HEVA Pro: A Next-Generation Sequencing Panel for Hereditary and Somatic Cancer Analysis

Introduction

HEVA Pro is an advanced targeted capture panel designed for the comprehensive analysis of hereditary and somatic cancer predisposition genes. Leveraging the power of next-generation sequencing (NGS), this panel enables the precise and efficient detection of both germline and somatic mutations across a wide spectrum of clinically relevant cancer-related genes. By offering high sensitivity and specificity, HEVA Pro provides valuable insights that support precision oncology, aiding in early diagnosis, Individualized patient management, and long-term genetic risk assessment. The panel's carefully curated gene selection is based on recommendations from established and widely recognized guidelines, including those provided by **the National Comprehensive Cancer Network (NCCN)**, ensuring its clinical relevance and reliability in diverse oncology settings. This makes HEVA Pro an essential tool for clinicians and researchers aiming to improve patient outcomes through targeted genetic analysis.

Gene Classification in HEVA Pro

The genes included in the HEVA Pro panel are categorized based on their association with specific hereditary cancer syndromes and pathways involved in tumorigenesis.

1. High-Penetrance Hereditary Cancer Genes

These genes are well-established for their role in hereditary cancer syndromes such as: *Breast and Ovarian Cancer Syndrome, Lynch Syndrome, Li-Fraumeni Syndrome, Familial Adenomatous Polyposis (FAP),Peutz-Jeghers Syndrome, Cowden Syndrome/PTEN Hamartoma Tumor Syndrome, Hereditary Diffuse Gastric Cancer (HDGC), Von Hippel-Lindau Syndrome, Hereditary Paraganglioma-Pheochromocytoma Syndrome;*

2. DNA Damage Repair and Genomic Instability Genes

Genes involved in homologous recombination repair (HRR) and mismatch repair;

3. Tumor Suppressor Genes

Genes with established tumor suppressor functions;

4. Oncogenes and Growth Pathway Regulators

Genes associated with tumorigenesis through oncogenic activation;

5. Chromatin Remodeling and Transcription Factors

Genes implicated in chromatin remodeling and transcriptional regulation,

6. DNA Repair and Replication Genes

Genes involved in maintaining genomic stability;

Clinical Utility and Applications

1. **Germline Testing for Hereditary Cancer Risk**
   Germline testing plays a crucial role in identifying individuals at an increased risk for hereditary cancers. By detecting pathogenic variants in well-established cancer predisposition genes, HEVA Pro enables informed genetic counseling and personalized risk management strategies. This approach allows for the stratification of individuals who may benefit from enhanced screening protocols, following NCCN guidelines. Additionally, the identification of hereditary mutations supports the implementation of prophylactic and preventive measures, such as risk-reducing surgeries and chemoprevention, ultimately improving patient outcomes and minimizing cancer incidence.
   Therefore, the main purposes of germline testing include:
   - **Identification of pathogenic variants for genetic counseling and risk management.**
   - **Stratification of individuals for enhanced screening protocols per NCCN guidelines.**
   - **Informing prophylactic and preventive strategies such as risk-reducing surgeries and chemoprevention.**
2. **Somatic Testing for Targeted Therapy Selection**

   In the context of somatic mutation analysis, HEVA Pro provides vital insights for guiding precision oncology. By identifying actionable mutations in oncogenes and tumor suppressor genes, the panel helps clinicians tailor targeted therapies to individual patient profiles.

For example, the detection of BRCA mutations can inform the use of PARP inhibitors, while EGFR mutations may indicate the suitability of tyrosine kinase inhibitors. Furthermore, ongoing somatic testing is essential for monitoring tumor evolution and identifying acquired resistance mechanisms, ensuring that treatment strategies remain effective and adaptable over time.

Importance of FFPE and Germline Sample Analysis with HEVA Pro An essential advantage of the HEVA Pro panel is its ability to efficiently analyze both germline and formalin-fixed, paraffin-embedded (FFPE) samples using its advanced targeted capture technology. FFPE samples, widely used in clinical pathology, often present challenges for molecular analysis due to DNA fragmentation and chemical modifications introduced during tissue processing. The HEVA Pro panel overcomes these obstacles with robust performance on degraded DNA, ensuring **high sensitivity and specificity** even with compromised sample quality. By accommodating both FFPE and germline DNA, HEVA Pro enables a more versatile and comprehensive approach to genetic testing, providing invaluable data for both hereditary cancer risk assessment and somatic mutation profiling.

The clinical benefits of this capability are far-reaching:

- In germline testing, the ability to detect pathogenic variants with high accuracy allows for more precise genetic counseling and risk management.
- For somatic testing, FFPE-derived DNA analysis is critical in identifying actionable mutations from biopsy samples, guiding targeted therapy decisions, and monitoring treatment response.

This dual-sample compatibility reduces the need for multiple testing platforms, streamlining workflows and minimizing turnaround time in clinical settings.

Therefore, the main purposes of somatic testing include:

- Identification of actionable mutations in oncogenes and tumor suppressors.
- Guiding precision oncology approaches (e.g., BRCA mutations for PARP inhibitors, EGFR mutations for tyrosine kinase inhibitors).
- Monitoring of tumor evolution and acquired resistance mechanisms.

Compliance with NCCN Guidelines

The genes included in HEVA Pro align with NCCN guidelines for hereditary cancer syndromes and targeted therapy recommendations. NCCN endorses the use of multi-gene panels for assessing cancer predisposition, particularly for individuals with a significant family history or personal cancer diagnosis. Specific references include:

- NCCN Guidelines for Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic (BRCA1/2, PALB2, ATM, CHEK2, etc.)
- NCCN Guidelines for Colorectal Cancer Screening (Lynch Syndrome Genes: MLH1, MSH2, MSH6, PMS2, EPCAM)
- NCCN Guidelines for Central Nervous System Cancers (SMARCB1, NF1, NF2)
- NCCN Guidelines for Soft Tissue and Bone Sarcomas (RB1, TP53, CDKN2A, etc.)

Analytical Validation - NA12878

Background

HEVA Pro utilizes NA12878 as an analytical validation sample to assess the technical performance of the panel. NA12878 is a widely used reference sample with a comprehensive, annotated genomic profile, allowing for precise validation of sequencing techniques and bioinformatics workflows.

Objectives

- Validate the analytical sensitivity and accuracy of HEVA Pro.
- Ensure high performance for accurate mutation detection, especially for low-frequency variants.

Methodologies

- Sample Selection: NA12878 was chosen due to its well-characterized and well-documented mutation profile.
- Sequencing: HEVA Pro was used to sequence this sample, followed by comparison with reference data to evaluate the detection capability.
- Performance Metrics: Metrics such as sensitivity, specificity, and coverage (Fig. 1) were used to assess the panel's analytical performance.

Conclusions

HEVA Pro's performance was verified against the NA12878 sample, demonstrating robust analytical sensitivity and accuracy for mutation detection, establishing it as a reliable tool for both clinical and research applications.

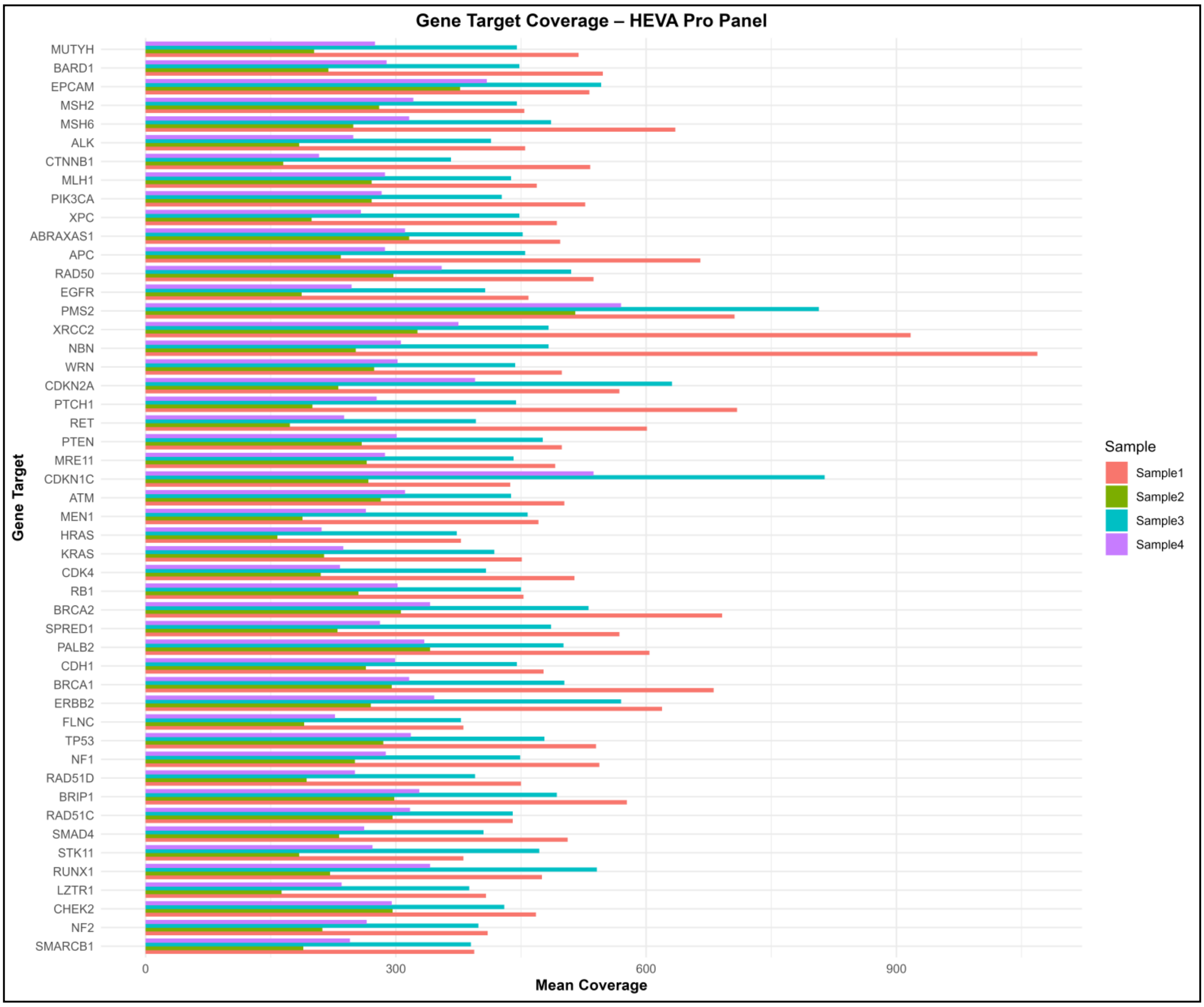

Figure 1. Gene Target Coverage Plot.

Average sequencing depth for each gene target across all samples. X-axis: Mean coverage (read depth) per gene target, computed from aligned sequencing reads. Y-axis: Gene targets.

Use Case 1: Hereditary Cancer Syndromes - Horizon and Coriell

Background

HEVA Pro is intended to identify genetic mutations in high-penetrance genes associated with hereditary cancer syndromes. To verify its effectiveness, we used Horizon reference standards (HD793, HD794, HD795) and Coriell samples (NA14094).

The analysis targets genes linked to common hereditary cancers, including BRCA1, BRCA2, MLH1, and APC.

Objectives

- Evaluate the ability of HEVA Pro to detect mutations associated with hereditary cancer syndromes.
- Calculate the panel's performance on both high-penetrance cancer genes and genes involved in DNA repair and mismatch repair (e.g., MLH1, MSH2).

Methodologies

- Sample Selection: Horizon samples (HD793, HD794, HD795) with known pathogenic variants, alongside Coriell samples like NA14094.
- Sequencing: The DNA samples were analyzed using next-generation sequencing (NGS), and the results were compared to known mutation databases.
- Analysis: Variants were called and compared to known mutations for confirmation.

Mutations to Identify

- BRCA1/2: Mutations leading to breast and ovarian cancer susceptibility.
- MLH1, MSH2, MSH6: Genes related to Lynch syndrome.
- APC: Mutations leading to Familial Adenomatous Polyposis (FAP).
- TP53: Linked to Li-Fraumeni syndrome.

Conclusions

The panel demonstrated high sensitivity in detecting pathogenic variants in BRCA1, BRCA2, MLH1, MSH2, and APC. This confirms HEVA Pro as an effective tool for identifying individuals at risk for hereditary cancers, supporting personalized care strategies.

Use Case 2: Somatic Mutations - Horizon and Coriell

Background

HEVA Pro also plays a key role in detecting somatic mutations that are pivotal for personalized oncology treatment. Horizon reference standards (HD793, HD794, HD795) and Coriell samples (NA14094, NA16659, NA11410, NA11254, NA21849) were selected to verify somatic mutation detection in oncogenes and tumor suppressor genes.

Objectives

- Assess HEVA Pro's ability to detect somatic mutations in key oncogenes such as KRAS, EGFR, PIK3CA, and TP53.
- Calculate the panel's performance for identifying actionable mutations that could guide precision treatment strategies, particularly for targeted therapies.

Methodologies

- Sample Selection: Tumor samples from Horizon (HD793, HD794) and Coriell (NA14094, NA16659, NA11410, NA11254, NA21849) were analyzed.
- Sequencing: Next-generation sequencing was employed to identify somatic mutations, and bioinformatics tools were used to compare these variants with clinical and research databases.
- Detection: Mutations were confirmed against established mutation panels and databases.

Mutations to Identify

- KRAS: Common somatic mutations involved in colorectal and pancreatic cancers.
- EGFR: Mutations important for the treatment of non-small cell lung cancer.
- PIK3CA: Mutations associated with breast cancer and other solid tumors.
- TP53: Frequent somatic mutations found in a variety of cancers.

Conclusions

The results demonstrated HEVA Pro's ability to accurately identify key somatic mutations in KRAS, EGFR, PIK3CA, and TP53. This study highlights the panel's potential to guide treatment decisions, monitor tumor evolution, and identify resistance mutations in clinical oncology.

Use Case 3: Multi-Gene Analysis for Tumor Profiling and Resistance Monitoring

Background

Somatic mutations are not only important for primary cancer diagnosis but also crucial for monitoring therapy resistance and tumor evolution. The NA27953 Coriell sample, alongside Horizon standards, were used to demonstrate HEVA Pro's ability to profile multiple genes in cancerous tissues for better decision-making.

Objectives

- Monitor tumor evolution and identify acquired mutations related to treatment resistance.
- Profile multiple genes related to oncogenic signaling pathways and resistance mechanisms, ensuring accurate and timely clinical decisions.

Methodologies

- Sample Selection: Coriell tumor samples like NA27953, NA14094were analyzed.
- Sequencing: Advanced sequencing techniques were used to detect mutations in both oncogenes and tumor suppressor genes.
- Analysis: Mutations were cataloged for actionable therapy options and potential resistance mechanisms, such as in EGFR, KRAS, and PIK3CA.

Mutations to Identify

- EGFR: Resistance mutations in lung cancer therapies.
- KRAS: Mutations indicating resistance to EGFR-targeted therapies.
- PIK3CA: Mutations related to targeted therapies in breast cancer.
- TP53: Mutations leading to tumor progression and resistance.

Conclusions

The use of HEVA Pro for multi-gene profiling confirmed its capability to monitor tumor evolution and detect mutations linked to resistance, providing an effective tool for personalized oncology treatment strategies.

Use Case 4: Detection of Copy Number Variations (CNVs) - Coriell Samples NA02718, NA14626, NA18949

Background

Copy Number Variations (CNVs) are large-scale genetic alterations where regions of the genome are duplicated or deleted. CNVs can be associated with various genetic disorders, cancer predisposition, and tumorigenesis. HEVA Pro is capable of detecting CNVs in addition to single-nucleotide mutations, making it a comprehensive tool for both inherited and somatic genetic analysis. This use case focuses on the detection of CNVs in NA02718, NA14626, and NA18949 from the Coriell repository.

Objectives

1. Verify HEVA Pro's ability to detect CNVs in addition to single nucleotide polymorphisms (SNPs) and small insertions/deletions (indels).
2. Assess the panel's performance in identifying clinically relevant CNVs associated with cancer and genetic disorders.

Methodologies

- Sample Selection: Coriell samples NA02718, NA14626, and NA18949, which are known to harbor CNVs, were selected for analysis. These samples are characterized by specific CNVs in regions associated with cancer susceptibility and other genetic disorders.
- Sequencing: DNA from these samples was processed using the HEVA Pro panel, and the CNV detection capability was assessed alongside standard mutation analysis.
- Bioinformatics and CNV Calling: Data from the sequencing run was processed using bioinformatics tools that allow for CNV detection based on read depth and comparison to reference genomes. CNVs were further validated using specialized algorithms for accurate detection and interpretation.
- Detection: The CNV results were compared against reference data and additional validation techniques

Conclusions

HEVA Pro demonstrated robust capability in detecting CNVs in the NA02718, NA14626, and NA18949 Coriell samples, offering accurate detection of clinically significant CNVs. The panel's ability to detect both mutations and CNVs in a single workflow enhances its utility in clinical settings, allowing for comprehensive genetic profiling. CNV detection by HEVA Pro was compared against traditional CNV detection methods, showing its potential for both diagnostic and prognostic applications in hereditary cancer risk assessment and personalized therapy planning.

Conclusion

The **HEVA Pro panel** represents a powerful tool for genetic analysis in both hereditary and somatic oncology. Its comprehensive design allows for simultaneous assessment of multiple cancer predisposition and tumor progression genes, improving patient management and personalized treatment strategies. With alignment to NCCN guidelines and a focus on precision medicine, HEVA Pro is a valuable asset for clinicians, geneticists, and researchers in the field of oncology.