Issue No. 5, 2001

QIAGEN News

Innovation Working for You

See the back cover for a full list of the articles featured in this issue!

New

QIAGEN® MagAttract™ 96 Miniprep System — walkaway template purification for high-throughput sequencing

QIAGEN introduces the MagAttract™ 96 Miniprep System for fully automated, high-throughput plasmid purification from bacterial cultures using the BioRobot® 8000. Using novel lysis chemistries to produce a clear lysate, MagAttract 96 technology offers the speed and efficiency of silica-based DNA purification, with the convenience of a fully automated, state-of-the-art magnetic bead separation protocol. Up to twenty 96-well plates can be processed per 16-hour working day.*

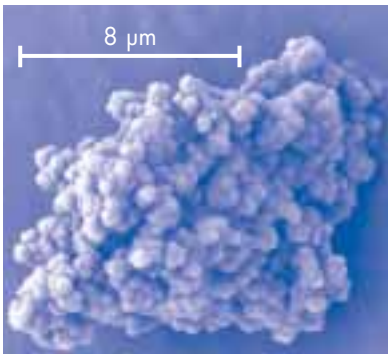
** Calculation is based on 4 x 96 samples per protocol on the QIAGEN BioRobot 8000 within a 16-hour working day, including one protocol running overnight.*

The MagAttract 96 Miniprep System provides:

- **Walkaway processing** — ready-to-run protocols at the click of a mouse
- **Economic pricing** — ideal for high-throughput genomics and screening
- **Reproducible yield** — 3 µg plasmid DNA from 1.25 ml culture
- **High purity** — reliable downstream performance

▶▶ High-throughput sequencing-template purification, page 10

MagAttract Particle



New

Bacterial RNA stabilization and isolation with RNeasy® Protect Bacteria Kits

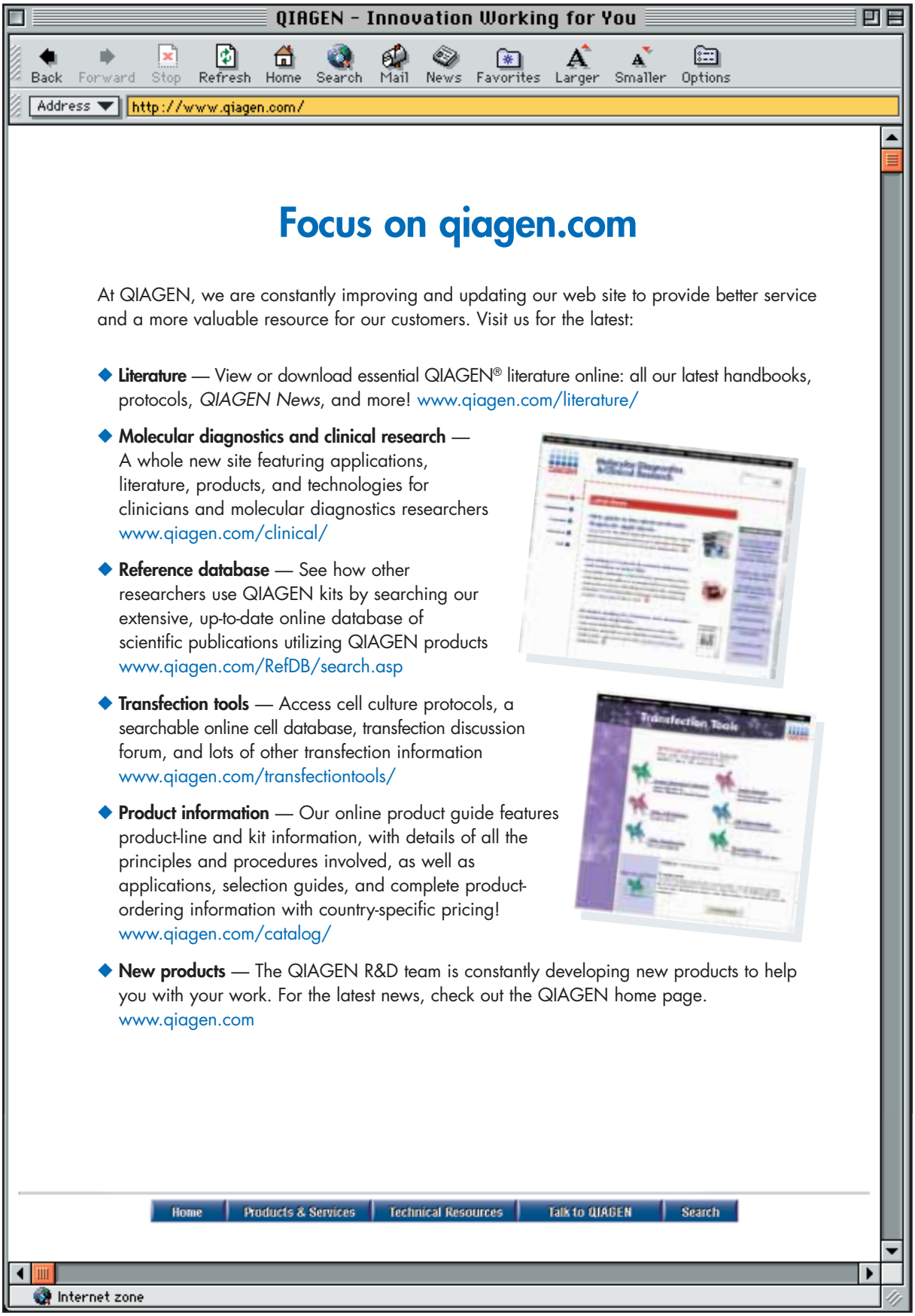
The new RNAprotect™ Bacteria System, developed by and available exclusively from QIAGEN, provides the complete solution for RNA stabilization and isolation, from bacterial cultures to pure RNA. The innovative RNAprotect Bacteria Reagent gives immediate stabilization of RNA in living bacteria for accurate gene-expression analysis. In addition, we've combined this new reagent with proven RNeasy® technology in RNeasy Protect Bacteria Kits for isolation of high-quality RNA ready to use in a wide range of downstream applications.

RNAprotect Bacteria Reagent provides:

- Immediate stabilization of bacterial gene-expression profiles prior to cell harvest and sample preparation
- Reliable gene-expression profiles in Gram-positive and Gram-negative bacteria
- Convenient procedure — simply add the reagent directly to bacterial cultures
- Improved sensitivity in any downstream application

▶▶ RNeasy Protect Bacteria Kits, page 18

QIAGEN



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New

QIAGEN broadens its spectrum of detection reagents — Penta·His™ antibody conjugates

QIAGEN introduces a new range of Penta·His™ antibody conjugates for the detection of 6xHis-tagged proteins. The proven monoclonal antibodies are available conjugated to a range of Alexa Fluor® fluorescent dyes for use in immunofluorescence procedures, and to biotin — which is used in conjunction with streptavidin or avidin conjugates — for a wide range of detection applications.

The mouse monoclonal antibodies are available as Alexa Fluor 488, 532, 555, and 647 conjugates, giving a range of highly specific reagents whose emission wavelengths cover a wide portion of the visible spectrum (Table 1, Figure 1). The conjugates can be used in all immunofluorescence procedures, such as fluorescence-activated cell sorting (FACS®), flow cytometry, confocal laser microscopy, immunocytochemistry and immunohistochemistry, protein localization, and targeting studies.

The Penta·His Biotin Conjugate can be used in conjunction with any streptavidin or avidin conjugate, delivering high flexibility in detection procedures. QIAGEN offers streptavidin conjugated to the intensely fluorescing algal pigment R-phycoerythrin (Streptvidin–R-PE).

Highly specific detection of 6xHis-tagged proteins

The Penta·His Antibody recognizes an epitope consisting of 5 consecutive histidine residues and binds with high specificity and an affinity markedly higher than other commercially available antibodies (1). The antibodies are produced under serum-free conditions and are free of viruses, mycoplasma, and contaminating immunoglobulins, making them suitable for even the most sensitive applications.

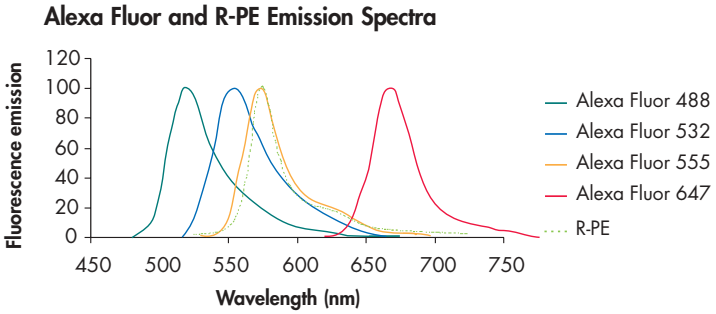


Figure 1 *Fluorescence emission spectra of Alexa Fluor dyes. Spectra were recorded in aqueous solution at pH 8.0 (Alexa Fluor 488), pH 7.2 (Alexa Fluor 532, 555, and 647), and pH 7.5 (R-PE).*

New Penta·His Alexa Fluor Conjugates offer:

- Highly specific detection of 6xHis-tagged proteins
- A range of colors from green to red
- Intense fluorescence over a wide pH range
- High photostability and instrument compatibility

Table 1. Spectral characteristics of dyes conjugated to Penta·His Antibodies

Dye	Excitation maximum (nm)	Emission maximum (nm)	Laser/light source
Alexa Fluor 488	494	519	Ar, Xe, ZnCdSe
Alexa Fluor 532	531	554	Ar, Xe, Nd-YAG
Alexa Fluor 555	555	565	Xe, HeNe, Hg-arc
Alexa Fluor 647	650	668	Kr, HeNe
R-phycoerythrin	480, 546, 565	578	Ar, Kr

References

1. *Monoclonal anti-His antibodies for sensitive detection of 6xHis-tagged proteins. QIAGEN News 1997 No. 3, 1.*
2. *Zola, H., Neoh, S.H., Mantzioris, B.X., Webster, J., and Loughnan, M.S. (1990) Detection by immunofluorescence of surface molecules present in low copy numbers. High sensitivity staining and calibration of flow cytometer. J. Immunol. Methods **135**, 247.*

Superior dye performance

Alexa Fluor dyes fluoresce more intensely and are more photostable than most other dyes, delivering bright signals and allowing more time for image capture. In addition, they provide high fluorescence intensities over a wide pH range. The absorption spectra of the dyes closely match the output wavelengths of the most commonly used excitation sources, making them compatible with a wide range of instruments. The high aqueous solubility of Alexa Fluor dyes enables them to be conjugated to antibodies without using organic solvents, and increases their resistance to precipitation during storage.

Penta·His Antibodies are available conjugated to the following Alexa Fluor dyes:

- **Alexa Fluor 488** — provides more intense fluorescence and is significantly more photostable than fluorescein conjugates. In addition, the fluorescence of Alexa Fluor 488 is independent of pH over the range pH 4–10, in contrast to fluorescein whose fluorescence is highly pH dependent.
- **Alexa Fluor 532** — an intermediate between the green-fluorescing Alexa Fluor 488 and the orange-fluorescing Alexa Fluor 555 dyes. Highly suited for use with 532 nm excitation sources, such as the frequency-doubled Nd-YAG laser.
- **Alexa Fluor 555** — fluoresces intensely in the orange region of the spectrum and is efficiently excited by the 546 nm spectral line of mercury-arc lamps. The excitation and emission spectra of Alexa Fluor 555 are virtually identical to those of the Cy®3 dye, allowing its use with optical filters designed for this dye.
- **Alexa Fluor 647** — excited by the 647 nm line of the krypton-ion laser or the 633 nm line of the He-Ne laser and shows very little change in spectral characteristics when conjugated to most proteins. The excitation and emission spectra of Alexa Fluor 647 virtually match those of the Cy5 dye, allowing its use with optical filters designed for this dye.

Penta·His Biotin and Streptavidin-R-PE Conjugates for higher sensitivity

Penta·His Biotin and Streptavidin-R-PE Conjugates are used to detect 6xHis-tagged proteins with very high sensitivity (Figure 2). By utilizing the extremely high affinity of the streptavidin-biotin interaction and the spectroscopic properties of R-phycoerythrin, the sensitivity of immunoassays can be increased some five- to tenfold over the corresponding Alexa 532 Conjugate. R-PE is among the best dyes currently available for high-sensitivity detection. Its high quantum yield (0.98) and extinction coefficient (2.4 x 10^6) allows the detection by flow cytometry of as few as 100 receptor-bound biotinylated antibodies per cell (2).

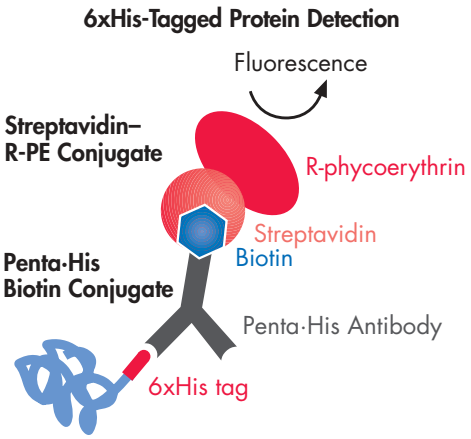


Figure 2 *Detection of 6xHis-tagged proteins using Penta·His Biotin Conjugate and Streptavidin–R-PE (not to scale).*

The high specificity and affinity of the Penta·His Antibody, combined with the superior performance offered by Alexa Fluor dyes and R-PE, make the new Penta·His Antibody Conjugates highly suitable for all immunofluorescence applications. To learn more about the QIA*express* Detection System visit us at **www.qiagen.com**, or contact QIAGEN Technical Services or your local distributor. ■

For ordering information, see page 15.

Reader Inquiry No. 01503

QIAGEN News

DNA prepared using QIAamp® protocols enables sensitive and specific PCR-based detection of *Babesia microti*

Bita Nakhai, Pinar Tuzmen, Nate Lawrence, and Mark Manak
BBI Biotech Research Laboratories, Gaithersburg, MD, USA

Manual and automated QIAamp® procedures were used to isolate high-quality DNA from blood. The DNA was used to develop a sensitive and specific assay for the detection of infection with Babesia microti*, the causative agent of babesiosis.*

Molecular diagnostic methods are highly sensitive, specific, and fast to carry out, and can therefore provide much more accurate and rapid diagnosis of infectious diseases than is possible with other diagnostic techniques. This enables patients to receive appropriate treatments promptly, which greatly improves their chances of recovery.

Babesia microti is a protozoan parasite that is the causative agent of babesiosis. Babesiosis is a parasitic tick-borne disease transmitted by the same tick vector and sharing the same host reservoir as Lyme disease. The disease occurs mainly in coastal areas of the north-eastern United States. Infections can occur in otherwise healthy individuals, but the most susceptible are the very young, the elderly, and the immunocompromized. These individuals suffer from a severe malaria-type illness, caused by attack of *B. microti* on red blood cells. A reliable molecular assay is useful in the detection of babesiosis, not only for detection of infection through ticks, but also because the disease can be transmitted through contaminated blood following a blood transfusion.

QIAamp technology provides good yields of highly pure nucleic acid from a wide range of clinical samples, ideally suited for use in sensitive downstream diagnostic assays. For high-throughput sample preparation, automated QIAamp protocols are available for use with BioRobot® workstations. We used the QIAamp DNA Blood Mini Kit and the QIAamp 96 DNA Blood BioRobot Kit for the isolation of DNA from blood samples for the diagnosis of patients infected with *Babesia microti*. Isolated DNA was amplified by PCR, using primers complementary to an rRNA gene of *B. microti*, and PCR products were analyzed by agarose gel electrophoresis and detected by Southern blotting. This method was simple and rapid to perform and yielded highly specific and sensitive results.

Materials and methods

Blood samples were taken into EDTA tubes from patients infected with *B. microti* and from healthy control individuals. DNA was isolated from 200 µl blood samples using manual QIAamp procedures (QIAamp DNA Blood Mini Kit) or QIAamp protocols automated on the BioRobot 9604 (QIAamp 96 DNA Blood BioRobot Kit).

DNA samples were amplified by PCR, using a primer pair (Bab F2 and Bab R2) specific for a fragment of an rRNA gene of *B. microti*. PCR products were analyzed by agarose gel electrophoresis. To confirm the identity of the products, the gels containing the products were Southern blotted, hybridized with an alkaline phosphatase-labeled probe specific to the *B. microti* genome, and visualized using CSPD, a chemiluminescent substrate.

To test the sensitivity of the method, serial dilutions either of hamster whole blood infected with *B. microti*, or of a cloned fragment of *B. microti* DNA were made in media, and DNA was isolated using QIAamp manual or automated protocols. Ten-fold serial dilutions of DNA samples were prepared down to a dilution of 10^{-10} and the DNA was amplified using Bab F2 and Bab R2 primers. ▶

The assay was validated by amplification and blind analysis of a number of patient samples together with control samples from healthy individuals. These analyses were repeated in an independent lab.

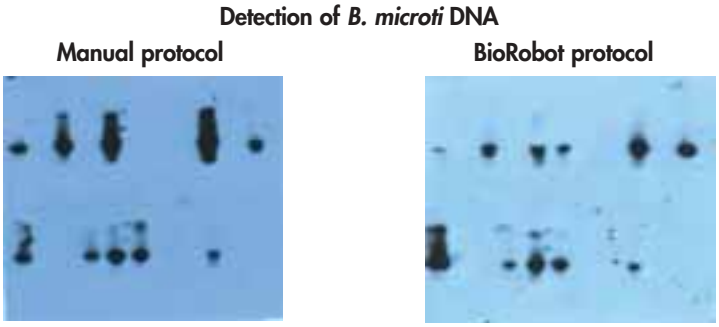


Figure 1 *DNA was isolated from clinical samples using QIAamp manual and automated protocols. Specific 240 bp products were amplified from* B. microti *DNA. Southern blots of the amplified products are shown. The assay correctly identified all clinical positive and negative samples.*

Results and discussion

Use of either the manual QIAamp DNA Blood Mini Kit protocol or the automated QIAamp 96 DNA Blood BioRobot Kit protocol yielded high-quality DNA. This was used to develop a molecular assay for detection of *B. microti* infection. PCR amplification of isolated DNA from a number of patient samples resulted in the production of specific 240 bp products (Figure 1). In contrast, PCR amplification of DNA isolated from uninfected control individuals consistently gave no products.

Hybridization of the PCR products amplified from patient samples with an alkaline phosphatase-labeled probe specific to the *B. microti* genome confirmed that they were amplified from *B. microti* DNA. Serial dilution experiments using DNA isolated from hamster cells infected with *B. microti* revealed that DNA could be detected down to a dilution of 10^{-8} (Figure 2 A). Comparison with PCR products amplified from cloned *B. microti* DNA shows that the intensity of the lowest dilution detected corresponds to an equivalent of 10 copies of Babesia DNA (Figure 2 B).

In blind testing, the assay successfully distinguished between samples taken from infected patients and those from healthy control individuals. Analysis of a number of samples in an independent laboratory yielded identical results, confirming the validity of the assay (Table 1).

Conclusions

Use of either the QIAamp DNA Blood Mini Kit or the QIAamp 96 DNA Blood BioRobot Kit provided good yields of high-quality *B. microti* DNA. This enabled the development of a PCR-based assay for highly specific and sensitive detection of *B. microti* infection. Serial dilution experiments revealed that the method was able to detect as few as 10 copies of *B. microti* DNA. QIAamp Kits therefore provide an efficient system for the isolation of highly pure *B. microti* DNA from blood that performs well in downstream PCR assays.

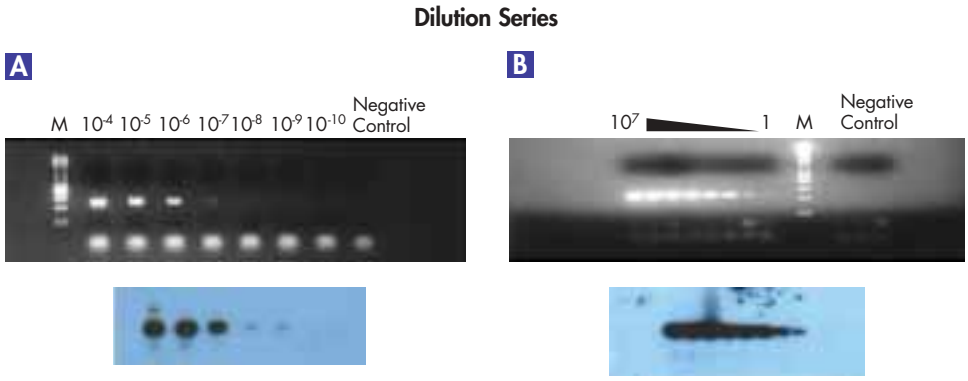


Figure 2 *Agarose gel analysis and Southern blotting of PCR products amplified using primers Bab F2 and Bab R2. Templates were* **A** *dilutions of hamster cell DNA infected with* B. microti *or* **B** *cloned* B. microti *DNA. In (A), products could be detected down to a dilution of* 10^{-8}*, which corresponds to 10 copies of* B. microti *DNA in (B).* ***M****: markers.*

Table 1. Blind testing by PCR of blood samples from a mixed group of patients and control individuals

Sample	Extraction using QIAamp DNA Blood BioRobot Kit	Extraction using QIAamp DNA Blood Kit	Results from independent laboratory
T1	+	+	+
T2	–	–	–
T3	+	+	+
T4	–	–	–
T5	+	+	+
T6	+	+/–	+
T7	–	–	–
T8	–	–	–
T9	+	+	+
T10	–	–	–
T11	+	+	+
T12	–	–	–
T13	–	–	–
T14	+	+	+
T15	+	+	+
T16	+	+	+
T17	–	–	–
T18	–	–	–
T19	+	+	+
T20	–	–	–

DNA extracted from 20 clinical samples was amplified by PCR. +: PCR product detected; –: no PCR product detected.

Reader Inquiry No. 01504

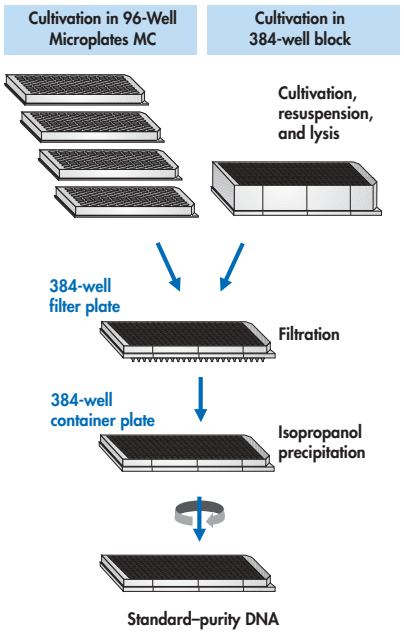
Ordering Information

Product	Contents	Cat. No.
QIAamp DNA Blood Mini Kit (50)*	For 50 DNA minipreps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
QIAamp 96 DNA Blood BioRobot Kit (12)	For 12 x 96 DNA preps: 12 QIAamp 96 Plates, Buffers, QIAGEN Protease, AirPore™ Tape Sheets, Tape Pad, S-Blocks, Racks with Collection Microtubes (1.2 ml), Caps	965142

** Larger kit sizes available; please inquire*

New *micro*R.E.A.L.™ Prep 384 Plasmid Kit — for very high-throughput isolation of plasmid DNA

_micro_R.E.A.L. Prep 384 Plasmid Kit Procedure



QIAGEN introduces the *micro*R.E.A.L.™ Prep 384 Plasmid Kit for rapid very high-throughput isolation of plasmid DNA from small-volume cultures (300–350 µl). Using a modified alkaline lysis procedure adapted for 384-well processing, the *micro*R.E.A.L. Prep 384 System yields plasmid DNA suitable for use in routine molecular biology applications. These include high-throughput automated screening and sequencing procedures, notably capillary electrophoresis sequencing.

The *micro*R.E.A.L. Prep 384 System offers:

- **Flexibility** — choice of 96- or 384-well culture formats
- **Reproducibility** — for reliable well-to-well sequencing results
- **Economy** — simple, cost-effective method
- **High speed** — up to 2 x 384 minipreps in 135 minutes

The *micro*R.E.A.L. Prep 384 System processes multiples of 384 samples using centrifugation for rapid, very high-throughput purification of plasmids from small-volume bacterial cultures.

Flexible procedure

Cultivation and lysis are carried out either in 96-well cultivation plates or in 384-well blocks (not supplied with the kit, please inquire). Lysates are then transferred to a 384-well filter plate (see flowchart) for further processing. For processing blocks and plates in 384-well format, QIAGEN recommends the use of multi-channel pipetting devices such as the BioRobot® RapidPlate™.

Denatured and precipitated genomic DNA, proteins, and carbohydrates are efficiently removed from the crude lysate by centrifugation through a 384-well filter plate. Plasmid DNA in the cleared lysate is then concentrated by isopropanol precipitation. After a single wash step, the precipitated plasmid DNA is resuspended in low-salt buffer and is ready for use in downstream applications.

Reproducible yields

The yield and quality of DNA obtained using the *micro*R.E.A.L. procedure depends on the cultivation conditions and the host–vector system. Yields of 1–1.5 µg plasmid DNA are reproducibly obtained from 300 µl (384-well block cultivation) or 350 µl (96-well microplate cultivation) bacterial cultures grown in 2x YT medium (Figure 1).

Reproducible Plasmid Purification Using the *micro*R.E.A.L. 384 Prep System

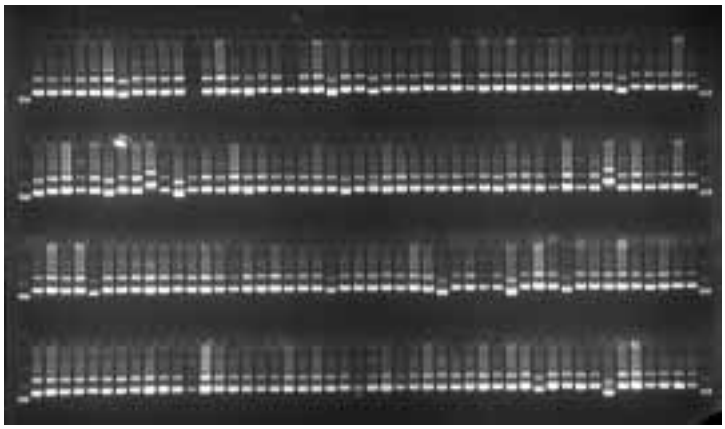


Figure 1 *Agarose gel analysis of shotgun clones purified using the microR.E.A.L. Prep 384 procedure. 20% (6 µl of a 30 µl sample) of the purified plasmid DNA was loaded per lane.* ***Outer lanes****: 100 ng marker plasmid DNA.*

DNA for high-throughput applications

The plasmid DNA obtained using the *micro*R.E.A.L. Prep 384 Plasmid Kit is highly suited for automated sequencing applications, such as high-throughput fluorescent sequencing using capillary or slab gel-based systems (Figure 2). For fluorescent sequencing reactions, QIAGEN recommends the use of BigDye™ Terminator chemistry or DYEnamic™ ET dye-terminator chemistry. Diluted sequencing-reaction premixes can be used for sequencing. Detailed protocols are available in the micro*R.E.A.L. Prep 384 Plasmid Handbook*.

For a free copy of this handbook or any QIAGEN literature, please contact QIAGEN Technical Services or your local distributor. Alternatively, the complete range of QIAGEN literature is available to download or view in a convenient PDF format 24 hours a day at **www.qiagen.com/literature**. Printed literature can also be requested via the website. ■

Reader Inquiry No. 01505

Phred Analysis of Bacterial Clones Prepared Using the *micro*R.E.A.L. Prep 384 Plasmid Kit

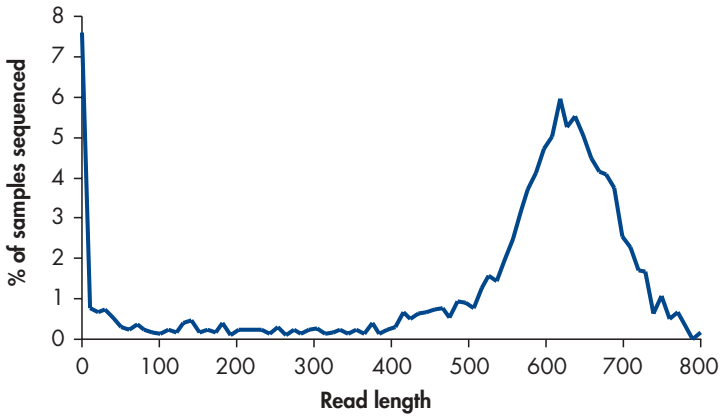


Figure 2 *Phred 20 analysis of bacterial clones prepared using the* micro*R.E.A.L. Prep 384 Plasmid Kit. 2323 plasmid shotgun sequences were derived from a bacterial genome project. Clones were sequenced using BigDye Terminator chemistry (quarter reactions) and analyzed using an ABI PRISM® 3700 DNA Analyzer with POP™-5 gel matrix. The analysis shows the percentage of sequencing reads, which were calculated using sequential windows of 10 bases.*

Ordering Information

Product	Contents	Cat. No.
***micro*R.E.A.L. Prep 384 Plasmid Kit — for very high-throughput plasmid minipreps**		
*micro*R.E.A.L. Prep 384 Plasmid Kit (20)	For 20 x 384 rapid extraction alkaline lysis minipreps	26363
Accessories		
96-Well Microplates MC (40)	40 x 96-well microplates for bacterial cultivation	19586
AirPore™ Tape Sheets (50)	Microporous tape sheets for covering 96-well blocks during bacterial cultivation	19571
Related products		
MagAttract™ Miniprep Core Kit — for high-throughput plasmid minipreps in a 96-well format		
MagAttract 96 Miniprep Core Kit (24)*	MagAttract Suspension (Miniprep) and buffers for 24 x 96 minipreps	120030
R.E.A.L.® Prep 96 Plasmid Kit — for economical, high-throughput rapid extraction alkaline lysis minipreps in a 96-well format		
R.E.A.L. Prep 96 Plasmid Kit (4)†	For 4 x 96 rapid extraction alkaline lysis minipreps	26171
QIAprep® 96 Turbo Miniprep Kit — for streamlined preparation of 96 high-purity plasmid minipreps		
QIAprep 96 Turbo Miniprep Kit (4)†‡	For 4 x 96 high-purity plasmid minipreps	27191

Note: *For growing bacterial cultures in 96-well format for the* micro*R.E.A.L. Prep 384 procedure, 96-Well Microplates MC are required. For cultivation in 384-well blocks, 384-well blocks and AirPore Tape Sheets are required.*

* *Designed for use with a robotic workstation; requires use of a 96-Well Magnet.*
† *Larger kit sizes available; please inquire.*
‡ *Requires use of QIAvac 96.*

Long Read-Length Using the MagAttract 96 Miniprep System

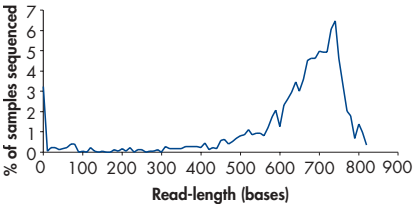
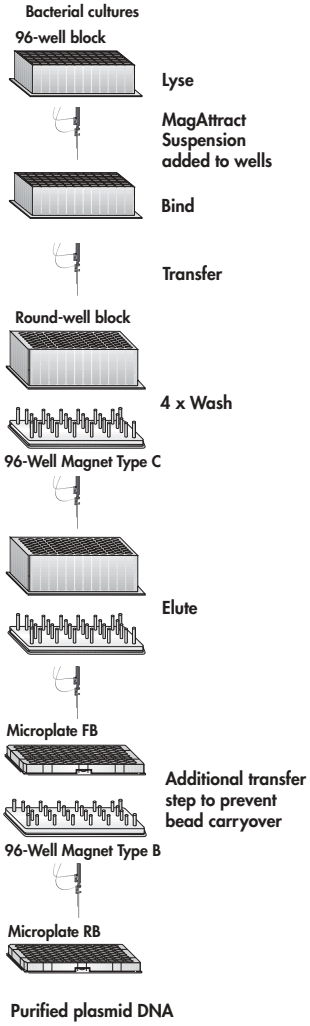


Figure 1 *Phred 20 analysis of 1722 shotgun clone sequences from plasmid DNA isolated for a bacterial genome project. Clones were prepared using the MagAttract 96 Miniprep System, sequenced using BigDye™ Terminator chemistry, and analyzed using an ABI PRISM® 3700 DNA Analyzer with POP™-5 gel matrix. The analysis shows the percentage of sequence reads, which were calculated using sequential windows of 10 bases.*

MagAttract 96 Miniprep Procedure



continued from page 1

Automation for walkaway purification of sequencing templates

Automation of standard applications will increase the throughput of your laboratory and the reproducibility of downstream applications. Levels of laboratory automation are increasing due to powerful new technologies and the increasing throughput of modern scientific techniques.

High-throughput methods in molecular biology, such as capillary sequencing, demand purification technologies that are non-labor-intensive, economical, reproducible, and provide high-purity nucleic acids. MagAttract 96 Miniprep technology gives reproducibly high yields of high-copy-number plasmids, ensuring that you only have to process samples once. With mean read-lengths of approximately 630 bases using capillary sequencing (Figure 1), the MagAttract 96 Miniprep System increases the speed and scope of your research.

High-throughput chemistries for plasmid purification

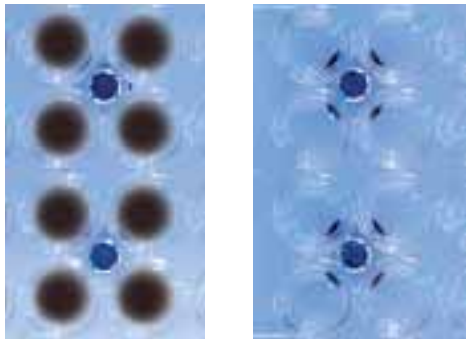
The MagAttract 96 Miniprep System provides a fully automated solution for high-throughput plasmid purification on the BioRobot 8000. The automation-compatible format of this magnetic separation technology allows the MagAttract Miniprep System to be used with other robotic systems.

The MagAttract principle relies on the high affinity of plasmid DNA for silica particles under specific denaturing conditions. MagAttract particles consist of a ferromagnetic core that is coated with silica using an innovative manufacturing process developed at QIAGEN. Each bead has a large surface area providing a high nucleic acid binding capacity — 3 µg per 20 µl MagAttract Suspension. MagAttract technology allows purification of plasmid DNA directly from crude bacterial lysates, eliminating the need for centrifugation or filtration steps.

In the MagAttract Miniprep protocol, harvested bacterial pellets are resuspended in lysis buffer. The novel lysis procedure is carried out in the 96-well block and ensures sufficient disruption of cells while providing optimal binding conditions. MagAttract bead suspension is added to the lysate and rapidly binds plasmid DNA, while all other cell components remain in solution.

96-Well Magnet

Rapid and Efficient Separation



A simple and effective protocol

MagAttract beads show a strong induced movement when the block is placed onto a 96-well magnet. Beads and associated plasmid DNA are rapidly concentrated at the side of the well nearest to the magnetic rod, even in viscous bacterial lysate, ensuring effective separation. MagAttract beads are quickly and easily resuspended in the absence of a magnetic field. Lysates are aspirated and beads are washed to remove contaminants. After washing, 50 µl of low–salt elution buffer is added. Beads are then concentrated using the magnet, and pure plasmid DNA is aspirated into a microplate. Eluate volume is then reduced to 20 µl by a heated incubation, yielding a high final concentration of approximately 150 ng/µl. Consistent yields of 3 µg per well (Figure 2) ensure that there is sufficient DNA for several successful sequencing reactions plus archiving.

Conclusions

The MagAttract 96 Miniprep System offers fully automated, high-throughput plasmid purification technology for state-of-the-art applications in the fields of genomics and screening.

- **Consistent yields** —3 µg of high-copy plasmid from 1.25 ml overnight culture
- **Automation compatible** — optimized and ready-to-run on the BioRobot 8000
- **Convenient high-throughput format** — 96-well format from start to finish

Reader Inquiry No. 01501

An Intuitive User Interface

Figure 4 *The fully automated MagAttract 96 Miniprep procedure in QIAsoft™, the BioRobot Operating System software. Protocol selection and execution is all at the click of a button, and intuitive user-prompts appear at the start of the procedure.*

Reproducible DNA Yields

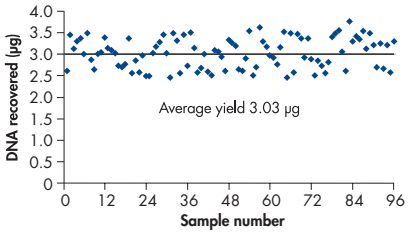


Figure 2 *DNA yields obtained using the MagAttract Miniprep procedure from 1.25 ml overnight culture of XL-1 Blue E.coli cells containing the plasmid pUC18. Cells were grown in a 96-well Flat Bottom Block with shaking at 300 rpm. Yields are based on A_{260} measurements of the individual samples.*

High-Purity DNA

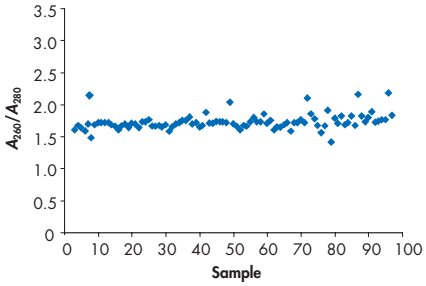


Figure 3 *Bacterial cultures were grown in a 96-well block. Plasmid pUC18 was purified using the MagAttract 96 protocol on the BioRobot 8000. Purified DNA was diluted in water and the A_{260}/A_{280} ratio was measured. A mean ratio of 1.72 was obtained.*

Ordering Information

Product	Contents	Cat. No.
MagAttract 96 Miniprep Core Kit (24)*	MagAttract Suspension (Miniprep) and buffers for 24 x 96 minipreps	120030
Accessories		
Flat-Bottom Blocks (24)	96-well blocks with 2 ml wells, 24 per case	19579
AirPore™ Tape Sheets (25)	Microporous tape sheets for covering 96-well blocks: 25 per pack	120001
Tape Pads (5)	Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack	19570
Round-Well Blocks (24)	96-well blocks with 1.2 ml wells, 24 per case	19576
96-Well Microplates FB (24)	96-well microplates with flat-bottom wells, 24 per case	36985
96-Well Microplates RB (24)	96-well microplates with round-bottom wells plus lids, 24 per case	19581

** The MagAttract 96 procedure requires the use of the following equipment which is not supplied with the kit: a robotic workstation e.g., BioRobot 8000, 96-well magnets Type B and Type C, a Reagent Holder (5-trough 80 ml) a MagAttract suspension reservoir (e.g., Disposable Troughs), 96-well flat-bottom blocks for optimal cell cultivation, 96-well round-well blocks, flat-bottom 96-well microplates (e.g., 96-Well Microplates FB), round-bottom 96-well microplates (e.g., 96-Well Microplates RB), and porous tape sheets for covering 96-well flat-bottom blocks during cell cultivation.*

Significantly higher yields from automated protein purification procedures

Ulla Römer, Julia Blümer, and Frank Schäfer
QIAGEN GmbH, Hilden, Germany

In response to customers' needs for larger amounts of protein from automated procedures, QIAGEN has developed an optimized Ni-NTA Superflow 96 BioRobot® protocol that allows the processing of larger culture volumes and the purification of milligram amounts of 6xHis-tagged protein per well. Increasing the amount of Ni-NTA Superflow resin used in the procedure and an optimized lysis buffer formulation enables up to 25 ml (purification under native conditions) or 15 ml (purification under denaturing conditions) of cell culture medium (LB) to be processed. The corresponding increase in biomass can deliver up to 4 mg of pure 6xHis-tagged protein per well (Table 1; Figure 1), allowing multiple assays to be carried out on the same batch of protein and reducing the total number of protein preps required. The single-step purification provides highly pure proteins over a wide range of yields, and the purification of large protein complexes is possible (e.g., the complete GroEL/GroES complex of 910 kDa can be isolated). Proteins immobilized during the purification procedure can be used to capture nontagged interaction partners, both when co-overexpressed (E lanes, Figure 1), or endogenously produced (S lanes, Figure 1). ■

Milligram Amounts of Pure 6xHis-Tagged Protein per Well

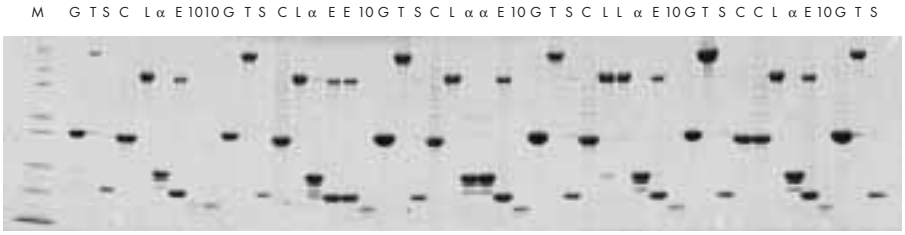


Figure 1 *Vector constructs for the expression of 6xHis-tagged proteins were transformed into* E. coli, *plated on selective medium, and colonies were picked for inoculating 25 ml cultures. Expression of 6xHis-tagged proteins was induced with IPTG for 2–4 hours. Cells were pelleted in 24-well blocks and processed on the BioRobot 3000 using 200 µl Ni-NTA Superflow resin per well. 5 µl (0.9%) of the first elution fraction was loaded for SDS-PAGE and proteins were visualized by Coomassie® staining.* ***G****: Green Fluorescent Protein (29 kDa);* ***T****: T7 RNA Polymerase (100 kDa);* ***S****:* E. coli *GroES (12 kDa). Some endogenous GroEL is copurified;* ***C****:* E. coli *chloramphenicol acetyltransferase (28 kDa);* ***L****:* E. coli *GroEL (60 kDa);* ***α****: human tumor necrosis factor α (18 kDa);* ***E****:* E. coli *GroES purified as a complex with co-overexpressed nontagged GroEL (12 and 60 kDa);* ***10****:* Saccharomyces cerevisiae *Cpn-10 (10 kDa).* ***M****: markers.*

Table 1. Yields of 6xHis-tagged proteins using the optimized Ni-NTA Superflow 96 BioRobot protocol

6xHis-tagged protein	Total yield per well (µg)*	Protein concentration (mg/ml)†
Green fluorescent protein	4000	6.0
T7 RNA polymerase	1000	1.4
GroES	300	0.4
Chloramphenicol acetyltransferase	2400	4.4
GroEL	740	1.0
Tumor necrosis factor α	1600	2.5
GroES/GroEL	1200	1.5
Cpn-10	170	0.3

** Yield obtained in two 550 µl elution fractions (average of 6 independent purifications). 80% of the protein elutes in the first 550 µl. † Protein concentration in the first 550 µl elution fraction.*

Reader Inquiry No. 01506

For more information on this application please contact your QIAGEN Sales Representative or visit us at **www.qiagen.com/automation**.

For ordering information, see page 15.

Important considerations for generating transgenic mice

The development of technology to manipulate the genome of animals has revolutionized biology, biomedical research, and biotechnology. The ability to add a specific gene to, or remove a specific gene from, a complex organism allows precise characterization of gene function, and allows generation of animal models for studying specific diseases and developmental processes. The animals can also be used for testing pharmacological molecules or gene therapy protocols.

This article gives a short overview of the procedure as well as important considerations for generating transgenic mice, in which an extra gene (transgene) is randomly inserted into the genome. The discussion applies equally to generation of other transgenic animals.

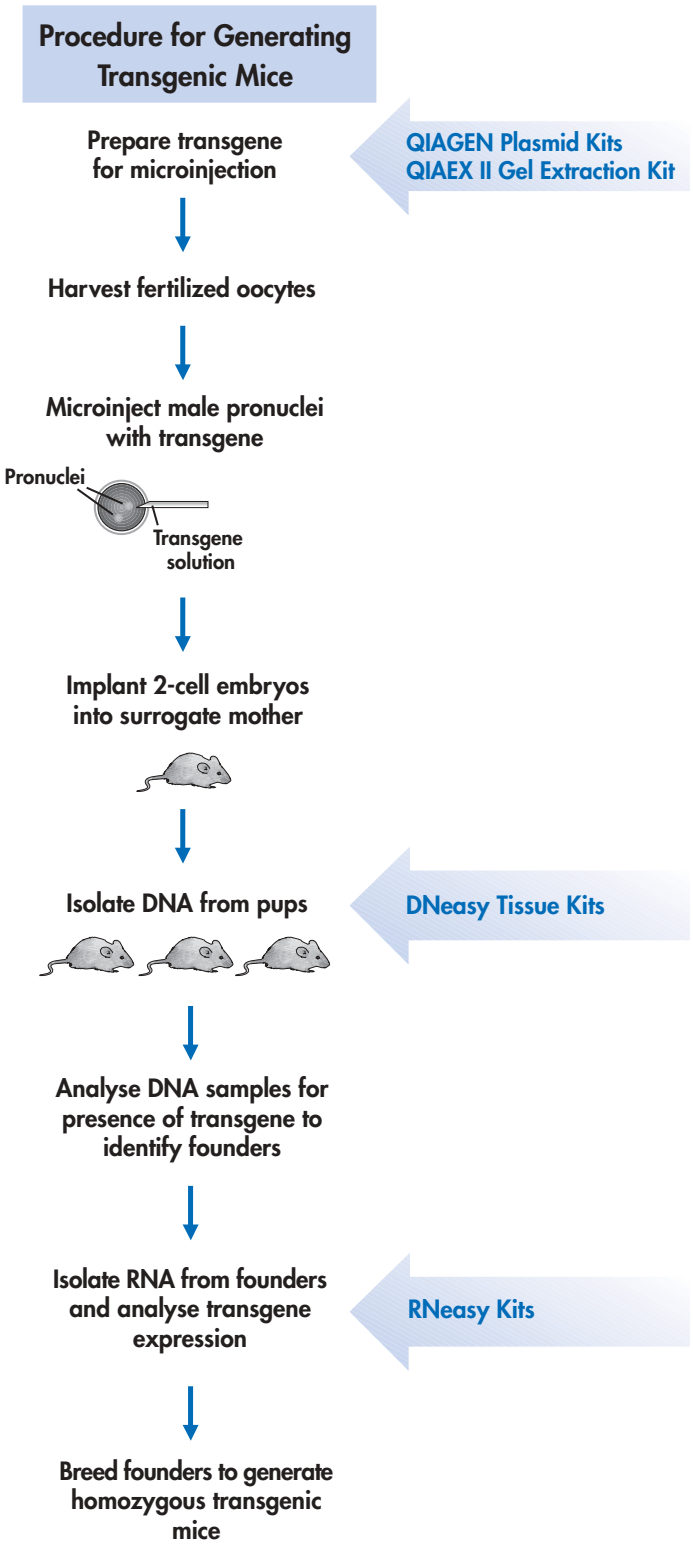
Generation of transgenic mice

Pronuclear DNA microinjection is the most commonly used method to generate transgenic mice (see flowchart). Fertilized oocytes are removed from the oviduct of a mouse, and the male pronucleus is microinjected with a solution containing the transgene. The injected eggs are cultured in vivo until the pronuclei have fused and the zygote has developed into a 2-cell embryo. The embryos are then transplanted into a surrogate mother, and pups are born 19–21 days later. Pups that developed from a zygote that successfully integrated the microinjected DNA will have the transgene in every cell of their body. These heterozygous animals (called founders) can then be bred to obtain homozygous mice.

Important considerations

Transgene preparation

Preparation of the transgene used for microinjection is critical for successful generation of transgenic animals. In general, transgenes derived from genomic DNA are expressed at higher levels than those derived from cDNA (1). The vector construct must contain the necessary promoter elements for expression of the transgene in the host cells. ▶



References

1. *Transgenic Core, Boston University School of Medicine (www.bu.edu/index.html)*
2. *NICHD Transgenic Mouse Development Facility, University of Alabama (main.uab.edu/sys/images/pdf/NICHD.pdf)*
3. *Transgenic Mouse Core Facility, University of Virginia Health System (www.med.virginia.edu/medicine/inter-dis/transgenic-mouse/DNAinj.html)*
4. *Blumberg, H. et al. (2001) Interleukin 20: discovery, receptor identification, and role in epidermal function. Cell* ***104****, 9.*
5. *Merscher, S. et al. (2000) TBX1 is responsible for cardiovascular defects in Velo-cardio-facial/DiGeorge syndrome. Cell* ***104****, 619.*
6. *Moeller, M.J., Kovari, I.A., and Holzman, L.B. (2000) Evaluation of a new tool for exploring podocyte biology: mouse* Nphs 1 *5' flanking region drives LacZ expression in podocytes. J. Am. Soc. Nephrol.* ***11****, 2306.*
7. *Transgenic animals (www.acs.ucalgary.ca/~browder/transgenic.html)*

Linearization of the vector and removal of unnecessary vector sequences through the use of appropriate restriction enzymes usually improves integration success and leads to higher expression rates (1, 2).

Vector DNA must be highly pure for successful generation of transgenic mice (1). Use of degraded DNA will result in lower numbers of transgenic pups, or none at all. Contaminants present in the DNA can be harmful to the embryo and prevent incorporation of the vector into the genome and/or expression of the transgene, and particulate matter can clog the microinjection pipettes. Several transgenic core facilities recommend the use of QIAGEN® Plasmid Kits for isolation of ultrapure plasmid DNA and the QIAEX® II Gel Extraction Kit for purification of DNA fragments from agarose gels following restriction digestion (e.g., 1, 2, 3).

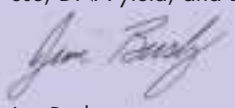
Identification of founders

Approximately 10–20% of pups can be expected to be transgenic. The litter must therefore be screened to identify founder animals. Both Southern-blot and PCR analysis can be used for screening. Southern-blot analysis is typically preferred for identifying founder animals (1) and allows determination of the number of integration sites, transgene copy number, and transgene integrity. PCR analysis is typically used for monitoring the breeding process once the founder(s) has been identified.

DNA for screening is usually isolated from tail samples and should be highly pure to avoid false positive and false negative results (1). Since only a small percentage of the pups is expected to be transgenic, the time required for screening should be minimized in order to keep animal housing costs low. DNeasy® Tissue Kits have been successfully used for isolation of DNA from mouse tails for screening in transgenic studies (4, 5, 6).

Analysis of transgene expression

Transgene expression can be influenced by the DNA surrounding the insertion site (7) and therefore must be thoroughly characterized before using animals in experimental systems. Transgene expression can be analyzed by northern-blot or RT-PCR analysis. To avoid false negative results, RNA should be intact and highly pure. RNeasy® Kits have been successfully used for isolation of RNA from mouse tissue for RT-PCR analysis of transgene expression (4).

> "We tested three different methods for isolation of genomic DNA from mouse tail samples. All samples were collected at the same time and processed according to the manufacturer's specifications. The DNeasy 96 Tissue Kit from QIAGEN yielded DNA of excellent quality, while DNA isolated using a home-made precipitation method and a kit from another supplier showed signs of degradation. I would recommend the DNeasy 96 Tissue Kit for ease of use, DNA yield, and DNA quality."
>
> 
>
> Jim Busby
> Research Associate
> Amgen, Inc., USA

Summary

- Vector DNA purity is a critical factor for successful generation of transgenic mice. Use of DNA purified with QIAGEN kits is recommended by several transgenic core facilities.
- Timely identification of founders requires high-purity DNA to avoid false-positive and false-negative results. DNeasy Tissue Kits provide a fast and convenient method for isolating DNA from mouse tails for use in PCR and Southern-blot analysis, and are highly suited for high-throughput applications.
- Analysis of transgene expression requires intact, pure RNA. RNeasy Kits allow fast and easy isolation of RNA that is suitable for all downstream applications, including northern-blot, slot-blot, and RT-PCR analysis.

A discussion of knock-out mice will appear in a future issue of *QIAGEN News*. ■

Reader Inquiry No. 01507

Ordering Information

Product	Contents	Cat. No.
EndoFree® Plasmid Kits — for purification of endotoxin-free ultrapure plasmid DNA		
EndoFree Plasmid Maxi Kit (10)*	10 QIAGEN-tip 500, 10 QIAfilter™ Maxi Cartridges, Buffers	12362
QIAEX II Gel Extraction Kits — for purification of DNA following agarose gel analysis		
QIAEX II Gel Extraction Kit (150)†	For up to 150 extractions: QIAEX II Suspension, Buffers	20021
DNeasy Tissue Kits — for isolation of genomic DNA from animal tissues and cells, yeast, or bacteria		
DNeasy Tissue Kit (50)†	50 DNeasy Spin Columns, Reagents and Buffers, Collection Tubes (2 ml)	69504
DNeasy 96 Tissue Kits‡ — for high-throughput DNA isolation from rodent tails and animal tissues		
DNeasy 96 Tissue Kit (4)†	For 4 x 96 DNA minipreps: 4 DNeasy 96 Plates, Reagents and Buffers, Collection Microtubes (1.2 ml), Collection Microtube Caps, 96-Well-Plate Registers	69581
RNeasy Kits — for isolation of total RNA from animal cells or tissues, yeast, or bacteria		
RNeasy Mini Kit (50)*†	50 RNeasy Mini Spin Columns, RNase-free Reagents and Buffers, Collection Tubes (2 ml)	74104

"QIAGEN broadens its spectrum of detection reagents — Penta·His™ antibody conjugates" (see page 3).

Product	Contents	Cat. No.
Penta·His Alexa Fluor® 488 Conjugate	125 µl Penta·His Alexa Fluor 488 Conjugate	35310
Penta·His Alexa Fluor 532 Conjugate	125 µl Penta·His Alexa Fluor 532 Conjugate	35330
Penta·His Alexa Fluor 555 Conjugate	125 µl Penta·His Alexa Fluor 555 Conjugate	35350
Penta·His Alexa Fluor 647 Conjugate	125 µl Penta·His Alexa Fluor 647 Conjugate	35370
Penta·His Biotin Conjugate	125 µl Penta·His Biotin Conjugate	34440
Streptavidin–R-PE	250 µl Streptavidin–R-phycoerythrin Conjugate	922721

"Significantly higher yields from automated protein purification procedures" (see page 12).

Product	Contents	Cat. No.
Ni-NTA Superflow™ 96 BioRobot® Kit (24)	For 24 x 96 6xHis-tagged protein preps: 24 QIAfilter 96 Plates, 24 TurboFilter 96 Plates, 3 x 100 ml Ni-NTA Superflow	969263
Ni-NTA Superflow (100 ml)§	100 ml nickel-charged resin (max. pressure: 140 psi)	30430

** Different kit formats available; please inquire*
† Larger kit sizes available; please inquire
‡ Requires use of the QIAGEN 96-Well-Plate Centrifuge System
§ Additional Ni-NTA Superflow resin required when using 200 µl per well, as described in the article (page 12).

Uncovering picoplankton biodiversity by sequencing of environmental rRNA genes

Klaus Valentin,* Khadidja Romari,† and Fabrice Not†

*Alfred Wegener Institute, Bremerhaven, Germany. E-mail: kvalentin@awi-bremerhaven.de

† Station Biologique, CNRS and Université Pierre et Marie Curie, Roscoff, France.

Picoplankton, small oceanic organisms that play a major role in the world's primary photosynthetic production, are not well characterized. This article describes the sequencing of marker genes, performed by QIAGEN Genomics Sequencing Services, as an efficient tool to examine the biodiversity of picoplankton.

Picoplankton

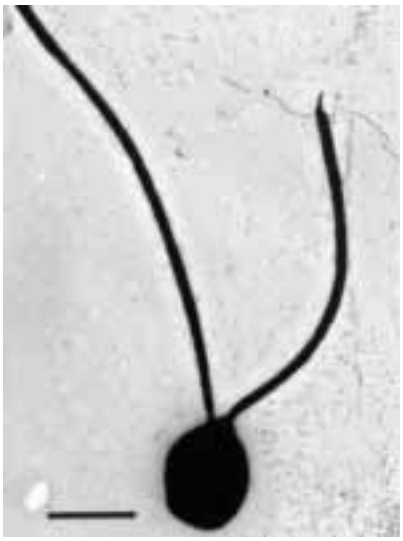


Figure 1 Pseudoscourfieldia marina, *one species of eukaryotic marine picoplankton. The bar represents 1 µm.*

Phytoplankton, microscopic unicellular algae found in the ocean, dominate the world's photosynthetic primary production. However, large areas of the open oceans are oligotrophic, i.e., lacking in nutrients, such as nitrogen, phosphorus, or iron, that are essential for algal growth. A morphological adaptation of the phytoplankton in such waters is a reduction in size, with the corresponding increase in the cell surface-to-mass ratio permitting easier uptake of nutrients. Indeed, in many oceanic regions small phytoplankton, 3 µm or less in size and called picoplankton (Figure 1), contribute up to 80% of the total chlorophyll (1, 2).

Despite their obvious importance, picoplankton have not been extensively studied until recently. Most picoplankton species are difficult to culture and, due to their small size, often lack characteristic features that can be distinguished by light microscopy. Molecular techniques such as the sequencing of marker genes or FISH (fluorescence in situ hybridization) can overcome these problems and help in the determination of picoplankton biodiversity. Recently published data based on molecular studies confirmed the presence of new eukaryotic groups in the picoplankton (3, 4, 5).

The aim of the European Union PICODIV project‡ is to analyze the biodiversity and abundance of picoplankton at various sites throughout the year. For these studies, molecular approaches are combined with "classical" methods such as light/electron microscopy, culturing, and pigment analysis. Here we describe the large-scale sequencing of environmental rRNA genes by QIAGEN Genomics Sequencing Services as an efficient way to examine the biodiversity of picoplankton.

Materials and methods

Samples were collected on a monthly or bimonthly basis at three sites in European coastal waters (Roscoff, France; Barcelona/Blanes Bay, Spain; and Helgoland, Germany). 5–10 liters of seawater was filtered through 3 µm filters, and this filtrate was then passed through 0.2 µm filters.

Environmental DNA was isolated from the 0.2 µm filters, and complete 18S and 16S rRNA genes were amplified by PCR using universal primers. PCR products were purified by agarose gel electrophoresis or using the QIAquick® PCR Purification Kit. Purified PCR products were cloned into a TA vector, generating at least 500 clones per sample. Clones were assessed by restriction fragment length polymorphism (RFLP) analysis of either reamplified inserts or of plasmid DNA isolated using QIAprep® columns. Unique clones (as judged by RFLP analysis) were sequenced by QIAGEN Genomics Sequencing Services using an internal and flanking primers.

Sequences provided by QIAGEN Genomics Sequencing Services were compared to each other (to sort out redundant clones) and to 18S/16S rRNA gene databanks (RDP, Genbank). Such comparisons allow a rough classification of sequences at the class or

‡ *www.sb-roscoff.fr/Phyto/PICODIV/index.html*

genus level and the identification of known species. Sequences were then placed into a universal tree using the ARB program (www.arb-home.de).

Results

Eukaryotic clone libraries were established and sequenced for 2 sites (Roscoff and Helgoland) over a full year. In total, about 1000 clones were partially sequenced, resulting in approximately 500 different sequences. The most predominant algal groups detected were Prasinophyceae, Bolidophyceae, Cryptophyta, Chrysophyceae, and Chlorarachniophyta. The first two groups were present in all libraries. Sequences for many heterotrophic eukaryotes (e.g., Alveolates, Stramenopiles, and Ciliates) were also recovered (Figure 2). The majority of sequences analyzed were previously unknown, potentially indicating the existence of new species of picoplankton.

Conclusions

- Large-scale sequencing of environmental rRNA gene clones is an efficient tool for uncovering the biodiversity of marine picoplankton. The picoplankton community in coastal waters contains an unexpected degree of biodiversity, with most algal classes represented in this group of organisms. Most of the picoplankton species still await description.
- Subcontracting the time-consuming sequencing efforts to QIAGEN Genomics Sequencing Services proved to be very cost-effective since it allowed the rapid and accurate acquisition of a large number of sequences.

Acknowledgements

The PICODIV program is supported by contract EVK3-CT1999-00021 from the European Union. The diversity work of the Roscoff team is also supported in part by the Région Bretagne (PRIR PicoManche) and the CNRS-Aventis fund. ■

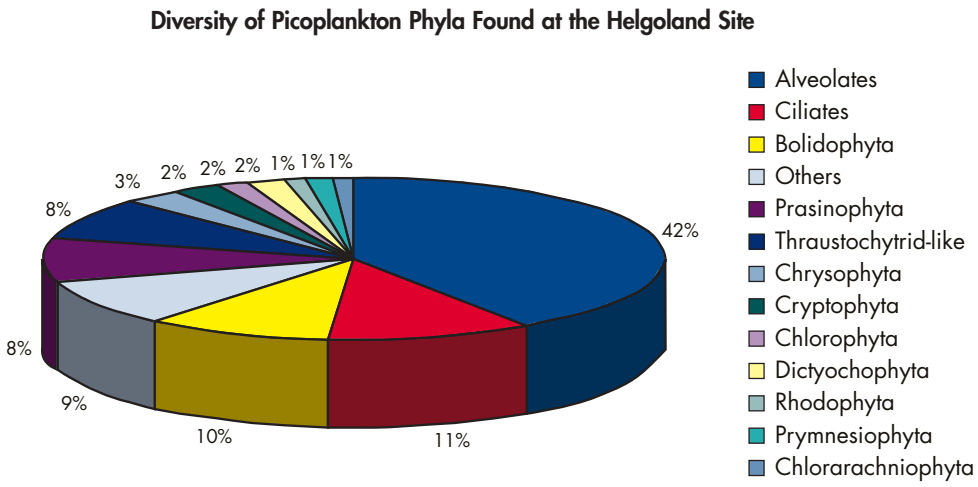


Figure 2 *Algal groups identified by sequencing of rRNA genes from picoplankton collected from the Helgoland site throughout 2000.*

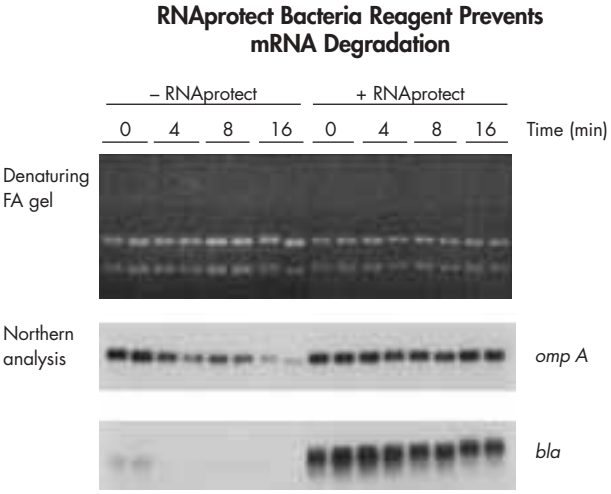
For information on QIAGEN Genomics Sequencing Services, please call QIAGEN Technical Services.

References

1. *Campbell, L., Nolla, H.A., and Vaulot, D. (1994) The importance of Prochlorococcus to community structure in the central North Pacific Ocean. Limnol. Oceanogr.* ***39****, 954.*
2. *Li, W.K.W. (1994) Primary production of prochlorophytes, cyanobacteria, and prokaryotic ultraphytoplankton: measurements from flow cytometric sorting. Limnol. Oceanogr.* ***39****,169.*
3. *Diez, B., Pedros-Alio, C., and Massana, R. (2001) Study of genetic diversity of eucaryotic picoplankton in different oceanic regions by small-subunit rRNA gene cloning and sequencing. Appl. Environ. Microbiol.* ***67****, 2932.*
4. *Guillou, L., Chretiennot-Dinet, M.J., Medlin, L.K., Claustre, H., Loiseaux-de Goer, S., and Vaulot, D. (1999) Bolidomonas: a new genus with two species belonging to a new algal class, the Bolidophyceae (Heterokonta). J. Phycol.* ***35****, 368.*
5. *Moon-van der Staay, S.Y., de Wachter, R., and Vaulot, D. (2001) Oceanic 18S rDNA sequences from Picoplankton reveal unsuspected eukaryotic diversity. Nature* ***409****,607.*

continued from page 1

Figure 1 *In order to monitor mRNA degradation only, transcription was stopped by adding the RNA polymerase inhibitor rifampicin to a growing culture of* E. coli. *The culture was split into two halves, and RNAprotect Bacteria Reagent was added to one half. Samples were left at room temperature for 0, 4, 8, and 16 minutes before centrifugation and RNA isolation. The resulting RNA was analyzed by agarose gel electrophoresis (**top panel**). Expression of two marker genes with different half lives was examined by northern blot analysis. **Middle panel**: ompA (half life of 15 minutes); **bottom panel**: beta lactamase (half life of 2–5 minutes).*



Immediate stabilization ensures reliable gene-expression analysis

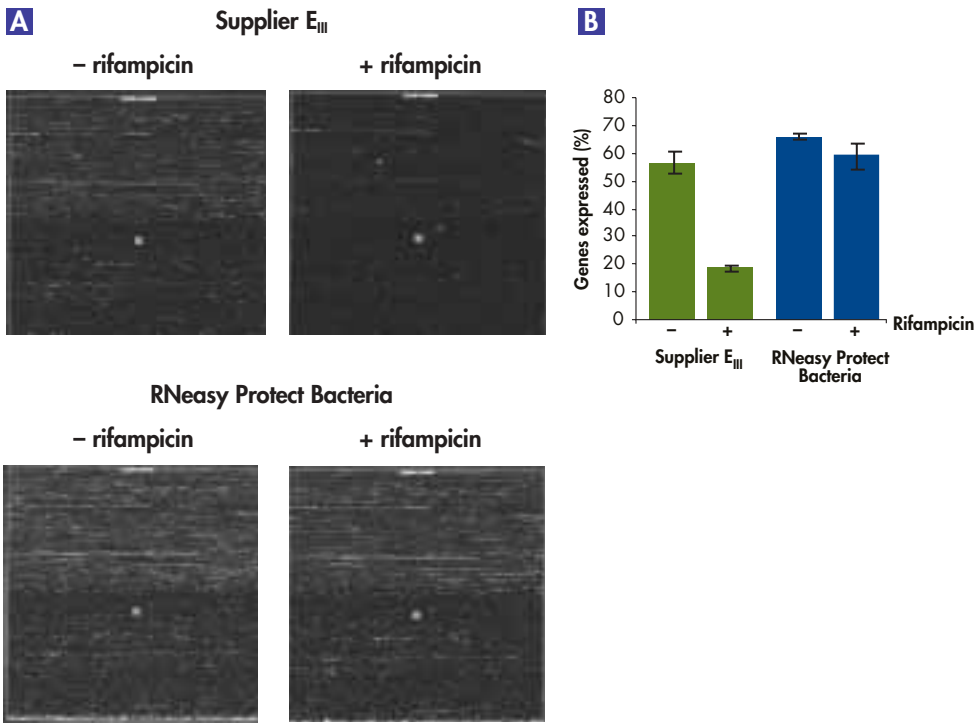
Using traditional methods for cell harvesting and RNA isolation, two major effects can lead to vast changes in bacterial expression profiles, causing artifacts in gene-expression analyses. Firstly, enzymatic degradation of RNA and/or down-regulation of genes leads to reduction or loss of many transcripts. The reduction is particularly significant with bacterial mRNA molecules because they usually have very short half lives of only a few minutes. These short-lived transcripts can degrade during the short times required for cell harvest before RNA isolation (Figure 1). Secondly, genes can be induced during handling and processing of samples, leading to higher expression levels of certain genes. Use of RNAprotect Bacteria Reagent overcomes these problems by providing immediate stabilization prior to RNA isolation. This ensures that the gene-expression profile is maintained and accurately reflects the state of the living bacteria (Figure 2).

Stabilization of RNA expression profiles

RNAprotect Bacteria Reagent has been used for stabilization of RNA in a wide range of bacterial species, including:

- Gram-positive species: *Staphylococcus aureus, Corynebacterium glutamicum, Bacillus subtilis, Clostridium difficile, Streptomyces coelicolor, Mycobactrium avium*

Figure 2 *Total RNA was isolated from RNAprotect stabilized* E. coli *cultures using the RNeasy Protect Bacteria Kit (**RNeasy Protect Bacteria**) or from unstabilized cultures using a commercial precipitation method (**Supplier E$_{III}$**). To distinguish between gene expression under defined culture conditions and effects of artifactual gene induction during harvesting and RNA isolation, the RNA polymerase inhibitor rifampicin was added to half of the culture prior to RNA isolation. Differences in transcript levels with and without rifampicin therefore generally reflect the degree of RNA degradation.* **A** *GeneChip® analysis of* E.coli *microarrays was carried out according to standard Affymetrix® protocols.* **B** *The percentage of genes expressed was estimated as the number of different transcripts determined present by GeneChip analysis divided by the total number of transcripts represented on the microarray. (Data from a collaborative study with Affymetrix.)*



- Gram-negative species: *Escherichia coli, Pseudomonas* sp., *Salmonella typhimurium, Rhizobium* sp.
- *Archaebacteria, Mycoplasma* sp.

Convenient, simple procedure

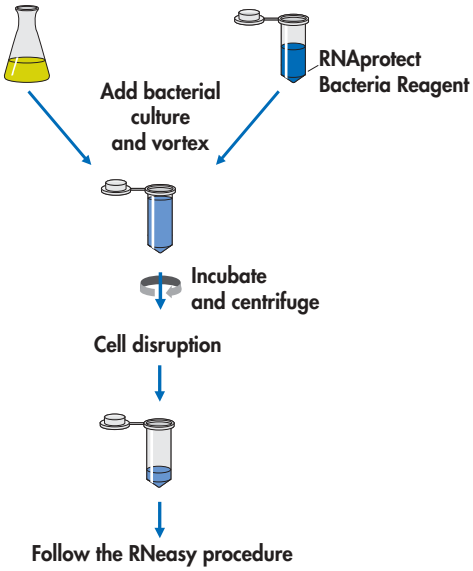
Although minimal medium is recommended for highly reproducible gene-expression analysis, the bacteria can be grown in either minimal or complex medium. Two volumes of reagent are added directly to 1 volume of bacterial culture, providing immediate stabilization of RNA. The stabilization allows time for efficient bacterial lysis using a choice of protocols: enzymatic lysis, mechanical disruption, or a combination of both methods. For best results, QIAGEN recommends using the Mixer Mill MM 300 for efficient mechanical disruption.

High-quality RNA for a wide range of applications

RNeasy Protect Bacteria Kits combine RNAprotect stabilization and RNeasy total RNA isolation to provide high-quality RNA that accurately reflects the expression profile of living bacteria. The resulting RNA has A_{260}/A_{280} ratios of 1.9–2.1* and is suitable for use in any downstream application. ■

** Measured in 10 mM Tris·Cl, pH 7.5.*

RNAprotect Bacteria Reagent Procedure



Reader Inquiry No. 01502

Ordering Information

Product	Contents	Cat. No.
RNeasy Protect Bacteria Kits — for RNA stabilization and isolation from bacteria		
RNeasy Protect Bacteria Mini Kit (50)†	RNAprotect Bacteria Reagent (2 x 100 ml) and RNeasy Mini Kit (50)	74524
RNeasy Protect Bacteria Midi Kit (10)‡	RNAprotect Bacteria Reagent (2 x 100 ml) and RNeasy Midi Kit (10)	75552
RNAprotect Bacteria Reagent — for stabilization of RNA in bacteria		
RNAprotect Bacteria Reagent	RNAprotect Bacteria Reagent (2 x 100 ml)§	76506
Related products		
Mixer Mill MM 300	Universal laboratory mixer mill	Inquire

† *For stabilization and isolation using up to 7.5 x 10^8 cells.*
‡ *For stabilization and isolation using 5 x 10^8 to 7.5 x 10^9 cells.*
§ *For stabilization of up to 100 ml bacterial culture.*

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Gene- and protein-expression analysis of factors involved in cardiovascular disease*

Thomas Skurk, Yu-Mi Lee, and Hans Hauner

Clinical Department, German Diabetes Research Institute, Düsseldorf, Germany

After total RNA isolation with the RNeasy® Mini Kit, Omniscript™ RT was used to generate cDNA from plasminogen activator inhibitor (PAI-1) mRNA. Semiquantitative real-time PCR and ELISA techniques allowed accurate measurement of changes in expression of PAI-1 in cultured human fat cells at the mRNA and protein level. PAI-1 is the main inhibitor of the fibrinolytic system and its increased production and release is associated with obesity.

Plasminogen activator inhibitor (PAI-1) inhibits the conversion of plasminogen to plasmin, the enzyme that breaks down fibrin in blood clots. Increased PAI-1 concentrations are associated with a higher risk of coronary heart disease, deep-vein thrombosis, thrombotic events in tumors, and obesity. Many studies have demonstrated that visceral fat mass and PAI-1 levels show a positive correlation. Studies have suggested that the vasopressor hormone angiotensin II (Ang II) — a peptide hormone that plays an integral part in the regulation of blood volume as part of the renin–angiotensin system — is a positive regulator of PAI-1. In this study, Ang II and its metabolites, Ang III, and Ang IV were added to human adipocytes in culture, and the effects on PAI-1 synthesis and release were measured. In addition, two angiotensin-receptor type 1 (AT_1) blockers were used to examine the role played by receptors on the surface of the cells.

Materials and methods

Adipocyte precursor cells were isolated and cultured, and adipose differentiation was induced by the addition of cortisol, triiodothyronine, insulin, troglitazone, and isobutyl-methylxanthine to the culture medium. Experiments were performed on day 16, when most cells in culture had acquired the adipocyte phenotype. Ang II and its metabolites were added on the day of the experiment as a fresh dilution in PBS to achieve the indicated final concentrations.

Total RNA was purified using the RNeasy Mini Kit, including the on-column DNA digestion step. Reverse transcription was carried out using Omniscript RT and 1 µg RNA. An RT-PCR product of the PAI-1 mRNA was cloned into a vector and used to generate a standard curve with copy numbers ranging from 200 to 20,000. Amplification was performed with LightCycler® technology, and the amount of amplification product after each cycle was measured by SYBR® Green I fluorescence at 530 nm. The sequence of the amplicon was confirmed by fluorescent dye-terminator sequencing. Primers for the ubiquitously expressed transcription factor Sp1 were included as an internal standard.

PAI-1 protein in the culture medium was assayed using an ELISA technique that measures both free and complexed human PAI-1 antigen.

Results

Addition of Ang II to differentiated adipocyte cells stimulated PAI-1 release into the culture medium in a dose-dependent manner (Figure 1). The maximum response was observed at an Ang II concentration of 10^{-5} M, and peak PAI-1 levels were reached after 48 hours. The Ang II metabolites Ang III and Ang IV were also found to have a stimulatory effect on PAI-1 release but to a lesser extent than Ang II. Addition of Ang II to adipocytes also had an effect at the transcriptional level, with steady-state levels of PAI-1 mRNA peaking ▶

Ang II Stimulates PAI-1 Protein Release

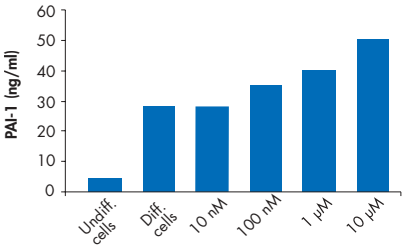


Figure 1 *Effect of adipose differentiation and addition of Ang II on PAI-1 protein release into culture medium from human preadipose and adipose cells in primary culture after a 24-hour incubation period. Ang II was added on day 16, when cells had acquired adipocyte phenotype. Results represent mean of 4 independent experiments in triplicate.* ***Undiff****: undifferentiated,* ***Diff****: differentiated.*

Ang II Increases PAI-1 mRNA Production

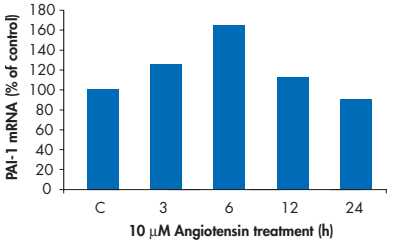


Figure 2 *Time course of effect of 10 µM Ang II on PAI-1 mRNA levels in in vitro-differentiated human adipocyte primary cultures. PAI-1 mRNA was quantified by LightCycler analysis. mRNA of control cultures was defined as 100%. Columns represent mean values of 4 separate experiments.* ***C****: Control cultures.*

* *Data excerpted from Skurk, T., Lee, Y.M., and Hauner, H. (2001) Angiotensin II and its metabolites stimulate PAI-1 protein release from human adipocytes in primary culture. Hypertension* ***37*** *(5), 1336–1340, with permission from Lippincott Williams & Wilkins.*

6 hours after addition of Ang II (Figure 2), corresponding to an increase of 65 ± 12% compared to control cultures. Addition of AT_1-receptor blockers led to a concentration-dependent reduction of PAI-1 release from adipocytes. At a concentration of 10^{-4} M the AT_1-receptor blocker candesartan abolished the increase in PAI-1 production produced by the addition of 10^{-5} M Ang II to the culture medium, indicating that the stimulatory effect of Ang II is mediated by the AT_1 receptor.

Discussion

The highly accurate and linear quantification delivered by the combination of Omniscript RT and LightCycler technology allowed the accurate and efficient determination of PAI-1 mRNA levels in cultured adipocytes. The increase in PAI-1 mRNA and protein levels seen upon Ang II addition demonstrates for the first time that Ang II and its metabolites are able to stimulate expression and release of PAI-1 from adipocyte cells. In addition, the dose-dependent reduction of PAI-1 release upon addition of AT_1-receptor blockers strongly suggests that this effect is mediated by the AT_1 receptor. These findings are important because they suggest that, in addition to their use as therapeutics in lowering blood pressure, AT_1-receptor blockers may also have a beneficial effect on the fibrinolytic system. Furthermore, PAI-1 production by adipose tissue may be a factor in the elevated risk of thrombosis observed in obesity. ■

Reader Inquiry No. 01509

Ordering Information

Product	Contents	Cat. No.
RNeasy Mini Kit — for total RNA isolation from a wide variety of sources		
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74104
Omniscript RT Kit — for highly sensitive and specific reverse transcription using 50 ng – 2 µg RNA		
Omniscript RT Kit (50)*	For 50 reverse-transcription reactions: 40 units Omniscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,† RNase-free water	205111
RNase-Free DNase Set — for on-column DNase digestion		
RNase-Free DNase Set (50)	1500 units RNase-free DNase I, RNase-free Buffer, and RNase-free water for 50 RNA minipreps	79254
Related products		
Sensiscript™ RT Kit — for reverse transcription using <50 ng RNA		
Sensiscript RT Kit (50)*	For 50 reverse-transcription reactions: Sensiscript Reverse Transcriptase, 10x Buffer RT, dNTP Mix,† RNase-free water	205211
QuantiTect™ SYBR Green PCR Kit — for quantitative, real-time PCR and two-step RT-PCR		
QuantiTect SYBR Green PCR Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml QuantiTect SYBR Green PCR Master Mix;‡ 2 x 2.0 ml RNase-free water	204143
QuantiTect SYBR Green RT-PCR Kit — for quantitative, real-time, one-step PCR		
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;‡ 1 x 100 µl QuantiTect RT Mix; 2 x 2.0 ml RNase-free water	204243

* *Larger kit sizes available: please inquire.*
† *Contains 5 mM each dNTP.*
‡ *Contains 5 mM $MgCl_2$.*

The effects of phenol on nucleic acid preparation and downstream applications

Margit Hiesinger, Dirk Löffert, Christoph Ritt, and Uwe Oelmüller
QIAGEN GmbH, Hilden, Germany

Phenol contamination of nucleic acid preparations (prepared using either a commercially available kit or a conventional method), can adversely affect the yield and quality of the nucleic acid obtained. Carryover of phenol also inhibits or reduces the efficiency of downstream applications such as PCR, sequencing, and screening.

Here we demonstrate how phenol contamination can affect both nucleic acid preparation and downstream reactions. We further show that QIAGEN® purification technologies allow the isolation of pure nucleic acids suitable for use in any downstream reaction, and save time when compared to purification methods using phenol.

Materials and methods

Nucleic acids were purified using conventional phenol–chloroform extraction (1), a commercial acid-phenol method, or the relevant QIAGEN kit (RNeasy® Kits for isolation of total RNA, QIAprep® Kits for purification of plasmid DNA, QIAamp® Kits for purification of genomic DNA, and MinElute™ and QIAquick® Kits for cleanup of DNA from enzymatic reactions). In some cases phenol was added to QIAGEN kit-purified nucleic acids, in order to assess the effects of phenol on downstream applications.

Results

Variable and reduced nucleic acid yields using phenol

UV scans of total RNA purified using an acid-phenol method show widely varying yields. The UV peaks are slightly shifted due to absorbance of phenol at 270 and 275 nm* (Figure 1A). There is also increased absorbance at 220–230 nm. In addition, variable and reduced yields are apparent on a formaldehyde agarose gel (Figure 1B). ▶

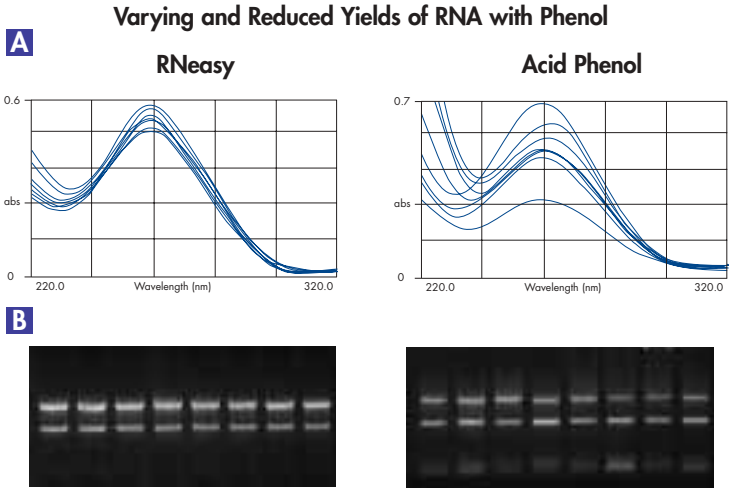


Figure 1 *Total RNA was isolated from1 x 10⁶ HeLa cells using the RNeasy Mini Kit (QIAGEN) or a commercial acid-phenol–extraction method (Supplier L). The RNA isolation was performed 8 times with each method. RNA was eluted (RNeasy) or resuspended after ethanol precipitation (phenol method) in 100 µl RNase-free water.* **A** *Aliquots were diluted in 10 mM Tris·Cl, pH 7.5, and analyzed by UV spectrophotometry.* **B** *10 µg of each sample, based on the A_{260} readings in (A), was analyzed on a formaldehyde agarose gel.*

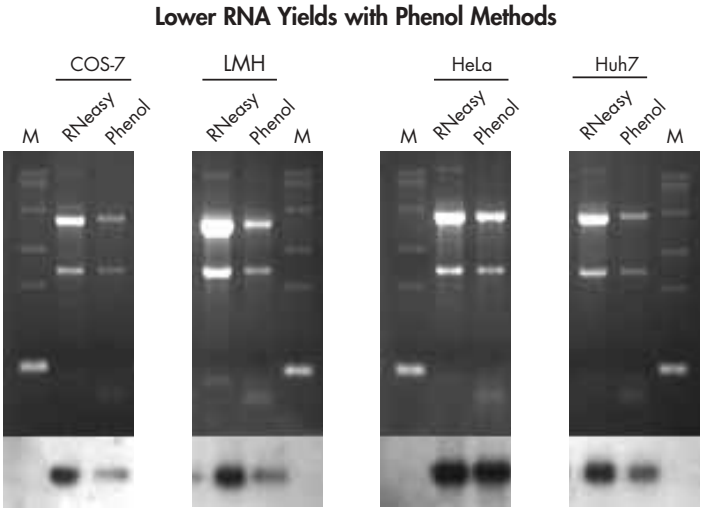


Figure 2 *Various cell lines grown in single wells of a 24-well plate (approximately 2.5 x 10⁵ cells). RNA was isolated using the RNeasy Mini Kit (QIAGEN;* ***RNeasy****) or a commercial acid phenol method (Supplier L;* ***Phenol****). RNA was eluted (RNeasy) or resuspended (Phenol) in 100 µl RNase-free water, and 20 µl was loaded per lane. Blots were hybridized with a ^{32}P-labeled GAPDH probe.* ***M****: 0.24–9.5 kb ladder.*

** In addition this can affect the A_{260}/A_{280} ratio (see reference 2).*

References

1. *Sambrook, J., Fritsch, E.G., and Maniatis, T., eds. (1989)* Molecular Cloning: A Laboratory Manual. *2nd ed., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.*
2. *Stulnig, T.M. and Amberger, A. (1994) Exposing contaminating phenol in nucleic acid preparations. BioTechniques* ***16****, 403.*

Formaldehyde agarose gels and northern blots show that consistently higher yields of RNA were obtained with RNeasy Kits than with acid-phenol methods (Figure 2). Higher yields were obtained with a variety of different cell lines using the same number of cells per prep (approximately 2.5 x 10^5). In the acid-phenol preparations, tRNAs and small rRNAs are seen (Figures 1 and 2). RNeasy purification provides an enrichment for mRNA, and small RNAs (i.e., 5S rRNA and tRNA) are selectively excluded. The presence of these small RNAs in the acid-phenol preps can contribute to mispriming during RT-PCR.

Phenol Shortens Read Lengths in Sequencing

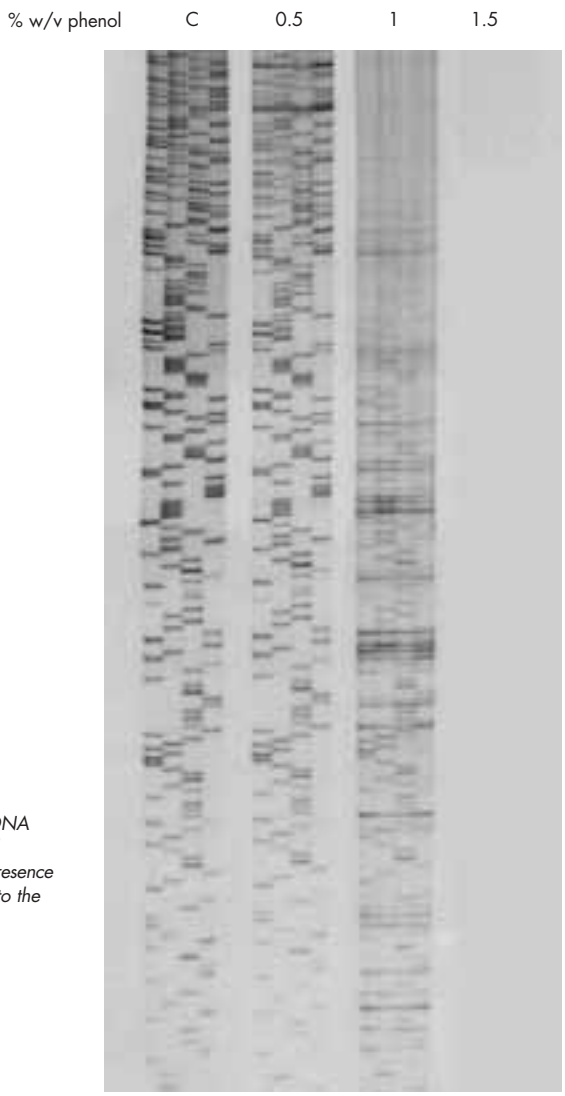


Figure 3 *Sequencing profile of plasmid DNA purified using the QIAprep procedure and sequenced in the absence (**C**: Control) or presence of phenol (concentrations indicated) prior to the sequencing reactions.*

Phenol contamination adversely affects sequencing and screening

The effect of phenol contamination on the quality of sequencing data was examined. Phenol was added to plasmid DNA prior to sequencing, so that the final phenol concentration in the sequencing reactions was between 0.1 and 2.25% (w/v). This corresponds to 0.01–0.25 µl saturated phenol solution in a 10 µl sequencing reaction. Above 1% (w/v) phenol, more compression bands were found in the sequence profiles, making reading of the sequence difficult or impossible (Figure 3). At phenol concentrations greater than 1.5% no sequence profiles could be obtained. Although phenol can be used efficiently to remove nuclease contamination from plasmid preparations, subsequent sequencing reactions are highly sensitive to traces of phenol contamination.

Phenol Decreases Accuracy in Screening

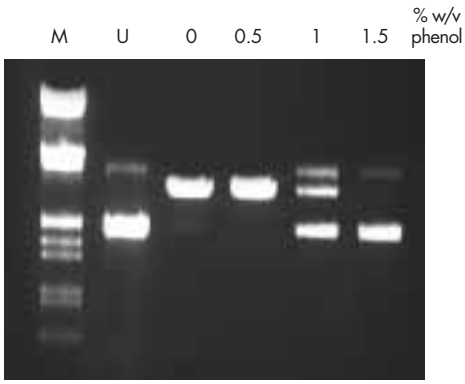


Figure 4 *Agarose gel analysis of DNA samples from Figure 3 digested with* EcoRI. ***U****: Undigested DNA;* ***M****: lambda* HindIII-EcoRI *markers.*

Analysis of plasmid DNA quality by agarose gel electrophoresis can be misleading since the phenol-contaminated plasmid DNA appears to be as pure as the uncontaminated DNA. Differences in plasmid quality due to phenol contamination can only be seen on agarose gels when the DNA is analyzed enzymatically, for example with a restriction enzyme (Figure 4). The phenol contamination leads to incomplete restriction digest and therefore to ambiguous screening results. The effects of phenol arise from its ability to denature proteins, thus inhibiting enzymatic reactions.

Phenol contamination affects PCR performance

Home-made PCR template preparation methods usually include a phenol extraction step. We examined the effects of phenol on PCR template preparation by purifying genomic DNA using the QIAamp DNA Blood Mini Kit and spiking preparations with final concentrations of 0.2% and 0.5% phenol.

Phenol, used for the removal of proteins such as proteases and nucleases, decreased the yield of PCR product when present at a final concentration of 0.2%. At 0.5% phenol, no PCR product was detectable (Figure 5).

Faster DNA cleanup with QIAquick and MinElute Systems

DNA cleanup of an enzymatic reaction or PCR sample was compared using QIAquick and MinElute DNA Cleanup Systems from QIAGEN with a conventional procedure using phenol–chloroform extraction and ethanol precipitation (1). Phenol extractions typically took 65 minutes from starting sample to ready-to-use DNA. In comparison, the MinElute procedure yielded ready-to-use DNA in only 6 minutes, and the QIAquick procedure in just 5 minutes — more than 10 times faster than phenol extractions. The QIAquick and MinElute DNA Cleanup Systems can be used for purifying DNA from all enzymatic reactions, including dephosphorylation, ligation, restriction digestion, end-labeling, primed synthesis, and nick-translation.

Conclusions

- Phenol can adversely affect the yield and quality of both RNA and DNA.
- Phenol carryover inhibits downstream enzymatic reactions. Nucleic acids purified using QIAGEN kits are suitable for even the most sensitive downstream applications
- QIAGEN kits are up to 12 times faster than phenol methods and yield reproducibly high quality nucleic acids
- QIAGEN offers fast, reliable, and convenient alternatives to phenol preps for all applications and throughput and purity requirements. ■

Effect of Phenol on Templates for PCR

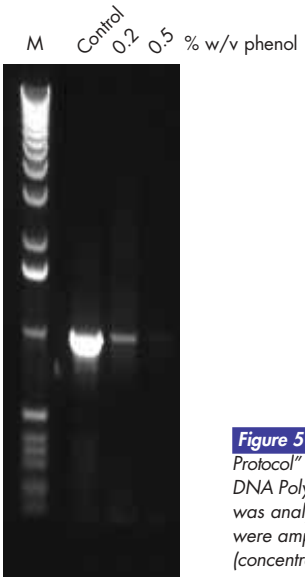
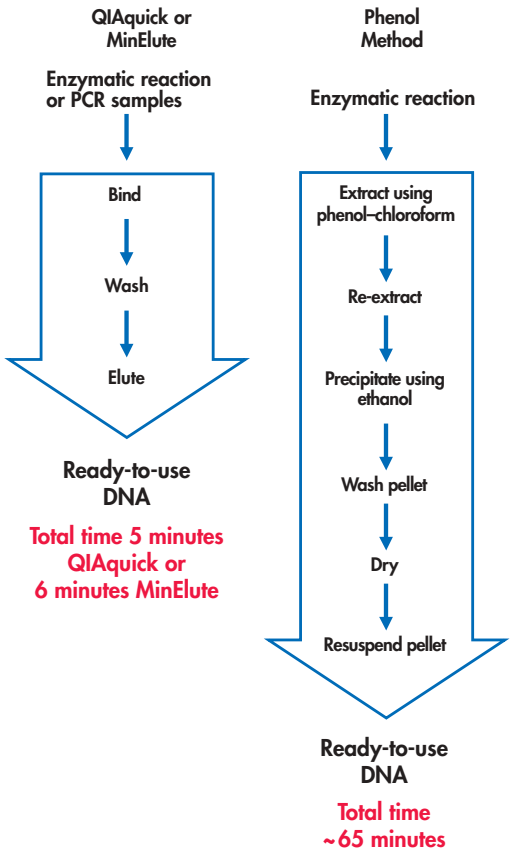


Figure 5 *PCR was carried out according to the "Standard PCR Protocol" in the Taq* PCR Handbook *provided with QIAGEN Taq DNA Polymerase and Taq PCR Core Kits. 10 µl of each sample was analyzed after PCR on a 1% agarose gel. PCR templates were amplified in the absence (**Control**) and presence of phenol (concentrations indicated). **M**: markers.*

Faster Cleanup with QIAquick and MinElute Kits



For ordering information, see next page.

Reader Inquiry No. 01510

Ordering Information

Product	Contents	Cat. No.
QIAprep Spin Miniprep Kit — for isolation of plasmid DNA from bacterial cultures		
QIAprep Spin Miniprep Kit (50)*	For 50 high-purity plasmid minipreps: 50 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2 ml)	27104
QIAquick and MinElute Kits — for purification of DNA fragments from PCR samples, enzymatic reactions, and agarose gels		
QIAquick PCR Purification Kit (50)*	For purification of 50 PCR reactions: 50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28104
QIAquick Gel Extraction Kit (50)*	50 QIAquick Spin Columns, Buffers, Collection Tubes (2 ml)	28704
MinElute PCR Purification Kit (50)*	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28004
MinElute Gel Extraction Kit (50)*	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28604
MinElute Reaction Cleanup Kit (50)*	50 MinElute Spin Columns, Buffers, Collection Tubes (2 ml)	28204
QIAamp Kits — for isolation of DNA and RNA from blood and other clinical samples		
QIAamp DNA Mini Kit (50)*	For 50 DNA preps: 50 QIAamp Spin Columns, QIAGEN Proteinase K, Reagents, Buffers, Collection Tubes (2 ml)	51304
QIAamp DNA Blood Mini Kit (50)*	For 50 DNA minipreps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
QIAamp Viral RNA Mini Kit (50)*	For 50 RNA preps: 50 QIAamp Spin Columns, Carrier RNA, Collection Tubes (2 ml), RNase-free Buffers	52904
QIAamp RNA Blood Mini Kit (50)*	For 50 RNA preps: 50 QIAamp Mini Spin Columns, 50 QIAshredder™ Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	52304
DNeasy® Kits — for isolation of genomic DNA from animal and plant cells and tissue		
DNeasy Tissue Kit (50)*	50 DNeasy Spin Columns, QIAGEN Proteinase K, Buffers, Collection Tubes (2 ml)	69504
DNeasy Plant Mini Kit (20)*	20 DNeasy Mini Spin Columns, 20 QIAshredder Spin Columns, RNase A, Buffers, Collection Tubes (2 ml)	69103
RNeasy Kits — for isolation of RNA from cells and tissues		
RNeasy Mini Kit (50)*	50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74104
RNeasy Plant Mini Kit (20)*	20 RNeasy Mini Spin Columns, 20 QIAshredder Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74903
RNeasy Protect Mini Kit (50)*	RNA*later*™ RNA Stabilization Reagent (50 ml), 50 RNeasy Mini Spin Columns, Collection Tubes (1.5 ml and 2 ml), RNase-free Reagents and Buffers	74124
RNeasy Protect Bacteria Mini Kit (50)	RNeasy Mini Kit (50) and RNAprotect™ Bacteria Reagent (2 x 100 ml)	74524

** Larger kit sizes available; please inquire.*

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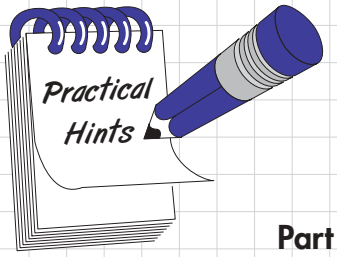

The QIAGEN Guide to Animal Cell Culture

Part I: Introduction to animal cell culture

Welcome to a new series of articles aimed at providing useful hints for culturing animal cells (i.e., cells derived from higher eukaryotes such as mammals, birds, and insects). This article introduces different types of animal cell cultures. The series will continue in future issues of *QIAGEN News* with considerations for cell culture and cell culture protocols.

Animal cell cultures

Depending on their origin, animal cells grow either as an adherent monolayer or in suspension.

Adherent cells are anchorage-dependent and propagate as a monolayer attached to the cell culture vessel. This attachment is essential for proliferation — 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 this confluent state for too long. Most cells derived from tissues are anchorage-dependent.

Suspension cells can survive and proliferate without being attached to a substratum. Hematopoietic cells (derived from blood, spleen, or bone marrow) as well as some transformed cell lines and cells derived from malignant tumors can be grown in suspension.

Primary cells, finite cultures, and continuous cell lines differ in their proliferative potential (see below). Different cell types vary greatly with respect to their growth behavior and nutritional requirements. Optimization of cell culture conditions is necessary to ensure that cells are healthy and in optimal condition for downstream applications.

Tip Extensive information on culturing cells can be found in reference 1.

Primary cell cultures

Primary cell cultures come from the outgrowth of migrating cells from a piece of tissue or from tissue that is disaggregated by enzymatic, chemical, or mechanical methods. Primary cultures are formed from cells that survive the disaggregation process, attach to the cell culture vessel (or survive in suspension), and proliferate.

Primary cells are morphologically similar to the parent tissue. These cultures are capable of only a limited number of cell divisions, after which they enter a nonproliferative state called

002

References

1. *Freshney, R.I. (1993)* Culture of Animal Cells, A Manual of Basic Technique, *3rd ed., New York: Wiley-Liss.*

senescence and eventually die out. Adherent primary cells are particularly susceptible to contact inhibition, that is, they will stop growing when they have reached confluency. At lower cell densities, however, the normal phenotype can be maintained. Primary cell culture is generally more difficult than culture of continuous cell lines.

Primary cell cultures are sometimes preferred over continuous cell lines in experimental systems. Primary cells are considered by many researchers to be more physiologically similar to in vivo cells. In addition, cell lines cultured for extended periods of time can undergo phenotypic and genotypic changes that can lead to discrepancies when comparing results from different laboratories using the same cell line. Furthermore, many cell types are not available as continuous cell lines.

Finite cell cultures

Finite cell cultures are formed after the first subculturing (passaging) of a primary cell culture. These cultures will proliferate for a limited number of cell divisions, after which they will senesce. The proliferative potential of some human finite cell cultures can be extended by introduction of viral transforming genes (e.g., the SV40 transforming-antigen genes). The phenotype of these cultures is intermediate between finite cultures and continuous cultures. The cells will proliferate for an extended time, but usually the culture will eventually cease dividing, similar to senescent primary cells. Use of such cells is sometimes easier than use of primary cell cultures, especially for generation of stably transfected clones.

Continuous cell lines

Finite cell cultures will eventually either die out or acquire a stable, heritable mutation that gives rise to a continuous cell line that is capable of unlimited proliferative potential. This alteration is commonly known as in vitro transformation or immortalization and frequently correlates with tumorigenicity.

Rodent primary cell cultures form continuous cell lines relatively easily, either spontaneously or following exposure to a mutagenic agent. In contrast, human primary cell cultures rarely, if ever, become immortal in this way and require additional genetic manipulation to form a continuous cell line. However, cell cultures derived from human tumors are often immortal.

Continuous cell lines are generally easier to work with than primary or finite cell cultures. However, it should be remembered that these cells have undergone genetic alterations and their behavior in vitro may not represent the in vivo situation.

The QIAGEN Guide to Animal Cell Culture will continue in future issues of *QIAGEN News* with tips for successful cell culture. 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.

Signature: Date:

Witness: Date:

QIA-Hints

QIAGEN Technical Services are always available to answer your questions!

TRANSFECTION

Q How can I adapt the PolyFect® transfection protocols for use with 96-well plates?

A We have developed protocols for transfection of COS-7, NIH/3T3, HeLa, 293, CHO, and HeLa-S3 cells in 96-well plates using PolyFect Transfection Reagent. You can view and download these protocols online at **www.qiagen.com/transfectiontools/prod_protocols/**. Alternatively, contact your QIAGEN Technical Service Department or local distributor.

PROTEIN

Q When I dialyze my 6xHis-tagged proteins, I sometimes see a precipitate form. What is the reason for this, and what can I do to prevent it?

A Precipitation of proteins during dialysis against phosphate or Tris buffers (e.g., PBS or TBS) is most likely caused by not having enough NaCl or KCl in the dialysis buffer. Precipitation can be due to salt concentrations that are too low, protein concentrations that are too high, abrupt pH changes, and/or using a pH that is close to the isoelectric point (pI) of the protein.

Dialysis is best performed using at least a 1000-fold excess of dialysis buffer, at 4°C, for at least 3 hours, followed by a change of buffer and a second dialysis. We recommend dialyzing against a buffer that contains at least 350 mM NaCl, 10% (w/v) glycerol, 50 mM NaH_2PO_4, and 5 mM $MgCl_2$. Salts and glycerol help to stabilize proteins and can be increased, if necessary, to higher concentrations. If the protein still precipitates, diluting it may also help.

Once a protein precipitates in PBS or TBS, it is often extremely difficult to refold it into an active form. If your protein precipitates, it is usually more efficient to simply repurify it from a new lysate and modify the dialysis conditions instead of trying to resolubilize the precipitated protein.

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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In North America

Canadian Association for Clinical Microbiology and Infectious Diseases (CACMID)	5–6 November, Victoria, BC
San Diego Conference (AACC), 8–10 November	San Diego, CA
Society for Neuroscience	10–15 November, San Diego, CA
Association for Molecular Pathology (AMP)	16–17 November, Philadelphia, PA
BIOPHEX	28–29 November, Long Beach, CA
10th International Conference on Gene Therapy of Cancer	6–8 December, San Diego, CA
American Society for Cell Biology (ASCB)	8–12 December, Washington, DC
Plant, Animal and Microbe Genomes X Conference (PAG-X)	12–16 January 2002, San Diego, CA
LabAutomation 2002	26–30 January, Palm Springs, CA
Advances in Genome Biology and Technology (AGBT)	6–9 February, Marco Island, FL
Microbial Genome Conference	8–12 February, Las Vegas, NV
American Academy of Forensic Science (AAFS)	11–16 February, Atlanta, GA
CHI — Genome Tri-Conference 2002	23 February – 1 March, Santa Clara, CA

In Europe

EMBL Ph.D. Student Symposium on Evolution	9–10 November, Heidelberg, Germany
Genes and Cancer (18th Molecular Biology and Cancer Network Meeting)	10–12 December, Coventry, UK
Swiss Society for Microbiology (SGM/SSM)	20–21 February 2002, Lucerne, Switzerland
Forum Labo Biotech	26–29 March, Paris, France

In Asia and Australasia

ASMR (Australian Society for Medical Research) National Scientific Conference	25–27 November, Surfers Paradise, Australia
24th Annual Meeting of the Molecular Biology Society of Japan	9–12 December, Yokohama, Japan
31st Annual Meeting of the Japanese Society for Immunology	11–13 December, Osaka, Japan

27th Annual Conference on Protein Structure and Function	10–14 February, Lorne, Australia
14th Lorne Cancer Conference	14–17 February, Lorne, Australia
23rd Lorne Genome Conference	17–21 February, Lorne, Australia

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