

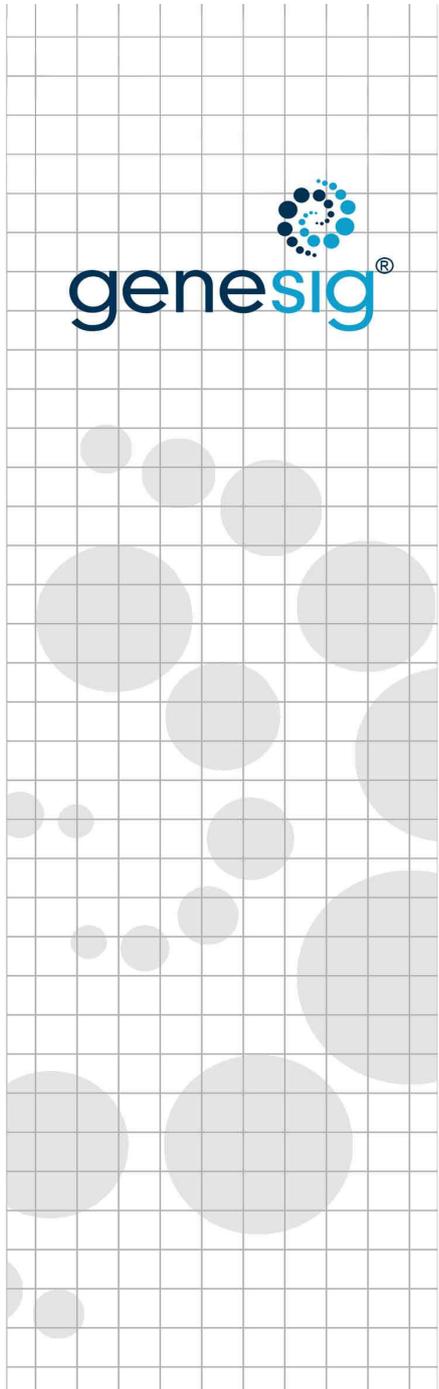


Primerdesign™ Ltd

# Love Bird (Agapornis) Sexing

genesig® Easy Kit  
2 Target Gene Kit  
for use on the genesig q16

50 reaction

A vertical decorative panel on the right side of the page features a light gray grid background. Scattered across the grid are several semi-transparent gray circles of various diameters. A solid blue horizontal bar is positioned at the bottom of this panel, with a small white downward-pointing arrowhead on its left side.

## DNA testing

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

For general laboratory and research use only

# genesig<sup>®</sup> Easy: at a glance guide

## For each DNA test

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

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

## For each positive control

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

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

## For each negative control

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

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

# Kit Contents



- **Agapornis\_spp-W and Agapornis\_spp\_Z specific primer/probe mixes (BROWN)**



- **Lyophilised oasis MasterMix**



- **Lyophilised oasis MasterMix resuspension buffer (BLUE lid)**



- **Agapornis\_spp-W and Agapornis\_spp\_Z positive control templates (RED lid)**



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



- **RNase/DNase free water (WHITE lid)**



- **100 x genesig q16 reaction tubes**

## Reagents and equipment to be supplied by the user

### **genesig® q16 instrument**

### **genesig® Easy DNA/RNA Extraction Kit**

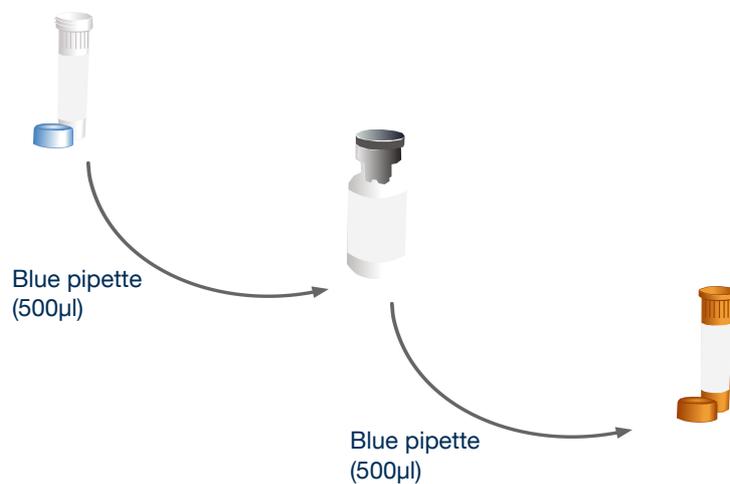
This kit is designed to work well with all processes that yield high quality DNA but the genesig Easy extraction method is recommended for ease of use.

### **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



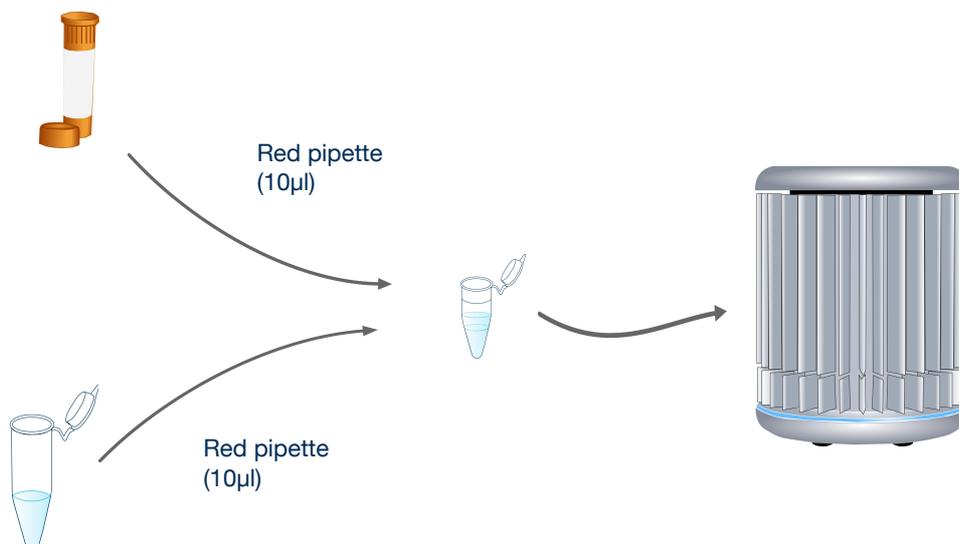
Use the blue pipette to transfer 500µl of the oasis mastermix re-suspension buffer in to the tube of lyophilised oasis mastermix. Then transfer all of that mastermix in to the brown tube labelled Love Bird sexing primers/probe.

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

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

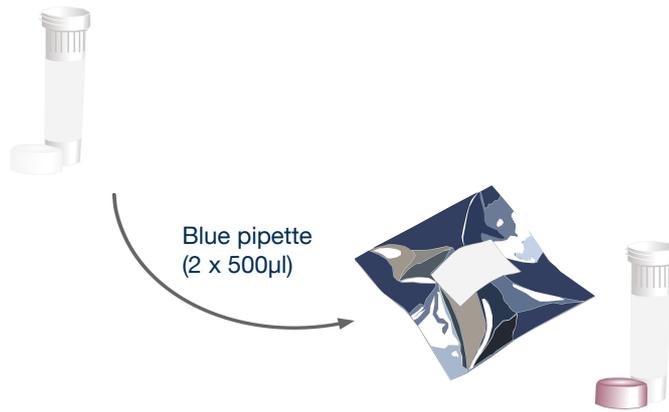
Store the reaction mix in the freezer from hereon.

## 2. Set up your test



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

### 3. Positive control

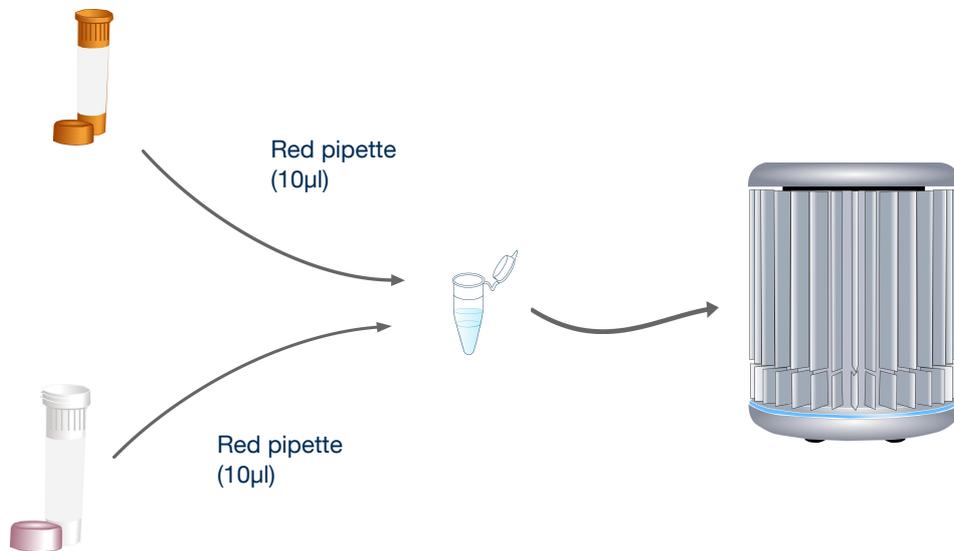


Use the blue pipette to transfer 1000 $\mu$ l (2 x 500 $\mu$ l) of water in to the positive control template tube. Cap and shake tube to mix.

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:

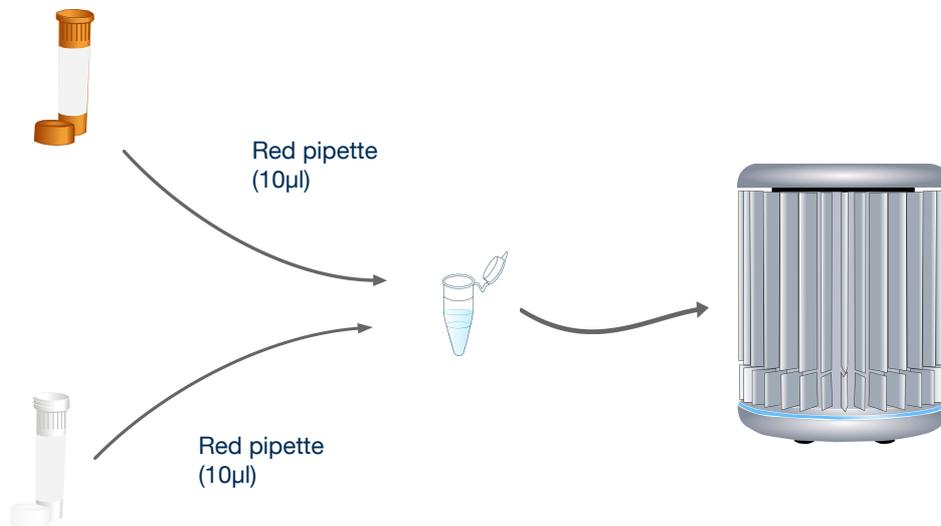
1. It will always test positive so it shows that everything is working as it should be.
2. The q16 software knows how much DNA is present in the positive control. So it can automatically compare your sample of interest with the positive control to calculate the amount of target DNA in your sample.

To create a positive control reaction, simply use 10 $\mu$ l 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.

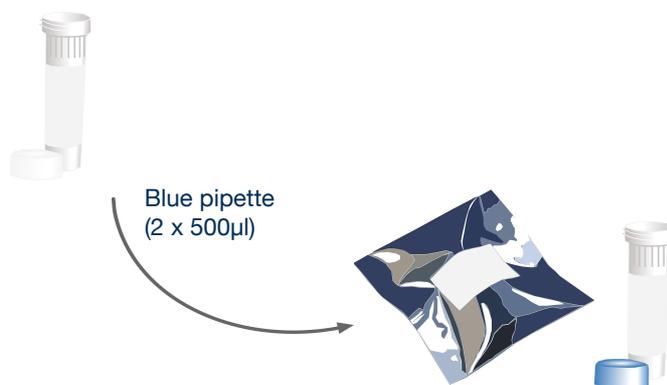
## 4. Negative control



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 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 in to account accordingly.

## 5. Internal extraction control



Use the blue pipette to transfer 1000µl (2 x 500µl) of water in to 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 using an alternative extraction kit:**

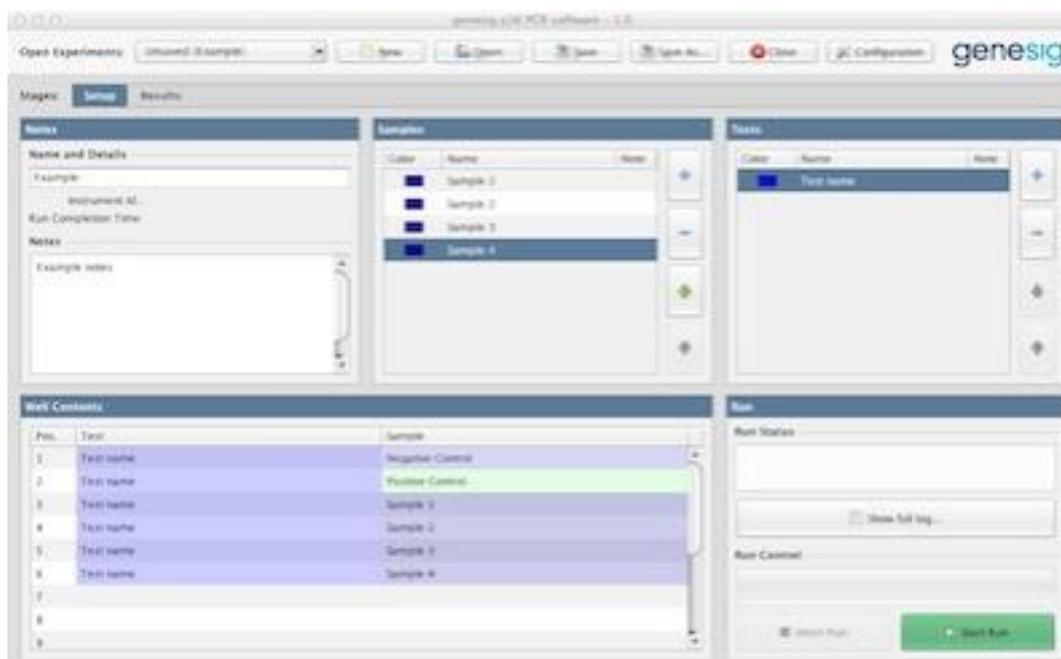
Use the red pipette to transfer 10µl of Internal Extraction Control DNA to your sample after the lysis buffer has been added then follow the rest of the extraction protocol.

**If using samples that have already been extracted:**

Use the grey pipette to transfer 5µl of Internal Extraction Control DNA to your extracted sample.

## 6. Running the test

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



# 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:

The primers/probes target the spindlin gene that is unique for each of the sex chromosomes and targets the variable region.

The kit contains two Agapornis primer and probe sets. The Agapornis\_spp-Z primer and probe set are designed to detect both male and female members of the Lovebird species. The Agapornis\_spp-W primer and probe set is specific to female lovebirds. Samples that test positive for both the Agapornis\_spp-W and Agapornis\_spp-Z kits can be confidently predicted to be female. Samples that only produce a strong positive signal for Agapornis\_spp-Z are male members of the species. Please refer to page 11 for further information about data interpretation.

## “Positive”

### **Explanation**

Your sample has produced a positive result. Your target of interest is present and you can use the reported quantity. As this is a two target gene kit, both target genes must be positive to confirm the test as a genuine positive.

## “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 templates 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 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 in to 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.

### **Solution**

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

genesig EASY extraction kit; you must add 5µl of Internal Extraction Control DNA in to 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.

### Solution

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 handling 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.

## About the test

Lovebirds are small parrots of the genus *Agapornis*, nine species of lovebird exist with eight being native to Africa and one species native to Madagascar. In the wild these species are separated geographically and possess different unique colourings. Determining the sex of lovebirds is difficult, in certain species colourings are identical between sexes. Behavioural cues can give an idea of sex but are not 100% reliable. Some experts may be able to determine sex by feeling birds beneath the body, however this becomes difficult with extremely young birds. The only reliable option is DNA testing, the rationale of this kit is to test for the Z chromosome and the W chromosome. Males are ZZ whilst females are ZW, this means that by testing for each chromosome individually we can determine the sex of the bird using real-time PCR.

## Data interpretation

Agapornis_spp-Z	Agapornis_spp-W	Sex
+ve	+ve	Female*
+ve	-ve	Male
-ve	+ve	Experiment fail
-ve	-ve	Not lovebird species

\*If the signal from *Agapornis\_spp-W* is delayed by more than 3 Cq relative to the *Agapornis\_spp-Z* (i.e. “*Agapornis\_spp-W*” CT value – “*Agapornis\_spp-Z*” CT value < 3), then the *Agapornis\_spp-W* signal should be disregarded and the sample should be confirmed as male. Please refer to Figure 1.

The Cq values for the q16 can be obtained by clicking on the “Details” tab (next to “Sample Results” on the Results page).

Figure 1.

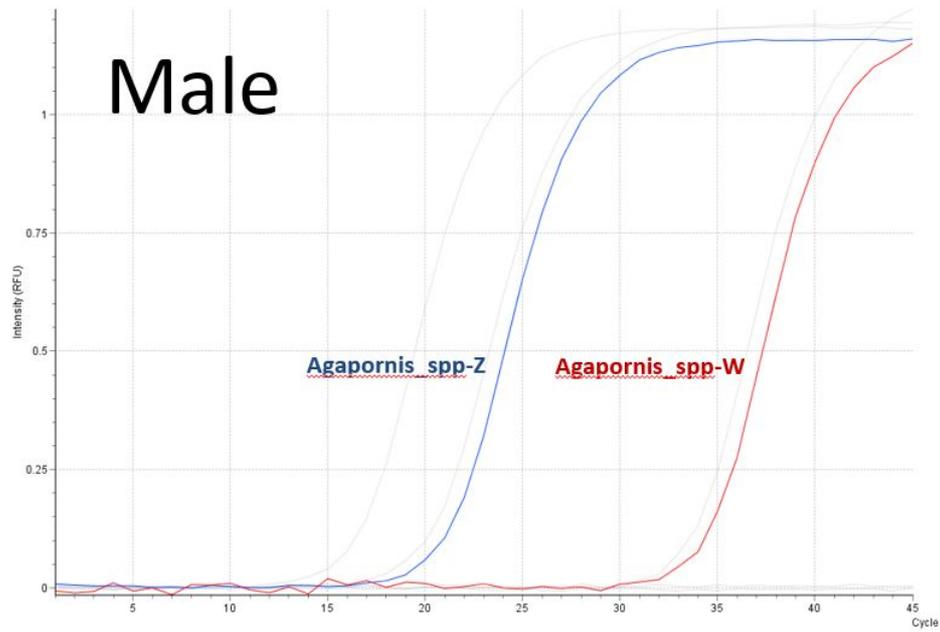
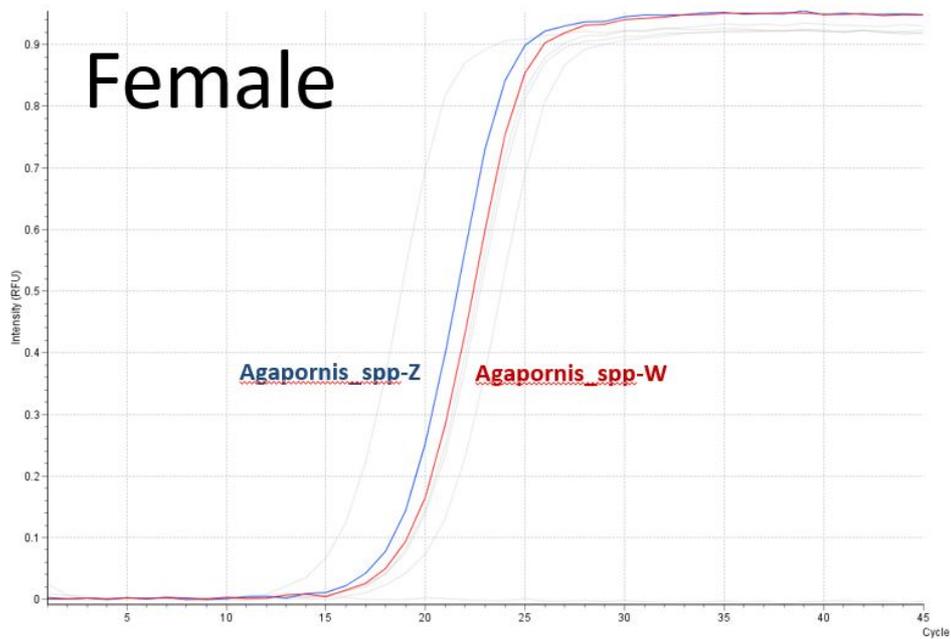


Figure 2.



## Kit storage and stability

This lyophilised kit is stable at room temperature but should be stored at -20°C on arrival. Primerdesign does not recommend using the kit after the expiry date stated on the pack. Once the lyophilised components have been re-suspended, unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

## 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® Love Bird sexing 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 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.

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