Quality metrics for enhanced performance of an NGS panel using single-vial amplification technology

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ABSTRACT

Aims Targeted next-generation sequencing (NGS) panels, which identify genomic alterations, are the stronghold of molecular oncology laboratories. In spite of technological advances, the quantity and quality of DNA from formalin-fixed paraffin-embedded tissue and paucicellular specimens are barriers to successful sequencing. Here, we describe an NGS assay employing single tube stem-loop inhibition mediated amplification technology that delivers highly accurate results with low input DNA. Rigorous quality metrics, regular monitoring and in-depth validation make the test attractive for clinical laboratories.

Methods The study used a customised NGS panel, targeting 48 genes across several solid tumour types. Validation, in accordance with guidelines from New York State, sequenced patient samples harbouring 136 known variants, including single-nucleotide variants (SNVs) and indels. Specimen types included formalin-fixed paraffin embedded blocks, core biopsies and cytology material. Neoplastic cellularity of the tumours ranged from 10% to 80%.

Results The assay was highly specific and sensitive with excellent accuracy, reproducibility and repeatability/precision. Concordant results for identification of SNVs and indels were obtained from specimens with DNA input of 2–3 ng, tissue with 10% neoplastic cellularity and variant allelic frequencies of 2.5%–3%. Over 99% of the target areas are shown to achieve at least 500X coverage when parsed through two bioinformatics pipelines. With over 2000 clinical specimens analysed, the success of the panel for reporting of results is 95.3%

Conclusions The advanced technology enables accurate identification of clinically relevant variants with uniformity of coverage and an impressive turn-around-time. The overall workflow and cost-effectiveness provide added value.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Detection of clinically relevant variants in solid tumours, using next-generation sequencing (NGS) panels, is used routinely in several laboratories. However, obtaining reliable results from low concentration DNA acquired from formalin fixed tissue, continues to pose a challenge in targeted NGS.

WHAT THIS STUDY ADDS

⇒ This study presents data on a targeted NGS panel that used a novel technology for detection of actionable and informative variants in specimens with 10% neoplastic cellularity and DNA input of 2–5 ng. Furthermore, the assay is able to successfully interrogate most paucicellular samples from core biopsies and cytology specimens.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The single tube workflow, favourable cost advantage and turn-around-time provide advantages for implementation of the assay in clinical laboratories. Automation will increase the adoption of NGS panels to smaller clinical laboratories.

INTRODUCTION

A challenging aspect in implementation of next-generation sequencing (NGS) with tumour tissue is compromised quality and limited DNA from formalin-fixed paraffin embedded (FFPE) samples.[1] While amplicon-based NGS technologies have unique challenges with sequencing of complex genomicalterations, the potential for accurate detection of hot-spot variants in samples with low DNA concentration is dependent on the technology.[2]

Modifications to amplicon-based NGS technologies have addressed limitations, including the minimisation of sequencing artefacts with low input DNA.[3–6] Here, we present the systematic validation of a solid tumour panel with NGS-centric stem-loop inhibition mediated amplification technology (SLIMamp Pillar Biosciencs, Massachusetts, USA).[6] This custom solid tumour panel (CSTP) interrogates 48 genes that harbour actionable variants relevant to oncological diagnosis, prognosis and therapy.

Best practices for validation of NGS assays have been discussed, and addressed by the joint recommendations of the Association for Molecular Pathology and College of American Pathologists.[7] For laboratories that operate within or test clinical samples originating in New York state, NGS assays should be validated as per guidelines from the New York State Department of Health (NYSDOH).[8]

This report establishes the performance characteristics of a new and novel NGS technology when challenged with low DNA input and limited neoplastic cellularity. In addition, we parsed the sequencing data through two bioinformatics pipelines and established rigorous quality metrics for accurate interpretation and reporting of clinically impactful variants. The 2-year performance data of the assay for detection of variants in tumour tissue attests to the enhanced utility of the actionable panel.

METHODS

Panel design

The custom panel includes tumour-relevant alterations including suppressors and oncogenes in 48 genes (*AKT1, ALK, ARAF, BRAF,CDKN2A, CTNNB1, CYSLTR2, DDR2, EGFR, EIF1AX, ERBB2, ERBB4, FBXW7, FGFR1, FGFR2, FGFR3, GNAQ, GNAS, GNA11, H3F3A, HRAS, HIST1H3B, IDH1, IDH2, KEAP1, KIT, KRAS, MAP2K1, MET, NRAS, NTRK1, PDGFRA, PLCB4, PIK3CA, POLD1, POLE, PTEN, PTPN11, RAC1, RAF1, RET, SF3B1, SMAD4, SRSF2, STK11, TERT, TP53* and *TSHR*) that are recurrently mutated in non-small cell lung carcinoma, metastatic and high-risk melanoma, colon cancers, gastrointestinal stromal tumours (GIST), pancreatic neoplasms, urothelial and thyroid tumours and gliomas. Complete coding regions were covered for *KEAP1, PTEN, STK11* and *TP53* genes. The panel comprised 247 amplicons corresponding to 26 745 base pairs (bp).

SAMPLE SELECTION

Controls

The positive control, SeraSeq Tri-Level Tumour Mutation DNA Mix v2 Low Concentration (SeraCare, Connecticut, USA) is a reference standard harbouring 40 variants in 28 genes, including multiple variants in *EGFR*, and *TP53* of which clinically relevant variants in 16 genes for a total of 22 genes/variants are detected by the assay. The variants are engineered to have variant allelic frequencies (VAF) of 4%, 7% or 10%. In addition, NA12878 (Coriell Institute of Medical Research, New Jersey USA), a well-characterised reference material, along with tumours with no known variants and normal tonsil were used to establish assay accuracy.

Patient samples

For validation, we identified 108 previously sequenced clinical samples from patients with lung adenocarcinoma (N=29), colorectal and pancreatic cancer (N=38), melanoma (N=12), GIST (N=9), thyroid cancer (N=11) and glioma (N=9). The orthogonal methods for identification of single-nucleotide variants (SNVs) and indels included Sanger sequencing, real-time PCR or validated NGS panels. Specimen types included FFPE tissues (N=85), fine needle aspiration (FNA) (N=19), cell smears (N=2) and cytolyt solution (N=2). Neoplastic cellularity of the tumours, estimated by a molecular pathologist, ranged from 10% to 80%. All specimens had at least 10% of tumour tissue in the area analysed. DNA recovered from the samples varied from 1.5 ng to 300 ng/uL and the DNA input for the assay was between 2.5ng and 20 ng per reaction.

For precision and reproducibility, patient samples that harboured different types of variants with known VAF (ranging from 15% to 50%) were identified. The samples were then combined to generate pools of 3 or 4 different variants. When pooled, the corresponding VAF ranged from 5% to 20%. The final pool had DNA concentrations that ranged between 2.5 and 5 ng/µL. The purpose of pooling was to enable the assessment for 3–4 different variants simultaneously.

Postvalidation, the assay was used routinely by our oncologists, for identification of actionable variants in cancer patients with tumour types indicated above, as well as for identification of relevant variants in patients with cholangiocarcinoma and urothelial tumours.

DNA extraction

The 5–8 FFPE tissue sections (5 µm thick) were obtained and the accompanying H&E was reviewed by a molecular pathologist to ensure tumour-cell content of at least 10%. DNA was extracted using the Qiagen FFPE kit and Qiacube protocol (QIAGEN, Hilden, Germany)[9] and quantified using the Qubit fluorometric system (Thermo Fisher Scientific, Massachusetts, USA).

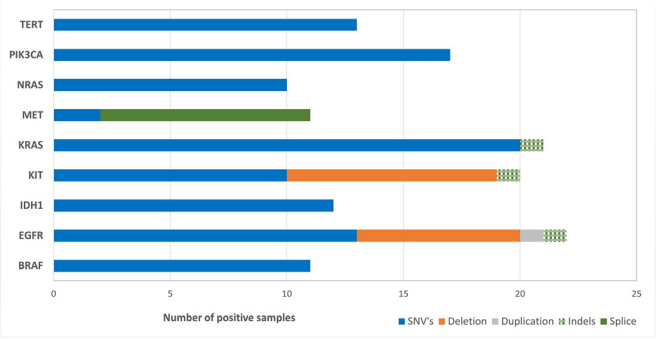

Figure 1 Distribution of 136 variant types identified in the validation cohort, including single nucleotide variants, splice/intronic, insertions, deletions and duplications. SNVs, single-nucleotide variants.

Library preparation and NGS

Total input DNA (range 2.5–20 ng) extracted from patient samples was used for library preparation as per manufacturer's recommendations (ONCO/Reveal Solid Tumour Panel, V.1.0, Pillar Biosciences)[10] and quantified on the Bioanalyzer system (Agilent, California, USA). Samples were normalised, pooled and run by using the MiSeq V.2 or MiSeq V.3 Reagent Kit with 2×150 paired end reads following the manufacturer's instructions (Illumina, California, USA). Data analysis including sequence alignment, variant calling and annotation was performed by using validated New York State approved software, NextGENe (SoftGenetics, Pennsylvania, USA)[9] and compared with PiVAT V.2.0.1 (Pillar Variant Analysis Toolkit, Pillar Biosciences, Massachusetts, USA).[11]

Data analysis

The absolute minimum average coverage of the assay at all target areas for passing the run was 500X. For interpretation and reporting of clinically relevant variants, the coverage at the hotspots was expected to be >1000X. Correspondingly, the mutant/alternate allele for variants at the limit of detection (LOD) (2.5%–3%) was seen in a minimum of 25 variant reads. Variants showed approximately the same read-ratio (ie, forward and reverse reads) and met the acceptable raw base call quality score thresholds for the assay. Variants were visualised using NextGENE or the Integrative Genomics Viewer and manually curated and filtered using COSMIC (http://cancer.sanger.ac.uk/cosmic), Varsome (https://varsome.com/) and ClinVar (http://

Table 1 Accuracy studies show that the specificity and sensitivity of the assay for the detection of variants is 100%

	Gene variant detected in CSTP asay N=136	Gene variant not detected in CSTP assay N=10
Gene variant detected by orthogonal method	136	0
Wild-type (no known variants)	0	10

CSTP, custom solid tumour panel.

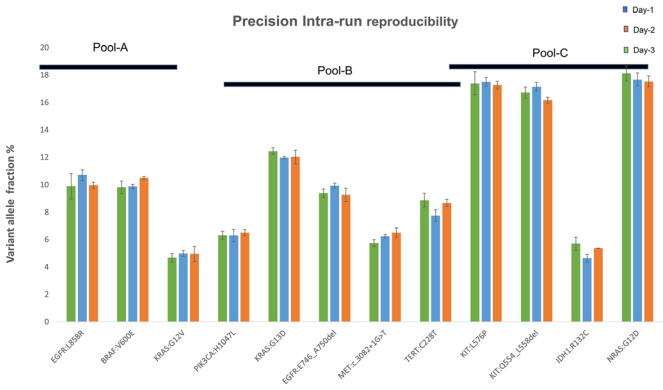

Figure 2 Intrarun reproducibility of the NGS panel. The SD of the VAF is seen. Patient specimens used for accuracy were combined to yield allele frequencies at or near the stated LOD of the assay (5%–15%). Each mixture of combined specimens contained three to five different variants—including SNV's and indels. The validation for precision was performed with three different mixtures labelled pool A; pool B and pool C using total DNA input of 2.5–5 ng, and three replicates on three different days. LOD, limit of detection; NGS, next-generation sequencing; VAF, variant allelic frequency.

www.ncbi.nlm.nih.gov/clinvar). Only non-synonymous variants were reported as either actionable variants (with known therapeutic significance) or clinically relevant variants (with diagnostic and/or prognostic significance).

RESULTS

Analytical validation of the panel

We sequenced 108 archived patient samples, known to harbour clinically relevant variants, along with 10 samples, with no known variants . A well-characterised control (NA12878) was run in triplicate in two different runs. In addition, a reference standard was included with each run. The performance characteristics of the assay were established as per the requirements of NYSDOH Clinical Laboratory Evaluation programme and Clinical and Laboratory Standards Institute.

Accuracy of the assay

The DNA samples used to establish accuracy came from 108 samples with multiple genomic alterations and at least 10% neoplastic cellularity. Following blinded analyses, the assay showed complete concordance for 136 actionable variants identified in 108 samples. The accuracy for actionable mutations, including, SNVs in *BRAF*, *EGFR*, *KIT*, *KRAS*, *IDH1*, *NRAS*, *PIK3CA* and *TERT* genes (N=106); indels in *EGFR* and *KIT* genes (N=20) and splice-site variants in *MET* (N=10), was established using 10 or more samples for each listed gene (figure 1). Variants were concordant in all specimen types. NA12878 was run in triplicate in two different runs. The average VAF (%) and the SD of the seven heterozygous and six homozygous variants harboured in NA12878 (online supplemental figure S1). In addition, eight tumour samples with no known variants and two samples of normal tonsil that were available in-house were run in duplicate in two different runs. Accuracy for the known actionable variants was established by calculating the total positive per cent agreement (PPA)/sensitivity and negative per cent agreement (NPA)/specificity for the different variants (N=136) and controls (N=10). The PPA and NPA were 100% for the assay (table 1).

Intrarun precision, inter-run reproducibility of the assay

Precision was examined for SNVs, splice-site variants, promoter variants and indels. Pool A (*EGFR*, *BRAF*, *KRAS*); Pool B (*PIK3CA*, *KRAS*, *EGFR*, *MET*, *TERT*) and Pool C (*IDH1*, *KIT*, *NRAS*), reflecting final VAFs of 5%–20% were formulated. Precision was determined with three libraries each, of pool A, B and C run in triplicate, while the reproducibility was confirmed by sequencing each pool A, B and C individually, on three different days (figure 2, table 2).

Limit of detection of the assay

DNA extracted from samples with known variants was serially diluted with DNA that did not harbour known variants, and the LOD was evaluated at VAF ranging from 20% down to 2.5%. Graphical representation of the LOD is seen in figure 3A–F. The LOD achieved by the assay for detection SNVs, indels and splice site variants in the *MET* gene was 2.5% at a minimum coverage of 1000X, Q30 >70 and on-target rate of 97%. The assay displayed the same LOD even when the DNA input was reduced to 2.5 ng (online supplemental figure S2).

Establishment of quality control metrics

Ongoing monitoring of assay performance

The performance of the assay was monitored with a Seraseq reference standard. The box and whisker plot (figure 4) shows the values obtained for the standard, monitored across 11 runs. The mean VAF±3 SD was used to set the acceptable range for the standard. The VAF for all 22 variants in the reference standard are expected to fall within the established range. These quality metrics are used to monitor the performance of every clinical run.

Coverage and sequencing quality metrics

We assessed coverage statistics for targeted amplicon when batches of 24 or 36 samples, with 15pM total library input, were sequenced on a V2 flowcell on the Miseq. Table 3 compares the quality control (QC) metrics for batches sequenced at the

Table 2 Reproducibility of the assay, shows the average VAF and SD between runs

	EGFR L858R	BRAF V600E	KRAS G12V	IDH1 R132C	NRAS G12D	EGFR E746_A750del	KIT Q554_L558del	MET c.3082+1G>T	TERT C228T
VAF(%) day 1	9.9	9.8	4.7	5.7	18.1	9.4	16.7	5.7	8.9
VAF(%) day 2	10.7	13.1	6.7	4.6	17.7	9.9	17.1	6.2	7.7
VAF(%) day 3	10.0	10.5	5.0	5.4	17.5	9.3	16.2	6.5	8.7
Average	10.2	11.1	5.4	5.2	17.8	9.5	16.7	6.2	8.4
SD	0.45	1.74	1.08	0.55	0.31	0.35	0.49	0.39	0.60

VAF, variant allelic frequency.

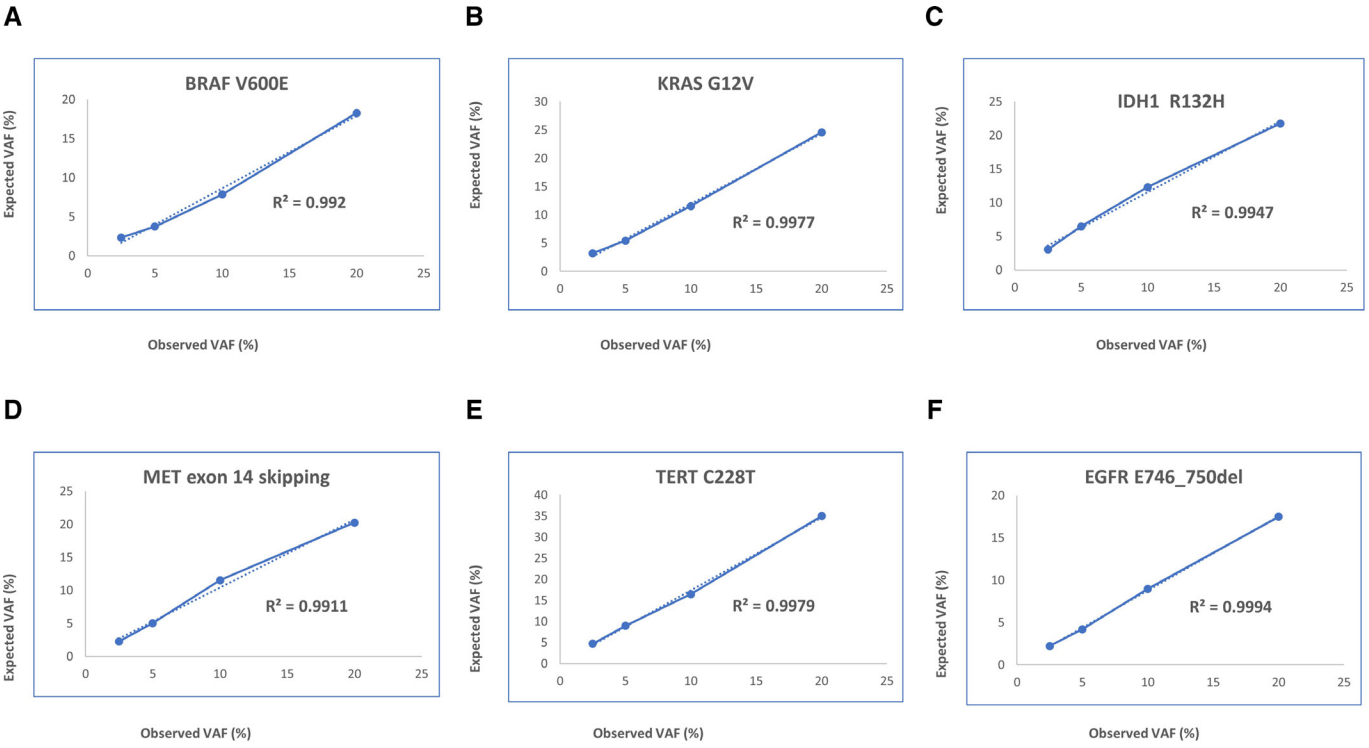

Figure 3 Performance characteristic of this custom solid tumour panel using known variants from patient samples and diluted with normal tonsil (WT). (A–F) demonstrates linearity and limit of VAF detection (2.5%–20%). VAF, variant allelic frequency; WT, wild type.

time of validation. Data analysis shows that 86% of specimens achieved 2000X, 96% achieved 1000X and over 99.5% of the target areas achieved at least 500X coverage (figure 5, online supplemental figure S3). Regions less than 2000X coverage were often intronic or DNA sequences with interspersed repeats that did not house known clinically relevant variants. The absolute minimum coverage at all target regions, to pass the run was set at 500X and the required coverage for reporting of variants near the LOD was set at 1000X. Sequencing metrics established for qualification of the run, included a minimum Q30 score for of >70 with an On-target rate of 97% and mapping score of 95% (box 1).

Comparison of bioinformatics approaches (NextGENe and PiVAT)

To investigate the robustness of technology, we analysed the data using variant output from two software systems NextGENe and

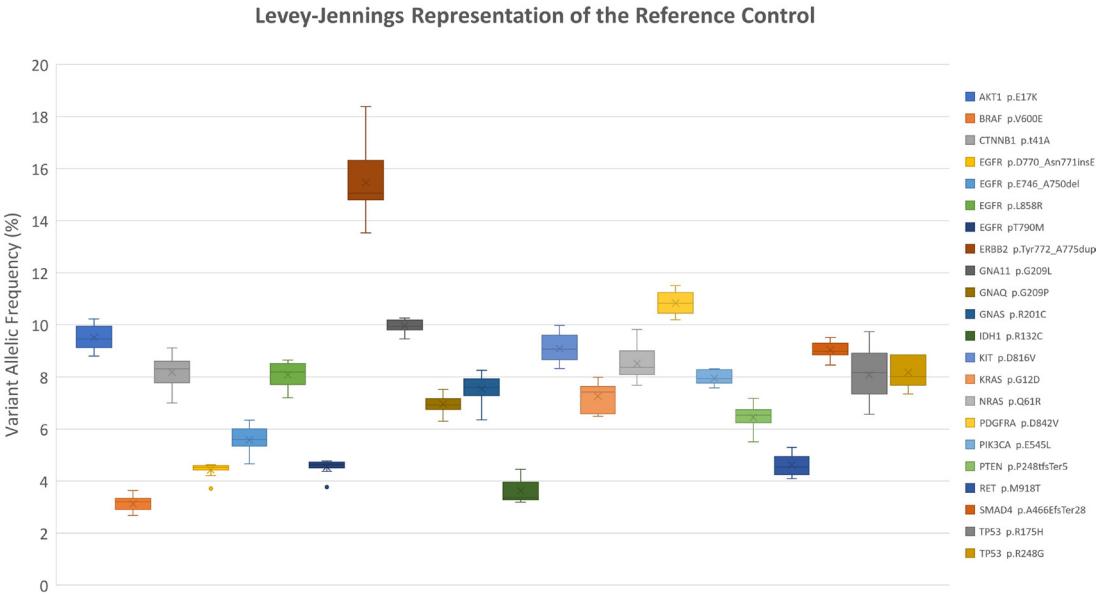

Figure 4 Monitoring the performance of the NGS assay using the positive control: The VAF% for each variant in the positive control was monitored as a QC metric. The Levey Jennings plot shows the average VAF for each variant for the 11 runs. The SD is seen. The range+3 SD are represented as (+3 SD) and (−3 SD), respectively. NGS, next-generation sequencing; QC, quality control; VAF, variant allelic frequency.

Table 3 QC metrics for representative batches of specimens sequenced at the time of validation

	Run-1	Run-2	Run-3	Run-4	Run-5	Run-6	Run-7	Run-8	Mean	SD
Total reads	2.90E+06	2.90E+06	1.77E+06	2.73E+06	2.31E+06	2.14E+06	2.20E+06	2.36E+06	2.48E+06	4.28E+05
Overall:Q=30	95.42	95.53	95.64	94.93	94.99	89.17	94.65	94.78	94.55	1.86
Mapping rate (%)	99.60	99.52	99.34	96.99	90.14	98.63	99.68	98.54	98.22	2.79
On-target rate (%)	99.26	99.39	99.47	99.25	98.76	99.08	99.44	99.14	99.21	0.21

QC, quality control.

PiVAT. While NextGENe was previously validated for use in clinical targeted amplicon based NGS panel, PiVAT is designed for accurate variant calling at low allelic frequencies. PiVAT also contains noise-weighted filtering that suppresses potential sequencing artefacts and errors. After demultiplexing and using the ≥2% LOD cut-off for VAF, we detected 136 variants in the 108 of patient samples with VAF % ranges from 2.5% to above 93% with both pipelines. The variants included 128 SNV's and 28 indels. There were five variants (four SNV's and one indel) where the VAF between the two pipelines showed a>5% variance (range 5.5% to 13.1%). PiVAT variant detection accuracy and LOD with SNVs that are part of homopolymers, small insertions and deletions, splice and promoter variants strongly correlated with the NextGENe variant output (figure 6).

Investigation of challenging genomic sequences

High degree of sequence similarity, existence of pseudogenes and other duplicated regions in the genome pose challenges in sequence analysis. In this panel, we found coverage of less than 2000X in segmental regions of *PTEN* and *STK11*. The graphical representation of the coverage for amplicons covering regions of *PTEN* are shown in figure 7A. *PTEN* has low coverage in regions encompassing intron 7 and exon 8 (chr10:89720581–89720829). Further inspection shows that poor mapping is likely due to the pseudogene *PTENP1* located on chromosome 9p13.3 (figure 7B). Similarly, this assay also had low coverage (<1500x) in the genomic region encompassing intron 4, exon 4 and 5 (chr19:1220484–1220602) of *STK11*. This was attributed to high GC content. Our investigations show that while the most common pathogenic *PTEN* (p.Q298*) and *STK11* (p.D194) reside in these regions, the overall coverage at these genomic coordinates has never been less than 1000X and therefore meets the QC metric of coverage.

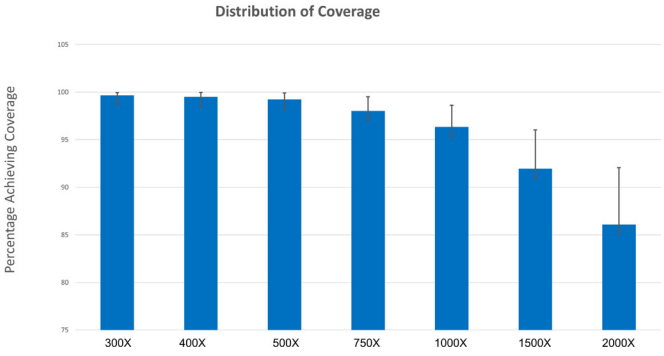

Figure 5 Distribution of percentage of amplicons at the target areas that achieved coverage between 300X and 2000X. The SD± is represented.

Clinical impact of the CSTP assay for patient management

For over 2 years, this panel has been routinely used for interrogation of actionable variants in patients with lung, colorectal, pancreatic, gall bladder, urothelial, brain, thyroid, skin, uveal and GIST. The panel is also run on some sarcomas, when requested by the oncologist, tumours when information on the primary is not available and carcinoma of unknown primary. These are collectively referred to as CSTP in figure 8. In select cases, requests for identification of mutations in single genes like *TERT* and *IDH* are also assayed. A total of 2032 tumours underwent analysis between 1 January 2020 and 31 December 2021, and more than 95% (1931/2032) of these were sequenced successfully. The primary reason for failure of 101 specimens were tissue with 10% or less neoplastic cellularity (N=47; 2.3%); Failure to amplify due to low input DNA (N=29; 1.42%). Interpretation and reports included information and comments on the variants relevant to the tumour. The pie chart in figure 8A shows the breakdown of the 1931 cases representing various tumour types that were successfully sequenced. Notably, 35% of the tumours were lung cancers and 29.8% were colorectal/pancreatic cancer cases. Clinically relevant variants were identified in 73% of the lung tumours, and 31% of these cases were actionable alterations with**Food and Drug Administration** (FDA)-approved therapeutic potential. The mutational landscape of the lung tumours sequenced is seen in figure 8B.

DISCUSSION

Current PCR-based target enrichment technologies for accurate detection of low-level variants require multiple primers and separate reactions to amplify overlapping amplicons that lead to complex workflows and increased turnaround time. Furthermore, large amplicon pools often generate non-specific products,

Box 1 Summary of quality control metrics for sequencing and interpretation of results set for the CSTP assay.

⇒ The minimum AQ30 score for all specimens in the target area is >70.
⇒ An 'on-target' rate of 97% and a mapping rate of 95% are required to pass the sequencing run.
⇒ The minimum coverage (total number of reads) for the assay at all target areas is 500X.
⇒ The lower limit of detection (LOD) of the variant allelic frequency is 2.5% when the coverage is at least 1000X.
⇒ The minimum number of variant reads for detection of a variant is 25.
⇒ The maximum ratio of variant to normal reads below which a sample will be called normal or not detected is approximately 0.025 (2.5% VAF)*

*This is supported by the LOD data where all variants with VAF of 2.5% were detected.

CSTP, custom solid tumour panel; VAF, variant allelic frequency.

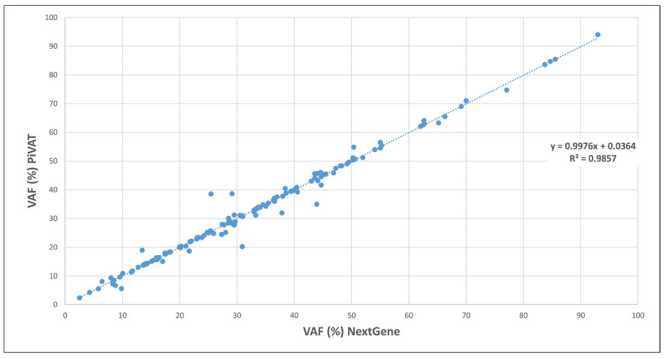

Figure 6 Graphical representation of the correlation of the variant allelic frequencies obtained between the NextGENe and PiVAT pipelines for 137 variants tested for accuracy. The data show excellent correlation between the two pipelines. VAF, variant allelic frequency.

resulting in background noise and PCR artefacts. NGS panels designed for enrichment are more successful when using novel technologies such as single-vial amplification with SLIMamp.[6]

The rigorous process of validation of our 48 gene panel was approved by NYSDOH for clinical use. With over 96% of target regions, including sequences with suboptimal G:C content achieving a coverage of over 1000X, the uniformity of coverage facilitates a low LOD of 2.5%–3% VAF for the targeted hotspot variants in all sample types. NGS testing of somatic variants at splice, coding and promoter regions of genes may present with intrinsically challenging G:C bearing sequences that lead to suboptimal data.[12] In addition, high degree of sequence similarity, existence of pseudogenes and other duplicated regions in the genome complicate the generation of quality sequences.[13 14] However, with almost 90% of the targeted regions achieving 2000X coverage, the CSTP assay is able to sequence the to meet the quality metrics eve in complex genomic regions. Notably, the low coverage regions (in *PTEN* and *STK11*), do not harbour clinically relevant variants reported the COSMIC database.[15] Such results verify that improved technology and established metrics can capture low-frequency variants present in clonal and heterogenous tumours without sacrificing the quality of results.

Establishing sound quality metrics for NGS assays is a key requirement for optimal performance and controls that harbour low VAF variants are crucial for quality assurance. Sequencing of histologically normal tissue is beneficial to identify potential population variants, thereby streamlining the bioinformatics output to verify and negate out any possible false positive call in this targeted panel. Notably, the assay did not have any amplicon drop-outs in targeted regions, as evidenced by uniformity and mapping on-target rates; over 99% of the amplicons having greater than 500x coverage. In addition, the high correlation of variant calls using two different bioinformatics pipelines further highlight the robustness of data.

Assay failures due to inadequate DNA quantity, suboptimal quality, tumour heterogeneity and paucity of neoplastic cells are universal limitations to optimal patient management.[16–20] While the FFPE samples used for routine clinical testing are fixed as per our standard protocol of under 30 hours, the validated assay is able to detect clinically relevant variants in samples that were fixed in formalin for up to 7 days.[21] Since its implementation for patient care, 253/2032 (12.4%) of the samples scored by a molecular pathologist had neoplastic cellularity of 10% or less (between 8% and 10%). Interestingly, the failure rate due to low tumour percentage was 2.3% along with 1.4% attributed to the quality of DNA. The data render the panel an attractive alternate to several current NGS hotspot assays. From the workflow point of view, the single-vial amplification favours the elimination of potential errors and reduction to 3–4 days in the turn-around-time.

With greater than 95% success rate, and an overall analytical failure rate of less than 3.75%, the CSTP assay has proven to

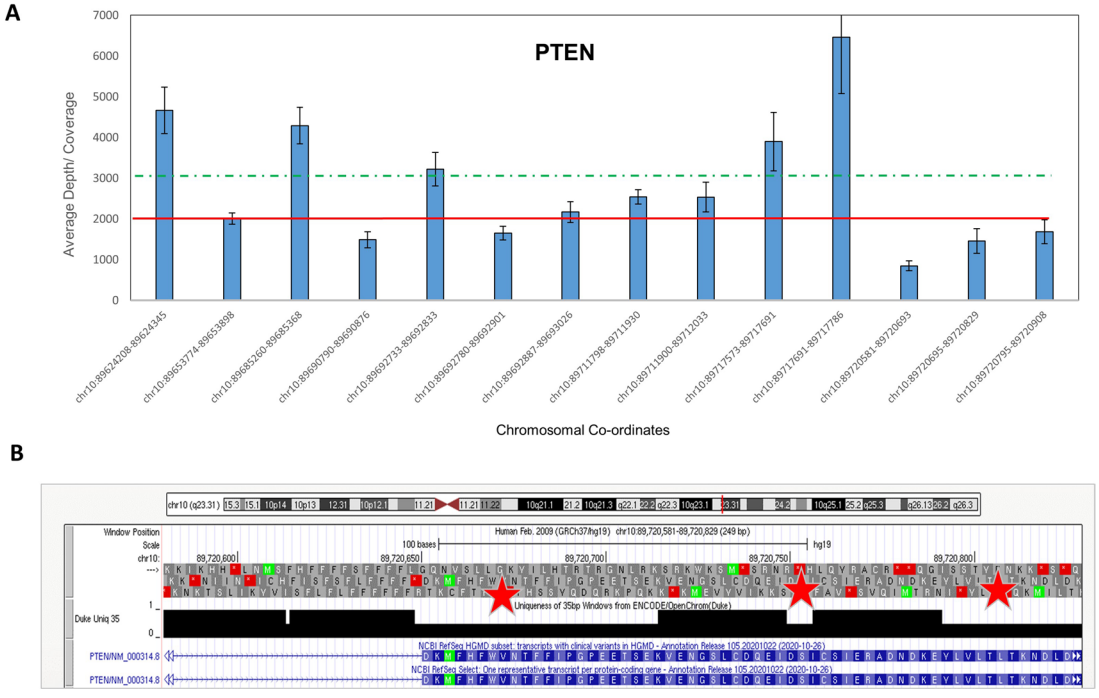

Figure 7 Evaluating the depth of coverage for the PTEN gene. (A). Graphical representation of the depth of coverage for the chromosomal coordinates covered in this panel. (B) A representative figure showing regions with low coverage having low mappability (★UCSC, hg19). LDT, laboratory developed test.

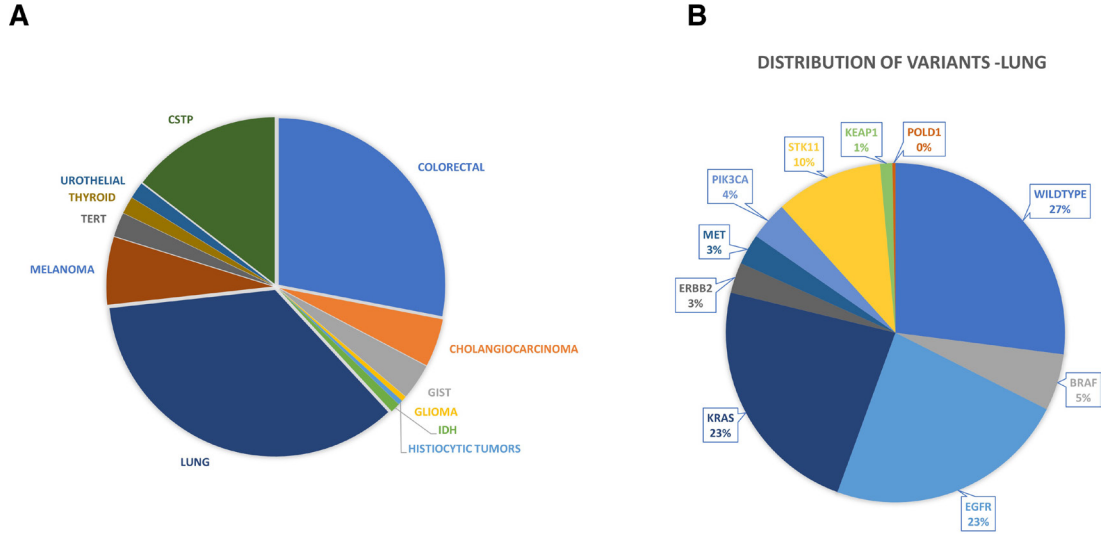

Figure 8 (A) Distribution of the 1931 cases representing various tumour types that were successfully sequenced. The figure includes cases where individual genes (*TERT* and/or *IDH*) were interrogated and sarcomas, carcinoma of unknown primary and tumours with no information on the primary site. These are collectively referred to as CSTP (custom solid tumour panel). (B) The mutational landscape of the lung tumours that were sequenced. CSTP, custom solid tumour panel.

be a robust and reliable targeted NGS panel for identification of therapeutic, diagnostic and prognostic biomarkers in several types of cancers. Finally, in conjunction with the RNA fusion panel, a comprehensive variant profile that interrogates almost all of the clinically relevant mutations in lung adenocarcinoma is obtained, suggesting the enhanced potential of using multiple small targeted NGS panels for molecular profiling.[22]

CONCLUSIONS

Targeted NGS panels for identification of variants in solid tumours has been adopted globally.[7 22] While the overall performance of these assays is good, QC measures are crucial for identifying potential problems.[13 23–25] Here, we document the clinical utility of a new and improved NGS technology with instituted quality metrics, that generates repeatable, robust and reproducible results with minimal DNA input and neoplastic cellularity for identification of actionable variants.

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