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 product 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

**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

**INCLUDED MUTATIONS**

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

**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 project\(^2\) and is originally derived from a participant in the Personal Genomes Project, public profile huAA53EO.\(^3\) 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.

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**FIGURE 1:** Design methodology for Seraseq Cardiomyopathy Reference Material v1

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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 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.

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**LABORATORY 1: HYBRIDIZATION CAPTURE METHOD FOLLOWED BY 150 BP PAIRED-END SEQUENCING**

![Graph](image1)

**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 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**

![Graph](image2)

**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 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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REFERENCES


   * Contributed equally to this work

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ORDERING INFORMATION

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<th>Material #</th>
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<td>Seraseq Cardiomyopathy Reference Material v1</td>
<td>1 vial x 200 μL per vial at 50 ng/μL concentration (10 μg total)</td>
</tr>
<tr>
<td>Coming soon (please inquire)</td>
<td>Seraseq Inherited Disease Reference Material</td>
<td>To be determined</td>
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