

## Tissue management for biomedical research

Tissues are increasingly becoming the biological material of choice for gene expression analysis studies in biomedical research. These include open-surgery biopsies, fine needle aspirates, and laser-microdissected tissues from human donors as well as organs and tissues from laboratory animals. In contrast to cultured cells, gene expression in tissue samples is closer to how genes are expressed in a living organism. However, working with tissues poses special challenges. Freshly harvested tissues must be immediately stabilized to prevent changes in the gene expression pattern and then thoroughly disrupted and homogenized to release RNA. Tissues archived by fixing and embedding require special procedures to overcome chemical modification of nucleic acids.

### Tissue Management System



Since tissues vary greatly in their properties, a standardized, universal procedure capable of tackling different types of tissue is required when studying gene expression in a variety of tissues. QIAGEN offers a range of products that form a Tissue Management system, streamlining the workflow from sample collection to data analysis. Products include kits for low- and high-throughput purification of high-quality RNA, optimized PCR master mixes, and validated gene expression assays.

New products are continually being developed to further streamline the workflow and to tackle a broader range of sample types (see next page). QIAGEN is also intensifying its collaboration with experts in biomedical tissue management to bring about further improvements in this important field. By mid-May, a worldwide Biomedical Tissue Management Advisory Panel will be established, with the aim of developing standard operating procedures to advance molecular-based medicine. ▶

### Inside

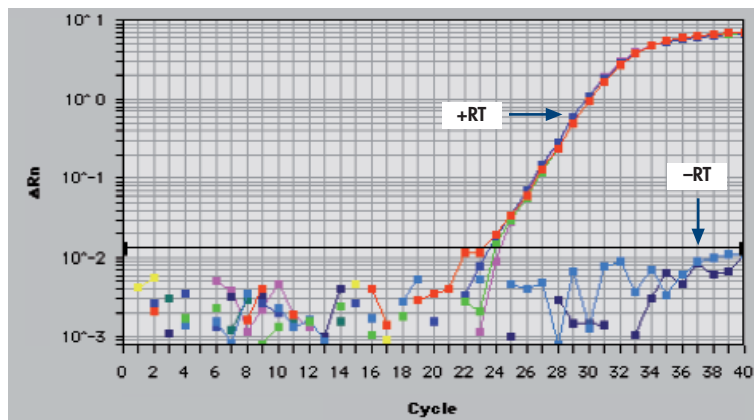
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## High yields of usable RNA from FFPE samples

Obtaining sufficient quantities of usable RNA from formalin-fixed, paraffin-embedded (FFPE) tissue sections for gene expression analysis represents a major challenge. The RNA molecules are often degraded and are crosslinked with protein and other RNA molecules. The new RNeasy FFPE Kit uses special lysis and incubation conditions to reverse formaldehyde modification and to efficiently release RNA without further RNA degradation. This allows the purification of high yields of usable RNA that delivers maximal performance in real-time RT-PCR and other downstream applications.

### Successful Real-Time Analysis



RNA purified in duplicate from FFPE rat liver was analyzed on the ABI PRISM<sup>®</sup> 7700 using the QuantiTect Probe RT-PCR Kit and primers and TaqMan<sup>®</sup> probe for c-jun. Reactions were performed in duplicate with (+RT) or without (-RT) reverse transcriptase. The -RT curves show no amplification even after 40 PCR cycles, demonstrating that the RNA is virtually free of genomic DNA.

## Getting the most out of precious tissue samples

For studies in biomedical research where DNA, RNA, and protein need to be obtained from the same tissue sample, the use of separate procedures to purify each of these biomolecules makes sample preparation time-consuming. The AllPrep DNA/RNA Mini Kit overcomes this bottleneck by allowing the purification of both DNA and RNA from the same tissue sample in one short, spin-column-based procedure. In addition, total protein can be acetone-precipitated from the lysate depleted of DNA and RNA. As the tissue sample does not have to be split for separate purification procedures, maximum yields of DNA, RNA, and protein can be achieved from the sample. DNA and RNA purified using the AllPrep DNA/RNA Mini Kit is ready to use in any downstream application, such as real-time RT-PCR and multiplex PCR. The precipitated protein is suitable for western blotting.

# Choosing endogenous controls for real-time RT-PCR

When analyzing gene expression in tissues and other biological samples by real-time RT-PCR, the expression of a target gene relative to that of an endogenous control can be determined. This is known as relative quantification and has the advantage of allowing the gene expression level of the target gene to be normalized to the amount of input RNA or cDNA. There is no need to determine the exact amount of template used in real-time RT-PCR. The use of an endogenous control corrects for variation in RNA content, variation in reverse-transcription efficiency, possible RNA degradation or presence of inhibitors in the RNA sample, variation in nucleic acid recovery, and differences in sample handling.

When performing relative quantification of the expression of a target gene, it is important to choose a suitable gene, usually a housekeeping or maintenance gene, to use as an endogenous control. An endogenous control is a gene whose expression level does not vary under experimental conditions, or in different states of the same tissue or cell line (e.g., “disease” versus “normal” samples). Endogenous controls commonly used for relative quantification include  $\beta$ -actin,  $\beta$ -2-microglobulin, peptidylprolyl isomerase A, and GAPDH mRNAs, and also 18S rRNA.  $\beta$ -actin is ubiquitously expressed and was one of the first genes to be used as an endogenous control. However, its transcription levels may vary and the presence of pseudogenes may mean that genomic DNA is detected during real-time RT-PCR, leading to inaccurate quantification.<sup>†</sup> GAPDH is a housekeeping gene commonly used as an endogenous control. However, GAPDH mRNA levels may vary between individuals, at different stages of the cell cycle, and following treatment with different drugs. Therefore, its use as an endogenous control must be carefully evaluated. As 18S rRNA is not an mRNA, its levels in the cell may not accurately reflect the cellular mRNA population. Therefore, careful testing of various endogenous controls or using a combination of endogenous controls may provide the most reliable solution when performing relative quantification.

A comprehensive range of ready-to-use primer sets and primer–probe sets for real-time RT-PCR analysis of housekeeping genes is available at [www.qiagen.com/GeneGlobe](http://www.qiagen.com/GeneGlobe) under “View Controls”. These include validated QuantiTect Primer Assays for SYBR® Green based detection and functionally validated QuantiTect Endogenous Control Assays for probe-based detection.

<sup>†</sup> QIAGEN offers two solutions for eliminating genomic DNA contamination: the RNeasy Plus Mini Kit (cat. no. 74134), which provides RNA purification using gDNA Eliminator columns, and the QuantiTect Reverse Transcription Kit (cat. no. 205311), which provides fast cDNA synthesis with integrated gDNA removal.



[www.qiagen.com/GeneGlobe](http://www.qiagen.com/GeneGlobe)

## Housekeeping Genes Commonly Used as Endogenous Controls

Gene name	Relative expression level*
18S ribosomal RNA	++++
Glyceraldehyde-3-phosphate dehydrogenase	+++
$\beta$ -Actin, cytoplasmic	+++
$\beta$ -2-microglobulin	+++
Phosphoglycerate kinase 2	+++
Peptidylprolyl isomerase A (cyclophilin A)	+++
Ribosomal protein, large, PO	+++
Hypoxanthine phosphoribosyl transferase 1	++
$\beta$ -Glucuronidase	+
TATA box binding protein	+
Transferrin receptor	+

\* “+” indicates relative abundance of the transcripts.

# Faster discovery by high-throughput RNAi screening of the mouse genome

QIAGEN has developed the Mouse Whole Genome siRNA Set V1.0, a powerful and flexible tool for drug discovery using high-throughput RNAi. Modules are available for druggable, predicted, phosphatase, kinase, and GPCR genes.

## Advantages of the Mouse Whole Genome siRNA Set V1.0:

- **Flexible formats** — choose the whole genome, druggable genome (Figure 1), or modules, individual siRNAs or pools, at different scales
- **High and specific knockdown** — using the innovative HiPerformance siRNA Design Algorithm
- **Faster discovery** — using off-the-shelf siRNAs targeting the whole range of mouse genes

## Flexible formats

Sets are available in a variety of modules (Table 1) and formats (Table 2), ensuring flexibility to suit specific screening requirements. Full siRNA sequence information and gene annotation are provided.

**Table 1. Modules of the Mouse Whole Genome siRNA Set**

Set name	Number of genes	Type
Mouse Whole Genome siRNA Set V1.0	~17,000	All known mouse genes (NM genes) from the RefSeq database
Mouse Predicted Genes siRNA Set V1.0	~5300	Computationally predicted genes (XM genes) from the RefSeq database
Mouse Druggable Genome siRNA Set V1.0	8353	Mouse druggable genes
Mouse Genome Supplement V1.0	~8700	siRNAs targeting genes from the Mouse Whole Genome siRNA Set that are not included in the Mouse Druggable Genome siRNA Set
Mouse Kinase siRNA Set V1.0	762	Mouse kinase genes
Mouse Phosphatase siRNA Set V1.0	294	Mouse phosphatase genes
Mouse GPCR siRNA Set V1.0	397	Mouse G protein-coupled receptor genes

For set format, choose between:

- **Individual siRNAs for each gene** to independently confirm phenotypes with multiple siRNAs. Either 2 or 4 individual siRNAs can be ordered for each gene at 0.25 nmol or 1 nmol scales.
- **A pool of siRNAs for each gene** to streamline screening and save time and resources. Positive results need further validation using individual siRNAs to exclude the possibility of off-target effects. Pools of 2 or 4 siRNAs can be ordered at a total of 0.5 nmol or 1 nmol respectively.

## High and specific knockdown using the innovative HiPerformance siRNA Design Algorithm

The HiPerformance siRNA Design Algorithm used at QIAGEN optimally combines design and homology analysis to ensure that siRNA is both potent and specific, providing high knockdown with minimal risk of nonspecific effects.

The HiPerformance siRNA Design Algorithm incorporates an innovative neural-network-based design algorithm, which has been licensed from Novartis (1). A proprietary homology analysis tool developed at QIAGEN and an up-to-date, internally curated, nonredundant sequence database are used to search for regions of homology in the genome.

## Freedom to discover

The Mouse Whole Genome siRNA Set V1.0 is provided with complete siRNA sequence disclosure at no extra cost. There are no restrictions on disclosure of individual sequences if required for publication. However, public disclosure of more than 0.5% of the sequences, in print or electronically, is prohibited without written permission from QIAGEN.

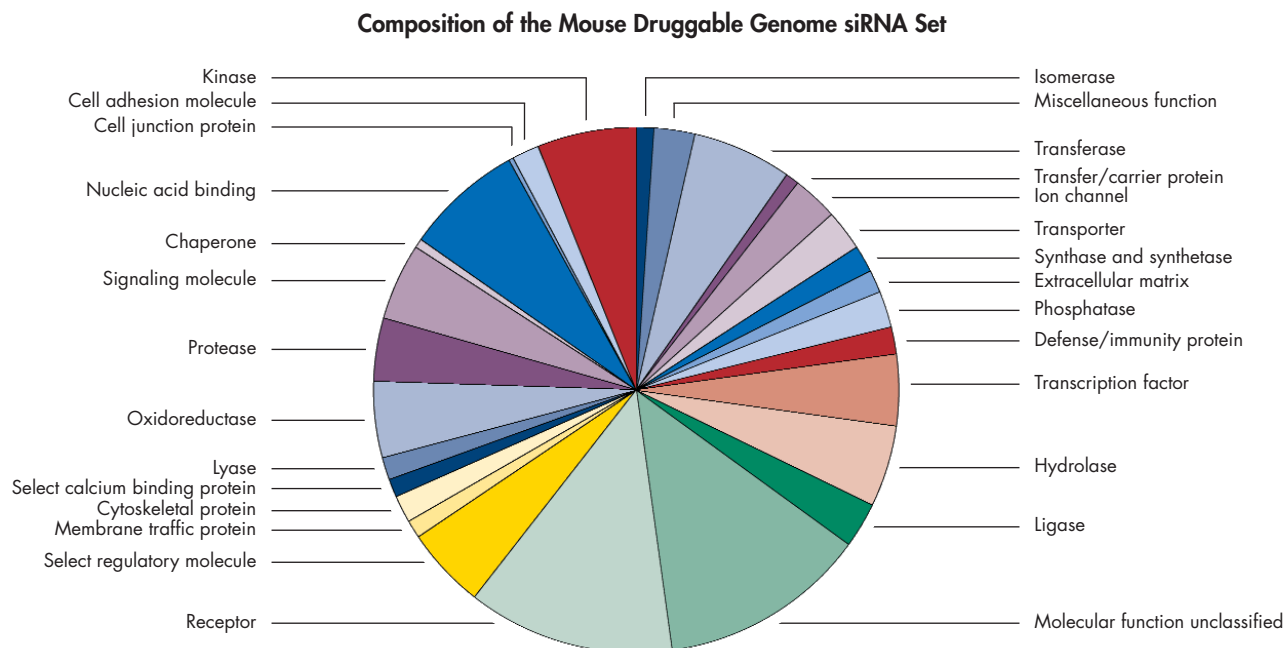
Although QIAGEN owns the siRNA designs in this set, all rights to any invention or discovery developed by researchers using these sequences belong to the researcher. This allows complete freedom for research and discovery.

**Table 2. Mouse Genome Set Formats**

Individual siRNAs	2 per gene (0.25 or 1.0 nmol)
	4 per gene (0.25 or 1.0 nmol)
Pooled siRNAs	2 per gene (0.5 nmol total)
	4 per gene (1.0 nmol total)

## Reference

- Huesken, D. et al. (2005) Design of a genome-wide siRNA library using an artificial neural network. *Nat. Biotechnol.* **23**, 995.



**Figure 1** Molecular function categories of the genes targeted in the Mouse Druggable Genome siRNA Set

**To find out more about high-throughput RNAi screening of the mouse genome, visit [www.qiagen.com/siRNA](http://www.qiagen.com/siRNA) !**

## Biomedical tissue management — join our worldwide Advisory Panel

We invite you to participate in our Biomedical Tissue Management Advisory Panel, starting mid-May. By joining, you benefit from:

- A regular exchange of ideas with fellow specialists using an exclusive online forum
- Exclusive access to an electronic information platform
- Product design and R&D influenced by your input
- Valuable personal incentives for responding to our request for scientific input

You can register from April 3, 2006 by visiting [www.qiagen.com/goto/JoinPanel](http://www.qiagen.com/goto/JoinPanel) !



## Ordering Information

Product	Contents	Cat. no.
RNeasy FFPE Kit (50)	50 RNeasy MinElute® Spin Columns, 50 gDNA Eliminator Spin Columns, Collection Tubes, Reagents and Buffers	74404
AllPrep DNA/RNA Mini Kit (50)	50 AllPrep DNA Spin Columns, 50 RNeasy Spin Columns, Collection Tubes, Reagents and Buffers	80204
QuantiTect Endogenous Control Assay (100)	For 100 x 50 µl reactions: 0.5 ml 10x QuantiTect Assay Mix (dyes available: FAM, Yakima Yellow™)	Varies*
QuantiTect Primer Assay (200)	For 200 x 50 µl reactions: 10x QuantiTect Primer Assay (lyophilized)	Varies*

\* Visit [www.qiagen.com/GeneGlobe](http://www.qiagen.com/GeneGlobe) to search for and order assays.

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