Seraseq® FFPE HRD Reference Material

0710-2643, 0710-2644, 0710-2645

This Product Data Sheet provides further details around the HRD assessment protocol method for Seraseq FFPE HRD High-Positive, Low-Positive and Negative Reference Material (RM).

DESCRIPTION

- Derived from tumor and matched-normal human cell lines, in fixed and paraffin embedded (FFPE) format
- Blended to ~65% tumor content, with SNP-matched normal cells
- 10 µm FFPE curl with DNA yield >100 ng per curl^

^Based on Qiagen QIAamp DNA FFPE Tissue Kit and the Qubit dsDNA HS Assay.
See the Technical Product Report for more details.

Gene ID	HGVS	Protein Variant
ATM	c.208A>T	p.K70*
ATM	c.557del	p.L186fs
BRIP1	c.107T>G	p.L36*
BRIP1	c.157dup	p.S53Kfs*16
RAD51C	c.242C>A	p.S81*
RAD51C	c.338dup	p.G114Wfs*25
RAD51D	c.271A>T	p.K91*
RAD51D	c.392dup	p.N131Kfs*23

See the Technical Spreadsheet for more details.

Table 1. Eight additional biosynthetic single-nucleotide variants (SNVs) of four homologous recombination repair (HRR) genes present in Seraseq FFPE HRD High-Positive RM and Seraseq FFPE HRD Negative RM.

DNA EXTRACTION

DNA was extracted from each FFPE material with Qiagen QIAamp DNA FFPE Tissue Kit, Maxwell RSC DNA FFPE Kit and Qiagen AllPrep Kit; yield was measured with the Qubit dsDNA HS Assay. The quality of DNA extracted with QIAamp DNA FFPE Tissue Kit was assessed using the Agilent gDNA ScreenTape Assay. The DNA integrity number (DIN) for each sample was calculated by TapeStation Analysis Software.

	Avg. Yield per 10 µm Curl (ng)		
Seraseq FFPE Product	**Qiagen QIAamp**	**Maxwell RSC**	**Qiagen AllPrep**
HRD High-Positive RM	165 ± 41	136 ± 55	161 ± 22
HRD Low-Positive RM	235 ± 68	122 ± 39	293 ± 44
HRD Negative RM	505 ± 191	200 ± 77	315 ± 10

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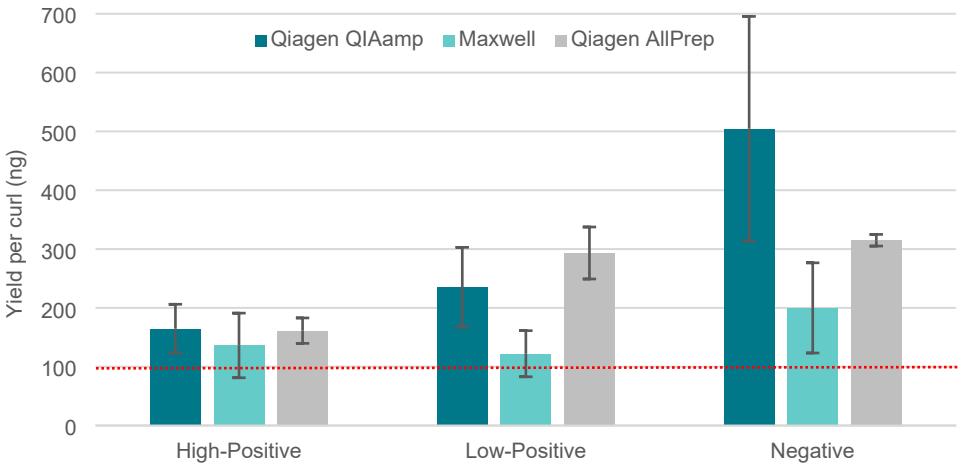

Table 2 and **Figure 1.** DNA yield per FFPE curl for each reference material as measured using the Qiagen QIAamp DNA FFPE Tissue Kit, the Maxwell RSC DNA FFPE Kit, the Qiagen AllPrep Kit for extraction, and the Qubit dsDNA HS Assay for concentration analysis. Average and standard deviation across 2-3 single curl extractions shown.

Seraseq FFPE Product	DIN
HRD High-Positive RM	4.2 ± 1.6
HRD Low-Positive RM	5.5 ± 1.2
HRD Negative RM	5.5 ± 1.1

Table 3. Quality of DNA extracted from Seraseq FFPE HRD Reference Materials using the QIAamp DNA FFPE Tissue Kit was assessed using the Agilent gDNA ScreenTape Assay for the TapeStation. Average DIN and standard deviation across 9 extractions shown for each material.

WHOLE GENOME SEQUENCING (WGS)

FFPE curls were extracted with the QIAamp™ DNA FFPE Tissue Kit (QIAGEN N.V.) using both the standard protocol and updated to account for the gentler fixation that is now used for molecular pathology as well at the Seraseq FFPE HRD reference materials. In the updated protocol, FFPE curls were extracted using the following two modifications: First, 56 °C proteinase K digestion was carried out overnight instead of for only 1 hour. Second, 90 °C formalin reversal was omitted.

For PCR-free WGS, ~400 ng of extracted DNA was purified with 0.8x AMPure™ XP beads (Beckman Coulter, Inc.) and eluted with 10 µl of 0.1x TE buffer with 5 mM potassium chloride (TEKCl). 7 µl thereof was fragmented for 15 minutes in a 10 µl reaction using the SureSelect™ Enzymatic Fragmentation Kit (Agilent Technologies, Inc.). This was then used as the input for the NxSeq™ UltraLow DNA Library Kit (LGC Biosearch Technologies). Two rounds of 0.8x AMPure XP purification were used to purify the libraries and elute them in 22 µl of TEKCl, of which 20 µl was recovered and quantified. Sequencing was carried out on a NextSeq™ 2000 (Illumina, Inc.) using a 300 cycle P1 flow cell for 3 such libraries. The resulting data was mapped to hg19 and reads mapping to 100 kbp bins across the genome were determined, which was then scaled to assess copies across the genome.

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As shown in Figure 2 below, when the Seraseq FFPE HRD RMs were extracted using the standard protocol that included the 90 °C formalin reversal step, a GC bias was introduced into the data that complicated the analysis of copies. On the other hand, when the updated protocol was used that avoided this step, the copies were much better resolved. The HRD Negative RM showed fewer transitions in copies than the Low-Positive and High-Positive RMs.

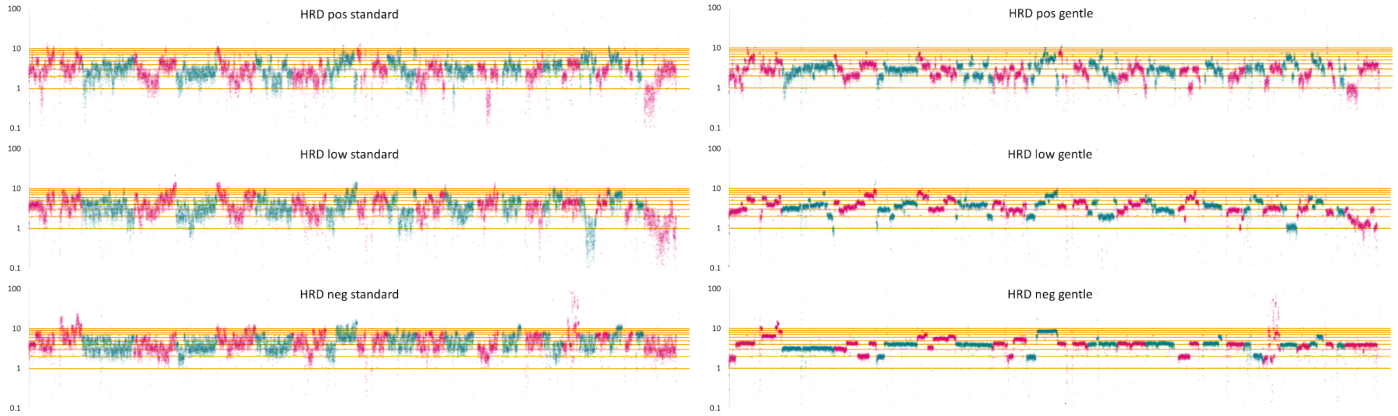

Figure 2. Two slightly different extraction methods were used to determine copies of different regions across the genome, where the updated method replaces the 90 °C formalin reversal step (standard) with an overnight 56 s°C proteinase K digestion (gentle). Chromosomes are colored alternating between magenta and teal. Compared to the HRD Negative RM (HRD neg), the Low-Positive (HRD low) and High-Positive RMs (HRD pos) show increased amounts of differences in copies.

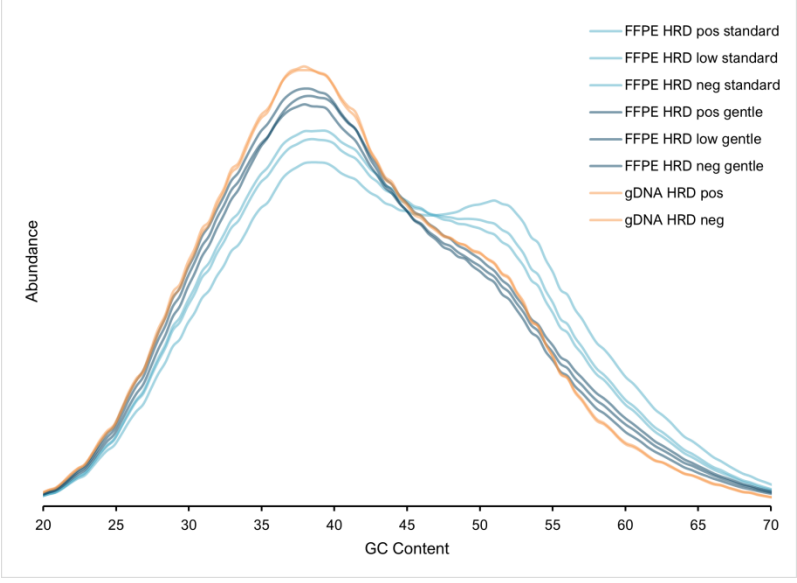

Figure 3. Relative WGS coverage (without extra steps to account for GC % effects) appears to be much cleaner when formalin reversal is skipped. Formalin reversal should not be needed when tissues are fixed using current recommendations for molecular pathology in vitro diagnostics (IVDs).
FFPE extracted with 90 °C formalin reversal, standard (light blue); FFPE extracted without formalin reversal, gentle (dark blue); gDNA (orange).

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MULTI-PLATFORM ANALYSIS

Seraseq FFPE Product	Illumina TSO 500 HRD	SOPHiA DDM HRD	**OncoScan CNV** with optimized EaCoN/ASCAT/scarHRD pipeline
HRD High-Positive RM	GIS 72 ± 3	GI 8.4	81
HRD Low-Positive RM	GIS 54 ± 2	GI 2.8	77
HRD Negative RM	GIS 31 ± 2	GI -8.4	30

Table 4.
Illumina TruSight™ Oncology (TSO) 500 HRD RUO assay calculates a Genomic Instability Score (GIS) using an algorithm licensed from Myriad Genetics, with the GIS cut-off at 42 to identify HRD positive samples. Illumina TSO 500 HRD is not available in the U.S. or Japan.

SOPHiA DDM HRD Solution genomic integrity (GI) indices are computed using a proprietary deep learning approach that takes as input the low pass WGS (lpWGS) coverage profile measured in each sample of interest.

Thermo Fisher OncoScan™ CNV Array (300kb genome-wide resolution) with an optimized EaCoN / ASCAT / scarHRD pipeline, uses a GIS cut-off at 42 to identify HRD positive samples.[1]

CONTACT US

WGS data files are available by contacting us at CDx-CustomerService@lgcgroup.com.

For additional information on these FFPE HRD reference materials, please visit our dedicated product page at https://www.seracare.com/HRD, or call us at **+1 800.676.1881**.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

[1] Kashofer, K; Jahn, S; Halbwedl, I; Hoetler, G. Beyond BRCA-HRD scoring using Affymetrix Oncoscan microarrays. Virchows Arch. 2016; 469: S203-S203. [Poster]