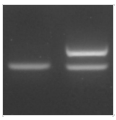

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Working

PCR Protocol for chicken sex identification.

PLOS One

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ABSTRACT

This is an invasive PCR based chicken gender determination protocol to identify the sex of fertilized egg at the early chicken's embryo states. It is based on the differences between males and females sexual chromosomes: ZZ for males and ZW for females.

Two set of primers have been developped for this method: SWIM and 12S. They amplify 2 amplicons for female samples and 1 amplicon for male samples.

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0213033

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

He L, Martins P, Huguenin J, Van T, Manso T, Galindo T, Gregoire F, Catherinot L, Molina F, Espeut J (2019) Simple, sensitive and robust chicken specific sexing assays, compliant with large scale analysis. PLoS ONE 14(3): e0213033. doi: 10.1371/journal.pone.0213033

PROTOCOL STATUS

Working

Lysis of chicken embryo's tissue and DNA extraction

1 **1.** Prepare the Lysis Buffer:

Composition for 100 ml Buffer	Quantity (Volume)	Final Concentration
Chelex Resin (Biorad Chelex 100)	10g	10%
SDS 20 %	1 ml	0.2%
Tris 1M pH8	1 ml	10 mM
Nuclease Free Water	qsp 100 ml	

2. Put 150 µl of lysis buffer in each 1.5 ml tube or well of 96-well plate for each sample to analyze.

3. Pipet 10~20 mg of embryo tissues from the embryo head (brain), membrane, chorion or others into the tube/well containing the lysis buffer.

4. Add 1.5 µl proteinase K at 20 mg/ml stock into each tube/well and vortex 3 seconds.

5. Incubate the tubes/plate in the thermocycler. **03:15:00**

Program:

· Heat lid at 60°C

· Incubate 3h at 55°C

· Incubate 15 min at 95°C

· Hold at 4°C

6. Centrifuge 5 minutes at 13000 rpm (or 15 mins at 8000 rpm) at room temperature. 00:05:00

7. Pipet 50 µl-100 µl of the supernatant and transfer to a new 96-well plate (this primary stock solution contains the template DNA at a concentration between 500 and 1000 ng/µl per tube) and store it:

- at 4°C if you are doing the PCR the same day.

- at -20°C if you are going to do the PCR another day (DNA is degraded with time in biological extracts).

8. Dilute the supernatant 10 times into a new 96-well plate (For example add 10 µl of the supernatant to 90 µl of nuclease free water). It will give a final concentration of prepared template DNA between 50 and 100 ng/µl.

Prepare the Primers Ddilutions

2 Two set of primers (12S & SWIM) are used in this protocol for positive control (12S) and female indication (SWIM).

SWIM Primers

SWIM Forward: GAG ATC ACG AAC TCA ACC AG

SWIM Reverse: CCA GAC CTA ATA CGG TTT TAC AG

12S Primers

12S Forward: CTA TAA TCG ATA ATC CAC GAT TCA

12S Reverse: CTT GAC CTG TCT TAT TAG CGA GG

Prepare a 10 µM primer working solution from 100 µM primer stock.

PCR Setup

3 **1.** Assemble all reaction components **on ice in this order:**

Component	25 µl reaction	50 µl reaction	Final Concentration
Nuclease Free Water	qsp 25 µl	qsp 50 µl	-
Platinium Green Hot Start PCR 2X Master Mix	12.5 µl	25 µl	1X
10 µM Forward Primer SWIM-F	0.5 µl	1 µl	0.2 µM
10 µM Forward Primer 12S-F	0.5 µl	1 µl	0.2 µM
10 µM Reverse Primer SWIM-R	0.5 µl	1 µl	0.2 µM
10 µM Reverse Primer 12S-R	0.5 µl	1 µl	0.2 µM
DNA extract	0.5 - 1 µl	1 µl	

The final concentration of MgCl2 is 1.5mM and each dNTP is at 0.2mM final.

2. Seal the plate, vortex and centrifuge briefly.

3. Transfer the PCR plate into the thermocycler.

Thermo-cycling conditions

4 01:00:00

Step		Temperature	Time
Preheat the lid		110°C	-
Initial Denaturation		94°C	2 min
35 Cycles	Denature	94°C	30 sec
	Anneal	55°C	30 sec
	Extend	72°C	30 sec
Final Extension		72°C	5 min
Hold		4°C	-

Results analysis

5 PCR amplifications can be analyzed by different manners:

- By electrophoretic migration on agarose gel:

For each sample, mix 10 µl of PCR product with 2 µl of 6X loading dye. Load them on a 2.5 % Gelgreen (Biotium, California USA) stained agarose gel in 0.5X TAE buffer and migrate DNA by electrophoresis at 100 Volts. 1 µg of DNA ladder (GeneRuler 50bp, Fisher Scientific, Illkirch France) can serve as a reference for the migration. Reveal the gel by Blue LED GelPicBox at 430nm (Nippongenetics, Düren Germany) or on a regular UV lamp.-

- By capillary electrophoresis:

Load 10 µl of the PCR reactions on a microfluidic capillary electrophoresis systems (Capillary LifeSciences, France) controlled by the Labchip GX version 4.1.1619.0 SP1 software. Capillary electrophoresis is the high throughput format of the traditional gel electrophoresis. It allows a rapid size-based separation and sensitive detection of specific DNA fragments via UV absorption or fluorescent labelling. The CE system is more advantageous than the conventional slab gel electrophoresis in terms of speed, high-throughput applicability, automated workflow, resolution, and sensitivity.

Results for males: When there is only one band at 131 bp appearing on the agarose gel, sample gender is male.

Results for females: When two bands at 131bp and 212bp are appearing on the agarose gel, sample gender is female.

Invalid results: If no band appear on the gel, it is invalidated. In this case, repeat the experience from PCR setup.

For more detail please look at our publication "Simple, sensitive and robust chicken specific sexing assays, compliant with large scale analysis" by Lyan He et al.