ctDNA Evaluation Set

Maximize insights from every sample with Sequins' internal standards

Background

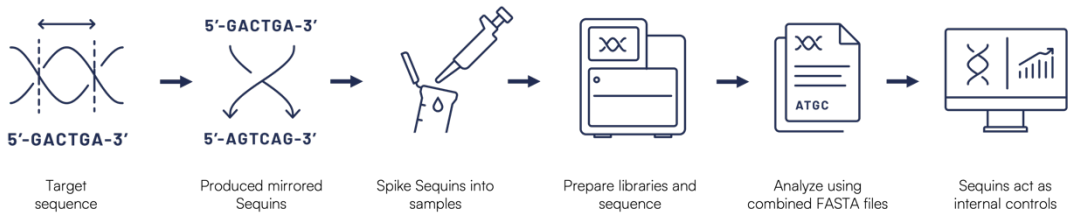
Next-generation sequencing (NGS) can be used to identify genetic variation and disease-associated mutations and has become a principal tool in biomedical research and clinical testing. However, numerous factors influence the accuracy of variant detection using NGS including sequencing depth, read length, sequencing errors, and PCR amplification biases introduced during library preparation. It is therefore imperative that a system of control standards is incorporated to correct for accumulated errors, improve data quality and interpretation, maximizing genomic insights.

Introduction to Sequins

Sequins™ (sequencing spike-ins) are synthetic nucleic acid reference controls that directly mirror naturally occurring sequences. Because Sequins retain the same nucleotide composition as the natural sequence, they enable accurate representation of genomic complexity without compromising integrity of the sample and results. Sequins perform equivalently throughout sequencing workflows, providing a true measure of control.

Sequins' innovative design enables production of synthetic mirrored sequences that directly represent almost any genomic feature, in any organism with a reference genome. This includes common and clinically relevant variants and analytically challenging regions of the genome. By combining sequins in precise ratios, quantitative features of genome biology, such as variant allele frequencies or copy-number variation, can also be emulated.

Sequins are simply 'spiked-in' to a sample prior to library preparation and together, progressed through a workflow. Sequins controls can then be distinguished from the native sample in the output library by virtue of their synthetic sequence enabling normalization and comparison between samples, runs, laboratories, chemistries, and sequencers.



Schematic showing the design and use of Sequins in an NGS workflow.

Sequins for Circulating Tumor DNA

Circulating tumor DNA (ctDNA) sequencing has fast become a powerful method for early detection, screening and recurrence monitoring of cancer. ctDNA testing leverages the ability to detect low concentration DNA fragments shed from cancer cells into the bloodstream. With minimal-invasiveness through blood draws and unbiassed by heterogeneity, “liquid biopsies” for ctDNA can quickly detect cancer-associated actionable variants, reveal information about cancer type and stage, and response to therapies. Using highly sensitive techniques, such as next-generation sequencing (NGS), ctDNA is analyzed to identify specific cancer-related mutations, genetic aberrations, and other molecular markers.

Liquid biopsies with ctDNA testing require high sequencing coverage and uniformity, coupled with high fragment depth. While ctDNA testing holds great promise, tumor type, mass, stage and background cell-free DNA, also make it challenging for accurate and reliable variant detection. Sequins internal control standards provide a powerful approach in addressing these challenges. By spiking in known quantities of synthetic controls representing target variants to individual samples, sequencing assays can be normalized and variant calls calibrated against internal benchmarks.

Benefits of Sequins for ctDNA

Quantification and Limit of Detection Calibration	Sequins representing synthetic cancer variants at varying allele frequencies provide a source of truth against which samples can be calibrated enabling confident and reliable identification of low-frequency variants.
Quality Control and Assay Validation	Sequins serve as an internal quality control and are subjected to the same technical influences and errors as the samples they are combined with, enabling evaluation and optimization of key parameters related to sequencing workflows and sample quality.
Standardization Across Labs and Studies	Spike-in controls enable normalization both within and between samples, workflows, and locations to enable unprecedented standardization and interoperability.

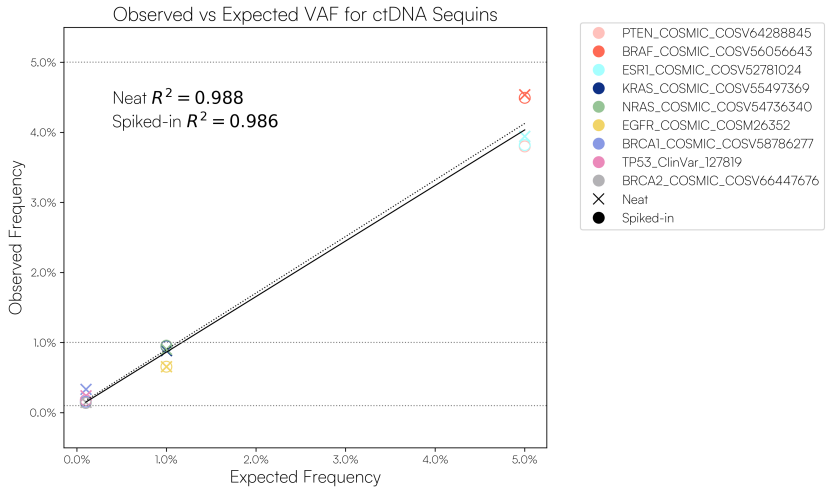
The Sequins ctDNA Evaluation Set targets nine clinically important cancer gene variants at precise variant allele frequencies and is applicable to a range of cancers. Sequins are pre-mixed in a single tube and can be spiked-in to each sample prior to library preparation and sequencing.

Sequins Evaluation Set content

Gene	COSMIC UID	Variant	VAF %
BRAF	COSV56056643	c.1799T>A (Substitution) | p.V600E Missense	5%
ESR1	COSV52781024	c.1613A>G (Substitution) | p.D538G Missense	5%
PTEN	COSV64288845	c.968dup (Insertion) | p.N323Kfs*2 Insertion/frameshift	5%
NRAS	COSV54736340	c.182A>G (Substitution) | p.Q61R Missense	1%
EGFR	COSM26352	c.? | p.V769_D770insASV (Insertion - In frame)	1%
KRAS	COSV55497369	c.35G>A (Substitution) | p.G12D Missense	1%
TP53	COSV52661282	c.659A>G (Substitution — Missense)	0.1%
BRCA1	COSV58786277	c.68_69del (Deletion) | pE23Vfs*17 (Frameshift)	0.1%
BRCA2	COSV66447676	c.5946del | p.S1982Rfs*22 (deletion — frameshift)	0.1%

The Sequins ctDNA Evaluation Set can be used as a backbone for further customization with additional gene targets through our on-demand program. We are releasing Sequins ctDNA Evaluation Set through our Evaluation Program. Please visit sequins.bio to find out more and register.

Sequins Performance



Observed versus expected variant allele frequencies (VAFs) for synthetic Sequin variants. Nine clinically important cancer gene variants representing varying allele frequencies (5%, 1% and 0.1% - see Sequins Evaluation Set content for details) were spiked into a contrived human cfDNA sample at 1% fractional abundance prior to library preparation. The ctDNA ladder delivers equivalent and reliable performance in both simple matrices (neat) and a complex human cfDNA background (spiked-in).

Contact Information

Ordering and Support

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Key Publications

Deveson, I.W., Gong, B., Lai, K. et al. Evaluating the analytical validity of circulating tumor DNA sequencing assays for precision oncology. *Nat Biotechnol* 39, 1115—1128 (2021).

Deveson, I.. Chen, W., Wong. T. et al. Representing genetic variation with synthetic DNA standards. *Nat Methods*. 13. 784- 791 (2016)

Hardwick SA, Deveson IW. Mercer TR. Reference standards for next-generation sequencing. *Nat Rev Genet*. 2017 Aug:18(8):473-484. doi: 10.1038/nrg.2017.44. Epub 2017 Jun 19. PMID: 28626224

Deveson, I.W., Madala, B.S., Blackburn, J. et al. Chiral DNA sequences as commutable controls for clinical genomics. *Nat Commun*. 10, 1342 (2019)

Blackburn, J., Wong. T., Madala, B.S. et al. Use of synthetic DNA spike-in controls (sequins) for human genome sequencing. *Nat Protoc*. 14, 2119-2151 (2019)