Development and correlative analysis of a TMB in specimens with reference MSI and somatic variant results

Abstract 124

Hestia Mellert PhD*, Leisa Jackson and Gary A. Pestano PhD

Biodesix, Inc., Boulder, CO 80301

Abstract

Background: Treatment of late-stage non-small cell lung cancer (NSCLC) has been greatly impacted by the advances in immunotherapy. Molecular biomarkers such as tumor mutation load/burden (TML or TMB) and micro-satellite instability (MSI) may identify patients with NSCLC that are more likely to respond to immune checkpoint inhibitors. In this report, we describe the development of an NGS-based assay for the robust detection of TMB and somatic variants simultaneously using a targeted assay that covers 1.7Mb of the genome (1.2Mb of which is exome).

Methods: Initial feasibility included reference control cell-lines, HCC1143 and NIST-8398, as well as comparisons between two alternative FFPE (formalin-fixed, paraffin-embedded) nucleic acid extraction methods. In development, eight FFPE specimens from patients with late stage cancer and a reference MSI status were evaluated. FFPE slides were extracted using the MagMAX™ FFPE DNA/RNA Ultra kit and nucleic acid quality was assessed using QPCR. Extracted DNA was processed through the Oncomine™ TML Assay with a minimum input of 20ng of DNA per specimen. Automated library and template preparation were performed followed by sequencing on the Ion GeneStudio™ S5 Plus system using replicate 540™ Chips. Eight samples with barcoded adapters were multiplexed per chip. Tumor mutation analysis and variant calling was performed using the Oncomine TML v2.0 workflow.

Results: The time from FFPE extraction to result was within 4 days and all samples passed QC metrics. TMB scores ranged from 14.4-36.04 mutations per megabase (Mus/Mb) for all samples with MSI-high reference results. A very high level of inter-chip concordance was also observed (R^2=0.990). Additionally, the variant caller function within the TMB workflow was used to detect BRAF V600E mutations with 100% concordance to reference testing results.

Conclusions: The accurate quantification of somatic mutations paired with fast turn-around time and robust automated workflow is optimal for assessing the tumor mutation burden as well as hotspot mutations from limited (FFPE) samples.

Materials and Methods

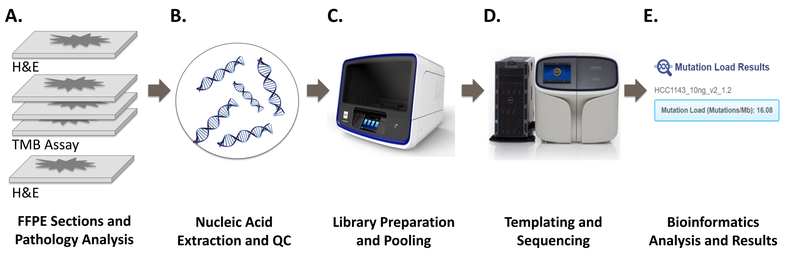

Figure 1. Tumor Mutation Burden Workflow Overview. A. Commercially sourced FFPE blocks were sectioned. Slides 1 and 5 were stained by H&E and reviewed by pathology for tumor content and level of necrosis. **B.** Nucleic acids were extracted from two or three 5-10μM unstained slides using the MagMAX™ FFPE DNA/RNA Ultra kit. Extracted DNA was subjected to QC analysis to determine quality and quantity using QPCR. **C.** Samples were assigned unique barcodes and library preparation was performed using an automated Ion Chef™ and the Oncomine™ Tumor Mutation Load assay. Pooled library concentration was determined using QPCR. **D.** The pooled libraries were templated using Ion Chef™ (540™ Chip) and sequenced on the Ion GeneStudio™ S5 Plus. Run and specimen level QC was completed using the TorrentSuite™ v.5.10.0. **E.** Bioinformatics analysis was implemented using the TML v2.0 workflow to determine TMB and SNVs on the Ion Reporter Software v 5.10.

Table 1. Turn Around Time for 16 Specimens.

	Day 1	Day 2	Day 3	Day 4
12:00 AM		6pm(D1)-4:30am(D2): Chip 1 Library Prep (Continued)	7:30pm(D2)-9:30am(D3): Chip 1 and Chip 2 Template Prep (Continued)	10pm (D3)-5:30am (D4): Chip 2 Data Analysis (Continued)
1:00 AM				
2:00 AM				
3:00 AM				
4:00 AM				
5:00 AM				
6:00 AM				
7:00 AM		7am-5:30pm: Chip 2 Library Prep (15 min hands-on)		7am-9am: Result Reporting
8:00 AM				
9:00 AM				
10:00 AM	10am-5pm: Extraction and QC (16 Samples)		9:30am-12pm: Chip 1 Sequencing (15 min hands-on)	
11:00 AM				
12:00 PM			12pm-2:30pm: Chip 2 Sequencing (15 min hands-on)	
1:00 PM				
2:00 PM				
3:00 PM				
4:00 PM				
5:00 PM	5pm-6pm: QC Data Interpretation		2:30pm-10pm: Chip 1 Data Analysis	
6:00 PM		5:30pm-7:30pm: Chip1 and Chip 2 Lib. QC		
7:00 PM				
8:00 PM	6pm(D1)-4:30am(D2): Chip 1 Library Prep (15 min hands-on)	7:30pm(D2)-9:30am(D3): Chip 1 and Chip 2 Template Prep (15 min hands-on)		
9:00 PM				
10:00 PM			10pm (D3)-5:30am (D4): Chip 2 Data Analysis	
11:00 PM				

Key: Hands-On Time; Hands-Off Time

Background

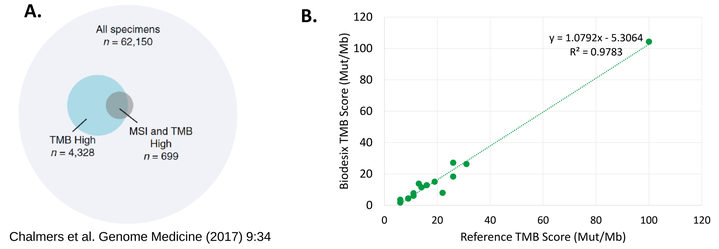

Figure 2. TMB association with MSI-high specimens and commercial TMB test comparison. A. The majority of specimens that are MSI high also harbor high levels of TMB. **B.** Mutation Burden scores - defined as the number of Mutations detected per Megabase of genome coverage (Mut/Mb) – Biodesix results are compared to Reference TMB Score (Caris MI Profile™ Test or FoundationOne® Test). Correlation was assessed using linear regression represented with the green dotted line. R-square=0.9783.

Results

Table 2. Specimen clinical characteristics and pathology review.
Note: Specimen 16-221 failed QC due to 100% necrotic tissue upon pathology review and was excluded from the study.

Case ID	Gender	Ethnicity	BMI	Smoking Status	Age at Sample Acquisition	Specimen Site	Histologic Type	T Stage	% Tumor (H&E)	% Necrosis (H&E)
14-231	M	White	Not Available	Not Available	55.7	Colon	Adenocarcinoma	3a	90	10
15-797	F	White	Not Available	Not Available	72.2	Ovarian	Endometrioid adenocarcinoma (not otherwise characterized)	3a	60	0
16-035	M	White	Not Available	Nonsmoker	91	Colon	Adenocarcinoma	4	80	10
17-270	F	White	34	Nonsmoker	76.2	Colon	Adenocarcinoma with mucinous features	4	90	0
17-464	F	White	20	Former smoker	90.3	Colon	Adenocarcinoma	4	90	40
17-690	M	White	23	Former smoker	83	Colon	Adenocarcinoma	4	90	20
18-364	F	White	21	Former smoker	84	Colon	Adenocarcinoma	4	60	40
18-522	F	White	30	Nonsmoker	82	Colon	Adenocarcinoma	4	80	5

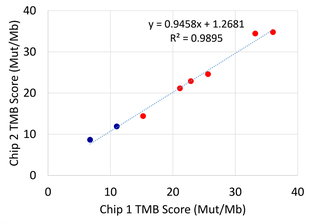

Figure 3. Inter-chip analysis of tumor mutation burden. TMB scores are compared between the same library run on two 540 chips. The correlation was assessed using linear regression analysis and is represented with the blue dotted line. Blue markers are MSI-low/stable; red markers are MSI-High.

Table 3. Comparison of TMB results with MSI reference results determined using PCR or IHC.

Case ID	MSI Status	TMB in Mut/Mb (Ave. Chip 1 and 2)
14-231	MSI-Low (BAT25+ only)	11.45
15-797	MSI-Stable (by IHC)	7.70
16-035	MSI-High	35.41
17-270	MSI-High	25.11
17-464	MSI-High	21.14
17-690	MSI-High	33.83
18-364	MSI-High	14.82
18-522	MSI-High	22.89

Table 4. Comparison of NGS variant caller results with BRAF V600E reference results (Ion Ampliseq NGS).

Case ID	BRAF V600E Reference	BRAF %MVF (Ave. Chip 1 and 2)
14-231	Negative	Not Detected
15-797	Not Reported	Not Detected
16-035	Positive	46.9%
17-270	Positive	27.5%
17-464	Positive	20.1%
17-690	Positive	31.3%
18-364	Negative	Not Detected
18-522	Positive	24.2%

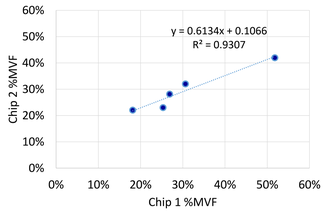

Figure 4. Inter-chip analysis of BRAF V600E SNVs detected by Variant Caller. The percent minor variant frequencies (%MVF) are compared between two 540 chips. The correlation was assessed using linear regression analysis and is represented with the blue dotted line.

Results (Run Metrics and Sequencing QC)

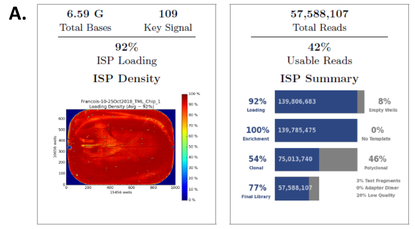

Figure 5. Sequencing Metrics. Sequencing metrics for **A.** Chip 1 and **B.** Chip 2 generated using TorrentSuite™ v.5.10.0 are shown. For this study, templating was performed using the Ion Chef (540 Chip) and the Ion S5 GeneStudio Plus sequencer.

QC Thresholds:
Mean Depth > 300
Uniformity >80%

Table 5a. Mapped read and uniformity metrics for Chip 1.

Barcode Name	Sample	Mapped Reads	On Target	Mean Depth	Uniformity
IonCode_0117	14_231	6,450,142	98.37%	406	97.00%
IonCode_0118	15_797	5,740,077	99.21%	363.7	80.99%
IonCode_0119	16_035	7,525,088	99.18%	478.2	93.66%
IonCode_0120	17_270	6,621,959	99.00%	426.7	96.70%
IonCode_0121	17_464	7,138,304	98.46%	456.5	96.89%
IonCode_0122	17_690	5,974,405	98.52%	382.4	96.46%
IonCode_0123	18_364	6,479,642	98.46%	416.5	96.88%
IonCode_0124	18_522	7,673,777	99.10%	498.5	96.21%

Table 5b. Mapped read and uniformity metrics for Chip 2.

Barcode Name	Sample	Mapped Reads	On Target	Mean Depth	Uniformity
IonCode_0117	14_231	6,508,974	98.26%	409.1	96.98%
IonCode_0118	15_797	5,854,948	99.15%	370.5	81.14%
IonCode_0119	16_035	7,600,804	99.13%	480.6	93.71%
IonCode_0120	17_270	6,691,027	98.94%	430.9	96.67%
IonCode_0121	17_464	7,190,792	98.35%	459	96.83%
IonCode_0122	17_690	6,035,482	98.43%	385.7	96.42%
IonCode_0123	18_364	6,560,651	98.41%	421.1	96.72%
IonCode_0124	18_522	7,858,173	99.04%	510	96.25%

Summary and Conclusions

- TMB performance at Biodesix is similar to on-market TMB assays
- TMB values were higher in MSI high specimens relative to low or stable MSI specimens (14.4-36.04 vs 7.7-11.4 mut/Mb respectively), aligning well with current literature
- Variant caller accurately detected reference BRAF mutations with 100% concordance to reference results
- The workflow provides a rapid turnaround time with results reported within 4 days of FFPE extraction

References

1. Chalmers et al. Genome Medicine (2017) 9:34
2. Janice Au-Young et al. AMP Annual Meeting 2018
3. Ruchi Chaudhary et al. SITC 2019
4. Snyder et al. NEJM, 371, 2014
5. Rizvi et al. Science, 348, 2015
6. Hause et al. Nature Med, 22, 2016
7. Fabrizio et al. J Gastrointest Oncology, 9, 2018

Acknowledgements

The authors gratefully acknowledge critical technical discussions with the team at ThermoFisher Scientific, including Ryan Meese, Ryan Peters, Lin Chen, Elaine Wong-Ho, Fiona Hyland, Jim Godsey.

*poster reprint requests: hestia.mellert@biodesix.com