

SPECIAL EDITION



THE  
NEW RNA  
WORLD

# The NEW RNA WORLD

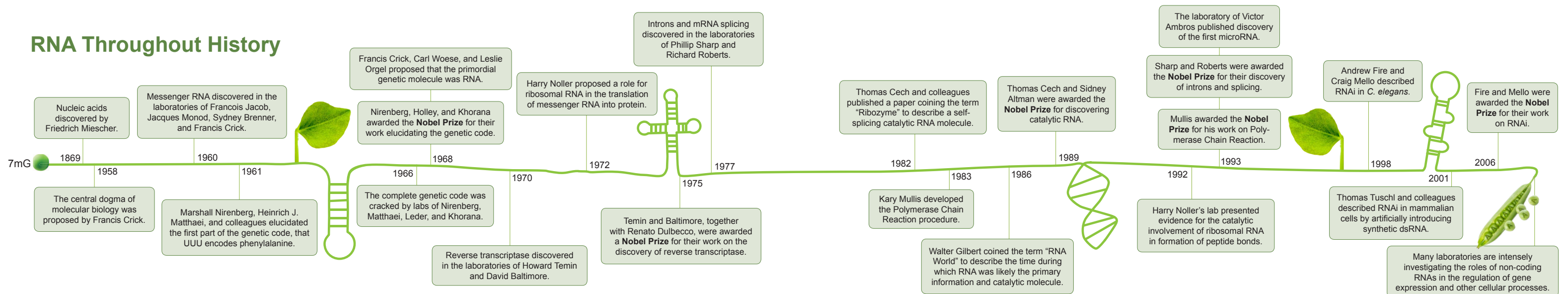
## Welcome to the New RNA World!

RNA has historically been thought of as an “intermediate” in the Central Dogma of molecular biology. The main function attributed to RNA in the past was to act as a messenger to deliver the instructions and information contained in DNA to encode proteins. We now know that there are also many different and unique classes of RNAs and that these RNA molecules can directly carry out many processes in the cell, including activating or repressing gene expression, catalyzing enzymatic reactions, modulating the activities of proteins and other RNAs, and acting as scaffolds or guides for large molecular complexes. RNA molecules perform a stunningly diverse and elegantly complex set of functions in all species of life, but there is almost certainly a vast amount that we still have to learn about RNA. Now, more than ever, it is evident that we are living in The New RNA World.

"We have fundamentally misunderstood the nature of the genetic programming of higher organisms because of the apparently reasonable but now evidently incorrect assumption that most genetic information is transacted by proteins. The vast majority of the human genome does not encode proteins, but is dynamically expressed as RNA, whose primary purpose appears to be to control the epigenetic processes that underpin development and brain function. Understanding these processes and their plasticity will occupy the next generation of molecular biologists and neuroscientists, and lead to an enlightened view of human biology and disease."

**-Professor John S. Mattick**  
Executive Director of the Garvan Institute of Medical Research  
Sydney, Australia

## RNA Throughout History



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# Epigenetic Regulation by Large Non-coding RNAs

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*It is now well accepted that mammalian genomes code for thousands of large and small non-coding RNAs (ncRNAs)<sup>7,16,21,23</sup>. Currently, there is great interest in the functional characterization of ncRNAs and their impact on development and potential involvement in human disease. This mini-review highlights the recent progress that has been made in the identification of large non-coding RNAs and provides insights into their mechanisms and functions.*

## Classifications of Non-coding RNAs

Non-coding RNAs come in many different sizes. For technical reasons, ncRNAs have been divided into small and large classes. RNAs that are less than 200 nucleotides (nt) in length are considered small ncRNAs and those larger than 200 nt are referred to as large ncRNAs (lncRNAs)<sup>31</sup>. Several types of small ncRNAs have been identified to date, including microRNAs (miRNAs) and piwi-interacting RNAs (piRNAs)<sup>34</sup>. Some of these small ncRNAs have been shown to be involved in the regulation of gene expression at the transcriptional, post-transcriptional and translational levels<sup>34</sup>.

MicroRNAs (generally 21-23 nt in length) can regulate gene expression by binding to complementary mRNAs leading to translational inhibition and mRNA decay<sup>2</sup>. Each microRNA can regulate up to 200-300 mRNAs in any given cell type<sup>30</sup>. Another class of small ncRNAs is referred to as piwi-interacting RNAs<sup>12,14,27</sup>. The piRNAs (generally 26-34 nt in length) are expressed in germ cells and interact with the Piwi family of proteins, namely, Miwi, Miwi2 and Mili. The piRNAs in total consist of more than 50,000 species and are produced from discrete loci 50-100 kb in size<sup>12,13,18,24</sup>. The function of piRNAs is not currently known, however, it is suggested they are involved in epigenetic regulation during germ cell development by as yet unknown mechanisms.

Non-coding RNAs that are larger than 200 nt in length are referred to as lncRNAs<sup>1</sup>. The lncRNAs are transcribed from several different genetic locations including antisense to protein-coding genes (Natural Antisense Transcripts or NATs)<sup>7,17,21</sup>, intergenic regions (large intergenic non-coding RNAs or lincRNAs)<sup>16,23</sup>, and introns of protein-coding genes<sup>42</sup>. With a few exceptions, it is only within the past few years that the functions and mechanisms of lncRNAs have begun to emerge. The lncRNAs that have been studied in detail were found to be involved in various biological processes including X chromosome inactivation, genomic imprinting, nuclear structure, and development. In the following sections, I will discuss some of the known functions of several lncRNAs; however, further research is needed to better understand the mechanisms and functions of these molecules.

## How are lncRNAs Discovered on a Large Scale?

Several efforts in the post-genomic era, including massive cloning and traditional sequencing

methods, indicated that the mammalian genome produces thousands of RNA transcripts in addition to protein-coding genes<sup>7,21</sup>. These studies changed our simplistic view of the genome and suggested the presence of thousands of previously unknown transcripts. One striking observation was that DNA sequences coding for protein-coding mRNAs also produced RNAs in the antisense direction and the majority of these antisense transcripts have no protein coding capacity. Although the functions of most of these transcripts are not known, several studies suggested a potential role for these non-coding antisense transcripts in the regulation of their protein-coding partners<sup>3,10,26,29,39,48</sup>.

Recently, we have discovered over 3,300 large, intergenic non-coding RNAs in human and mouse by taking advantage of clues from chromatin modifications<sup>16,23</sup>. It is well known from ChIP-Seq data that transcribed protein-coding genes have characteristic chromatin modifications. Specifically, they have trimethylation of histone H3 at lysine 4 (H3K4me3) at the promoter region and trimethylation of histone H3 at lysine 36 (H3K36me3) in the body of the gene<sup>35</sup>. Regions that have this chromatin signature are referred to as K4-K36 domains. By eliminating K4-K36 domains that correspond to protein-coding genes we identified greater than 3,300 intergenic K4-K36 domains that are transcribed into lincRNAs. Bioinformatic analyses have confirmed that the majority of these tran-

scripts have no protein-coding potential. Furthermore, we found many of these lincRNAs to interact with multiple chromatin-modifying complexes in several human and mouse cell types suggesting that lincRNAs may be involved in epigenetic regulation<sup>23</sup>.

Recent advances in RNA sequencing technology (RNA-Seq) have validated these observations and allowed further interrogation of the total cellular RNA, or transcriptome, at a much higher resolution<sup>37</sup>. Thousands of transcripts, in addition to protein-coding mRNAs and microRNAs, have been found to be expressed in a wide range of tissues and cell types<sup>47</sup>.

## Epigenetics

Many of the lncRNAs appear to be involved in epigenetic mechanisms of gene regulation, making introduction of this field prudent before delving into proposed functions and mechanisms<sup>5,9,24</sup>. Although the field of epigenetics earned its name over 50 years ago, it was not until this past decade that the significance of epigenetics has been recognized in human health and disease<sup>9</sup>. The term epigenetics refers to changes in gene expression and/or phenotype that can be heritable without a change in the underlying DNA sequence itself. Several factors contribute to epigenetic mechanisms of gene regulation including DNA methylation, histone modifications, and non-coding RNAs. DNA methylation in the promoter region of

genes is typically associated with transcriptional repression. Several mammalian enzymes are responsible for establishing and maintaining DNA methylation in the genome<sup>20</sup>; however, no DNA demethylase has been identified to date despite evidence that DNA demethylation can be an active process in mammalian cells at specific stages of development.

The modification of histone proteins has also been shown to be involved in epigenetic regulation<sup>25</sup>. Histones are highly conserved proteins that package DNA in the nucleus and modulate the accessibility of transcription factors and RNA polymerases to DNA. Histone modifications typically take place at amino acids located in the N-termini of histones such as lysine, arginine and serine residues which can be acetylated or methylated (lysine and arginine) and phosphorylated (serine). Histone modifications are placed on and removed from histone residues by numerous enzymes that usually work as part of multi-protein complexes<sup>4</sup>.

A major question in biology is how chromatin-modifying complexes are targeted to specific genomic loci since many of these enzymes lack DNA binding capacity. Recent studies suggest a potential role for non-coding RNAs in the guidance of chromatin-modifying complexes to genomic loci<sup>15,23,42,46</sup>. However, the mechanism by which non-coding RNAs guide protein complexes is not known and is currently under intense investigation.

## Functions of Large Non-coding RNAs

Based on our current understanding of lncRNAs, it appears that such molecules are involved in many different aspects of cellular functions<sup>33,34</sup>. The recent observation that numerous lncRNAs interact with chromatin-modifying complexes suggest a role for these molecules in epigenetic regulation<sup>23</sup>.

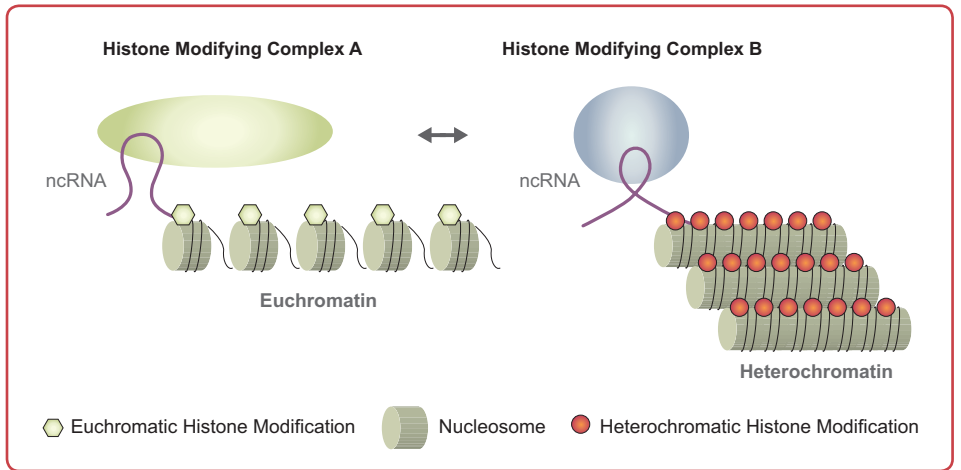
One of the best known examples of a functional lncRNA is the X-inactive specific transcript (Xist)<sup>11</sup>. The large non-coding Xist RNA is involved in the silencing of an entire X chromosome in female somatic cells<sup>6</sup>. Mammalian females have two X chromosomes while males have one X and one Y chromosome (mostly heterochromatic). In order to achieve dosage compensation between males and females, one X chromosome in female cells, starting in early stages of development, is randomly inactivated. This inactivation requires the expression of Xist and is maintained in all of the somatic cells of the female. Although the exact mechanism of action of Xist is not completely understood, it is thought that Xist mediates its silencing effects by interacting with repressive chromatin-modifying complexes such as the polycomb repressive complex 2 (PRC2). Xist-PRC2 complexes are thought to be recruited to the inactive X chromosome to confer repressive chromatin marks<sup>60</sup>. Interestingly, Xist expression is itself regulated by a large non-coding RNA expressed from the opposite antisense DNA strand overlapping with

Xist and is known as antisense to Xist, or Tsix<sup>28</sup>. This large non-coding RNA is expressed from the active X chromosome and also interacts with chromatin-modifying complexes.

Another well-studied lncRNA is HOTAIR, which was discovered with tiling arrays that spanned the human HOX loci<sup>43</sup>. HOTAIR is expressed from the HOXC locus and marks a boundary of active and inactive chromatin. Furthermore, HOTAIR, similar to Xist, interacts with chromatin-modifying complexes such as PRC2 and the corepressor complex CoREST<sup>23,43</sup>, and may guide these complexes to genomic loci. A recent study found HOTAIR to be involved in cancer metastasis via reprogramming of chromatin<sup>15</sup>. Overexpression of HOTAIR in cells injected into mice became metastatic

identified in human and mouse cells<sup>7,17,21</sup>. Functional studies have shown that many of these antisense transcripts are involved in the regulation of their protein-coding counterparts. For example, the non-coding RNA AIR is responsible for the allele specific expression of several protein-coding genes in the mouse placenta via its interaction with the histone H3K9 methyltransferase G9a<sup>38,44</sup>. Another lncRNA known as Kcnq1ot1 is involved in the repression of several protein-coding genes in *cis* through interaction with repressive chromatin-modifying complexes<sup>40</sup>. A recent study found some of the lncRNAs to also have an enhancer-like function in human cells<sup>39</sup>.

Although many lncRNAs have been shown to regulate gene expression only a few have been



Non-coding RNAs have been shown to interact with multiple chromatin-remodeling and histone-modifying proteins, including both euchromatin- and heterochromatin-promoting complexes, and play a role in targeting these complexes to specific sites in the genome.

compared to control cells with an empty vector. Furthermore, HOTAIR may serve as a scaffold for targeting chromatin modifying complexes to chromatin<sup>46</sup>. It was recently reported that the 5' end of HOTAIR interacts with PRC2 and the 3' end of HOTAIR interacts with LSD1/CoREST/REST suggesting that HOTAIR may serve as a scaffold to tether multiple chromatin-modifying complexes to chromatin<sup>46</sup>. It is possible that other lncRNAs that interact with chromatin-modifying complexes also function in a manner similar to HOTAIR.

Recently, a lincRNA was shown to be regulated by p53 (a gene mutated in most human can-

cers)<sup>19</sup>. This lincRNA is activated in response to DNA damage through the binding of p53 to its promoter. Subsequently, the lincP21 transcript binds to hnRNPk and may guide this protein to its target loci. Another study identified a non-coding RNA, pRNA, that is required for the recruitment of the DNA methyltransferase DNMT3b to the promoter region of rDNA genes<sup>44</sup>.

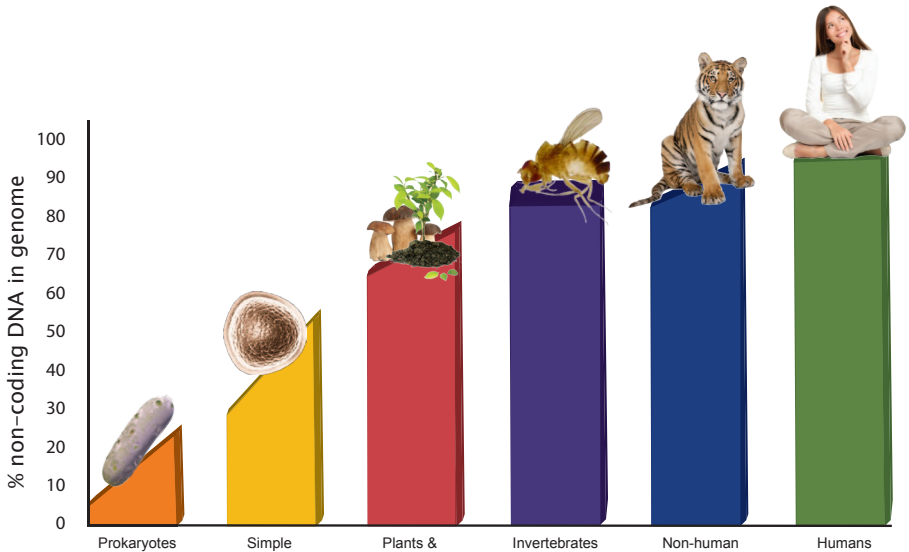
Genome-wide analysis of the mammalian transcriptome has now provided direct evidence that thousands of large non-coding RNAs are transcribed.

Also, many of the lncRNAs that are transcribed antisense to protein-coding genes have been

## Misregulation of Large Non-coding RNAs in Human Disease

As the functions and mechanisms of large non-coding RNAs are beginning to emerge, there is an intense interest in identifying any potential role of these molecules in human disease. Several studies have shown that lncRNAs are misregulated in human disease; however, it has yet to be shown that these molecules are sufficient to drive the disease state.

The expression of multiple lncRNAs has been shown to be altered in several human neurological disorders. Two lncRNAs (FMR4 and FMR1AS) expressed from the FMR1 locus have been previously shown to be silenced in



While the number of protein-coding genes an organism expresses does not scale with organism complexity (humans have approximately the same number as worms!), the amount of non-protein-coding DNA does. Therefore, it is likely that RNA transcribed from these non-protein-coding regions allows for complex developmental and differentiation patterns, as well as advanced cognitive potential. Figure adapted from Mattick, J.S. (2004) *Sci. Am.*



fragile X patients similar to the protein-coding gene FMR1<sup>22,26</sup>. Depletion of FMR4 using small interfering RNAs increased cell death, suggesting that FMR4 may play a role in protecting neuronal cells<sup>22</sup>. Also, a lncRNA antisense to the BACE1 gene, a critical gene in Alzheimer's disease, has been shown to regulate BACE1 at the mRNA and protein levels both *in vitro* and *in vivo*<sup>10</sup>. Interestingly, this lncRNA is elevated in the brain of Alzheimer's patients relative to control subjects. Additionally, the expression of several lncRNAs have been shown to be altered in schizophrenia, for example the lncRNA PSZA11q14 shows reduced expression in brains from patients with schizophrenia<sup>41</sup>.

Furthermore, two lncRNAs were previously found to be misregulated in heart disease. The expression of the long non-coding RNA MIAT has been shown to be associated with

increased risk of myocardial infarction, and the lincRNA ANRIL was found to be associated with increased risk to coronary heart disease. Recently, many lincRNAs were found to be induced in a p53 dependent manner suggesting a potential role for these transcripts in cancer<sup>15,19,23</sup>. Also, the lincRNA HOTAIR was shown to be required for cancer metastasis *in vivo*<sup>15</sup>.

### Conclusions and Future Directions

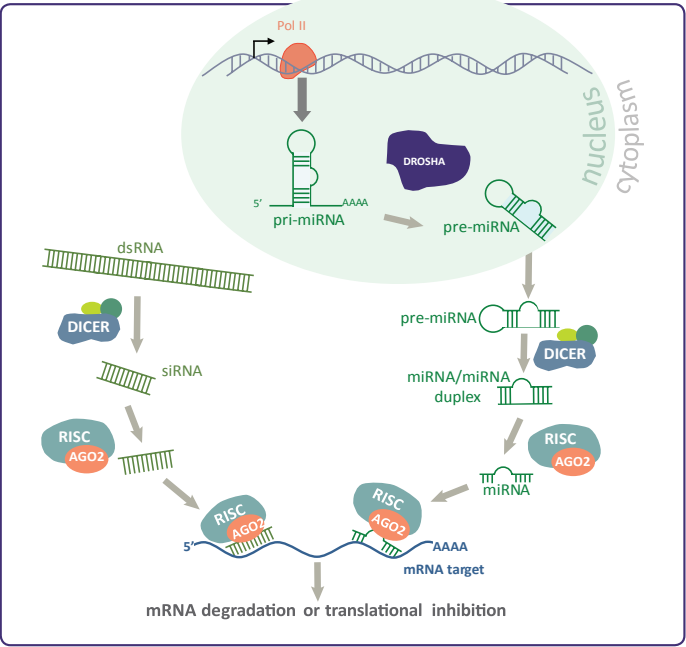
Genome-wide analysis of the mammalian transcriptome has now provided direct evidence that thousands of large non-coding RNAs are transcribed. Currently, there is great interest in identifying the functions and mechanisms of these novel transcripts. There is now strong evidence that many of these molecules are biologically significant, with a large percentage

of these molecules functioning through their interactions with chromatin-modifying complexes to alter gene expression<sup>23</sup>. These findings are beginning to shed light on how chromatin-modifying complexes, many of which lack DNA binding capacity, are targeted to specific genomic loci and suggest the tantalizing idea that lncRNAs are in some way guiding chromatin-modifying complexes to genomic loci. Misregulation of these lncRNAs has already been reported in a wide range of human diseases suggesting that their activity is critical for human health. Finally, I hypothesize that many large non-coding RNAs function through their interaction with protein partners in currently uncharacterized ribonucleoprotein complexes. Addressing such lncRNA-protein complexes will add a new dimension to our understanding of biology, epigenetics, and medicine.

ally regulate gene expression by RNA-induced transcriptional silencing (RITS) or by mediating the degradation of messenger RNAs.

In the years following the discovery of RNAi, conserved RNAi pathways were found to exist in most eukaryotic organisms, and even some prokaryotic organisms were found to use related regulatory mechanisms. This led to the

revolutionary observation from the laboratory of Thomas Tuschl that synthetic siRNA molecules could be artificially introduced into mammalian cells to silence genes of interest in a controlled manner for many experimental needs<sup>8</sup>. Using RNAi, every gene can theoretically be specifically silenced, permitting researchers to investigate its function more quickly and easily than previously possible. The ability to perform RNAi screens with large siRNA libraries, including whole-genome screens, allowed for high-throughput unbiased determination of factors required for many different cellular pathways.



Schematic diagram of small RNA biogenesis. Adapted from He and Hannon<sup>9</sup>

### Functions & Applications of Small RNAs

Both miRNAs and siRNAs can exhibit cell- or tissue-specific expression patterns, highlighting their roles in cellular development and differentiation and also indicating their importance to cellular fate and identity decisions. miRNAs and siRNAs regulate the expression of their target genes primarily through post-transcriptional mechanisms, either by inhibiting the translation of messenger RNA or by promoting its degradation. However, more recent reports demonstrate that miRNAs can control gene expression by mediating the epigenetic modification of regulated genes, including both DNA methylation and histone modification<sup>6</sup>. Additionally, the expression of miRNAs themselves can be regulated by epigenetic mechanisms<sup>7</sup>, clearly establishing an intimate relationship between small RNAs and epigenetics.

mature miRNAs then associate with the Argonaute protein in the RNA-Induced Silencing Complex (RISC), which guides the miRNAs to their target sites to regulate gene expression, primarily by either repressing translation or promoting transcript degradation.

A process termed RNA interference (RNAi) mediated by small interfering RNAs (siRNAs) was discovered in the late 1990's (also in *C. elegans*) in the laboratories of Andrew Fire and Craig Mello<sup>4</sup>. The discovery and characterization of RNAi led to Fire and Mello being awarded the Nobel Prize in Physiology or Medicine in 2006.

siRNAs are generated by Dicer-dependent cleavage of double-stranded RNA (rather than the hairpin structures of pre-miRNAs), and mature siRNAs associate with the RISC and are directed to their target sites. siRNAs gener-

In addition to being a useful tool in basic science research, RNAi also holds tremendous promise as a novel therapeutic strategy for treating human diseases. Using the principles of RNAi, scientists can design molecules to si-

## YOU ARE WHAT YOU EAT!

*Some of the early research on the characterization of RNAi was done in plants and recent work suggests that the plant RNAi mechanisms can regulate gene expression in people and animals that eat the plants*<sup>5</sup>.

..... lence genes responsible for certain diseases, potentially resulting in effective treatments and cures. RNAi-based therapies are currently in clinical trials for treatment of many human diseases, including multiple types of cancers, eye and retina disorders, kidney disorders, and viral infections<sup>9</sup>.

While RNAi-based therapies have the potential to completely transform modern medicine, products of the RNAi pathway can also be exploited to detect and diagnose human disorders. Expression of miRNAs and other small RNAs can be dysregulated in diseases such as

cancer, and, in fact, these RNA molecules can act in a manner similar to classic tumor suppressors and oncogenes<sup>10</sup>. Small RNAs can be secreted from cells and detected in blood and/or urine, and therefore their altered expression patterns observed in patients may serve as useful biomarkers for a wide variety of diseases<sup>10</sup>.

The recent discovery of stable miRNA biomarkers in circulating blood represents a rapidly growing field. New and improved RNA biomarkers are constantly being described and validated. Detection of miRNAs in blood and urine provides a way to diagnose diseases that is non-invasive and more sensitive than many current methods. Although RNA purification from sample sources such as blood and urine can often be difficult, improved methods for recovery of miRNAs and RNAs that are low in abundance from these sources are now available.

### Conclusions & Future Directions

Small RNAs have quickly gone from being thought of as "junk RNA", transcriptional noise, and RNA degradation intermediates to being considered some of the most important and most intensely studied molecules. Because miRNAs are now known to play such critical roles in biology, it is very hard to believe that less than 20 years ago their existence was essentially unknown. Just 10 years ago it was nearly impossible to knock down your favorite gene using RNAi! Although a lot has been discovered about the functions and activities of miRNAs, there is still certainly much more to learn. To keep up in this rapidly advancing field, it is critical to use the best, unbiased methods to recover the highest amount of small RNAs from your samples, and to use the best purification methods for each type of sample source. For more information on industry-leading products available from Zymo Research for purification and clean-up of small RNAs, please read more on the following pages.

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### April showers bring displays of RNAi in flowers!

The white regions in this beautiful petunia are the result of RNAi-mediated silencing of the genes responsible for pigmentation! Plant crops have also been engineered to express siRNAs against pests such as beetles and viruses. Other important uses of RNAi in plants include making vegetables more healthy, decaffeinating coffee beans, and producing allergy-free fruit.

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# The Rapidly Expanding Small RNA World

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*The discovery of small regulatory RNAs was one of the greatest advancements in biology in the last 20 years. The notion that these previously unrecognized small RNA molecules were playing important roles in the regulation of gene expression resulted in a paradigm shift in molecular biology that is still being felt today. It is now generally accepted that small non-coding RNAs are likely to regulate the expression of the majority of mammalian genes<sup>1</sup> and new regulatory RNAs are still being discovered from essentially all species of life.*

### Biogenesis and Processing of Small RNAs

MicroRNAs (miRNAs) were first discovered in the early 1990's in the laboratory of Victor Ambros and were found to regulate gene expression in the nematode *Caenorhabditis elegans*<sup>2</sup>. Since then, hundreds of miRNAs have been discovered, and the list of known miRNAs is still growing. miRNAs are generally first transcribed as primary-miRNA transcripts (pri-miRNAs), which are processed (capped, polyadenylated, spliced) similarly to messenger RNAs (refer to the figure on the next page for a schematic diagram of miRNA biogenesis

and processing or reference 3 for a more detailed description).

Pri-miRNAs are further processed by the Microprocessor protein complex, which consists of the RNase III enzyme Drosha and the double-stranded RNA-binding protein Pasha, to generate pre-miRNA molecules, which are ~70 nucleotides in length and adopt an imperfect hairpin structure. These pre-miRNAs are transported from the nucleus to the cytoplasm where they are cleaved by the enzyme Dicer to produce the mature miRNAs, which are usually 21-23 nucleotides in length. The

The research characterizing RNAi in *Caenorhabditis elegans* in the laboratories of Craig Mello and Andrew Fire resulted in them being awarded the Nobel Prize in Physiology or Medicine in 2006, just 8 years after their work was published in *Nature* in 1998<sup>4</sup>.



# Winning the War Against Viruses

Kevin F. Bryant  
Zymo Research Corporation

*Although viruses are so small that they can only be seen with the most powerful microscopes, their devastating effects are impossible to ignore. As societies continue to evolve, so do viruses, potentially becoming even more infectious and dangerous. Many viruses contain rapidly mutating RNA genomes, thus allowing them to potentially evade immune responses and acquire resistance to antiviral drugs. Additionally, many viruses, including both RNA and DNA viruses, encode newly discovered regulatory RNAs, such as miRNAs. Therefore, viruses should be considered major players in The New RNA World. To keep up with the constant threat of emerging and re-emerging viral infections, it is necessary to identify and monitor possible sources of outbreaks worldwide in order to stop small clusters of infections from becoming deadly pandemics.*

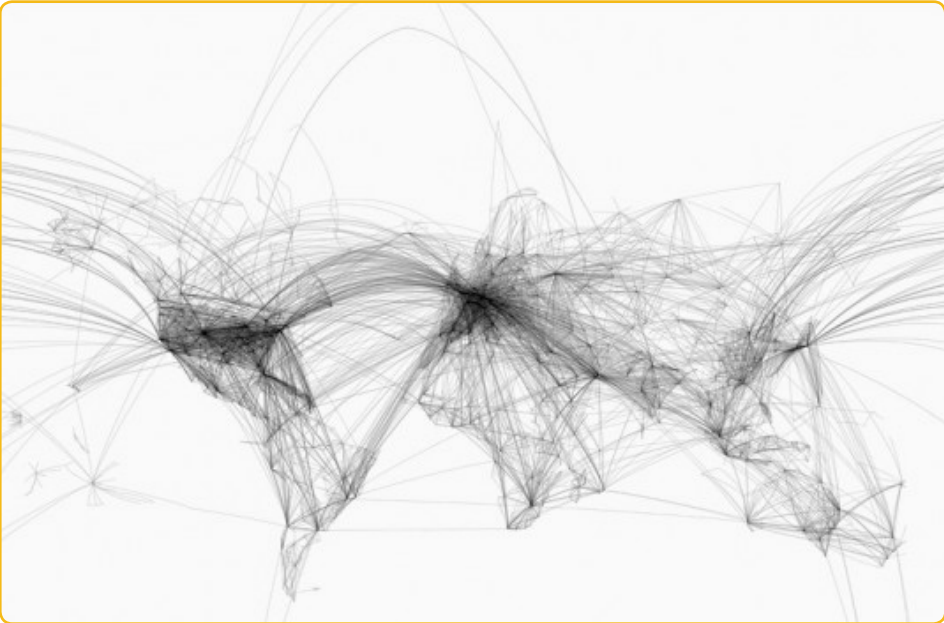
### World At Risk

The constant and immediate threat of viral outbreaks and pandemics is not just the stuff of science fiction. New or mutant viruses are only a flight away from reaching virtually any community in the world. In many densely populated urban areas, infected individuals can come in contact with and infect many other people in a short period of time, quickly spreading the virus before authorities have the chance to react and respond.

While most of us sit in offices or laboratories, and work at universities or companies, some researchers are out in the field, in both remote areas of the developing world and the downtown areas of modern cities, surveying and taking samples to identify possible sources of viral outbreaks (the same is true for bacterial infections, but we will focus on viral infections in this article). Their mission is straightforward – discover any potential sources of viral outbreaks, and stop them before they threaten lives and become global pandemics. In order to accomplish this mission, researchers have to be out in the field, outside of the lab, where new epidemics have the chance to develop and spread. Secondly, and equally important, researchers have to collect samples of potentially infectious material and safely transport them back to the laboratory for analysis, allowing early detection and responses, including preparation and dissemination of appropriate vaccines.

Not worried about the threat of significant and devastating viral outbreaks? As a brief reminder, the current HIV/AIDS pandemic that began in the early 1980's has already led to greater than 25 million deaths worldwide, and that number will likely surpass 100 million in the next 10 years (based on UNAIDS estimates), making it one of the worst pandemics in history – and HIV/AIDS has only

The first cases of **“The 1918 Spanish Flu”** were in the United States and parts of Europe, before there were any reports of influenza in Spain. However, this outbreak of influenza started during World War I, and **many countries were censoring the news of influenza deaths due to fears of decreased morale among soldiers and the portrayal of weakness to the enemy.** Because Spain was a neutral country during the war, the news of influenza outbreaks in Spain were not blocked, which resulted in the worldwide pandemic becoming known as the **“Spanish Flu”**.



Schematic of domestic and international flight patterns depicting how quickly infected individuals can spread viruses across the world.

been around for ~30 years. We need only look back a few years to remember the most recent influenza pandemic. Luckily, the 2009 H1N1 flu pandemic was not the huge public health disaster many feared. This was, at least in part, due to effective virus forecasting and subsequent vaccinations. About 10 years ago the deadly SARS outbreak caused quite a scare, but due to rapid detection and international response, this viral disease was stopped from becoming a pandemic.

### How Viruses Shaped History

The commonly-used phrase “going viral” refers to the rapid spread of videos and YouTube clips, but has its origin in the manner in which viral outbreaks rapidly spread through communities around the world. Viral

pandemics have played significant roles in shaping civilizations and societies throughout history. For example, based on some recent estimates, more people are thought to have

**A major disease outbreak, which was probably influenza, was recorded by Hippocrates in 412 BC. The term “influenza” (from Italian; influence) was coined in 1357 AD when the development of flu was blamed on the influence of the stars.**

died from influenza during World War I than from combat. As bad as influenza pandemics have been in the past, smallpox is thought to have had an even greater effect on civilizations. Smallpox infections have likely been responsible for killing more people throughout history than all wars combined!

Many native and indigenous “New World” populations were ravaged by viruses introduced by European explorers because their immune systems were completely naïve to these “Old World” viruses. Smallpox infections of the Aztec and Inca populations are attributed to being one of the primary reasons for their conquest by the Spaniards. Old World viral diseases, including smallpox, influenza, and measles, are also estimated

to have killed as many as 95% of the Native American population! Despite the devastating consequences of smallpox in the past, the global smallpox vaccination program resulted in the best success story so far in our fight against viral diseases, and the virus causing smallpox is the only human virus officially declared eradicated.

### Current Threats

The threat of entire populations being wiped out by viral infections is as great today as it has ever been. The emergence of deadly new and mutant viruses is a constant part of viral evolution, and with air travel making the world a global civilization, these viruses can spread to naïve people much faster than in the past. Ebola virus and other viruses causing hemorrhagic fever are highly infectious, deadly, and have the potential to cause the next pandemic. Viruses like Ebola also have the potential to be weaponized for use in bio-warfare or be released by terrorists.

Additionally, the re-emergence of viral infections and pandemics is also a constant threat. For example, there are seasonal influenza outbreaks every year, which are always a threat to become pandemics. Norovirus outbreaks routinely occur in places like cruise ships and elementary schools, and the threat of West Nile Virus and Eastern Equine Encephalitis Virus re-emerge every year when mosquitoes hatch.



Even Kings need vaccines. Rapid and robust virus detection in the field allows quick vaccine responses to stop the spread of infection and disease.

To keep up with the constant threats of viral diseases and to stop the next pandemic before it starts, it is necessary to use state-of-the-art technologies and procedures to acquire and analyze viral samples in the field. The most sensitive and specific methods to detect viruses in samples are nucleic acid-based, and therefore reagents that stabilize the otherwise potentially unstable RNA genomes of many viruses must be used to ensure that the samples remain stable in the time between when they are collected in the field and when they can be purified for analysis in the laboratory. High quality, inhibitor-free nucleic acids are also necessary to sequence viral genomes to search for new mutations in viruses that may increase their pathogenicity, thus increasing their public health risks.

The **largest** virus discovered to date is *Megavirus chilensis* (or just Megavirus, for short), with a linear double-stranded genome of greater than 1.2 megabases encoding over 1200 protein-coding genes. The **smallest** virus discovered to date is the Porcine circovirus type 1, with a single-stranded DNA genome of just over 1,700 nucleotides.

## UP NEXT:

**“It’s a brave new RNA world as the form, function, and cellular utilization of RNAs never ceases to amaze.”**

Kevin V. Morris, Ph.D.  
The Scripps Research Institute



### New Solutions to Challenges in RNA Sample Preparation

Investigating the functions and activities of RNA represents one of the fastest growing and most intense areas in biological research. Because every experiment investigating RNA requires preparation of high-quality RNA samples, the success of these experiments absolutely depends on using the best technology and RNA purification procedures. Due to both the presence of ubiquitous and stable RNase enzymes, and the perceived unstable properties of RNAs, handling and processing RNA is often thought to be more difficult than most other sample types. However, RNA research can be simplified if the correct tools are used. At Zymo Research, we believe that *The Beauty of Science is to Make Things Simple*, and our innovative products and technologies for RNA purification and sample preparation are **Making RNA Simple!** Read more about our complete portfolio of products to use in your RNA research on the following pages, and for more information, please visit [www.ZymoResearch.com](http://www.ZymoResearch.com).

In most cases, it will be necessary to quickly and completely inactivate the infectivity of viruses in samples collected in the field to protect the health of the researchers collecting and analyzing these samples. The same challenges also exist when collecting, storing and transporting samples from patients involved in clinical trials testing new vaccine candidates and antiviral drugs. This can be especially difficult in developing parts of the world, which are in the greatest need of vaccine and antiviral drug interventions.

### Conclusions & Future Directions

The only way to win the war against viruses is to have an effective proactive system in place to identify outbreaks early and have rapid and effective response plans. We need to know where the viruses are, where they are going, and how they are changing. The best way to track viruses is to go where they are, collect samples, and bring them back to the laboratory for study. This type of field research is critical to the continued advancements of societies, and is literally of life-and-death importance. To ensure that viruses do not win, it is necessary that the best available methods are used to study them. To learn more about the industry-leading products developed by Zymo Research to inactivate viruses and purify viral genomes, please read more on the following pages.





NEVER PHASE SEPARATE AGAIN!

RNA Directly from TRIzol® and TRI Reagent®

The **Direct-zol™ RNA MiniPrep** facilitates efficient and consistent broad size-range purification (including miRNAs) of high quality (DNA-free) total RNA directly from samples stored in TRIzol®, TRI Reagent®, and all other acid-guanidinium-phenol based reagents. The innovative Direct-zol™ procedure bypasses phase separation and precipitation steps with a spin column format, saving time and also eliminating phenol carryover without compromising RNA quality.

The Direct-zol™ technology couples the effectiveness of TRI Reagent® for infectious agent inactivation and sample preservation with a convenient no hassle, no mess procedure for DNA-free RNA. The Direct-zol™ procedure is ideal for both routine lab use and high-throughput and automated applications.

Squeeze the most from your next RNA prep!

**HISTORY:** Acid-guanidinium-phenol based reagents for RNA isolation were introduced in the mid-1980s by Chomczynski and Sacchi<sup>1,2</sup>. Chomczynski's protocol is the most cited method for isolating high quality RNA.

Reference  
1. Chomczynski, P. & Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162, 156-159 (1987)  
2. Chomczynski, P. & Sacchi, N. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nature Protocols 1(2), 581-585 (2006)

GET IT DIRECT!



- ✓ **No phase separation**
- ✓ **No precipitation**
- ✓ **No phenol carryover**
- ✓ **Broad range recovery**
- ✓ **DNA-free**

SAMPLE INPUT

- TISSUE
  - Adipose
  - Brain
  - Bursa of Fabricius
  - Colon
  - Cortical
  - Duodenum
  - Embryos
  - Heart
  - Intestine
  - Joint
  - Kidney
  - Liver
  - Lung
  - Mammary
  - Muscle
  - Ovary
  - Oral cavity
  - Pancreas
  - Pituitary
  - Placenta
  - Replacenta
  - Spinal Cord
  - Tumor
  - Umbilical Cord
  - Uterine
- PLANT TISSUE
  - Callus
  - Leaves
  - Meristem
  - Root
  - Rosettes
  - Stem
  - Woody Tissue
- ORGANISM
  - Apicomplexa
  - Arachnids
  - Astrocytes
  - Bacteria
  - Bovine
  - Chicken
  - Clean
  - Coral
  - Drosophila
  - Fish
  - Fungi
  - Geranium
  - Human
  - Hydra
  - Insect
  - Lolium
  - Nematode
  - Mollusks
  - Mouse/Rat
  - Nicotiana
  - Trifolium
  - Yeast

**Reagent Compatibility:**  
TRIzol®, TRI Reagent®, RNAzol®, QIAzol®, TriPure™, TriSure™, and all other acid-guanidinium-phenol based reagents

**Sample Types:**  
Cells, tissue, plasma, serum, whole blood, and *in vitro* processed RNA (e.g., transcription products, DNase-treated or labeled RNA)

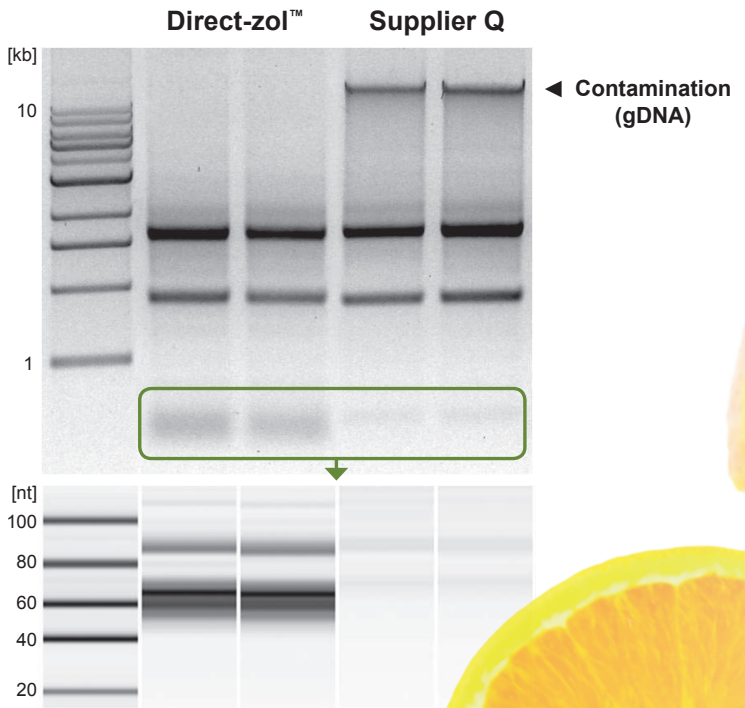
**High-Quality RNA for All Downstream Applications:**  
RT-PCR, next-gen sequencing, microarray, Northern blot, miRNA-seq, and more!

\*Compatibility is not limited to the samples & reagents listed.

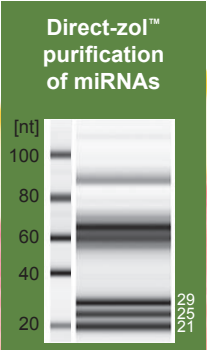


SPIN  
WASH  
ELUTE

Efficient Recovery of Small & Large RNAs



(Top) High quality broad size-range DNA-free RNA is purified from human epithelial cells using the Direct-zol™ RNA MiniPrep compared to a preparation from Supplier Q (1% agarose/TAE gel).  
(Bottom) Small RNAs are efficiently recovered with the Direct-zol™ procedure. However, this is not the case with Supplier Q's prep (Bioanalyzer, Small RNA Chip).



MicroRNAs are effectively recovered from TRIzol® extracts using the Direct-zol™ procedure. miRNAs (21-29 nt) "spiked" into the extract are evidenced by a Bioanalyzer (Small RNA Chip).

What scientists are saying about Direct-zol™ RNA kits:

"Direct-zol is the most excellent kit for RNA isolation that I ever used in the past 20 years."

—H.Z., Joslin Diabetes Center, Harvard Medical School

"[The Direct-zol™ RNA MiniPrep] worked well for our specific need for isolation of very small and small RNAs in a single preparation. Yields were very good. [I was able] to isolate very small (16 nt) and small (200 nt) RNAs with the same protocol from the same sample."

—K.W., Bacteriology, University of Wisconsin

"I just got the results from a two sample test of your kit and wow. We're seeing a 5-10 fold increase in extraction efficiency of RNA from mixed viral/host samples. We are now excited to re-extract our remaining samples and use all as template for cDNA synthesis and sequencing."

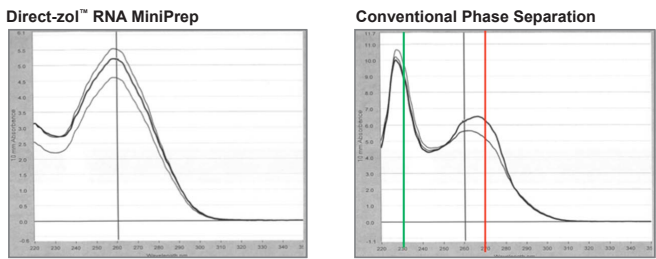
—S.J., Genome Science, Los Alamos National Laboratory

"The ratio 260/280=1.98, 260/230=1.89, RIN=9. Excellent for expression array!"

—J.W., The Methodist Hospital Research Institute, Houston, TX



Phenol-free RNA



RNA purified with Direct-zol™ RNA MiniPrep is phenol-free and (RT)PCR-ready. Phase separated samples show phenol (TRIzol®) contamination as measured and indicated by absorbance at 230 nm (green) and peak shift 260→270 nm (red).

DIRECT-ZOL™ RNA

Product	Format	Preps	Cat. No.
Direct-zol™ RNA MiniPrep	Spin Column	50 200	R2050, R2051* R2052, R2053*
Direct-zol™-96 RNA	96-well Plate	2 x 96 4 x 96	R2054, R2055* R2056, R2057*
Direct-zol™-96 MagBead RNA	Magnetic Beads	2 x 96 4 x 96 8 x 96	R2100, R2101* R2102, R2103* R2104, R2105*

All Direct-zol™ RNA Kits are supplied with DNase I  
\*Supplied with TRI Reagent®

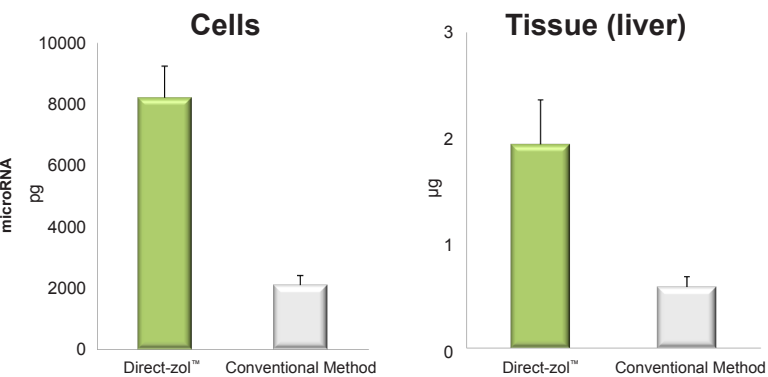


Get your **FREE** sample today!  
[www.zymoresearch.com/Direct-zol](http://www.zymoresearch.com/Direct-zol)



# GET IT ALL! The Best Method for Small RNA Recovery

## 4X More miRNAs with Direct-zol™



The Direct-zol™ RNA MiniPrep recovered ~4-fold more miRNAs (<40 nt) than conventional methods. miRNAs purified from cells and tissue were quantified using the Bioanalyzer, small RNA chip.

*“The Direct-zol RNA MiniPrep showed the highest recovery [of miRNA] from both cell culture and frozen post-mortem human brain tissue when compared to miRNeasy, mirVana and RNeasy Plus.”*

—O. E., USC, Los Angeles, USA

The importance of miRNAs and other small RNAs in regulating gene expression and other cellular processes is clear. Many small RNAs are expressed at low abundance, or only in specific cell types, so to obtain results you can trust, you need to recover ALL small RNAs during your purification. Ensure that you recover the highest yields of high-quality small RNAs from your samples with Zymo Research's RNA purification technologies.

**Get miRNAs Direct** from samples in TRI Reagent® without phase separation (page 10-11). **Direct-zol™ RNA** recovers the most miRNAs and small RNAs!

### ORGANIC PURIFICATION OF SMALL RNAs

Product	Preps	Cat. No.
Direct-zol™ RNA MiniPrep	50	R2050, R2051*
	200	R2052, R2053*
Direct-zol™-96 RNA	2 x 96	R2054, R2055*
	4 x 96	R2056, R2057*
Direct-zol™-96 MagBead RNA	2 x 96	R2100, R2101*
	4 x 96	R2102, R2103*
	8 x 96	R2104, R2105*

All Direct-zol™ RNA Kits are supplied with DNase I  
\*Supplied with TRI Reagent®



Get your **FREE** sample today!  
[www.zymoresearch.com/Direct-zol](http://www.zymoresearch.com/Direct-zol)

### NON-ORGANIC PURIFICATION OF SMALL RNAs

Product	Preps	Cat. No.
RNA Clean & Concentrator™-5	50 200	R1015 R1016
RNA Clean & Concentrator™-25	50 100	R1017 R1018
RNA Clean & Concentrator™-100	25	R1019
ZR-96 RNA Clean & Concentrator™	2 x 96	R1080
DNA-Free RNA Kit™	50 200	R1013 R1014
ZR small-RNA™ PAGE Recovery Kit	20	R1070
ZR RNA MicroPrep™	50 200	R1060 R1061
ZR RNA MiniPrep™	50 200	R1064 R1065
ZR-Duet™ DNA/RNA MiniPrep	50	D7001
ZR Urine RNA Isolation Kit™	20 50	R1038 R1039
ZR Fungal/Bacterial RNA MicroPrep™	50	R2010
ZR Fungal/Bacterial RNA MiniPrep™	50	R2014
ZR Tissue & Insect RNA MicroPrep™	50	R2030

# Selective Purification of Micro, Small & Large RNAs



Clean-up and purification methods vary in their capability to effectively isolate a broad size range of RNA. While column based procedures generally simplify the RNA purification, many columns/kits from other suppliers do not provide for efficient recovery of both small and large RNA molecules and often result in the loss of small RNA species.

The **RNA Clean & Concentrator™** clean-up and **ZR RNA** purification systems have been fine tuned for broad size-range recovery of small and large RNAs. Both have been optimized for quick, specific isolation of total (>17 nt), large (>200 nt), or small (17-200 nt) RNA species. The included Zymo-Spin™ column and plate technologies enable unprecedented sample concentration with elution volumes as little as 6 µl (page 19).

The **RNA Clean & Concentrator™** and **ZR RNA** purification systems feature convenient and efficient in-column DNase treatment. DNA-free RNA is ideal for all downstream applications including next generation sequencing platforms, hybridization techniques, RT-qPCR, RNA-Seq and other advanced platforms (page 17).

**Tip:** For simultaneous purification of DNA and RNA (including small RNAs) from the same cell sample, see **ZR-Duet™** kit (page 23).

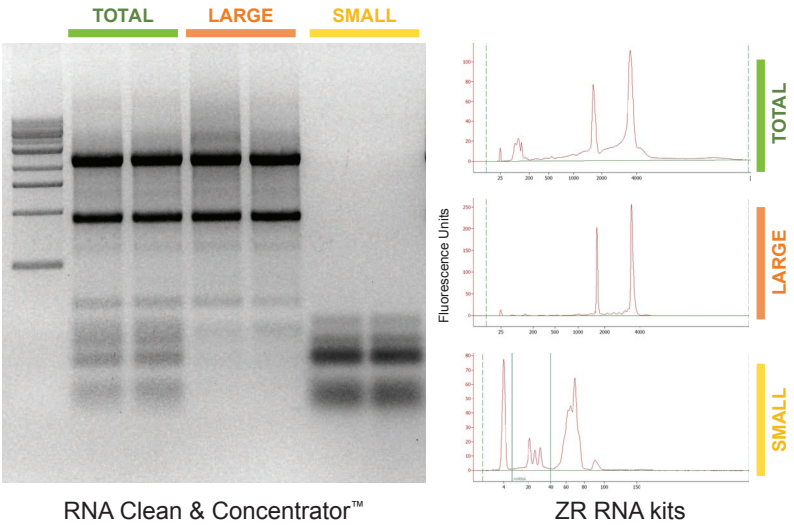


**Did you know?**  
The **RNA Clean & Concentrator™** is recommended in Illumina's work flows, including Next-Gen sequencing library prep.

### KITS FOR SELECTIVE RNA PURIFICATION

Product	Preps	Cat. No.
RNA Clean & Concentrator™-5	50 200	R1015 R1016
RNA Clean & Concentrator™-25	50 100	R1017 R1018
RNA Clean & Concentrator™-100	25	R1019
ZR-96 RNA Clean & Concentrator™	2 x 96	R1080
ZR RNA MicroPrep™	50 200	R1060 R1061
ZR RNA MiniPrep™	50 200	R1064 R1065
ZR-Duet™ DNA/RNA MiniPrep	50	D7001

### Purify the RNA You Need



Total RNA (>17 nt), large (>200 nt) or small RNAs (17-200 nt) are efficiently concentrated and purified with the ZR kits.

(Left) RNA clean-up with the **RNA Clean & Concentrator™-5** (duplicate samples resolved in a 1% agarose gel.)

(Right) RNA purified from human cells with the **ZR RNA MicroPrep™** (Bioanalyzer profiles).





# 30-Day Worry-free Sample Storage at Ambient Temperature

## RNA Shield™

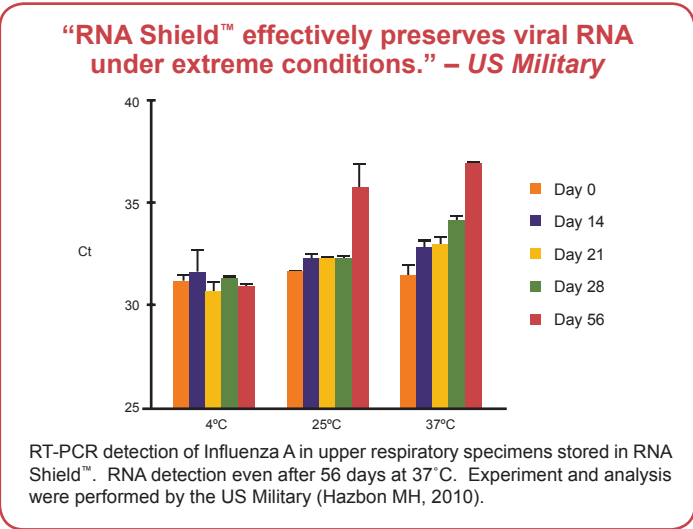
One Reagent for:

- ✓ Storage with simultaneous viral inactivation of swab samples, cells, tissue, biological liquids and more!
- ✓ Direct spin column purification of RNA without the need for reagent removal

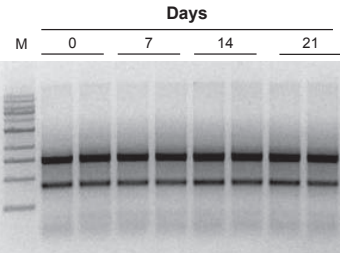
RNA is susceptible to degradation by exposure to ubiquitous ribonucleases, elevated temperatures, pH extremes, and other harsh conditions. The stabilization of intact RNA is a key factor that determines the quality and consistency of downstream analyses. Improper sample collection and storage can dramatically and irreversibly compromise the integrity of RNA.

**RNA Shield™** instantly lyses cells, ensures RNA stability and preserves expression profiles during transport or long-term storage at ambient temperature without the need for cold storage or specialized equipment. RNA Shield™ effectively inactivates RNases and infectious agents. Simply add a sample to RNA Shield™ and store/transport for later processing of high-quality RNA. Samples in RNA Shield™ can be added directly to most of Zymo Research's RNA purification kits, unlike other reagents, which require complicated and time-consuming removal steps.

\* RNA Shield™ stabilizes both RNA and DNA at ambient temperature.

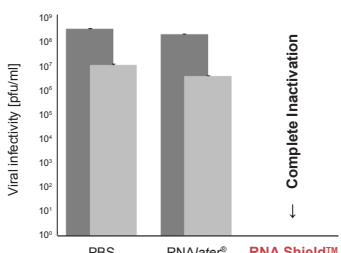


### Stabilization of Cellular RNA



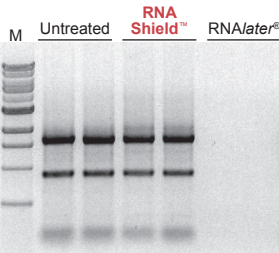
Cellular RNA is effectively stabilized in RNA Shield™ at ambient temperature. RNA was purified directly using the RNA Shield™ Purification Kit.

### Complete Viral Inactivation



Herpes Simplex Virus is completely inactivated in RNA Shield™. HSV-1 (dark grey) & HSV-2 (light grey) inactivation following a 5 minute incubation in RNA Shield™ reagent (*H. Oh, F. Diaz and D. Knipe; Harvard Medical School*)

### Direct RNA Purification



RNA can be purified directly from RNA Shield™ without reagent removal. Cellular RNA was extracted from samples stabilized in RNA Shield™ with TRIzol® and purified with the Direct-zol™ RNA MiniPrep. Conversely, RNA/ater® did not facilitate direct purification.

	RNA Shield™	RNA/ater®
For cells, biological liquids & tissue	YES	Tissue only
Complete viral inactivation	YES	NO
Direct purification	YES	NO

### RNA STORAGE SOLUTION

Product	Volume/Preps	Cat. No.
RNA Shield™ (reagent only)	50 mL 250 mL	R1100-50 R1100-250
RNA Shield™ Purification Kit (supplied with the RNA Shield™ reagent)	50	R1100

**Did you know?** “Biological samples with RNA/ater® should be considered as potentially infectious even after prolonged storage or transportation time at ambient temperature, because virus infectivity of non-enveloped as well as of enveloped viruses is stabilized, and appropriate precautions should be taken when handling this material.”

-C. Uhlenhaut, Journal of Virological Methods 128 (2005), 189-191

# Co-purification of Viral Genomes—Any Virus, Any Size

ZR viral kits feature a one-step buffer system for effective sample lysis, viral inactivation, nucleic acid stabilization and purification. This, coupled with Zymo-Spin™ column technology, ensures reliable processing of both common and the most complex samples, including plasma and serum. The ZR Viral RNA or DNA kits facilitate sensitive detection by recovering nucleic acids from samples with extremely low viral load, without the need for a carrier. The one-step buffer system makes the procedure ideal for high-throughput and automated applications.

Co-purification of viral RNA and DNA genomes poses challenges for conventional column purification techniques. The **ZR Viral RNA/DNA Kits™** feature a unique spin column technology for exceptional capture and release of both small and large nucleic acids. This enables effective screening and identification of DNA and RNA viruses from the same sample.

The ZR viral kits were used for a large scale population study during the 2009 influenza A (H1N1) pandemic<sup>1,2</sup>.

References  
1. Lee, S.H., Park, B.H., Kang, H.Y., Lee K.O. and Kim, G.Y. (2010). Interim report on pandemic H1N1 influenza virus infections in Korea, August to October, 2009. ESCMID, Vienna, Austria, 10-13 April 2010, P1095.  
2. Ducatez, M.F., Sonnerberg, S., Hall, R.J., Peacey, M., Ralston, J., Webby, R.J., and Huang, Q.S. (2010). Genotyping assay for the identification of 2009-2010 pandemic and seasonal H1N1 influenza virus reassortants. J Virol Methods. 168, 78-81.

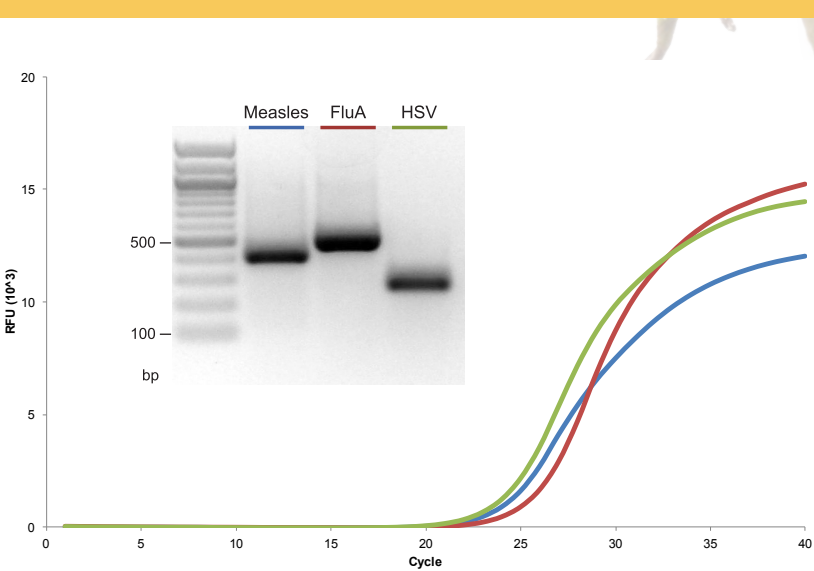
### ZR VIRAL RNA & DNA KITS

Product	Preps	Cat. No.
ZR Viral RNA Kit™	50 200	R1034 R1035
ZR-96 Viral RNA Kit™	2 x 96 4 x 96	R1040 R1041
ZR Viral DNA/RNA Kit™	25 100	D7020 D7021



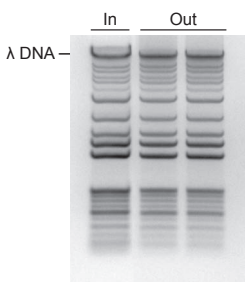
**Do you have viral samples in TRIzol® or TRI Reagent®?** Look no further than the Direct-zol™ RNA MiniPrep – an innovative method that bypasses phase separation, lengthy precipitations, and significantly improves viral genome detection (page 10).

### Co-purification of DNA & RNA Viral Genomes from a Sample



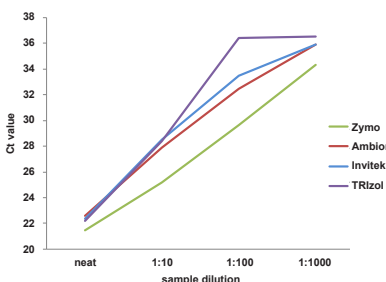
Detection of viral RNA and DNA co-purified from the same sample with the ZR Viral DNA/RNA Kit. HSV ~150 kb DNA; Measles ~16 kb RNA; FluA ~14 kb RNA

### Broad Range Recovery



Small and large nucleic acids are efficiently co-purified with the Zymo-Spin™ XL column technology. Lambda DNA (~50 kb), 1 kb and 50 bp DNA ladder were recovered from a mixture using the ZR Viral DNA/RNA Kit.

### Sensitive, Linear Detection



Sensitive detection of H1N1 influenza virus with ZR Viral RNA Kit™ (signal detected at lower Ct; *M. Peacey, ESR, NCBID*).



# Targeted RNA Isolation—Tissue, Blood & Urine

Separation of a sample may be required to purify RNA from desired components (or parts) of a specific source material. RNA purified from separated samples can be used for downstream applications such as biomarker discovery and detection of diseases. Zymo Research has developed specialized, innovative products for tissue, blood and urine that allow easy sample separation and targeted RNA purification.

## High Quality RNA from Your Partitioned Samples for:

- ✓ Biomarker Discovery & Analysis
- ✓ Gene Expression Studies
- ✓ And More!

### Plasma · Serum WBCs

- ✓ Partitioned or whole blood processing
- ✓ Compatible with PAXgene®

### Tissue Sections · Fingerprints

- ✓ RNA from a specific region of fresh or FFPE tissue
- ✓ Portable LCM alternative

### Exosomes · Cells · Sediment

- ✓ Total RNA, including miRNA

## KITS FOR TARGETED RNA ISOLATION

Product	Preps	Cat. No.
ZR Urine RNA Isolation Kit™ (cells - sediment - exosomes)	20 50	R1038 R1039
ZR Whole-Blood RNA MiniPrep™ (whole blood - WBCs - plasma - serum)	50 100	R1020 R1021
Pinpoint® Slide RNA Isolation System I (frozen tissue sections)	50	R1003
Pinpoint® Slide RNA Isolation System II (FFPE tissue sections)	50	R1007

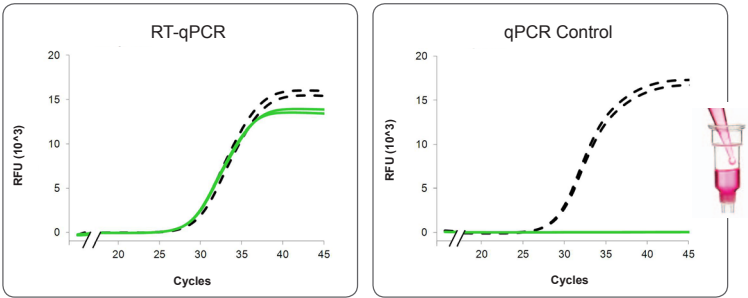
**CSI: RNA** Pinpoint® RNA Systems can purify RNA from cells left behind in fingerprints. “The Pinpoint™ method proved to be the only one that allowed reproducible detection of skin-targeted mRNA markers.”

—Visser, et al. (2011), Int J Legal Med

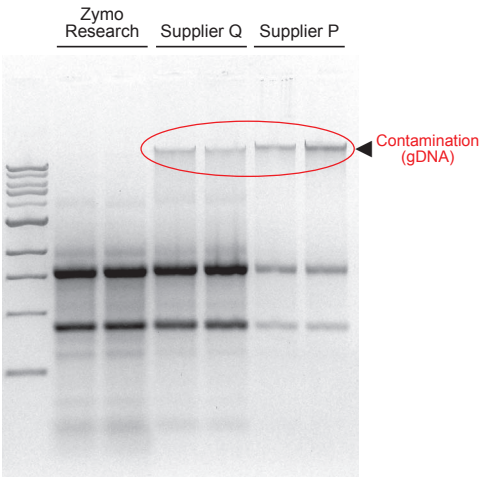
# DNA-free RNA—Right Away!

For most downstream applications, it is essential to obtain RNA that is free of contaminating genomic DNA. Zymo Research's RNA kits remove the vast majority of genomic DNA and feature convenient in-column DNase I treatment. Also, like the Direct-zol™ RNA MiniPrep (below), some kits include DNase I for *DNA-free RNA - Right Away!*

## Reliable RT-PCR



DNA-free RNA isolated with the Direct-zol™ RNA MiniPrep (green). Non-DNase treated samples (black) provided for comparison.



High levels of genomic DNA contamination present in supplier Q & P preps are eliminated with ZR kits. Total RNA isolated from human epithelial cells (without DNase treatment).

**BORN TO BE  
DNA FREE**

Direct-zol™ RNA

In-column DNase I treatment is an integral component of nearly all Zymo Research's RNA purification kits. For in-column treatment, simply add the DNase I mixture to the column containing your RNA samples and your RNA is DNA-free in just minutes!

## DNA-FREE RNA - RIGHT AWAY!

Product	Preps	Cat. No.
Direct-zol™ RNA MiniPrep	50 200	R2050, R2051* R2052, R2053*
Direct-zol™-96 RNA	2 x 96 4 x 96	R2054, R2055* R2056, R2057*
Direct-zol™-96 MagBead RNA	2 x 96 4 x 96 8 x 96	R2100, R2101* R2102, R2103* R2104, R2105*
DNA-Free RNA Kit™	50 200	R1013 R1014

Kits supplied with DNase I  
\*Supplied with TRI Reagent®



Get your **FREE** sample today!  
[www.zymoresearch.com/Direct-zol](http://www.zymoresearch.com/Direct-zol)



When RNA is contaminated with genomic DNA, simply use the DNA-Free RNA Kit™ to perform an in-tube DNase treatment and clean-up.

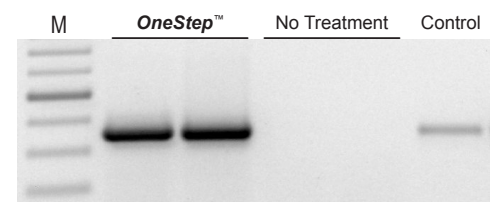


# Boost Your RT-PCR in *OneStep*™ with Ultra-Pure Inhibitor-free RNA

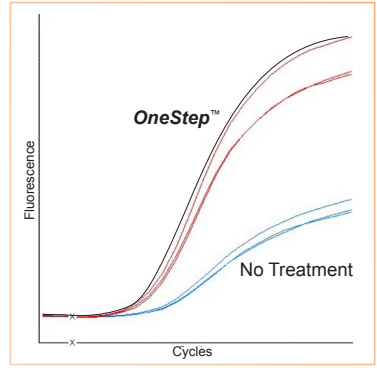
Is your RNA inhibitor-free? RNA purified from difficult source materials, such as environmental samples, plants, soil, and feces, often contain compounds that can significantly inhibit RT-PCR and PCR and confound your results. Zymo Research has developed streamlined solutions for efficient inhibitor removal and RNA clean-up from difficult source materials.

The unparalleled performance of the *OneStep*™ kits ensure quick and complete removal of RT-PCR inhibitors such as polyphenolic compounds, including humic/fulvic acids, tannins, melanin and other pigments. The *OneStep*™ technology is also an integral component of the ZR Plant RNA and ZR Soil/Fecal RNA kits. Inhibitor-free RNA can now be conveniently isolated from sources like soil, feces, plant and pigmented animal tissue.

Quick and complete removal of a wide variety of RT-PCR inhibitors, including:  
polyphenolics ▪ humic acids ▪ fulvic acids  
tannins ▪ melanin pigments



RT-PCR amplification of a eukaryotic transcript isolated from sludge.



Humic acid removal with the *OneStep*™ technology as indicated by the early amplification and increase in fluorescence signal for the *OneStep*™-treated samples compared to the non-treated samples Cp = [30 vs. 31], respectively (RT-qPCR data shown). RNA was isolated from plant leaf samples (*Nicotiana sp.*) "spiked" with humic acid (final A<sub>230nm</sub> = 0.2) using the ZR Plant RNA MiniPrep™.



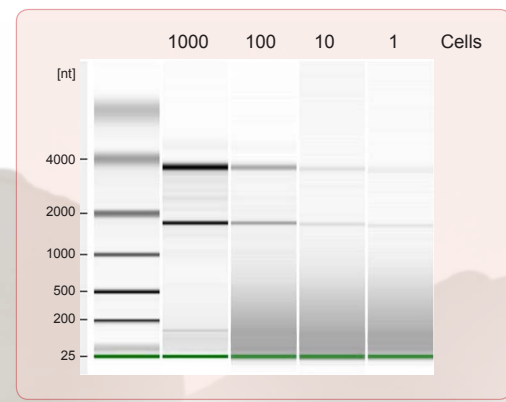
*OneStep*™ RT-PCR Inhibitor Removal

Inhibitor-free RNA

## INHIBITOR-FREE RNA

Product	Preps	Cat. No.
<i>OneStep</i> ™ PCR Inhibitor Removal Kit	50	D6030
<i>OneStep-96</i> ™ PCR Inhibitor Removal Kit	2 x 96	D6035
ZR Plant RNA MiniPrep™ ( <i>OneStep</i> ™ technology included)	50	R2024
ZR Soil/Fecal RNA MicroPrep™ ( <i>OneStep</i> ™ technology included)	50	R2040

# RNA from a Single Cell with Zymo-Spin™ Columns



ZR MicroPrep™ kits facilitate purification of picogram amounts of high-quality RNA. RNA was purified from human epithelial cells using the *Quick-RNA*™ MicroPrep (Bioanalyzer profile).

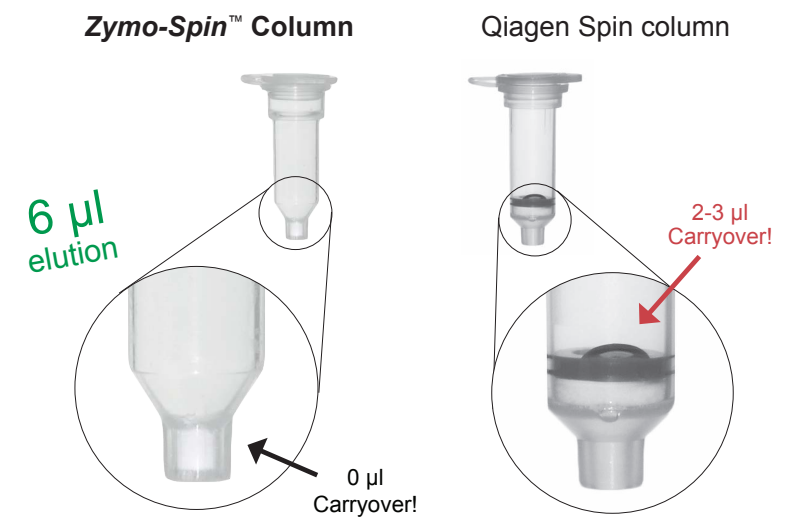
*"Since no commercial kits are available for single bacterial cell RNA isolation/purification, we adapted several kits designed for RNA isolation/purification from mammalian/eukaryotic cells. We found that ZR RNA MicroPrep kit was capable of RNA isolation/purification of both Gram-positive and Gram-negative bacterial cells without modifications."*

- (Gao W. J Microbiol Methods. 2011 Jun;85(3):221-7)

Most current purification methods have proven unsuitable for the isolation of high-quality RNA from small input samples. The MicroPrep™ series of RNA kits from Zymo Research feature exclusive **Zymo-Spin™ IC** column technology for the recovery of RNA from individual cells. The Zymo-Spin™ columns, in combination with optimized buffers, allow purification of picogram amounts of high-quality RNA from a wide variety of sample sources.

Many sensitive RNA applications require ultra-pure, concentrated RNA. The innovative Zymo-Spin™ IC column from Zymo Research allows elution of inhibitor-free RNA in just 6 µl, without buffer carryover or residue left in the column.

## Question Your Spin Column!



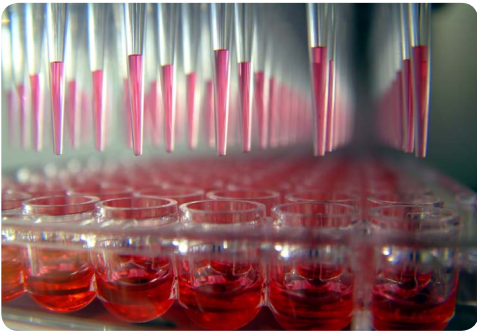
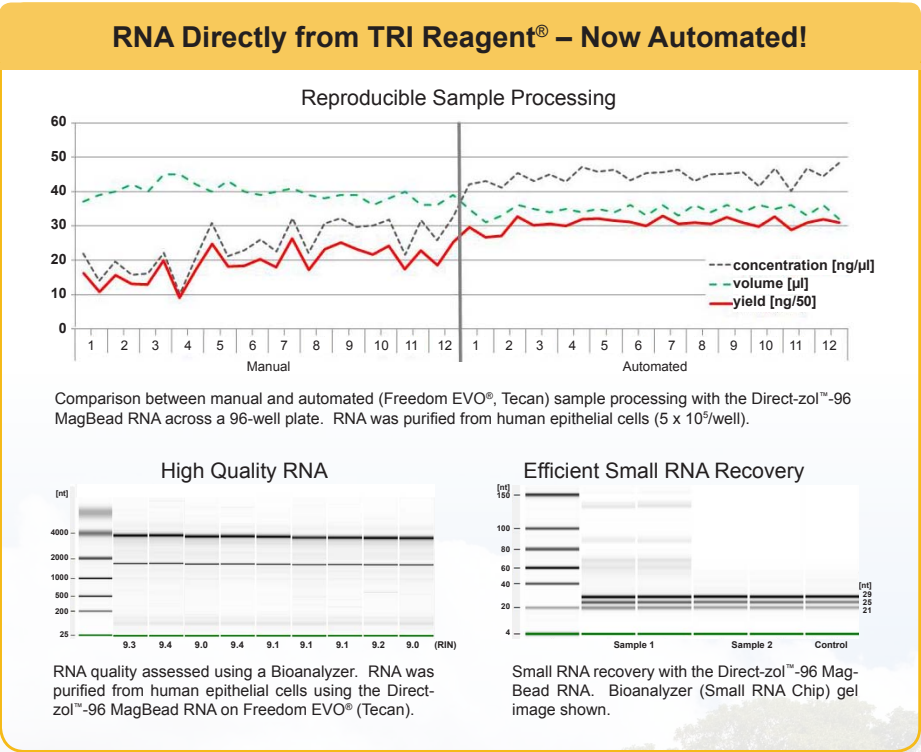
## KITS FOR SMALL INPUT SIZES

Product	Preps	Cat. No.
<i>Quick-RNA</i> ™ MicroPrep	50 200	R1050 R1051
ZR RNA MicroPrep™	50 200	R1060 R1061
ZR Viral RNA Kit™	50 200	R1034 R1035
ZR Urine RNA Isolation Kit™	20 50	R1038 R1039
ZR Tissue & Insect RNA MicroPrep™	50	R2030
ZR Soil/Fecal RNA RNA MicroPrep™	50	R2040
ZR Fungal/Bacterial RNA MicroPrep™	50	R2010
YeaStar™ RNA Kit	40	R1002
Pinpoint® Slide RNA Isolation Kit I & II	50 50	R1003 R1007
RNA Clean & Concentrator™-5	50 200	R1015 R1016
Oligo Clean & Concentrator™-5	50 200	D4060 D4061
Zymoclean™ Gel RNA Recovery Kit	50	R1011
ZR small-RNA™ PAGE Recovery Kit	20	R1070
DNA-Free RNA Kit™	50 200	R1013 R1014



# High-Throughput & Automated Purification of High-Quality Total RNA

For rapid, high-throughput isolation of ultra-pure RNA, Zymo Research offers RNA purification kits in 96-well and magnetic bead formats. These products (listed in the chart below) allow for high-throughput and automated purification of high-quality RNA, and provide consistent results from each well for all downstream standard and Next-Gen applications.



The Direct-zol™-96 RNA kits introduce fast, streamlined methods for isolating RNA from a variety of samples in TRI Reagent® without messy phase separation and precipitation steps. Kits are available in two 96-well formats: Zymo-Spin™ plate and magnetic bead. The magnetic bead format is completely adaptable for automated robotic protocols.

## HIGH-THROUGHPUT & AUTOMATED KITS

Product	Preps	Cat. No.
ZR-96 RNA Clean & Concentrator™	2 x 96	R1080
ZR-96 Oligo Clean & Concentrator™	2 x 96 4 x 96	D4062 D4063
Direct-zol™-96 RNA	2 x 96 4 x 96	R2054, R2055* R2056, R2057*
Direct-zol™-96 MagBead RNA	2 x 96 4 x 96 8 x 96	R2100, R2101* R2102, R2103* R2104, R2105*
ZR-96 Quick-RNA™	2 x 96 4 x 96	R1052 R1053
ZR-96 Viral RNA Kit™	2 x 96 4 x 96	R1040 R1041

All Direct-zol™ RNA Kits supplied with DNase I  
\*Supplied with TRI Reagent®

## High-Throughput Capability for Everyday Use!

**No additional special equipment required with Zymo-Spin™ plates**



# FAQs

### My samples are in TRIzol®, can I use your Direct-zol™ RNA kit?

Yes. Direct-zol™ RNA kits are fully compatible with samples in TRIzol®, TRI Reagent® and all acid-guanidinium-phenol based reagents (see page 10).

### What kit do you recommend for purification of miRNAs and other small RNAs?

For small RNA purification from a wide variety of samples use the Direct-zol™ RNA kits (page 12).

### How efficient is the recovery of small RNAs with the Direct-zol™ RNA kit?

The Direct-zol™ RNA MiniPrep recovers >99% of "spiked" small RNAs from cellular lysates. Recoveries surpass conventional methods and commercially available kits from other providers (mirVana, miRNeasy, RNeasy® Plus).

### Should I use the ZR RNA or the Quick-RNA™ kit?

For cells and biological liquids use the ultra fast Quick-RNA™ system. The ZR RNA kits are ideal for tissue input, samples stored in RNA/ater® and allow for small RNA enrichment if needed.

### Can Zymo kits be used with samples stored in RNA/ater®?

Yes. Most kits, including Direct-zol™ RNA, RNA Clean & Concentrator™ and the ZR RNA kits, are fully compatible with samples stored in RNA/ater® without the need for reagent removal.

### Can your lysis/extraction buffers be used for sample storage?

Samples may be stored in lysis/extraction buffers and RNA will be stabilized for up to 5 days at room temperature (up to 25°C) or long-term when frozen. For prolonged storage at room temperature, we recommend RNA Shield™ (page 14).

### Do you provide DNase I with your RNA kits?

DNase I is included with the Direct-zol™ RNA kits and the DNA-Free RNA Kit™. The protocols for our other RNA purification kits also include instructions for in-column DNase treatment steps that are fully compatible with our DNase I products.

### How can I clean up my previously isolated impure RNA preps for sensitive downstream analyses such as RNA-seq, microarrays, etc.?

For salt, protein (enzyme) and phenol removal or just to concentrate diluted samples, use the RNA Clean & Concentrator™. For removal of polyphenolics, humic/fulvic acids, tannins and melanin use the OneStep™ PCR Inhibitor Removal Kit.

### I have really low inputs (<1000 cells), what kit should I use?

All MicroPrep™ format kits feature the Zymo-Spin™ IC columns for recovery of picogram amounts of RNA that can be eluted into a ≥6 μl volume (page 19). An RT reaction cocktail may also be used to elute the RNA from the column.

### How can I maximize my RNA recovery?

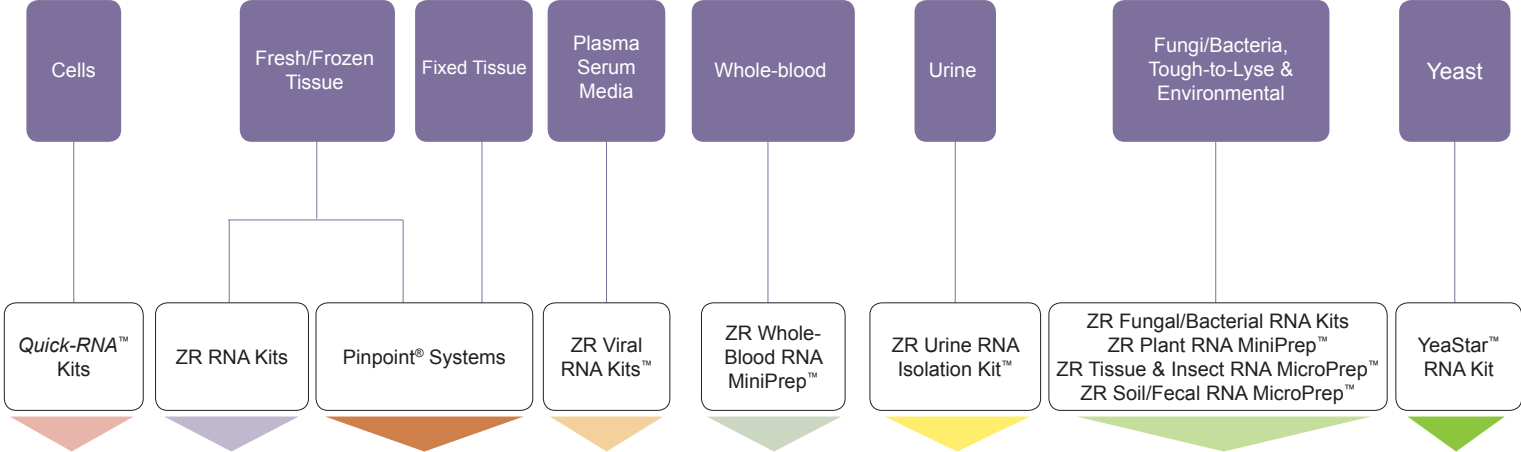
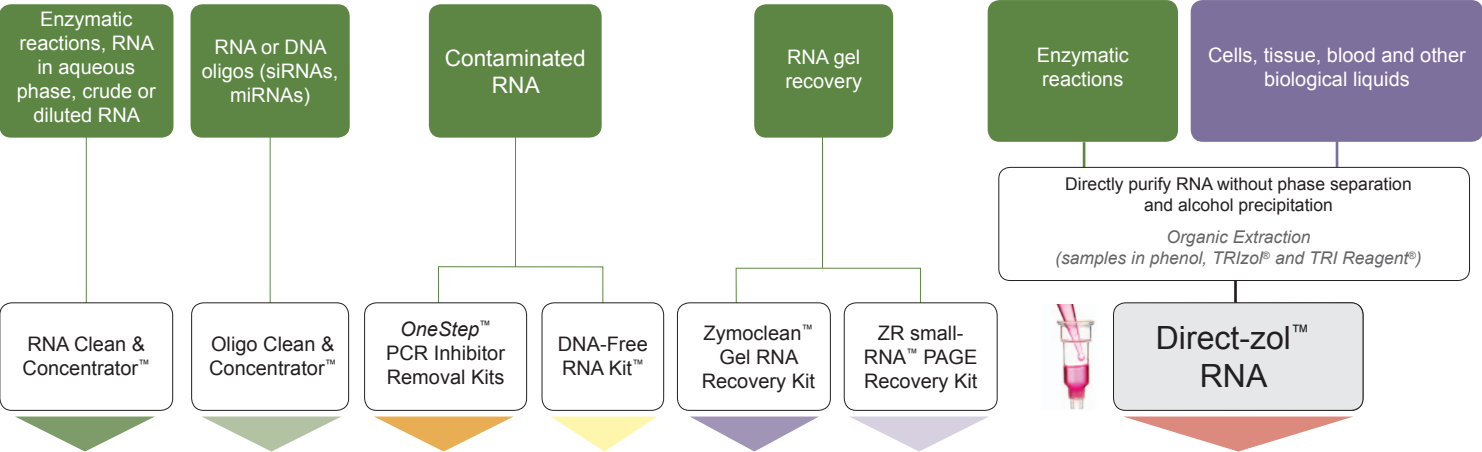
Pre-heated (95°C) elution buffer (water) will increase the efficiency of the elution. If acceptable, an increased elution volume and/or repeated elution may also improve the elution efficiency.

### Can I evaluate one of your kits before placing an order?




Please visit the specific product page on our website ([www.zymoresearch.com](http://www.zymoresearch.com)) to see if there is a sample available or contact us ([info@zymoresearch.com](mailto:info@zymoresearch.com) or 1-888-882-9682) for more details.

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RNA Products Available from Zymo Research

Cat. No.	Product	Application	Prep/Format	Price	
RNA Clean-up					
R1015 R1016	RNA Clean & Concentrator™-5 	Cleanup and concentration of modified, labeled, impure, diluted, DNase treated RNA (>17 nt) and purification of RNA from aqueous phase of organic extracts.  <i>Note: DNA-Free RNA Kit™ includes DNase I</i>	50/column 200/column	\$129.00 \$441.00	
R1017 R1018	RNA Clean & Concentrator™-25		50/column 100/column	\$129.00 \$231.00	
R1019	RNA Clean & Concentrator™-100		25/column	\$171.00	
R1080	ZR-96 RNA Clean & Concentrator™		2 x 96/plate	\$391.00	
R1013 R1014	DNA-Free RNA Kit™		50/column 200/column	\$150.00 \$536.00	
D4060 D4061	Oligo Clean & Concentrator™	Cleanup and concentration of RNA or DNA oligos (& miRNAs and siRNAs).	50/column 200/column	\$79.00 \$299.00	
D4062 D4063	ZR-96 Oligo Clean & Concetrator™		2 x 96/plate 4 x 96/plate	\$199.00 \$387.00	
D7010 D7011	ssDNA/RNA Clean & Concentrator™	Separation of short ssRNA and ssDNA (up to 200 nt) from double stranded species.	20/column 50/column	\$80.00 \$160.00	
R1011	Zymoclean™ Gel RNA Recovery Kit	Recovery of RNA from agarose gels.	50/column	\$116.00	
R1070	ZR small-RNA™ PAGE Recovery Kit	Small RNA (>17 nt) recovery from polyacrylamide gels.	20/column	\$128.00	
D6030	OneStep™ PCR Inhibitor Removal Kit	Removal of polyphenolics, humic/fulvic acids, tannins, melanin etc. from RNA.	50/column	\$102.00	
D6035	OneStep™-96 PCR Inhibitor Removal Kit		2 x 96/plate	\$312.00	
RNA from Samples in TRI Reagent® (Small RNA Recovery)					
R2050 R2052	Direct-zol™ RNA MiniPrep 	RNA (>17nt) from TRI Reagent®, TRIzol® and all other acid-guanidinium-phenol based reagents without phase separation.	50/column 200/column	\$160.00 \$511.00	
R2051 R2053	Direct-zol™ RNA MiniPrep w/ TRI Reagent®		50/column 200/column	\$226.00 \$621.00	
R2054 R2056	Direct-zol™-96 RNA		2 x 96/plate 4 x 96/plate	\$392.00 \$632.00	
R2055 R2057	Direct-zol™-96 RNA w/ TRI Reagent®		2 x 96/plate 4 x 96/plate	\$592.00 \$1,032.00	
R2100 R2102 R2104	Direct-zol™-96 MagBead RNA	RNA (>17nt) from TRI Reagent®, TRIzol® and all other acid-guanidinium-phenol based reagents without phase separation. These kits are in a magnetic bead format that is adaptable for high-throughput and automated protocols.	2 x 96/plate 4 x 96/plate 8 x 96/plate	\$392.00 \$632.00 \$1,012.00	
R2101 R2103 R2105	Direct-zol™-96 MagBead RNA w/ TRI Reagent®		2 x 96/plate 4 x 96/plate 8 x 96/plate	\$592.00 \$1,032.00 \$1,812.00	
Enzymes, Reagents, and Markers					
E1009	DNase I w/ 10X Reaction Buffer	Lyophilized	250 U	\$52.00	
R1090	ZR small-RNA™ Ladder	ssRNA (17, 21, 25, 29 nt)	10 µg	\$83.00	
R1100-50 R1100-250	RNA Shield™ 	Cell, biological liquid, tissue storage and RNA purification	50 mL 250 mL	\$62.00 \$221.00	
R1100 R1101	RNA Shield™ Purification Kit	<i>Note: Supplied with the RNA Shield™ reagent.</i>	50/column 50/column	\$182.00 \$120.00	
S6010	ZR BashingBead™	Lysis/Filtration tubes w/ 0.5 mm beads (50 mL)	25 pack	\$184.00	

Cat. No.	Product	Application	Prep/Format	Price
RNA from Cells				
R1050 R1051	Quick-RNA™ MicroPrep	Total RNA from cells.	50/column	\$150.00
			200/column	\$477.00
R1054 R1055	Quick-RNA™ MiniPrep		50/column	\$150.00
			200/column	\$477.00
R1056	Quick-RNA™ MidiPrep		25/column	\$252.00
R1052 R1053	ZR-96 Quick-RNA™		2 x 96/plate	\$372.00
			4 x 96/plate	\$717.00
RNA from Tissue				
R1060 R1061	ZR RNA MicroPrep™	RNA (>17 nt) from tissue.	50/column	\$191.00
			200/column	\$611.0
R1064 R1065	ZR RNA MiniPrep™		50/column	\$191.00
			200/column	\$611.00
D7001	ZR-Duet™ DNA/RNA MiniPrep	Parallel purification of DNA/RNA from cells.	50/column	\$297.00
R1003	Pinpoint® Slide RNA Isolation System I	RNA from frozen tissue sections.	50/column	\$149.00
R1007	Pinpoint® Slide RNA Isolation System II	RNA from FFPE tissue.	50/column	\$237.00
RNA from Biological Liquids				
R1034 R1035	ZR Viral RNA Kit™	RNA (DNA) from body fluids (plasma, serum, CSF, urine).	50/column	\$129.00
			200/column	\$441.00
R1040 R1041	ZR-96 Viral RNA Kit™		2 x 96/plate	\$359.00
			4 x 96/plate	\$644.00
D7020 D7021	ZR Viral DNA/RNA Kit™		25/column	\$129.00
			100/column	\$441.00
R1020 R1021	ZR Whole-Blood RNA MiniPrep™	RNA from whole or partitioned blood.	50/column	\$208.00
			100/column	\$373.00
R1038 R1039	ZR Urine RNA Isolation Kit™	Cellular and endosomal RNA from urine.	20/column	\$111.00
			50/column	\$254.00
RNA from Tough-to-Lyse Samples				
R2010	ZR Fungal/Bacterial RNA MicroPrep™	RNA from bacteria, yeast and fungi.	50/column	\$222.00
R2014	ZR Fungal/Bacterial RNA MiniPrep™		50/column	\$222.00
R2024	ZR Plant RNA MiniPrep™	RNA from leaves, stems, buds, flowers, fruits, seeds, etc.	50/column	\$254.00
R2030	ZR Tissue & Insect RNA MicroPrep™	RNA from insects, arthropod specimen and small tissue.	50/column	\$222.00
R2040	ZR Soil/Fecal RNA MicroPrep™	RNA from soil, sludge, sediments and feces.	50/column	\$412.00
R1002	YeaStar™ RNA Kit	RNA from yeast strains susceptible to zymolyase.	40/column	\$135.00



wide DNA methylation and hydroxymethylation analysis, Zymo Research provides the most consolidated and highly regarded products in the industry.

Since 1994, Zymo Research has been offering innovative, quality, and easy-to-use tools for DNA/RNA purification. Now, as “The Epigenetics Company”, Zymo Research offers a wide range of products and services for epigenetics research. From industry-leading bisulfite technologies to state of the art services for genome-

100% Satisfaction Guaranteed. Zymo Research is committed to the highest standard of quality and assures your satisfaction with its products.

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