

# Spotlight 59<sup>TM</sup>

## ERASE-Seq Cloud-based Analysis

### Instructions for Use

Document No. 630-0145

Revision A

For Research Use Only

Not intended to treat or diagnose any disease condition



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## INTENDED USE

The ERASE-Seq cloud based analysis has been designed for the ultra-sensitive detection of variants present in liquid biopsy samples, including CTCs and ctDNA. It is needed for the analysis of data generated using the Spotlight 59 Oncology Panel. Please contact [support@fluxionbio.com](mailto:support@fluxionbio.com) for instructions on how to apply the analysis pipeline to data generated using panels other than Spotlight 59.

The ERASE-Seq Cloud-based analysis is for Research Use Only.

## SUMMARY AND EXPLANATION

ERASE-Seq is a computational pipeline designed to work with Spotlight 59 data. After customer registration, secure storage containers are provided for fastq data upload, and, after analysis completion, for results file download.

The computation is performed in a secure and HIPAA compliant cloud based analysis system. The ERASE-Seq caller relies on a large number of healthy normal controls that established background error rates at each position in the Spotlight 59 panel. Values from sample sequencing runs are compared by the caller to the background error map values, eliminating both recurring artifacts and stochastic errors.

For more detailed information, please find links to our Spotlight 59 application note here: <http://fluxionbio.com/isoflux-products/>

## FLUXION PART NUMBERS USED:

### Spotlight 59 Oncology panel

## REQUIRED MATERIALS

- Fastq NGS result files
- Internet connectivity with upload speeds > 5MBPS

## WARNINGS AND PRECAUTIONS

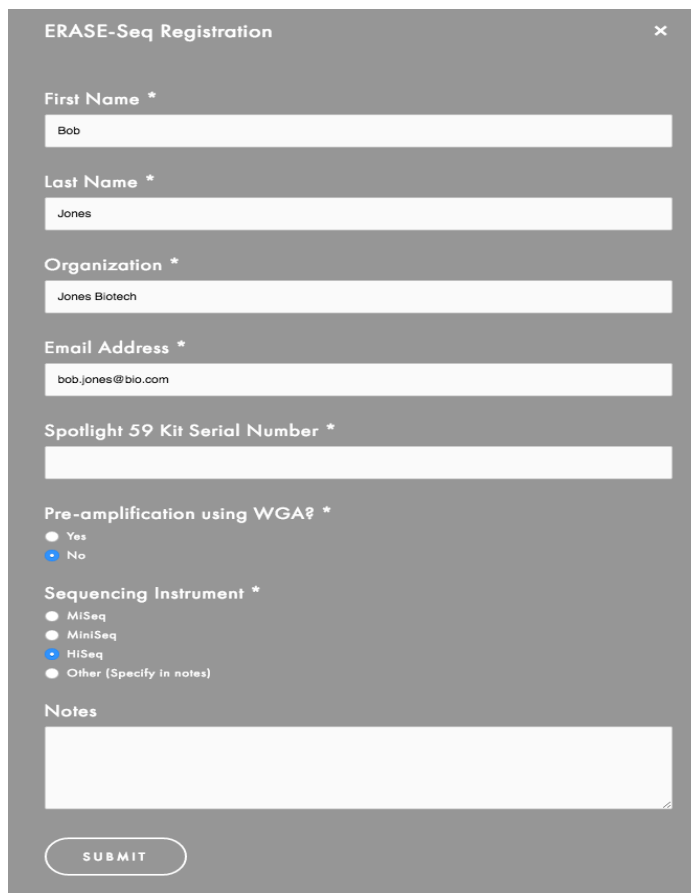
- For Research Use Only
- Please read the entire contents of the Instructions for Use before processing samples.

**DATA UPLOAD****1. Registration**

- 1.1. Navigate to: [www.fluxionbio.com/erase-registration](http://www.fluxionbio.com/erase-registration)
- 1.2. Hit the 'ERASE-SEQ REGISTRATION' button:



- 1.3. Enter the registration information:



ERASE-Seq Registration

First Name \*  
Bob

Last Name \*  
Jones

Organization \*  
Jones Biotech

Email Address \*  
bob.jones@bio.com

Spotlight 59 Kit Serial Number \*

Pre-amplification using WGA? \*  
 Yes  
 No

Sequencing Instrument \*  
 MiSeq  
 MiniSeq  
 HiSeq  
 Other (Specify in notes)

Notes

SUBMIT

1.4. All except the 'Notes' fields are required. The email provided will be used for notification and retrieval of the pipeline results (i.e. variant report). Explanations for some of the fields can be found below:

**I. Spotlight 59 Kit Serial Number:**

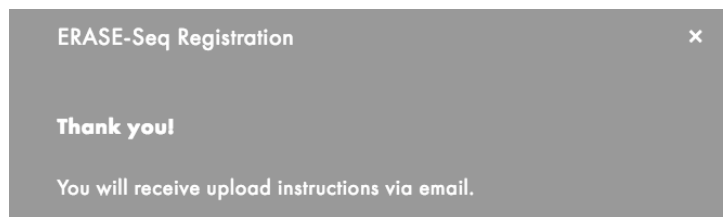
- Kit customers can access the computation pipeline free of charge; this number is checked against our database of kits sold
- **Please note:** data obtained using different amplification panels has different noise criteria; data obtained without the use of our Spotlight59 panel (and /or not following our instructions for use for the panel) will yield erroneous results. If you'd like to use ERASE-Seq but have data from a modified panel or protocol, please contact Fluxion support

**II. Pre-amplification using WGA?**

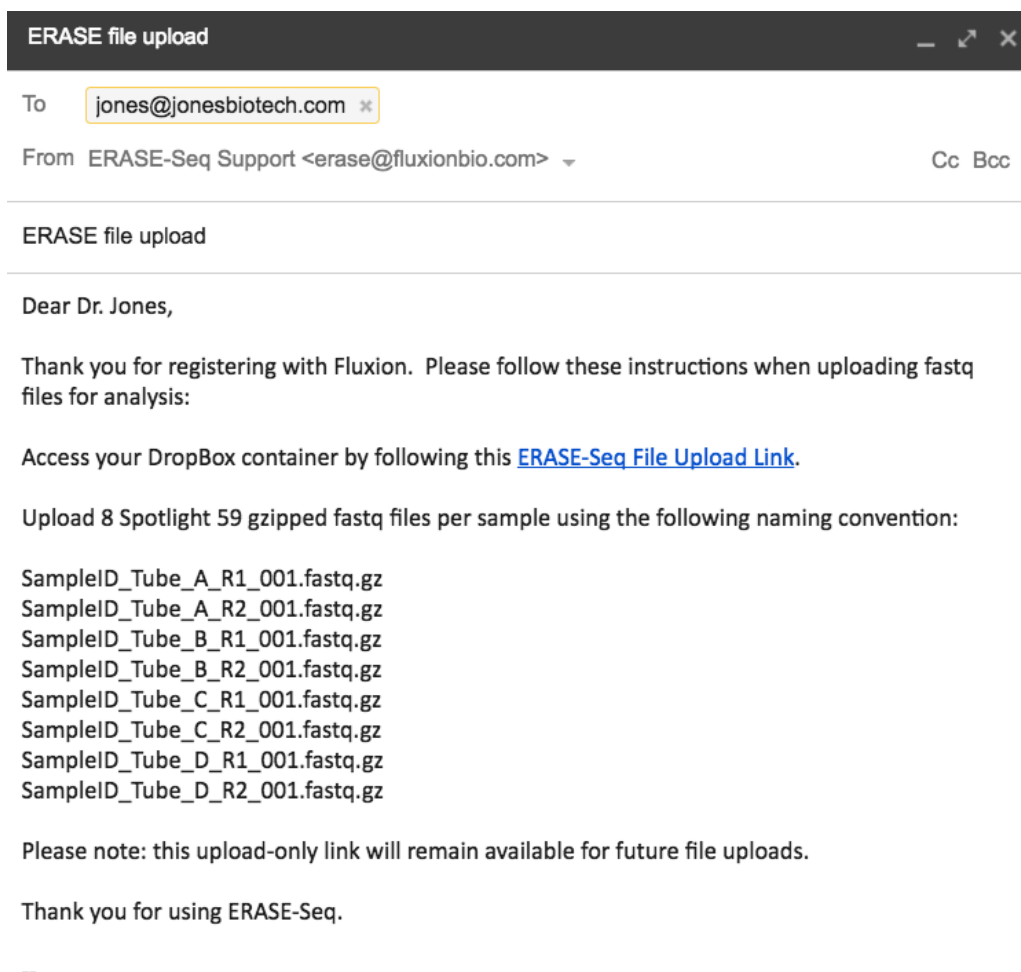
For samples that have been pre-amplified using a WGA reagent (for example, samples resulting from the use of the IsoFlux NGS Kit) both the computational parameters and pre-determined error map are different, choose 'Yes'. Choose 'No' for all other samples (that required no pre-amplification before the Spotlight 59 targeted amplification step).

### III. Sequencing Instrument.

The Spotlight 59 pipeline has only been validated for the instruments listed. Please choose the one used to generate the data. For other instruments, additional information may be required, and the performance may differ. Please specify the instrument used in the 'Notes' section in this case.



- 1.5. Once the registration is complete, a private DropBox storage folder will be created that is customer specific and will be associated with the email you provided.
- 1.6. You will receive an email request for fastq files to be uploaded to your storage area:



## 2. File upload

- 2.1. Upload all 8 fastq files per sample, using the appropriate file name nomenclature:

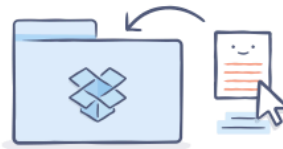


Sign in ▾

 ERASE Seq is requesting

Jones\_JBiotech

 Only ERASE Seq will see these files unless they choose to share them.



Choose files

[How does this work?](#)

- 2.2. Dropbox is a leader in cloud storage security, and has all of the safeguards in place to be HIPPA compliant. Fluxion and DropBox have a signed BAA on file. All data is encrypted during the upload process.


- 2.3. Multiple files can be uploaded simultaneously:



Sign in ▾

 ERASE Seq is requesting

Jones\_JBiotech

 Only ERASE Seq will see these files unless they choose to share them.

8 files · 1 GB



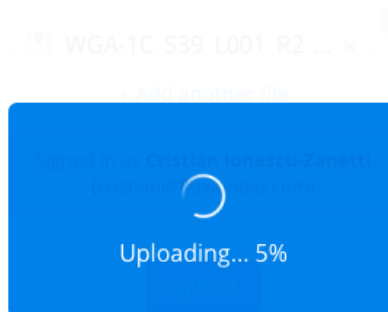
[+ Add another file](#)

First name  Last name

Email address

Upload

- 2.4. For a typical run, upload times will be between 20 minutes to 1 hour per sample, depending on your local upload speed:



- 2.5. We recommend letting the upload complete in one session. If the upload is interrupted for any reason, it will resume when the computer re-connects to the internet.

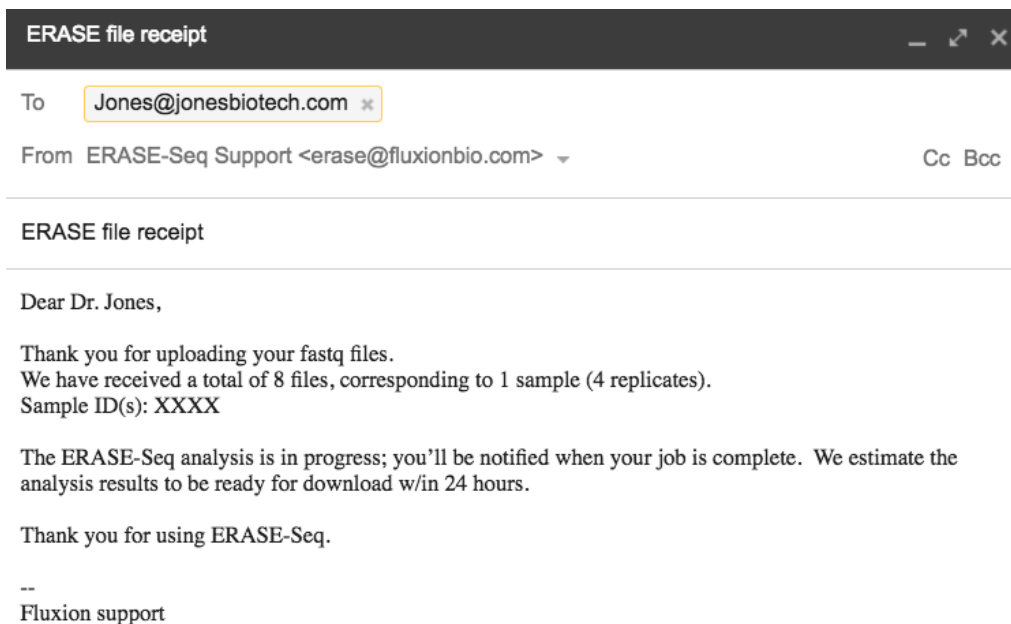
Thanks! You're all done.  
Cristian Ionescu-Zanetti will be notified of your submission. Want to request files from people you know?  
[Learn more about file requests](#)



- 2.6. Please note that this email link can only be used to upload data. A separate, limited time link, will be provided for downloading the resulting reports. After report retrieval, the raw data originally will be erased.

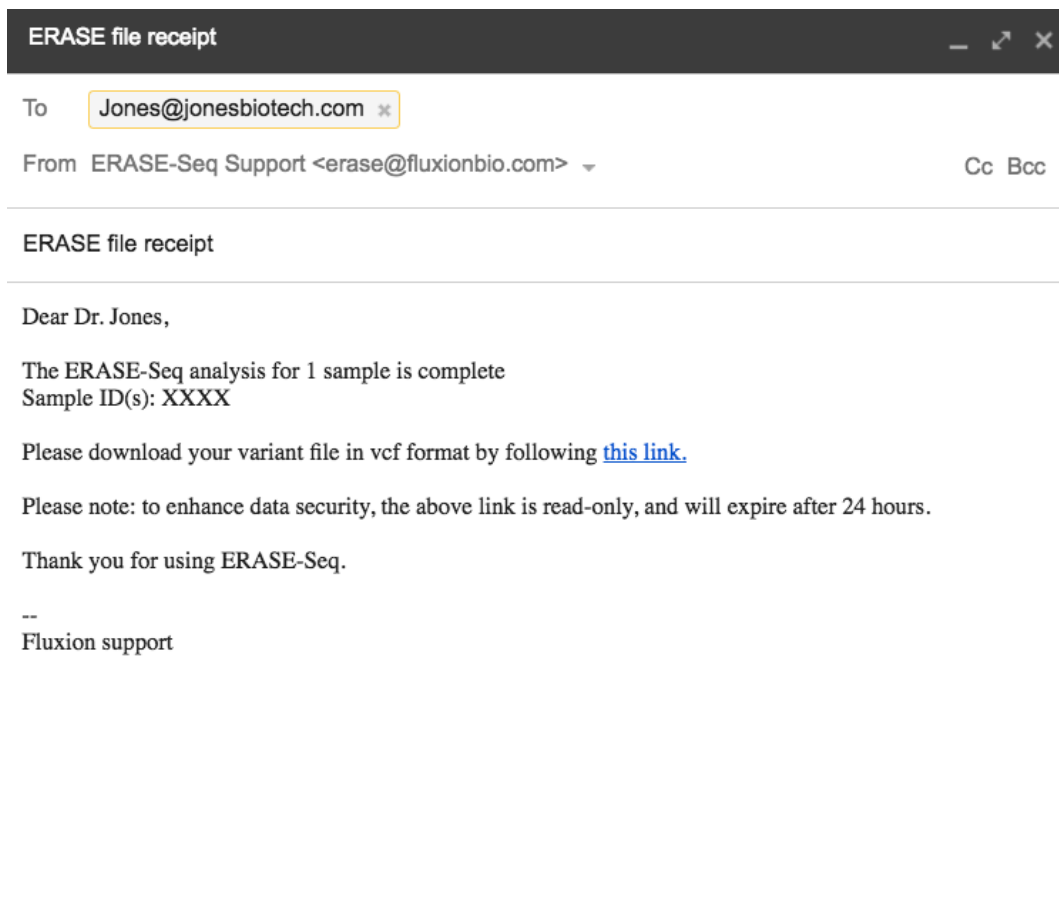
## 2. Retrieving the run results

- 2.1. Within one working day of upload completion, you will receive a message acknowledging receipt of your files and an expected timeline for ERASE-Seq result file generation:



- 2.2. When the analysis is complete, you'll receive a second email containing a link for file download. The link will only be valid for 24 hours:





2.3. Your results delivery area will contain two different one or more .vcf files containing a list of the variants detected. Each vcf file corresponds to one of the uploaded samples. The .vcf file can be used to import variants into third party reporting software. The data Quality Control results (coverage, uniformity) are included in the .vcf file header.

2.4. For any questions about the process, please feel free to contact Fluxion support, or [erase@fluxionbio.com](mailto:erase@fluxionbio.com)

**SUPPORT**

For assistance with this product, please contact Fluxion Biosciences through one of these methods:

**Email:** [support@fluxionbio.com](mailto:support@fluxionbio.com)

**Phone:**

Toll Free USA +1 (866) 266-8380

International +1 (650) 241-4777

**WWW:**

[www.fluxionbio.com](http://www.fluxionbio.com)