Applied Biosystems

ViroSeq™ HIV-1 Genotyping System
For the Analysis of HIV-1 Drug Resistance Mutations

- **Complete, integrated system**
- **Optimized reagents with contamination control**
- **Dedicated analysis software**
- **Multiple throughput options**
- **From the leader in DNA sequencing and analysis**

Introduction

"Increasing evidence, however, indicates that viral resistance and drug failure are closely linked."[1]

Applied Biosystems introduces ViroSeq™ HIV-1 Genotyping System for improved analysis of HIV-1 mutations in drug resistance research testing.

Genotypic information on HIV-1 resistance mutations is generally recognized as an integral part in the development of new therapeutic agents. The high viral turnover of HIV-1 and an error prone reverse transcriptase can result in numerous mutations generated with each replication cycle. Identifying and discerning the significance of each mutation in an ever-changing HIV-1 virus can be a challenge.

Why Genotype?

" …HIV-1 genotype is predictive of virologic response to subsequent drugs."[2]

Treatment alternatives rely, in large part, upon changes in the HIV-1 genome. Research has established that some single-base mutations can confer resistance to certain therapies. In other cases, combinations of mutations can confer resistance. Compensatory mutations restoring drug effectiveness can further complicate the viral profile.

"Other causes are related to the pre-existence of drug resistant variants within HIV-1 quasispecies and the transmission of HIV-1 resistant variants at the time of the infection."[3]

With an increasing number of drug resistant mutations, genotyping is rapidly becoming a necessary tool. Genotyping in research studies, particularly sequencing-based genotyping, can help to maximize the efficiency and cost-effectiveness of clinical development programs for new, antiretroviral therapies. Applied Biosystems, the world leader in both PCR and DNA sequencing, has simplified the HIV-1 genotyping process to help optimize drug research and development.

An Integrated System

Sequencing-based genotyping of retroviral RNA is a complex multi-step process. Our integrated and optimized system streamlines this process. The ViroSeq™ HIV-1 Genotyping System includes sample preparation, reverse transcriptase-

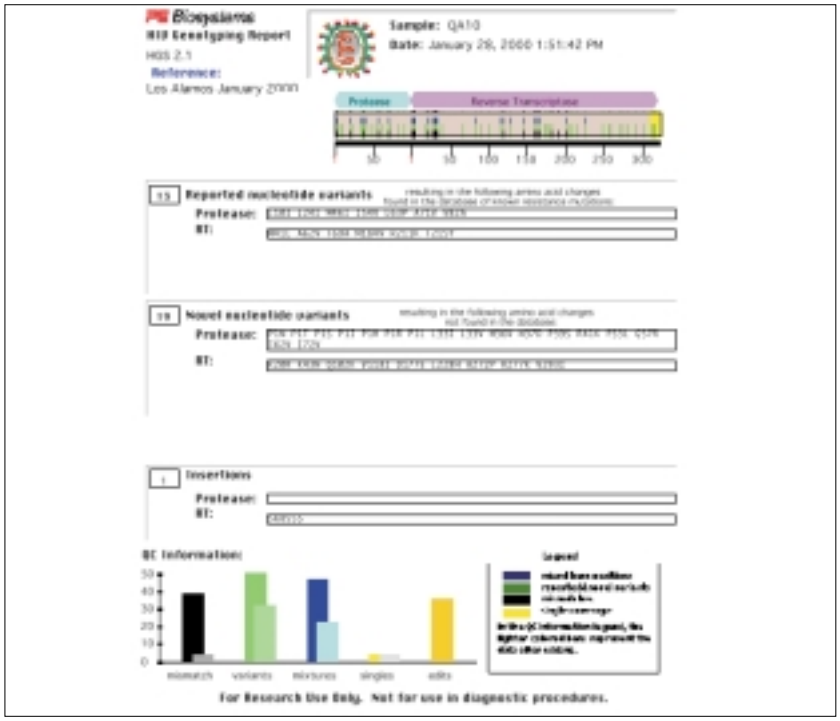

Figure 1. The ViroSeq™ HIV-1 Genotyping Report summarizes key information in a user-friendly format. The navigation bar provides a schematic representation of all positions of interest, including: reported variants (those found in the Los Alamos HIV-1 resistance database, http://HIV-1-web.lanl.gov), and novel variants (those amino acid changes that differ from the reference sequence, which do not appear in the reported database). Tables list reported variants, novel variants and insertions. Quality control information provides a view of overall data quality.

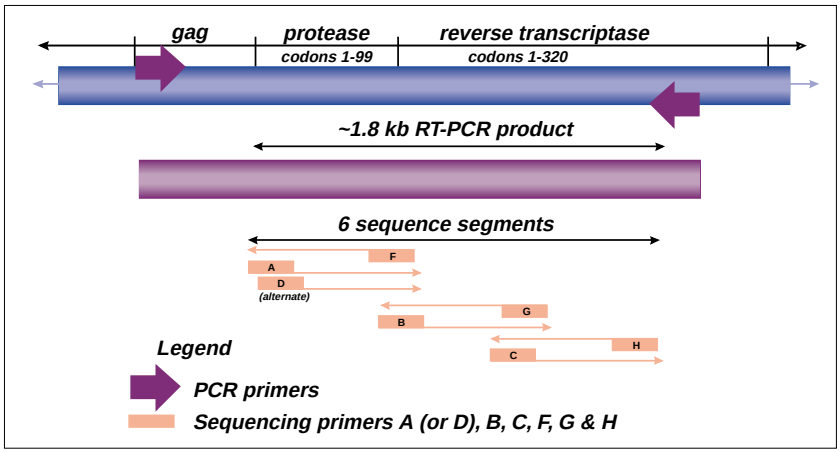

Figure 2. This diagram outlines the RT-PCR and sequencing strategy utilized in the ViroSeq™ HIV-1 Genotyping System. A RT-PCR product of approximately 1.8 kb is generated, followed by high specificity PCR with AmpliTaq Gold® DNA Polymerase. Six sequencing reactions, with BigDye™ terminator chemistry, provide full coverage in forward and reverse orientations of sequence data for codons 1–99 of protease and 1–320 of reverse transcriptase.

polymerase chain reaction (RT-PCR), positive controls, and a sequencing module, as well as dedicated, user-friendly software that provides the HIV-1 Genotyping Report (Figure 1).

The system runs on all Applied Biosystems genetic analysis instrumentation and thermal cyclers providing a complete range of automation and throughput options.

Making It Simple

Applied Biosystems has simplified cutting-edge technology. The latest improvements in PCR and sequencing chemistry are incorporated into the ViroSeq™ HIV-1 Genotyping System.

Using the proven AmpErase® UNG/dUTP contamination control reagents, the risk of contamination from one sample to another is minimized. In addition, AmpliTaq Gold® DNA Polymerase, our premier PCR enzyme, delivers a highly specific RT-PCR product. The unique heat-activated hot start capability of the enzyme results in fewer undesirable, non-specific PCR products, which can interfere with sequencing.

The ViroSeq™ HIV-1 Genotyping System combines AmpliTaq® DNA Polymerase, FS with BigDye™ terminators. Together, these technological advancements allow higher sample throughput using a single lane, four-color fluorescence detection without compromising the level of accuracy. This results in labor savings and confidence in your sequencing results.

Improved Sensitivity

The ViroSeq™ HIV-1 Genotyping System specifications indicate sensitivity down to 2000 copies/mL (cpm). Research studies indicate that the system can reproducibly genotype samples with 1000 copies of HIV-1. Additional research studies using the ViroSeq™ HIV-1 Genotyping System have shown successful genotyping of Clade A-G (Figure 3).

Providing Hardware Options

Applied Biosystems offers a complete line of thermal cyclers and genetic analysis instruments. All of these platforms support the advanced chemistries, which are incorporated into the ViroSeq™ HIV-1 Genotyping System. The GeneAmp® PCR System 9700 and 9600 thermal cyclers deliver the precision and accuracy required to perform optimized PCR.

The 310 and 377 genetic analyzers provide automated fluorescent sequencing (Table 1). The 377 genetic analyzer is designed for high throughput. It accommodates 18 to 96 lanes and can analyze up to 16 plasma samples per run and up to 48 plasma samples in 24 hours. The auto-loading feature of the capillary electrophoresis-based 310 genetic analyzer minimizes hands-on labor.

Applied Biosystems 3100 genetic analyzer, with 16 capillaries, offers the total automation of the 310 genetic analyzer and the exceptional throughput of the 377 genetic analyzer. This combination of automation and throughput provides researchers with the most cost effective and convenient method for genotyping the HIV-1 virus. The 3100 genetic analyzer can analyze up to 24 plasma samples per day.

Automating Analysis

Collection, editing and analysis of sequencing data can create delays. The ViroSeq™ HIV-1 Genotyping System's dedicated software streamlines all of these steps and minimizes the amount of hands-on time required for data analysis. The software automates the assembly process and

Performance of the ViroSeq™ HIV-1 Genotyping Kit, Version 2 with a HIV-1 Subtype (Clade) Panel

Subtype	A	B	C	D	E	F	G
Number of isolates	3	7	5	3	7	3	1

- All samples were successfully amplified and sequenced at viral loads of 25,000 viral particles per mL.
- In addition, 1 subtype A, 1 subtype C, 1 subtype E and 1 subtype F samples were successfully amplified and sequenced at viral loads of 5000 and 1000 particles per mL.[4]

Figure 3. Unpublished data generated by Dr. Nathalia Marlowe and Brad Hoo (Applied Biosystems) in collaboration with Dr. Tarek Elbeik (UCSF Clinical Microbiology Research Laboratory).

reduces hands-on time by focusing manual editing on defined positions of interest. The user-friendly editing palette, navigation bar and edit window help users quickly master the editing process (Figure 4).

Quality Checks

The integrated ViroSeq™ HIV-1 Genotyping System Software does the quality checks for you. Both forward and reverse orientations of the DNA sequence are checked for nucleotide base call agreement. Any base mismatch between these segments is automatically defined as a position of interest to allow manual editing of that position. The final report indicates the total number of mismatches before and after editing, as a snapshot of the data's overall quality.

Warnings are displayed to bring attention to any abnormalities, such as areas of single-stranded coverage, primer orientation disagreement or issues with data collection. These features allow laboratories to track and document the high-quality data that is consistently produced from run to run or among operators.

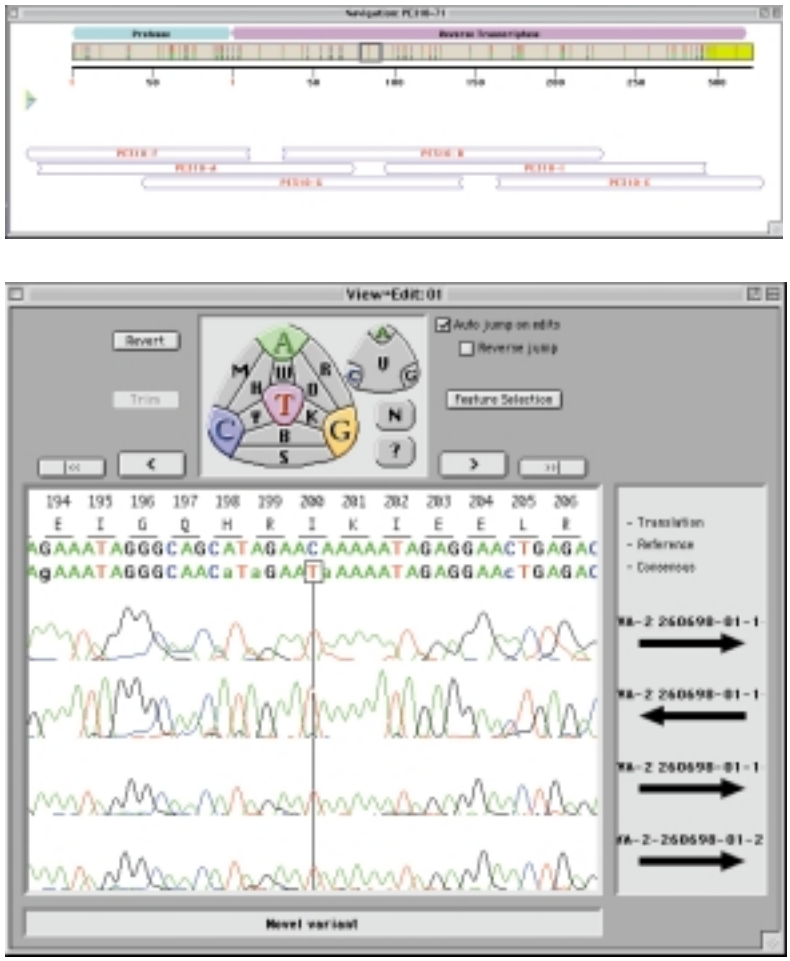

Figure 4. During an editing session, the navigation bar displays a real-time representation of positions of interest. A black box outlines the cursor position. Reported and novel variants are indicated by green bars, mixed base positions by blue bars and mismatches by black bars. If there are any areas with only single coverage, they are displayed in yellow.

The user-friendly editing window displays and labels (from top to bottom) the codon position, the amino acid translation, the reference sequence and the project consensus sequence, as well as the electropherograms for the forward and reverse orientations.

The cursor outlines a particular nucleotide, defined as a position of interest. The editing palette, with IUB and color-coding, provides an efficient means to streamline editing. Once a position is edited, the cursor can be set to sequentially "auto-jump" to the next position of interest in order to further speed the editing process. In the lower portion of the editing window, a position information box describes the position currently displayed by the cursor.

377 System → High Throughput

- Gold standard in automated sequencing
- Maximum throughput
- Convenient batch processing

Number of lanes	Genotypes per run	Run time	Genotypes per shift
96	12-16	7 hrs	**12-16**

310 System → Automation

- Proven capillary technology
- Continuous data collection
- Walk-away operation

Number of lanes	Runs per Genotype	Run time	Genotypes per day
single capillary	6	2 hrs	**2**

3100 System → Automation

- Proven capillary technology
- Walk-away operation
- Reduced editing time

Number of lanes	Genotypes per run	Run time	Genotypes per day
16 capillary	20–24	24 hrs	**20–24**

Table 1. Options in electrophoresis instrumentation

Relevant Genotypic Information

A comprehensive summary of pertinent research information of each analysis is contained in the ViroSeq™ HIV-1 Genotyping Report. The ViroSeq™ HIV-1 Genotyping System Software automatically converts the edited sequencing data into a table of genotypic information with reported and novel variants, and insertions. Reported variants are those base changes reported in the Los Alamos HIV-1 resistance database that confer an amino acid change and are associated with HIV-1 resistance. Embedded

into the software is the HIV-1 pNL4-3 sequence for use as a comparative sequence reference. Any change in the sample sequence from the pNL4-3 sequence is reported as a novel variant. This system of capturing both reported and novel variants expands the utility of the research data by providing a means to record any emerging trend in the frequency of occurrence of novel variants.

The Whole Product Solution

The ViroSeq™ HIV-1 Genotyping System represents our ongoing commitment to deliver an integrated, whole product solution for genotyping HIV-1. The quality control, standardization, and cost-effectiveness delivered by this research system allow more effective use of your resources, higher throughput, and faster data analysis. The system is supported by Applied Biosystems extensive worldwide service organization.

For more information, please contact your Applied Biosystems sales representative or your local sales office.

References

1 Hirsch, M.S., et al. Antiretroviral drug resistance testing in adults with HIV infection: implication for clinical management. *JAMA* 1998; 279:1984–1991.

2 Zolopa, A.R., et al. HIV-1 Genotypic Resistance Patterns Predict Response to Saquinavir-Ritonavir Therapy in Patients in Whom Previous Protease Inhibitor Therapy Had Failed. *Annals of Internal Medicine.* 1999; 131:821–831.

3 Yerly, S., et al. Transmission of antiretroviral-drug-resistant HIV-1 variants. *The Lancet.* 1999; 354:729–733.

4 Michael, N.L., et al. Development of calibrated Viral Load Standards for Group M Subtypes of Human Immunodeficiency Virus Type 1 and Performance of an Improved AMPLICOR HIV-1 MONITOR Test with Isolates of Diverse Subtypes. *Journal of Clinical Microbiology.* August 1999; p. 2557–2563.

Ordering Information

Description	P/N
ViroSeq™ HIV-1 Genotyping System Version 2, 48 samples includes: Sample Preparation Module RT-PCR Module Version 2, Prt/5'RT Sequencing Module, Prt/5'RT RNA Control Module, Prt/5'RT MicroAmp® Reaction Tubes Microcon® YM-100 Microconcentrators	4315425
ViroSeq™ HIV-1 Genotyping System Software	4307816
ViroSeq™ HIV-1 Genotyping Demonstration Software (Software expires 60 days from first use.)	4311573
ViroSeq™ HIV-1 Genotyping System User's Manual	4315267
Components Available Separately:	
Sample Preparation Module, 48 samples	4306027
Sequencing Module, Prt/5'RT, 48 samples	4305611
RNA Control Module, Prt/5'RT, 5 reactions	4315236
HIV-1 Control (8E5 non-infectious virus)	Coming soon

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