Microsatellite instability

Microsatellite instability is caused by a defect in the DNA mismatch repair (MMR) pathway. MSI is becoming an important biomarker for immune checkpoint inhibition. The Oncomine Comprehensive Assay Plus determines MSI status by measuring instability of 76 different microsatellite markers using a numeric score. Samples with high MSI scores are considered MSI-high, and samples with low MSI scores are considered microsatellite stable (MSS).

Tumor mutational burden (TMB)

TMB is a metric for assessing the number of mutations within a tumor genome and is often measured as the number of mutations per coding area in the tumor genome. Research has shown that a high TMB may correlate with a response to immune checkpoint inhibitor therapy [1-3]. The Oncomine Comprehensive Assay Plus is designed to provide an accurate assessment of TMB (mutations/Mb) with 1 Mb of exonic coverage.

Bioinformatics solution for visualizing mutational signatures

Mutational signatures are important tools in precision oncology research that provide insight into the biological mechanisms involved in carcinogenesis (e.g., UV damage, deficiency in DNA repair). The Oncomine Comprehensive Assay Plus provides you with a comprehensive genomic profile. As part of the streamlined bioinformatics workflow, the mutational signature plot is automatically generated and does not require additional analysis with third-party software.

References:

1. Rizvi et al. Science 2015 348(6230), Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer.
2. Snyder et al. N Engl J Med. 2014 371(23), Genetic basis for clinical response to CTLA-4 blockade in melanoma.
3. Chalmers et al. Genome Medicine 2017 9:34, Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden.

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Molecular Pathology

Tumour tissue

Biopsy material

Liquid Biopsy

Liquid Profiling

HRD research

HRD is becoming an important biomarker in precision oncology. HRD is the inability to repair double-strand DNA breaks using the HRR pathway. The understanding of HRR genes such as BRCA1 and BRCA2 has driven the development of different approaches for targeting HRD, such as poly (ADP-ribose) polymerase (PARP) inhibitors via synthetic lethality. There are two key approaches for assessing HRD status: detecting mutations in HRR genes that may cause HRD and measuring the consequences of HRD by the presence of genomic scarring/instability. The Oncomine Comprehensive Assay Plus enables detection of HRR gene mutations that may cause HRD as well as reporting the consequence of genomic scarring using the GIM.

Causes: mutation detection of HRR pathway genes
The significant role of HRR genes in maintaining genome stability and tumor suppression has been studied extensively, especially in the BRCA1 and BRCA2 genes. The status of HRR genes is now considered a new potential biomarker for precision oncology. Figure 3A shows the HRR genes covered in the Oncomine Comprehensive Assay Plus.
Consequences: genomic instability measurement
The Oncomine Comprehensive Assay Plus uses hetero-zygous population SNPs to determine the ploidy levels of genomic segments. The genomic segmentation is then used to infer tumor cellularity, loss of heterozygosity (LOH) for each segment, and the GIM.

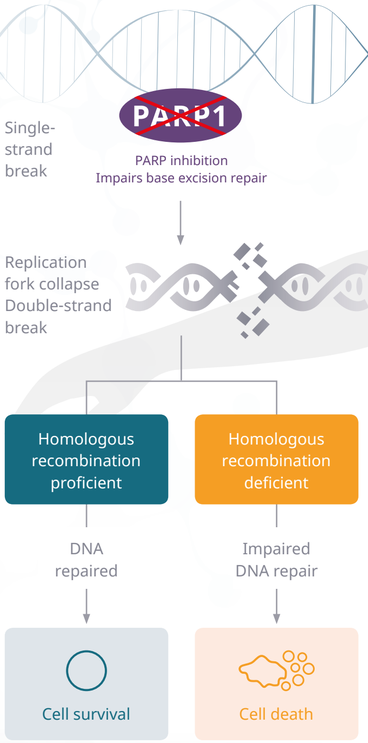


Figure 2. **Synthetic lethality concept.**

A comparative analysis was evaluated for concordance between the Oncomine Comprehensive Assay Plus, a targeted NGS assay for genomic segmentation, and the Applied Biosystems™ OncoScan™ CNV Assay. The OncoScan CNV Assay is a microarray-based assay with more than 200,000 SNPs that provides higher resolution for genomic segmentation. Percent Loss of Heterozygosity (LOH) is highly concordant between the two methods (r = 0.95; Figure 3B), which suggests that genomic segmentation is not sensitive to the resolution of SNPs.

The Oncomine Comprehensive Assay Plus measures genomic instability using the GIM. The GIM is a numeric value between 0 and 100 that summarizes unbalanced copy number changes using genomic segmentation. Higher GIM values correlate with the observation of more genomic instability in the sample. GIM values are higher in HRD-positive samples than they are in HRD-negative samples in ovarian cancer (Figure 3C).

Comprehensive genomic profiling without compromise

The Ion Torrent™ Oncomine™ Comprehensive Assay Plus

Performance:

using commercially available reference controls and clinical research formalin-fixed, paraffinembedded (FFPE) samples, assay sensitivity and specificity ranged from 93 % to 100 %, with averages of 97.0 % sensitivity and 98.3 % specificity across all variants, with CNV gain and CNV loss demonstrating exceptional 100 % specificity

Single-gene biomarkers:

detect all types of single-gene variants, such as single-nucleotide variants (SNVs), insertions and deletions (indels), novel and known fusions, splice variants, and copy number variants (CNVs), including copy number gains and losses

Multi-gene biomarkers:

study potential responses to immunotherapy with measurement of tumor mutational burden (TMB) and predisposition to genetic hypermutability by comparing microsatellite instability (MSI) regions, and analyze mutational signatures for insights into etiological factors in tumorigenesis

HRD research:

detect mutations in 46 homologous recombinantion repair (HRR) genes, including BRCA large gene rearrangements, and assess genomic scarring with the genomic instability metric (GIM)

Low input requirements:

FFPE sample inputs of 20 ng DNA or RNA are sufficient to profile more than 500 genes, which helps ensure more samples can be analyzed

High testing success:

sequencing success rates of up to 95 % and low quantity not sufficient (QNS) results help ensure more samples are successfully tested

Bioinformatics solution:

streamlined bioinformatics analysis pipeline is optimized for this assay and packaged in a user-friendly experience with Ion Torrent™ Oncomine™ Reporter software, which gives fully annotated results

Highly automated workflow:

hands-on time of ~1 hour supports lab efficiency and helps reduce the risk of errors due to handling

End-to-end solution

Automated GeneStudio S5 Prime workflow

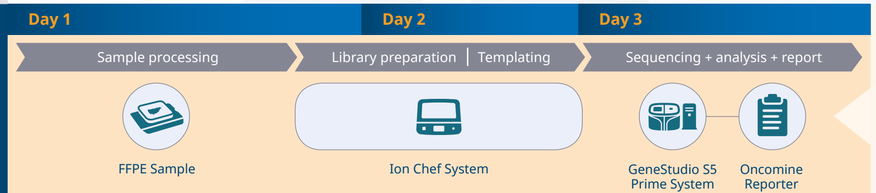


Figure 1. The Oncomine Comprehensive Assay Plus workflow is compatible with manual or automated library preparation. This provides the flexibility to integrate the workflow that best meets the needs of your lab.

FusionSync technology

Key considerations for optimal fusion detection:
- Detection of fusions in low-input samples
- Detection of low-level fusion transcripts
- Detection of novel fusions in driver genes

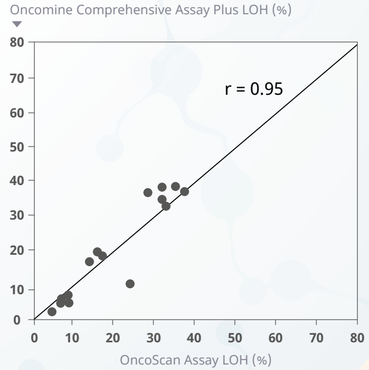
With Ion Torrent™ FusionSync™ technology, the Oncomine Comprehensive Assay Plus covers >1,300 isoforms across 49 fusion drivers. This enables highly sensitive and robust detection of known fusions and novel combinations of known fusion partners. The exon-tiling imbalance approach simultaneously enables detection of novel fusions with key fusion driver genes, such as ALK, FGFR2, NTRK1, NTRK2, NTRK3, and RET.

For each driver gene in which a fusion is detected, the software also predicts with high confidence the position of the fusion breakpoint relative to the kinase domain. This is critical, as an intact kinase domain is essential for the pathogenicity of a fusion event.

A 46 HRR pathway genes covered by the Oncomine Comprehensive Assay Plus

ABRAXAS1	*ATM*	*ATR*	*BAP1*	*BARD1*	*BLM*	*BRCA1*	*BRCA2*	*BRIP1*	*CDK12*	*CHEK1*	*CHEK2*
FANCA	*FANCC*	*FANCD2*	*FANCE*	*FANCF*	*FANCG*	*FANCI*	*FANCL*	*FANCM*	*MRE11*	*NBN*	*PALB2*
PARP1	*PARP2*	*PARP3*	*POLD1*	*POLE*	*PPP2R2A*	*PTEN*	*RAD50*	*RAD51*	*RAD51B*	*RAD51C*	*RAD51D*
RAD52	*RAD54L*	*RNASEH2A*	*RNASEH2B*	*RNASEH2C*	*RPA1*	*SLX4*	*TP53*	*XRCC2*	*XRCC3*		

B Sample-level LOH comparison



C GIM stratification of HRD-positive samples

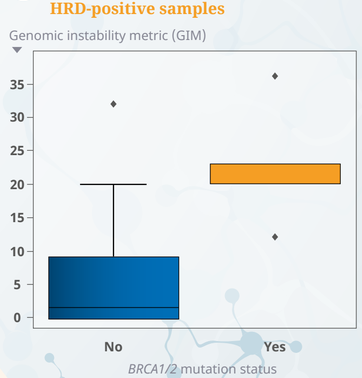


Figure 3. **HRR pathway research using the Oncomine Comprehensive Assay Plus.** **(A)** The 46 HRR pathway genes covered in the Oncomine Comprehensive Assay Plus. **(B)** Oncomine Comprehensive Assay Plus sample-level LOH estimates (y-axis) correlate favorably with the orthogonal OncoScan assay (x-axis), for the same FFPE samples. The test sample set consisted of FFPE samples from various solid-tumor tissue types. The Pearson correlation coefficient (r) is shown as the measure of association. **(C)** Comparison of GIM for *BRCA*-positive and *BRCA*-negative ovarian cancer samples (n = 46). **No:** no pathogenic *BRCA1* or *BRCA2* mutation present. **Yes:** pathogenic *BRCA1* or *BRCA2* mutation present. **Source:** internal R&D data.