

# HLA Sequencing-Based Typing Systems

## Introduction

Long acknowledged as the gold standard for genetic analysis, automated multicolor fluorescent sequencing is now accepted as the definitive method for high-resolution HLA typing research. Applied Biosystems has incorporated the latest advancements of AmpliTaq Gold® DNA Polymerase-mediated hot start PCR and BigDye™ terminator sequencing chemistry into a complete sequencing-based typing (SBT) system for high-resolution typing of HLA-A, HLA-B, and HLA-DRB genes.

Applied Biosystems HLA Typing Systems provide more than just high-resolution typing results. Our BigDye™ terminator sequencing reagents are designed for ease-of-use to allow increased throughput with minimal labor costs. MatchMaker™ software completes the system by providing automated allele calling and editing functions.

## Robust and Efficient PCR

High-resolution typing is brought to a new level of performance and simplicity with the Applied Biosystems HLA SBT Systems. Ready-reaction PCR mixes provide high specificity amplification with AmpliTaq Gold® DNA Polymerase. Optimized primer design combined with the enzyme's unique invisible hot start capability ensure robust and reproducible amplification in every run. The PCR products are used as templates for subsequent sequencing reactions.

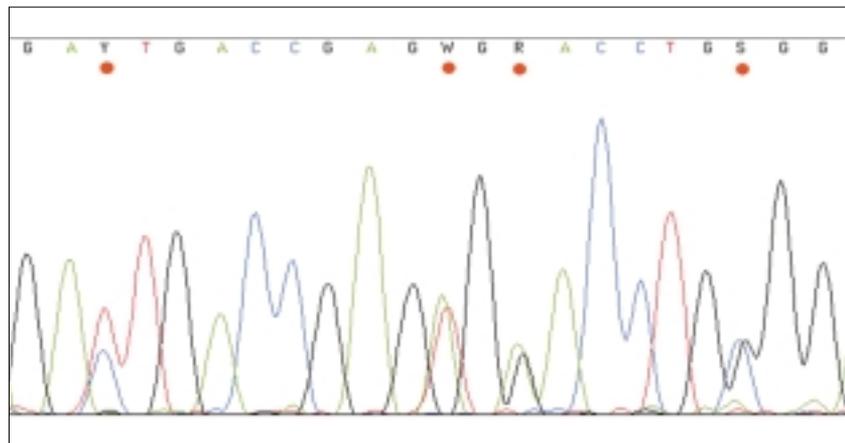


Figure 1. Representative data generated from an HLA-A\*0211/2402 sample. This sequence is from positions 288-308 at the 3' of exon 2. Note the even base incorporation at the four heterozygote positions, the low background noise, and baseline resolution of the peaks.

## Reliable Allele Identification

Four-color dye primer sequencing has been the universally accepted method for HLA sequencing-based typing. Now BigDye™ terminators have greatly simplified sequencing while maintaining a level of precision and reliability that is essential in HLA typing. This advanced chemistry produces sequences having even base incorporation and low background noise that facilitate heterozygote detection (Figure 1). BigDye™ terminators are clearly the chemistry of choice for reliable HLA typing.

## Minimized Labor and Cost

The combination of BigDye™ terminators and AmpliTaq® DNA Polymerase, FS provides the most convenient sequencing chemistry available for routine HLA typing research. Ready-reaction sequencing mixes are prepared with custom sequencing primers that generate complete sequences in both forward

and reverse directions for all regions of interest. Relative to dye primer sequencing, the dye terminator-based chemistry provides a fourfold reduction in the number of reactions. Fewer sequencing reactions required per sample translates directly into increased sample throughput with lower reagent and labor costs. All steps can be performed in a 96-well format with minimal manipulations, allowing a single technician to easily perform several hundred sequencing reactions in one day.

## Class I: HLA-A and HLA-B

Applied Biosystems HLA-A and HLA-B SBT systems permit simpler, more informative high-resolution typing of Class I genes than any other method. The strategy is unique with respect to both the PCR and the sequencing. The ready-reaction PCR mixes provide high specificity amplification with

AmpliTaq Gold® DNA Polymerase. A single PCR product encompassing exons 1 through 5 allows complete sequencing of exons 2, 3, and 4 using state-of-the-art BigDye™ terminators (Figure 2). Analysis of all three exons minimizes ambiguities and ensures that rare and null alleles are also identified. In addition, the ready-reaction sequencing mixes are designed to provide complete sequence information in both orientations of each exon. This is essential for correctly identifying every base position and ensuring correct allele calls.

**Class II: HLA-DRB**

Low and high resolution typing combine in this complete, two-step system for HLA-DRB typing research. The 12 ready-reaction PCR mixes provide high specificity amplification using allele group-specific primers for exon 2 of HLA-DRB1, DRB3, DRB4, and DRB5. The PCR products are subsequently sequenced to generate high-resolution allele assignments. The system is designed to ensure complete sequencing in both orientations for all regions of interest, ensuring absolute confirmation of the allele type. Greater ease-of-use and higher throughput result in reduced labor and processing time.

**Codon 86 Module Resolves Ambiguous DRB Typings**

A majority of samples with ambiguous DRB typings are heterozygous at codon 86. Using our unique codon 86 ready-reaction sequencing mixes, the two alleles in a heterozygous PCR product are resolved, allowing unambiguous, high-resolution allele typing (Figure 3). Optimized to work with our DRB PCRs, this new sequencing module makes our HLA-DRB SBT System the most comprehensive stand-alone high-resolution typing method available.

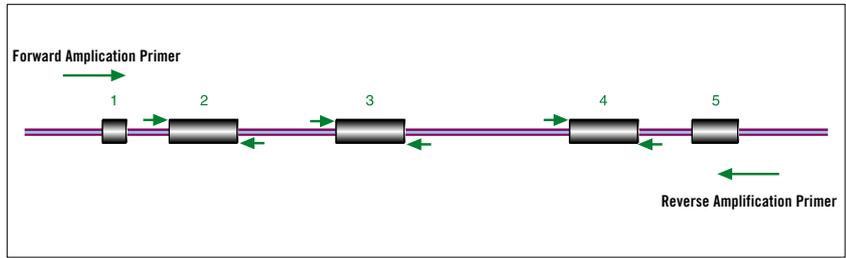


Figure 2. Schematic diagram of the HLA-A and HLA-B amplification and sequencing strategy. A single 2 kb PCR amplicon is generated at each locus with six subsequent sequencing reactions (three forward and three reverse, as indicated by green arrows) for a complete sequence of exons 2, 3, and 4.

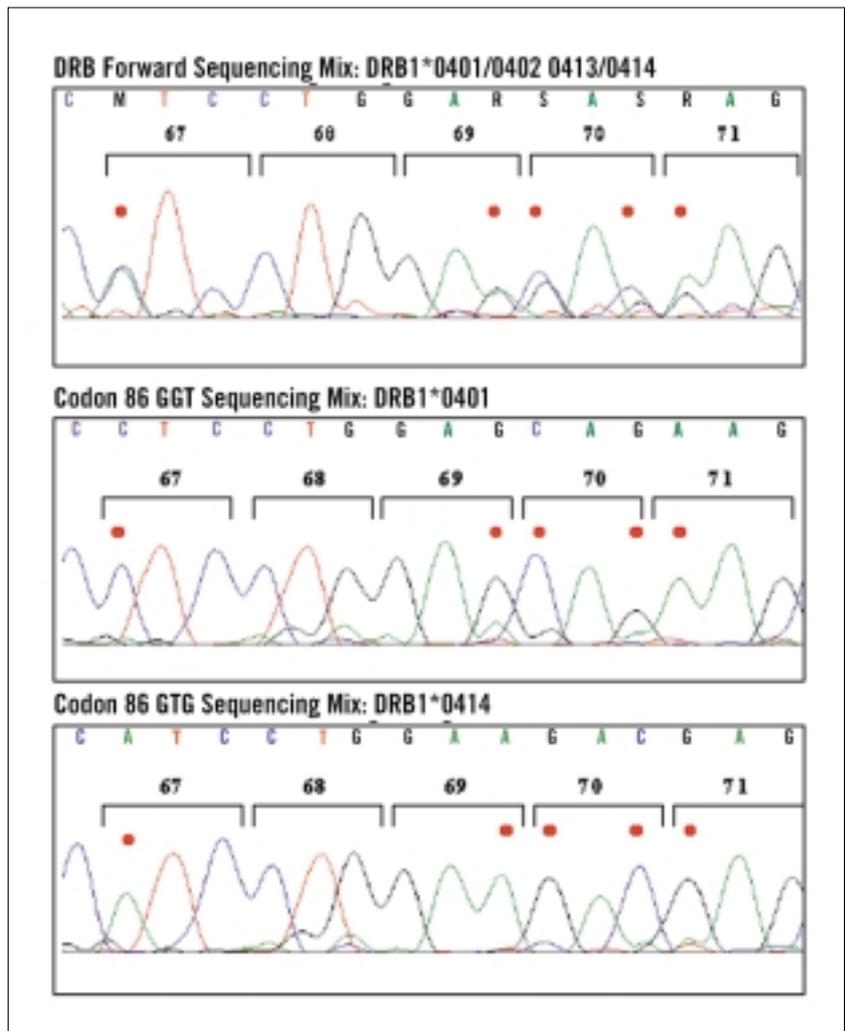
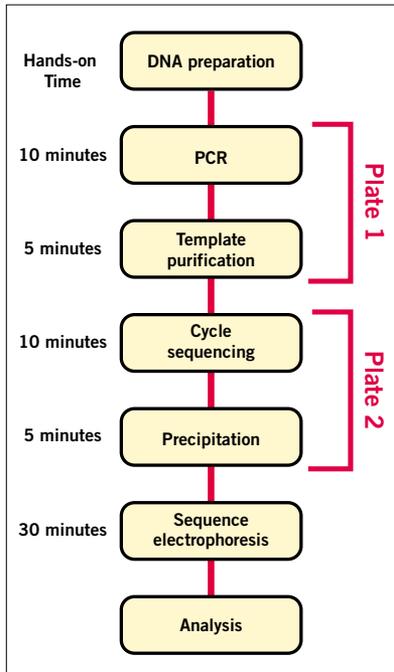


Figure 3: These sequences demonstrate the utility of the codon 86 sequencing primers for resolving ambiguous heterozygote combinations. In this sample, the original heterozygote sequence was an exact match to both 0401/0402 and 0413/0414 (top sequence). Processing the original heterozygote PCR with the two codon 86 sequencing mixes resulted in homozygous sequences and definitive allele assignments of DRB1\*0401 and DRB1\*0414 (lower two sequences). In this allele combination, there are five heterozygote positions between codons 67-71 (identified with red dots). In the two lower sequences, each heterozygote is clearly resolved into homozygote base calls, demonstrating the specificity of the codon 86 sequencing mixes.



**Figure 4:** Schematic representation of the HLA sequencing-based typing protocol performed in a 96-well format. All steps can be limited to two 96-well plates, lessening sample transfers and reducing the assay time. The “hands-on” technician time is described for each step in the process. A single technician can perform the assay, from PCR to completion of sequencing, in a single working day.

### Allele Identification with MatchMaker™ Software

Matchmaker™ software completes this integrated system. This software allows batch processing for high sample throughput. It automatically identifies critical information and allows editing of the HLA sequence data. It compares the analyzed sequences to a database of known alleles and generates final HLA typings.

### Sample Processing

Incorporating four-color terminator sequencing chemistry into each HLA kit has greatly simplified sequencing-based typing of HLA-A, HLA-B, and HLA-DRB genes. This has facilitated implementation of common protocols irrespective of HLA locus, allowing simultaneous sequencing of HLA-A, HLA-B, and HLA-DRB genes within a single 96-well plate.

The combination of four-color terminator chemistry and a 96-well format has significantly reduced the amount of sample manipulations and sample transfers. Figure 4 provides a schematic description of the necessary steps and “hands-on” time required to perform this assay. This simple protocol translates into a reduced labor requirement while maximizing the high-quality data generated.

### Conclusion

Applied Biosystems HLA Sequencing-Based Typing Systems provide a simple, integrated approach to research high-resolution typing of Class I and II HLA genes. Our streamlined approach allows sample processing at many different levels of throughput to accommodate the needs of any size laboratory. You can rely on Applied Biosystems to bring your laboratory a broad range of integrated products, including instruments, reagent systems, software, and worldwide support for comprehensive and efficient sequencing-based HLA typing research.

### References

1. Lee, L., et al., 1997. New Energy Transfer Dyes for DNA Sequencing, *Nucleic Acids Research* 25:14.
2. Krausa, P., et al., 1998. High Resolution Typing at the HLA-A Locus: Improvements in Sequencing-Based Typing Protocols, 24th Annual ASHI Meeting, *Human Immunology* 59:150.
3. Krausa, P., et al., 1998. Sequencing the HLA-B Locus, A Flexible Approach to High Resolution SBT, 24th Annual ASHI Meeting, *Human Immunology* 59:150.
4. Stein, J., et al., 1998. High Resolution Class II HLA-DRB Typing of 50 DNA Samples Using BigDye Terminator Sequencing Chemistry, 24th Annual ASHI Meeting, *Human Immunology* 59:153.

## Ordering Information

First-time users should order a starter kit, which includes all reagents and software needed for sample analysis. The protocol with supplemental diskette must be ordered separately.

Repeat users should order a reagent kit only, as this contains all reagents necessary for sample analysis.

Description	P/N
HLA-DRB Sequencing-Based Typing Starter Kit with BigDye™ Terminators Includes: HLA-DRB Amplification Module, 48 samples HLA-DRB Sequencing Module, 96 reactions HLA Control and Loading Buffer Module HLA-DRB Sequencing-Based Typing Protocol HLA-DRB and Codon 86 Supplemental Diskette MatchMaker™ Allele Identification Software and User's Manual	4305026
HLA-DRB Reagent Kit with BigDye™ Terminators Includes: HLA-DRB Amplification Module, 48 samples HLA-DRB Sequencing Module, 96 reactions	4305213
HLA-DRB Codon 86 Sequencing-Based Typing Kit, 24 samples	4305023
HLA-DRB/Codon 86 Protocol and Supplemental Diskette Includes: HLA-DRB SBT Protocol HLA-DRB Codon 86 Supplemental Diskette HLA-DRB Codon 86 SBT User Bulletin	4305027
HLA-A Sequencing-Based Typing Starter Kit Includes: HLA-A Amplification and Sequencing Module, 24 samples HLA Control and Loading Buffer Module HLA-A/HLA-B Sequencing-Based Typing Protocol HLA-A/HLA-B Supplemental Diskette MatchMaker™ Allele Identification Software and User's Manual	4303557

HLA-A Amplification and Sequencing Module, 24 samples 4303554

HLA-B Sequencing-Based Typing Starter Kit 4307643

Includes:

HLA Amplification and Sequencing Module, 24 samples

HLA Control and Loading Buffer Module

HLA-A/HLA-B Sequencing-Based Typing Protocol

HLA-A/HLA-B Supplemental Diskette  
MatchMaker™ Allele Identification Software and User's Manual

HLA-B Amplification and Sequencing Module, 24 samples 4307642

HLA-A/HLA-B Protocol and Supplemental Diskette 4307644

Includes:

HLA-A/HLA-B SBT Protocol

HLA-A/HLA-B Supplemental Diskette

Components Sold Separately:

HLA-DRB Sequencing Module, 96 reactions 4305022

HLA Control and Loading Buffer Module M0028

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