

# Fully automated DNA purification and efficient multiplex PCR for analysis of microsatellite loci

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Microsatellites — highly polymorphic DNA markers comprised of nucleotides that are repeated in tandem arrays and distributed throughout the genome — are a useful tool for genetic identification of individuals. The microsatellites most frequently used in PCR analysis are short tandem repeats (STRs). Typical applications for the analysis of STRs are forensics, identity and paternity testing, and genetic-linkage analysis. In such investigations, simultaneous analysis of multiple microsatellite loci by multiplex PCR is highly desirable.

Important factors for successful multiplex PCR amplification are the quality and concentration of PCR template. For medium and high throughput, automated methods provide efficient nucleic acid purification with minimal handling variability, leading to more consistent results. The BioRobot® MDx workstation provides walkaway automated purification of genomic and mitochondrial DNA from blood using QIAGEN® certified ready-to-run QIAamp® protocols. The protocol is rapid and efficient, requiring only 2.5 hours preparation time for 96 samples (including a detailed load check of 20 minutes). Automated reaction setup for downstream applications ensures maximal reliability.

The new QIAGEN Multiplex PCR Kit is the first commercially available kit specifically developed for multiplex PCR and provides a fast, efficient, and reliable procedure. The simple multiplex master-mix solution eliminates the need for lengthy optimization procedures, such as adjusting the amounts of Mg<sup>2+</sup>, enzyme, or primers. The *QIAGEN Multiplex PCR Handbook* contains a specific protocol for the amplification of microsatellite loci that was used for this study.

In this study we demonstrate that genomic DNA isolated using the QIAamp DNA Blood BioRobot MDx Kit is highly suited for direct use as a template in multiplex PCR of microsatellite loci using the QIAGEN Multiplex PCR Kit.

## Materials and methods

Blood samples were collected from 12 healthy donors in spray-dried K-EDTA collection tubes and were stored at 2–8°C until preparation. Genomic and mitochondrial DNA was purified from 200 µl whole blood using the QIAamp DNA Blood BioRobot MDx Kit. The BioRobot MDx workstation required 2.5 hours (including a load check of 20 minutes) for preparation of 96 samples in a fully automated procedure. Yield and purity of the purified human DNA were determined photometrically. Isolated DNA was stored at –20°C until used in multiplex PCR.

Multiplex PCR of the STR markers vWA, D7S820, F13A1, HUMTH01, FES/FPS, and amelogenin was performed using the QIAGEN Multiplex PCR Kit and the protocol for amplification of microsatellite loci contained in the *QIAGEN Multiplex PCR Handbook*. Each reaction contained 5 µl of a primer mix containing all primers at 2 µM (final primer concentration = 0.2 µM), 25 µl of 2x QIAGEN Multiplex PCR Master Mix, and 19 µl water. Four samples were chosen at random from 4 donors, and 1 µl of eluate (18–43 ng DNA) was used as template for multiplex PCR. PCR was carried out for 24 cycles using the conditions shown in Table 1. For visualization of PCR products, one primer from each pair was fluorescently labeled at the 5' end with either 6-FAM (vWA, D7S820, F13A1, and amelogenin) or HEX (HUMTH01 and FES/FPS). A 1 µl aliquot of the multiplex PCR product was used for analysis on the ABI PRISM® 377 sequencer.

**Table 1. PCR cycling protocol for microsatellite analysis**

Step	Time	Temperature
Initial HotStarTaq® DNA Polymerase activation step	15 min	95°C
Denaturation	30 s	94°C
Annealing	90 s	60°C
Extension	60 s	72°C
Final extension	30 min	60°C

## Results and discussion

### Reliable DNA quality and quantity using the QIAamp DNA Blood BioRobot MDx Kit

Isolation of whole blood genomic DNA from eight 200 µl whole blood replicates from 12 different donors with the QIAamp DNA Blood BioRobot MDx Kit provided high-purity DNA with an average yield of 5.1 µg (Figure 1) and an average  $A_{260}/A_{280}$  ratio of 1.9. Real-time PCR amplification of the human  $\beta$ -actin gene was successful in all 96 samples and gave an average threshold cycle of 19.81 (SD = 1.08; data not shown).

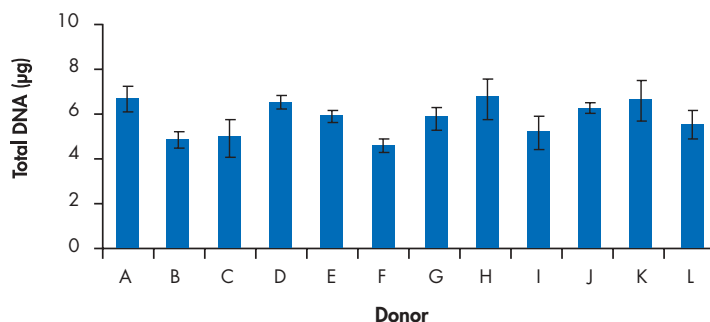
The amount of DNA obtained from each replicate from a given donor was highly reproducible. The  $A_{260}/A_{280}$  ratios and successful real-time PCR amplification show that DNA obtained using the QIAamp DNA Blood BioRobot MDx Kit is highly pure and suited for direct use in sensitive downstream applications.

### Reliable multiplex PCR of STR loci using the QIAGEN Multiplex PCR Kit

Multiplex (6plex) PCR amplification of 5 STR loci and the amelogenin gene from 4 individuals was successful and highly reproducible, giving clear peaks in electropherograms and allowing identification of individual genotypes (Figures 2 and 3). All 6 targets were well amplified from all DNA samples, irrespective of whether the locus was homozygous (both alleles generate a fragment of the same size) or heterozygous (fragments of different size are generated from each allele).

Satisfactory results were obtained using the same number of PCR cycles and the same amount of PCR product for each sample, indicating that minor differences in DNA concentration do not significantly influence multiplex PCR of microsatellite loci. ▶

**Reproducible DNA Yields from Whole Blood**

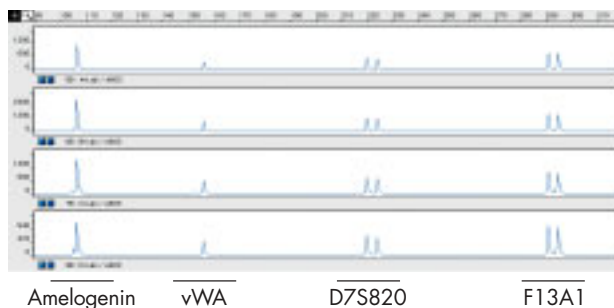


**Figure 1** Genomic DNA was isolated from ninety-six 200 µl whole blood samples (8 replicates from 12 different donors A–L) using the QIAamp DNA Blood BioRobot MDx Kit and the BioRobot MDx workstation. Yields varied depending on the cell content of blood from each donor.

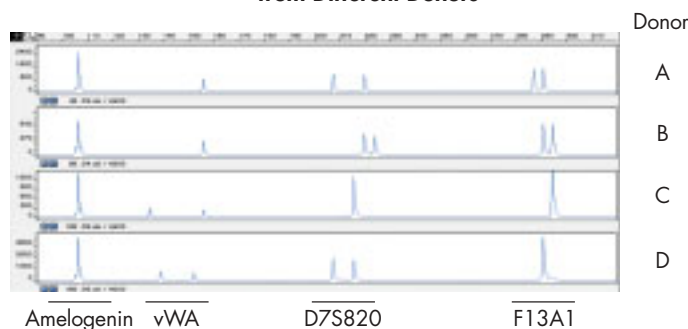
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## Highly Reproducible Results from Replicate Samples



## Accurate Microsatellite Analysis of Genomic DNA from Different Donors



**Figure 2** Multiplex PCR analysis of microsatellite loci. Equal volumes (1  $\mu$ l) of DNA preparations were used as template for a 6plex PCR assay using the QIAGEN Multiplex PCR Kit. Fluorescently labeled PCR products were detected using an ABI PRISM 377 Sequencer. Data for the four 6-FAM-labeled PCR products are shown. Homogenous signals were obtained from 4 different DNA preparations from the same donor.

**Figure 3** Experimental details were as in Figure 2 except that DNA from 4 different donors was used for multiplex PCR.

### Conclusions

- ◆ Standardized processing using the BioRobot MDx together with QIAamp chemistry results in highly consistent yields and high purity of genomic DNA from individual whole blood samples.
- ◆ Without any adjustment, such as equalizing sample concentrations, genomic DNA was effectively amplified in multiplex PCR using the QIAGEN Multiplex PCR Kit. ■

### Ordering Information

Product	Contents	Cat. No.
BioRobot MDx	System includes: robotic workstation with 8 dilutor units, variable spacing system, sample tracking system, computer controlled vacuum pump, automated vacuum manifold, cooling and heating unit, QIAsoft™ MDx Operating System, computer, laboratory cabinet, accessory cabinet, 1 year warranty on parts and labor	900600
QIAamp DNA Blood BioRobot MDx Kit (12)*	For 12 x 96 DNA preps: 12 QIAamp 96 Plates, Buffers, QIAGEN Protease, Elution Microtubes CL, Caps, S-Blocks, Disposable Troughs, Top Elute Fluid, Tape Pad	965152
QIAGEN Multiplex PCR Kit (100)	For 100 x 50 $\mu$ l multiplex PCR reactions: 2x QIAGEN Multiplex PCR Master Mix (providing a final concentration of 3 mM MgCl <sub>2</sub> , 3 x 0.85 ml), 5x Q-Solution (1 x 2.0 ml), distilled water (2 x 1.7 ml)	206143

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