desalted oligonucleotides can be used directly in the kinase reaction, and full-length radiolabeled probes can be recovered by gel purification (see section "Gel Purification of Probe" on page 23). This strategy is not preferred because smaller products will compete for labeling with the full-length oligonucleotides, potentially reducing specific activity.

Substrates must have a 5'-OH

Any nucleic acid with a 5'-OH (e.g. RNA or DNA oligonucleotides, PCR products) can be phosphorylated by T4 PNK. DNA cut with most restriction enzymes, IVT RNAs, or small RNAs purified from biological samples have a 5'-PO_4 and, unless they are treated enzymatically to remove the phosphate first, are not substrates for T4 PNK.

2. Chemical inhibitors to avoid

   Since ammonium ions, inorganic phosphate, and pyrophosphate, inhibit T4 PNK, it is important that the nucleic acid substrate not contain these compounds. For example, DNA or RNA should not be precipitated with, or dissolved in, reagents containing ammonium salts prior to T4 PNK reactions. A monovalent cation concentration ≥100 mM can also compromise T4 PNK reactions because the K_m for ATP of T4 PNK is decreased as the monovalent cation concentration is increased.

   If there is a chance that your substrate could contain any of these chemical inhibitors, precipitate it to remove these compounds before using it in a *mir*Vana Probe & Marker Kit end labeling reaction (precipitation instructions are provided in section "Precipitation of Oligonucleotides" on page 24).

3. Calculate the molar amount of nucleic acid substrate and [γ-^{32}P]ATP

   For optimal labeling of nucleic acid probes with T4 PNK, the reaction should contain a 2–5 molar excess of radiolabeled ATP over substrate (5' nucleic acid ends). Instructions for calculating the molar concentration of radiolabel and substrate are shown below.

   [γ-^{32}P]ATP

   Calculate the molar concentration of radiolabeled ATP from its specific activity (we recommend 4000–7000 Ci/mmol) and concentration (typically 10–150 mCi/mL).

$$\text{molar concentration (μM or pmol/μL)} = \frac{\text{concentration (mCi/mL)} \times 1000}{\text{specific activity (Ci/mmol)}}$$

   For example, 1 μL of [g–32P]ATP at 10 mCi/mL and

$$\frac{10\ \text{mCi/mL} \times 1000}{6000\ \text{Ci/mmol}} = 1.67\ \mu\text{M or } 1.67\ \text{pmol/}\mu\text{L [g–32P]ATP}$$

Oligonucleotide substrate

- Oligonucleotides are usually supplied dehydrated. We recommend resuspending them at ~100 μM (100 pmol/μL) in nuclease-free water or TE buffer (10 mM Tris-HCl pH 7–8, 0.1 mM EDTA). Then check the concentration of the stock solution by diluting a sample 1:50 to 1:500 in water and reading its absorbance in a spectrophotometer at a wavelength of 260 nm. Multiply the reading by the dilution factor to give A260, then calculate the concentration with the following equation:

$$\text{Concentration (μM or pmol/μL)} = \frac{A_{260} \times 10^6}{(e \times L)}$$

  Where:

  e = extinction coefficient (L x mol–1 x cm–1)

- Finally, prepare a 0.5–10 pmol/µL working solution of the nucleic acid substrate for end labeling with this kit. The two positive control oligonucleotides provided with the kit are at 5 pmol/µL.

5' End Labeling Reaction

From 0.1–20 pmol of 5' ends can be efficiently end labeled per 10–20 µL reaction. Use the amount of material within the range that suits your needs. In general, a labeling reaction containing 2–5 pmol of substrate will provide enough labeled material (after purification) for many different downstream experiments, including Northern blot analysis. We recommend including a 2–5 molar excess of labeled ATP over molecules of substrate to obtain probe with the maximum specific activity. If desired, as little as a 1–2 fold molar excess of ATP can be used in the reaction, but we do not recommend using less than an equal molar amount of ATP over substrate (see step 3. on page 11 for instructions on calculating molar amounts of substrate and labeled ATP).

1. Thaw the frozen reaction components at room temp

   Thaw the following reaction components and reagents at room temp, then briefly vortex each before use.

   - DNA or RNA substrate
   - [γ–32P]ATP (4000–7000 Ci/mmol, 10–150 mCi/mL)
   - 10X Kinase Buffer
   - Nuclease-free Water

   IMPORTANT! Keep the tube of T4 PNK at –20°C and do not vortex it.

2. Assemble the reaction at room temp

   a. In a microfuge tube, mix the following at room temp:

Amount		Component
to 10	µL	Nuclease-free Water
0.1–20	pmol	DNA or RNA substrate
1–40	pmol	[γ-32P]ATP
1	µL	10X Kinase Buffer
1	µL	T4 PNK (10 U/µL)

   Note: The reaction can be assembled in a final volume of 20 µL if required to accommodate the substrate and/or [γ-^{32}P]ATP volume. For 20 µL reactions, use 2 µL of 10X Kinase Buffer (1X final concentration).

   b. Gently mix by pipetting or gentle vortexing. Centrifuge briefly to collect the mixture at the bottom of the tube.

3. Incubate at 37°C for 1 hr

   Incubate the 5' end labeling reaction for 1 hr at 37°C.

4. After the labeling reaction

   Storage

   Store radiolabeled nucleic acids at –20°C or –80°C, and keep them on ice when in use. Because of radiolytic decay, they have a limited shelf life — typically less than 1 week.

No purification

For many applications (e.g. Northern blots) end labeled nucleic acid can be used directly without any purification. However, if the T4 PNK must be inactivated (e.g. for subsequent enzymatic reactions), add EDTA to 1 mM and heat the reaction at 95°C for 2 min.

Purification options

Below are listed common cleanup options and when they might be appropriate.

- If it is important to remove the kinase reaction reagents and the free nucleotides (e.g. to accurately quantitate the radiolabeled probe or marker) purify the labeled nucleic acid immediately after step 3., following the procedure described in section "Column Purification to Remove Free Nucleotides" on page 14.
- If the oligonucleotide substrate did not consist of primarily full-length material (see "Gel Analysis of Oligonucleotides and Labeling Products" on page 21), the full-length radiolabeled probes can be recovered by gel purification (see section "Gel Purification of Probe" on page 23).

Specific activity

The specific activity of the reaction product will depend primarily on the specific activity of the [γ-^{32}P]ATP. Other factors such as the current date in relation to the reference date for the radionucleotide, and the quality of the substrate will also have some impact.

To measure the specific activity of the probe, reserve a 1 µL aliquot of the reaction before column or gel purification. Measure and compare the total amount of radiolabel present in the reaction mixture with the trichloroacetic acid (TCA) precipitable counts as described in section "Determining Percent Incorporation and Specific Activity" on page 21.

Preparation of Radiolabeled Decade Markers

Radiolabeled Decade Markers are prepared from the Decade Marker RNA substrate provided with the kit. The RNA is first 5' end labeled, and the reaction is then diluted into the 10X Cleavage Reagent that generates the molecular weight marker set in a 5 minute reaction.

The resulting Decade Markers can be used without any purification, or they can be purified to remove unincorporated [γ-^{32}P]ATP and allow precise quantitation by scintillation counting. This is advantageous for some experiments because it provides a way to determine the volume of marker to load on a gel for a given exposure time. The purification should be done immediately after the labeling reaction, and the cleavage reaction is then carried out using purified 5' end labeled transcript.

1. Assemble the 5' end labeling reaction

Amount		Component
to 10	µL	Nuclease-free Water
1	µL	Decade Marker (~1 pmol)
≥1	pmol	[γ–32P]ATP†
1	µL	10X Kinase Buffer
1	µL	T4 PNK (10 U/µL)

† For example 1 µL of 6000 Ci/mmol [γ-^{32}P]ATP at a concentration of 10 mCi/mL provides ~1.67 pmol.

2. Incubate 1 hr at 37°C

   Incubate the 5' end labeling reaction for 1 hr at 37°C.

   - If you do not plan to remove unincorporated [γ–^{32}P]ATP, proceed to the next below.
   - To make purified Decade Markers, complete the column purification described in section "Column Purification to Remove Free Nucleotides" on page 14, and then proceed to the next step below.

3. Add 10X Cleavage Reagent

   Unpurified labeling reactions

   Add 8 µL of Nuclease-free Water and 2 µL of 10X Cleavage Reagent.

   Purified labeling reactions

   After completing step 6. on page 16 of the column purification:

   - Measure the exact volume of eluted material with a pipet
   - Add 1/10 volume of 10X Cleavage Reagent.

4. Mix thoroughly

   Gently mix by pipetting or slow vortexing. Centrifuge briefly to collect the mixture at the bottom of the tube.

5. Incubate 5 min at room temp

   Incubate the cleavage reaction for 5 min at room temp.

6. Add an equal volume Gel Loading Buffer II, incubate 3 min at 95°C

   Stop the cleavage reaction by adding an equal volume of Gel Loading Buffer II and heating at 95°C for 3 min. The Decade Markers are now ready for gel electrophoresis.

7. Storage and usage instructions

   - Store the Decade Markers at –20°C for up to 10 days.
   - To use the Decade Markers, thaw the frozen solution, remove an appropriate volume, and heat at 95°C for 3 min before loading on the gel.
   - The Decade Markers can be used in any denaturing acrylamide gel, however we find that using a denaturing (7 M urea) 10–20% acrylamide gel provides the best resolution of the ten Decade Marker fragments. An 11th band (150 nt) is also visible; its intensity will be about 2–5-fold higher than the rest of the markers. The appropriate volume of Decade Markers to load on a gel will depend on the size of the wells, the age of the radioisotope, and the exposure time.

Column Purification to Remove Free Nucleotides

The purification procedure was designed to quickly remove unincorporated radiolabeled nucleotides and other labeling reagents from end labeled RNA. It is convenient because only a single tube is used, and a minimal amount of radioactive waste is produced. The procedure can also be used to efficiently purify radiolabeled RNA molecules prepared by in vitro transcription, for example with the mirVana miRNA Probe Construction Kit or the MAXIscript® Kit. An RNA Carrier Solution is mixed with the labeled RNA for optimal recovery (up to 100%) of small amounts of very short RNAs.

The protocol can also be used to clean up DNA labeling reactions. However, the recovery will not be as efficient as for RNA (typically less than 50% of the RNA recovery). The RNA Carrier Solution must not be used to purify radiolabeled DNA oligonucleotides as it will further reduce their recovery. Start at step 2. for labeled DNA.

Add 15.4 mL of 100% ethanol to the Binding/Washing Buffer Concentrate before use.

Use ACS grade or better 100% ethanol to make a working solution of the Binding/Washing Buffer, and mix well.

1. Add 10 µL of RNA Carrier Solution to RNA labeling reactions

   Start here for purification of RNA. (Go to step 2. for purification of DNA.)

   Add 10 µL of RNA Carrier Solution to:

   - RNA 5' end labeling reactions, immediately after the kinase reaction (section "5' End Labeling Reaction" on page 12)
   - Decade Marker 5' end labeling reactions, immediately after the kinase reaction (step 3. on page 14)
   - In vitro transcription reactions, immediately after the DNase treatment (e.g. step 5. on page 14 in the *mir*Vana Probe Construction Kit User Guide)

   Note: The RNA Carrier Solution contains sheared yeast RNA (1 mg/mL) that will be recovered with the purified RNA at the end of the procedure. Yeast RNA is often used as a nonspecific competitor in molecular biology procedures, and will be compatible with most downstream applications. However, omit the RNA Carrier Solution if yeast RNA is expected to interfere with subsequent enzymatic reactions or functional assays.

2. Add 350 µL Binding/Washing Buffer with ethanol

   Start here for purification of DNA.

   IMPORTANT! Add 15.4 mL of 100% ethanol, ACS grade or better, to the Binding/Washing Buffer Concentrate before use.

   Add 350 µL Binding/Washing Buffer to each sample and mix thoroughly by pipetting a few times, or by inverting the tube several times.

3. Draw the mixture through a Purification Cartridge

   a. Apply the mixture to a Purification Cartridge pre-assembled in one of the 2 mL Collection Tubes provided.

   b. Centrifuge 15–30 sec at ~10,000 x g.

4. Wash with 300 µL Binding/Washing Buffer

   a. Apply 300 µL Binding/Washing Buffer to the same Purification Cartridge and Collection Tube (the capacity of the Collection Tubes is 700 µL).

   b. Centrifuge at least 1 min at ~10,000 x g.

5. Elute nucleic acid with 10–40 µL of ~95°C Elution Buffer

   Elution Buffer is nuclease-free 10 mM Tris pH 7, 0.1 mM EDTA.

   a. Transfer the Purification Cartridge into a fresh Elution Tube.

b. Apply preheated (~95–100°C) Elution Buffer to the Purification Cartridge.

   The exact volume of Elution Buffer is not critical. For optimal recovery of nucleic acid we recommend eluting with two sequential elutions of 20 µL Elution Buffer each.

c. Recover the nucleic acid by centrifugation at ~10,000 x g for ~30 sec.

6. Elute with a second 10–20 µL aliquot of ~95°C Elution Buffer

   Apply a second aliquot of hot Elution Buffer to the filter and respin for ~30 sec. This second elution is collected in the same tube as the first elution.

7. After the purification

   To make purified Decade Markers:

   To continue preparation of labeled Decade Markers, proceed to "Purified labeling reactions" on page 14.

   Storage

   Store radiolabeled nucleic acids at –20°C or –80°C, and keep them on ice when in use. Because of radiolytic decay, they have a limited shelf life — typically less than 1 week.

   Determine cpm per µL

   Scintillation count 1 µL of the purified material to determine the amount of radioactivity present (cpm/µL).

Troubleshooting

Using the Control Substrates

Description

There are Control RNA and Control DNA Substrates (5 µM) for 5' end labeling reactions provided with the *mir*Vana Probe & Marker Kit. The Control RNA Substrate is a 28 nt RNA oligonucleotide that is complementary to miR-16 miRNA. Once labeled, it can be used as a probe for both solution hybridization with the mirVana miRNA Detection Kit, and for Northern blotting (procedure in section "Precipitation of Oligonucleotides" on page 24). The Control DNA Substrate is a 16 nt DNA oligonucleotide that is complementary to 5S rRNA and can be used for Northern blotting.

Instructions

To do the positive control reaction, use 1 µL or less of either the Control RNA or DNA Substrate (2–5 pmol) in a 5' end labeling reaction with at least 5 pmol of fresh radiolabeled ATP (e.g. 3 µL of [γ–32P]ATP at 6000 Ci/mmol and 10 mCi/mL). Follow the instructions in section "5' End Labeling Reaction" on page 12. If desired, the labeled Control RNA can be purified following the instructions in section "Column Purification to Remove Free Nucleotides" on page 14.

Expected result

Positive control reactions using either the RNA or DNA Control Substrate should yield labeled nucleic acid with a specific activity of at least 10^6 cpm/pmol (see section "Determining Percent Incorporation and Specific Activity" on page 21 to determine percentage of incorporation and specific activity).Representative examples of radiolabeled probes and their usage in a solution hybridization assay or in a Northern blot are shown in Figure 1 and Figure 2 on page 9.

Troubleshooting Poor Labeling

1. Try the positive control

   The first step in troubleshooting poor labeling is to check that the kit components are functioning properly by using the Decade Marker RNA or one of the Control Substrates in a 5' end labeling reaction as described in section "Using the Control Substrates" on page 16 above. If your experiments involve labeling RNA, we recommend doing the control reaction with the Control RNA Substrate. If you will be labeling DNA, the Control DNA Substrate is probably a more pertinent control.

2. The positive control works, but my samples don't label well

3. There is an inhibitor in the nucleic acid substrate.

   As discussed in step 2. on page 11, the quality of the substrate can be a significant factor in the performance of the kit. Consider whether your preparation contains any of the following reaction inhibitors, and if necessary, clean up the nucleic acid you want to label:

   - residual ammonium salts
   - inorganic phosphate and/or pyrophosphate
   - monovalent cation concentration 100 mM

4. Only nucleic acids with a 5'-OH are a substrate for T4 PNK.

   Nucleic acids such as PCR products and chemically synthesized RNA or DNA oligonucleotides typically have a 5'-OH (hydroxyl group). DNA from restriction enzyme digestion, nucleic acids purified from biological samples, and in vitro transcribed RNAs typically do not have a 5'-OH, and thus are not substrates for T4 PNK.

5. Check the concentration of nucleic acids and [g-32P]ATP.

   For optimal labeling of nucleic acid probes with T4 PNK, a 2–5 molar excess of ATP over 5' nucleic acid ends is recommended. Determine the exact concentration of each substrate following the instructions in step 3. on page 11.

6. 5' end of oligonucleotide is inaccessible because of tertiary structure.

   The 5' end of an oligonucleotide may be occluded by its tertiary structure, reducing the efficiency of the kinase reaction. To work around this problem, heat the sample to 90°C for 5 min, then place it immediately on ice just before the reaction. To start the reaction, add the denatured oligonucleotide directly to a mixture of the rest of the reagents.

A Materials Not Provided with the Kit

Materials Not Provided with the Kit

Radiolabeled ATP

- [γ–^{32}P]ATP 4000–7000 Ci/mmol, 10–150 mCi/mL

General laboratory equipment and supplies

- Constant temperature heat block (37°C and 95–100°C)
- Microcentrifuge
- RNase-free 1.5 mL or 0.5 mL polypropylene microfuge tubes, adjustable pipettors and RNase-free tips
- 100% ethanol (ACS grade or better)

Related Products Available from Life Technologies

*mir*Vana™ miRNA Probe Construction Kit Part no. AM1550	This kit is designed to produce short (<100 nt) labeled RNA transcripts for use in hybridization assays to detect small RNAs, including miRNA and siRNA. The kit supplies reagents for both transcription template preparation and RNA probe synthesis. Radiolabeled probes made with the kit are ideal for use with the mirVana miRNA Detection Kit.
mirVana™ miRNA Detection Kit Part no. AM1552	The mirVana miRNA Detection Kit provides an extremely sensitive solution hybridization assay capable of detecting attomole amounts of RNA. In addition, it can be used to simultaneously detect several small RNAs such as miRNA and siRNA, or both small RNA and long RNA species in the same sample. For a complete solution for small RNA analysis, use this kit in conjunction with the mirVana miRNA Probe Construction Kit and/or the *mir*-Vana Probe & Marker Kit.
mirVana™ miRNA Isolation Kit Part no. AM1560	The *mir*Vana miRNA Isolation Kit is designed especially for the isolation of small RNAs, such as microRNA (miRNA), small interfering RNA (siRNA), and small nuclear RNA (snRNA), from tissues and cells. The kit uses a fast and efficient glass fiber filter (GFF) based procedure to isolate total RNA ranging in size from kilobases down to 10-mers. It also includes a procedure to enrich the population of RNAs that are 200 bases and smaller, which enhances the sensitivity of small RNA detection by solution hybridization and Northern blot analysis.
NorthernMax® Kits Part no. AM1940, AM1946	Life Technologies's NorthernMax Kits: NorthernMax, and NorthernMax-Gly, combine ultrasensitive, reliable Northern blot protocols with unsurpassed quality control to ensure optimal results in less time.
Electrophoresis Reagents	Life Technologies offers gel loading solutions, agaroses, acrylamide solutions, powdered gel buffer mixes, nuclease-free water, and RNA and DNA molecular weight markers for electrophoresis.
RNase-free Tubes & Tips	Life Technologies RNase-free tubes and tips are available in most commonly used sizes and styles. They are guaranteed RNase- and DNase-free.

miRNA Certified FirstChoice® Total RNA	All of Life Technologies's high quality total RNA from normal human, mouse, and rat tissue is prepared by methods that quantitatively recover microRNAs. The entire line of FirstChoice Total RNAs are free of DNA and shown to be intact by stringent quality control standards.

B Additional Procedures

Gel Analysis of Oligonucleotides and Labeling Products

The best way to quickly analyze RNA or DNA oligonucleotides either before or after a labeling reaction is to electrophorese a sample on a denaturing 12% or 15% polyacrylamide gel (see section "Additional Recipes" on page 25 for gel recipes). The procedure described here can be used to check the quality of chemically synthesized DNA or RNA oligonucleotides, and to analyze radiolabeled DNA or RNA oligonucleotides before or after column purification (to verify their integrity).

1. Mix a sample of the oligonucleotide with Gel Loading Buffer II in a final volume of 5–10 µL.
   - Use 1–5% of the labeling reaction for radiolabeled probes.
   - Use 50 to 200 pmol for oligonucleotides (e.g. 0.5–2 µL of a 100 µM stock).
2. Heat the mixture for 3 min at 95–100°C
3. Load the sample on a denaturing 12% or 15% polyacrylamide gel and electrophorese at 20–25 mA.
4. Stop electrophoresis when the bromophenol blue dye front has migrated to the bottom of the gel.
5. Expose to X-ray film for one to several minutes for radiolabeled oligonucleotides, or stain the gel with ethidium bromide as described below for unlabeled oligonucleotides.
   - Soak the gel for 5 min in a 0.5–1 µg/mL solution of ethidium bromide in 1X TBE.
   - Wash the gel for 2–5 min in 1X TBE.
   - Visualize oligonucleotides using a UV transilluminator.

Determining Percent Incorporation and Specific Activity

The specific activity of a labeled nucleic acid reflects the efficiency of the kinase reaction and provides a useful number for subsequent experiments. Specific activity is simply cpm/pmol, calculated before purification. Doing the trichloroacetic acid (TCA) precipitation and scintillation counting described below will also tell you how many cpm/µL are in the tube, and the percentage of the radiolabel that was incorporated into TCA precipitable material.

You will not be able to determine the specific activity of radiolabeled nucleic acid that has been purified away from unincorporated nucleotides. You can, however, assay a small aliquot of the labeled nucleic acid after purification to determine its cpm/µL.

1. Dilute the labeled nucleic acid into carrier nucleic acid
   a. Mix 1 µL of the labeling reaction or purified reaction with 9 µL nuclease-free water in a fresh microfuge tube (1:10 dilution).

b. Dispense 198 µL carrier DNA or RNA (1 mg/mL) into a nuclease-free 1.5 mL microfuge tube. (Life Technologies Sheared Salmon Sperm DNA Part no. AM9680 can be used for this.) Add 2 µL of the diluted *mir*Vana Probe & Marker Kit reaction to the carrier DNA or RNA and mix thoroughly.

2. Measure the total amount of radiolabel in the reaction mixture

   Transfer 100 µL of the mixture from above step to aqueous scintillation cocktail and count in a scintillation counter. This will measure the total amount of radiolabel present in the reaction mixture (unincorporated and incorporated counts).

3. Measure TCA-precipitable counts

   a. Transfer another 100 µL of the mixture from step 1.b. to a 12 x 75 mm glass tube, and add 2 mL of cold 10% TCA. Mix thoroughly and place on ice for 10 min. This will precipitate nucleic acids, but not free nucleotides.

   b. Collect the precipitate via vacuum filtration through a Whatman GF/C glass fiber filter (or its equivalent).

   c. Rinse the tube twice with 1 mL of 10% TCA and then rinse once with 3–5 mL of 95% ethanol. Pass each of the rinses through the GF/C filter.

   d. Place the filter in a scintillation vial, add aqueous scintillation cocktail, and count in a scintillation counter. The number will reflect radiolabel that was incorporated.

4. Calculate incorporation and specific activity

   Divide the cpm in step 3.b. by the cpm in step 2. to determine the fraction of label incorporated (multiply by 100 for percent incorporation).

   To determine the specific activity of the labeled nucleic acid, calculate the total cpm incorporated in the reaction and divide it by the pmol amount of nucleic acid substrate in the reaction.

   Total cpm incorporated =
   cpm from step 3.d. dilution factor (10) x kinase reaction volume (10 or 20)

   $$\text{Specific activity} = \frac{\text{total cpm incorporated}}{\text{pmol substrate in the reaction}}$$

Alkaline Hydrolysis of RNA Probes

Purified 5' end labeled RNA (unincorporated nucleotides removed) can be rapidly hydrolyzed to generate a gel electrophoresis “ladder” of 5' end labeled RNA fragments. This procedure suggests using 3 different hydrolysis times. After the experiment, select the ladder that provides the best distribution of nucleic acids over the size range best suited to your experiments. At least one of the time points should yield a significant amount of full-length RNA and a single base ladder of RNA bands below.

1. In an RNase-free microfuge tube, mix 5 µL of purified 5' end labeled RNA with 10 µL Alkaline Hydrolysis Buffer.

   Note: The total amount of probe is not critical. If desired, the probe can be diluted with the provided Nuclease-free Water to a final volume of 5 µL.

2. Split the mixture into 3 tubes containing 5 µL each.

3. Place the tubes in a 95°C incubator.

4. Stop the reactions at three different time points by adding 10 µL of Gel Loading Buffer II.

   The optimal incubation time must be determined empirically, for example by testing 5, 10 and 15 min. With the Control RNA probe we typically stop the reactions at 6, 8 and 10 min.

5. Separate the hydrolysis products on a denaturing gel, and visualize them by autoradiography.

Load 3–5 µL of each reaction on a denaturing 12–15% polyacrylamide gel (recipe in section "Additional Recipes" on page 25) according to the size range appropriate for your experiment. It is a good idea to run an untreated control RNA sample next to the RNA ladder for comparison.

Gel Purification of Probe

Specific applications, such as ribonuclease protection assays, S1 nuclease assays, and DNA primer extension require full-length probes. If the oligonucleotide substrate in the kinase reaction was not comprised primarily of full-length material, we recommend gel purifying the probe to separate full-length oligonucleotides from shorter products as well as from unincorporated nucleotides. This procedure can be done directly after the labeling reaction, or after column purification.

1. Separate products on an acrylamide gel

   Add an equal volume of Gel Loading Buffer II to the sample and heat for 3 min at 95–100°C. Load the products on a denaturing polyacrylamide gel and electrophorese the gel at ~10–25 mA until the bromophenol blue reaches the bottom of the gel. (See section "Additional Recipes" on page 25 for acrylamide concentration guidelines and gel recipes.) An entire labeling reaction (10 µL plus 10 µL Gel Loading Buffer II) can be loaded in a single standard 10 x 5 x 0.75 mm well. For larger volumes use a "preparative scale" comb with wide teeth that will form large capacity wells.

2. Excise the gel fragment containing the full-length nucleic acid

   After electrophoresis, remove one glass plate from the gel, cover the gel with plastic wrap, and expose it to X-ray film for 30 sec to several minutes; the exposure time will depend on the specific activity of the probe. The goal is to get an autoradiograph with a faint or "grey" signal so that a small discrete gel fragment can be excised. Glow-in-the-dark stickers are the easiest way to orient the film with the gel to cut out the band. Once exposed, develop the film and identify the full-length probe; it is usually the most slowly migrating, most intense band on the autoradiograph. Now, align the exposed X-ray film with the gel, and cut out the area of the gel that contains the full-length labeled probe with a razor blade or a scalpel, and transfer it to an RNase-free microfuge tube. Remove the smallest possible fragment of gel that contains the full length probe.

3. Elute nucleic acid from acrylamide gel slices

   To elute the full-length probe, add 100–150 µL probe elution buffer (0.5 M ammonium acetate, 1 mM EDTA, 0.2% SDS) to the gel slice and incubate at 37°C for 30 min. Transfer the buffer, which contains the eluted probe, to a clean microfuge tube and repeat with 50–100 µL of probe elution buffer. Pool the two elution fractions and determine the cpm/µL of the recovered probe by scintillation counting using 1–2 µL of the eluted material.

Since longer probes elute more slowly from the gel, we recommend increasing the elution time for probes longer than 40 nt to at least 1 hr. For convenience, or to maximize recovery from the gel, incubation can be carried out overnight with ~200 µL or more of Probe Elution Buffer. The Probe Elution Buffer contains EDTA and SDS which will inactivate low levels of nuclease contamination.

If your application requires it, salt and SDS can be removed by ethanol precipitation (see section below).

Precipitation of Oligonucleotides

1. Add 1/10th volume 3M sodium acetate and ≥3 volumes 100% ethanol and mix well.
2. Chill at or below –20°C for ≥30 min.
3. Spin at top speed in a microfuge for ≥20 min and discard the supernatant.
4. Rinse the pellet with cold 75% ethanol and recentrifuge at top speed, for ≥5 min.
5. Air dry the pellet.

Northern Blot Analysis of Small RNA Molecules

Northern blots can provide qualitative and quantitative data for relatively abundant small RNAs. For less abundant RNAs, such as specific miRNAs, we recommend using the more sensitive mirVana miRNA Detection Kit procedure or analyzing RNA fractions that are enriched in small RNAs species with the mirVana miRNA Isolation Kit.

Specific small RNAs can be detected on Northern blots using either antisense RNA probes prepared by in vitro transcription or 5' end labeled antisense RNA or DNA probes. Below we provide a procedure using Life Technologies optimized NorthernMax® reagents for RNA probes, and a procedure adapted from Patterson and Guthrie for DNA probes (Patterson 1987).

1. Run RNA sample on an acrylamide gel

   Run 1–50 µg of total RNA or an RNA fraction enriched in small RNAs on a denaturing 15% polyacrylamide gel (see section "Additional Recipes" on page 25 for recipe). Stop electrophoresis when the bromophenol blue dye front has migrated to the bottom of the gel. Stain the gel with ethidium bromide, and examine it on a transiluminator to assess the quality of the samples and to make sure that there is good separation of the RNA.

2. Transfer RNA to hybridization membrane

   After staining, transfer the RNA to a nylon membrane by electroblotting (Life Technologies offers BrightStar®-Plus Nylon membrane Part no. AM10100). This procedure can be performed in a semi-dry apparatus using a stack of three sheets of blotting paper soaked in 0.25X TBE placed above and below the gel/membrane. For 0.75 mm gels, transfer at 200 mA for at least 0.2 A-hr (A x hr). Extending this time does not result in loss of sample. After blotting, keep the membrane damp and UV crosslink the RNA to the membranes using a commercial crosslinking device (120 mJ burst over 30 sec).

3. RNA probes

   a. Prehybridize membrane in ULTRAhyb®-Oligo Hybridization Buffer (Part no. AM8663) for at least 1 hr at 65°C.

   b. Add 1–5 x 10^5 cpm RNA probe per mL of ULTRAhyb-Oligo.

   c. Hybridize 8–24 hr at 42°C.

d. Wash 3 times for 5 min each at room temp with NorthernMax Low Stringency Wash Buffer (Part no. AM8673).

e. Wash 15 min at 42°C with NorthernMax Low Stringency Wash Buffer.

f. DNA probes

g. Prehybridize membrane in prehybridization solution (recipe in section "Additional Recipes" on page 25) for at least 1 hr at 65°C.

h. Discard the prehybridization solution.

i. Add 1–5 x 10^5 cpm DNA probe per mL of hybridization solution (recipe in section "Additional Recipes" on page 25).

j. Hybridize 8–24 hr at room temp.

k. Wash 3 times for 5 min each at room temp with Northern wash solution (recipe in section "Additional Recipes" on page 25).

l. Wash 15 min at 42°C with Northern wash solution.

4. Expose the membrane

   After the final wash, wrap the blot in plastic wrap and expose to X-ray film or a phosphorimager screen according to the manufacturer instructions. The latter method allows quantification of the amount of signal present in each band.

5. Detecting larger RNA species

   To look for larger RNAs (e.g. mRNAs which are not resolved on an acrylamide gel) in the same RNA samples, you would have to make a Northern blot using an agarose gel system (NorthernMax® Kits Part nos. AM1940, AM1946 are ideal for this).

Additional Recipes

1. Recommended polyacrylamide concentration

Nucleic Acid Size	Polyacrylamide Concentration	Xylene Cyanol Position	Bromophenol Blue Position
>30 nt	12%	~ 40 nt	~15 nt
<30 nt	15%	~ 30 nt	~10 nt

2. 10X TBE

   TBE is generally used at 1X final concentration for preparing gels and/or for gel running buffer.

IMPORTANT! Do not treat TBE with diethylpyrocarbonate (DEPC).

Concentration		Component	for 1 L	
0.9	M	Tris base	109	g
0.9	M	Boric Acid	55	g
20	mM	0.5 M EDTA solution	40	mL

Dissolve with stirring in about 850 mL nuclease-free water. Adjust the final volume to 1 L.

Alternatively, Life Technologies offers nuclease-free solutions of 10X TBE (Part nos. AM9863, AM9865) and ready-to-resuspend powdered 10X TBE packets (Part no. AM9864). Both are made from of ultrapure molecular biology grade reagents.

3. Denaturing acrylamide gel mixes

   Use the following instructions to prepare 15 mL of gel mix with the indicated percentage of acrylamide and 8 M urea. 15 mL is enough gel solution for one 13 x 15 cm x 0.75 mm gel. Life Technologies offers reagents for acrylamide gel preparation; see our latest catalog or our web site for specific information.

Amount				
12% gel		15% gel		Component
7.2	g	7.2	g	Urea
1.5	mL	1.5	mL	10X TBE
4.5	mL	5.6	mL	40% acrylamide (acrylamide:bis acrylamide = 19:1)
to 15	mL	to 15	mL	Nuclease-free water
Stir to mix, then add:				
75	µL	75	µL	10% ammonium persulfate
15	µL	15	µL	TEMED

Mix briefly after adding the last 2 ingredients, and pour gel immediately.

4. 50X Denhardt's Solution

Amount		Component
10	g	Ficoll 400
10	g	bovine serum albumin
10	g	polyvinylpyrrolidone
to 1	L	nuclease-free water

5. 20X SSC

Amount		Component
175.3	g	NaCl
88.2	g	sodium citrate
800	mL	nuclease-free water
pH to 7.0 with HCl		
to 1	L	nuclease-free water

Alternatively, Life Technologies offers ready-to-use 20X SSC (Part no. AM9763, AM9765, AM9770) or ready-to-resuspend powder (Part no. AM9764).

6. Northern blot prehybridization solution for DNA probes

Amount	Component
6X	SSC
10X	Denhardt's solution
0.2%	SDS

7. Northern blot hybridization solution for DNA probes

Amount	Component
6X	SSC
5X	Denhardt's solution
0.2%	SDS

Add 1–5 x 10^6 cpm/mL 5' end labeled antisense probe. Filter solution with 0.45 µm pore filter before use to remove background flecks.

8. Northern wash solution for DNA probes

Amount	Component
6X	SSC
0.2%	SDS

C

Safety

General Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:
Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.).
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of

according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:
- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/ 29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:
- Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Bibliography

Carrington JC and Ambros V (2003) Role of microRNAs in plant and animal development. *Science* **301(5631)**: 336–8.

Lagos-Quintana M, Rauhut R, Lendeckel W and Tuschl T (2001) Identification of novel genes coding for small expressed RNAs. *Science* **294(5543):** 853–8.

Pasquinelli AE and Ruvkun G (2002) Control of developmental timing by micrornas and their targets. *Annu Rev Cell Dev Biol.* **18**: 495–513.

Patterson B and Guthrie C (1987) An essential yeast snRNA with a U5-like domain is required for splicing in vivo. Cell 49(5): 613–24.

Documentation and Support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/sds**

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

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