PRODUCT SHEET

Seraseq® Cardiomyopathy Reference Material

MULTIPLEXED REFERENCE MATERIAL TO ASSESS HYPERTROPHIC CARDIOMYOPATHY ASSAY PERFORMANCE

HIGHLIGHTS

EXPERT-DESIGNED, MULTIPLEXED CONTENT

10 UNIQUE VARIANTS QUANTITATED WITH DIGITAL PCR; ASSURES PRECISE DETECTION OF INHERITED VARIANTS.

HIGH-QUALITY MANUFACTURED REFERENCE MATERIAL ELIMINATES THE NEED TO PROCURE DIFFICULT-TO-FIND VARIANTS.

INTRODUCTION

Next Generation Sequencing (NGS) is increasingly being used to discover causative variants for a growing number of inherited disorders, such as cardiomyopathy, by analyzing multiple genes in targeted gene panels. As such panels continue to expand, there is a growing demand for multiplexed reference materials that cover a broad range of prevalent pathogenic variants (SNV's & Indels) to expedite test development, provide more comprehensive analytical validation, as well as to monitor routine assay performance. However, the traditional practice of using genomic reference materials (e.g. NA12878) or remnant patient samples covering a small subset of target variants is not informative for the disorder being tested, robust or sustainable over the long-term, especially in an environment of growing regulatory oversight.

Seraseq Cardiomyopathy Reference Material v1 addresses the lack of multiplexed reference materials with an expert-designed product[1] for targeted NGS assays focused on hypertrophic cardiomyopathy (HCM). This unique product combines ten actionable HCM mutations in a well-characterized genomic background at a 50% target allele frequency that can be used for assay development, analytical validation, or routine monitoring of assay performance.

Product Benefits:

- Single vial format for 10 unique variants for HCM across 5 genes
- Save time and cost by using this highly multiplexed configuration for validation and routine QC

Product Features:

- Mutation targets precisely quantitated with digital PCR (dPCR)
- Well-characterized GM24385 human genomic DNA as background 'wild-type' material
- Formulated at a 50 ng/µL concentration in a fill volume of 200 µL
- Manufactured under cGMP compliance in ISO 13485 certified facilities

Product Design:

This product uses SeraSeq engineered biosynthetics technology. Each individual mutation (as shown in Figure 1) is engineered approximately in the middle of a ~1KB construct flanked by wild-type sequence that identically matches the reference genome. The constructs are precisely quantitated by a digital PCR assay and mixed in a single genomic DNA background (GM24385) to ensure a target allele frequency of 50%. The GM24385 genomic DNA has been extensively characterized by the Genome in a Bottle project[2] and is originally derived from a participant in the Personal Genomes Project, public profile huAA53E0[3]. This technology offers significant advantages over single variant genomes or mixtures of various unrelated genomes while performing identical to authentic patient genomic DNA in NGS-based inherited disease assays.

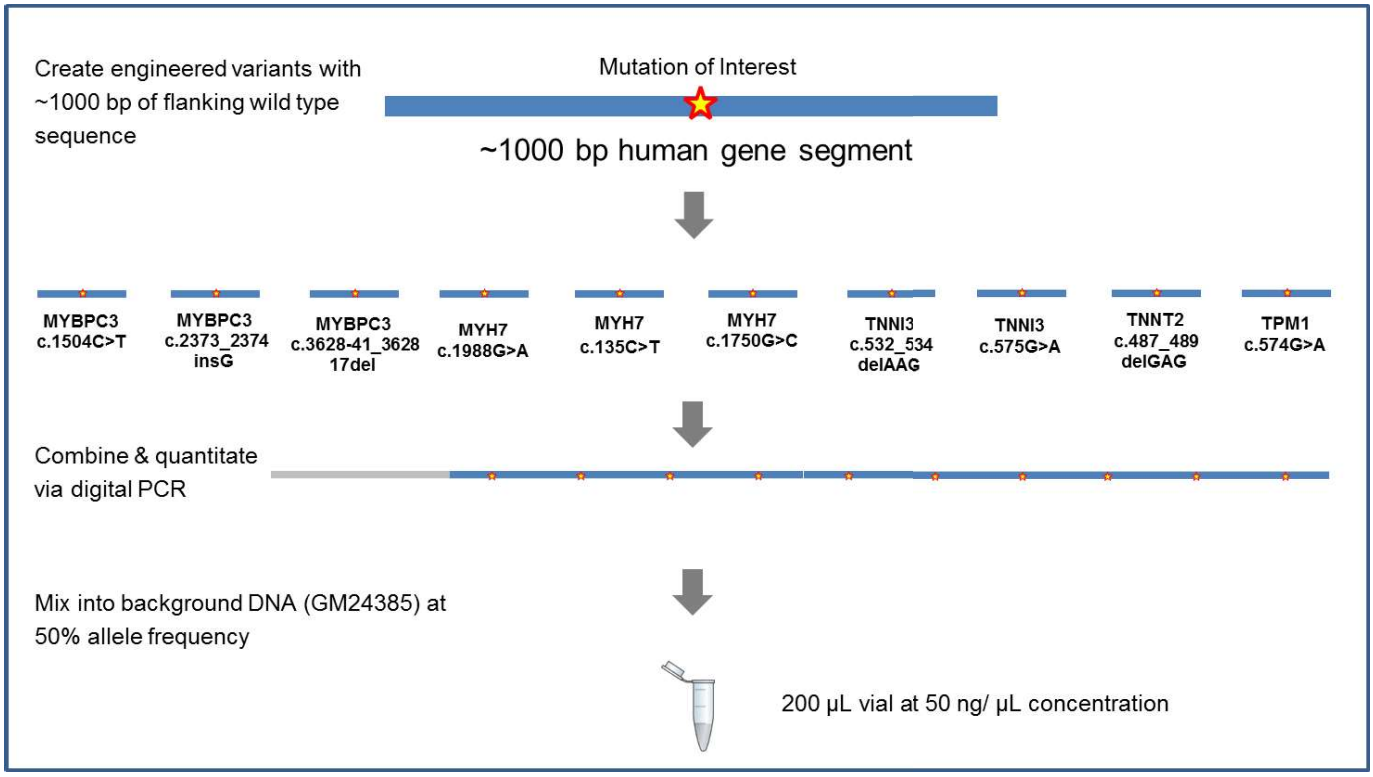

FIGURE 1: Design methodology for Seraseq Cardiomyopathy Reference Material v1

Mutations included in the Seraseq Cardiomyopathy Reference Material

Gene ID	Genomic DNA (hg19/GChr37)	HGVS Nomenclature	Amino Acid Change	Class	Target allele frequency
MYBPC3	g.47364249G>A	NM_000256.3:c.1504C>T	p.Arg502Trp	Pathogenic	50%
MYBPC3	g.47359280_47359281insC	NM_000256.3:c.2373dupG	p.Trp792ValfsX41	Pathogenic	50%
MYBPC3	g.47353826_47353850del	NM_000256.3:c.3628-41_3628-17delAGCCTGGATGGCTTCCCTCCCTCTC	NA	Likely Pathogenic	50%
MYH7	g.23896042C>T	NM_000257.2:c.1988G>A	p.Arg663His	Pathogenic	50%
MYH7	g.23898214G>A	NM_000257.2:c.1357C>T	p.Arg453Cys	Pathogenic	50%
MYH7	g.23896932C>G	NM_000257.2:c.1750G>C	p.Gly584Arg	Likely Pathogenic	50%
TNNI3	g.55665413_55665415delCTT	NM_000363.4:c.532_534delAAG	p.Lys178del	Pathogenic	50%
TNNI3	g.55663260C>T	NM_000363.4:c.575G>A	p.Arg192His	Pathogenic	50%
TNNT2	g.201332505_201332507delCTC	NM_001276345.1:c.517_519delGAG	p.Glu163del	Pathogenic	50%
TPM1	g.63353922G>A	NM_001018005.1:c.574G>A	p.Glu192Lys	Likely Pathogenic	50%

TABLE 1: List of mutations included in the Seraseq Cardiomyopathy Reference Material. This does not include the variants present in the GM24385 cell line.

ROBUST NEXT-GENERATION SEQUENCING REFERENCE MATERIAL

Example sequencing results from two high-profile laboratories offering NGS Cardiomyopathy panels are shown below (Figures 2 & 3). Samples were processed using their respective standard commercial assays under the same workflow, protocol conditions and analysis parameters as those used for routine samples.

These results cumulatively demonstrate that the Seraseq Cardiomyopathy Reference Material v1 can be used as a powerful and highly-multiplexed reference standard to assess HCM assay performance across a broad range of common and difficult to sequence alleles.

Laboratory 1: Hybridization capture method followed by 150 bp paired-end sequencing

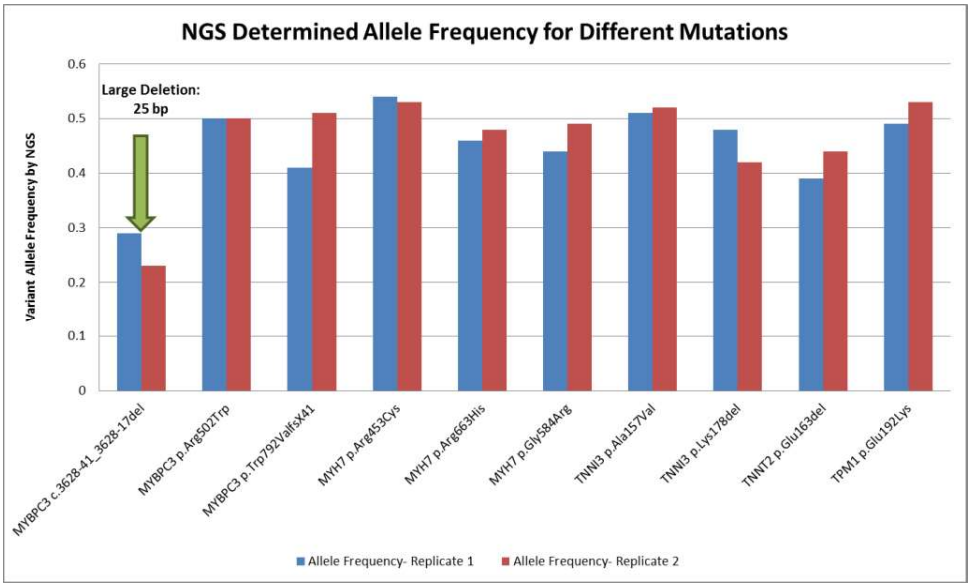

FIGURE 2: NGS allele frequency for two replicates of samples using Seraseq Cardiomyopathy Reference Material v1. Results indicate target values are close to 50% (as expected) for nine of the ten variants. The large 25 bp deletion (green arrow) was detected at a much lower frequency (around 25-30%) and reflects recognized difficulty with the mapping of such large deletions via NGS.

Laboratory 2: Hybridization capture method followed by 150 bp paired-end sequencing

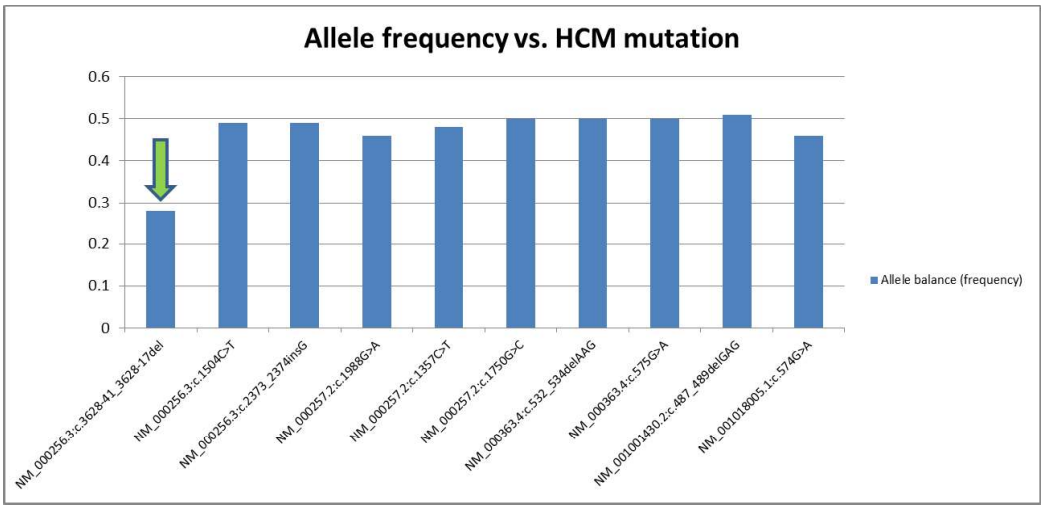

FIGURE 3: Detected NGS allele frequency using Seraseq Cardiomyopathy Reference Material v1. Results indicate target values are close to 50% (as expected) for nine of the ten variants. The large 25 bp deletion (green arrow) was detected at a much lower frequency (around 25-30%) and reflects recognized difficulty with the mapping of such large deletions via NGS.

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ORDERING INFORMATION

Material #	Product	Fill Size
0740-0021	Seraseq™ Cardiomyopathy Reference Material v1	1 vial x 200 µL per vial at 50 ng/ µL concentration (10 µg total)
N/A	The above product can be customized with additional mutations.	Custom fill size

REFERENCES

1. Emily M Kudalkar[A*], Naif AM Almontarishi[A,B,C*], Catherine Huang [D], Bharathi Anekella [D], Mark Bowser[A], Elizabeth Hynes[A], Russell Garlick [D], Birgit H. Funke[A,B]. Multiplexed reference materials as controls for diagnostic next generation sequencing – a pilot investigating applications for hypertrophic cardiomyopathy, The Journal of Molecular Diagnostics (2016) 18: 882-889.

2. Zook, J., Catoe, D., McDaniel, J. *et al.* Extensive sequencing of seven human genomes to characterize benchmark reference materials. Sci Data 2016**; 3,** 160025 doi.org/10.1038/sdata.2016.25

3. Personal Genome Project. Public Profile–huAA53E0. Available at: https://my.pgp-hms.org/profile/huAA53E0. Accessed 01 May 2020.

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