Analyzing Genetic Differences

Second edition — innovative tools

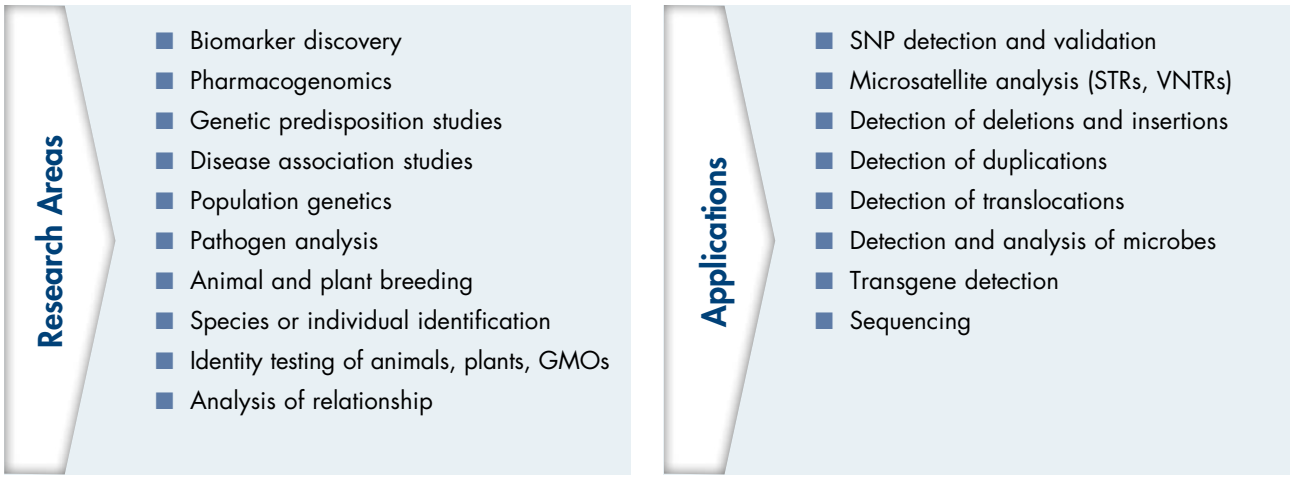
QIAGEN®

Sample & Assay Technologies

Genotyping applications and their challenges

Genotyping is the synonym for a variety of applications used to analyze differences in genomic DNA between individuals. These applications are routinely used in almost all areas of research, applied testing, and diagnostics (Figure 1A). Although these areas are different, the challenges associated with genotyping applications are similar and consist of important factors such as sample quality and quantity, sensitivity, reliability, time to result, and overall costs (Figure 1B).

A



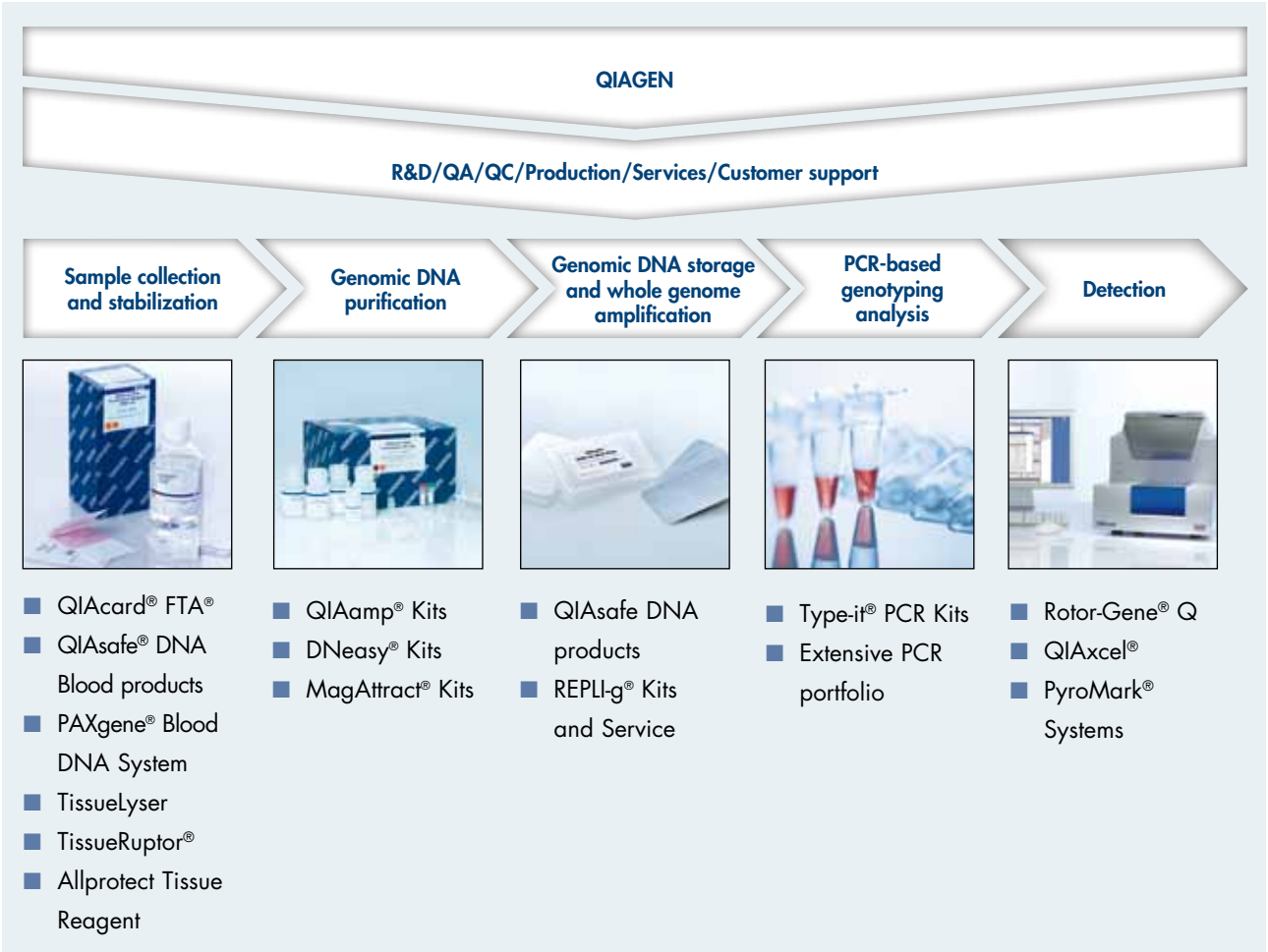
B

Figure 1. **A** **Applications in genotyping research areas and** **B** **associated challenges.**

QIAGEN, as a sample and assay technology provider, offers a wide range of specialized and innovative solutions that are designed specifically to overcome challenges associated with genotyping studies. Our tools enable our customers to reliably and reproducibly genotype:

- Humans
- Animals
- Plants
- Microorganisms
- Viruses

As outlined on page 3, QIAGEN offers an extensive product portfolio which covers the complete genotyping workflow, from sample to result. QIAGEN's state-of-the art research and development departments are continuously working on developing new tools to address the changing needs of our genotyping customers. Our Technical Service Departments are staffed by experienced scientists who have extensive practical and theoretical expertise in sample and assay technologies as well as the use of QIAGEN products and are happy to assist you with technical support. In addition, our service department offers contract whole genome amplification services and sequencing services.



Analyzing genetic differences

The analysis of DNA differences between individuals has become increasingly important in all areas of biological and medical research and diagnostics. The term "genotyping", which is one of the most frequently cited terms in scientific publications, is used as a synonym for a wide range of applications associated with human, animal, plant, microbial, or viral samples.

Although the techniques and platforms used for genotyping are extremely diverse, they all rely on the principles of a few basic technologies:

- Fragment length polymorphism analysis
- Hybridization technologies including arrays
- Sequencing
- PCR-based methods and primer extension technologies

From typing of disease or cancer loci to biomarker discovery and pathogen detection, ensure success at the first attempt and trust QIAGEN for all your genotyping requirements.

This brochure gives an overview of the workflows associated with genotyping applications and the dedicated solutions offered by QIAGEN targeting the different challenges associated with each step.

Sample collection and stabilization

The integrity of the DNA sample material is critical for achieving accurate and reliable results in genotyping assays. If samples are collected, transported, and stored inadequately, the integrity of DNA is compromised, leading to degradation and affecting subsequent downstream analysis. The need for resampling dramatically increases the costs of a project and often the same sample is impossible to obtain again. QIAGEN, as part of PreAnalytiX®, has developed the PAXgene Blood DNA System; an integrated blood collection and DNA purification system (storage for up to 14 days at room temperature) (Figure 2).

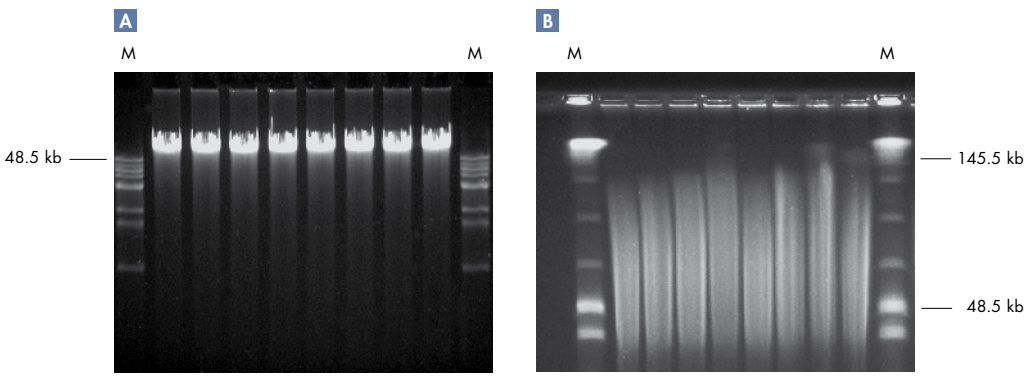


Figure 2. High-quality and high-molecular-weight genomic DNA. Genomic DNA isolated from 8 blood donors using the PAXgene Blood DNA System. A Agarose gel analysis. B Pulsed-field gel electrophoresis for enhanced separation of high-molecular-weight genomic DNA. **M**: markers.

QIAsafe DNA Blood Tubes and 48-Well Plates — room-temperature blood storage

QIAsafe DNA Blood Tubes and 48-Well Plates provide innovative QIAsafe matrix technology for dry storage of 50–200 µl blood samples at room temperature, without degradation of DNA. Refrigeration costs are eliminated and samples may be conveniently shipped at ambient temperatures. Blood samples are easily recovered by simple rehydration and used in DNA purification protocols for subsequent genotyping applications.

Figure 3. QIAsafe DNA Blood Tubes enable dry storage of blood samples at room temperature.

QIAcard FTA Spots — room-temperature sample collection and storage

QIAcard FTA Spots represent a sample collection system that utilizes proven Whatman® FTA technology to simplify the handling and processing of nucleic acids. After applying samples to QIAcard FTA Spots, genomic DNA is stabilized in situ for years at room temperature. QIAcard FTA Spots can be easily transported, archived, or processed immediately. DNA purified from samples stored on QIAcard FTA Spots is of high quality, for use in a variety of sensitive genotyping applications (Figure 4).

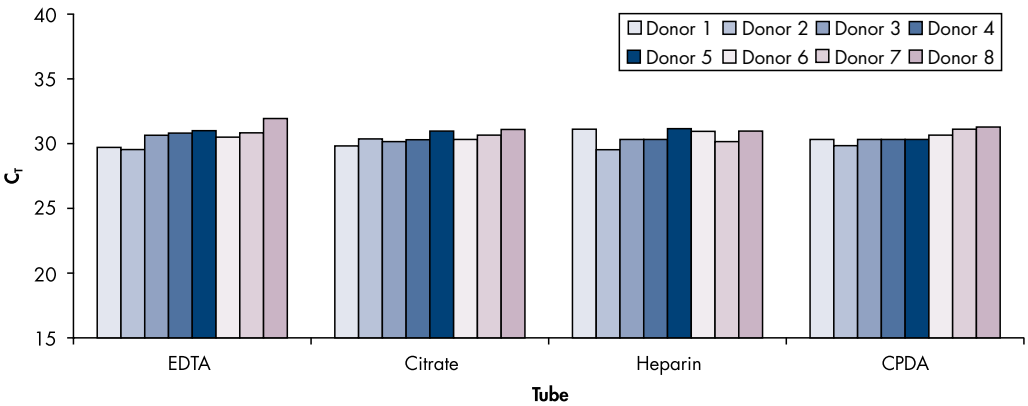


Figure 4. High-quality DNA from blood with different anticoagulants, spotted on QIAcard FTA Spots. Blood from 8 donors was collected in 4 different blood-draw tubes containing the indicated anticoagulants. Blood (125 µl) was spotted onto QIAcard FTA Spots and stored overnight at room temperature. DNA was purified from punches of the QIAcard FTA Spots using the QIAamp DNA Micro Kit. Purified DNA was amplified using the QuantiTect® Probe PCR Kit on the ABI PRISM® 7900HT SDS, with primers and probe specific for a 294 bp fragment of the β-actin gene.

Genomic DNA purification

Genomic DNA purification is a crucial step in all genotyping workflows. Even the best and most robust detection technology can only provide reliable results if the template DNA is of adequate quality and purity. QIAGEN's diverse portfolio of DNA purification technologies is specially adapted to overcome all the challenges in DNA purification, such as low yields and poor quality, and to provide the best DNA quality possible from nearly all samples derived from humans, plants, animals, and microbes (see page 7).

QIAGEN solutions for genomic DNA purification

Sample material	Critical factors: Removal of PCR inhibitors	Limited amounts of DNA	DNA recovery	Variability of sample types	Difficult to lyse	Short DNA fragments	Nonhomogenous sample materials	High cell density	QIAGEN solutions: Manual	Automated systems: QIAamp and DNeasy Kits	QIAcube®	EZ1® Advanced instruments	QIAsymphony® SP	BioRobot® MDx and BioRobot Universal System	BioSprint® 96	Autopure LS®
Whole blood	■								■	■	■	■	■	■	■	■
Buffy coat								■	■	■	■	■	■	■	■	■
Plasma and serum		■							■	■	■	■	■			
Cell-free body fluids		■							■	■	■	■	■			
Urine	■	■							■	■		■	■			
Saliva	■								■	■	■	■				■
Bone marrow									■	■	■					■
Dried blood spots			■						■	■	■		■	■		
Tissue					■		■		■	■	■	■		■		
Fixed tissue						■	■		■	■	■					
Bones and teeth	■	■			■				■	■	■					
Cultured cells									■	■	■	■		■	■	
Swabs							■		■	■	■	■	■	■	■	■
Forensic casework samples		■		■					■	■			■			
Hair		■							■	■	■		■			
Stool	■						■		■	■		■	■			
Insects					■				■	■						
Mouse tails					■				■	■				■		
Plant materials	■								■	■				■		

Our purification products can be used with manual spin or vacuum based procedures in single spin-column or 96-well formats or can be fully automated on one of QIAGEN´s proprietary automated systems — providing increased flexibility and compatibility with all downstream challenges. Our fully automated systems such as the QIAcube or the QIAsymphony SP provide walkaway DNA purification, which saves valuable time and increases the reproducibility of the results by eliminating human error across all throughput requirements (Figure 5).

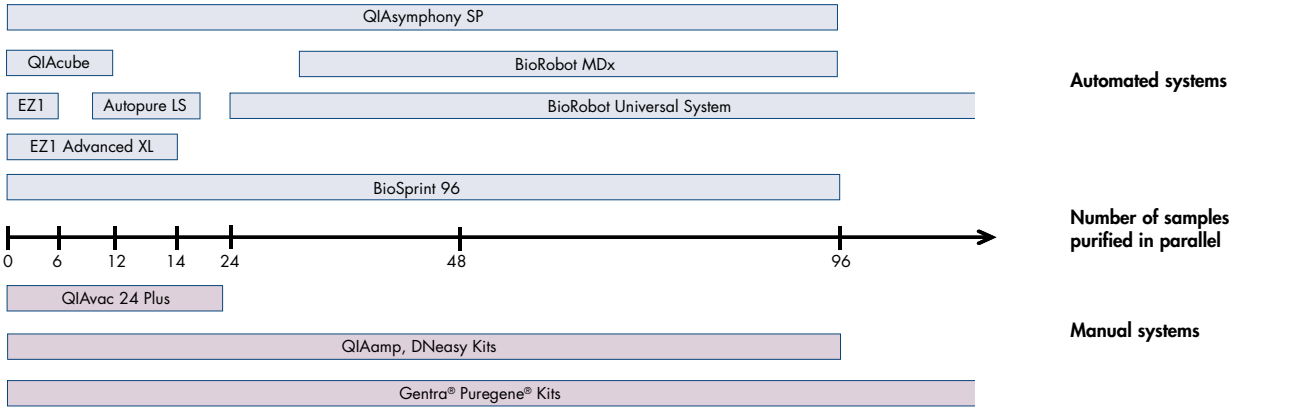


Figure 5. DNA purification systems suitable for all throughput requirements for genotyping applications. This figure shows automated and manual DNA purification systems grouped by the number of samples that can be processed in parallel. All systems are optimized to provide high-quality DNA with fast, easy, and convenient handling.

If certain sample materials require specific treatments for efficient purification, we have dedicated kits and optimized protocols to achieve this (Figure 6). The online ProductFinder (www.qiagen.com/Products/ProductFinder) enables fast and easy selection of the most appropriate system to suit your requirements.

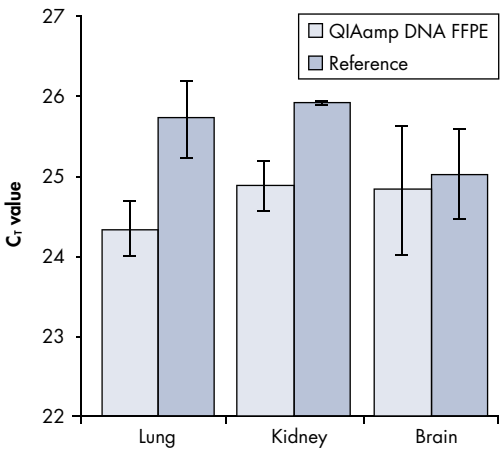


Figure 6. Efficient lysis procedure for FFPE samples. Genomic DNA was purified from different FFPE rat tissue samples using the QIAamp DNA FFPE Tissue Kit or a standard overnight proteinase K digest as reference followed by DNA purification using the QIAamp DNA Mini Kit. DNA was quantified photometrically, and 20 ng was used as a template in a real-time PCR analysis. Lower C_T values are achieved with the QIAamp DNA FFPE Tissue Kit, indicating greater recovery of amplifiable DNA compared to the reference.

Genomic DNA storage and whole genome amplification

It is often necessary to store DNA samples following collection, especially in cases where assays are performed at a later stage or for long-term DNA sample archiving. DNA sample stability is of extreme importance when performing sensitive genotyping assays to ensure valid interpretation of the experimental data. Inadequate storage can affect the overall integrity of the sample and ultimately affect downstream applications as well as leading to increases in cost. QIAGEN provides a cost-effective solution for genomic DNA transport, storage, and archiving at ambient temperatures.

Researchers often face the problem of limited or insufficient DNA quantity for downstream analysis. QIAGEN's REPLI-g Kits and Service solve this problem by providing high yields of whole genomic DNA from small or precious samples.

QIAsafe — no freezer required, DNA storage at room temperature

QIAsafe DNA Tubes and 96-Well Plates provide innovative anhydrobiosis-based technology for stable, room-temperature transport and storage of purified DNA (Figure 7). Room-temperature storage saves on refrigeration costs and eliminates the risk of sample loss due to freezer breakdown. Sample recovery from the QIAsafe matrix is as easy as "just add water". For room-temperature blood storage and DNA stabilization, we offer QIAsafe DNA Blood Tubes and 48-Well Plates (see page 5).

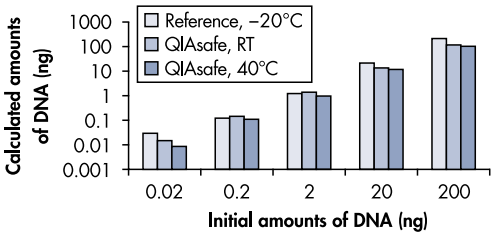


Figure 7. High recovery of genomic DNA stored for 1 year. Differing amounts of genomic DNA were stored for 1 year in solution at –20°C **(Reference)**, or in QIAsafe DNA Tubes at room temperature **(RT)** or 40°C. After rehydration of the QIAsafe samples, DNA was quantified using real-time PCR of a fragment of the β-actin gene. DNA amounts were calculated relative to the reference samples.

▶ REPLI-g Kits and Service — unlimited amounts of DNA

Limited or insufficient DNA quantity for downstream analysis can become a challenge in genotyping applications. QIAGEN's Multiple Displacement Amplification (MDA) technology for whole genome amplification solves this problem by providing high yields of whole genomic DNA from small or precious samples. REPLI-g Kits and Service enable reliable and accurate amplification of the original source DNA — without bias. The amplified DNA can be directly used in a range of applications. REPLI-g amplified DNA was evaluated and tested using all methods listed in Table 1. For more information, please visit the REPLI-g Web Resource page at www.qiagen.com/goto/wga.

Table 1. Evaluation of REPLI-g amplifed DNA

Application	Platform	Kit/assay
SNP genotyping	Any thermal cycler	Type-it Fast SNP Probe PCR Kit
SNP genotyping	Affymetrix®	GeneChip® Mapping System 10k, 100k, 500k
SNP genotyping	Affymetrix	GenomeWide SNP Arrays 5.0, 6.0
SNP genotyping	Affymetrix	Targeted Genotyping System
SNP genotyping	Illumina	GoldenGate Assay
SNP genotyping	Illumina	Infinium II Assay
SNP genotyping	Real-time PCR thermal cycler (ABI)	SNPlex Genotyping System
SNP genotyping	Sequenom	Mass Array, HotStarTaq® DNA Polymerase
SNP genotyping	High-resolution melting (HRM®)	Type-it HRM PCR Kit
STR analysis	Any sequencer	Type-it Microsatellite PCR Kit
Microsatellite analysis	Gel-based	Type-it Microsatellite PCR Kit
Detection of mutations	QIAxcel	Type-it Mutation Detect PCR Kit
Detection of mutations	Gel-based	Type-it Mutation Detect PCR Kit
Detection of mutations	High-resolution melting (HRM)	Type-it HRM PCR Kit

PCR-based genotyping analysis

PCR amplification techniques have become essential for many procedures directed at detection and analysis of genetic differences such as identifying mutations in diagnostics research, typing of disease loci, and investigating relationship and paternity patterns, while facing method-specific challenges (see Table 2).

Table 2. Increasing success with challenging PCR-based genotyping applications

Application	Amplification method	Challenge
Microsatellite analysis	Highly specific multiplex PCR Fluorescent primers required	Parallel amplification of multiple products High resolution detection platform
Detection of mutations	Highly specific multiplex PCR Innovative HRM technology	Parallel amplification of multiple products Specific amplification in high background Accuracy, speed
Single-cell PCR	Highly specific hot-start PCR	High sensitivity
Detection of SNPs	SNP genotyping (using TaqMan® probes) SNP genotyping (using HRM)	High call rates Throughput Accuracy, speed

Type-it PCR Kits — the fast and easy way to reliable genotyping results

Accurate genotypic analysis often requires extensive optimization of experimental parameters. Sample materials may be limiting in genotyping studies, for example, when large numbers of SNPs need to be analyzed or when working with sample materials such as biopsies or FFPE tissue. In addition to the challenges listed in Table 2, some studies require analysis of a large number of different mutations of a certain gene related to a disease (e.g., deletions, translocations, or insertions). Including the necessary internal controls, a large number of reactions are required when performing singleplex or low-plex-grade PCR analysis, leading to increases in both costs and analysis time. QIAGEN recognizes these challenges and has developed reliable PCR-based kits that are dedicated for genotyping applications ranging from identification of microsatellite loci and analysis of SNPs to detection of mutations using different detection technologies (Table 3).

Table 3. Applications of Type-it PCR Kits

Kit	Dedicated application	Field of research
Type-it Microsatellite PCR Kit	Microsatellites STR (short tandem repeats) VNTR (variable number of tandem repeats) SSR (simple sequence repeat)	Species or individual identification Analysis of relationship Microsatellite instability Population genetics Lineage analysis
Type-it Mutation Detect PCR Kit	Deletions Insertions Duplications Translocations SNP preamplification (SNaPshot Multiplex Kit*)	Typing of disease loci Typing of transgenic plants or animals GMO analysis
Type-it Fast SNP Probe PCR Kit	Detection of SNPs using TaqMan MGB™ Probes	Typing of disease loci Biomarker discovery and validation
Type-it HRM PCR Kit	Detection of SNPs and mutations Mutation scanning Pathogen detection	Typing of disease loci Biomarker discovery and validation

* Available from Applied Biosystems.

Type-it Microsatellite PCR Kit

The Type-it Microsatellite PCR Kit is dedicated for fast and reliable microsatellite analysis by multiplex PCR. Highly accurate genotypic analysis of humans, animals, plants, and bacteria is achieved using microsatellites, STR, or VNTR markers (Table 3). The Type-it Microsatellite PCR Kit eliminates the need for optimization of PCR parameters and ensures high product yields for all fragments in parallel. It also enables subsequent analysis on capillary sequencers. This makes it highly suitable for routine analysis as well as for the development of new assays (Figure 8).

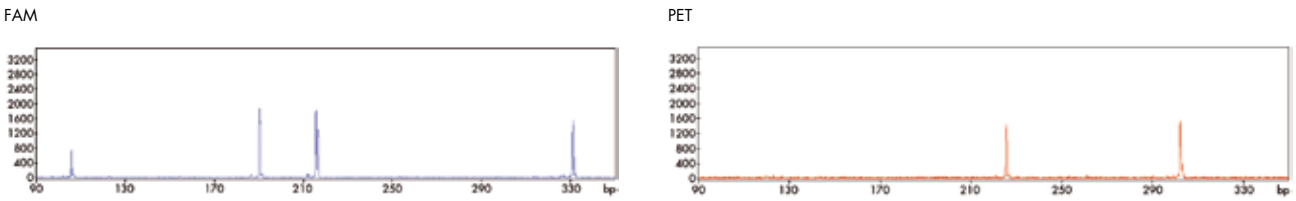


Figure 8. Successful 13-plex STR analysis using the Type-it Microsatellite PCR Kit. Only the FAM and PET channel representing 6 of 13 analyzed loci of the respective four channels of a 3730 xl Capillary Sequencer (ABI) are shown.

Type-it Mutation Detect PCR Kit

The Type-it Mutation Detect PCR Kit is especially designed to enable fast and reliable detection of mutations such as deletions, insertions, and translocations (Table 3). It is also highly suited for multiplex PCR-based preamplification of SNPs as preparation for genotyping systems such as the SNaPshot Multiplex Kit.* The Type-it Mutation Detect PCR Kit ensures comparable amplification efficiency for all amplicons in a high-plex-grade multiplex experiment and allows more mutation targets to be combined without the loss of amplification efficiency for any of the targets (Figures 9 and 15).

Type-it Fast SNP Probe PCR Kit

The Type-it Fast SNP Probe PCR Kit provides the ultimate solution for accurate SNP genotyping, even for difficult templates and also for low template amounts (Table 2). Outstanding separation and tight allele clustering ensure high call rates and accurate, reproducible, and reliable genotyping results (Figure 10). Significantly reduced PCR run times are achieved not only on all standard cyclers, but also on fast cyclers with rapid ramping rates, resulting in greater flexibility combined with substantial time and cost savings. The Type-it Fast SNP Probe PCR Kit is functionally verified using TaqMan SNP Genotyping Assays on suitable cyclers such as the Rotor-Gene Q (see page 15).

Type-it HRM PCR Kit

The Type-it HRM PCR Kit enables highly accurate genotyping using innovative HRM technology (see pages 14 and 15).

A vast range of PCR and RT-PCR products

In addition to the latest developments in the QIAGEN genotyping PCR portfolio — the Type-it PCR product range — QIAGEN offers a wide range of PCR and RT-PCR products that have been specifically developed to provide accurate results at the first attempt. Our range of products offer convenient features such as master mix formats, gel loading PCR buffers, and streamlined protocols highly suited for detection platforms such as agarose gels, capillary sequencers, or automated electrophoresis systems like the QIAxcel system. Whatever your PCR application, QIAGEN provides a kit to suit your needs. For more information, visit www.qiagen.com/goto/pcr.

* Available from Applied Biosystems.

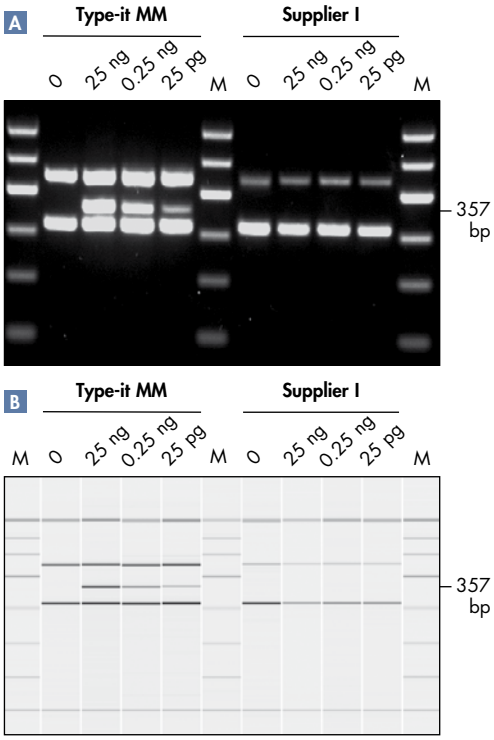


Figure 9. Sensitive detection of a mutated cancer-related gene. The indicated amounts of DNA extracted from a lymphoma related cell line (Ramos) were spiked into human leukocyte DNA and the mutated Ramos target was detected together with 2 internal controls. Using the Type-it Mutation Detect PCR Kit, the mutated gene was detected even when only 25 pg of DNA was present. A Electrophoresis was performed on a 1.3% agarose gel. B Electrophoresis was performed on the QIAxcel System using the QIAxcel High Resolution cartridge. Marker: 100 bp ladder.

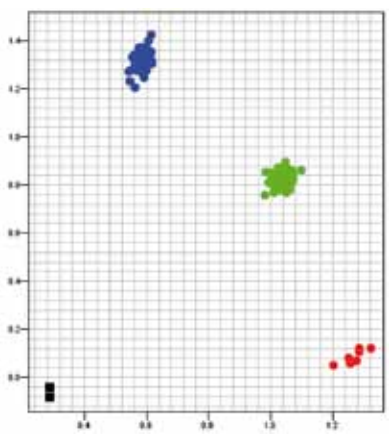


Figure 10. The Type-it Fast SNP Probe PCR Kit provides outstanding separation and tight allele clustering. Cluster plot analysis using a TaqMan MGB-based SNP genotyping assay and a panel of different genomic DNAs (10 ng each in 10 µl reactions) and two no template controls (NTCs) using the StepOnePlus™ real-time PCR instrument. Each dot represents one sample. Black: NTCs. Blue: homozygous DNAs for FAM fluorescence (T allele). Green: heterozygous samples. Red: homozygous for VIC® fluorescence (A allele).

HRM technology — the fast, sensitive, and cost-effective approach to genotyping

HRM characterizes double-stranded PCR products based on their melting behavior as they transition from double-stranded DNA to single-stranded DNA with increasing temperature. PCR products can be easily discriminated according to sequence, length, GC content, or strand complementarity, down to single base pair changes. Understanding the genetic differences associated with human disease or cancer as well as linking them to biomarkers, such as SNPs or deletions, is crucial for the prognosis and future treatment of genetic disorders. QIAGEN's HRM hardware, software, and kits deliver highly specific, sensitive, and accurate results, without time-consuming optimization, and outperform other available solutions.

PCR-based genotyping analysis and versatile detection platforms

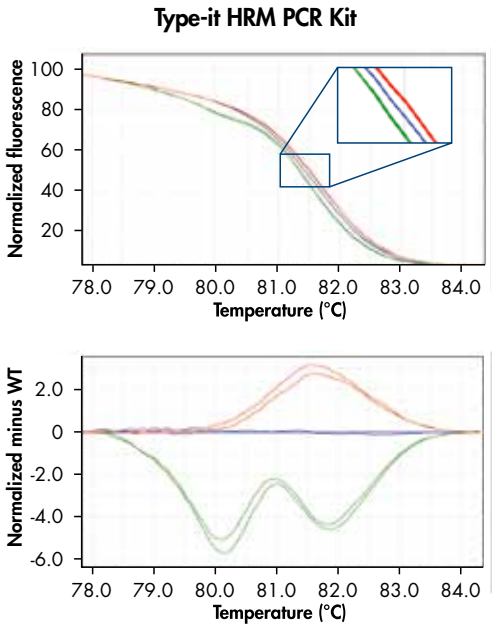


Figure 11. Successful genotyping of an A/T Class IV SNP using the Type-it HRM PCR Kit. Typing the SNP (rs2270938) in the human GYS1 gene using the Type-it HRM PCR Kit results in highly reproducible and accurate results. The normalized melting curve and difference plot show successful and reliable discrimination of all 3 genotypes (wild-type, heterozygote, and mutant) of a Class IV SNP. Blue: wild-type; Green: heterozygous; Red: mutant. Analysis was performed on the Rotor-Gene Q 5plex HRM intrument.

Table 4. Typical HRM based genotyping applications

Dedicated application	Description
Detection of SNPs	Class I–IV (most difficult and rarest)
Mutation detection	Deletions, insertions, translocations, point mutations
Mutation scanning	Screening of unknown samples for new mutations
Pathogen typing/detection	Screening for microbiology research

Accurate genotyping with the Type-it HRM PCR Kit

The latest addition to the dedicated genotyping product line, Type-it, is the Type-it HRM PCR Kit. The optimized kit ensures accurate resolution of sequence variations and is the preeminent tool for unambiguous allelic discrimination using HRM technology. The kit enables successful analysis of genomic loci that are difficult to amplify and is compatible with all real-time instruments suitable for HRM analysis, such as the Rotor-Gene Q, the LightCycler® 480, and the Applied Biosystems® 7500 and 7900 Fast Systems. Novel EvaGreen® fluorescent dye contained in the master mix ensures distinct melting curves. In contrast to kits from other suppliers, the Type-it HRM PCR Kit requires no optimization in the development of new HRM assays. Due to the unique master mix chemistry and optimized HRM buffer, specific amplification products and reliable results are consistently ensured, even when analyzing Class IV SNPs (Figure 11).

Outstanding real-time results with the Rotor-Gene Q

QIAGEN's real-time PCR cycler, the Rotor-Gene Q (Figure 12), combines multiple optimized design features to provide the consistent performance and reliable results that your research demands. The rotary design of the Rotor-Gene Q and its outstanding thermal and optical performance are highly suited to HRM. The Rotor-Gene Q is the only real-time cycler currently capable of accurately deciphering the most difficult Class IV SNPs by HRM. The HRM option for the Rotor-Gene Q includes a specially tuned high-intensity optical HRM channel and a thermal resolution of 0.02°C, as well as powerful new HRM analysis software, ensuring straightforward and successful results.

Figure 12. Rotor-Gene Q.

Efficient data analysis with Rotor-Gene ScreenClust HRM Software

Rotor-Gene ScreenClust HRM Software uses an innovative mathematical approach and advanced statistical methods to differentiate between different alleles in an HRM experiment (Figure 13). By grouping samples into clusters, Rotor-Gene ScreenClust HRM Software enables applications such as genotyping and mutation scanning. Analysis with the software is rapid, highly specific, and sensitive, giving you data you can trust and faster results. The powerful software exceeds the analytical performance of other currently available HRM software packages.

A winning combination for HRM based genotyping

The Type-it HRM PCR Kit, together with the Rotor-Gene Q and Rotor-Gene ScreenClust HRM Software, provides a complete solution for reliable and accurate HRM based genotyping. Our products ensure highly specific amplification combined with maximum thermal resolution and straightforward software analysis and outperform other currently available HRM solutions.

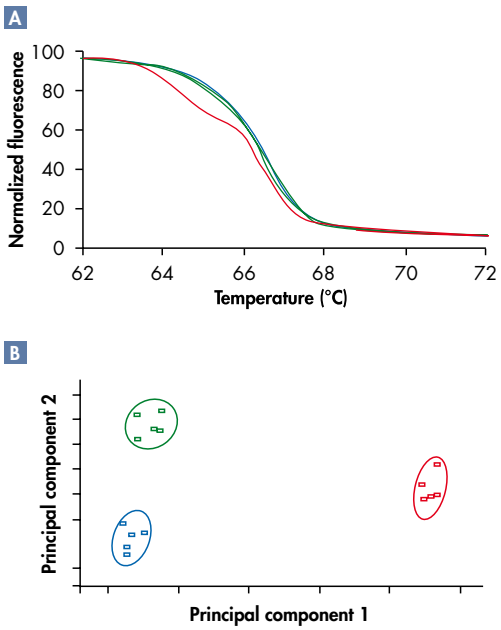


Figure 13. Accurate identification of difficult A/T Class IV SNPs. The AHRR7 gene was analyzed with the Type-it HRM PCR Kit on the Rotor-Gene Q. Rotor-Gene ScreenClust HRM Software correctly identified wild-type (blue), homozygous mutant (green), and heterozygous (red) alleles. The difference between homozygote alleles in this experiment was less than 0.1°C. A A normalized melting curve. B A principal component plot showing the samples grouped into clusters.

Detection of end-point PCR products with the QIAxcel

The most commonly used method for readout of end-point PCR-based genotyping assays is gel electrophoresis, using manually poured slab gels. This method is highly labor-intensive and exposes users to hazardous chemicals such as ethidium bromide. In addition, thorough analysis of the data in terms of fragment sizes and concentration can be tedious — especially when the data are to be compared with previously analyzed PCR products. The QIAxcel system fully automates high-resolution capillary electrophoresis of up to 96 samples per run (Figures 14 and 15). Analysis of DNA fragments from PCR or multiplex PCR reactions (Figure 15) derived, for example, from PCR-based genotyping applications can be analyzed in less than 10 minutes (12 samples). It also ensures increased standardization as well as greater safety as exposure to hazardous chemicals such as ethidium bromide is minimized.

Figure 14. QIAxcel system. The QIAxcel analyzer and QIAxcel gel cartridges eliminate tedious agarose gel preparation.

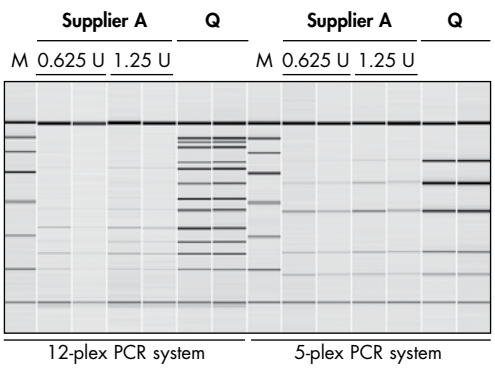


Figure 15. Reliable preamplification of SNPs using the Type-it Mutation Detect PCR Kit analyzed on the QIAxcel. Two different PCR systems (12-plex and 5-plex), both including the SNP of interest were amplified using the standard protocol for the Type-it Mutation Detect PCR Kit (Q) or using preoptimized conditions with a hot-start DNA polymerase from Supplier A. With the Type-it Mutation Detect PCR Kit, all fragments of interest were amplified without any optimization of PCR parameters and could successfully be used as input for the SNaPshot PCR system. Amplification using the kit from Supplier A resulted in missing fragments.

Detection using PyroMark Systems

Sample collection and stabilization → Genomic DNA purification → Genomic DNA storage and whole genome amplification → PCR-based genotyping analysis → **Detection**

Pyrosequencing® — the definitive detection platform for genetic analysis

Pyrosequencing ensures ultimate precision and accuracy when performing highly sensitive mutational analysis or when quantifying alleles in mixed cell populations. Data are presented in a sequence context which serves as a built-in quality control of the results. Pyrosequencing may also be performed after an HRM experiment. This combines fast and effective mutational screening by HRM with detailed sequence analysis and verification by Pyrosequencing (Figure 17).

Figure 16. PyroMark Q24 instrument with PyroMark Q24 Vacuum Workstation.

▶ PyroMark Q24

The PyroMark Q24 uses proven Pyrosequencing technology for mutational analysis of 1–24 samples in as little as 15 minutes. The PyroMark Q24 consists of the PyroMark Q24 instrument, the PyroMark Q24 Vacuum Workstation for preparation of single-stranded DNA, reagents and controls, as well as PyroMark Q24 Software for analysis (Figure 16).

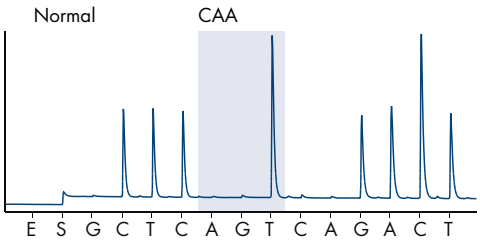


Figure 17. Results from mutation analysis of codon 61 using the PyroMark Q24 KRAS v2.0 test and PyroMark Q24. Analysis of codon 61 is set up as a reverse assay, which means that CAA is read as TTG. The Pyrogram® trace shows a sample with a normal genotype. Light blue areas indicate the variable position.

▶ PyroMark Q96 MD

For higher throughput needs, we offer the PyroMark Q96 MD, which is highly suitable for real-time, sequence-based detection of SNPs and mutations, and allele frequencies. It enables analysis of up to 96 samples. The PyroMark Q96 MD instrument in combination with the PyroMark Q96 Vacuum Workstation, application software, reagents, and controls provides a complete solution for Pyrosequencing analysis.

A complete workflow

The term genotyping encompasses a range of applications used to detect genetic differences between individuals and is used in several different areas of biological research. Sensitivity, reliability, and reproducibility are prerequisites for successful genotyping analysis. QIAGEN provides innovative solutions for a complete genotyping workflow. Our specialized technologies are designed to overcome the challenges associated with genotyping, enabling highly accurate and reproducible results to be achieved faster — without the need for tedious optimization. From sample collection and purification to analysis and detection — we have optimized kits to suit all your needs and help streamline your workflow.

Table 5. Overview of relevant Web addresses

Web page	Overview	Link
Genotyping application page	QIAGEN genotyping resource center with overview on applications, products, and resources on genotyping	www.qiagen.com/goto/genotyping
ProductFinder	Online tool to find the most suitable QIAGEN products based on applications	www.qiagen.com/Products/ProductFinder
Automated solutions application page	QIAGEN automation resource center with overview on automated systems and related resources	www.qiagen.com/goto/automation
Whole Genome Amplification application page	QIAGEN Whole Genome Amplification resource center with product overview and technical resources	www.qiagen.com/goto/wga
Epigenetics application page	QIAGEN epigenetics resource center with overview on products and resources for methylation research	www.qiagen.com/goto/epigenetics
PCR and RT-PCR overview page	Overview on QIAGEN's vast PCR and RT-PCR portfolio	www.qiagen.com/goto/pcr
HRM genotyping page	Overview on QIAGEN's HRM based solutions for genotyping	www.qiagen.com/goto/HRMgenotyping

Ordering Information

Product	Contents	Cat. no.
▶ QIAamp DNA Mini Kit (50)*	For 50 DNA preps from genomic, mitochondrial, bacterial, parasite, or viral samples	51304
▶ QIAamp DNA FFPE Tissue Kit (50)	For purification of genomic FFPE tissues	56404
▶ QIAcard FTA One Spot (100)*	For room-temperature sample collection and storage	159201
▶ QIAsafe DNA Tubes (50)*	For room-temperature storage of DNA	159104
▶ QIAsafe DNA Blood Tubes (50)*	For room-temperature blood storage with DNA stabilization	159134
▶ REPLI-g Mini Kit (25)*	For genomic DNA preparation using whole genome amplification	150023
▶ Type-it Microsatellite PCR Kit (200)*	For multiplex PCR analysis of microsatellite loci	206243
▶ Type-it Mutation Detect PCR Kit (200)*	For multiplex PCR analysis of mutations	206343
▶ Type-it Fast SNP Probe PCR Kit (800)*	For SNP genotyping using TaqMan or TaqMan MGB probes	206045
▶ Type-it HRM PCR Kit (100)*	For genotyping using HRM technology	206542
▶ Rotor-Gene ScreenClust HRM Software	For HRM data analysis	9020147
▶ Rotor-Gene Q	For outstanding performance in real-time PCR	Inquire
▶ PyroMark Q24	For Pyrosequencing in 24-well format	9001514
▶ PyroMark Q96 MD	For Pyrosequencing in 96-well format	9001526
▶ QIAxcel System	For automated DNA fragment analysis	9001421

*Additional kit sizes and/or formats available; see www.qiagen.com.

PyroMark Q24 KRAS v2.0 is intended for research use only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease. For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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Purchase of this product is accompanied by a limited, non-transferable immunity from suit to use it with detection by a dsDNA-binding dye as described in U.S. Patents Nos. 5,994,056 and 6,171,785 and corresponding patent claims outside the United States for the purchaser's own internal research. No real-time apparatus or system patent rights or any other patent rights, and no right to use this product for any other purpose are conveyed expressly, by implication or by estoppel. The QIAGEN silver logo is exclusively licensed to Corbett Research. NOTICE TO PURCHASER: The purchase of this product (Rotor-Gene Q, Rotor-Disc) includes a limited, non-transferable license to certain patents (see details below) surrounding rapid polymerase chain reaction (PCR) methods and instrumentation, the use of SYBR Green I in PCR reactions, melting curve analysis, analysis methods of DNA melting data, specifically high resolution melting (HRM) and others. The purchase of this product (Rotor-Gene Q, Rotor-Disc) includes a limited, non-transferable license to one or more of US Patents Nos 6,787,338; 7,238,321; 7,081,226; 6,174,670; 6,245,514; 6,569,627; 6,303,305; 6,503,720; 5,871,908; 6,691,041; 7,387,887; and U.S. Patent Applications Nos. 2003-0224434 and 2006-0019253 and all continuations and divisionals, and corresponding claims in patents and patent applications outside the United States, owned by the University of Utah Research Foundation, Idaho Technology, Inc., and/or Roche Diagnostics GmbH, for internal research use or for non-in vitro diagnostics applications. No right is conveyed, expressly, by implication or estoppel, for any reagent or kit, or under any other patent or patent claims owned by the University of Utah Research Foundation, Idaho Technology, Inc., and/or Roche Diagnostics GmbH, or by any other Party. For information on purchasing licences for in-vitro diagnostics applications or reagents, contact Roche Molecular Systems, 4300 Hacienda Drive, Pleasanton, CA 94588, USA. This real-time thermal cycler (Rotor-Gene Q) is licensed under U.S. Patent No. 6,814,934 and corresponding claims in any foreign counterpart patent thereof owned by Applied Biosystems LLC, in all fields, including research and development, all applied fields, and human and animal in-vitro diagnostics. No rights are conveyed expressly, by implication or estoppel to any patents on real-time methods, including but not limited to 5' nuclease assays, or to any patent claiming a reagent or kit. For further information on purchasing additional rights, contact the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California, 94404, USA.

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Australia ▪ Orders 03-9840-9800 ▪ Fax 03-9840-9888 ▪ Technical 1-800-243-066

Austria ▪ Orders 0800/28-10-10 ▪ Fax 0800/28-10-19 ▪ Technical 0800/28-10-11

Belgium ▪ Orders 0800-79612 ▪ Fax 0800-79611 ▪ Technical 0800-79556

Brazil ▪ Orders 0800-557779 ▪ Fax 55-11-5079-4001 ▪ Technical 0800-557779

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

China ▪ Orders 021-3865-3865 ▪ Fax 021-3865-3965 ▪ Technical 800-988-0325

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

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

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

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

Hong Kong ▪ Orders 800 933 965 ▪ Fax 800 930 439 ▪ Technical 800 930 425

Ireland ▪ Orders 1800 555 049 ▪ Fax 1800 555 048 ▪ Technical 1800 555 061

Italy ▪ Orders 800-789 544 ▪ Fax 02-334304-826▪ Technical 800-787980

Japan ▪ Telephone: 03-6890-7300 ▪ Fax 03-5547-0818 ▪ Technical 03-6890-7300

Korea (South) ▪ Orders 1544 7145 ▪ Fax 1544 7146 ▪ Technical 1544 7145

Luxembourg ▪ Orders 8002-2076 ▪ Fax 8002-2073 ▪ Technical 8002-2067

Mexico ▪ Orders 01-800-7742-639 ▪ Fax 01-800-1122-330 ▪ Technical 01-800-7742-639

The Netherlands ▪ Orders 0800-0229592 ▪ Fax 0800-0229593 ▪ Technical 0800-0229602

Norway ▪ Orders 800-18859 ▪ Fax 800-18817 ▪ Technical 800-18712

Singapore ▪ Orders 65-67775366 ▪ Fax 65-67785177 ▪ Technical 65-67775366

Spain ▪ Orders 91-630-7050 ▪ Fax 91-630-5145 ▪ Technical 91-630-7050

Sweden ▪ Orders 020-790282 ▪ Fax 020-790582 ▪ Technical 020-798328

Switzerland ▪ Orders 055-254-22-11 ▪ Fax 055-254-22-13 ▪ Technical 055-254-22-12

UK ▪ Orders 01293-422-911 ▪ Fax 01293-422-922 ▪ Technical 01293-422-999

USA ▪ Orders 800-426-8157 ▪ Fax 800-718-2056 ▪ Technical 800-DNA-PREP (800-362-7737)

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