

Seraseq™ Cardiomyopathy Reference Material v1

MULTIPLEXED REFERENCE MATERIAL TO ASSESS 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.

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 (SNVs & indels) to expedite test development, perform analytical validation, and 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, 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 product¹ for targeted NGS assays focused on hypertrophic cardiomyopathy (HCM). This unique product combines 10 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 FEATURES

- 10 variants considered pathogenic or likely pathogenic for HCM
- Mutation targets precisely quantitated with digital PCR
- Well-characterized GM24385 human genomic DNA as background 'wild-type' material
- Manufactured under cGMP compliance in ISO 9001 and ISO 13485 certified facilities

INCLUDED MUTATIONS

Gene ID	Mutation Type	HGVS Nomenclature	Amino Acid Change	Class	Target Frequency
MYBPC3	Substitution	c.1504C>T	p.Arg502Trp	Pathogenic	50%
MYBPC3	Small insertion	c.2373_2374insG	p.Trp792ValfsX41	Pathogenic	50%
MYBPC3	Large deletion (in repetitive region)	c.3628-41_3628-17 del	NA	Likely pathogenic	50%
MYH7	Substitution	c.1988G>A	p.Arg663His	Pathogenic	50%
MYH7	Substitution	c.1357C>T	p.Arg453Cys	Pathogenic	50%
MYH7	Substitution	c.1750G>C	p.Gly584Arg	Likely pathogenic	50%
TNNI3	Small deletion	c.532_534delAAG	p.Lys178del	Pathogenic	50%
TNNI3	Substitution	c.575G>A	p.Arg192His	Pathogenic	50%
TNNT2	Deletion (in highly repetitive region)	c.487_489delGAG	p.Glu163del	Pathogenic	50%
TPM1	Substitution	c.574G>A	p.Glu192Lys	Likely pathogenic	50%

TABLE 1: List of mutations included in the Seraseq Cardiomyopathy Reference Material v1

SERASEQ ENGINEERED BIOSYNTHETIC TECHNOLOGY

Each individual mutation (Table 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 project² 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 identically to authentic patient genomic DNA in NGS-based inherited disease assays.

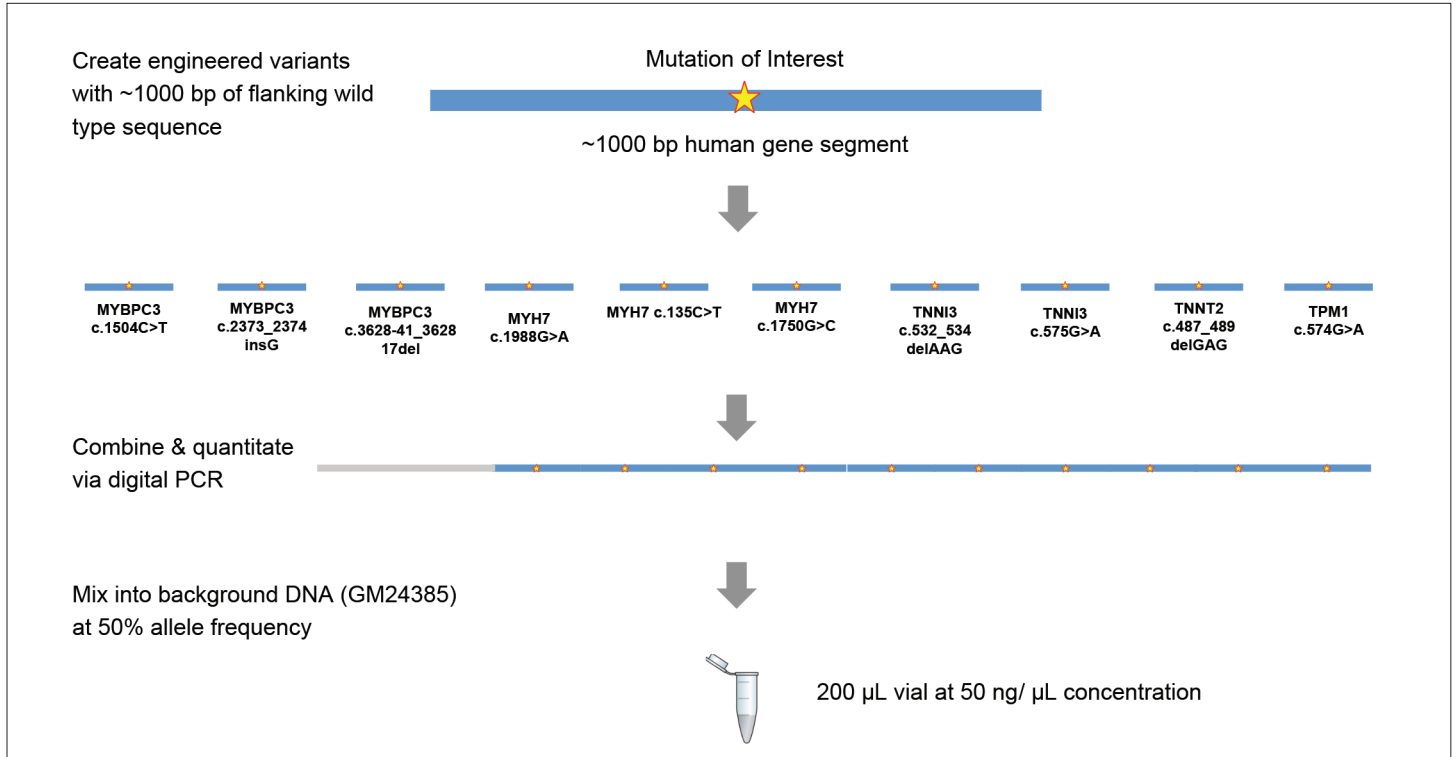


FIGURE 1: Design methodology for Seraseq Cardiomyopathy Reference Material v1

ROBUST NEXT-GENERATION SEQUENCING REFERENCE MATERIAL

Example sequencing results from two high-profile laboratories offering NGS 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 for routine samples.

These results cumulatively show that the SereSeq 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

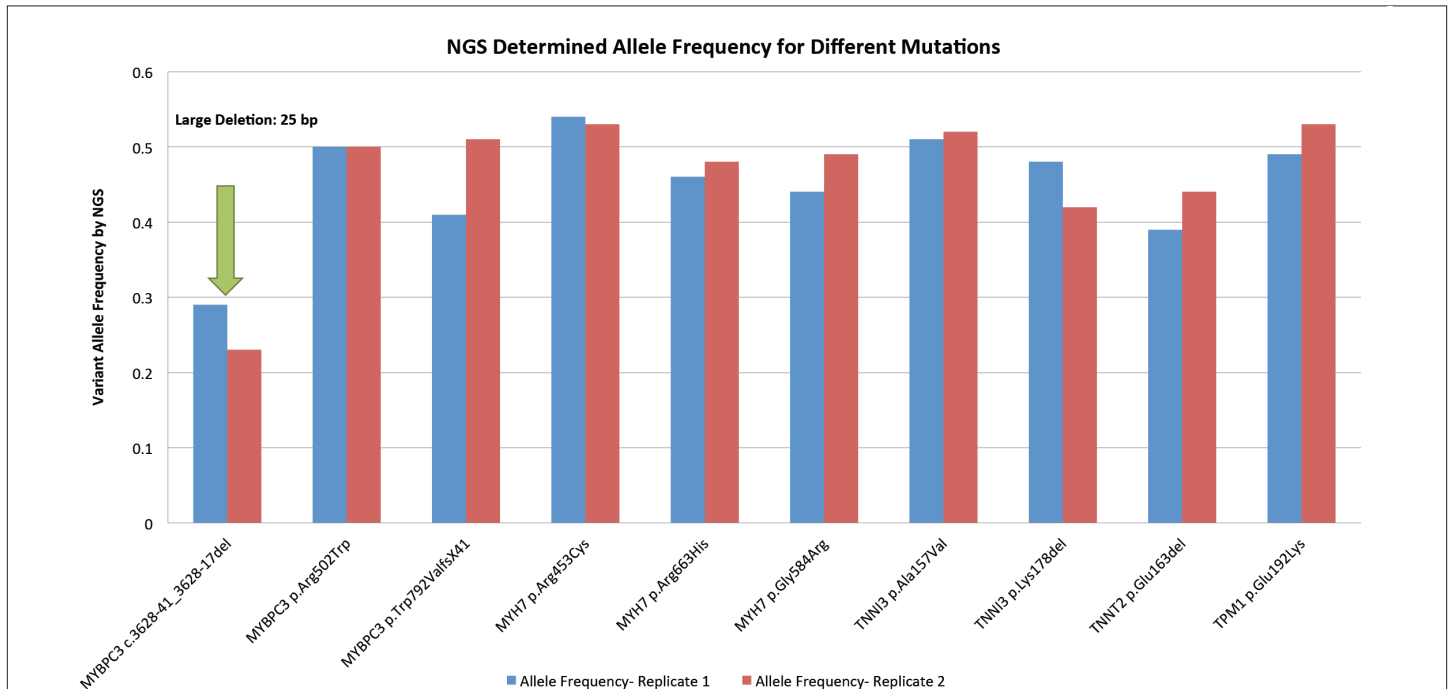


Figure 2: NGS allele frequency for two replicates of samples using SereSeq Cardiomyopathy Reference Material v1. Results indicate target values are close to 50% (as expected) for nine of the 10 variants. The large 25 bp deletion (green arrow) was detected at a much lower frequency (around 25-30%), and reflects recognized difficulty with the mapping of such large deletions via NGS.

LABORATORY 2: HYBRIDIZATION CAPTURE METHOD FOLLOWED BY 150 BP PAIRED-END SEQUENCING

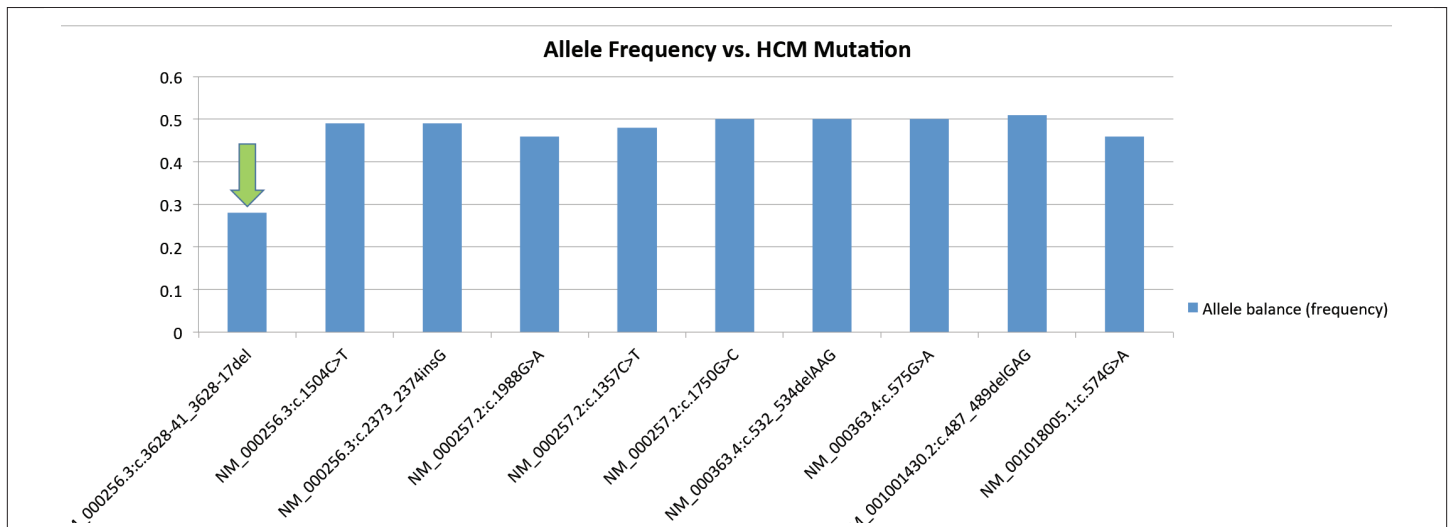


Figure 3: Detected NGS allele frequency using SereSeq Cardiomyopathy Reference Material v1. Results indicate target values are close to 50% (as expected) for nine of the 10 variants. The large 25 bp deletion (green arrow) was detected at a much lower frequency (around 25-30%), and reflects recognized 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)
Coming soon (please inquire)	Seraseq Inherited Disease Reference Material	To be determined

LEARN MORE

To learn more about Seraseq Cardiomyopathy Reference Material v1 and SeraCare's products for precision inherited disease diagnostics, visit www.seracare.com.

Contact us at +1.508.244.6400 and 800.676.1881 or email us at info@seracare.com.

REFERENCES

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2. Stanford University. GIAB Reference Materials and Data. Available at: <https://sites.stanford.edu/abms/content/giab-reference-materials-and-data>. Accessed 13 April 2016.
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