MyGo | Pro

PERFORMANCE MADE EASY

USER GUIDE

WELCOME

Thank you for choosing the MyGo Pro.

The MyGo Pro provides unmatched performance in a convenient format. Novel Full Spectrum Optics deliver 120 optical channels of fluorescence data from every tube for exceptional multiplex PCR. High-performance Peltier elements, combined with solid silver blocks, provide both speed and proven world-leading thermal uniformity.
Performance made easy…

CONTENT

This user guide will teach you what you need to know to start running your MyGo Pro. It describes everything from connecting your instrument to the network to data analysis for your qPCR experiments.

Contents include...

SETUP

Learn how to install your MyGo Pro - from setting it up on your workbench, to configuring the instrument and installing the software.

TIPS

Learn some useful tips for using your instrument.

EXPERIMENT

Learn the basics of setting up an experiment on your MyGo Pro.

ANALYSIS

Learn how to easily analyse your results.

EXPORT

Learn how to export your data for use with other software.

MAINTENANCE

Learn how to look after your MyGo Pro.

TROUBLESHOOTING

If you ever have a problem with your instrument, learn how to troubleshoot it here.

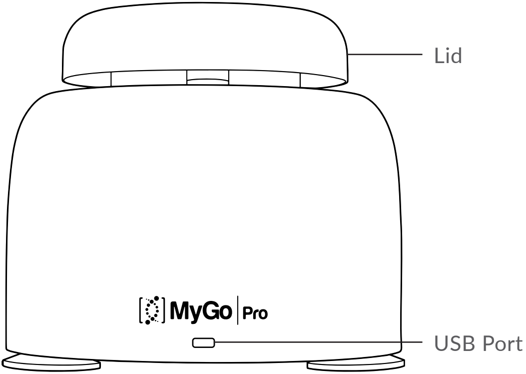
SETUP

INSTALLING YOUR MYGO PRO

You should have the following items in the MyGo Pro flight case:

1. MYGO PRO QPCR INSTRUMENT
2. MYGO PRO USER GUIDE
3. POWER SUPPLY UNIT AND MAINS CABLE
4. ETHERNET CABLE
5. USB DRIVE

Please keep the flight case and outer box the MyGo Pro came in, in case you need to protect your MyGo Pro during storage or shipment in future.



LID

The lid ensures that tubes are seated correctly in the wells, provides optical isolation, includes a heated lid to reduce condensation, and prevents dust falling into empty wells.

USB PORT

Insert the USB drive here to run an experiment from the USB drive.

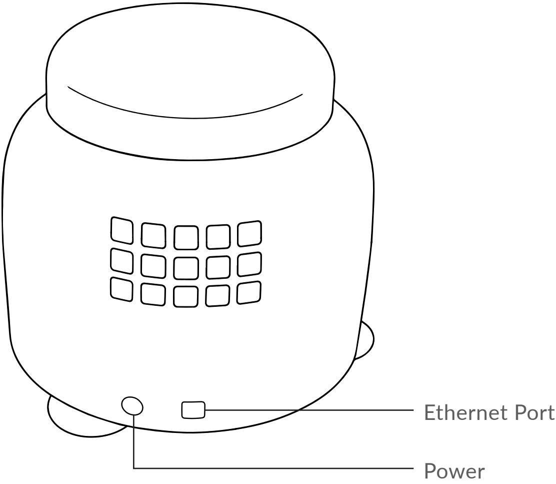
DISPLAY LEDs

The MyGo Pro uses display LEDs to help you understand what the instrument is doing. Here is a summary of these display LEDs.

Blue	Initialising
Green	Idle
Red	Lid open - Please close the lid
Yellow	Tubes loaded
Blue	Running, low temperature
Pink	Running, medium temperature
Red	Running, high temperature
Cyan	Experiment complete
Red-Flashing	Fault detected

MYGO PRO CONNECTIONS

The MyGo Pro has three connections. One in the front and two in back as shown below:



POWER

This is to connect your MyGo Pro to the provided power supply unit.

ETHERNET PORT

This is for connecting your MyGo Pro to your LAN or computer.

POWERING ON YOUR MYGO PRO

Place the MyGo Pro on your lab bench, and then connect AC power to the power supply unit. Your instrument will now turn on after a few seconds. The display LEDs will light up blue, and then turn green if a lid is closed or red if not. Your MyGo Pro is ready to run. The MyGo Pro uses a 3-pin IEC mains connector. If you are not using an earthed supply then you must provide an additional earth connection.

CONNECTING YOUR MYGO PRO

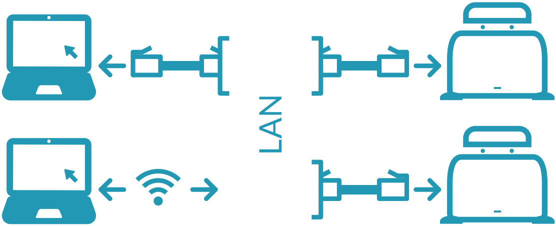
TO A NETWORK, PC OR LAPTOP

Connect one end of the Ethernet cable to the instrument and the other end to a LAN port, or directly to your computer. Once your MyGo Pro is connected to a LAN, you can connect to your instrument via Wi-Fi if your network supports it.

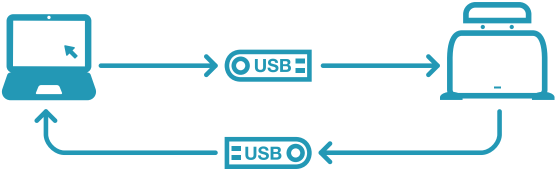
A summary of these connections is shown on the following page...

MyGo Pro Connection Modes

1. Direct network connection



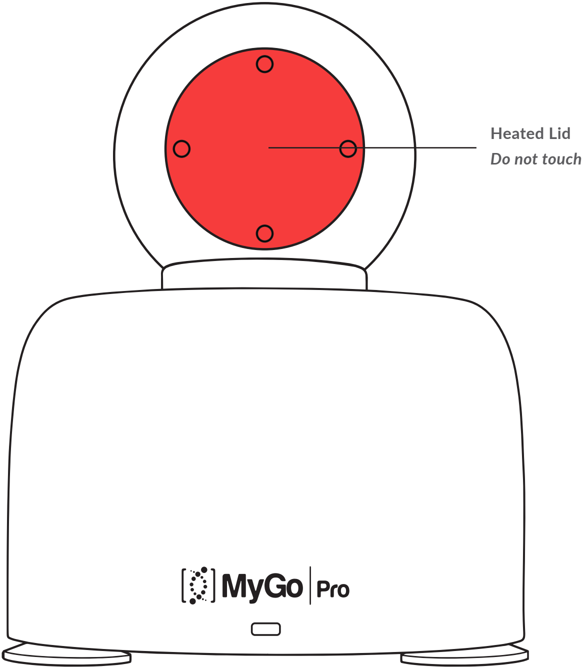
2. Local area network connection



3. USB drive connection

MYGO PRO HEATED LID

The MyGo Pro heated lid will get hot. Please do not touch it. The heated lid will be preheated to 105°C if user activity is detected. This enables your run to start as soon as possible. After 5 minutes of inactivity the heated lid will be turned off to conserve energy.



INSTALLING YOUR MYGO PRO SOFTWARE

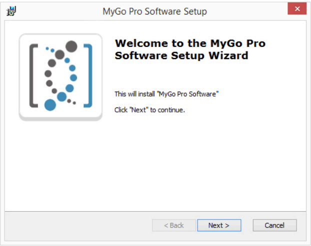
Your MyGo USB drive contains software for Windows, Mac OS X, and Linux operating systems. Please open the software file matching your chosen operating system. The latest version of the software can also be downloaded from our website mygopcr.com.

WINDOWS

Double-click on the Windows installer and follow the on-screen instructions to install your MyGo Pro software on Windows.

MAC OS X

Double-click on the disk image and follow the on-screen instructions to install your MyGo Pro software on your Mac PC.


MyGo Pro Software Setup

Welcome to the MyGo Pro Software Setup Wizard

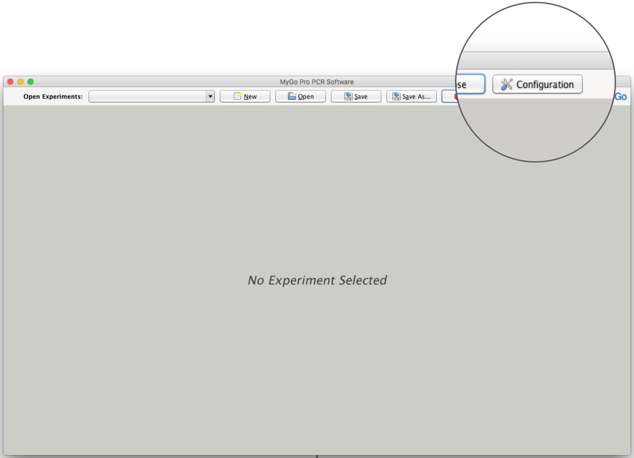
This will install "MyGo Pro Software"

Click "Next" to continue.


MyGo Pro Software

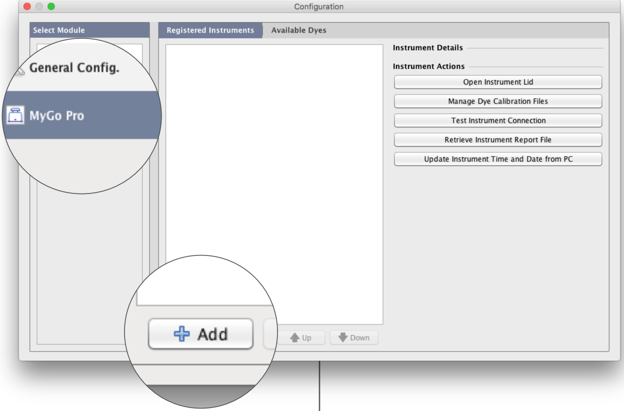
CONFIGURING YOUR MYGO PRO

With your MyGo Pro software open, and your MyGo Pro connected to the network, please connect to your MyGo Pro Instrument.

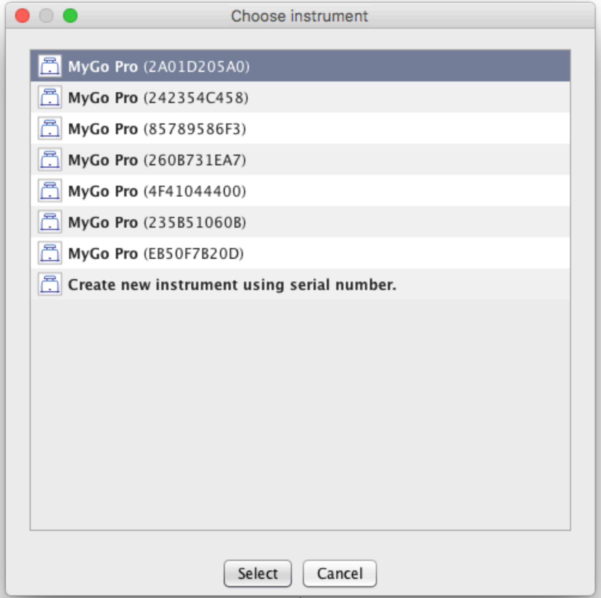


Please select Configuration

Please select MyGo Pro



To add your new MyGo Pro select the **Add** button. You will now be presented with a list of available instruments.



MyGo Pro (2A01D205A0)
MyGo Pro (242354C458)
MyGo Pro (85789586F3)
MyGo Pro (260B731EA7)
MyGo Pro (4F41044400)
MyGo Pro (235B51060B)
MyGo Pro (EB50F7B20D)
Create new instrument using serial number.

Double-click on the instrument you wish to connect to, or press **Select** with the instrument selected. The instrument will now be added to the list of **Registered Instruments** for use in the software.

TIPS

Here are some great tips to keep in mind whilst running your MyGo instrument.

1. LID GETS WARM
2. DO NOT LEAVE THE LID OPEN
3. SPIN YOUR TUBES
4. REMOVE ALL BUBBLES
5. KEEP YOUR LAB CLEAN
6. KEEP YOUR INSTRUMENT CLEAN
7. PC SETTINGS

LID GETS WARM

The MyGo Pro lid can get warm during operation, this is completely normal.

DO NOT LEAVE THE LID OPEN

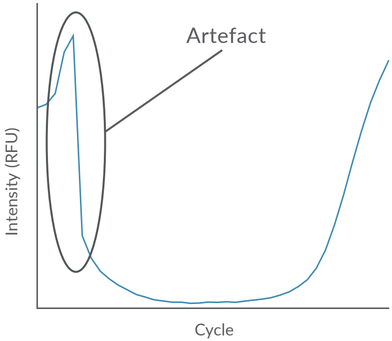
If the lid is open dust may fall into the wells and affect the performance of your instrument.

SPIN YOUR TUBES

This should ensure that all contents are at the bottom of the tubes, and there are no bubbles present. Reaction mixtures which are viscous, or contain high levels of detergents, will require stronger centrifugation to remove bubbles. Please use sufficient g-force to ensure that no bubbles are present.

REMOVE ALL BUBBLES

Bubbles can cause optical artefacts as shown in the graph below. Ensure that no bubbles are present in reaction volumes.



KEEP YOUR LAB CLEAN

Please keep your work space clean including all lab equipment like surfaces, pipettes, and tube racks. This will keep the instrument clean and help maintain good results.

KEEP YOUR INSTRUMENT CLEAN

We recommend a routine cleaning of your instrument. To do so follow the cleaning guide in the **Maintenance section.**

PC SETTINGS

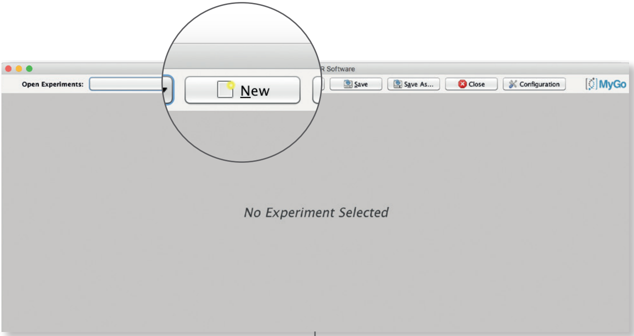
Please disable all power saving settings e.g. sleep and hibernate.

EXPERIMENT

This section will teach you everything you need to know to get started with MyGo Pro experiments. You will learn how to create, save, open, and close experiments. You will also learn how to set up an experiment, including thermal profile, sample information, and optical settings. Finally we will show you how to run your new experiment from the software, and from the USB drive provided.

CREATE A NEW EXPERIMENT

Below is the MyGo Pro home screen.

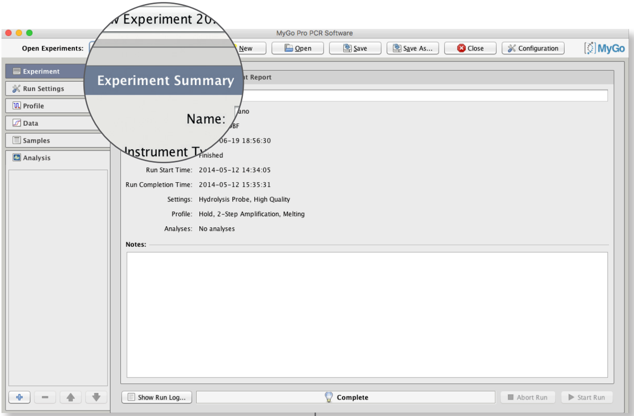


No Experiment Selected

Click **New** to create a new experiment.

EXPERIMENT SUMMARY

Once you have created a new experiment the **Experiment Summary** will be displayed as shown below:



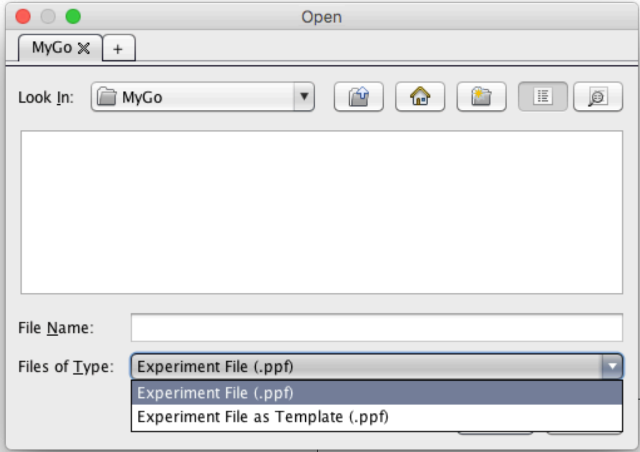
ano
BF
06-19 18:56:30
Finished
Run Start Time: 2014-05-12 14:34:05
Run Completion Time: 2014-05-12 15:35:31
Settings: Hydrolysis Probe, High Quality
Profile: Hold, 2-Step Amplification, Melting
Analyses: No analyses

By default the experiment name will be "**New Experiment**" with a date and time stamp, which can be edited.

The summary will give you information about the instrument you are running, the settings chosen, and any useful notes you choose to add about your experiment.

OPEN AN EXPERIMENT

By selecting **Open** you can open an experiment as shown below:



FILES OF TYPE

The **Files of Type** drop down menu gives you the option to open an experiment normally, or as a template that can be used for a new run.

EXPERIMENT FILE

An experiment file will contain all the data and analyses, but can not be re-run.

To open as an **Experiment File** select the first option shown on the opposite page.

EXPERIMENT FILE AS TEMPLATE

Opening an experiment file as a template allows you to use the same profile, samples, settings, and analyses to re-run a previous experiment, generating new data. When an experiment file is opened as a template, it has the same:

1. **Temperature profile.**
2. **Optical settings.**
3. **Samples and targets.**
4. **Analyses, including settings.**

If you wish to open an **Experiment File** as **Template** select the second option.

USB RUN

To open a USB run navigate to the USB location using the **Open** dialog window shown to the left. Select **Experiment File** in the **Files of Type** drop down menu, and then double click on the USB run.

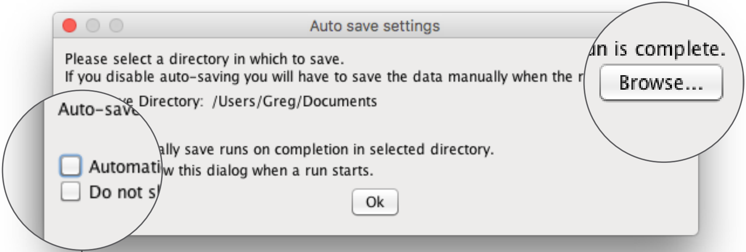
SAVING AN EXPERIMENT

Once an experiment has completed it can either be automatically saved to a pre-set location on your computer or manually saved by you. The experiment must be saved before the software is closed to ensure that data is not lost.

AUTOMATIC SAVING

When starting a run you will be prompted with the window displayed below.

To automatically save your data when the run has finished, firstly click **Browse** and choose the location to save your run to.



Please select a directory in which to save.
If you disable auto-saving you will have to save the data manually when the r
un is complete.
Auto-save Directory: /Users/Greg/Documents
☐ Automati ally save runs on completion in selected directory.
☐ Do not s w this dialog when a run starts.

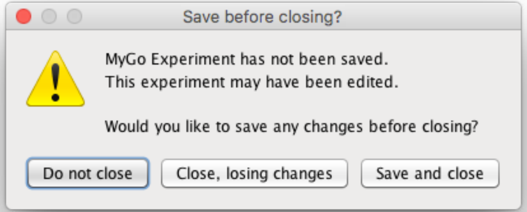
Make sure the first checkbox is selected and your experiments will be automatically saved. To prevent the software from showing this dialog again, select the second checkbox. You can change these settings later by clicking **Configuration** and selecting **General Config.**

MANUAL SAVING

Once your run has finished you can save your experiment manually by clicking **Save** or **Save As**. The first time an experiment is saved, you will be prompted to select a location and filename to use. After this you can click **Save** to use the same location, overwriting the previous version, or **Save As** to choose a new location.

CLOSING AN EXPERIMENT

To close an experiment select **Close**. If the experiment has not been saved the following dialog box will appear:



MyGo Experiment has not been saved.
This experiment may have been edited.

Would you like to save any changes before closing?

SETTING UP A PROFILE

In this section we will cover Hold Times, Programs and setting up a temperature profile.

HOLD TIMES

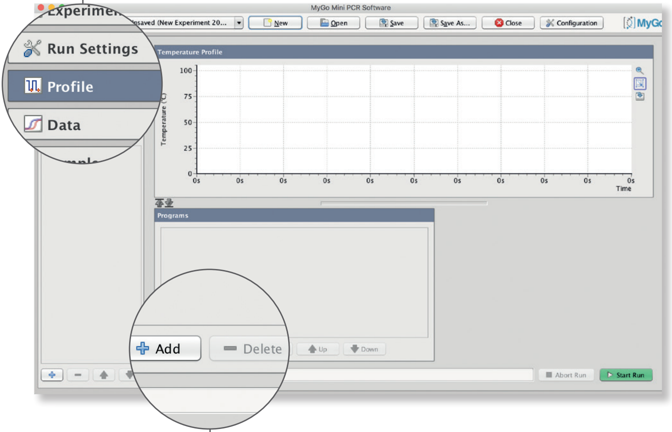
During thermal cycling protocols hold times should be chosen to allow for the following: thermal equilibration of reaction volumes; completion of biochemical reactions, for example full length strand extension; and optical data acquisition.

Typically, optical data is acquired at the same temperature that strand extension occurs. By default this time is set to 30s, However, this time can be reduced to a minimum of 17s, to save time, or increased to more than 30s in order to increase PCR efficiency and optical sensitivity.

For other hold times during cycling protocols, by default times are set to 10s. However, this time can be reduced to a minimum of 3s, to save time, or increased to more than 10s in order to increase PCR efficiency.

A thermal profile is required to perform an experiment.

Select the profile tab as shown below:

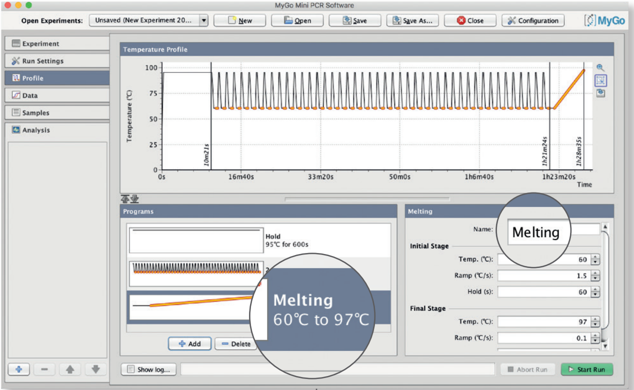


PROGRAMS

To add a program to your experiment click **Add** in the **Programs** pane. Programs can be deleted by selecting them in the list then clicking **Delete**. The program order can also be altered by selecting the program to move then clicking the **Up** and **Down** arrows.

TEMPERATURE PROFILE

Once programs have been added to the experiment they will appear in the **Temperature Profile** pane in the order they are present in the **Programs** pane as shown below:



Open Experiments: Unsaved (New Experiment 20...

Hold
95°C for 600s

Melting
60°C to 97°C

Name: Melting

Initial Stage
Temp. (°C): 60
Ramp (°C/s): 1.5
Hold (s): 60

Final Stage
Temp. (°C): 97
Ramp (°C/s): 0.1

PROGRAM SETTINGS

To alter the settings of a particular program you should first select it, then refer to the right hand pane. In the example above the **Melting** program is selected and can be edited in the right hand pane.

SAMPLES

You will now learn how to set up samples and targets which can be defined as follows:

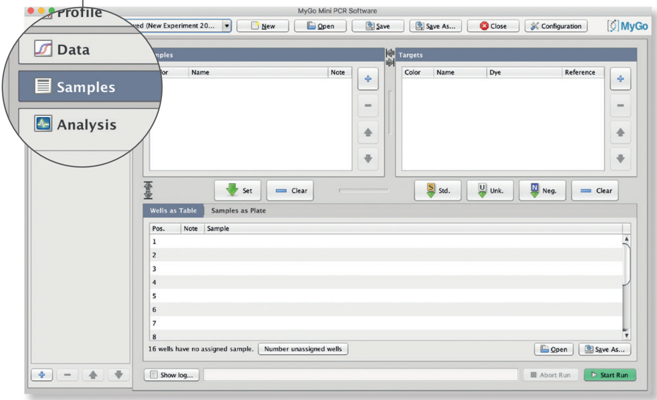
SAMPLES

A description of the specimen being analysed e.g. “Mouse #5”

TARGETS

A molecular target e.g. “x174”, detected with a specified fluorescent reporter e.g. “FAM”

Samples or targets do not need to be defined before starting a run. They can be added to an experiment, whilst it is running, or once it has finished. With a new experiment created, please select the **Samples** tab as shown below:



16 wells have no assigned sample.

ADDING SAMPLES AND TARGETS

Samples and targets can be added and removed from the experiment by selecting **+** and **-** in the **Samples** and **Targets** panes.

The order of samples and targets can be altered by clicking the **Up** and **Down** arrows in the **Samples** and **Targets** panes.

ASSIGNING SAMPLES TO WELLS

Assign wells by selecting them and clicking **Set**. Samples can also be unassigned by selecting wells you wish to un-assign and clicking **Clear**.

ASSIGNING TARGETS TO WELLS

Targets can be assigned to wells as a Standard, Unknown or Negative by clicking **Std.**, **Unk.**, or **Neg.**, respectively. Assignments can be cleared by clicking **Clear** with the targets and wells you wish to clear selected. For more information about different types of standard controls please contact technical support.

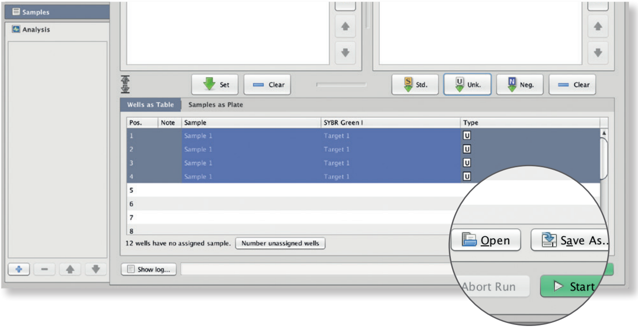
DYE COMPATIBILITY

The MyGo Pro comes pre-calibrated for over 20 different fluorescent labels, and the software enables users to use any dye with an emission maximum between 510nm and 750nm. A list of the available dyes on the MyGo Pro can be viewed below. The **Auto Dye File Generation** software analysis module enables you to calibrate your MyGo Pro for additional fluorophores if required.

Factory Calibrated Dyes	SYBR Green I, ResoLight, FAM, VIC, HEX, Yellow 555, Red 610, TexasRed, Cy5, CAL 540, CAL 560, CAL 590, CAL 610, CAL 635, JOE, Pulsar 650, Quasar 570, Quasar 670, Quasar 705, ROX, TAMRA, TET

SAVING AND OPENING A SAMPLE SETUP

If you wish to save or open sample and target information setup you can select **Save As.**, or **Open**, respectively.



Pos.	Note	Sample	SYBR Green I	Type
1		Sample 1	Target 1	U
2		Sample 1	Target 1	U
3		Sample 1	Target 1	U
4		Sample 1	Target 1	U
5				
6				
7				
8				

12 wells have no assigned sample.

Sample and target setups can be saved in the following formats:

CSV	Editable .csv files.
PSD	A proprietary locked file format.
RDML	The Real-time PCR Data Markup Language (RDML) is a structured and universal data standard for exchanging quantitative PCR (qPCR) data.

RUN SETTINGS

The MyGo Pro run settings tab is where you will determine the optical settings, view your dye calibrations and access the advanced settings.

OPTICS SETTINGS

The MyGo Pro will aquire optical data during the hold times. The longer the hold times are the better your optical data will be. Different reagents can require different optical settings.

INTERCALATING DYES

These dyes, for example SYBR Green I, ResoLight and EvaGreen, are bright reagents and require a shorter integration time.

SEQUENCE-SPECIFIC PROBES

These dyes, for example FAM, VIC and HEX, are commonly used for TaqMan genotyping or multiplex experiments. Hydrolysis probes are not as bright as Intercalating dyes and therefore require a longer integration time.

INTEGRATION TIME

Integration Time is the exposure time, in seconds, of the optical sensor inside the MyGo instrument during thermal cycling. Brighter reagents require shorter integration times than dimmer reagents.

DYE CALIBRATIONS

Here is where you can review the current dyes present in your experiment. You have the following options when working with dyes in your MyGo Pro software:

OPEN

Open dye files from a file to be added to this experiment.

SAVE

Save a dye file from your experiment to a file on your computer. These can then be used on multiple computers and MyGo Pro instruments.

REMOVE

Remove dye files from this experiment. Factory calibrated dye files can not be removed.

RENAME

Rename your dye files to your liking.

UPDATE FROM DEVICE

Upload dye files from your MyGo Pro to your new experiment.

ADVANCED SETTINGS

Here, if necessary, you can tailor optical settings to the characteristics of your assay.

You can adjust Amplification Integration Time to increase, or decrease the sensitivity of the MyGo Pro fluorescence detection system. Longer integration times will provide improved signal to noise with dim fluorescent reporters. Short integration times will reduce the risk of the fluorescence detection system being saturated by very bright fluorescent reporters.

For your convenience, at the end of every run, your MyGo Pro will record a Recommended Amplification Integration Time in the Run Log. Runs where every well contains a strong positive sample will enable your MyGo Pro to estimate the most accurate Recommended Integration Time for your assay.

OPTIMISE MELT ACQUISITIONS

By default your MyGo Pro will use automatically optimised integration time settings during melting analysis. If you want your MyGo Pro to use the Amplification Integration Time during both amplification and melting analysis, deselect this tick box.

STARTING A RUN FROM THE SOFTWARE

To start a run from the MyGo Pro software select **Start Run**. You will then be presented with the auto save options (unless you have chosen not to be prompted) and then be asked to choose an instrument from the list of **Registered Instruments**. Select an instrument and press **Select** to begin the run.

STARTING A RUN FROM A USB DRIVE

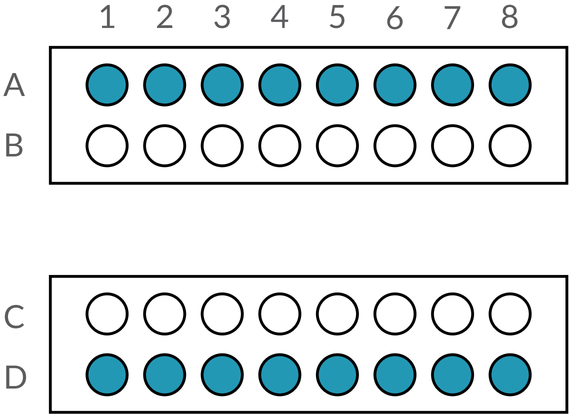
To start a run using a USB drive select **Start Run**. Then, when you are asked to choose an instrument, select **Start run from USB**. You will then be prompted to find the location of your USB drive. Once selected press **Save** and safely remove your USB drive as normal. The USB drive can now be placed into the instrument and your experiment will start automatically.

LABELLING YOUR STRIPS

You can write on the lids of your stip caps. **Please do not write on the sides of your strip tubes.** Ink can transfer from the sides of tubes to the walls of the wells, and this can affect the performance of your instrument.

LOADING STRIPS INTO YOUR MYGO PRO

In order to ensure that the heated lid is balanced, please make sure that the mount contains at least a strip in rows A and D (as shown in blue below) or single tubes in wells A1, A8, D1 and D8. These positions can be filled with tubes containing reagents, or empty tubes.



ANALYSIS

This section will teach you about different types of analysis available. These can be grouped into the following groups. For information about more analysis options please contact technical support.

QUANTIFICATION

Determine accurate quantities of template or relative expression levels of genes using **Absolute Quantification** or **Relative Quantification.**

GENOTYPING

Use TaqMan probes or High Resolution Melting to perform SNP genotyping using **Automatic Endpoint Genotyping** or **Automatic High Resolution Melt.**

MELTING

Automatically determine the melting temperature of your amplicons using **Automatic Tm Calling.**

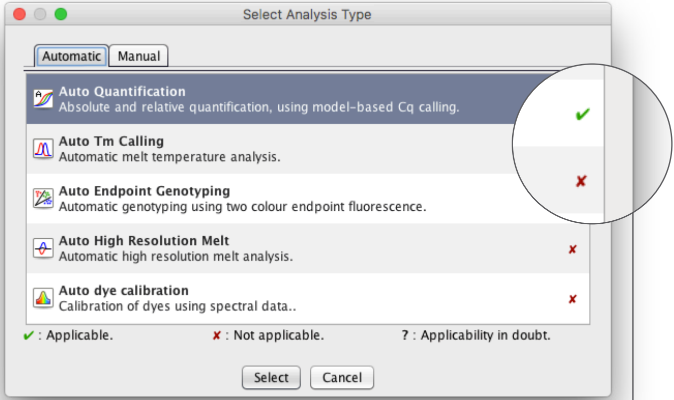
RUN FUSION

Combine data from multiple runs on your MyGo Pro for analysis together.

SELECT ANALYSIS TYPE

Once your experiment has completed you can add an analysis type by selecting the “+” button in the bottom left of the software window.

Analysis types are separated into **Automatic** and **Manual** sections. **Automatic** analysis types use automated data processing to provide accurate results with minimal user intervention. **Manual** analysis types enable users to apply traditional analytical methods.



Auto Quantification ✓
Absolute and relative quantification, using model-based Cq calling.

Auto Tm Calling ✗
Automatic melt temperature analysis.

Auto Endpoint Genotyping
Automatic genotyping using two colour endpoint fluorescence.

Auto High Resolution Melt ✗
Automatic high resolution melt analysis.

Auto dye calibration ✗
Calibration of dyes using spectral data..

✓ : Applicable. ✗ : Not applicable. ? : Applicability in doubt.

Ticks and crosses indicate which analyses can be applied to the data in your experiment.

AUTOMATIC QUANTIFICATION

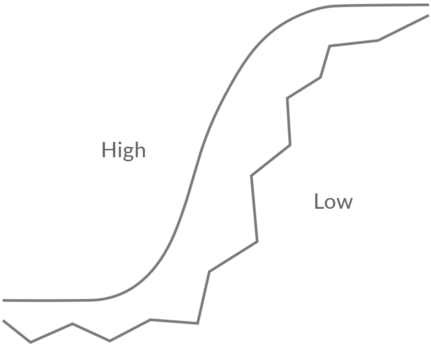
POSITIVE / NEGATIVE CALLING

The first step in amplification analysis is to determine which targets have produced a positive amplification curve, and which amplifications have produced a negative amplification curve.

For every amplification curve the software calculates three metrics, these metrics are used to determine which amplifications are positive. These metrics are:

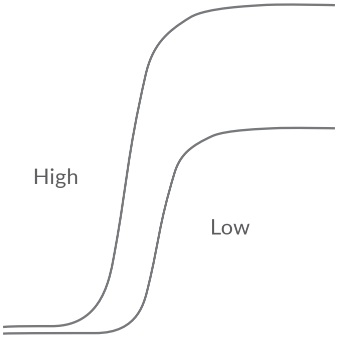
QUALITY

Quality is a measure of how clean an amplification appears. This value will tend to be higher for amplifications with a clear exponential phase and steady baseline, and lower for noisier or flatter curves.



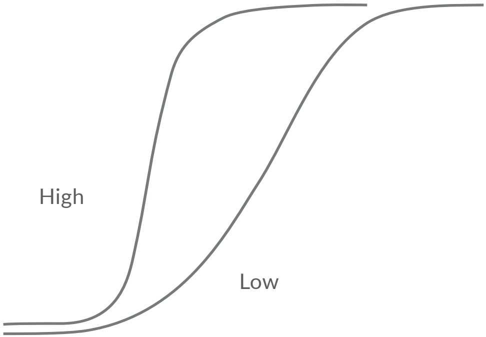
INTENSITY

Intensity is a measure of the size of the exponential phase of the amplification. Note that this may be smaller for amplifications with a negative baseline drift.



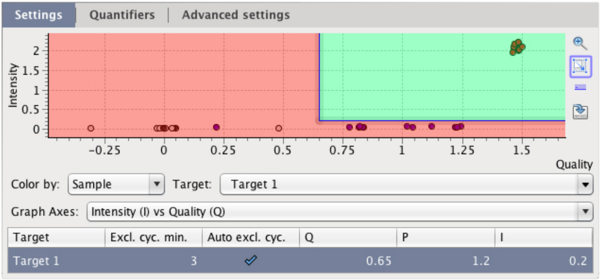
PERFORMANCE

Performance is a measure related to the observed efficiency of amplification at later cycles. This value will tend to be lower for inhibited or out-competed reactions, and higher for efficient reactions.



SETTINGS

The software uses a combination of Quality (Q), Performance (P), and Intensity (I) thresholds to determine if an amplification is positive (green zone) or negative (red zone). All values (Q, P and I) must be above the relevant threshold for an amplification to be called positive. The Settings tab enables you to review metrics for amplifications and set appropriate thresholds.



Color by: Sample

Target: Target 1

Graph Axes: Intensity (I) vs Quality (Q)

Target	Excl. cyc. min.	Auto excl. cyc.	Q	P	I
Target 1	3	✓	0.65	1.2	0.2

The dots represent individual amplification curves in your experiment. All dots that are within the top right of the graph are called positive and will be subject to normalization determined in **Advanced Settings.**

To switch between "Intensity vs Quality" and "Performance vs Quality" select the Graph Axes drop down menu. All 3 thresholds are used for calling even if they are not currently displayed on the graph.

ARTEFACT FILTERING

Biochemical and physical factors can cause fluorescence levels to change during a run. These are often observed during early cycles. Filtering out these artefacts can improve the accuracy of amplification analysis. Two settings enable you to control the process of filtering out such artefacts.

EXCL. CYC. MIN.

The software will exclude at least this number of cycles from data before performing analysis.

AUTO EXCL. CYC.

By default the software will automatically determine which cycles at the beginning of a run exhibit artefacts, and exclude this number of cycles from data before performing analysis. This feature can be disabled by deselecting this option.

NORMALISATION OPTIONS

Here you can choose the way your data is normalised before display in the amplification graph. By default Background normalisation is applied to assist data visualisation when troubleshooting challenging assays. Full normalisation enables the discrimination of subtle differences in amplification kinetics.

You can choose between the following modes:

	Normalisation Mode		
	None	Background	Full
Background Correction	✗	✓	✓
Normalisation	✗	✗	✓

BACKGROUND CORRECTION

Background fluorescence, including drift, is estimated and removed. There are many potential sources of background fluorescence, e.g. emissions from unquenched probes.

NORMALISATION

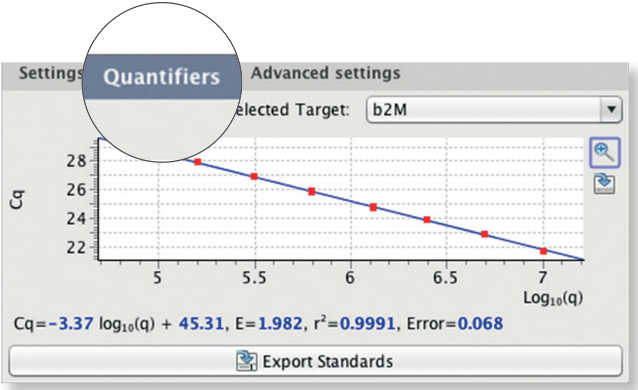
Positive amplifications are rescaled to account for intensity variation between wells. Uncorrected intensity can be affected by a number of factors, e.g. the optical properties of disposables. Normalised amplifications are rescaled to a nominal value of 1 relative fluorescent unit, with exact endpoint values a function of curve shape.

RESULTS AS TABLE

The results of amplification analysis are presented in the Results as Table view.

QUANTIFIERS

If you have assigned standards with different known quantifiers to your experiment, for example a dilution series, you will be able to see your reaction efficiency by selecting **Quantifiers.** Here you will be shown the relationship between Cq and input template quantity, plus a variety of statistics derived from the standards.



elected Target: b2M

$Cq=-3.37 \log_{10}(q) + 45.31$, $E=1.982$, $r^2=0.9991$, Error=0.068

RELATIVE QUANTIFICATION

If you are performing relative quantification analysis then select **Relative Quantification** at the top of the **Auto Quant**. pane.

MULTIPLEXING

The MyGo Pro supports multiplexing with a broad variety of dyes.

FOR BEST RESULTS:

- Ensure that there is no competition between the different PCR in the multiplex reaction.
- Ensure that probes have low background fluorescence, for example by using dual-quenched probes.
- Ensure that probe signal strength is balanced, by using an appropriate concentration of primers and probes.
- Make sure to select the correct dye for each Target.
- Generate assay specific dye files if necessary.

TIPS

Spectral properties of fluorophores can vary as a result of many factors, including stereochemistry, sequence context and buffer chemistry. If you observe spectral crosstalk, generate assay specific dye files to compensate for these effects.

DYE FILE GENERATION

DYE EXPERIMENT

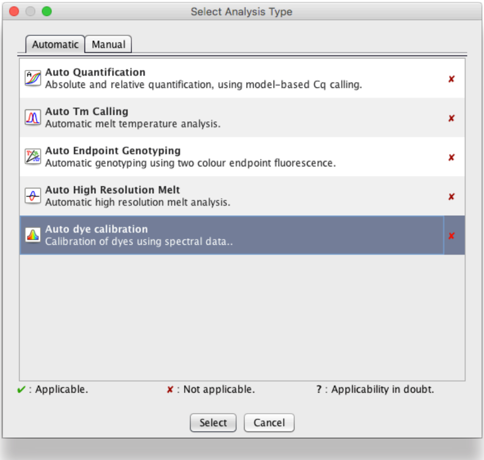
Dye file generation allows you to calibrate your MyGo Pro for new dyes that are not included in the factory-calibrated list. This calibration is based on a PCR amplification using the relevant dye or labelled probe.

DYE CALIBRATION RUN

You will need to perform a real time PCR run in order to generate a dye file. Every well of your MyGo Pro should contain the same reagents. These reagents should produce a single PCR product, and a strong signal for the dye that you are calibrating. Create a sample named after the dye that you are calibrating, and assign that sample to all wells.

AUTO DYE CALIBRATION

Select the **Auto Dye Calibration** option in the Select Analysis Type window. The **Auto Dye Calibration** analysis module will analyse changes in fluorescence during the dye calibration run to create a new dye file.



Auto Quantification
Absolute and relative quantification, using model-based Cq calling. ✘

Auto Tm Calling
Automatic melt temperature analysis. ✘

Auto Endpoint Genotyping
Automatic genotyping using two colour endpoint fluorescence. ✘

Auto High Resolution Melt
Automatic high resolution melt analysis. ✘

Auto dye calibration
Calibration of dyes using spectral data.. ✘

✔ : Applicable. ✘ : Not applicable. ? : Applicability in doubt.

DYE DATA

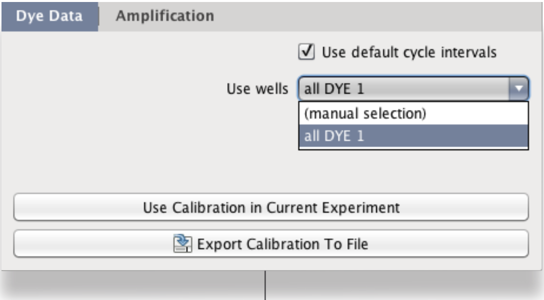
Select this tab to view the wells that will be used in the calibration.

AMPLIFICATION

Select this tab to view the cycles that will be used in the calibration.

EXPORT CALIBRATION

Select the **Dye Data** tab and you can choose to export the calibration to a file, or, use it in your current experiment.



Select the Use Wells drop down menu and you will see the list of samples present in the experiment. Select the sample you wish to create a dye from.

USE CALIBRATION IN CURRENT EXPERIMENT

By selecting this option your new dye file will appear in the target list in this experiment. This option will not save the dye file to your computer.

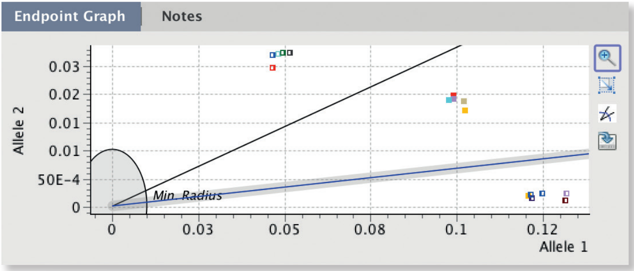
EXPORT CALIBRATION TO FILE

Alternatively you can save this new dye file to your Mac or PC.

GENOTYPING

AUTOMATIC ENDPOINT GENOTYPING

TaqMan genotyping experiments can be analysed automatically using **Automatic Endpoint Genotyping.** Genotypes of samples are determined by the ratio of endpoint fluorescence between two TaqMan probes. Thresholds for the fluorescence ratios are generated automatically as shown below:



AUTOMATIC HIGH RESOLUTION MELTING

Automatic HRM can be performed by using the **Automatic HRM** module as shown on the following page:

DIFFERENCE, NORMALIZED AND MELT

Once opened the analysis module will automatically determine optimised parameters and generate a set of **Difference**, **Normalized** and **Melt** graphs.

CLUSTERING

The difference curves will then be converted into a clustering graph showing clusters of different genotypes.

GENOTYPES

The software will automatically determine **Inferred genotypes** based on the clustering graph. You can define your control Genotypes using the **Genotypes** pane.

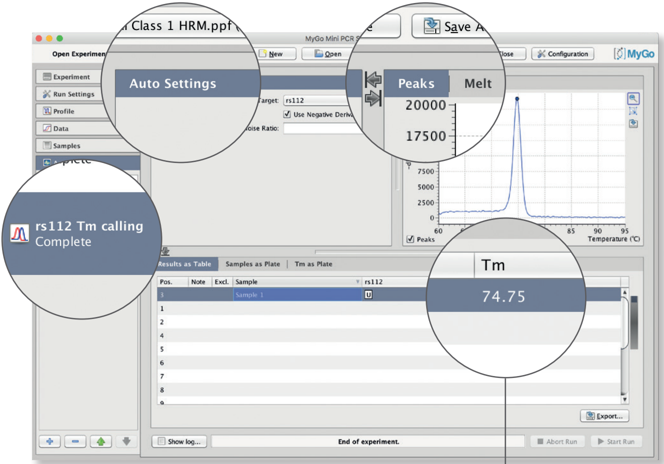
Pos.	Excl.	Sample	Assigned genotype	Inferred genotype
1		Heterozygote		● Cluster 1
2		Homozygote 2		■ Cluster 2
3		Homozygote 1		◆ Cluster 3
4		Heterozygote		● Cluster 1
5		Homozygote 2		■ Cluster 2
6		Homozygote 1		◆ Cluster 3
7		Heterozygote		● Cluster 1
8		Homozygote 2		■ Cluster 2
9		Homozygote 1		◆ Cluster 3

End of experiment.

MELTING

AUTOMATIC TM CALLING

Add an **Automatic Tm Calling** analyses to identify and characterise melting peaks. You can see the results below.



arget: rs112

☑ Use Negative Deriv

oise Ratio:

rs112 Tm calling
Complete

Pos.	Note	Excl.	Sample	rs112	Tm
3			Sample 1	U	74.75
1					
2					
4					
5					
6					
7					
8					

End of experiment.

Tm values will be presented in the table.

AUTO SETTINGS

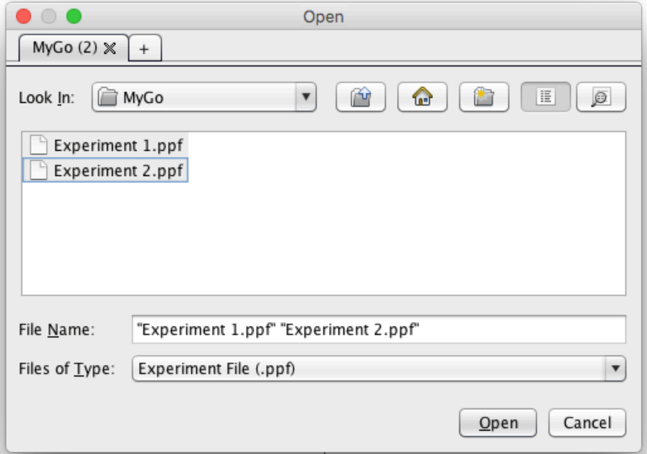
The user has the option to change the target if the experiment contains more than one target. The signal-to-noise ratio setting determines how big a peak should be before it is called. Increasing this value will increase specificity of peak detection. Decreasing this value will increase the sensitivity of peak detection.

PEAKS AND MELTS

The **Peaks** pane will show you the melt peaks with melting point indicators. The **Melts** pane will show you the melting curves of the same reactions.

RUN FUSION

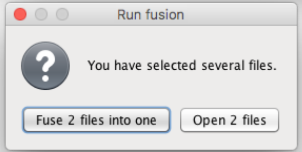
Run Fusion enables you to combine data from multiple runs from your MyGo Pro together for analysis. To fuse multiple experiments together hold down **cmd** on a Mac or **Ctrl** on a PC and select the files you wish to fuse from the open dialog window as shown below.



Look In: MyGo

File Name: "Experiment 1.ppf" "Experiment 2.ppf"

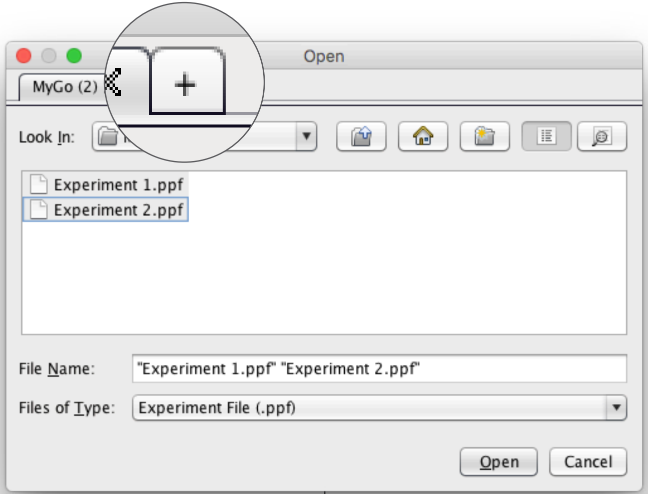
Files of Type: Experiment File (.ppf)



You have selected several files.

Select **Open** and then from the next dialog select **Fuse 2 files into one.** You can also open files as separate experiments using **Open 2 Files**.

If you are fusing experiments from multiple file locations you must select the first experiment and then select the “+” from the top left of the open file dialogue window as shown below.



File Name: "Experiment 1.ppf" "Experiment 2.ppf"

Files of Type: Experiment File (.ppf)

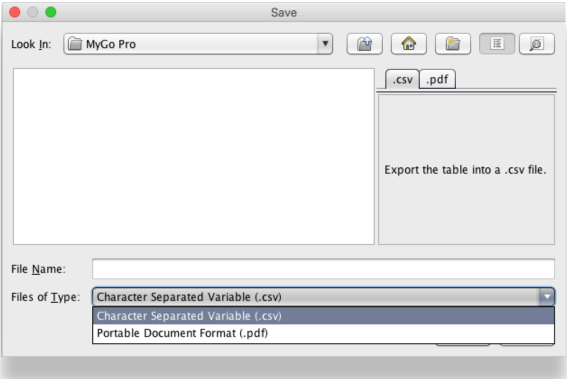
Now hold **cmd/Ctrl** and select the next experiment. Select **Open** to fuse your experiments together.

EXPORT

When you have finished analysing your data in the MyGo software, you can export the results in a variety of ways, from raw data to user defined custom reports. This section will take you through the steps you need in order to do this.

TABLE EXPORT

From panes showing results in tabular format you can export data using the **Export** button.



Look In: MyGo Pro

Export the table into a .csv file.

File Name:

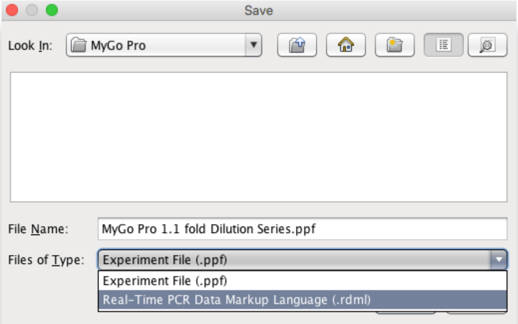
Files of Type: Character Separated Variable (.csv)

To export data click the **Export** button, shown on the previous page. Data can be exported in the following formats:

CSV	This is an editable data file that can be opened in many spreadsheet applications.
PDF	This file format is suitable for archiving, printing and presentations, but cannot be edited.

RDML EXPORT

The software is able to export Cq values and amplification curves from **Auto Quantification** and melt curves from **Automatic Tm Calling** in RDML format. To do this select **Save as**, as if you were saving your experiment. Now from the **Files of Type** drop down menu, select **Real-Time PCR Data Markup Language** as shown below:



File Name: MyGo Pro 1.1 fold Dilution Series.ppf

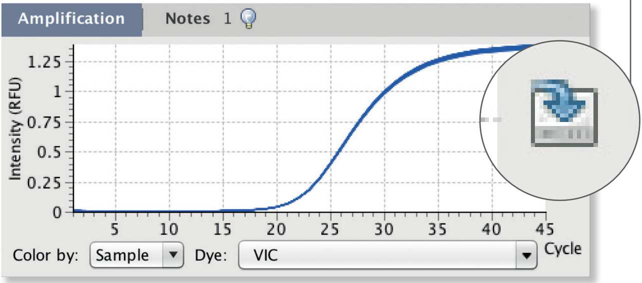
Files of Type: Experiment File (.ppf)

Experiment File (.ppf)

Real-Time PCR Data Markup Language (.rdml)

GRAPH EXPORT

From panes showing results as graphs, such as amplification and melt curve data can be exported by selecting the button from the below example.

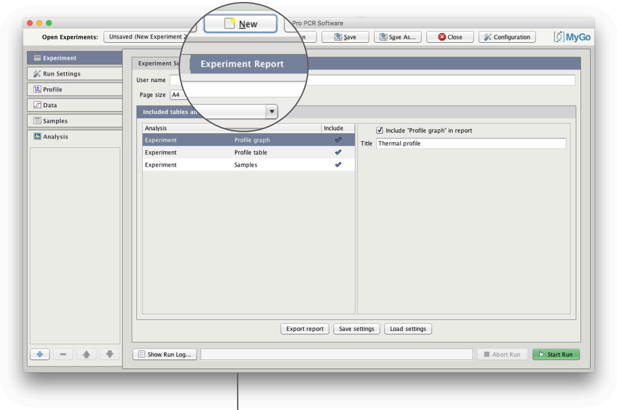


The user can export information as bitmap images (.PNG), Scalable Vector Graphics (.SVG), Character-Separated Variable (.CSV), or Portable Document Format (.PDF).

PNG	This is a bitmap image.
SVG	A Scalable Vector Graphic is an XML-vector based graphic that can be used where a high resolution scalable graph is required, for example for papers or web design.
CSV	This contains information about the graph you are exporting in spreadsheet format.
PDF	This contains the graph you are exporting in the Portable Document Format. The graph is in vector format for higher quality.

REPORT GENERATION

Customisable reports can be generated from your experiment by selecting **Experiment Report** under the **Experiment** tab. This provides you with the ability to choose which parts of the data to include in a report. For example, items such as thermal profiles, sample information and various analysis results, e.g. Tm values or quantities can be included, or excluded from reports.



User name

Page size A4

Analysis		Include
Experiment	Profile graph	✓
Experiment	Profile table	✓
Experiment	Samples	✓

☑ Include "Profile graph" in report

Title Thermal profile

Please select Experiment Report.

MAINTENANCE

This section will help you take care of your MyGo Pro. It covers the following areas:

1. CLEANING
2. DISPOSABLES
3. ENVIRONMENTAL CONDITIONS
4. DISASSEMBLY

CLEANING

DAY-TO-DAY

For day-to-day cleaning wipe the external surface of your instrument with a damp, soft, lint-free cloth. Then dry your instrument with another soft, lint-free cloth.

Notes: Avoid abrasive cloths, towels, paper towels, and similar items that might cause damage. Before cleaning your instrument unplug all external power sources, devices, and cables. Do not get moisture into any openings.

CLEANING WELLS

It is important to keep the wells of your instrument clean. Automated background subtraction processes mean that low levels of fluorescence contamination in the wells of your instrument will not affect system performance. Dirty wells however, can result in lower fluorescence readings, and can affect heat transfer from wells to tubes. Regular cleaning of your instrument's wells will help prevent dirty wells affecting the performance of your instrument. The MyGo Cleaning Kit has been designed to enable you to clean the wells of your instrument.

**For details of the MyGo Cleaning Kit please visit:
http://www.mygopcr.com/cleaning-kit/**

Notes: Do not use any other method or materials to clean the wells of your instrument. Doing so may damage your instrument.

DECONTAMINATION

If your instrument needs decontaminating please follow the instructions contained in the MyGo Decontamination Guide.

For details of the MyGo Decontamination Guide please visit:
http://www.mygopcr.com/decontamination/

Please note that for health and safety reasons you must print and complete a physical copy of the decontamination form, and include this with any instrument or lid that is returned. The form must be sent with the instrument, and we can not accept electronic versions of the form. Failure to comply with these guidelines will result in a charge for decontamination.

DISPOSABLES

We recommend the use of MyGo disposables for optimal results. If you wish to use third party disposables please note the following:

A. Physical dimensions should be the same as the MyGo disposables to ensure that they fit into your instrument, without damaging it.

B. Caps should seal tubes effectively in order to prevent variability caused by evaporation, and to minimise the risk of contamination with PCR products.

C. Disposables should have reproducible wall thicknesses, which are thin enough to ensure rapid temperature equilibration, but thick enough to avoid breakages.

In many cases third party disposables will have inferior thermal and optical characteristics, which will reduce the quality of results obtained from the system.

ENVIRONMENTAL CONDITIONS

YOUR WORK SPACE

Your MyGo Pro should be placed on a surface that is flat, dry, and not subject to draughts. Do not install your MyGo Pro instrument directly in the flow of air from an air conditioner or fan. Do not install your MyGo Pro instrument in a dusty environment.

PREVENTING CONTAMINATION

Prevent contamination by wearing gloves, using clean tube racks, and filter tips. Make sure tubes are sealed, PCR product is disposed of, and leaks are cleaned immediately.

ENVIRONMENTAL OPERATING CONDITIONS

Humidity
MAX: 80% at +32°C
MIN: 30% at +15 to +32°C

Temperature
+15°C to +32°C

Pressure
0 to 2000 MAMSL
80 to 106Kpa

ENVIRONMENTAL STORING/ TRANSPORTING/PACKING CONDITIONS

Humidity
10% to 95%
No condensation

Temperature
-20°C to +60°C

Pressure
0 to 3000 MAMSL
70 to 106Kpa

DISASSEMBLY

Please note that your MyGo Pro contains no user-serviceable components inside. Any disassembly of your MyGo Pro instrument will void all warranties.

TROUBLESHOOTING

This section will help you troubleshoot your MyGo Pro if you think something is wrong.

FAQ

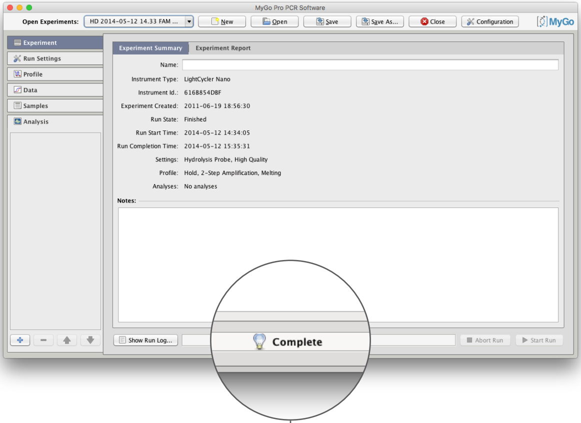
Here are some frequently asked questions, and the answers.

How do I create a template?	All experiments can be used as a template. Click "Open" and then using the "file type" selection in the file dialog to select the "Template" option, then select the file to open as a template.
What is the minimum hold time for thermal cycling?	For holds during thermal cycling with no optical acquisitions the minimum hold time is 3s. For holds during thermal cycling with optical acquisitions the minimum hold time is 17s.
Can I leave my MyGo Pro powered on overnight?	Yes. It is safe to leave your MyGo Pro overnight, it will enter a low power standby mode.

Where is the threshold for determining Cq values in Auto Quant?	Modern methods of determining Cq values are not based on simple thresholds. Modern methods of Cq determination are model based. Auto Quant fits a model of a PCR amplification to the fluorescence data observed. This model fit then enables the estimation of a number of important parameters including Cq values.
Why do some of my amplification curves drop down before rising in Auto Quant?	Correct background removal in Auto Quant relies on correct qualitative calling. Check that appropriate thresholds are set so that positive amplifications are called positive.
Why is my Cy5 reaction not working?	Cy5 is a relatively dim reporter, you may need to increase Integration Time to improve signal to noise with Cy5 labelled hydrolysis probes.

ERROR MESSAGES

Your MyGo Pro will let you know when something is wrong by displaying an error. Most errors are reported as messages in the Status Bar in the MyGo software. Some errors are reported by the instrument display LEDs.



Open Experiments: HD 2014-05-12 14.33 FAM ...

Name:
Instrument Type: LightCycler Nano
Instrument Id.: 616B854DBF
Experiment Created: 2011-06-19 18:56:30
Run State: Finished
Run Start Time: 2014-05-12 14:34:05
Run Completion Time: 2014-05-12 15:35:31
Settings: Hydrolysis Probe, High Quality
Profile: Hold, 2-Step Amplification, Melting
Analyses: No analyses

Status Bar.

If you encounter an error, please make sure that the instrument is running in a lab within the specified environmental conditions, tubes have been loaded correctly, the lid has been fitted correctly, and all cables are attached correctly and securely. If the error still occurs, you may need to contact technical support with the following actions described below.

ERROR MESSAGES - DISPLAYED IN STATUS BAR

Message	Action
Saturation Warning.	Please use the log button to view the detailed message - this will tell you which wells saw a saturated reading. To correct, please reduce the integration time setting, or reduce the concentration of dye or probes.
Run Prepare Error (followed by one of the following messages) **a) No tubes loaded.** **b) Instrument has had a hardware error, and is in failsafe mode.**	Please follows the instructions in the error message and continue as normal.

c) Instrument is not yet ready to run. **d) Instrument has finished a run and is waiting for tubes to be removed.** **e) Open lid, remove tubes and then add new ones.**	
FAILURE	This message will be followed by a short code. Please contact Technical Support.
Instrument connection too slow.	May be reported at the start of a run. Please check that your network is properly configured and is operating at 100Mb/s or more.
Network error.	Please check that your network is properly configured and is operating at 100Mb/s or more. Always leave your laptop or PC turned on throughout the run, and do not use any sleep, power-save or hibernate function. Do not close laptop lid. Alternatively, perform the run using a USB drive.

ERRORS REPORTED BY THE MYGO PRO DISPLAY LEDS

Some errors are reported by the instrument display LEDs flashing red. If your instrument display LEDs are flashing red, please contact technical support.

ERROR LOG FILES

In the unlikely event that you encounter a problem the software can produce log files. These files help to diagnose the problem.

SOFTWARE ERRORS

For software issues go to **Configuration** > **About** and select **Save Software Logs to File**.

HARDWARE ERRORS

For Hardware faults go to **Configuration** > **MyGo Pro** and select **Retrieve Instrument Report Files**. Once exported, please make sure you send the error file, the serial number, and the experiment file that contained the error to Technical support. It is OK to send experiments that have not completed and/or were aborted.

NOTES

MyGo | Pro

www.mygopcr.com

Version 003 (EN)