Table 1 Assignment of equine clock genes by FISH.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences (5'-3')</th>
<th>PCR product (bp)</th>
<th>CHORI-241 clone</th>
<th>Co-localized marker</th>
<th>ECA</th>
<th>Conserved synteny to HSA¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARNTL</td>
<td>F: ggc cct gtt gac ttt agt gac ttg c</td>
<td>270</td>
<td>91:D20</td>
<td>LYVEY¹⁰</td>
<td>7q16-q18</td>
<td>11p15</td>
</tr>
<tr>
<td></td>
<td>R: atc cac agc tag ccc aat aat cca tga</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PER2</td>
<td>F: cca gca aat att tct gaa gca tgc a</td>
<td>127</td>
<td>44:A20</td>
<td>INHA¹³</td>
<td>6P12-P13</td>
<td>2q37</td>
</tr>
<tr>
<td></td>
<td>R: ggc atc agc agc cag aca gg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLOCK</td>
<td>F: agt atc tag aca aat tca tga aaa gaa aac gag tag a</td>
<td>124</td>
<td>11:A9</td>
<td>ADH1C¹²</td>
<td>3q21</td>
<td>4q12</td>
</tr>
<tr>
<td></td>
<td>R: gct ctt ctc tag aac agt aca ttt gtc cat c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRY1</td>
<td>F: tgg gca act gtt atg gcc tga att tt</td>
<td>242</td>
<td>73:13</td>
<td>MGE¹³</td>
<td>28q15-q16</td>
<td>12q23</td>
</tr>
<tr>
<td></td>
<td>R: ccc gtt tca gga agc aag cca ct</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

¹Conserved synteny to the human whole-genome sequence (Build 36.1).

Exclusion of Wilms tumour (WT1b) and ovarian cytochrome P450 aromatase (CYP19A1) as candidates for sex determination genes in Nile tilapia (Oreochromis niloticus)

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Source description: The Wilms tumour (WT1) and cytochrome P450 aromatase (CYP19A1) genes are involved in mammalian sex differentiation, and are considered to be candidate genes for sex determination in those vertebrates in which major sex-determining genes have not been identified. WT1 encodes a zinc-finger DNA-binding protein that plays a critical role in kidney and gonadal development in mammals. Recent evidence suggests that, during gonadal development, WT1 upregulates SRY transcription by directly binding to DNA.¹ Cytochrome P450 aromatase is a steroidogenic enzyme responsible for the conversion of androgens to estrogens, which is a key reaction during sex differentiation in vertebrates. Tilapia ovarian aromatase appears to play an important role in sex differentiation and ovarian development.²,³ The 5' flanking region of the tilapia CYP19A1 gene has binding sites for SF-1, WT1 and SRY, all of which are key sex-determining factors in mammals.³

Primer design: Primers WT1_2F (5'-AACCTGTACCAATGG-CATC-3') and WT1_2R (5'-GATGGGACAGTTTGAAGTAT-3''), which amplify an intron of tilapia WT1, were designed from an EST (NM0513.39_D11_07.seq) derived from tilapia gonad cDNA library (http://reprobio.nibb.ac.jp/). Primers tCYP19aF (5'-AAATCTGGAACTATGTTAATGGA-3') and tCYP19aR (5'-GCAGAGATCAGATCCATGAGA-3'), which amplify the promoter region of tilapia CYP19A1, were designed by using a complete coding sequence deposited in GenBank (AF472620).⁴

Sequencing and identification of SNPs: Parental DNA samples from two Nile tilapia families (F5 and F7) were sequenced to identify SNPs in each locus. For tilapia CYP19A1, the tCYP19aF primer was used for cycle sequencing and a SNP (AF472620:g.2124G>A) was found. Sires were g.2124AG heterozygotes, while dams were g.2124AA homozygotes. WT1 is apparently duplicated in the tilapia genome. WT1a and WT1b have similar putative amino acid sequences, but they have introns of different sizes and belong to two different tilapia BAC contigs. WT1b shows greater coding-sequence similarity to the human WT1 than does WT1a. Locus WT1a locus was previously mapped on LG7 in the interspecific map of O. aureus and O. niloticus.⁵ To map WT1b, we sequenced the intron spanned by the WT1b primers (EF026800). Sires were EF026800:g.686CG heterozygotes, whereas dams were g.686CC homozygotes.

Genotyping and linkage analysis: Thirty-nine individuals of family F5 and 43 individuals of family F7 were genotyped for CYP19A1, WT1b and 13 microsatellites on tilapia LG1, which was identified as the sex chromosome in previous work.⁶

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References
7 Bellone R. et al. (2006) Cytogenet Genome Res 114, 93A.

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Linkage analysis was performed by using Joinmap (version 3.0) with a LOD-score threshold of 3.0. CYP19A1 and WT1b were mapped at 7.8 cM and 32.4 cM respectively, on LG1 (Fig. 1). LOD scores for linkage of ovarian CYP19A1 and WT1b with SEX were 1.44 and 11.34 respectively.

Comment: Our previous work showed that the SEX locus in Nile tilapia lies at 34.9 cM on LG1, between UNH995 and GM201.6 Tilapia CYP19A1 is located at the proximal end of LG1, 27.1 cM away from SEX. WT1b maps just 2.5 cM from SEX and 2.4 cM from GM201. However, breakpoint analysis identified two recombinants that excluded WT1b as a candidate for sex determination (data not presented).

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References

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Cost-effective parentage verification with 17-plex PCR for goats and 19-plex PCR for sheep

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Description: The use of microsatellite markers for parentage verification is well established. SNPs are in discussion too but not yet an alternative for systematic use. Microsatellites can be multiplexed into a single PCR, creating an easy and cost-effective assay. However, multiplex systems containing a large number of microsatellites are not generally available for small ruminants. For parentage testing in goat, Luikart et al.2 developed a two-set system, each containing 11 microsatellite markers. Each set is amplified in three multiplex groups and run in one gel lane (on an ABI 377 DNA sequencer; ABI). There is also a parentage testing system for goats that includes two sets of 6 and 8 microsatellites respectively and another parentage testing system for sheep that includes two sets of 7 and 8 microsatellites respectively. These later two systems were developed by Laboratorio Gruppi Sanguigni (LGS, Cremona, Italy) and used in the ISAG Comparison Test (http://www.isag.org.uk/journal/comparisonguide.asp). Herein we describe robust and reproducible multiplex PCRs that contain 17 microsatellites for goats (BM1258, BM1329, BOBF24A, CSRD247, ETH110, HSC, ILSTS005, INRA005, INRA040, INRA063, INRA132, OarFCB20, OarFCB128, SPS113, SPS115, SRCRSP001 and SRCRSP008) and 19 microsatellites for sheep (CSRD247, ETH110, HSC, ILSTS005, ILSTS011, INRA040, INRA063, INRA132, OarFCB20, OarFCB128, SPS113, SPS115, TCRGC4 and TCRV6B). Details of these multiplexes are given in Tables S1 and S2 respectively. A total of 426 goats belonging to 11 breeds and 316 sheep belonging to 10 breeds were included (Table 2) in tests to verify these multiplexes.

PCR conditions and genotyping: Each reaction was performed in a total volume of 15–16 µl containing 10–50 ng template DNA, 3 µl of distilled water, 8.3 µl of Qiagen Multiplex PCR Master Mix and primers (Tables S1 and S2). The cycling conditions were as follows: initial activation step at 95 °C for 15 min; 30 cycles of 94 °C for 30 s, 58 °C for 90 s and 72 °C for 60 s; and final extension at 60 °C for 30 min. PCR products were diluted with 50 µl of distilled water, and then 1.2 µl of diluted PCR was mixed with 0.4 µl of Genescan-500 LIZ Size Standard (Applied Biosystems) and 10.6 µl of Hi-Di formamide (Applied Biosystems). The samples were run on an ABI Prism® 3100 Genetic Analyzer (POP-4; run temperature 45 °C). Data collection and analysis of genotypes were performed using the 3100 Data Collection Software.