Bacteria Hemorrhagic Septicemia

Easy Kit

for use on the q16

50 reaction

For general laboratory and research use only

Easy: at a glance guide

For each DNA test

Component	Volume	Lab-in-a-box pipette	
A.hydrophila reaction mix	10 µl		
Your DNA sample	10 µl		

For each positive control

Component	Volume	Lab-in-a-box pipette	
A.hydrophila reaction mix	10 µl		
Positive control template	10 µl		

For each negative control

Component	Volume	Lab-in-a-box pipette	
A.hydrophila reaction mix	10 µl		
Water	10 µl		

Kit Contents

- **A.hydrophila specific primer/probe mix (BROWN)**
  Once resuspended the kits should remain at -20ºC until ready to use.

- **Lyophilised oasig Mastermix**

- **Lyophilised oasig Mastermix resuspension buffer (BLUE lid)**

- **A.hydrophila positive control template (RED lid)**

- **Internal extraction control DNA (BLUE lid)**

- **RNAse/DNAse free water (WHITE lid)**

- **50 x q16 reaction tubes**

Reagents and equipment to be supplied by the user

q16 instrument

Easy DNA/RNA Extraction Kit

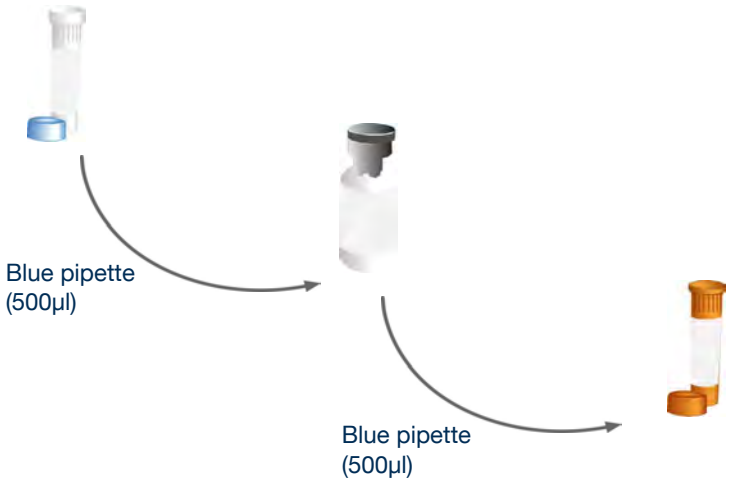
This kit is designed to work well with all processes that yield high quality DNA but the Easy extraction method is recommended for ease of use.

Lab-In-A-Box

The Lab-In-A-Box contains all of the pipettes, tips and racks that you will need to use a 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 mastermix resuspension buffer into the tube of lyophilised oasig mastermix and mix well by inversion. Then transfer all of that mastermix into the brown tube labelled A.hydrophila primers/probe.

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.**

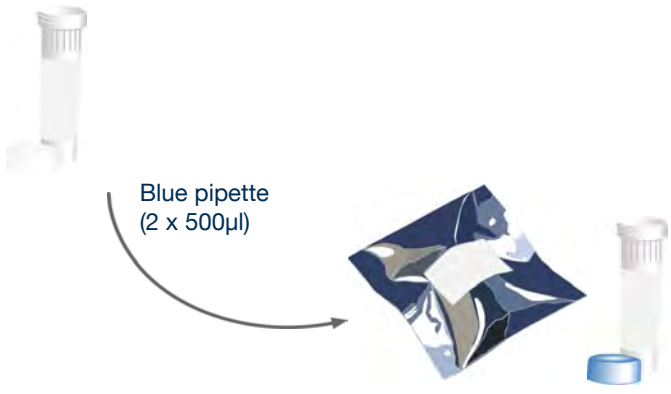
Leave to stand for 5 minutes. Now your reaction mix is ready to use.

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 EASY kit to temperatures above -20°C for longer than 30 minutes at a time.

2. Internal extraction control



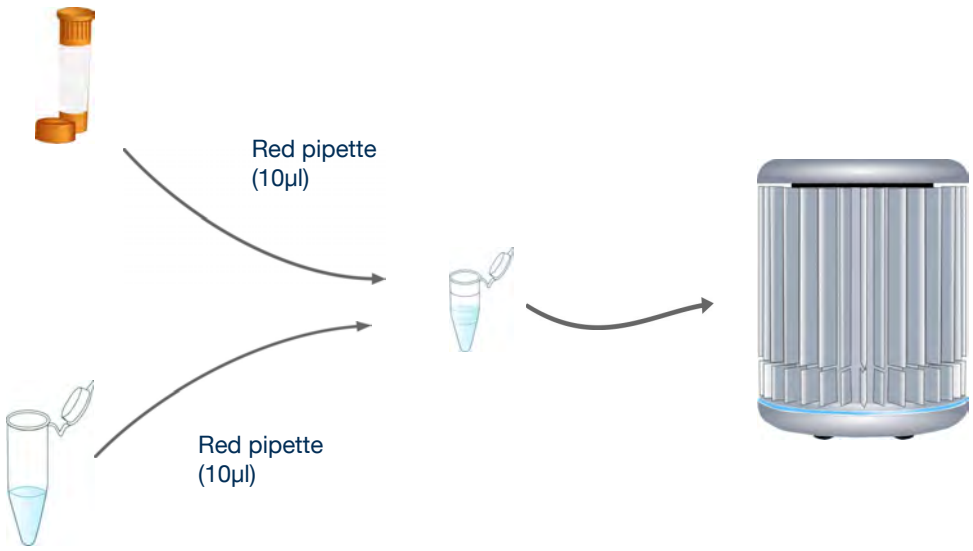
Use the blue pipette to transfer 1000µl (2 x 500µl) of water into the Internal Extraction Control DNA tube. Cap and shake tube to mix.

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. Set up a test

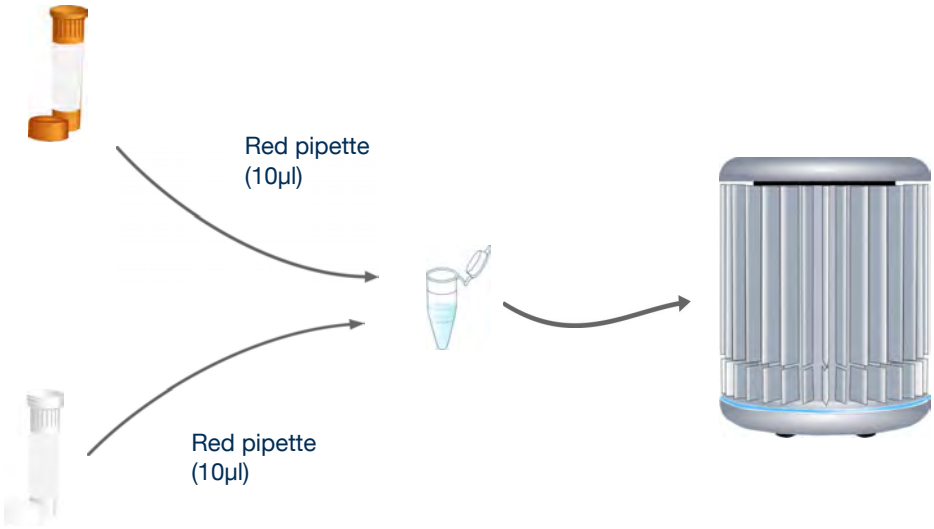


For each sample you wish to analyse, use the red pipette to combine 10µl of your A.hydrophila reaction mix with 10µl of your DNA sample in the reaction tubes provided. Always change pipette tips between samples.

Top tip

- Always pipette the reaction mix directly into the bottom of the tube adding the DNA sample to the side of the tube to reduce the introduction of bubbles.
- Flick the bottom of the tubes to remove any bubbles.
- Apply centrifugal force with a sharp wrist action to ensure all solution is at the bottom of the reaction tube.
- You can label the tube lids to aid your reaction setup but avoid labelling tube sides.

4. Negative control



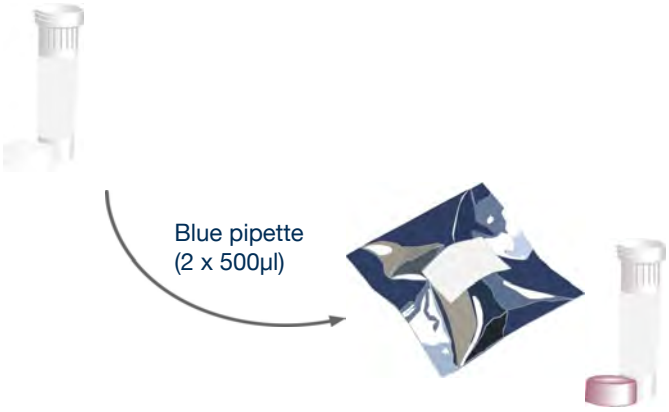
For each test you will require a negative control. Instead of DNA, water is used. This sample should prove negative thus proving that all of your positive samples really are positive.

Because some 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 pipette the reaction mix directly into the bottom of the tube adding the water to the side of the tube to reduce the introduction of bubbles.
- Flick the bottom of the tubes to remove any bubbles.
- Apply centrifugal force with a sharp wrist action to ensure all solution is at the bottom of the reaction tube.
- You can label the tube lids to aid your reaction setup but avoid labelling tube sides.

5. Positive control

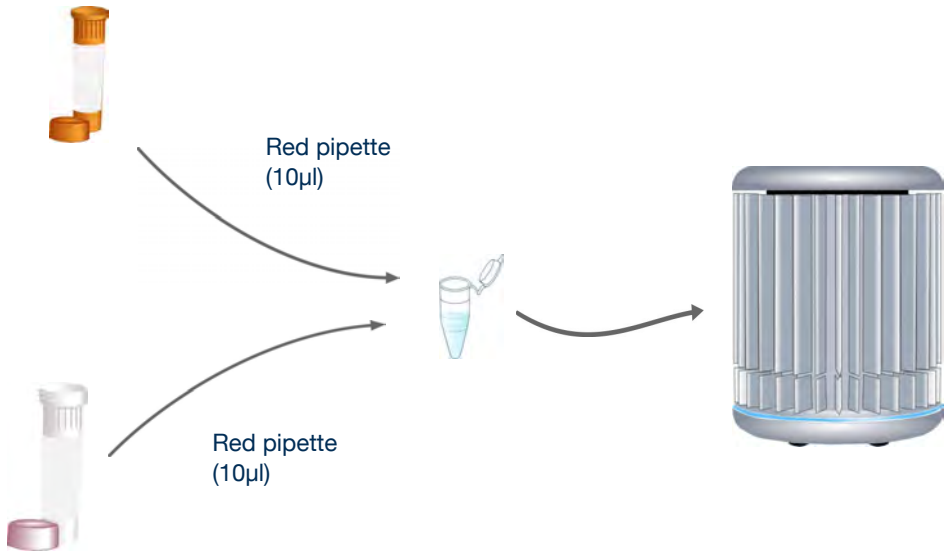


Use the blue pipette to transfer 1000µl (2 x 500µl) of water into the positive control template tube. Cap and shake tube to mix.

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.



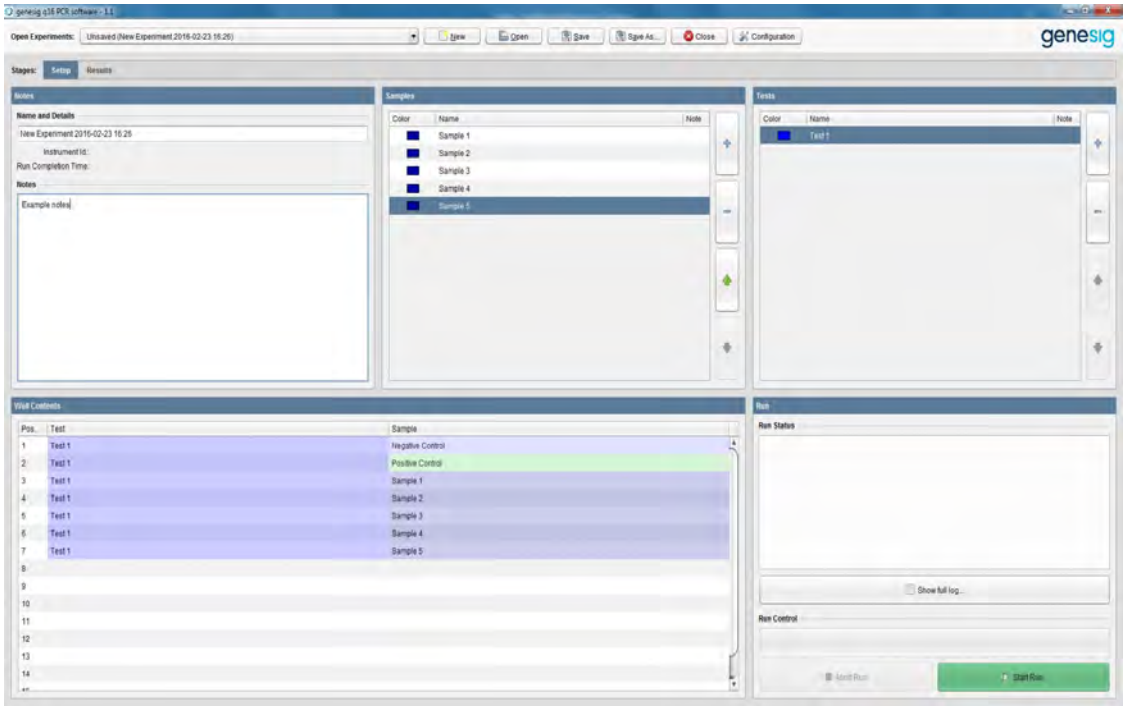
Take great care when setting up your positive control. The positive control template has the potential to give you a false positive signal in your other samples. Set positive controls up last after all other sample tubes are closed. Always change pipette tips between samples. You may even choose to set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

Top tip

- Always pipette the reaction mix directly into the bottom of the tube adding the positive control to the side of the tube to reduce the introduction of bubbles.
- Flick the bottom of the tubes to remove any bubbles.
- Apply centrifugal force with a sharp wrist action to ensure all solution is at the bottom of the reaction tube.
- You can label the tube lids to aid your reaction setup but avoid labelling tube sides.

6. Running the test

Place the tubes into the correct positions in your q16 as defined by the software and start run.



Top tip

- 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

What do my results mean?

Analysis of your data is carried out automatically by the 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”

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 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 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.

“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 secure as a positive test. 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 not therefore of high enough quality. The exact copy number of DNA/RNA present cannot be accurately calculated in this instance. If you require quantitative information for your sample then proceed with the solutions below.

Solutions

1. Check the DNA/RNA extraction 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 “positive result, poor quality sample”. 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 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 as a whole 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.

Bacteria Hemorrhagic Septicemia

Aeromonas hydrophila is a heterotrophic, gram-negative bacterium of the Aeromonadaceae family. This bacterium has a rod shape with rounded ends reaching 1-3µm in length. Polar flagella allow motility. 3 enterotoxins encoded by the 5Mbp genome are thought to be responsible for making this bacterium pathogenic. These entereotoxins are namely: cytotoxic (act); heat-labile cytotonic (alt) and heat-stable cytotonic (ast) of which the former is thought to contribute most to toxicity. A. hydrophila can be considered as an opportunistic pathogen which is linked to infections in fish and amphibians as well as humans, but may not be the cause.

Infection most usually occurs when an organism is dealing with environmental change. This could be a change in temperature or when infected with a different bacterium. In fish, the bacteria mainly infect the kidneys and liver resulting in disease that is thought to be the result of stress. In amphibians, the infection can cause internal heamorrhaging. Infection in humans can be caused by ingestion of infected food such as fish or meat. Once ingested the bacterium progresses to an organ via the bloodstream. Alternatively the bacterium can infect open wounds. The bacteria have pili which they utilise in attachment to host organism and invasion of the cells or organs. Colonisation occurs by asexual reproduction. Upon cell invasion, the enterotoxins are activated causing cell damage.
Transmission in fish occurs when infected fish are moved into new fisheries without sanitation.
The bacteria are largely antibiotic resistant, can survive refrigeration and are also able to grow at temperatures in a wide range up to 37°C. However, a sodium/calcium hypochlorite solution can kill this bacterium.

Infection in fish may cause diseases including tail and fin rot, heamorrahagic septicemia and ulcer disease. In amphibians this bacterium is linked to a disease called red leg which is a result of internal heamorrhaging. Infection in humans can lead to a number of diseases including septicemia, meningitis, pneumonia and gastroenteritis. The bacteria can also infect open wounds causing cellulitis, myonecrosis and ecthyma gangrenosum. The latter two diseases occur more commonly in the immunocompromised, although infections do also occur in immunocompetent people.

Specificity

The Kit for Bacteria Hemorrhagic Septicemia (A.hydrophila) genomes is designed for the in vitro quantification of A.hydrophila genomes. The kit is designed to have the broadest detection profile possible whilst remaining specific to the A.hydrophila genome.

The primers and probe sequences in this kit have 100% homology with a broad range of A. hydrophila sequences based on a comprehensive bioinformatics analysis.

The primers have 100% homology with >95% of sequenced Aeromonas hydrophila isolates in the NCBI database. Due to the high sequence homology, the primers also have a high level of homology to other closely related Aeromonas sp. The primers therefore may also detect A. salmonicida, A. caviae and A. sobria.

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.

We do 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 A.hydrophila 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 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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