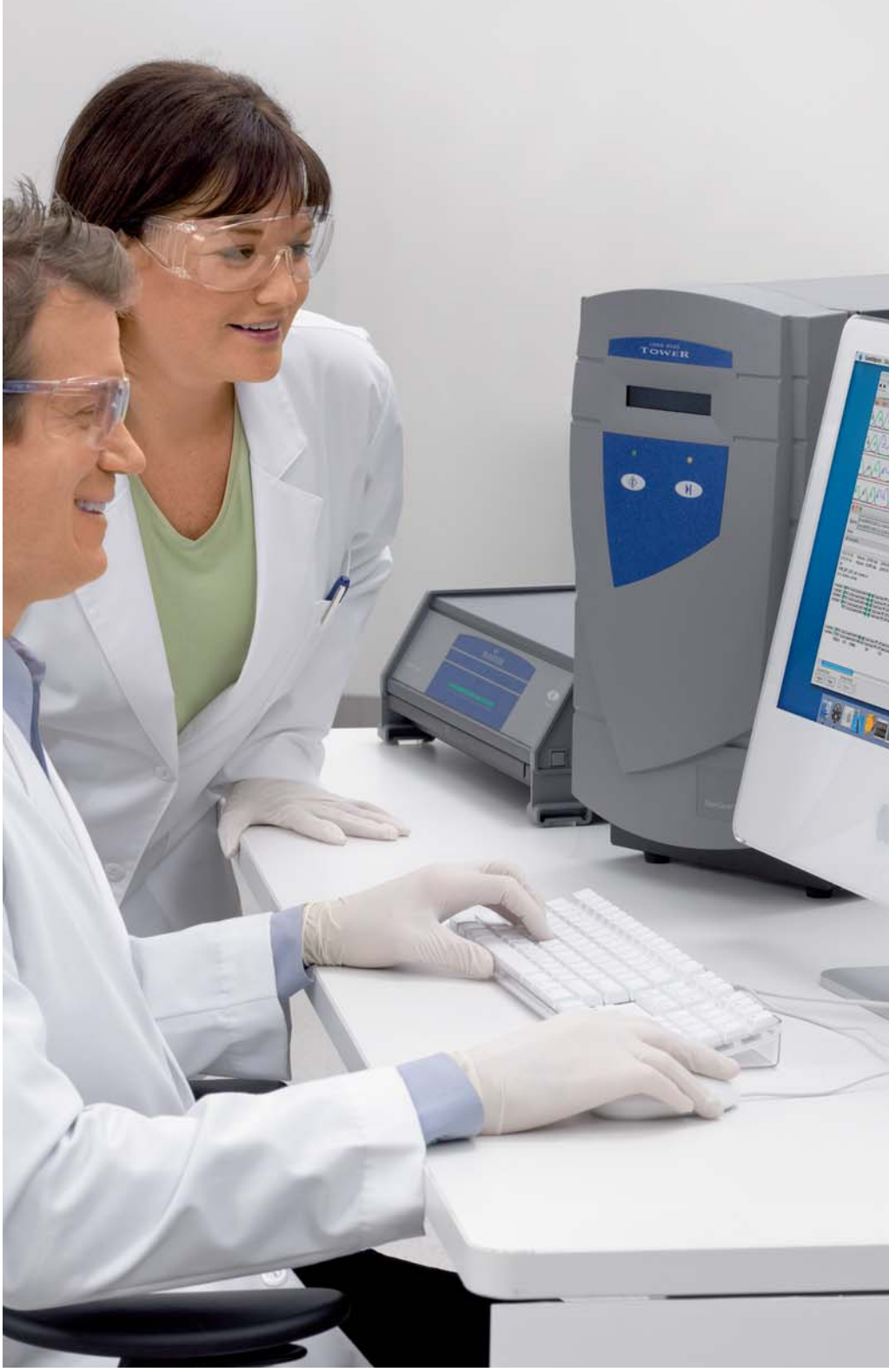
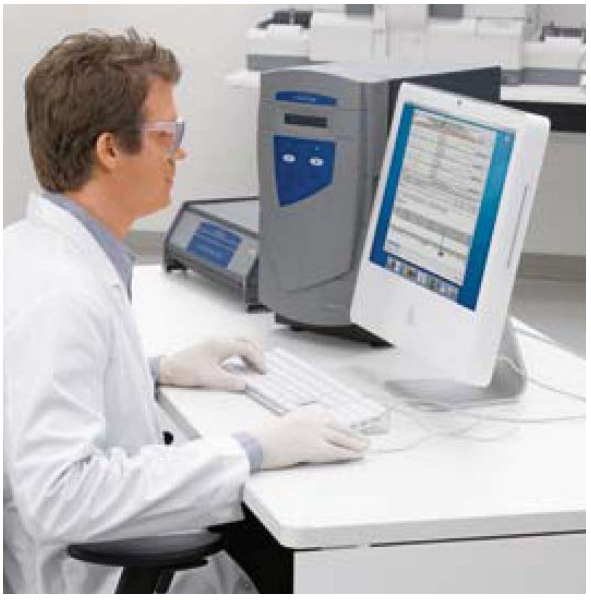


TRUGENE HBV Genotyping Assay

Identify Viral Genotype and Interrogate Hepatitis B Viral Mutations

Answers for life.

SIEMENS



Introduction

The Hepatitis B viral genome is a partially double-stranded DNA molecule, approximately 3200 bases in length, packed inside a nucleocapsid structure composed of the viral core (C) protein (Figure 1). This nucleocapsid is surrounded by the viral envelope, comprising of a mixture of lipids and viral structural proteins or surface antigens (HBsAg, Pre-S1 and Pre-S2).

Virus replication is carried out through enzymatic activity of the viral polymerase or P protein.

This multifunctional protein has domains possessing DNA synthesis priming, reverse transcriptase (RT)/DNA polymerase and ribonuclease (RnaseH) activities[1]. Within the RT region, the protein can be further subdivided into several functional domains (A – F), based on analogy with other viral polymerases. The organization of the HBV genome is such that the same region that codes for the viral P protein also codes for the viral structural proteins, but in an alternate translational reading frame[2] (Figure 2).

TRUGENE® HBV Module 2.0.

Identify and Interrogate Hepatitis B Viral Mutations

Antiviral Drug Development

The P protein and the reverse transcriptase/DNA polymerase domain are the major functional units for viral replication, and as such have been targets in HBV antiviral drug development. A number of new therapeutic agents that inhibit viral replication are currently approved and in clinical trials.

Given the resistance issues identified with lamivudine therapy and the beneficial experience of using combinations of several drugs in the HIV setting, it is likely that new agents will be used in combination with lamivudine to attempt to suppress the development of resistance.

Resistance to lamivudine is a consequence of the low fidelity of the viral polymerase, the high viral load, high viral replicative capacity and the long course of therapy.[10,11,12] The incidence of drug resistance increases with length of therapy, so that typically a significant number of patients will have resistant virus after a year, rising after three years.

Molecular Variants of HBV

Originally, there were two different numbering schemes for assigning mutations within the reverse transcriptase (RT) domain of HBV. These schemes are known in the scientific literature as the genotype-dependent numbering and the genotype-independent numbering scheme.

The lack of a universally accepted numbering convention led to inconsistencies in the published scientific literature. As a result, the genotype-independent scheme has emerged as a method to standardize the way in which HBV mutations in the RT domain are numbered.[3]

The TRUGENE HBV Module 2.0 has adopted that widely accepted nomenclature. This classification system is based on analysis of the complete genomic sequence of the virus. Eight discrete genotypes (A-H) have been identified.[4,5,6,7]

It has been suggested that the viral genotype may correlate with differences in clinical features of HBV infection.[8,9]

The region of the genome that is used to assign viral genotype is also the region that is associated with the development of drug resistance.

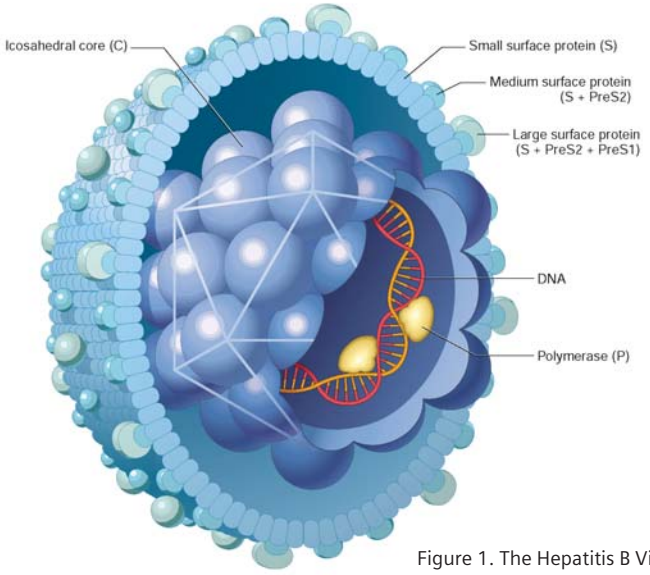


Figure 1. The Hepatitis B Virus.

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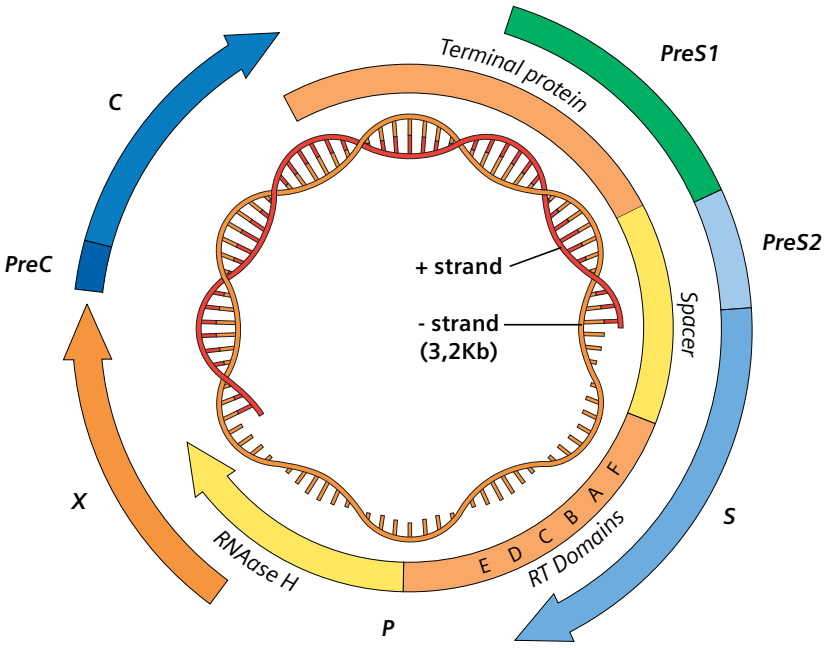

Figure 2. The HBV Genome.

Reverse Transcriptase Mutations Identified by the TRUGENE HBV Genotyping Assay
A181T
A181IV + M204I
A181V/T
I169T + V173L + L180M + **T184G** + **S202I** + M204V
I169T + V173L + L180M + M204V + **M250V**
I169 T + V173L + L180M + M204V
L180M + M204V/I/S
L180M + A194T + M204V
L180M + M204V/I
L180V/I + M204I [non-A genotype]
M204I
N236T
Q215S + L180M + M204V
T184S + L180M+ M204V
V173L + L180M + M204V
V214A / Q215S
Various combinations of mutations at codons **184, 202** and **250**

List is not comprehensive. Any mutation that occurs within the primer region (Rt99-rt280 and (s101-s237) will be identified and listed in one of the four Mutation Profile columns.

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The OpenGene DNA Sequencing System.

TRUGENE HBV Genotyping Assay – Two Tests in One Kit

The TRUGENE HBV Assay from Siemens Healthcare Diagnostics allows researchers to identify the HBV genotype and HBV mutations at the same time using direct sequence analysis. This improves workflow efficiency and reduces overall costs normally associated with seperate HBV genotyping and mutational analysis. The assay runs on Siemens' OpenGene® DNA Sequencing System, offering a sequence-based solution for determination of:

- The genotype
- Viral mutations, highlighting those that differ from a consensus sequence and that current scientific literature has identified

This Research Use Only assay amplifies the viral genome directly from plasma or serum samples and sequences the region coding for the viral RT gene and the central portion of HBsAg. Amplified material is then added to a set of four CLIP™ sequencing reaction tubes and labeled via the CLIP sequencing reaction (Figure 3).

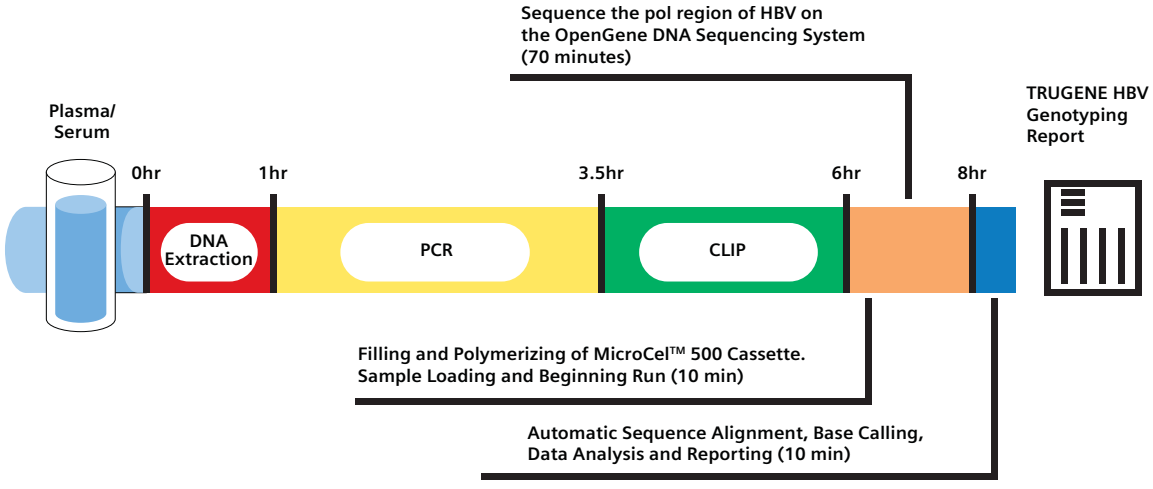

Figure 3. Timeline for TRUGENE HBV genotyping.

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A fully integrated HBV Genotyping Solution: Hardware, Chemistry, Software and Report

Traditional approaches to HBV genotyping have involved "home brew" sequence-based phylogenetic analysis[11], differential hybridization[12], fragment length polymorphism[13], or subjective hybridization based assays. The TRUGENE HBV Genotyping Assay is a standardized and validated kit allowing researchers to directly sequence and interrogate the virus for a more comprehensive viral analysis. This provides the laboratory more confidence in the data generated for HBV genotyping and mutational analysis.

CLIP Sequencing

CLIP is a proprietary sequence chemistry that produces bi-directional sequences using two fluorescently-labeled DNA primers which increases the overall confidence of the base calls. The sequencing reaction is initiated with the addition of the sample and a thermostable DNA polymerase. As the reaction mixture proceeds through each thermal cycle, primers hybridize to template DNA and are extended, then terminated along the target DNA sequence allowing bi-directional sequence in the same tube.

Analysis

The OpenGene DNA Sequencing System acquires sequence data in real time, and each pair of forward and reverse sequences are combined and aligned with stored reference sequences. The TRUGENE HBV Genotyping Kit Includes reagents to PCR amplify a portion of the HBsAg (s101-s237) and overlapping polymerase protein (rt99-rt280). Software assigns the viral genotype and the mutations and polymorphisms present in the sample.

The TRUGENE HBV software module contains sequences that correspond to the surface antigen and polymerase regions for genotype A through H and a universal mutation reporting reference sequence for comparison.

Reporting

The TRUGENE HBV Genotyping Report shows the closest consensus sequence allowing the determination of the viral genotype and a mutation table showing the published mutations from key scientific journal articles.

Conclusion

The TRUGENE HBV Assay from Siemens Healthcare Diagnostics runs on the OpenGene DNA Sequencing System and uses sequencing technology that overcomes the traditional issues faced when performing DNA sequencing.

The assay provides a determination of HBV viral genotype and interrogates known mutations – essentially providing two answers from one test.

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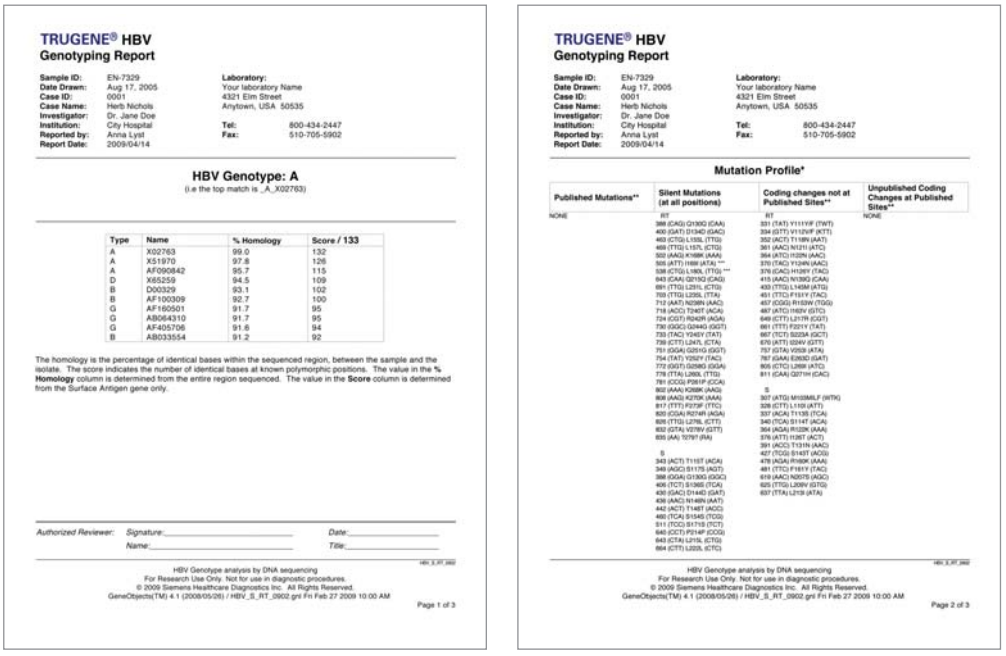

TRUGENE® HBV
Genotyping Report

Sample ID: EN-7329
Date Drawn: Aug 17, 2005
Case ID: 0001
Case Name: Herb Nichols
Investigator: Dr. Jane Doe
Institution: City Hospital
Reported by: Anna Lyst
Report Date: 2009/04/14

Laboratory:
Your laboratory Name
4321 Elm Street
Anytown, USA 50535
Tel: 800-434-2447
Fax: 510-705-5902

HBV Genotype: A
(i.e the top match is _A_X02763)

Type	Name	% Homology	Score / 133
A	X02763	99.0	132
A	X51970	97.8	126
A	AF090842	95.7	115
D	X65259	94.5	109
B	D00329	93.1	102
B	AF100309	92.7	100
G	AF160501	91.7	95
G	AB064310	91.7	95
G	AF405706	91.6	94
B	AB033554	91.2	92

The homology is the percentage of identical bases within the sequenced region, between the sample and the isolate. The score indicates the number of identical bases at known polymorphic positions. The value in the **% Homology** column is determined from the entire region sequenced. The value in the **Score** column is determined from the Surface Antigen gene only.

Authorized Reviewer: Signature:_____ Date:_____
Name:_____ Title:_____

HBV Genotype analysis by DNA sequencing
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TRUGENE® HBV
Genotyping Report

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Laboratory:
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Mutation Profile*

Published Mutations**	Silent Mutations (at all positions)	Coding changes not at Published Sites**	Unpublished Coding Changes at Published Sites**
NONE	RT 399 (CAG) Q130Q (CAA) 400 (GAT) D134D (GAC) 460 (CTG) L153L (TTG) 469 (TTG) L157L (CTG) 502 (AAG) K168K (AAA) 505 (ATT) I169I (ATA) *** 538 (CTG) L180L (TTG) *** 643 (CAA) Q215Q (CAG) 691 (TTG) L231L (CTG) 703 (TTG) L235L (TTA) 712 (AAT) N238N (AAC) 718 (ACG) T240T (ACA) 724 (GGT) R242R (AGA) 730 (GGC) G244G (GGT) 735 (TAC) Y245Y (TAT) 739 (CTT) L247L (CTA) 751 (GGA) G251G (GGT) 754 (TAT) Y252Y (TAC) 772 (GGT) G258G (GGA) 778 (TTA) L260L (TTG) 791 (CCG) P261P (CCA) 802 (AAG) K268K (AAG) 808 (AAG) K270K (AAA) 817 (TTT) F273F (TTC) 820 (CGA) R274R (AGA) 826 (TTG) L276L (CTT) 832 (GTA) V278V (GTT) 835 (AA) 70797 (RA) S 343 (ACT) T115T (ACA) 349 (AGC) S117S (AGT) 398 (GGA) G133G (GGC) 406 (TCT) S136S (TCA) 430 (GAC) D144D (GAT) 438 (AAC) N146N (AAT) 442 (ACT) T148T (ACC) 460 (TCA) S154S (TCG) 511 (TCG) S171S (TCT) 640 (CCT) P214P (CCG) 643 (CTA) L215L (CTG) 664 (CTT) L222L (CTC)	RT 331 (TAT) Y111YF (TWT) 334 (GTT) V112VF (KTT) 352 (ACT) T118N (AAT) 361 (AAC) N121I (ATC) 364 (ATC) I122N (AAC) 370 (TAC) Y124N (AAC) 376 (CAC) H126Y (TAC) 415 (AAC) N139Q (CAA) 433 (TTG) L145M (ATG) 451 (TTC) F151Y (TAC) 457 (CGG) R153W (TGG) 467 (ATC) I156V (GTC) 649 (CTT) L217P (CGT) 661 (TTT) F221Y (TAT) 667 (TCT) S223A (GCT) 670 (ATT) I224V (GTT) 757 (GTA) V253I (ATA) 767 (GAA) E263D (GAT) 805 (CTC) L268I (ATC) 811 (CAA) Q270H (CAC) S 307 (ATG) M103MILF (WTK) 328 (CTT) L110I (ATT) 337 (ACA) T113S (TCA) 340 (TCA) S114T (ACA) 364 (AGA) R122K (AAA) 376 (ATT) I126T (ACT) 391 (ACC) T131N (AAC) 427 (TCG) S143T (ACG) 478 (AGA) R160K (AAA) 481 (TTC) F161Y (TAC) 619 (AAC) N207S (AGC) 625 (TTG) L209V (GTG) 637 (TTA) L213I (ATA)	NONE

HBV Genotype analysis by DNA sequencing
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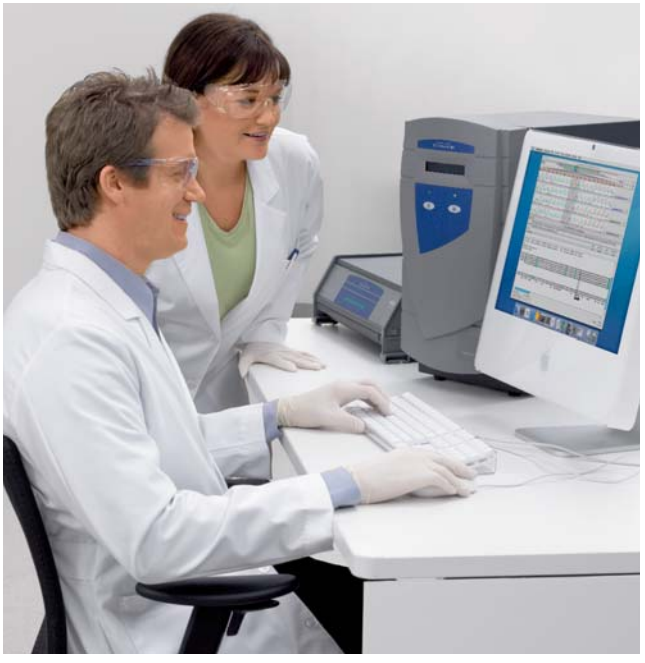
TRUGENE HBV reporting.

OpenGene DNA Sequencing System

Our OpenGene DNA Sequencing System runs a panel of TRUGENE Genotyping Assays to facilitate semi-automated DNA sequencing and/or genotyping.

- Fully integrated, dynamic genotype determination
- Accessible – allows research laboratories to initiate DNA sequencing operations with minimal equipment and a small footprint
- Scalable – accommodates up to eight Long-Read Towers per MAC OS®X unit for easy scale-up
- Quality – CLIP sequencing chemistry is optimized to improve workflow by eliminating several steps associated with other sequencing methodologies
- MAC OSX operating system and OpenGene software provide enhanced data management and easy networking
- Backward compatibility of data for easy conversion from OpenStep (UNIX-based system)



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