

Primerdesign™ Ltd

Speciation demonstration kit

genesig® Easy Kit

for use on the genesig® q16

50 reactions

GENESIG

Kits by Primerdesign


For general laboratory and research use only.

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genesig[®] Easy: at a glance guide


For each DNA test

Component	Volume	Lab-in-a-box pipette	
Demo species reaction mix	10µl	●	
Demo species sample	10µl	●	

For each positive control

Component	Volume	Lab-in-a-box pipette	
Demo species reaction mix	10µl	●	
<u>Positive control template</u>	10µl	●	

For each negative control

Component	Volume	Lab-in-a-box pipette	
Demo species reaction mix	10µl	●	
<u>Water</u>	10µl	●	

Kit contents



- **Demo species primer/probe mix (BROWN)**
Once resuspended the kits should remain at -20°C until ready to use
- **Lyophilised oasisig™ Master Mix**
- **Lyophilised oasisig™ Master Mix resuspension buffer (BLUE)**
- **Demo species positive control template (RED)**
- **4 Demo species samples (RED)**
- **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® 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.

Kit introduction

This kit is a speciation demonstration kit for use on the q16 instrument. The core components of this kit are identical to those in the standard genesigEASY kit range.

The kit includes 4 demonstration samples that are carefully designed to give a range of test results that will be representative of the results that will be achieved by the real genesig Easy kits.

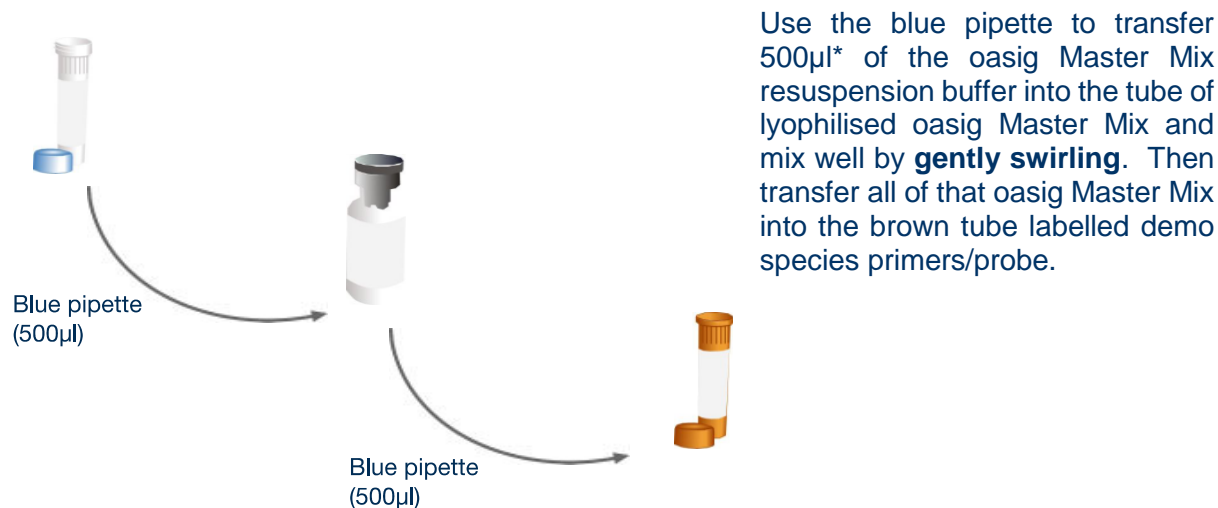
Simply use these 4 demonstration samples as if they were “real-world” samples.

The 4 samples are as follows:

1. Demo species Fail sample: This sample is designed to mimic a sample of poor quality and will give a failed test result.
2. Demo species Neg sample: This sample is designed to mimic a negative sample and will give a negative result
3. Demo species Low sample: This sample is designed to mimic a positive sample with a low level of DNA present.
4. Demo species High sample: This sample is designed to mimic a strong positive sample.

Step-by-step guide

1. Create your reaction mix



* Transferring 525µl of the oasis Master Mix resuspension buffer to your oasis 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.**

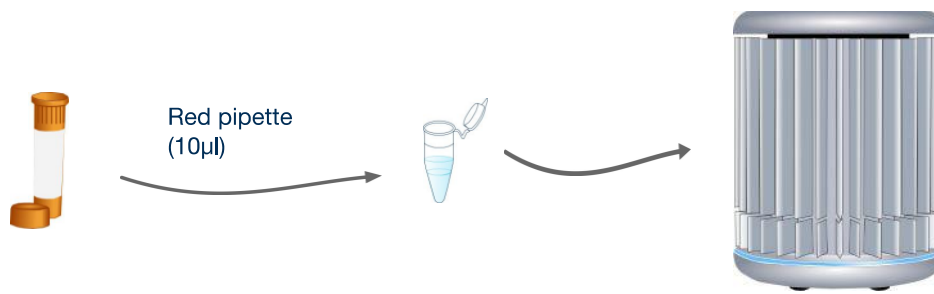
Leave to stand for 5 minutes. Now your reaction mix is ready to use.

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. Add reaction mix to all reaction tubes

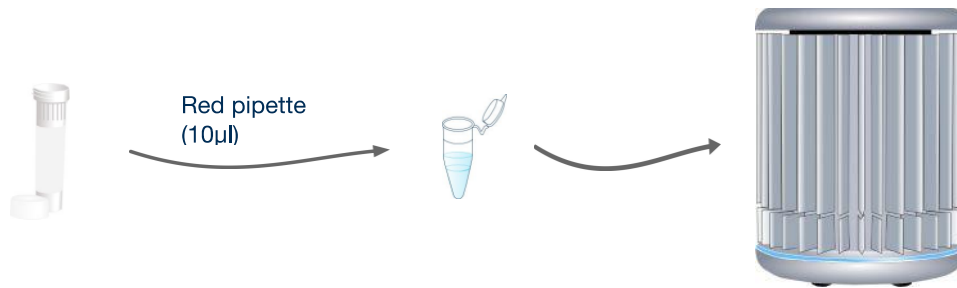


For every reaction to be run, use the red pipette to add 10µl of your demo species reaction mix to every tube.

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.

3. 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.

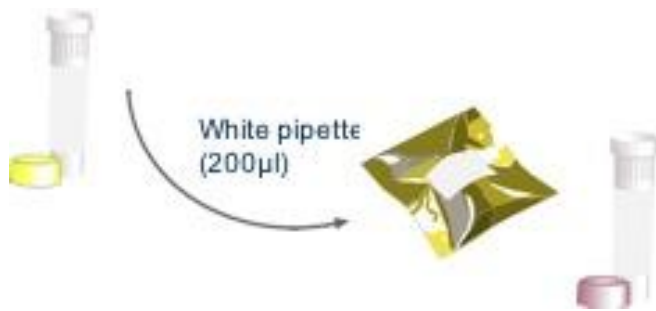
To create a negative control reaction, simply use the red pipette to add 10µl of the water to the required reaction tubes. Close these tubes after adding the water.

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.

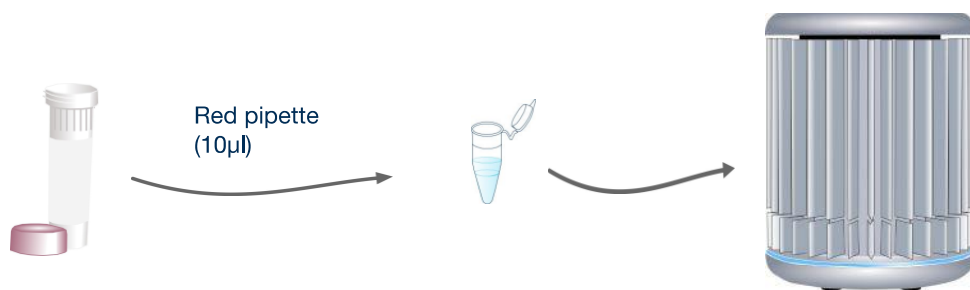
Top tip

- Always add the water to the side of the tube to reduce the introduction of bubbles.

4. Set up your test



Use the white pipette to transfer 200µl of template preparation buffer into each of the species test samples supplied (red lid). Cap and shake the tubes to mix. Leave to stand at room temperature for five minutes.

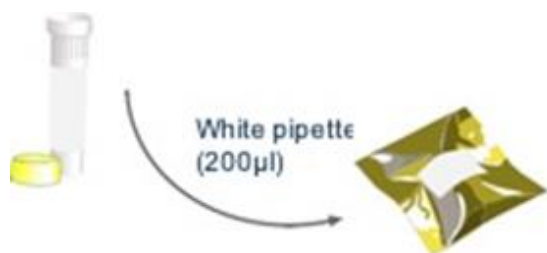


For each of the four test samples, use the red pipette to add 10µl of the demo species samples to the required reaction tubes. Always change pipette tips between samples.

Top tip

- Always add the DNA sample to the side of the tube to reduce the introduction of bubbles.

5. Positive control

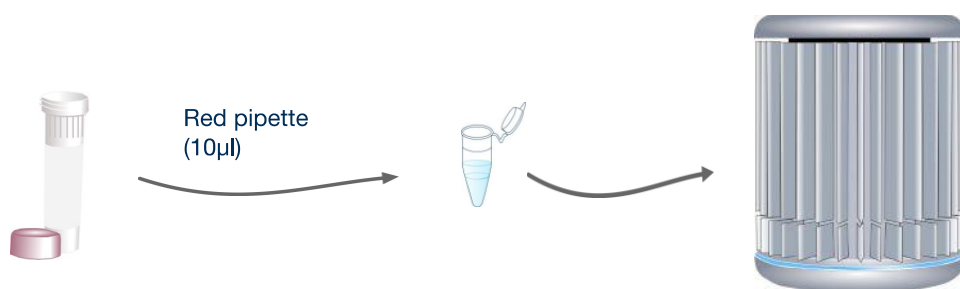


Use the white pipette to transfer 200µl of template resuspension buffer into the positive control template tube. Cap and shake tube to mix.

Each time you run a test you will require a positive control. This is a small portion of DNA from your species 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. It uses this information to internally calibrate every test. This is essential to give accurate information about the percentage of DNA in your sample that is from your species of interest.

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



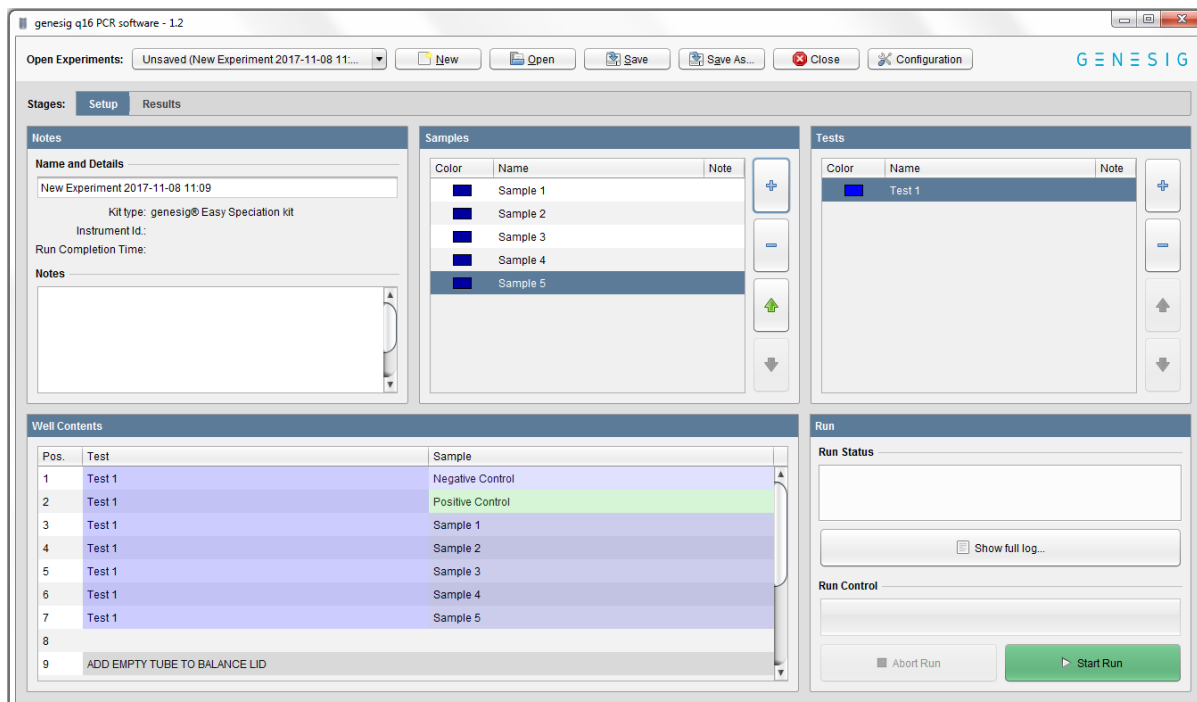
Take great care when setting up your positive control. The positive control template has the potential to give you a false positive signal in your other samples. Set positive controls up last after all other sample tubes are closed. Always change pipette tips between samples. You may even choose to set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

6. Running the test

Open a new experiment and select “Species detection test”. Use the sample section “+” sign to add 4 samples, edit sample names accordingly. Place the tubes into the correct positions in your q16 as defined by the software, this may include the positioning of empty tubes to ensure 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

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”

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 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 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 samples and the positive control template 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”

Explanation

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, the negative control and one ‘mock sample’. For the ‘mock sample’ add internal control template instead of any sample DNA. If the positive control works, the mock sample will now be called as a negative result.

“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 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 concentration of sample that is added to the sample prep solutions 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.

“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.

“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. 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.

“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 universal target was present in the starting material.

Solutions

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

Kit storage and stability

This lyophilised kit is stable at room temperature but should be stored at -20°C 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.

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 Applied Biosystems 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.

Trademarks

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