Automated haplotype-specific separation and allele-level typing of ambiguous allele pairs

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This study demonstrates that haploid components of DNA could be purified from genomic DNA using HaploPrep™ reagents and the QIAGEN® BioRobot® EZ1® workstation, a liquid-handling robot that has the ability to capture and manipulate magnetic beads. Automation of the haplotype-specific extraction ensures efficient, parallel, and reliable processing of multiple samples.

Haplotype-specific extraction (HSE) establishes haplotypes from individual patient's samples without knowledge of familial information by physically separating a diploid sample into its haploid components (1). Magnetic beads are selectively attached to polymorphic sites and used to isolate the targeted fragments from a heterozygous mixture. The process does not require amplification. HaploPrep separated DNA is directly analyzed with kits and assays already in use for HLA typing.

Automated HaploPrep separations were carried out on the QIAGEN BioRobot EZ1 workstation, capable of handling up to 6 samples. This enables efficient, parallel, and reliable processing of multiple samples.

The method permits the unambiguous typing of diploid allele pair combinations that fail to be resolved by conventional HLA typing techniques. Shown are haplotype-specific separations targeting the major allele groups of the HLA-B locus, performed on genomic DNA.

The purification of alleles allows the unambiguous identification of a patient's haplotype and allele pair combination.

Figure 1. The BioRobot EZ1 workstation processes up to 6 haplotype-specific extractions.

Materials and methods

The purpose of this study was to automate the HaploPrep procedure on a robotic platform. This was successfully achieved on the QIAGEN BioRobot EZ1 workstation.

The same HaploPrep probes and reagents developed for manual extractions were used following the automated HaploPrep procedure (Figure 2). All probes were designed to target HLA-B polymorphisms. Some of the HLA-B probes used required modifications to the PCR protocol. These modifications included the following: HaploPrep extracted DNA was substituted for genomic DNA, the number of PCR cycles was increased, and/or the amount of *Taq* DNA polymerase was increased.

Results

Numerous samples were separated into haploid fractions at QIAGEN laboratories. Haplotype-specific extractions have been confirmed using INNO-LiPA HLA-B primers and probes and Olerup SSP™ typing.

Three samples were HaploPrep extracted at the American Red Cross National Histocompatibility Laboratory. Figure 3 shows the sequencing results for one of the diploid samples and its two haploid components.

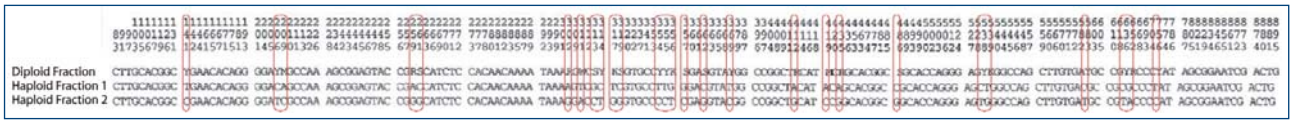


Figure 3. Heterozygote positions in diploid sample resolved into homozygote positions in 2 haploid fractions through the use of HaploPrep haplotype-specific extraction. The samples were sequenced using reagents from Forensic Analytical HLA B reagents. HaploPrep extracted DNA was substituted for genomic DNA, and the number of PCR cycles was increased from 35 cycles to 45 cycles.

One of the five sequencing reactions failed for reason unrelated to the HaploPrep procedure. The heterozygote positions called by the ABI™ Basecaller software were resolved to be homozygote positions in the HaploPrep extraction although there are traces of the second allele.

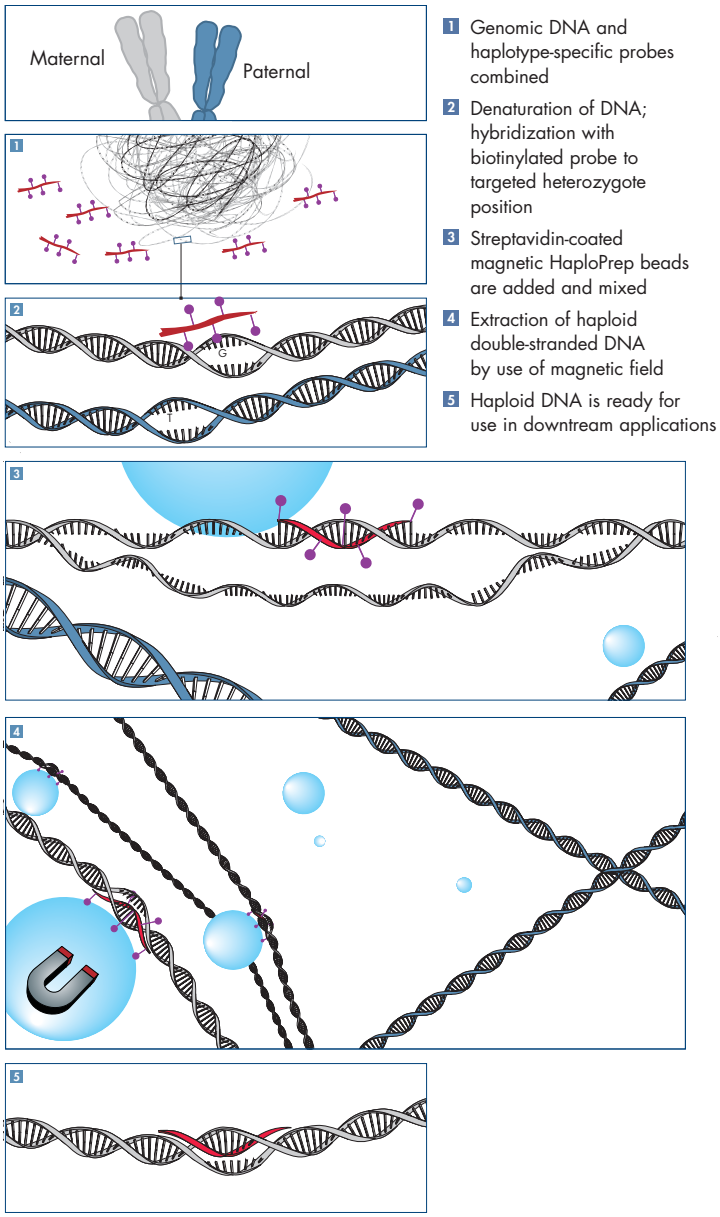
Examples:

Sample 34482 diploid typing had the following possible allele combinations.

- HLA B*1501101/5107
- HLA B*1501102N/5107
- HLA B*1515/52012

All heterozygote positions were resolved to homozygote positions, and the final typing of the sample was a HLA B*15010101/5107.

On the 3 diploid samples sequenced there were a total of 102 heterozygote positions in the polymorphic sites sequenced. The sequencing results of the 5 haploid extractions of these samples were uniformly all called homozygotes by the ABI Basecaller software.



1. Genomic DNA and haplotype-specific probes combined
2. Denaturation of DNA; hybridization with biotinylated probe to targeted heterozygote position
3. Streptavidin-coated magnetic HaploPrep beads are added and mixed
4. Extraction of haploid double-stranded DNA by use of magnetic field
5. Haploid DNA is ready for use in downtream applications

Figure 2. HaploPrep haplotype-specific extraction — the easy 5-step method.

Conclusions

- Diploid allele pair combinations that fail to be resolved by conventional sequencing-based typing (SBT) or sequence-specific oligonucleotide probes (SSOP) can be typed unambiguously using HaploPrep separated DNA.
- The separation of the alleles can be done using a robotic platform.
- The HaploPrep separated DNA can be used directly with kits and assays already in use for HLA typing.

Acknowledgements

We would like thank Baiba Poore and Debra Kukuruga for the sequencing-based typings performed at the American Red Cross National Histocompatibility Lab in Baltimore, MD.

Reference

1. Nagy, M., Entz, P., Otremba, P., Schoenemann, C., Murphy, N., and Dapprich, J. (2007) Haplotype-specific extraction: a universal method to resolve ambiguous genotypes and detect new alleles — demonstrated on HLA-B. Tissue Antigens **69**, 176.

Ordering Information

Product	Contents	Cat. no.
BioRobot EZ1	Robotic workstation for automated purification of nucleic acids using EZ1 kits, installation, 1-year warranty on parts and labor*	9000705
EZ1 HaploPrep Card	Preprogrammed card for EZ1 HaploPrep protocols	9018113
EZ1 HaploPrep Kit	For 48 haplotype-specific extractions: HaploPrep Cartridges, HaploPrep Hybridization Buffer, Disposable Filter-Tips, Disposable Tip Holders, Microcentrifuge Tubes	4340004
HaploPrep HLA Locus Probes	Probe mixtures for specific HLA alleles	Varies†

* Warranty PLUS 2 (cat. no. 9237720) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and parts.

† See probe lists at www.qiagen.com/goto/HaploPrepProbes to choose the appropriate locus-specific probe.

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