

# Genie®II

# USER MANUAL

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+1 11

# SAFETY NOTICES

# Please read the following notices carefully before using the Genie<sup>®</sup> II system.

The equipment supplied has been designed to consider the normal likely operation and features have been designed in to avoid risks associated with this use. However to avoid any risk to the safety of the equipment, operator or those in the same area as the equipment please read this chapter with care before unpacking and using the instrument. If you are in any doubt as to the correct use of the equipment please contact the vendor.

## Notices



Using the instrument in a manner not specified by OptiGene Ltd may result in personal injury or damage to the instrument.



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 the surface is suitable for the weight and size of the instrument. If the instrument is dropped it may cause harm.



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 it off at the mains outlet 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 over the instrument they may get inside and cause a dangerous situation with respect to the voltage within the enclosure.



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

Normal safe local operating standards should be applied in general terms. The items above are for guidance and are not a definitive list.

Please consult the instrument supplier if you are in any doubt.

## **Disconnection Method**



The Genie<sup>®</sup> 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 lid are hot, please allow to cool before touching the surfaces.



Safe removal of fluids from the Genie<sup>®</sup> 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 COSH regulations. If in doubt consult the person responsible for the equipment in the laboratory.

## SUPPORT

## HOW TO OBTAIN SUPPORT

For the latest services and support information go to <u>www.optigene.co.uk/support.htm</u> *IMPORTANT!* When directed to do so, contact OptiGene to schedule maintenance or calibration of the Genie<sup>®</sup> II instrument

## SUPPORTED CONSUMABLES

*IMPORTANT!* Genie<sup>®</sup> II<sup>™</sup> uses a proprietary tube strip that maximise optical and thermal efficiencies. **Other tubes and strips will not fit.** 

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

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

Catalogue number	Description
ISO-001	Isothermal Master Mix
OP-0008-50	Genie <sup>®</sup> II tubes pack size 50 strips
OP-0008-500	Genie <sup>®</sup> II tubes 500 strips pack size

# **BOX CONTENTS**

Following is a list of contents in the Box for Genie<sup>®</sup> II

- Genie<sup>®</sup> II instrument
- Power supply
- Power Lead
- USB connection Lead
- Manual PDF version on memory stick
- USB memory stick

## SITE PREPARATION

## HOW TO SET UP THE Genie<sup>®</sup> 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 inlet at the rear of the instrument and the outlet vent at the front of the instrument must not be covered. Restricting airflow by placing objects in front of these areas will impede operation and significantly 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<sup>®</sup> II is an electrical instrument and care should be taken not to operate if there is a risk of water damage.

## CONNECTIONS

Once un-packed, Genie<sup>®</sup> II is ready for use.

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

Located to the rear of the instrument is an on/off power button, switch to on and Genie<sup>®</sup> II will power up and progress through its checks.



## OPENING AND CLOSING THE LID

To open and close the instrument lid to add or remove samples, gently lift the lid and it should open upwards. Close the lid by lowering gently.



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

## **INSERTING TUBES**

You will notice that each of the heating blocks has locating pins

The Genie II strips have holes that locate on these pins, the strips will only fit in one orientation.

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

### BATTERY

The Genie<sup>®</sup> II has an internal rechargeable battery. The battery is fully charged and ready for use.

The battery monitor is on the status bar next to the time and date

	ወ	A+B Idle	1 Sep 2011 18:30 🧯
--	---	----------	--------------------

To activate the battery monitor, press the battery icon and the monitor will appear as a popup.

To remove the pop up simply press on the status indicator

Battery Status Charging 100% 4800mAh 16.4V +0.0A 3h 6min active time

Battery status pop-up

*IMPORTANT!* The Genie<sup>®</sup> II internal battery will only charge when the instrument is plugged into mains electricity and the instrument is switched on. Genie<sup>®</sup> II can then be shut down using the 'turn off' command within the utilities menu. The LED will glow brightly until the battery is fully charged. At this point Genie<sup>®</sup> II can be switched off using the switch on the rear.

## **BATTERY MONITOR**



 Run
 Active
 View
 Notes
 Utilities
 A/B

 Genie II

 Isothermal Amplification Made Simple

 Battery Status

 Charging 62%

 3832mAh
 15.6V +1.8A

 15.6V +1.8A
 15.ev 2011 18:10

 ©
 A+B Idle
 1 Sep 2011 18:10





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

Here the pop-up shows that the battery is charging 62%

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

The pop-up shows that the instrument is in use and the battery is at 99%

# EMBEDDED SOFTWARE

The Genie<sup>®</sup> II uses a touchscreen for viewing and inputting data.

Simply touch the screen gently and press the keys required.

*IMPORTANT!* Do not use a pen or any other implement upon the screen otherwise damage could occur.

## 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 simply touch the screen to access the main menu.



## MAIN MENU



To begin a new run click run To view profiles or previous runs click view To access the utilities click utilities

## INITIAL SET UP

When running for the first time check the time and date on the lower status bar.

ا	A+B Idle	1 Sep 2011 18:30	

*If either are incorrect then proceed to change the time and date.* 

The time and date settings are in the utilities menu.

## UTILITIES

Run	Active	View	Notes	Utilities	A/B
	Touchscreen				
	Time & Date				
	Turn off				Close
ወ	A+B Idle			8 Sep 2011	12:18 📋

To access the utilities screen click the utilities tab on the menu bar, you are then given several options.

### TOUCHSCREEN CALIBRATION

## **Touchscreen calibration** Touching anywhere on the screen with Touch screen to start the exception of Test or Skip will invoke the touchscreen calibration Test Skip **Touchscreen calibration** Here you are required to touch the Touch the target points target points shown on the screen, this will calibrate the touchscreen Close Clear To check the sensitivity of the screen Points press test. **Touchscreen Test** Any point now pressed on the screen will be displayed. Version 1.05 S/N GEN2-1005

### SET TIME AND DATE

Set Date a	nd Time			
Date 08/09	/11	7	8	9
Time 12:19	:01	4	5	6
Okay	Cancel	1	2	3
C A+B Idle			8 Sep	2011 12:19 📋

Click in the white box for date and enter the date. The date should be in the format DD/MM/YY.

Click in the white box to enter the time. The format should be HH:MM:SS

### TURN OFF

Pressing turn off, will place the Genie® II into 'standby'. Genie® II can also be placed into

standby mode by pressing the O symbol.

Note if pressing this button during a run Genie<sup>®</sup> II will not enter standby mode.

Normal operation can be resumed by pressing anywhere on the screen.

### RUN

$\land$	File	Date	LAMP default.prof		
$\wedge$	LAMP default.prof	29/03/2011 11:01	29/03/2011 11:01		
	18S Product Melt.prof	01/04/2011 09:03	65,65,98-80@0.050		
	LAMP Anneal only.prof	04/04/2011 12:56			
V			New Load Cancel		
Φ	·		· · · · · · · · · · · · · · · · · · ·		

When you choose run you will be taken to the profile window. Here you can choose to create a new profile, load an existing profile or cancel and return to the menu screen.

### TO CREATE A NEW PROFILE

Run	Active	View	Notes	Utilities	А/В
	Preheat		<ul> <li>Amplification</li> </ul>	🗸 🗸	nneal
Preheat:	65 °C	Amplificatio	on: 65 °C	Anneal:	98 °C
for	0:00 mm:ss	f	for 30:00 mm	n:ss to:	80 °C
Description:				at 🦲	0.05 °C/s
80					
60- 40-	<u>+ · · · · </u>	<u></u>	···		····
Save	Low gain for	Calcein	We	lls Start	Cancel
Ð	A+B Idle			28 Oct 2011 1	3:29 🧯

Press the New button the run screen. Adjust the profile by touching the temperature box or time box that you wish to change. Inputs are made using the onscreen keyboard, which will appear, as in the example below.

Run	Active	View	Notes	Utilities	A/B
	reheat		Amplification		Anneal
Preheat:	40 °C	Amplification	n:65 ℃	Anneal: (	98 °C
for	0:00 mm:ss	fc	or <u>30:00</u> mr	n:ss to:(	80 °C
Description:				at (	0.05 °C/s
1 2	3 4	5 6	7 8	9 0	back
Q	W E R S D F	Τ \ G	IUV HCH	0 ( L	P enter
CAPZ	XC	<b>V</b> В	NM	, .	<>
shift	sym			- =	Cancel

Once you have made the required changes press Enter and now you can save the profile. Name the profile, click save and it will be saved within the directory allowing you to load it for future runs.

Save profile						
	A LAMP default.prof 29/03/2011 11:01					
	18S Product Melt.prof 01/04/2011 09:03					
	LAMP Anneal only.prof 04/04/2011 12:56					
New						
Okay Cancel	×					
1 2 3 4 5 6	7 8 9 0 back					
Q W E R T Y U I O P A S D F G H J K L : enter						
CAP Z X C V B	N M , . <>					
shift sym	- = Cancel					

Name the profile, press Okay and it will be saved within the directory allowing you to load it for future runs.

### TO LOAD A SAVED PROFILE

Run

Preheat:

Description:

Save

¢

for

Preheat

40 °C

0:00 mm:ss

Low gain for Calcein

A+B Idle



View

Amplification:

for

Notes

Amplification

65 °C

30:00 mm:ss

Utilities

Anneal:

to:

at

🗸 Anneal

Start Cancel

8 Sep 2011 12:21 📋

98) °C

80 °C

0.05 °C/s

From the menu on the left choose the protocol that you wish to load and press the load key.

The profile will be loaded and you will see the profile in the next screen

To begin the run alter the profile if required and press start.

Run	Active	View	Notes	Utilities	A/B
F	Preheat	(	Amplification	🗸 A	nneal
Preheat:	40 °C	Amplification	n: 65 °C	Anneal:	98 °C
for	0:00	Stari		to:	80 °C
Description:		Start		at	0.05 °C/s
80	A	В	Both Ca	ncel	
	1 · · · · · · · · · · · · · · · · · · ·	15	<u> </u>	30	<u> </u>
Save	Low gain fo	or Calcein		Start	Cancel
ወ	A+B Idle			8 Sep 2011 1	2:21

You will be prompted to choose Block A, Block B or both

### CALCEIN DETECTION

Low gain for Calcein

Low gain for Calcein

If you are running experiments with Calcein rather than Fluorescein

Click on the tick box for Calcein.

## WELL NAMES

Run	Active	View	Notes	Utilitie	s A/B
	Preheat		<ul> <li>Amplifica</li> </ul>	tion	Anneal
Preheat:	65 °C	Amplificat	ion: 6	5)℃ Anne	eal: 98 °C
for	0:00 mm:ss		for 30:0	mm:ss	to: 80 °C
Description:					at 0.05 °C/s
80					
60 40					
لف	<u>ś</u> iù	15	20	25	30 35
Save	Low gain for	Calcein		Wells	Start Cancel
Q	A+B Idle			28 Oct	2011 13:29 🧯

Click on the wells button

Wells

1	First Block we	ll names & abbrevi	iations	
1		5		More
2		6		Okay
3		7		
4		8		Cancel
1 2 3 Q W E A S [	4 5 R T D F G	6 7 8 Y U H J	90 I O P K L :	back
CAP Z X	c v	BNM	, . [	<>
shift sym			- =	

Second Block well names & abbreviations 1 Well 9 5 Well 13 Ш More 6 Well 14 2 Well 10 1ſ ][ Okay 3 Well 11 7 Well 15 Cancel 4 Well 12 8 Well 16 1 2 3 4 5 6 7 8 9 0 back R Y I Q w Е т U 0 Ρ DF J к ∥ А s G H L : CAP z x || c 📗 ٧ в Ν м <--> , . shift -= sym

Wells button will load the wells screens

For The first block click the well that you wish to insert a name into and type the name you wish.

More switches to the second block

Okay continues back to the run screen

Cancel returns to the run screen

Second block well names

If the run is saved at this point the well names and abbreviations will also be saved as part of the profile

The wells screen can be accessed at any time the instrument is running by returning to the profile screen and pressing the wells button.

## ACTIVE SCREENS

Once the run has started you will be able to access the active screens.

Run	Active	View	Notes	Utilities	A/B	
Profile	Temperature	Amp	lification	Anneal	Results	

The software will automatically go to the temperature screen, however you can access other screens using the tabs below active.

Run	Active	View	Notes	Utilities	A/B
	Preheat		<ul> <li>Amplification</li> </ul>	🗸 An	neal
Preheat:	65 °C	Amplificati	on: 65 °C	Anneal:	98 °C
for	0:00 mm:ss		for 30:00 mm:s	s to:	80 °C
Description:				at 🦲	0.05 °C/s
80					
40		15	20 25	30	· · · · · ·
Save	Low gain for	<sup>-</sup> Calcein	Wells	5 Start	Cancel
ወ	A+B Idle			28 Oct 2011 13	:29 🎝

### PROFILE

This shows you the profile that is running.

Run	Active	View	Notes	Utilities	A/B
Profile	Temperature	a Amplifica	ition	Anneal	Results
80-					B 65.0
60	_				Extend Advance
					Stop
<u></u>	A+B Einished	20	25	30 35 8 Sen 201	11 10:57

### TEMPERATURE

This shows you the temperature of the block (s) as the experiment is progressing.

		Active	View	Notes	Utiliti	es	A/B	
Ţ	Profile	Temperature	Amplificati	on	Anneal		Results	$\mathcal{T}$
80- 60-		Ex (	tend Amplif Yes	ication? No			A Exter Advan Stop	65.0
20	5	10 15	20	25	30	35	Clos	e
0	A	+B Amplification			8 Sep	2011	11:16 🧯	

### EXTEND

This allows 10 minutes to be added to the Amplification phase by clicking the button



### ANNEAL

Advances the programme from amplification to the Anneal phase by clicking the button



### AMPLIFICATION.

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



### ANNEAL.

This shows you the fluorescent data that is being acquired during the anneal phase of the experiment.

Run	Active Vi	iew Notes	Utilities	A/B
Profile	Temperature	Amplification	Anneal	Results
Well		Amplification mm:ss	Anneal °C	
A1		8:00	86.61	]
A2		9:00	86.61	]
A3		10:00	86.56	]
A4		11:00	86.61	]
A5		13:45	86.61	]
A6		15:00	86.61	]
A7		13:30	86.61	]
A8				Close
<u>۵</u>	+B Idle		8 Sep 201	1 12:31 🧯

### RESULTS.

This shows you the results of the experiment. You will see the sample name and then its respective amplification time and Anneal temperature.



When Genie II is running you can switch the views for Temperature, Amplification, Anneal and Results between block A and Block B by pressing the A/B button on the top menu bar.

## STOPPING A RUN



If you need to stop a run in progress, return to the Temperature Screen and press the stop button. A confirmation pop up box will prompt yes or no.

## NOTES SCREEN

On the notes screen you can make notes about the experiment that you are performing and they will be stored as part of the log file

## VIEWING PREVIOUS RUNS

To view previous runs click the view button on the top menu.



	Run		View	Notes	Utili	ties A/B
J	Profile	Temperatur	e Amplifi	ation	Anneal	Results
	GEN_0067.GE GEN_0068.GE GEN_0069.GE GEN_0070.GE		.oading file	Please wait		/20-05 <u>(20,000</u> )
× ≫						Open Cancel
ወ	A٠	+B Idle			8 Se	p 2011 12:50 📋

Please wait for the file to load and then you will be able to view the data

You are now able to view the profile that was run, the temperature log, the amplification, the anneal and the results table.

# **CONNECTING TO A COMPUTER**

Genie<sup>®</sup> II is a standalone instrument however for software updates and other applications you may wish to connect to a computer.

*IMPORTANT!* Do not plug Genie<sup>®</sup> II into the computer before installing software.

The PC software is preloaded on the USB stick.

Insert USB memory stick into computer.

Labview Software FULL File Edit New Favorites	INSTALL Version 22 Tools Help	
🔾 Back 🔹 🌍 🔹 🏂	🔎 Search 😰 Folders 💷 -	Addres
File and Folder Tasks	bit     b	
Removable Disk (F:)     My Documents     My Computer     My Network Places	_	
Details		
	1 99 MP	

Cente
 Destination Directory
 Select the primary installation directory.

 All activase will be installed in the following location(s). To install software into a
 different location(s), click the Browse button and select another directory.

 Directory for Genie
 C:\Phogram Files\OptiGene\Genie\
 Directory for National Instruments
 Directory for National Instruments
 C:\Phogram Files\OptiGene\Genie\
 C:\Phogram Files\OptiGene\Genie\Cenie\
 C:\Phogram Files\OptiGene\Genie\Ce

Choose 'Set up installer'

Choose a location for installation to take place.



Genie	
Start Installation Review the following summary before continuing.	
Adding or Changing • Garge Files • NAMSE 4.2 • NAMSE 4.2 Film Time Support	
Click the Next button to begin installation. Click the Back button to change the installation settings.	
Save File << Back	Cancel

Genie Genie	
Current Deserver 10% Constable	
<< Back Next>>	Cancel

🐙 Genie				
Installatio	on Complete			
The installer h	as finished updating your syst	tem.		
		<< Back	Next >>	<u> </u>

Accept the License Agreement

The software will inform of the processes being performed

#### **Overall progress**

Installation complete, click Finish



System must be restarted

Genie II can now be connected to the computer. When connected via USB and switched on, the Genie<sup>®</sup> II will appear as a USB device. Device driver software will be installed from the Genie<sup>®</sup> II

Driver Software Installation		<b>X</b>
Installing device driver softwa	ire	
USB Composite Device Genie USB Mass Storage Device OptiGene Genie II USB Device Obtaining device driver software from	Ready to use Searching Windows Update Ready to use Ready to use Windows Update might take a while.	
Skip obtaining driver software from Wi	ndows Update	Close

**Device Driver installation** 



C GEN2-1005 (F:)

File Edk View Favorites Tools Help

Dack
Dack
Dack
C Back
D Search
Folders
Folders
File and Folder Tasks
File and Folder Tasks
File and Folder
Folder
File and Folder
Folder
File and Folder
Folder
File and Folder
Folder
Folder
File and Folder
File and Folder
Folder
File and F

Genie<sup>®</sup> II is now visible as a USB device and the name appears as the instrument's serial number.

Log files and Profile files can be copied from the Genie<sup>®</sup> II to the computer and vice versa

Genie<sup>®</sup> II is now ready to be used and can be controlled from the computer.

## GENIE II FIRMWARE UPDATES

First check which versions of Firmware and FPGA are installed on your Genie.

They are shown in the bottom left hand corner of the Main Menu screen



For Versions 0.99 or earlier please <u>contact</u> OptiGene directly for the correct upgrade files. It is highly recommended to upgrade.

For Versions 1.00 and later go to the OptiGene <u>website</u> and check on the Support page if there is a newer version of firmware available.



Click on the link and download and save the zip file.

Open the zip file and extract the contents to a new folder.

The contents of the new folder will include this guide and the latest firmware and FPGA code.

If your Genie already has the latest FPGA code then you only need to upgrade the firmware code.

If you need to upgrade both the FPGA code and the firmware code then upgrade the FPGA code first, followed by the firmware code using the Genie<sup>®</sup> II software on your computer.

To install code updates, Connect your Genie<sup>®</sup> II to your computer, open the Genie<sup>®</sup> II software on your computer and choose software update from the options menu

🔛 G	enie		X			
Eile	Options	<u>W</u> indows				
	<u>S</u> oftw	are update	L .			
W	<u>T</u> ermir	nal	e			
	Buttor	าร				
	Empty	window				
	<u>R</u> esto	re default files				
	Is	othermal				
		Anneal				
	Other					
0.	2.0.3					

### Click on software update

🔛 Genie update	
Connect to	×
Instrument	Find
OK Cancel	
<	>
1	
	Cancel

Genie is detected and you click OK to accept



Navigate to the folder where you have extracted the downloaded zip file and choose the file to upload and click send

The file will be uploaded.

Genie® II will only restart after installation of a firmware file has completed.

It will not restart if an FPGA file has been uploaded.

The new FPGA code will show on the Main Menu upon a Genie<sup>®</sup> II restart.

# PC SOFTWARE

## WELCOME SCREEN

🖪 Genie 📃 🗖 🔀
<u>File Options Windows</u>
Welcome to Genie
Preheat
Isothermal
Anneal
Other
0.2.0.3

From this screen you are able to choose to start a new experiment or review data saved from previous runs.

If you wish to carry out an experiment make sure that Genie<sup>®</sup> II is connected to the computer and switched on.

To start a new isothermal protocol, simply click the Isothermal button.

## QUICK START BUTTONS

The Quick Start Buttons are the buttons on the front welcome screen. Normally they state 'Preheat', 'Isothermal', 'Anneal' and 'Other...' These buttons can be modified very easily, so frequently run experiments can be stored as a button and run right from the welcome screen.

Simply save your run file as you need it then simply go to <u>Options</u> on the welcome screen and click buttons.

Then type in the name you wish to call that run and tell the button where the run file is located.

The Welcome screen should then be updated with your new button.

## SETTING UP A RUN

### **PROFILE SCREEN**

In this screen you are able to modify all the experiment parameters

Notes Acco	we Wale 1	Profile Graphs	Raak classificatio	n Genotuner	Rup	
ASS	iys   weas	Profile   Graphs	Peak classificado	In Genocypes	Rull	
Enter inforr	nation here abo	ut the type of expe	eriment			
Experiment I	lame IsoThern	al				
Experiment I	lates					
File						
File % C:1Decur	nents and Settin	ins)Steve]Annicati	Inn Data)OntiGene10	Senie) Tsothermal, ex	nt	
File B C:\Docur	nents and Settin	gs\Steve\Applicati	ion Data\OptiGene\0	Senie\Isothermal.ex	pt	
File B C:\Docur	nents and Settin	gs\Steve\Applicati	ion Data\OptiGene\(	5enie\Isothermal.ex	pt	

Notes

- Assays
- Wells
- Profile
- Graphs
- Peaks Classification
- Genotypes

Simply click upon the tab in which you want to add information into. We recommend however that you work through the tabs in order Notes, Assays, Wells and Profile.

This will allow you to add all the information necessary before starting the run.

### NOTES TAB

Genie profile - I	xperiment 1	Crache Dask discrification	Construes )	Dup	
Assays	weis Frome		denocypes	Kun	
Enter information	here about the type	of experiment			
Experiment Name	Experiment 1				
Experiment Notes					
This is where yo	i can make notes rele	want to the experiment being p	performed		
This is where you	i can make notes rele	want to the experiment being p	performed		
File	and Settings(Steve)	wank to the experiment being p	ie/New.expt		

If you click the notes tab, this allows you to make any experimental notes relevant to the assay you are running.

### WELLS TAB

	Assays no	in line	nie   G	apins	Peak Liass	icadori Genocypes Ron		
8)	i wells 🚫 16 we	ds De	rine the h	ame and i	contents o	each weil and now it is displayed on the graphs.		
	Name	Assay	Colour	Point	Line	Note	Visible	
1	sample 1						<b>~</b>	
2	sample 2			11			<b>V</b>	
3	3			100			<b>~</b>	
4	4			- 616			<b>V</b>	
5	5			12			<b>V</b>	
6	6			- 616			<b>V</b>	
7	7			- 616			<b>V</b>	
8	8			10			<b>V</b>	

This tab allows you to input specific sample information.

Choose if you are running 8 or 16 wells (default is 8)

You have the ability to enter names, change plot colours, line styles and choose to make the sample visible on the display or not.

### **PROFILE TAB**

🖬 Genie profile - IsoThermal	
Notes Assays Wells Profile Graphs Peak classification Genotypes Run	
Basic Options Estimated run time 38:13 mmss Direbeat / Activation S 5 °C for 10 s Block Gradent 0 °C Bran rate 0.05	~ ~ ~ ~ ~ ~ ~
Load Save Cancel	Run

The Profile tab allows you to set the thermal profile that you wish to use and gives you an estimated run time for your desired profile.

Prel	heat	: / Ac	tiva	ation		
)	95	°⊂ f	or	÷)	10	s

Isothermal		
Temperature 🕣	65	°C
Duration 쉬	30:00	mm:ss
Block Gradient	0	°C

If you require activation for the enzyme then tick the Preheat/Activation checkbox. Now set the required temperature and time for the Preheat/Activation.

Make sure the Isothermal checkbox is ticked and then alter the temperature and time to suit your assay conditions.

Anneal		
Start Temperature 🕣	99	°C
End Temperature 🕣	80	°C
Ramp rate 🕣	0.1	°⊂/s

If you are carrying out an Anneal curve at the end of the reaction, then tick the checkbox for Anneal and alter the start and end temperature. This dialogue box shows how to set an annealing curve from high temperature to low temperature. The melt Tm peak will be a different temperature from an annealing Ta peak. This is normal.

### **BLOCK GRADIENT**

The Block Gradient facility enables a gradient to be set across the blocks. This is especially useful when setting up assays for optimisation of the correct temperature.

Isothermal		
Temperature 分	63.5	∘⊂
Duration 分	30:00	mm:ss
Block Gradient	7	∘⊂

To set a gradient.

Set centre temperature as  $63.5^{\circ}$ C with a gradient of 7°C.

That will set from  $60^{\circ}$ C in well 1 to  $67^{\circ}$ C in well 8.

### **OPTIONS DIALOGUE BOX**

If you click the options button



You will see the following screen, which allows changes to be made to the protocol parameters

Ramp rate	1 °C/s N	1easure period ()	15		
- Anneal	1 °C/s	Settling time	60	Measure period	0
Initial rate 🗍	1 43	Settling time 🕣	60	Measure period 🕣	0

#### **GRAPHS TAB**



The graphs tab allows the criteria for different graphs to be adjusted

Simply click on the tabs for each graph and alter settings, as you require.

For more information see Analysis and Graph Options Chapter

#### PEAK CLASSIFICATION TAB



This tab allows you to identify peaks for genotyping. Define and enter peaks into the table and the software will automatically label matching peaks.

### **GENOTYPE TAB**

9 Genie profile - IsoThermal	
Notes Assays Wells Profile Graphs Peak classification Genotypes Run	
This table defines which peaks must or must not be present for each genotype, and which can be ignored.	
Name Abbr	<b>•</b>
	~
<u>s</u>	>
Load Save	Cancel Run

The genotype tab allows the user to define peaks, which can be used for automated genotyping by the software

### **RUN DIALOGUE BOX**

🚾 Start Run		×
Instrument 싉	OptiGene:Genie I:GEN1-003	Find
Block	C A 🖲 B C Both	
Run Name	IsoThermal	
Data file	% IsoThermal_031.genie	
	OK Cancel	

When you click Run, you will see the start run dialogue appear. This allows the user to choose which Block you would like to run and also allows you to save the run file under a name of your choice. You can create a new folder or simply navigate to an existing folder. We advise that you only save files in folders/subfolders in the Genie<sup>®</sup> II folder under My Documents.

Check that you are running the assay on Block A or Block B or Both.

Symbols in the run name get converted to underscore in the file name

If you do not give a file name the File name is created from run name + unique number in the target directory.

### RUN TAB

Run Name 28.09.10_NDM_LAMP				
Started	Ended	Instrument	Operator	
28/09/2010 11:13:46	28/09/2010 11:52:36	OptiGene:Genie I:GEN1-003		
Run Notes				
RunFile				

The run tab is the experiment information screen. It displays the run name, profile running and run date. It allows you to input the operator name, make experimental notes.

## OBSERVING A RUN

### **PROFILE TAB**



When running, the profile screen allows you to observe the temperatures of the blocks and the heated lids plotted against time. It acts as a thermal record of the run.

### ISOTHERMAL TAB



When running, the Isothermal screen allows you to observe the fluorescence of each well plotted against time.

### **ISOTHERMAL RATIO TAB**



## Isothermal Ratio peak time is equivalent to the Ct or Cq in qPCR assays.

Our method identifies a consistent point within the exponential region without user intervention. This amplification ratio generates several measurements of amplification including time of crossing and relative measures of amplification efficiency and curve shape. This allows the ratio method to achieve highly reliable determination along with quantitative evaluation. Isothermal Ratio is unaffected by drifting fluorescent baseline, unnormalised baseline and odd jumps in the amplification plot due to bubbles in a tube etc. Genie®II software can detect the peaks and call them automatically.



Select the Peak detect option (None Simple or Full), check the default Peak criteria and click Okay.

The peak criteria allows the software to 'call the peak' in the results table.

### ANNEAL TAB



The anneal tab plots the fluorescence of each well versus temperature in a annealing curve

### ANNEAL DERIVATIVE TAB



The Anneal Derivative tab shows the derivative fluorescence of each well plotted against temperature, thereby giving the data as a peak.



If the Peak detect box is set on Simple or Full then the peaks will be displayed in the results table.

Check that the peak criteria are set.

### PEAKS TAB

le Isothermal	Isothe	rmal Ratio 🛛 Anneal	Anneal Deriva	ative Peaks	Results						
'eaks											Settings.
Graph	Well	Name	Peak Position	Value	Width	Height	d²Y/dX²	Relative	Class	A	
Isothermal Ratio	A1_	10E7 - KHV1+tRNA	8:13	0.05475	0:30	0.05522	+2.003E+5	1.000			
Isothermal Ratio	A2_	10E6 - KHV1+tRNA	9:25	0.05316	0:30	0.05514	-1.916E-5	1.000			
Isothermal Ratio	A3_	10E5 - KHV1+tRNA	10:48	0.04727	0:45	0.05011	-1.604E-5	1.000			
Isothermal Ratio	A4_	10E4 - KHV1+tRNA	12:19	0.04103	0:44	0.04480	-9.833E-6	1.000			Protok and
Isothermal Ratio	AS_	10E3 - KHV1+tRNA	15:42	0.02640	1:45	0.02946	-5.540E-6	1.000			rinsned
Isothermal Ratio	A6_	10E2 - KHV1+tRNA	22:12	0.02183	0:00	0.01726	-9.082E-6	1.000			
Anneal Derivative	A1_	10E7 - KHV1+tRNA	89.72	1.429E+4	0.20	1.400E+4	-1.967E+5	1.000			Extend
Anneal Derivative	A2_	10E6 - KHV1+tRNA	89.66	1.261E+4	0.20	1.233E+4	-1.723E+5	1.000			
Anneal Derivative	A3_	10E5 - KHV1+tRNA	89.55	1.322E+4	0.25	1.293E+4	-1.707E+5	1.000			
Anneal Derivative	A4_	10E4 - KHV1+tRNA	89.56	1.267E+4	0.25	1.235E+4	-1.664E+5	1.000			Advance
Anneal Derivative	AS_	10E3 - KHV1+tRNA	89.55	1.307E+4	0.25	1.279E+4	-1.661E+5	1.000			
Anneal Derivative	A6_	10E2 - KHV1+tRNA	89.55	1.317E+4	0.25	1.288E+4	-1.680E+5	1.000			
											Stop
										* 1	
-										4	

This page shows the results in a tabular format and displays the peaks for the different profiles used within the experiment, the position of peaks, the peak width and height.

### **RESULTS TAB**

Is	othermal Isothermal	Ratio Anneal	Annea	al Derivative	Peaks	Results					
sults											Settin
Vell	Name	Genotype	Abbr.	Peaks						A	
1_	10E7 - KHV1+tRNA	AA BB		8:13	89.72						
2_	10E6 - KHV1+tRNA	AA BB		9:25	89.66						
3_	10E5 - KHV1+tRNA	AA BB		10:48	89.55						
4_	10E4 - KHV1+tRNA	AA BB		12:19	89.56						10000
5_	10E3 - KHV1+tRNA	AA BB		15:42	89.55						Finishe
6_	10E2 - KHV1+tRNA	AA BB		22:12	89.55						
7_	10E1 - KHV1+tRNA	AA BB									Exte
8_	10E0 - KHV1+tRNA	AA BB									
-											
					-						Adva
								-			
											Sto
			-			-		-			
			-			-		-		_	
								-			
						_			 		
					-				 	_	
		-			-						
		-				-	-	-		-	
		-		-				-		Ψ.	
										P-	Expor

This page shows the results in a tabular format and displays the peaks and genotypes

## ANALYSIS AND GRAPH OPTIONS

### **GENIE®II GRAPH OPTIONS WINDOW**

Click the settings button and it displays the Genie<sup>®</sup> II Graph Options window. From this window several aspects of the graphical information can be modified.

There are four tabs on this page and each tab allows the user to check and modify settings appropriate for that tab i.e. Isothermal, Isothermal Ratio, Anneal & Anneal Derivative.

Isoti	ermal Isothermal Ratio Anneal Anneal Derivative
	Name Isothermal X axis () Time Phase () Isothermal Y axis () Fluorescence
	Normalisation V from 120 to 240 s Peak detect None Smoothing 0 points Peak criteria
	Fit width () 0 Min width () 0 Min height () 0
	Peak markers Peak labels

Normalisation allows all the fluorescent signals to be normalised/back grounded over a defined period of time.

Normalisation 🗹 from 🗍 120 to 🗍 240 5

Tick the normalisation checkbox and then set the time for normalisation from say 60 seconds to 240 seconds. Then click Okay.



The screenshot below shows data that has not been normalised; the fluorescent signals are different for each well and look untidy.



### With Normalisation

### PEAK DETECT OPTIONS

Isothermal Isothermal Ratio Anneal Anneal Derivative
Name Anneal Derivative X axis Temperature
Phase Anneal Y axis J -Derivative
Normalisation from () 0 to () 0 °⊂ Peak detect ()None Smoothing () 25 Points Peak criteria
Fit width 🗍 7 Min width 🗍 0.25 Min height 🌖 7000 Min ratio 🗍 0
Peak markers Peak labels

Isother	rmal Isothermal Ratio Anneal Anneal Derivative
Pt No Pe	Name     Anneal Derivative     X axis     Temperature       hase     Anneal     Y axis     Derivative       ormalisation     from     0     to     0     °C       eak detect     Simple     Smoothing     25     points
F M	Fit width () 7 Min width () 0.25 Min height () 7000 Min ratio () 0 Peak markers Peak labels

Isothermal Isothermal Ratio Anneal Anneal Derivative
Name     Anneal     Derivative     X axis     Temperature       Phase     Anneal     Y axis     Derivative       Normalisation     from     0     to     0     °C       Peak detect     Full     Smoothing     25     points       Peak riteria     7     Min width     0.25     Min height     7000
Min ratio 🗧 0
Peak markers Peak labels

None: no peaks are detected

Simple: The software looks at the peak and assigns the peak to the highest plotted point. Multiple peaks can be detected.

Full: The software curve fits and assigns the centre of a peak and can report multiple peaks. Also does sub-point interpolation.

## **OPENING PREVIOUS RUN FILES**

To view previously run data simply go to File, Log and open the file you require.

Files are saved by default in a Genie® folder in My Documents. The run file is encrypted.



The requested file will be loaded back into the software and you will be able to analyse the data as required.

### ADDITIONAL FEATURES



**Extend**: If you are running an isothermal assay and find more time is required press the extend button and the run will extend by 10 minutes. If the button is pressed 3 times it will extend the run by 3 x 10 minutes. A box will pop up every time Extend is pressed and the Run profile will be updated to show the new time.

**Advance**: if you are happy that the amplification has completed and you would like to go to the annealing curve, you can press the advance button and it will stop the isothermal phase and go onto the next stage specified

**Stop**: Stop will stop the run in progress, <u>DO NOT</u> press unless you want the run to stop

**Export**: when you have finished the analysis, you can export the results into a txt file. Simply name and save the file. This allows you to import the results into Excel or other spread sheet programmes.

### **EXPORT FUNCTION**

The Export function allows that data from each graph can be individually exported as a tabdelimited .txt file that can be imported into a spreadsheet for additional end-user manipulation.



The example below shows that if you export the Isothermal graph it will export time versus fluorescence for each well.

Time	Fluorescence	Time	Fluorescence	Time	Fluorescence	Time
80.6	-238.3	80.6	-194.8	80.6	-340.2	80.6
95.6	-213.3	95.6	-148.8	95.6	-275.2	95.6
110.6	-160.3	110.6	-91.83	110.6	-198.2	110.6
125.6	-127.3	125.6	-75.83	125.6	-158.2	125.6

The data exported is the data from that graph; if changes are made to the graph for analysis purposes then the graph will need to be re-exported for the changes to be reported.

### FOR EACH GRAPH

Profile Graph	Export time versus temperature for each well plus heated lid i.e. 10 temperature readings four times every second throughout the run.			
Isothermal graph	Export time versus fluorescence for each well.			
Isothermal Ratio graph	Export time versus fluorescence ratio for each well.			
Anneal graph	Export temperature versus fluorescence for each well.			
Anneal Derivative graph	Export temperature versus fluorescence derivative for each well.			
Peaks graph	Export the Well, Name, Peak Position, Value, Width, Weight, d <sup>2</sup> Y/dX <sup>2</sup> , Relative and Class for each well.			
Results graph	Export Well, Name, Genotype, Abbreviation, Peaks for each well.			

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