AccuGenomics

A higher standard of accuracy

PRODUCT INFORMATION

Accukit™ Myeloid DNA IS

SNAQ™-SEQ Spike-in Standards for MRD

Standardized Nucleic Acid Quantification for Sequencing (SNAQ™-SEQ) is a critical QC method to provide variant specific sensitivity controls for therapeutically relevant Acute Myeloid Leukemia (AML) targets in each sample. Mixtures of synthetic DNA created to mimic the sample regions of interest are added to each sample prior to NGS library preparation; they covary through the complex chemistry, flow cell detection and bioinformatics workflow, mirroring the samples native target template to provide the ideal assay run control. Internal standards (IS) bring the reference material directly into the sample, eliminating the need for external run controls for more cost efficient reagent and flow cell utilization, while also providing direct QC of the sample.

Accukit™ Myeloid DNA IS are added to each sample as a single reagent addition, represent genomic regions with clinically relevant cancer driver variants, and are compatible with hybrid capture and amplicon-based library prep chemistries. Accukits are available pre-fragmented for use with blood and plasma (cfDNA), or unfragmented for FFPE or tissues; they are easily compatible with your existing workflow, platform, and analysis pipeline.

Accukit Myeloid DNA IS Targets:

ABL1
DNMT3A
FLT3
FLT3-ITD
IDH1/2
JAK2
KRAS
NRAS
TET2
TP53
NPM1
JAK2

Actionable AML Internal Standards validate assay and sample performance to confirm that assay sensitivity is being met for every sample tested

Accurate and quantitative NGS assays are essential for monitoring disease progression, assessing treatment efficacy, and detecting measurable residual disease (MRD); combined, these will lead to improved diagnosis, treatment, and patient outcomes. Use of the SNAQ™-SEQ mixture of internal standards provides variant level sensitivity for key actionable Myeloid cancer variant regions of interest.

To achieve MRD level sensitivities, NGS technologies follow technically complex and rigid protocols, often running an external reference material as an indirect assay performance QC in parallel to a batch of patient samples. The external positive control material is treated like a sample, so it consumes valuable reagents, processing time, flow cell throughput and downstream bioinformatic analysis. Additionally, an external batch control does not monitor or reflect the complex individual interactions of each sample matrix with the test reagents and instrumentation. SNAQ™-SEQ functions similarly to internal standards being used in many other analytic methodologies by providing an internal sample matrix that is designed to mimic the conversion and detection efficiency of hotspot regions. For NGS MRD, each sample is spiked with a mixture of internal standards at Limit of Detection levels, which provides the *ideal sample internal reference* sensitivity QC.

Accukit™ Myeloid DNA IS was developed to support the FNIH MRD in AML Consortia, and was validated by participating FNIH laboratories.

Accurate and standardized NGS data for the most critical AML targets

Analytical test sensitivity is critical to NGS MRD performance, and Internal Standards support rapid assay development and validation. IS also standardize the reporting of accurate results during routine use, to enable comparison between different studies, panels and timelines.

Based on the genomic test input and IS concentration, the IS are added to each sample to achieve the desired limit of detection variant allele fraction. For example, 30 ng gDNA (8300 copies) would be mixed with 41 copies Accukit™ Myeloid DNA IS to yield a 0.5% IS variant allele frequency.

SNAQ™-SEQ Internal Standards are designed, manufactured, and released to provide a consistent ground truth LoD for each target in the assay. Each IS concentration is confirmed by two independent methods, and the conversion rate and transferability has been demonstrated with all of the major NGS testing pipelines. They improve the accuracy and standardization of variant detection in NGS assays, which leads to better treatment response monitoring.

Accukit™ Myeloid DNA IS improve the accuracy of measurable residual disease (MRD) detection for Acute Myeloid Leukemia (AML).

Accukit™ Myeloid DNA IS

The Accukit™ Myeloid DNA IS contains a blend of 15 DNA Internal Standards (IS) to clinically relevant targets in monitoring treatment response by MRD (Table 1). The mixtures are suitable for assay development and validation, and also serve as a direct sample reference QC when added to each sample.

Additional variant targets can be designed and included in a custom Accukit™ to meet the specific needs of any testing laboratory.

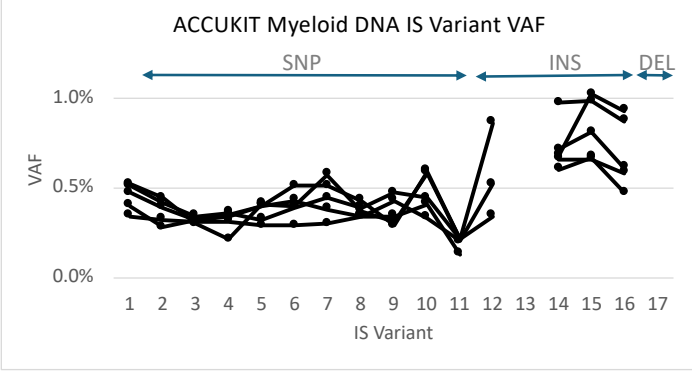
Table 1:

VARIANT	#	CHROM	POS	REF	ALT	Gene	Mutation-nt	Mutation-aa
SNP	1	chr9	133748281	C	T	ABL1	c.942C>T	p.Ile314
	2	chr2	25457241	G	A	DNMT3A	c.2646C>T	p.Arg882
	3	chr13	28592644	C	A	FLT3	c.2501G>T	p.Arg834Leu
	4	chr2	209113116	C	T	IDH1	c.391G>A	p.Gly131Ser
	5	chr15	90631841	C	T	IDH2	c.512G>A	p.Gly171Asp
	6	chr9	5073774	G	T	JAK2	c.1853G>T	p.Cys618Phe
	7	chr12	25398288	C	T	KRAS	c.31G>A	p.Ala11Thr
	8	chr1	115252199	C	T	NRAS	c.441G>A	p.Lys147
	9	chr1	115256531	T	A	NRAS	c.180A>T	p.Gly60
	10	chr4	106164918	G	A	TET2	c.3786G>A	p.Arg1262
	11	chr17	7577562	C	T	TP53	c.719G>A	p.Ser240Asn
INS	12	chr13	28608248	C	C..29..T	FLT3	c.1808insA..28..T	p.Lys602_Trp603insTyr..Lys
	13	chr13	28608315	C	C..125..T	FLT3	c.1741insA..124..G	p.Gln580_Val581insLys..Gln
	14	chr5	170837550	A	AGTCT	NPM1	c.866_867insGTCT	p.Gln289Cfs*12
	15	chr5	170837550	A	AGCAT	NPM1	c.866_867insGCAT	p.Gln289Cfs*12
	16	chr5	170837550	A	AGCCT	NPM1	c.866_867insGCCT	p.Gln289Cfs*12
DEL	17	chr9	5070014	TGGTGTT	T	JAK2	c.1605_1610delGGTTGT	p.Met535_Val536delinsIleHis

Figure 1 depicts an example following the addition of Accukit™ Myeloid DNA IS at limiting levels to 5 patient samples. The SNV IS variants were detected as expected. FLT3-ITD detection was highly variable, with two dropouts likely missed due to the challenges associated with detecting a lengthy insertion by short read NGS. The NMP1 variants were all detected, but at 2-3 fold higher VAF than expected, which is likely a result of bioinformatic inaccuracies when processing insertions.

Overall, Accukit™ Myeloid DNA IS was used as a per sample sensitivity QC, indicating nominal performance for SNV and short indels.

Figure 1:



IS Variant VAF in Patient Samples Spiked With AccuGenomics SNAQ™-SEQ Accukit Myeloid DNA IS. Purified leukocyte genomic DNA (120 ng) was mixed with 150 copies AccuGenomics SNAQ™-SEQ Myeloid DNA Internal Standards Accukit (Cat #2023), and sequenced using the ThermoFisher Oncomine Myeloid MRD Assay on a ThermoFisher Genexus instrument, the resulting VAF were analized for IS variants. IS variants were unique SNP and indels (variants described, Table 1); the expected IS VAF was 0.4%.

Notes:
1. FLT3-ITD long was not reported by the NGS method (variant #13).
2. FLT3-ITD short was not reported in two of five samples (variant #12).
3. The reported INS VAF were often higher than expected and with less precision.
4. The NGS panel used was not designed to detect the deletion variant #17.

References:

1. Cescon DW, Bratman SV, Chan SM, Siu LL. Circulating tumor DNA and liquid biopsy in oncology. Nat Cancer 2020;1:276–90.

2. Izevbaye I, Li Y Liang LY, Cheryl Mather C, et al. Clinical Validation of a Myeloid Next-Generation Sequencing Panel for Single-Nucleotide Variants, Insertions/Deletions, and Fusion Genes. J Mol Diagn. 2020;22(2):208-219.

3. Heitzer E, Haque IS, Roberts CES, Speicher MR. Current and future perspectives of liquid biopsies in genomics-driven oncology. Nat Rev Genet 2019;20:71–88.

4. Abbosh C, Birkbak NJ, Swanton C. Early stage NSCLC - challenges to implementing ctDNA-based screening and MRD detection. Nat Rev Clin Oncol 2018;15:577–86.

AccuGenomics Support of the FNIH MRD in AML Consortia:

AccuGenomics is proud to be a support partner in the Foundation for the National Institutes of Health (FNIH) MRD in AML project, led by the FNIH Biomarkers Consortium. This research initiative is investigating the use of genetic tests to improve the accuracy of measurable residual disease (MRD) detection for Acute Myeloid Leukemia (AML). The project will help to establish MRD as a biomarker in AML and will generate important molecular information that may help inform treatment decisions, and ultimately improve patient outcomes in the future.

Part Number	Description	Samples and Format	Application Compatibility
2140	Accukit™ Myeloid DNA IS, (fragmented)	100 samples 100ul @ 300 copies/ul	liquid biopsy or cfDNA
2023	Accukit™ Myeloid DNA IS, (unfragmented)	100 samples 100ul @ 300 copies/ul	bone marrow or FFPE

For more information: info@accugenomics.com
FOR RESEARCH USE ONLY

AccuGenomics Inc.
1410 Commonwealth Dr., Suite 105,
Wilmington, NC 28403
www.accugenomics.com
info@accugenomics.com
910.332.6522

AccuGenomics
A higher standard of accuracy

v3.04-2024