QIAGEN News
Innovation Working for You
3
June
2002

New Fully automated, high-throughput RNA isolation using the RNeasy® 96 BioRobot® 8000 Kit

The new RNeasy® 96 BioRobot® 8000 System provides a standardized, reliable method for isolation of high-quality RNA from large numbers of samples. Sample sizes range from 1 to 5 x 10^5 cells. The system provides fast and reproducible total RNA purification for high-throughput gene expression profiling using sensitive applications such as quantitative real-time RT-PCR. Variations are low throughout the entire purification process, giving TaqMan® threshold-cycle values with a coefficient of variation (CV) less than 3%.

The RNeasy 96 BioRobot 8000 System provides:

- **Walkaway automation** of front-end tasks in gene expression analysis
- **High-throughput purification and reaction setup** of 96 samples in under 90 minutes on a single workstation
- **Reproducible yields** of high quality RNA for reliable results
- **Cross-contamination–free processing** for the most sensitive applications

▶▶ Fully automated RNA isolation, page 10

New MinElute™ 96 UF PCR Purification Kit — high-throughput PCR purification for microarray analysis and sequencing

The new MinElute™ 96 UF PCR Purification Kit provides fast, high-throughput PCR purification, in a manual or fully automated procedure. The advanced ultrafiltration plates in the kit are designed to allow final elution volumes as low as 20 µl, delivering highly concentrated, purified DNA.

MinElute 96 UF PCR Purification Kits offer:

- **Elution volumes reduced to a minimum** — high DNA concentrations in as little as 20 µl
- **Fast procedure** — allowing high throughput
- **Fully automatable** — walkaway processing on the BioRobot® workstations and other automated systems
- **High, reproducible recovery** — 90% for fragments larger than 100 bp
- **Cost-effective** — well suited for high-throughput projects

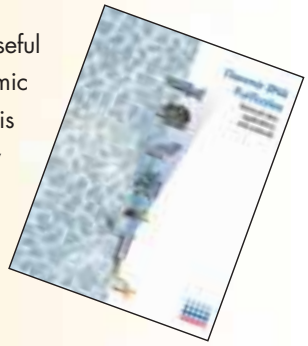
▶▶ High-throughput PCR purification, page 8

QIAGEN

What's New?

- QIAGEN provides all you need for **highly sensitive DNA array analysis** — from sample preparation to oligos and enzymes to make probes for self-spotted arrays — as well as customized chips and **two new, highly sensitive detection technologies**. Ask about our **new DNA microarray systems** for sensitive and reliable detection of the true gene expression pattern without bias due to amplification.

- All you need for success in molecular diagnostics! The new **BioRobot® MDx** will be available shortly. This instrument is designed for fully automated analysis of nucleic acids for clinical applications and offers a number of advantages over earlier models (see page 16 for further details).



- The QIAGEN® ***Genomic DNA Purification*** brochure provides useful information and detailed protocols for researchers isolating genomic DNA from a wide variety of sample sources. Detailed information is presented on current isolation methods and molecular biology applications. The guide offers help on maximizing DNA yield, purity and performance in downstream applications. To order your free copy, contact QIAGEN Technical Services or your local distributor, or visit **www.qiagen.com**.

- New **RNA*later*™ TissueProtect Tubes** provide RNA*later* RNA Stabilization Reagent in easy-to-handle, reclosable tubes for immediate RNA stabilization at sample collection. Simply excise the tissue sample and place it in the tube for storage. RNA*later* TissueProtect Tubes will be available soon; please inquire.

- We welcome your feedback on *QIAGEN News*. Please send your comments or suggestions to **news.editor@qiagen.com**.

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New

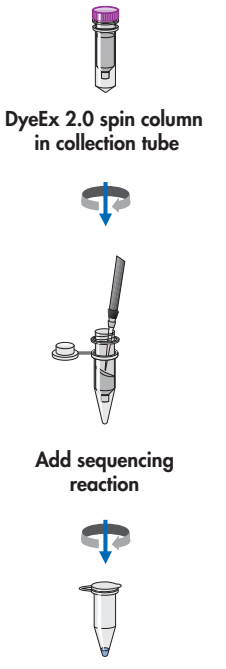
DyeEx™ 2.0 Spin Kits — added convenience for fast, efficient dye-terminator removal from sequencing reactions

QIAGEN introduces DyeEx™ 2.0 Spin Kits, for fast and efficient removal of all unincorporated dye terminators, including BigDye™ Terminators v 3.0 from sequencing reactions. In addition to the fast and easy microspin format, DyeEx 2.0 Spin Kits bring a number of new advantages to the well-established gel-filtration technology of the DyeEx System.

DyeEx 2.0 Spin Kits offer:

- **Easy handling** — a clear plastic tube and higher gel bed for accurate sample loading
- **Added convenience** — easy-to-snap-off bottom closure
- **High performance** — optimized for efficient removal of all unincorporated dye terminators, including BigDye Terminators v 3.0
- **Fast procedure** — 7 minute protocol

DyeEx 2.0 Spin Procedure



DyeEx 2.0 spin column in collection tube

Add sequencing reaction

Purified sequencing reaction

The DyeEx 2.0 procedure is based on gel-filtration chromatography, which separates molecules according to their molecular weight. DyeEx 2.0 Spin Kits use gel-filtration material consisting of spheres with uniform pore sizes. When sequencing reactions are applied onto DyeEx 2.0 spin columns, impurities such as dye terminators and salts diffuse into the pores and are retained in the gel-filtration material while DNA fragments, which are too large to enter the pores, are eluted in the flow-through.

Fast and easy procedure

DyeEx 2.0 spin columns use prehydrated gel-filtration technology to combine efficient dye-terminator removal with fast and convenient handling. In contrast to other commonly used dye-terminator removal methods, DyeEx 2.0 Spin Kits require no hydration, precipitation, or multiple pipetting steps — only two quick centrifugations are needed to purify the sequencing reaction. In contrast, sequencing reaction cleanup by ethanol precipitation is very time-consuming and inefficient (Table 1). Incomplete removal of dye terminators may lead to the appearance of dye-blobs in sequencing data, making stretches of sequence unreadable. Using DyeEx 2.0 Spin Kits to clean up sequencing reactions ensures that reactions loaded onto sequencing instruments are free of dye terminators. Without dye-blobs the sequence is easily readable throughout. ▶

Table 1. Comparison of the DyeEx 2.0 Spin procedure with ethanol precipitation

	DyeEx 2.0 Spin procedure	Ethanol precipitation
Time required (12 samples)	10 minutes	≥45 minutes
Handling	Ready-to-use microspin format	Multiple pipetting steps
Sequence quality	++	+

Table 2. DyeEx 2.0 Spin Kit specifications

Specification	DyeEx 2.0 Spin Kit
Sample volume:	10–20 µl
Dye terminators removed	
◆ BigDye (including all BigDye Terminators v 3.0)	✔
◆ dRhodamine	✔
◆ Rhodamine	✔
◆ DYEnamic ET	✔
◆ WellRED	✔
Compatible DNA sequencers	
◆ ABI PRISM® 377, 373, 310, 3100, 3700	✔
◆ MegaBACE™ 1000	✔
◆ CEQ 2000	✔

Remove any dye terminator

DyeEx 2.0 Spin Kits are suitable for removal of any unincorporated dye terminator molecules. All BigDye, dRhodamine and ET terminators are efficiently removed to yield pure DNA products (Table 2 and Figure 1).

DyeEx product line

New DyeEx 2.0 Spin Kits belong to the QIAGEN® DyeEx product line, which also includes the DyeEx 96 Kit. The DyeEx 96 Kit allows purification of up to 96 sequencing reactions in parallel, and is also suitable for the removal of all unincorporated dye terminators, including BigDye Terminators v 3.0.

Summary

The new DyeEx 2.0 Spin Kit combines easy handling and added convenience with fast and efficient removal of all unincorporated dye terminators from sequencing reactions. ■

High-Quality Sequencing Following DyeEx Cleanup

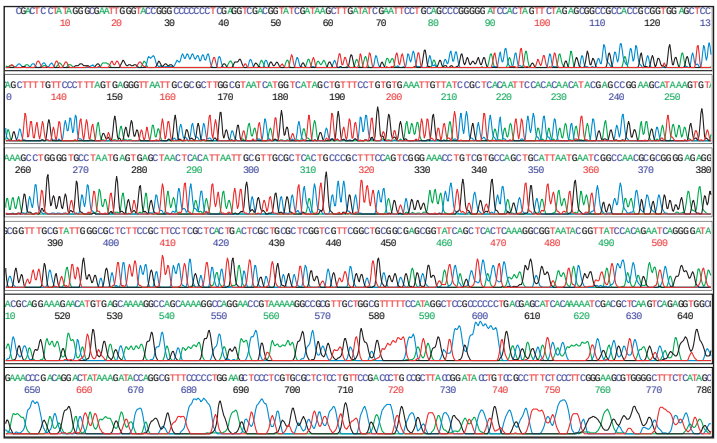


Figure 1 *Sequence of a 3 kb plasmid sequenced using the ABI PRISM BigDye v 3.0 Cycle Terminator Sequence Kit and purified using the DyeEx 2.0 Spin Kit.*

Ordering Information

Product	Contents	Cat. No.
DyeEx 2.0 Spin Kit (50)	50 DyeEx 2.0 Spin Columns, Collection Tubes (2 ml)	63204
DyeEx 2.0 Spin Kit (250)	250 DyeEx 2.0 Spin Columns, Collection Tubes (2 ml)	63206
Related products		
DyeEx 96 Kit (4)	4 DyeEx 96 Plates, 48-Well	63181
DyeEx 96 Kit (24)	24 DyeEx 96 Plates, 48-Well	63183

Increasing efficiency of cloning PCR products

Ralf Peist, Dorothee Honsel, Thea Rütjes, and Dirk Löffert
QIAGEN GmbH, Hilden, Germany

UA-based cloning is widely used for fast, easy, and highly efficient cloning of PCR products. The extra A added by *Taq* DNA polymerase hybridizes to the complementary U of the pDrive Cloning Vector, included in QIAGEN® PCR Cloning Kits. The PCR product is efficiently ligated in the vector without the need for complicated primer design or time-consuming restriction digestion to generate sticky or blunt ends.

In rare cases, the cloning efficiency of certain PCR products can be low for no apparent reason. Here, we demonstrate that the 5'-terminal base of the PCR primer can significantly affect the A-addition activity of *Taq* DNA polymerase. Sometimes only a small fraction of PCR products contain the extra A, leading to low cloning efficiency. We provide simple guidelines to increase the cloning efficiency of these difficult-to-clone PCR products.

Materials and methods

GeneScan® analysis to determine length of PCR products

Plasmid DNA was isolated using the QIAprep® Spin Miniprep Kit. PCR was carried out using a non-proofreading DNA polymerase with primers specific for 112 bp, 213 bp, or 363 bp fragments of the pTZ19 R plasmid. Each PCR product was amplified in 4 separate reactions, using a fluorescently labeled forward primer and one of 4 reverse primers, identical except for a different base (A, C, G, or T) at the 5' terminus. The PCR products were analyzed on an ABI PRISM® 377 Sequencer, and the size of each product was determined using ABI™ GeneScan Analysis Software.

Analysis of cloning efficiency

Mouse and human genomic DNA were isolated using the DNeasy® Tissue Kit and the QIAamp® DNA Blood Mini Kit, respectively. PCR was carried out using QIAGEN *Taq* DNA Polymerase or HotStarTaq™ DNA Polymerase with primers specific for a 500 bp fragment of the murine p53 gene, a 750 bp fragment of the human prion protein gene, or a 1000 bp fragment of the human interleukin 9 gene. Each PCR product was amplified in 4 separate reactions, using one of 4 pairs of primers, identical except for a different base (A, C, G, or T) at the 5' terminus of both primers (Table 1).

Table 1. Combinations of primers used to generate a 1000 bp fragment of the human interleukin 9 gene

PCR fragment	Forward primer	Reverse primerA
A	5'-ACTC…TGC-3'	5'-ACGC…TGT-3'
C	5'-CCTC…TGC-3'	5'-CCGC…TGT-3'
G	5'-GCTC…TGC-3'	5'-GCGC…TGT-3'
T	5'-TCTC…TGC-3'	5'-TCGC…TGT-3'

The PCR products were cloned into the pDrive Cloning Vector using the QIAGEN PCR Cloning*plus* Kit. Numbers of colonies were normalized to the number obtained when both primers contained a 5'-terminal A. ▶

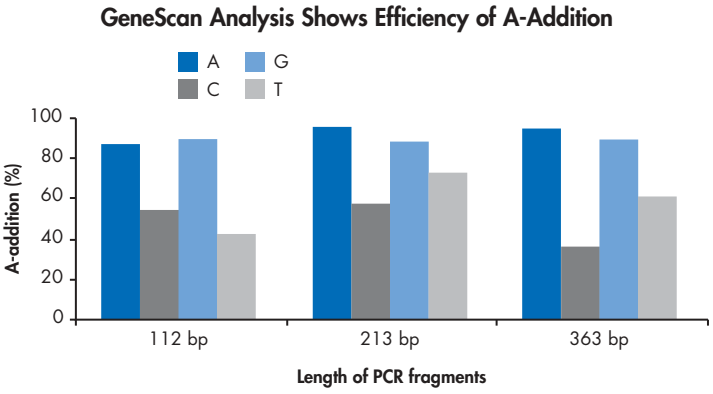


Figure 1 The effect of the primer's 5' terminus on A-addition was investigated using 3 different PCR products. Efficiency of A-addition was determined by analyzing the PCR product length on an ABI PRISM 377 Sequencer. Absolute percentages of A addition for each fragment are shown.

Results and discussion

Effect of primer's 5'-terminal base on A-addition

Non-proofreading DNA polymerases, such as *Taq* DNA polymerase, add an extra A to the 3' end of PCR products, which is not encoded in the template. In order to investigate the effect of the primer's 5'-terminal base on A-addition, we compared the size of 3 different PCR products on an ABI PRISM 377 Sequencer, which can resolve single-base differences in product size (Figure 1). The highest efficiency for adding a single A occurred when using a primer with a 5'-terminal A. Using a primer with a 5'-terminal G, A-addition efficiency was high, but longer reaction times led to addition of two extra As, which could affect subsequent cloning efficiency (data not shown).

Cloning Efficiencies with Different 5' Ends of Primers

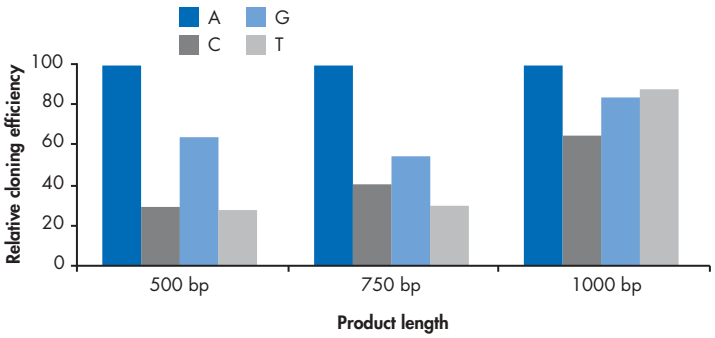


Figure 2 *The effect on the cloning efficiency for primers with different 5' ends was investigated using 3 different PCR products. Each of the 4 reverse primers was identical except for a different base (A, C, G, or T, as indicated) at the 5' terminus. The PCR products were cloned into the pDrive Cloning Vector using the QIAGEN PCR Cloning^{plus} Kit. Numbers of colonies were normalized to the number obtained when both primers contained a 5'-terminal A.*

Effect of primer's 5'-terminal base and ligation time on PCR cloning

Since the primer's 5'-terminal base affected the efficiency of A-addition, we then investigated how this affects subsequent cloning of the PCR product. PCR products were amplified using 4 different pairs of primers, differing only in their 5'-terminal bases. The cloning efficiency correlated with the efficiency of single-A addition; PCR using primers with 5'-terminal As led to the highest number of insert-bearing colonies (Figure 2). These results were confirmed by cloning experiments with different PCR fragments (data not shown). Therefore, primers should be designed so as to contain an A at their 5' ends whenever possible.

If it is not possible or desirable to include a primer 5'-terminal A, ligation times can be increased to compensate for the lower cloning efficiency. Ligation for 30 minutes provided efficient cloning of PCR products generated using a primer with a 5'-terminal A. Increasing the ligation time to 2 hours significantly improved the cloning efficiency of PCR products generated using a primer with a 5'-terminal C (Figure 3).

Effect of Ligation Time on Cloning Efficiency

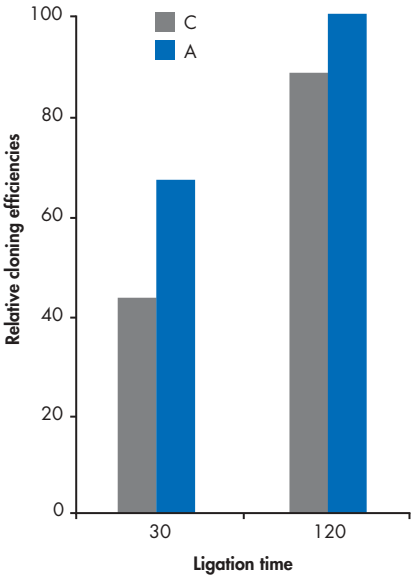


Figure 3 *The effect of ligation time on cloning efficiency was determined for two 1000 bp PCR fragments, generated using primers containing either 5'-terminal Cs or 5'-terminal As. The PCR products were cloned using the QIAGEN PCR Cloning^{plus} Kit with the indicated ligation times. Numbers of colonies were normalized to the number obtained using primers containing a 5'-terminal A and a 120-minute ligation time.*

Conclusions

- QIAGEN PCR Cloning Kits provide a simple and efficient method for direct cloning of PCR products in just 40 minutes, from PCR product to plated cells.
- In rare cases, cloning efficiency is unexpectedly low. The efficiency can be significantly improved by using primers containing an A at their 5' terminus. This is due to the increased efficiency of A-addition by *Taq* DNA Polymerase when using these primers.
- If including a primer 5'-terminal A is not feasible, moderately increasing the ligation time can significantly improve the cloning efficiency. ■

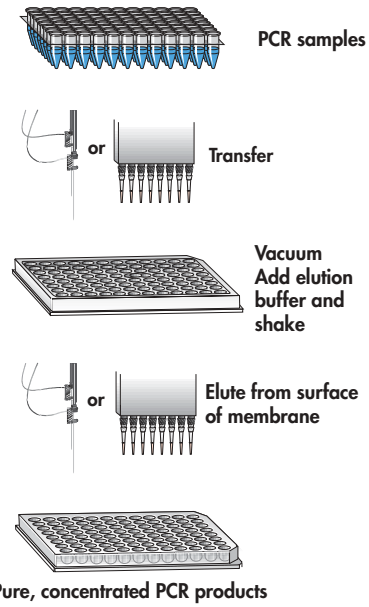
Ordering Information

Product	Contents	Cat. No.
QIAGEN PCR Cloning Kits — for direct cloning of PCR products generated by *Taq* and other non-proofreading DNA polymerases		
QIAGEN PCR Cloning Kit (10)*	For 10 reactions: 2x Ligation Master Mix (50 µl), pDrive Cloning Vector (0.5 µg), distilled water (1.7 ml)	231122
QIAGEN PCR Cloning^{plus} Kit (10)*	For 10 reactions: 2x Ligation Master Mix (50 µl), pDrive Cloning Vector (0.5 µg), distilled water (1.7 ml), QIAGEN EZ Competent Cells (10 tubes, 50 µl each), SOC medium (2 x 1.9 ml)	231222
***Taq* DNA Polymerase — for standard and specialized PCR applications**		
Taq DNA Polymerase (250 U)*	250 units *Taq* DNA Polymerase, 10x PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$	201203
HotStarTaq DNA Polymerase — for highly specific hot-start PCR		
HotStarTaq DNA Polymerase (250 U)*	250 units HotStarTaq DNA Polymerase, 10x PCR Buffer,† 5x Q-Solution, 25 mM $MgCl_2$	203203
QIAprep Spin Miniprep Kit — for isolation of high-purity plasmid DNA for routine molecular biology applications		
QIAprep Spin Miniprep Kit (50)*	For 50 high-purity plasmid minipreps: 50 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27104
DNeasy Tissue Kit — for isolation of genomic DNA from animal tissues and cells, yeast, or bacteria		
DNeasy Tissue Kit (50)*	50 DNeasy Spin Columns, Proteinase K, Buffers, Collection Tubes (2 ml)	69504
QIAamp DNA Blood Mini Kit — for isolation of genomic, mitochondrial, or viral DNA from blood and related body fluids		
QIAamp DNA Blood Mini Kit (50)*	For 50 DNA minipreps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
Related products		
QIAGEN Operon® Oligonucleotide Synthesis Service — high-quality oligos, modified oligos, and longmers		
Oligonucleotide Synthesis Service	Custom-made oligonucleotides up to 100 nt and a wide range of modified oligos, including Molecular Beacons, dual-labeled probes, and many more	Inquire

* *Larger kit sizes available; please inquire*

† *Contains 15 mM $MgCl_2$*

MinElute 96 UF Procedure



96 samples in 18 min (manual procedure)

continued from page 1

Highly concentrated DNA eluates in a high-throughput format

Purified PCR products are often used in downstream applications that require high concentrations of DNA, such as spotting on slides for microarray analysis and sequencing. QIAGEN has designed the first ultrafiltration-plate products that allow final elution volumes as low as 20 µl in the manual procedure or 30 µl on BioRobot workstations. MinElute 96 UF purified DNA fragments are approximately threefold more concentrated than DNA obtained using other commercially available plates based on ultrafiltration, which require elution volumes between 50 and 100 µl (Figure 1). Other commercially available silica-based plate format products typically require elution volumes between 80 and 100 µl. The concentrated DNA obtained in the MinElute 96 UF procedure often eliminates the need for a time-consuming extra concentration step before spotting for microarray analysis and sequencing.

MinElute 96 UF PCR purification procedure

MinElute 96 UF PCR Purification Kits utilize the convenience of multiwell technology for high-throughput purification. PCR products are loaded into the wells of the ultrafiltration plates, and a vacuum is applied. While small molecules such as primers, salts, and unincorporated nucleotides run through the membrane, PCR products ≥100 bp are retained. Purified PCR products are eluted directly from the surface of the membrane in small volumes (as little as 20 µl in the manual procedure), leading to highly concentrated eluates (see flowchart). Water, DMSO, or buffers such as SSC can be used for elution. DNA recoveries of 85–95% are typical (Table 1 and Figure 2).

Highly Concentrated DNA Eluates

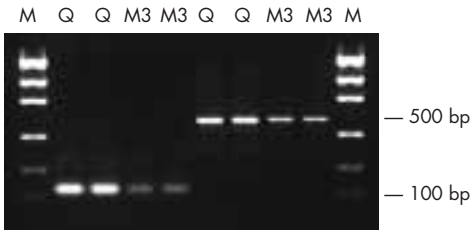


Figure 1 *DNA fragments (100 bp and 500 bp) were purified using either the MinElute 96 UF Kit (20 µl elution volume) or from an ultrafiltration-based kit from Supplier M3 (50 µl elution volume). As examples, two wells were each loaded with 5 µl aliquots of eluate (**M**: markers; **Q**: QIAGEN; **M3**: Supplier M3). MinElute 96 UF-purified 100 bp fragments are threefold more concentrated than fragments from Supplier M3.*

Manual or fully automated procedure

MinElute 96 UF Purification Kits offer two convenient handling options; manual processing using a commercial vacuum manifold, or walkaway processing on the BioRobot workstations and other automated systems.

Summary

The new MinElute 96 UF Purification Kit delivers high-throughput manual or fully automated PCR purification in a 96 well format. The ultrafiltration plates are designed to offer minimal elution volumes and high end-concentrations of high-purity DNA, ensuring optimal results in downstream applications. ■

Table 1. Typical results

DNA recovery for fragments ≥100 bp	85–95%
Reproducibility of recovery	CV ≤5%
Elution volume	20 µl
Volume of eluate	20 µl
Primers (≤20mer)	Removed
DNA binding capacity per well	15 µg
Recommended PCR sample volume	≤150 µl

Table refers to manual procedure. Results may vary if using an automated procedure.

High Reproducibility

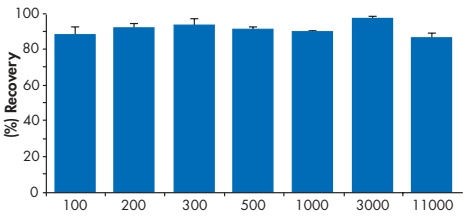


Figure 2 *DNA fragment size (bp) of recovery using the MinElute 96 UF PCR Purification Kit.*

Ordering Information

Product	Contents	Cat. No.
MinElute 96 UF PCR Purification Kit (4)	4 MinElute 96 UF PCR Purification Plates	28051
MinElute 96 UF PCR Purification Kit (24)	24 MinElute 96 UF PCR Purification Plates	28053
QIAvac Multiwell Unit	Vacuum manifold for processing QIAGEN 96-well plates	9014579
Related products		
BioRobot 3000	System includes: custom-designed robotic workstation comprised of 2–4 dilutor units and selected system components, QIAsoft™ 4.1 Operating System, installation and training, 1 year warranty on parts and labor, worktables of various sizes available	900400
BioRobot 8000	System includes: robotic workstation comprised of 2–4 dilutor units and selected system components, variable spacing system, QIAsoft 4.1 Operating System, installation and training, 1 year warranty on parts and labor	900500

Ordering Information

For "6xHis tag minireview: novel applications of QIA*express* technology" (see page 14)

Product	Contents	Cat. No.
Ni-NTA Agarose (25 ml)	25 ml nickel-charged resin (max. pressure: 2.8 psi)	30210
Ni-NTA Agarose (100 ml)	100 ml nickel-charged resin (max. pressure: 2.8 psi)	30230
Ni-NTA Agarose (500 ml)*	500 ml nickel-charged resin (max. pressure: 2.8 psi)	30250
Ni-NTA Superflow (25 ml)	25 ml nickel-charged resin (max. pressure: 140 psi)	30410
Ni-NTA Superflow (100 ml)	100 ml nickel-charged resin (max. pressure: 140 psi)	30430
Ni-NTA Superflow (500 ml)*	500 ml nickel-charged resin (max. pressure: 140 psi)	30450
QIA*express* Type IV Kit†	5 µg each: pQE-30, pQE-31, pQE-32 (N-terminal 6xHis); 10 ml Ni-NTA Agarose	32149
QIA*express* Type ATG Kit†	5 µg each: pQE-60, pQE-70 (C-terminal 6xHis); 10 ml Ni-NTA Agarose	32169
Ni-NTA HisSorb Strips (24)	2 racks of 12 x Ni-NTA–coated 8-well strips in 96-well format	35023
Ni-NTA HisSorb Plates (5)‡	5 Ni-NTA–coated, transparent 96-well plates	35061
Ni-NTA HisSorb Plates, white (5)	5 Ni-NTA–coated, opaque, white 96-well plates	35081

* *Also available in bulk quantities. Please inquire.*

† *Each QIA*expressKit *contains 1µg pREP4, 1 µg control expression plasmid,* E.coli *host strains M15 [pREP4] and SG13009 [pREP4], 5 x 1 ml and 5 x 5 ml bed-volume disposable plastic columns, reagents, and a comprehensive manual, The QIA*expressionist™.

‡ *Please inquire about customized assay products available in minimum orders of 100 plates (allow 6 weeks for delivery). Customized products available include Ni-NTA HisSorb Plates (transparent, white, or black) in 96- and 384-well format, as well as plates coated with any of the QIA*express *Anti·His Antibodies in 96- or 384-well format.*

continued from page 1

Fully automated RNeasy 96 technology and RT-PCR setup on the BioRobot 8000 workstation

In gene expression analysis, the quality of results is only as good as the quality of the RNA purification and reaction setup methods. QIAGEN® automation expertise accelerates high-throughput gene expression projects by combining proven RNeasy silica-gel–membrane chemistry with walkaway automation on the BioRobot 8000. The procedure starts with automated removal of the medium from cells in 96-well cell culture plates. The automated procedure lyses cells directly in the plate and transfers the samples to the wells of the RNeasy 96 plate. RNA binds to the silica-gel membrane, and contaminants are removed using optimized buffers and automated vacuum processing. Highly pure RNA is then eluted in a small volume of RNase-free water to individual collection tubes. The RNA is ready for immediate use in sensitive downstream applications (Figure 1) as well as long-term archiving.

High-Quality RNA for Sensitive Analysis of a Low-Copy Transcript

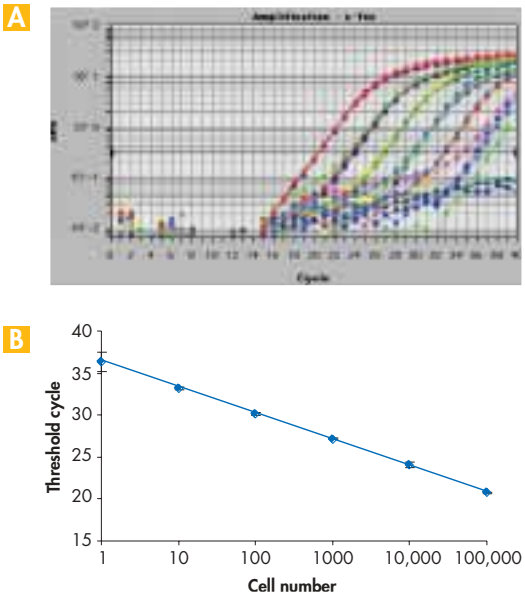


Figure 1 *RNA was isolated from 1 to 1 x 10^5 HeLa cells using the RNeasy 96 BioRobot 8000 procedure. Total RNA was eluted in 100 µl RNase-free water, and 5 µl was used for RT-PCR. Quantitative, real-time, one-step RT-PCR analysis was carried out on an ABI™ Sequence Detection System using the QuantiTect Probe RT-PCR Kit with primers and probe specific for the low-copy c-fos transcript.* **A** *Amplification plot* **B** *C_T values. Error bars represent standard deviation from 8 different samples for each cell number.*

No Cross-Contamination between Wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	18	X	18	X	19	X	18	X	18	X	18	X
B	X	18	X	18	X	18	X	18	X	18	X	18
C	18	X	19	X	19	X	19	X	19	X	19	X
D	X	19	X	19	X	19	X	19	X	19	X	18
E	19	X	19	X	19	X	19	X	19	X	18	X
F	X	18	X	19	X	19	X	19	X	18	X	18
G	18	X	18	X	18	X	18	X	18	X	19	X
H	X	18	X	18	X	18	S	S	S	S	S	S

Figure 2 *Alternating wells of 96-well cell culture plates contained either 5 x 10^5 HeLa S3 cells (**white**) or water (**blue**). RNA purification was carried out for all wells using the RNeasy 96 BioRobot 8000 procedure. Total RNA was eluted in 100 µl RNase-free water, and 5 µl was used for quantitative, real-time RT-PCR on the ABI Sequence Detection System using the QuantiTect Probe RT-PCR Kit with QIAGEN Operon primers and dual-labeled probe specific for the β-actin transcript. C_T values are indicated.* **X**: *No signal was detected after 40 PCR cycles.* **S**: *Standards (not included in the analysis).*

High-throughput quantitative RT-PCR

The RNeasy 96 BioRobot 8000 procedure consistently provides high-quality RNA for sensitive, real-time applications. Following RNA isolation, the same automated system performs reaction setup for quantitative, real-time RT-PCR. Filter-tips prevent cross-contamination (Figure 2). Precision robotic handling provides minimal well-to-well variation and high repeatability. Highly sensitive results are achieved in real-time RT-PCR using the QuantiTect™ Probe RT-PCR Kit with QIAGEN Operon® primers and fluorescent probes (Figure 3).

Automation expertise for successful real-time gene expression analysis

The BioRobot 8000 workstation is optimized for high-throughput RNA purification and RT-PCR setup, making it the instrument of choice for front-end tasks in real-time gene expression analysis projects. The BioRobot 8000 worktable (Figure 4) has the capacity to purify RNA from up to 192 samples. With the 1-plate protocol, the system can carry out both RNA purification and reaction setup in a single run — from living cell cultures in 96-well plates, to RT-PCR mixtures, ready to use in real-time gene expression analysis.

The RNeasy worktable configuration includes the cooling and heating system, which provides temperature control for master-mix solutions containing heat-labile reagents such as primers, enzymes, real-time labels and dyes.

Repeatability of Fully Automated RNA Purification and TaqMan RT-PCR Setup

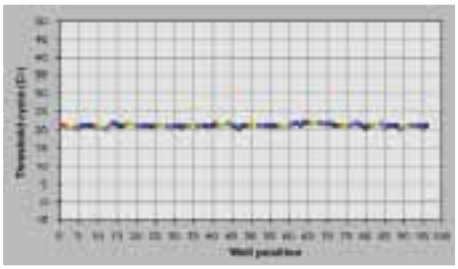


Figure 3 *RNA was isolated from 96 aliquots (5 x 10^4 cells each) of a HeLa S3 cell culture using the RNeasy 96 BioRobot 8000 procedure. Quantitative, real-time, one-step RT-PCR was set up in the same protocol on the BioRobot 8000 workstation, using the QuantiTect Probe RT-PCR Kit with QIAGEN Operon primers and dual-labeled probe specific for the low-copy c-myc transcript. Threshold cycles (C_T) are shown for all 96 samples. The mean C_T was 21.34 ± 0.34 (mean ± standard deviation), representing a CV of 1.6%.*

BioRobot 8000 RNeasy configuration systems and components:

- **Robotic handling system** — allowing walkaway processing using the automated vacuum manifold, which replaces tedious centrifugation steps
- **8-probe pipetting system** — providing precise liquid handling for tasks such as removal of culture media and RT-PCR setup (Figure 5)
- **High-Speed Dispensing System** — providing rapid distribution of buffers during RNA purification for fast processing
- **300 µl and 1100 µl disposable filter-tips** — ensuring cross-contamination–free aspiration and dispensing for reliable results
- **Protective worktable hood** — reducing environmental contamination, important for sensitive RNA applications

Easy-to-use software and QIAGEN certified protocols

QIAsoft™ Operating System software provides ready-to-run QIAGEN protocols for fully automated purification of RNA and subsequent reaction setup. Protocols are easily customized to suit your individual application requirements.

QIAsoft Operating System software enables integration of external instruments, including cooled plate storage and plate sealing instruments, for further walkaway automation of front-end tasks in gene expression analysis.

BioRobot 8000 Worktable for RNeasy 96 Chemistries

Figure 4 *A high-capacity worktable for walkaway processing.*

Precise Liquid-Handling Optimized for Real-Time Analysis

Figure 5 *Cross-contamination–free liquid handling.*

Ordering Information

Product	Contents	Cat. No.
RNeasy 96 BioRobot 8000 Kit — for high-throughput, walkaway RNA isolation on the BioRobot 8000		
RNeasy 96 BioRobot 8000 Kit (12)	For 12 x 96 total RNA preps on the BioRobot 8000: 12 RNeasy 96 Plates, Elution Microtubes CL (1.2 ml), Caps, Square-Well Blocks, RNase-free Reagents and Buffers	967152
BioRobot 8000* — for high-throughput, walkaway nucleic acid purification		
BioRobot 8000	System includes: robotic workstation comprised of 8 dilutor units and selected system components; variable spacing system; QIAsoft 4.1 Operating System; 1 year warranty on parts and labor	900500
Related products		
RNeasy 96 Kit — for high-throughput manual RNA isolation		
RNeasy 96 Kit (4)†	For 4 x 96 total RNA preps: 4 RNeasy 96 Plates, Elution Microtubes CL (1.2 ml), Caps, RNase-free Reagents and Buffers	74181
RNeasy 96 Kit (12)†	For 12 x 96 total RNA preps: 12 RNeasy 96 Plates, Elution Microtubes CL (1.2 ml), Caps, RNase-free Reagents and Buffers	74182
QuantiTect Probe PCR and RT-PCR Kits — for quantitative, real-time PCR and RT-PCR using sequence-specific probes		
QuantiTect Probe RT-PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe RT-PCR Master Mix,‡ 100 µl QuantiTect RT Mix, 2 x 2.0 ml RNase-free water	204443
QuantiTect SYBR® Green RT-PCR Kit — for quantitative, real-time RT-PCR using SYBR Green		
QuantiTect SYBR Green RT-PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect SYBR Green RT-PCR Master Mix,§ 100 µl QuantiTect RT Mix, 2 x 2.0 ml RNase-free water	204243
QIAGEN Operon Oligonucleotide Synthesis Service — high-quality oligos, modified oligos, and longmers		
Oligonucleotide Synthesis Service	Custom-made oligonucleotides and a wide range of modified oligos, including Molecular Beacons, dual-labeled probes, and many more	Inquire

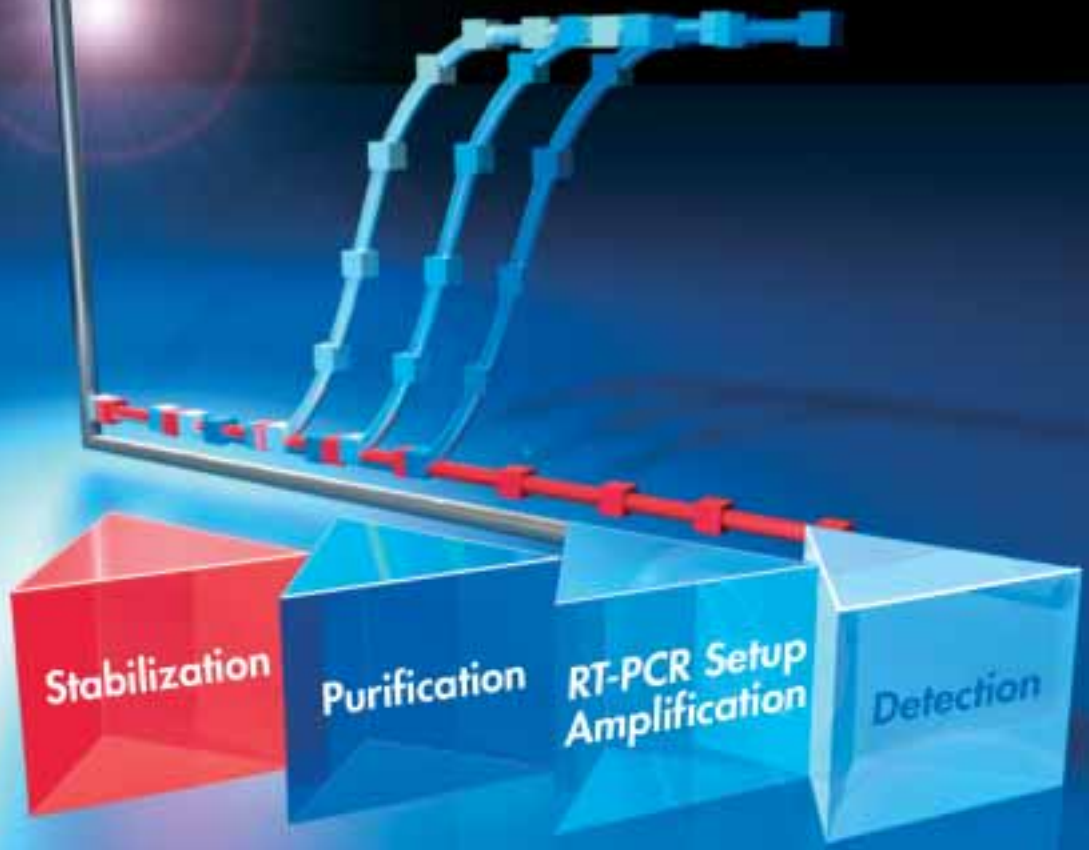
* *QIAGEN Robotic Systems are not available in all countries; please inquire.*

† *Requires use of either QIAvac 96 or the QIAGEN 96-Well-Plate Centrifugation System*

‡ *Contains 8 mM $MgCl_2$*

§ *Contains 5 mM $MgCl_2$*

QIAGEN — All You Need for Success in Real-Time Gene Expression Analysis



Need advanced solutions for real-time gene expression analysis?

QIAGEN has everything you need — from RNA stabilization to automated and manual RNA purification and PCR setup — plus primers, probes, enzymes, and complete kits for quantitative PCR and RT-PCR!

With QIAGEN's complete portfolio of products for real-time gene expression analysis, you can develop your assays faster and with less cost.

Choose QIAGEN for success in real-time gene expression analysis!

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CRTA01 2002SWW

6xHis tag minireview: novel applications of QIA*express*® technology

For over a decade, the QIA*express*® System has been the method of choice for expression, purification, detection, and assay of recombinant proteins. The system is based on the remarkable selectivity of Ni-NTA (nickel-nitrilotriacetic acid) for an affinity tag of 6 consecutive histidine residues (the 6xHis tag). The small size of the 6xHis tag and its neutral charge at physiological pH means that it rarely interferes with protein structure, function, or immunogenicity. In addition to routine applications of the Ni-NTA–6xHis-tag system, researchers have adapted the unique properties of this interaction to a wide variety of protein studies. Here we describe a selection of recent publications that illustrate the usefulness of the Ni-NTA–6xHis-tag interaction for:

- Binding probe molecules in a highly sensitive fluorescent biosensor
- Ordered orientation of cytochrome c oxidase in lipid vesicles
- Localization of a chloroplast protein within a large complex

Sensitive and specific fluorescent detection of activated Ras*

Christian F.W. Becker et al.
Max-Planck-Institut für molekulare Physiologie, Dortmund, Germany

Reversible phosphorylation of the protein Ras·GDP to an activated GTP form plays a central role in eukaryotic cell signaling. Ras mutations that stabilize the activated form lead to a continuous stimulation of downstream signaling factors, cell proliferation, and in some cases, cancer. A biosensor that distinguishes between Ras·GDP and Ras·GTP would therefore be a useful tool in cancer diagnostics and drug screening.

Previous studies have shown that a sequence of 81 amino acids from the Ras·GTP-binding effector molecule c-Raf1 is sufficient for tight Ras binding. In this study, the Ras binding domain (RBD) fragment of c-Raf1 was reconstituted by ligation of two synthetic peptides, one of which contained a fluorescently labeled amino acid close to the Ras binding site. The full-length peptide, which carried a 6xHis tag at its C-terminus, was immobilized on opaque Ni-NTA HisSorb™ Plates. Addition of Ras complexed to a non-hydrolyzable triphosphate group (GppNHp) led to a 20% change in fluorescence intensity. The system was made more sensitive by addition of small amounts of organic solvents to samples. In both cases, addition of Ras·GDP had no effect on fluorescence intensity.

The ability to discriminate between Ras·GDP and Ras·GTP — a differentiation not possible using antibodies due to the small conformational differences between the two forms — demonstrates the usefulness of this biosensor in applications for which a normal ELISA procedure is not possible.

Efficient purification and ordered reconstitution of phospholipid vesicles†

Carrie Hiser, Denise Mills, Michael Schall, and Shelagh Ferguson-Miller
Department of Biochemistry and MSU-NIH Mass Spectrometry Facility, Michigan State University, East Lansing, MI, USA

The final electron acceptor in the respiratory chain of eukaryotes and many prokaryotes is the protein cytochrome c oxidase (CcO). CcO catalyzes the reduction of oxygen to water and uses energy from the reaction to pump protons across the membrane in which it is situated. The pumping mechanism can be studied using native and mutant forms of CcO reconstituted into lipid vesicles.

* *Excerpted from Becker, C.F.W., Hunter, C.L., Seidel, R.P., Kent, S.B., Goody, R.S., and Engelhard, M. (2001) A sensitive fluorescence monitor for the detection of activated Ras: total chemical synthesis of site-specifically labeled Ras binding domain of c-Raf1 immobilized on a surface. Chem. Biol.* ***8****, 243.*

† *Excerpted from Hiser, C., Mills, D.A., Schall, M., and Ferguson-Miller, S. (2001) C-terminal truncation and histidine-tagging of cytochrome c oxidase subunit II reveals the native processing site, shows involvement of the C-terminus in cytochrome c binding, and improves the assay for proton pumping. Biochemistry* ***40****, 1606.*

The very small and rapid external pH changes caused by proton pumping are followed using a pH-sensitive dye. However, measurement of proton pumping across a membrane is especially problematic, as the proteins must first be reconstituted into a lipid bilayer, and measurements taken in the presence of buffering and light-scattering lipid vesicles.

In this study, placing a 6xHis tag at the C-terminus of cytochrome c oxidase (CcO) allowed overexpressed protein to be directly purified from a bacterial membrane fraction on Ni-NTA Agarose. After mixing with lipids, reconstituted cytochrome oxidase-containing phospholipid vesicles (COVs) were purified, again using Ni-NTA Agarose. This method of purification led to a more concentrated sample of vesicles that contained correctly oriented CcO and removal of vesicles that contained no CcO. The increased protein-to-lipid ratio in the Ni-NTA–purifed vesicles allowed increased amounts of oxidase to be used in spectrophotometric measurements of proton pumping. In time-resolved kinetic measurements, significantly higher rates of proton transfer were also observed. This was attributed to a homogeneous, correctly oriented vesicle population and reduced buffering and light-scattering effects from vesicles containing no protein.

This study shows that, in addition to the benefit of efficient protein purification, the 6xHis tag can be used to purify vesicles containing 6xHis-tagged proteins in the correct orientation, facilitating investigations into the respiratory process.

Locating a specific protein in a multisubunit complex: identification of PsbH in PSII*

Claudia Büchel, Edward Morris,
Elena Orlova, and James Barber
Wolfson Laboratories, Department of Biology and Biochemistry, Imperial College of Science, Technology and Medicine, London, UK

Photosystem II (PSII) is a dimeric membrane protein complex containing over 25 subunits per monomer. The complex, which is found in the thylakoid membranes of higher plants, algae, and cyanobacteria, catalyzes the light-driven production of oxygen during photosynthesis. While several three-dimensional models have been derived from electron and X-ray crystallographic analysis, the resolution of the current models does not allow assignment of some small subunits. The identification and location of these small subunits is vital for a full understanding of the structural–functional relationship of the whole complex.

In this study, PSII core dimers were isolated from a green alga that was genetically altered to express a PsbH protein with a 6xHis tag at its N-terminus. This protein of 87 amino acids, about which little is known, forms part of the PSII complex. It is predicted to have one membrane-spanning α-helix close to its C-terminus. To identify the position of PsbH in the multisubunit complex, PSII dimers containing 6xHis-tagged PsbH were isolated from crude algal membrane preparations using Ni-NTA Agarose. Purified protein complexes were then immobilized on carbon-coated electron microscopy grids and labeled using a Ni^{2+}-NTA–gold cluster. The location of the gold-labeled PsbH subunit was identified by statistical analysis of electron micrographs. Comparison of the data obtained in this study and existing electron- and X-ray crystallographic data indicated that the N-terminus of PsbH is close to the two transmembrane helices of cytochrome b_{559}.

This highly flexible and powerful method allows the precise location of subunits in large protein complexes where the resolution of crystallographic analysis is insufficient to identify specific amino acid side chains.

Summary

These studies demonstrate not only the power and flexibility of the QIA*express* System but also the ingenuity of our customers who are constantly discovering new and exciting applications for QIA*express* technology. To learn more about the QIA*express* System, visit us on the web at **www.qiagen.com** or contact QIAGEN Technical Services. ■

For ordering information, see page 9.

** Excerpted from Buchel, C., Morris, E., Orlova, E., and Barber, J. (2001) Localisation of the PsbH subunit in photosystem II: a new approach using labelling of His-tags with a $Ni^{(2+)}$-NTA gold cluster and single particle analysis. J. Mol. Biol.* ***312****, 371.*

Improved performance in automated isolation of viral nucleic acids from plasma

Thomas Hanselle, Michael Otte, Dirk Freystadt, and Frank Krieg-Schneider
QIAGEN GmbH, Hilden, Germany

QIAGEN continuously strives to improve and extend its technologies for automated isolation of viral nucleic acids. In this article we describe the development of current technology and outline recent and upcoming improvements.

QIAamp® 96 Viral RNA BioRobot® Kit

The first kit from QIAGEN for automated viral RNA purification, the QIAamp® 96 Viral RNA BioRobot® Kit, was introduced over 4 years ago for use with the BioRobot 9604. The kit provides generic purification of RNA from a wide range of viruses. It is validated by the Paul-Ehrlich Institute (PEI) in Germany (Federal Agency for Sera and Vaccines) for use in blood safety testing of HCV, when used in combination with the Cobas® Amplicor® v.2.0 HCV kit from Roche Molecular Systems, Inc. An internal control is added to the lysis buffer to monitor the purification process and amplification reaction. An extensive study using the BioRobot 9604 showed a PCR failure rate of less than 0.4% when using the internal control provided with the Amplicor kit. Figure 1 shows performance improvements in successive versions of the kit.

Improved Performance of the QIAamp 96 Viral RNA BioRobot Kit

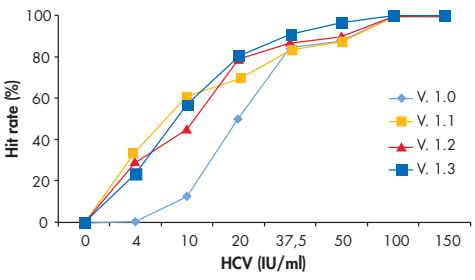


Figure 1 *Comparison of detection of RNA from HCV, using four versions of the QIAamp 96 Viral RNA BioRobot Kit together with the Cobas Amplicor v.2.0 Kit. Version 1.0 (V.1.0) was used for field tests. Version 1.1 (V.1.1) was improved by changes introduced to obtain PEI validation. Version 1.2 (V.1.2) incorporated optimized washing procedures into the protocol. Version 1.3 (V.1.3) achieved excellent performance through improved centrifugation conditions.*

Improved Performance of the QIAamp 96 Virus BioRobot Kit

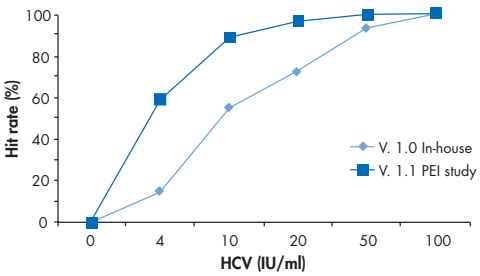


Figure 2 *Comparison of detection of RNA from HCV, using two versions of the QIAamp 96 Virus BioRobot Kit together with the Cobas Amplicor v.2.0 Kit. Version 1.0 (V.1.0) was used for field tests. Version 1.1 (V.1.1) showed excellent performance, achieved by improved centrifugation conditions.*

Since the introduction of the QIAamp 96 Viral RNA BioRobot Kit, additional kits have been developed, featuring a range of improvements. Improvements in HCV purification using the QIAamp 96 Virus BioRobot Kit are shown in Figure 2. Optimized lysis conditions and purification efficiency led to the higher viral detection rates in the current version of the kit.

When an internal control was used in the HCV purification procedure, the PCR failure rate was only 0.1%, demonstrating the high purity of the purified nucleic acid. In further experiments using this kit, plasma samples containing both HCV and HBV were used for purification. Each 50 µl eluate was split into two 25 µl portions. One portion was used to amplify HBV, and the other to amplify HCV. These experiments showed that both DNA (HBV) and RNA (HCV) viruses could be co-purified with similar efficiencies. Furthermore, use of 25 µl eluate volumes per amplification provided the same detection sensitivity as was achieved using 50 µl eluate volumes in the QIAamp 96 Viral RNA BioRobot Kit. The improved sensitivity of the QIAamp 96 Virus BioRobot Kit was further confirmed by hit rate studies (summarized in Table 1).

QIAamp Virus BioRobot 9604 Kit

The QIAamp Virus BioRobot 9604 Kit is the kit most recently developed for viral nucleic acid isolation on the BioRobot 9604. This kit provides a generic purification system for purification of viral RNA or DNA from a wide range of species. The performance of the kit is similar to the QIAamp 96 Virus BioRobot Kit. Two major improvements are included in this kit. Firstly, new QIAamp 96 plates are supplied, made from a softer plastic to minimize the chance of cracking during centrifugation. Secondly, new elution microtubes are used. These are bar-coded and have individually labeled wells to prevent mix-up of samples, are individually packed for contamination-free use, and have a new "pipetting-friendly" design.

HCV purification using the QIAamp Virus BioRobot 9604 Kit has been tested using blood collected in BD Vacutainer™ Brand PPT Plasma Preparation Tubes (PPT tubes). PPT tubes are evacuated tubes for blood collection, containing EDTA as an anticoagulant and a material that on centrifugation forms a barrier between the plasma and the cellular fraction of blood. PPT tubes were used to collect blood that was used for HCV detection. Whole blood samples collected into PPT tubes were spiked with different amounts of PEI HCV standard reference material #75/98, generating a dilution series. After centrifugation of the PPT tubes, viral RNA was isolated from the plasma fraction of whole blood using the QIAamp method. The 95% limit of detection in a Probit analysis was approximately 11 IU/ml of whole blood. This high sensitivity showed that all viral particles were concentrated in the plasma fraction of whole blood, and significant amounts were not retained in the barrier or the cellular fraction. Use of PPT tubes in combination with the QIAamp Virus BioRobot 9604 Kit provides a highly sensitive method for HCV detection.

Coming soon — the new BioRobot MDx!

QIAGEN will launch the BioRobot MDx, designed for fully automated analysis of nucleic acids for clinical applications, in the next few months. This instrument offers a number of advantages over earlier models:

- **Walkaway automation** — newly designed vacuum chamber (patent pending), eliminating manual centrifugation steps
- **Closed system with hood** — no access to the worktable is possible during runs, providing improved user safety and security during runs
- **Complete loading check** — system checks that all components are present before a run begins
- **Easy recognition of modules** — each module is color-coded and unique in shape to prevent mixing up consumables
- **Convenient tip drawer** — users do not need to lean over the worktable to replace tips, improving safety
- **Optimized protocols** — protocols for purification of viral nucleic acids from plasma and serum and purification of genomic DNA from blood available when the instrument is launched
- **Ready-to-use protocols for reaction setup** — using Cobas Amplicor A-rings

Table 1. Improved sensitivity in QIAamp Kits

Kit and version		Probit analyses: 95% Probit values (IU/ml)	Probit analyses: Confidence interval	Probit analyses: Number of replicates per titer
QIAamp 96 Viral RNA BioRobot Kit	V.1.0	61.8	43.7–118.7	24
	V.1.1	57.8	36.2–124.2	48*
	V.1.2	53.5	35.3–111.8	32
	V.1.3	45.1	37.5–56.7	144*
QIAamp 96 Virus BioRobot Kit	V.1.0	23.6	18.8–32.5	144–296*
	V.1.1	19.7	15.4–28.4	104*

Studies were performed using plasma spiked with PEI HCV standard reference material #75/98. For each kit version, RNA from 6 dilutions of the reference material in HCV-negative plasma, ranging from 0–100 IU/ml HCV, were purified on the BioRobot 9604 and amplified using Amplicor Cobas v.2.0 kits. A positive PCR result was scored as a hit. Statistical software was used to determine the HCV titer at which a hit would be scored with a 95% probability. Sensitivity approximately doubled between the QIAamp 96 Viral RNA BioRobot Kit, V.1.3, and the QIAamp 96 Virus BioRobot Kit, V.1.1.

** Results from experiments performed in 3 labs on 3 different instruments*

Conclusions

- QIAGEN systems for viral nucleic acid purification allow highly sensitive viral detection. They are designed to provide a generic sample preparation method for use with detection systems for a wide range of RNA and DNA viruses, and show outstanding performance for HCV, HBV, and HIV.
- High purity DNA and RNA are produced, suitable for use in sensitive downstream applications.
- Continuous monitoring and ongoing research into viral nucleic acid isolation ensures that QIAGEN regularly provides improved kits and instruments. ■

Ordering Information

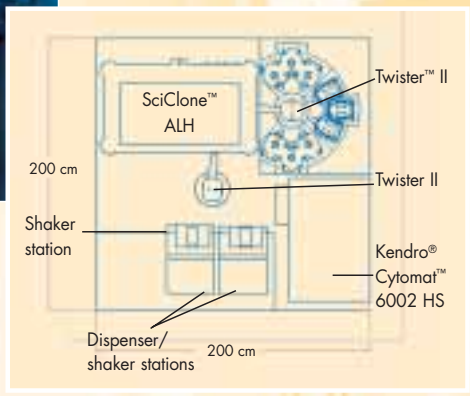
Product	Contents	Cat. No.
QIAamp Virus BioRobot 9604 Kit (12)*	For 12 x 96 nucleic acid preps: 12 QIAamp 96 Plates, RNase-free Buffers, QIAGEN Protease, AirPore Tape Sheets, Tape Pad, S-Blocks, Racks with Elution Microtubes CL, Carrier RNA, Caps	965662
BioRobot 9604†	System includes: robotic workstation with 4 dilutor drives; microprocessor-controlled vacuum pump; vacuum manifold; High-Speed Pipetting System; Tip-Change System; QIAsoft™ 3.0 Operating System, Basic Edition; computer; installation and training; 1 year warranty on parts and labor	900300

* *For research use only. Not for use in diagnostic procedures.*

† *The BioRobot 9604 is not available in all countries. Please inquire.*

Walkaway automation of front-end tasks in genomics projects

The BioRobot® HT Plasmid System performs fully automated plasmid purification and sequencing-reaction setup, allowing overnight processing of up to 60 x 96-well plates. Cost-effective MagAttract® purification chemistries provide highly pure plasmid DNA for accurate sequencing and long read-lengths.



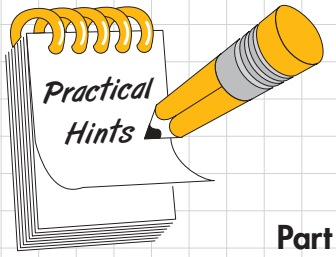
The QIAGEN BioRobot HT Plasmid System delivers:

- **High-quality DNA** — highly pure plasmid DNA for optimal sequencing results
- **Unattended reaction setup** — with purified template in 96- or 384-well formats
- **Overnight operation** — efficient workflow for early completion of projects
- **High throughput** — over 5000 samples purified in a single 16.5 h run
- **Optimized hardware** — designed for MagAttract technology to ensure quick startup and continued success

001

Reference

1. *Freshney, R.I. (1993)* Culture of Animal Cells: a Manual of Basic Technique. *3rd ed. New York: Wiley-Liss.*
2. *Ausubel, F.M. et al., eds. (1991)* Current Protocols in Molecular Biology. *New York: Wiley Interscience.*
3. *Spector, D., Goldman, R.R., and Leinwand, L.A., eds. (1998)* Cells: a Laboratory Manual. *Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.*



The QIAGEN Guide to Animal Cell Culture

Part IV: Essential protocols for animal cell culture

Welcome to the next of our series of articles aimed at providing useful hints for culturing animal cells. This article contains useful protocols for animal cell culture. The series will continue in future issues of *QIAGEN News*, followed by hints for successful transfection.

Maintaining cell cultures

Establishment and maintenance of animal cell cultures require standardized approaches for media preparation, feeding, and passaging (or subculturing) of the cells. Cultures should be examined regularly to check for signs of contamination and to determine if the culture needs feeding or passaging.

The cell culture protocols below have been adapted from the following sources: *Culture of Animal Cells; a Manual of Basic Technique* (1), *Current Protocols in Molecular Biology* (2), and *Cells: A Laboratory Manual* (3). These protocols are examples of methods for general cell culture, and have not been rigorously validated and optimized by QIAGEN. There are many alternative protocols in current use.

IMPORTANT: Potentially biohazardous materials (e.g., cells, culture medium, etc.) should be sterilized before disposal, and disposed of according to your institution's guidelines.

Cell thawing

1. Heat a water bath to 37°C, and warm the growth medium into which the cells will be plated.

2. Add prewarmed growth medium to an appropriately sized cell culture vessel.

3. Remove a vial of frozen cells from liquid nitrogen, and place in the water bath until thawed.

IMPORTANT: Wear protective goggles and gloves when thawing vials that have been stored in liquid nitrogen. Vials may explode when removed from liquid nitrogen.

IMPORTANT: Proceed to step 4 as soon as the cells have thawed. Do not allow the cells to warm up before transferring them into growth medium.

4. Wash the outside of the vial with 70% ethanol or another suitable disinfectant.

5. Slowly pipet the thawed cell suspension into the cell culture vessel containing prewarmed growth medium. Swirl the vessel gently to mix the cells with the medium.

Tip Immediate removal of DMSO may sometimes be necessary, especially for suspension cells, primary cells, and sensitive cell types. For such cell types, pipet the thawed cell suspension into a sterile centrifuge tube containing prewarmed medium, centrifuge at 200 x *g* for 2 min, aspirate the supernatant, resuspend the cells in fresh growth medium, and then transfer to an appropriate cell culture vessel.

IMPORTANT: Thoroughly mix the cells in the cell culture vessel to ensure even distribution of the cells throughout the vessel.

6. Incubate cells overnight under their usual growth conditions.

7. The next day, replace the growth medium

Trypsinizing cells

Trypsinization is a technique that uses the proteolytic enzyme trypsin to detach adherent cells from the surface of a cell culture vessel. This procedure is performed whenever the cells need to be harvested (e.g., for passaging, counting, or for nucleic acid isolation).

1. Aspirate the medium and discard.

2. Wash cells with PBS or HBSS (see Table 1), aspirate, and discard. Repeat.

Tip The volume of PBS or HBSS should be approximately the same as the volume of medium used for culturing the cells.

3. Add enough warmed 1x trypsin–EDTA solution (see Table 1) to cover the monolayer, and rock the flask/dish 4–5 times to coat the monolayer.

4. Place the flask/dish in a CO_2 incubator at 37°C for 1–2 min.

5. Remove flask/dish from incubator and firmly tap the side of the flask/dish with palm of hand to assist detachment.

Tip If cells have not dislodged, return the flask/dish to the incubator for a few more minutes.

IMPORTANT: Do not leave cells in 1x trypsin–EDTA solution for extended periods of time. Do not force the cells to detach before they are ready to do so, or clumping may occur. ▶

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Tip Overly confluent cultures, senescent cells, and some cell lines may be difficult to trypsinize. While increasing the time of trypsin exposure may help to dislodge resistant cells, some cell types are very sensitive to trypsin and extended exposure may result in cell death. In addition, some cell lines will resist this treatment and will produce cell clumps.

6. Once dislodged, resuspend the cells in growth medium containing serum.

Tip Use medium containing the same percentage of serum as used for growing the cells. The serum inactivates trypsin activity.

7. Gently pipet the cells up and down in a syringe with a needle attached to disrupt cell clumps.

Tip If pipetted too vigorously, the cells will become damaged. Ensure that pipetting does not create foam.

8. Proceed as required (e.g., with passaging, freezing, nucleic acid isolation, etc).

Table 1. Composition of solutions for animal cell culture protocols

Solution	Composition and storage
1x PBS (phosphate-buffered saline)	137 mM NaCl; 2.7 mM KCl; 4.3 mM Na_2HPO_4; 1.47 mM KH_2PO_4 The pH should be 7.4 without adjustment. Store at room temperature.
1x HBSS (Hanks balanced salt solution)	5 mM KCl; 0.3 mM KH_2PO_4; 138 mM NaCl; 4 mM $NaHCO_3$; 0.3 mM Na_2HPO_4; 5.6 mM D-glucose The pH should be 7.4 without adjustment. Store at room temperature.
1x trypsin–EDTA solution	0.05% (w/v) trypsin; 0.53 mM EDTA; Dissolve trypsin and EDTA in a calcium- and magnesium-free salt solution such as 1x PBS or 1x HBSS.*

** Store 1x trypsin-EDTA solution at –20°C. Small aliquots can be stored at 2–8°C for 1–2 weeks. Work quickly when using trypsin during cell culture, since trypsin degrades and enzymatic activity declines at 37°C.*

Passaging cells

Many adherent cell cultures will cease proliferating once they become confluent (i.e., when they completely cover the surface of cell culture vessel), and some will die if they are left in a confluent state for too long. Adherent cell cultures therefore need to be routinely passaged, that is, once the cells are confluent, a fraction of the cells need to be transferred to a new cell culture vessel. Suspension cells will exhaust their culture medium very quickly once the cell density becomes too high, so these cultures similarly require regular passaging.

IMPORTANT: Although regular passaging is necessary to maintain animal cell cultures, the procedure is relatively stressful for adherent cells as they must be trypsinized. We do not recommend passaging adherent cell cultures more than once every 48 h.

1. Harvest the cells, either by trypsinization (adherent cell cultures) or by centrifugation at 200 x *g* for 5 min (suspension cell cultures). Resuspend the cells in an appropriate volume of prewarmed growth medium containing serum.

Tip The volume of medium used to resuspend the cells depends on the split ratio required (see step 2) and the size of the cell culture vessel. If too small a volume is used, it may be difficult to accurately pipet the desired volume to the new culture vessel. Conversely, if too large a volume is used, the culture vessel may be too full following transfer of the cells.

Tip Removal of trypsin may sometimes be necessary following harvesting of adherent cells, especially for primary and sensitive cell types. Centrifuge the cells at 200 x *g* for 5 min, carefully aspirate the supernatant, and resuspend the cells in an appropriate volume of prewarmed medium containing serum.

2. Transfer an appropriate volume of the resuspended cells to a fresh cell culture vessel containing prewarmed growth medium. Swirl the vessel gently to mix the cells with the medium.

IMPORTANT: Thoroughly mix the cells in the cell culture vessel to ensure even distribution of cells.

IMPORTANT: Some cell types will not survive if too few cells are transferred. We do not recommend high split ratios for primary cells, sensitive cell types, or senescent cultures.

Tip For adherent cells, we recommend adding enough cells so that the culture takes approximately one week to reach confluence again. This minimizes the number of times the cells are trypsinized as well as the handling time required to maintain the culture.

Tip When determining how many cells to transfer to the new cell culture vessel, it can be helpful to think in terms of how many cell divisions will be required for the culture to reach confluence again. For example, if half the cells are transferred, then it will take the culture one cell division to reach confluency again; if a quarter of the cells are transferred then it will take 2 cell divisions, and so on. If a culture divides once every 30 h or so, then in one week it will undergo approximately 5 cell divisions. A split ratio of 1:32 ($1:2^5$) should therefore be appropriate for the cells to reach confluency in about one week. In step 1, resuspend the cells in 8 ml medium, and transfer 0.25 ml to the new cell culture vessel.

3. Incubate cells under their usual growth conditions.

The QIAGEN Guide to Animal Cell Culture will continue in future issues of *QIAGEN News*. If there is any other information you would like to see on these pages of *QIAGEN News*, please let us know by calling QIAGEN Technical Services or your local distributor.

QIA-Hints

QIAGEN Technical Services are always available to answer your questions!

DNA isolation

Q What kit can I use to isolate DNA from food products to test for genetically modified organisms (GMOs) or for other types of analysis?

A That depends on the type of food to be analyzed. For plant-based foods, such as soy, tofu, and cookies, DNA isolation has been successfully carried out using the DNeasy® Plant Mini Kit. The QIAamp® DNA Stool Mini Kit has been used for isolation of genomic DNA from highly processed foods and foods that contain high levels of PCR inhibitors, such as chocolate. For meat and processed meats, such as sausage, we recommend the DNeasy Tissue Kit.

These guidelines should help you choose what kit to use for different types of food products. See also the article "Detection of genetically modified soybean and maize in raw and processed foodstuffs" and the accompanying editorial in the last issue of *QIAGEN News* (2002 No. 2, pages 14–18)

RNA isolation

Q I would like to isolate RNA from small numbers of cells. Can I use the RNeasy® Mini Kit or RNeasy 96 Kits with fewer than 100 cells?

A Yes. The RNeasy Mini Kit and RNeasy 96 Kits have been used successfully to isolate RNA from fewer than 10 cells, and even from single cells. We recommend adding 20 ng of carrier RNA to the cell lysate before loading it onto the RNeasy membrane. The carrier RNA will co-purify with the RNA from the cells, so make sure that it won't interfere with any downstream analyses, such as RT-PCR.

Nearly any carrier RNA can be used, except for tRNA and other RNAs <200 nt. These small RNAs will not bind to the RNeasy membrane. We commonly use poly(C) as a carrier (e.g., from Amersham Biosciences, cat. no. 27-4220-02).

Protein expression

Q I am having difficulty making a bacterial expression construct that encodes a highly toxic gene product. What should I do?

A It is often difficult to propagate plasmids that express toxic gene products in *E. coli*. "Leaky" expression of extremely toxic gene products can kill bacteria and create selective pressure for bacteria harboring mutated non-expressing plasmids, especially in early growth or after transformation. Therefore, for expression of very toxic proteins, a higher level of *lac* repressor may be required.

We recommend using the pQE-80L series of expression vectors in the M15[pREP4] host strain. The pQE-80L series of vectors carry the *lacIq* repressor gene, and the *E. coli* strain M15[pREP4] carries the pREP4 plasmid, which constitutively express the *lac* repressor at high levels in cis and trans, respectively.

This combination of two repressor modules results in highly efficient suppression of recombinant protein expression prior to induction and gives the best chance of successful expression of highly toxic proteins.

Please do not hesitate to call your local QIAGEN Technical Service Department if you have any questions or require further information regarding any QIAGEN products.

QIAGEN® Challenge 2002 — congratulations to this year's winners!

Our thanks to everyone who entered this year's QIAGEN® Challenge, which accompanied the QIAGEN Product Guide 2002. The Grand Prize winners for all participating countries are listed below.

In the USA:

- Karen Marquardt, University of Wisconsin, Madison, WI
- Apostolos Angelidis, University of California, Davis, CA
- Ronald Somerville, Purdue University, West Lafayette, IN

In Australia:

- Shauna Brown, Australian Wine Research Institute, Urrbrae
- Kathryn Hall, The Australian Museum, Sydney
- Naomi Komadina, WHO Influenza Centre, Victoria

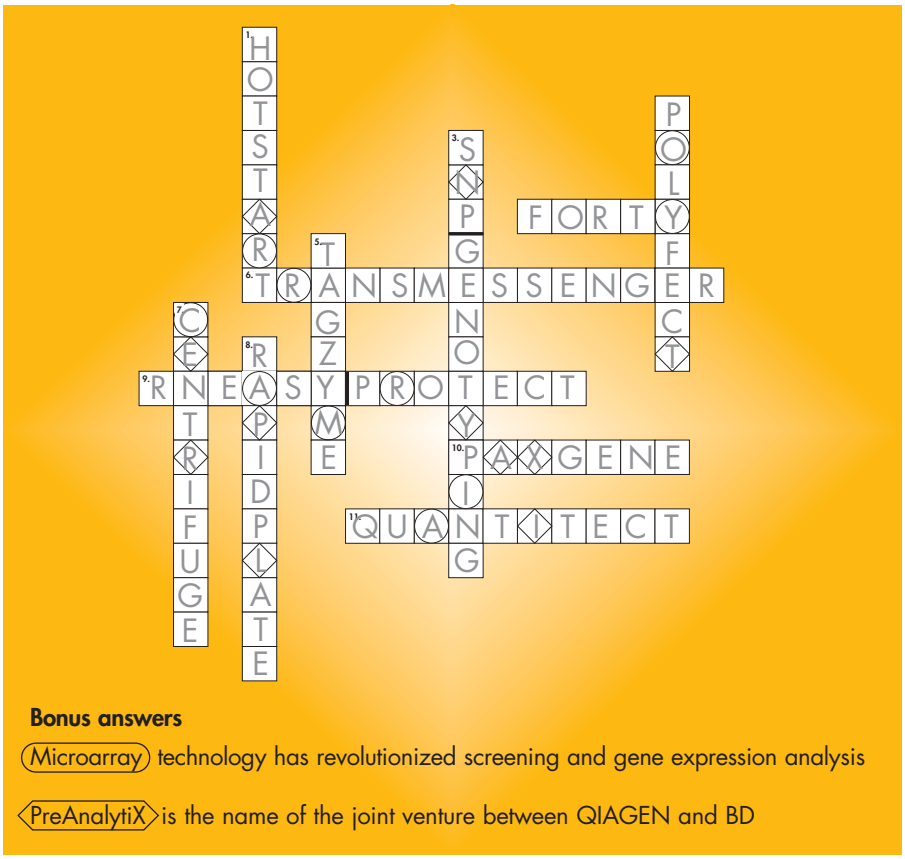
In Switzerland (QIAGEN Sweepstakes Promo):

- Kathrin Dresen, Aprentas, Muttenz
- Bénédicte Baisse Agushi, CHUV, Lausanne
- Kerstin Grosdemange, CMU, Geneva
- Michal Krawczyk, CMU, Geneva
- Reto Portmann, Universität Bern, Bern
- Yong-Ming Yuan, Université de Neuchâtel, Neuchâtel

In France:

- Isabelle Bourget, Nice
- Nicole Guiraud, Bergerac
- Christophe Parizot, Paris

The solution to the QIAGEN Challenge 2002



Bonus answers

Microarray technology has revolutionized screening and gene expression analysis

PreAnalytiX is the name of the joint venture between QIAGEN and BD

In Germany:

- Nicole Wendler, Universität München, Institut für Immunologie, Munich

In the UK and Ireland:

- Andrew Farenbach, University of Dundee, Scotland
- Keith Davidson, The Babraham Institute, Cambridge
- Helen Shaw, Royal Manchester Children's Hospital, Manchester

Come and See Us

QIAGEN exhibits and presents at many venues throughout the year. During the next few months, you can visit us at any of the meetings listed below. Come and see what's new, or talk to a company representative.

In North America

American Association for Clinical Chemistry — 2000 Clin Lab Expo	26–28 July, Chicago, IL
Drug Discovery Technology 2002	4–9 August, Boston, MA
16th Symposium of the Protein Society	17–21 August, San Diego, CA

In Europe

PHLS Annual Scientific Conference	9–11 September, Warwick, UK
British Society for Histocompatibility & Immunogenetics 13th Conference and AGM	16–18 September, Glasgow, UK

In Asia

8th Annual Meeting of JSGT	18–20 July, Tokyo, Japan

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Purchase of QuantiTect Probe Kits does not provide a license to use patented technology covered by U.S. Patents 5,210,015, 5,487,972, and 5,538,848.

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Hoffmann-La Roche owns patents and patent applications pertaining to the application of Ni-NTA resin (Patent series: RAN 4100/63: USP 4.877.830, USP 5.047.513, EP 253 303 B1), and to 6xHis-coding vectors and His-labeled proteins (Patent series: USP 5.284.933, USP 5.130.663, EP 282 042 B1). All purification of recombinant proteins by Ni-NTA chromatography for commercial purposes, and the commercial use of proteins so purified, require a license from Hoffmann-La Roche.

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1020129 06/2002