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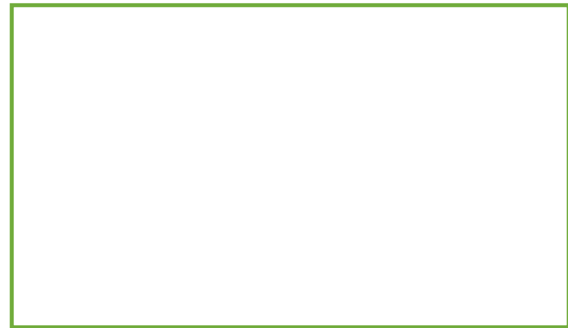
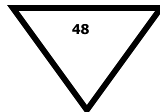


FAST Avian Sexing PCR Kit

REF **MBK0102**



48 TESTS

INTENDED USE

The FAST Avian Sexing PCR kit is intended for gender identification of monomorphic birds.

INTRODUCTION

Gender identification of species is very important for veterinary, medical, and ecological sciences. Nevertheless, sexing based on external morphology is often impossible, because more than 50% of bird species are monomorphic (Vucicevic et al., 2012). Traditional methods for sexual identification as the observation of sex-specific behaviour, laparoscopy, laparotomy or cloacal examination are time consuming, expensive, and, in some case, invasive and dangerous. To avoid this limitation, molecular sexing-based methods using non-invasive means, such as feathers, were improved (Morinha et al., 2012).
The most common method for avian sexing is based on amplification and electrophoresis of the Chromo-helicase-DNA binding protein (CHD) gene on the W and Z chromosomes (Griffiths et al., 2008). The detection of these genes allows for sexing of unknown samples.

PRINCIPLE OF THE ASSAY

The FAST Avian Sexing PCR kit is a PCR-based assay that enables rapid avian molecular sexing using a ready-to-use amplification mix containing specific primer pairs for amplification of the CHD-W and CHD-Z genes, specific for several avian species listed in Table I.

KIT CONTENTS

- 1X Avian Mastermix: 1 x 1000 µL
- Male Control: dried - 50 µL
- Female Control: dried - 50 µL
- Positive Control Reconstitution Buffer: 400 µL

ADDITIONAL EQUIPMENT AND MATERIAL REQUIRED

- Powder free gloves
- Vortex
- Tabletop centrifuge
- Micropipettes and filter tips
- Sterile 1.5 mL vials and sterile 0.2 mL PCR vials
- Thermalcycler
- Agarose gel electrophoresis apparatus

SHIPPING CONDITIONS

Shipping at room temperature has no detrimental effect on the performance of this kit.

STORAGE

Upon arrival, store at -20°C. If stored at recommended temperature all reagents are stable until the expiration date. The performance of kit components is not affected for up to 5 freezing and thawing. If the reagents are to be used only intermittently, they should be stored in aliquots.

GENERAL PRECAUTIONS
The operator should always pay attention to:

- MAINTAIN STRICTLY SEPARATE WORKING AREAS FOR DNA EXTRACTION AND PCR SET-UP
- use pipette tips with filter
- store positive material (specimens and amplicons) separately from all other reagents and, if possible, add it to the reaction mix in a facility separated space
- thaw all components and samples at room temperature before starting an assay

PROTOCOL

1. SAMPLE PREPARATION AND DNA EXTRACTION

Crude total DNA can be obtained by FAST Avian Extraction Kit (Prod. Code: MBK0101, Diatheva). Purified total DNA can be obtained by Genomic DNA Isolation Kit MBK0069 (Prod. Code: Kit MBK0069, Diatheva), please follow the procedure described in Annex A. Other commercial DNA extraction or extraction/purification systems can be used. However, the compatibility must be verified by the end user. Please refer to Diatheva technical support for additional information.

2. AMPLIFICATION STEP

IMPORTANT NOTE: The FAST Avian Sexing PCR kit has been formulated for testing several avian species. Be sure to use the correct thermal protocol based on species to be tested. Please refer to Table I for the distinct species to analyse.

2.1 PCR SET UP

- Thaw the **1X Avian Mastermix**, vortex for 15 seconds and briefly spin vial in a microcentrifuge before opening
- Aliquot 18 µL of **1X Avian Mastermix** in the PCR tubes
- In a separate area, add **4 µL** of the crude DNA samples or **1 µL** of the purified DNA samples to be tested into the corresponding PCR vial containing PCR mix and centrifuge briefly
- In the same separate area, reconstitute Male Control and Female Control (dried) by adding 50 µL of Positive Control Reconstitution Buffer, vortex for 60 seconds and centrifuge briefly. Add **4 µL** of Male and Female Control into the corresponding PCR vial containing amplification mix (It is possible to add **1 µL** of Male and Female Control if purified DNA samples are analysed)
- NOTE: Store the reconstituted Male and Female Controls at -20°C

2.2 PCR RUN

- Program the PCR thermal cycler with the following parameters:

Important note: Different species require different annealing temperatures

1 cycle	95°C for 3 min
35 cycles	95°C for 30 sec 50-62°C***** for 15 sec 60°C for 30 sec
1 cycle	72°C for 5 min
	cool down to 4°C

*****for appropriate annealing temperature (**See Table I**)

- Set the reaction volume to **22 µL** if testing crude DNA or **19 µL** if testing purified DNA
- Perform the PCR run
- When the run is completed, proceed immediately to the next step (Paragraph 3: Agarose Gel Electrophoresis) or store the reaction at +4°C for 24-48 hours or at –20°C for a longer time.

3. AGAROSE GEL ELECTROPHORESIS

- Add 5 µL of DNA loading buffer directly to amplified samples
- Load 15 µL of amplicons on a 2.5% agarose gel containing ethidium bromide or any other stain gel agent, in the presence of a DNA standard specific for the low range (100-1000 bp).
- To correctly identify all obtained PCR products, run the gel as long as amplicons are well distinguishable as shown below (Fig.1).

4. INTERPRETATION OF RESULTS

If Male and Female Control have been included check the validity of results according to the following scheme:
Male Control: presence of one band with size corresponding to 380 bp (indicating a successfully performed PCR)
Female Control: presence of two bands with size corresponding to 380 & 480 bp (indicating a successfully performed PCR)

PCR products loaded on 2.5% agarose gel will show only one band in male and two bands in female samples (Fig.1 and 2).
Note: DNA samples obtained from Female birds extracted using purification column can generate a third band 20 bp longer than male band, anyway, there is no ambiguity in results interpretation (Fig.2)

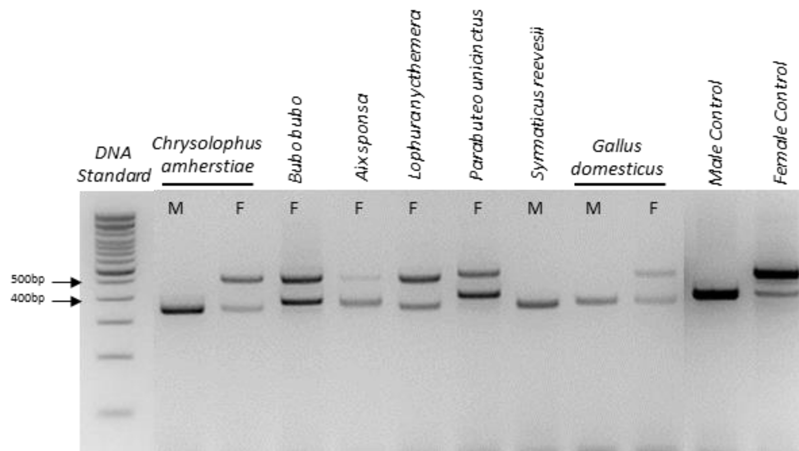


Fig.1: Representative agarose gel electrophoresis analysis of amplicons obtained through the amplification of specific avian species and Male and Female Controls using FAST Avian Sexing PCR Kit. Different PCR product sizes (bp) are obtained for any bird species.

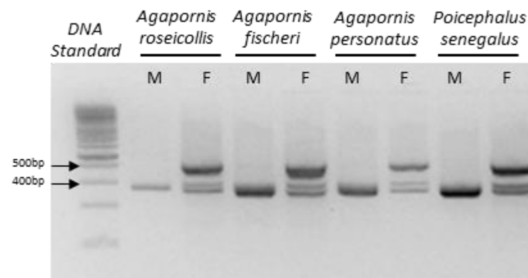


Fig.2: Representative agarose gel electrophoresis analysis of three amplicons obtained through the amplification of female birds

For the interpretation of results please refer to Table I:

Table I: Optimal annealing temperature and sizing of PCR product

Order	Family	Latin name	Annealing temperature	PCR product sizes (bp)
Accipitriformes	Accipitridae	*Aquila chrysaetos*	55°C	M (380 bp); F (380 & 480 bp)
		Aquila nipalensis	55°C	M (400 bp); F (400 & 500 bp)
		Geranoaetus melanoleucus	55°C	M (380 bp); F (380 & 480 bp)
		Parabuteo unicinctus	58°C	M (380 bp); F (380 & 480 bp)
	Cathartidae	*Cathartes aura*	58°C	M (380 bp); F (380 & 480 bp)
Anseriformes	Anatidae	*Aix sponsa*	55°C	M (380 bp); F (380 & 480 bp)
		Anser anser domesticus	55°C	M (380 bp); F (380 & 480 bp)
		Anser cygnoides	55°C	M (380 bp); F (380 & 480 bp)
		Anas platyrhynchos	55°C	M (380 bp); F (380 & 480 bp)
		Cairina moschata	55°C	M (380 bp); F (380 & 480 bp)
		Tadorna ferruginea	55°C	M (360 bp); F (360 & 460 bp)
Columbiformes	Columbidae	*Columba livia (pigeon)* [a]	50°C	M (380 bp); F (380 & 480 bp)
Coraciiformes	Coraciidae	*Coracias garrulus*	55°C	M (380 bp); F (380 & 480 bp)
Falconiformes	Falconidae	*Crested caracara*	55°C	M (380 bp); F (380 & 480 bp)
		Falco biarmicus feldeggii	58°C	M (400 bp); F (400 & 500 bp)
		Falco cherrug	55°C	M (380 bp); F (380 & 480 bp)
		Falco peregrinus	55°C	M (380 bp); F (380 & 480 bp)
		Falco tinnunculus	58°C	M (400 bp); F (400 & 500 bp)
Galliformes	Numididae	*Numida meleagris*	55°C	M (380 bp); F (380 & 480 bp)
	Phasianidae	*Pavo cristatus*	58°C	M (360 bp); F (360 & 460 bp)
	Phasianidae	*Chrysolophus amherstiae*	55°C	M (380 bp); F (380 & 480 bp)
		Chrysolophus pictus	58°C	M (380 bp); F (380 & 480 bp)
		Chrysolophus pictus luteus	58°C	M (380 bp); F (380 & 480 bp)
		Gallus domesticus	55°C	M (360 bp); F (360 & 460 bp)

		Lophura nycthemera	55°C	M (380 bp); F (380 & 480 bp)
		Phasianus colchicus mongolicus	55°C	M (380 bp); F (380 & 480 bp)
		Syrmaticus reevesii	55°C	M (380 bp); F (380 & 480 bp)
Passeriformes	Fringillidae	*Carduelis carduelis* [b]	52°C	M (360 bp); F (360 & 460 bp)
		Carduelis major [b]	52°C	M (360 bp); F (360 & 460 bp)
		Chritagra mozambica [c]	52°C	M (380 bp); F (380 & 480 bp)
		Eophona personata	55°C	M (380 bp); F (380 & 480 bp)
		Loxia leucoptera	50°C	M (380 bp); F (380 & 480 bp)
		Serinus canaria [d]	52°C	M (360 bp); F (360 & 460 bp)
	Oriolidae	*Oriolus chinensis*	55°C	M (380 bp); F (380 & 480 bp)
	Pycnonotidae	*Spizixos semitorques*	60°C	M (380 bp); F (380 & 480 bp)
	Sylviidae	*Paradoxornis gularis o Psittiparus gularis*	55°C	M (380 bp); F (380 & 480 bp)
	Thraupidae	*Dacnis cayana*	55°C	M (380 bp); F (380 & 480 bp)
		Tangara arthus	55°C	M (380 bp); F (380 & 480 bp)
		Tangara Cyanocephala	55°C	M (380 bp); F (380 & 480 bp)
		Tangara guttata	55°C	M (380 bp); F (380 & 480 bp)
		Tangara nigroviridis	55°C	M (380 bp); F (380 & 480 bp)
	Turdidae	*Turdus iliacus*	55°C	M (380 bp); F (380 & 480 bp)
		Turdus merula	55°C	M (380 bp); F (380 & 480 bp)
		Turdus philomelos	55°C	M (380 bp); F (380 & 480 bp)
		Turdus pilaris	55°C	M (380 bp); F (380 & 480 bp)
Psittaciformes	Cacatuidae	*Nymphicus hollandicus*	55°C	M (380 bp); F (380 & 480 bp)
	Psittacidae	*Agapornis fischeri*	58°C	M (380 bp); F (380 & 480 bp)
		Agapornis nigrigenis	58°C	M (380 bp); F (380 & 480 bp)
		Agapornis personatus	58°C	M (380 bp); F (380 & 480 bp)
		Agapornis roseicollis	55°C	M (380 bp); F (380 & 480 bp)
		Amazona aestiva xanthopteryx	60°C	M (380 bp); F (380 & 480 bp)
		Amazona amazonica[e]	58°C	M (380 bp); F (380 & 480 bp)
		Amazona ochrocephala	60°C	M (380 bp); F (380 & 480 bp)
		Ara ararauna	55°C	M (380 bp); F (380 & 480 bp)
		Ara chloropterus	55°C	M (380 bp); F (380 & 480 bp)
		Aratinga pertinax	55°C	M (380 bp); F (380 & 480 bp)
		Aratinga solstitialis	55°C	M (380 bp); F (380 & 480 bp)
		Forpus coelestis	58°C	M (380 bp); F (380 & 480 bp)
		Myiopsitta monachus	62°C	M (380 bp); F (380 & 480 bp)
		Pionites heine	58°C	M (380 bp); F (380 & 480 bp)
		Pionites leucogaster xanthomeria	55°C	M (420 bp); F (420 & 520 bp)
		Pionites melanocephalus	55°C	M (420 bp); F (420 & 520 bp)
		Pionus maximiliani	55°C	M (380 bp); F (380 & 480 bp)
		Poicephalus senegalus	58°C	M (380 bp); F (380 & 480 bp)
		Psittacula krameri	55°C	M (380 bp); F (380 & 480 bp)
		Psittacus erithacus	58°C	M (380 bp); F (380 & 480 bp)
		Pyrrhura molinae	55°C	M (420 bp); F (420 & 520 bp)
		Trichoglossus haematodus	55°C	M (380 bp); F (380 & 480 bp)
	Psittaculidae	*Chalcopsitta atra*	58°C	M (380 bp); F (380 & 480 bp)
		Chalcopsitta duivenbodei	55°C	M (380 bp); F (380 & 480 bp)
		Melopsittacus undulatus	55°C	M (380 bp); F (380 & 480 bp)
		Platycercus eximius	55°C	M (380 bp); F (380 & 480 bp)
		Polytelis anthopeplus	55°C	M (380 bp); F (380 & 480 bp)
		Pseudeos fuscata	55°C	M (380 bp); F (380 & 480 bp)
		Trichoglossus rubritorquis	55°C	M (380 bp); F (380 & 480 bp)
Strigiformes	Strigidae	*Athene noctua*	58°C	M (380 bp); F (380 & 480 bp)
		Bubo africanus	55°C	M (380 bp); F (380 & 480 bp)
		Bubo bengalensis	55°C	M (380 bp); F (380 & 480 bp)

		Bubo bubo	55°C	M (380 bp); F (380 & 480 bp)
		Bubo bubo sibiricus	55°C	M (380 bp); F (380 & 480 bp)
		Bubo scandiacus	55°C	M (380 bp); F (380 & 480 bp)
		Ninox boobook	58°C	M (380 bp); F (380 & 480 bp)
		Ninox novaeseelandiae	58°C	M (380 bp); F (380 & 480 bp)
		Strix ocellata	55°C	M (380 bp); F (380 & 480 bp)
		Strix aluco	55°C	M (380 bp); F (380 & 480 bp)
		Surnia ulula	58°C	M (380 bp); F (380 & 480 bp)
	Tytonidae	*Tyto alba*	58°C	M (380 bp); F (380 & 480 bp)

a) Presence of 600 bp non-specific band that does not compromise the correct result
b) Presence of non-specific bands, if the bird is male there is a 360 bp more intense band, if it is female there is a 460 bp more intense band
c) Presence of a 900 bp non-specific band that does not compromise the correct result
d) Presence of non-specific bands in purified DNA extracts, if the bird is male there is a 360 bp more intense band
e) Presence of a 700 bp non-specific band that does not compromise the correct result

TROUBLESHOOTING

Observations	Possible cause	Suggested solution
Absence of PCR product	Error in adding nucleic acid to the amplification tube	Check the correct sample addition to the corresponding amplification tube according to the extraction method used
	Incorrect preparation of the amplification mix	Confirm the addition of all the components to the reaction mix. All the reagents should be well mixed and centrifuge before the use
	Incorrect setup of the PCR instrument	Check the thermal protocol settings and annealing temperature for the specific avian species and repeat the test using the correct settings
	Poor quality template	DNA extraction should be performed according to the manufacturer's instructions of the recommended extraction kit (as indicated above). Repeat the extraction and amplification step
	Insufficient starting template	Repeat the extraction phase by increasing the material to be processed to 2 or more feathers. Repeat the test
	Wrong reagent conservation or use of the reagents beyond the expiration date	Check the storage conditions and use a new kit if necessary
	Presence of PCR inhibitors	Dilute DNA starting template
Non-specific extra bands on gel	High concentration of nucleic acid	Dilute DNA starting template
	Cross-reaction with non-target sequences	Run the amplicons for a longer time to distinguish any non-specific bands from target amplicons.
Presumed error in the result: "female" sample tested with "male" result	Error in adding nucleic acid to the amplification tube	Check the correct sample addition to the corresponding amplification tube according to the extraction method used
	Incorrect preparation of the amplification mix	Confirm the addition of all the components to the reaction mix. All the reagents should be well mixed and centrifuge before the use
	Incorrect setup of the PCR instrument	Check the thermal protocol settings and annealing temperature for the specific avian species and repeat the test using the correct settings
	Poor quality template	DNA extraction should be performed according to the manufacturer's instructions of the recommended extraction kit (as indicated above). Repeat the extraction and amplification step
	Insufficient starting template	Repeat the extraction phase by increasing the material to be processed to 2 or more feathers. Repeat the test
	Wrong reagent conservation or use of the reagents beyond the expiration date	Check the storage conditions and use a new kit if necessary
	Presence of PCR inhibitors	Dilute DNA starting template
Presumed error in the result: "male" sample tested with "female" result	Contamination	Decontaminate all the surfaces and work areas with sodium hypochlorite. Repeat the entire procedure starting from the extraction step
	Error in adding nucleic acid to the amplification tube	Check the correct sample addition to the corresponding amplification tube according to the extraction method used
	Incorrect preparation of the amplification mix	Confirm the addition of all the components to the reaction mix. All the reagents should be well mixed and centrifuge before the use
	Incorrect setup of the PCR instrument	Check the thermal protocol settings and annealing temperature for the specific avian species and repeat the test using the correct settings
	Wrong reagent conservation or use of the reagents beyond the expiration date	Check the storage conditions and use a new kit if necessary
	Too much starting template	Dilute DNA starting template
	Cross-reaction with non-target sequences	Run the amplicons for a longer time to distinguish any non-specific bands from target amplicons.

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