PRIMER
DESIGN

genesig® q32 Software Guide Overview

The genesig q32 Real-Time PCR Instrument provides fast results with genesig kits

The genesig q32 provides unmatched performance in a convenient format. High-performance Peltier elements, combined with solid silver blocks, provide both speed and proven world-leading thermal uniformity.

This user guide will teach you what you need to know to start running your genesig q32.

It describes everything from connecting your instrument to the network to data analysis for your qPCR experiments.

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Content

This user guide will teach you what you need to know to start running your genesig q32. 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 genesig q32 - from setting it up on your workbench, to configuring the instrument and installing the software.

Tips

Learn some useful tips when using your instrument.

Experiment

Learn the basics of setting up an experiment on your genesig q32.

Export

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

Maintenance

Learn how to look after your genesig q32.

Troubleshooting

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

Setup

Installing your genesig q32

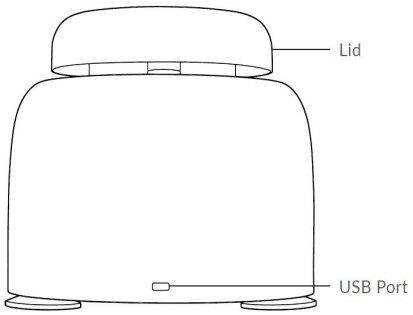
You should have the following items in your genesig q32 flight case:

1. genesig q32 Real-Time PCR Instrument

2. Power supply unit and mains cable

3. Ethernet cable

4. USB drive



Please keep the flight case and outer box the genesig q32 came in, in case you need to protect your genesig q32 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.

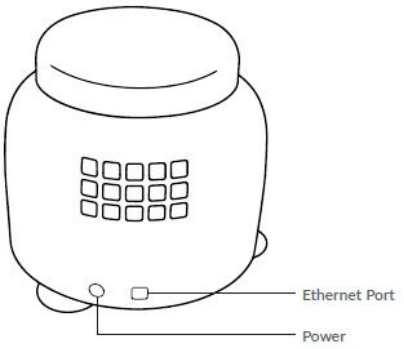
Display LEDs

The genesig q32 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

genesig q32 connections

The genesig q32 has three connections. One in the front and two in back as shown.



Power

This is to connect your genesig q32 to the provided power supply unit.

Ethernet port

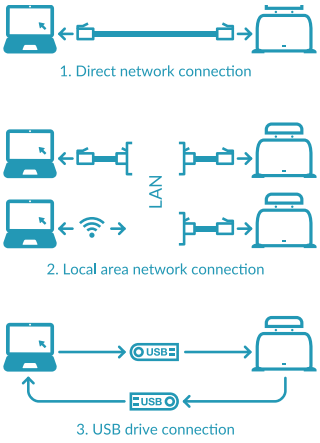
This is for connecting your genesig q32 to your LAN or computer.

Powering on your genesig q32

Place the genesig q32 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 genesig q32 is ready to run. The genesig q32 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 genesig q32

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 genesig q32 is connected to a LAN, you can connect to your instrument via Wi-Fi if your network supports it.



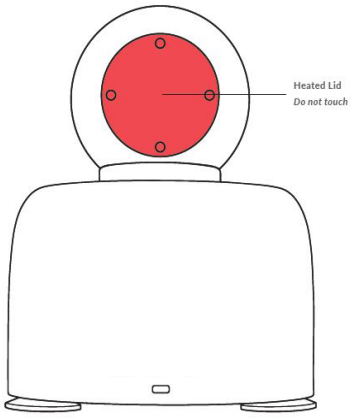
1. Direct network connection

2. Local area network connection

3. USB drive connection

genesig q32 heated lid

The genesig q32 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 genesig q32 software

Your genesig q32 USB drive contains software for Windows, and Mac OS X 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 Primerdesign.co.uk.

Windows

Double-click on the Windows installer and follow the on-screen instructions to install your genesig q32 software on Windows.

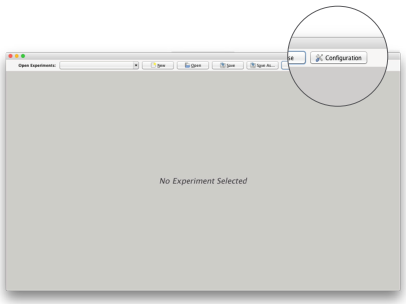
Mac OS X

Double-click on the disk image and follow the on-screen instructions to install your genesig q32 software on your Mac PC.

Configuring your q32

With your genesig q32 software open, and your q32 connected to the network, please connect to your genesig q32 instrument.

To add your new genesig q32, select configuration. This will produce a pop-up screen, at the left-hand side of this pop-up, select genesig q32. There will now be an “Add” button available at the bottom of the pop-up screen, select this “Add” option. You will now be presented with a list of available instruments.



Tips

Here are some great tips to keep in mind whilst running your genesig q32 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 genesig q32 lid can get warm during operation, this is completely normal.

Do not leave the lid open

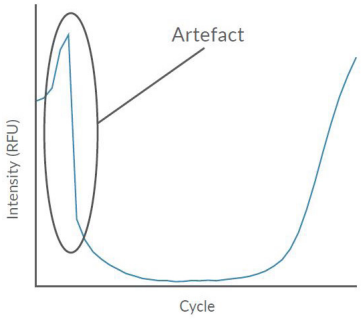
If the lid is open dust may fall into the wells and affect the performance of your instrument.

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. 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.

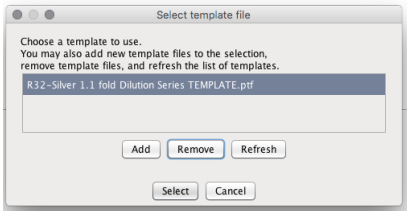
PC Settings

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 genesig q32. 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.

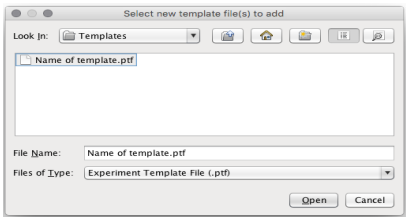
New button



Clicking the 'New' button will display a selection box showing available templates.

To load a template, either double-click it, or select it then click "Select" at the bottom of the dialog. You may also click "Cancel" to exit without using a template.

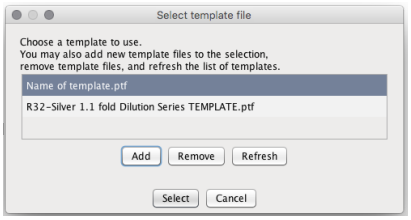
Clicking "Add" will display a file dialog (see below) - use this to navigate to any new ".ptf" files you would like to use.



To the right you can see an example "Name of template" template window. The genesig q32 software comes with pre-created templates for you to open and use. Select the template you wish to use and click "Open".

This will return you to the template selection dialog, with the new template added to the list.

To remove a template you are no longer using, select it in the list and click Remove.



Templates are stored in a ".pecan/var_templates" directory in the your home directory, in a sub-directory named after the genesig q32.

Any valid .ptf files for the genesig q32 will be available for use as templates. If you add new files manually (e.g. using the file browser), click Refresh to add them to the dialog.

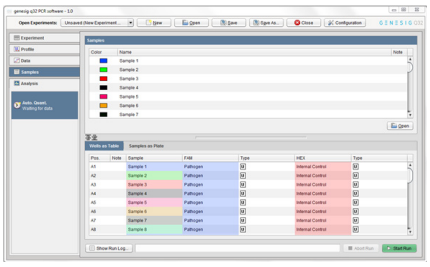
Samples

You will now learn how to set up samples and targets. The approach slightly differs depending on whether you are using a raw template or a preloaded template to run your new experiment.

- Raw templates: useful for qualitative detection or where quantification will be performed manually from Cq values
- Preloaded templates: useful for automated copy number quantification when using a genesig Pathogen Detection Kit

Wells do not need to be labelled before starting a run. This can be performed whilst an experiment is running, or once it has finished.

Raw templates

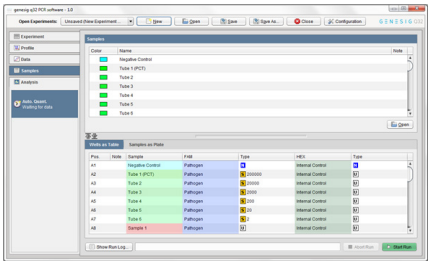


Raw template files come with all wells unlabelled:

- FAM and HEX channels are already active in each well
- Just label each well with the name of the sample and target to be assigned to that well (e.g. "Mouse #5 HIV1" or "Positive Control HIV1")

Preloaded templates

Preloaded templates come with wells A1 – A7 preloaded with a negative control and serial dilution standards for one assay, all other wells are unlabelled:

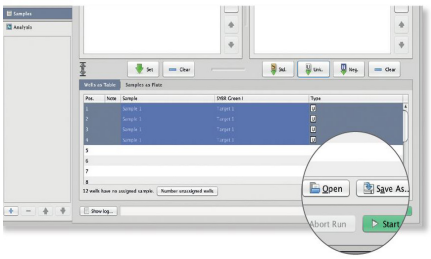


- FAM and HEX channels are already active in each well
- Standards represent a tenfold serial dilution from 200000 to 2 copies per µl
- Just label each well with the name of the sample and target to be assigned to that well (e.g. “Mouse #5 HIV1”)
- Results include efficiency calculations and automated copy number results for the samples
- Please load the correct tubes into each well or your data will not be analysed appropriately

If you wish for be provided with a customised Preloaded Template setup (e.g. 2 separate standard curves for 2 different assays), please contact your Primerdesign representative.

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.



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.

Starting a run from the software

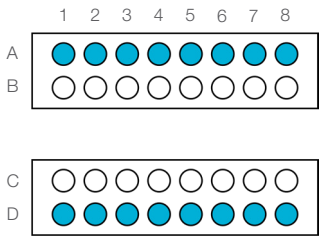
To start a run from the genesig q32 software select Start Run. You will then be presented with the auto save options (unless you have chosen not to be prompted) and then be asked to choose an instrument from the list of Registered Instruments. Select an instrument and press Select to begin the run.

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.

Loading strips into your genesig q32

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.



Using genesig Easy Real-Time PCR Kits on the genesig q32

Running the test

Before using a genesig Easy kit on the q16, please ensure you are using the correct template file for your experiment:

- genesig Easy Target detection assays template file: q32-Easy-Pathogen-DNA 2.0.pft; q32-Easy-Pathogen-RNA 2.0.pft
- genesig Easy Speciation assays template file: q32-Easy-Speciation.pft
- genesig Easy AnimalFINDER template file:q32-AnimalFINDER.pft

Place the tubes into the correct positions in your q32 as defined by the template file, this may include positioning of empty tubes to ensure that the q32 lid is balanced. The run can then be started.

Interpreting results

Before interpreting sample results, it is necessary to verify the positive and negative controls. If the controls do not fulfil the following criteria the testing needs to be repeated.

- genesig Easy target detection assays: negative control must be flat or present with a FAM Cq > 35 and the positive control must present with a FAM Cq between 14 and 22 (inclusive).
- genesig Easy speciation assays: negative control must be flat or present with a FAM Cq > 35 and the positive control must present with a FAM Cq < 31.
- genesig Easy AnimalFINDER assays: negative control must be flat or present with a FAM Cq > 35 and the positive control must present with a FAM Cq between 14 and 22 (inclusive).

Use the appropriate matrix below to analyse your samples:

genesig Easy Target detection and AnimalFINDER sample anlysis

Sample	Internal control	Negative control	Interpretation
+	+*	-	**POSITIVE RESULT**
-	+*	-	**NEGATIVE RESULT**
+ / -	-	+ / -	**SAMPLE PREPARATION FAILED**
+	+*	>35	†
+ / -	+ / -	≤35	**EXPERIMENT FAILED** Due to test contamination

genesig Easy speciation sample analysis

Sample	Universal target	Negative control	Interpretation
+	+	-	**POSITIVE RESULT**
-	+	-	**NEGATIVE RESULT**
+	-	+ / -	**POSITIVE RESULT - CAUTION, LOW LEVEL OF UNIVERSAL TARGET DNA**
-	-	+ / -	**LOW LEVEL OF UNIVERSAL TARGET DNA**
+	+	>35	†
+ / -	+ / -	≤35	**EXPERIMENT FAILED** Due to test contamination

*The internal control is expected to produce a HEX Cq < 32 in your samples. An internal control Cq > 32 is strong evidence of a sample preparation failure, however be aware that high concentrations of your FAM target can delay the internal control amplification. As a rule, if the FAM target produces a Cq < 30, the internal control signal does not need to be analysed.

†In the event of a negative control amplification Cq > 35, a sample can still be analysed. However, a sample must produce a Cq that is at least 5 Cqs earlier than the negative control to be deemed positive. If this is not achieved, the sample should be reported as inconclusive.

Calculating quantitative results

Only calculate results from samples which achieve a 'POSITIVE RESULT' designation from the matrices above:

genesig Easy target detection and AnimalFINDER

Calculate copy numbers using the following formula:

Copy number=$2^{-(\text{Sample Cq - Positive Control Cq})} \times 1{,}000{,}000$

genesig Easy speciation calculations

First calculate the delta Cqs:

- **Speciation Target ΔCq** = Speciation Target Sample Cq – Speciation Target Positive Control Cq
- **Universal Target ΔCq** = Universal Target Sample Cq – Universal Target Positive Control Cq

Secondly calculate the percentage using the following formula:

Speciation %=$2^{-(\text{Speciation Target } \Delta Cq - \text{Universal Target } \Delta Cq)} \times 100$

If you are not comfortable using the formula, please contact your Primerdesign representative who can provide an applet to automate speciation calculations.

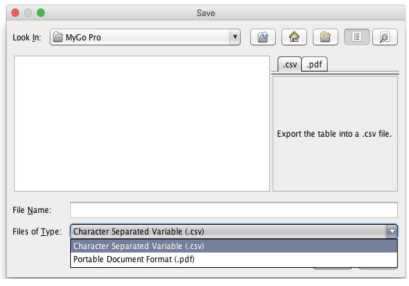
Export

When you have finished analysing your data in the genesig q32 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.

To export data click the Export button, 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.

Maintenance

Here are some great tips to keep in mind whilst running your genesig q32 instrument.

1. Cleaning
2. Disposables
3. Environmental conditions

Cleaning

Day-to-day

For day-today 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. Don't get moisture into any openings.

Deep cleaning wells

Automated background subtraction processes mean that low levels of fluorescence contamination in the wells of your instrument will not affect system performance. Should one or more wells of your instrument become contaminated with high levels of fluorescent substances, and need cleaning, please contact Technical Support for guidance.

Disposables

We recommend the use of genesig q32 disposables for optimal results:

genesig 0.1ml Tubes, 500	**Cat. no. Z-genesig-0.1**
genesig 8 Strip Tubes, 120	**Cat. no. Z-genesig-8STRIP**

If you wish to use third party disposables please note the following:

A. Physical dimensions should be the same as the genesig q32 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 thickness, 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 genesig q32 should be placed on a surface that is flat, dry, and not subject to draughts. Do not install your genesig q32 instrument directly in the flow of air from an air conditioner or fan. Do not install your genesig q32 instrument in a dusty environment.

Do not cover or obstruct air flow around any part of the instrument, including the heated lid and the vents on the instrument base and back.

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

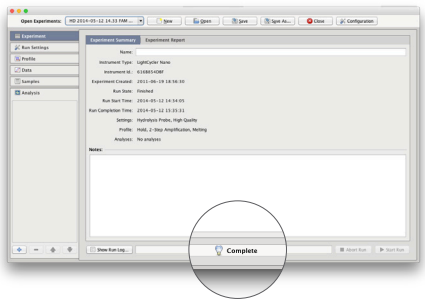
Troubleshooting

This section will help you troubleshoot your genesig q32 if you think something is wrong.

Error messages

Your genesig q32 will let you know when something is wrong by displaying an error. Most errors are reported as messages in the Status Bar in the genesig q32 software. Some errors are reported by the instrument display LEDs.

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. Please contact Technical Support with reference to this message.
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.

Error messages - displayed in status bar *continued*

Message	Action
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 genesig q32 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 will log this information into two separate files. These files help to diagnose the problem. 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 okay to send experiments that have not completed and/or were aborted.

Software errors

For software issues click on Show Run Log, and click on Export. Please contact Technical Support with the exported information.

Hardware errors

For Hardware faults go to Configuration > genesig q32 and select Retrieve Instrument Report Files.