Design of a universal primer pair for the identification of deer species

Liuwei Xie, Yongyan Deng, Xiqun Shao, Pengfei Hu, Dawei Zhao, Chunyi Li & Hengxing Ba

Conservation Genetics Resources

ISSN 1877-7252
Volume 13
Number 1

Conservation Genet Resour (2021)
13:9-12
DOI 10.1007/s12686-020-01176-2

Springer

Your article is protected by copyright and all rights are held exclusively by Springer Nature B.V.. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Author's personal copy

Conservation Genetics Resources (2021) 13:9–12
https://doi.org/10.1007/s12686-020-01176-2

TECHNICAL NOTE

Design of a universal primer pair for the identification of deer species

Liuwei Xie[1] · Yongyan Deng[2] · Xiqun Shao[1] · Pengfei Hu[2] · Dawei Zhao[2] · Chunyi Li[2] · Hengxing Ba[1,2]

Received: 17 August 2020 / Accepted: 1 October 2020 / Published online: 7 October 2020
© Springer Nature B.V. 2020

Abstract

Deer species has both scientific research and economic value, and half of these species, however, are listed as endangered animals. For the conservation purpose, we designed a novel universal deer-specific PCR primer pair based on an evolutionarily conservative coding sequence (i.e., CEP295NL gene) across some deer species. This primer pair was successfully amplified and sequenced, showing around ~540 bp in cervids. Validation results showed that it can be utilized to develop a reliable and simple diagnostic tool for distinguishing other closely related species, as well as possibly interspecific identification amongst cervids.

Keywords Deer · Universal primer · Nuclear gene · CEP295NL · Diagnostic tool · Species identification

Cervidae is the second largest family in Ruminantia (second to Bovidae) and consists of 56 species (Geist 1998). Half of these deer species are today red listed by the IUCN as vulnerable, endangered or extinct in the wild. Deer are excellent models for studying evolution, biodiversity, interspecies hybridization (Abernethy 2008; Derr et al. 1991), social organization (i.e. hierarchical status) (Bartos and Bubenik 2011), unique organ development (i.e. fully regenerable antlers) (Li et al. 2009) and habitat selection (Blix 2016; Qiao et al. 2006). Moreover, some species (such as sika deer and wapiti) have the more economically values than others (such as roe deer and muntjac). Deer meat with a low fat content is appreciated for its characteristic taste, and its consumption has been associated with positive health effects (Kaltenbrunner et al. 2018b). Velvet antlers are widely used as a famous Traditional Chinese Medicine in East Asian countries for many centuries. However, before applicable for their scientific research, conservation and detection of adulteration of deer production, a reliable and simple diagnostic tool must be developed. Here, we developed and validated a PCR-based assay with a universal primer pair from a single copy nucleus gene. Compared with the deer species-specific PCR primers (Kaltenbrunner et al. 2018a, 2018b; Yang et al. 2020), our universal PCR primer has the advantage of saving time and more widely application for identification of deer species.

In order to establish this assay, we used 12 published deer genomes consisting of all subfamilies, including red deer (Ba et al. 2020; Bana et al. 2018), Eurasian elk, roe deer (de Jong et al. 2020), Pere David's deer (Zhang et al. 2018), Chinese water deer (Chen et al. 2019), white-lipped deer (Chen et al. 2019), hog deer (Wang et al. 2019), mule deer (Russell et al. 2019), black muntjac (Chen et al. 2019), reeves muntjac (Mudd et al. 2020), white-tailed deer and reindeer (Li et al. 2017), and three closer non-deer species (i.e., cattle, sheep and goat) to retrieve evolutionary conservative coding genes for designing universal PCR primers across deer genomes. We obtained 4,603 orthologous coding sequences among these 15 species by using reciprocal BLASN match. These orthologs were aligned using the ClustalX v1.83 (Larkin et al. 2007). Next, to design PCR primers, with the aid of an in-house script, we manually screened these multiple

Liuwei Xie and Yongyan Deng contributed to this manuscript equally.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12686-020-01176-2) contains supplementary material, which is available to authorized users.

✉ Chunyi Li
Lichunyi1959@163.com

✉ Hengxing Ba
bahengxing@caas.cn

[1] Institute of Special Wild Economic Animals and Plants, Chinese Academy of Agricultural Sciences, No.4899 Juye Street, Jingyue District 130112, Changchun, China

[2] Institute of Antler Science and Product Technology, Changchun Sci-Tech University, No.1345 Pudong Road, Nanguan District 130000, Changchun, China

Author's personal copy

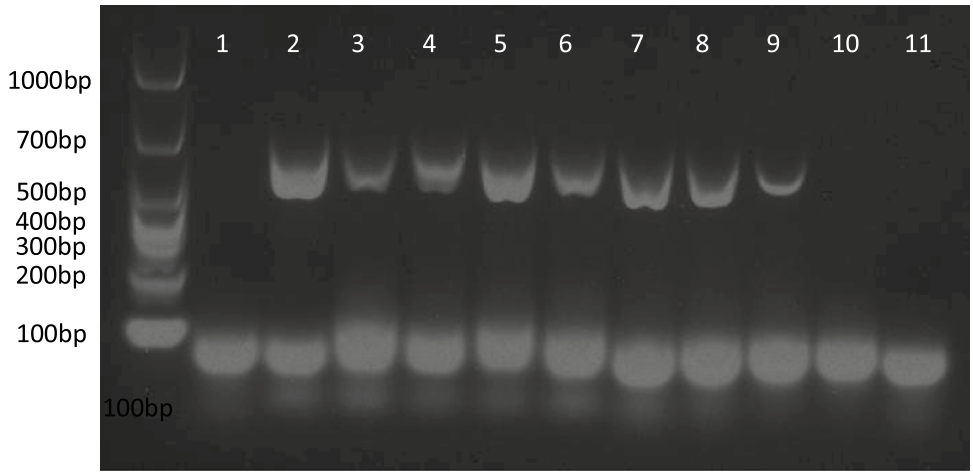

Fig. 1 PCR amplification for 10 species. 1: no DNA template control; 2: sika deer; 3: wapiti; 4: reindeer; 5: Chinese water deer; 6: samba; 7: muntjac; 8: Pere David's deer; 9: roe deer; 10: cattle; 11: goat

alignments according to three criteria: (1) Regions for PCR primer design are located inside a single exon, in so doing it can eliminate effects of intron sequences in the actual application (DNA samples), because a gene coding sequence is usually consisted of multiple exons that are separated by introns. Therefore, the single exon sequences to be selected are essential to have sufficient length (≥ 1000 bp) for designing the PCR primer pairs; (2) the orthologs for designing PCR primers must be present in all these 12 deer species; (3) the selected PCR primer regions must be evolutionarily conserved within deer species, but not with other non-deer species. Based on these criteria, we focused on a gene named CEP295NL, consisting of only one exon with the length of 1677 bp, and this gene is evolutionarily conservative across deer species. Finally, we designed a PCR primer pair based on two conserved regions, although they include some distinguishing polymorphisms between deer species (F: 5′-ACG AGCGCCTTCCATGAAGACYGGT-3′; R: 5′-AGGTGG GCTTCCATCCCCGCTGAACTT-3′) (Fig. S1).

We selected eight deer species (i.e., sika deer, wapiti, reindeer, Chinese water deer, samba, muntjac, Pere David's deer and roe deer) that span all subfamilies and their closer non-deer species (i.e., cattle and goat) to test the PCR amplification of this primer pair. We extracted total genomic DNA using the DNeasy Blood and Tissue extraction kit (Qiagen, Valencia CA, USA). PCR amplification was carried out in a final reaction volume of 25 µL composed of 2.0 µL of 10 × PCR buffer, 1.6 µL $MgCL_2$ (25 mM), 1.2 µL of dNTP mixture (25 mM), 2 µL each of universal primers (10 mM), 1 U of Taq polymerase (Takara Biotech Co. Ltd., Dalian, China), 2 µL DNA template (about 30 ng) and ultrapure water to 25 µL. The PCR cycler conditions used were an initial denaturation at 95 °C for 5 min, followed by 32 cycles of 95 °C for 30 s, 67 °C for 30 s and 72 °C for 45 s with a final extension at 72 °C for 10 min. After resolution by 2% agarose gel electrophoresis and staining in ethidium bromide, the resulting amplicons were visualized under UV light. As expected, the PCRs successfully amplified a fragment of ~ 540 bp in all tested deer species without any other visible non-specific bands (Fig. 1). For all mutations of the primer regions, there was one specific mutation (T536A) (Fig. S1) in both mule and white-tailed deer, but the mutation was not confirmed by our PCR testing due to lacks of their DNA samples. Therefore, it is no guarantee if our designed primer pair could amplify these two deer species. Chinese water deer were also found to have two specific mutations (C530T and A1048G), but their DNA samples were available and successfully PCR-amplified, suggesting that these species-specific mutations inside our primer binding regions could be PCR-amplified. Finally, these PCR products were subjected to sequencing in both directions by Sangon Biotech (Shanghai, China) Co., Ltd on an ABI 3500x sequencer (Applied Biosystems, USA).

Using the BLAST algorithm, we found that the nearest 100 matches for these amplicon sequences were all from the CEP295NL genes, further confirming that our PCR amplifications are right products. Next, a phylogenetic tree of 19 species (including cattle, sheep and goat as outgroups) was inferred by using the Maximum Likelihood method and Hasegawa-Kishino-Yano model (Hasegawa et al. 1985) employed in MEGA X (Kumar et al. 2018). The bootstrap consensus tree was inferred from 1000 replicates. Initial tree for heuristic search was obtained automatically by applying Neighbor-Join/BioNJ algorithms (Saitou and Nei 1987) to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach (Tamura et al. 2012), and then selecting the topology with superior log likelihood value (− 1531.53). Our results yielded an essentially identical tree topology (Fig. 2) to the previous studies (Frank et al. 2016; Hassanin et al. 2012; Randi et al. 1998), confirming that these sequences perfectly match the phylogeny of deer species and is likely to be as an interspecific discriminating molecular marker.

Author's personal copy

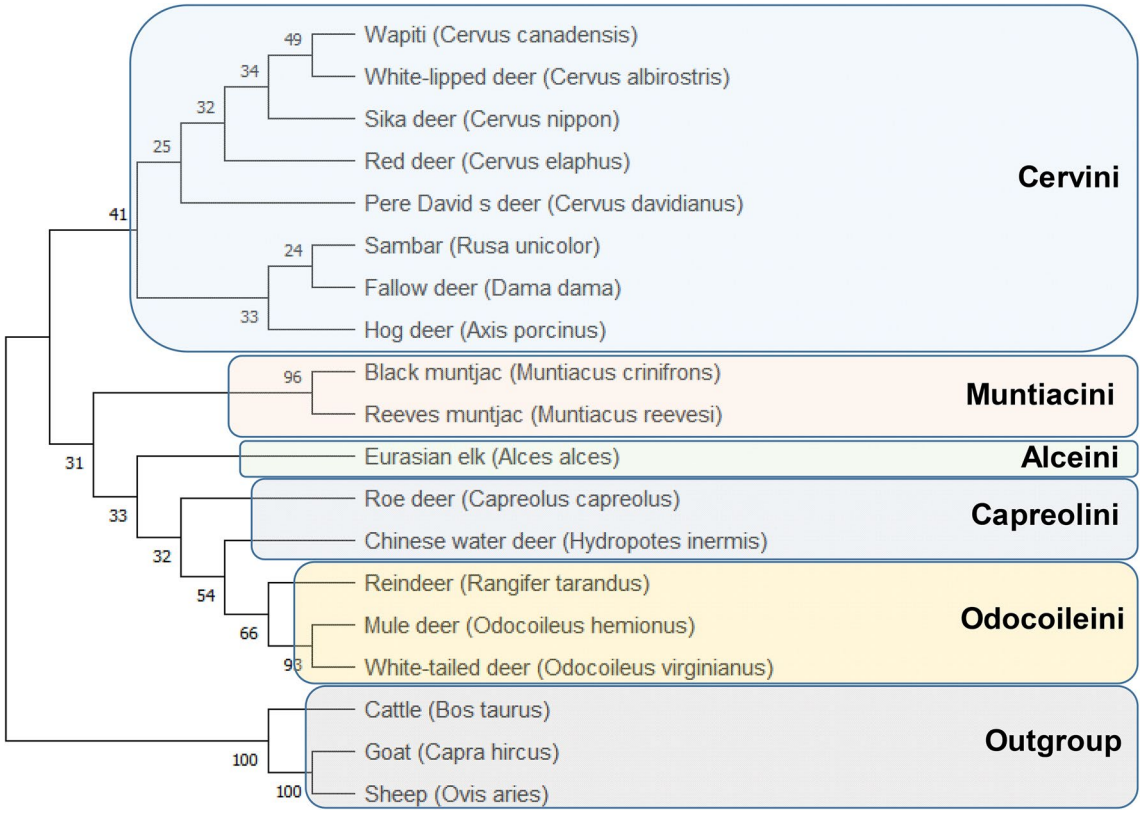

Fig. 2 Phylogenetic tree of 19 species (including cattle, sheep and goat as outgroup) was inferred by using the Maximum Likelihood method and Hasegawa-Kishino-Yano model based on the partial CEP295NL sequences (~540 bp). The bootstrap values are indicated for each nodes

In conclusion, utilizing our universal PCR primer pair designed for the CEP295NL gene, we provided a good genetic resolution between cervids and other closely related species. With the additional application of sequencing technology, BLAST match and phylogenetic analysis, our approach could also be helpful for developing a reliable and simple diagnostic tool for the identification of deer species in Cervidae.

Acknowledgements This work was funded by National Natural Science Foundation of China (No. 31402035) and Science and Technology Foundation of Jilin Province of China (No. 20200602013ZP). We wish to thank Dr. Zhipeng Li for his deer DNA samples.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Abernethy K (2008) The establishment of a hybrid zone between red and sika deer (genus Cervus). Mol Ecol 3:551–562

Ba H et al (2020) Chromosome-level genome assembly of Tarim red deer *Cervus elaphus* yarkandensis. Sci Data 7:187. https://doi.org/10.1038/s41597-020-0537-0

Bana NA et al (2018) The red deer *Cervus elaphus* genome CerEla1.0: sequencing, annotating, genes, and chromosomes. Mol Genet Genomics 293:665–684. https://doi.org/10.1007/s00438-017-1412-3

Bartos L, Bubenik G (2011) Relationships between rank-related behaviour, antler cycle timing and antler growth in deer behavioural aspects. Anim Prod Sci 51:303–310

Blix AS (2016) Adaptations to polar life in mammals and birds. J Exp Biol 219:1093–1105. doi:https://doi.org/10.1242/jeb.120477

Chen L et al (2019) Large-scale ruminant genome sequencing provides insights into their evolution and distinct traits. Science. https://doi.org/10.1126/science.aav6202

de Jong MJ, Li Z, Qin Y, Quemere E, Baker K, Wang W, Hoelzel AR (2020) Demography and adaptation promoting evolutionary transitions in a mammalian genus that diversified during the Pleistocene. Mol Ecol. https://doi.org/10.1111/mec.15450

Derr JN, Hale DW, Ellsworth DL, Bickham JW (1991) Fertility in an F1 male hybrid of white-tailed deer (*Odocoileus virginianus*) × mule deer (*O. hemionus*. J Reprod Fertil 93:111–117. https://doi.org/10.1530/jrf.0.0930111

Frank K, Barta E, Bana NA, Nagy J, Horn P, Orosz L, Steger V (2016) Complete mitochondrial genome sequence of a Hungarian red deer (*Cervus elaphus* hippelaphus) from high-throughput sequencing data and its phylogenetic position within the family Cervidae. Acta Biol Hung 67:133–147. https://doi.org/10.1556/018.67.2016.2.2

Author's personal copy

Geist V (1998) Deer of the world, their evolution, behavior, and ecology. Stackpole Books, Mechanicsburg

Hasegawa M, Kishino H, Yano T (1985) Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J Mol Evol 22:160–174. https://doi.org/10.1007/BF02101694

Hassanin A et al (2012) Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. C R Biol 335:32–50. https://doi.org/10.1016/j.crvi.2011.11.002

Kaltenbrunner M, Hochegger R, Cichna-Markl M (2018) Development and validation of a fallow deer (Dama dama)-specific TaqMan real-time PCR assay for the detection of food adulteration. Food Chem 243:82–90. https://doi.org/10.1016/j.foodchem.2017.09.087

Kaltenbrunner M, Hochegger R, Cichna-Markl M (2018) Sika deer (*Cervus nippon*)-specific real-time PCR method to detect fraudulent labelling of meat and meat products. Sci Rep 8:7236. https://doi.org/10.1038/s41598-018-25299-7

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23(21):2947–2948

Li C, Yang F, Sheppard A (2009) Adult stem cells and mammalian epimorphic regeneration-insights from studying annual renewal of deer antlers. Curr Stem Cell Res Ther 4:237–251

Li Z et al (2017) Draft genome of the reindeer (*Rangifer tarandus*). Gigascience 6:1–5. https://doi.org/10.1093/gigascience/gix102

Mudd AB, Bredeson JV, Baum R, Hockemeyer D, Rokhsar DS (2020) Analysis of muntjac deer genome and chromatin architecture reveals rapid karyotype evolution. Commun Biol 3:480. https://doi.org/10.1038/s42003-020-1096-9

Qiao J, Yang W, Gao X (2006) Natural diet and food habitat use of the Tarim red deer, *Cervus elaphus* yarkandensis. Chin Sci Bull 51:147–152

Randi E, Mucci N, Pierpaoli M, Douzery E (1998) New phylogenetic perspectives on the Cervidae (Artiodactyla) are provided by the mitochondrial cytochrome b gene. Proc Biol Sci 265:793–801. https://doi.org/10.1098/rspb.1998.0362

Russell T, Cullingham C, Kommadath A, Stothard P, Herbst A, Coltman D (2019) Development of a novel mule deer genomic assembly and species-diagnostic SNP panel for assessing introgression in mule deer, white-tailed deer, and their interspecific. hybrids. G3 (Bethesda) 9:911–919. https://doi.org/10.1534/g3.118.200838

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Tamura K, Battistuzzi FU, Billing-Ross P, Murillo O, Filipski A, Kumar S (2012) Estimating divergence times in large molecular phylogenies. J Proc Nat Acad Sci 109:19333–19338. https://doi.org/10.1073/pnas.1213199109

Wang W et al (2019) The sequence and de novo assembly of hog deer genome. Sci Data 6:180305. https://doi.org/10.1038/sdata.2018.305

Yang Y et al (2020) Rapid identification of cervus antlers by species-specific PCR assay. Nat Prod Res 34:1315–1319. https://doi.org/10.1080/14786419.2018.1560285

Zhang C et al (2018) Draft genome of the milu (*Elaphurus davidianus*). Gigascience. https://doi.org/10.1093/gigascience/gix130

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.