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SUPPORTING INFORMATION

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Sexing assay for chickens and other birds for large-scale application based on a conserved sequence variant in *CHD1* genes on W and Z chromosomes

Several PCR-based methods are known for sexing of chickens and other birds (Çakmak et al., 2017; Eiras et al., 2018; Gruszczyńska & Grzegrzółka, 2021; Morinha et al., 2012). While some methods for bird sexing are suitable for large-scale analyses (Chen et al., 2012; Clinton et al., 2016; He et al., 2019; Margulis & Danielli, 2019; Morinha et al., 2013; Rosenthal et al., 2010), research is still ongoing because most of these methods are costly and time consuming. Here we report a newly developed, easy to use competitive allele-specific PCR (KASP) assay that is suitable for large-scale sexing in chickens and other birds. The KASP assay is based on an A/G difference in exon 17 between the W- and Z-chromosomal variants of the conserved *chromodomain helicase DNA binding protein 1* (*CHD1*) in exon 17 (Figure S1). Sex-specific primers were designed up- and downstream of this variant in *CHD1* genes to amplify a product of 46 bp (Figure 1; Table S1, Figure S1). The amplicon overlaps with the PCR product for sex genotyping of the method of Fridolfsson and Ellegren (1999) (Figure S1). Furthermore, similar sequences were obtained from NCBI databases using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for duck, goose, quail and turkey and were aligned with the chicken sequence (Figure 1).

In total, 734 chicken samples with known sex were analysed (Table S2). In addition, 55 samples of other species of the orders Galliformes and Anseriformes were analysed (Table S2). If the sex of non-chicken samples was unknown it was verified by multiplex PCR modified from Fridolfsson and Ellegren (1999) (Table S1, Figure S1). Samples were mainly taken from an extensive DNA collection, which was set up within the framework of the projects AVIANDIV (Lyimo et al., 2014) and SYNBREED (www.synbreed.tum.de).

Each KASP reaction contained 20–50 ng of template DNA, KASP v. 4.0 2× Master mix standard ROX and the KASP-by-Design assay mix (LGC Genomics). The standard KASP thermal cycling conditions according to LGC protocols were performed in an Eppendorf Mastercycler (Eppendorf). After amplification, microplates were analysed with FLUOstar Omega (BMG Labtech) using excitation and emission values of 485/520 nm for the FAM-labelled-FRET cassette, 530/560 nm for the HEX-labelled-FRET cassette and 584/620 nm for the ROX standard.

All chickens, ducks, geese, quails and turkeys were correctly assigned to their sex using the newly developed KASP assay (Table S2). This newly developed KASP assay is well suited to chicken, duck, goose, quail and turkey for efficient sex determination on a larger scale.

KEYWORDS

bird, *CHD1*, chicken, KASP, sexing

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Z chromosome
KASP Primer FZ      CTTGACAAGCTACTGATTCGTCTG
KASP Primer R                                GAACGTGGCAACAGAGTWC
Chicken             CTTGACAAGCTACTGATTCGTCTGCGAGAACGTGGCAACAGAGTTC
Duck                CTTGACAAGCTACTGATTCGTCTGAGAGAACGTGGCAACAGAGTTC
Goose               CTTGACAAGCTACTGATTCGTCTGAGAGAACGTGGCAACAGAGTTC
Quail               CTTGACAAGTTACTGATTCGTCTGCGAGAACGTGGCAACAGAGTTC
Turkey              CTTGACAAACTACTGATTCGGCTGCGAGAACGTGGCAACAGAGTTC

W chromosome
KASP Primer FW      CTTGACAAGTTACTGATTCGTCTA
KASP Primer R                                GAACGTGGCAACAGAGTWC
Chicken             CTTGACAAGTTACTGATTCGTCTACGAGAACGTGGCAACAGAGTAC
Duck                CTTGACAAGCTACTGATTCGTCTACGAGAACGTGGCAACAGAGTAC
Goose                       GTTACTGATTCGTCTACGAGAACGTGGCAACAGAGTAC
Quail                       GTTACTGATTCGTCTACGAGAACGTGGCAACAGAGTAC
Turkey              CTTGACAAGTTACTGATTCGTCTACGAGAACGTGGCAACAGAGTAC
                    ********  ********** **↑ ******************** *
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FIGURE 1 Sequence alignment of W and Z chromosomes of the amplicon amplified by the sex KASP assay developed here. Included are the primers (KASP primer FZ, FW and R), parts of chicken (*Gallus gallus*) *CHD1*_Z (NC_052572.1:51980340–51980385) and corresponding chicken (*G. gallus*) *CHD1*_W sequences (NC_052571.1:5554074–5554119 (reversed)). Furthermore, sequences for duck (*Anas platyrhynchos*; W, XM_038169386.1:2924–2969; and Z, LS423640.1:50147602–50147647), goose (*Anser cygnoides*; W, HQ423306.1:1–38; and Z, XM_013193416.1:2522–2567), quail (*Coturnix*; W, FJ937780.1:409–446; and Z, NC_029547.1:45496568–45496613) and turkey (*Meleagris gallopavo*; W, XM_019610844.2:99–144; and Z, XM_031557424.1:1669–1714) were aligned with chicken. We observed a sex-specific allele in the chicken *CHD1* gene (Z, NC_052572.1:g.51980364; and W, NC_052571.1:g.5554096 (reversed)) that seems to be highly conserved in bird species (indicated by an arrow). A 'G' was detected in the sequences of the Z chromosomes and an 'A' was detected in the corresponding sequences of the W chromosomes at this position. Asterisks indicate conserved bases between both sex chromosomes as well as between the species included here

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

The data supporting the findings of the present study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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Evaluation of truncating variants in the *LCORL* gene in relation to body size of goats from Switzerland

BACKGROUND

More than 200 years ago, the formation of goat breeds began through morphological standardisation, especially of coat colour and body size, and systematic selection to improve production traits (Burren et al., 2016). In Switzerland, breed formation, followed by various breeding objectives and selection programmes, resulted in 10 modern goat breeds (Henkel et al., 2019). Recently, the VarGoats project generated individual whole genome sequencing data from representatives of these Swiss breeds beside 116 further *Capra hircus* breeds, including the Boer goat originating from Africa, to understand the consequences of domestication and breeding (Denoyelle et al., 2021).

Variation in stature or body size in domestic animals such as cattle or dogs is generally controlled by fewer genes with greater effects than in humans (Bouwman et al., 2018; Plassais et al., 2019). The *ligand-dependent nuclear receptor corepressor-like* gene (*LCORL*) gene encoding a transcription factor has been repeatedly found to be associated with measures of skeletal frame size and adult height in humans and dogs (Plassais et al., 2019; Soranzo et al., 2009). Alternative splicing results in multiple *LCORL* transcript variants. Similar to humans, in goats, one transcript is long encoding isoform X1 (1864 aa, XP_017904811.1) and several that are significantly shorter (e.g. 601 aa, XP_017904814.1), differing significantly in the sequence of the last exons. Alignment of the human (NP_001381375.1) and caprine (XP_017904811.1) LCORL protein sequences revealed a strong 82% match. Recently, a search for signatures that are shared across large-sized goat breeds revealed that five medium-to-large-sized Pakistani goat breeds had a common selection signature on chromosome 6 in a region harbouring the *LCORL* gene (Saif et al., 2020). Subsequent sequencing analyses proposed a frameshift variant in *LCORL* exon 7 (p.Ser277fs) as potentially causal variant mediating the body size-increasing effect (OMIA 002246-9925). The long LCORL isoform X1 contains a DUF4553 DNA-binding domain from amino acid position 1404 to 1860 within the deleted segment of the derived caprine allele. Due to strong conservation of this DNA-binding domain across mammals it could be speculated that, in large goats, the truncation may disrupt transcription factor binding of LCORL with its target. The same was reported for dogs, as a single nucleotide insertion in the last exon of the long isoform of *LCORL*, resulting in a premature stop codon after amino acid 1221 and a significantly truncated protein, has never observed small breeds, whereas it is present in medium and large breeds (OMIA 002246-9615) (Plassais et al., 2019).