Primerdesign™ Ltd

Pollachius pollachius Pollock speciation kit

genesig® Easy Kit

for use on the genesig® q16

50 reactions

For general laboratory and research use only

DNA testing

Everything...
Everyone...
Everywhere...

genesig® Easy: at a glance guide

For each DNA test

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

For each positive control

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

For each negative control

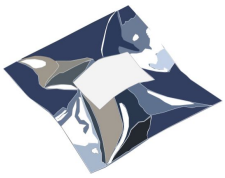
Component	Volume	Lab-in-a-box pipette	
Pollock reaction mix	10 µl		
Water	10 µl		

Kit Contents

- **Pollachius pollachius species 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)**



- **Pollachius pollachius species positive control template (RED lid)**

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

- **50 x genesig® q16 reaction tubes**

Reagents and equipment to be supplied by the user

genesig® q16 instrument

genesig® Easy DNA/RNA Extraction Kit

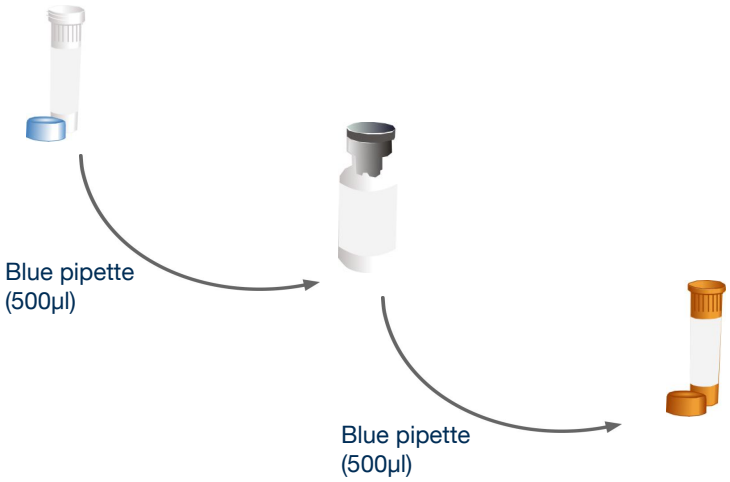
This kit is designed to work well with all processes that yield high quality DNA but the genesig® Easy extraction method is recommended for ease of use.

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 then 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 Pollock 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.**

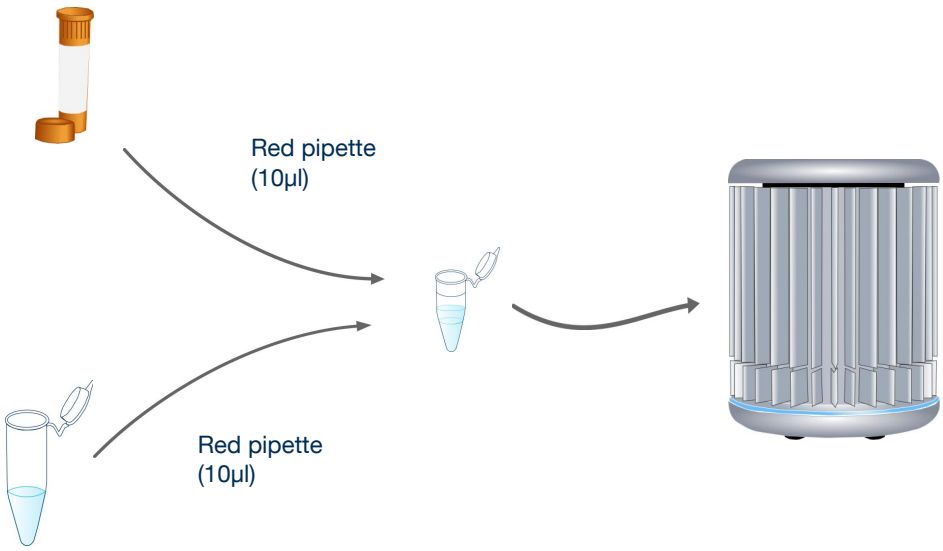
Leave to stand for 5 minutes. Now your reaction mix is ready to use.

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

2. Set up a test

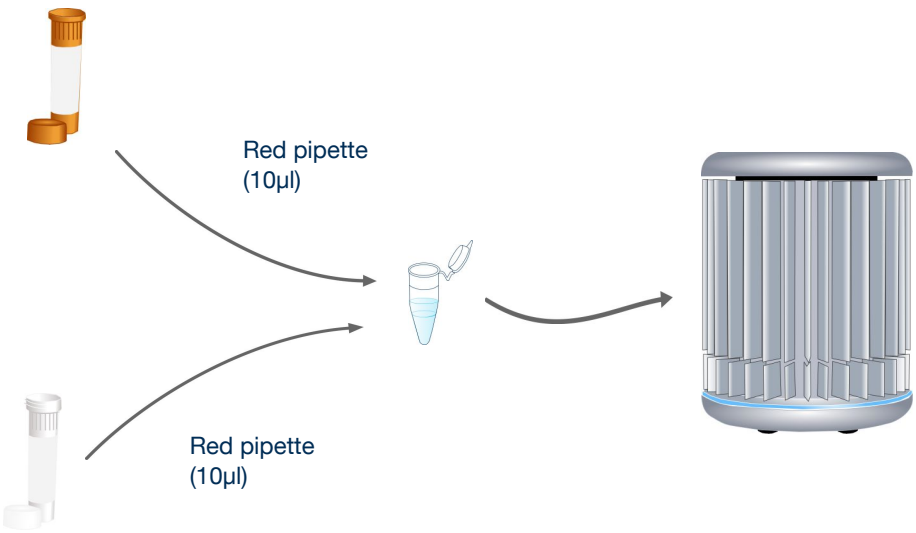


For each sample you wish to analyse, use the red pipette to combine 10µl of your Pollock reaction mix with 10µl of your DNA sample in the reaction tubes provided. Always change pipette tips between samples.

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.

3. Negative control



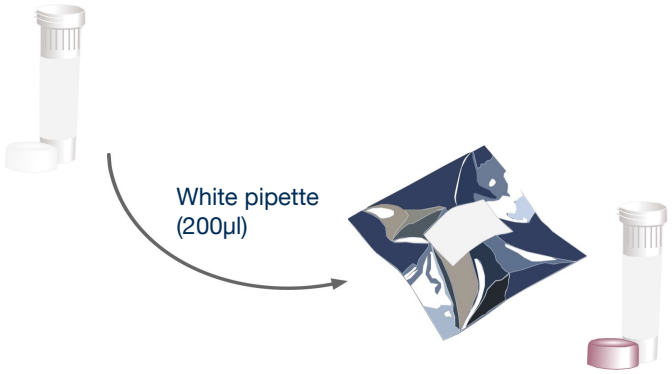
For each test you will require a negative control. Instead of DNA, water is used. This sample should prove negative thus proving that all of your positive samples really are positive.

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 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.

4. Positive control

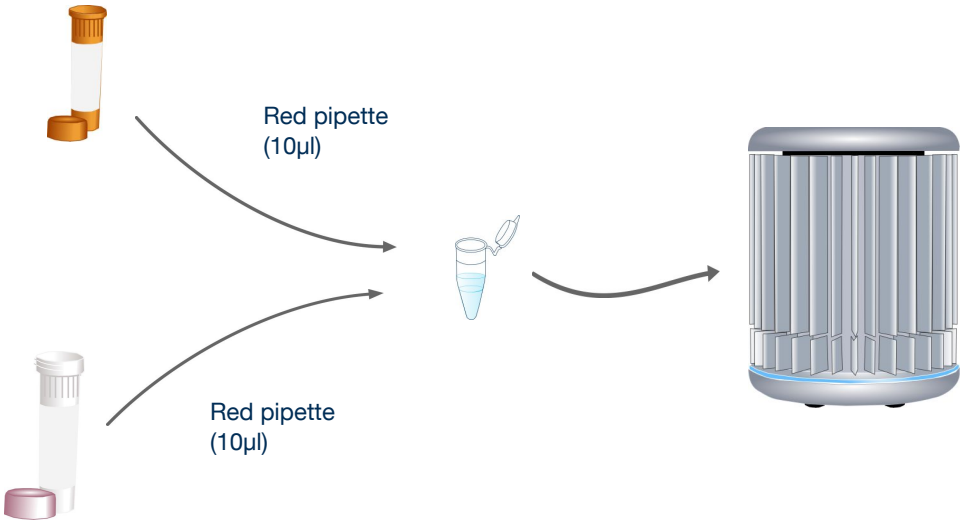


Use the white pipette to transfer 200µl of water into the positive control template tube. Cap and shake tube to mix.

Each time you run a test you will require a positive control. This is a small portion of DNA from your 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.



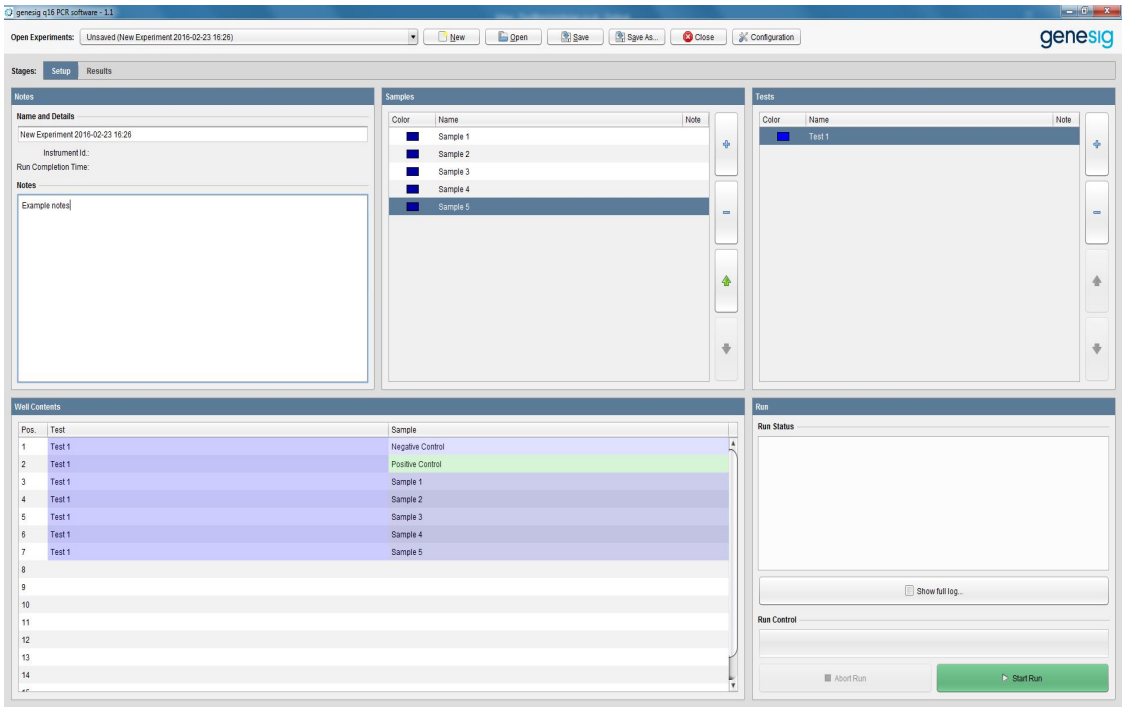
Take great care when setting up your positive control. The positive control template has the potential to give you a false positive signal in your other samples. Set positive controls up last after all other sample tubes are closed. Always change pipette tips between samples. You may even choose to set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

Top tip

- Always 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.

5. 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 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 percentage.

“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 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.

“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 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.

“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 secure as a positive test. 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 “test sensitivity” column. e. g. If the “test sensitivity” is reported at “1%” then the sample can be reported as “positive test with less than 1% meat adulteration.

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 reaction.
2. If this is not possible, check the DNA/RNA extraction protocol for any user errors during preparation and repeat the DNA/RNA extraction.
3. 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 extraction.

“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 sample DNA ”

Explanation
The test has failed because neither the sample quality nor quantity was high enough therefore the sample will not be fit for analysis.

Solutions

1. Try increasing the concentration of the DNA sample that is added to the reaction.
2. If this is not possible, check the DNA/RNA extraction protocol for any user errors during preparation and repeat the DNA extraction.
3. Poor samples can result from overloading the DNA extraction with too much starting material. Try reducing the amount of starting material and repeat the DNA extraction.

Speciation by qPCR

This kit provides a method for detecting Pollachius pollachius mitochondrial DNA that may be present in a food sample. The kit is based on the PCR amplification of a unique species specific tag present in the mitochondrial genome of that species. The mitochondrial genome is an ideal target since it has been sequenced for many different species. This allows comprehensive bioinformatics analysis followed by careful design to ensure specific detection of the desired species whilst excluding detection of other related species. Furthermore, since there are multiple copies of each mitochondrial genome within each cell, the detection sensitivity for this kit is up to 100 times greater than that of a test which targets a single copy locus within the nuclear DNA genome.

The test works by detecting the level of DNA present from the animal of interest and then compares that to the total level of animal DNA in the sample.

Sensitivity

Under optimal conditions, the kit provides exceptional sensitivity and will detect adulterated foodstuffs. Detection of less than 0.1% adulteration is possible.
The sensitivity depends on the quality of the DNA sample that you use. Greater sensitivity is possible with higher levels of good quality DNA. The genesig® q16 will automatically assess the DNA quality and report on the sensitivity of each test on a case-by-case basis.

Specificity

The kit is designed to specifically detect Pollock species that are relevant to the food industry and to give negative detection on other possible animal species.
If you have a query about the detection status of a specific species or sub-species please enquire: enquiry@primerdesign.co.uk

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® Pollock 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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