ST-19

Evolution of a Comprehensive Genomic Profiling (CGP) Kit to Simplify Workflows and Detect Homologous Recombination Deficiency (HRD)

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Summary

The AVENIO Tumor Tissue CGP Kit V2 (Research Use Only. Not for use in diagnostic procedures.) was optimized to include fast workflows, higher throughput, and robust bioinformatics algorithms. Homologous Recombination Deficiency (HRDsig) Positive Percent Agreement (PPA) was assessed, as well as Microsatellite Instability (MSI), Tumor Mutation Burden (TMB), genomic Loss of Heterozygosity (gLOH), and 4 alteration classes (SNVs, Indels, Rearrangements, and Copy Number Alterations (CNA)). High agreement to FoundationOne® CDx (F1CDx®) was achieved, demonstrating high performance of an easy to use CGP assay.

Introduction

- Comprehensive Genomic Profiling (CGP) is the use of NGS to detect alterations and important biomarkers in cancer related genes.
- The AVENIO Tumor Tissue CGP Kit V2 (a distributed Research Use Only assay) sequences 335 oncology related genes – with content aligned with the FDA- approved FoundationOne® CDx (F1CDx) (Foundation Medicine, Inc., Cambridge, MA, USA).
- Roche Diagnostics and Foundation Medicine have updated to a V2 kit, with faster wet-lab procedures, improved extraction efficiency, optimized bioinformatics, cheaper sequencing, and expanded gene content. A new capability is added **to detect HRD, through the new HRDsig score.**

Methods

- Extraction optimization: 16 FFPE samples were extracted with 1 or 3 hour incubations. The extracted DNA was evaluated for concentration and quality, using an included qPCR.
- Ligation study: A shortened procedure was tested and key sequencing metrics were analyzed against the original condition, to reduce the library preparation by 1 day.
- Higher throughput study: 12 samples were pooled, sequenced and compared to 8 samples.
- Variant sensitivity: 317 FFPE DNA samples were sequenced with the AVENIO CGP Kit V2. Variant detection was compared with the reference method, F1CDx. The samples included a diverse range of DNA qualities, assessed by qPCR. Sequencing was performed on Illumina NextSeq sequencers with >60M reads per sample. Data Analysis was performed using the FoundationOne® Analysis Platform.
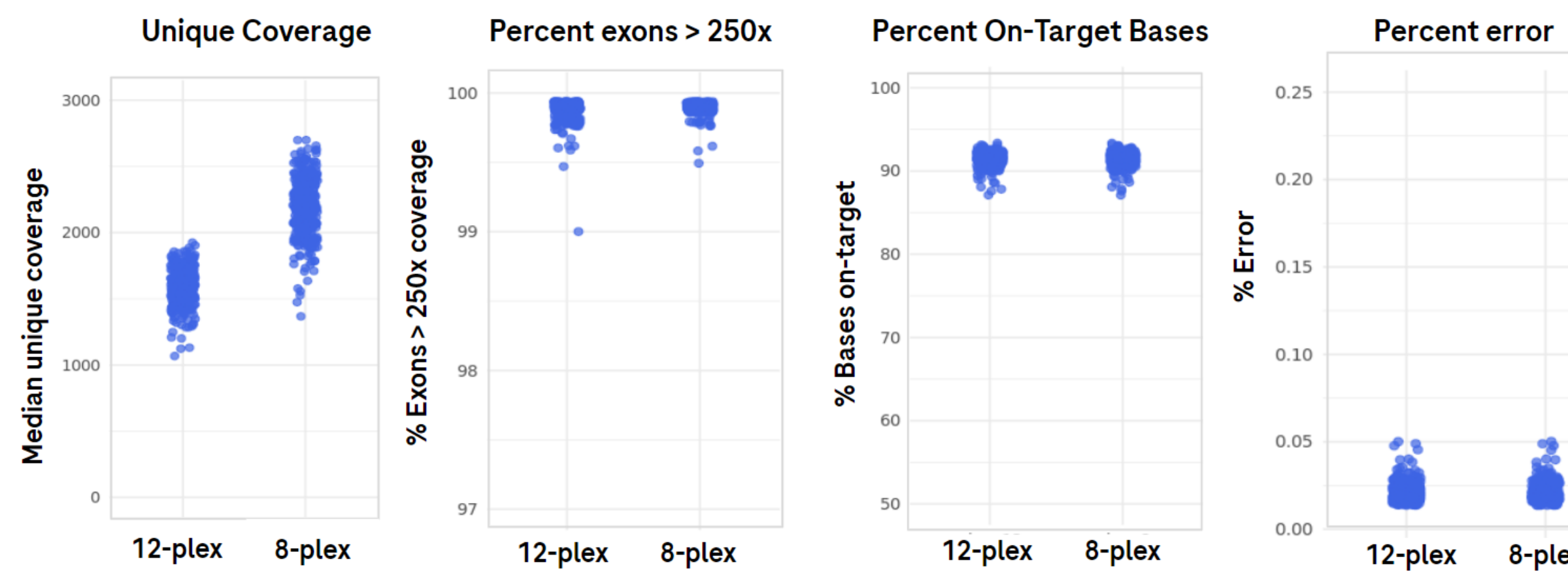
Results

Higher extraction yield

- The optimized 3 hr extraction condition increased both yield and quality of the extracted DNA. Challenging, degraded FFPE tissues showed improvement of DNA acceptance (80.3% from 20.9%).
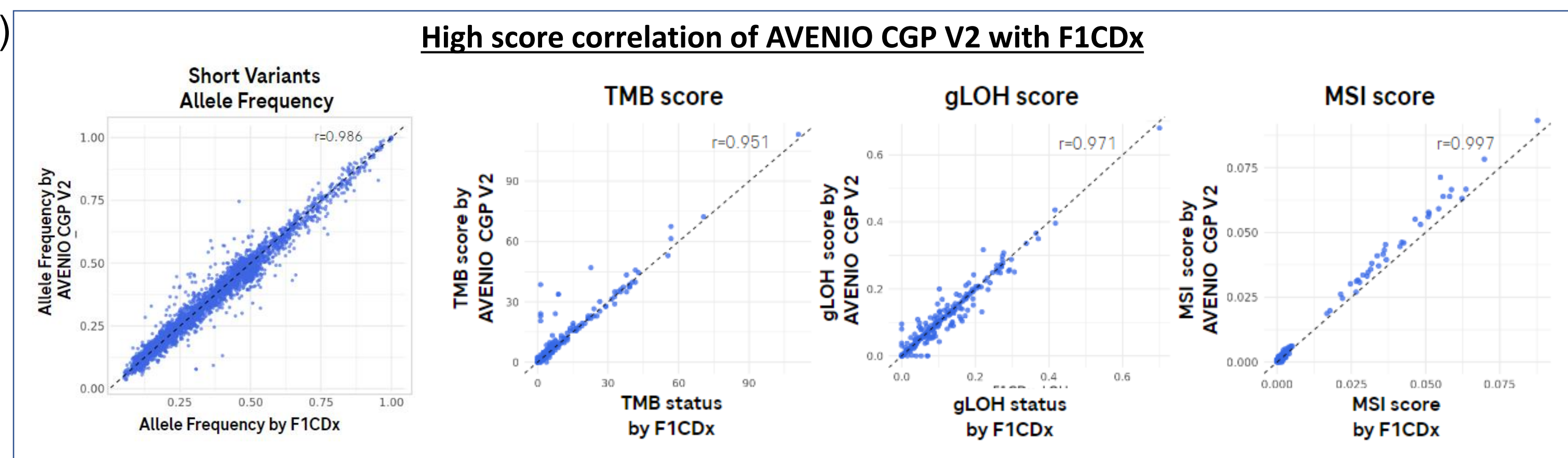- Improved conditions can be used on challenging samples (old, poor quality, limited tissues).

Extraction yield / Extracted DNA quality (qPCR)
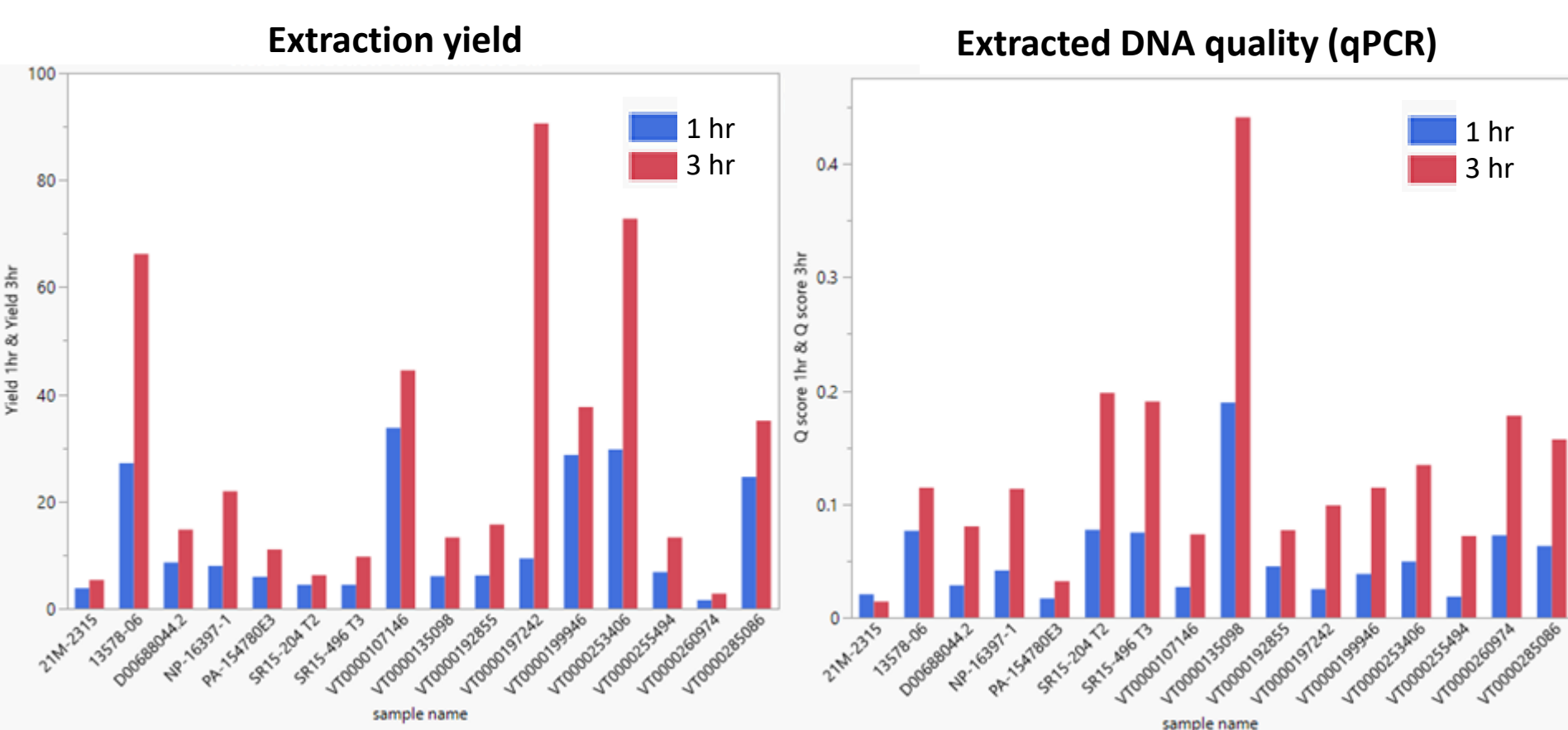
Higher throughput and sequencing performance

Unique Coverage / Percent exons > 250x / Percent On-Target Bases / Percent error

- 99.68% pass rate was achieved with AVENIO CGP V2 (316/317 FFPE DNA). High sequencing coverage was achieved with all samples, >1500x with 8-plex sequencing.
- Increased multiplexing from 8 samples to 12 on a Illumina NextSeq 550 System was observed to achieve >60M reads, maintaining SNV PPA of 99.5% and reducing sequencing costs by 33%.
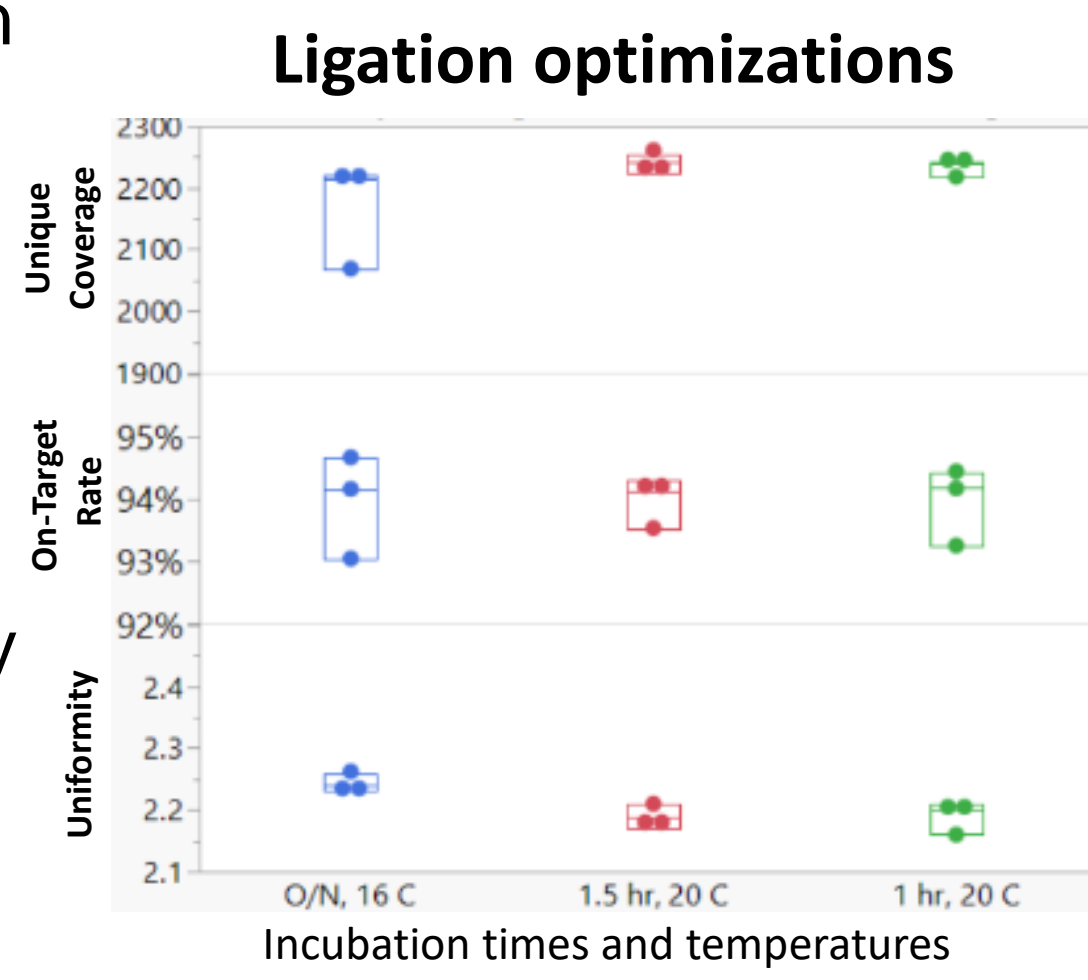
High Variant and signature detection alignment with F1CDx

- Short variant (SNVs and Indels) allele frequency was highly concordant to F1CDx values
- Signature scores were highly concordant to F1CDx scores*.
- High PPA for AVENIO CGP V2 to F1CDx was achieved with 8-plex and 12-plex sequencing of 316 FFPET samples

High score correlation of AVENIO CGP V2 with F1CDx — Short Variants Allele Frequency (r=0.986), TMB score (r=0.951), gLOH score (r=0.971), MSI score (r=0.997)

Classification	Percent Positive Agreement (PPA) to F1CDx		
	AVENIO CGP V1	AVENIO CGP V2	
	8 - plex	8 - plex	12-plex
Short Variants	98.2%	99.7%	99.7%
Rearrangements	90.5%	90.8%	89.9%
CNA	94.8%	95.6%	99.8%
MSI high	100%	100%	100%
TMB high	100%	100%	100%
LOH high	96.8%	97.1%	100%
HRD positive	N/A	91.7%	91.7%

High Positive Agreement of AVENIO CGP V2 with F1CDx — TMB agreement, gLOH agreement, MSI agreement, HRDsig agreement

* For PPA analysis purposes, AVENIO CGP V2 signature cut-offs were: TMB High ≥12 mutations/MB, gLOH high ≥0.18, MSI high ≥ 0.0124, HRD positive ≥ 0.7 HRDsig score
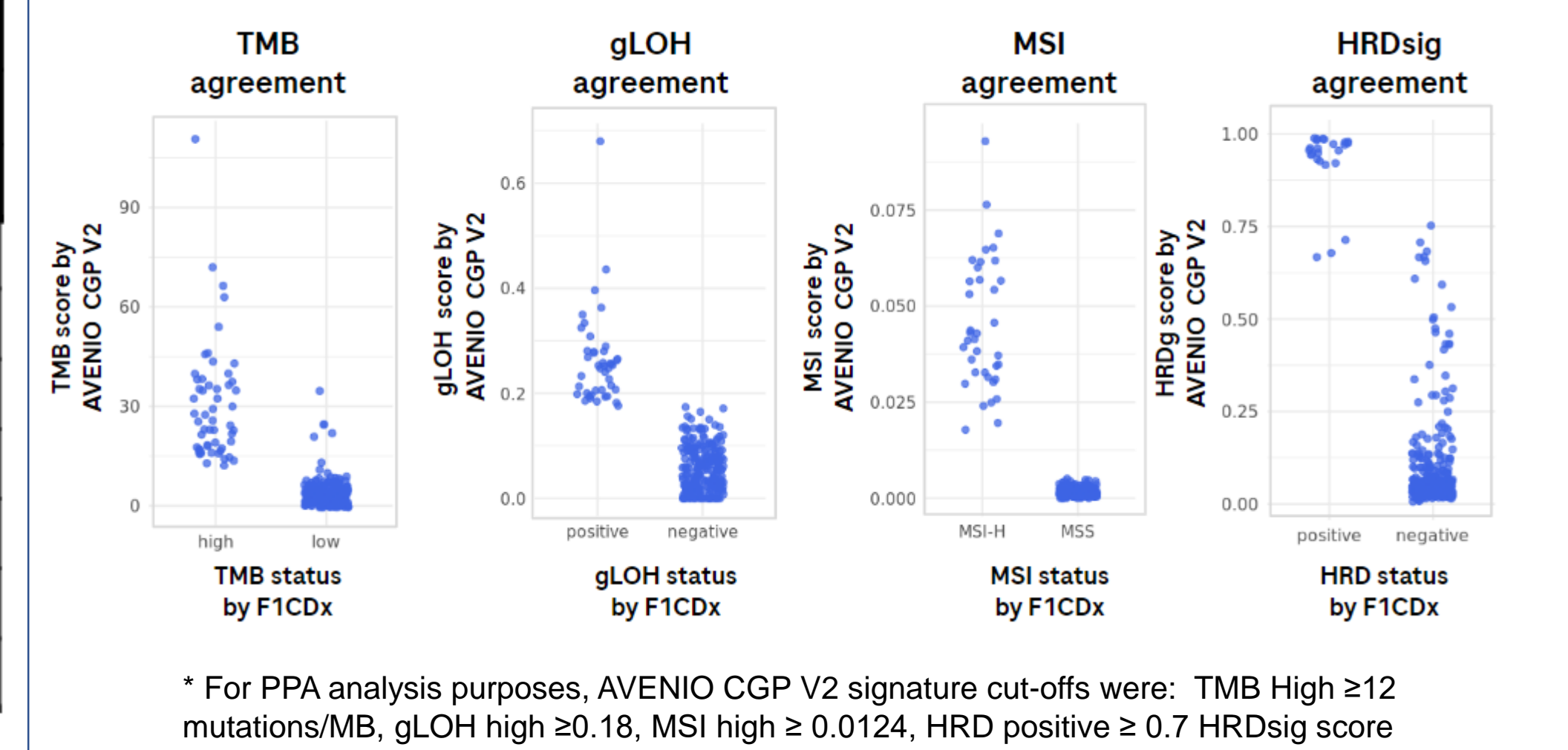
Shortened and flexible workflow

- 1 hour 20°C and overnight 16°C ligation condition had comparable sequencing results.
- The AVENIO CGP V2 2-day shortened library preparation performed equivalently with the 3 day workflow, achieving >85% on-target rate, and 1800-2500x coverage.
- Both 3 day and 2 day library prep options are available allowing schedule flexibility

Ligation optimizations — Incubation times and temperatures (O/N, 16 C; 1.5 hr, 20 C; 1 hr, 20 C)

3 day library prep: Day 1 Extract (1 hr) — Day 2/3 Library prep/Target Enrich. (2 overnight steps) — Day 4 Sequencing — Day 5 Analysis

2 day library prep: Extract (Option: 1 or 3 hrs) — Library prep/Target Enrich. (1 overnight step) — Sequencing — Analysis

- Important oncology variants: 100% PPA of AVENIO CGP V2 to F1CDx

Disease Ontology	Genes	Mutations	No. Samples in F1CDx	No. Samples in AVENIO CGP V2	Measured Variant Allele Frequency, Copy Number or Breakpoint Reads
non-small cell lung carcinoma	EGFR	T790M	6	6	8.4% - 51.8%
non-small cell lung carcinoma	EGFR	L858R	11	11	8.6% - 34.0%
non-small cell lung carcinoma	EGFR	Exon 19 deletion	9	9	15.0% - 61.7%
non-small cell lung carcinoma	EGFR	G719A	1	1	29.8%
non-small cell lung carcinoma	MET	Exon 14 splice mutation	2	2	21.9% - 83.3%
non-small cell lung carcinoma	BRAF	V600E	7	7	7.6% - 17.6%
colon adenocarcinoma (crc)	BRAF	V600E	9	9	8.1% - 51.5%
melanoma	BRAF	V600E/V600K	11	11	8.7% - 65.7%
colon adenocarcinoma (crc)	KRAS	Codon 12 mutation	9	9	13.0% - 47.4%
colon adenocarcinoma (crc)	KRAS	Codon 13 mutation	4	4	35.1% - 60.5%
colon adenocarcinoma (crc)	KRAS	Codon 61 mutation	3	3	29.8% - 34.2%
colon adenocarcinoma (crc)	NRAS	Codon 61 mutation	2	2	16.5% - 43.9%
breast cancer	PIK3CA	C420R/E542K/E545D/Q546K/H1047R/H1047L	14	14	1.3% - 61.9%
breast cancer	ERBB2	ERBB2 amplification	8	8	5 - 147 copies
non-small cell lung carcinoma	ALK-ELM4	ALK-EML4 fusion	5	5	3.2% - 7.6% / 18 - 41 reads

PPA of CGP V2 and V1 kits

- AVENIO CGP V2 adds HRD and workflow updates, while maintaining performance of the original kit (V1).

Classification	AVENIO CGP V2 (12-plex) vs V1
Short Variants	98.5%
Rearrangements	94.4%
CNA	96.9%
MSI high	100%
TMB high	100%
gLOH high	100%

Conclusions

- Comprehensive Genomic Profiling (CGP) through NGS requires high performing, reliable assays in translational research labs.
- The new AVENIO Tumor Tissue CGP Kit V2 demonstrates high performance (when compared to F1CDx and original CGP kit, V1), while incorporating faster workflows, higher throughput, improved bioinformatics algorithms, and successful incorporation of a new HRD signature.

Abbreviations:
CGP – Comprehensive Genomic Profiling, F1CDx – FoundationOne CDx, CNA – Copy Number Alternation, RE – Rearrangements, MSI – Microsatellite Instability, TMB – Tumor Mutation Burden, gLOH – genomic Loss of Heterozygosity, HRD – Homologous Recombination Deficiency, HRDsig – HRD signature, SNV – Short Nucleotide Variations, and Indels – Insertions and deletions

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Presented at AMP EU 2024