# Analyzing Gene Expression and Regulation


QIAGEN®

Sample & Assay Technologies

# Important mechanisms of regulating gene expression

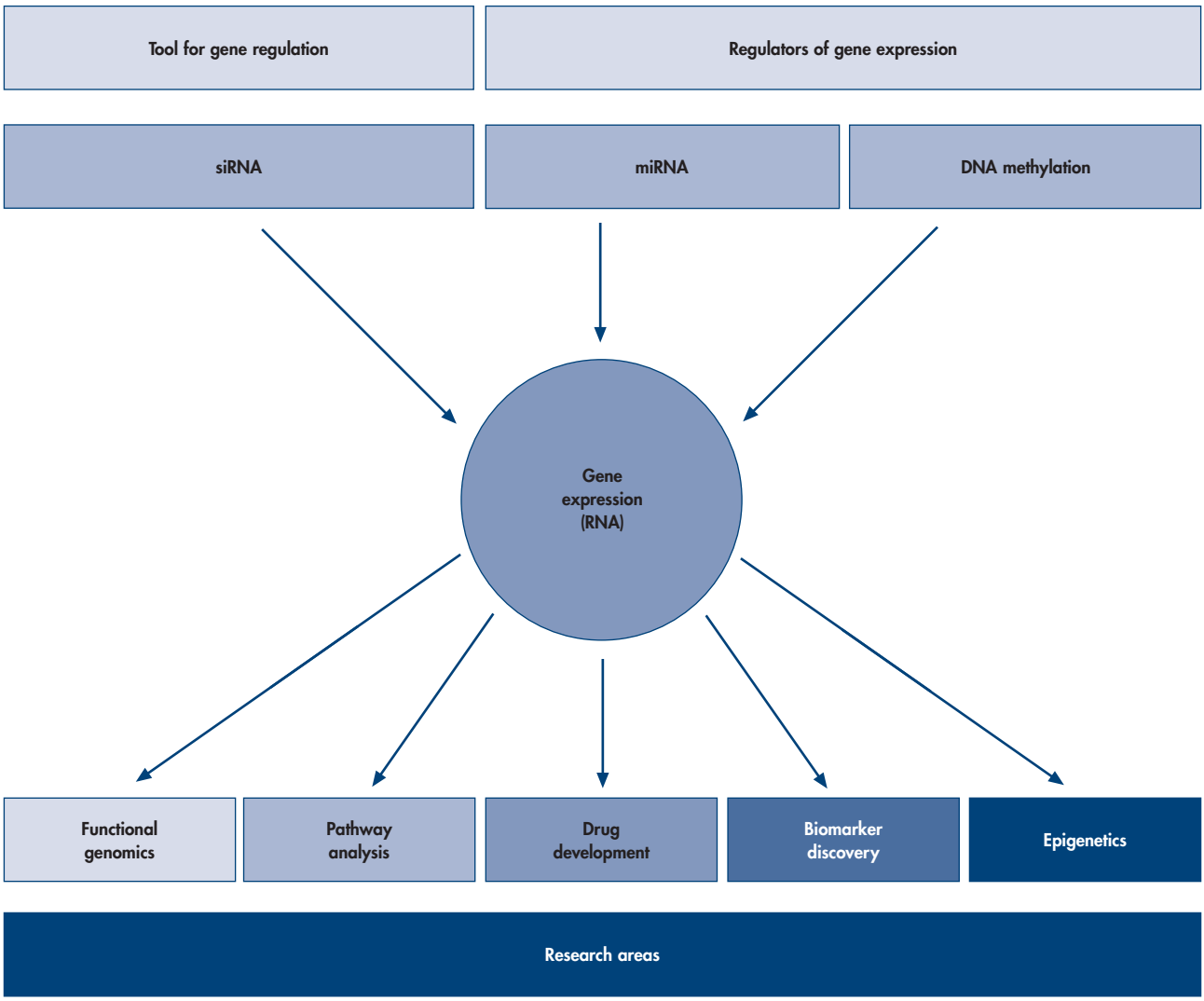



**Figure 1. Gene expression and gene regulation.** Expression of genes can be regulated artificially by siRNA-mediated RNAi, a powerful tool for gene knockdown. Mechanisms of gene regulation include gene silencing by miRNAs, which are naturally occuring short RNAs, and DNA methylation of genomic DNA, which can occur as a result of environmental stimuli. RNAi and the study of miRNAs and DNA methylation are of importance in a variety of research areas.

# Broad range of solutions for studying genes

The ground-breaking invention of siRNA-mediated RNAi and the important discoveries of miRNA and DNA methylation open up many possibilities for researchers. RNAi provides a powerful tool for studying gene function, while miRNA and DNA methylation represent exciting areas of research into diseases such as cancer. QIAGEN recognizes the importance of these scientific advances, and offers a broad portfolio of products that overcomes the major challenges in gene silencing and gene expression analysis and in research into gene regulation by miRNA and DNA methylation (Table 1).

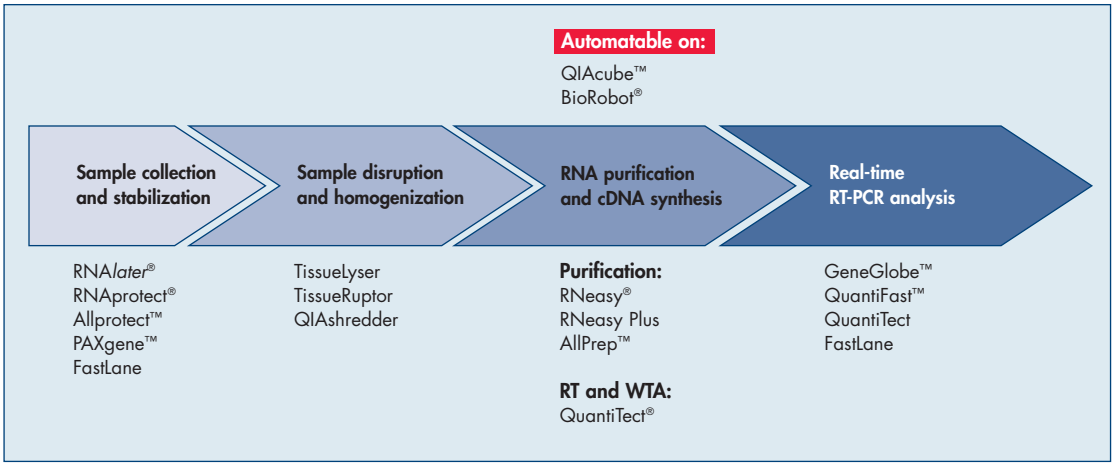
**Table 1. QIAGEN solutions to challenges in studying genes**

| Application | Challenge | QIAGEN solution |
|---|---|---|
| **Gene expression analysis** | Artifical changes in transcript levels upon sample collection | Reagents for immediate and convenient sample stabilization |
| | Working with limited amounts of sample material | Uniform whole transcriptome amplification with minimal sequence bias |
| | Designing optimal gene expression assays | Bioinformatically validated, genomewide primer sets |
| | Achieving efficient PCR amplification | Optimized real-time PCR master mixes for all cyclers |
| | Carrying out high-throughput analysis | Real-time RT-PCR analysis without RNA purification |
| **RNAi/miRNA research** | Sourcing reagents for high knockdown | Predesigned, potent, specific siRNAs, available online |
| | Minimizing off-target effects | Highly specific and potent siRNA, and reagent to transfect low siRNA amounts |
| | Efficient siRNA delivery | Transfection reagent validated for broad range of cell types |
| | Lack of proven tools in the new field of miRNA research | Tools for purification and real-time PCR analysis |
| **Epigenetics** | Accurate and reproducible DNA methylation analysis | Complete bisulfite conversion (>99% of unmethylated cytosines) |
| | Sensitive results even from small samples | Unique DNA protection during bisulfite treatment |
| | Fast and easy bisulfite conversion | Streamlined 6-hour procedure with prealiquoted buffers; automated protocol available |
| | DNA methylation analysis from FFPE tissue samples | Dedicated DNA purification and bisulfite conversion methods |

## Real-time PCR

**QIAGEN sets the standard for gene expression analysis, providing:**

- Purification of RNA that accurately represents the in vivo gene expression profile
- Highly specific and sensitive quantification of transcripts with no need for optimization
- Standardized, streamlined workflows to ensure comparable and reproducible data




**For more details on standardized gene expression analysis, visit www.qiagen.com/geneXpression !**

## Gene expression analysis applications

Gene expression analysis plays an important role in areas such as cancer research, enabling biomarker identification, target validation, and drug development. Of importance to researchers is the ability to achieve accurate real-time PCR with the minimum of time and effort. Real-time PCR with SYBR® Green detection in just 45 minutes while maintaining high specificity and sensitivity can be achieved by combining genomewide, predesigned primer sets with a patent-pending buffer system optimized for fast-cycling PCR (Figure 2). This innovative fast real-time PCR technology provides speed and accuracy not only on fast cyclers but also on standard cyclers, whether detection is done with SYBR Green or with sequence-specific probes (Figure 3). For applications such as RNAi validation, the entire real-time PCR workflow can be streamlined by eliminating the need for RNA purification and allowing analysis of multiple targets in one tube (Figure 4). All these PCR technologies avoid tedious optimization steps through the use of ready-to-use master mixes.

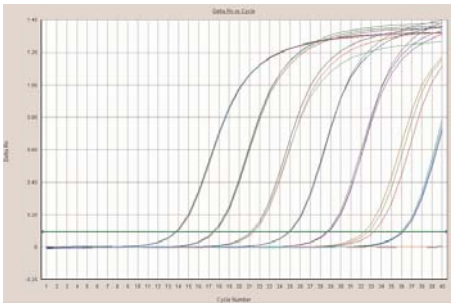


**Figure 2. Wide dynamic range in SYBR Green based real-time RT-PCR.** The QuantiFast SYBR Green RT-PCR Kit provided accurate quantification of HSP89 (a heat shock protein) transcript over 7 logs of template when used with the corresponding QuantiTect Primer Assay. Reactions were run in triplicate on the Applied Biosystems® 7500 Fast System using 10-fold dilutions of in vitro transcript ($10^7$ down to 10 copies).

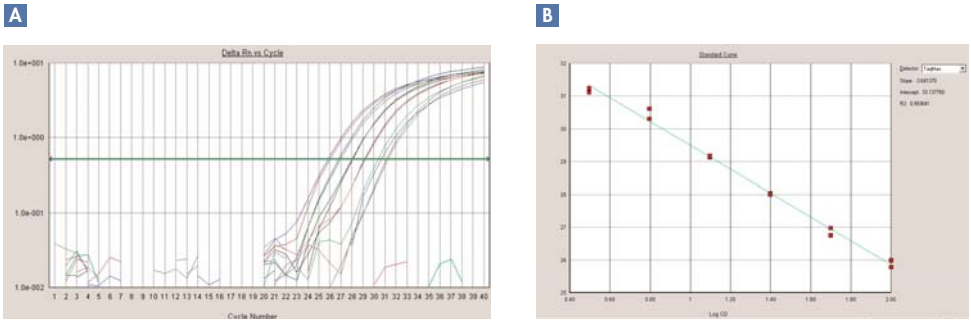



**Figure 3. Fast and accurate probe-based real-time RT-PCR.** The QuantiFast Probe PCR Kit provided A clearly distinguishable $C_T$ values when two-fold dilutions of cDNA (100 ng to 3.13 ng) from skeletal muscle were analyzed and B a PCR efficiency of 90%. Reactions were run in duplicate on the ABI PRISM® 7000 using a TaqMan® gene expression assay for NFkB. (Data kindly provided by Dr. Despina Constantin and Dr. Tim Constantin, University of Nottingham Medical School, United Kingdom.)

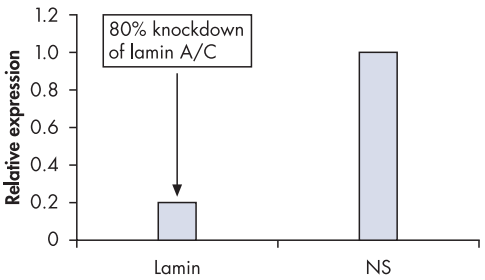



**Figure 4. Reliable validation of gene silencing.** In a 96-well plate of HCT116 cells, 5 wells were transfected with lamin A/C siRNA **(Lamin)** or nonsilencing siRNA **(NS)**. Cells were analyzed by multiplex, real-time RT-PCR using the FastLane Cell Multiplex Kit and TaqMan assays for lamin A/C (structural protein) and 18S rRNA (endogenous control). (Data kindly provided by Angela Quinn, Genzyme Corporation, USA.)

## siRNA and miRNA


**World-class RNAi solutions from QIAGEN provide:**

- Innovative siRNA design ensuring potent siRNA with minimal off-target effects
- Maximum flexibility to choose siRNA scales, formats, and plate layout
- Exceptionally efficient transfection at low siRNA concentrations

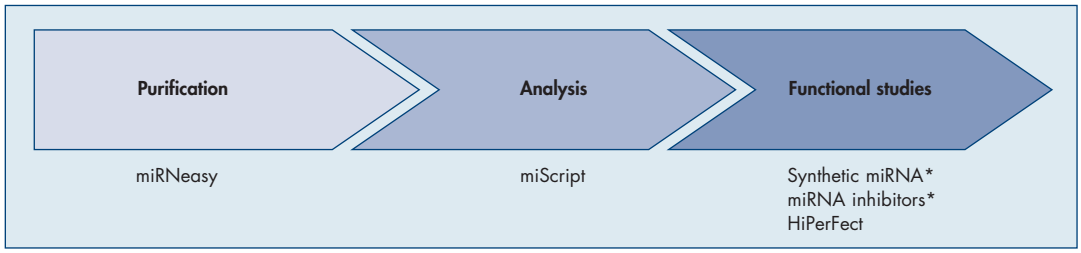
**Automatable on:**
QIAcube
BioRobot

| siRNA | Transfection | Purification | Analysis | In vivo studies |
|---|---|---|---|---|
| FlexiPlate siRNA<br>FlexiTube siRNA<br>HP Genomewide siRNA<br>HP Validated siRNA<br>QIAGEN® AllStars Controls<br>HP siRNA Sets | HiPerFect<br>TransMessenger® | RNeasy<br>TurboCapture<br>AllPrep | FastLane<br>QuantiFast<br>QuantiTect | Large-Scale siRNA<br>Synthesis |

**For world-class RNAi solutions you can rely on, visit www.qiagen.com/siRNA !**

**QIAGEN solutions for advancing miRNA research ensure:**

- Effective purification and miRNA enrichment from all types of animal tissues and cells
- Sensitive and specific detection and quantification of miRNA
- Fast, simple quantification of multiple miRNAs from a single cDNA synthesis reaction
- Quantification of both mRNA and miRNA from the same cDNA synthesis reaction




* Please inquire.

**Discover more about how to advance your miRNA research at www.qiagen.com/miRNA !**

## RNAi and miRNA applications

RNAi has a wide variety of applications, including analyzing groups of genes involved in specific pathways and high-throughput screening for drug discovery. For example, RNAi sreening experiments have identified kinases that could potentially be targeted in anticancer therapies (Figure 5). RNAi analysis is also used in follow-up investigations of genes found to be upregulated or downregulated in microarray experiments. In miRNA research, purification and real-time PCR technologies can be used to characterize multiple miRNAs (Figures 6 and 7).

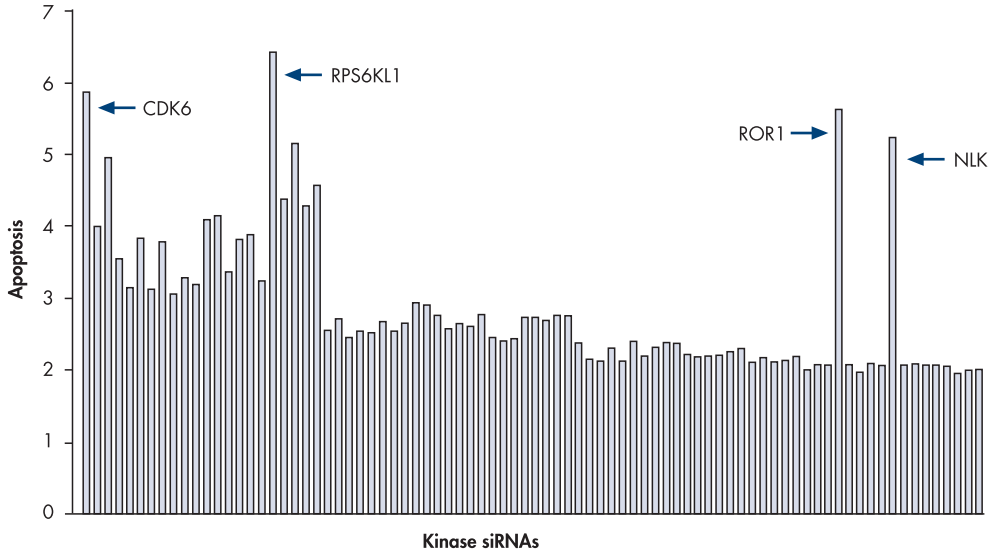



**Figure 5. siRNA screening of kinase genes reveals novel anticancer targets.** These data show a portion of the results achieved when cells were transfected with siRNAs from QIAGEN's Human Kinase siRNA Set followed by measurement of apoptosis using a histone-DNA fragmentation ELISA. Silencing of the 4 survival kinases indicated causes increased apoptosis. Of these 4 kinases, 2 are novel and of unknown function (RPS6KL1 and ROR1). (Data kindly provided by Jeffrey P. MacKeigan, Leon O. Murphy, and John Blenis, Harvard Medical School, USA.) For full details of this research, refer to MacKeigan, J.P. et al. (2005) Sensitized RNAi screen of human kinases and phosphatases identifies new regulators of apoptosis and chemoresistance. Nat. Cell Biol. **7**, 591.

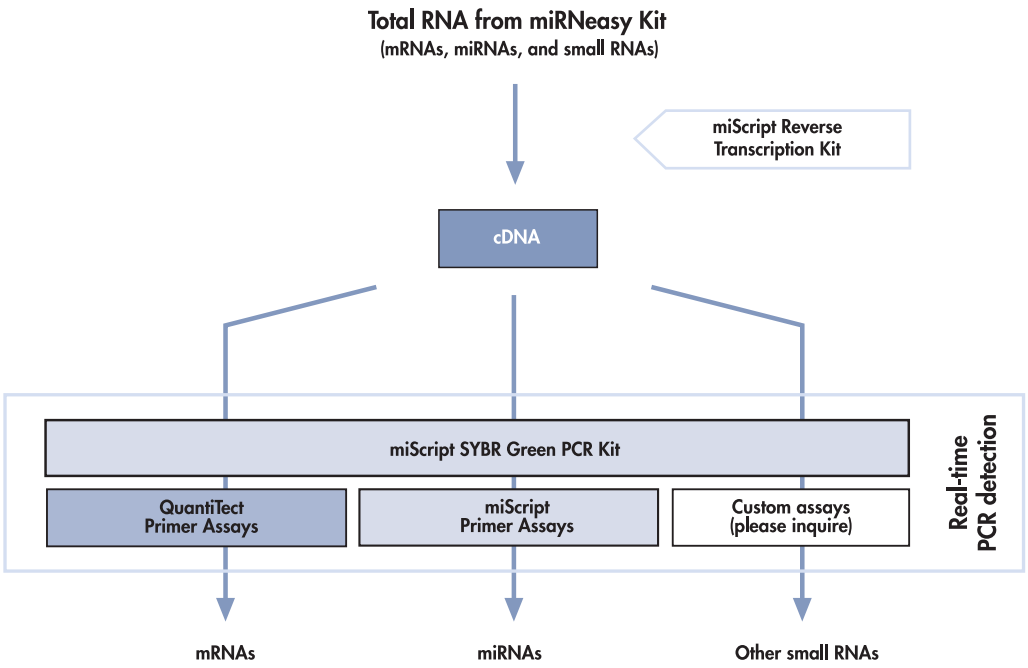



**Figure 6. miRNA characterization using miRNeasy and miScript technologies.** Combined miRNeasy and miScript technologies enable purification, detection, and quantification of miRNAs, small RNAs, and mRNAs.

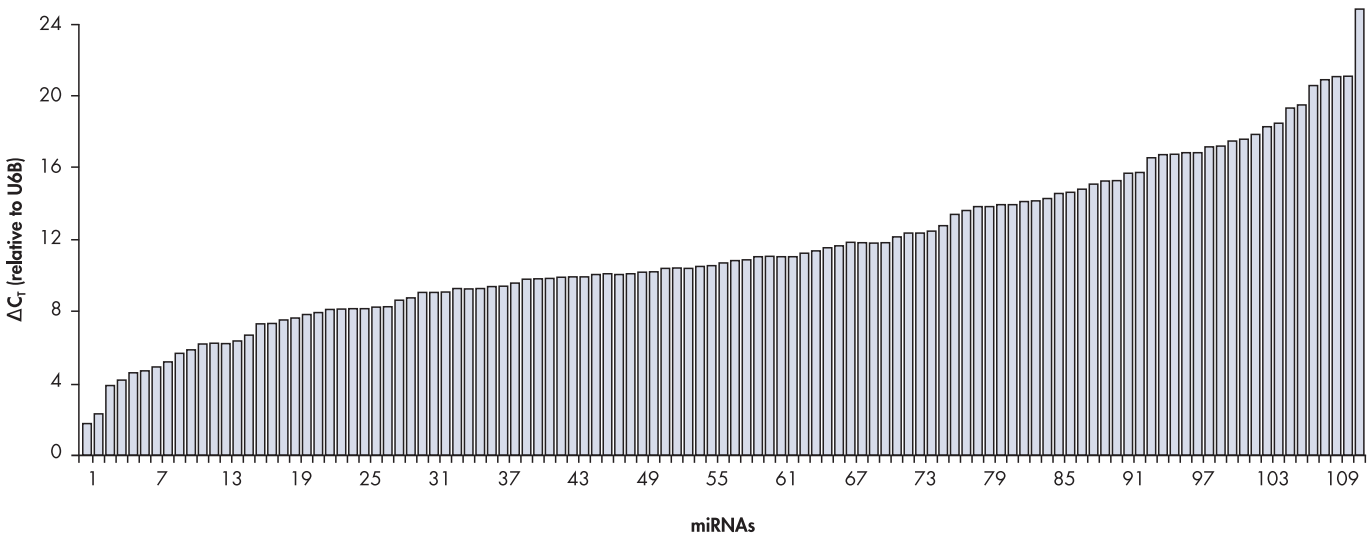
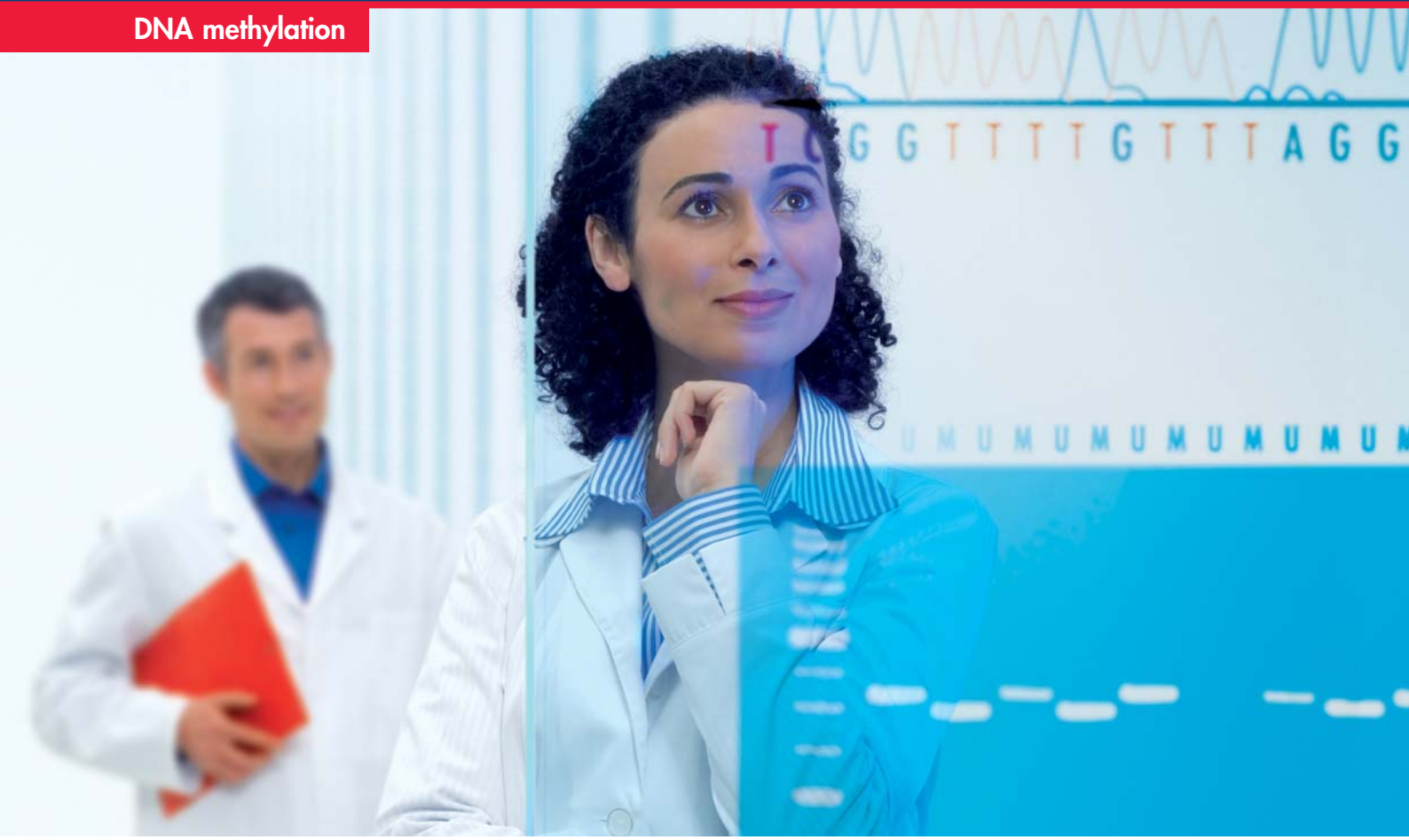


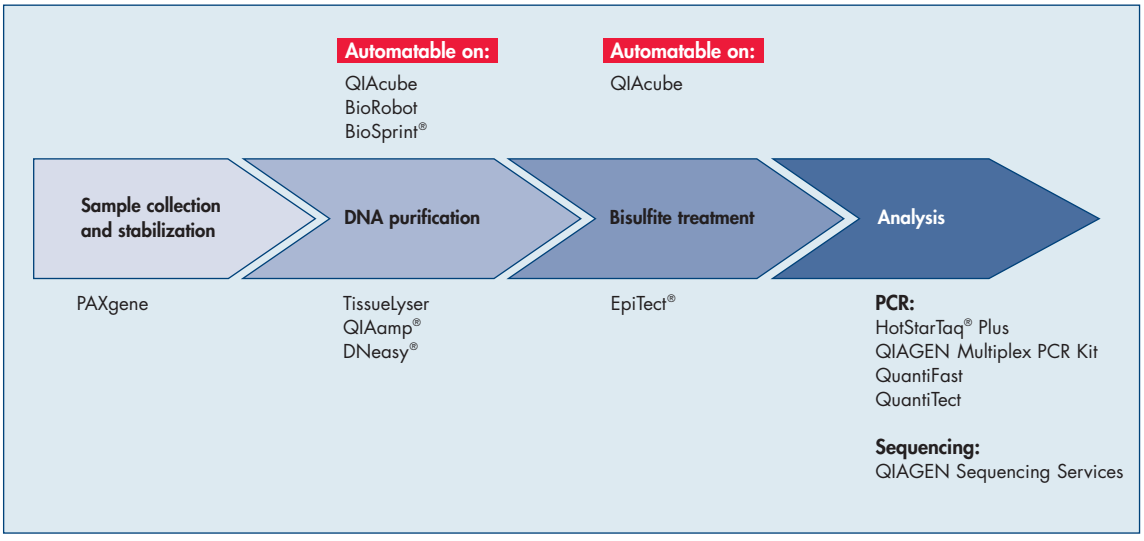

**Figure 7. Detection of multiple miRNAs in Jurkat cells.** Total RNA was prepared from Jurkat cells using the miRNeasy Mini Kit. The miScript System was used for real-time PCR analysis of 328 miRNAs. Of these, 111 miRNAs were expressed.

## DNA methylation



**Innovative QIAGEN solutions for epigenetics include:**

- Purification of high-quality DNA from any sample type, including FFPE tissue sections
- Complete bisulfite conversion and protection of even low DNA amounts (Figures 8 and 9)
- Robust and specific PCR technologies for accurate results




**For more on epigenetics research, visit www.qiagen.com/epigenetics !**

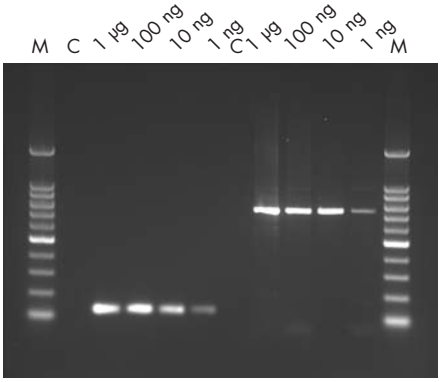



**Figure 8. Amplification of large PCR products from minimal amounts of bisulfite treated DNA, indicating DNA protection.** Human genomic DNA was purified from blood using the QIAamp DNA Blood Mini Kit, and various amounts (1 ng – 1 µg) were converted using the EpiTect Bisulfite Kit, which includes a novel DNA protection solution for bisulfite treatment. PCR was performed using the HotStarTaq *Plus* Master Mix Kit and 2 sets of primers designed to amplify converted DNA. 5 µl of each PCR was loaded onto a 1.3% agarose gel. As little as 1 ng DNA is sufficient for conversion using the EpiTect Bisulfite Kit. **C:** untreated genomic DNA (negative control). **M:** marker.

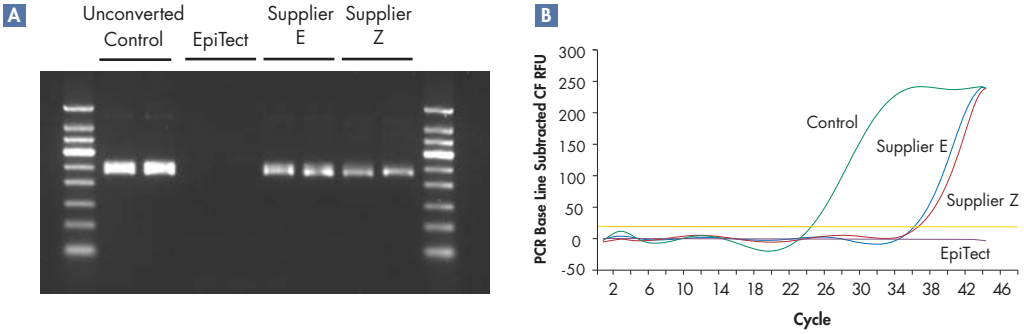



**Figure 9. Highly efficient cytosine conversion, leading to absence of genomic DNA detection.** 1 µg genomic DNA was converted using the EpiTect Bisulfite Kit or bisulfite kits from alternative suppliers (**Supplier E** and **Supplier Z**) according to the manufacturers' instructions. Next, the presence of unconverted DNA in comparable amounts of each sample was determined by **A** end-point PCR using the HotStarTaq *Plus* Master Mix Kit and **B** SYBR Green based real-time PCR using the QuantiTect SYBR Green PCR Kit. The EpiTect Bisulfite Kit treated DNA showed complete conversion while DNA treated using kits from Suppliers E and Z exhibited a significant proportion of unconverted DNA.

## Epigenetics applications

DNA methylation is of interest in many areas of research, including DNA repair, cell cycle control, developmental biology, cancer, and other diseases. Many historical tissue samples are stored in paraffin, and these sample types can prove difficult to analyze using conventional techniques. Using the EpiTect Bisulfite Kit, even FFPE samples can be reliably analyzed in applications such as Pyrosequencing® analysis, providing results that are identical to those from frozen tissue samples (Figure 10).

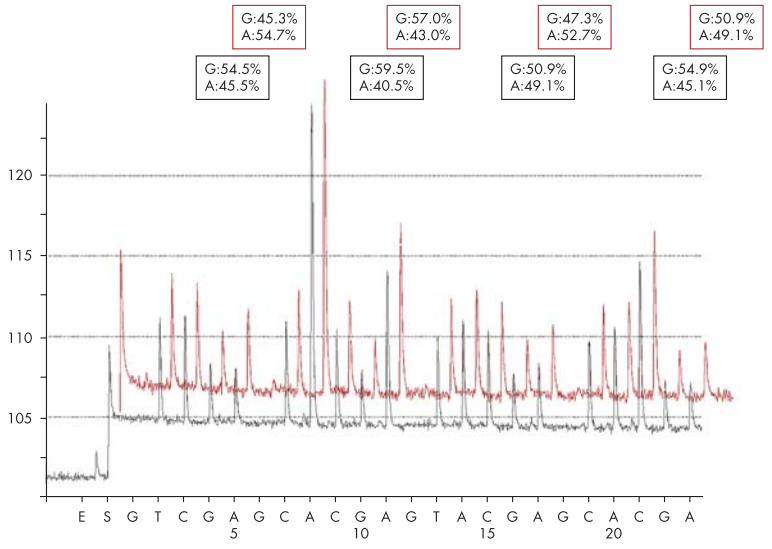



**Figure 10. Comparable Pyrosequencing results from frozen and FFPE tissue samples.** Pyrograms were obtained from matched frozen (black) and FFPE (red) tissues. Virtually identical results were obtained, opening up the possibility to accurately investigate methylation of the MGMT (O-6-methylguanine-DNA methyltransferase) gene in archival tissues. (Data kindly provided by Dr. Thomas Mikeska, Department of Neuropathology, University of Bonn, Germany.)

## More Web links

### General

- GeneGlobe Web portal (real-time RT-PCR assays and siRNAs) www.qiagen.com/GeneGlobe
- Literature, journal references, tips and tools, and more www.qiagen.com/support
- QIAGEN ProductFinder www.qiagen.com/ProductFinder

### Gene expression analysis

- SYBR Green based real-time RT-PCR www.qiagen.com/SYBRGreen
- Multiplex, real-time RT-PCR www.qiagen.com/multiplex
- Fast, real-time RT-PCR www.qiagen.com/fastPCR
- Real-time RT-PCR without RNA purification www.qiagen.com/FastLane
- Critical Factors for Successful Real-Time PCR [PDF] www.qiagen.com/criticalfactors
- Critical Factors for Success in Real-Time, Multiplex PCR [PDF] www.qiagen.com/criticalfactorsMP

### RNAi

- Transfection cell database www.qiagen.com/TransfectionTools
- High-Throughout RNAi User Forum www.qiagen.com/htRNAi

### Automated solutions

- Automation products page www.qiagen.com/automation
- QIAcube www.qiagen.com/myQIAcube

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**Canada** ▪ Orders 800-572-9613 ▪ Fax 800-713-5951 ▪ Technical 800-DNA-PREP (800-362-7737)

**China** ▪ Orders 021-51345678 ▪ Fax 021-51342500 ▪ Technical 021-51345678

**Denmark** ▪ Orders 80-885945 ▪ Fax 80-885944 ▪ Technical 80-885942

**Finland** ▪ Orders 0800-914416 ▪ Fax 0800-914415 ▪ Technical 0800-914413

**France** ▪ Orders 01-60-920-920 ▪ Fax 01-60-920-925 ▪ Technical 01-60-920-930

**Germany** ▪ Orders 02103-29-12000 ▪ Fax 02103-29-22000 ▪ Technical 02103-29-12400

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