My Nonacus User Guide V3.3

This document guides users through setting up and using MyNonacus GUI for sample processing and result management in an organized and step-by-step manner.

Table of contents

GALEAS™ / nonacus

1. Introduction

The document explains how to setup on the MyNonacus software and submit samples for processing. It covers:

Initial One-Time Setup:
- Account Registration: Guidance on creating an account on the MyNonacus including login procedures.
- Software Installation: Steps to download and install the necessary desktop applications, such as the MyNonacus Uploader and file upload/download scripts.
- Configuration: Instructions for completing/creating required files, installing python scripts, prerequisites like Python and necessary libraries and preparing templates like the Analysis Request File (ARF).

Batch Sample Submission:
- Detailed procedures for preparing and submitting batches of samples using the GALEAS software.
- Instructions on how to complete and use the ARF for batch uploads.
- Steps for monitoring batch processing, receiving email notifications, and downloading results once processing is complete.
- Guidelines on using the Batch Management tab, downloading individual results or automating downloads with the download script.

One Time Steps
• Create a MyNonacus account • Download the Desktop sample upload applciation • Download example ARF template • Download File upload script • Download Batch result files download script

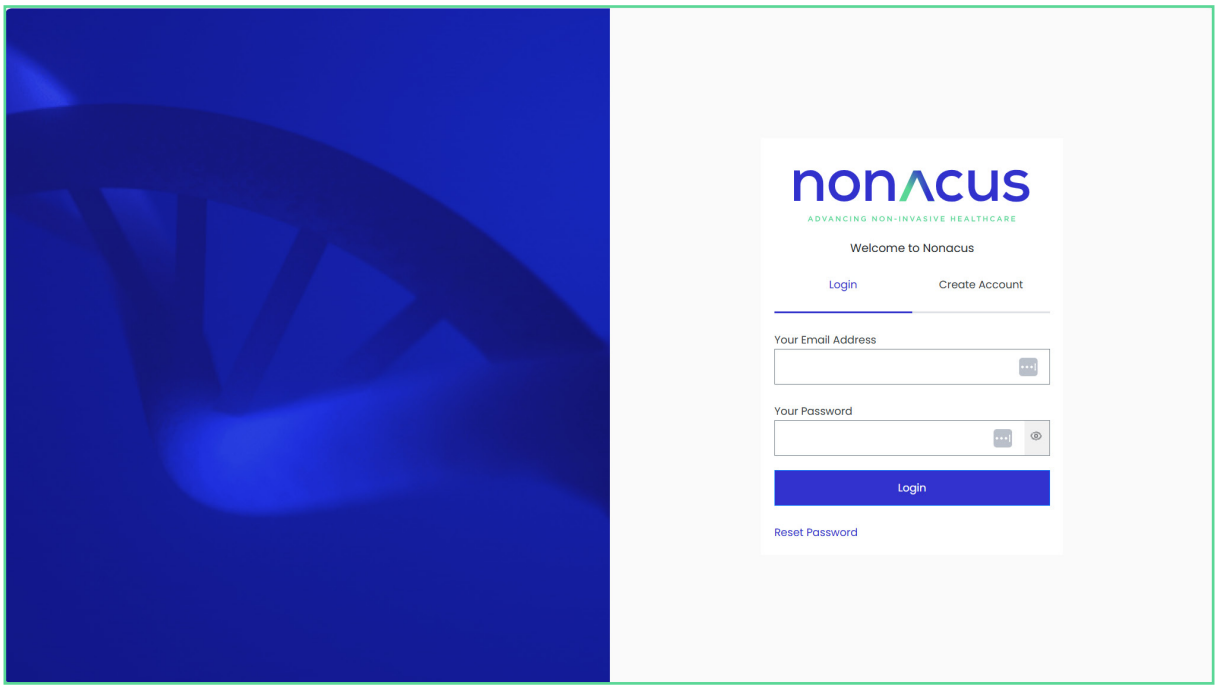
Batch Sample Processing
• Login via Desktop sample upload application • Create an ARF for the batch of samples • Use the Dekstop application to upload samples • Read the Batch notification email • Login in to MyNonacus • Download the results of the sample processing

2. Account Registration

To register for a 'MyNonacus' account on www.nonacus.com, open your preferred web browser and navigate to the site. At the top right of the homepage, click the 'My NONACUS' logo.

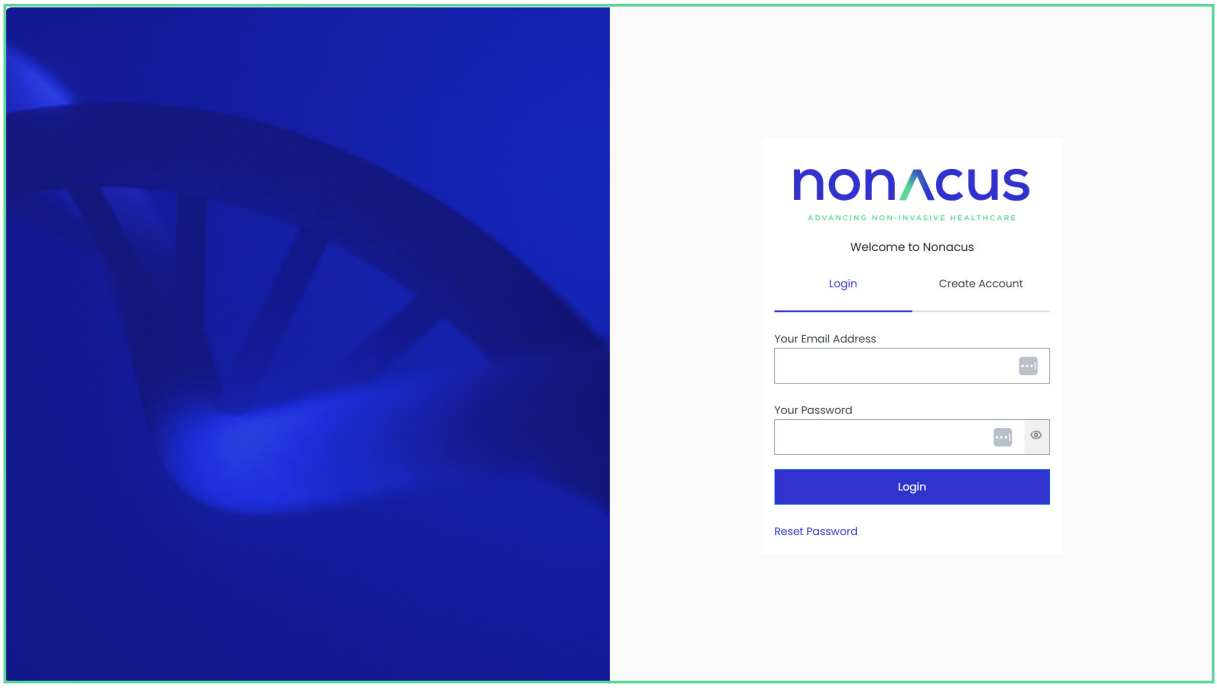
Select the Create Account Tab. Fill out the registration form with your personal details, such as your name, email address, and a secure password, then submit the form. Follow any subsequent prompts, such as email verification, to complete your registration.

After you have registered, you can request access to GALEAS in your Account by sending a mail to **support@nonacus.com.** Your account will be associated with the GALEAS software, which enables you to upload & process patient samples (in FASTQ format) and download the bioinformatic results.



3. Login to MyNonacus

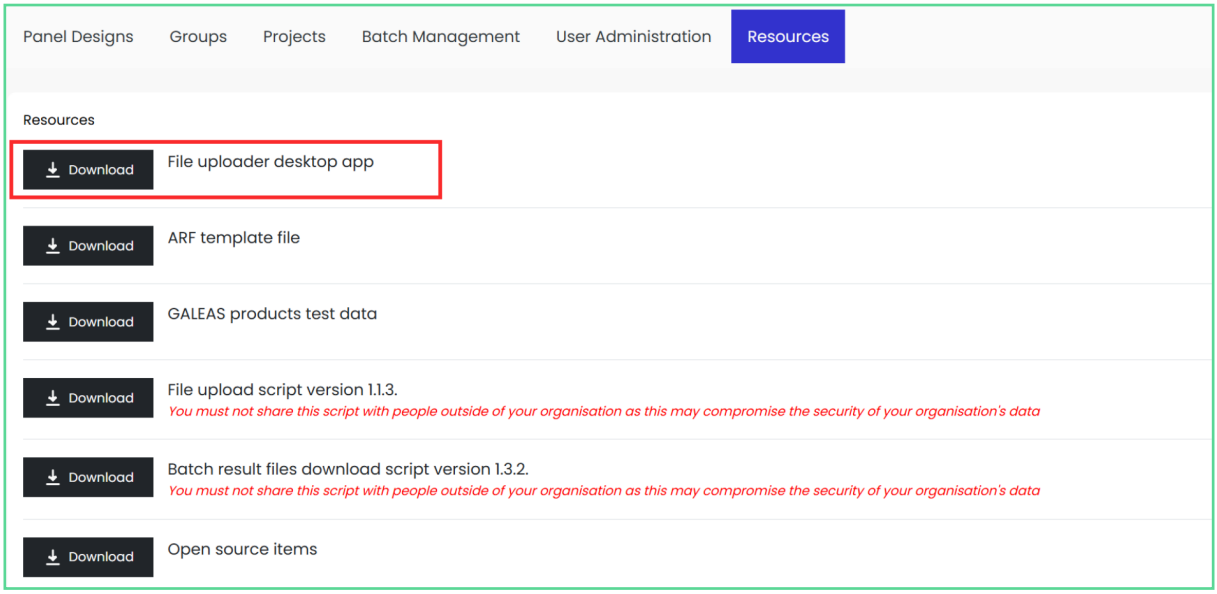
Once the registration complete, go to **mynonacus.nonacus.com** via your preferred web browser to login, where you can design Custom Panels, view Catalogue Panels and analyse patient samples.



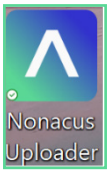
4. Software and Analysis Request File (ARF) Download

4.1. Download and Install the File uploader desktop app

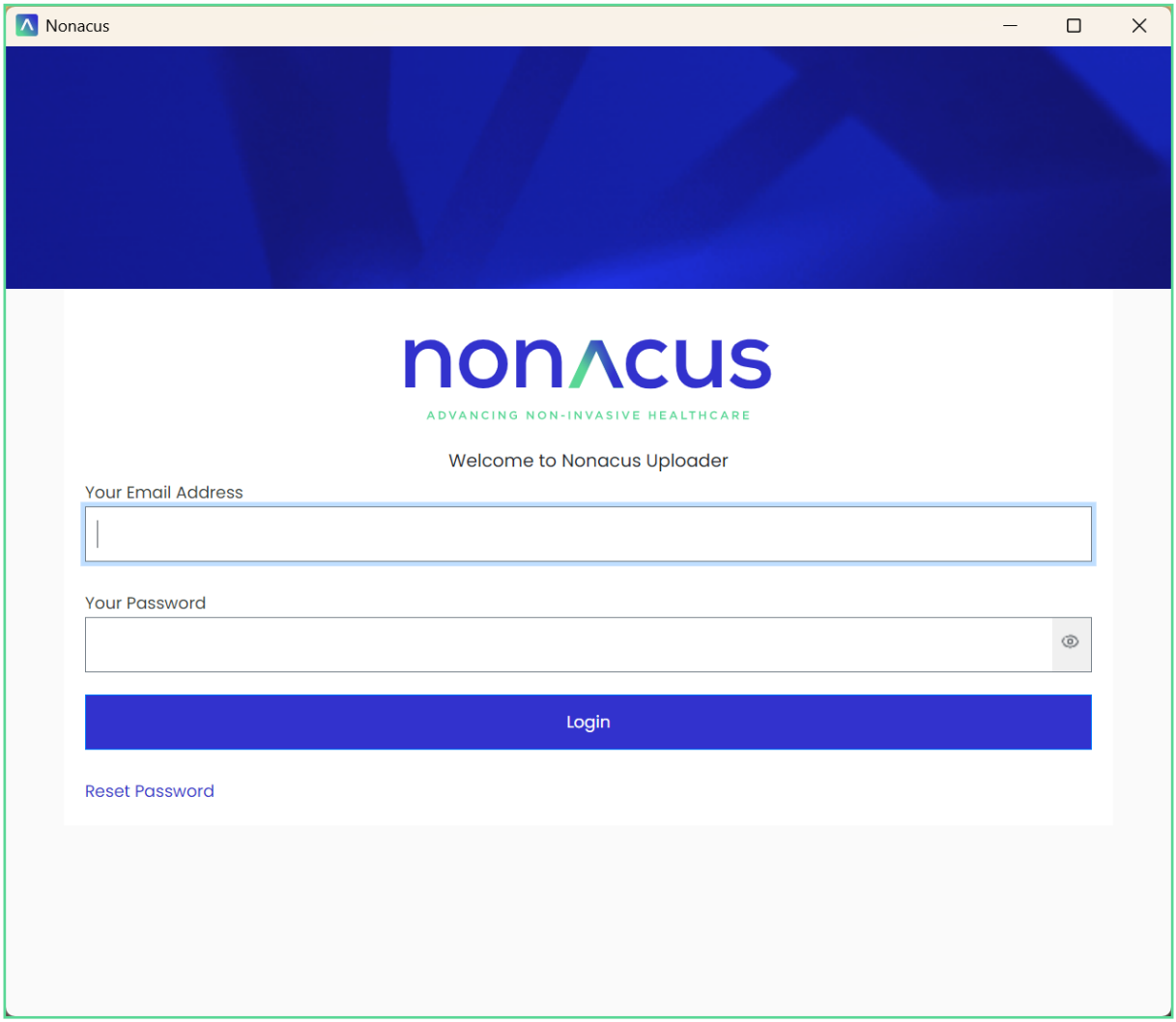
To upload FASTQ files, start by downloading the Desktop File Uploader from the Resources tab on the MyNoncus GUI, Open the Resources tab and locate the Download button for File uploader desktop app. Click **Download** to begin downloading the installation file to your computer. Please note that this application only works on Microsoft Windows platforms.



Once the download is complete, open the file to start the installation process. Follow the on-screen prompts to install the Nonacus Uploader application onto your PC. After installation, you can launch the application by clicking the shortcut icon on your PC:

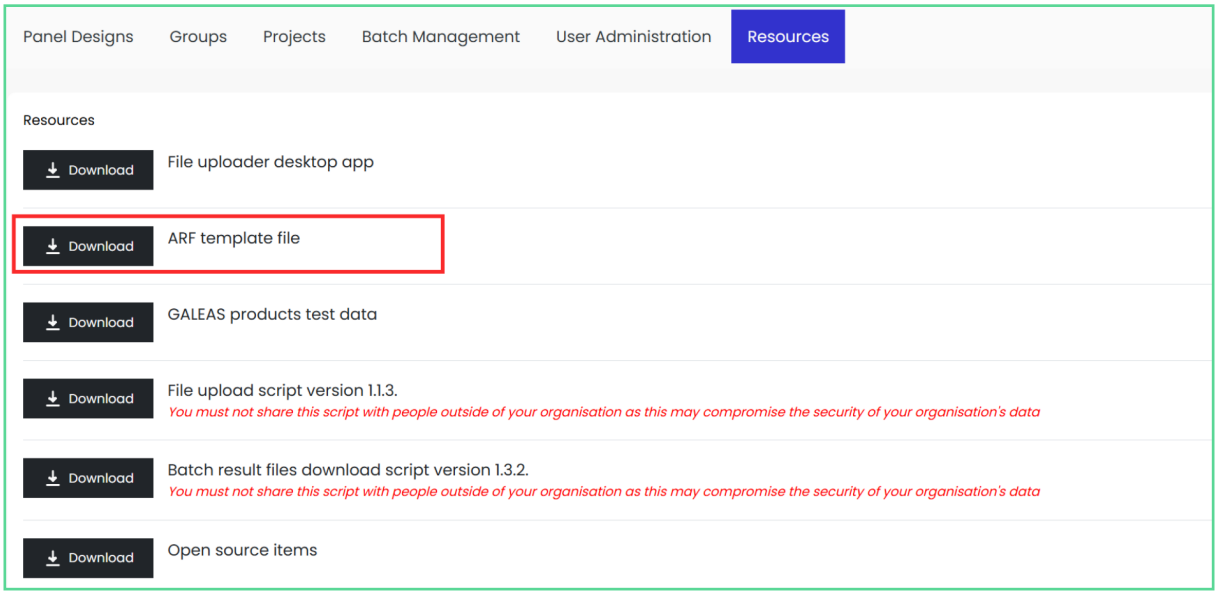


Login page for Nonacus Uploader as below, same login credentials used for MyNonacus.

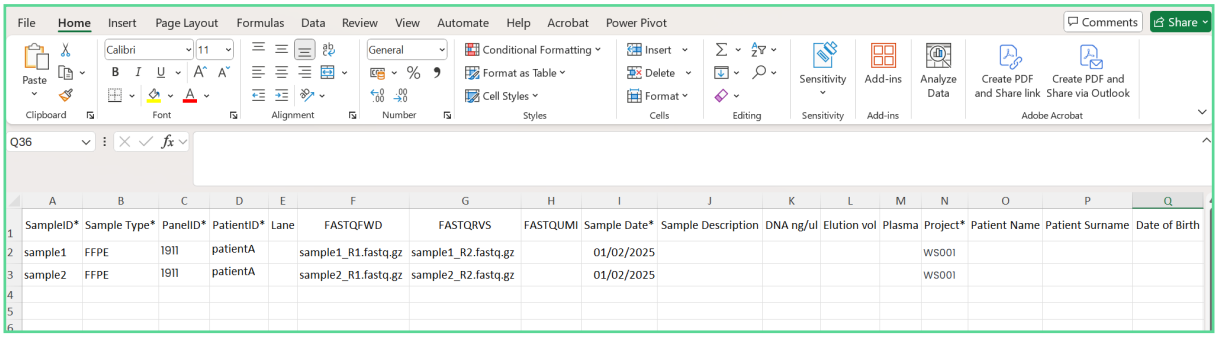


4.2. Download Example Analysis Request File (ARF) file

A SampleSheet instructs an Illumina sequencer on how to process physical samples, whereas the Analysis Request File (ARF) informs the GALEAS software which FASTQ files to upload, how to associate them with specific patients in the system and run the appropriate bioinformatics pipeline. The ARF file is essential for performing a batch upload. An example of an ARF file can be downloaded from the MyNonacus GUI, by clicking on the resources tab and then clicking on "ARF Template file" 'download' link. An ARF file is required for a batch upload.



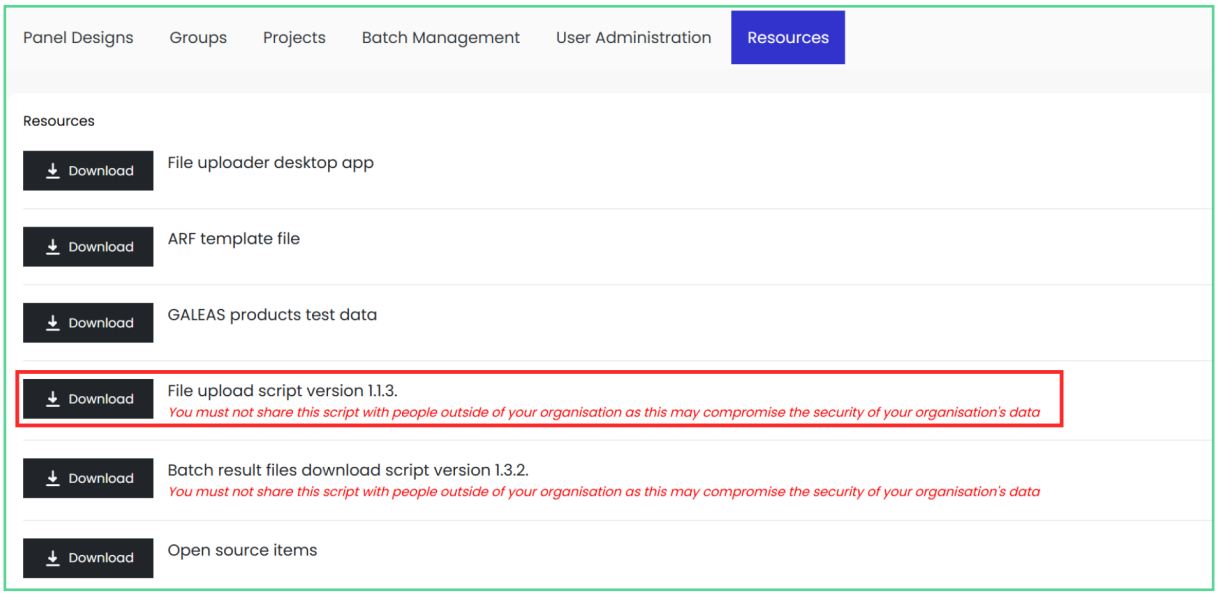
A screenshot of the ARF is as follows;



SampleID*	Sample Type*	PanelID*	PatientID*	Lane	FASTQFWD	FASTQRVS	FASTQUMI	Sample Date*	Sample Description	DNA ng/ul	Elution vol	Plasma	Project*	Patient Name	Patient Surname	Date of Birth
sample1	FFPE	1911	patientA		sample1_R1.fastq.gz	sample1_R2.fastq.gz		01/02/2025					WS001			
sample2	FFPE	1911	patientA		sample2_R1.fastq.gz	sample2_R2.fastq.gz		01/02/2025					WS001			

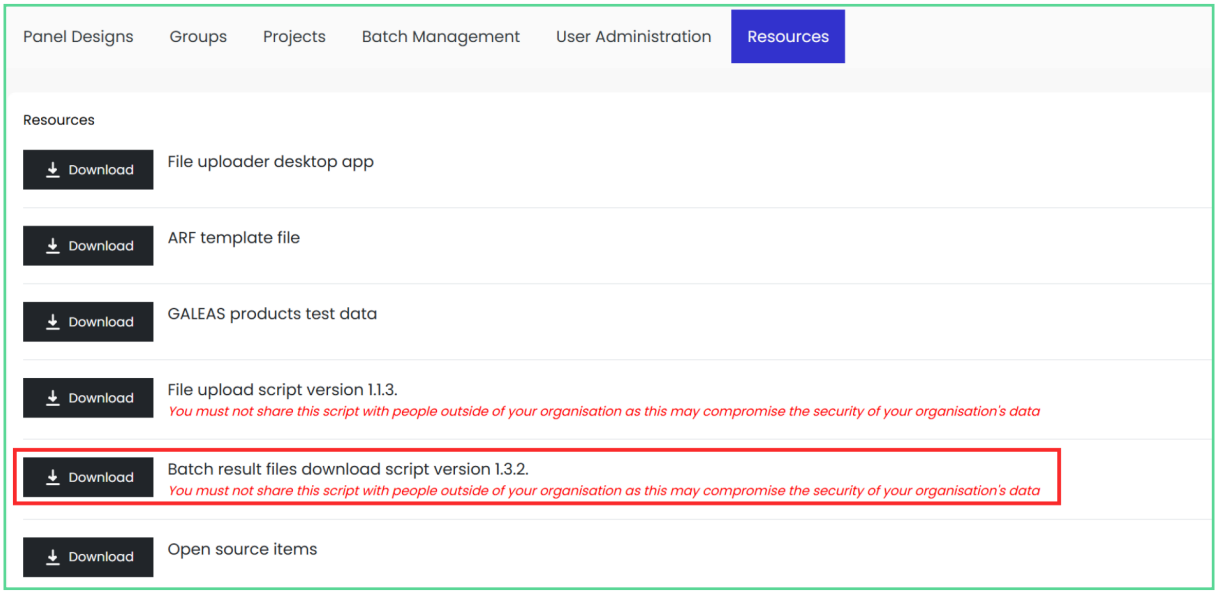
4.3 Download File Upload Script: **ARFUpload.py**

- Click Download button as shown below for File Upload script (ARFUpload.py).
- Depending on your browser settings, you may be prompted to choose a location to save the file or it may automatically download to your default Downloads folder. If prompted, select a preferred directory on your computer where you want to save the script.
- Click Save to confirm the download location.



4.4 Download File download script: **GaleasDownload.py**

- Click Download button as shown below for Batch result files download script GaleasDownload.py

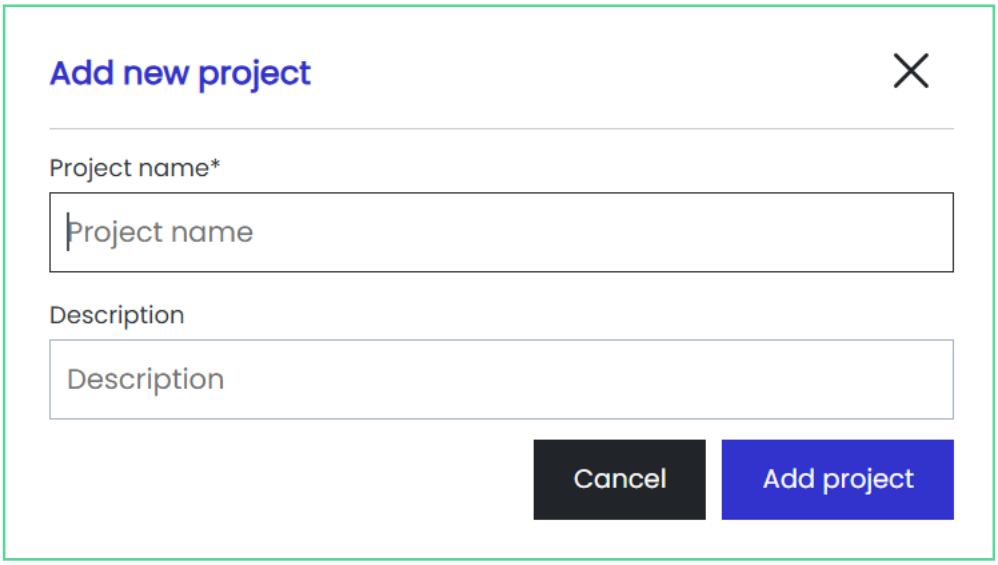


- Depending on your browser settings, you may be prompted to choose a location to save the file or it may automatically download to your default **Downloads** folder. If prompted, select a preferred directory on your computer where you want to save the script.
- Click **Save** to confirm the download location.

5. Create a project

Projects group patients together and are associated with a customer in the GALEAS system, allowing any user belonging to that customer to view all related projects. Each patient can belong to **ONLY** one project, and while a 'default' project exists for each customer, it is recommended to create additional projects to keep patients well organized.

To create a new project, click on the "Projects" tab. On this page, you will see a list of any existing projects as well as a list of patients for the most recently selected project, if applicable. Locate and click the "+ Add Project" button to start creating a new project. Follow the on-screen prompts to name and configure the project as needed. This process will help you effectively group and manage patients under the appropriate projects within the system.



5.1 Create an ARF for Batch Sample Processing

Overview ARF File
The Analysis Request File (ARF) instructs the MyNonacus software which FASTQ files to upload and how to associate them with a specific patient in the system. Below is a detailed explanation of the ARF format, including its structure and required fields, followed by an example of a completed ARF.

Patient Privacy
It is essential to exclude any Personally Identifiable Information (PII) from the ARF. Use only nonidentifiable IDs to protect patient privacy and comply with data protection regulations.

ARF File Generation
The ARF follows a specific format with defined fields.

- Use the ARF template downloaded in section 4.2. By ensuring that all mandatory fields are included and correctly formatted.
- Save file as with excel format (.xlsx)

An format ARF is shown below.

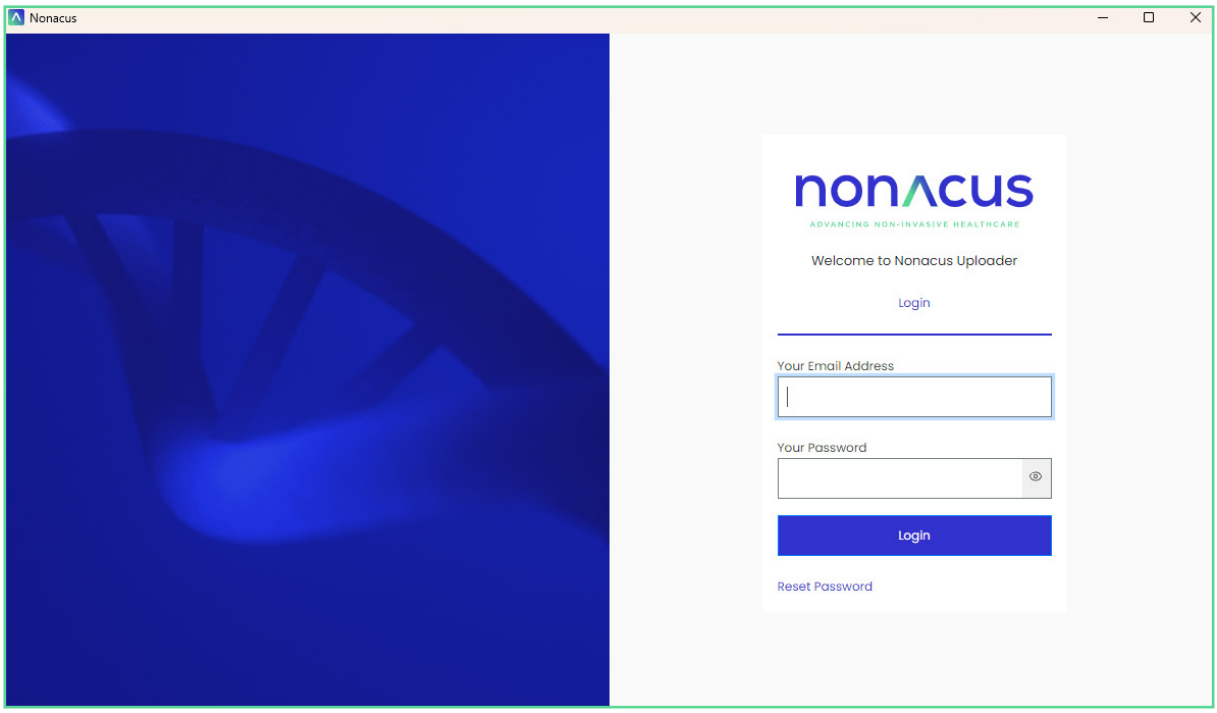
Column Name	Mandatory / Optional	Description
SampleID*	Mandatory	A mandatory field, that must be unique, to identify the sample in the system. The FASTQ filename must contain the sampleID in it, to enable the application to 'find' the FASTQ file(s) and associate it with this sample when the filenames are not explicitly provided in the 3 filename columns below (FASTQ Forward filename etc.). The system will automatically work out whether the R1, R2 (and R3) are forward, reverse or UMI files.
Sample Type*	Mandatory	A mandatory field which must be one of the following; • FF • FFPE • Blood • Urine Pellet • Saliva Depending on the Panel being used
PanelID*	Mandatory	A mandatory field, this is the panel associated with the sample, the ID's are as follows; **1821** for GALEAS Bladder **1969** for GALEAS Hereditary Plus **1911** for GALEAS Tumor **2359** for GALEAS Tumor HRD
PatientID*	Mandatory	A mandatory field this is the unique ID for the patient – it can be an existing ID, in which case the sample will be associated with that patient in the system, or it can be a new ID, in which case a new patient will be created with the details supplied in the ARF.
Lane	Optional	Optional field to be used when the sequencer produces FASTQ files for each lane, in which case the filenames below must be provided for each lane specified here (lanes 1-4). If the sequencer produces multi-lane files (most common) then this field can be left blank.
FASTQ Forward filename (.1)	Optional	Optional field which specifies the filename of the FASTQ file for forward reads e.g.Sample01.1.fastq.gz
FASTQ Reverse filename (.2 or .3)	Optional	Optional field which specifies the filename of the FASTQ file for reverse reads e.g. Sample01.3.fastq.gz
FASTQ UMI filename (.2)	Optional	Optional field which specifies the filename of the FASTQ file for UMI e.g. Sample01.2.fastq.gz

Column Name	Mandatory / Optional	Description
SampleDate	Mandatory	Mandatory field to specify when the sample was taken
Sample Description	Optional	Optional description field for the sample
DNA ng/ul	Optional	Optional field specifying the amount of DNA in ng per ul
Elution vol	Optional	Optional field specifying the elution volume
Plasma	Optional	Optional field specifying the plasma volume
Project*	Mandatory	This field is optional if a patientID is already associated with a Project. If the patientID is new (i.e. not an existing patient) then this is a Mandatory field specifying the project to associate the patient with. The project must be created in the system prior to sample upload & processing.
First Name	Optional	Optional field specifying patient first name
Surname	Optional	Optional field specifying patient surname
Date of Birth	Optional	Optional field specifying the DoB of the patient
Cancer Diagnosis/ Conditions	Optional	Optional field, specifying the disease/condition of the patients
Comment	Optional	Optional field for a comment
Clinician Forename	Optional	Optional field for the requesting clinician first name
Clinician Surname	Optional	Optional field for the requesting clinician surname
Clinician Address Line 1	Optional	Optional field for the requesting clinician address (1st line)
Clinician Address Line 2	Optional	Optional field for the requesting clinician address (2nd line)
Clinician City	Optional	Optional field for the requesting clinician city
Clinician Country Name	Optional	Optional field for the requesting clinician country
Clinician Postcode	Optional	Optional field for the requesting clinician postcode
Received Date	Optional	Optional field specifying the date the sample was received at the lab

5.2 Upload Sample to MyNonacus

Using Nonacus Uploader requires you to login refer to steps in section 4.1 in this document.

The desktop application will then allow you to upload a single sample ("Single Sample Upload tab") or a batch of samples using the Batch Upload tab – see next section "Choose the 'batch-upload' tab for multiple files using the ARF".



5.3 Uploading a Batch of Samples using Nonacus Uploader

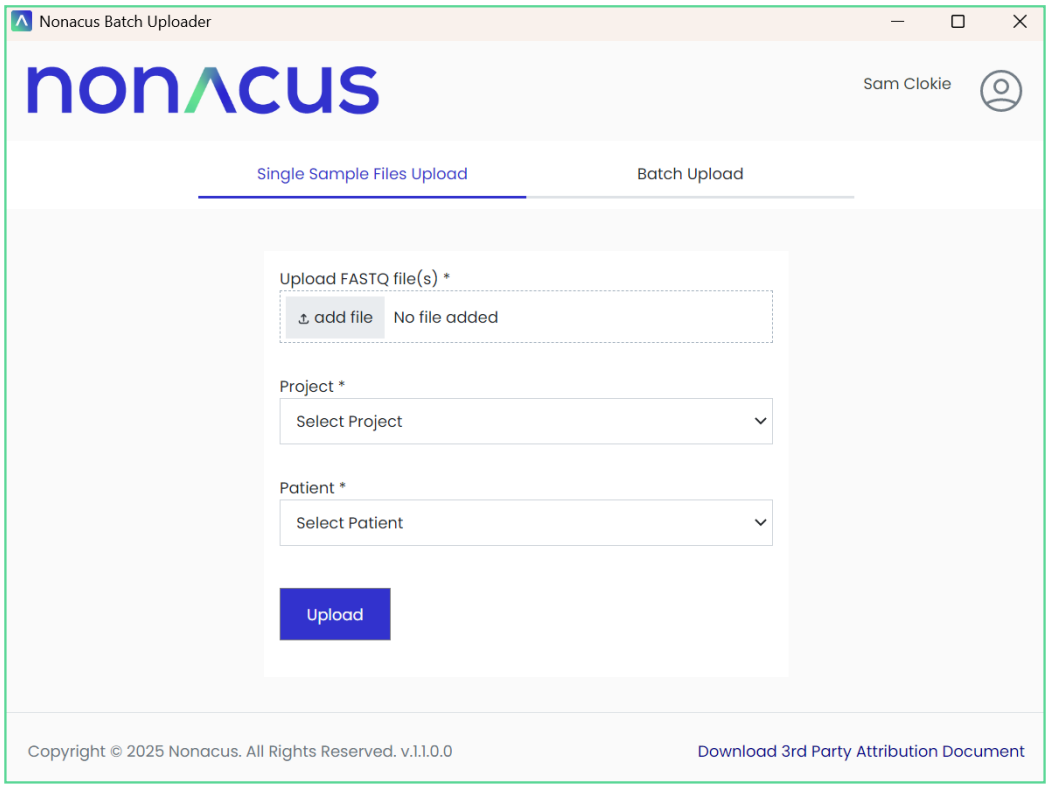
Choose the **Batch Upload** tab in the Nonacus Batch Uploader

Under **Analysis Request File—add file,** select your ARF to upload. Then under **Sample Files Folder –add file** to select the folder of the fastq files for uploading.

Finally click on **Upload** button. MyNonacus will then perform some checks, such as ensuring all the samples you've listed in the ARF have corresponding FASTQ file(s) in the location you've specified. If there are any errors, a message will be displayed informing you what the issue is and how to fix it.

Uploading even a small number of FASTQ files can take hours, depending on the nature of the samples (e.g. small panel versus Exome) and your network/internet speed. Please leave the application running until it says it has completed the upload.

Once the samples have been uploaded, the MyNonacus system will process the files, and again this process could take several hours.
You will receive an email notification when the processing is complete – see section 6 "Batch Notification Email."



5.4. Batch upload using the File upload script

To streamline and automate sample processing, you can use the File Upload Script obtained in section 4.3.
This script can be executed from the command line, allowing you to process batches of samples without manual interaction with the desktop application.

NOTE: The script is designed to work on both Windows and Linux machines

5.4.1. Pre-requisites for Running the File Upload Script

5.4.1.1 Install Python (v3 or later)

Download and install the latest version of Python from https://www.python.org/downloads/
Follow the installation instructions for your specific operating system (Windows, macOS, or Linux).

5.4.1.2 Install Required Python Dependencies

Install the necessary Python packages using pip if not already installed in your system, run the following commands:

```
python -m pip install requests
pip install openpyxl
pip install numpy
pip install boto3
```

After completing these steps, your environment should be ready to run the File Upload Script with the ARF file and sample folder paths as arguments.

5.4.2. Get the Script

The script is customer specific for security reasons, so to get your copy refer to section 4.3 in this document, you need to
- Login to the customer site
- Go to the resources tab
- Click the download button

5.4.3. Run the Script

5.4.3.1 Prepare Your Files:

- Ensure you have a completed Analysis Request File (ARF) that specifies which FASTQ files to upload and how to associate them with patients.
- Place all relevant sample files in a designated folder on your computer.

5.4.3.2 Open the Command Line Interface:

- On Windows, you might use Command Prompt or PowerShell.
- On macOS or Linux, open the Terminal.

5.4.3.3 Navigate to the Script Location:

- Use cd to navigate to the directory where the File Upload Script is located, or ensure the script's directory is included in your system's PATH for easier access.

```
cd /path/to/ARFUpload.py
```

5.4.3.4 Execute the Script with Required Arguments:

- Run the script by providing two arguments:
- The path to the ARF file.
- The path to the folder containing your sample files.

```
python ARFUpload.py -a /path/to/your/ARF_file.xlsx /path/to/your/sample_folder -s path_to_sample_files_folder
```

5.4.4 Monitor the Process:

- After executing the command, the script will begin processing the samples as defined in the ARF file and uploading the corresponding FASTQ files.

```
GALEAS UPLOAD SCRIPT, VERSION 1.1.2
Uploading ARF file…
Uplading samples…
Getting remote file locations
Checking if files have been upladed for sample sample_name
Updating sample file paths for sample sample_name
Sample sample_name uploaded to GALEAS successfully, 1 sample now uploaded .
Processing started.
```

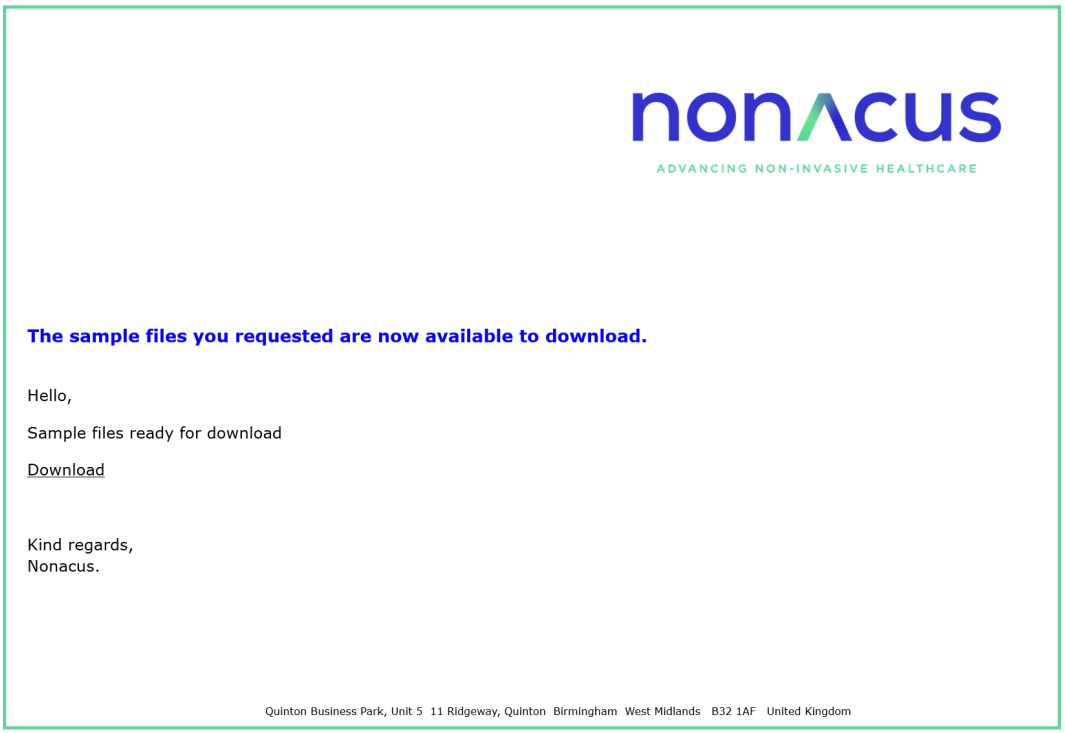
- Watch the command line output for any messages or errors that might require your attention.

5.4.5 Verify Upload Completion:

- Once the script finishes executing, verify that all intended files have been uploaded correctly via logging in to the MyNonacus GUI and that the sample associations in the system are correct.

6. Batch notification email

Once the GALEAS solution has finished processing your batch of samples, you will receive an email notification. This email contains a link that directs you to the “Batch Download Pages” within GALEAS. Clicking the link may prompt you to log in; once authenticated, the link will take you directly to the appropriate page. On this page, you can download the processing results, such as the generated report, for your batch of samples.



nonacus

ADVANCING NON-INVASIVE HEALTHCARE

The sample files you requested are now available to download.

Hello,

Sample files ready for download

Download

Kind regards,
Nonacus.

Quinton Business Park, Unit 5 11 Ridgeway, Quinton Birmingham West Midlands B32 1AF United Kingdom

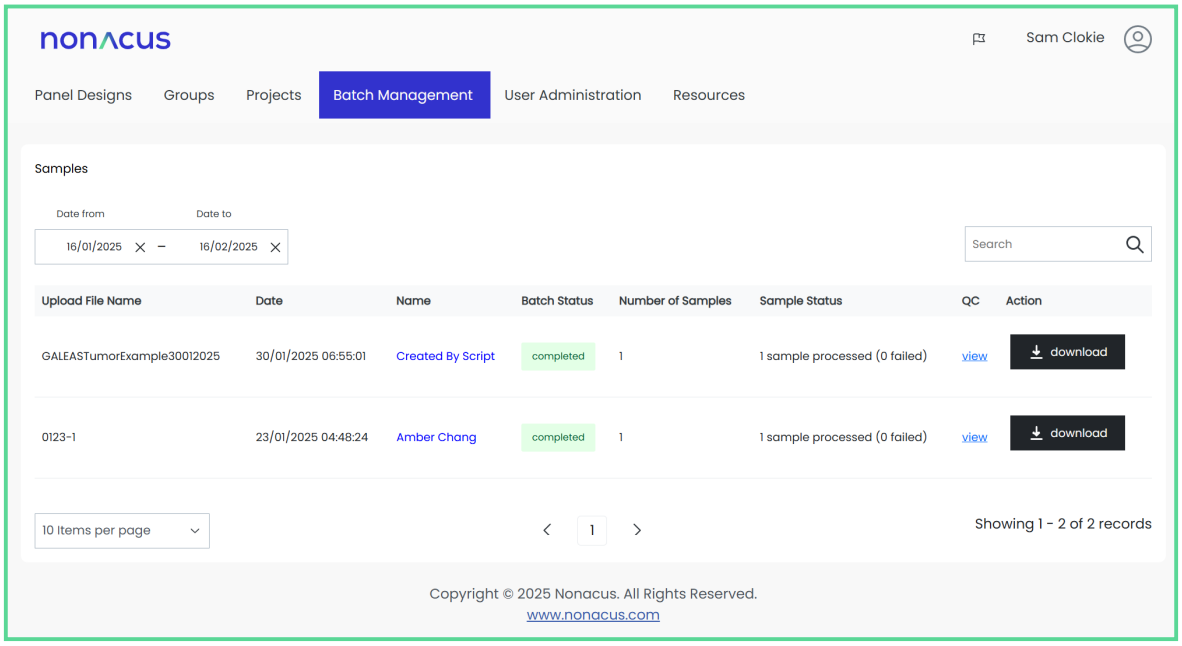
7. Download the Result for the batch(es)

7.1. Download via MyNonacus GUI

Once the processing is complete, the link provided in the email will take you directly to the "Batch Management" tab in the GALEAS software. This page displays all the batches that have been processed for your customer, with each row representing a single sample batch processing run.

To download the results for a specific batch, locate the desired row and click the **Download** button. This action will download all the results for each sample within that batch to your computer's default 'Downloads' folder.

For each successfully processed sample, the downloaded contents will vary depending on the panel used. For detailed information on what files and data to expect for different panel being used – see the annex for a description of the contents for GALEAS Bladder, Hereditiary Plus and Tumor.



7.2. Download Using Download Script

The download script enables users to automatically download the results of a specific batch run, streamlining and automating their Next-Generation Sequencing (NGS) workflows. This tool simplifies retrieving data for further analysis and integration into existing pipelines. For detailed instructions on how to download and set up the script, please refer to section 4.4 of this document.

7.3. Running the script

7.3.1. Instructions for Preparing and Using the Download Script

- Move the Python script into your desired folder. The script saves downloaded files in the same directory where it resides. For example, if you place the GaleasDownload.py file in your Documents folder, all downloads executed by the script will also be saved in the Documents folder.
- Ensure that the batch you intend to download is in a completed state. The script can only download results for batches that have finished processing. Batches still in the processing stage cannot be downloaded.

7.3.2 Prerequisites for running Python, please refer to section 5.4.1.1 and 5.4.1.2

7.3.3. Execute the Script with Required Arguments:

Run the script by providing two arguments:

- Batch name: the name of the ARF that was uploaded for this batch.
- <file types>: a list of file types to be included in the download (case insensitive). The following file types are supported:

```
python GaleasDownload.py -b “<batch name>” <file types>

python GaleasDownload.py -b “Batch upload1” PDF JSON MAF

#For download BAM files
python”GaleasDownload.py” -b “<batchName>” -s “<sampleID>” BAM
```

The following file types are supported:
PDF VCF BAM JSON QC MAF TSV TXT ALL (include all files)

8. Description of Download contents

8.1. GALEAS Bladder

The download bundle for a GALEAS Bladder sample contains the following;

• PDF of the report

• JSON encoding of the report

• multiQC.html – web page containing a large variety of sample QC data obtained from multiQC

8.2 GALEAS Hereditary Plus

File	File Type	Description
$sampleID.bam **$sampleID.bam.bai**	bam, bai	BAM and BAM index file
$sampleID.HRDreport.pdf	PDF	HRD Reprot in PDF format
$sampleID.germline.vcf.gz	Compressed VCF	SNV/Indel germline VCF file.
$sampleID.germline.vcf.gz.tbi	VCF Index	Index for the SNV/Indel germline VCF file.
$sampleID.germline.vep.vcf.gz	Compressed VCF (VEP Annotated)	Ensembl VEP annotated SNV/Indel germline VCF file.
$sampleID.germlineCNV.vcf.gz	Compressed VCF	germline CNV VCF file.
$sampleID.germlineCNV.vcf.gz.tbi	VCF Index	Index for the germline CNV VCF file.
$sampleID. germlinecnvplots.zip	Zip folder	one .html file per gene with CNV calls in the zip
multiQC_report.html	HTML File	A multiQC report summarizing quality control metrics.

NOTE: SNV/Indel calling is by Sentieon, with extra annotations by Ensemble VEP, CNV calling is by cn.mops, ExomeDepth and Sentieon CNV callers.

8.3 GALEAS Tumor

The download bundle for a GALEAS Tumor sample contains the following:

• Tumor Download Folder Contents

File	File Type	Description
multiQC_report.html	HTML File	A multiQC report summarizing quality control metrics.
GALEAS Tumor-$sampleID.zip	ZIP Archive	A zip folder per sample: GALEAS Tumor-$sampleID.zip

Contents of GALEAS Tumor-$sampleID.zip

File	File Type	Description
MSI scores (*.msi files)	Multiple MSI Data Files	Contain 3 data points: • Total Number of Sites, • Number of Somatic Sites, • MSI percentage.
$sampleID.BAFLog2.scatter.pdf	PDF	Ratio CNV scatter image.
$sampleID.germline.vcf.gz	Compressed VCF	Germline VCF file.
$sampleID.germline.vcf.gz.tbi	VCF Index	Index for the SNV/Indel germline VCF file.
$sampleID.HRDreport.pdf	PDF	HRD report in PDF format.
$sampleID.HRDreport.html	HTML Document	HRD report in HTML format.
$sampleID.somatic.maf	MAF File (Mutation Annotation Format)	SNV/Indel somatic mutation annotation file.
$sampleID.somatic.vcf.gz	Compressed VCF	SNV/Indel somatic VCF file.
$sampleID.somatic.vcf.gz.tbi	VCF Index	Index for the SNV/Indel somatic VCF file.
$sampleID.somatic.vep.vcf.gz	Compressed VCF (VEP Annotated)	Ensembl VEP annotated SNV/Indel somatic VCF file.
$sampleID.somaticCNV.vcf.gz	Compressed VCF	Somatic CNV VCF file.
$sampleID.somaticCNV.vcf.gz.tbi	VCF Index	Index for the somatic CNV VCF file.
$sampleID.cns	cns (a TSV file)	cns (Copy Number Segments) files contain detailed results of the CNV calls. Each row represents a segment with fields like chromosome, start and end positions, log2 copy ratio, segment mean, and optionally p-values
$sampleID.cns.seg	.seg file	seg (Segmented Copy Number Data) contains a standardized format used to visualize copy number segments in genome browsers like IGV (Integrative Genomics Viewer). Includes sample ID, chromosome, start and end positions, segment mean, and sometimes additional metadata.
$sampleID.somaticSV.vcf.gz	Compressed VCF	Somatic SV (Structural Variant) VCF file.
$sampleID.somaticSV.vcf.gz.tbi	VCF Index	Index for the somatic SV VCF file.
$sampleID_TMB_summary.tsv	TSV File	TMB summary with 4 data points: • Variants remaining after filtering, • Total length of target regions (bp), • Tumour mutational burden (Variants/Mb), • Panel size warning (true/false).

8.4 GALEAS Tumor HRD

The download bundle for a GALEAS Tumor HRD sample contains the following:

• Download folder contains HRD-$sampleID folder for each sample, in each HRD-$sampleID folder, it has $sampleID HRDreport.pdf:

File	File Type	Description
$sampleID.HRDreport.pdf	PDF	HRD report in PDF format

• Tumor Download Folder Contents

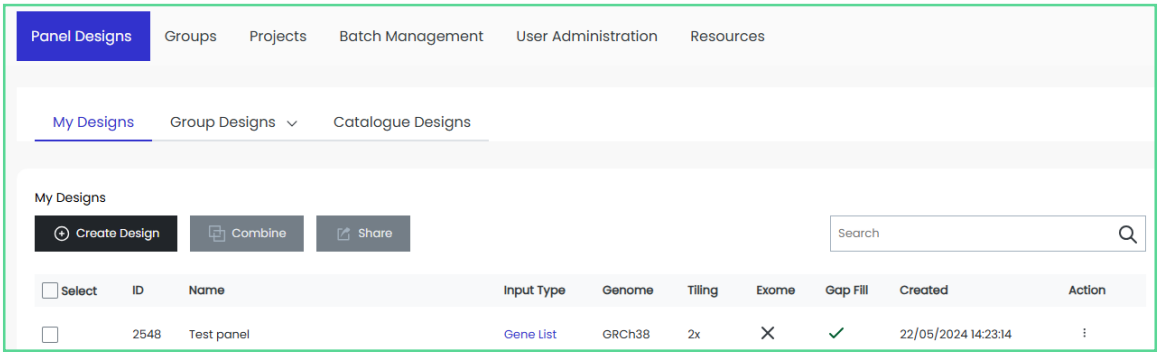
File	File Type	Description
multiQC_report.html	HTML File	A multiQC report summarizing quality control metrics.
GALEAS Tumor HRD-$sampleID.zip	ZIP Archive	A zip folder per sample: GALEAS Tumor HRD-$sampleID.zip

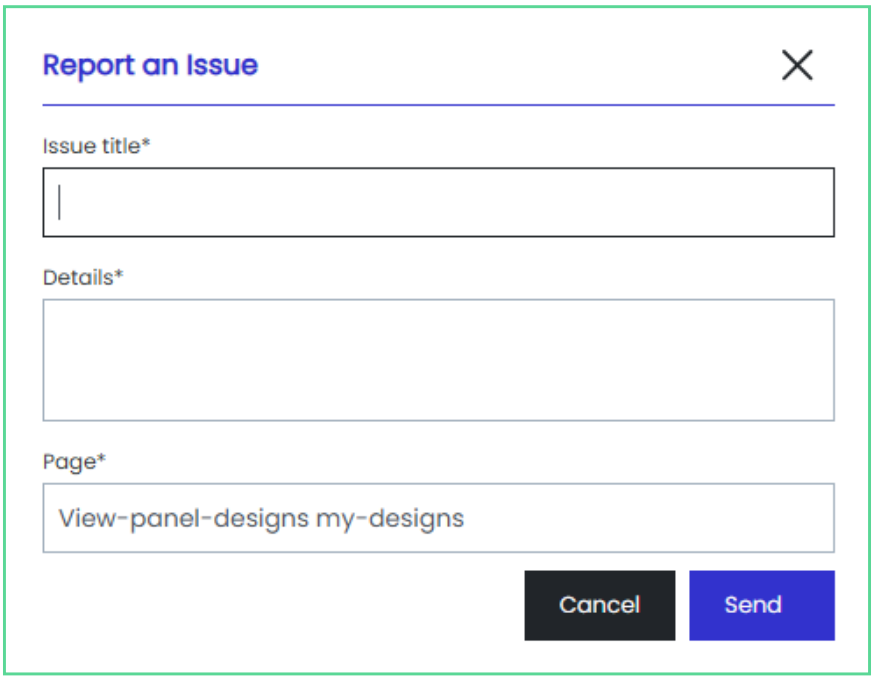
Contents of GALEAS Tumor HRD-$sampleName.zip

File	File Type	Description
MSI scores (*.msi files)	Multiple MSI Data Files	Contain 3 data points: • Total Number of Sites, • Number of Somatic Sites, • MSI percentage.
$sampleID.BAFLog2.scatter.pdf	PDF	Ratio CNV scatter image.
$sampleID.germline.vcf.gz	Compressed VCF	Germline VCF file.
$sampleID.germline.vcf.gz.tbi	VCF Index	Index for the germline VCF file.
$sampleID.HRDreport.pdf	PDF	HRD report in PDF format.
$sampleID.HRDreport.html	HTML Document	HRD report in HTML format.
$sampleID.somatic.maf	MAF File (Mutation Annotation Format)	SNV/Indel somatic mutation annotation file.
$sampleID.somatic.vcf.gz	Compressed VCF	SNV/Indel somatic VCF file.
$sampleID.somatic.vcf.gz.tbi	VCF Index	Index for the SNV/Indel somatic VCF file.
$sampleID.somatic.vep.vcf.gz	Compressed VCF (VEP Annotated)	Ensembl VEP annotated SNV/Indel somatic VCF file.
$sampleID.somaticCNV.vcf.gz	Compressed VCF	Somatic CNV VCF file.
$sampleID.somaticCNV.vcf.gz.tbi	VCF Index	Index for the somatic CNV VCF file.
$sampleID.somaticSV.vcf.gz	Compressed VCF	Somatic SV (Structural Variant) VCF file.
$sampleID.somaticSV.vcf.gz.tbi	VCF Index	Index for the somatic SV VCF file.
$sampleID_TMB_summary.tsv	TSV File	TMB summary with 4 data points: • Variants remaining after filtering, • Total length of target regions (bp), • Tumour mutational burden (Variants/Mb), • Panel size warning (true/false).

9. Report Issues with MyNonacus GUI

For reporting any issues with using MyNonacus GUI, click the Flag icon on the top right of the webpage and submit issue to MyNonacus.





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