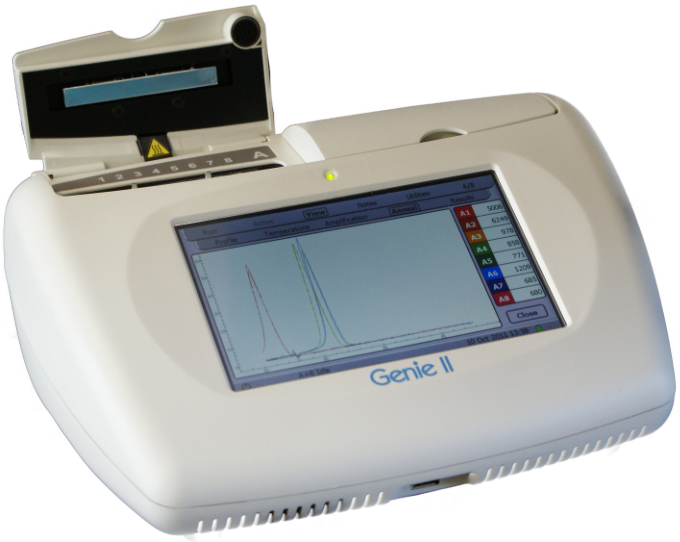

Genie® II

User Manual

(Instrument Software Version v2.34.3)

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feedback@optigene.co.uk

Table of Contents

Chapter 1

SAFETY NOTICES

Please read the following notices carefully before using Genie® II.

The Genie® II is specifically designed to run any isothermal amplification method that employs target detection by fluorescence measurement. Genie® II will detect all dyes that can be excited from a blue light source and with an emission above 510 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.

While every effort has been made to protect the inside of the instrument against splashes, the instrument carries no IP rating. If fluids are spilt on the instrument 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® II 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 blocks and heated lids are hot, so allow to cool before touching the surfaces.

Safe removal of fluids from Genie® II 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® II can be disinfected using the following procedure. This same procedure 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® II 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 bleach or alcohol solution to enter the wells as this can cause damage.

Chapter 2

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® II instrument.

IMPORTANT! If a Genie® II 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® II 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.

Chapter

3

BOX CONTENTS

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

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

Chapter

4

SITE PREPARATION

HOW TO SET UP GENIE® II

The laboratory bench should be level and stable. The instrument should be placed centrally on the lab bench 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 front of the instrument and 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.

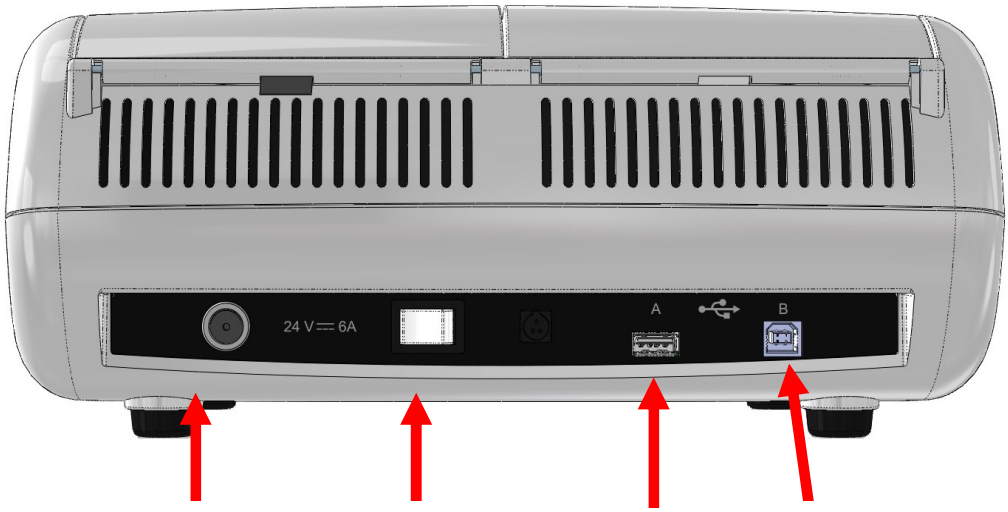
It is recommended that the instrument is kept away from sinks and other wet areas. Genie® II is an electrical instrument and care should be taken not to operate if there is a risk of water damage.

CONNECTIONS

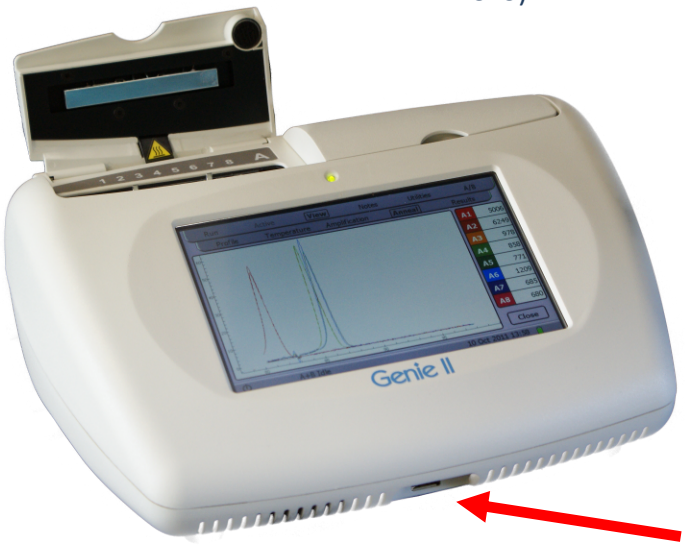
Genie® II is ready to use straight out of the box without any external connections. It can be operated standalone, taking power from its internal battery. In order to charge the battery or to use Genie® II with a computer or external devices, such as a printer, barcode scanner or pendrive, some connections 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® II will power up and progress through its checks.



POWER INLET POWER SWITCH USB (TO EXTERNAL DEVICES) USB (TO PC)



USB (TO EXTERNAL DEVICES)

OPENING & CLOSING THE LIDS

Gently lift the lids upwards. Close the lids by lowering gently.

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

INSERTING TUBES

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

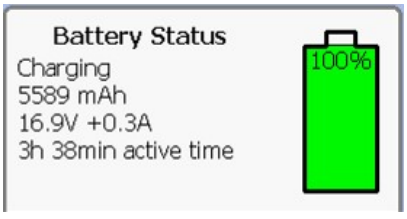
IMPORTANT! The shape of the tubes is such that they will only fit in one way round. The locating pins on the block have corresponding holes in the strips.

BATTERY

Genie® II has an internal rechargeable battery. When Genie® II 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 pop-up in the bottom right hand corner of the screen. To remove the pop-up press on the status bar indicator again.



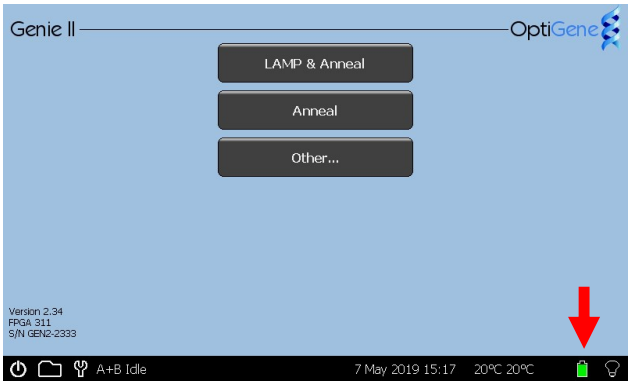
Battery monitor pop-up

IMPORTANT! Genie® II's internal battery will only charge when the instrument is plugged into mains electricity and the instrument is switched on. Genie® II can be placed into standby using the power button in the bottom left corner. The LED above the display will glow brightly until the battery is fully charged. At this point Genie® II can be switched off using the switch on the rear.

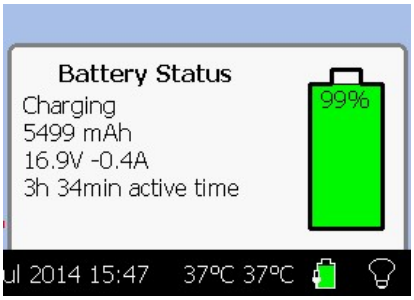
Note: If this button is pressed during a run, Genie® II will not enter standby.

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

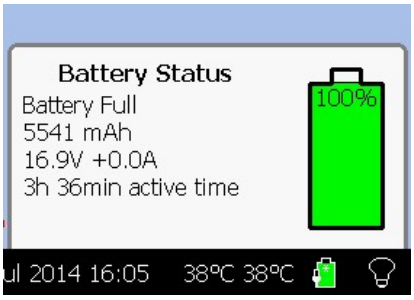
BATTERY MONITOR



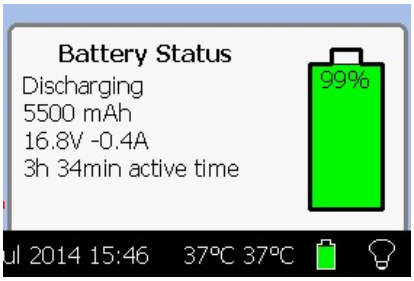
The battery status can be seen but there is no pop-up.



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



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

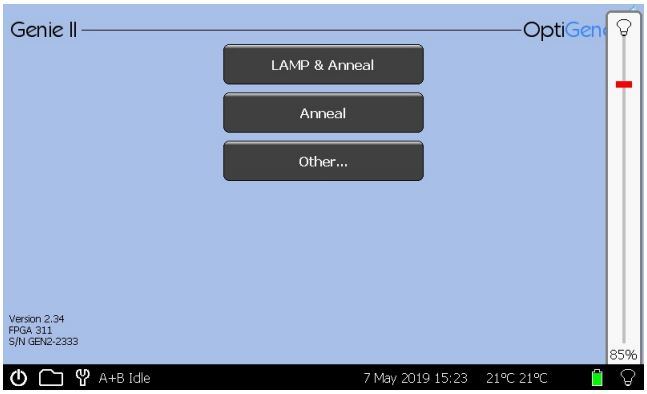


Here the pop-up shows that the instrument is discharging.

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.

Chapter

5

USER INTERFACE

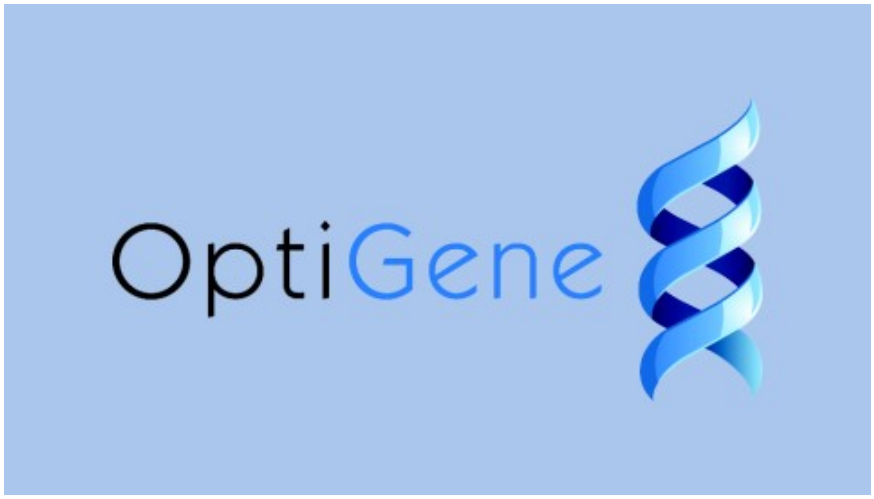
Genie® II uses a touchscreen for viewing and inputting data.

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.

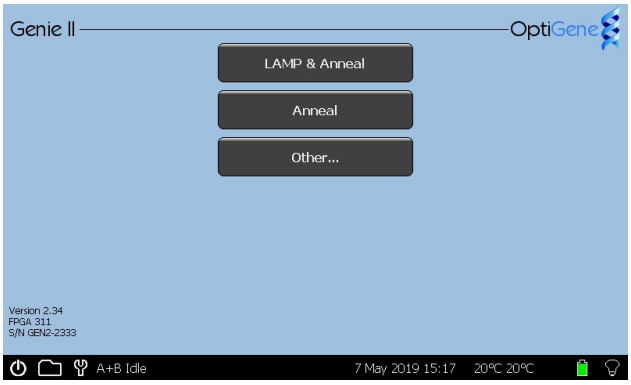
IMPORTANT! Do not use a pen or any other sharp implements to touch the screen.

GENIE® II 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.

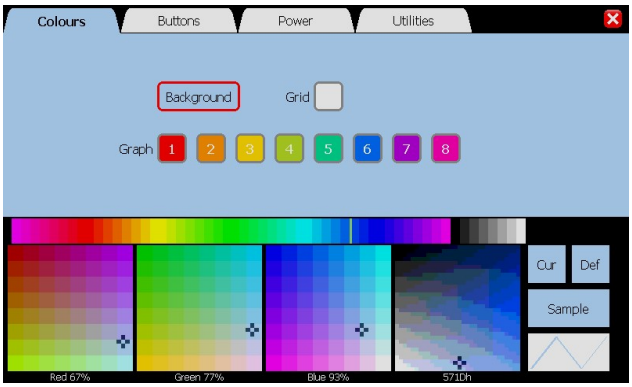
To access the toolbox touch the spanner icon on the status bar.

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

TOOLBOX

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

COLOURS

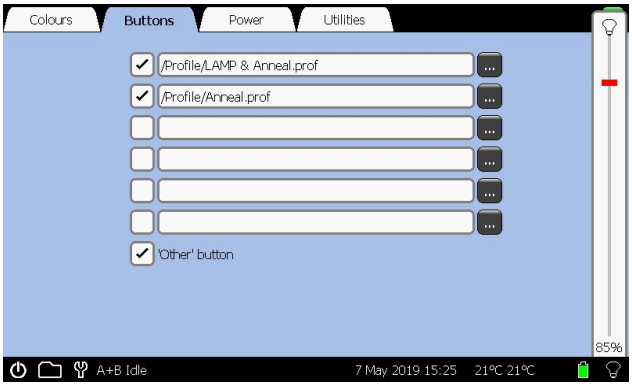


The background colour and the default colours of the lines on graphs can be changed here. Click the background button or a graph number and then drag the cursor on the colour chart to select a colour.

The two small coloured boxes on the right show the colour that is currently set (Cur.) and the default (Def.) colour. Pressing on either will set the colour.

The 'Grid' box alters the colour used for the gridlines on all graph screens.

BUTTONS

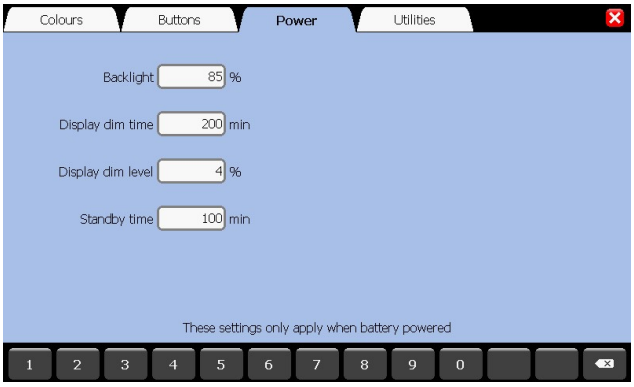


Set up the quick start buttons on the main screen.

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.

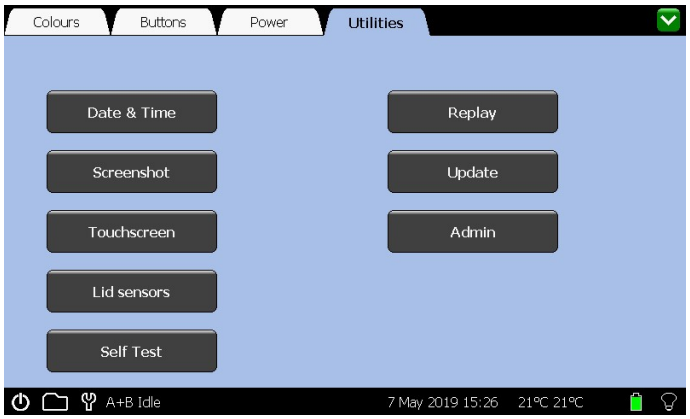
Backlight: An alternative way to set the backlight power. It is not recommended to set the brightness at 100% for long periods of time, as this will significantly decrease battery life.

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.

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 below).

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.

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

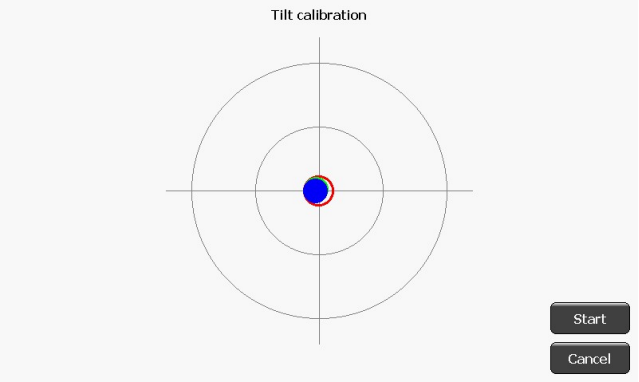
Touching anywhere on the screen with the exception of 'Test' or 'Skip' will invoke the touchscreen calibration.

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

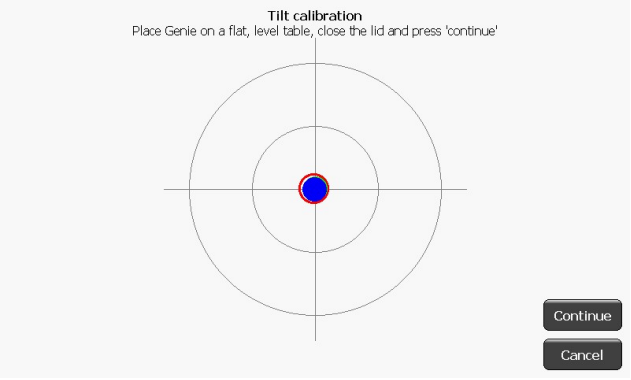
Calibrate the touchscreen by touching the target points shown on the screen (the stylus should be used for this).

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

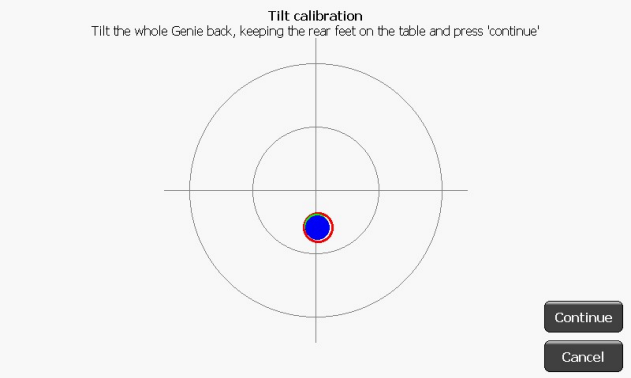


Touching ‘Start’ on the screen will start the calibration.



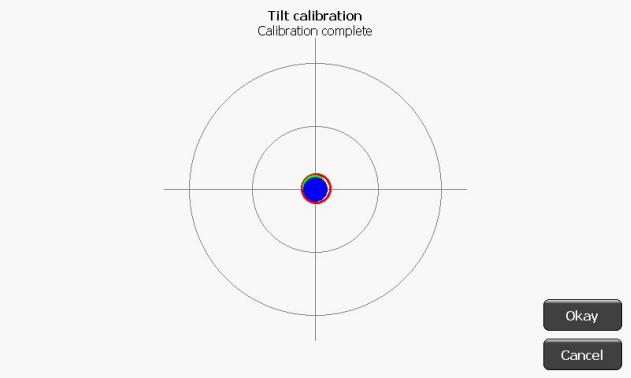
Follow the instructions at the top of the screen.

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



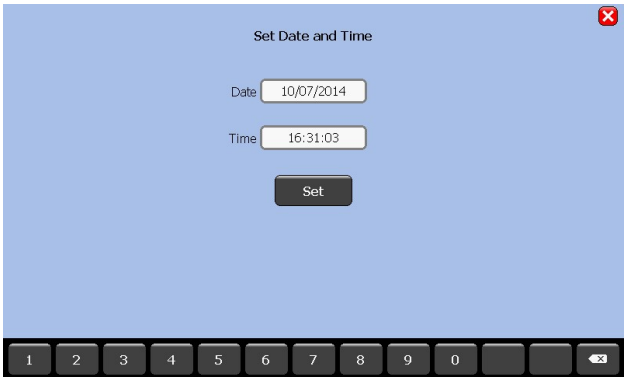
Follow the instructions at the top of the screen.

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 and blue circles should remain inside the red circle as it moves around. Press ‘Okay’ to save the calibration or Cancel to disregard changes.

DATE AND TIME

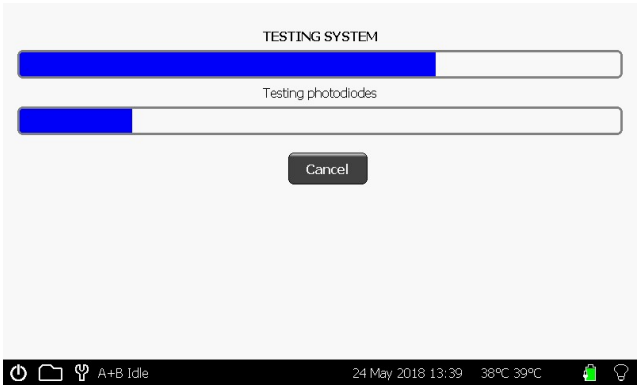


Date: click in the white box for date and enter in the format DD/MM/YY.

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.

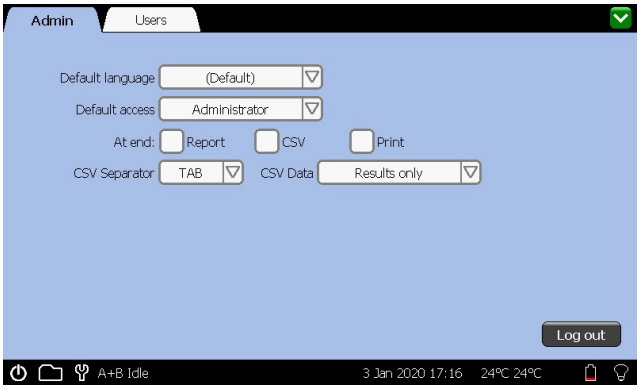
SELF TEST



The ‘Self Test’ function allows the Genie® to check that it is fully working correctly and check for any faults, and if so report them to the user who should send the result back to OptiGene Ltd for analysis.

Part of this procedure includes allowing checking of the calibration which can be run with additional kits from OptiGene (contact us for more information).

ADMIN

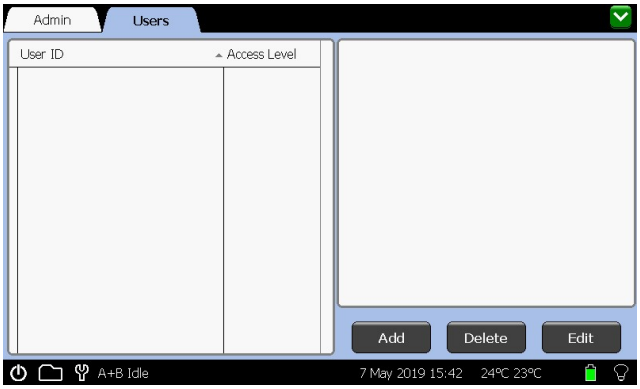


Default language: This sets the default language of the instrument.

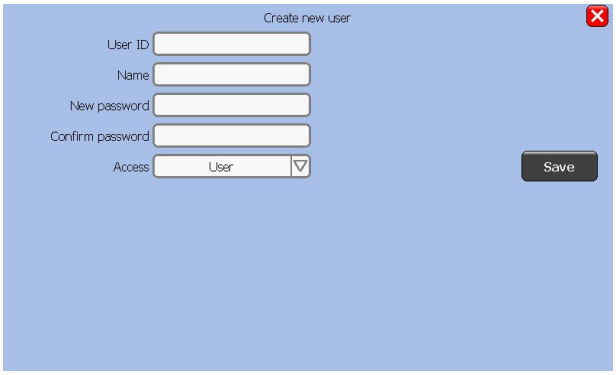
Default access: This sets the default access when the instrument is turned on. By default the instrument will be in Admin mode. In this mode all features are fully available. When Login Required is selected, the instrument will not be able to be used until a user has logged in.

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.

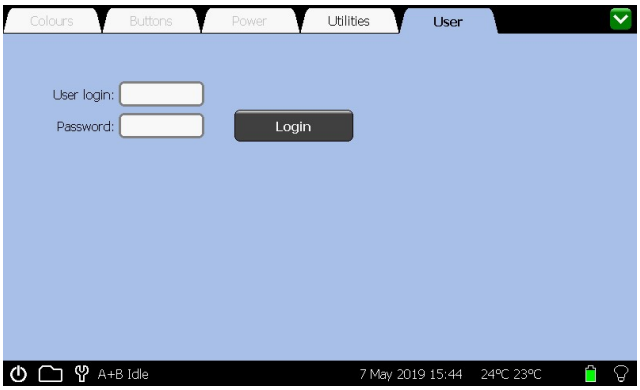
The levels of access are as follows:

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

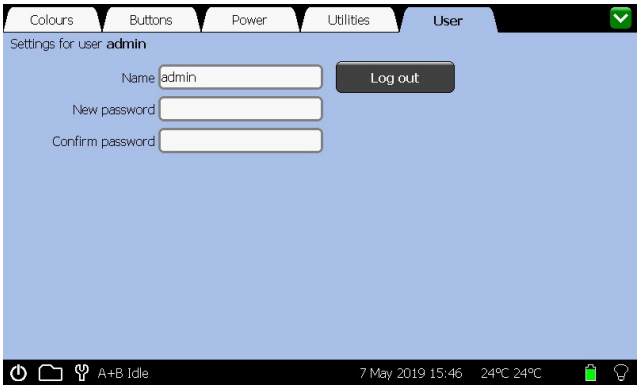
User: Can run any profiles on the instrument, and can change colours, power settings and the screen capture functionality.

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.

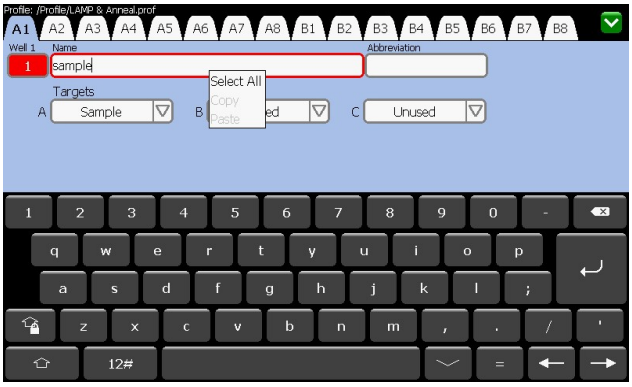


In order to log in to a certain level that may be above the default access level, the user can log in on the User tab in Settings. When logged in, this screen changes to allow the user to change their password.



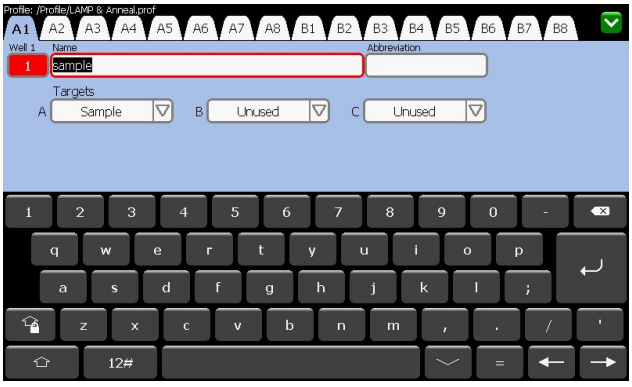
COPY & PASTE

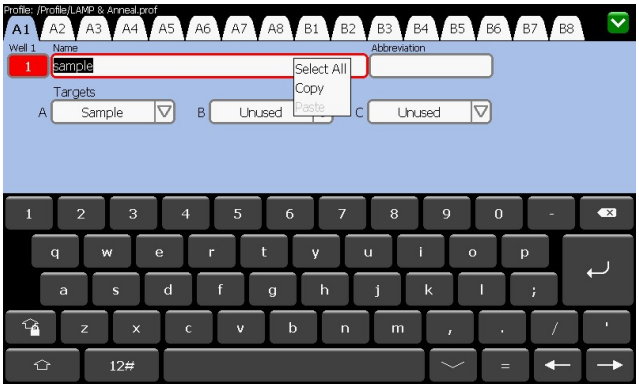
Users are able to copy and paste in any text box which can save time in entering well names or run names.

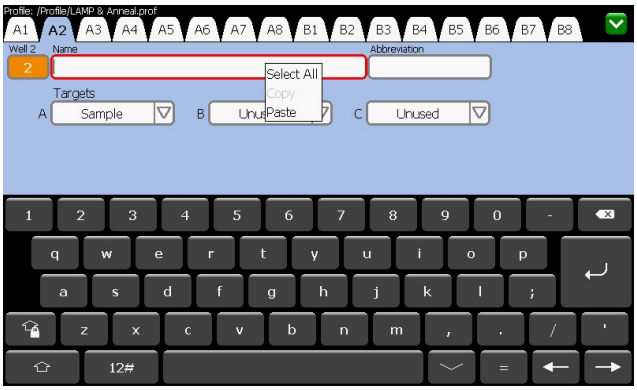


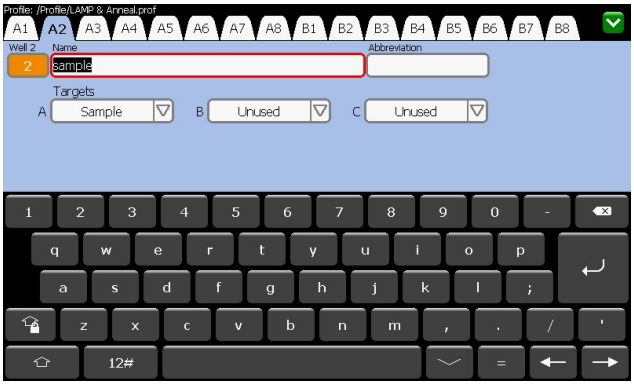
To copy:

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:

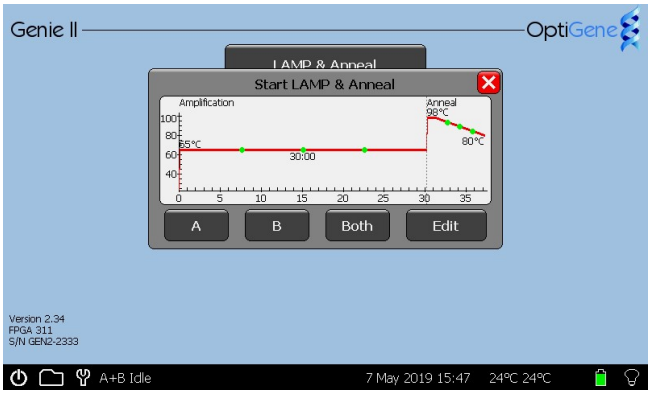
Touch the text box you wish to paste into, and then hold down on the box until a pop-up appears and select ‘Paste’.

RUN

On Genie® II, there are two ways to start a run: either quick start, by touching one of the predefined profiles saved on the instrument or by creating a new profile. Predefined profiles can only be selected for quick start if they are saved anywhere on the instrument file system.

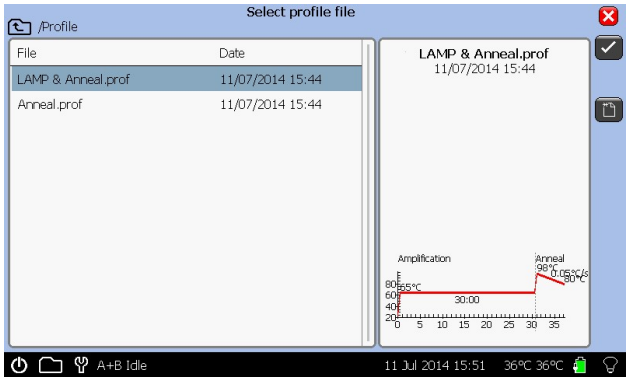
QUICK START

Up to six saved profiles can be shown on the main screen to allow quick starting of an assay. The six 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

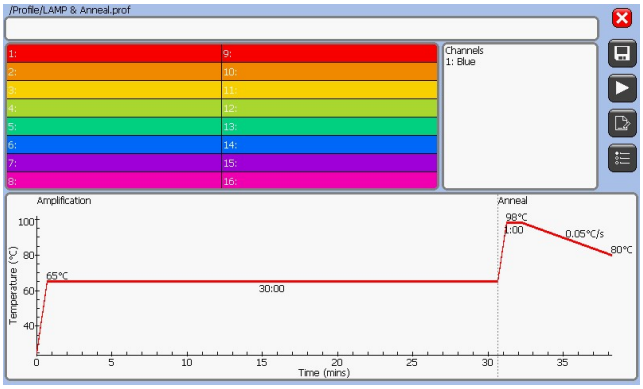


To create a new profile, press the 'Other…' button on the main screen. This allows access to any predefined profiles or creation of a new one. Cancel to return to the main menu.

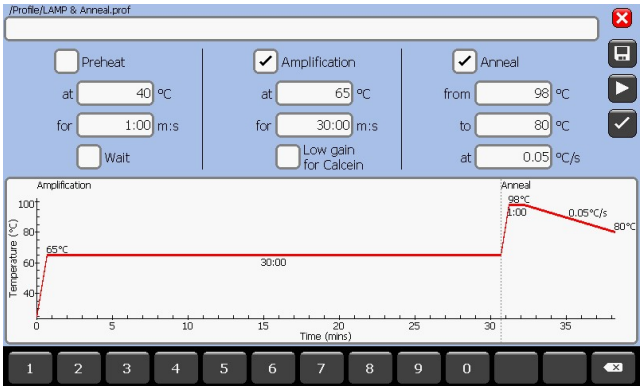
Press the tick to select a file, or  to create a new profile.

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

TO CREATE A NEW PROFILE

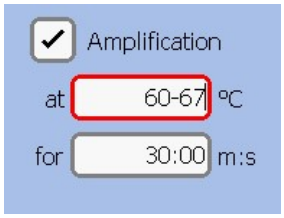


Click the graph to adjust the profile by touching the appropriate temperature or time box.

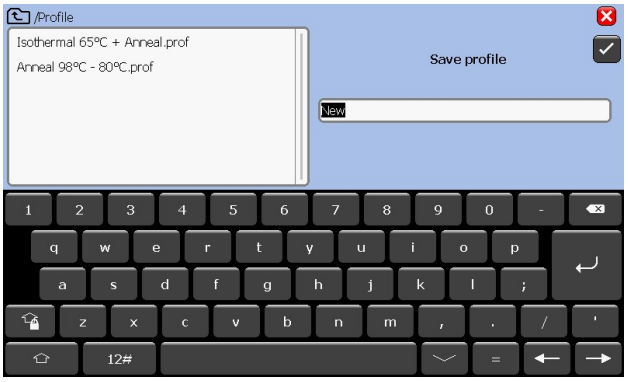


Once the required changes have been made, pressing the tick button will accept the changes. Clicking the cross in the top corner will cancel any changes.

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

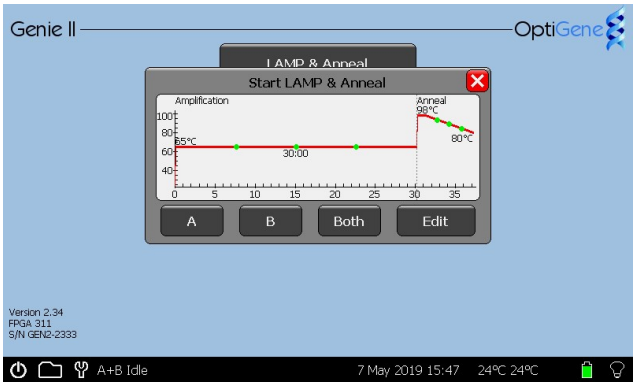


To set a thermal gradient across a block, enter a range of temperatures in the ‘Amplification’ temperature box. The range of temperatures should be entered separated by a hyphen, as shown below.



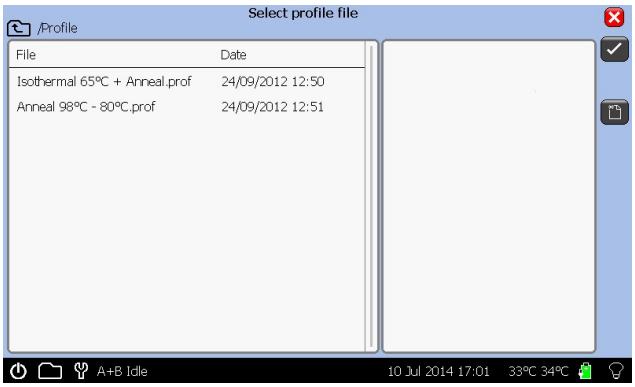
Pressing the save icon will save the profile. Name the profile, press the tick button and it will be saved within the ‘PROFILE’ directory in the on-board memory allowing it to be loaded for future runs.

TO LOAD A SAVED PROFILE

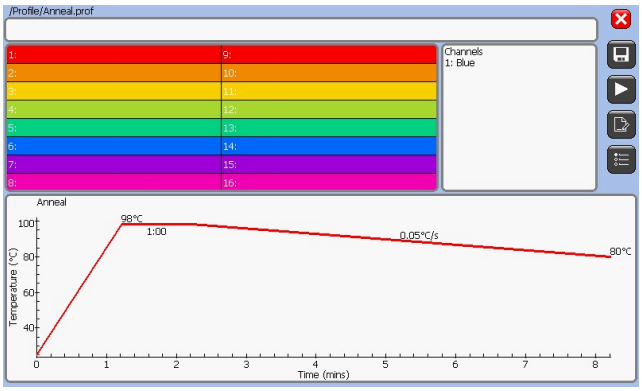


Pressing one of the quick start buttons on the main menu will pop-up a preview of the profile and give the user the choice to start, edit or cancel the run.

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

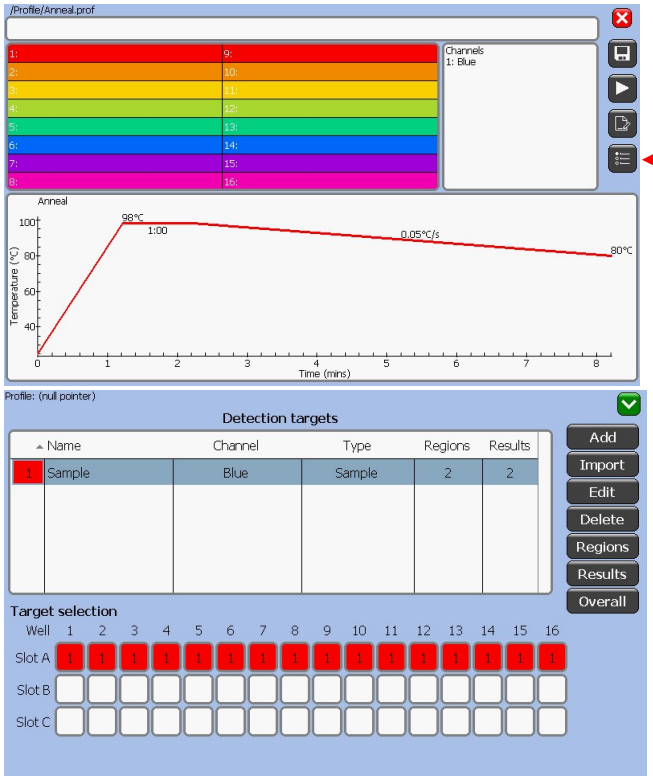


A preview graph will appear, and allow the user to start, edit or cancel the run. Pressing ‘Edit’ will open the main profile screen.

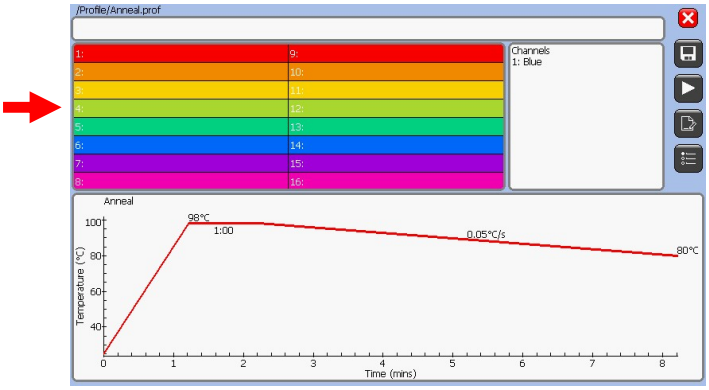


Changes can be made as required. To begin the run, press the play icon.

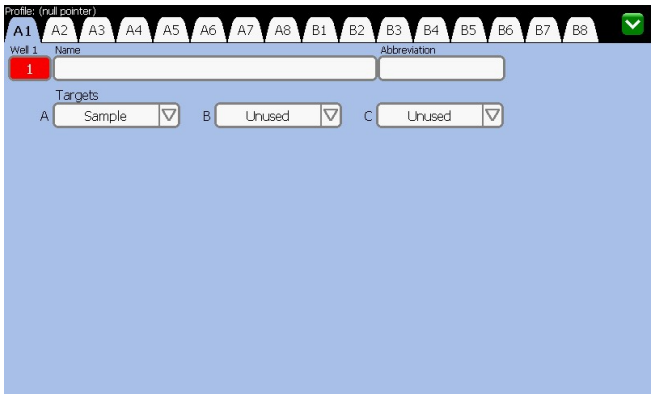
A prompt will be displayed. Select ‘A’, ‘B’ or ‘Both’ to run or ‘Cancel’ to abort.



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.



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.

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

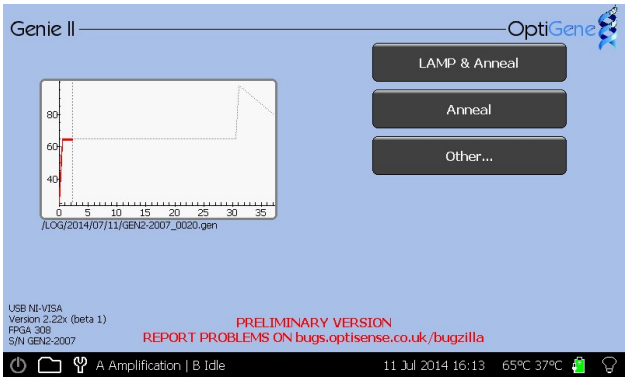
There is a tab for all 16 wells, click on the required tab and make changes.

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.

PREVIEW WINDOW



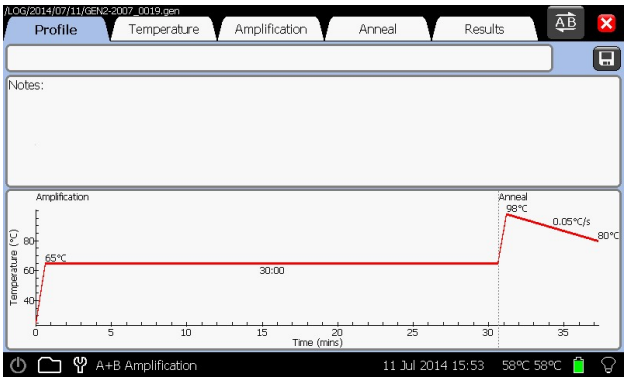
When a run is started, the main screen will display a preview of the run that is active. As the run progresses, the grey line showing the profile will turn red.

Block A will appear on the left, and Block B on the right. If both are started, then the preview will appear in the middle of the page.

Touching the graph will take you to the active screens.

ACTIVE

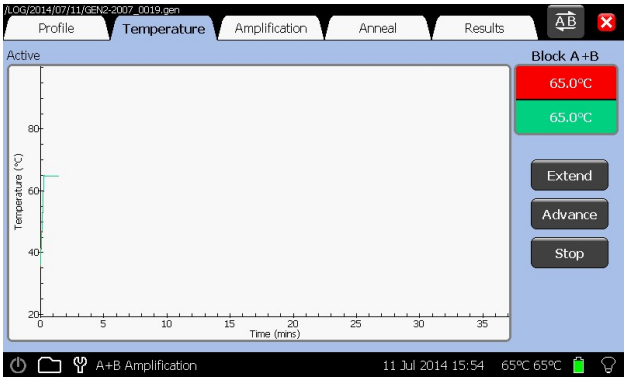
Once a run is started, the software will go to the 'Temperature' screen initially. The other screens can be accessed using the tabs.



PROFILE

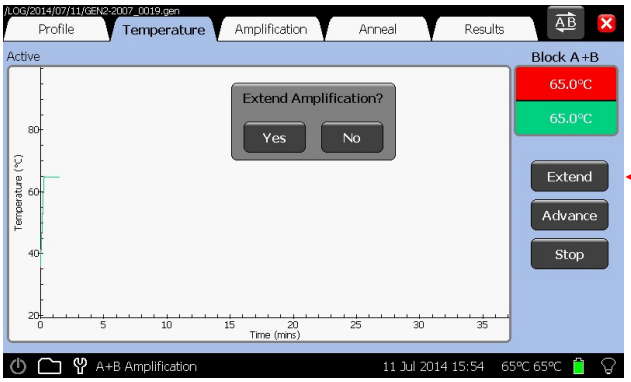
This shows the temperature profile that is running.

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.



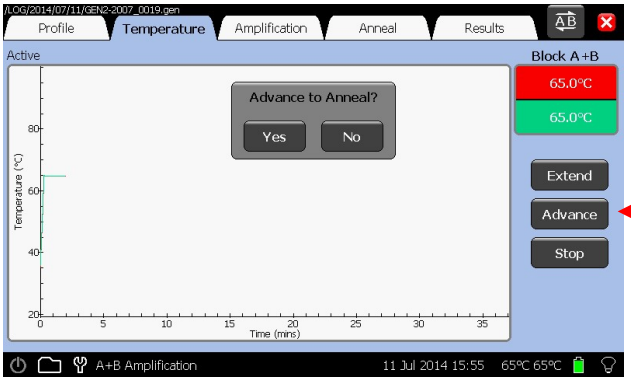
TEMPERATURE

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



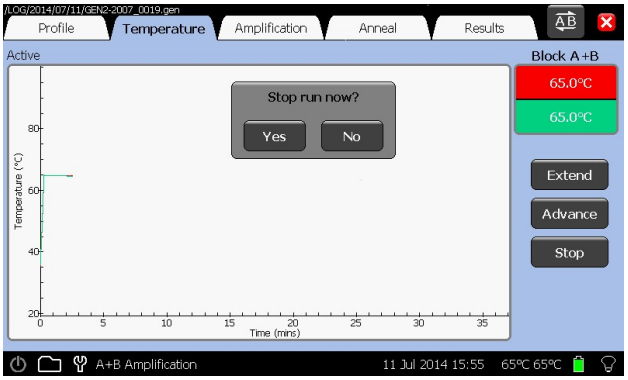
EXTEND

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



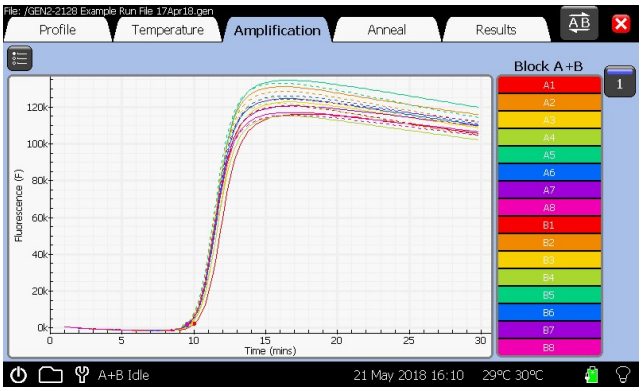
ADVANCE

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



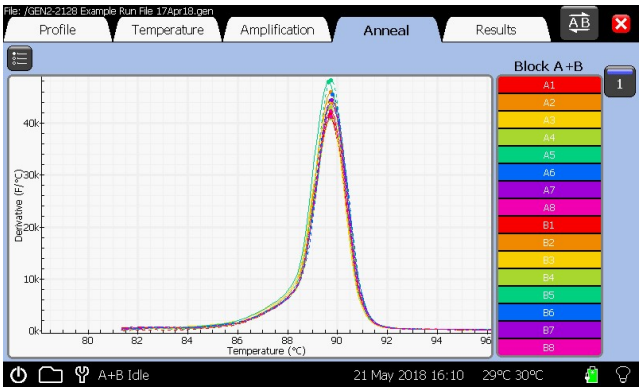
STOP

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



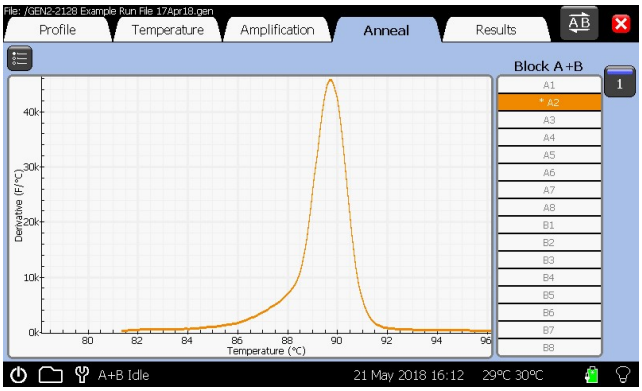
AMPLIFICATION

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



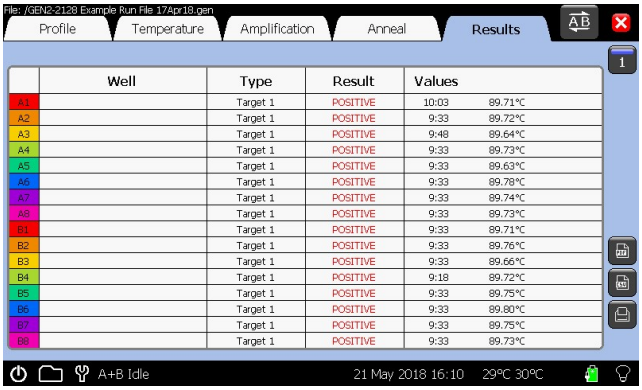
ANNEAL

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



SELECTION OF GRAPHS

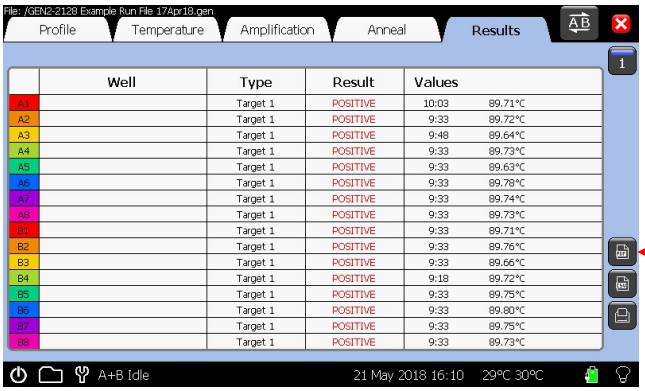
Pressing the well name on either the ‘Amplification’ or the ‘Anneal’ page cycles the state of the related curve on the graph between normal, highlighted and off.



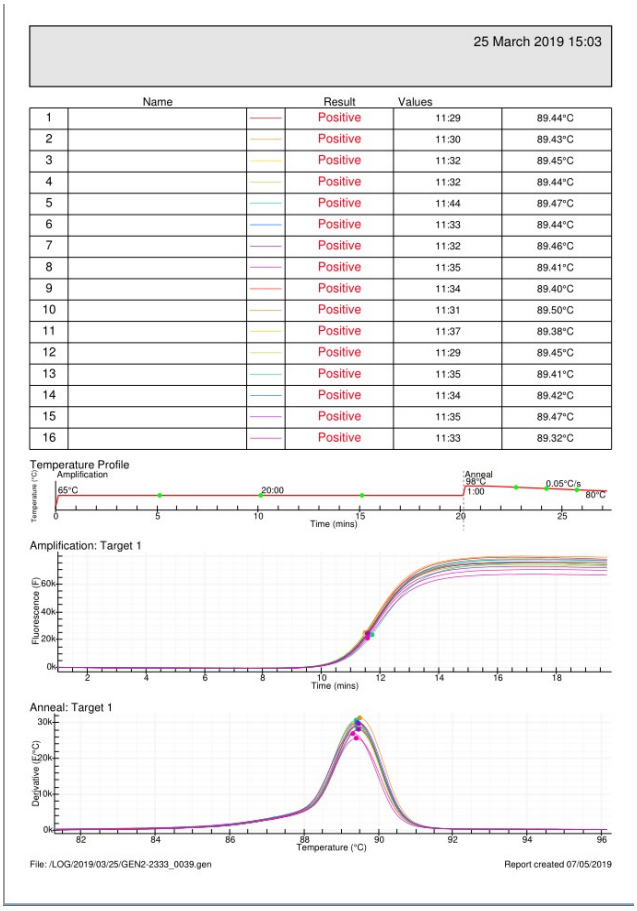
RESULTS

This shows the results of the experiment. Each sample name is shown along with amplification time and anneal temperature and any results that have been defined (see chapter 6).

REPORT GENERATION



Pressing the button shown on the results page will generate a 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.

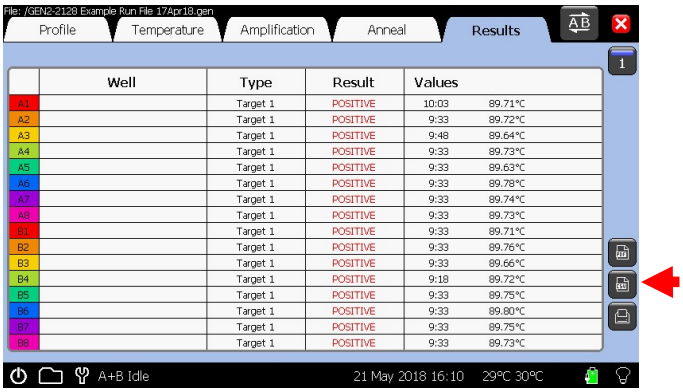


25 March 2019 15:03

	Name		Result	Values	
1			Positive	11:29	89.44°C
2			Positive	11:30	89.43°C
3			Positive	11:32	89.45°C
4			Positive	11:32	89.44°C
5			Positive	11:44	89.47°C
6			Positive	11:33	89.44°C
7			Positive	11:32	89.46°C
8			Positive	11:35	89.41°C
9			Positive	11:34	89.40°C
10			Positive	11:31	89.50°C
11			Positive	11:37	89.38°C
12			Positive	11:29	89.45°C
13			Positive	11:35	89.41°C
14			Positive	11:34	89.42°C
15			Positive	11:35	89.47°C
16			Positive	11:33	89.32°C

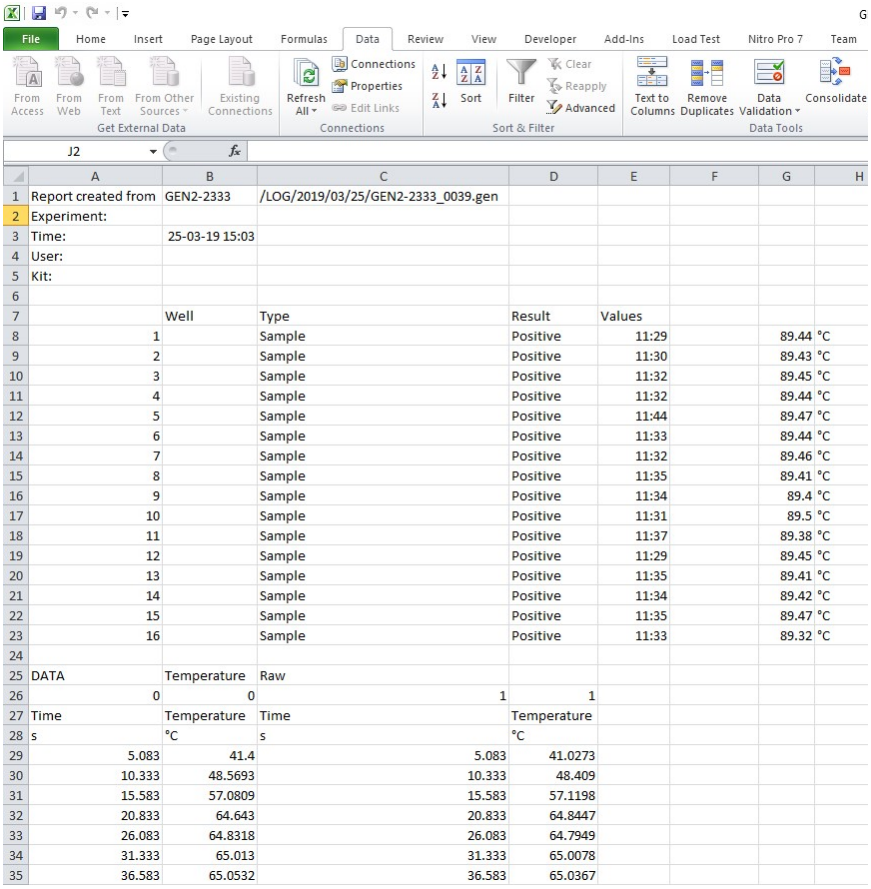
An example of a generated report file.

EXPORT TO CSV



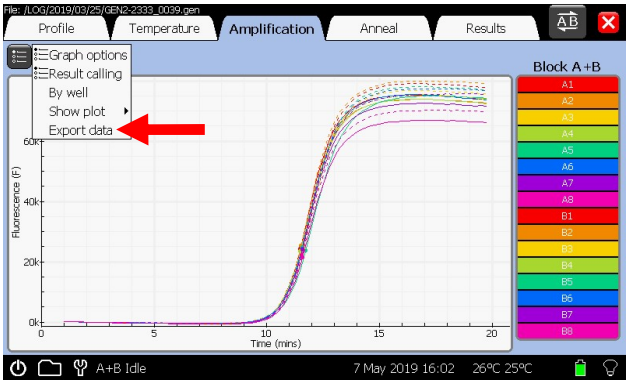
Pressing the button shown on the results page will generate a CSV (comma separated values) file with the results in and save it in the ‘Reports’ directory on the Genie instrument.

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.



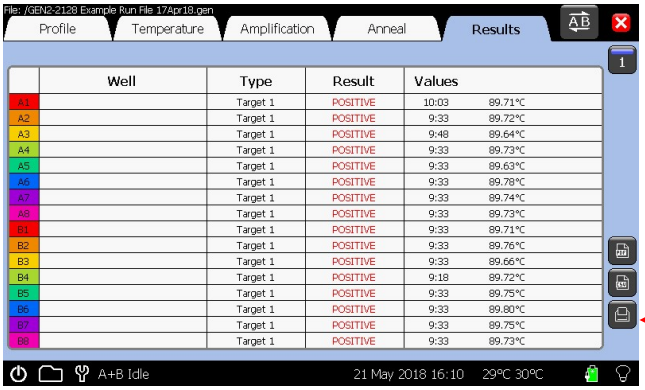
	A	B	C	D	E	F	G	H
1	Report created from	GEN2-2333	/LOG/2019/03/25/GEN2-2333_0039.gen					
2	Experiment:							
3	Time:	25-03-19 15:03						
4	User:							
5	Kit:							
6								
7		Well	Type	Result	Values			
8	1		Sample	Positive	11:29		89.44	°C
9	2		Sample	Positive	11:30		89.43	°C
10	3		Sample	Positive	11:32		89.45	°C
11	4		Sample	Positive	11:32		89.44	°C
12	5		Sample	Positive	11:44		89.47	°C
13	6		Sample	Positive	11:33		89.44	°C
14	7		Sample	Positive	11:32		89.46	°C
15	8		Sample	Positive	11:35		89.41	°C
16	9		Sample	Positive	11:34		89.4	°C
17	10		Sample	Positive	11:31		89.5	°C
18	11		Sample	Positive	11:37		89.38	°C
19	12		Sample	Positive	11:29		89.45	°C
20	13		Sample	Positive	11:35		89.41	°C
21	14		Sample	Positive	11:34		89.42	°C
22	15		Sample	Positive	11:35		89.47	°C
23	16		Sample	Positive	11:33		89.32	°C
24								
25	DATA	Temperature	Raw					
26	0	0	1	1				
27	Time	Temperature	Time	Temperature				
28	s	°C	s	°C				
29	5.083	41.4	5.083	41.0273				
30	10.333	48.5693	10.333	48.409				
31	15.583	57.0809	15.583	57.1198				
32	20.833	64.643	20.833	64.8447				
33	26.083	64.8318	26.083	64.7949				
34	31.333	65.013	31.333	65.0078				
35	36.583	65.0532	36.583	65.0367				

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



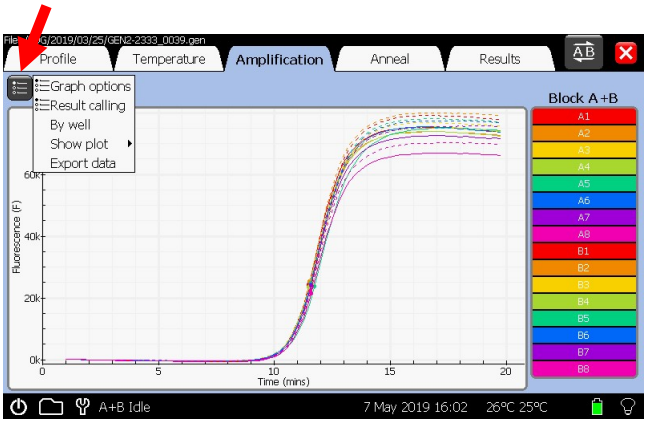
Any graph can have its data exported to a CSV file also by clicking on Export data in the Graph Options menu.

PRINT RESULTS TABLE



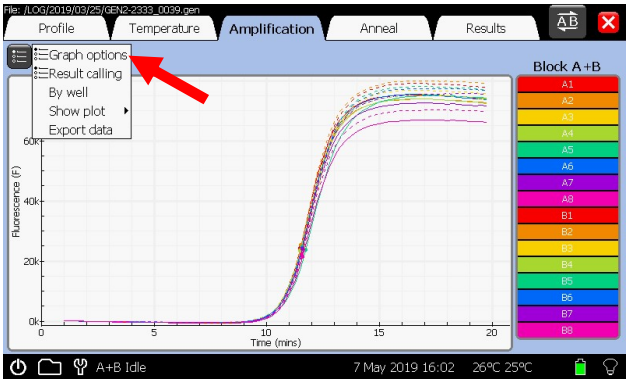
Pressing the button shown on the results table will print the table onto a label if the correct printer is attached to the Genie® instrument via the USB port on the front or rear (contact OptiGene for more information regarding a printer).

ADDITIONAL OPTIONS

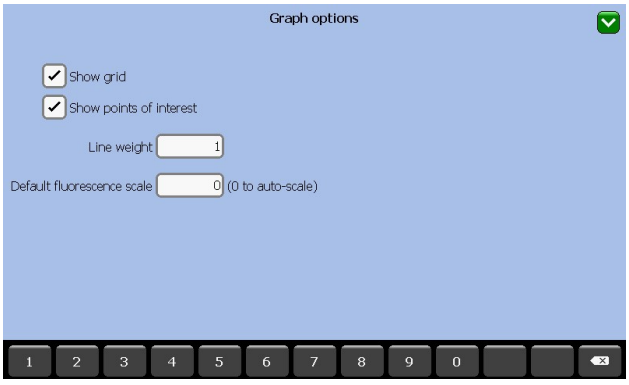


Touching the button shown on the amplification or anneal plots will show a drop down menu with some additional options.

GRAPH OPTIONS



Select Graph options



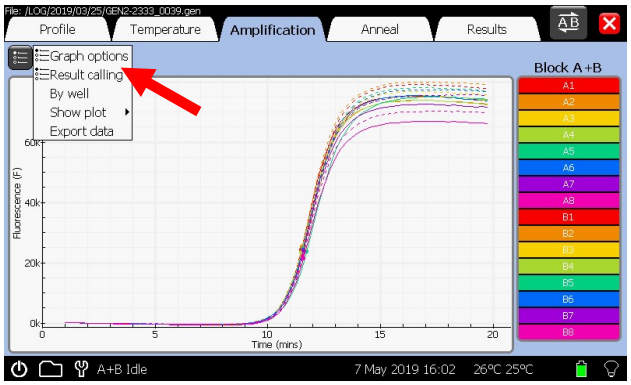
Show grid: This will enable/disable the grid behind the plots

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)

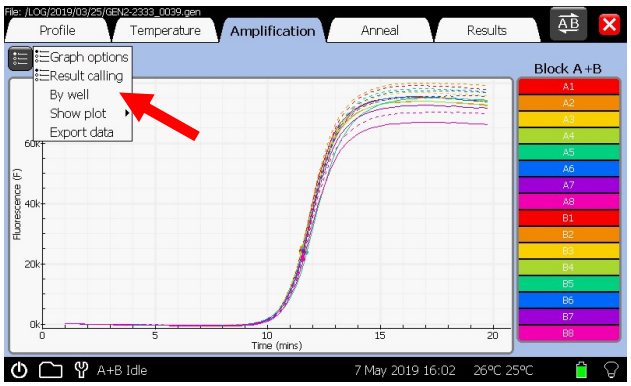
Default fluorescence scale: Set the Y axis upper scale on the graph. Set this value to 0 to auto-scale.

RESULT CALLING

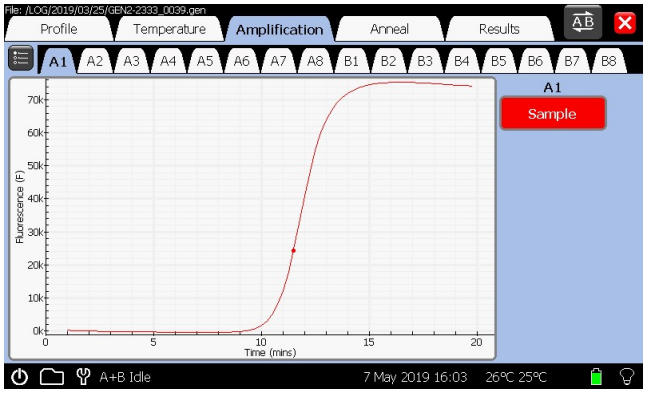


Result calling is explained in further detail in Chapter 6.

BY WELL

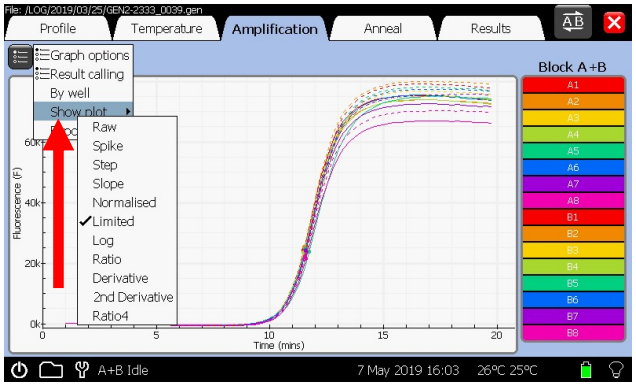


Selecting 'By well' will show all fluorescence plots by well rather than by fluorescence channel.



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

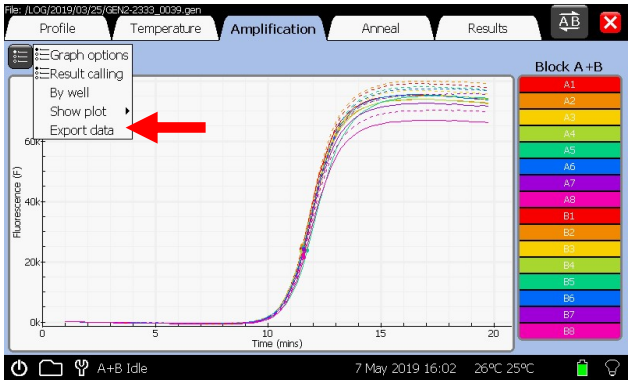
SHOW PLOT



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
Step	The data after step removal
Slope	The data after slope correction
Normalised	The data after normalisation
Log	The 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 ($(F\text{-}1\text{-}F1)/F0^2$) that gives an earlier indication of amplification.

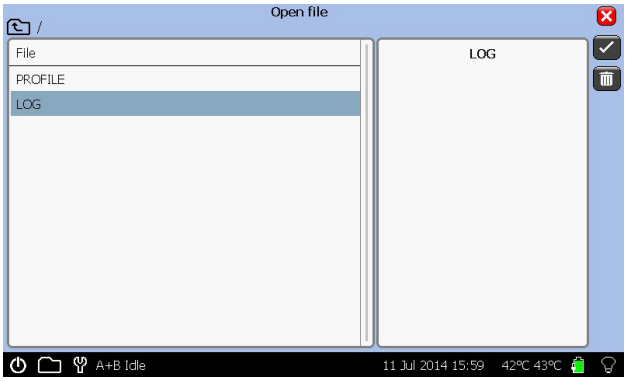
EXPORT DATA



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

VIEW

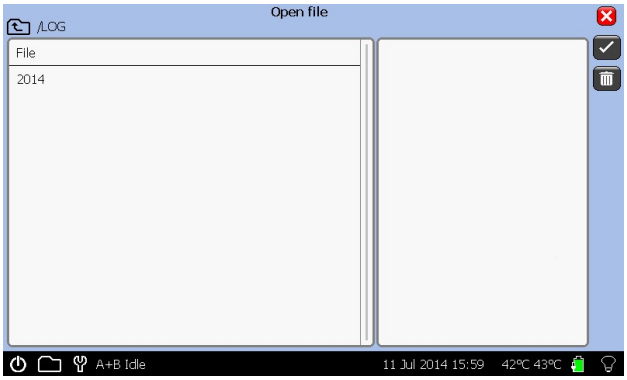
To view previous runs press the folder icon on the status bar.



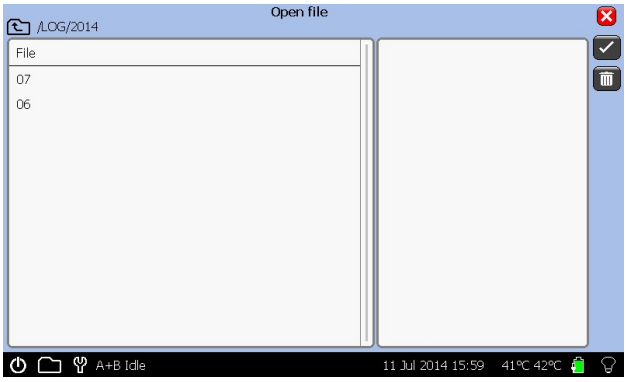
This will display a file browser window.

All Genie® II runs are saved in the 'LOG' folder.

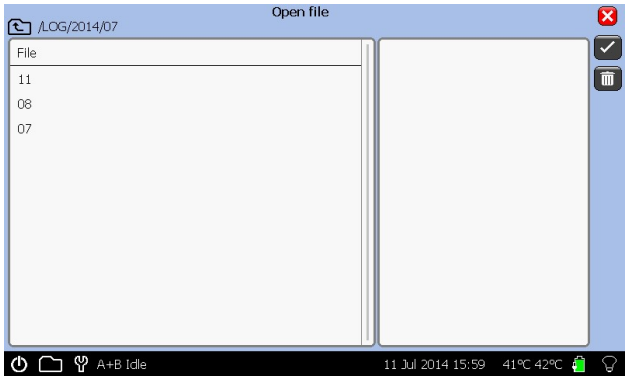
To open, click on 'LOG' and then tick icon, or double press on the folder name.



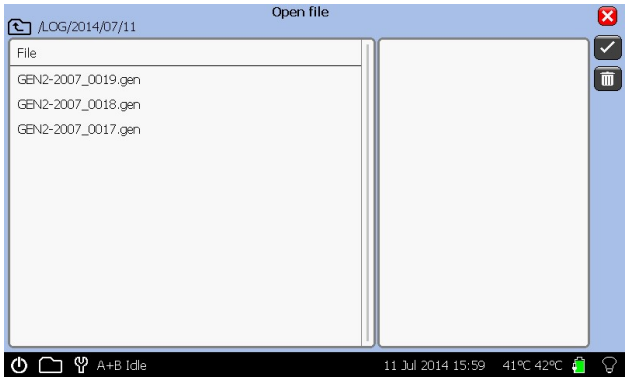
Each run is stored in a folder by date order: **Year**/Month/Day.



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.

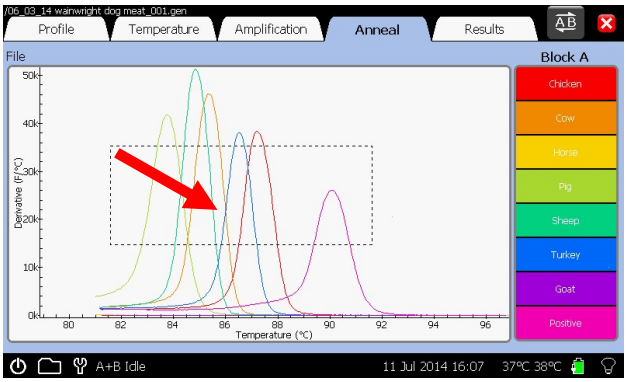
Select a file, a preview will appear in the right hand pane, then touch the tick button to load it.

To delete a file, touch the trash can icon.

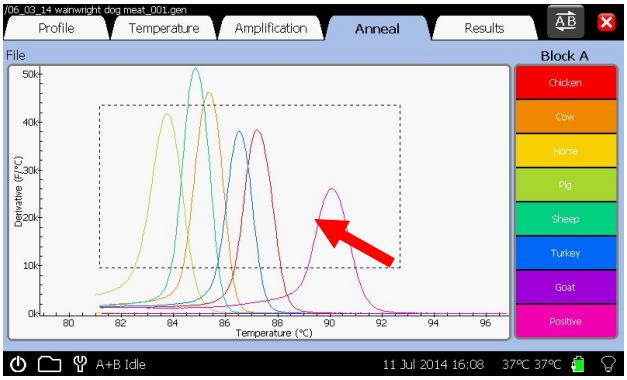
When the file has opened, Genie® II will display the profile that was run, the temperature log, amplification data, anneal data and the results table.

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.

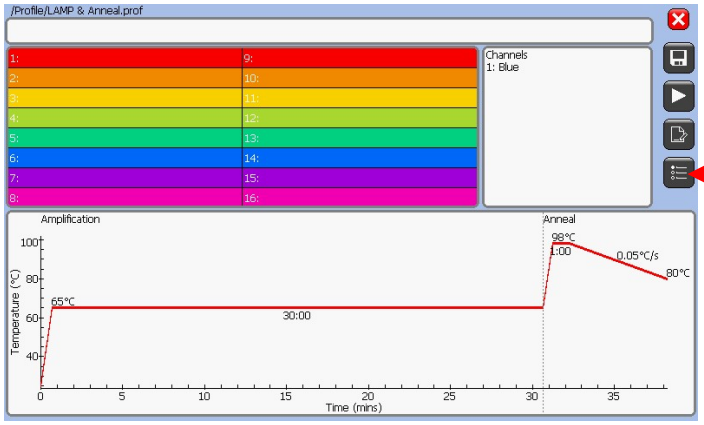
Detection starts with examining ***regions*** of interest on various graphs for required ***features***.

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.

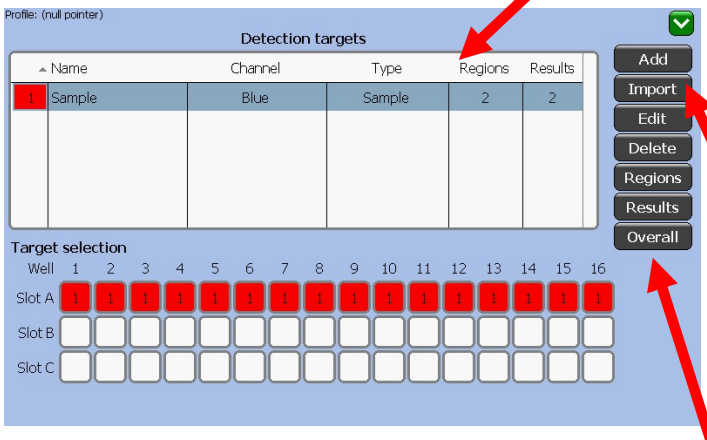
Advanced features allow regions to be defined relative to other regions or the results of other targets.

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.

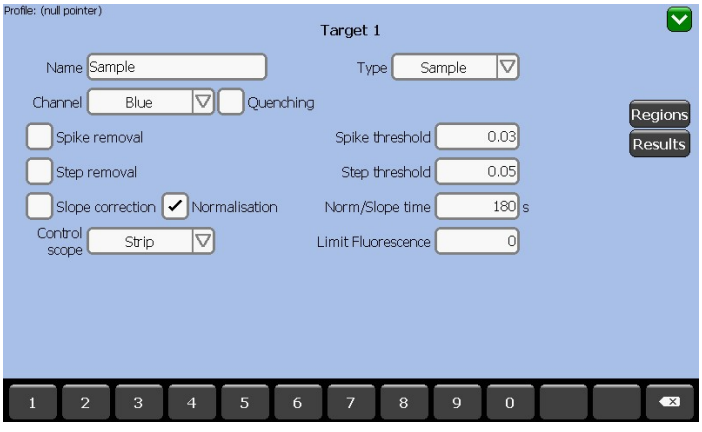


This screen shows an overview of the 'Detection targets', showing the number of regions of interest, the number of results, and which targets are assigned to which well. From here new targets can be added, imported from other runs or profiles, edited or deleted.

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.

UPDATE SCREENSHOT

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 factors.

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.

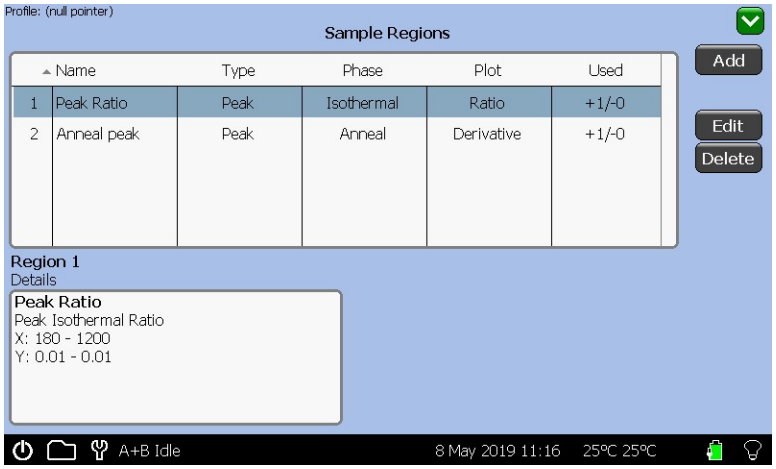
The state of the control is determined by the final result call.

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.

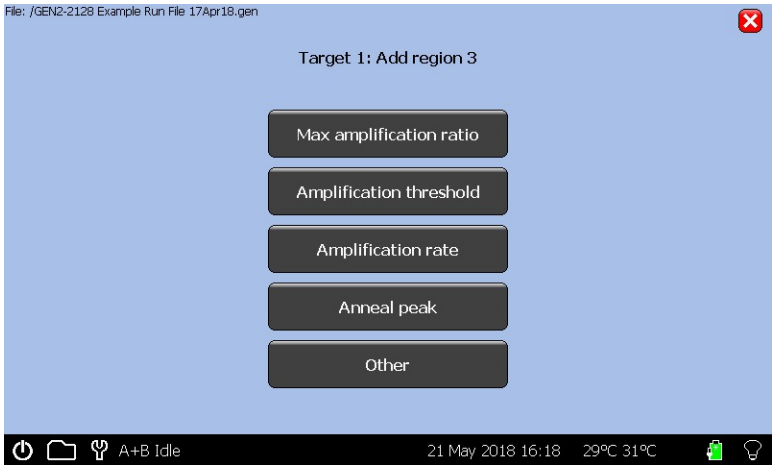
REGIONS OF INTEREST



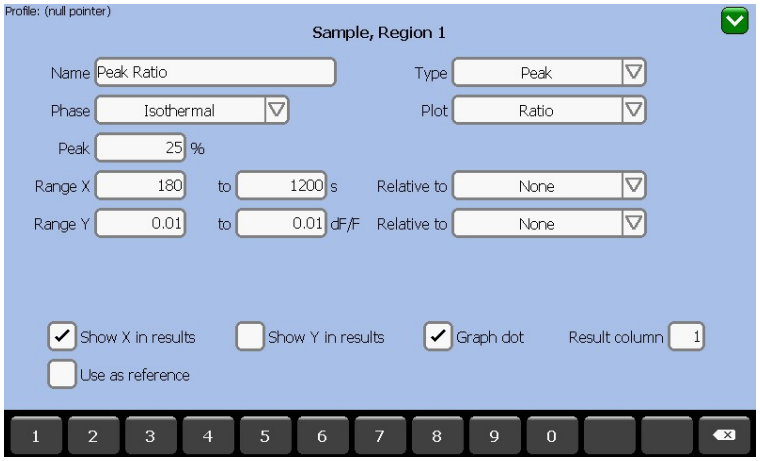
This screen shows an overview of all the regions of interest for the selected target.

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.



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.

Raw	Raw unprocessed fluorescence
Spike	The data after spike removal
Step	The data after step removal
Slope	The data after slope correction
Normalised	The 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)/$F0^2$) 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).

Show X/Y in result Displays the X and/or Y value found on the results table, provided the region is detected and used in the result.

Graph dot 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.

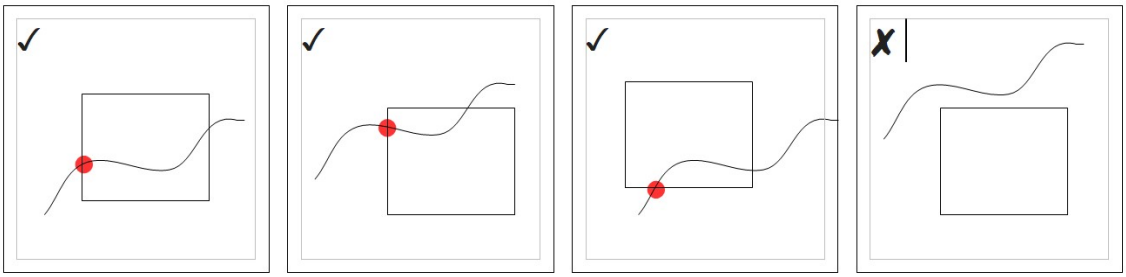
Additional parameters will be displayed depending on the feature type selected. These are explained further later.

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

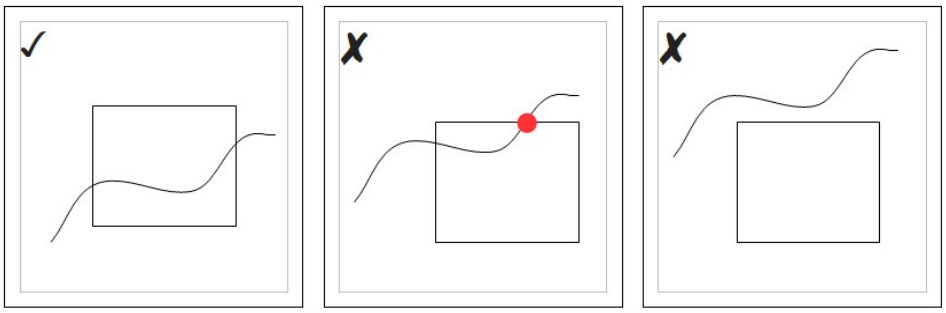
Feature type: Any

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.



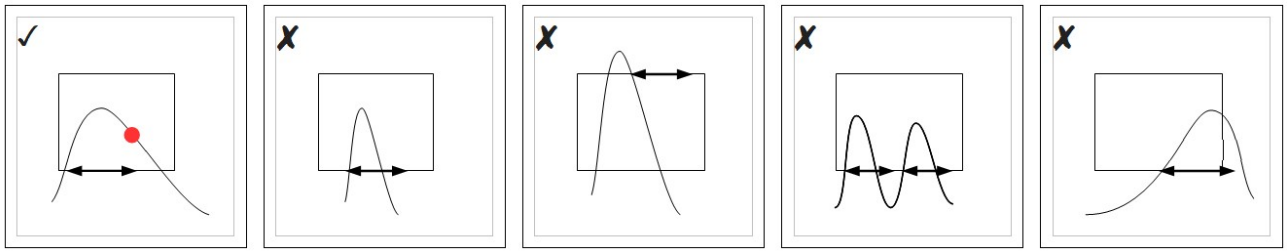
Feature type: All

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



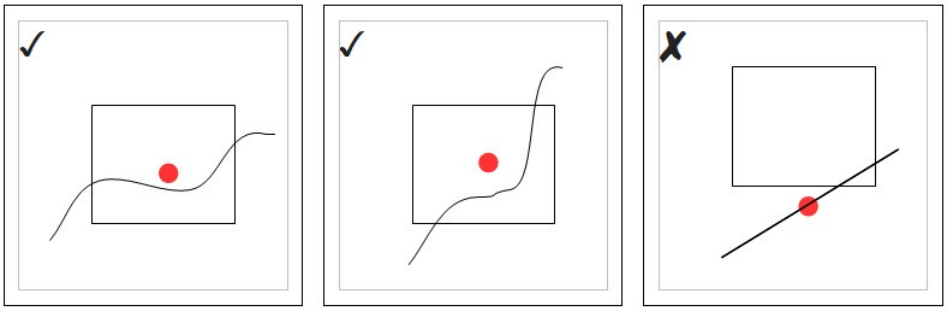
Feature type: Min

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

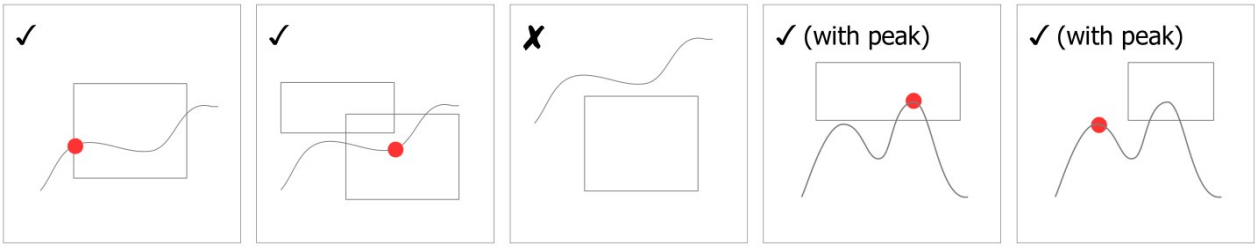


Feature type: Threshold

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

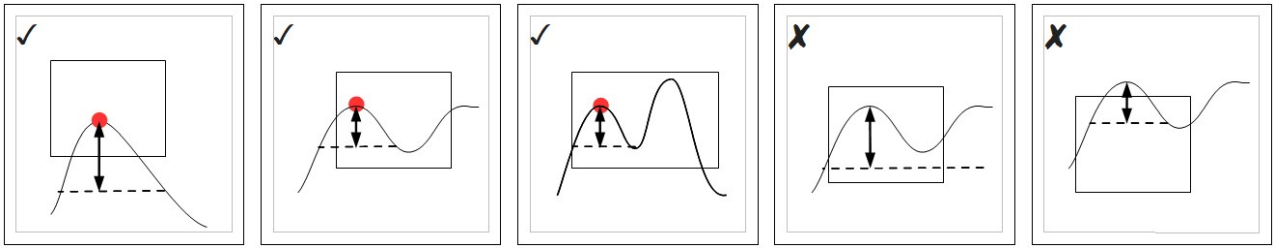
If peaks (or dips) are required, each peak is checked against all required thresholds.

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 Plot - Has the Reference option set.	
Additional parameters:	*Well:*	Well number reference.
	In:	Relative location of reference well
	Same:	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 pair
	Half:	Well 1-4 in the current half-strip
	Strip:	Well 1-8 in the current strip
	Target:	Target slot to examine
Unused parameters:	Window range parameters.	
Uses:	Comparison of amplification times, anneal temperatures, SNP target alignment, extra controls.	

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.

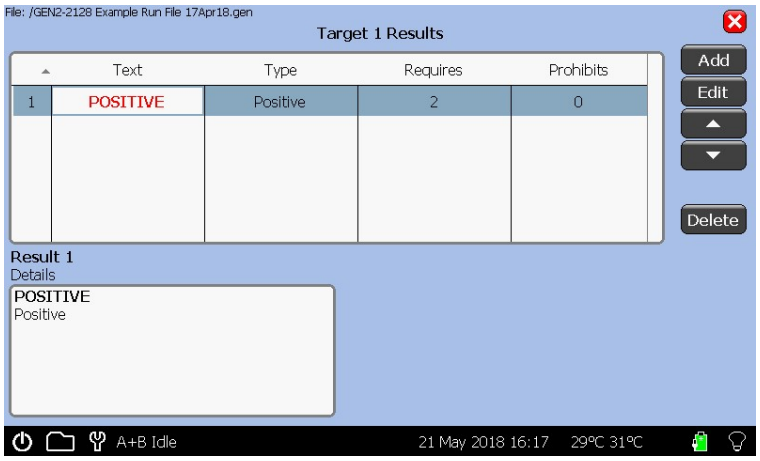
1. Create an 'any' region, testing normalised fluorescence
2. Create a 'peak' region, testing smoothed ratio; X range 0-0, relative to region 1

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.

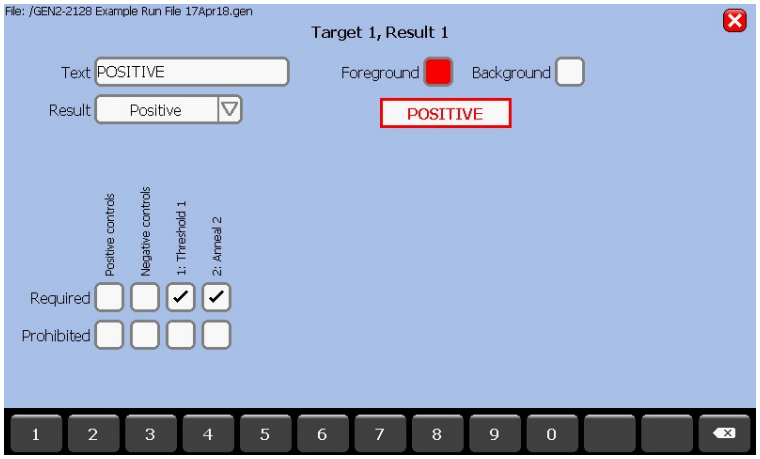
RESULT DEFINITIONS



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

Foreground/Background These are the foreground and background colour of the displayed result.

Result The type of the result

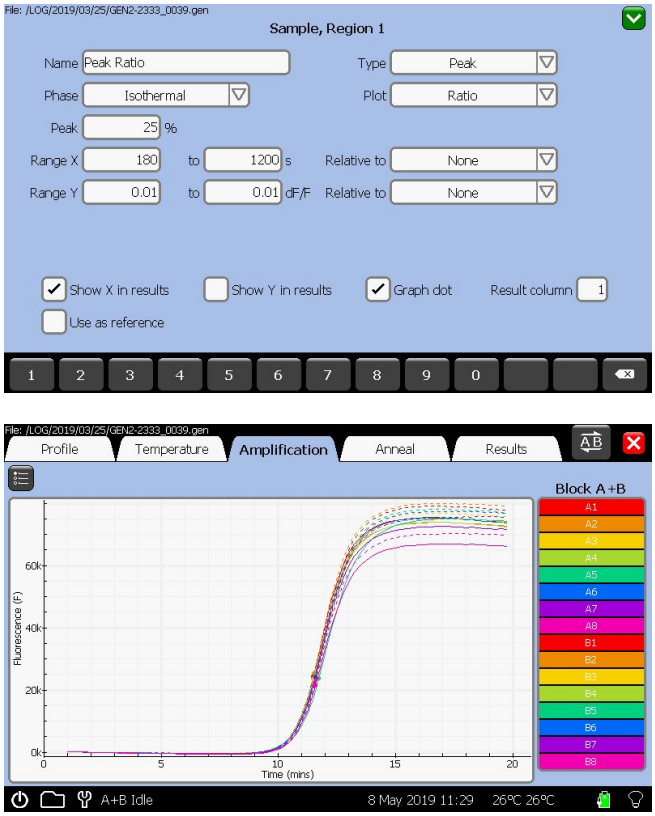
None:	An unused result that will block later results until determined.
Provisional:	An intermediate result that will be shown until/unless overridden.
Invalid:	A final result that cannot be determined
Positive:	A final result determined as positive
Negative:	A final result determined as negative

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.

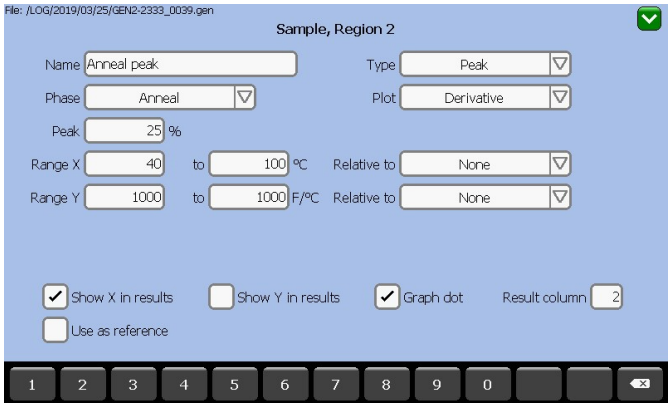
Unselected regions and controls have no effect on the result. Regions that are both required and prohibited should be avoided – this combination may be given a special meaning in the future.

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.

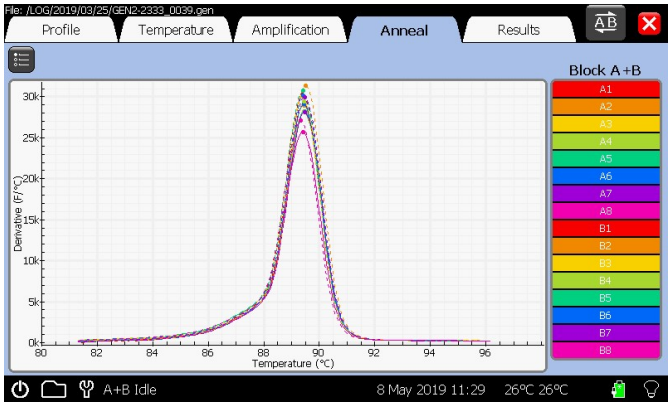


In this example all the wells have met the criteria defined in the ‘Peak Ratio’ region.

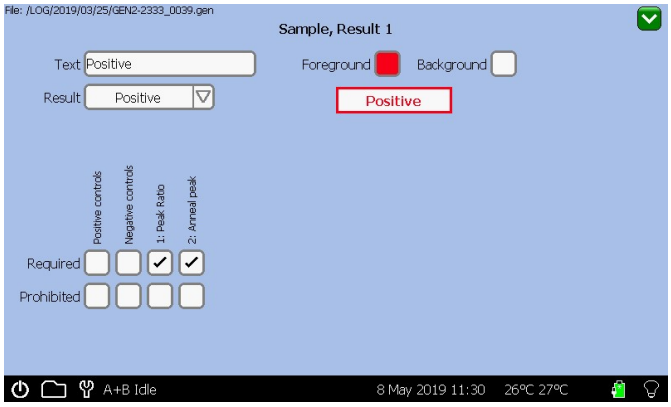
The points at which the criteria are met are marked by a dot on the graph line.



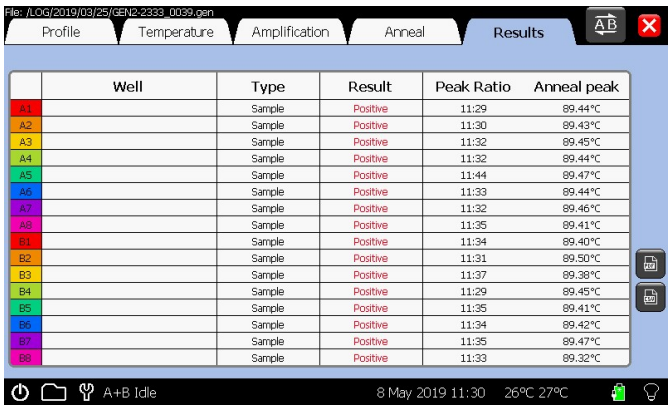
All wells have met the criteria defined in the 'Anneal peak' region.



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



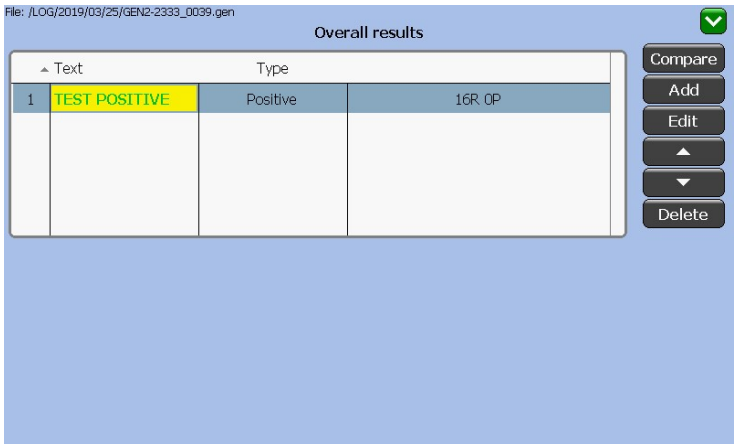
The result defined is shown in the Results table.



	Well	Type	Result	Peak Ratio	Anneal peak
A1		Sample	Positive	11:29	89.44°C
A2		Sample	Positive	11:30	89.43°C
A3		Sample	Positive	11:32	89.45°C
A4		Sample	Positive	11:32	89.44°C
A5		Sample	Positive	11:44	89.47°C
A6		Sample	Positive	11:33	89.44°C
A7		Sample	Positive	11:32	89.46°C
A8		Sample	Positive	11:35	89.41°C
B1		Sample	Positive	11:34	89.40°C
B2		Sample	Positive	11:31	89.50°C
B3		Sample	Positive	11:37	89.38°C
B4		Sample	Positive	11:29	89.45°C
B5		Sample	Positive	11:35	89.41°C
B6		Sample	Positive	11:34	89.42°C
B7		Sample	Positive	11:35	89.47°C
B8		Sample	Positive	11:33	89.32°C

OVERALL RESULT

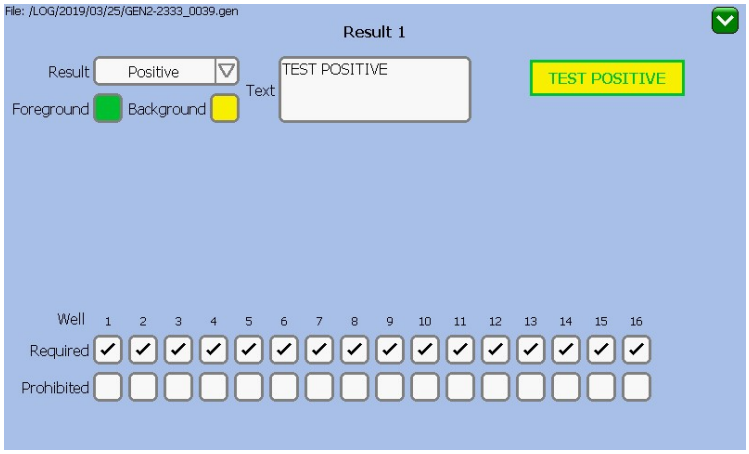
Overall result calling takes the results for each well and combines then to give a result for the whole strip.



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.

Foreground/Background The foreground and background colour of the displayed result.

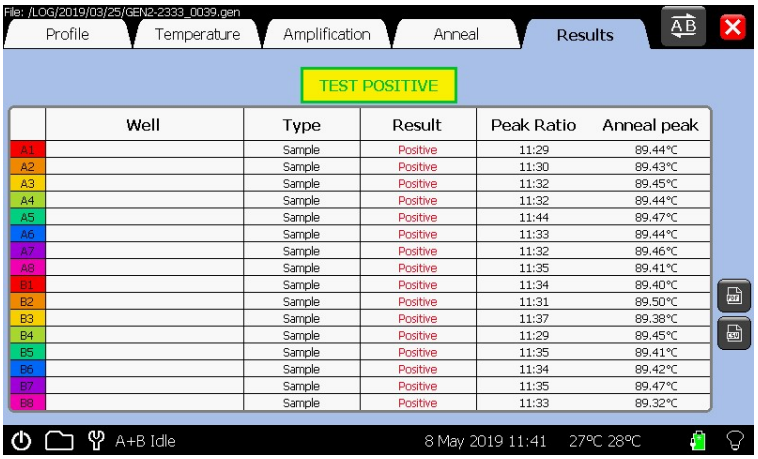
Required Select all wells and comparisons that must be positive to satisfy this result

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.

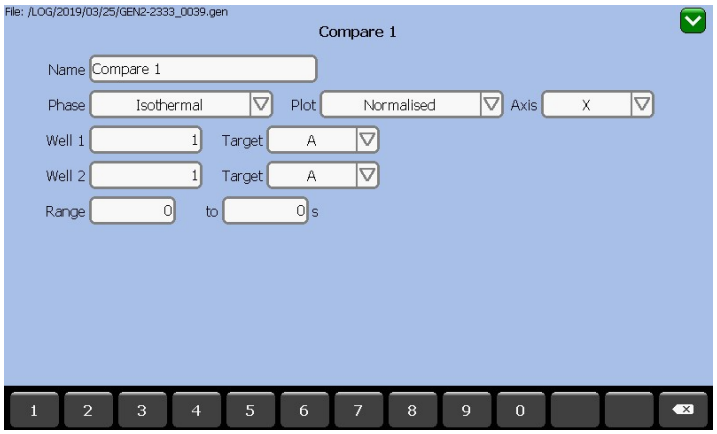
A well is negative if any target produces a **negative** result type.



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 7

CONNECTING TO EXTERNAL DEVICES

Genie® II 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 to a PC or via a pendrive to the USB socket on the front or the rear of the instrument. Genie® can also be connected to a barcode scanner and a printer for text input and output (contact OptiGene Ltd for more information).

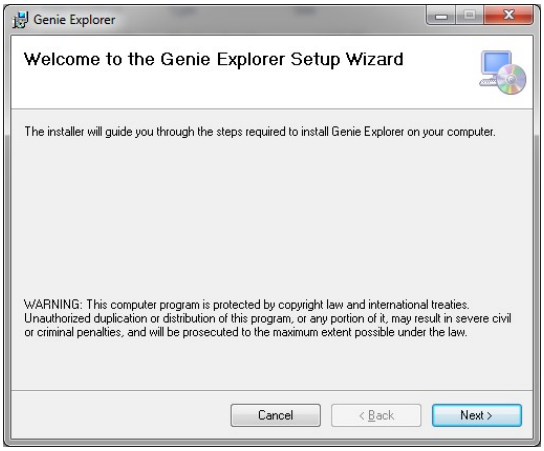
PENDRIVE

A pendrive can be plugged into the USB A socket in the rear or the front 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.

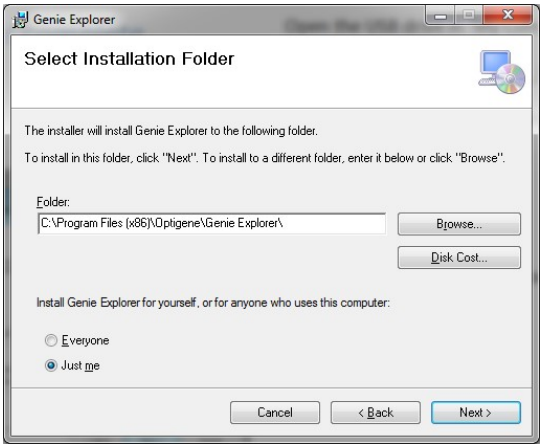


Open the USB drive in ‘My Computer’.

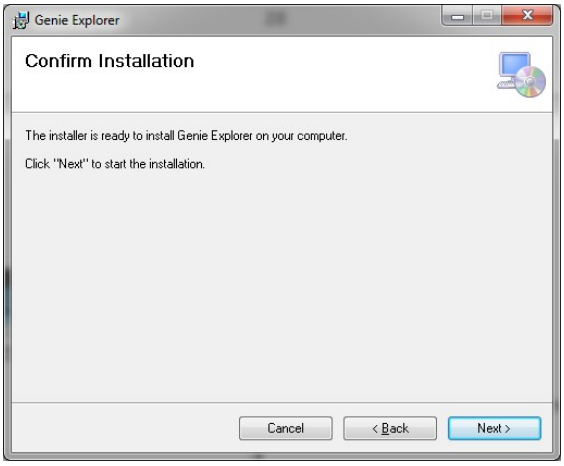
Run the file ‘GenieInstall.msi’.

Follow the onscreen instructions.

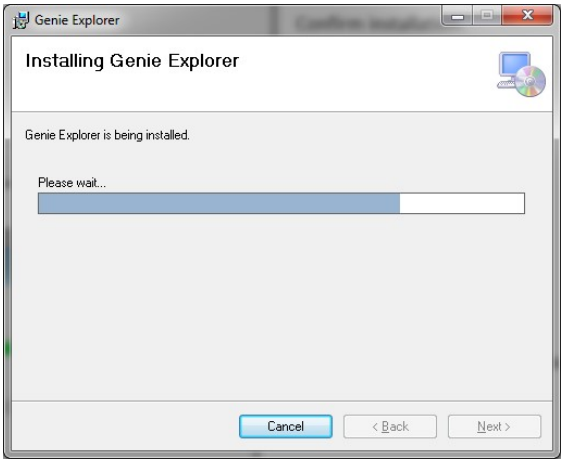
*A prompt may appear requesting installation of .NET Framework 4.0. This must be installed prior to installation. Follow the link to the Microsoft download page.
http://www.microsoft.com/en-gb/download/details.aspx?id=17718



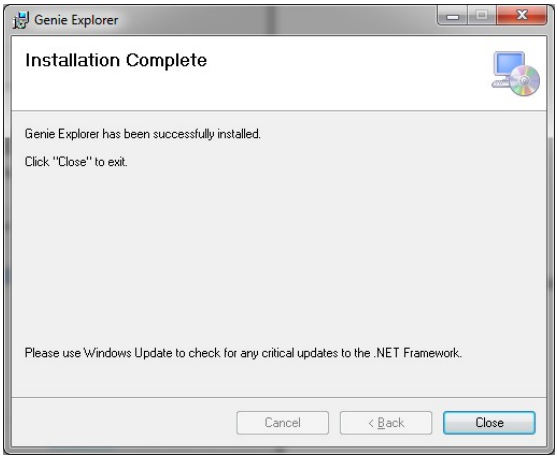
Choose a location for the installed program.



Confirm to start the installation.



The installer will copy all necessary files to the computer.



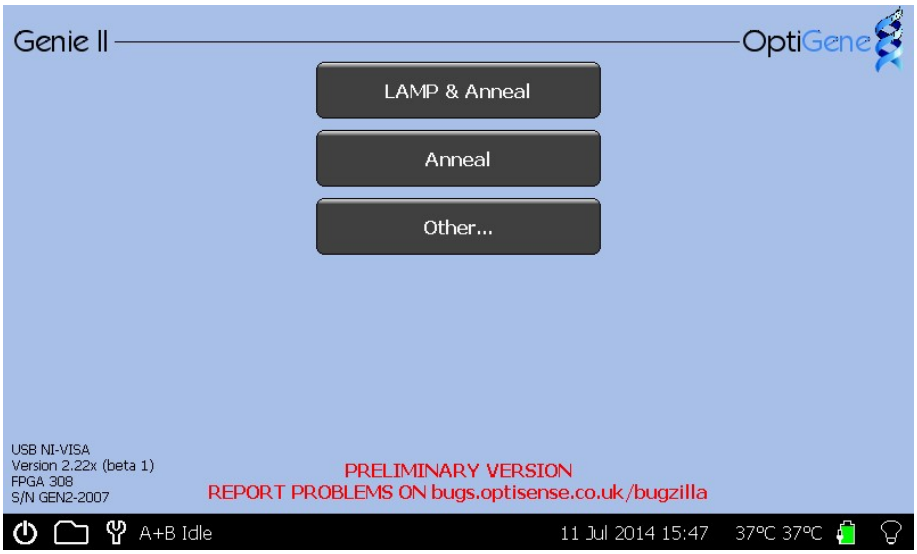
Once the installation is complete, exit by clicking ‘Close’.

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.

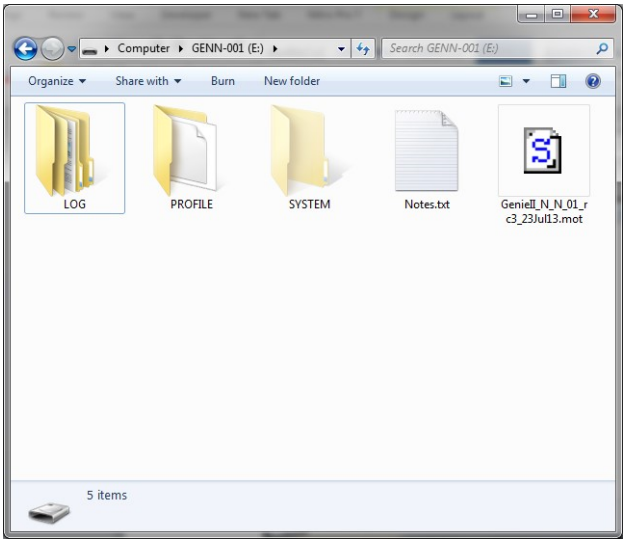
GENIE® II SOFTWARE UPDATES

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

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

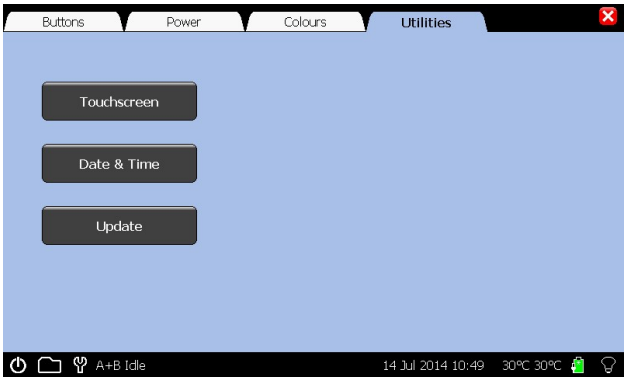


- To download the latest firmware, visit the OptiGene website (http://www.optigene.co.uk). Click on 'Support' and click on the appropriate link on the right hand side of the page and download the '.zip' file.
- Open the '.zip' file and extract the contents to a new folder. The contents of the folder will include the latest firmware, FPGA software and this manual.
- If Genie® II 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.

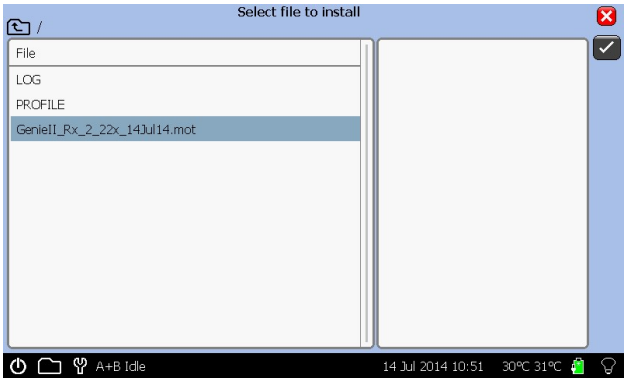


To install the updates, connect Genie® II to a computer. Navigate to 'My Computer' and open the Genie® II drive. The drive is named with the instrument serial number, e.g. GEN2-2001. Copy and paste (or drag) the firmware or FPGA software files onto the Genie® II drive.

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



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



Touch on the file, and touch 'Open'. Genie® II 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® II will restart when completed. If an FPGA software update was performed, Genie® II will require a manual restart by turning the instrument off and on from the switch on the rear of the unit.

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

Chapter

8

GENIE® II TECHNICAL SPECIFICATION

Sample Number	16 wells (2x8 strips)
Sample Volume	10 µl to 150 µl
Touchscreen	High-brightness TFT / LCD module (800x480)
Heater technology	Ceramic substrate with resistive coating
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 LED with high-quality interference filter 40 nm band pass
Detection optics	Photodiode with high-quality interference filter 510 nm long pass
Operating temperature	10°C - 40°C
Storage Temperature	20°C - 70°C
Approvals	CE
Dimensions	20cm (H) X 21cm (D) X 30cm (W)
Weight	2kg / 4.4 lb
Connections	1 x USB 'B' 2 x USB 'A' 1 x Power
Power supply	Input: 100-240V AC / Output: 24V DC - 150W
Battery Type	Lithium Polymer

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If you have any feedback or comments about the instrument please email:

feedback@optigene.co.uk

Version	Date	Changes	Approved
V1.00	10/07/14	Initial Release	SUL
V1.01	01/04/15	Added disclaimer about Genie Explorer.	SUL
V1.02	17/09/15	Added instrument cleaning guidelines and feedback email address.	SUL
V1.03	03/11/15	Separating Genie Explorer into own manual	SUL
V1.04	18/02/16	Updated Technical Specification	SUL
V1.05	21/05/18	Updated to match latest software updates.	SUL
V1.06	08/05/19	Updates for result calling	SUL
V1.07	12/12/19	Updates for new features, copy&paste, smoothing	SUL