

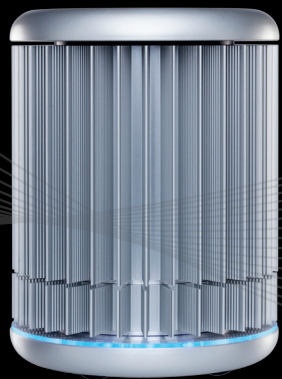
The genesis[®] q16

Instruction Manual

PRIMER
DESIGN

The genesis[®] q16 instruction manual

Congratulations on purchasing the genesis q16! The q16 is a revolutionary instrument that brings the 21st century technology of DNA testing to everyone. As well as being beautiful and highly portable, the q16 is powerful. In combination with the genesis Easy kit range, the genesis q16 can be used to detect and quantify over 550 different targets.



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Connecting the machine

Unpack your genesis q16 and remove all packaging. Insert the power cable (supplied) into the power jack in the base of the instrument and plug into a power source.

The q16 can be operated in three ways.

Direct connection to Mac/PC

If you are planning to run the instrument via a direct connection to Mac/PC, plug the Ethernet cable (supplied) into the base of the instrument and connect to a spare Ethernet port on your Mac/PC. Ideally your Mac/PC should run the most recent version of the operating system. The genesis q16 will run on OS X version 10.7.3 and newer releases for Mac and Windows Vista or 7, 8, 8.1 and 10 for PC.

If you have multiple genesis q16 instruments you can connect them all to the same Mac/PC via a network switch.

Direct connection to a local area network

If you are planning to run the instrument via a local area network, plug the Ethernet cable into the base of the instrument and connect to a spare Ethernet port on your network.

Standalone operation via a USB drive

If you are planning to run the instrument in standalone mode no Ethernet cable connection is required. Instead a USB drive is loaded with the experiment setup data and then inserted into the base of the q16 via an extension cable (supplied) to initiate a run.

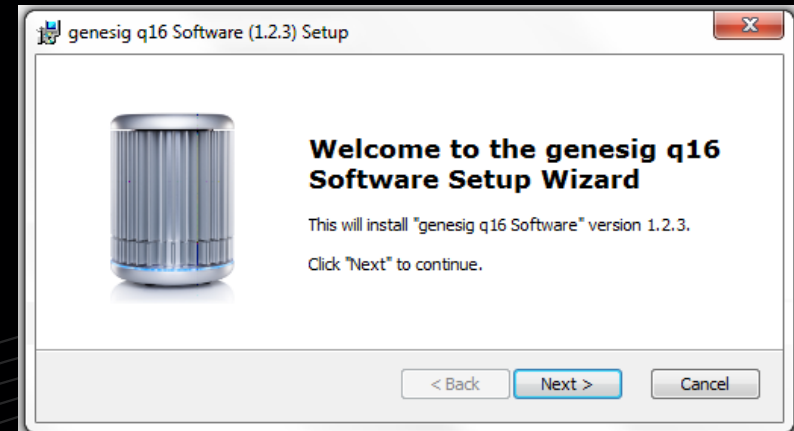
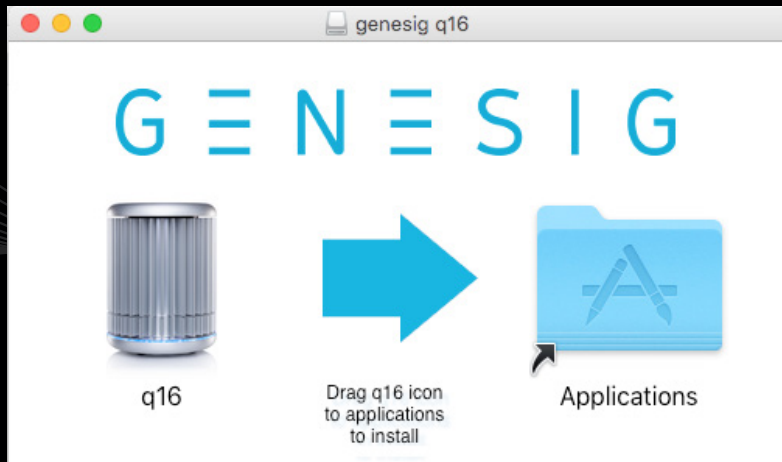
Please ensure that you use the USB drive provided with your instrument.

If using a USB drive from an alternative supplier the strict minimum speed limit for compatibility with the q16 software is 0.2MiB/s average over 10MiB of data (a 10MiB file should be written in 50 seconds or less).

Installing the software

The software requires a JavaScript runtime environment to operate successfully. If your Mac/PC does not support a current Java runtime environment you will be prompted to download and install an update. Follow the on-screen instructions to download and install the update and then re-start your computer.

The q16 software is provided on the USB drive shipped with your instrument. The latest software is always available from <http://www.genesig.com/q16-software>. Open the installer file and follow the on-screen instructions.



General operation of the instrument

To open the q16 twist the lid a quarter turn anti-clockwise and lift. The lid is replaced by repeating this operation in reverse.

The genesis q16 uses low profile 0.2ml reaction tubes that are supplied as part of each genesis Easy kit. Do not use alternative reaction tubes as this may damage your instrument. If additional reaction tubes are required please contact Primerdesign.

NEVER open a genesis q16 reaction tube after an analysis run has completed. Dispose of them carefully and ensure that they do not open by accident, as this risks contaminating your working area and may affect future test results.

What is my q16 saying?

The q16 emits a variety of lights at its base to communicate the instrument's current status. Please pay attention to these lights according to the guide below to ensure that the q16 is used appropriately:

- Amber flashing light = Lid off
- Blue static light = Standby - ready for run
- Blue circling light = Cycling - warming up
- Green circling light = Run starting

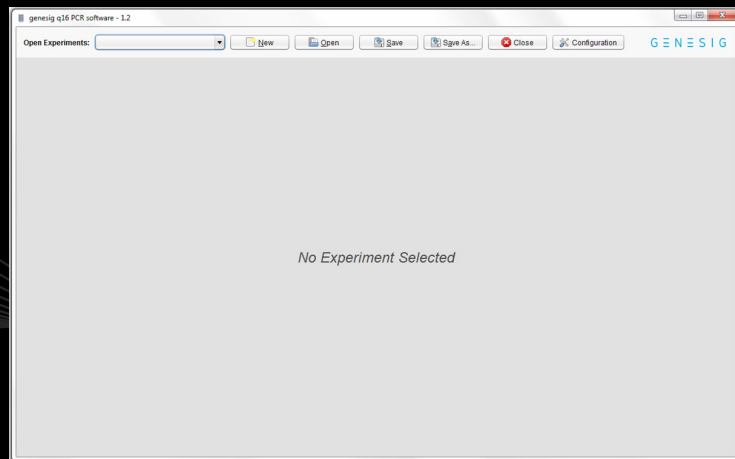
- Red circling light = Cycling - at maximum temperature
- Purple circling light = Cycling - cooling down
- Rainbow circling light = Run complete - remove and safely dispose of q16 tubes
- Red flashing light = Machine Error



Opening the software



Once installed double click on the q16 software icon to open the software.



Click the 'New' button to begin a new test.

Selecting a test type

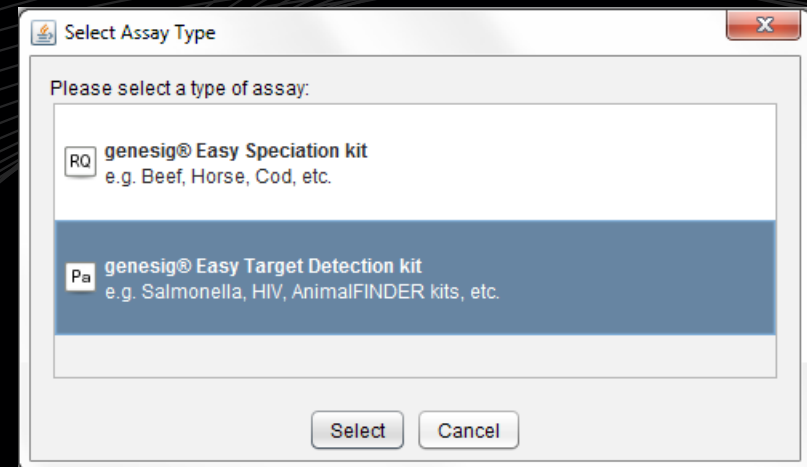
Select the type of test you wish to perform:

genesig® Easy Speciation Kit

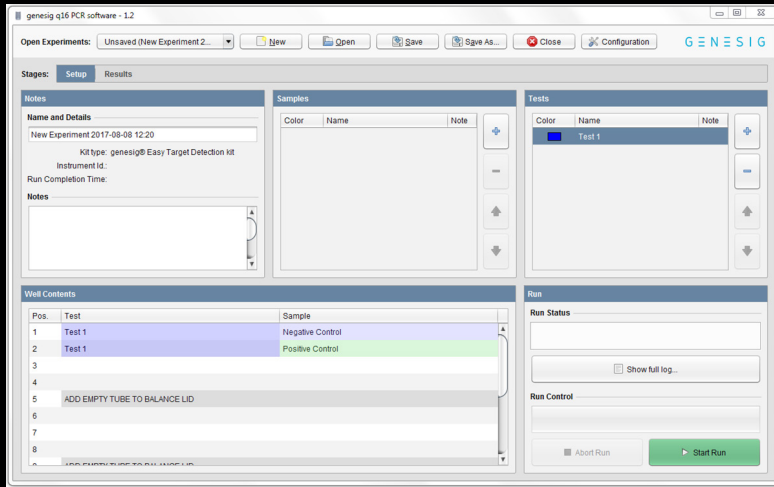
Select this if you wish to use a genesig Easy speciation kit. This test will calculate the percentage of your sample that is from a particular species e.g. you can calculate what percentage of your beefburger is horse meat.

genesig® Easy Target Detection Kit

Select this if you are using a genesig Easy kit for the detection of a particular target e.g. you can test for the presence of Salmonella or HIV. Also select this option if you are using a genesig Easy AnimalFINDER kit. These tests approximate the copy number of the target DNA present.



The main screen layout



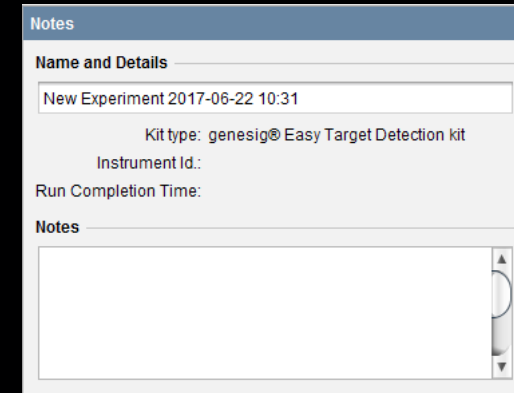
There are 2 tabs in the view. One for setting up your test and a second where your results will be displayed after the analysis is complete:



In the setup screen there are 4 simple set-up panels to consider;

1. Notes:

Your test will be given a default name but this can be changed by clicking in the “Name” field and entering new text. The “Notes” field allows you to make notes on your test if required.

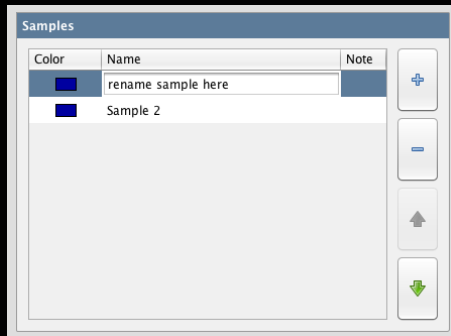


2. Samples:

In this panel you can define how many samples you wish to test. Use the + button to add more, and the – button to remove them. You can alter the order of the samples using the up and down arrows. The software automatically limits the number of samples you can have dependent on the capacity of the instrument.



The main screen layout

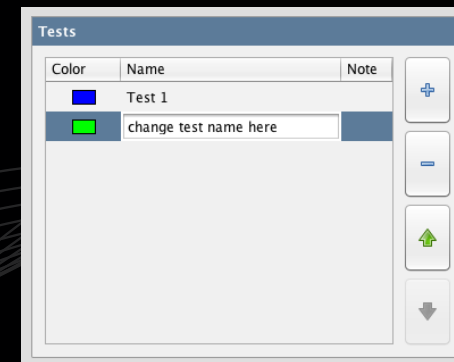
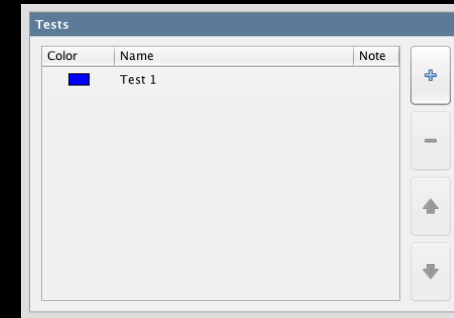


You can alter the name of your samples by clicking in the “Name” field and adding new text. You can also attach more detailed notes to a sample by clicking in the “Notes” field and adding text.

Samples are coloured to make visual identification easier. You can alter the colour associated with each sample by double-clicking on the coloured square next to it.

3. Tests:

You can perform multiple tests on the genesig q16. In this panel you can define how many tests you wish to perform. Use the + button to add more and the – button to remove them. You can alter the order of the tests using the up and down arrows. The software automatically limits the number of tests you can perform dependent on the capacity of the instrument.



You can alter the name of your tests by clicking in the “Name” field and adding new text. You can also attach more detailed notes to a test by clicking in the “Notes” field and adding text.

Tests are coloured to make visual identification easier. You can alter the colour associated with each test by double-clicking on the coloured square next to it.

The main screen layout

4. Well contents:

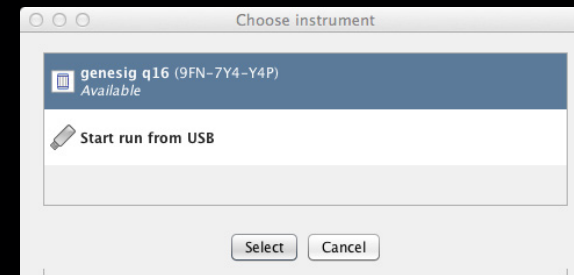
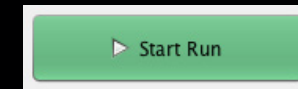
Pos.	Test	Sample
1	Test 1	Negative Control
2	Test 1	Positive Control
3		
4		
5	ADD EMPTY TUBE TO BALANCE LID	
6		
7		
8		

Taking into consideration the number of tests and the number of samples you have specified, the software automatically allocates the well position within the genesis q16 in which each sample is to be placed. Similarly the well-position of the Positive and Negative Controls are also defined automatically. Be sure to follow the allocated well positions carefully when loading your samples into the instrument.

Starting a q16 run

1. Starting the analysis

Once all of your samples have been loaded into the instrument and the lid closed, click the “Start Run” button.



From the list select the q16 instrument that you are using and analysis will begin automatically.

The status of the run and the remaining time is displayed in this panel during the analysis.

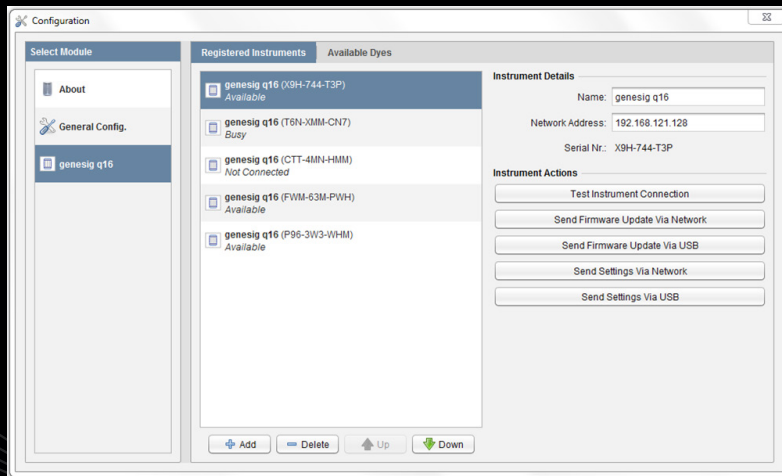
The run can be stopped at any time by clicking the “Abort Run” button. If you abort the run, discard your samples as they may be affected by the run beginning.

If your instrument is not displayed you will need to manually register the q16. This can be done using the instructions overleaf:

Starting a q16 run

2. Manually registering the q16

- Click “Configuration” at top right hand corner of the software screen.
- Select the “genesig q16” tab at the left hand side of the pop-up window.
- Click on the “Add” button now displayed at the bottom of the pop-up window.



- A list of all registered q16 instruments should appear, choose the instrument you wish to connect.
- If the software does not detect any registered instruments, you will be prompted to manually input the 9 digit/letter

serial id no. of your q16 (this can be found on the underside of the machine).

- Wait for a few minutes. If your q16 becomes designated as “Available”, then close the configuration pop-up window. You will now be able to start a run.
- If your instrument does not become “Available”, and is designated as “Not Connected” instead, please check the wires and ports are all connected properly, then repeat all of the steps above.
- If your instrument continues to be displayed as “Not Connected”, please see the Troubleshooting section of this handbook and contact your network administrator as it is likely that your network security is preventing a proper connection.
- Primerdesign can provide your I.T. department with the recommended protocols for manually configuring the instrument connection, please contact us.

3. Standalone operation from a USB drive

In order to conduct a successful USB run, please follow these steps:

Starting a q16 run

- Setup your experiment as normal.
- Connect the USB drive to the Mac/PC.
- Click “Start Run” then select “Start run from USB”.
- In the navigation window that appears, select the drive associated with the USB.
- Wait until the “Testing USB drive speed” pop-up disappears.
- Safely remove USB drive from Mac/PC (use proper USB eject protocol to ensure that the file ‘USB RUN’ has been transferred to your USB)
- Connect the USB drive to the q16 and the run should start automatically. The q16 will say ‘analysis starting’ and the LED light indicators will change to green.
- During the run the LEDs will hold red for several minutes then begin cycling between blue, red and purple until the analysis is complete.
- The USB drive should not be removed until the run is completed as indicated by the q16 saying ‘analysis complete’ and the LED light indicators changing to rainbow colours.

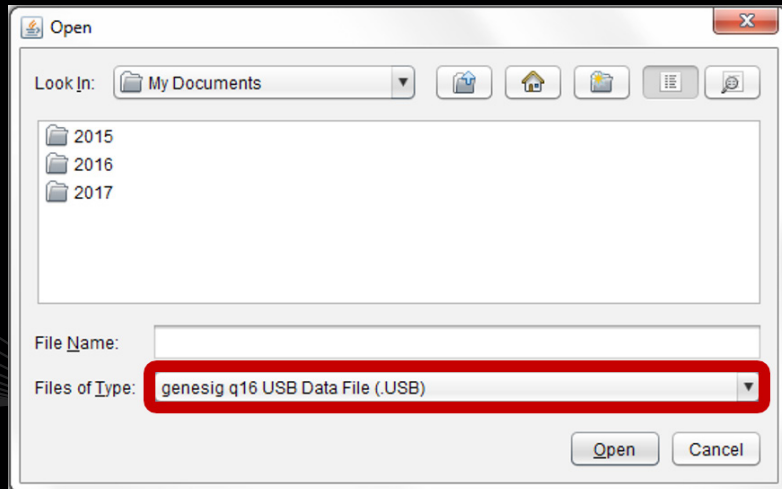


Results

If you are running the instrument via a direct connection or over a LAN simply click the “Results” tab to view the results.



If you are running the instrument direct from a USB drive, the results will be stored as a .USB file on the USB drive. This can be opened in the software on your Mac/PC. Insert the USB drive into your Mac/PC. Open the genesig q16 software and click “Open” then change the “Files of Type” selection (underneath the file name) from ‘Experiment File (.ppf)’ to ‘genesig q16 USB Data File (.USB)’.



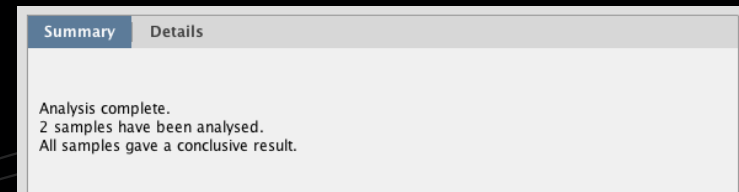
Your USB run will now be viewable in the appropriate folder (the first USB run is typically named ‘PCR01.USB’).”



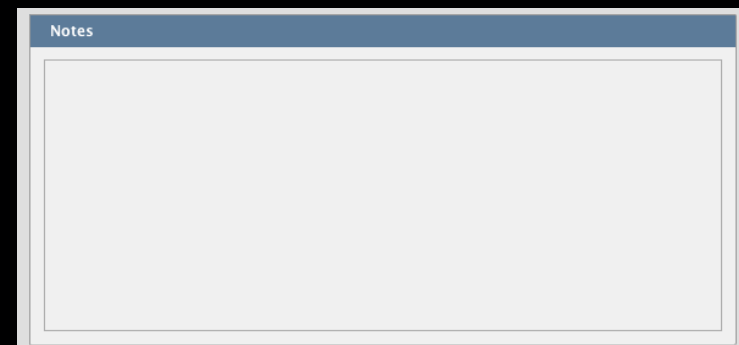
The results window has 2 further tabs. The first shows a summary of the results complete with automated analysis. The second gives a more detailed view showing the underlying raw data and is designed for advanced users or for troubleshooting.

Summary results view:

A text based summary of the entire analysis is displayed in the top left panel.



More detailed information is displayed in the top right “Notes” panel.



Results

Sample Results		Details	
#	Test	Sample	Status
1	Test 1	Negative Control	
2	Test 1	Positive Control	
3	Test 1	Sample 1	POSITIVE
4	Test 1	Sample 2	POSITIVE
5			

In the summary view the samples are listed exactly as you set them up in the previous screen. The status field shows the test result as a simple, automatically analysed status.

Species test results

%	% Sensitivity
90.21	1.00
100.00	1.00
52.83	1.00
12.66	0.10
31.01	1.00
28.05	1.00

When carrying out a genesig Easy speciation test, the quantitative analysis is carried out automatically. The percentage of your sample that is from the species of interest is displayed e.g. if your beefburger sample contains 10% horse meat, a result of 10% will be displayed.

The q16 also automatically calculates the sensitivity of the test. This allows you to determine how reliable your results are. The quality of the DNA samples directly affects the sensitivity of testing. A good quality sample can yield highly sensitive testing whereas a poor quality sample will result in less sensitive testing. The figure displayed in the “% Sensitivity” field is the lowest speciation percentage that could be reliably reported from your sample.

For example:

- If the speciation percentage of a sample is greater than the reported % sensitivity - The speciation percentage reported is reliable. The sample result is reported by the q16 because the result is above the sensitivity threshold and can therefore be reliably interpreted.
- If the speciation percentage of a sample is lower than the reported % sensitivity – The speciation percentage cannot be reliably reported. The sample result is withheld by the q16 because the result is below the sensitivity threshold and can therefore not be reliably interpreted. In this situation please refer to the troubleshooting section “What Do My Results Mean?”

Results

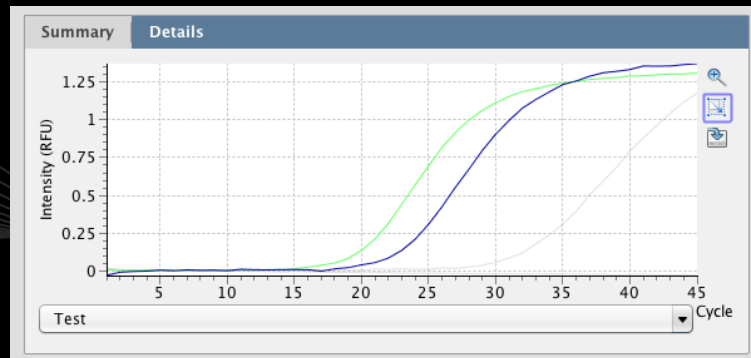
Detection test results

When carrying out a genesig Easy target detection test, quantitative analysis is carried out automatically. The number of copies of the target genome for each positive sample is calculated and displayed in the “Copy Number” field.

Copy Number
7,430
5
1,421,005
3
24

Detailed results view:

The detailed results view shows the raw underlying data and is designed for advanced users and for troubleshooting.



Amplification plots are visible in the top left panel. The drop down menu allows you to view the amplification plots derived from the test reaction or the Internal Extraction Control/Universal target reaction.



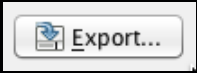
Selecting amplification plots: Amplification plots can be selected by clicking and dragging the cursor over the plot of interest. Alternatively individual tests and samples can be selected from the table below to highlight the amplification plot for a given reaction.



Zooming: Click the magnifying glass icon to select it. To zoom into the graph, move the mouse to the top left of the area you wish to zoom in on. Hold down the left mouse button, and move the mouse to the bottom right of the area you wish to zoom in on and release. To zoom out again, move the mouse anywhere over the data area of the graph, then hold down the left mouse button, move the mouse a short distance up and to the left, and release the mouse button.

Cq values can be read by hovering the cursor over a given amplification plot. Cq values can also be seen in the export file.

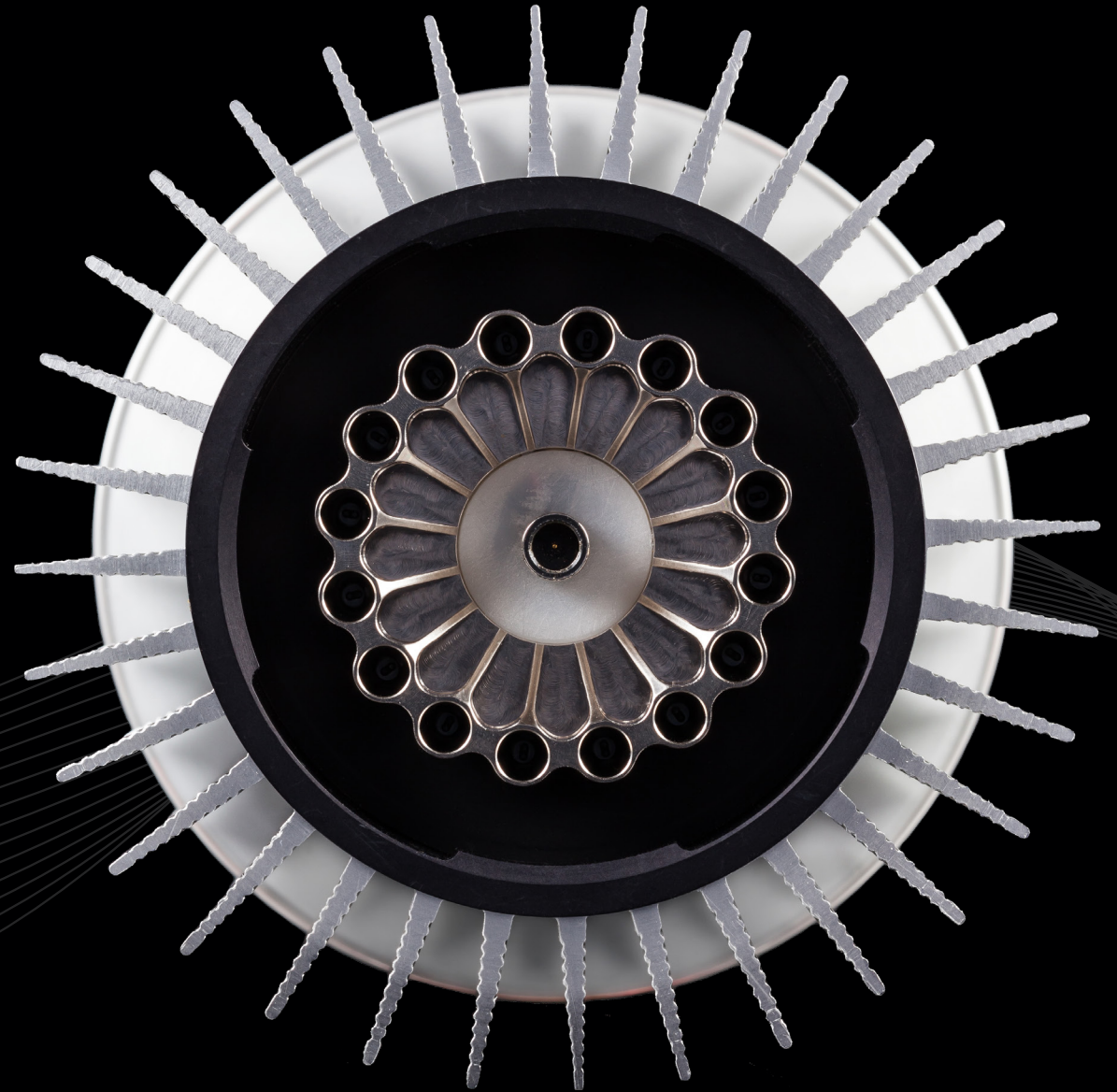
Exporting results



Detailed results can be exported by clicking the export button. A choice of export formats are available, e.g. .pdf .csv.



An image file version of the amplification plots can be exported by clicking the graph export icon.



What do my results mean?

Analysis of your data is carried out automatically by the genesig q16. The information in this section is designed to help you fully understand a result.

The following potential results may be seen when using any genesig® Easy kit:

“Positive”

Explanation

Your sample has produced a positive result. Your target of interest is present and you can use the reported copy number/percentage.

“Negative”

Explanation

Your sample has produced a negative result. Your target of interest is not present in your sample.

“Test failed”

Explanation - if positive control has failed

The test has failed because the positive control has not worked. The Positive Control is present to show that all aspects of the test are working correctly together. When this control test fails the test as a whole is invalidated. This finding indicates that a problem has occurred in the reaction set-up part of the experiment and has nothing to do with sample preparation.

Solutions

1. Check the entire workflow and test set-up to look for any user errors, then repeat the test e.g. have the right colour pipettes and solutions been used with the correct tubes?
2. Ensure the positive and negative controls are inserted into the correct wells of your q16.
3. A component of the test may have ‘gone off’ due to handling errors, incorrect storage or exceeding the shelf life. When you open a new kit, run a simple test to show that changing the kit has solved the problem. Prepare a test which includes only the Positive Control and the Negative Control. If the Positive Control works, the sample result in the summary view will specify ‘Pass’ for this component. If you are using a genesig Easy target detection kit also include one ‘mock sample’. For the ‘mock sample’ add internal control template instead of any sample DNA/RNA. If the Positive Control works, the mock sample will now be called as a negative result.

“Test Contaminated”

Explanation

The Negative Control should be completely free of any DNA/RNA. If you see this warning message it means that at some point during the setup, the Negative Control has been contaminated with DNA/

What do my results mean?

RNA and has given a positive signal. This contamination has invalidated the test. The Positive Control and your test samples are both possible sources of contaminating DNA/RNA. The PCR tubes from previous runs will also contain very high amounts of DNA so it is important that these are carefully disposed of after each run is completed and NEVER OPENED. It may be the case that your kits have become contaminated which will lead to the same problem occurring repeatedly.

Solutions

1. Clean your working area using a commercial DNA remover solution to ensure the area is DNA free at the start of your run and re-run the test.
2. If the problem persists then the kit has become contaminated and it will have to be discarded and replaced with a new kit. When you open the new kit, run a simple test to show that changing the kit has solved the problem: Prepare a test which includes only the Positive Control, the Negative Control and one 'mock sample'. For the 'mock sample' add water (supplied with the genesig Easy kit) instead of any sample DNA/RNA. The result for the Negative Control and the mock sample should be negative indicating that contamination is no longer present.

Preventive action

An ideal lab set-up has a 'Clean area' where the test reagents are prepared and a 'sample area' where DNA/RNA samples and the

Positive Control DNA are handled. The best workflow involves setting up all the test components (excluding the positive control template) in the clean area and then moving the tests to the sample area for sample and Positive Control addition. If this method is followed then the kit components are always kept away from possible sources of contamination. For extra security the Negative Control can be completely prepared and sealed in the clean area. All work areas should be decontaminated regularly with DNA remover.

“Test failed and is contaminated”

Explanation

The Positive Control is indicating test failure, and the Negative Control is indicating test contamination.

Solutions

1. For appropriate solutions, read both the “Test failed” and “Test Contaminated” sections of this handbook.

What do my results mean?

The following potential results are specific for your **genesig®** Easy speciation kit:

“Positive result lower than test sensitivity”

Explanation

The test is positive so if you are only interested in obtaining a ‘present’ or ‘absent’ answer for your sample then your result is reliable. However, if the calculated percentage falls outside the accurate range for the test the exact percentage cannot accurately be calculated. Nonetheless a semi-quantitative result can be reported based on the “% sensitivity” column. e.g. if the “% sensitivity” is reported at “1%” then the sample can be reported as “positive test with less than 1% presence of species of interest”. If you require more accurate quantitative information for your sample then proceed with the solutions below.

Solutions

1. A higher quality of sample containing more DNA will yield a more sensitive test. Try increasing the amount of sample that is added to the Sample Prep Solution stage during the extraction.
2. If you cannot increase the amount of sample, check the sample preparation protocol to look for any user errors then repeat.

3. Poor quality samples can result from overloading the sample preparation protocol with too much starting material. Try reducing the amount of starting material then repeat.

“Low level of universal target DNA”

Explanation

The test has failed because either the sample quality or quantity was too low. This has been identified by the universal target and may be caused by the sample having been compromised or a low amount of the universal target being present in the starting material (e.g. a low amount /absence of universal meat/fish DNA in a non-animal sample). In either case the sample will not be fit for analysis.

Solutions

1. Try increasing the amount of sample that is added to the Sample Prep Solution stage during the extraction.
2. If you cannot increase the amount of sample, check the sample preparation protocol to look for any user errors then repeat.
3. Poor quality samples can result from overloading the sample preparation protocol with too much starting material. Try reducing the amount of starting material then repeat.

What do my results mean?

4. Check the sample is suitable for speciation analysis. For example, if testing a non-animal sample with a speciation kit, the genesig Easy AnimalFINDER kits will be more appropriate as they do not require the detection of the universal meat/fish target endogenous control.

“Positive – Caution, low level of universal target DNA”

Explanation

The test is positive so if you are only interested in obtaining a ‘present’ or ‘absent’ answer for your sample then your result is reliable. However, the quantification has failed because either the sample quality or quantity was too low. The signal produced by the universal target acts as an endogenous control, identifying the quantity of DNA in the sample from which the species percentage is calculated. In this case the total level of DNA was not high enough. This will occur if the sample quality has been compromised or if a low amount of the universal target was present in the starting material (e.g. low amount/absence of universal meat/fish in a non-animal sample).

Solutions

For appropriate solutions read the “Low level of universal target DNA” section of this handbook.

“Test failed”

Explanation - if positive control has passed

The test has failed because the sample is not suitable for qPCR testing. This particular sample has failed because it contains one or more factors that are inhibitory to PCR. This has compromised accuracy and precision of the quantitative reporting, resulting in a reported speciation percentage greater than 100%.

Solutions

1. Dilute the extracted sample 1:10 in water to “dilute out” the PCR inhibitors.
2. Check the sample preparation protocol to look for any user errors then repeat.
3. PCR inhibitors can result from overloading the DNA/RNA sample preparation protocol with too much starting material. Try reducing the amount of starting material (by a factor of 2) then repeat.

What do my results mean?

The following potential results are specific for your genesig® Easy target detection kit:

“Sample preparation failed”

Explanation

The test has failed because the quality of the sample was not high enough. The Internal Extraction Control component identifies whether the sample has been prepared correctly and is of suitable quality. This error message means that this quality control test has failed and the sample quality is not high enough for analysis.

Solutions

1. Check the sample preparation protocol to look for any user errors then repeat.
2. Poor quality samples can result from overloading the sample preparation protocol with too much starting material. Try reducing the amount of starting material then repeat.
3. Failing to add the Internal Control Template to your sample during the sample preparation protocol can also lead to this error. Ensure that this step has not been overlooked or forgotten. If your samples are derived from an archive store or from a process separate from your genesig Easy DNA/RNA extraction kit; you must add 5µl of Internal Control Template into each 500µl of your sample to make it suitable for use on the q16.

“Positive result, poor quality sample”

Explanation

The test is positive so if you are only interested in obtaining a ‘present or absent’ answer for your sample then your result is reliable. However, the test contains an Internal Extraction Control component that identifies if the sample is of high quality. This quality control test has failed and the sample is therefore not of high enough quality to accurately calculate the exact copy number of DNA/RNA present. If you require quantitative information for your sample then proceed with the recommended solutions.

Solutions

For appropriate solutions, read the “Sample preparation failed” section of this handbook.

Troubleshooting

Network connection problems:

USB run fails to start:

Solution

The software can connect to one or more instruments via an Ethernet network. There are two ways of configuring the network to allow this:

Standard Network: The software and instruments will connect automatically to a standard network, where TCP/IP addresses are assigned automatically by a DHCP server. Connect the instrument to the network in the same way you would connect a printer, using the supplied Ethernet cable to connect to a wall socket or network switch/hub. The software will automatically connect to the instrument when it has been turned on.

Direct Network: The software and instruments can also be used on a local network that does NOT have automatic address assignment. Ask your network administrator to help you set up in this instance. IP address "169.254.88.1" / Subnet Mask "255.255.0.0".

The full protocol on manually configuring q16 instruments can be found at <http://www.genesig.com/q16-troubleshooting>.

Check that you have the latest version of the q16 software. To do this click 'Configuration' at the top right hand corner of the software screen. Underneath the q16 insignia the version number of the software will be displayed. Check that this matches the version number specified on the genesig website: <http://www.genesig.com/q16-software>. If it does not then the latest version of the software can be downloaded from this page.

If the experiment still fails to start check that your USB drive has not been corrupted. If it has been then use a different USB drive.

Troubleshooting

Unusual looking data:

If your test results are not as you expect and you can see that the raw data looks unusual – poor quality amplification plots etc.

Failed results:

The instrument is reporting failed results.

Solution

Bubbles in your reaction tubes can cause problems. Tap the tubes on a work surface or shake them down to burst any bubbles before placing them in to the q16. Make sure that the contents of each tube have collected at the base of the tube.

Check that you have put all reaction tubes in the correct wells. If the positive and negative controls are in the wrong place this will cause problems. Please contact Primerdesign technical support for advice or manual data interpretation.

For more information please visit www.primerdesign.co.uk

genesig kits are sold for general laboratory and research use only. Please feel free to contact us for free advice or technical support.

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