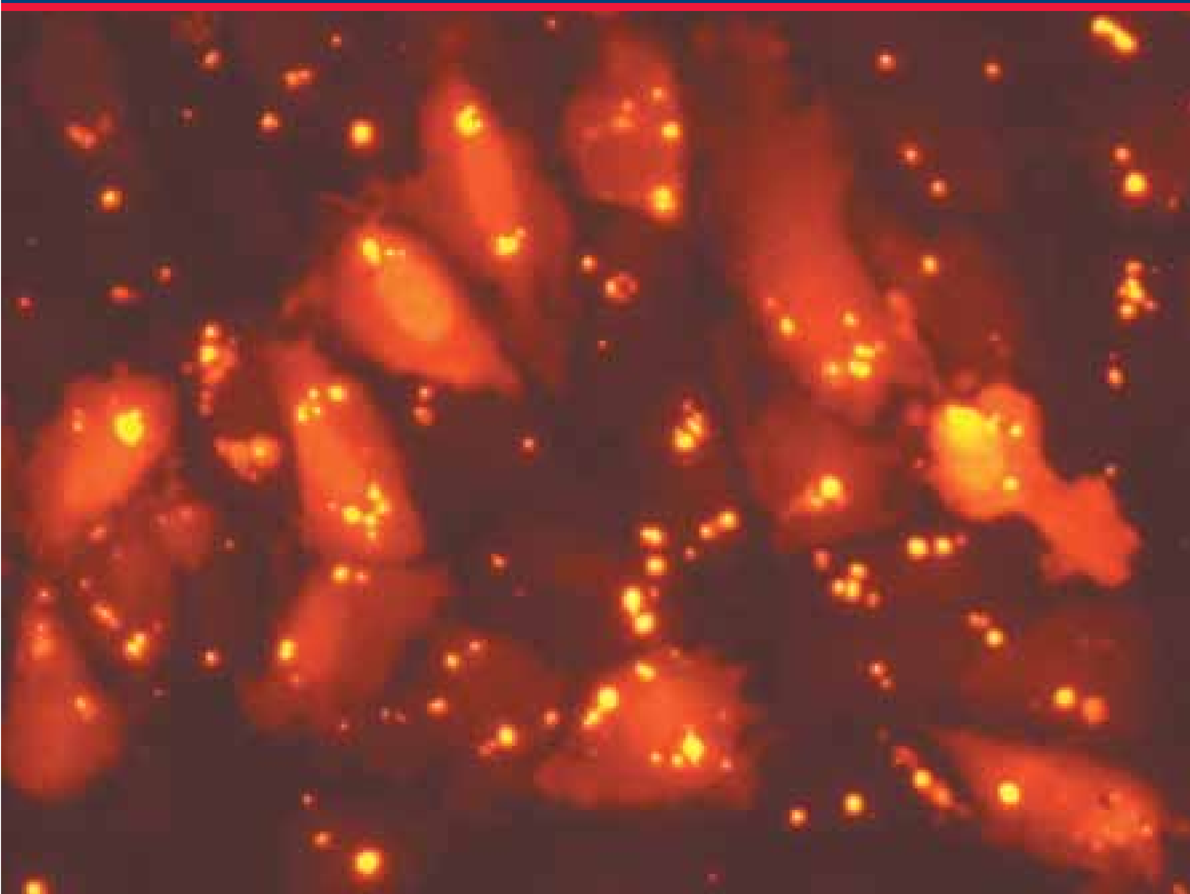
QIAGEN News | 1 April 2004



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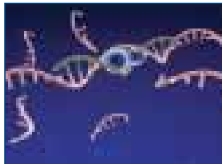
What's New?



- 2-for-Silencing siRNA Duplexes are designed using a revolutionary new algorithm and stringent homology analysis. Efficient knockdown of your target gene is guaranteed with just two siRNA duplexes, providing optimal results with less effort and expense. Visit www.qiagen.com/siRNA to find out how 2-for-Silencing siRNA Duplexes can save you time and money in RNAi experiments!

- As sample throughput demands for forensic science continue to increase due to the introduction of DNA databanks and expanding numbers of casework samples, automating time-consuming and labor-intensive sample prep can increase both reproducibility and throughput. The new EZ1 DNA Forensic Card enables automated purification of DNA from forensic samples such as cigarette butts, hairs, and dried blood. Visit www.qiagen.com/goto/EZforensics and discover how to automate your forensic research!

- QuantiTect® Custom Assays are easily designed using free Web-based software and are suitable for use on any real-time cycler. Assays are compatible with one-step and two-step RT-PCR, and are supplied as ready-to-use primer and probe solutions. Visit www.qiagen.com/goto/assays to find out how easy it is to design a custom assay for your gene of interest!

- The BioRobot® Gene Expression — GeneChip® Target Prep provides walkaway preparation of targets for Affymetrix® GeneChip arrays in 96-well format. By automating workflow from cDNA synthesis to cRNA fragmentation, hands-on time is greatly reduced, saving time and speeding up analyses. As with all BioRobot workstations, comprehensive support — including installation, performance testing, and on-site software training — comes as standard.

- High-throughput RNA purification from animal tissues just got easier! The new RNeasy® 96 Universal Tissue Kit enables purification of high-performance RNA from your choice of tissue — all in a 96-well format. The easy-to-follow protocol integrates QIAzol lysis and RNeasy purification technologies with high-throughput disruption and homogenization on the new TissueLyser system (see page 3).

- A new online resource lets you enjoy the advantages offered by QIAGEN® technologies by adapting them to your own specific applications! Visit www.qiagen.com/goto/userprotocols to find additional protocols for purification of plasmid DNA from yeasts and a wide range of bacteria, plus many more special applications!

- Get more *QIAGEN News* online! Check www.qiagen.com for a new electronic article every week!

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High-throughput disruption of a wide range of biological samples

New

The TissueLyser system provides high-throughput processing for simultaneous, rapid, and effective disruption of up to 192 biological samples, including animal and plant tissues, bacteria, and yeast.

TissueLyser with TissueLyser Adapter Set (96-well)

Benefits of the TissueLyser system:

- **Fast** — disruption of up to 192 samples in as little as 2–4 minutes
- **Cross-contamination–free** — closed system prevents tube-to-tube carryover
- **Reproducible** — highly standardized disruption method
- **Flexible** — compatible with different sample types and disruption buffers
- **Integrated** — a key component of the QIAGEN® tissue-management system, from sample collection and stabilization to RNA and DNA purification

Rapid, simultaneous disruption of up to 192 samples

The TissueLyser provides rapid and efficient disruption in a wide range of applications and at various levels of throughput. Processing of up to 2 x 96 samples takes as little as 2–4 minutes. Disruption and homogenization using the TissueLyser gives yields comparable to or better than with traditional rotor–stator homogenization methods (Figure 1).

Disruption is achieved through the beating and grinding effect of beads on the sample material as they are shaken with the sample in a variety of grinding vessels. The optimized design of TissueLyser Adapter Sets, for simultaneous disruption of up to 48 or 192 samples, ensures that tubes remain securely sealed during disruption. This prevents cross-contamination, which is especially important for highly sensitive downstream applications, such as real-time RT-PCR or microarray analysis.

TissueLyser Adapter Set (24-well)

Reproducible results using a variety of samples

The TissueLyser is well-suited for high-throughput processing of animal tissues (Figure 2). This is in contrast to rotor–stator homogenization, where samples must be processed individually, and the rotor–stator homogenizer must be cleaned after each sample to prevent cross-contamination.

For highly reproducible results and higher RNA yields, stabilization of tissues in RNA*later*™ RNA Stabilization Reagent or RNA*later* TissueProtect Tubes is an effective method to prevent RNA degradation during sample processing (Figure 2).

The TissueLyser is especially well-suited for disruption of difficult-to-lyse tissues, including fiber-rich tissues (Figure 3) and fatty tissues (Figure 4), with subsequent total RNA purification using RNeasy® Fibrous Tissue Kits and RNeasy Lipid Tissue Kits. ▶

Comparable or Higher RNA Yields Using the TissueLyser

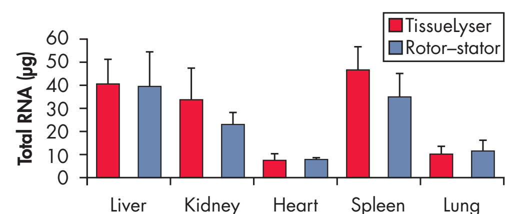

Figure 1 The indicated rat tissues (10 mg each, frozen) were disrupted and homogenized in lysis buffer using the standard TissueLyser protocol or for 1 minute each using a rotor–stator homogenizer. Total RNA was purified using the RNeasy Mini Kit or, for heart tissue, the RNeasy Fibrous Tissue Mini Kit. RNA yields were determined by absorbance at 260 nm.

Efficient Disruption and Homogenization of Animal Tissues

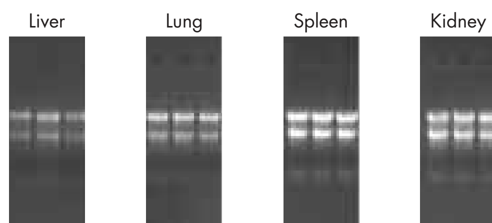

Figure 2 Rat liver, lung, spleen, and kidney tissue was stabilized in RNA*later* RNA Stabilization Reagent for 1 year at –20°C and then processed in lysis buffer using theTissueLyser. Total RNA was purified using the RNeasy Mini Kit and analyzed by formaldehyde agarose gel electrophoresis.

High-Quality RNA from Fiber-Rich Tissues

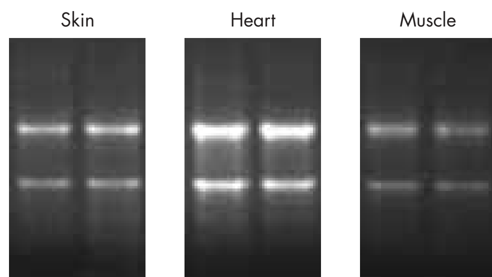

Figure 3 Rat skin, heart, and muscle tissue was flash-frozen in liquid nitrogen and processed in lysis buffer using the TissueLyser (2 x 2 min at 20 Hz). Total RNA was purified using the RNeasy Fibrous Tissue Mini Kit and analyzed by formaldehyde agarose gel electrophoresis.

Reliable Purification of Total RNA from Fatty Tissues

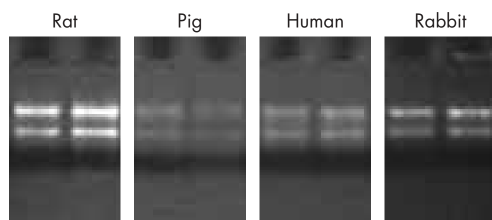

Figure 4 Adipose tissue from rat, pig, human (breast), and rabbit was flash-frozen and processed in QIAzol Lysis Reagent (included in RNeasy Lipid Tissue Kits) using the TissueLyser (2 x 2 minutes at 20 Hz). Total RNA was purified using the RNeasy Lipid Tissue Mini Kit and analyzed by formaldehyde agarose gel electrophoresis.

The TissueLyser provides efficient disruption and homogenization of biological material in each sample vessel for reproducible results in many downstream applications, such as purification of genomic DNA from plants (Figure 5) and total RNA purification from bacteria.

Flexible system with integrated purification protocols

The TissueLyser system is a key component of QIAGEN's complete solution for tissue management — from sample collection and stabilization to RNA and DNA purification. Over 20 optimized disruption and homogenization protocols integrate the TissueLyser system with QIAGEN RNeasy, DNeasy®, QIAamp®, MagAttract®, and EZ1 systems for purification of high-performance nucleic acids from a wide range of sample types. The TissueLyser system is scalable, from single samples up to 2 x 96 samples in parallel.

Complete disruption and homogenization system

The TissueLyser system includes a number of different accessories for ease of use with different sample sizes and throughputs. TissueLyser Adapter Sets are available for processing up to 2 x 96 samples in collection microtubes or up to 2 x 24 samples in 2.0 ml microcentrifuge tubes. TissueLyser Bead Dispensers are available to conveniently deliver single beads into microcentrifuge tubes or to deliver 96 beads in parallel into collection microtubes.

Reproducible Genomic DNA Purification from Plants

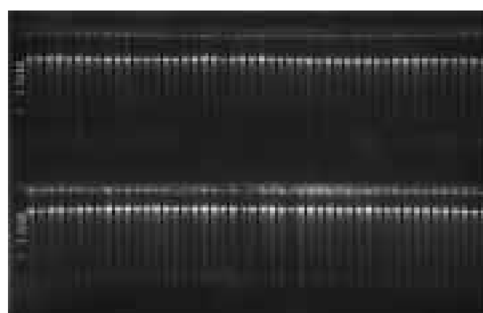

Figure 5 Ninety-six individual wheat samples were processed in liquid nitrogen using the TissueLyser. Genomic DNA was purified using the MagAttract 96 DNA Plant Kit on the BioRobot® Plant Science System — Genotyping. DNA was analyzed on an agarose gel.

"RNA*later*™" is a trademark of AMBION, Inc., Austin, Texas and is covered by various U.S. and foreign patents.

Ordering Information

Product	Contents	Cat. no.
TissueLyser (220–240V, 50/60 Hz)	Universal laboratory mixer-mill disruptor, 220–240 V, 50/60 Hz	85220
TissueLyser Adapter Set 2 x 96	2 Sets of Adapter Plates for use with collection microtubes (racked, 1.2 ml) on the TissueLyser	69984
TissueLyser Adapter Set 2 x 24	2 Sets of Adapter Plates and 2 Racks for use with 2.0 ml microcentrifuge tubes on the TissueLyser (use of 2.0 ml safe-lock tubes is strongly recommended)	69982
Stainless Steel Beads, 5 mm (200)	Stainless Steel Beads, suitable for use with 1.2 ml collection microtubes and and 2.0 ml microcentrifuge tubes on the TissueLyser	69989
Tungsten Carbide Beads, 3 mm (200)	Tungsten Carbide Beads suitable for use with 1.2 ml collection microtubes and 2.0 ml microcentrifuge tubes on the TissueLyser	69997
TissueLyser Single-Bead Dispenser, 5mm	For dispensing individual beads (5 mm diameter)	69965
TissueLyser Single-Bead Dispenser, 7 mm	For dispensing individual beads (7 mm diameter)	69967
TissueLyser 3 mm Bead Dispenser, 96-Well	For dispensing 96 beads (3 mm diameter) in parallel	69973
TissueLyser 5 mm Bead Dispenser, 96-Well	For dispensing 96 beads (5 mm diameter) in parallel	69975
Collection Microtubes (racked)	Nonsterile polypropylene tubes (1.2 ml), 960 in racks of 96	19560
"Sensitive, specific microarray analysis of the human and mouse genomes", page 6		
Human Genome Oligo Set Version 3.0*	34,580 optimized 70mers plus 24 controls in 384-well plates	810521
Human Genome Sample Oligo Set Version 3.0*	96 optimized 70mers†	810520
Human Genome Set Controls*	12 unique positive controls and 12 unique negative controls provided in 16 replicates in a 384-well plate	810599
Mouse Genome Oligo Set Version 3.0*	31,769 optimized 70mers plus 24 controls in 384-well plates	810617
Mouse Genome Sample Oligo Set Version 3.0*	96 optimized 70mers†	810616
Mouse Genome Set Controls*	12 unique positive controls and 12 unique negative controls provided in 16 replicates in a 384-well plate	810699

* 600 pmol each. Please inquire about Array-Ready Oligo Sets available for other organisms or currently in development.

† Please inquire about plate formats.

Highly sensitive microarray analysis of the human and mouse genomes

New

Array-Ready Oligo Sets are ready-to-print, optimized 70mer probe sets that use state-of-the-art bioinformatics to allow accurate microarray analysis. The updated version 3.0 sets for the human and mouse genomes use sequence information from public open-source Ensembl databases, and probe design that allows detection of all known alternative splice variants. The Human Genome Oligo Set Version 3.0 contains 34,580 probes, representing 24,650 genes and 37,123 gene transcripts, and the Mouse Genome Oligo Set Version 3.0 contains 31,769 probes, representing 24,878 genes and 32,829 gene transcripts. By providing probes that represent all known and predicted genes and transcripts, Array-Ready Oligo Sets allow gene expression analysis over an entire genome.

Version 3.0 Human and Mouse Genome Oligo Sets offer:

- Probes representing all known and predicted genes and transcripts
- Cutting-edge probe design using Ensembl database resources
- Detection of alternative splice variants using common, partial common, and individual transcript probes
- Complete annotation on the QIAGEN® Oligo Microarray Database (OMAD) with direct links to Ensembl

Comprehensive probe sets enable detection of splice variants

The Ensembl Human Database Version 13.31 and the Ensembl Mouse Database Version 14.30 are based on the respective genome sequencing projects. These databases provide not only the best non-redundant gene sets available but also exon and intron coordinates, making these databases the tools of choice for designing probes to detect alternative splice variants. A total of 7027 genes in the human and 4692 genes in the mouse Ensembl databases have more than one transcript. Version 3.0 Human and Mouse Genome Oligo Sets allow detection of alternative splice variants using common, partial common, or individual transcript oligos (Figure 1). This reveals important experimental data, as alternative splicing is a key source of diversity and complexity in the human and mouse transcriptomes. This provides an important advantage over probe sets designed using UniGene databases, since UniGene does not contain information about intron/exon structure or splice variants.

Comprehensive, easy-to-access annotation

Detailed information for each oligo is available from OMAD, a relational database maintained by QIAGEN. This database is linked to Ensembl, giving access to comprehensive annotation information, including oligo chromosome coordinates, Gene Ontology (GO), comparative genome analysis, and functional annotation information (InterPro, Online

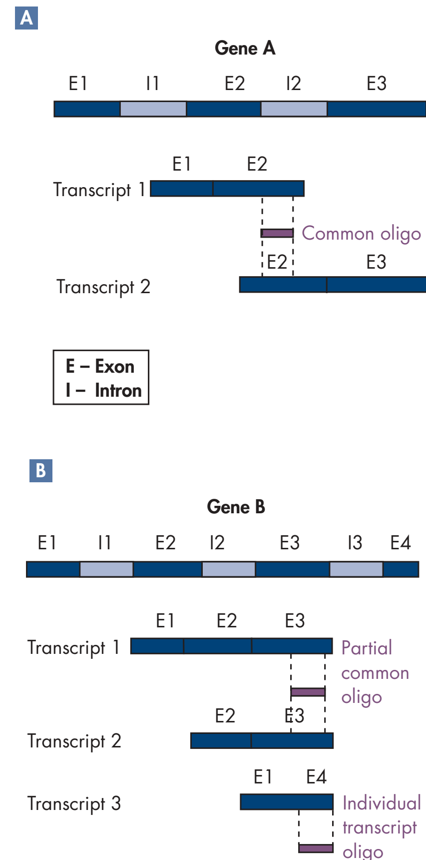

Figure 1 A Common oligo B Partial common oligo and individual transcript oligo.

Mendelian Inheritance in Man (OMIM), and protein families). The gene list, gene ontology information, and transcript list for each set can be downloaded from the OMAD download center. To visit OMAD, go to www.qiagen.com/goto/omad .

Optimized probe design for accurate microarray analysis

Array-Ready Oligo Sets are designed according to strict criteria that ensure accurate microarray results and offer:

- Melting temperatures normalized within optimal T_m ± 5°C
- GC contents normalized to within 40–60%
- Probes located within 2000 bases from the 3' end of the available transcript sequence (Figure 2)
- Probe sequences that cannot have more than 8 contiguous single nucleotide repeats, potential hairpin stem lengths longer than 9 bases, or more than 20 contiguous bases in common with another gene
- Probes that have 100% homology to the target gene and minimal cross-hybridization (<70% homology) to other genes, transcripts, or open reading frames (checked by BLAST® search) ▶

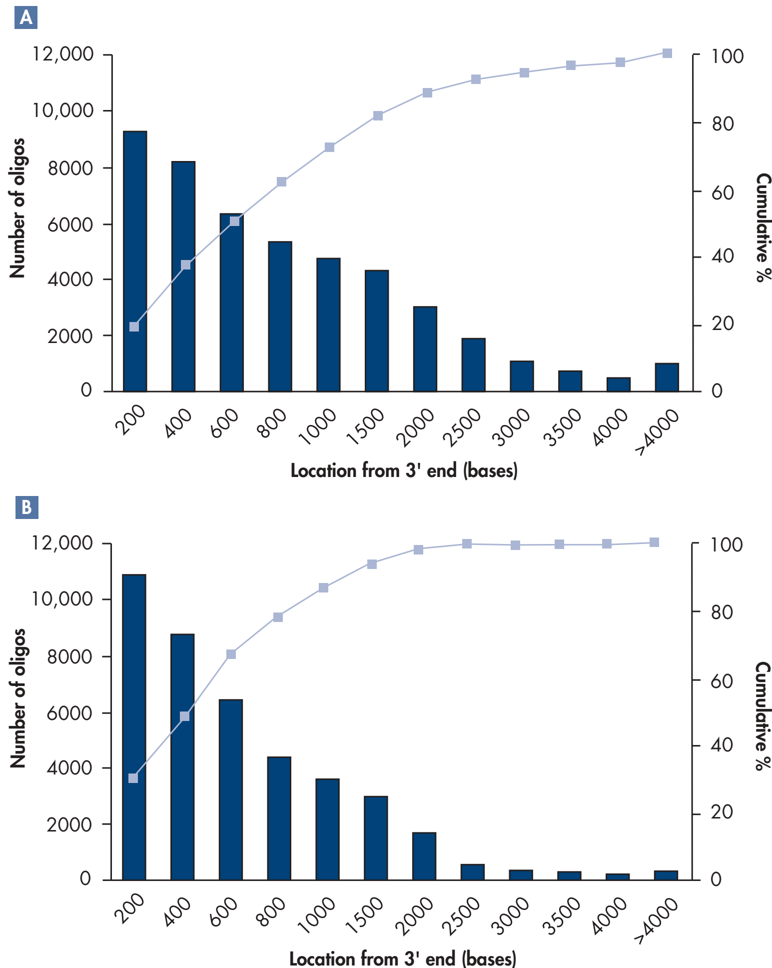

Figure 2 Location from 3' end for all oligos of **A** the Human Genome Oligo Set Version 3.0 and **B** the Mouse Genome Oligo Set Version 3.0.

Comparison of 50mer, 70mer, and cDNA Probes

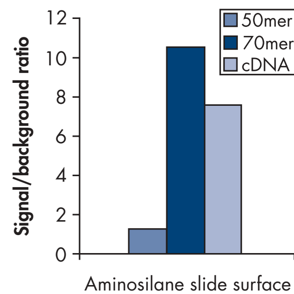

Figure 3 Signal to background ratios obtained using 50mer, 70mer, and cDNA probes printed on aminosilane slides. Data kindly provided by Olaf Thürigen, DKFZ, Heidelberg, Germany.

Comparison of 50mer, 70mer, and Q-PCR Data

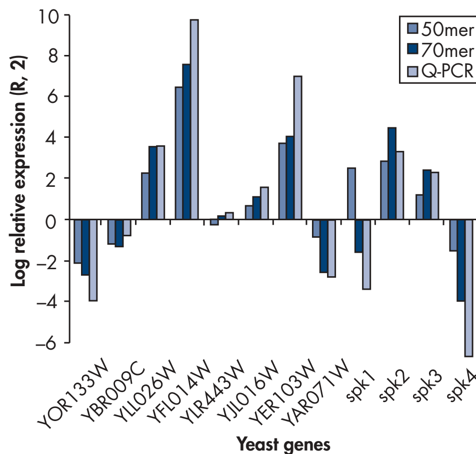

Figure 4 Gene expression results for a variety of yeast genes from 50mer and 70mer probes were compared with quantitative real-time PCR (Q-PCR) data. The samples spk1–4 are spiking controls.

70mer probes provide high specificity

The 70mer probes that are used in Array-Ready Oligo Sets provide specificity and sensitivity comparable to cDNA probes but without the drawbacks of PCR amplification. Several studies have shown that 70mer probes are an optimal length for accurate microarray analysis. Expression analysis comparing 50mer probes, 70mer probes, and cDNA probes printed on aminosilane slides showed that 70mers gave the highest signal to background ratio (Figure 3).

For a range of yeast genes, gene expression analysis comparing use of 50mer probes, 70mer probes, and quantitative real-time PCR data showed that 70mers provided superior detection and high correlation with quantitative PCR data (Figure 4).

An independent study, conducted by scientists at The Institute for Genomic Research (TIGR) and other organizations, compared microarray data obtained using 70mer probes and cDNA probes. The results showed a high correlation between data achieved using 70mers and cDNA probes and demonstrated that 70mers gave sensitive, specific measurements for most of the genes studied (1).

In another study, microarray analysis of 7344 genes was carried out to evaluate 70mer probes and in situ-synthesized 25mer probes. The results suggested that spotted 70mers were highly suitable for large-scale gene expression analysis (2).

Stringent quality control

To ensure high quality, Array-Ready Oligo Sets undergo rigorous quality control (QC) procedures. Oligos that fail to meet QC criteria are resynthesized.

To find out more about Array-Ready Oligo Sets for the human and mouse genomes and a wide range of other organisms, visit www.qiagen.com/goto/oligosets .

References

1. Wang, H-Y. et al. (2003) Assessing unmodified 70-mer oligonucleotide probe performance on glass-slide microarrays. Genome Biol **4**, R5.
2. Barczak, A. et al. (2003) Spotted long oligonucleotide arrays for human gene expression analysis. Genome Res. **13**, 1775.

For ordering information, see page 5.

Validated RNAi control kit for gene silencing applications

New

Many factors influence the success of an RNAi experiment including siRNA design, siRNA transfection conditions, and methods of downstream analysis. A factor that can be overlooked is the inclusion of appropriate control experiments. A non-silencing control, consisting of fluorescently labeled siRNA with no homology to mammalian genes, is useful for optimization of transfection conditions and to eliminate the possibility of nonspecific silencing effects. A positive control, consisting of siRNA that is known to provide high levels of gene knockdown, can be routinely transfected in parallel with the siRNA under study to ensure that optimal conditions are maintained, and for confirmation of experimental results. The new siRNA Human/Mouse Control Kit allows easy control experiments in both human and mouse cell lines. Integrated products for western blot or quantitative RT-PCR analysis are also available for downstream validation.

The new RNAi Human/Mouse Control Kit provides:

- A universal positive control siRNA used with the same protocol and reagents for both human and mouse cell lines
- A fully validated protocol for an immediate start to RNAi experiments
- Positive and non-silencing control siRNAs for transfection optimization, troubleshooting, and confirmation of data
- Flexibility in control validation, using additional tools from QIAGEN for western blot analysis or quantitative real-time RT-PCR

RNAiFect™ Transfection Reagent ensures high transfection efficiency

The RNAi control kit contains RNAiFect Transfection Reagent, which is based on a lipid formulation and has been specifically developed for transfection of eukaryotic cells with siRNA. Transfection of HeLa S3 cells with fluorescently labeled non-silencing siRNA shows that RNAiFect Reagent provides high transfection efficiency (Figure 1).

Easy control of transfection efficiency

The RNAi control kit contains a fluorescently labeled non-silencing siRNA, which serves as a control of transfection efficiency. The siRNA has no homology with any mammalian mRNA sequence, and therefore shows no gene silencing activity. The fluorescent label enables transfection to be easily followed by fluorescence microscopy.

Using MAPK1 siRNA as a positive control in RNAi experiments

The RNAi Human/Mouse Control Kit contains a validated siRNA directed against a sequence common to both human and mouse MAPK1 mRNA. When transfected into mouse NIH/3T3 and human HeLa S3 cells, expression of MAPK1 protein is almost eliminated, showing the high efficiency of gene silencing (Figure 2). This siRNA is used as a positive control of the gene silencing effect in RNAi experiments. Expression analysis of the protein kinase MAPK1 (also called Erk2 and MAPK2) in a wide range of human and mouse cell lines showed that it is highly expressed, making it a suitable candidate for use as a control of gene silencing efficiency (Figure 3). ▶

Efficient Transfection Using RNAiFect Reagent

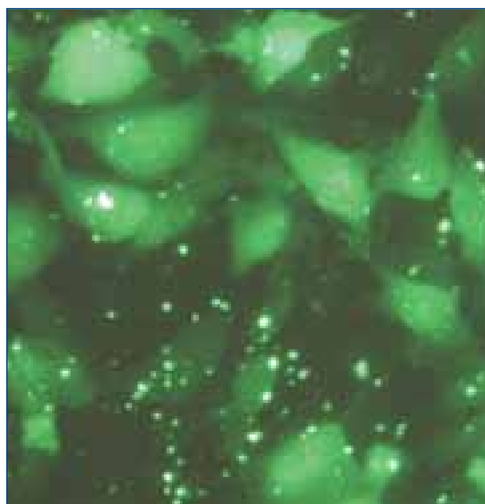

Figure 1 HeLa S3 cells transfected with non-silencing, fluorescently labeled control siRNA.

Analyzing the gene silencing effect

After transfection of siRNA targeting MAPK1 and western blotting, the silencing effect can be followed at the protein level using the MAPK1-specific Tag·100 mouse monoclonal antibody, which recognizes both human and mouse MAPK1 (Figures 2 and 3). QIAGEN also offers ready-to-use validated and custom primer–probe sets and reagents for quantitative real-time RT-PCR on any real-time cycler. The QuantiTect® Hs_MAPK1 Assay and the QuantiTect Mm_Mapk1 Assay can be used for monitoring gene silencing of human and mouse MAPK1 respectively. These reagents enable the gene silencing effect to be followed at the mRNA level.

Summary

- By providing a positive control siRNA targeted against both human and mouse MAPK1, fluorescently labeled non-silencing siRNA, and RNAiFect Transfection Reagent, the RNAi Human/Mouse Control Kit contains everything needed for a complete set of controls in RNAi experiments in human and mouse cells.
- The RNAi Human/Mouse Control Kit can be used in combination with QIAGEN® solutions for downstream analysis by western blot or quantitative real-time RT-PCR.
- The RNAi Control Kit is a useful tool for rapid siRNA assay setup, optimization, and troubleshooting in both human and mouse cell lines.

MAPK1 Gene Silencing in HeLa S3 and NIH/3T3 Cells

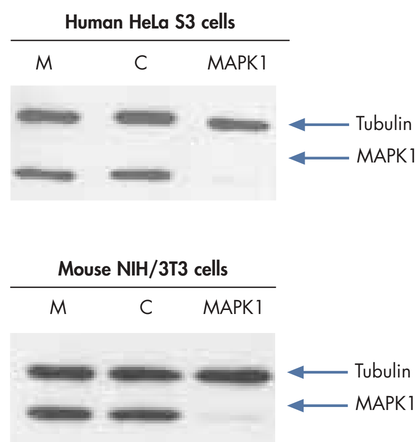

Figure 2 HeLa S3 (human) and NIH/3T3 (mouse) cells were transfected with siRNA targeted against a sequence common to human and mouse MAPK1 mRNA (**MAPK1**), or a non-silencing control siRNA (**C**). Cells were also mock transfected without siRNA addition (**M**). After transfection and incubation, cell lysates were separated by SDS-PAGE, and subjected to western blot analysis. Blots were probed using MAPK1-specific Tag·100 and tubulin-specific primary antibodies.

Expression of MAPK1 Protein in Several Cell Lines

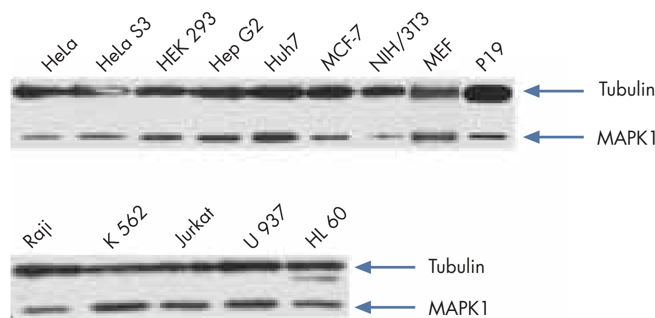

Figure 3 Western blot analysis showing expression of MAPK1 in a variety of human and mouse cell lines. MAPK1 was detected using the Tag·100 Antibody, which reacts specifically with an epitope common to both human and mouse MAPK1. Tubulin was also detected using anti-tubulin antibody and was used as an internal loading control.

Related articles in this issue

RNAi in adherent and suspension cells (page 12)
Screening large numbers of human genes using RNAi (page 20)

Ordering Information

Product	Contents	Cat. no.
RNAi Human/Mouse Control kit	RNAiFect Reagent, buffers, MAPK1 siRNA, non-silencing fluorescently labeled control siRNA	Inquire
Tag·100 Antibody, BSA-free (100 µg)	100 µg mouse anti-Tag·100 antibody that recognizes endogenous mammalian MAPK1	34680
QuantiTect Hs_MAPK1 Assay	For 100 x 50 µl reactions (for use in a 96-well plate or single tubes) or 250 x 20 µl reactions (for use in a 384-well plate or single capillaries): 0.5 ml 10x QuantiTect Assay Mix (dyes available: FAM)	Inquire
QuantiTect Mm_Mapk1 Assay	For 100 x 50 µl reactions (for use in a 96-well plate or single tubes) or 250 x 20 µl reactions (for use in a 384-well plate or single capillaries): 0.5 ml 10x QuantiTect Assay Mix (dyes available: FAM)	Inquire
For full details about QuantiTect Gene Expression Assays and Quantitect Custom Assays, please visit www.qiagen.com/goto/assays .		
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 Probe PCR Kit (200)*	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect Probe PCR Master Mix, 2 x 2.0 ml RNase-free water	204343
Related products		
HPP Grade siRNA (20 nmol)	siRNA purified to >90%[†]	–
RNAiFect Transfection Reagent (1.0 ml)*	RNAiFect Reagent and buffer, for up to 170 transfections in 24 well plates; up to 500 transfections in 96-well plates	301605
For full details of custom and library siRNA available, please visit www.qiagen.com/siRNA .		
"High protein yields from in vitro translation reactions", page 17		
EasyXpress Linear Template Kit (20)	For 20 two-step PCRs: ProofStart® DNA Polymerase, buffer, RNase-free water, Q-solution, XE-Solution, positive-control DNA, and PCR primers	32703
EasyXpress Protein Synthesis Mini Kit	For 20 x 50 µl reactions: *E. coli* extract, reaction buffer, RNase-free water, and positive-control DNA	32502
EasyXpress Protein Synthesis Maxi Kit	For reactions up to 4000 µl: 4 x 350 µl *E. coli* extract, reaction buffer, RNase-free water, and positive-control DNA	32506

* Larger sizes available, please inquire.

† Available in guaranteed yields of 20 and 40 nmol. Also available in 96-well plates; please inquire.

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RNAi-mediated gene silencing has greatly facilitated studies of gene knockdown in mammalian cells. However, the efficient delivery of siRNA to some difficult-to-transfect cells, notably primary cells, is sometimes problematic. In this study, a range of adherent and suspension cells were transfected with QIAGEN® HPP (High-Performance Purity) Grade siRNA using RNAiFect™ Transfection Reagent and Nucleofector® technology from amaxa, and the efficiency of gene silencing was analyzed.

Nucleofector technology is an efficient non-viral method for transfection of a wide variety of primary cells and difficult-to-transfect cell lines. It is based on two components: the Nucleofector Device that delivers unique electrical parameters, and Nucleofector Kits that contain cell-type–specific solutions. Many different substrates, such as DNA, RNA, siRNA duplexes, or DNA oligos, can be effectively transfected. For further details, go to www.amaxa.com/RNAi .

Materials and methods

Human and mouse adherent cell lines HeLa S3, AGS, HEK 293, Huh7, NIH/3T3, MEF, and HUVEC were transfected with siRNA targeted to either lamin A/C or ferritin heavy chain mRNA and with non-silencing, control siRNA using RNAiFect Reagent (see figure legends for details).

The suspension cell types Jurkat, HL-60, K562, THP-1, U937, and primary human T-cells were transfected with siRNA targeted to vimentin, CD2, or CD4 mRNA using Nucleofector technology. The gene silencing effect was determined by quantitative, real-time RT-PCR or flow cytometry. HPP Grade siRNA was used for all experiments.

Results

RNAiFect Transfection Reagent was shown to be effective for siRNA transfection into adherent cell lines and primary adherent cells. Figure 1A shows HUVEC cells transfected with rhodamine-labeled siRNA using RNAiFect Reagent. Results of quantitative real-time RT-PCR analysis in each adherent cell line transfected using RNAiFect Reagent are shown in Figure 2. The level of gene knockdown observed ranged from 70 to 90%.

Nucleofector technology was found to provide efficient transfection of siRNA into suspension cells. Jurkat cells transfected with rhodamine-labeled siRNA using Nucleofector technology are shown in Figure 1B. Efficient knockdown of the housekeeping gene vimentin was achieved in human and mouse suspension cell lines (Figure 3). In K562 cells, knockdown of vimentin gene expression of up to 93% was achieved.

High Transfection Efficiency

Figure 1 A HUVEC cells transfected with rhodamine-labeled siRNA using RNAiFect Transfection Reagent B Jurkat cells transfected with rhodamine-labeled siRNA using Nucleofector technology.

Knockdown of the CD2 gene encoding the T-cell receptor accessory protein and the CD4 gene encoding the T-cell co-receptor protein was shown in a variety of cell lines, including primary human T-cells. The promyelocytic suspension cell line HL-60 and the monocytic leukemia cell line THP-1 were transfected with siRNA targeted to the CD4 mRNA. The results were analyzed by flow cytometry. In HL-60 cells different amounts of siRNA duplexes were used for transfection, and down regulation of CD4 expression occurred in a dose-dependent manner. The most pronounced gene silencing effects were achieved with 1.4 µg siRNA (Figure 4). In THP-1 cells, 1.4 µg siRNA was transfected and effective knockdown of CD4 expression was achieved (Figure 5). Transfection of control siRNA duplexes did not influence the expression of CD4. Primary human T-cells were transfected with siRNA targeted to the T-cell receptor gene CD2. The gene silencing effect was shown by flow cytometry (Figure 6). Control staining of the CD4 receptor demonstrated that the gene silencing effect achieved was CD2-specific. ▶

Efficient Gene Silencing in Adherent Cells Using RNAiFect Reagent

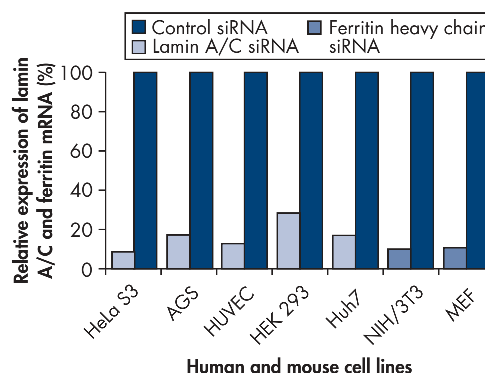
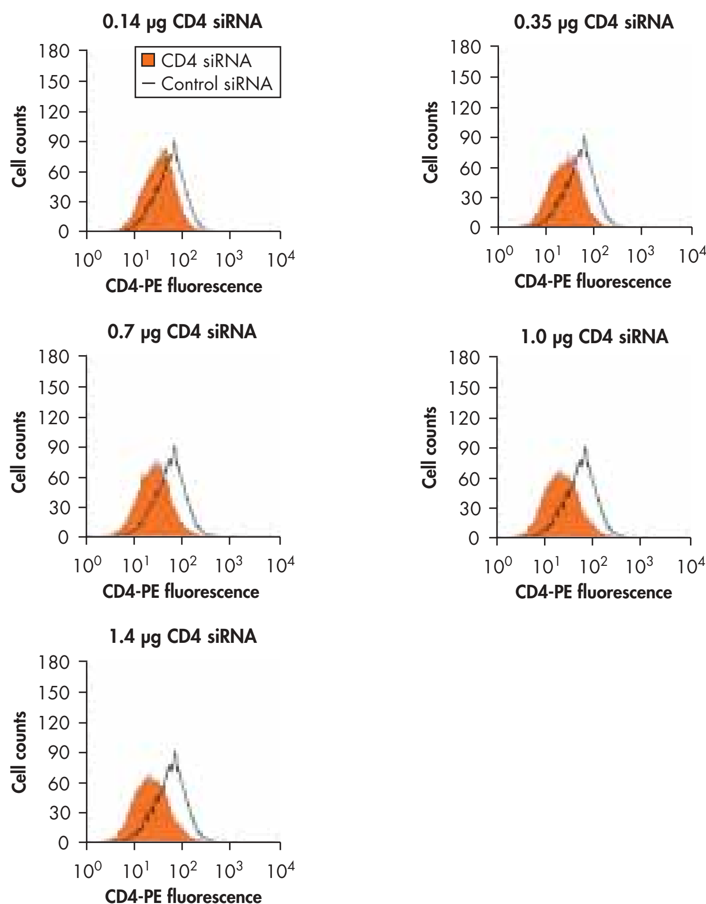


Figure 2 Cells were plated 24 hours before transfection. Cells were transfected with control siRNA or siRNA targeted to lamin A/C or ferritin heavy chain mRNA using RNAiFect Reagent. After 48 hours, cells were lysed and RNA was purified using the RNeasy® system. The mRNA was reverse transcribed using Omniscript® Reverse Transcriptase and the resulting cDNA was amplified using the QuantiTect® SYBR® Green PCR Kit with primers targeting lamin A/C, ferritin heavy chain, and GAPDH. Expression of lamin A/C and ferritin heavy chain were normalized to the expression of GAPDH and quantified using a standard curve.

Optimization of siRNA Amounts in HL-60 Cells



Efficient Gene Silencing in Suspension Cells Using Nucleofector Technology

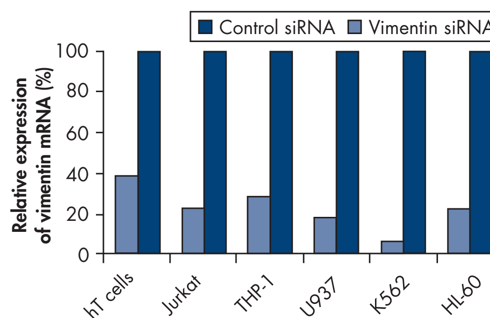
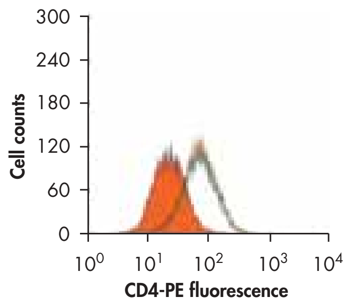


Figure 3 Six different suspension cell types were transfected with 1.4 µg siRNA targeted to vimentin or with 1.4 µg control siRNA using Nucleofector technology. After 48 hours, total RNA was prepared from cells and used for quantitative real-time RT-PCR analysis. The expression level of vimentin mRNA was normalized to the expression of a housekeeping gene and quantified using a standard curve.

Figure 4 Five different amounts of siRNA duplexes targeting human CD4 were transfected into HL-60 cells using Nucleofector technology. Cells were stained with anti-CD4 antibody, and analyzed by flow cytometry, 48 hours after transfection. siRNA targeted against vimentin was also transfected as a control. Peaks to the left of the histogram indicate less bound antibody due to lower gene expression. **PE**: phycoerythrin

CD4 Gene Silencing in THP-1 Cells



CD4 siRNA + Nucleofection — No siRNA + Nucleofection
— Vimentin siRNA + Nucleofection — CD4 siRNA, no Nucleofection

Figure 5 THP-1 cells were transfected with 1.4 µg siRNA targeted to CD4 using Nucleofector technology. Cells were stained with anti-CD4 antibody, and analyzed by flow cytometry, 48 hours after transfection. Control transfections were carried out as indicated. **PE**: phycoerythrin

Discussion

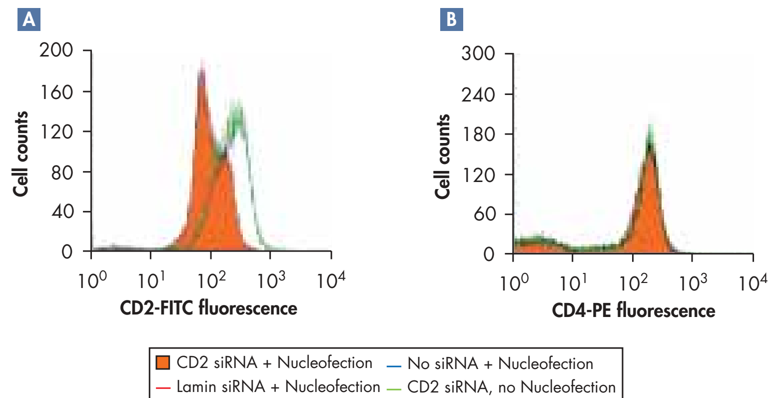
RNAi-meditated gene silencing has become a valuable tool in functional genomics research, target validation, and gene-specific therapeutics. RNAiFect Transfection Reagent, a lipid formulation, has been specifically developed for transfection of cells with siRNA. By combining high transfection efficiency with low cytotoxicity, using RNAiFect ensures successful transfection of sensitive cells, including primary cells. The combination of QIAGEN siRNA and amaxa Nucleofector technology was shown to provide efficient transfection of suspension cells. For many primary cells and cell lines, ready-to-go protocols are available so that no further optimization is required.

In these experiments, QIAGEN HPP Grade siRNA was shown to provide highly efficient specific gene knockdown of up to 93%.

Conclusions

- Effective gene silencing can be achieved in a wide range of adherent cells, including sensitive primary cells, using RNAiFect Transfection Reagent and QIAGEN HPP Grade siRNA
- Effective gene silencing can be achieved in a wide range of suspension cells, including sensitive primary cells, using amaxa Nucleofector technology and QIAGEN HPP Grade siRNA
- HPP Grade siRNA provides highly efficient and highly specific RNAi-mediated gene silencing

CD2 Gene Silencing in Primary Human T-Cells



CD2 siRNA + Nucleofection — No siRNA + Nucleofection
— Lamin siRNA + Nucleofection — CD2 siRNA, no Nucleofection

Figure 6 Primary human T-cells were transfected with 1.4 µg siRNA targeted to CD2 using Nucleofector technology. Cells were stained with A anti-CD2 antibody or B anti-CD4 antibody as a control, and analyzed by flow cytometry 48 hours after transfection. Control transfections were carried out as indicated. **PE**: phycoerythrin; **FITC**: fluorescein isothiocyanate

Product Guide 2004 questionnaire

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☐ Essential ☐ Very useful ☐ Useful ☐ Limited use ☐ Not useful

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Comprehensive ordering information	☐	☐	☐	☐	☐	☐
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Links to Web	☐	☐	☐	☐	☐	☐
Comprehensive ordering information	☐	☐	☐	☐	☐	☐
Price on same page as item description	☐	☐	☐	☐	☐	☐
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Physical dimensions of the catalog	☐	☐	☐	☐	☐	☐

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☐ A more detailed catalog every other year, with yearly price-list updates

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☐ Very convenient ☐ Convenient ☐ Inconvenient ☐ Very inconvenient

☐ I don’t use online resources

8. Which of the new products listed on pages 3–9 of the QIAGEN *Product Guide 2004* do you feel would be most useful for your work? ____________________

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Online product guide	☐	☐	☐
Other printed material (e.g., flyers, ads)	☐	☐	☐
Other electronic sources (e.g., e-mailers)	☐	☐	☐
Personal contact (e.g., QIAGEN representative)	☐	☐	☐

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Thank you for taking the time to complete the QIAGEN *Product Guide 2004* questionnaire. Your input is extremely valuable to us and will help us to make future improvements, to provide the information you want about QIAGEN products.

High protein yields from in vitro translation reactions

New

The new EasyXpress™ Protein Synthesis System provides large quantities of recombinant protein from in vitro translation–transcription reactions in just 1 hour. Using plasmid DNA and the optimized *E. coli* lysates in the EasyXpress Protein Synthesis Kit, synthesis reactions deliver up to 600 µg protein per milliliter reaction volume. If no plasmid is available, the EasyXpress Linear Template Kit can be used to generate an expression template in a 2-step PCR procedure, providing recombinant protein from a gene within a single working day. This is in contrast to the laborious procedures usually associated with producing recombinant proteins, such as cloning, transfection or transformation, clone selection, cell culture, and subsequent cell lysis and purification.

The EasyXpress Protein Synthesis System provides:

- High yields in just 1 hour — up to 600 µg protein per milliliter reaction volume
- Streamlined procedure — from gene to purified protein in a single day
- Significant time- and cost-savings — no cloning, transformation, fermentation, or specialized equipment required
- Simple and efficient protein purification — total flexibility in addition of affinity tag sequences to expression templates
- Pre-aliquoted color-coded reagents — fast and easy reaction setup

High yields on any scale from optimized *E. coli* extracts

The EasyXpress Protein Synthesis Kit uses highly productive *E. coli* extracts that contain all transcriptional and translational machinery components required for efficient protein synthesis using plasmid or PCR product expression templates (Figure 1). The extracts are supplemented with T7 RNA polymerase, enabling use of expression templates containing promoters for either T7 or *E. coli* RNA polymerases (such as the T5 promoters in pQE vectors) for protein synthesis. Proteins are synthesized in a single-tube reaction in which up to 600 µg/ml biologically active protein can be synthesized within 1 hour. Although yields from reactions using a plasmid as template tend to be higher than those using PCR products, yields of up to 400 µg/ml can be obtained using linear templates (Figure 2). Reactions can be easily scaled up or down without any reduction in translation efficiency (Figure 3) making the EasyXpress Protein Synthesis Kit highly suitable for both small-scale expression screening and large-scale synthesis. ▶

From DNA to Protein

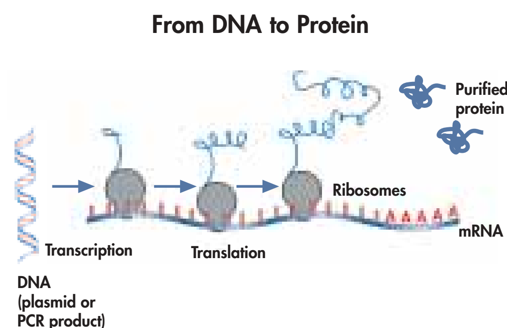

Figure 1 Schematic showing transcription and translation of proteins.

Highly Productive *E. Coli* Extracts Provide High Protein Yields

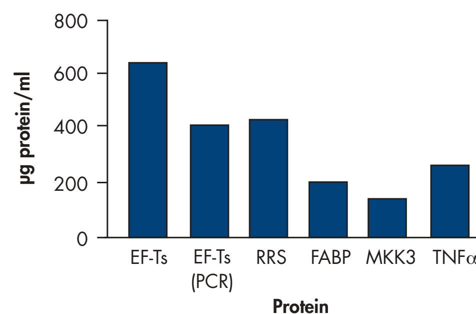

Figure 2 Yields of different *E. coli* and mammalian 6xHis-tagged proteins from in vitro translation reactions using the EasyXpress Protein Synthesis Mini Kit. Except where indicated, plasmid DNA was used as template in 50 µl reactions incubated for 1 hour at 37°C. After synthesis, protein was purified using Ni-NTA Magnetic Agarose Beads, and yield was determined via Bradford assay or on the basis of ^{14}C-leucine incorporation into TCA-insoluble reaction products. **EF-Ts**: *E. coli* elongation factor Ts; **EF-Ts (PCR)**: *E. coli* elongation factor Ts, PCR template; **RRS**: *E. coli* arginyl tRNA synthetase; **FABP**: bovine fatty acid binding protein; **MKK3**: human dual specific mitogen-activated protein kinase kinase 3 (plasmid DNA kindly provided by Axxima AG); **TNFα**: human tumor necrosis factor α.

Fast, flexible generation of expression templates by PCR

The easiest way to generate in vitro expression templates is by using a 2-step PCR procedure and the EasyXpress Linear Template Kit, which contains enzymes, nucleotides, buffers, and primers designed to add 6xHis or *Strep*-tag® coding sequences to either terminus of expressed proteins (Figure 4). Once added to constructs, the small 6xHis tag and *Strep*-tag can be used to quickly and efficiently purify proteins using Ni-NTA and/or *Strep*-Tactin® affinity purification matrices (1). Adding a C-terminal tag ensures that only full-length protein is purified using the relevant purification matrix.

Robust protein expression

The EasyXpress Protein Synthesis Kit is not optimized for expression of a certain class of protein and therefore provides consistently high yields over a much wider range of proteins than other kits, and in some cases, produces protein where others fail (Figure 5).

Reference

1. Two steps to ultrapure protein preparations. QIAGEN News **2003**, *57*.

High, Consistent Yields on any Reaction Scale

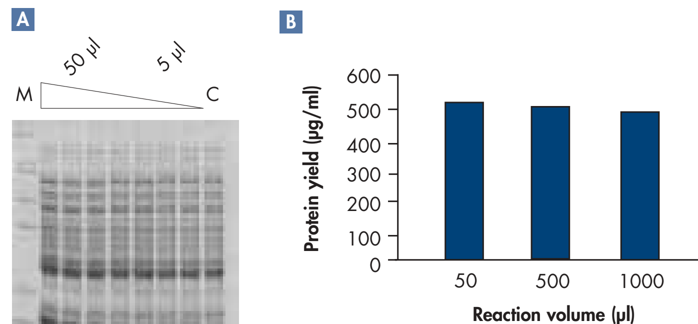

Figure 3 EF-Ts protein was expressed in in vitro translation reactions performed in the indicated reaction volume for 1 hour. **A** Coomassie®-stained SDS-PAGE gel. **C**: No template control reaction. **M**: markers. **B** After expression, protein was purified using Ni-NTA Magnetic Agarose Beads and yield determined via Bradford assay.

Generation of Expression Constructs by 2-Step PCR

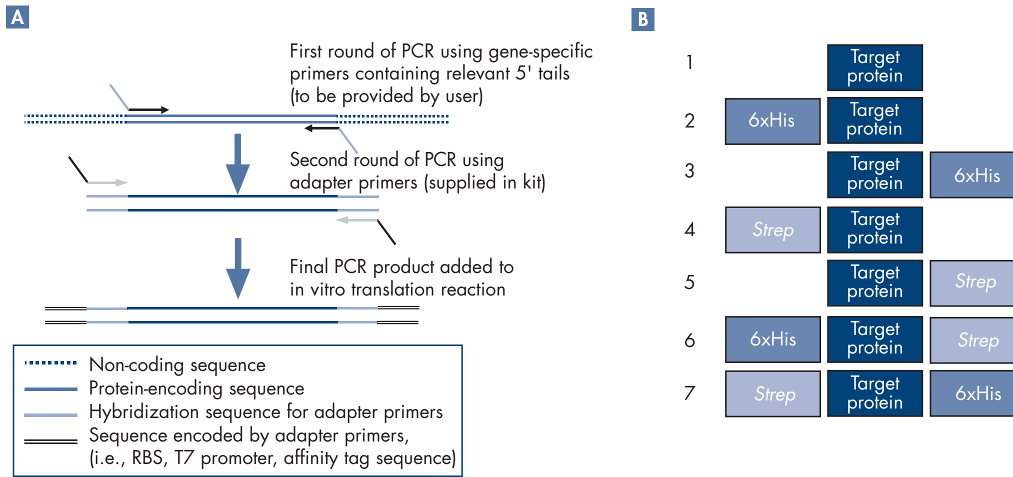

Figure 4 **A** Schematic showing generation of expression constructs by two-step PCR. **B** The seven different expression constructs that can be generated using primers supplied with the EasyXpress Linear Template Kit.

Robust Protein Expression Using the EasyXpress System

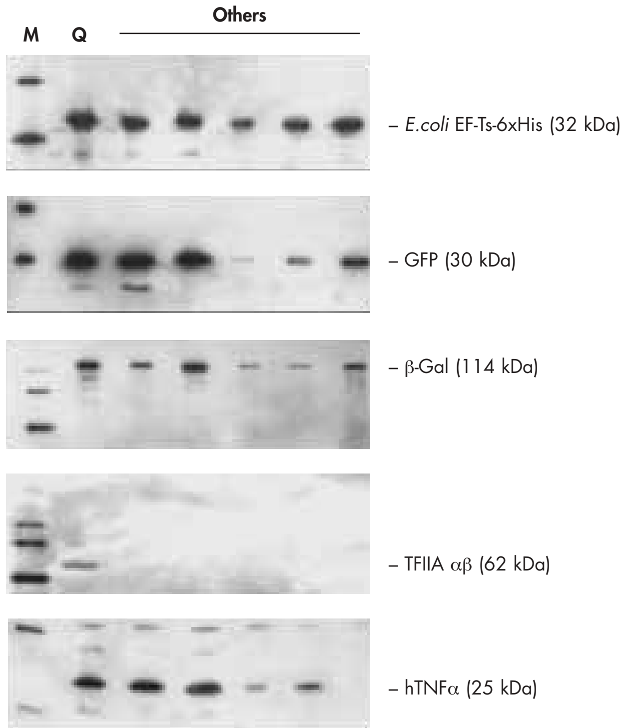

Figure 5 Western blots of different in vitro translated 6xHis-tagged proteins synthesized using the EasyXpress Protein Synthesis Kit (**Q**) and other commercially available kits. Plasmid constructs contained T7 promoter/terminators except hTNFα which was expressed using a TAGZyme™-pQE-2 construct carrying the T5 promoter. **M**: 6xHis Protein Ladder

For ordering information, see page 11.

Screening large numbers of human genes using RNAi

New

The Human Druggable Genome siRNA Set V 1.0 is the first large publicly available short interfering RNA (siRNA) set specific for druggable targets. This comprehensive discovery tool enables high-throughput gene silencing studies of 5000 potential human druggable targets using RNAi.

The Human Druggable Genome siRNA Set V 1.0 offers:

- **siRNA targeting a wide range of genes** — to provide a powerful tool for target validation studies
- **Fully annotated sequence information** — to give complete freedom for discovery
- **siRNA designed using a state-of-the-art algorithm** — to maximize gene silencing potential
- **High-purity HPP Grade siRNA** — for efficient gene silencing

Gene selection

The field of RNAi has the potential to revolutionize drug discovery. Chemically synthesized siRNA is rapidly becoming one of the major tools of choice for pharmaceutical and biotech companies who employ high-throughput screening approaches for target validation. Currently, a distinct subset of human genes are of therapeutic interest and this subset is referred to as the druggable genome. The QIAGEN® Human Druggable Genome siRNA Set, comprising 10,000 duplexes designed against 5000 specifically chosen druggable targets, provides a means of studying large numbers of human genes. This comprehensive discovery tool provides siRNAs targeting kinases, proteases, G-protein–coupled receptors, oncogenes and tumor suppressors, nuclear receptors, structural proteins, cell-surface receptors, ion channels, transcription factors, cytokines, cell-cycle control genes, genes involved in apoptosis, hypothetical open reading frames derived from the human genome project, and other sequences that are potential targets for small molecule or nucleic acid therapeutics. All duplexes in the set are fully annotated and all sequence information is disclosed (Table 1). QIAGEN makes no claim to any discoveries made using this set, giving researchers complete freedom. Purchase of the set confers a research-use license from the Massachusetts Institute of Technology.

Table 1. Examples of siRNA Duplex Annotation*

Genbank ID	Symbol	Chr	Cyto location	Gene name	Locus ID
NM_000014	A2M	12	12p13.3-p12.3	Alpha-2-macroglobulin	2
NM_012089	ABCB10	1	1q42	ATP-binding cassette, sub-family B, member 10	23456
NM_000022	ADA	20	20q12-q12.11	Adenosine deaminase	100
NM_000025	ADRB3	8	8p12-p11.2	Adrenergic, beta-3-, receptor	155
NM_001148	ANK2	4	4q25-q27	Ankyrin 2, neuronal	287
NM_032043	BRIP1	17	17q22-q24	BRCA1 interacting protein 1 C-terminal helicase	83990
NM_001223	CASP1	11	11q23	Caspase 1	834
NM_006725	CD6	11	11q13	CD6 antigen	923
NM_021951	DMRT1	9	9p24.3	Doublesex and mab-3 1 related transcription factor	1761
NM_001999	FBN2	5	5q23-q31	Fibrillin 2 (congenital contractual arachnodactyly)	2201
NM_002025	FMR2	X	xq28	Fragile X mental retardation 2	2334
NM_002026	FN1	2	2q34	Fibronectin 1	2335
NM_000160	GCGR	17	17q25	Glucagon receptor	2642

* Full siRNA sequence information, including position in the gene, will be provided upon purchase of the Human Druggable Genome siRNA Set.

siRNA sequence design

The Human Druggable Genome siRNA Set is designed using a proprietary state-of-the-art algorithm and stringent homology analysis to give a high success rate for gene silencing. QIAGEN has tested the efficiency of the design algorithm using more than 100 siRNAs of the Human Druggable Genome siRNA Set. To increase the likelihood of efficiently silencing target gene expression, two duplexes are provided for each gene. In silencing experiments, 46% of the siRNA duplexes give ≥90% knockdown and 75% of the siRNA duplexes give ≥70% knockdown of the corresponding target mRNA (Figure 1).

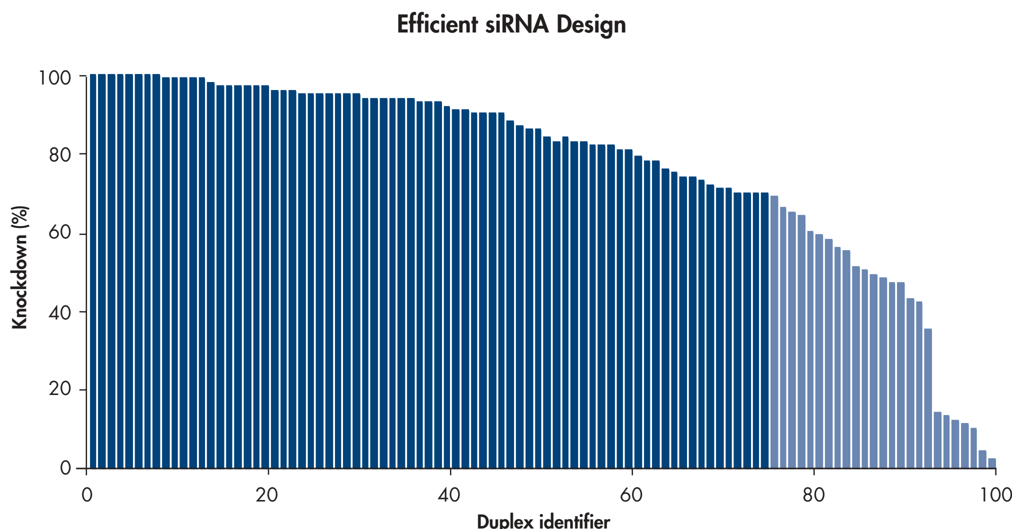

Figure 1 HeLa S3 cells were co-transfected with siRNA duplexes and luciferase-tagged target plasmid constructs. Cells were incubated for 48 hours before luciferase activity was analyzed. Luciferase activity was normalized against that in cells that were transfected with non-silencing control siRNA and a tagged construct. All experiments were performed in duplicate and the data were averaged.

HPP Grade siRNA for efficient gene silencing

All HPP Grade siRNA is produced using QIAGEN's patented TOM-amidite chemistry. The proprietary HPP synthesis process improves yields and ensures high coupling efficiency. Using our state-of-the-art, high-throughput synthesis facilities, each set is synthesized and provided in standard 96-well microplate format. Each duplex is delivered as 2 nmol of >90% pure HPP Grade siRNA. Stringent quality control, including MALDI-TOF analysis, is performed on each duplex. Annotation data for each 96-well plate are conveniently stored on a CD accompanying the set.

Ordering Information

Product	Contents	Cat. no.
Human Druggable Genome siRNA Set	10,000 duplexes of HPP Grade siRNA (2 nmol)	1022561

Visit www.qiagen.com/siRNA or contact QIAGEN today to discover more about gene silencing!

Sensitive quantification of a broad range of kinase activities

New

Specific phosphorylation of serine, threonine, and tyrosine residues is the most common mechanism for the regulation of cellular protein activity and plays a vital role in cell signaling, oncogenesis, apoptosis, and immune disorders. Two new LiquiChip™ assay kits facilitate research into these cellular processes by enabling screening of a wide range of serine/threonine and tyrosine kinase activities in a single assay.

LiquiChip Broad-Range Kinase Kits offer:

- Substrates optimized to enable detection of a wider range of Tyr and Ser/Thr kinase activities
- Fast analysis of the inhibitory effect of drug candidates on kinase activity
- Sensitive quantitative kinase assays without the need for radioactivity
- Ready-to-run kits containing all you need for assay and detection

Kinase assays without the need for radioactivity

LiquiChip assays measure the interaction of immobilized, bead-bound assay components with reaction partners in solution. In LiquiChip Broad-Range Kinase Kits, the components immobilized on LiquiChip beads are peptides or proteins that serve as Ser/Thr or Tyr kinase substrates. Substrate-coated beads are incubated with a sample containing one or more active kinases and a non-radioactive Mg^{2+}-ATP cocktail, which serves as a phosphate donor. After reaction, phosphorylation sites are detected by primary antibodies that specifically recognize phosphorylated serine, threonine, or tyrosine residues. Primary antibodies are detected using a biotinylated secondary anti-mouse antibody. Secondary antibodies are detected in turn using streptavidin conjugated to highly fluorescent phycoerythrin (Figure 1).

Kinase Detection without the Need for Radioactivity

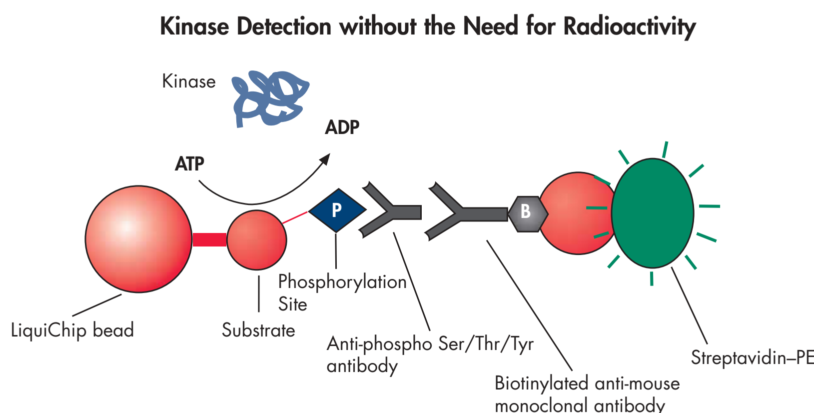

Figure 1 Detection of kinase activity using LiquiChip Broad-Range Kinase Kits.

Optimized substrates enable detection of more kinases

LiquiChip Broad-Range Tyr beads are coated with a tyrosine-rich oligopeptide that is a substrate for a wide range of tyrosine kinases, including cytosolic tyrosine kinases (e.g., members of the Src family, Zap70, Csk, and Abl-1) and receptor tyrosine kinases (e.g., EGFR, InsR, and PDGFR alpha). Examples of kinase activities that have been analyzed using the LiquiChip Broad-Range Tyr Kinase Kit are shown in Table 1. Figure 2 shows activity assays for proteins from the cytosolic and receptor classes of tyrosine kinase, demonstrating the sensitivity and broad-range applicability of the LiquiChip Broad-Range Tyr Kinase Kit.

Table 1. Activity of Selected Tyrosine Kinases Assayed Using LiquiChip Broad-Range Tyr Beads

Class	Level of phosphorylation
Src Kinases	
Src (p60-src, c-Src)	+++
Fyn	++
Lyn	+++
Lck (Lsk)	+++
Yes	+++
Hck (B-cell/myeloid kinase, Bmk)	+++
Other cytosolic kinases	
Csk (c-Src kinase)	+++
Fes/Fps	++
Zap70 (70 kD Zeta associated protein)	++
Abl-1	++
Receptor kinases	
EGFR	++
InsR (insulin receptor)	+++
PDGFR alpha	++
FGFR3	+++

Sensitive Detection of Tyrosine Kinases from Different Classes Using a Single Substrate

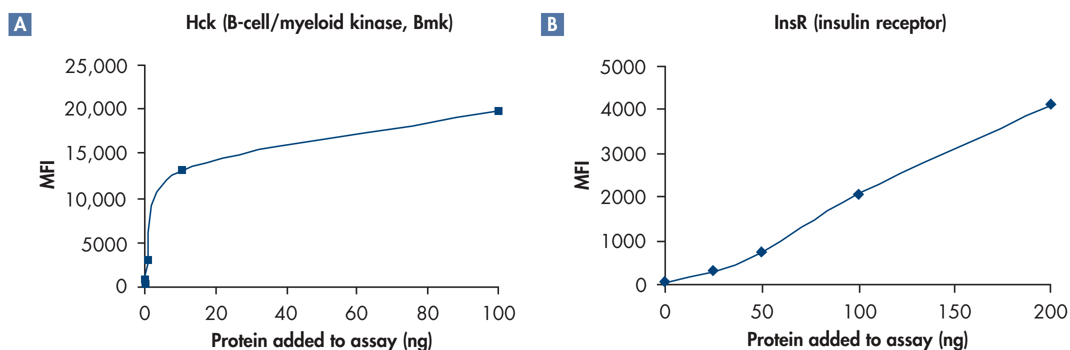

Figure 2 Activities of exemplary A cytosolic and B receptor kinases measured using the LiquiChip Broad-Range Tyr Kinase Kit.

The LiquiChip Broad-Range Ser/Thr Kinase Kit contains 4 differently coded bead sets each coated with a different Ser/Thr kinase substrate (myelin basic protein [MBP], histone H1, and two peptides). In contrast to other commercially available *x*MAP™-based Ser/Thr kinase assay kits, the range of substrates immobilized on LiquiChip Broad-Range Ser/Thr beads allows sensitive analysis of virtually all Ser/Thr kinase activities including those listed in Table 2. Another advantage of the multiple substrates included in the LiquiChip Broad-Range Ser/Thr Kinase Kit is the generation of a "phosphorylation profile" which can be used characterize kinase activities (Figure 3). ▶

Table 2. Activity of Selected Ser/Thr Kinases Assayed Using LiquiChip Broad-Range Ser/Thr Beads

	Substrate			
Ser/Thr kinase	**MBP (bead code 18)**	**Histone H1 (code 20)**	**PKA substrate (code 25)**	**CKII substrate (code 27)**
Proline-directed kinases				
Mitogen-activated protein kinase (MAPK/Erk2)	++	–	–	–
Cyclin dependent kinase (Cdc-2/Cdk-1)	++	–	–	–
p38δ/SAPK4	++	–	–	–
p38α/SAPK2α	++	–	–	–
Basophilic kinases				
Protein kinase A (PKA)	–	–	++	–
Protein kinase C (PKC)	+	++	++	+
p70S6 kinase	–	–	++	–
Acidophilic kinases				
Glycogen synthase kinase 3β (GSK3β)	+	+	+	+
Casein kinase II (CKII/CK2)	–	–	–	++
Other kinases				
Checkpoint kinase (CHK1)	++	++	++	++
JNK1α1/SAPK1c	++	–	+	–
Protein kinase Bα (PKBα/Akt1)	++	++	++	++

Applications

Reversible protein phosphorylation has been known for some years to control a wide range of cellular processes and activities such as transmembrane signaling, intracellular amplification of signals, and cell-cycle control. LiquiChip Broad-Range Ser/Thr and Tyr Kinase Kits can be used for sensitive measurement of a wide range of kinase activities in:

- Cell signaling and cellular pathway studies
- Drug screening
- Kinase inhibitor analysis
- Kinase activity profiling

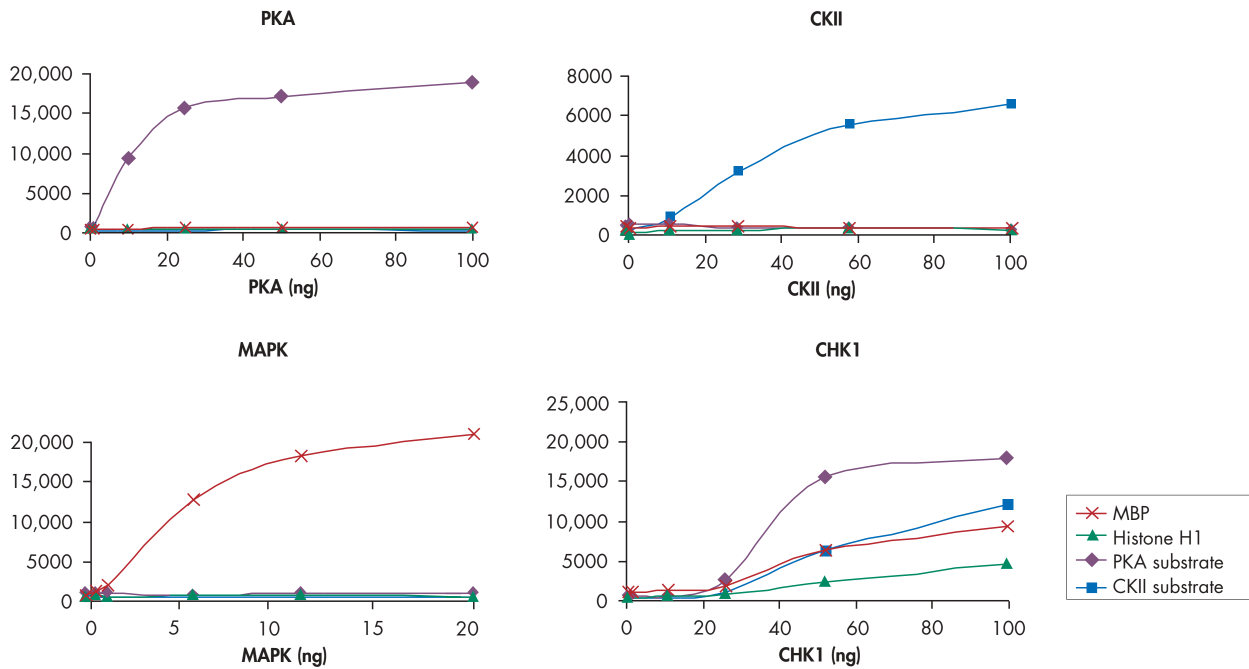

Figure 3 Multiplex assays of Ser/Thr kinases using the 4 differently coded LiquiChip beads coated with different substrates.

Ordering Information

Product	Contents	Cat. no.
LiquiChip Broad-Range Ser/Thr Kinase Kit	For 100 kinase assay points: substrate-coated LiquiChip Beads, anti-phosphoserine/threonine antibodies, biotinylated anti-mouse IgG, Streptavidin-PE, stop solution, 96-well test plate, assay template software	922081
LiquiChip Broad-Range Tyr Kinase Kit	For 100 kinase assay points: substrate-coated LiquiChip Beads, anti-phosphotyrosine antibodies, biotinylated anti-mouse IgG, Streptavidin-PE, stop solution, 96-well test plate, assay template software	922083

Integrated Solutions — Automated Nucleic Acid Prep

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New

BioRobot EZ1 — 1–6 samples

BioRobot M48 — 6–48 samples

BioRobot MDx — up to 96 samples

QIAGEN provides a range of automated workstations to standardize sample prep for clinical research applications. Whatever your throughput requirements, QIAGEN delivers trusted solutions that are tailored to your needs:

- **Purify 1–6 samples per run** — and get high-quality DNA or RNA at concentrations optimized for your applications, as easy as A B C!
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PCR

I've used your protocol for amplification of long PCR products, described in the *ProofStart® PCR Handbook*. Can I clone the amplicon directly into UA cloning vectors without further A-addition steps?

Yes. We have successfully cloned long PCR products using QIAGEN® PCR Cloning Kits, without the need for A-addition or further manipulation. The pre-optimized ratio of ProofStart and *Taq* DNA Polymerases specified in the protocol provides both proofreading and A-addition by the respective enzymes. The protocol is specifically designed for use with these QIAGEN enzymes and buffers, providing robust amplification without the need for optimization.

DNA purification

QIAprep® and QIAquick® spin columns are both for DNA purification. Can I use them interchangeably?

We do not recommend this. These spin columns have different silica-gel membranes, which are optimized for purification of different sizes and amounts of DNA from different sources. QIAprep spin columns are optimized for purification of plasmid DNA from *E. coli*, with a maximum binding capacity of 20 µg. In contrast, QIAquick spin columns are designed for DNA cleanup from PCR or gels, with a 10 µg binding capacity. For best results, we recommend using the QIAGEN kit that is designed for your specific application.

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.

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Purchase of QIAGEN products for PCR containing *Taq* DNA Polymerase, HotStarTaq DNA Polymerase, or ProofStart DNA Polymerase is accompanied by a limited license to use them in the Polymerase Chain Reaction (PCR) process for research and development activities in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e. an authorized thermal cycler. The PCR process is covered by U.S. Patents 4,683,195 and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. The purchase of QIAGEN's DNA oligos conveys no license, either expressly or by implication, to the purchaser or subsequent user to use the PCR process.

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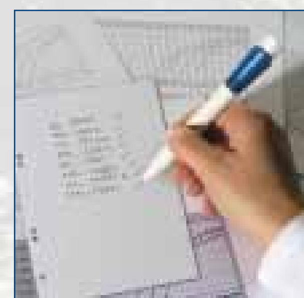

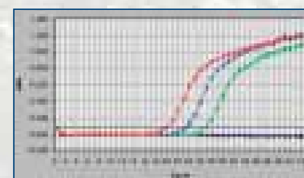

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