Primerdesign™ Ltd

Eubacteria

genesig® Easy Kit
for use on the genesig® q16

50 reactions

GENESIG
Kits by Primerdesign

For general laboratory and research use only

genesig® Easy: at a glance guide

For each DNA test

Component	Volume	Lab-in-a-box pipette	
Eubacteria reaction mix	10 µl	●	
Your DNA sample	10 µl	●	

For each positive control

Component	Volume	Lab-in-a-box pipette	
Eubacteria reaction mix	10 µl	●	
Positive control template	10 µl	●	

For each negative control

Component	Volume	Lab-in-a-box pipette	
Eubacteria reaction mix	10 µl	●	
Water	10 µl	●	

Kit contents

- **Eubacteria primer/probe mix (BROWN)**
  Once resuspended the kits should remain at -20ºC until ready to use.
- **Lyophilised oasig™ Master Mix**
- **Lyophilised oasig™ Master Mix resuspension buffer (BLUE)**
- **Eubacteria positive control template (RED)**
- **Internal extraction control DNA (BLUE)**
- **RNase/DNase free water (WHITE)**
- **Template preparation buffer (YELLOW)**
- **54 x genesig® q16 reaction tubes**

Reagents and equipment to be supplied by the user

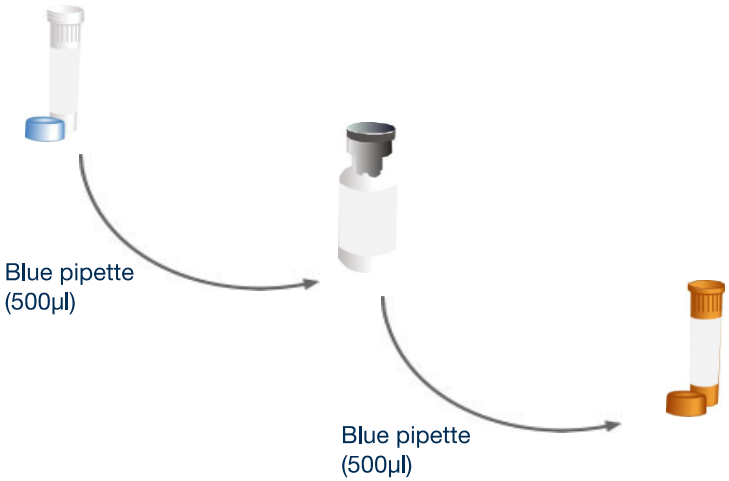
genesig® q16 instrument

genesig® Easy DNA/RNA extraction Kit
This kit is designed to work well with all processes that yield high quality DNA but the genesig Easy extraction method is recommended for ease of use.

genesig® Lab-In-A-Box
The genesig Lab-In-A-Box contains all of the pipettes, tips and racks that you will need to use a genesig Easy kit. Alternatively, if you already have these components and equipment these can be used instead.

Step-by-step guide

1. Create your reaction mix



Use the blue pipette to transfer 500µl***** of the oasig Master Mix resuspension buffer into the tube of lyophilised oasig Master Mix and mix well by **gently swirling**. Then transfer all of that master mix into the brown tube labelled Eubacteria primers/probe.

* Transferring 525µl of the oasig Master Mix resuspension buffer to your oasig Master Mix (instead of the 500µl recommended above) will enable you to take full advantage of the 50 reactions by accounting for the volume losses during pipetting. In order to do so with the genesig Easy fixed volume pipettes use 1 x blue, 2 x red and 1 x grey pipettes to make the total volume. Please be assured that this will not adversely affect the efficiency of the test.

Cap and shake tube to mix. A thorough shake is essential to ensure that all components are resuspended. **Failure to mix well can produce poor kit performance.**

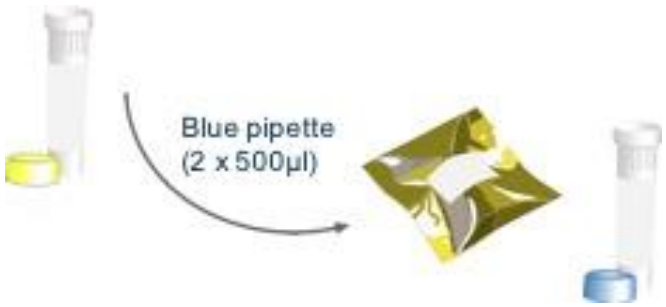
Leave to stand for 5 minutes. Now your reaction mix is ready to use.

Store the reaction mix in the freezer from hereon.

Top tip

- Ensure that the reaction mix is mixed thoroughly before each use by shaking.
- Once resuspended do not expose genesig Easy kit to temperatures above -20°C for longer than 30 minutes at a time.

2. Internal extraction control



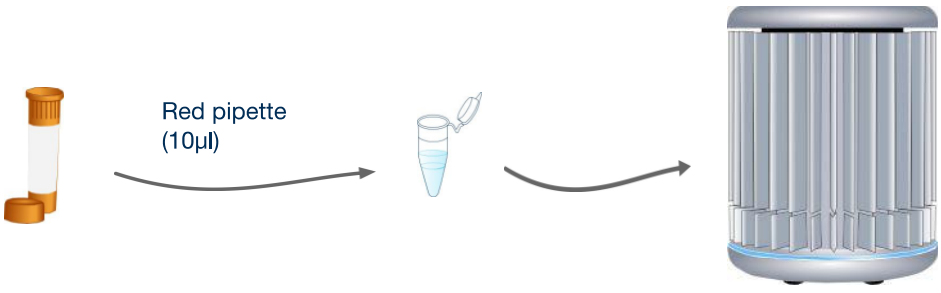
Use the blue pipette to transfer 1000µl (2 x 500µl) of template preparation buffer into the internal extraction control DNA tube. Cap and shake tube to mix.

Your kit contains internal extraction control DNA. This is added to your biological sample at the beginning of the DNA extraction process. It is extracted along with the DNA from your target of interest. The q16 will detect the presence of this internal extraction control DNA at the same time as your target. This is the ideal way to show that your DNA extraction process has been successful.

If you are using an alternative extraction kit:
Use the red pipette to transfer 10µl of internal extraction control DNA to your sample **after** the lysis buffer has been added then follow the rest of the extraction protocol.

If you are using samples that have already been extracted:
Use the grey pipette to transfer 5µl of internal extraction control DNA to your extracted sample.

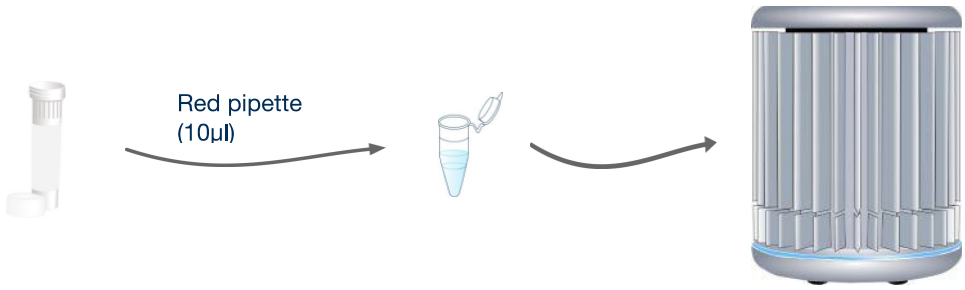
3. Add reaction mix to all reaction tubes



For every reaction to be run, use the red pipette to add 10µl of your Eubacteria reaction mix to every tube.

Top tip
• Always pipette the reaction mix directly into the bottom of the tube. • You can label the tube lids to aid your reaction setup but avoid labelling tube sides.

4. Negative control



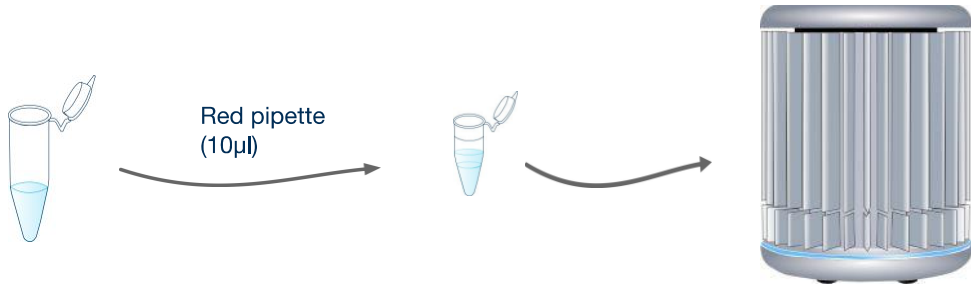
For each test you will require a negative control. Instead of DNA, water is used. This sample should prove negative thus proving that all of your positive samples really are positive.

To create a negative control reaction simply use the red pipette to add 10µl of the water to the required reaction tubes. Close these tubes after adding the water.

Because some genesig kit targets are common in the environment you may occasionally see a “late” signal in the negative control. The q16 software will take this into account accordingly.

Top tip
• Always add the water to the side of the tube to reduce the introduction of bubbles.

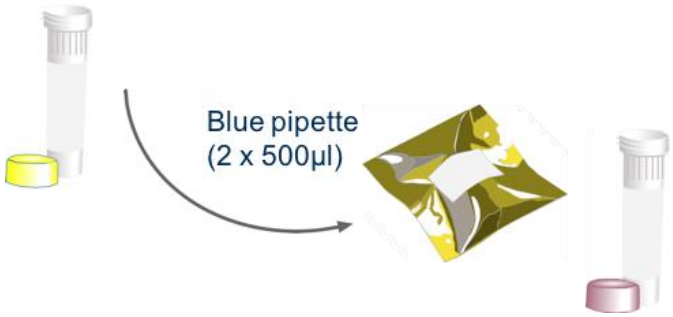
5. Set up a test



For each sample you wish to analyse, use the red pipette to add 10µl of your DNA sample to the required reaction tubes. Close these tubes after adding the sample. Always change pipette tips between samples.

Top tip
• Always add the DNA sample to the side of the tube to reduce the introduction of bubbles.

6. Positive control

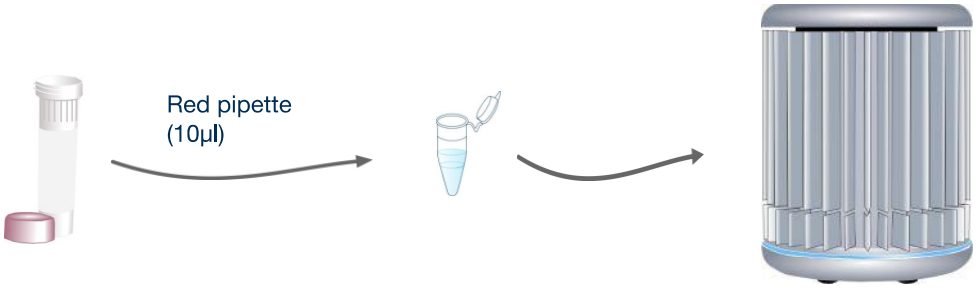


Use the blue pipette to transfer 1000µl (2 x 500µl) of template preparation buffer into the positive control template tube. Cap and shake tube to mix.

Each time you run a test you will require a positive control. This is a small portion of DNA from your target of interest. It serves two purposes:

1. It will always test positive so it shows that everything is working as it should be.
2. The q16 software knows how much DNA is present in the positive control. So, it can automatically compare your sample of interest with the positive control to calculate the amount of target DNA in your sample.

To create a positive control reaction, simply use 10µl of the positive control instead of your DNA sample.

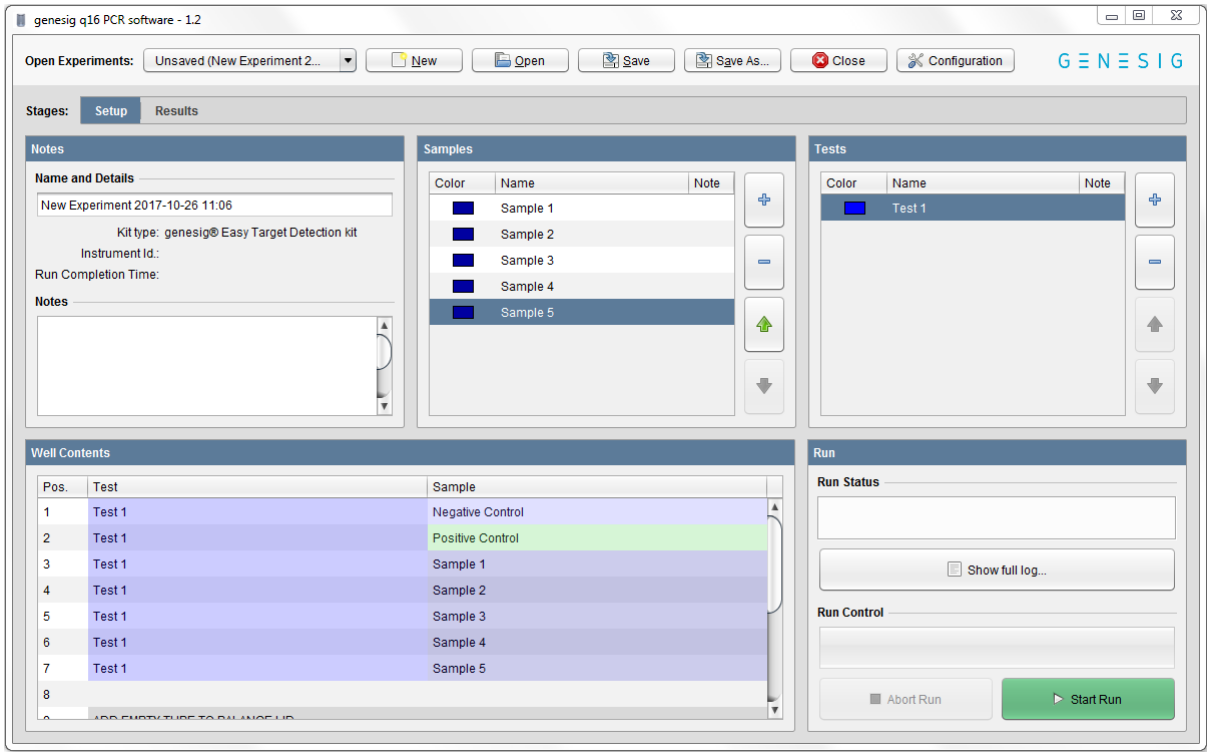


Take great care when setting up your positive control. The positive control template has the potential to give you a false positive signal in your other samples. Set positive controls up last after all other sample tubes are closed. Always change pipette tips between samples. You may even choose to set up positive controls in a separate room.

Top tip
• Always add the positive control to the side of the tube to reduce the introduction of bubbles.

7. Running the test

Place the tubes into the correct positions in your q16 as defined by the software, this may include positioning of empty tubes to ensure that the q16 lid is balanced. The run can then be started.

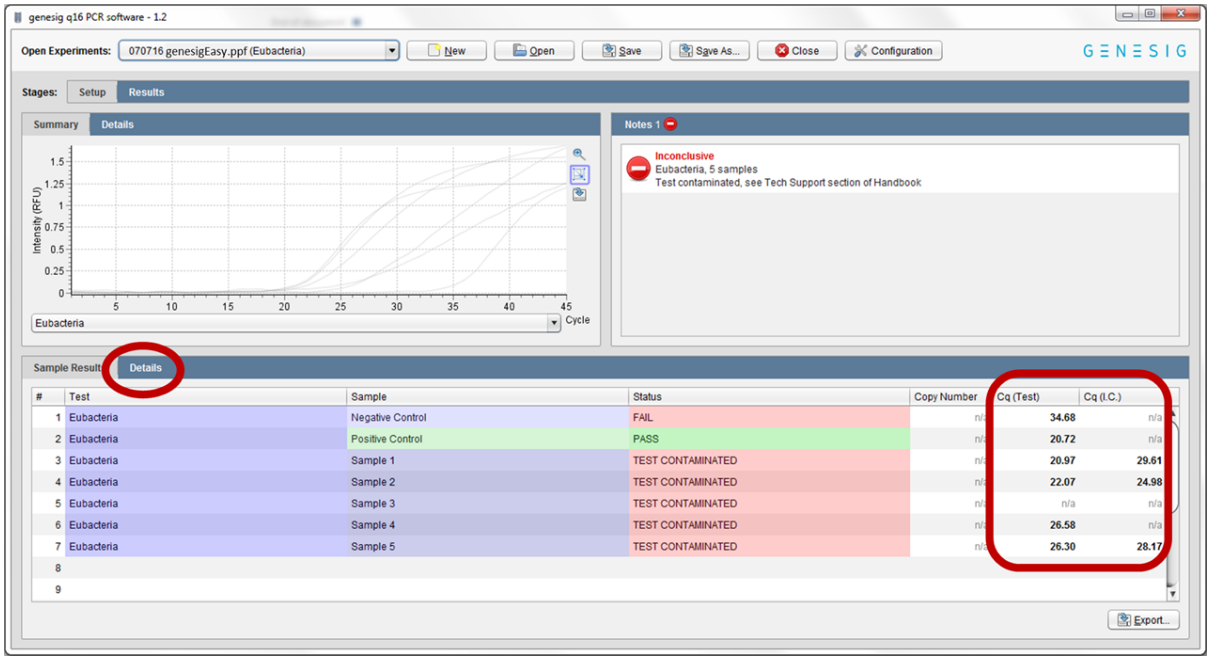


Top tip

- Before loading tubes into the q16, check for bubbles! Flick the bottom of the tubes to remove any bubbles that may have formed during the test setup.
- Apply centrifugal force with a sharp wrist action to ensure all solution is at the bottom of the reaction tube.
- When repeating a test you can use a previous file as a template by clicking ‘open’ then selecting File name > Files of Type > Experiment file as template

8. Interpreting results

Due to the ubiquitous nature of Eubacteria the negative control is likely to show amplification. This typically means that the q16 will automatically fail the test and you will be presented with the report “TEST CONTAMINATED” (see below). In this event you should manually interpret the results using the following instructions:



1. Click on the “Details” tab above the sample results table (indicated by the red circle in the figure above)
2. This will display the Cq values produced by the experiment on the right-hand side of the sample results table (indicated by the red square).
3. Use these Cq values and the following results interpretation instructions to interpret your results (please note: absence of a Cq value in the sample results table indicates absence of amplification).

Manual interpretation

A. Controls

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.

i) Negative Control

Negative control	Interpretation	Next step
Cq ≤ 30	**TEST CONTAMINATED**	Go to the "what do my results mean?" section of this handbook
Cq > 30	**PASS**	Continue to the next step

ii) Positive Control

Positive control	Interpretation	Next step
14 ≤ Cq ≤ 22	**PASS**	Continue to the next step
Cq > 22	**TEST FAILED**	Go to the "what do my results mean?" section of this handbook
Cq < 14		

B. Samples

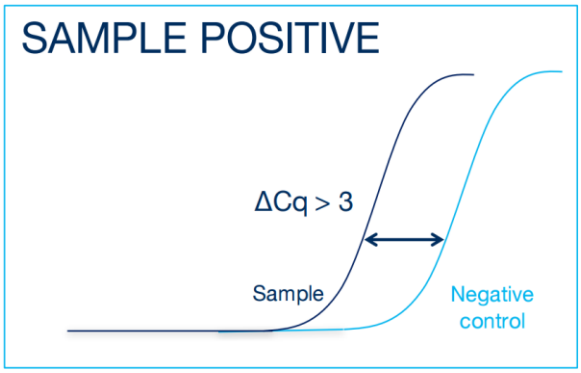
Sample	Interpretation	Next step
Cq ≤ 25	**POSITIVE**	Go to "E. Calculating copy number"
Cq > 25	**POTENTIAL POSITIVE**	Continue to next step
No amplification	**POTENTIAL NEGATIVE**	Continue to next step

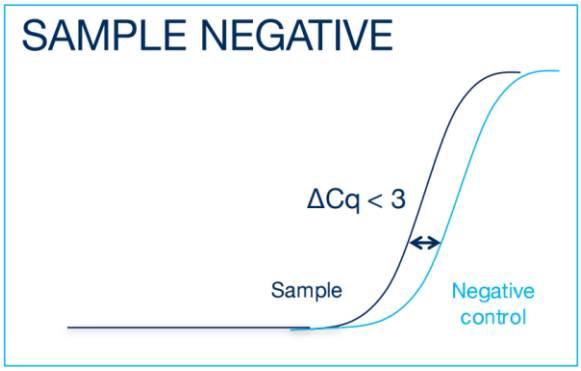
C. Checking the internal control

Unconfirmed Interpretation	Internal control	Interpretation	Next step
POTENTIAL POSITIVE	Cq ≤ 32	**POTENTIAL POSITIVE**	Continue to the next step
	Cq > 32	**SAMPLE PREPARATION FAILED**	Go to the "what do my results mean?" section of this handbook
POTENTIAL NEGATIVE	Cq ≤ 32	**NEGATIVE**	Report results
	Cq > 32	**SAMPLE PREPARATION FAILED**	Go to the "what do my results mean?" section of this handbook

D. Confirmation of positive signal

A potential positive signal must be reinterpreted based on the relative signal strength of the sample and the negative control:



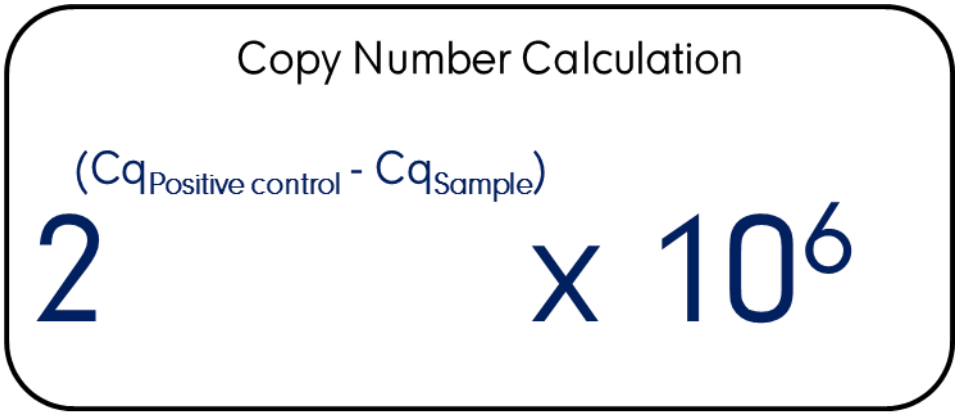


If the sample amplifies > 3 Cq earlier than the negative control then the sample is positive and the copy number calculated.

If the sample amplifies < 3 Cq earlier than the negative control then a negative call is the correct result which should be reported.

E. Calculating copy number

The following formula should be used only on samples which have been confirmed as positive by following all the manual interpretation steps:



What do my results mean?

Analysis of your data is carried out automatically by the genesig q16. The following information is designed to help you fully understand a result or to troubleshoot:

“Positive”

Explanation
Your sample has produced a positive result. Your target of interest is present and you can use the reported quantity.

“Negative”

Explanation
Your sample has produced a negative result. The target is not present in your sample.

“Test contaminated”

Please note: Eubacteria DNA is known to be highly prevalent within the air and environment generally and the negative control may therefore give a late positive signal due to environmental contamination. The interpreting results section of this handbook gives guidance on how to interpret results where environmental contamination is evident.

Explanation
The negative control should be completely free of any DNA. If you see this error message it means that at some point during the setup, the negative control has been contaminated with DNA 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. The genesig q16 reaction tubes from previous runs will also contain very high amounts of DNA so it is important that these are carefully disposed of after the 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 solution such as “DNA remover” 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 experiment 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 instead of any sample DNA. 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 template are handled. The best workflow involves setting up all the test components 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. The clean area should be decontaminated regularly with DNA remover to keep it clean.

"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 or if the sample is of low quality. This error message means that this quality control test has failed and the sample is not fit for analysis.

Solutions
1. Check the sample preparation protocol for any user errors during preparation and repeat the DNA/RNA extraction.
2. Poor samples can result from overloading the DNA/RNA extraction with too much starting material. Try reducing the amount of starting material and repeat the DNA/RNA extraction.
3. Failing to add the internal extraction control DNA to your sample during the DNA/RNA extraction process can also lead to a reported result of "sample preparation failed". 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 extraction kit; you must add 5µl of internal extraction control DNA into each 0.5ml of your sample to make it suitable for use on the q16.

"Test failed"

Explanation
The positive control is present to show that all aspects of the test are working correctly together. This error message shows that the quality control test has failed and the test is invalidated. This finding indicates that a problem has occurred in the test set-up part of the experiment and has nothing to do with DNA/RNA extraction.

Solutions
1. Check the entire workflow to look for any user errors during test set-up and repeat the test e.g. have the right colour pipettes and solutions been used with the correct tubes?
2. A component of the test may have 'gone off' due to handing errors, incorrect storage or exceeding the shelf life. Open a new kit and run a simplified test which includes only the positive control, the negative control and one 'mock sample'. For the 'mock sample' add water instead of any sample DNA. If the positive control works, the mock sample will now be called as a negative result indicating that all the components of this kit are working correctly.

“Test failed and is contaminated”

Explanation

The positive control is indicating test failure, and the negative control is indicating test contamination. Please read the “Test Failed” and “Test contamination” sections of this technical support handbook for a further explanation.

Solution

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

Eubacteria

The term Eubacteria encompasses all species within the Bacteria domain. This group of unicellular organisms has a wide range of morphologies, habitats and metabolisms, with some being of high significance in animal and plant pathology.

The majority of bacteria are between 0.5-5µm in length with a lipid membrane surrounding a cytoplasm lacking organelles. Around the cell membrane is a cell wall made of peptidoglycans. The structure of this cell wall varies between bacterial species and is used to separate bacterial species into two groups: Gram-negative and Gram-positive. This categorisation can be made by applying a staining technique to a bacterial sample and differentiating between the results.
On the outer bacterial surface there may be one or more of several appendages such as fimbriae, pili or flagella that have different roles. Many bacteria have fimbriae over the cell surface that are several µm in length and are thought to be used in attachment to surfaces such as other cells. Some bacteria also have flagella which are rigid protein structures up to 20µm in length and are used for motility.

Within the bacterial cell, genomes are not stored within a nucleus and are most commonly found as a circular chromosome within the nucleoid. Bacterial genomes can range in size from 160Kbp to 12.2Mbp depending on the species. Virulence factors and antibiotic resistance are often encoded by DNA found in plasmid formation in addition to the main chromosome.
Bacterial reproduction is by binary fission, where a single bacterium forms two identical daughter cells first by replication of the genome followed by division of the cell.

Specificity

If you require further information, or have a specific question about the detection profile of this kit then please send an email to enquiry@primerdesign.co.uk and our bioinformatics team will answer your question.

Kit storage and stability

This lyophilised kit is stable at room temperature but should be stored at -20ºC on arrival. Once the lyophilised components have been resuspended they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.
Primerdesign does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA/DNA integrity.

Dynamic range of test

Under optimal PCR conditions genesig Eubacteria detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primerdesign genesig® detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied Biosystems business group of the Applera Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U. S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

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