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Variant call concordance between two laboratory-developed, solid tumor targeted genomic profiling assays using distinct workflows and sequencing instruments

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ABSTRACT

Targeted genomic profiling (TGP) using massively parallel DNA sequencing is becoming the standard methodology in clinical laboratories for detecting somatic variants in solid tumors. The variety of methodologies and sequencing platforms in the marketplace for TGP has resulted in a variety of clinical TGP laboratory developed tests (LDT). The variability of LDTs is a challenge for test-to-test and laboratory-to-laboratory reliability. At the University of Vermont Medical Center (UVMMC), we validated a TGP assay for solid tumors which utilizes DNA hybridization capture and complete exon and selected intron sequencing of 29 clinically actionable genes. The validation samples were run on the Illumina MiSeq platform. Clinical specificity and sensitivity were evaluated by testing samples harboring genomic variants previously identified in CLIA-approved, CAP accredited laboratories with clinically validated molecular assays. The Molecular Laboratory at Dartmouth Hitchcock Medical Center (DHMC) provided 11 FFPE specimens that had been analyzed on AmpliSeq Cancer Hotspot Panel version 2 (CHPv2) and run on the Ion Torrent PGM. A Venn diagram of the gene lists from the two institutions is shown. This provided an excellent opportunity to compare the inter-laboratory reliability using two different target sequencing methods and sequencing platforms. Our data demonstrated an exceptionally high level of concordance with respect to the sensitivity and specificity of the analyses. All clinically-actionable SNV and InDel variant calls in genes covered by both panels ($n = 17$) were identified by both laboratories. This data supports the proposal that distinct gene panel designs and sequencing workflows are capable of making consistent variant calls in solid tumor FFPE-derived samples.

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1. Introduction

The advent of massively parallel sequencing methods has made it possible to interrogate large genomic regions for clinically actionable variants (Goodwin et al., 2016; Pfeifer, 2013; Rehm et al., 2013; Wong et al., 2011). This increase in sequencing power at a lower cost and the ability to capture and sequence only clinically actionable regions of a genome have led to many clinical laboratories implementing in-house sequencing practices (Cheng et al., 2015; Cottrell et al., 2014; Pritchard et al., 2014; Singh et al., 2013; Tsongalis et al., 2014). Guidelines for validation and production of these tests have been published by a number of regulatory and professional organizations (Aziz et al., 2015; Gargis et al., 2012; Hagemann et al., 2013; Rehm et al., 2013; State, 2015; Strom, 2016). Understandably the guidelines emphasize documenting and reporting the limits of variant call specificity and sensitivity. Lacking, however, is nuanced guidance regarding different TGP methodologies and workflows. This is important given that different methodologies have distinct limitations. It benefits clinical laboratory professionals to understand the limitations of different methods before investing valuable resources for test development and validation. Analysis of variant calling comparisons between divergent workflows and sequencing instruments is, therefore, of great value.

The focus of this work is the comparison of variant calls made using two different solid tumor sequencing library preparation workflows and sequencing platforms. DHMC uses the AmpliSeq™ Cancer Hotspot Panel V2 (CHPv2) and sequences the libraries on an Ion PGM™ (ThermoFisher). UVMMC uses SureSelect XT target enrichment (Agilent) and sequences the libraries on a MiSeq System (Illumina). The Ion PGM and Illumina MiSeq are arguably the most common massively parallel sequencing instruments in clinical use today and each has well-described advantages and disadvantages (Loman et al., 2012; Quail et al., 2012). These instruments have similar overall operational

Abbreviations: TGP, targeted genomic profiling; LDT, laboratory developed test; FFPE, formalin-fixed paraffin-embedded; SNV, single nucleotide variant; InDel, insertion or deletion; VAF, variant allele fraction.

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costs, but the MiSeq has been shown to have the greater sequencing output per run and lower error rates. Amplicon sequencing has several advantages over target-enrichment sequencing including generally lower genomic DNA input amounts, cost and turnaround time and greater on-target coverage (Chang and Li, 2013; Samorodnitsky et al., 2015). Lowering the DNA input requirements reduces the possibility that a patient will be subjected to additional invasive procedures to acquire tissue and therefore improves quality of care and lowers cost of care. Reduced turn-around times can have obvious impact on patient treatment. Target-enrichment approaches are, however, capable of identifying variants that are outside of amplicon hotspots, and in some cases these variants have clinical value even if they are not actionable with respect to therapeutic response.

Central to the development of any new clinical assay is determining the analytical specificity of the method compared to that of orthogonal methods on identical samples. Here we describe a comparison of massively parallel sequencing results on samples shared between UVMMC and DHMC as part of the GenePanel Solid Tumor assay validation study at UVMMC. Where the sequence design coverage overlapped between the DHMC and UVMMC's TGP assays, all variants calls were made by both workflows. The data herein provide very strong evidence that somatic variant analysis of tumor FFPE tissue can have high analytical specificity and sensitivity across very different target capture and sequencing methodologies.

2. Material and methods

2.1. Clinical samples and DNA extraction

Eleven FFPE samples previously sequenced at DHMC between 2013 and 2015 were shared for TGP assay validation at UVMMC. UVMMC obtained these samples as 5 μm thick unstained recuts from DHMC. Extraction of DNA at UVMMC was carried out without prior micro-dissection of tumor. A flat-edge razor was used to macro-dissect tissue-containing sections from each slide and place them in a 1.6 mL microfuge tube. Samples were incubated in 1 mL of xylene substitute (Shandon) for 20 min at 37 °C and then centrifuged at 20,000*g* for 10 min to pellet tissue. The supernatant was removed and the pellets were washed twice with 1 mL of 100% ethanol and centrifugation at 20,000*g* for 5 min. Final tissue pellets were air dried to remove traces of ethanol and then DNA extraction was carried out with the QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer's instructions. Extracted DNA was quantified by fluorescence spectroscopy using the Qubit dsDNA High Sensitivity Assay Kit (Life Technologies). DNA quality was assessed by the Kapa hgDNA Quality Control and Quantification Kit (Kapa Biosciences). Quantity and quality metrics are presented in Table 1.

Sample processing at DHMC was initiated with pathologist review of one H&E slide per sample that is marked for tumor content and tumor area. Each sample was then submitted to histology for eight unstained slides each composed of a 5 μm thick section. Each slide was manually macrodissected and deparaffinized using xylene. DNA extraction is performed using Gentra PureGene Kit (Qiagen), and quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen) according to the manufacturer's recommendations. DNA qualities of samples sequenced in 2015 were assessed using the KAPA hgDNA Quantification and QC Kit (KAPA Biosystems). Samples received prior to 2015 were not assessed for DNA quality.

Table 1
Summary of UVMMC DNA extraction yield and qPCR-based quality metrics.

Sample name	Extraction yield, ng	Kapa 129/41 QC ratio	Input amount, ng
DHMC01	36	0.41	33
DHMC02	265	0.64	243
DHMC03	527	0.39	483
DHMC04	539	0.12	494
DHMC05	2316	0.51	1000
DHMC06	3636	0.55	1000
DHMC07	1848	0.44	1000
DHMC08	2328	0.56	1000
DHMC09	107	0.55	98
DHMC10	3036	0.44	1000
DHMC11	140	0.49	129

2.2. DNA sequencing library preparation

The GenePanel Solid Tumor assay at UVMMC employs an Agilent Sure Select XT target probe library custom designed to capture exons and selected intronic regions of the following 29 genes, *AKT1*, *ALK*, *BRAF*, *CDKN2A*, *CTNNB1*, *DDR2*, *EGFR*, *ERBB2*, *ESR1*, *FGFR1*, *FGFR2*, *GNA11*, *GNAQ*, *IDH1*, *IDH2*, *KIT*, *KRAS*, *MAP2K1*, *MAP2K2*, *MAPK1*, *MET*, *MTOR*, *NRAS*, *PDGFRA*, *PIK3CA*, *PTEN*, *RET*, *ROS1* and *STK11* (Fig. 1). Target capture for UVMMC sample library preparation was performed according to the manufacturer's protocol for Sure Select XT (Agilent) without deviation. DNA fragmentation was carried out using a Covaris S series sonicator.

Samples were barcoded for sequencing using single index 6 base-pair barcodes and 6 samples in the validation study were multiplexed for each MiSeq v2 150 bp paired-end sequencing V2 flow cell (Illumina). Multiplexed denatured libraries were applied to the flow cell at a concentration of 12.5 nM. Pooled and denatured libraries were quantified prior to loading the flow-cell using Kapa Library Quantification Kit for Illumina® Platforms (Kapa Biosystems).

At DHMC, the AmpliSeq™ Cancer Hotspot Panel V2 (CHPv2) was designed to amplify 207 amplicons mapped in hotspot regions of 50 oncogenes and tumor suppressor genes. Library preparation was performed using at least 10 ng of DNA. Samples were barcoded, quantified, normalized, pooled, and sequenced on an Ion 318 or 316 Chip using the Ion PGM System (de Abreu et al., 2016; Tsongalis et al., 2014).

2.3. Data analysis and reporting

UVMMC data analysis was carried out on the Clinical Genomicist Workstation (Pierian Diagnostics) (Sharma et al., 2013). All analysis was based on the human reference sequence UCSC build hg19 (NCBI build 37.2). Read alignment was performed using Novoalign (version 3.02.00). Samtools (version 0.1.19) was used to provide input for the SNV caller Varscan2 (version 2.3.6). Genome Analysis Toolkit (version 1.2) and Pindel (version 0.2.4d) were the variant callers used to identify insertions, deletions, and complex InDels.

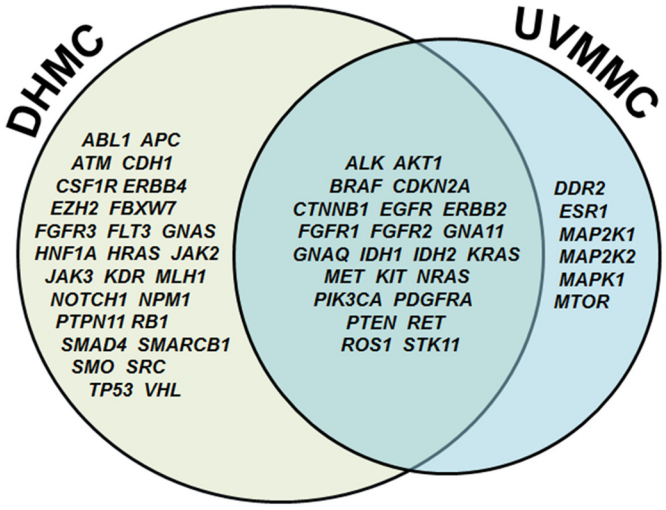

Fig. 1. Venn diagram for the solid tumor assay gene lists from DHMC and UVMMC.

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