

Genie[®] III User Manual

(Instrument Software Version v3.18.2)



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SAFETY NOTICES

Please read the following notices carefully before using Genie[®] III.

The Genie® III is specifically designed to run any isothermal amplification method that employs target detection by fluorescence measurement. Genie® III will detect all dyes that can be excited from a blue light source and with an emission between 510 and 560 nm and all dyes excited by a yellow light source with an emission above 620 nm. Further uses include enzyme kinetic analysis and protein denaturation analysis using fluorescent dyes.

The equipment supplied has been designed to be completely safe to use. However to avoid any risk to the safety of the equipment, operator, or anybody in the vicinity of the equipment, please read this chapter before unpacking and using the instrument. If there is any doubt as to the correct use of the equipment contact the vendor.

Notices



Using the instrument in a manner not specified by OptiGene may result in personal injury or damage to the instrument and the protection provided by the equipment may be impaired.



Always ensure that the surface on which the instrument is placed is level and stable and will not cause the instrument to topple over. Ensure that the surface is suitable for the weight and size of the instrument. If the instrument is dropped it may be damaged.



The instrument should never be lifted by its covers. Always ensure that the base or sides are used as the lifting point.



The instrument is electrically powered. Please ensure that the correct voltage settings have been applied before applying power to the instrument. If in doubt consult a qualified electrician. The instrument has a rating label affixed to the rear. Please consult this if needed.



Always disconnect the equipment before moving or removing any guards or covers. Switch off at the mains, remove the mains plug from the wall socket and remove the cable from the inlet socket on the rear.



The instrument carries an IP60 rating. This means it is protected from total dust ingress. This only applies when the lid on the top and the lip on the back are closed. If fluids are spilt on the instrument when the lid is open they may cause damage and cause an electrical hazard.



If a spill occurs, remove power from the instrument. Do not touch the instrument or any fluid flowing from it while it is connected to the mains supply. Always follow local health and safety guidelines.

Normal safe local operating standards should be applied at all times. The warnings above are for guidance only. Please consult the instrument supplier if there is any doubt.

Disconnection Method



Genie® III is disconnected by removal of incoming mains power source to the unit. Following disconnection the unit should be left for a period of at least 5 minutes before any internal assemblies are removed or examined.



When in use the heating block and heated lid are hot, so allow to cool before touching the surfaces.



Safe removal of fluids from Genie[®] III will depend on the chemistry used. This will also require knowledge of the fluids used in the system to adhere with local health and safety and COSHH regulations. If in doubt, consult the person responsible for the equipment in the laboratory.

Cleaning Method

The Genie® III can be disinfected using the following procedure which can be used as a safety measure if this equipment is routinely exposed to bio-hazardous materials.

- 1. Wipe all outside surfaces of the Genie® III with a 10% bleach solution.
- 2. After 10 minutes wipe all the same surfaces with a 70% ethanol solution.

CAUTION: Do not allow any of the liquid solutions to enter the wells as this can cause damage.

SUPPORT

HOW TO OBTAIN SUPPORT

For the latest services and support information go to http://www.optigene.co.uk/support.htm

IMPORTANT! When directed to do so, contact OptiGene Ltd. to schedule maintenance or calibration of a Genie® III instrument.

IMPORTANT! If a Genie® III instrument is kept in a very cold environment, the battery will not begin charging until the internal temperature has reached 15°C.

SUPPORTED CONSUMABLES

IMPORTANT! Genie[®] III uses a proprietary tube strip that maximises optical and thermal efficiencies. **Other tubes and strips will not fit.**

IMPORTANT! Forcing non-supported consumables will cause damage to the instrument and invalidate the warranty.

IMPORTANT! The shape of the tubes is such that they will only fit in one way round. The locating pins on the block have corresponding holes in the strips.

BOX CONTENTS

The following is a list of contents in the box for Genie® III:

- Genie® III instrument
- Power supply
- Power lead
- USB connection lead
- Stylus
- USB memory stick containing Genie® Explorer and this manual as a '.PDF' file

SITE PREPARATION

HOW TO SET UP GENIE® III

Genie® III has been designed to be used at point-of-care, and so not necessarily in a laboratory. When it is being used, the instrument should be placed on a level and stable surface and the surfaces surrounding the instrument must be clear of obstructions at all times.

Care must be taken not to unduly restrict the air at the outlet vents at the rear. Restricting airflow may impede operation and could affect performance.

Electrical points should be close to the instrument to avoid injury from trailing wires.

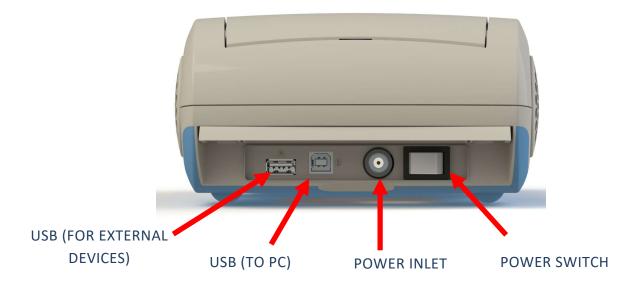
Genie® III is rated to IP60 which means that the internal electronics and optical block is protected from dust, but only when the lid on top and the lip on the back are closed. It is recommended that the instrument is kept away from sinks and other wet areas when running.

CONNECTIONS

Genie[®] III is ready to use straight out of the box without any external connections. It can be operated standalone, taking power from its internal battery. In order to charge the battery or to use Genie[®] III with a computer, some connections must be made.

Connect the power supply plug into the back of the instrument and then attach the power cable to the supply.

Located at the rear of the instrument is an on/off power switch. When in the on position Genie® III will power up and progress through its checks.



OPENING & CLOSING THE LID

Press the button on the front of the unit and the lid should open upwards. Close the lid by lowering and pressing down firmly.



Care must be taken to ensure that objects are not obstructing the lid when trying to close it and under no circumstances should the lid be forced open or closed.

INSERTING TUBES

IMPORTANT! Genie® III uses a proprietary tube strip that maximises optical and thermal efficiencies. Other tubes and strips will not fit.

IMPORTANT! The shape of the tubes is such that they will only fit in one way round. The locating pins on the block have corresponding holes in the strips.

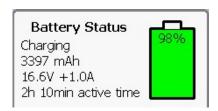
BATTERY

Genie[®] III has an internal rechargeable battery. When Genie[®] III is delivered the battery should be fully charged by the user.

The battery monitor is on the status bar next to the block temperature reading.

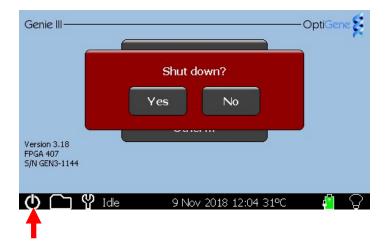


To see more details on the battery, press the battery icon and the monitor will appear as a popup in the bottom right hand corner of the screen. To remove the pop-up press on the status bar indicator again.



Battery monitor pop-up

IMPORTANT! Genie® III's internal battery will only charge when the instrument is plugged into mains electricity and the instrument is switched on. Genie® III can be shut down using the power button in the bottom left corner. At this point Genie® III can be switched off using the switch on the rear.



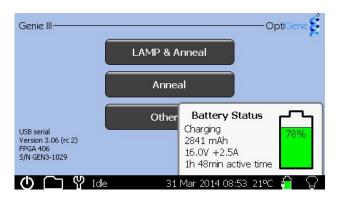
Note: If this button is pressed during a run, Genie® III will not enter standby.

When in standby, normal operation can be resumed by pressing anywhere on the screen.

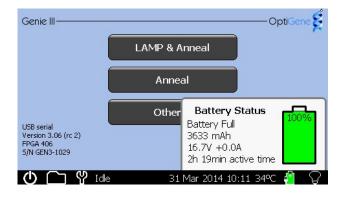
BATTERY MONITOR



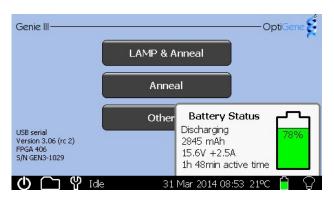
The battery status can be seen but there is no pop-up.



Here the pop-up shows that the battery is currently charging.



Here the pop-up shows that the battery is fully charged.



Here the pop-up shows that the instrument is discharging.

SCREEN BRIGHTNESS CONTROL

Next to the battery icon is the brightness control.



Touch the icon and a slider will appear on the right hand side of the screen. Move the slider to the desired position. Press the icon again to remove the slider.



It is not recommended to set the brightness at 100% for long periods of time, as this will significantly decrease battery life.

USER INTERFACE

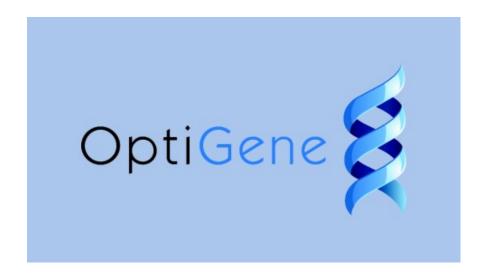
Genie® III uses a touchscreen for viewing and inputting data.

Touch the screen gently and press the appropriate keys when required. The touch screen can be operated while wearing protective gloves or by using the stylus included with the instrument.

IMPORTANT! Do not use a pen or any other sharp implements to touch the screen.

GENIE® III WELCOME SCREEN

When switching on, the LED above the screen will be **amber** in colour. Wait for the light to change to **green**, then touch the screen to access the main menu.



MAIN MENU



To start a predefined run, touch the name of the assay and it will begin. Alternatively, touch 'Other...' to create a new profile, or open a saved profile.

To view profiles or data from previous runs touch the folder icon on the status bar.

To access the toolbox touch the spanner icon on the status bar.

IMPORTANT! When running for the first time check that the time and date on the status bar are correct. These can be changed from the 'Utilities' screen in the toolbox.

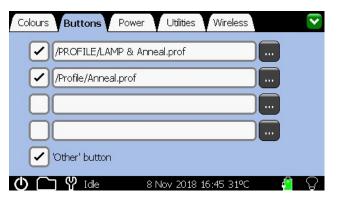


TOOLBOX

Pressing the spanner icon in the taskbar will load the toolbox.



BUTTONS

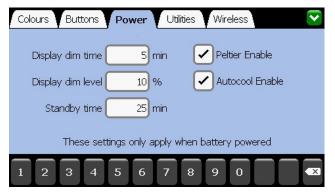


Set up the quick start buttons on the main screen.

Touching '...' will allow the user to browse the saved profiles on the instrument and assign that profile to the corresponding quick start button on the main screen.

To remove the buttons, untick the box next to the profile name.

POWER



These settings only apply when battery powered.

Display dim time: how long the instrument waits without input before dimming the display.

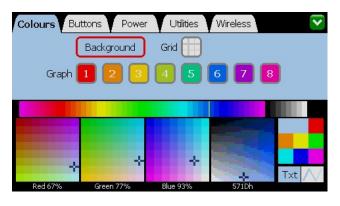
Display dim level: the brightness level the instrument will use after the specified idle time.

Standby time: how long the instrument waits before turning off the display.

Peltier Enable: Enable active cooling of the block during a run. Turning this off will save power when running on battery.

Autocool Enable: Enable active cooling of the block after a run has finished. Turning this off will save power when running on battery.

COLOURS

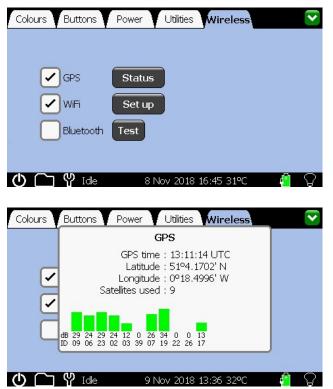


The background colour and the default colours of the lines on graphs can be changed here.

Click the background button or a graph number and then drag the cursor on the colour chart to select a colour.

The small table of coloured boxes on the right show the colour that is currently set, the default colour and the previous colours that have been used. Pressing on any of them will set the colour.

WIRELESS

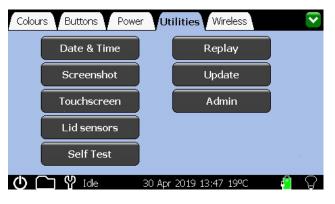


GPS, WiFi & Bluetooth: Ticking the corresponding box will turn this feature on.*

Click the View button next to GPS and the status will be shown, giving universal identities of time, latitude, longitude and the current satellites being used.

How to use the WiFi is explained in chapter 7.

UTILITIES



Date & Time: Set the time and date within the instrument.

Screenshot: A small yellow, moveable icon will appear on the screen, which, when pressed, will take a screenshot of the current screen. This will be saved into the CAPTURE directory on the instrument's internal memory.

Touchscreen: This will start the touchscreen calibration (see next section).

Lid Sensors: Allows the user to recalibrate the lid sensors.

Self Test: This will run a diagnostics on the instrument to see if there are any problems and report back if there is.

^{*} Bluetooth features are currently in development.

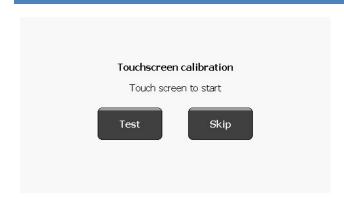
Please contact OptiGene Ltd. if so on what to do next.

Replay: This will replay a run file as if it were running in real-time

Update: Allows updating of the instrument software (See Chapter 7).

Admin: This allows Administrators to set up different levels of user access as well as language.

TOUCHSCREEN CALIBRATION



Touching anywhere on the screen with the exception of 'Test' or 'Skip' will invoke the touchscreen calibration.

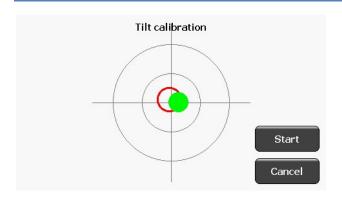
To check the accuracy of the screen calibation press 'Test'. Any point pressed on the screen will then be marked.



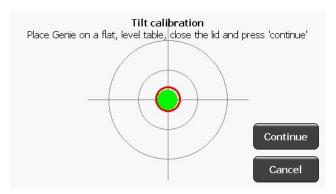
Calibrate the touchscreen by touching the target points shown on the screen (the stylus should be used for this).

It is also possible to run the touchscreen calibration when the instrument first starts. Press and hold down on the welcome screen for 5 seconds to initiate.

LID SENSORS

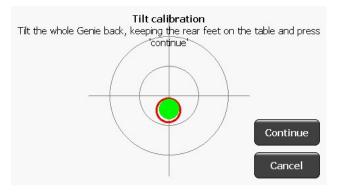


Touching 'Start' on the screen will start the calibration.



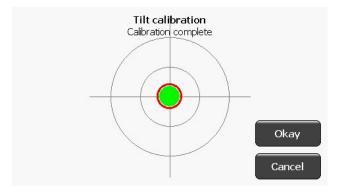
Follow the instructions at the top of the screen.

Place Genie on a flat, level table, close the lid and press 'continue'.



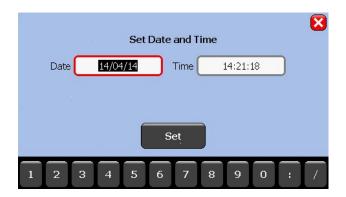
Follow the instructions at the top of the screen.

Tilt the whole Genie back, keeping the rear feet on the table and press 'continue'



The calibration is complete. The calibration can be tested by tilting the Genie in different directions. If done correctly, the green circle should remain inside the red circle as it moves around. Press 'Okay' to save the calibration or Cancel to disregard changes.

DATE AND TIME

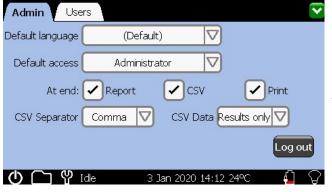


Date: click in the white box for date and enter in the format DD/MM/YY.

Time: click in the white box for time and enter in the format HH:MM:SS.

Press 'Set' to save or click the cross to cancel.

ADMIN

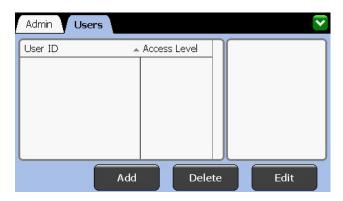


Default language: This sets the default language of the instrument.

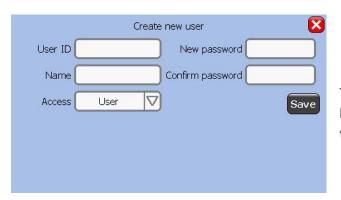
Default access: This sets the default access when the instrument is turned on. By default the instrument will be in Admin mode. In this mode all features are fully available. When Login Required is selected, the instrument will not be able to be used until a user has logged in

If any level is selected and the instrument is restarted. This is the level that the instrument will default to on startup.

At end: At the end of a run, if the boxes are ticked, the Genie will automatically create a PDF report, a CSV (comma separated value) file, and print the result table to the connected printer (accessory). The separator of the CSV file can be set between a tab and a comma, depending on what is required, and the CSV data can be the results only, the raw data, all data (including all signal processing steps) and data



On the Users tab, users can be added or removed with different levels of access.



To create a new user, click on the Add button, and then enter the details for the user and select an access level.

The levels of access are as follows:

No access: The user is disabled (can be used to temporarily revoke access to a user).

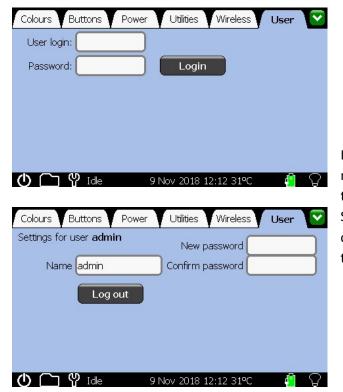
User: Can run any profiles on the instrument, and can change colours, power settings

and the screen capture functionality.

Expert: The user will have access to editing profiles and viewing and editing result

calling and can change the buttons

Administrator: The user will have full unrestricted access to all the instruments settings and can add users and set language.



In order to log in to a certain level that may be above the default access level, the user can log in on the User tab in Settings. When logged in, this screen changes to allow the user to change their password.

COPY & PASTE

Users are able to copy and paste in any text box which can save time in entering well names or run names.



To copy:

In a text box, if text is not highlighted, highlight the text by holding down on the text box until a pop-up appears, and select 'Select All' and then hold down again and select 'Copy'.





To paste:

Touch the text box you wish to paste into, and then hold down on the box until a pop-up appears and select 'Paste'.

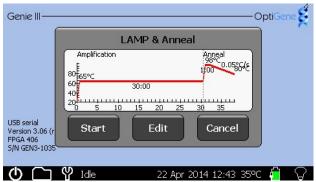
RUN

On Genie® III, there are two ways to start a run: either quick start, by touching one of the predefined profiles saved on the instrument (or an attached USB pendrive) or by creating a new profile. Predefined profiles that are saved on the instruments internal memory can be selected for quick start.

QUICK START



Up to four saved profiles can be shown on the main screen to allow quick starting of an assay. The four profiles are selected from the 'Buttons' page in the Toolbox.



Pressing one of buttons on the main menu will pop-up a preview of the profile, and allows the user to start, edit or cancel the run.

PROFILE SCREEN

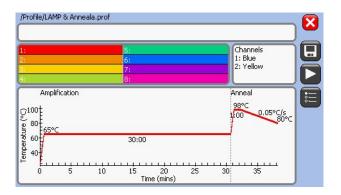


To create a new profile, press the 'Other...' button on the main screen. This allows access to any predefined profiles or creation of a new one. Cancel to return to the main menu.

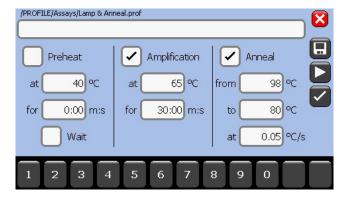
Press the tick to select a file, or to create a new profile.

If there are many profiles, scroll down by dragging on the window.

TO CREATE A NEW PROFILE

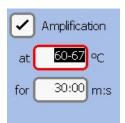


Press the 'New' button on the profile screen. Click the graph to adjust the profile by touching the appropriate temperature or time box.

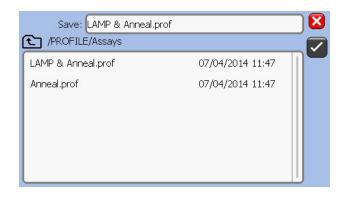


Once the required changes have been made, pressing the tick button will accept the changes. Clicking the cross in the top corner will cancel any changes.

Pressing the play icon will accept the changes and prompt the user to start the run.

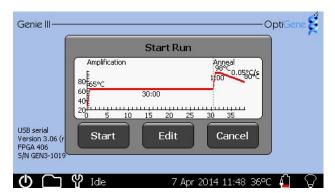


To set a thermal gradient (maximum of 7 degrees) across a block, enter a range of temperatures in the 'Amplification' temperature box. The range of temperatures should be entered separated by a hyphen, as shown below.



Pressing the save icon will save the profile. Name the profile, press the tick button and it will be saved within the 'PROFILE' directory in the on-board memory allowing it to be loaded for future runs.

TO LOAD A SAVED PROFILE



Select profile file

PROFILE/ASSAYS

Anneal.prof

LAMP & Anneal.prof

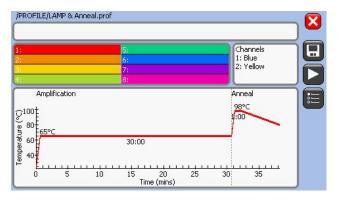
07/04/2014 11:47

07/04/2014 11:47

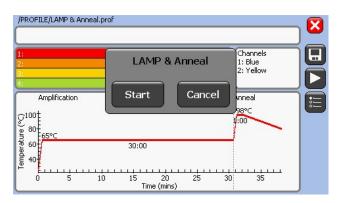
Pressing one of the quick start buttons on the main menu will pop-up a preview of the profile and give the user the choice to start, edit or cancel the run.

Alternatively, press the 'Other...' button and a file browser will be displayed. Choose the profile to be loaded and press the 'Open' button.

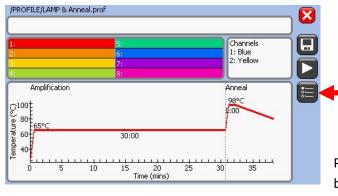
A preview graph will appear, and allow the user to start, edit or cancel the run. Pressing 'Edit' will open the main profile screen.

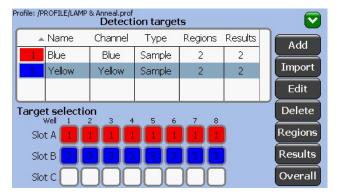


Changes can be made as required. To begin the run, press the play icon.



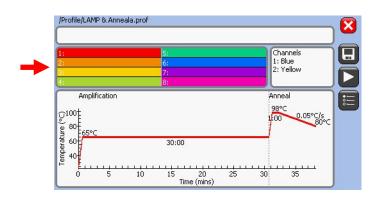
A prompt will be displayed. Select 'Start' to run or 'Cancel' to abort.





Pressing the Result Calling Options button will display more options. This is further explained in Chapter 6. If no result calling is set up then the instrument will use pre-set defaults.

WELL NAMES



To assign names to the block wells, click on the well names area.



To change a well name, press on the text box and type a name and abbreviation if desired.

'Next' switches to the next name and saves the current well name.

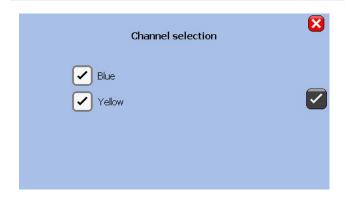
Pressing the return button on the keyboard or the green arrow returns to the run screen with changes saved.

It is possible to select the detection target for each well on this page too (see chapter 6 for details).

If the profile is saved at this point the well names, abbreviations and all result calling will also be saved as part of the profile.

The well names screen can be accessed at any time the instrument is running by clicking on the 'Results' tab and clicking anywhere on the well names column.

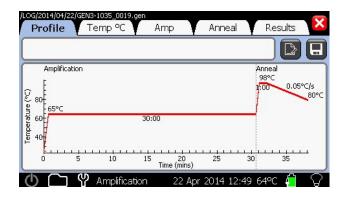
CHANNELS



Touching the 'Channels' area will bring up this window allowing selection of the channels to be used.

ACTIVE

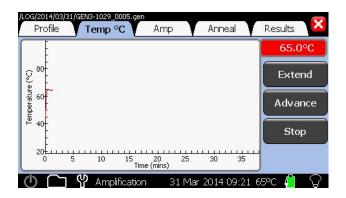
Once a run is started, the software will go to the 'Temperature' screen initially. The other screens can be accessed using the tabs.



PROFILE

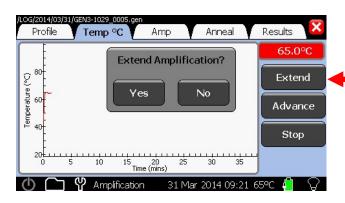
This shows the temperature profile that is running.

At the top of this screen, there is a text box to edit the run description, a button to add notes about the run and a button to save the profile.



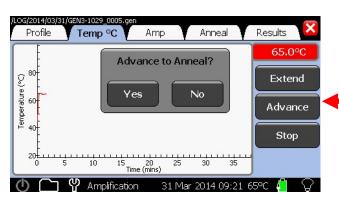
TEMPERATURE

This shows the current temperature of the block as the experiment is progressing.



EXTEND

This adds 10 minutes to the current phase of the run.



ADVANCE

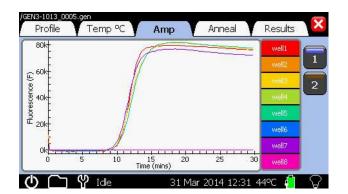
Advances to the next phase of the run (Preheat to Amplification or Amplification to Anneal).



STOP

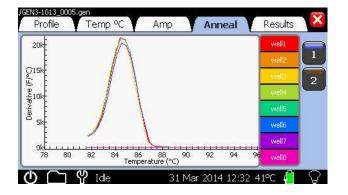
The 'Stop' button will abort a run in progress. A confirmation pop up box will prompt 'Yes' or 'No'.





AMPLIFICATION

This shows the fluorescence data that is being acquired during the amplification phase of the experiment.



ANNEAL

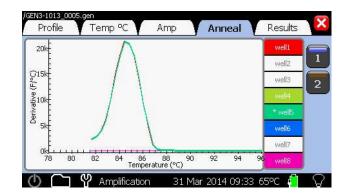
This shows the fluorescence derivative data that is being acquired during the anneal phase of the experiment.



MULTIPLE CHANNEL OPTIONS

Genie® III uses two different channels depending on the profile being run.

These buttons allow either the first or second channel to be selected. The graphs will update by pressing the relevant button on the 'Amplification' and 'Anneal' pages.



SELECTION OF GRAPHS

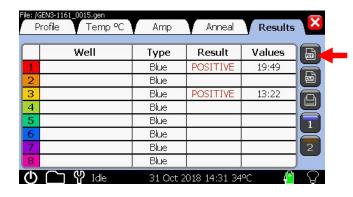
Pressing the well name on either the 'Amplification' or the 'Anneal' page cycles the state of the related curve on the graph between normal, highlighted and off.



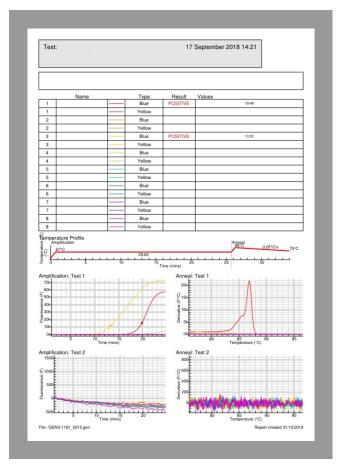
RESULTS

This shows the results of the experiment. Each sample name is shown as well as amplification time and annealing temperature.

REPORT GENERATION



Pressing the button shown on the results page will generate an A4 PDF report. This will be a single page report showing the amplification graph, the anneal graph and the results table. These reports will be saved into a 'Reports' directory on the internal storage.



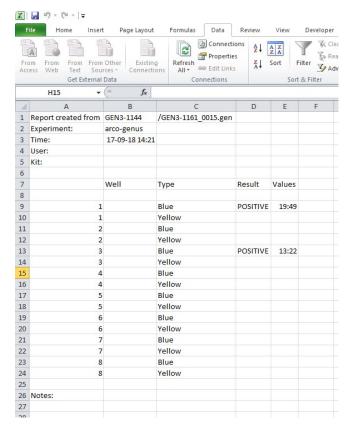
An example of a generated report file.

EXPORT TO CSV

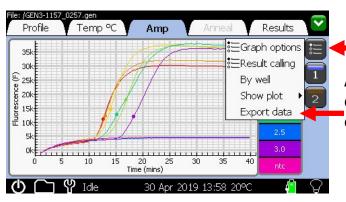


Pressing the button shown on the results page will generate a CSV (comma separated values) file with the results in and save it in the 'Reports' directory on the Genie instrument.

The formatting can be switched between tab or comma separated using the setting in 'Admin' in Settings along with what data is put into the CSV.



An example of a generated CSV file opening in Microsoft Excel.



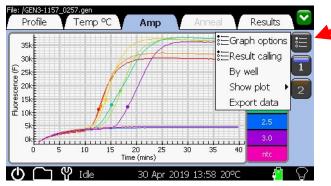
Any graph can have its data exported to a CSV file also by clicking on Export data in the Graph Options menu.

PRINT RESULTS TABLE



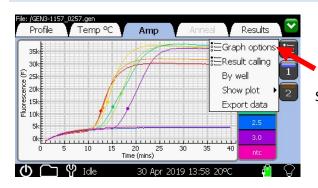
Pressing the button shown on the results table will print the table onto a label if the correct printer is attached to the Genie® instrument via the USB port on the rear.

ADDITIONAL OPTIONS

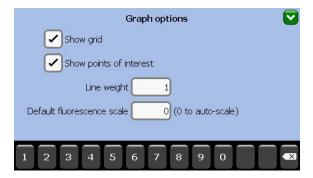


Touching the button shown on the amplification or anneal plots will show a drop down menu with some additional options.

GRAPH OPTIONS



Select Graph options



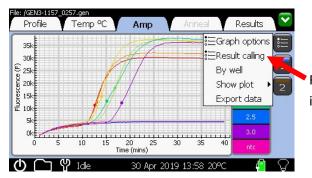
Show grid: This will enable/disable the grid behind the plots

Show points of interest: Enable/disable any dots on the plots that have been generated by the result calling.

Line weight: set the line weight of all plots (default 1)

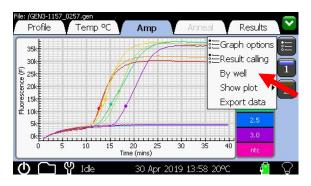
Default fluorescence scale: Set the Y axis upper scale on the graph. Set this value to 0 to auto-scale.

RESULT CALLING

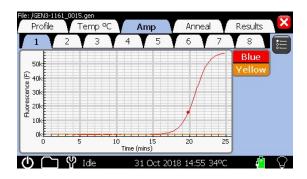


Result calling is explained in further detail in Chapter 6.

BY WELL

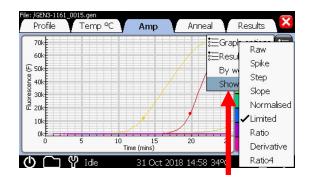


Selecting 'By well' will show all fluorescence plots by well rather than by fluorescence channel.



This allows comparison of both channels at the same time. This can be reversed by the same process.

SHOW PLOT



Selecting 'Show plot' will produce a second menu to select which plot to display on the screen depending on which signal processing step is wanting to be viewed. These are explained below and further in Chapter 6.

Raw Raw unprocessed fluorescence
Spike The data after spike removal

StepThe data after step removalSlopeThe data after slope correctionNormalisedThe data after normalisationLogThe log of the normalised data.

Ratio The ratio (dF/F) of adjacent points (after step removal). This plot is

smoothed with an averaging filter.

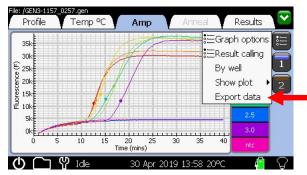
Derivative The gradient of the data (generated with a differentiating filter)2nd Derivative The gradient of the derivative (a second application of the same

filter)

Ratio4 An alternative ratio ((F-1-F1)/F0²) that gives an earlier indication of

amplification.

EXPORT DATA

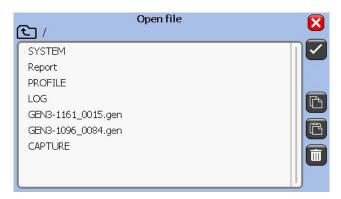


Any graph can have its data exported to a CSV file also by clicking on Export data in the Graph Options menu.

VIEW

To view previous runs press the folder icon on the status bar.





This will display a file browser window.

All Genie[®] III runs are saved in the 'LOG' folder.

To open, click on 'LOG' and then tick icon, or double press on the folder name.



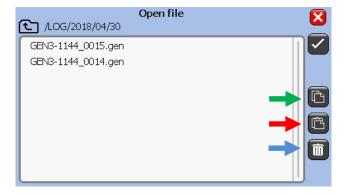
Each run is stored in a folder by date order: Year/Month/Day.



Each run is stored in a folder by date order: Year/Month/Day.



Each run is stored in a folder by date order: Year/Month/Day.



The default filename for the each run is the instrument serial number followed by a sequential number.

Select a file, then touch the tick button to load the file.

To copy a file, touch the copy icon, shown with the green arrow.

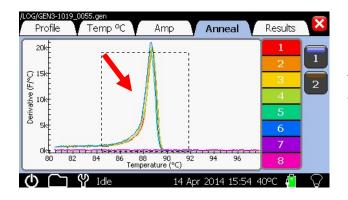
To paste a file, touch the paste icon, shown with a red arrow.

To delete a file, touch the trash can icon, shown with a blue arrow.

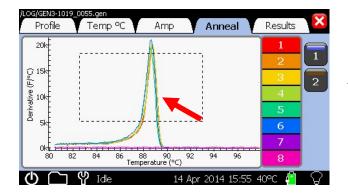
When the file has opened, Genie® III will display the profile that was run, the temperature log, amplification data, anneal data and the results table.

ZOOMING FUNCTION

Zooming is available on temperature, fluorescence and anneal graphs.



To zoom in on the area of interest, touch the plot area and drag to the right and/or down.



To zoom out, touch on the plot area and drag to the left and/or up.

A double press on the screen will zoom out to the full extent of the graphs.

Chapter 6

GENIE RESULT CALLING

OVERVIEW

Genie instruments can generate results according to detected amplification times, anneal peaks and other features. Due to the varying requirements of differing applications, setting up the parameters is highly flexible.

Analysis is based on *targets*, where each target has its own set of parameters and possible results. Each well can have up to three targets assigned for multiplex applications.

The target parameters specify the fluorescence channel, target type and various signal processing options.

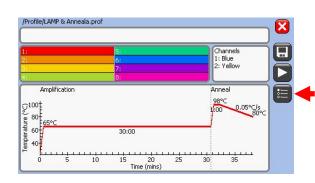
Detection starts with examining *regions* of interest on various graphs for required *features*.

The presence or absence of features in the regions determine which *result* is displayed for the well. The presence or absence of control targets in other wells can also be included in the determination.

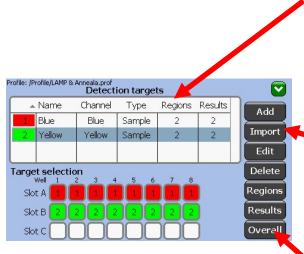
Advanced features allow regions to be defined relative to other regions or the results of other targets.

For multi-well assays, that may use the whole strip and where more than positive & negative controls are needed, an overall result can be determined.

RESULT CALLING INTERFACE



Pressing this button on the profile screen will allow the user to change the parameters for result calling for the profile. All the parameters are stored within the profile and the run file.

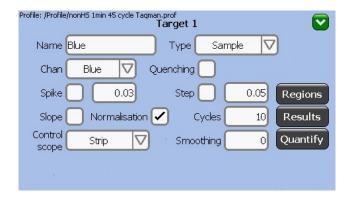


This screen shows an overview of the 'Detection targets', showing the number of regions of interest, the number of results, and which targets are assigned to which well. From here new targets can be added, imported from other runs or profiles, edited or deleted.

If you wish to import targets from another profile or run file, touch 'Import' and then select the file you wish to import from, you can then select which target from that file you wish to import.

The 'Overall' button allows the user to create an overall result based on the results of multiple wells.

TARGET PARAMETERS



This screen shows the Target parameters. The explanation of the different options is below.

Name The name of the target is displayed in the results table and various reports.

Type The target type determines the purpose of the target:

Sample A normal sample for analysis

Pos control A positive control that is expected to amplify.

Neg control A negative control that should not amplify.

Reference Some other secondary target

Chan The fluorescence channel / colour to examine. All regions for a target use the same

channel.

Quenching Indicates that amplification causes a decrease in signal. Ratios and derivatives are

inverted for quenching.

Spike removal

Enable/disable spike removal. Spike removal identifies single-point spikes in fluorescence and replaces them with the average of the two adjacent points.

Spike removal analyses the data to remove rogue spikes from the fluorescence data. Spikes can be caused by particles or bubbles in the well and by external environmental

Spike threshold Spike detection finds points in fluorescence that are proportionately higher or lower than the points on either side. The threshold should exclude normal noise in the signal. Peak noise in the unsmoothed ratio plot with spike and step removal turned off gives a good indication of the minimum threshold.

> The spike removal threshold is target-dependant and is affected by noise:signal ratio of the assay.

Step removal

Enable step removal. Step removal identifies sudden steps in fluorescence and subtracts the step height from all subsequent fluorescence values. Steps can be caused by particles, bubbles, drips and settling of well contents. Step removal occurs after spike removal.

Step threshold

Step detection is approximately based on detecting single point spikes in the ratio (unsmoothed, with step removal turned off) exceeding the threshold on both sides. Set the threshold to clearly exceed the peak-to-peak noise.

Excessive step removal will cause flat areas and shifted amplification.

The step removal threshold is target-dependant and is affected by noise:signal ratio of the assay.

Slope

Slope correction adjusts for drift by examining the initial fluorescence and removing the slope from subsequent fluorescence data after a given time period (Time). Slope correction occurs after spike and step removal.

Normalisation

Normalisation removes the average initial fluorescence from all fluorescence data after a given time period (S/N time). Slope correction and normalisation both use the same time.

Control Scope

When the target type is 'Pos Control' or 'Neg Control', this indicates which group of wells the control is related to.

None	The control is stand-alone and does not contribute to the result of other
targets	
Well	The control only applies to other targets in the same well

Pair Applies to targets in the same pair (1-2, 3-4, 5-6, 7-8) Half Applies to targets in the same half-strip (1-4, 5-8) Strip Applies to all targets in the same strip (1-8).

Running controls in a different strip is of dubious validity, so is not directly supported.

Smoothing

This setting adjusts the amount of smoothing applied to anneal derivative plots. If this value is set to 0, the default smoothing settings in the instrument is used. If this is set to a value between 1 and 8, the instrument uses an alternative smoothing function which increases the amount of smoothing as the number is increased.

CONTROL TARGETS

Control targets allow easy configuration of two control types (positive & negative).

A control has a scope which indicates which other targets / wells can be affected. Control scope is relative to the part of the strip that the sample well is in.

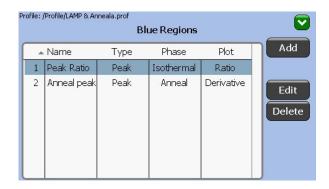
The state of the control is determined by the final result call.

For each well, controls within scope are collected and tested with the required / prohibited options.

Positive and negative controls are treated identically – just collected separately. It is therefore possible to reverse the meaning or use two positive controls that are handled differently.

Advanced users can create additional controls using references.

REGIONS OF INTEREST



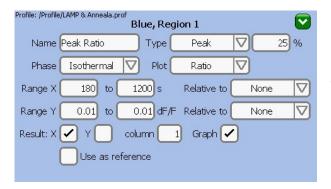
This screen shows an overview of all the regions of interest for the selected target.

The type of region, as well as which phase of the assay and plot is summarised as well as the more specific details in the box below.

The regions can be edited and deleted from this screen.



Touching 'Add' will display the screen shown. These are some generic preset regions to help get the user set up quicker. Alternatively touching 'Other' will allow the user to create something custom.



This is the set up screen for the region. The explanation of the different parameters is below.

Regions of interest define which features to examine to generate a result.

Name The region name appears in the results set-up.

Type Selects the *feature type* to examine (explained further below in the section titled

Features).

Phase Selects the measurement phase to examine.

Plot The graph to examine. Any step of the signal processing can be examined.

RawRaw unprocessed fluorescenceSpikeThe data after spike removalStepThe data after step removalSlopeThe data after slope correctionNormalisedThe data after normalisation

Limited The data after limiting (value set in the Target parameter screen)

Ratio The ratio (dF/F) of adjacent points (after step removal). This plot is

smoothed with an averaging filter.

Derivative The gradient of the data (generated with a differentiating filter)2nd Derivative The gradient of the derivative (a second application of the same

filter)

Ratio4 An alternative ratio ((F-1-F1)/F0²) that gives an earlier indication of

amplification.

Amplification phases use the steps Raw, Spike, Step, Slope, Normalised, Ratio, Derivative, Ratio4.

Anneal / melt phases use the steps Raw, Spike, Step, Derivative, 2nd Derivative.

Range X/Y to Specify the 'window' to examine. If the limits are the same, it is treated as a threshold.

Units vary according to phase and plot selected. The meaning can vary according to

type. Limits are inclusive.

Relative to Adjusts the range 'window' relative to the value found by another region.

BE AWARE: Compatibility of units is not checked – it is possible to make nonsensical selections (e.g. by making a time range (in seconds) on an amplification plot relative to

an anneal temperature in °C).

Result X/Y Displays the X and/or Y value found on the results table, provided the region is

detected and used in the result.

Result column Specifies which column(s) of the results table to put the X/Y value in (default 1). If both

X & Y are selected the X value is in the column specified and Y is in the following

column.

Graph Show the point found on the graphs, provided the region is detected and used in the

result.

Use as reference Advanced feature. Allows the point found to be referenced by another target, provided

the region is detected and required by the result.

Additional parameters will be displayed depending on the feature type selected. These are explained further later.

Regions (other than Threshold type) are independent unless they explicitly use relative ranges; in which case the region is undefined until the region(s) it is dependent on is/are fully determined. Be careful not to create circular references as all regions will remain undefined.

FEATURES

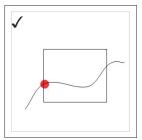
Feature type: Any

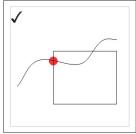
Positive if: Any point on the graph is within the window. Negative if: All points in X range are outside Y range.

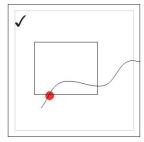
Complete when: X position passes the window or a point in range is found Point identified: The first point that falls in the window (without interpolation)

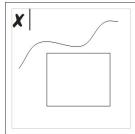
Additional parameters: None

Uses: Crossing threshold, Progress check.









Feature type: All

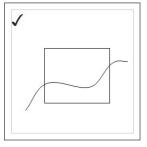
Positive if: All points in the X range fall within the Y range
Negative if: Any point in the X range falls outside the Y range

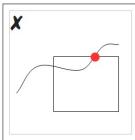
Complete when: X position passes the window or a point in X range falls outside Y range

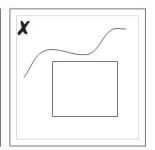
Point identified: The first point in X range that falls outside Y range (if any)

Additional parameters: None

Uses: Range check







Feature type: Min

Positive if: A minimum width in X range falls within Y range Negative if: No group of points in window cover width.

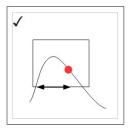
Complete when: Minimum width found or X position passes window.

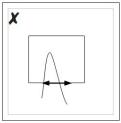
Point identified: The point where the minimum width is satisfied (i.e. width after crossing

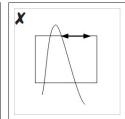
point)

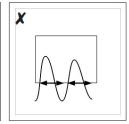
Additional parameters: Width: in the same units as the X axis

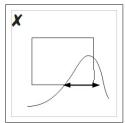
Uses: Amplification rate check, anneal peak width, noise rejection.











Feature type: Average

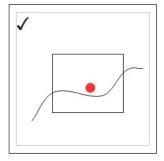
Positive if: The average of all points in X range falls within Y range
Negative if: The average of all points in X range falls outside Y range

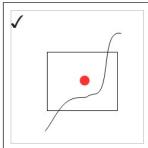
Invalid if: There are no points in X range Complete when: X position passes window

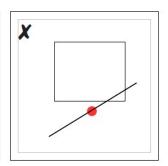
Point identified: Y: Average Y value within X range

X: Midpoint of X range

Additional parameters: None
Uses: Level check







Feature type: Threshold

All thresholds required or prohibited by a result are considered together. A threshold that is *prohibited* has its Y range inverted.

If peaks (or dips) are required, each peak is checked against all required thresholds.

Positive if: There is a point/peak where all thresholds that are in X range are also in Y

range or there is a peak where no thresholds are in X range.

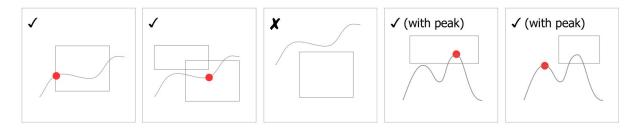
Negative if: There are no points/peaks where all thresholds are in Y range

Complete when: A point/peak is found or X position passes window Point identified: The first point/peak that satisfies all thresholds

Additional parameters: None

Uses: Crossing threshold with multiple conditions or regions

Additional peak detection criteria (e.g. minimum fluorescence)



Feature type: Peak

Positive if: A peak is detected in X range, with peak point in Y range (before interpolation)

All required thresholds that are in X range must also be satisfied.

Negative if:

No peak detected or all peaks out of Y range or thresholds are not satisfied

Complete when:

Peak found or peak tracking point passes window (hysteresis threshold can be

outside X range)

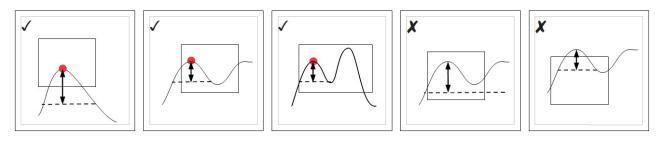
Point identified: Peak position, if found, interpolated with 3-point quadratic fit.

Additional parameters: Peak %: Detection hysteresis – signal must drop specified amount on both

sides for a peak to be detected. Lower values are more sensitive to local

maxima.

Uses: Amplification time, Anneal temperature.



Feature type: Dip

Identical to Peak type, except minima are identified instead of maxima.

Feature type: Reference

This is an advanced calling feature to allow results from one well to affect others.

Positive if: The referenced target result is positive
Negative if: The referenced target result is negative
Invalid if: The referenced target result is invalid
Complete when: The referenced target produces a result

Point identified: The point identified by a target region that matches all the following criteria:

- Is required by the result of the target

- Has a valid point

Uses matching Phase and PlotHas the Reference option set.

Additional parameters: Well: Well number reference.

In: Relative location of reference wellSame: in the same well as the current target

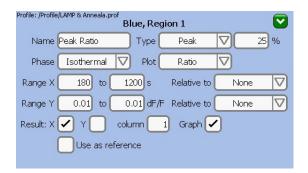
Adjacent: in the well adjacent to the current well (other well of pair)

Pair:Well 1 or 2 in the current pairHalf:Well 1-4 in the current half-stripStrip:Well 1-8 in the current stripTarget:Target slot to examine

Unused parameters: Window range parameters.

Uses: Comparison of amplification times, anneal temperatures, SNP target

alignment, extra controls.



In this example wells 1 and 3 have met the criteria defined in the region above.

The points at which the criteria are met are marked by a dot on the graph line.

RELATIVE REGIONS

Relative regions allow features to be aligned to other features.

Example 1: Find the first peak ratio after normalised fluorescence has passed a threshold.

- 1. Create an 'any' region, testing normalised fluorescence
- 2. Create a 'peak' region, testing smoothed ratio; X range 0-0, relative to region 1

Example 2: Test the fluorescence at a peak ratio location.

- 1. Create a 'peak' region, testing smoothed ratio
- 2. Create an 'average' region, testing normalised fluorescence; X range -30 to +30, relative to region 1.

Regions can be relative to the values found by other targets, by using references.

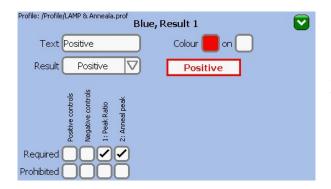
RESULT DEFINITIONS



This screen shows an overview of the target results. The type and how many regions required/prohibited are shown.

The order of which the results are called is important. This can be changed by selecting a result and using the up and down arrows. The first matching result will be displayed in the result table.

New results can be added and results can be deleted from this screen.



Adding or editing a result will show this screen. The explanation of the different parameters is below.

Result definitions specify which features are required or prohibited for each possible result. Result definitions are tested in order. The first matching result is displayed in the result overview table.

Result checking waits for all dependant regions to complete, or until no pending regions can affect the result (e.g. if a prohibited region is positive, the state of other regions is irrelevant).

Text This is the result message seen by the user on the results table and recorded in reports.

Colour/ on These are the foreground and background colour of the displayed result.

Result The type of the result

None: An unused result that will block later results until determined.

Provisional: An intermediate result that will be shown until/unless overridden.

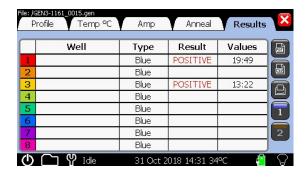
Invalid: A final result that cannot be determined Positive: A final result determined as positive Negative: A final result determined as negative

Required Select all regions and controls that must be positive to satisfy this result.

Prohibited Select all regions and controls that must be negative to satisfy this result.

Unselected regions and controls have no effect on the result. Regions that are both required and prohibited should be avoided – this combination may be given a special meaning in the future.

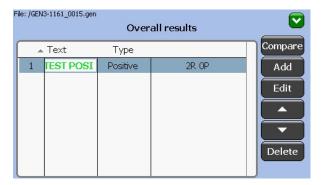
All positive control targets whose scope includes a particular well are collected together in one pair of required/prohibited tick boxes (likewise for negative control targets). Fine-grained use of controls can be achieved by advanced users by using reference regions.



This shows the result table showing the called result.

OVERALL RESULT

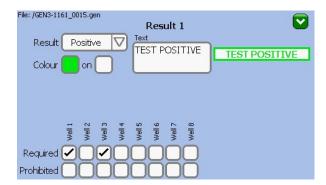
Overall result calling takes the results for each well and combines then to give a result for the whole strip.



This screen shows the defined overall results. Result definitions are tested in order. The first matching result is displayed. This order can be changed using the up and down arrow buttons.

The Compare feature is explained below.

OVERALL RESULT DEFINITIONS



The result definition set up screen. The explanation of the different parameters is below.

Overall result definitions specify which wells are required or prohibited for each possible result.

Result checking waits for all dependant wells to complete, or until no pending wells can affect the result (e.g. if a prohibited well is positive, the state of other wells is irrelevant).

Text This is the result message seen by the user and recorded in reports.

Colour/ on The foreground and background colour of the displayed result.

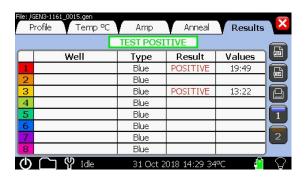
Required Select all wells and comparisons that must be positive to satisfy this result

Prohibited Select all wells and comparisons that must be negative to satisfy this result

Unselected wells have no effect on the result. Wells that are both required and prohibited should be avoided – this combination may be given a special meaning in the future.

A well is positive if any target produces a **positive** result type.

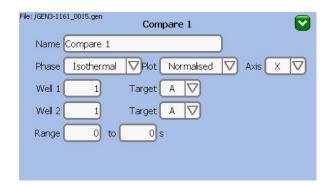
A well is negative if any target produces a **negative** result type.



An overall result is displayed at the top of the results table, as shown in the screenshot.

WELL COMPARE

Values calculated for wells can be compared and included in the overall result.



The compare set up screen. The explanation of the different parameters is below.

Name The compare name appears in the results set-up.

Phase Select the phase to compare (e.g. isothermal or anneal)

Plot Select which data step to compare (e.g. normalised fluorescence)

Axis Select which axis to compare (X or Y)

Well 1/2 Set which wells to compare

Target The targets to compare

Range The comparison is positive if the difference between well values is in the

specified range. (Well 2 value - Well 1 value)

A region that is set to 'Use as reference', tests the selected phase and plot, and is required by the well target result, is needed before the comparison is made.

Chapter

CONNECTING TO EXTERNAL DEVICES

Genie® III is a standalone instrument; however, it can be connected to external devices for software updates, data upload and further analysis. Files can be transferred to a PC running Microsoft Windows (XP, Vista,7, 10) via the USB connection on the back of the instrument or via WiFi to a phone/mobile device with a network connection or via a pendrive to the USB socket on the rear of the instrument. Genie® can also be connected to a barcode scanner for text input.

PENDRIVE

A pendrive can be plugged into the USB A socket in the rear of the unit. This allows files and software updates to be transferred to and from the unit without it needing to be connected to a PC. The pendrive can be accessed via any file manager screens, including the software update screen, allowing updates to be performed at a site without needing a computer.

WIRED CONNECTION

Disclaimer: Genie® Explorer is an additional tool and should not be used for patient care or clinical analysis.

IMPORTANT! Do not plug any Genie® instrument into the computer before installing Genie® Explorer. Genie® Explorer can be installed from the USB drive included with any Genie® Instrument.



Open the USB drive in 'My Computer'.

Run the file 'GenieInstall.msi'.

Follow the onscreen instructions.

*A prompt may appear requesting installation of .NET Framework 4.0. This must be installed prior to installation. Follow the link to the Microsoft download page.

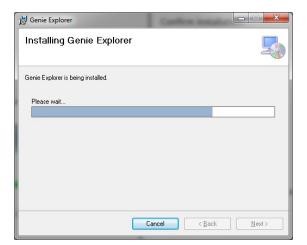
http://www.microsoft.com/engb/download/details.aspx?id=17718



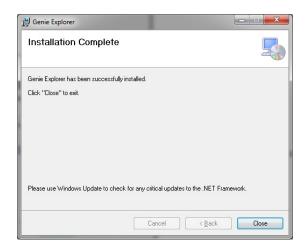
Choose a location for the installed program.



Confirm to start the installation.



The installer will copy all necessary files to the computer.



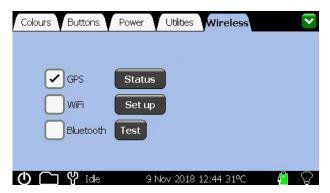
Once the installation is complete, exit by clicking 'Close'.

A Genie® instrument can now be connected to the computer. When connected via USB and switched on, the Genie® instrument will appear as a USB drive and will be accessible from Genie® Explorer.

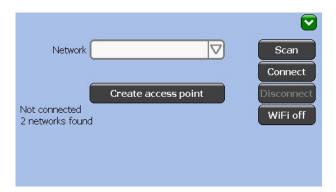
WIRELESS CONNECTION

Genie® III is WiFi enabled. When connected to a wireless network or a mobile hotspot, files can be transferred to your phone or device quickly and easily to be shared via email or downloaded onto a computer without the need to have the instrument physically connected or even in the same room. It is also possible to view live updates from the instrument to know when a test is complete without physically having to look at the instrument.

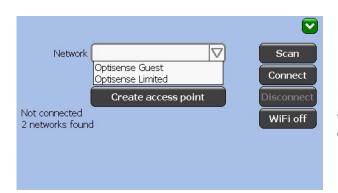
CONNECTING GENIE® TO A WIRELESS NETWORK



To enable the WiFi, touch the Settings button, the Wireless tab and click the tick box next to WiFi. Touch 'Set up' to set up a connection.



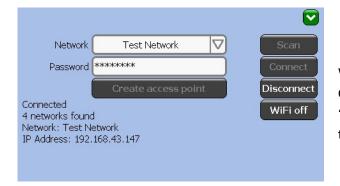
Touch the 'Scan' button to search for wireless networks nearby. This can be a wireless network or a mobile hotspot created from a phone.



Press the drop down arrow to see available networks and select one to connect to by touching its name.

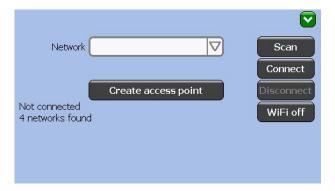


Type in the wireless network key/password and touch 'Connect' to join the network.

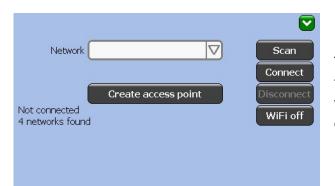


When connected to a network the Genie® will display the word 'Connected' and display the name of the network and an IP Address.

Once connected to a network the files on the Genie® III can be accessed and downloaded from either a web browser on the same network or via the 'Genie' app when using a mobile hotspot (currently only available on Android).



Touching 'Disconnect' will disconnect from the current network and the instrument will forget the password.



Touching 'WiFi off' will disconnect from the current network turn off the wireless functionality. Pressing 'WiFi on' will turn the WiFi back on.



To create an Access Point, to allow direct communications between Genie and a wireless device without the need for an existing network, touch 'Create access point'.

This will create an open network which can be accessed from another wireless device.

ACCESSING GENIE® VIA WEB BROWSER

To access files via a web browser the device connecting to the Genie® must be on the same network. Once connected, open a browser and type in http:// followed by the serial number of the instrument or the IP address displayed. For example, http://gen3-1024. This can be done on any device (phone/tablet/computer) running any operating system (Android/Windows/iOS) with a web browser.

The following screen will be displayed:

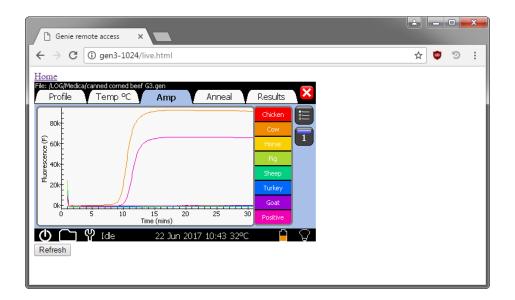


Log Files: This will give access to the LOG folder on the instrument. The file structure is as it is on the instrument and is sorted by date. Clicking on a .gen file will download it to your device.



Report Files:* This will give access to the Report folder on the instrument. Clicking on a .pdf file will download it to your device. *Functionality coming soon

Live view: Clicking on this will allow the user to see what is happening live on the instrument. It will display a screenshot from the instrument which can be manually refreshed. This will allow users to check what status their assay is at without having to physically go and check their instrument if it is in a different room or laboratory.



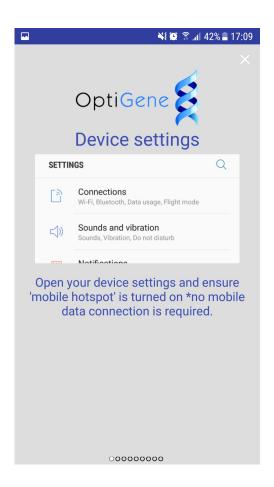
ACCESSING GENIE® VIA GENIE® III APP (ANDROID)

The Genie® III app on Android allows quick access to files on a Genie device that is connected via a mobile hotspot, which is ideal for use when there is no wireless network available or the instrument is being used outdoors. It allows users to access files and reports and stores them on the device for email or sharing with others via mobile networks or later via WiFi.

The app is available on the Google Play Store.



Open the app.



It will detect if a mobile hotspot is enabled and if not ask the user to create one using the phone's settings (follow instructions in app by pressing the '?' button). No mobile data connection is required to do this.

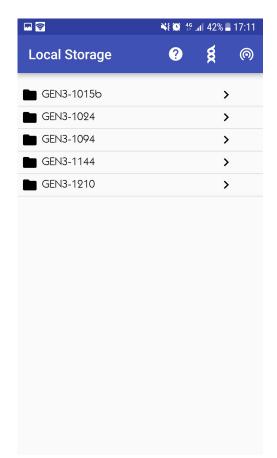


Once a hotspot is created and the Genie® device is connected to the network, press the button and the app will display the serial number in a panel on the right. Clicking on the serial number of the instrument will display its 'log' folder and allow the user to download the files to the device.

Multiple instruments can be connected to the same device.



Navigate to the log file that is required and touch the file name to download to the device.



It is possible to see what files are currently already on the device as well by clicking on the button. Every Genie that has been accessed will be displayed as its own folder.



Once downloaded the user can send the files via other applications by navigating to the file on the local storage and touching the tick box next to the file (multiple can be selected) and then pressing the button to share with other applications (email/instant messengers).

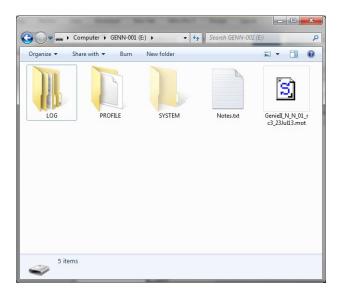
GENIE® III SOFTWARE UPDATES

It is recommended to keep the software on Genie[®] III up-to-date. Upgrading may improve performance and add new features to Genie[®] III.

There are two types of software on Genie[®] III; the firmware, and the FPGA software. The current versions of firmware and FPGA software that are installed on Genie[®] III are displayed in the bottom left hand corner of the main menu screen.

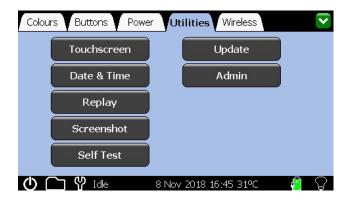


- To download the latest firmware, visit the OptiGene website
 (http://www.optigene.co.uk). Click on 'Support' and click on the appropriate link on the right hand side of the page and download the '.zip' file.
- Open the '.zip' file and extract the contents to a new folder. The contents of the folder will include the latest firmware, FPGA software and this manual.
- If Genie® III already has the latest FPGA software only the firmware will need to be updated.
- If both firmware and FPGA software updates are required, update the FPGA software first, followed by the firmware.
- If it is a firmware update, the file will be a '.mot' file, whereas if it is an FPGA software update it will be an '.rbf' file.

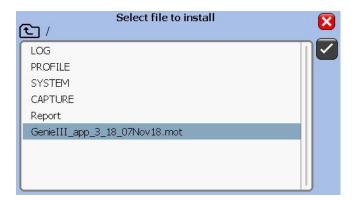


To install the updates, connect Genie[®] III to a computer. Navigate to 'My Computer' and open the Genie[®] III drive. The drive is named with the instrument serial number, e.g. GEN3-1001. Copy and paste (or drag) the firmware or FPGA software files onto the Genie[®] III drive.

The files can also be loaded to a pendrive which can be plugged into the instrument to install updates.



Now on Genie® III select the Toolbox, touch the 'Utilities' tab and press 'Update'. Genie® III will prompt for a file to use for the update.



Touch on the file, and touch the tick button. The Genie® III will then install the updated software. Please wait for it to finish before trying to do anything else.

If the update was a firmware update, Genie[®] III will restart when completed. If an FPGA software update was performed, Genie[®] III will require a manual restart by turning the instrument off and on from the switch on the rear of the unit.

Genie® III will automatically delete the files when the update has completed.



GENIE® III TECHNICAL SPECIFICATION

Sample Number	8 wells
Sample Volume	10 µl to 150 µl
Touchscreen	High-brightness TFT / LCD module (480x272)
Heater technology	Ceramic substrate with resistive coating with peltier devices
Cooling method	Forced convection
Temperature sensor	High-precision thermistor
Temperature control type	Multi-zone independent digital PID
Temperature control range	ambient - 100°C
Temperature accuracy	±0.1°C
Temperature uniformity across block	±0.2°C
Temperature gradient	Programmable up to 8°C
Optics source	470 nm & 590nm dual colour LED with high-quality interference filter 40 nm band pass
Detection optics	Photodiodes with high-quality interference filters 510-560 nm band pass & 620 nm long pass
Operating temperature	0°C - 40°C
Storage Temperature	20°C - 70°C
Approvals	CE
Dimensions	250mm (L) X 165mm (W) X 85mm (H)
Weight	1.75kg / 4 lb
Connections	1 x USB 'B' 1 x USB 'A' 1 x Power in
Environmental protection	IP60
Positioning	GPS
Wireless Connections	Bluetooth & WiFi
Power supply	Input: 100-240V AC / Output: 24V DC - 150W
Battery Type	Lithium Polymer



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Version	Date	Changes	Approved
V1.00	10/02/14	Initial Release	SUL
V1.01	14/03/14	Spelling errors.	SUL
V1.02	10/06/14	Updated Genie Explorer	SUL
V1.03	10/11/14	Additional features added to main software	SUL
V1.04	01/04/15	Added disclaimer about Genie Explorer.	SUL
V1.05	17/09/15	Added instrument cleaning guidelines and	SUL
		feedback email address.	
V1.06	03/11/15	Separating Genie Explorer to own manual	SUL
V1.07	18/02/16	Updated Technical Specification	SUL
V1.08	02/03/16	Updated IP Rating	SUL
V1.09	22/06/17	WiFi and latest functionality added	SUL
V1.10	30/04/19	Result calling added	SUL
V1.11	12/12/19	Copy and paste and other new feature added	SUL

Version 1.1