Instructions for Use

IdentiClone® RUO *TCRG* 2.0 Software

For automated analysis of *TCRG* capillary electrophoresis data obtained from ABI 3500 instruments.

RUO For RESEARCH USE ONLY. Not for use in diagnostic procedures.

Manufactured in U.S.A.

Product Compatibility

IdentiClone RUO *TCRG* 2.0 Software (REF S100001) was developed specifically for and is compatible with only the following Invivoscribe assays:

Catalog Number		Products	Quantity
REF	12070101	T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 - ABI Fluorescence Detection	33 reactions
REF	12070111	T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 MegaKit - ABI Fluorescence Detection	330 reactions

invivoscribe

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1. Assay Use

This Research Use Only (RUO) software is intended for the analysis of raw FSA files obtained from ABI 3500 and ABI 3500xL instruments in conjunction with the T-Cell Receptor Gamma Gene Rearrangement Assay 2.0 to identify T-cell receptor gamma (*TCRG*) chain gene rearrangements and is useful for the identification of clonal T-cell populations and evaluation of new research and methods in malignancy studies.

2. Glossary and Abbreviations

2.1. Glossary

Table 1. Glossary Terms

Term	Definition
Amplicon	A DNA fragment created during the replication of genetic material.
Assay	T-cell Receptor Gamma Gene Rearrangement Assay 2.0
Clonal	The aggregate of genetically identical cells or organisms produced from a single progenitor cell. • A *Sample Name* result (final call) in which Clonality is detected.
Final call / Final Clonality call	The final *Sample Name* clonality result
Injection	Set of up to 24 samples simultaneously analyzed on the ABI 3500xL instrument. These may include run controls from one or more PCR runs.
Invalid	A sample result which does not meet the validity criteria.
Master Mix	Amplification reagent with primers to amplify specifically targeted regions.
Negative Control	A buffer solution containing polyclonal DNA; this control is expected to generate a Non-Clonal result.
No Template Control	A buffer solution (or water) containing no template; this control is intended to detect the presence of contamination during PCR setup.
Non-Clonal	A *Sample Name* result in which clonality is not detected.
Platemap / Plate Map	Visual representation of a detection plate which gets imported to the ABI Genetic Analyzer. It provides a 96-well plate layout containing associated run information, including *Run number, Sample Name*, and *Sample Type* for each well location.
Positive Control	A buffer solution containing DNA used to assess assay validity; this control is expected to generate a Clonal result.
RBP	Regression Based Predictor value is a software-generated value derived from electropherogram data, serving as a potential indicator of repeated sequences.
Run	At least one sample processed together with a set of run controls (Positive Control, Negative Control, NTC) through amplification and detection, using same MM.
Sample ID	Unique identification associated with a subject sample assigned by the software.
Sample Name	Unique identification associated with a subject sample assigned by the user.
Software	IdentiClone RUO *TCRG* 2.0 Software
System	The whole package of software, hardware and assay (as applicable) that make up the medical device.

2.2. Abbreviations

Table 2. Abbreviations defined

Abbreviation	Definitions
ABI	Applied Biosystems Instruments, a Life Technologies brand of Thermo Fisher Scientific
CE	Capillary electrophoresis; an electrokinetic method used to separate amplicons by size.
RUO	Research Use Only
DNA	Deoxyribonucleic Acid

Table 2. **Abbreviations defined**

Abbreviation	Definitions
EULA	End User License Agreement
FNC	File naming convention
FSA	Fragment analysis data file created by the capillary electrophoresis instrument.
IFU	Instructions for use
LIVS	A file format that gets generated while annotating a plate. Also known as an annotated plate map file.
MM	Master mix
PC	Positive control
NC	Negative control
NTC	No template control
OS	Operating system
PCR	Polymerase chain reaction
PDF	Portable document format
QC	Quality Control
RBP	Regression Based Predictor
UI	User Interface

3. Principles of the Procedure

3.1. Gene Rearrangement Assay

Invivoscribe's T-Cell Receptor Gamma (*TCRG*) Gene Rearrangement Assay 2.0 contains a single multiplex master mix that targets all conserved regions within the variable (V) and the joining (J) region genes described in lymphoid malignancies. This is critical for more comprehensive analysis of samples, as some T-cell lymphoproliferative disorders involve V and J segments that would not be identified with a single Vγ (1-8) and Jγ1/2 primer set. Amplification with all primers in a single tube has several additional important advantages over existing methods. The polyclonal background that results from the combination of all primers in a single tube produces a more robust and easily interpreted signal with capillary electrophoresis, which aids in the interpretation of small peaks. The average size of the *TCRG* gene rearrangement PCR product is 190 bp, with a normal distribution of product sizes between 159 and 207 bp. This protocol leads to improved PCR product formation from paraffin-embedded samples when compared to other protocols that yield products of 260 bp or longer. Positive and negative DNA controls, as well as a Specimen Control Size Ladder master mix are included. Clonality is indicated if a dominant amplicon is detected.

3.2. Differential Fluorescence Detection

Differential fluorescence detection is commonly used to resolve the different-sized amplicon products using a capillary electrophoresis instrument. Primers can be conjugated with several different fluorescent dyes (fluorophores) so that they can produce different emission spectra upon excitation by a laser in the capillary electrophoresis instrument. In this manner, different fluorescent dyes can correspond to different targeted regions. This detection system results in unsurpassed sensitivity, single nucleotide resolution, differential product detection and relative quantification. In addition, the use of agarose and polyacrylamide gels, as well as the use of carcinogens such as ethidium bromide, can virtually be eliminated. Further, differential detection allows accurate, reproducible and objective interpretation of primer-specific products and automatic archiving of data. Inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately one to two nucleotides.

3.3. Software Interpretation

The IdentiClone RUO *TCRG* 2.0 Software provides an objective interpretation of raw data files (FSA) generated by ABI 3500 and ABI 3500xL instruments to identify clonal gene rearrangements in unknown samples. The software allows the user to upload a plate map for sample traceability in addition to configuring multiple ABI instrument injections for high-throughput laboratories.

4. Minimum System Requirements

- **Processor:** Intel® X64 compatible CPU (Core 2 Duo or newer) with a clock speed of at least 1GHz
- **Hard Drive:** At least 50 GB of free disk space is required; 250 GB recommended.
- **RAM:** 4 GB required; 8 GB or more recommended.
- **Operating System:** Windows (64-bit) 10 Pro or 11 Pro is required.
- A PDF reader to visualize data reports generated by the IdentiClone RUO *TCRG* 2.0 Software.

5. Warnings and Precautions

- **System font**. User interface is designed to use the default system font settings on a Windows computer.
- **Compatible files.** IdentiClone RUO *TCRG* 2.0 Software is compatible with FSA files generated by the ABI 3500xL and ABI 3500xL Dx Genetic Analyzers.
- **Characters in pathname and file name.** It is important that the filenames only contain the following characters (A-Z, a-z, 0-9, ., _ (underscore), - (hyphen)). If the Software encounters a character not within this set, it may fail.
- **End-user and in-use environment.** The Software is for Research use only (RUO) in a clinical laboratory setting. Use of this product must be limited to trained personnel.
- **Security.** Use of endpoint protection software is highly recommended to protect the computer running the Software.
    - Ensure no unauthorized devices are plugged into the workstation on which the Software is running, physically block all USB and other communication ports when not in use.
    - Endpoint protection software installed on the workstation containing the Software should be monitored for alerts so that action can be taken when the endpoint is compromised.
- **Network settings.** This software does not communicate over a network and no software specific firewall settings are needed.
    - Ensure the operating system firewall is turned on and that any network ports not needed for the functioning of the workstation are closed.
    - If the workstation containing the software is connected to a network, ensure all appropriate network security safeguards are in place, e.g., ensuring the workstation cannot be reached directly from the internet.
- **Access controls.** This Software supports account-based access controls. Passwords and account information must not be shared amongst users. If more users need to use the software, new accounts can be created.
    - Do not provide regular Software users administrator rights on the workstation, following the principle of least privilege. This Software does not require administrator rights to run.
    - Each Software user on the workstation must have the minimal access rights needed to perform their assigned tasks following the principle of least privilege.
    - When a new user account is created in the Windows OS, the password associated with the account must be changed before the account is used.
- **Software updates.** Invivoscribe, Inc will offer updated software for download via its software portal. When installing the software update, Microsoft Windows will show the organization that created the software, ensure this reads "Invivoscribe, Inc.". Only install software updates downloaded from the Invivoscribe, Inc download portal.
    - Customers will be notified when Invivoscribe issues any update. The update will be made available via the Invivoscribe Software Portal download portal and is installed just like the initial software installation. Double-click the installer executable file, verify the software was created by "Invivoscribe, Inc." by reading prompt shown by Microsoft Windows and follow the instructions on screen.
- **License Key**. A license key is required for the software to function and will be provided at the time of purchase of software.
- **Backup precautions.** The software has functionality to save a backup of the generated results to a pre-specified directory (see Section 13.8). It is recommended that this directory be itself backed-up as part of the normal backup procedure as used by the implementing organization. Ensure the workstation is backed up in its entirety so it can be quickly restored in case of an emergency.

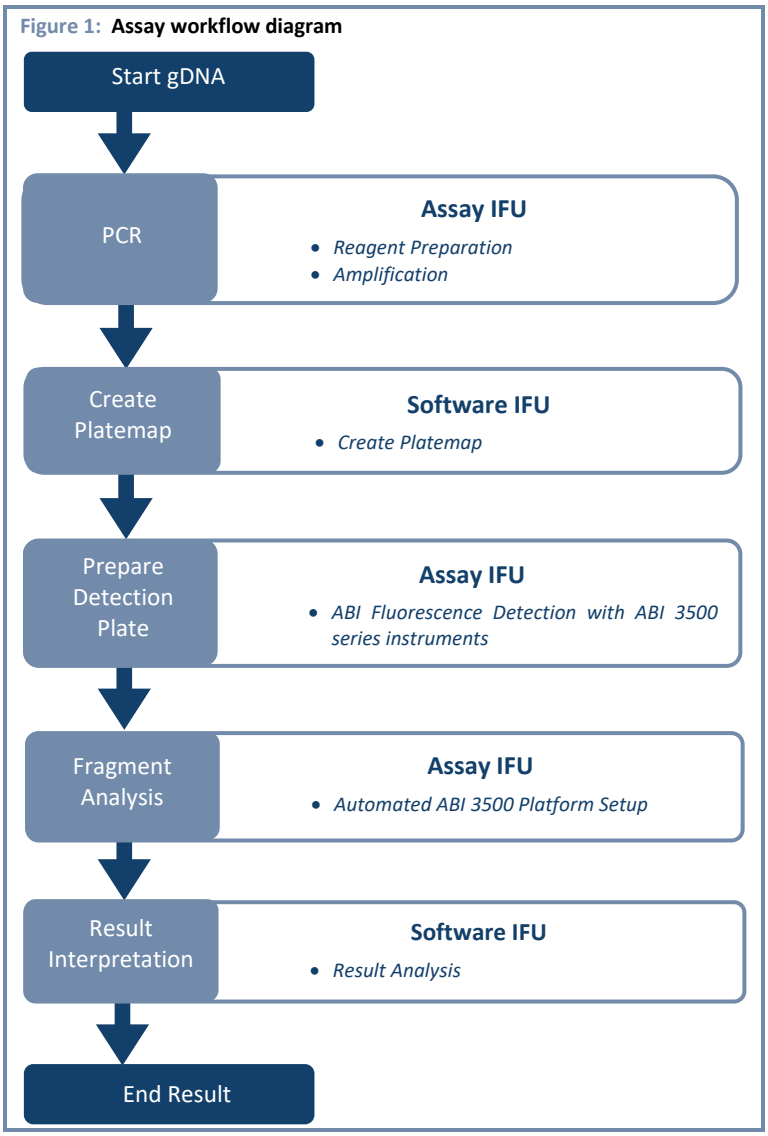
IMPORTANT! Do NOT change or alter any files generated by the Software.

- Editing the Platemap files generated by the Software using ABI Genetic Analyzer software will lead to file corruption and an inability to perform analysis.

6. Software Procedure

Note: **The Software requires utilizing this document in conjunction with the T-Cell Gamma Gene Rearrangement Assay 2.0 IFU (📖: 280288).**

Figure 1: **Assay workflow diagram**



6.1. Download the software from Invivoscribe Software Portal

6.1.1. Using any web browser, navigate to https://catalog.invivoscribe.com/softwareportal/

6.1.2. Complete the following text fields:

6.1.2.1. Email: Enter a valid email address. A link to the software download will be sent to this address.

6.1.2.2. Customer Account Number: Enter your unique ID used when placing orders with Invivoscribe.

6.1.2.3. Software Code: Enter the software code found on your sales order.

6.1.3. Check the *Terms and Conditions* box to proceed.

6.1.4. Click the **Request Software** icon.

6.1.5. Following valid input text into the above fields, a link to the software download will be sent to the provided email address.

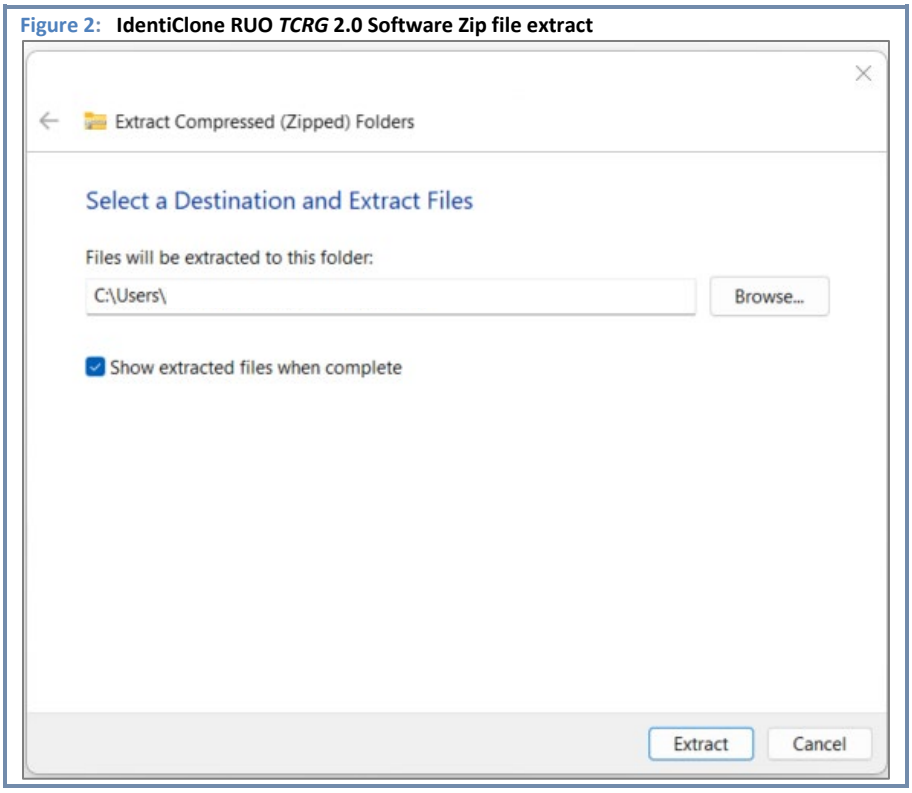
6.1.6. Click the link or copy+paste into a web browser; the software will automatically download.

6.2. Install the IdentiClone RUO *TCRG* 2.0 Software

6.2.1. Right-click ***S100001_y.zip*** file; click **Extract All…**, then select the desired folder to extract all the contents of the zip file, which include:

- ***IdentiClone-RUO-TCRG-2.0-Software-1.2.x.RUO.msi*** – the software application
- ***IC_RUO_TCRG_FNC.xml*** – the file name convention settings, see section 8.3. in the Assay IFU
- ***TCRG Instrument Parameters.xml*** – the instrument parameters, see section 8.3. in the Assay IFU
- ***IC_RUO_TCRG_RG.xml*** – the results group parameters, see section 8.3. in the Assay IFU

Figure 2: IdentiClone RUO *TCRG* 2.0 Software Zip file extract



6.2.2. Navigate to the location where the zip file contents were extracted.

6.2.3. Double-click the ***IdentiClone RUO TCRG 2.0 Software-1.2.x.RUO.msi*** file.

6.2.3.1. Follow the on-screen steps in the Windows setup wizard; first click **More Info**, then click **Run anyway.**

6.2.3.2. Follow the Windows setup Wizard installation prompts, select the directory file path to save the software in the desired location and click **Install**.

6.2.4. Once installation is complete, click **Finish**.

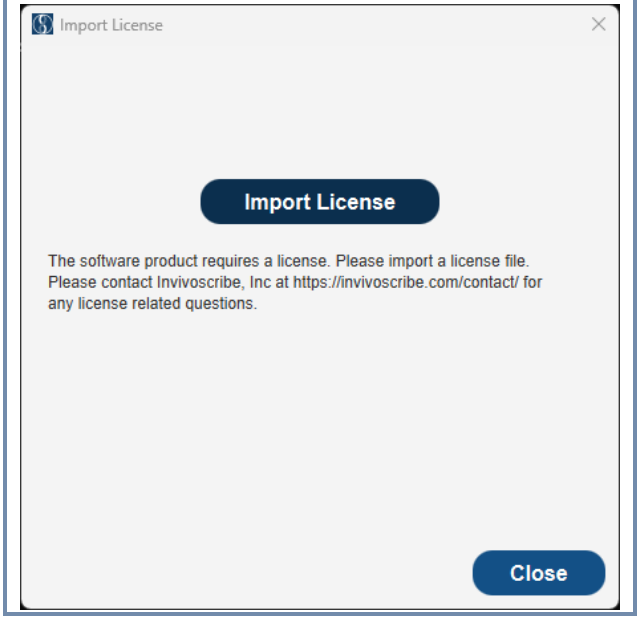
Note: **x in *IdentiClone RUO TCRG 2.0 Software-1.2.x.RUO*.msi indicates software version number 1, 2, 3, etc.; and y in *S100001_y.zip* indicates software zip package revision letter A, B, C, etc.**

6.3. Enter the Software License Key Information

Note: **License keys are customer and product-specific, and must be current in order for the software to function. The license keys are checked at software startup, after successful login, and before every analysis.**

6.3.1. While accessing the software for the first time, a screen with an “Import License” button will appear. (Figure 3)

Figure 3: IdentiClone RUO *TCRG* 2.0 Import License Screen



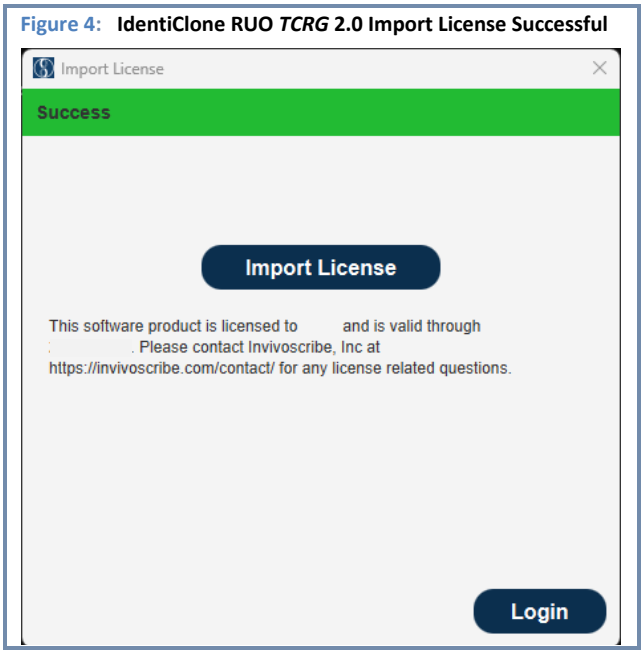
6.3.2. Click on the “Import License" button and navigate to the directory file path where the license key file is saved.

Note: **The license keys will be provided at the time of software purchase in a file called “licenseKey-Customer Name-Software Name-License expiration date.”**

6.3.3. Select the appropriate “licenseKey” file to import into the software.

6.3.4. Once successful, the following screen will appear displaying the name to which the license was issued and the license expiration date. (Figure 4)

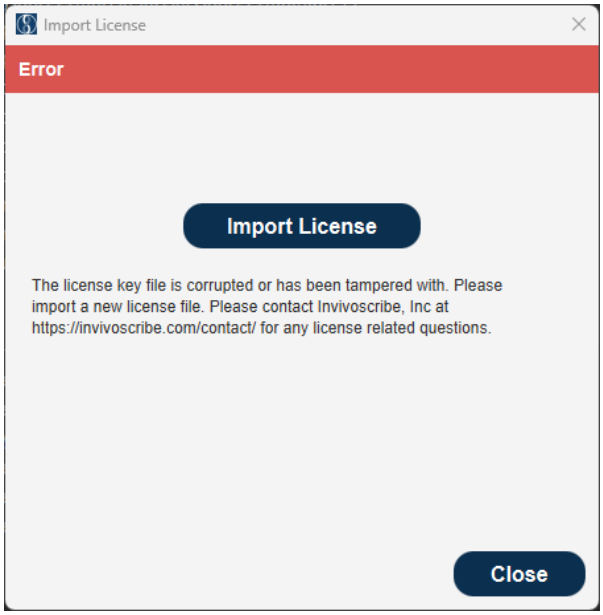
Figure 4: IdentiClone RUO *TCRG* 2.0 Import License Successful



- The software is now ready to use and can be accessed by navigating to the directory file path selected in step 6.2.3.2 and opening the executable. Alternatively, the software can also be accessed via the *Start Menu* shortcut under an *Invivoscribe* folder or via the Desktop shortcut.

6.3.5. Invalid license keys will cause an error message as shown. If you experience such error, please contact https://invivoscribe.com/contact/ for assistance with license related queries. (Figure 5)

Figure 5: IdentiClone RUO *TCRG* 2.0 Import License Error

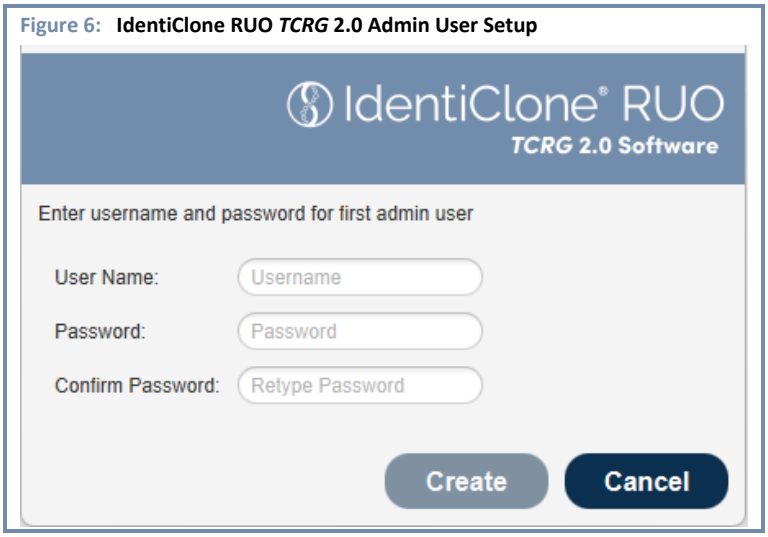


6.4. Administrative Configuration

6.4.1. Create the Admin user account.

- If using the IdentiClone RUO *TCRG* 2.0 Software for the first time, a prompt will appear to create the first Admin user. (Figure 6)
- The first Admin user must create subsequent non-admin users. See *13.2 Create User* to create new users.

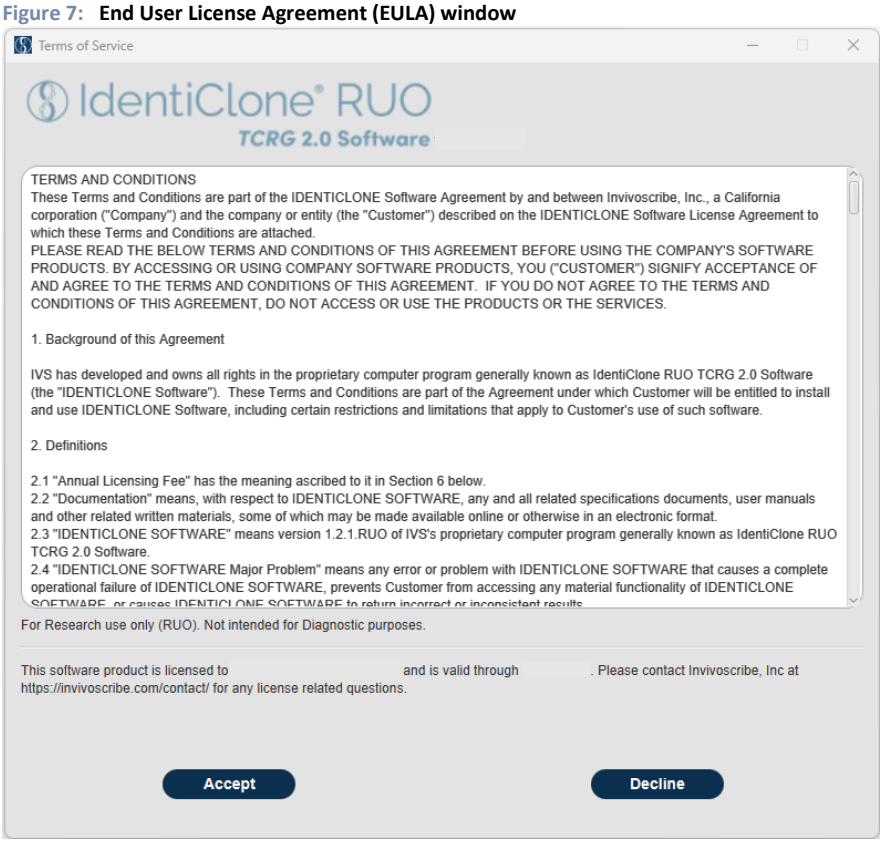
Figure 6: IdentiClone RUO *TCRG* 2.0 Admin User Setup



6.4.2. During the first successful login, the Software End User License Agreement (EULA) will be displayed. (Figure 7)

- The software EULA must be accepted in order to continue on to the main application.

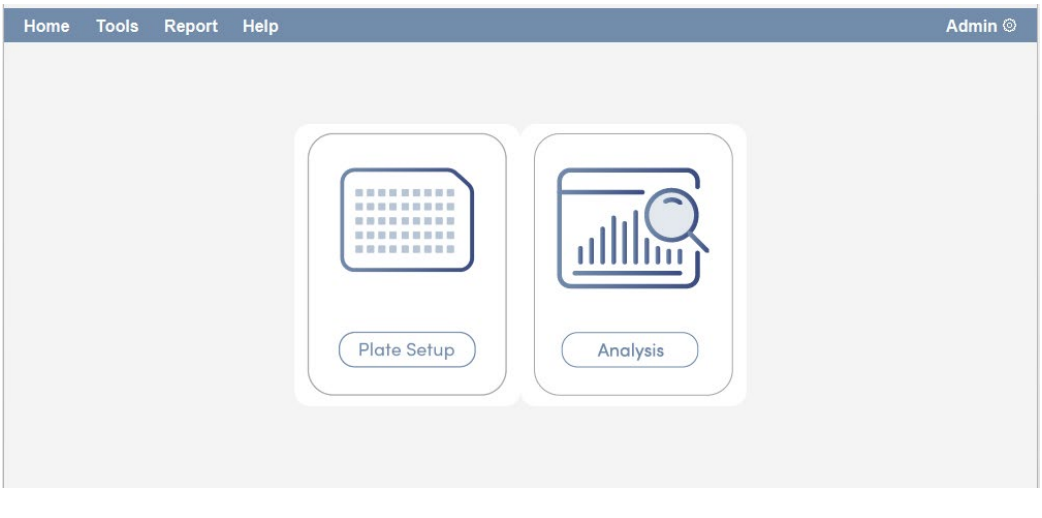
Figure 7: End User License Agreement (EULA) window



6.4.2.1. The IdentiClone RUO *TCRG* 2.0 Software will always open to the home page after the EULA is accepted. (Figure 8)

- The home page is used to navigate to the main features of the applications like *Plate Setup* and *Analysis*. (Figure 8)

Figure 8: IdentiClone RUO *TCRG* 2.0 Software Home user interface



6.4.2.2. To access as a regular user (non-admin), Enter login credentials and click **Login**. (Figure 9)

- Click **Log Out** to logout from the application; the Software will automatically logout any user after 5 minutes of inactivity.

Figure 9: **IdentiClone RUO *TCRG* 2.0 Software login window**

6.5. Create Platemap

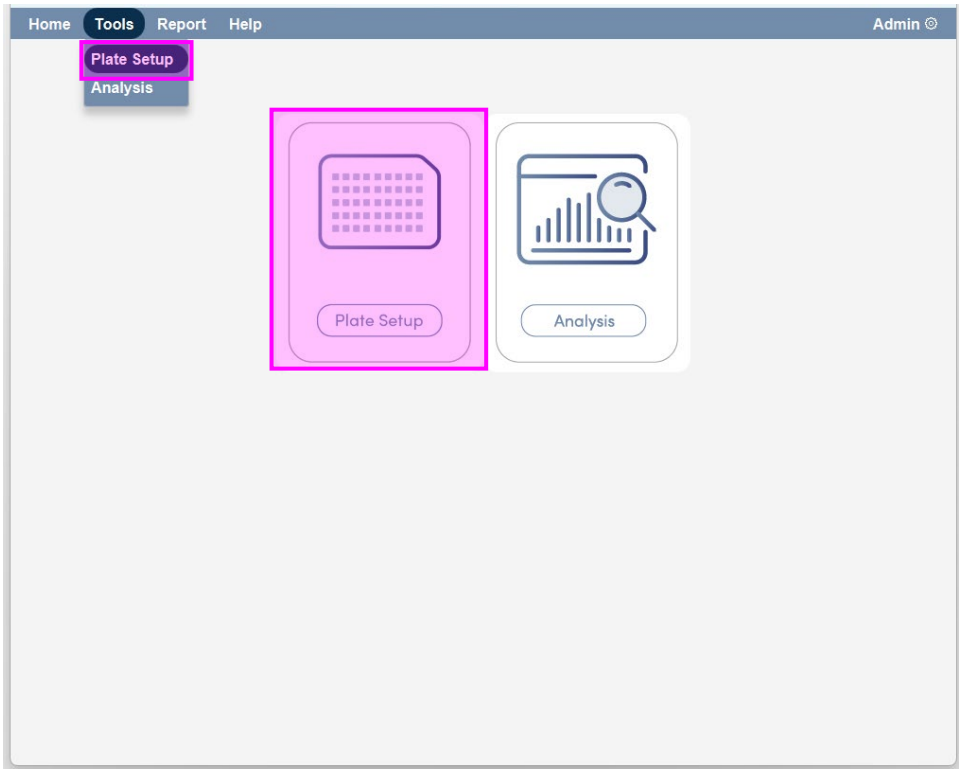
Note: To create a new platemap with the Software, go to section 6.5.1. To create a platemap using a previously saved platemap (and make modifications), follow section 6.5.3.

6.5.1. Create a platemap using the *Plate Setup* function

6.5.1.1. Click **Plate Setup** from the home user interface. (Figure 10)

6.5.1.1.1. Alternatively, *Plate Setup* can also be accessed by navigating to **Tools** → **Plate Setup**.

Figure 10: **Plate Setup Selection**



6.5.1.2. The IdentiClone RUO *TCRG* 2.0 Software defaults to **one** run. To add additional runs, click **Configure Runs**, then click **Add Run.** (Figure 11)

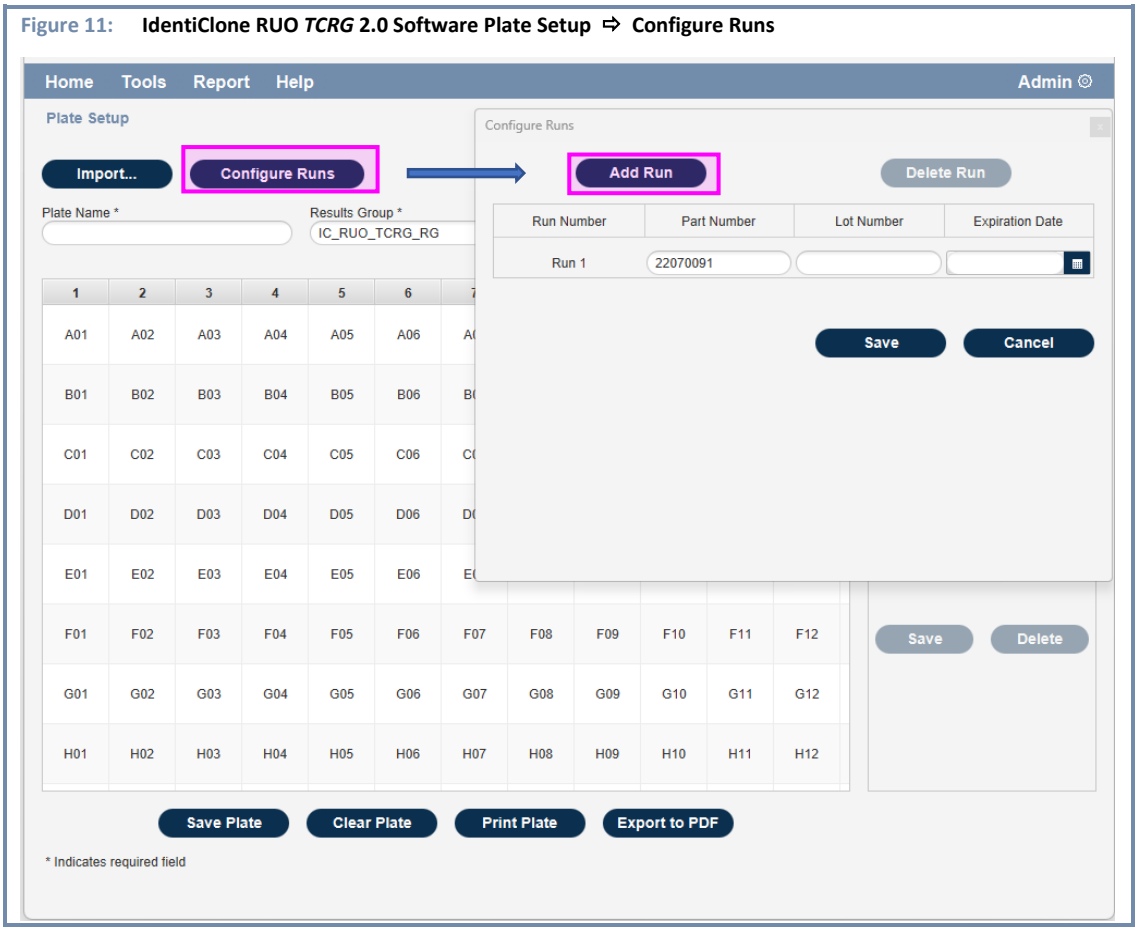
Note: Each Run must include a Positive, Negative and No Template Control; these controls will be indicated on the platemap as PC (Positive Control), NC (Negative Control), and NTC (No Template Control).

- Runs must be configured before annotating the plate.
- Up to 24 runs can be configured per plate (A run represents a set of controls and corresponding samples).

6.5.1.2.1. (Optional) Manually enter the associated master mix information, then click **Save**.

- The Part Number must contain 6-12 alphanumeric characters.
- The Lot Number must contain 6-10 alphanumeric characters.
- The Expiration Date can be selected from a calendar dropdown view.

Figure 11: IdentiClone RUO *TCRG* 2.0 Software Plate Setup ⇨ Configure Runs



6.5.1.3. To remove a run, navigate to **Configure Runs**, then click **Delete Run**.

- By default, the most recent run created will be deleted first; a run can be deleted only if there are no wells assigned to the run.

6.5.2. Configure and save a new plate

6.5.2.1. Navigate to *Plate Setup* and enter information into the 4 fields located above the plate map: *Plate Name*, *Results Group*, *File Name Convention, and Plate Barcode (Optional).* (Figure 12)

- These fields are limited to 50 characters or less and may only include letters (A-Z, a-z), numbers (0-9), hyphen (-) and underscore (_). No spaces or special characters (other than specified) will be accepted.
- File Name Convention and Results Group will be automatically populated with default settings.

6.5.2.1.1. *Plate Name* refers to the user designated plate name and must be populated before proceeding to the next step.

6.5.2.1.2. *Results Group* indicates the FSA file save location and must be populated before proceeding to the next step.

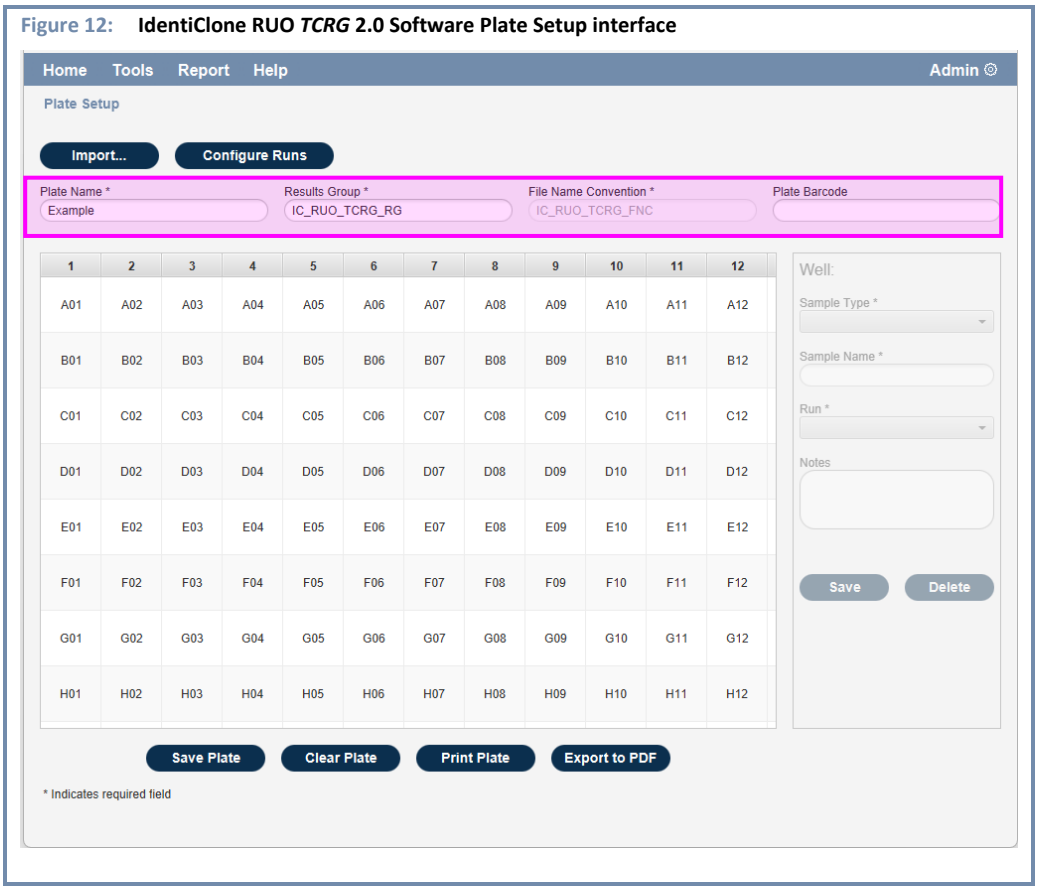
6.5.2.1.3. *File Name Convention* defines the FSA file naming convention and must be populated before proceeding to the next step.

6.5.2.1.4. *Plate Barcode* is optional but, is displayed on analysis run reports.

6.5.2.1.5. *Results Group* and *File Name Convention* entries must match the names of the corresponding entries on the ABI 3500/3500xL instrument.

- Refer to the Assay IFU for the correct naming convention for the *Results Group* and *File Name Convention* (: 280288, section 8.3).

Figure 12: IdentiClone RUO *TCRG* 2.0 Software Plate Setup interface



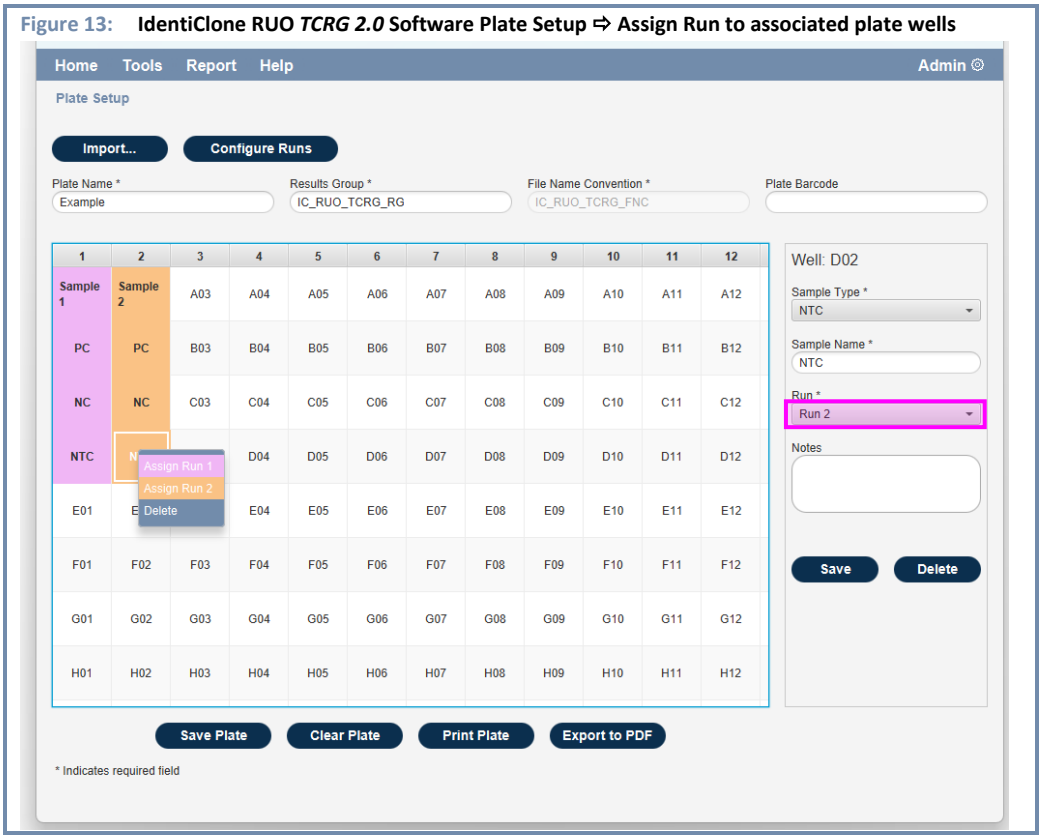
6.5.2.2. Select the well(s) to be used (i.e., to be loaded with diluted amplicon).

- **Shift + LeftClick**: selection of multiple adjacent cells;
- **Ctrl + LeftClick**: selection of multiple individual cells.

6.5.2.2.1. **Right-click** over the well selection to prompt the run assignment window, then assign a run. (Figure 13)

- The run can be assigned by individual wells or by selecting a group of wells.

Figure 13: IdentiClone RUO *TCRG 2.0* Software Plate Setup ⇨ Assign Run to associated plate wells



6.5.2.3. Select each well assigned to a run and enter required information on the right side of the window. (Figure 14)

6.5.2.3.1. Select the appropriate *Sample Type* from the designated dropdown box: PC, NC, NTC or Sample.

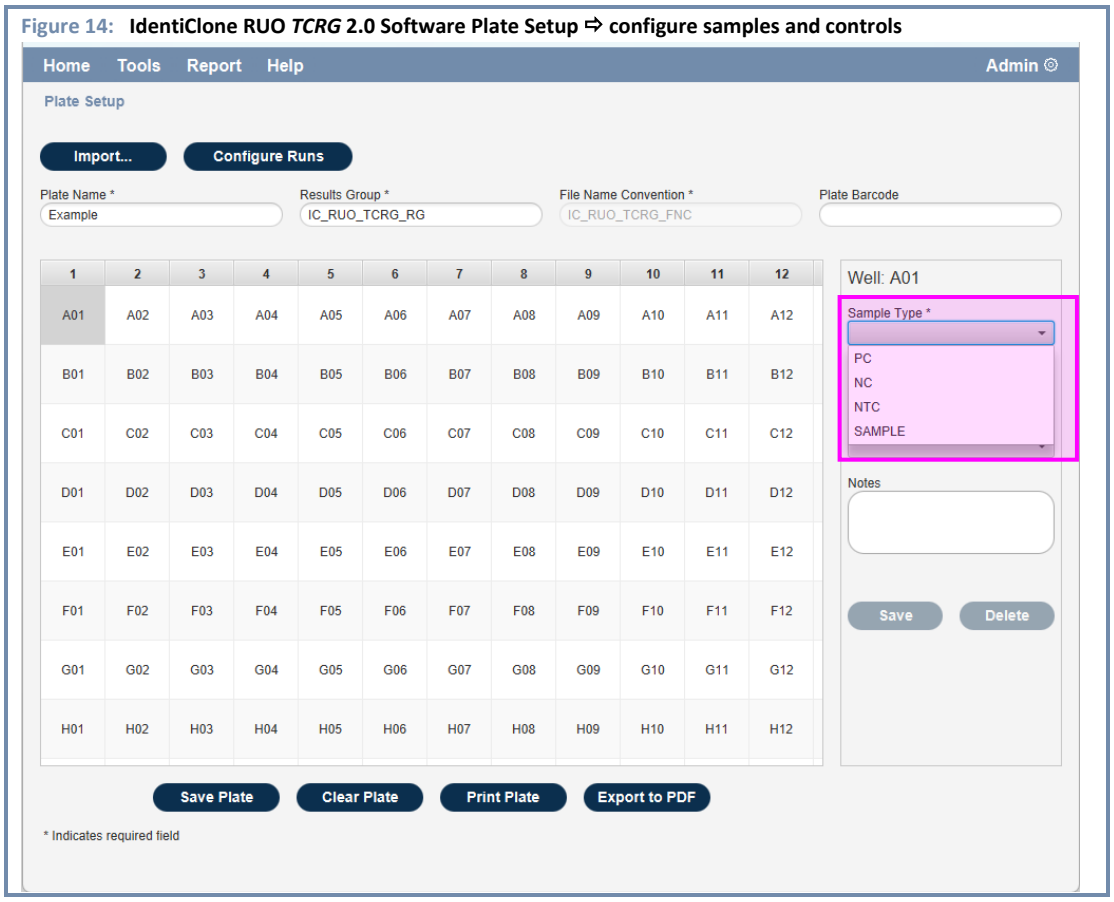
6.5.2.3.2. Enter *Sample Name*, a unique identification associated with a patient sample.

Note: **Sample Name can also be populated by using an external barcode scanner.**

6.5.2.3.3. Confirm that the correct *Run* is assigned to the sample.

6.5.2.3.4. (Optional) Enter *Notes,* if any.

Figure 14: **IdentiClone RUO *TCRG* 2.0 Software Plate Setup ⇨ configure samples and controls**



Platemap rules:

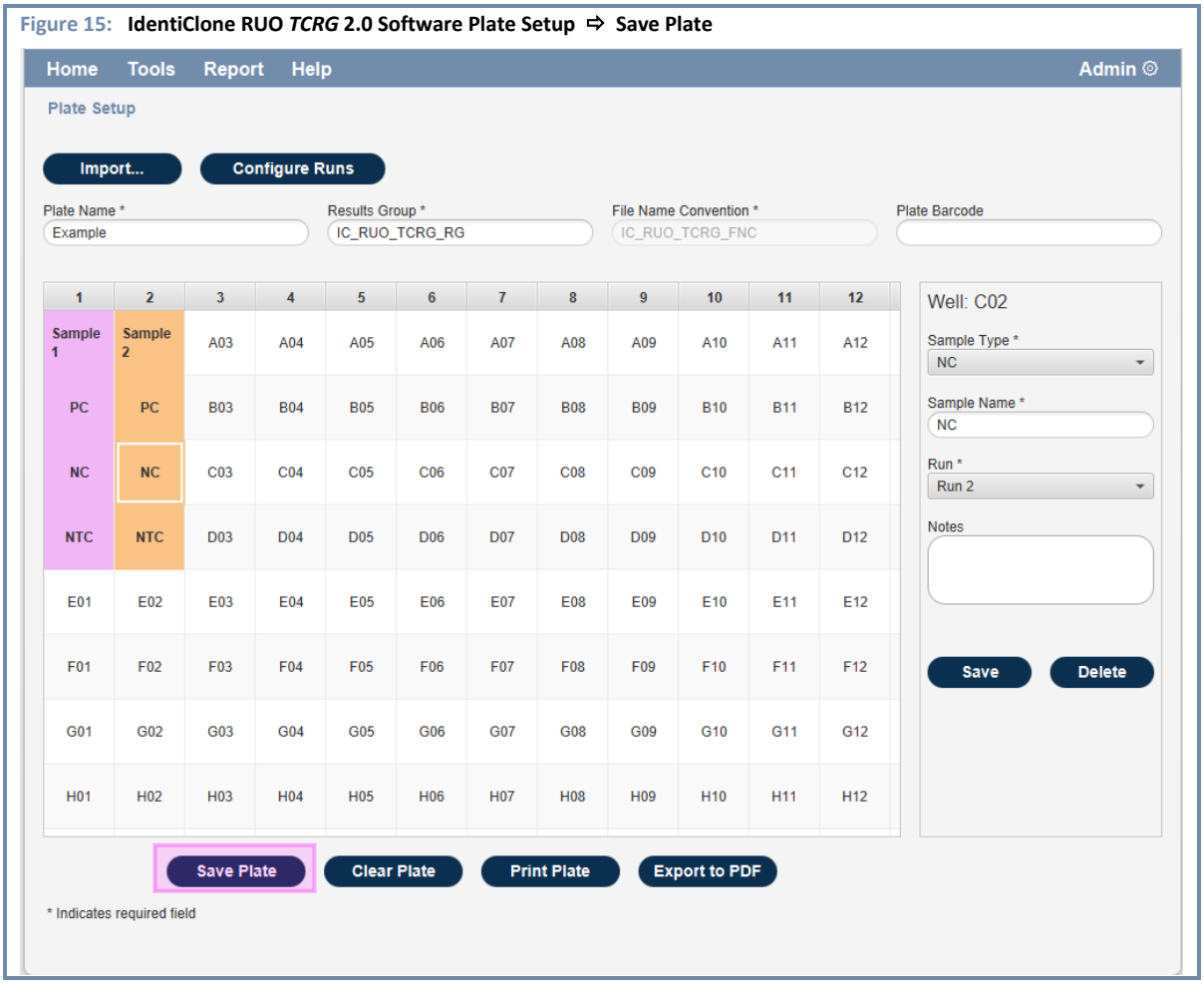
- **Each run must include a Positive, Negative and No Template Control; these controls will be indicated on the platemap as *PC* (positive control), *NC* (negative control), and *NTC* (no template control)**
- **Fields marked with asterisk (*) are required to save a well.**
- **The fields *Sample Name* and *Notes* may only contain 50 characters or less.**
- ***Sample Name* can only include letters (A-Z, a-z), numbers (0-9), hyphens (-) and underscores (_). No spaces or special characters (other than specified) are allowed.**
- **The highlighted well position in the *Plate Setup* will become bold once all parameters are defined and saved for a particular sample or control.**

6.5.2.4. Click **Save** to complete configuration of the well; repeat step 6.5.2.3.3 for each assigned well on the plate.

6.5.2.5. Upon defining and saving all samples and controls for every run in the plate, click **Save Plate.**

- Different runs will be highlighted and displayed in various colors on the plate map. (Figure 15)

Figure 15: IdentiClone RUO *TCRG* 2.0 Software Plate Setup ⇨ Save Plate



6.5.2.6. Select the directory filepath for the output files. (Figure 16)

6.5.2.6.1. Once the platemap is saved, a paired set of output files will be generated, as CSV and LIVS file formats.

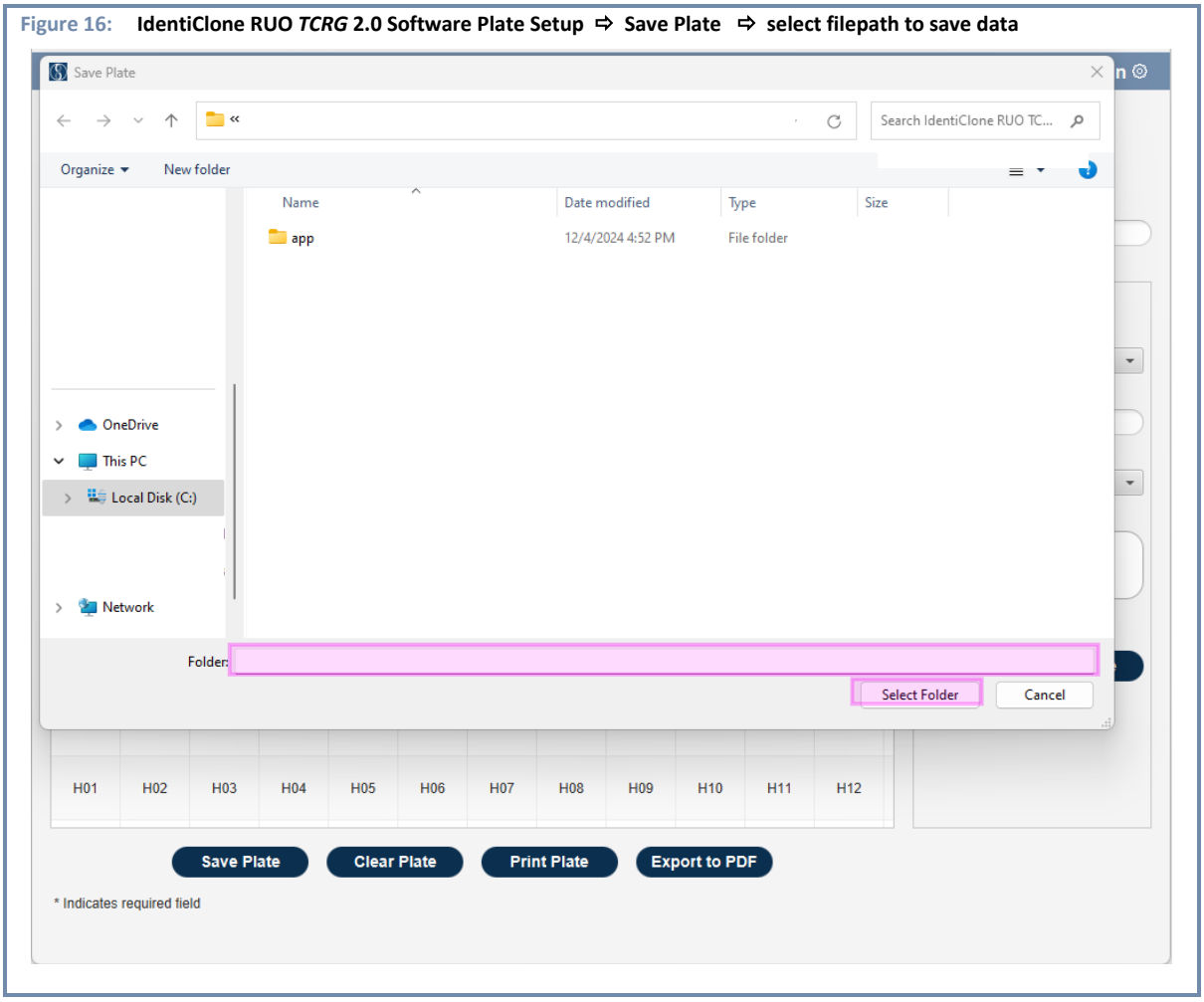
- The CSV file contains platemap information and will be imported to the ABI 3500xL or ABI 3500xL Dx Genetic Analyzer.

Important: **The CSV import file may not be compatible with old versions of ABI 3500 Data Collection Software.**

- The LIVS file contains platemap specific data required for the analysis and will be used in conjunction with the respective ABI 3500xL or ABI 3500xL Dx result files (i.e., FSA file).

Important: **Once generated, do NOT alter CSV and LIVS. If any further modifications are needed, refer back to Section 6.5 and generate a new platemap to obtain a new set of paired CSV and LIVS output files.**

Figure 16: **IdentiClone RUO *TCRG* 2.0 Software Plate Setup ⇨ Save Plate ⇨ select filepath to save data**



6.5.3. Alternatively, a user may create a platemap using a previously saved platemap file through excel.

- Using a previously used platemap will not replace existing results; every saved platemap possesses a unique identifier and is paired with unique LIVS file.

IMPORTANT! **Import Feature only permits the use of previously configured platemap to be used as a template for creating the new plate. Previously configured platemap cannot be modified using this feature.**

6.5.3.1. Manually set up the plate using a spreadsheet application, then import the resulting CSV file using the **Import** button (Figure 17).

6.5.3.1.1. Follow the CSV format (Figure 17) and platemap rules defined above; the CSV format and columns mapping includes:

- User Defined Field 1 => *Sample Type*, which can include SAMPLE, PC, NC or NTC
- User Defined Field 2 => *Run Number* requires a value from Run 1 to Run 24

Figure 17: **Example CSV file layout**

- ***Template CSV File* indicates the template file prior to any modifications. This file can be used as a template for generating a new platemap. Once the file is updated and saved, a unique SID number will be assigned at the end of the *Sample Name* (column B) in the newly generated CSV file.**
- ***New CSV File* indicates the file after modification and saving.**

Template CSV File (Before Save)

	A	B	C	D	E	F	G	H
1	3500 Plate Layout File Version 1.0							
2								
3	Plate Name	Application Type	Capillary Length (cm)	Polymer	Number of Wells	Owner Name	Barcode Number	Comments
4	Example	Fragment	50	POP7	96			
5							Sample Type	Run Number
6	Well	Sample Name	Assay	Results Group	File Name Conventi	Sample Type	User Defined Field 1	User Defined Field 2
7	A01	Sample1	TCRG Instrument Parameters	IC_RUO_TCRG_RG	IC_RUO_TCRG_FNC	Sample	SAMPLE	Run 1
8	A02	Sample2	TCRG Instrument Parameters	IC_RUO_TCRG_RG	IC_RUO_TCRG_FNC	Sample	SAMPLE	Run 2

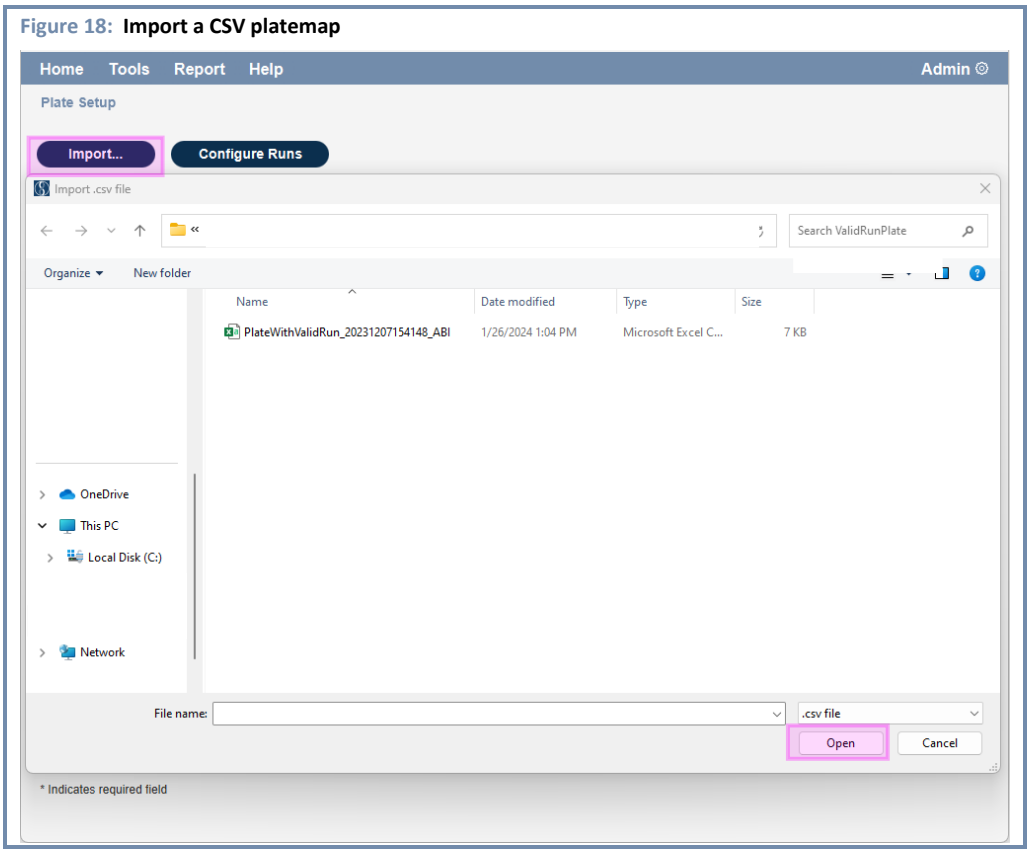
New CSV File (After Save)

	A	B	C	D	E	F	G	H
1	3500 Plate Layout File Version 1.0							
2								
3	Plate Name	Application Type	Capillary Length (cm)	Polymer	Number of Wells	Owner Name	Barcode Number	Comments
4	Example	Fragment	50	POP7	96			
5								
6	Well	Sample Name	Assay	Results Group	File Name Conventi	Sample Type	User Defined Field 1	User Defined Field 2
7	A01	Sample1_SIDd195682ca608	TCRG Instrument Parameters	IC_RUO_TCRG_RG	IC_RUO_TCRG_FNC	Sample	SAMPLE	Run 1
8	A02	Sample2_SIDc029313bc1d5	TCRG Instrument Parameters	IC_RUO_TCRG_RG	IC_RUO_TCRG_FNC	Sample	SAMPLE	Run 2

Note: SID Tag Added

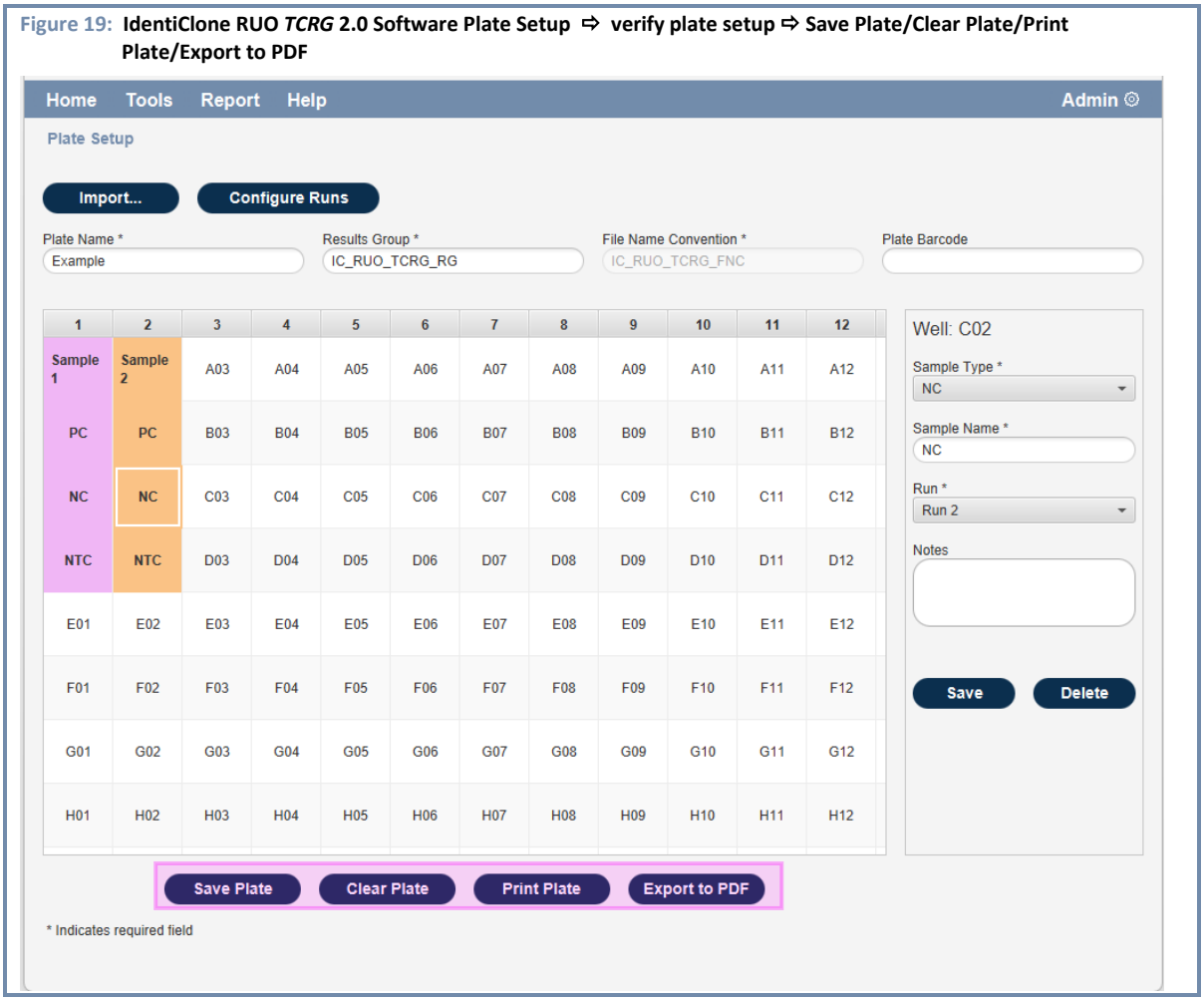
6.5.3.1.2. Click **Import**, then select the corresponding CSV file. (Figure 18)
6.5.3.1.3. Verify the correct CSV file populates the *File name* box and click **Open**.

Figure 18: Import a CSV platemap



6.5.3.2. Confirm that each data field for the plate and each sample is populated correctly. (Figure 19)

Figure 19: IdentiClone RUO *TCRG* 2.0 Software Plate Setup ⇨ verify plate setup ⇨ Save Plate/Clear Plate/Print Plate/Export to PDF



6.5.3.3. After confirming that all samples and controls are populated correctly, click **Save.**

- Refer to section 6.5.2.6 and Figure 16 to save the newly generated CSV and LIVS files.
- To clear all information stored on the plate map, click **Clear Plate.**

6.5.4. Save/Print PDF template of plate map.

6.5.4.1. Print plate map to local printer.

6.5.4.1.1. To generate a hard copy of the plate map displaying Sample Name, well information, and assay information click **Print Plate.**

6.5.4.1.2. Select the appropriate printer to print. Adjust the printing properties/settings as needed, and click **OK** to print.

6.5.4.2. Save plate map as PDF. (Figure 20)

6.5.4.2.1. To save a PDF version of the plate map displaying Sample Name, well information, and assay information click **Export to PDF.**

6.5.4.2.2. Select desired file directory to save PDF file, click **Select Folder.**

Figure 20: IdentiClone RUO *TCRG* 2.0 Software Printable Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample1 Run 1	Sample2 Run 2										
B	PC Run 1	PC Run 2										
C	NC Run 1	NC Run 2										
D	NTC Run 1	NTC Run 2										
E												
F												
G												
H												

Example 2024-12-17 14:25

6.5.5. Proceed to fragment analysis by capillary electrophoresis (refer to the *TCRG* 2.0 Assay IFU, : 280288, sections 7.5.3: *ABI Fluorescence Detection with ABI 3500 series instruments* and 8.3: *Sample Analysis and Interpretation (Automated)*).

IMPORTANT! Do not edit the platemap on the ABI 3500xL instrument following importing of the CSV file onto the device. If additional changes are required, recreate the platemap in the IdentiClone RUO *TCRG* 2.0 Software and save as a new plate. Use the CSV file generated from the new plate to import onto the device. If the software platemap and ABI platemap do not match, software analysis cannot proceed.

7. Result Analysis

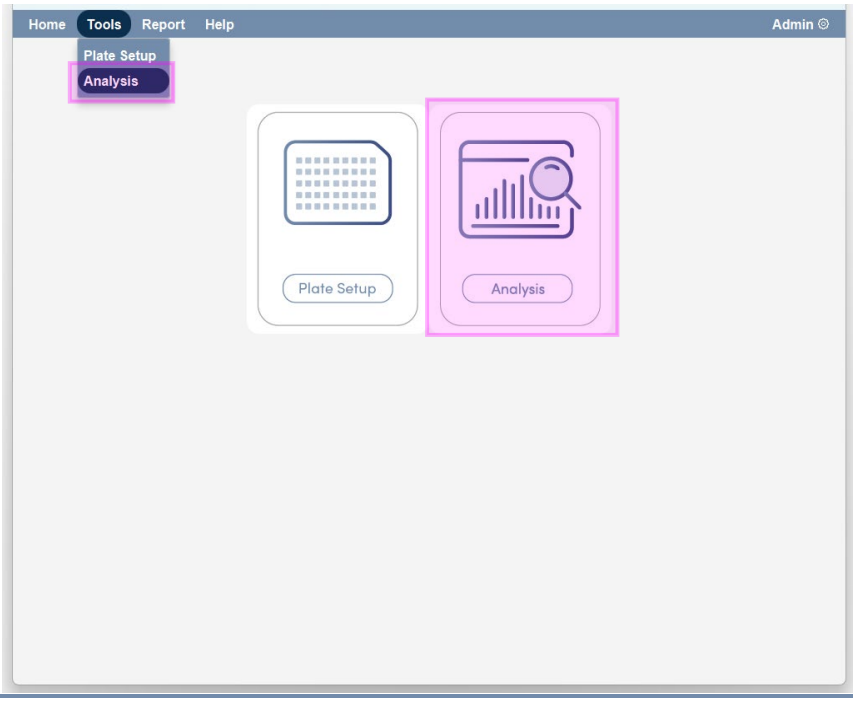
7.1. Select Data for Analysis

7.1.1. Select the *Analysis* tool from the home user Interface.

- Alternatively, *Analysis* can be accessed by navigating to **Tools → Analysis**. (Figure 21)

7.1.1.1. Verify that both FSA and LIVS files for the associated run(s) and plate(s) are located in the same directory filepath.

Figure 21: IdentiClone RUO *TCRG* 2.0 Software home user interface ⇨ Analysis



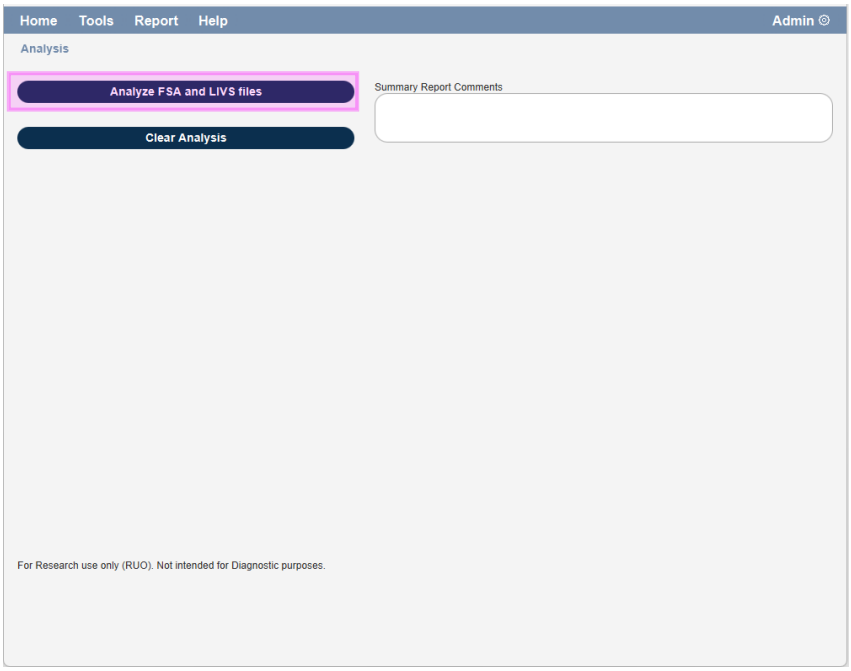
7.1.2. Click Analyze FSA and LIVS files. (Figure 22)

7.1.2.1. Navigate to the directory filepath containing the FSA and LIVS files, select the folder, and confirm the selection.

- Multiple plates can be processed simultaneously, provided all corresponding LIVS and FSA files are present within the selected folder, including those in nested folders.

Note: **Do not edit FSA files generated by the ABI 3500xL platform. These FSA files are validated with LIVS files from the software. Uploading files incorrectly will halt analysis process. An error message window will pop-up with a corresponding error code. (Table 4)**

Figure 22: **IdentiClone RUO *TCRG* 2.0 Software Analysis**



Important: **Sample results are generated at this time. These results are for Research Use Only purposes and should not be used for any determination of medical diagnosis.**

7.2. Select Samples for Analysis

7.2.1. Select samples for report generation (☑). (Figure 23)

- By default, all runs and samples from valid runs are preselected to generate corresponding PDF reports.

7.2.1.1. Click checkbox (☑) to toggle the selection of any *Run Report* or *Sample Report*.

7.2.1.1.1. Click the checkbox by Run Report ☑ to toggle the selection of all run reports.

7.2.1.1.2. Click the checkbox by Sample Report ☑ to toggle the selection of all selectable sample reports.

7.2.1.1.3. Click the checkbox (☑) under the *Sample Report* column, within a row corresponding to a run to select all samples included in that run. (Figure 24)

Figure 23: IdentiClone RUO *TCRG* 2.0 Software ⇨ Intermediate Sample Results

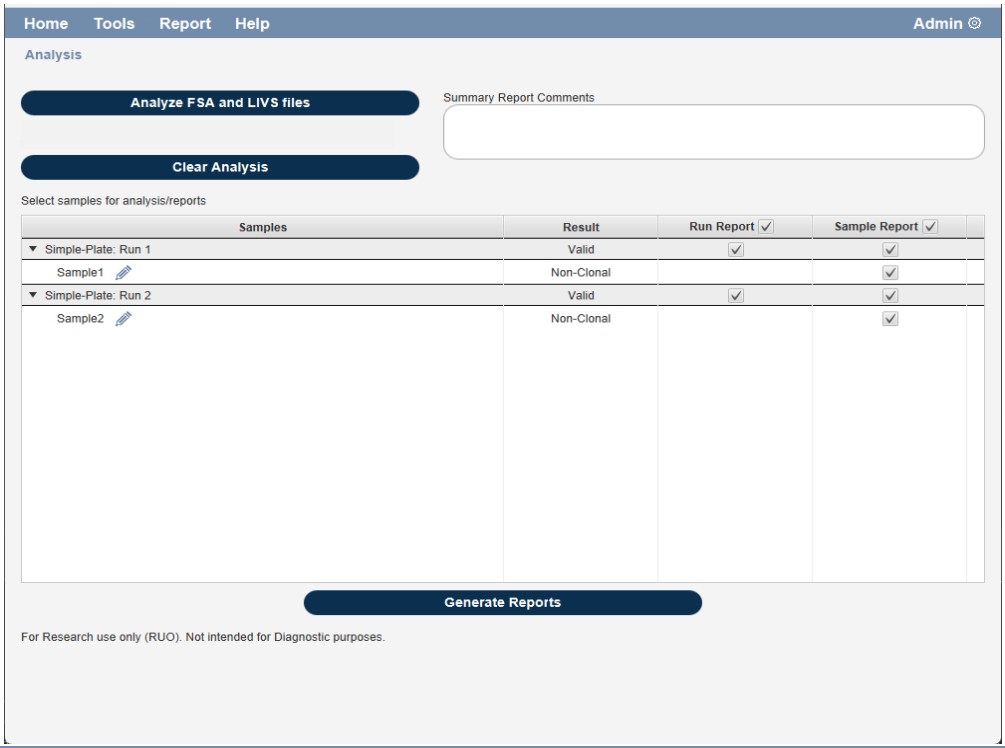
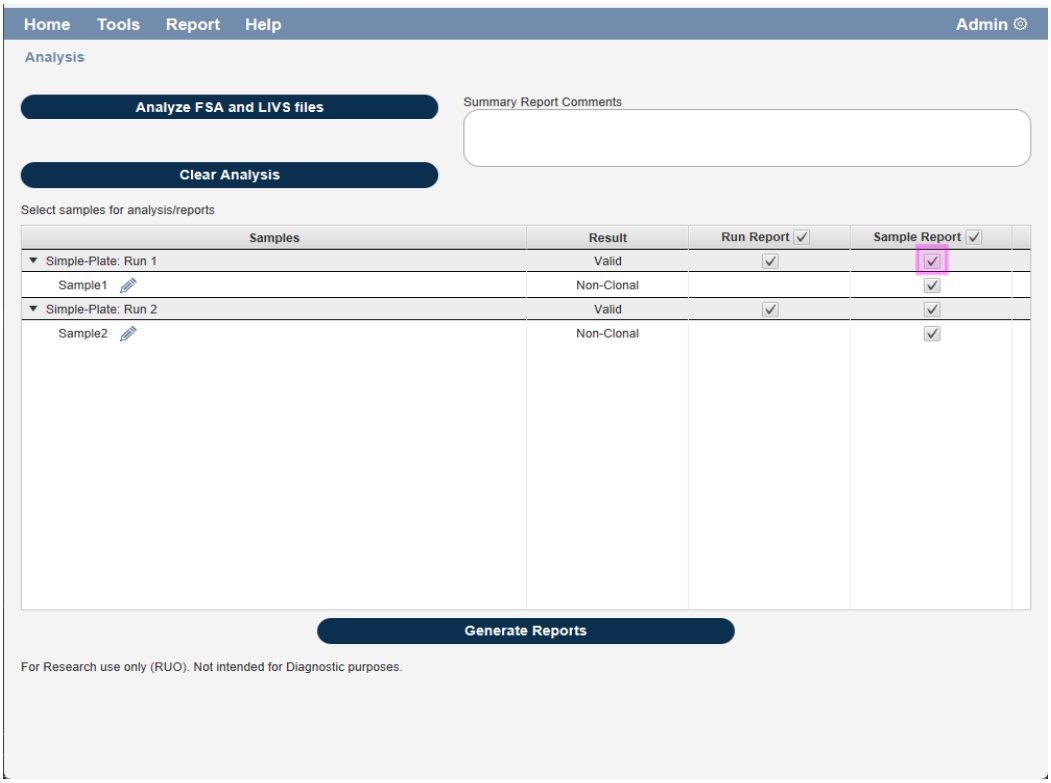


Figure 24: IdentiClone RUO *TCRG* 2.0 Software ⇨ Generate Sample Reports for Runs



Note: **If a run is invalid, associated sample reports cannot be selected from that run.**

7.3. Generate Reports

7.3.1.1. Click Generate Reports

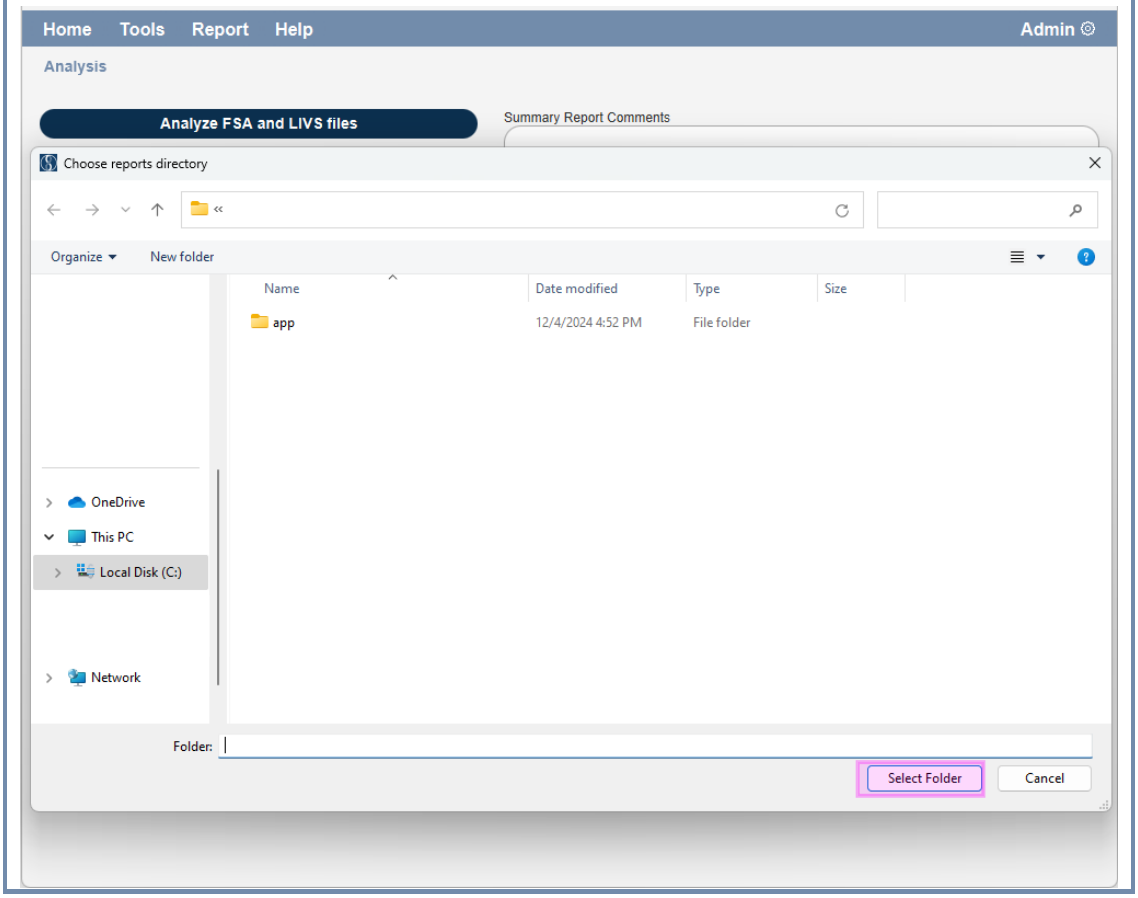
7.3.1.1.1. Select the directory location for the reports to be saved; click **Select Folder.** (Figure 25)

- An *Analysis Completed* pop-up window will appear if analysis was successful. Click **Open Folder** to open the designated file directory. Click **Close** to dismiss window.

7.3.1.2. After the reports are generated, a prompt will appear, providing the option to open the folder containing the reports.

- Results are grouped by *Sample Name.*

Figure 25: IdentiClone RUO *TCRG* 2.0 Software ⇨ Select Directory for Reports



7.3.2. Software Report Interpretation

7.3.2.1. A *Run Report* is generated by the software for every run selected following analysis.

- A *.csv* format run report including traceable run information as well as a summary of controls and samples is generated by the software. (Figure 26)
- Traceable run information and control validity is recorded on Page 1 of the Run Report generated by the software. (Figure 27)
- A summary of samples included in a run is included starting on Page 2 of the Run Report generated by the software. (Figure 28)

Figure 26: *.csv* Run Report

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Sample Name	Sample Type	Sample Result	RBP	Error Code	Well	Run Result	Run ID	Run Number	Plate Barcode	Plate Name	Sample Filename	Software Version	Part Number	Lot Number	Expiration	ABI Instrument	ABI Serial Number	Run Start Date	Sample ID	Sample Notes
2	NTC	NTC	Valid			A03	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	NTC_SIDcd4b65138916.fsa	v1.2.0.RUO				3500	33873-040	1/31/2023 10:05	SIDcd4b65138916	
3	PC	PC	Valid	4.99		A01	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	PC_SID1c8345569b75.fsa	v1.2.0.RUO				3500	33873-040	1/31/2023 10:05	SID1c8345569b75	
4	NC	NC	Valid	-3		A02	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	NC_SIDa06424e18100.fsa	v1.2.0.RUO				3500	31815-051	1/18/2023 15:26	SIDa06424e18100	
5	Sample10	SAMPLE	Non-Clonal	-3.1		B01	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	Sample10_SIDbdcf9007bcf9.fsa	v1.2.0.RUO				3500	33870-040	6/5/2023 13:13	SIDbdcf9007bcf9	
6	Sample11	SAMPLE	Invalid		AN04.03	B02	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	Sample11_SID6d63957c4149.fsa	v1.2.0.RUO				3500	33873-040	1/31/2023 10:05	SID6d63957c4149	
7	Sample12	SAMPLE	Non-Clonal	-3.1		B03	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	Sample12_SIDfd6806effdcf.fsa	v1.2.0.RUO				3500	33870-040	6/5/2023 13:13	SIDfd6806effdcf	
8	Sample1	SAMPLE	Non-Clonal	-3.1		A04	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	Sample1_SID0b165027d37d.fsa	v1.2.0.RUO				3500	33870-040	6/5/2023 13:13	SID0b165027d37d	
9	Sample2	SAMPLE	Non-Clonal	-3.1		A05	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	Sample2_SID7f3c1a0dcef2.fsa	v1.2.0.RUO				3500	33870-040	6/5/2023 13:13	SID7f3c1a0dcef2	
10	Sample3	SAMPLE	Non-Clonal	-3.1		A06	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	Sample3_SID348704aa5952.fsa	v1.2.0.RUO				3500	33870-040	6/5/2023 13:13	SID348704aa5952	
11	Sample4	SAMPLE	Non-Clonal	-3.1		A07	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	Sample4_SID13db8712d11c.fsa	v1.2.0.RUO				3500	33870-040	6/5/2023 13:13	SID13db8712d11c	
12	Sample5	SAMPLE	Non-Clonal	-3.1		A08	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	Sample5_SIDe2f813764d47.fsa	v1.2.0.RUO				3500	33870-040	6/5/2023 13:13	SIDe2f813764d47	
13	Sample6	SAMPLE	Non-Clonal	-3.1		A09	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	Sample6_SID3cc6c4d5fd63.fsa	v1.2.0.RUO				3500	33870-040	6/5/2023 13:13	SID3cc6c4d5fd63	
14	Sample7	SAMPLE	Non-Clonal	-3.1		A10	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	Sample7_SID19fc29c241b3.fsa	v1.2.0.RUO				3500	33870-040	6/5/2023 13:13	SID19fc29c241b3	
15	Sample8	SAMPLE	Non-Clonal	-3.1		A11	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	Sample8_SID939f279aa213.fsa	v1.2.0.RUO				3500	33870-040	6/5/2023 13:13	SID939f279aa213	
16	Sample9	SAMPLE	Clonal	5.62		A12	Valid	1e2f06e9-af45-4a2d-a5ec-82e12da0bccd	1	A123456789	SamplePlate	Sample9_SIDcebc57f0a2c7.fsa	v1.2.0.RUO				3500	33870-040	6/5/2023 13:13	SIDcebc57f0a2c7	

Figure 27: **IdentiClone RUO *TCRG* 2.0 Software Run Report Page 1**

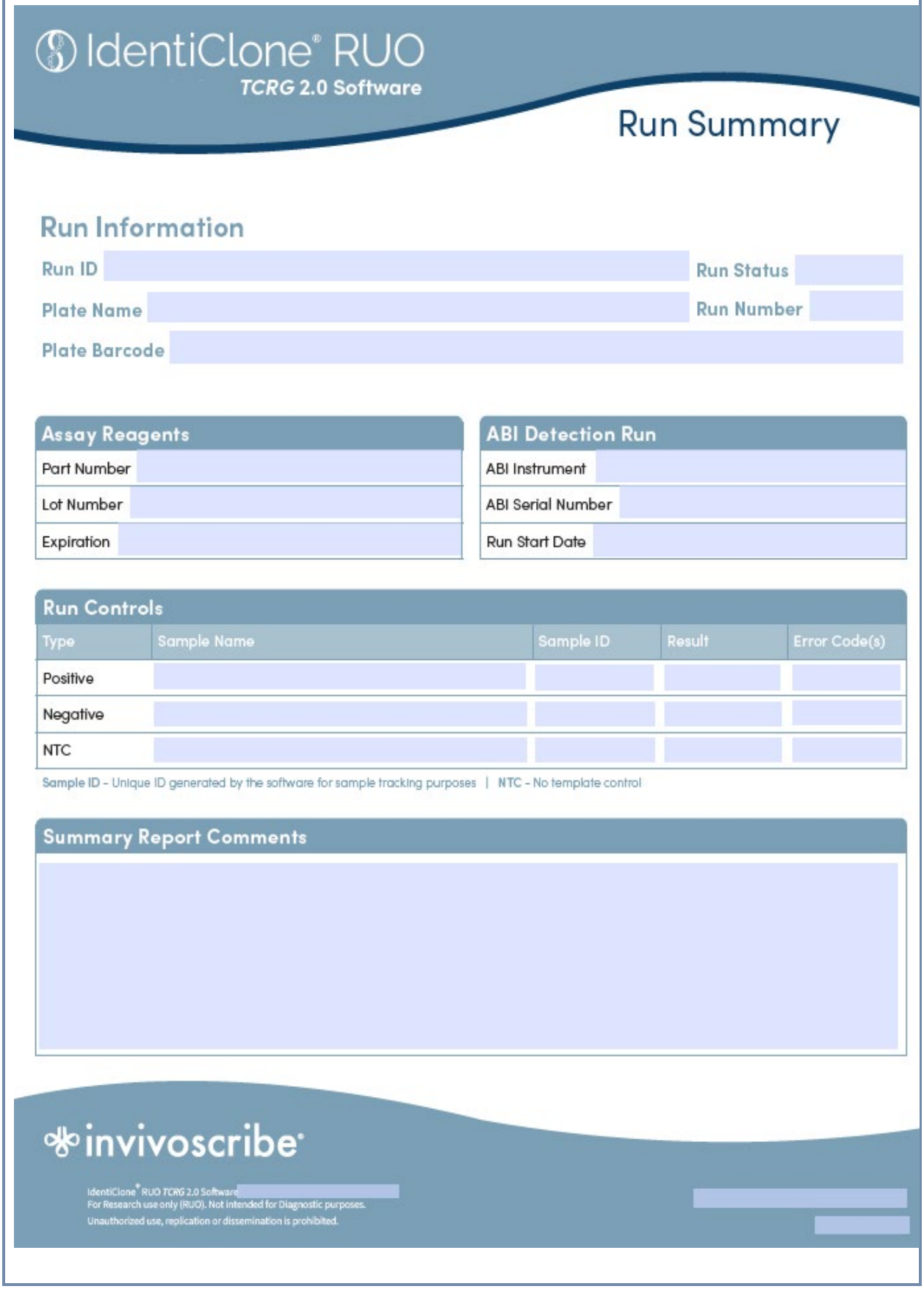


Figure 28: **IdentiClone RUO *TCRG* 2.0 Software Run Report Page 2**

7.3.3. A *Sample Report* is generated by the software for every sample selected following analysis (Figure 29); information in the report includes:

- Sample information, such as the sample result, RBP, unique identifiers (e.g., Sample Name, Sample ID) and associated notes; and
- Run information contains traceability records, e.g., Run ID and Run Number, as well as optional fields such as Assay Reagent and ABI instrument data.

Figure 29: **IdentiClone RUO *TCRG* 2.0 Software Sample Report**

IMPORTANT! **RBP values are included for information only. These values have not been validated to have a direct quantitative correlation with clonality levels.**

8. Software Error Messages and Corrective Action(s)

8.1. Plate Map (PM) Errors

Table 3 includes potential error codes associated with step 6.5 *Create Platemap*. Follow the indicated corrective action in the event that any of these error codes appear during plate mapping.

Table 3: Plate Map Error Codes and Associated Corrective Actions

Error Code	Error Message	Corrective Action
PM04	Plate name cannot be blank	Verify the *Plate Name* field is populated.
PM05	Plate name contains illegal characters	Verify *Plate Name* contains only letters (A-Z, a-z) numbers (0-9), underscores (_) and hyphens (-). No spaces are permitted.
PM07	Results group cannot be blank	Ensure the *Results Group* field is not blank.
PM11	Plate contains no samples	Ensure each plate has at least one run containing one set of controls and at least one sample.
PM12	Sample name contains illegal characters	Verify *Sample Name* contains no more than 50 characters, and only includes letters (A-Z, a-z), numbers (0-9), underscores (_), and hyphens (-). No spaces are permitted.
PM14	Sample name cannot exceed 50 characters	Shorten *Sample Name* to be less than or equal to 50 characters.
PM15	Invalid sample type detected	Ensure the rules below are followed before importing a CSV file representing a platemap (this is created using a CSV file from a previous run): • The *Sample Type* column can only include values = *SAMPLE, PC, NC or NTC.* • The *Sample Name* and *User Defined Fields 1* and *2* in the CSV file must be empty or all the fields must be entered following the platemap rules. • *User Defined Field 1* => *Sample Type* with values = *SAMPLE, PC, NTC, NC.* • *User Defined Field 2* => *Run number* from *Run 1* up to *Run 24.*
PM16	• Sample name cannot be blank; OR • Run number cannot be blank; OR • Sample must have a sample type assigned	Verify each well contains the necessary information fields prior to saving.
PM22	Run is missing a positive/negative/no template control or Run has too many positive/negative/no template controls	When adding a *Run*, verify it contains exactly one set of controls, i.e., one NC, one PC and one NTC.
PM24	Import file contains no samples	Verify the import file is properly formatted with the appropriate *Sample Name* field.
PM28	Plate name cannot exceed 50 characters	Decrease *Plate Name* to be less than or equal to 50 characters.
PM29	Sample notes contains illegal characters	Verify *Sample Notes* does not contain commas.
PM30	Barcode contains illegal characters	Verify the *ABI instrument Barcode* is correct.
PM34	Well is assigned a run, but is missing a sample information	Save the *Wells* assigned to a run with the associated sample or control information.
PM35	Sample notes cannot exceed 50 characters	Verify *Sample Notes* only include up to 50 characters.
PM36	Invalid run number detected	Run Number can only be a numeric character from 1 - 24.

8.2. File Validation (FV) Errors

The error codes listed in Table 4 can occur while performing step 7.1 *Select Data for Analysis*; if any of these error codes appear while selecting data for analysis follow the indicated corrective action.

Table 4: File Validation Error Codes and Associated Corrective Actions

Error Code	Error Message	Corrective Action
FV03.5	Invalid FSA file	Repeat the Assay beginning from Fragment Analysis by Capillary Electrophoresis. • Do not edit the LIVS files after saving the plate • Do not edit FSA file output from ABI 3500
FV04.1	The format of the LIVS file is invalid	
FV06.1	Some samples in the list of FSA files have no match in the LIVS file. Ensure the ABI platemap is not manually edited.	
FV06.2	LIVS file cannot be located	Upload the corresponding LIVS file generated by the Software (containing the annotated plate information) in conjunction with the FSA files for analysis.
FV06.3	Path provided is not a directory	Ensure the correct directory is selected containing FSA and LIVS files.
FV06.4	Multiple LIVS files representing the same plate found	Only use LIVS files generated by the Software; do NOT duplicate any LIVS files – they include annotated plate information that allows traceability of samples to a plate.
FV06.5	Maximum LIVS files limit reached	Limit the number of LIVS files in the directory to 5
FV06.6	Invalid ABI settings detected. Please confirm the ABI settings used match those specified in the IFU. Refer to IFU for further instructions.	Reset the ABI settings as recommended, then repeat the Assay beginning from Fragment Analysis by Capillary Electrophoresis. Only the FSA files generated by the ABI instrument using the recommended ABI settings can be uploaded to perform the analysis.
FV07.1	• The format of the import file is invalid; OR • There was a problem importing the plate.	Verify the correct CSV file was selected for importing into the plate map setup.

8.3. Analysis (AN) Errors

Table 5 includes error codes that can occur during data analysis. Follow the indicated corrective action below if any of the error codes below appear during data analysis.

Table 5: Analysis Error Codes and Associated Corrective Actions

Error Code	Error Description	Corrective Action
AN01.01	NTC invalid	Re-test entire run starting from Fragment Analysis by Capillary Electrophoresis.
AN01.02	NTC invalid	Re-test entire run starting from PCR Amplification.
AN02.01	PC invalid	Re-test entire run starting from Fragment Analysis by Capillary Electrophoresis.
AN02.02		
AN02.03		
AN02.04	PC invalid	Re-test entire run starting from PCR Amplification.
AN02.05		
AN02.06	PC invalid	Re-test entire run starting from PCR Amplification. If the problem persists, contact IVS Customer Support.
AN02.07		
AN03.01	NC invalid	Re-test entire run starting from Fragment Analysis by Capillary Electrophoresis.
AN03.02		
AN03.03		

Table 5: Analysis Error Codes and Associated Corrective Actions

Error Code	Error Description	Corrective Action
AN03.04	NC invalid	Re-test entire run starting from PCR Amplification.
AN03.05		
AN03.06	NC invalid	Re-test entire run starting from PCR Amplification. If the problem persists contact IVS Customer Support.
AN04.01	Sample invalid	Re-test sample starting from Fragment Analysis by Capillary Electrophoresis.
AN04.02		
AN04.03		
AN04.04	Sample invalid	Re-test sample starting from PCR Amplification.
AN04.05	Sample invalid	Re-test sample starting from PCR Amplification.
AN04.08	Sample invalid	Refer to *Run Report* to check for run failure error code.

8.4. Other (OT) Errors

Error codes listed in Table 6 are categorized as "other" and can occur at any time while using software. Follow the corrective action indicated for the specified error code.

Table 6: Other Error Codes and Associated Corrective Actions

Error Code	Error Message	Corrective Action
OT01	Not enough disk space available in chosen result output location	Verify the output file location selected for file export has enough space (at least 10 MB).
OT02	Output location (file path) for results file is not writable	Verify the directory file path selected has write permissions.
OT03	Input location is not readable	Verify the directory file path selected has read permissions.

9. References

1. van Dongen, J, et al. (2003) "Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936." *Leukemia,* 17:2257–2317.
2. Langerak, AW, et al. (2012) "EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations." *Leukemia,* 26:2159-2171.
3. Miller, JE, et al. (1999) "An automated semiquantitative B- and T-cell clonality assay." *Molecular Diagnostics,* 4(2):101-117.

- T-cell Receptor Gamma Gene Rearrangement Assay 2.0 Instructions for Use (Invivoscribe : 280288)
- ABI 3500 and 3500xL Genetic Analyzer User Manual (Obtain applicable user manual from equipment manufacturer)

10. Technical and Customer Service

We appreciate your business. We are happy to assist you with understanding this software, and will provide ongoing technical assistance Monday through Friday to ensure our assays are performing efficiently in your laboratory.

Contact Information

Invivoscribe, Inc
10222 Barnes Canyon Road | Building 1 | San Diego | California 92121-2711 | USA
Phone: +1 858 224-6600 | Fax: +1 858 224-6601 | Business Hours: 7:00AM - 5:00 PM PST/PDT
Technical Service: support@invivoscribe.com | Customer Service: sales@invivoscribe.com | Website: www.invivoscribe.com

11. Symbols

The following symbols are used in Invivoscribe RUO product labeling.

REF	Catalog Number		Expiration Date
	Storage Conditions	RUO	Research Use Only
	Manufacturer		Expiration Date
VOL	Reagent Volume		Consult Instructions for Use

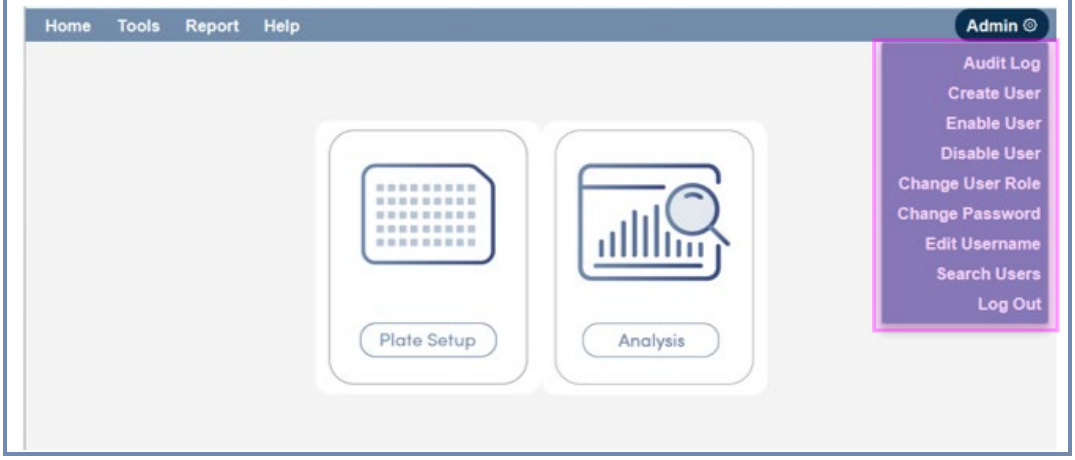
12. Legal Notice

For Legal Notices related to this product, visit: https://invivoscribe.com/legal-notices/

13. Appendix A : Admin User Access

The Admin user has additional privileges as compared to a basic user, including additional menu options (Figure 30) to allow multiple user management features to be accessed.

Figure 30: **Additional Admin menu options**

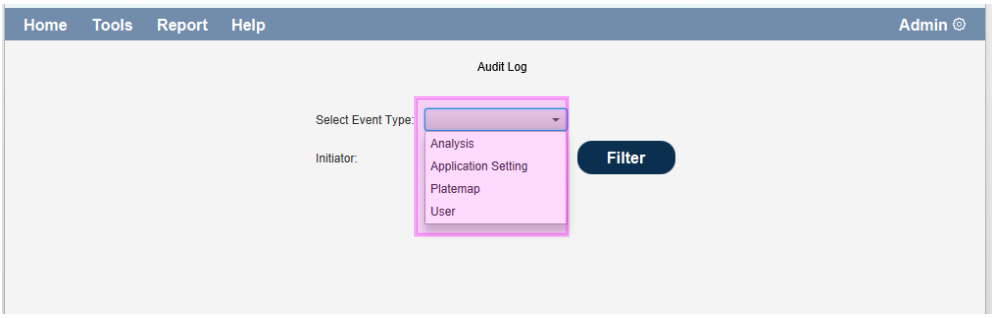


13.1. Audit log

Only users with Admin privileges have access to view the Audit logs, which allows all activities performed with the Software to be viewed by category based on the event type and corresponding action(s). (Figure 31)

13.1.1. **Analysis Event Type:** All analysis activity is logged from start of analysis to the report generation activities.
13.1.2. **Application Setting**: The setting activity that applied throughout the application; e.g., setting backup location activities are logged.
13.1.3. **PlateMap**: All plate setup activities, e.g., saving a platemap or importing a CSV file for plate setup.
13.1.4. **User**: All user activities, such as editing username, change role, login, logout etc., are logged.

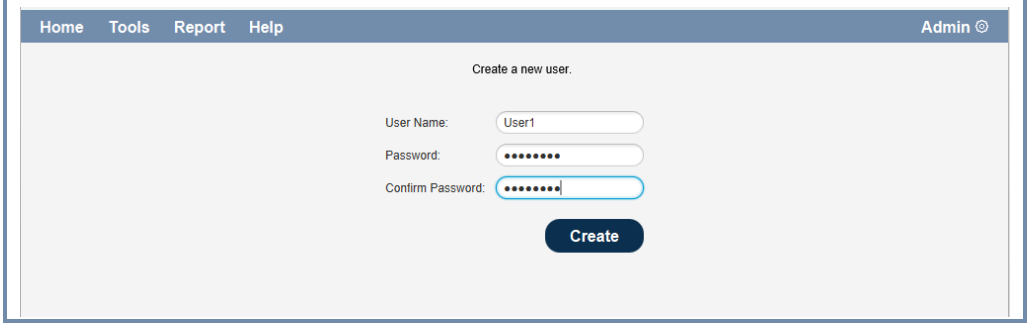
Figure 31: Audit Log categories



13.2. Create User

Only users with Admin privileges have the ability to create other users with basic role privileges; this requires a username and password. (Figure 32)

Figure 32: Create a new user



13.3. Enable and disable a User

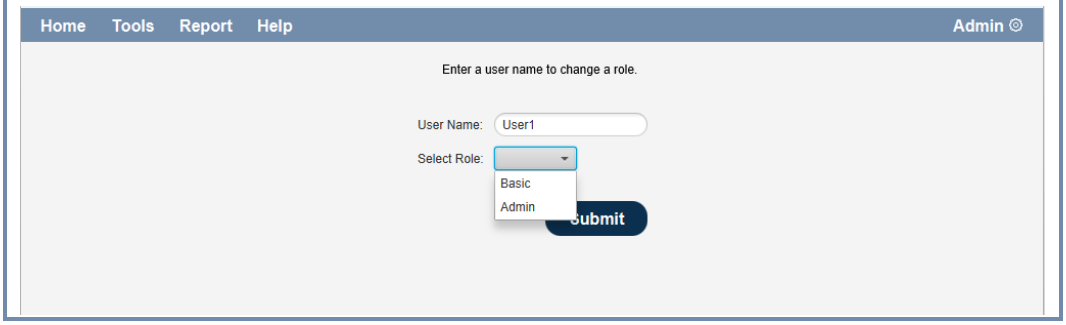
Only users with Admin privileges have the ability to enable and disable users assigned a basic role. Admin level users cannot be disabled. If a user is disabled or deactivated, they cannot login to the Software until the user is enabled and activated. (Figure 33)

Figure 33: Enable / Disable a user

13.4. Change User Role

Only users with Admin privileges have the ability to change a user role from *Basic* to *Admin* or vice versa. (Figure 34)

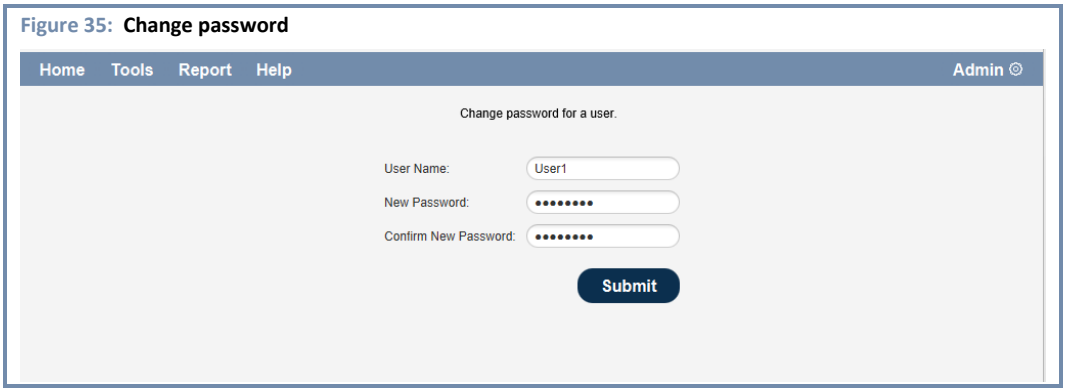
Figure 34: Change user role



13.5. Change User Password

Only users with Admin privileges have the ability to change their own and other user passwords by providing a username and a new password. (Figure 35)

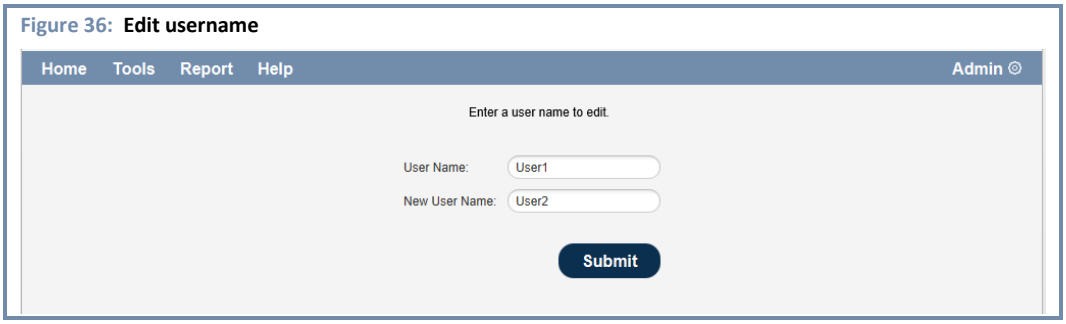
Figure 35: Change password



13.6. Edit Username

Only users with Admin privileges have the ability to edit their own and other usernames by providing the old (former) username and new username. (Figure 36)

Figure 36: Edit username



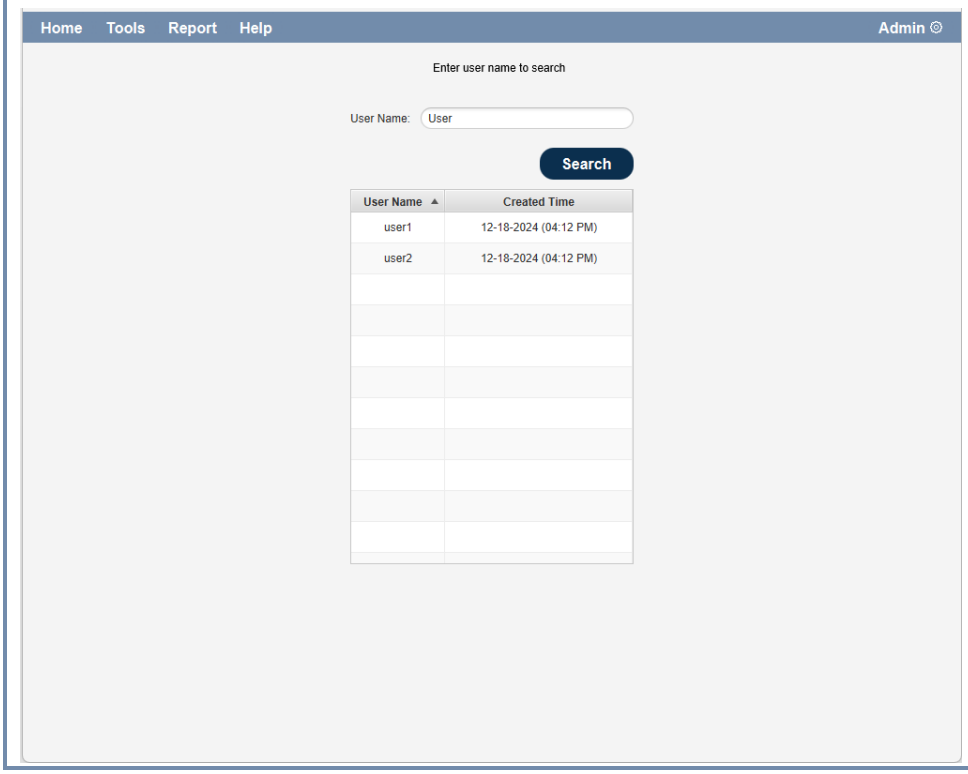
13.7. Search Users

Only users with Admin privileges have the ability to search for the users with access to the Software. (Figure 37)

13.7.1. Click **Admin** and select **Search** from the dropdown menu, then search by entering part of a username.

13.7.2. The software returns the list of users matching the username search criteria.

Figure 37: Search for user



13.8. Set Back up location

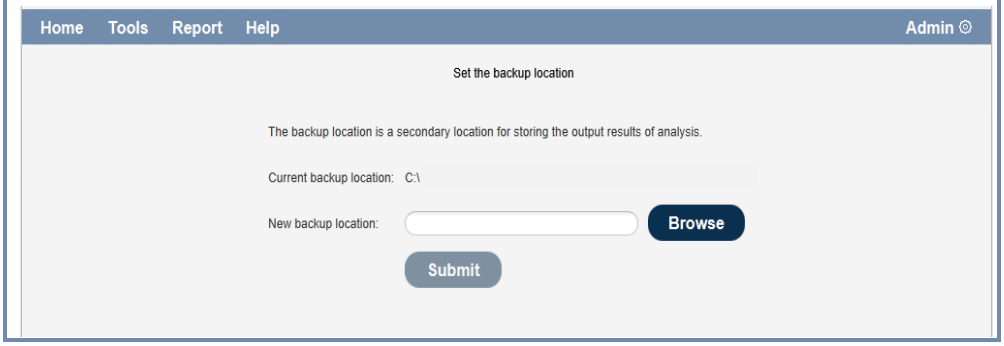
Only users with Admin privileges have the ability to use the *Set Backup Location*, which allows configuration of the directory filepath to for the PDF reports. The first Admin user can set the PDF report backup location prior to using the *Analysis* function.

13.8.1. Click the **Report** menu and select **Set Backup Location** from the dropdown menu.

13.8.2. Click the **Browse** button and navigate to the directory filepath for the PDF report backup files, then click **Submit**. (Figure 38)

- The filepath for a previously configured backup location will display in the *Current Backup Location* field.
- The Software will default to use the install directory.

Figure 38: Set Backup location function



13.9. Basic user access

The role of a basic user includes limited permissions, allowing access to perform *Plate Setup*, *Analysis, Edit Username* and *Change Password*.

13.9.1. User management features are accessed under the *Settings* menu. (Figure 39)

13.9.2. Change a basic user password or username by clicking on the respective options in the dropdown menu.

Figure 39: Basic User settings

13.10. User Inactivity

The Software application is programmed to provide a warning after 5 minutes of inactivity, which includes a prompt to *Continue* or *Cancel* the session. If this prompt is ignored, the Software will log out the user and return to the *Login* screen. (Figure 40)

- When the user logs in again, the Software will continue to the same screen / function before the user was logged out.

Figure 40: Inactivity warning and prompt