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Extended RAS Panel

The first Food and Drug Administration (FDA)-approved next-generation sequencing (NGS) *in vitro* diagnostic for metastatic colorectal cancer (mCRC) for use in establishing eligibility of patients for treatment with Vectibix®.

Highlights

- **First Fully Validated NGS-Based Companion Diagnostic for Vectibix**
  FDA-approved test for detection of RAS mutations
- **Integrated, Streamlined Workflow**
  Comprehensive diagnostic solution includes library prep, sequencing, and clinical report
- **Extended Gene Coverage**
  Simultaneous detection of 56 RAS mutations contraindicated for Vectibix therapy

Introduction

Colorectal cancer is the third leading cause of cancer-related deaths in the US.[1] Mortality has decreased with improvements in both screening and treatment methods.[1] The epidermal growth factor receptor (EGFR), which is overexpressed in mCRC, has become an effective therapeutic target.[2] Clinical trials demonstrated prolonged survival in mCRC patients treated with Vectibix (panitumumab), a fully-human monoclonal antibody that blocks the EGFR signaling pathway.[3] Discovery of activating mutations in *Kirsten rat sarcoma viral oncogene homolog* (*KRAS*) (exon 2, codons 12 and 13) that affect the efficacy of treatment, led to the use of biomarkers to predict patient response to Vectibix therapy.[4,5] Subsequent studies have identified other activating mutations in exons 3 and 4 of *KRAS* and a related gene, *neuroblastoma rat sarcoma viral oncogene homolog* (*NRAS*). Genetic testing recommendations have expanded to include these mutations, referred to here as extended RAS coverage, to improve diagnostic evaluations for selection of appropriate therapy candidates.[6-9]

To aid in the identification of patients eligible for treatment with Vectibix, Illumina offers the Extended RAS Panel. As the first FDA-approved, NGS-based *in vitro* diagnostic for mCRC, the Extended RAS Panel is used to simultaneously evaluate 56 mutations contraindicated for Vectibix therapy. The Extended RAS Panel covers 12 codons in *KRAS*/*NRAS* that may contain activating mutations (Figure 1). The panel is intended to be used on the MiSeqDx® instrument. On-instrument software delivers an easy-to-interpret report to guide therapy decisions accurately and efficiently.

Comprehensive Content Design

Content for the Extended RAS Panel was developed according to guidelines from the American Society for Clinical Pathology (ASCP), the Association for Molecular Pathology (AMP), the College of American Pathologists (CAP), and the American Society of Clinical Oncology (ASCO).[6-9] Within a single assay, the Extended RAS Panel targets 12 codons in two RAS genes (*KRAS* and *NRAS*), simultaneously detecting all 56 mutations that are contraindicated for Vectibix therapy (Table 1).

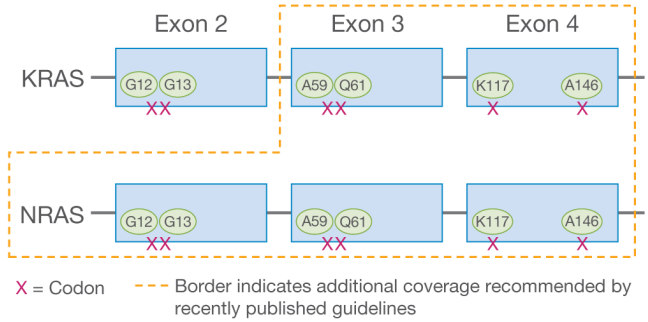


Figure 1: Comprehensive Mutation Detection by the Extended RAS Panel — Society guidelines (ASCP, CAP, AMP, and ASCO)[6-9] recommend confirmation of *KRAS*/*NRAS* mutational status before treatment of mCRC with Vectibix therapy.[5] Targeted sequencing provides deep coverage of each relevant codon and detects the presence of activating mutations that are contraindicated for Vectibix treatment, as compared to no mutations (wild-type).

Table 1: Summary of Activating Mutations Detected by Extended RAS Panel

KRAS				*NRAS*			
Exon	Wild-Type Codon	Wild-Type Amino Acid	No. of Mutations Covered	Exon	Wild-Type Codon	Wild-Type Amino Acid	No. of Mutations Covered
2	c.34-36	p.Gly12	9	2	c.34-36	p.Gly12	9
2	c.37-39	p.Gly13	6	2	c.37-39	p.Gly13	6
3	c.175-177	p.Ala59	2	3	c.175-177	p.Ala59	2
3	c.181-183	p.Gln61	6	3	c.181-183	p.Gln61	6
4	c.351-353	p.Lys117	2	4	c.351-353	p.Lys117	2
4	c.437-439	p.Ala146	3	4	c.437-439	p.Ala146	3

For *In Vitro* Diagnostic Use.

Fully Integrated, Streamlined Workflow

The Extended RAS Panel is a fully integrated testing solution with an easy-to-perform protocol. The workflow involves three main stages (Figure 2). The first is manual preparation of the samples for sequencing (library preparation), which involves hybridizing indexed oligonucleotides to genomic DNA, followed by enzymatic extension and amplification of targeted sites. In the second stage, the prepared sample is loaded onto the MiSeqDx instrument and sequenced. The third stage, analysis, occurs automatically on the MiSeqDx instrument.

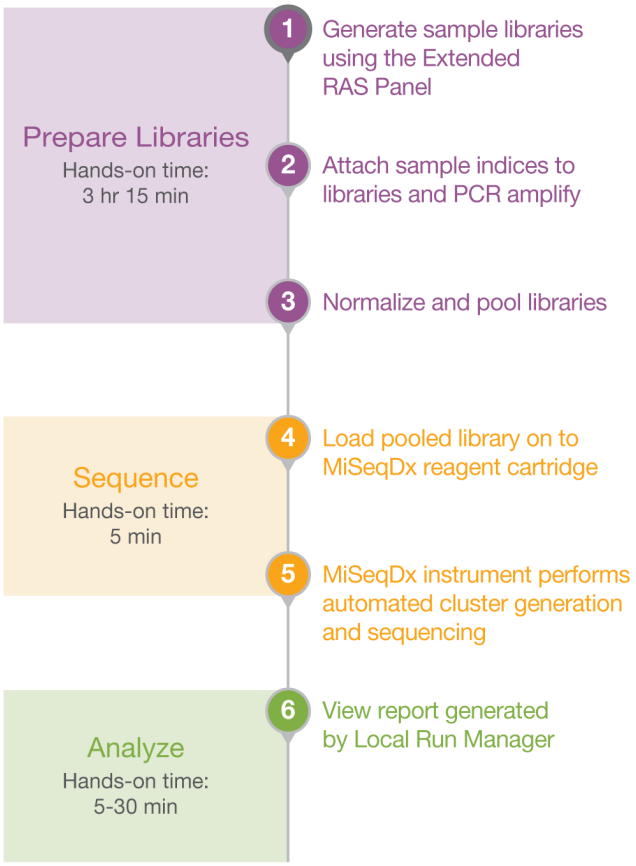


Figure 2: Integrated Clinical Assay Workflow—The Extended RAS Panel offers a comprehensive solution for identifying mutations in *KRAS*/*NRAS* that are contraindicated for Vectibix therapy.

Easy-to-Interpret Results

On-instrument software integrated into the MiSeqDx instrument provides a user-friendly interface to set up and run assays, monitor progress in real time, perform automated analysis, and view clinical reports. For each sample, the clinical report includes sample ID, quality control valid/invalid indication, and an interpretation of mutation status (Figure 3).

Clinical Metrics

To achieve full validation, critical metrics were established for the Extended RAS Panel. To assess clinical accuracy, high concordance was demonstrated between the Extended RAS Panel and Sanger sequencing, a validated reference standard for clinical testing (Table 2). Reproducibility of the panel was established in three independent testing sites (Table 3). Importantly, the assay accurately identified a patient population that demonstrated a progression-free survival benefit, and a trend in the positive direction for overall survival, when treated with Vectibix.[10]

Table 2: Clinical Accuracy

Characteristic	Positive % Agreement[b]	Negative % Agreement[c]
Concordance[a]	98.7% (227/230)	97.6% (206/211)

a. Compared against bidirectional Sanger sequencing as a validated reference standard
b. Number of samples with a RAS mutation as detected by the Extended RAS Panel divided by number of samples with RAS mutations as detected by Sanger
c. Number of wild-type RAS samples as indicated by the Extended RAS Panel divided by number of wild-type samples indicated by Sanger

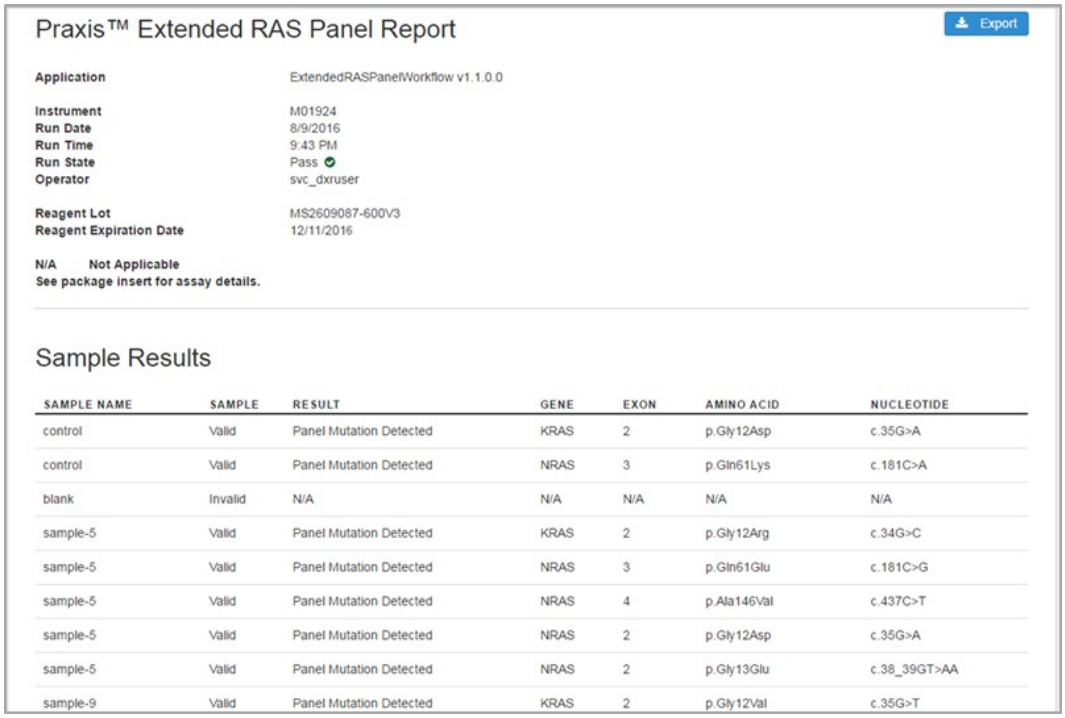
Table 3: Reproducibility[a]

Characteristic	Mutant Samples	Wild-Type Samples	All Variants
Percent Correct Call[b]	99.6% (905/909)	100% (142/142)	99.9% (58,580/58,856)

a. Across three external testing sites (2 operators/site), one reagent lot, three nonconsecutive days, using six well-characterized DNA sample panels
b. Percent Correct Call is calculated as 100% multiplied by the number of correct calls divided by the number of calls attempted

Summary

The Extended RAS Panel is the first FDA-approved, NGS-based *in vitro* diagnostic for mCRC for use in establishing eligibility of patients for treatment with Vectibix. Including extended coverage of RAS mutations according to published guidelines from ASCP, CAP, AMP, and ASCO,[6] the panel targets 12 codons in two different genes (*KRAS* and *NRAS*) for the simultanous detection of 56 mutations contraindicated for Vectibix therapy. The assay is part of an integrated workflow that includes deep targeted sequencing and automated data analysis for production of a clinical report. With ≥ 99.6% reproducibility and high clinical accuracy, the Extended RAS Panel enables appropriate selection of candidates for Vectibix treatment, which may lead to improved outcomes in mCRC patients.



Praxis™ Extended RAS Panel Report

Export

Application ExtendedRASPanelWorkflow v1.1.0.0

Instrument M01924
Run Date 8/9/2016
Run Time 9:43 PM
Run State Pass
Operator svc_dxruser

Reagent Lot MS2609087-600V3
Reagent Expiration Date 12/11/2016

N/A **Not Applicable**
See package insert for assay details.

Sample Results

SAMPLE NAME	SAMPLE	RESULT	GENE	EXON	AMINO ACID	NUCLEOTIDE
control	Valid	Panel Mutation Detected	KRAS	2	p.Gly12Asp	c.35G>A
control	Valid	Panel Mutation Detected	NRAS	3	p.Gln61Lys	c.181C>A
blank	Invalid	N/A	N/A	N/A	N/A	N/A
sample-5	Valid	Panel Mutation Detected	KRAS	2	p.Gly12Arg	c.34G>C
sample-5	Valid	Panel Mutation Detected	NRAS	3	p.Gln61Glu	c.181C>G
sample-5	Valid	Panel Mutation Detected	NRAS	4	p.Ala146Val	c.437C>T
sample-5	Valid	Panel Mutation Detected	NRAS	2	p.Gly12Asp	c.35G>A
sample-5	Valid	Panel Mutation Detected	NRAS	2	p.Gly13Glu	c.38_39GT>AA
sample-9	Valid	Panel Mutation Detected	KRAS	2	p.Gly12Val	c.35G>T

Figure 3: Easy-to-Interpret Clinical Report—The on-instrument software analyzes the sequence data and assesses each sample independently. A clinical report is automatically generated, providing a simplified view of sample validity and mutation presence/absence. If one or more mutations are detected, location and resultant amino acid changes are provided.

Intended Use

The Praxis Extended RAS Panel is a qualitative *in vitro* diagnostic test using targeted high throughput parallel sequencing for the detection of 56 specific mutations in RAS genes [KRAS (exons 2, 3, and 4) and NRAS (exons 2, 3, and 4)] in DNA extracted from formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) tissue samples. The Praxis Extended RAS Panel is indicated to aid in the identification of patients with colorectal cancer for treatment with Vectibix (panitumumab) based on a no mutation detected result. The test is intended to be used on the Illumina MiSeqDx instrument.

Ordering Information

Product	Catalog No.
Extended RAS Panel Configured for 2 runs with up to 10 samples plus 2 controls per run, or 20 samples per kit	20012431

Learn More

To learn more about the Extended RAS Assay, visit www.illumina.com/ExtendedRASPanel

References

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