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Harbinger Health's Technical Optimization and Assessment of xGen™ Hybridization and Wash v3 Kit Support Application for High-Throughput Targeted Methyl Sequencing

Harbinger Health

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BACKGROUND

Hybrid capture (HC)-based enrichment of bisulfite-treated cell-free DNA (cfDNA) is an established approach for targeted analysis of methylation signal. The Harbinger Health HHx assay applies this approach with next-generation sequencing and machine learning (ML), using proprietary cancer-associated methylation markers, for early cancer detection.

Integrated DNA Technologies (IDT) recently released the xGen Hybridization & Wash v3 Kit (V3 kit), designed to streamline the enrichment process. This study evaluates the xGen™ Hybridization and Wash v3 (V3) kit, optimizing its workflow against the established v2 (V2) kit within the HHx workflow.

OBJECTIVES

- Assess and optimize the xGen V3 kit for HC workflows compared to V2.
- Evaluate reductions in processing time and handling complexity.
- Compare sequencing metrics (on-target rates, coverage, uniformity) and cancer detection performance between kit versions.

METHODS & MATERIALS

- Parameters were evaluated to adapt the V3 kit for methylation applications, including hybridization duration and wash steps.
- cfDNA extracts were bisulfite-converted, converted to sequencing libraries, and independently enriched via HC using both V2 and V3 kits. Libraries from 476 cfDNA samples (45 cancer, 166 non-cancer, 265 blinded) were processed.
- All samples underwent enrichment with a 48kb panel; a subset of 141 samples was also enriched with a 156kb panel using both kits.
- Comparative analysis included on-target rate, median target coverage, coverage uniformity (90/10 ratio), and tumor-content correlation (Pearson r).
- Binary ML classifiers for cancer detection were trained and cross-validated on 179 (42 cancer and 147 non cancer) paired V2/V3 samples (identical bisulfite-converted libraries split into parallel hybrid capture workflows) to provide unbiased estimates of sensitivity and specificity across panel-chemistry combinations.

RESULTS

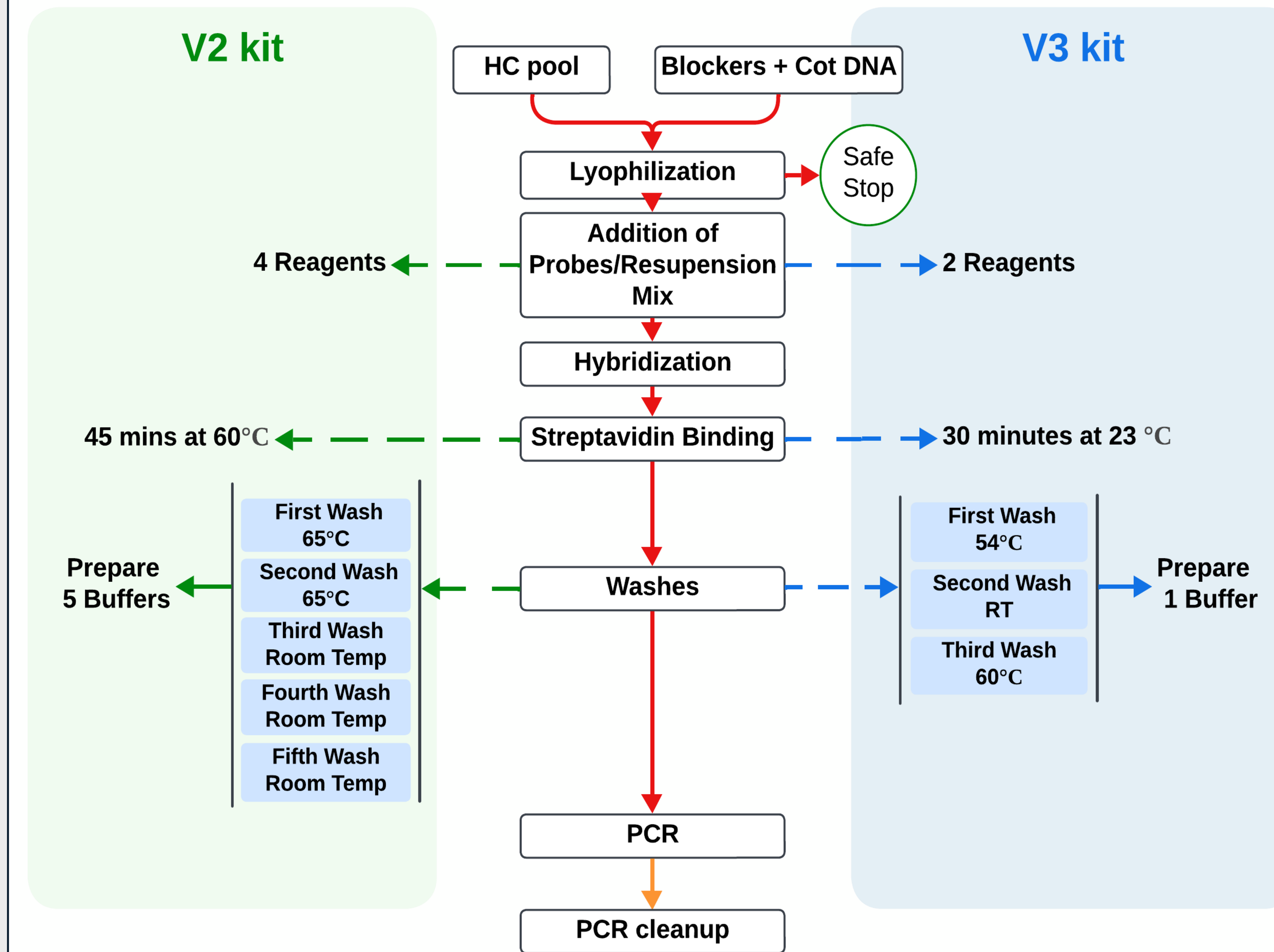


Figure 1. Workflow Comparison of V2 vs V3 Kits. Flowchart showing key steps (hybridization time and washes) for V2 (left) and V3 (right) kits used for cfDNA methylation processing. The V3 kit included fewer reagents (from 4 to 2), reduced steps, and >1 hour time savings based on optimizations for 476 samples and the xGen V3 protocol.[1]

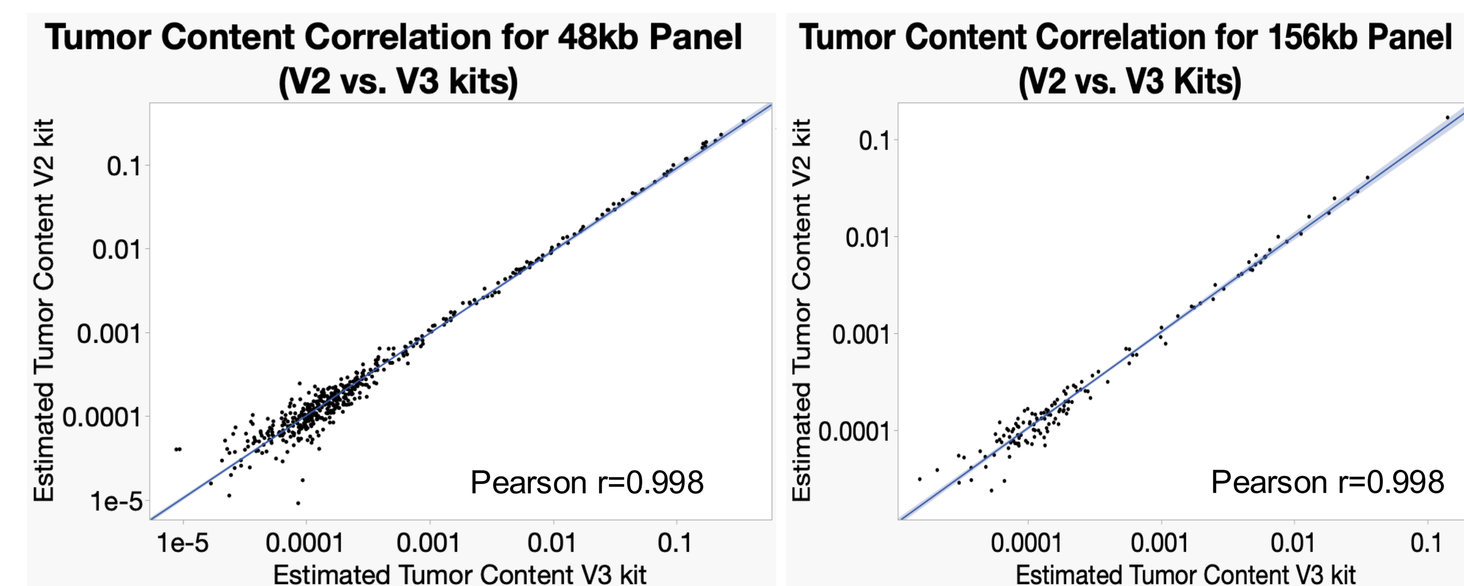


Figure 2. Tumor Content Correlation. Data show high correlation (Pearson r = 0.998) across both methylation panels, confirming that the updated chemistry preserves biological signal fidelity and quantitative performance.

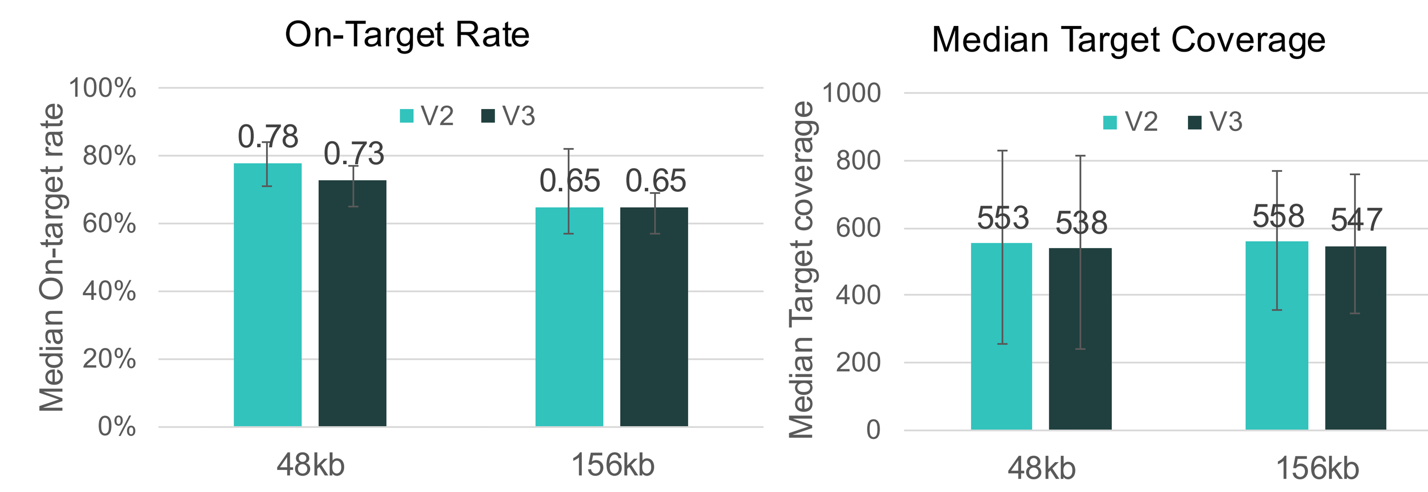
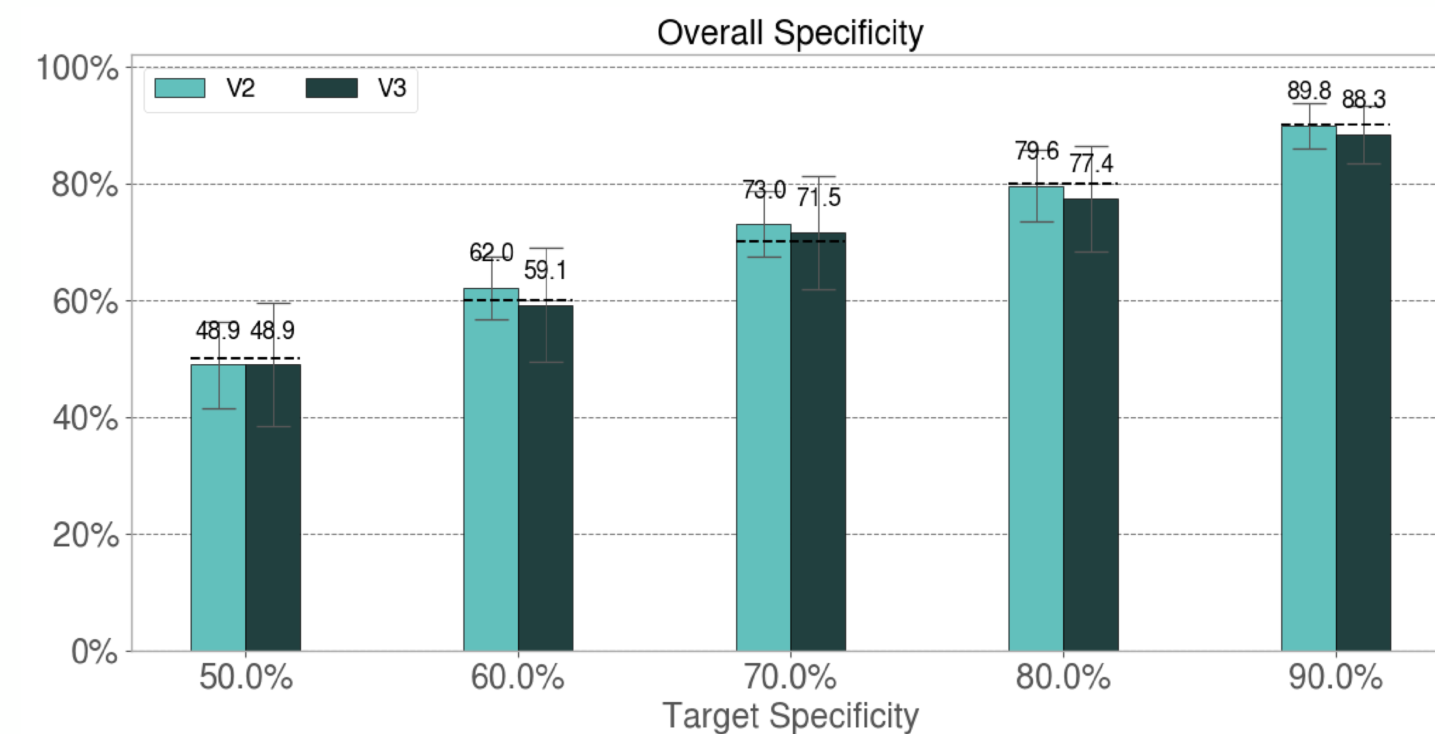


Figure 3. On-Target Rate and Median Target Coverage. The V3 chemistry achieved comparable enrichment efficiency and sequencing depth to V2.



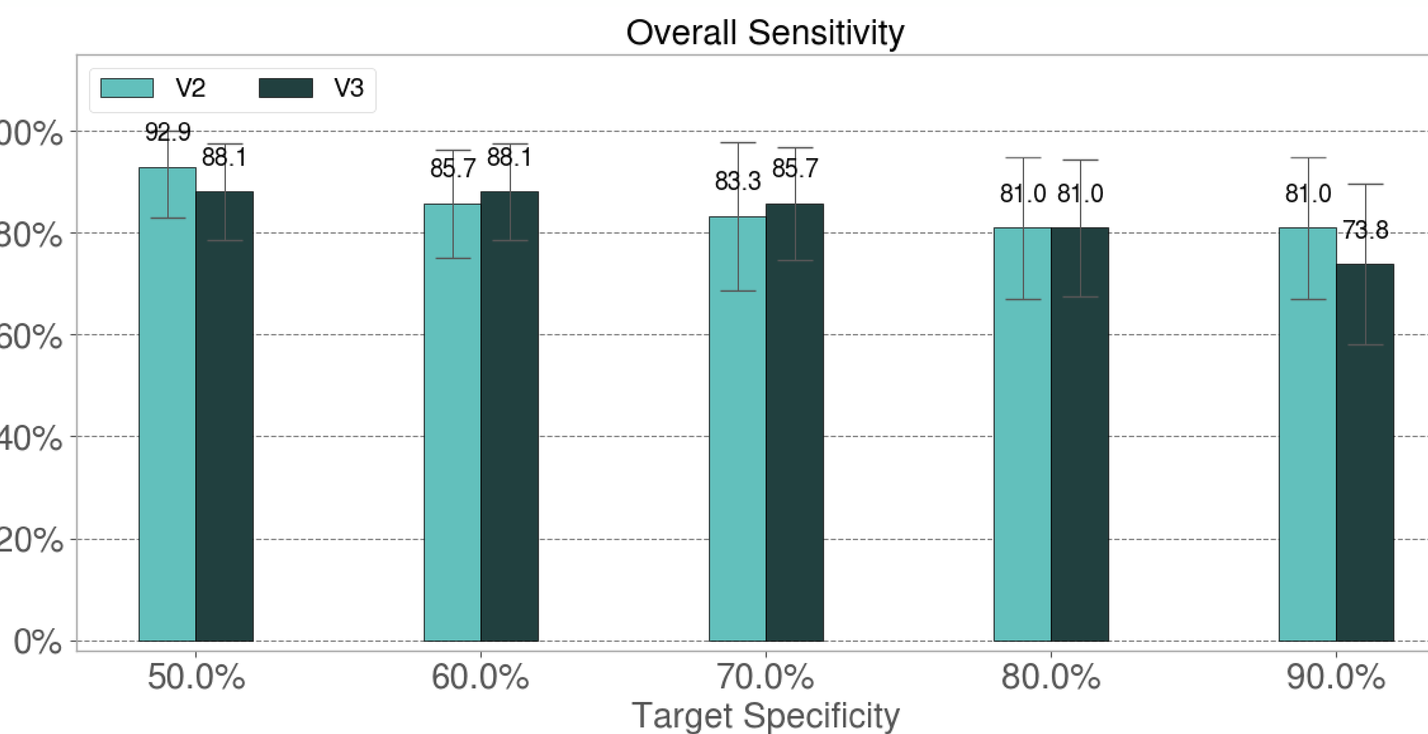


Figure 4. Machine-Learning Classifier Performance on 48 kb Panel. Bar chart showing sensitivity and specificity of classifiers trained and cross-validated on 179 paired V2/V3 samples (42 cancer, 137 non-cancer) within each kit version. Performance was consistent across kits: V3 achieved 81.0% sensitivity at 77.4% specificity (target: 80%), closely matching V2 (81.0% sensitivity at 79.6% specificity). Similar performance was observed for the 156kb panel. Minor kit-specific bias was observed only when models were cross-applied between versions.

DISCUSSION

Workflow Efficiency

- The optimization of hybridization time, wash steps, and HC plexity with the V3 kit reduced processing time by over 1 hour and simplified handling complexity compared to the V2 kit, markedly improving workflow efficiency for high-throughput applications.

Assay Performance

- The V3 kit exhibited comparable performance to V2, with median on-target rates of 73% (V3) vs. 78% (V2) for the 48kb panel and 65% for both kits on the 156kb panel.

Uniformity & Data Quality

- Tumor content correlation surpassed 99% (Pearson r=0.998 for both panels), and median target coverage was robust at 547× (V3) and 558× (V2) for the 156kb panel, and 538× (V3) and 553× (V2) for the 48kb panel.
- Coverage uniformity remained consistent, with a 90/10 ratio of 2.2-2.4 across all comparisons, ensuring high sequencing quality. Despite differences in panel size and biomarker composition, these key sequencing metrics were comparable, indicating V3's flexibility for future assay designs.

Cancer detection performance

- ML classifiers trained on 179 paired cfDNA samples further validated V3's efficacy. While minor kit-specific bias was observed when cross-applying models between kits, performance within each kit was stable, suggesting that subtle chemistry differences (likely due to differences in overall kit chemistry, including wash buffers, binding times, and temperatures), do not affect downstream predictive accuracy.

Clinical & Operational Implications

- This parity in performance, alongside workflow enhancements, positions V3 as a suitable alternative to V2.
- Future efforts could leverage expanded training datasets to minimize cross-kit bias, enhancing model generalizability and broadening V3's clinical utility in early cancer detection.

CONCLUSIONS

Evaluation of xGen V3 for cfDNA methylation shows parity with the V2 kit in enrichment, sequencing quality, and tumor-signal detection within established and optimized HHx workflow. While expanded training data would enhance clinical model development, findings support V3 kit integration with potential to improve scalability and efficiency in high-throughput methylation-based early cancer detection applications.

REFERENCES

1. xGen Hyb and Wash Reagents v3 Kit protocol.

DISCLOSURES

This study was sponsored by Harbinger Health. Cambridge, MA.

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