PRODUCT SHEET

Seraseq® HIV-1 Drug Resistance and Tropism Reference Materials

EXPEDITING VALIDATION AND TESTING OF HIV-1 ASSAYS BY NEXT-GENERATION SEQUENCING

HIGHLIGHTS

SINGLE-VIAL FORMAT FOR EACH MUTATION FREQUENCY. IDEAL FOR ASSAY OR BIOINFORMATIC PIPELINE DEVELOPMENT AND ESTABLISHING ASSAY SENSITIVITY

49 UNIQUE VARIANTS, QUANTITATED WITH DIGITAL PCR. ASSURES PRECISE DETECTION OF DRUG RESISTANCE MUTATIONS

HIGH-QUALITY MANUFACTURED REFERENCE MATERIAL SAVES TIME AND COST PROCURING SAMPLES OR PRODUCING HOMEBREW REAGENTS

INTRODUCTION

Developing and optimizing NGS HIV-1 assays can be challenging. Assay developers, clinical labs and researchers rely on positive disease state materials that can effectively challenge all elements of an assay from sample preparation through amplification, detection and analysis. Valuable reference materials mimic natural samples and go through extraction, reverse transcription, sequencing and data analysis. Remnant patient samples rarely contain more than a handful of mutations of interest. Successful assays require accuracy throughout the entire process, from sample RNA purification and quantitation, to library construction and template preparation, through bioinformatics parameters and variant annotation.

The Seraseq® HIV-1 Drug Resistance and Tropism v2 Reference Materials were developed using our AccuPlex™ recombinant viral technology. This method results in recombinant viral particles which are efficiently packaged in host cells, but lack the genes required to produce new viral particles and are therefore replication defective[1]. For Research Use Only. Not for In vitro Diagnostic Use.

These reference materials allow users to assess performance of NGS or Sanger sequencing-based HIV-1 drug resistance assays. SeraCare's two products are based on an HXB2 HIV-1 reference strain (Genbank K03455)[2]. They include 4000 bp region of gag and pol sequences, and 1525 bp of the env region (Figure 1).

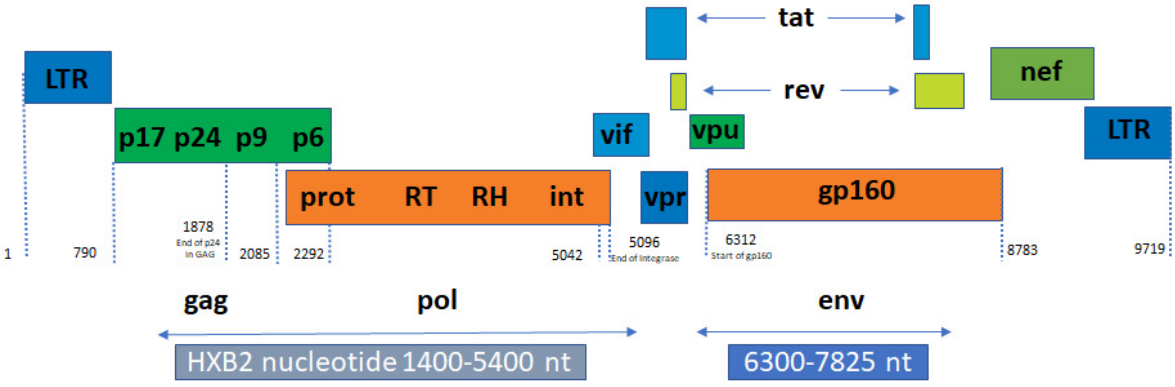

FIGURE 1: Regions of HIV-1 strain HBX2 included in SeraCare's Seraseq HIV-1 Drug Resistance and Tropism v2 Reference Materials. These reference materials contain two fragments of HIV genome corresponding to gag and pol region as well as env region, including the variable loops.

The sequences of the recombinant viruses in these products, contain:

- variants in the group specific antigen (gag), HIV protease (PRO), reverse transcriptase (RT) and integrase (IN) gene regions to detect drug resistance
- envelope sequences in the reference materials are indicative of CCR5 or CXCR4 co-receptor usage and can be used to monitor tropism determination

Product Benefits

- Ensures lab safety with replication defective and non-infectious virus
- Highly stable with no loss in titer after multiple freeze-thaws
- HIV-1-like viral structure, mammalian-based recombinant virus
- Suitable for multiple applications — development, evaluation, monitoring, and troubleshooting NGS or Sanger HIV-1 drug resistance assays

Product Features

- Two different product formats aimed for NGS or Sanger sequencing
- Single-vial format for each mutation frequency MF (1%, 5%, 20% or 40%) at 50,000 viral copies/mL
- 49 unique mutation variants (Table 1)
- Allele frequency verified by digital PCR analysis
- Tested by NGS using both Illumina® MiSeq® and Ion TorrentTM PGM platforms
- Manufactured under cGMP compliance in ISO 13485 certified facilities

PRECISELY QUANTITATED MUTATION MIX

With the accuracy of digital PCR, Seraseq HIV-1 Drug Resistance and Tropism Reference Materials v2 provides a precisely quantitated mixture of mutations at a specific mutation frequency which is useful for NGS assay development, optimization and validation. This accuracy was further confirmed by NGS on Illumina MiSeq and Thermo Fisher Ion PGM™. sequencers (Figure 2). The material configuration allows users to ensure identification of minority drug resistant and non-R5 HIV-1 variants at low frequencies (less than 2%).

To help meet different needs of NGS and Sanger sequencing, Seraseq HIV-1 Drug Resistance and Tropism Reference Materials v2 is offered as two products: mutational frequency (MF) 1-20% for NGS and MF of 40% for Sanger sequencing. Both materials are offered at 50,000 viral copies per mL.

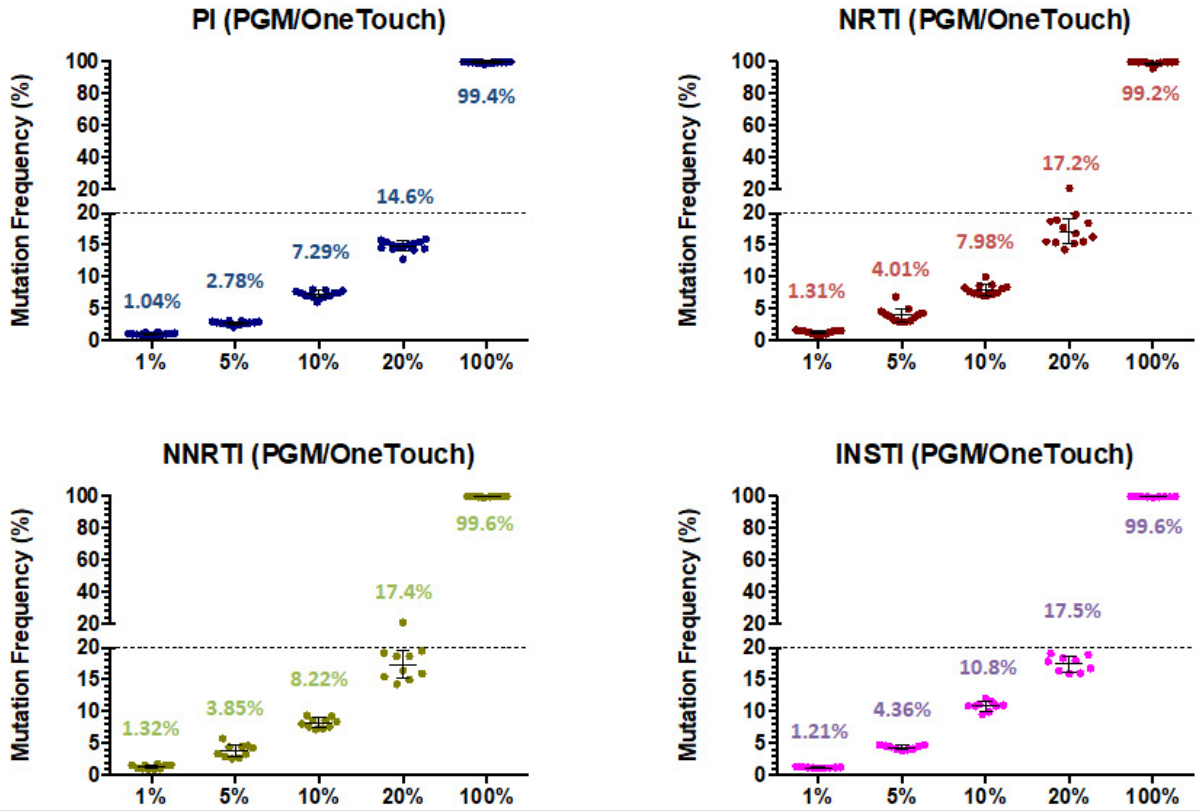

FIGURE 2: Performance of the Seraseq HIV-1 Drug Resistance and Tropism v2 Reference Materials (MF1-20) on MiSeq and Ion Torrent. Data courtesy of Miguel E. Quiñones-Mateu. University Hospital Case Medical Center.

EXPANDED LENGTH OF THE CONSTRUCTS AND LIST OF MUTATIONS

In the predecessor product, Seraseq HIV-1 Drug Resistance and Tropism Reference Materials v1, the HIV-1 sequence started at 1900 bp mark of the HXB2 reference strain and included 47 mutations based on their key role in antiretroviral drug resistance[3]. This design, however, did not allow for testing of mutations in the gag gene responsible for resistance to maturation inhibitors. Hence, the 5' end of the recombinant virus was extended in the Seraseq HIV-1 Drug Resistance and Tropism Reference Materials v2 by 500 bp and includes the gag/pol gene regions which correspond to nucleotides 1400-5400 in the HXB2 reference strain as shown in Figure 1. The new version 2 materials contain two additional mutations in the gag gene (Table 1). The 3' end of the constructs remained unchanged and contains the envelope regions corresponding to 6300-7825 bp.

Gag	Protease Gene	Reverse Transcriptase Gene (NRTI)	Reverse Transcriptase Gene (NNRTI)	Integrase Gene
H219Q (CAT to CAA)	L24I (TTA to ATA)	M41L (ATG to TTG)	L100I (TTA to ATA)	T66I (ACA to ATA)
G381S (GGC to AGC)	D30N (GAT to AAT)	K65R (AAA to AGA)	K101E (AAA to GAA)	L74M (CTG to ATG)
	V32I (GTA to ATA)	D67N (GAC to AAC)	K103N (AAA to AAC)	E92Q (GAA to CAA)
	M46I (ATG to ATA)	T69S (ACT to TCT) with the insertion of two additional Serines (TCCTCC)	V108I (GTA to ATA)	T97A (ACA to GCA)
	I47L (ATA to CTA)	K70R (AAA to AGA)	V106A (GTA to GCA)	E138A (GAA to GCA)
	G48V (GGG to GTG)	L74V (TTA to GTA)	Y181C (TAT to TGT)	G140S (GGA to TCA)
	I50V (ATT to GTT)	F77L (TTC to CTC)	Y188L (TAT to TTA)	Y143R (TAC to CGC)
	I54M (ATC to ATG)	Y115F (TAT to TTT)	G190A (GGA to GCA)	Q148H (CAA to CAC)
	G73S (GGT to GCT)	F116Y (TTT to TAT)	P225H (CCT to CAT)	N155H (AAT to CAT)
	L76V (TTA to GTA)	Q151M (CAG to ATG)	M230L (ATG to CTG)	
	V82A (GTC to GCC)	M184V (ATG to GTG)		
	I84V (ATA to GTA)	L210W (TTG to TGG)		
	N88D (AAT to GAT)	T215Y (ACC to TAC)		
	L90M (TTG to ATG)	K219Q (AAA to CAA)		

TABLE 1:
List of mutations included in the Seraseq HIV1 Drug Resistance and Tropism v2 Reference Materials (MF1-20 and MF40).

ABOUT SERACARE

TRUSTED SUPPLIER TO THE DIAGNOSTIC TESTING INDUSTRY FOR OVER 30 YEARS.

HIGH-QUALITY CONTROL PRODUCTS, RAW BIOLOGICAL MATERIALS, AND IMMUNOASSAY REAGENTS.

INNOVATIVE TOOLS AND TECHNOLOGIES TO PROVIDE ASSURANCE IN DIAGNOSTIC ASSAY PERFORMANCE AND TEST RESULTS.

FOR MORE INFORMATION, PLEASE VISIT OUR WEBSITE: WWW.SERACARE.COM.

RELIABLE, CONSISTENT CONTROL MATERIAL

As a manufactured control material, developed under cGMP compliance in ISO 13485 certified facilities, Seraseq HIV-1 Drug Resistance and Tropism v2 Reference Materials provide a consistent source of reference materials for your NGS assay. These products not only ensure a reliable supply which is consistent lot-to-lot; but also eliminates the need to obtain, characterize, blend, and document your own mixes of viral strains, saving you time and resources in your assay development and validation efforts.

ORDERING INFORMATION

Material Number	Product	Fill Size
0740-0026	Seraseq HIV-1 Drug Resistance and Tropism v2 Reference Material (MF1-20)	3 vials x 1 mL each
0740-0025	Seraseq HIV-1 Drug Resistance and Tropism v2 Reference Material (MF40)	1 vial x 1 mL

LEARN MORE

To learn more about Seraseq HIV-1 Drug Resistance and Tropism Reference Materials and SeraCare's product offering for infectious diseases diagnostics, visit https://www.seracare.com/Controls---Reference-Materials-Infectious-Disease/.

Contact us at 508.244.6400 and 800.676.1881 or email info@seracare.com.

REFERENCES
1. Schlesinger S. 2000. Alphavirus expression vectors. Adv Virus Res. 55:565-77.
2. http://www.hiv.lanl.gov/content/sequence/HIV/MAP/annotation.html
3. Johnson VA, Calvez V, Günthard HF, Paredes R, Pillay D, Shafer RW, Wensing AN, and Richman DD. 2013. Update of the Drug Resistance Mutations in HIV-1: March 2013 Topics in Antiviral Medicine. Volume 21 Issue 1 pp 6-14.

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