FLUXION

DATA SHEET

ERASE-Seq™ Variant Caller

Achieve unparalleled sensitivity and specificity from your liquid biopsy NGS data

Designed specifically for detection of mutations from liquid biopsy samples (ctDNA, CTCs), ERASE-Seq is a bioinformatics software solution that provides ultra-sensitive variant detection from sequencing data sets, including off-the-shelf and custom targeted panels. Currently validated on three commercial panels (Spotlight 59, Swift 56G, and Illumina TruSight Tumor 15), ERASE-Seq can be applied to any existing or custom-developed panel. Custom panels are easily validated via Fluxion's ERASE-Seq Validation Program (EVP).

How Does ERASE-Seq Work?

ERASE-Seq (Elimination of Recurrent Artifacts and Stochastic Errors) functions by quantitatively modeling the background error profile of normal controls that have gone through the library prep and sequencing process in order to establish the inherent noise distribution for each possible variant. The background modeling is done once and then embedded in the ERASE-seq algorithm. Variant calls are then made by comparing sample sequencing data to the background error distribution, providing large increases in both sensitivity and specificity. To further improve detection sensitivity, samples can be split into two or more reactions to allow statistical analysis between the replicates as well as to the background model.

Key Benefits

- Detection to <0.1% allele frequency
- False positive rates <0.1 FP per sample
- No MID (molecular IDs) required; increases sensitivity, reduces bias and assay complexity
- Can be adapted to any panel; use the same panel for both tissue biopsy and liquid biopsy samples
- Ideal for challenging sample types, including ctDNA, CTCs, and FFPE
- Flexible: results can be tailored based on desired performance, DNA input, and sequencing costs
- Simple cloud-based interface; upload fastq files and retrieve an Excel report and VCF file within 24 hours

The use of this inter-sample statistical analysis, as opposed to the more typical intra-sample QC metrics, provides substantial performance gains. This approach eliminates both random errors and systematic biases inherent in all sequencing workflows. ERASE-Seq delivers superior detection sensitivity to 0.05% allele frequency (AF), with a false positive rate 10-100X lower than leading molecular barcode approaches[1]. ERASE-Seq analysis is accessed by uploading raw fastq files via secure upload to a HIPAA-compliant cloud-based server. VCF and Excel variant reports are available for download within 24 hours. Local software installations are also available.

ERASE-Seq Performance

ERASE-Seq can be adapted to the desired level of detection sensitivity, DNA input, or sequencing data limits. A validated panel run in "standard" 2-replicate mode will deliver 90% sensitivity at 0.1-0.2% AF depending on the variant, with a false positive rate below 0.1 FP per sample. ERASE-Seq significantly outperforms typical molecular barcode approaches, which achieve 80% sensitivity and contain 3-5 SNV and 5-10 indel false positive calls at 0.5% AF. See the typical ERASE-Seq performance table on page 2 for details. ERASE-Seq is also easily configurable to focus on specific variant targets to further improve sensitivity and specificity.

Typical ERASE-Seq Performance

	Standard	Background Correction Only	Ultra-sensitive
90% Sensitivity LOD	0.1-0.2%	0.25-0.5%	0.05-0.1%
False Positives at LOD	0.1	0.1	0.1
DNA Input Requirement	20ng	10ng	40ng
Replicates (reaction tubes)	2	1	4
Overall Sequencing Depth	10,000X	5,000X	20,000X

Product Specifications

Feature	Specification
Input Data Required	fastq files at 5000x mean read depth
DNA input quantity	10ng per replicate (single-tube reaction panels)
Replicates	1, 2, or 4 technical replicate runs per sample, see performance table
Coverage Uniformity Required	Uniformity of > 90% at > 20% of mean, 5,000x mean read depth
Sensitivity (for base substitutions and indels)	>90% at 0.25% allele frequency, single replicate >90% at 0.1% allele frequency, 2 replicates >90% at 0.05% allele frequency, 4 replicates
Specificity	<0.1 false positives per sample (specificity >99.99%), for standard 2 replicate ERASE-Seq
Commercially-Available Validated Panels*	Fluxion Spotlight 59, Swift 56G, Illumina Trusight Tumor 15 (validated for use on Illumina MiSeq, HiSeq, NextSeq, ISeq)
Turnaround time & reporting	24 hours; pdf report is provided along with VCF variant file

*Other panels can be validated through Fluxion's ERASE-Seq Validation Program.

Ordering Information

Description	Part Number
ERASE-Seq Analysis (10 sample pack)	990-0038
ERASE-Seq EVP Validation (Validation of standard or custom panel for ERASE-Seq pipeline analysis. Requires submission of normal control NGS data of expected sample type- FFPE, ctDNA, or gDNA)*	990-0040

*Contact Fluxion for details. Custom panel development is also available.

1. Kamps-Hughes et al. "ERASE-Seq: Leveraging replicate measurements to enhance ultralow frequency variant detection in NGS data." *PloS One* 13.4 (2018): e0195272.

FLUXION

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