ion torrent

Prediction of DDR and other mutation signatures using targeted panels

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Background

Cancer genomes are subject to diverse mutational processes that generate recognizable mutational signatures. Mutational processes can cause driver mutations and are considered the primary cause of tumorigenesis. Cosmic signatures classify these mutational processes, whether environmental or intrinsic. Identification of signatures including DNA Damage Response (DDR) signatures enables research into the origin of cancer mutagenesis and potentially treatment optimization. Typically, Mutation signatures are identified using Whole Genome Sequencing or Whole Exome Sequencing. We demonstrate signature prediction of mutation signatures with two amplification-based targeted panels, which have a high sequencing success rate for FFPE samples.

Methods

2050 FFPE Samples were identified from a pan solid tumor cohort, run on a targeted panel of 1.7 Mb (Oncomine Tumor Mutation Load or OCAPlus) with an AmpliSeq-based enrichment that is robust with 20ng of input DNA. We filtered out germline mutations, removing variants at appreciable frequency in population databases, to generate a set of somatic SNVs. Single base change substitution (SBS) matrix for these somatic mutations was constructed. Cancer signatures described in COSMIC Mutational Signatures v3.1 (Released on June 2020), were characterized and the cosine similarity between the normalized sample and SBS COSMIC signatures was measured. Signatures with a strong match (> 0.7) to the normalized sample were selected. We also used an orthogonal approach to impute the signatures using a reduced candidate set. In this approach, the DeconstructSigs R package was used to determine the weights of each mutational signature contributing to an individual tumor sample.

Content

Ion Torrent Oncomine Tumor Mutation Load Assay (TML)
The Oncomine Tumor Mutation Load Assay™ is a targeted next-generation sequencing (NGS) assay that provides an assessment of tumor mutation load and mutation signatures in a simple workflow. The assay measures TMB (from 1.2Mb of coding region) and detects mutations in 409 cancer genes.

Ion Torrent Oncomine Comprehensive Assay Plus (OCAPlus)
The Oncomine Comprehensive Assay Plus™ is a targeted next-generation sequencing (NGS) assay that provides a comprehensive genomic profiling solution appropriate for formalin-fixed paraffin-embedded (FFPE) tissues. The assay addresses multiple biomarkers covering over 500 genes, including targets that are relevant in cancer. This assay enables analysis of variants across 500+ genes and detection of SNVs, CNVs, In-Dels, TMB, MSI, and gene fusions.

Figure 1. Differential rates of mutations within trinucleotides is a characteristic of mutational signatures.

Prevalence of Mutational Signatures

Two thousand fifty samples from a wide range of solid tumor cancer types were analyzed; 57.7% of samples had at least 1 recognizable mutational signature. If a sample had more than one signature, it was counted twice.

Mutational Signatures associated with Base Excision Repair, APOBEC mutations, MMR and others were observed.

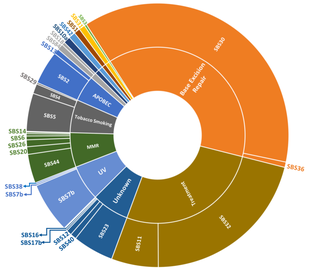


Figure 2. Signature prevalence among samples sequenced with TML assay. N = 1072 samples

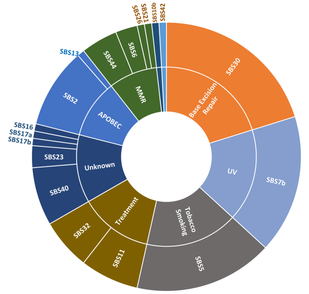


Figure 3. Signature prevalence among samples sequenced with OCAPlus assay. N = 114 samples

Sample with an MMR signature – exome and panel

Table 4a. Signatures identified in sample

Signatures in Panel	Signature in Exome
SBS44 (0.778)	SBS44 (0.734)
	SBS6 (0.873)
	SBS15 (0.785)

Figure 4b. Sample mutational profile from Exome sequencing

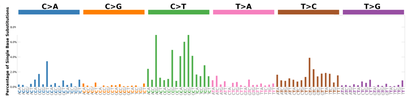


Figure 4c. Sample mutational profile from OCA Plus Panel sequencing

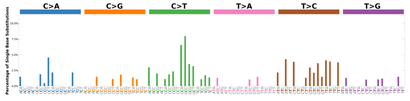


Figure 4e. Signature contribution in sample from Panel sequencing

Figure 4d. COSMIC Mutational Signature Profiles

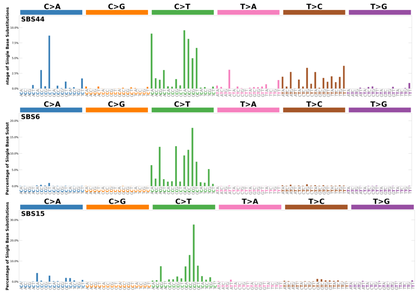
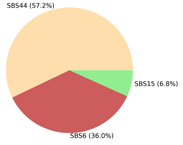


Figure 4f. Signature contribution in sample from Exome sequencing



Sample has a mutation in MLH1 and SBS44 signature

Figure 5a. Representation of Mutational profile of a cancer sample sequenced using TML panel.

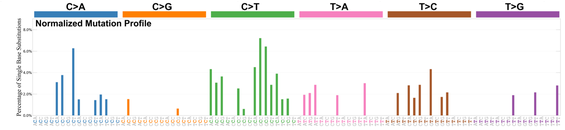


Figure 5b. Representation of Mutational profile of Cosmic Signature SBS44

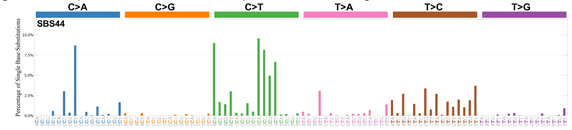
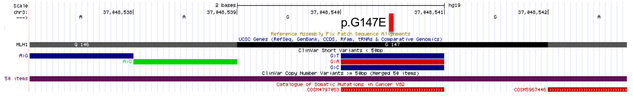
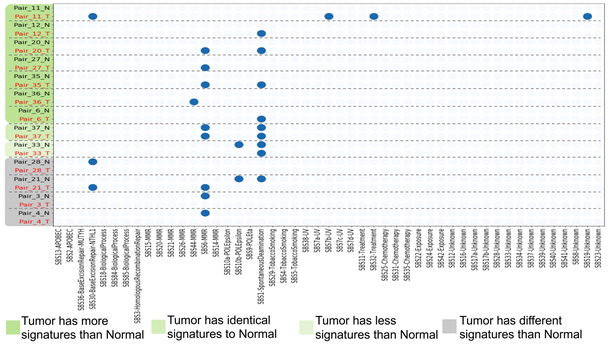


Figure 5c. Missense mutation in signature relevant MLH1 gene was identified in the sample



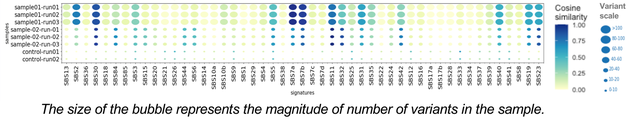
Tumor samples have more mutational signatures than their matched normal samples

Figure 6. Heatmap depicting signatures identified in Tumor/Normal samples pairs sequenced using OCAPlus panel



Reproducibility of mutation signatures with targeted sequencing

Figure 7. Representation of cosine similarity predictions of two tumor samples and a control. The tumor samples were each run thrice, and the control was run twice.



The size of the bubble represents the magnitude of number of variants in the sample.

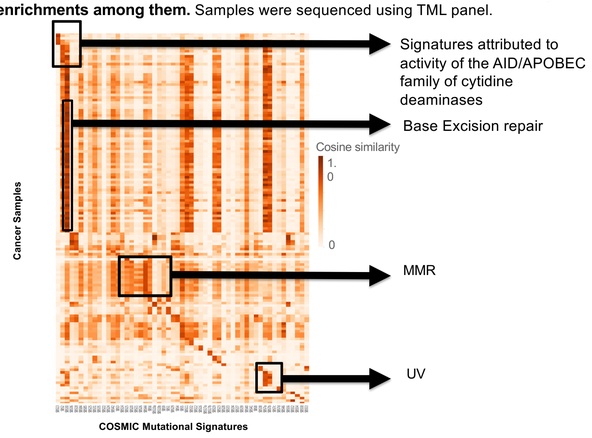
DNA Damage Repair and associated Mutational Signature abundances

Table 8. Prevalence of Base Excision Repair, MMR, APOBEC and HRR signatures in OCAPlus and TML datasets

Category	Signature	Samples sequenced with OCAPlus Panel		Samples sequenced with TML Panel	
		Samples with signatures	Samples with signature relevant gene mutations	Samples with signatures	Samples with signature relevant gene mutations
APOBEC	SBS13	1	0	7	0
APOBEC	SBS2	11	0	44	0
Base Excision Repair	SBS30	23	1	401	59
Base Excision Repair	SBS36	0	0	7	1
HRR	SBS3	0	0	3	0
MMR	SBS14	0	0	2	1
MMR	SBS15	0	0	1	0
MMR	SBS20	0	0	10	2
MMR	SBS21	0	0	3	3
MMR	SBS26	1	1	9	9
MMR	SBS44	5	5	38	17

Heatmap: Samples and Mutational Signature

Figure 9. Heatmap showing a subset of **samples analyzed and patterns of signature enrichments among them.** Samples were sequenced using TML panel.



Conclusions

This research demonstrates that mutation signatures can be identified using amplification-based targeted sequencing data with two Oncomine panels. This approach expands the availability of mutation signatures in clinical research samples because of the limited availability of DNA in FFPE samples and the higher success rates of targeted amplicon-based sequencing.

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