ABOUT OMNISEQ COMPREHENSIVE℠

OmniSeq Comprehensive℠ is a next generation sequencing assay that uses multiplexed PCR-based DNA-seq and RNA-seq technologies to detect somatic variants in tumors (mutations, copy number variants and fusions) for 144 genes (118 oncogenes and 26 tumor suppressor genes) to guide cancer therapeutic management.

The DNA-seq component detects mutations (single nucleotide variants, insertions and deletions) and copy number variants in both oncogenes and tumor suppressor genes, while the RNA-seq component performs fusion analysis in oncogenes. DNA-seq mutational analysis detects gain-of-function mutations in oncogenes using a hotspot coverage strategy while copy number analysis detects high level amplification. DNA-seq mutational analysis also detects loss-of-function mutations in tumor suppressor genes using a complete coding sequence coverage strategy, while copy number analysis detects homozygous deletions. The RNA-seq component is focused on fusion analysis.

OmniSeq Comprehensive℠ Genes and Next Generation Sequencing Technologies

DNA-Seq							RNA-Seq
Mutations (SNVs and Indels)				Copy Number			Fusions
Hotspot			Coding Sequence	Loss	Gain		
ABL1	GNA11	MYD88	APC	APC	ACVRL1	IL6	ABL1
AKT1	GNAQ	NFE2L2	ATM	ATM	AKT1	KIT	AKT3
ALK	GNAS	NPM1	BAP1	BAP1	APEX1	KRAS	ALK
AR	HNF1A	NRAS	BRCA1	BRCA1	AR	MCL1	AXL
ARAF	HRAS	PAX5	BRCA2	BRCA2	ATP11B	MDM2	BRAF
BRAF	IDH1	PDGFRA	CDH1	CDH1	BCL2L1	MDM4	EGFR
BTK	IDH2	PIK3CA	CDKN2A	CDKN2A	BCL9	MET	ERBB2
CBL	IFITM1	PPP2R1A	FBXW7	FBXW7	BIRC2	MYC	ERG
CDK4	IFITM3	PTPN11	GATA3	GATA3	BIRC3	MYCL	ETV1
CHEK2	JAK1	RAC1	MSH2	MSH2	CCND1	MYCN	ETV4
CSF1R	JAK2	RAF1	NF1	NF1	CCNE1	MYO18A	ETV5
CTNNB1	JAK3	RET	NF2	NF2	CD274	NKX2-1	FGFR1
DDR2	KDR	RHEB	NOTCH1	NOTCH1	CD44	NKX2-8	FGFR2
DNMT3A	KIT	RHOA	PIK3R1	PIK3R1	CDK4	PDCD1LG2	FGFR3
EGFR	KNSTRN	SF3B1	PTCH1	PTCH1	CDK6	PDGFRA	MET
ERBB2	KRAS	SMO	PTEN	PTEN	CSNK2A1	PIK3CA	NTRK1
ERBB3	MAGOH	SPOP	RB1	RB1	DCUN1D1	PNP	NTRK2
ERBB4	MAP2K1	SRC	SMAD4	SMAD4	EGFR	PPARG	NTRK3
ESR1	MAP2K2	STAT3	SMARCB1	SMARCB1	ERBB2	RPS6KB1	PDGFRA
EZH2	MAPK1	U2AF1	STK11	STK11	FGFR1	SOX2	PPARG
FGFR1	MAX	XPO1	TET2	TET2	FGFR2	TERT	RAF1
FGFR2	MED12		TP53	TP53	FGFR3	TIAF1	RET
FGFR3	MET		TSC1	TSC1	FGFR4	ZNF217	ROS1
FLT3	MLH1		TSC2	TSC2	FLT3		
FOXL2	MPL		VHL	VHL	GAS6		
GATA2	MTOR		WT1	WT1	IGF1R		

OmniSeq Comprehensive℠ reports mutations at the protein level based on the Life Technology Oncomine™ Assay Knowledgebase. Standard Human Genome Variation Society (HGVS) nomenclature (http://www.hgvs.org/varnomen) is used in reporting coding DNA and predicted protein changes.

OmniSeq Comprehensive℠ reports detected variants with therapeutic associations for the tumor type tested and for other tumor types as described by the OmniSeq Knowledgebase℠, which contains industry leading third party

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scientific publications from sources such as PubMed, international and US practice guidelines from sources such as NCCN and ASCO, approved FDA drug label content, and genomic content from multiple sources such as COSMIC, 1000 Genomes Project, dbSNP, SIFT, PolyPhen, and ClinVar. This information is proprietarily curated by OmniSeq for final clinical and genomic content. While OmniSeq reviews this information to help ensure accuracy, decisions about patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community. There is no guarantee that detection of any variant by this test will result in therapeutic efficacy or lack of efficacy. The absence of detected variants by this test does not confer a lack of therapeutic efficacy for any drug or therapy known to target genes in this test. It is possible that therapeutic implications associated with variants detected by this test are not suitable for a specific patient.

OmniSeq Comprehensive℠ reports detected variants as having unknown therapeutic significance when they meet one of the following criteria: 1) the detected variants are identified in the OmniSeq Knowledgebase℠ as having prognostic associations in the tumor type tested; 2) other variants in the same genes as the detected variants have been identified in the OmniSeq Knowledgebase℠ as having prognostic or therapeutic associations, or; 3) the detected variants are in tumor suppressor genes that are not in the OmniSeq Knowledgebase℠, are deleterious in at least one protein modeling database (SIFT or PolyPhen), and are not reported in the 1,000 Genomes database at a prevalence of 1% or greater. OmniSeq Comprehensive℠ reports wild type for some genes and tumor types where appropriate, such as EGFR for NSCLC, KIT/PDGFRA for GIST, and NRAS/KRAS for colorectal cancer. When wild type analysis does not meet criteria for 95% confidence in the wild type call for a specific variant position, the results are reported as indeterminate.

OmniSeq Comprehensive℠ testing includes mutational analysis of 15 genes (APC, BRCA1, BRCA2, MLH1, MSH2, NF2, PTEN, RB1, RET, STK11, TP53, TSC1, TSC2, VHL, and WT1) designated by the American College of Medical Genetics and Genomics (ACMG) as harboring potential germline mutations that may result in incidental findings related to hereditary susceptibility to disease. While OmniSeq Comprehensive℠ does not sequence matching non-tumor tissue from tested patients, it is possible that germline mutations can be identified from tumor-only sequencing results without direct analysis of germline DNA. OmniSeq Comprehensive℠ reports detected mutations in ACMG genes as potentially hereditary when they are identified as pathogenic or likely pathogenic in ClinVar (http://www.ncbi.nlm.nih.gov/clinvar) at the nucleotide level. If clinically applicable, these results should be further investigated by additional germline testing at the discretion of the ordering or treating physician.

OmniSeq Comprehensive℠ has an assay sensitivity and PPV of 97.0% and 97.9%, respectively, for formalin fixed paraffin embedded specimens. For insertions and deletions, OmniSeq Comprehensive℠ has an assay sensitivity and positive predictive value (PPV) of 82.0% and 96.7%, respectively. OmniSeq Comprehensive℠ detects single nucleotide variants, insertions and deletions with 95% sensitivity at a minimum VAF of 14.6% and an analytical sensitivity of 79.8% at a VAF of 5%. OmniSeq Comprehensive℠ can reliably detect single nucleotide variants, insertions and deletions in samples with 20% or greater neoplastic nuclei. For copy number variants, OmniSeq Comprehensive℠ has an assay sensitivity and PPV of 93% and 90%, respectively. OmniSeq Comprehensive℠ can reliably detect copy number variants in samples with 50% or greater neoplastic nuclei. For gene fusions, OCP has an assay sensitivity and PPV of 100% and 100%, respectively, for the known fusions in this assay. Knowledge of known fusion partners is required for detection by OmniSeq Comprehensive℠. Percent neoplastic nuclei has minimal to no influence on the detection of gene fusions in OmniSeq Comprehensive℠ due to the RNA-based method of detection.

The performance characteristics of OmniSeq Comprehensive℠ were analytically validated by OmniSeq Laboratories under the requirements of the Clinical Laboratory Improvement Amendments of 1988. The U.S. Food and Drug Administration (FDA) has not approved or cleared this test; however, FDA approval or clearance is currently not required for clinical use of this test. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions. This test should not be regarded as investigational or for research use. OmniSeq, LLC is authorized under the Clinical Laboratory Improvement Amendments and by the New York State Clinical Laboratory Evaluation Program to perform high-complexity testing.