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SNAQ-SEQ™: Use of synthetic internal standards in conjunction with Poisson Exact Test to call variants in contrived circulating tumor DNA specimens

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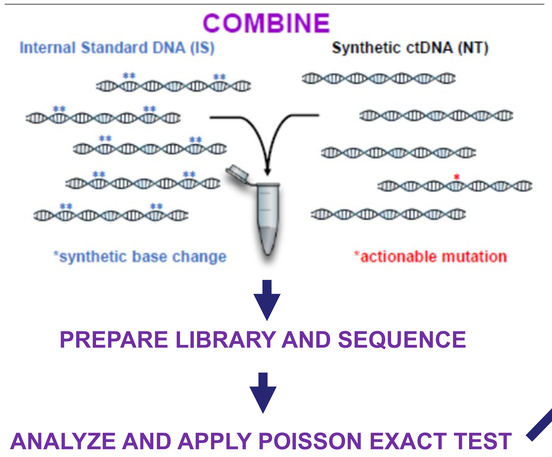
BACKGROUND

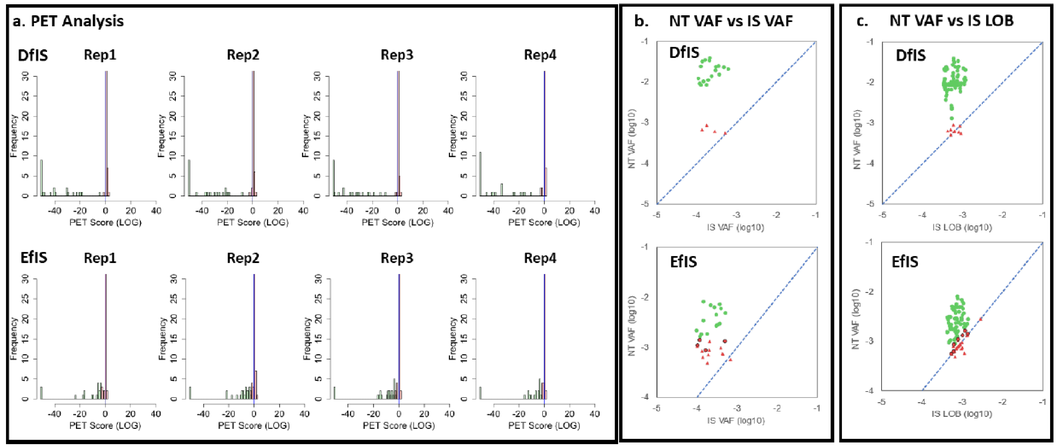
Targeted next generation sequencing (NGS) analysis of circulating tumor (ct) DNA promises to significantly advance targeted therapy of cancer. However, targeted NGS has poor accuracy for calling known variants with VAF below 0.5%. The goal of this study from the FDA-Sequencing and Quality Control Phase 2 (SEQC2) consortium was to a) develop and validate bioinformatic and biostatistical methods that incorporate synthetic competitive internal standards (IS) in targeted NGS analysis of actionable tumor mutations, and b) assess utility in analysis of low VAF actionable mutations in ctDNA.

METHODS

A synthetic IS spike-in was designed for each actionable mutation target, suitable for use in NGS following targeted PCR or hybrid-capture enrichment and either with unique molecular index (UMI) or non-UM I library preparation. Contrived ctDNA reference samples developed by the SEQC2 consortium (Jones et al, Genome Biol. 2021) containing actionable mutations at known variant allele fraction were used. An aliquot of each sample was mixed with a mixture of IS. Following Illumina TST170 enrichment and library preparation, each library was sequenced, then native template (NT) sequences were separated from IS sequences bioinformatically. In the SNAQ-SEQ™ method, Poisson Exact Test (PET) analysis was used to calculate the significance of difference between the VAF for each sample variant and VAF for corresponding variant in IS variant. Analysis was based on NT variant count and position coverage (i.e., copies recovered in library preparation) and IS count and position coverage. PET performed an exact test of a simple null hypothesis about the ratio between two rate parameters in Poisson distribution.



RESULTS



- Panel a: SNAQ-SEQ analysis of panel provider VCF PASS and Low-Support variants–SNAQ-SEQ called variants indicated as green columns, not called as red columns
- Panel b and c: Panel provider VCF PASS and LowSupportvariants displayed–Panel b: NT variant VAF on y-axis and matched IS variant VAF on x-axis.
- Panel c: NT variant VAF on y-axis and matched IS determined LOB on x-axis

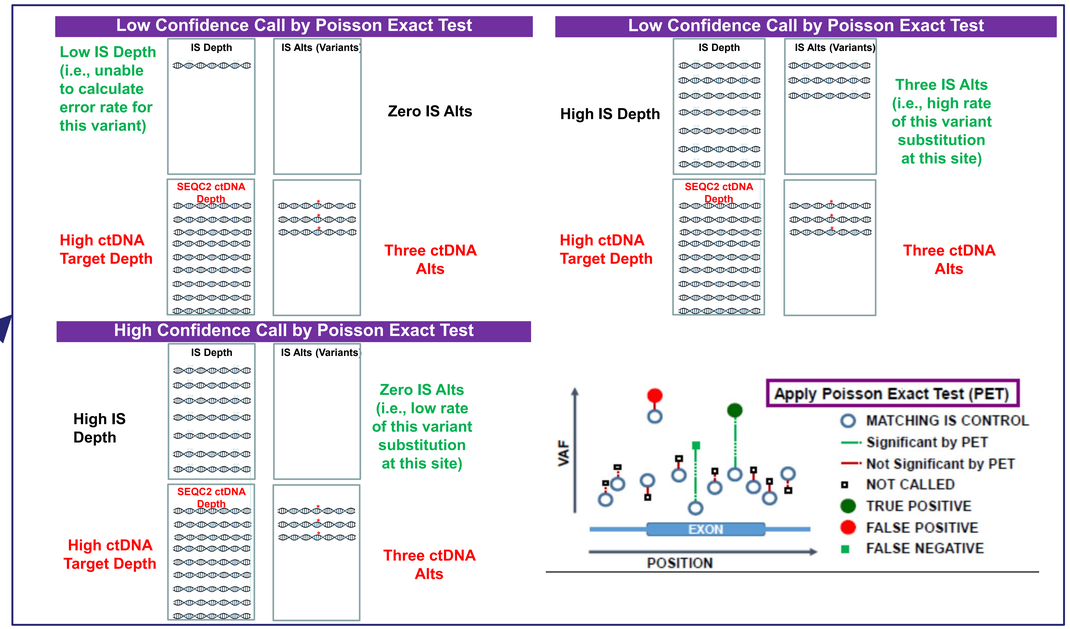


Table 1. Effect of SNAQ-SEQ QC on ILM UMI NGS Analysis of ground truth true positive variants in *Sample EfIS* replicate libraries

True Positive Variant Annotation					True Positive Variant VAF			
CHROM	POS	REF	ALT	GENE	Rep1	Rep2	Rep3	Rep4
chr1	115256529	T	A	NRAS	0.4%	0.2%	0.2%	0.2%
chr1	115258748	C	A	NRAS	0.1%		0.2%	
chr12	112888162	G	C	PTPN11	0.1%	0.3%	0.2%	
chr15	66729162	C	T	MAP2K1		0.1%	0.4%	0.2%
chr17	7576569	G	A	TP53		0.3%	0.2%	0.2%
chr17	7577022	G	A	TP53	0.3%	0.2%	0.2%	0.1%
chr17	7577085	C	T	TP53	0.1%	0.2%	0.1%	0.1%
chr17	7577118	C	A	TP53	0.5%	0.5%	0.3%	0.5%
chr17	7577529	A	T	TP53	0.6%	0.4%	0.5%	0.2%
chr17	7578211	C	T	TP53	0.4%	0.3%	0.4%	0.4%
chr17	7578671	C	T	TP53	0.2%	0.2%	0.5%	
chr17	7578679	A	G	TP53	0.3%	0.5%	0.7%	0.5%
chr17	37880987	C	T	ERBB2		0.2%	0.2%	0.2%
chr3	178936091	G	A	PIK3CA	0.2%	0.2%	0.3%	0.1%
chr4	1803153	G	A	FGFR3	0.2%	0.2%	0.2%	0.2%
chr4	1803172	G	A	FGFR3	0.2%	0.2%	0.2%	0.2%
chr4	1803173	C	T	FGFR3	0.3%	0.1%	0.1%	0.2%
chr4	1803385	G	C	FGFR3	0.2%	0.2%	0.2%	0.3%
chr4	1803704	T	C	FGFR3	0.8%	0.7%	0.6%	0.6%
chr9	139399344	A	G	NOTCH1	0.3%	0.3%	0.5%	

Table 2. Summary: SNAQ effect on detection sensitivity

Variant VAF Range	0.10% ≤ VAF ≤ 0.3%		VAF > 0.3%		All VAF	
Variant Caller	ILM	SNAQ	ILM	SNAQ	ILM	SNAQ
Expected TP calls	56	56	24	24	80	80
Observed TP calls	41	48	23	23	64	71
Sensitivity	73%	86%	96%	96%	80%	89%
SNAQ Sensitivity Increase		13%		0%		9%

CONCLUSIONS

- Following mixture of contrived ctDNA reference samples with IS, PET analysis enabled calculation of technical error rate, limit of blank, and limit of detection for each variant at each nucleotide position, in each sample.
- Using this SNAQ-SEQ™ analysis, true positive mutations with variant allele fraction too low for detection by current practice were detected with this method, thereby increasing sensitivity.
- SNAQ-SEQ™ provides QC that will improve accuracy and inter-lab harmony in measurement of actionable mutations in ctDNA clinical specimens

AUTHOR DISCLOSURES

JCW has 5–10% equity interest in and serves as a consultant to Accugenomics, Inc. Technology relevant to this manuscript was developed and patented by JCW, ELC and DJC and is licensed to Accugenomics. These relationships do not alter our adherence to all policies on sharing data and materials.
JX: None. NH: None. JL: None. TM: None.