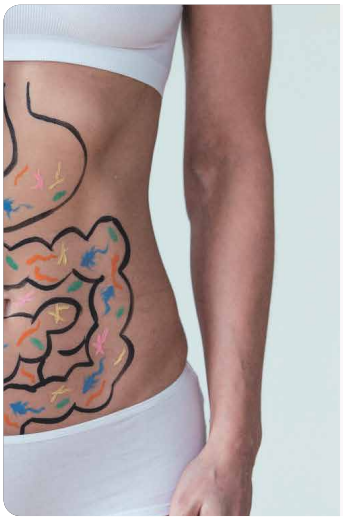
DIACARTA®

OptiSeq™ Colorectal Cancer Panel 43-Gene Detection Test

Find Gene Mutation Using NGS

Key Features



- For biomedical research, cancer biomarker and drug/therapeutic target discoveries
- For LDT service, patient genetic profiling and patient selection for targeted therapies
- 43 genes, 537 genetic alterations
- Single nucleotide variants (SNV), insertion and deletions (Indels), and copy number variants (CNV)
- Blood sample/cell-free DNA (cfDNA) input at 10 to 40 ng
- Analytical sensitivity: 0.25% VAF (10 ng DNA input) or 0.1% VAF (40 ng DNA input)
- Clinical sensitivity and specificity: 88% and 100%, respectively

Colorectal cancer (CRC) is the second cause of cancer death in the United States, next to lung and bronchus cancer. According to the American Cancer Society, the estimated death for CRC patients is 52,580 in 2022. There is a trend that CRC is developed at a younger age and the American Cancer Society has changed from the start screening age from 50 to 45. However, only 2/3 of the population from 45 to 75 are up to date for CRC screening aiming for early cancer detection; many people missed the opportunity to get detected and treated early and therefore lost their lives.

Multiple somatic mutations associated with CRC have been identified and they serve as biomarkers providing diagnostic and therapeutic values. We have used next-generation sequencing (NGS) to reveal the CRC-associated mutations as a molecular profiling tool for guiding personalized treatment and therapy monitoring.

Use of the OptiSeq™ Colorectal Cancer Panel 43-Gene Detection Test

When the product is used in clinical research, the OptiSeq™ Colorectal Cancer Panel can be used to profile 457 CRC alterations/mutations in 43 genes and study the mechanism of CRC related to these mutations or other biomarkers. When the product is used in clinical studies or trials for drug responses, the CRC subjects can be profiled to study the relationship between the mutations with drug responses. When the product is validated in a clinical lab for CRC patient profiling or CRC mutation detection, the actionable mutation information can be used as a guide for physicians for targeted drug therapies; the other mutation information may provide information for the physicians to identify what clinical trials are going on for the therapies against these mutations, especially when patients have exhausted standard care options.

The 43 Genes Associated with CRC in this NGS Pane

Compared to whole-exome sequencing (WES) or whole-genome sequencing (WGS), the benefit of a targeted NGS panel is to focus on a specific group of mutations related to a particular disease, such as CRC. Therefore, the sequencing depth can be increased for better accuracy without sequencing unrelated genes. The 43 genes in our CRC NGS panel are listed in the table on the right side.

AKT1	APC	ARID1A	ATM	BAT25
BAT26	BAT34C4	BAT40	BRAF	CTNNB1
EGFR	ERBB2	FBXW7	GNAS	HSP110
KMT2C	KMT2D	KRAS	MET	MLH1
MLH3	MONO27	MSH2	MSH3	MSH6
MTHFR	MYC	NR21	NR22	NR24
NRAS	PIK3CA	PMS1	PMS2	POLE
PTEN	PVT1	RNF43	SMAD4	STK11
TP53	VEGFA	ZFHX3		

These gene mutations include both the somatic mutations such as BRAF and KRAS as well as inherited mutations (passed from family members) such as APC for Familial adenomatous polyposis (FAP), attenuated FAP (AFAP), Gardner syndrome, and MLH1, MSH2, MSH6, PMS2 for Lynch syndrome. The NCCN (National Comprehensive Cancer Network) guideline for color cancer highly recommend that the exon 2,3,and 4 of KRAS and NRAS and V600E of BRAF to be determined at the diagnosis of the stage VI. All these mutations have been included in this panel.

The 43-gene panel detects the following alterations:

437 nucleotide (SNVs) substitutions, insertions, deletions (indels)

11 copy number variants (CNVs)

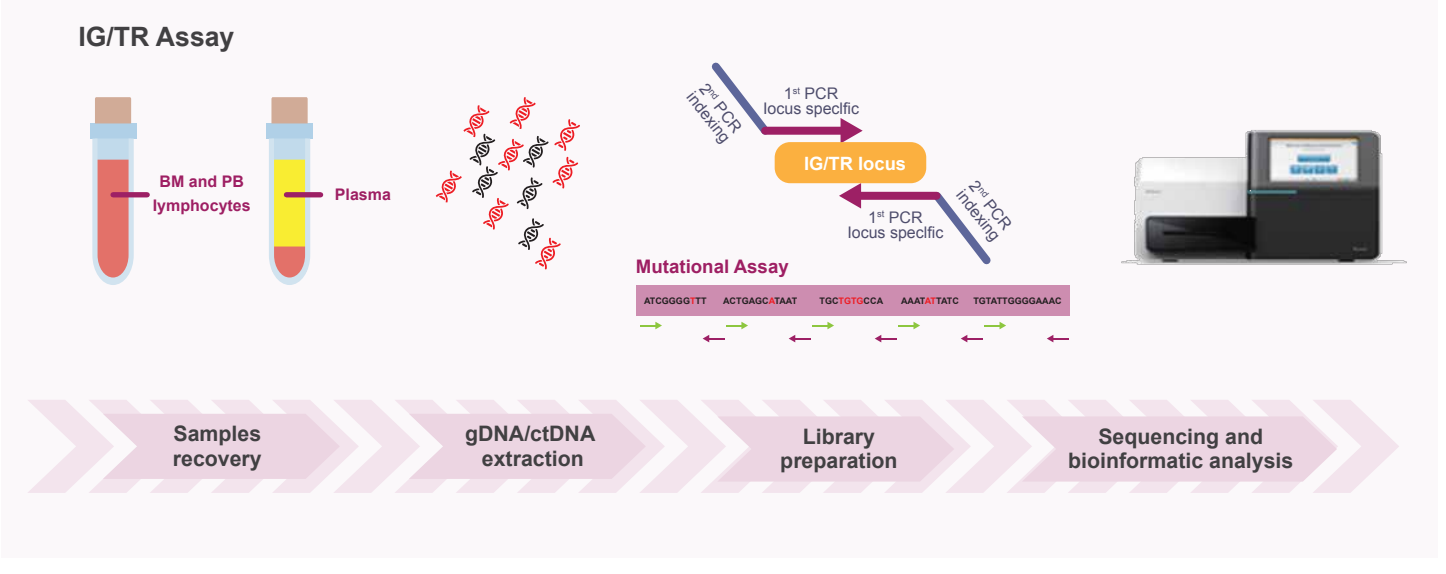
Selected gene rearrangements

Genomic signatures including microsatellite instability (MSI) in 9 regions

The Sequencing Workflow

Sequencing of the selected 43 genes includes sample recovery or plasma isolation from blood samples, cell-free DNA (cfDNA) extraction from plasma or genomic DNA (gDNA)preparation from the white blood cells, library preparation from cfDNA or gDNA, and sequencing and bioinformatics analysis.

Workflow of the 43-gene NGS panel for CRC mutation detection



Assay Performance

Analytical Sensitivity and Limit of Detection (LoD)

The LoD for 61 representative genomic alterations (SNVs and indels) using the OptiSeq™ Colorectal Cancer Panel 43-Gene Detection Test was calculated. The established median LoD for 10ng input was 0.25% VAF and the median LoD for 40ng input was 0.1% VAF. Regardless of the input of cfDNA >95% (59 out of 61) of representative targets were reliably identified at 0.25%VAFs. CNV level of detection of the kit was tested using representative CNVs using 10ng of input since it represents the most challenging conditions. Reference CNV alterations were detected with different levels: gain of copy number was identified at >10% and copy number loss at >20%.

Alteration	LoD, 10ng input, median of the means (VAF%)	LoD, 40ng input, median of the means (VAF%)
SNVs and indels	0.25%	0.1%
CNVs gain	≥10%	Not tested
CNVs loss	≥20%	Not tested
MSIs	≥5%	Not tested

Clinical Performance of the Assay

To validate this kit's clinical performance with clinical samples, 25 CRC and 42 health donator individuals' whole blood were collected and isolated for cfDNA samples with Qiagen kit (CRC cohort). The samples were tested with the OptiSeq™ Colorectal Cancer Panel 43-Gene Detection Teston the MiSeq instruments. 22 out of 25 CRC samples were identified as having mutants and therefore as positive. Three samples were identified as negative having mutation levels below a threshold. All health samples have no mutants detectable. The result indicates that the clinical sensitivity for CRC is 88% (95% CI: 67.7-96.8) and specificity 100% (95% CI: 89.5-100).

Summary of OptiSeq™ Colorectal Cancer Panel 43-Gene Detection Test

Sample Status	OptiSeq™ Colorectal Cancer Panel 43-Gene Detection Test			
	Positive	**Negative**	**Sensitivity (%)**	**Specificity (%)**
Positive	22	3	88% (95% CI: 67.7-96.8)	100% (95% CI: 89.5-100)
Negative	0	42		
Total	22	45		

Ordering Information

Product Name: OptiSeq™ Colorectal Cancer Panel 43-Gene Detection Test

Pack Size: 24 Reactions **Intended Use:** Research Use Only **Catalog:** DC-50-0005R