SAMPLE(S)

Shallow sample ID	**sample123-HRDShallow**
Panel sample ID	**sample123**
Panel Manifest	**BRCA_V4**

ANALYSIS

Date	**08/11/2022**
User	**SeqOne**
Workset	**somaHRD v1.0.2**

Test(s) performed **HRD Status detection based on shallow WGS and BRCA1/2 mutations.**

SeqOne HRD Status[1] : NEGATIVE

SAMPLE QUALITY

NAME	VALUE		DESCRIPTION
% of tumoral cells	**40**	*(>= 30%)*	Low value of tumoral cells is likely to generate false negative results.
Coverage	**1,2**	*(>= 0,1X)[2]*	Average sequencing coverage of the genome
% correct mapping	**97,8**	*(>= 50%)*	Proportion of reads correctly mapped

GENOMIC INSTABILITY FEATURES

The following variables, are used to determine the SeqOne HRD Score.

NAME	VALUE	CLASS[3]	DESCRIPTION
LGA Status	**8**	HRDeficiency	Large Genomic Alteration Status
LPC Status	**9**	HRDeficiency	Loss of Parental Copy status
CCNE1 Amplification	**0,00**	HRProficiency	Copy number variation for CCNE1

BRCA1/BRCA2 MUTATIONS

No pathogenic mutations have been identified on BRCA1 and BRCA2 genes.

SeqOne HRD Score[1]	VALUE	STATUS	CLASS	METHOD
	0,14	**NEGATIVE**	**Very low**	**SHALLOW WGS + BRCA1/2 GENE PANEL**

[1] **HRD positive status is defined** by at least one of the following: (1) At least one pathogenic BRCA1/2 mutation and/or (2) a SeqOne HRD score greater than 50%. Altough SeqOne HRD score includes LGA, LPC, CCNE1/RAD51B amplification, and the number of pathogenic BRCA1/2 mutations, the decision rule described above ensures that all patients with a pathogenic mutation in BRCA1/2 are HRD positive.

[2] The **minimum coverage required** to run the test is 0.1X but we rather recommend a coverage of 1X. When decreasing the coverage to 0.1X, rate of misclassification increases by 5%.

[3] **Class for the variable** was determined based on a cohort of ovarian cancer patients. Very high values correspond to the top 20%, high values to the top 20-40%, medium values are in the range 40-60% of the distribution, low values in the range 60-80% and very low values are in the lowest 20% of the distribution. High values of LGA and LPC increase the probability to be HRD whereas high values of CCNE1 and RAD51 amplification increase the probability to be HRP.

METHODOLOGY

The HRD score correspond to the probability that the patient is HRD positive and is obtained from five features determined with two sources of data. The first source of data is a gene panel, which is used to provide the number of pathogenic mutations in the BRCA1 and BRCA2 genes. If the HRD score is determined with sWGS only, computation of the score assumes that their are no pathogenic mutation in BRCA1 and BRCA2 genes. The second source of data arises from a shallow Whole Genome Sequencing (sWGS), which consists of two Genomic Instability (GI) features and two features of Copy Number Variation (CNV). The Genomic Instability features consist of the number of a Large Genomic Alterations (LGA) and a Loss of Parental Copy feature (LPC). LGA is defined as the number of copy number breakpoints. We consider a breakpoint if we observe a Copy Number alteration between two genomic segments of at least 10Mb long and at most 3Mb apart. LPC is defined as the number of haploid segments of at least 10Mb long. The CNV features consist of gene amplifications measured with sWGS at the CCNE1 and RAD51B genes. The HRD score consists of a linear combination of the five features which is converted with a logit link to map those values to the scale of a probability, i.e., between 0 and 1.

QC CHECKS

Average coverage of sWGS should be at least 0.1X. Proportion of properly **mapped reads** for paired-end sequencing should be **at least 50%.** Coverage and proportion of properly mapped reads are computed after trimming sequencing adapters.

LIMITATION OF SEQONE HRD TESTING

Percentage of tumor cells needed for sequencing should be **at least 30%.**

SENSITIVITY

We performed in silico downsampling experiments based on 45 sequences with a coverage of 3x. We found that decreasing the coverage to 0.1x decreases the misclassification rate from 93% (42/45) to 89% (40/45). The misclassification rate is the percentage of samples classified differently by SeqOne HRD and Myriad myChoice™ HRD.