Seraseq®

Inherited Cardiovascular DNA Mix

Package Insert

NAME AND INTENDED USE

The Seraseq® Inherited Cardiovascular DNA Mix is a reference material intended for use in the development, validation, and evaluation of routine performance of Next Generation Sequencing (NGS) assays (and other molecular assays) that identify inherited (germline) variants in genes associated with cardiovascular diseases such as cardiomyopathies and arrythmias among others. This reference material is suitable for use by clinical laboratories, research institutions, and diagnostic assay developers to ensure consistent and reliable results across different sequencing runs and laboratory conditions.

The Seraseq Inherited Cardiovascular DNA Mix is not intended for use in patient diagnosis, treatment, or in any therapeutic procedures. It is intended for use by trained laboratory personnel proficient in NGS technologies and familiar with proper laboratory practices and quality control procedures.

For Research Use Only (RUO). Not for use in diagnostic procedures.

REAGENT PROVIDED

Seraseq Inherited Cardiovascular DNA Mix is a mixture of synthetic DNA constructs and genomic DNA extracted from the human cell line GM24385. It contains 61 synthetic mutations in 56 genes (not including those present in the GM24385 background) associated with autosomal recessive disorders (Table 2). The product is formulated to simulate a heterozygous state for each mutation at a 50% variant allele frequency (VAF) confirmed by droplet digital PCR and measured by NGS.

Table 1. Seraseq Inherited Cardiovascular Mix

Material No.	Product	Format
0740-0132	Seraseq® Inherited Cardiovascular DNA Mix	1x 25 µL

One (1) vial, 25 µL per vial, 250 ng total mass, at a nominal concentration of 10 ng/µL is provided. The product is formulated in a 1 mM Tris / 0.1 mM EDTA pH 8.0 aqueous buffer. Refer to the batch-specific Technical Product Report for exact concentration and VAF measured. Manufactured in the USA.

WARNINGS AND PRECAUTIONS

Safety and Handling Precautions

Handle Seraseq Inherited Cardiovascular DNA Mix and all materials derived from human blood products as though it is capable of transmitting infectious agents. Use Centers for Disease Control and Prevention (CDC) recommended universal precautions for handling reference materials and human specimens[1]. Do not pipette by mouth; do not smoke, eat, or drink in areas where specimens are being handled. Clean any spillage by immediately wiping it up with 0.5% sodium hypochlorite solution. Avoid contamination of the product when opening and closing the vials. Dispose of all specimens and materials appropriately.

STORAGE INSTRUCTIONS

Store Seraseq Inherited Cardiovascular DNA Mix frozen at -20 °C or colder. Once opened, a vial can be thawed and re-frozen up to five (5) times. Sub-aliquoting of the product into low DNA binding tubes may be advisable to limit the number of freeze/thaw cycles to five (5) or less.

INDICATIONS OF REAGENT INSTABILITY OR DETERIORATION

Seraseq Inherited Cardiovascular DNA Mix should appear as a clear liquid. Alterations in this appearance may indicate instability or deterioration of the product and vials should be discarded.

PROCEDURE

Materials Required but not Provided

Refer to instructions supplied by manufacturers of the test kits to be used.

Instructions for Use

Allow the product vial to come to room temperature before use. Mix by vortexing to ensure a homogeneous solution and spin briefly. Seraseq Inherited Cardiovascular DNA Mix should be integrated into library preparation after the DNA isolation step; no further purification or DNA isolation is needed. If a DNA shearing step is part of the workflow, the reference material should be sheared and go through the target selection and library preparation in parallel with test specimens. Refer to standard assay procedures in order to determine the amount of material to use.

EXPECTED RESULTS & INTERPRETATION OF RESULTS

Table 2 indicates each of the mutations represented in the Seraseq Inherited Cardiovascular DNA Mix. While the presence and frequency of each variant in this product was confirmed during manufacture using digital PCR assays and NGS, there may be differences in observed allele frequencies due to assay characteristics. The Seraseq Inherited Cardiovascular DNA Mix does not have assigned values for allele frequencies of the variants present. Furthermore, specific detection of variants and variant allele frequencies within the product will vary among different assays, different procedures, different lot numbers, and different laboratories.

Each laboratory must establish an assay-specific expected value and acceptance range for each variant and lot of the Mutation Mix prior to its routine use. When results for the product are outside of the established acceptance range, it may indicate unsatisfactory test performance. Possible sources of error include: deterioration of test kit reagents, operator error, faulty performance of equipment, contamination of reagents or change in bioinformatics pipeline parameters.

LIMITATIONS OF THE PROCEDURE

SERASEQ INHERITED CARDIOVASCULAR DNA MIX MUST NOT BE SUBSTITUTED FOR THE MANDATORY POSITIVE AND NEGATIVE CONTROL REAGENTS PROVIDED WITH MANUFACTURED TEST KITS.

Seraseq Inherited Cardiovascular DNA Mix is not compatible with MLPA (Multiplex ligation-dependent probe amplification) assays and NGS analysis methods based only on coverage depth, since the large genomic rearrangements do not reflect copy losses or gains across the whole DNA sequence.

TEST PROCEDURES and *INTERPRETATION OF RESULTS* provided by manufacturers of test kits must be followed closely. Deviations from procedures recommended by test kit manufacturers may produce unreliable results. Seraseq Inherited Cardiovascular DNA Mix is not a calibrator and should not be used for assay calibration. Adverse shipping and storage conditions or use of expired product may produce erroneous results.

SPECIFIC PERFORMANCE CHARACTERISTICS

Seraseq Inherited Cardiovascular DNA Mix has been designed for use with NGS sequencing procedures for the purposes of evaluating assay performance. Seraseq Inherited Cardiovascular DNA Mix does not have assigned values. Procedures for implementing a quality assurance program and monitoring test performance on a routine basis must be established by each individual laboratory.

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14527-01 February 2025

Table 2. List of Mutations

Gene	Protein change	Cytogenetic Location	Type of nucleotide alteration	Variant size (bp)	Molecular Consequence	Classification	Conditions
ABCC9	R1116H	12p12.1	SNV	1	Missense variant	** Pathogenic	Hypertrichotic osteochondrodysplasia Cantu type; Dilated cardiomyopathy 1O
ACTC1	E101K	15q14	SNV	1	Missense variant	** Pathogenic/Likely pathogenic	Dilated cardiomyopathy 1R; Hypertrophic cardiomyopathy 11; Atrial septal defect 5
ACTN2	A119T	1q43	SNV	1	Missense variant 5 prime UTR variant	** Pathogenic	Primary dilated cardiomyopathy; Ventricular fibrillation; Noncompaction cardiomyopathy; Atrial septal defect 7; Primary dilated cardiomyopathy; Hypertrophic; Dilated cardiomyopathy 1AA; ACTN2-related disorder
ALPK3	N/A	15q25.3	Indel	2	Nonsense variant	** Pathogenic/Likely pathogenic	Cardiomyopathy; Cardiovascular phenotype
BAG3	G118fs	10q26.11	Large Microsatellite	142	Frameshift variant	* Pathogenic	Dilated cardiomyopathy 1HH; Myofibrillar myopathy 6
CACNA1C	R518H	12p13.33	SNV	1	Missense variant	** Pathogenic/Likely pathogenic	Hypertrophic cardiomyopathy 1, Long QT syndrome 8, Cardiac arrhythmia; Timothy syndrome
CALM1	N98S	14q32.11	SNV	1	Missense variant	** Pathogenic/Likely pathogenic	Catecholaminergic polymorphic ventricular tachycardia 4, Long QT syndrome 14
CALM2	N98S	2p21	SNV	1	Missense variant	** Pathogenic	Long QT syndrome 15
CASQ2	S113fs	1p13.1	Deletion	16	Frameshift variant	** Pathogenic	Catecholaminergic polymorphic ventricular tachycardia 1 & 2
CDH2	Splice variant	18q12.1	SNV	1	Splice donor variant	** Pathogenic	Arrhythmogenic right ventricular dysplasia, familial, 14; Agenesis of corpus callosum
CTNNA3	L766del	10q21.3	Microsatellite	3	Inframe deletion	Pathogenic	Arrhythmogenic right ventricular cardiomyopathy
CRYAB	L49fs	11q23.1	Deletion	1	Frameshift variant	* Pathogenic	Dilated cardiomyopathy 1II
DES	R406W	2q35	SNV	1	Missense variant	** Pathogenic/Likely pathogenic	Desmin-related myofibrillar myopathy; Arrhythmogenic right ventricular cardiomyopathy; Cardiomyopathy
DMD	R2205fs	Xp21.1	Microsatellite	2	Frameshift variant 5 prime UTR variant	** Pathogenic	Duchenne muscular dystrophy; Becker muscular dystrophy; Dilated cardiomyopathy 3B
DSC2	P729fs	18q12.1	Deletion	1	Frameshift variant	** Pathogenic	Arrhythmogenic right ventricular dysplasia 11
DSG2	K346del	18q12.1	Microsatellite	3	Inframe deletion Analytic detection difficult	** Pathogenic	Arrhythmogenic right ventricular dysplasia 10; Dilated cardiomyopathy 1BB
DSP	N/a	6p24.3	Microsatellite	2	Genic downstream transcript variant	** Pathogenic	Arrhythmogenic cardiomyopathy with wooly hair and keratoderma; Arrhythmogenic right ventricular dysplasia 8; Primary dilated cardiomyopathy
	N/a	6p24.3	Large Indel	4738	Genic downstream transcript variant	** Pathogenic/Likely pathogenic	Arrhythmogenic cardiomyopathy with wooly hair and keratoderm; Arrhythmogenic right ventricular dysplasia 8
EMD	S52fs	Xq28	Duplication	1	Frameshift variant	** Pathogenic	X-linked Emery-Dreifuss muscular dystrophy

Gene	Protein change	Cytogenetic Location	Type of nucleotide alteration	Variant size (bp)	Molecular Consequence	Classification	Conditions
FKTN	S152fs	9q31.2	Duplication	1	Frameshift variant Non-coding transcript variant	** Pathogenic	Walker-Warburg congenital muscular dystroph; Dilated cardiomyopathy 1X; Muscular dystrophy-dystroglycanopathy (congenital with brain and eye anomalies), type A, 4
FLNC	R1426fs	7q32.1	Deletion	1	Frameshift variant	** Pathogenic	Dilated Cardiomyopathy, Dominant Distal myopathy with posterior leg and anterior hand involvement; Hypertrophic cardiomyopathy 26; Myofibrillar myopathy 5
GAA	E176fs	17q25.3	Deletion	1	Frameshift variant	*** Pathogenic Reviewed by expert panel. FDA RECOGNIZED DATABASE	Glycogen storage disease, type II; Dilated cardiomyopathy
GATA6	P234fs	18q11.2	Deletion	1	Frameshift variant	* Likely pathogenic	Primary dilated cardiomyopathy
GLA	N215S	Xq22.1	SNV	1	Missense intronic variant Non-coding transcript variant	** Pathogenic	Hypertrophic cardiomyopathy ; Fabry disease
HCN4	A485V	15q24.1	SNV	1	Missense variant	** Pathogenic/ Likely pathogenic	Brugada syndrome 8; Left ventricular noncompaction cardiomyopathy
JPH2	T161K	20q13.12	SNV	1	Missense variant	** Pathogenic/ Likely pathogenic	Primary familial hypertrophic cardiomyopathy
JUP	W680fs	17q21.2	Deletion	2	Frameshift variant	** Pathogenic	Arrhythmogenic right ventricular dysplasia 1; Naxos disease
LAMP2	R293*	Xq24	SNV	1	Nonsense variant	** Pathogenic	Hypertrophic cardiomyopathy; Danon disease
LDB3	F575I	10q23.2	SNV	1	Missense variant	** Pathogenic	Dilated cardiomyopathy 1C
LMNA	R190W	1q22	SNV	1	Missense variant	** Pathogenic	Primary dilated cardiomyopathy; LMNA-related disorder; Charcot-Marie-Tooth disease type 2
MYBPC3	W792fs	11p11.2	Duplication	1	Frameshift variant Seen in >50 probands	**Pathogenic Major contributor to disease	Hypertrophic cardiomyopathy
	N/a	11p11.2	SNV	1	Splice donor intronic variant. Seen in >20 probands. “Not difficult technically but often outside the region labs call variants in.”	** Pathogenic/Likely pathogenic	Hypertrophic cardiomyopathy; Left ventricular noncompaction 10
MYH6	splice variant	14q11.2	SNV	1	Splice donor variant	** Pathogenic	Hypertrophic cardiomyopathy 14

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MYH7	P838L	14q11.2	SNV	1	Missense variant	*** Pathogenic Reviewed by expert panel. FDA RECOGNIZED DATABASE	Restrictive cardiomyopathy
	R723C	14q11.2	SNV	1	Missense variant	*** Pathogenic Reviewed by expert panel. FDA RECOGNIZED DATABASE	Hypertrophic cardiomyopathy
	R904H	14q11.2	SNV	1	Missense variant	*** Pathogenic Reviewed by expert panel. FDA RECOGNIZED DATABASE	Dilated cardiomyopathy
MYL2	R58Q	12q24.11	SNV	1	Missense variant	** Pathogenic	Hypertrophic cardiomyopathy 10
MYL3	M149V	3p21.31	SNV	1	Missense variant	** Pathogenic/Likely pathogenic	Hypertrophic cardiomyopathy 8
MYPN	S1043fs	10q21.3	Deletion	1	Frameshift variant Non-coding transcript variant	** Pathogenic/Likely pathogenic	Dilated cardiomyopathy 1KK
NEXN	M513fs	1p31.1	Duplication	1	Frameshift variant	* Pathogenic	Dilated cardiomyopathy 1CC; Hypertrophic cardiomyopathy 21
NKX2-5	Y237*	5q35.1	SNV	1	3 prime UTR nonsense variant	** Pathogenic	Primary dilated cardiomyopathy; Ventricular fibrillation; Noncompaction cardiomyopathy; Atrial septal defect 7
PKP2	Q323fs	12p11.21	Indel	4	Frameshift variant	** Pathogenic/Likely pathogenic	Arrhythmogenic right ventricular dysplasia 9
		12p11.21	Inversion	8933	Splice donor impacting exons 5-6. Analytic detection difficult	* Pathogenic	Arrhythmogenic right ventricular dysplasia 9
PLN	R14del	6q22.31	Microsatellite	3	Intronic variant	* Pathogenic - Major contributor to disease - Founder variant Europe/North America	Dilated cardiomyopathy 1P; Arrhythmogenic right ventricular dysplasia 9; Hypertrophic cardiomyopathy 18; Sudden Infant Deaths Syndrome
PPA2	E172K	4q24	SNV	1	Missense intronic variant	** Pathogenic/Likely pathogenic	Inborn genetic diseases; Sudden cardiac failure, infantile
PRDM16	1-BP DUP	1p36.32	Duplication	1	Frameshift variant	** Pathogenic	Left ventricular noncompaction cardiomyopathy 8
PRKAG2	R302Q	7q36.1	SNV	1	Missense variant	** Pathogenic	Familial Hypertrophic Cardiomyopathy with Wolff-Parkinson-White Syndrome; Hypertrophic cardiomyopathy 6; Lethal congenital glycogen storage disease of heart
PTPN11	T468M	12q24.13	SNV	1	Missense variant	*** Pathogenic Reviewed by expert panel. FDA RECOGNIZED DATABASE	Noonan syndrome with multiple lentigines; LEOPARD syndrome 1; RASopathy; Hypertrophic Cardiomyopathy

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Gene	Protein change	Cytogenetic Location	Type of nucleotide alteration	Variant size (bp)	Molecular Consequence	Classification	Conditions
RAF1	L613V	3p25.2	SNV	1	Missense variant Non-coding transcript variant	*** Pathogenic Reviewed by expert panel. FDA RECOGNIZED DATABASE	Noonan syndrome 5 with multiple lentigines; LEOPARD syndrome 2; RASopathy; Hypertrophic cardiomyopathy 1
RBM20	R634W	10q25.2	SNV	1	Missense variant	** Pathogenic	Dilated cardiomyopathy 1DD
RYR2	G357S	1q43	SNV	1	Missense variant	** Pathogenic	Cardiomyopathy; Catecholaminergic polymorphic ventricular tachycardia 1
SCN5A	F851fs	3p22.2	Microsatellite	2	Frameshift variant	** Pathogenic	Dilated cardiomyopathy 1E; Cardiac arrhythmia; Long QT syndrome 3|Atrial fibrillation, familial, 10; Sick sinus syndrome 1; Brugada syndrome 1; Progressive familial heart block, type 1A; SUDDEN INFANT DEATH SYNDROME; Ventricular fibrillation, paroxysmal familial, type 1
TMEM43	S358L	3p25.1	SNV	1	Missense variant	** Pathogenic	Primary dilated cardiomyopathy; Arrhythmogenic right ventricular cardiomyopathy, type 5
TNNC1	A8V	3p21.1	SNV	1	Missense variant	** Pathogenic/Likely pathogenic	Dilated cardiomyopathy 1Z; Hypertrophic cardiomyopathy 13
TNNI3	R162Q	19q13.42	SNV	1	Missense variant Seen in >20 proband	** Pathogenic Major contributor to disease	Hypertrophic cardiomyopathy 7; Dilated cardiomyopathy 1FF; Familial Restrictive Cardiomyopathy 1; TNNI3-related disorder
TNNI3K	E768K	1p31.1	SNV	1	Missense intronic variant	** Pathogenic/Likely pathogenic	Atrial conduction disease; Dilated cardiomyopathy
TNNT2	E173del	1q32.1	Microsatellite	3	Inframe deletion in highly repetitive region - Analytic detection difficult	** Pathogenic/Likely pathogenic	Dilated cardiomyopathy 1D; Hypertrophic cardiomyopathy 2
TPM1	D230N	15q22.2	SNV	1	Missense variant	** Pathogenic	Hypertrophic cardiomyopathy 3; Dilated cardiomyopathy 1Y
TTR	V50M	18q12.1	SNV	1	Missense variant	** Pathogenic	TTR-related disorder; Familial amyloid neuropathy; Charcot-Marie-Tooth disease; Cardiomyopathy; Carpal tunnel syndrome 1; Hyperthyroxinemia, dystransthyretinemic; Familial amyloid neuropathy
TTN	L26789fs	2q31.2	Large Deletion	68	Frameshift variant	** Pathogenic/Likely pathogenic	Cardiovascular phenotype
SHOC2	S2G	10q25.2	SNV	1	Missense variant	*** Pathogenic Reviewed by expert panel. FDA RECOGNIZED DATABASE	Noonan syndrome-like disorder with loose anagen hair 1; RASopathy

NOTE: Above list does not include variants present in the GM24385 background. Target variant allele frequency is 50%. Substitution refers to a single nucleotide variant; Indel is defined as a deletion/insertion less than 10 base pairs, and large deletions or insertions are defined as longer than 10 base pairs.

REFERENCES

1. Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee, 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings.
Human Genetic and Genomic Testing Using Traditional and High-Throughput Nucleic Acid Sequencing Methods. Third Edition. CLSI guideline MM09, 2023.

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