Comparative Analysis Of Multiple Copy Number Alteration Tools In The Detection Of Amplifications And Deletions On Both Whole-exome And Targeted NGS Panel Platforms

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Introduction

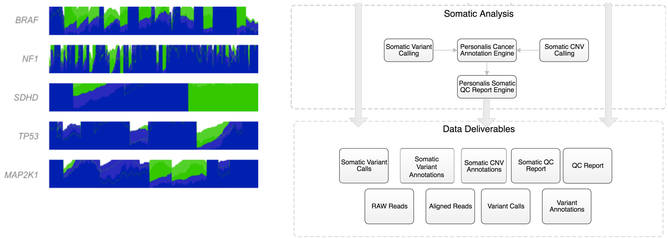
Somatic copy number variations, or CNVs, are frequent occurrences in the tumor landscape and the detection of these events remains a challenge. Whole-genome sequencing is well suited for detection of CNVs but is not always an option, especially in the clinical setting where whole exome and targeted panels are both faster and more cost effective. FACETS is a recently released tool that supports CNV identification in both targeted panels and whole exome data. We sought to determine the performance of this tool in identifying known CNVs across the two platforms as well as in comparison to methods using separate tools for amplifications and deletions. Using this approach, we were able to identify clinically relevant CNVs on both targeted panel and whole-exome platforms.

Methods

ACE Exome, ACE Cancer Panel, and DNA Cancer Pipeline

Many canonical cancer genes contain gaps in sequencing coverage through standard NGS. We developed an augmented target enrichment strategy (ACE) optimized for even coverage across the entire span of gene content. Sequencing coverage of select cancer genes with a standard exome (blue) and with our ACE exome (green) as shown in Figure 1. An optimized cancer bioinformatics pipeline was used to analyze samples in this study. This pipeline was designed to detect somatic variants including SNVs, small insertions/deletions, and copy number changes.

Figure 1: ACE Exome Enhancements and Somatic Pipeline Overview



Cell Lines and Copy Number Analysis

Cancer cell lines representing multiple cancer types including breast, colorectal, and lung were sequenced using ACE Cancer Panel and/or ACE Exome platforms. Exome tumor samples were analyzed with the matched normal whereas the panel samples were analyzed with a proxy normal sample. Our somatic pipeline utilizes multiple tools for identifying CNVs, with some tools used exclusively for amplifications or deletions. For exome data, Sequenza and Control FREEC were used for amplifications and deletions, respectively. For panel data, Excavator with OncoCNV were used for amplifications and OncoCNV for deletions. Analytical sensitivity was calculated as follows: True Positives * 100/(True Positives + False Negatives). Threshold for amplification was copy number >= 8.

Results

Analysis of panel data produced divergent results between tool sets

Table 1: Copy Number Analysis for ACE Cancer Panel

Cancer Cell Line	Gene	CNA Type	Excavator/OncoCNV Copy Number	FACETS Copy Number
A431	CDKN2B	DEL	0	6
A498	CDKN2B	DEL	0	4
BT-474	AURKA	AMP	13	11
BT-474	ERBB2	AMP	22	21
HCC1395	CDKN2A	DEL	0	0
HCC1395	CDKN2B	DEL	0	0
HCC1599	AKT2	AMP	7	5
HCC1599	CCNE1	AMP	8	5
HCC1954	ERBB2	AMP	104	32
HCC1954	FGFR4	AMP	5	7
HCC1954	RAD21	AMP	8	9
HCC1954	TERT	AMP	16	19
K562	CDKN2A	DEL	0	1
K562	CDKN2B	DEL	0	1
K562	MAPK1	AMP	11	7
K562	NUP214	AMP	14	11
NCI-H2122	CDKN2B	DEL	0	1
NCI-H2126	CDKN2A	DEL	0	1
NCI-H2126	CDKN2B	DEL	0	1
OVCAR-3	AKT2	AMP	9	8
OVCAR-3	CCNE1	AMP	8	10
U87MG	CDKN2A	DEL	0	1
U87MG	CDKN2B	DEL	0	1
U87MG	MLLT3	DEL	0	1

Analytical Sensitivity for ACE Cancer Panel: The results of the panel analysis differed greatly from exome in regards to agreement between the tool sets, with more than half of the events not identified by FACETS. Analytical sensitivity for Excavator/OncoCNV and FACETS was 92% and 41%, respectively.

Analysis of exome data produced comparable results between tools

Table 2: Copy Number Analysis for ACE Exome

Cancer Cell Line	Gene	CNA Type	Sequenza/Control FREEC	FACETS Copy Number
HCC1395	CDKN2A	DEL	0	0
HCC1395	CDKN2B	DEL	0	0
HCC1599	AKT2	AMP	18	14
HCC1599	CCNE1	AMP	18	17
HCC1954	ERBB2	AMP	20	58
HCC1954	FGFR4	AMP	16	11
HCC1954	RAD21	AMP	18	14
HCC1954	TERT	AMP	20	27
NCI-H2122	CDKN2B	DEL	0	1
NCI-H2126	CDKN2A	DEL	0	0
NCI-H2126	CDKN2B	DEL	0	0

Analytical Sensitivity for ACE Exome: The exome analysis produced nearly identical results from the two CNV methods, with the sole difference being FACETS inability to identify the CDKN2B deletion in NCI-H2122. Analytical sensitivity for Sequenza/Control FREEC and FACETS was 99.9% and 91%, respectively.

Conclusion

The need for the development of robust tools for identification of CNVs cannot be understated. As new methods are developed, the rigorous evaluation of their strengths and weaknesses will facilitate continued improvement. All tools show reduced sensitivity in panel data compared to exome, a factor contributing to this is likely the greater representation of known SNVs in the exome data – which are used in detection of copy number change points.

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