



Article

Copy Number Variations of Four Y-Linked Genes in Swamp Buffaloes

Ting Sun ¹, Quratulain Hanif ² , Hong Chen ¹, Chuzhao Lei ¹  and Ruihua Dang ^{1,*} 

¹ Key Laboratory of Animal Genetics, Breeding and Reproduction of Shaanxi Province, College of Animal Science and Technology, Northwest A&F University, Yangling 712100, Shaanxi, China; sunting_sim07@163.com (T.S.); chenhong1212@126.com (H.C.); leichuzhao1118@nwfau.edu.cn (C.L.)

² National Institute for Biotechnology and Genetic Engineering, Pakistan Institute of Engineering and Applied Sciences, Faisalabad 577, Pakistan; micro32uvas@gmail.com

* Correspondence: dangruihua@nwsuaf.edu.cn; Tel.: +86-153-8862-7637

Received: 19 November 2019; Accepted: 18 December 2019; Published: 22 December 2019



Simple Summary: The amplification of the male-specific region of the Y chromosome was a unique phenomenon during mammalian sex chromosome evolution. The Y-linked copy number variations of many species have been confirmed. However, the Y-linked copy number variations (CNVs) in water buffalo are still unknown. In this study, we investigated the copy number variations of four Y-linked genes (*SRY*, *UTY*, *DBY*, and *OFD1Y*) in buffalo. Our results showed that *UTY* was a single-copy gene in buffalo, while *DBY*, *OFD1Y*, and *SRY* exhibited copy number variations in buffalo.

Abstract: Copy number variation (CNV), a significant source of genetic diversity in the mammalian Y chromosome, is associated with the development of many complex phenotypes, such as spermatogenesis and male fertility. The contribution of Y-linked CNVs has been studied in various species, however, water buffalo has not been explored in this area and the genetic information still remains unknown. The aim of the current study was to investigate the CNVs of four Y-linked genes, including, *sex determining Region of Y-Chromosome (SRY)*, *ubiquitously transcribed tetratricopeptide repeat gene protein on the chromosome Y (UTY)*, *DEAD-box helicase 3 Y-linked (DDX3Y)*, also known as *DBY*, and *oral-facial-digital syndrome 1 Y-linked (OFD1Y)* in 254 swamp buffaloes from 15 populations distributed across China, Vietnam, and Laos using quantitative real-time PCR (qPCR). Our results revealed the prevalence of a single-copy *UTY* gene in buffaloes. The *DBY* and *OFD1Y* represented CNVs among and within different buffalo breeds. The *SRY* showed CNVs only in Vietnamese and Laotian buffaloes. In conclusion, this study indicated that *DBY*, *OFD1Y*, and *SRY* showed CNVs, while the *UTY* was a single-copy gene in swamp buffaloes.

Keywords: swamp buffalo; CNVs; Y-linked genes

1. Introduction

Copy number variation (CNV) is a significant source of genetic diversity, comprised of varying copy numbers of DNA segments (at least 50 bp in size) within species [1]. CNVs are known to be associated with gene expression, phenotypic variation, adaption, and the development of various diseases [2,3]. In cattle, the CNV is associated with resistance or susceptibility to gastrointestinal nematodes [4], production traits [5,6], and muscle [7]. Studies showed that the CNV also plays a key role in the coat color in goat [8], pig [9], and horse [10].

The mammalian Y chromosome stands out from rest of the genome because it is constitutively haploid and escapes recombination for most of its length, leading to a correspondingly unusual genomic landscape [11]. Chromosome Y is rich in segmental duplications and provides a rich environment for

the generation of CNVs [11–13]. The male-specific region of the Y chromosome (MSY) accounts for 95% of the Y chromosome, consisting of three regions: ampliconic, X-transposed, and X-degenerate. The MSY is enriched with multi-copied genes and contains different and some specific MSY genes in different species [11,14–17].

Water buffalo (*Bubalus bubalis*), an important livestock in tropical and subtropical climates, can provide meat, milk, and hides for people [18]. Water buffalo can be divided into two types according to body size, outward appearance, biological characteristics, and chromosome karyotype: swamp (2n = 48) and river buffalo (2n = 50) [19,20]. The CNVs of river buffalo on the autosomal and X chromosomal have been investigated [21,22]. However, there are few studies about the Y-linked CNV genes in buffalo. Only Zhang et al. [23] detected the CNVs in two multicopy-Y-linked genes (*HSFY* and *ZNF280BY*) in buffaloes, where swamp buffalo showed significant CNV abundance. To date, there is no research about CNVs of X-degenerate genes in buffaloes. In the current study, four X-degenerate genes, *sex determining Region of Y-Chromosome (SRY)*, *ubiquitously transcribed tetratricopeptide repeat gene protein on the chromosome Y (UTY)*, *DEAD-box helicase 3 Y-linked (DDX3Y, also known as DBY)*, and *oral-facial-digital syndrome 1Y-linked (OFD1Y)*, were selected to determine their copy numbers on buffalo Y chromosome. *SRY* gene, mainly associated with sex differentiation and male sexual development, was used as a candidate marker for sperm quality and fertility in bulls [24]. Studies showed that *SRY* is present as a single-copy gene in human, mouse, chimpanzee, horse, and cattle [14,16,25,26], while as a multi-copy gene in domestic cat and rabbit [27,28]. *DBY* and *UTY* are known to play key roles in spermatogenesis and bull fertility [29,30]. *DBY* and *UTY* were also identified as single copy genes in human, chimpanzee, horse, cattle, and mouse [14,16,25,26]. *OFD1Y* may play an important role in sperm development, which is associated with infertility in cattle [31], and highly expressed in porcine testis [32]. *OFD1Y* is a single-copy gene in cattle whose function is still unknown [16,33,34], but there are at least 14 and 18 copies in chimpanzee and human, respectively [33].

In our previous studies, several mutation sites in *SRY*, *DBY*, and *OFD1Y* genes were identified [35]. Then, the authors chose one sample to perform the TA cloning for further verification. Finally, 40 cloning sequences were obtained in *SRY*, *DBY*, and *OFD1Y* genes of the sample, respectively, and showed several mutation sites. These genes were located in the MSY region which is a non-recombining region with no X–Y crossing over [11], but single nucleotide polymorphisms (SNPs) were in the sample [35]. So, the authors deduced that *SRY*, *DBY*, and *OFD1Y* were multi-copy genes in buffaloes [35]. However, their CNVs were unknown. The aim of the study was to investigate the CNVs of *SRY*, *DBY*, *OFD1Y*, and *UTY* of two river buffaloes and 254 swamp buffaloes from 15 populations distributed in China, Vietnam, and Laos using qPCR.

2. Materials and Methods

In total, 254 male buffalo samples (including 204 ear tissues and 50 blood samples) were collected from 15 swamp buffalo populations (including 254 swamp buffaloes) in China, Vietnam, and Laos. The study was approved by the Institutional Animal Care and Use Committee of Northwest A&F University. The genomic DNA was extracted from the ear tissues or the blood using a standard phenol-chloroform method [36]. PCR primers were designed using the Primer Premier 5.0 (Table S1). *Basic transcription factor 3 (BTF3; XM_006062116)*, a two-copied gene in bovine, was chosen as a reference gene [37,38].

To validate the male specificity of primers used in this study, a routine PCR was performed. Five female buffaloes were used as negative controls, and water was used as a blank control. The PCR protocol was as follows: each 12.5 μ L reaction contained 1 μ L of genome DNA (10 ng/ μ L), 0.5 μ L of each primer (10 pmol/ μ L), 6.25 μ L of 2 \times PCR Mix buffer (CWBIO), and 4.25 μ L of distilled water. Thermocycling consisted of an initial denaturation at 95 $^{\circ}$ C for 5 min, followed by 35 cycles at 94 $^{\circ}$ C for 30 s, annealing temperature (Table S1) for 40 s, and 72 $^{\circ}$ C for 30 s, followed by a final extension at 72 $^{\circ}$ C for 10 min. The PCR products were visualized on 1% agarose gel electrophoresis.

qPCR was used to measure the *SRY*, *DBY*, *UTY*, and *OFD1Y* copy numbers using the Roche LightCycler 480. Standard curves were generated from male buffalo DNA diluted to 40, 20, 10, 5, 2.5, 1.25, and 0.625 ng/μL for *SRY*, *DBY*, *UTY*, and *OFD1Y*. Primer efficiencies were calculated based on the slopes of standard curves and the equation $E = 10^{[1/\text{slope}]}$ [39]. For the test samples, DNA was concentrated to 5 ng/μL. The qPCR reaction contained 5 μL of SYBR Green PCR Master Mix (TAKARA), 0.5 μL of primers (10 pmol/μL), 3 μL of distilled water, and 1 μL of DNA template. Each sample and calibrator were run in triplicate using a standard shuttle PCR protocol (pre-denaturation at 95 °C for 30 s followed by denaturation at 95 °C for 5 s and annealing at 60 °C for 20 s for 40 cycles). A melting curve was then generated by taking fluorescent measurements every 0.1 °C from 65–95 °C.

The DNA sample of a Yibin buffalo was used to calibrate cycle threshold (C_T) variations among the plates. The C_T value of the calibrator for each gene was determined using average C_T values of all the plates for the corresponding sample. The copy numbers were estimated using the following three equations:

$$\text{Copy number calibrator} = \frac{(E_{\text{reference}})^{C_{T \text{ reference}}}}{(E_{\text{target}})^{C_{T \text{ target}}}} \quad (1)$$

$$\text{Ratio} = \frac{(E_{\text{target}})^{\Delta C_{T \text{ target}} (\text{calibrator} - \text{sample})}}{(E_{\text{reference}})^{\Delta C_{T \text{ reference}} (\text{calibrator} - \text{sample})}} \quad (2)$$

$$\text{Copy number test sample} = (\text{Copy number}_{\text{calibrator}}) \times (\text{ratio}) \times 2.$$

In order to minimize technical errors and to have accurate copy number estimates, 11 samples with high variation in raw C_T values (CV >1%) among the triplicates were excluded from further analysis.

To verify the accuracy of qPCR results, TA cloning was performed for these four genes with the samples exhibiting different copy numbers from the others. PCR products were purified using Universal DNA Purification Kit (TIANGEN, Beijing, China), then ligated into the cloning vector pGEM-T Easy and transformed into *Escherichia coli* DH-5α (CWBio, Beijing, China). Then, 10–15 clones per sample were picked and amplified by PCR method. PCR products were sequenced on an ABI PRISM 377 DNA sequencer (Perkin-Elmer, Foster City, CA, USA) (Shanghai Sangon Biotech Company, Shanghai, China).

3. Results and Discussion

The results showed that each pair of primers amplified a male specific band with the expected fragment size, confirming that the designed primers were male-specific and could be used for the qPCR analysis in this study (Figure 1). The primer efficiency of *SRY*, *DBY*, *UTY*, and *OFD1Y* were 1.98, 1.96, 2.07, and 2.02, respectively (Table S1).

Our results showed that the median copy numbers (MCNs) of *SRY*, *DBY*, *OFD1Y*, and *UTY* were 1, 2, 1, and 1 with CNVs ranges of 1–4, 1–8, 1–4, and 1, respectively (Table 1). The MCN and copy number of *SRY* gene was 1 in 13 Chinese native buffalo populations. Yet the MCN was 2 with the CNVs ranging from 1 to 4 in Laotian and Vietnamese buffalo, indicating that the *SRY* is a multi-copy gene in buffalo. The MCN of *DBY* was 2 with its copy number ranging from 1 to 8. Thus, we defined that *DBY* is a multi-copy gene in buffalo. The MCN and copy number of *DBY* is variable among individuals and populations. The Fuling population possessed the highest MCN (6), while the Guangxi and Yibin populations exhibited the lowest MCN (1). The MCN and the copy number of *UTY* gene was 1 in all tested buffaloes, which indicated that *UTY* was a single-copy gene in buffalo. The MCN of *OFD1Y* gene was 1 with the copy number ranging from 1 to 4 in several populations. So, we concluded that *OFD1Y* is a multi-copy gene in buffalo populations.

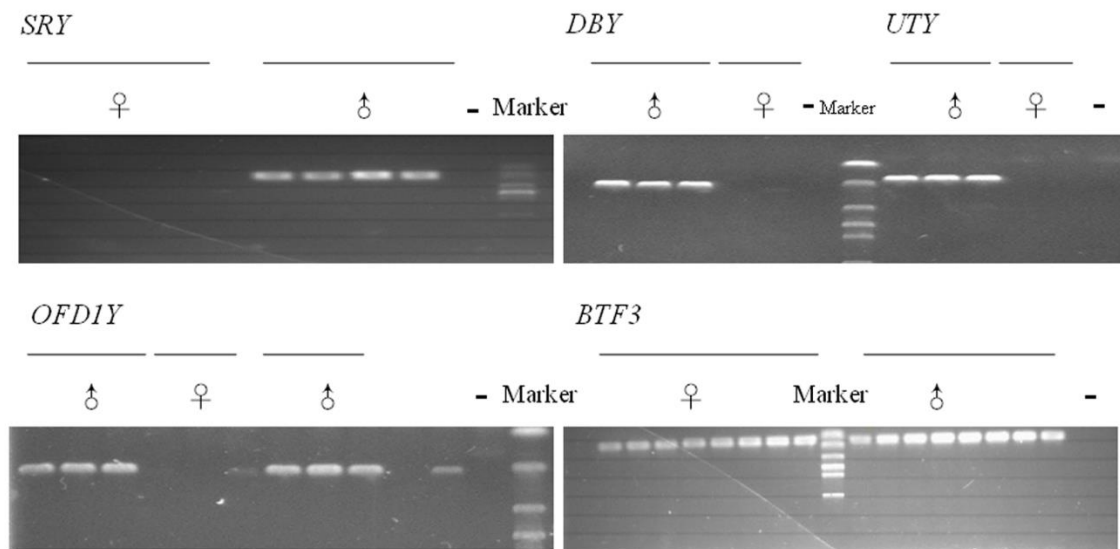


Figure 1. Gel electrophoresis of PCR products of the buffalo sex determining Region of Y-Chromosome (*SRY*), ubiquitously transcribed tetratricopeptide repeat gene protein on the chromosome Y (*UTY*), DEAD-box helicase 3 Y-linked (*DDX3Y*, also known as *DBY*), and oral-facial-digital syndrome 1 Y-linked (*OFDIY*) genes. The primers of the *SRY*, *DBY*, *UTY*, and *OFDIY* genes amplified male-specific band. Marker, D2000 DNA ladder; the “♂” represents the male buffalo genomic DNA; the “♀” represents the female buffalo genomic DNA; the “-” represents the blank control (distilled water).

Table 1. The median copy number of *SRY*, *DBY*, *UTY*, and *OFDIY* genes in 16 buffalo populations.

Population	Abbreviation	Sample Size	Median Copy Number			
			<i>SRY</i>	<i>DBY</i>	<i>UTY</i>	<i>OFDIY</i>
Dehong	DH	20	1	2 (1–4)	1	1
Diandongnan	DDN	7	1	2 (1–2)	1	1 (1–2)
Guizhou	GZ	4	1	2 (1–2)	1	1
Guangxi	GX	11	1	1 (1–2)	1	1
Fuling	FL	7	1	6 (2–8)	1	1
Xinyang	XY	33	1	2 (1–3)	1	1 (1–4)
Jianghan	JH	14	1	3 (1–4)	1	1 (1–2)
Guangyuan	GY	27	1	2 (1–3)	1	1
Yibin	YB	28	1	1 (1–2)	1	1 (1–2)
Haizi	HZ	4	1	2 (1–3)	1	1
Xiajiang	XJ	25	1	2 (1–4)	1	1
Poyanghu	PYH	24	1	2 (2–3)	1	1 (1–2)
Jianghuai	JHU	14	1	2 (1–3)	1	1 (1–2)
Vietnamese	VN	26	2 (1–4)	2 (1–4)	1	2 (1–4)
Laotian	LA	12	2 (1–4)	2 (1–4)	1	1 (1–2)
Total		254	1	2	1	1

Note: The numbers in the “()” represent the range of copy numbers of *SRY*, *DBY*, *UTY*, and *OFDIY* genes.

The results of qPCR indicated that *SRY*, *DBY*, and *OFDIY* showed CNVs within buffalo populations, which were in accordance with our previous cloning results [35]. In different species, the copy number of the Y-linked genes may be different [15]. *SRY*, *DBY*, and *UTY* act as single-copy genes in human, mouse, chimpanzee, horse, and cattle [14,16,25,26], while *UTY* is a multi-copy gene in domestic cat and rabbit [33]. *OFDIY* showed copy number variations in chimpanzee and human, but demonstrated to be a single-copy gene in cattle [16,33,34]. Studies also showed that the copy number of genes varied from individual to individual [11]. The male-specific region is enriched in multi-copy gene families that are all predominantly expressed, or solely in testis, and involved in spermatogenesis and male

fertility [11,16,39,40]. Previous studies have demonstrated that the Y-linked CNVs are associated with male reproductive traits [39], testis size, and bull fertility in cattle [40]. Although CNVs of the Y-linked gene families and their function have not been well-studied in buffalo due to the unavailability of the Y chromosome sequences, we boldly speculate that these four genes may also play an important role in the spermatogenesis and male fertility in buffaloes; yet, more work should be done to verify this corollary in future.

4. Conclusions

In conclusion, we found that the buffalo *UTY* gene was a single-copy gene on the Y chromosome, while the copy numbers of *SRY*, *DBY*, and *OFDIY* were variable among buffalo populations. These three genes (*SRY*, *DBY*, and *OFDIY*) demonstrated large-scale CNVs among individuals and populations, which provide great genetic resources for assessing their association with male fertility in future work.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2076-2615/10/1/31/s1>. Table S1: The information of primers used in qPCR.

Author Contributions: Conceptualization, T.S. and R.D.; methodology, R.D.; software, T.S.; validation, R.D.; formal analysis, Q.H.; investigation, H.C.; resources, C.L.; data curation, T.S.; writing—original draft preparation, T.S.; writing—review and editing, Q.H.; visualization, T.S.; supervision, Q.H.; project administration, C.L.; funding acquisition, R.D. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by National Natural Science Foundation of China (31872317) and the National Beef Cattle and Yak Industrial Technology System (CARS-37).

Acknowledgments: We would like to thank Xiangpeng Yue for his good suggestions and technical support.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Mills, R.E.; Walter, K.; Stewart, C.; Handsaker, R.E.; Chen, K.; Alkan, C.; Abyzov, A.; Yoon, S.C.; Ye, K.; Cheetham, R.K. Mapping copy number variation by population-scale genome sequencing. *Nature* **2011**, *470*, 59–65. [[CrossRef](#)] [[PubMed](#)]
2. Beckmann, J.S.; Estivill, X.; Antonarakis, S.E. Copy number variants and genetic traits: Closer to the resolution of phenotypic to genotypic variability. *Nat. Rev. Genet.* **2007**, *8*, 639. [[CrossRef](#)] [[PubMed](#)]
3. Redon, R.; Ishikawa, S.; Fitch, K.R.; Feuk, L.; Perry, G.H.; Andrews, T.D.; Fiegler, H.; Shaperro, M.H.; Carson, A.R.; Chen, W. Global variation in copy number in the human genome. *Nature* **2006**, *444*, 444. [[CrossRef](#)] [[PubMed](#)]
4. Hou, Y.; Liu, G.E.; Bickhart, D.M.; Matukumalli, L.K.; Li, C.; Song, J.; Gasbarre, L.C.; Van Tassell, C.P.; Sonstegard, T.S. Genomic regions showing copy number variations associate with resistance or susceptibility to gastrointestinal nematodes in Angus cattle. *Funct. Integr. Genom.* **2012**, *12*, 81–92. [[CrossRef](#)] [[PubMed](#)]
5. Prinsen, R.T.M.M.; Rossoni, A.; Gredler, B.; Bieber, A.; Bagnato, A.; Strillacci, M.G. A genome wide association study between CNVs and quantitative traits in Brown Swiss cattle. *Livest. Sci.* **2017**, *202*, 7–12. [[CrossRef](#)]
6. Zhou, Y.; Connor, E.E.; Wiggans, G.R.; Lu, Y.; Tempelman, R.J.; Schroeder, S.G.; Chen, H.; Liu, G.E. Genome-wide copy number variant analysis reveals variants associated with 10 diverse production traits in Holstein cattle. *BMC Genom.* **2018**, *19*, 314. [[CrossRef](#)]
7. Zhou, Y.; Utsunomiya, Y.T.; Xu, L.; Hay, E.H.A.; Bickhart, D.M.; Alexandre, P.A.; Rosen, B.D.; Schroeder, S.G.; Carneiro, R.; de Rezende Neves, H.H.; et al. Genome-wide CNV analysis reveals variants associated with growth traits in *Bos indicus*. *BMC Genom.* **2016**, *17*, 419. [[CrossRef](#)]
8. Fontanesi, L.; Beretti, F.; Riggio, V.; Gómez González, E.; Dall'Olio, S.; Davoli, R.; Russo, V.; Portolano, B. Copy Number Variation and Missense Mutations of the Agouti Signaling Protein (ASIP) Gene in Goat Breeds with Different Coat Colors. *Cytogenet. Genome Res.* **2009**, *126*, 333–347. [[CrossRef](#)]
9. Rubin, C.-J.; Megens, H.-J.; Barrio, A.M.; Maqbool, K.; Sayyab, S.; Schwochow, D.; Wang, C.; Carlborg, Ö.; Jern, P.; Jørgensen, C.B.; et al. Strong signatures of selection in the domestic pig genome. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 19529–19536. [[CrossRef](#)]

10. Sundström, E.; Imsland, F.; Mikko, S.; Wade, C.; Sigurdsson, S.; Pielberg, G.R.; Golovko, A.; Curik, I.; Seltenhammer, M.H.; Sölkner, J.; et al. Copy number expansion of the STX17 duplication in melanoma tissue from Grey horses. *BMC Genom.* **2012**, *13*, 365. [[CrossRef](#)]
11. Skaletsky, H.; Kuroda-Kawaguchi, T.; Minx, P.J.; Cordum, H.S.; Hillier, L.; Brown, L.G.; Repping, S.; Pyntikova, T.; Ali, J.; Bieri, T. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. *Nature* **2003**, *423*, 825–837. [[CrossRef](#)] [[PubMed](#)]
12. Quintanamurci, L.; Fellous, M. The Human Y Chromosome: The Biological Role of a “Functional Wasteland”. *J. Biomed. Biotechnol.* **2001**, *1*, 18–24. [[CrossRef](#)] [[PubMed](#)]
13. Graves, J.A. Sex chromosome specialization and degeneration in mammals. *Cell* **2006**, *124*, 901–914. [[CrossRef](#)] [[PubMed](#)]
14. Hughes, J.F.; Skaletsky, H.; Pyntikova, T.; Graves, T.A.; Daalen, S.K.M.V.; Minx, P.J.; Fulton, R.S.; Mcgrath, S.D.; Locke, D.P.; Friedman, C. Chimpanzee and human Y chromosomes are remarkably divergent in structure and gene content. *Nature* **2010**, *463*, 536. [[CrossRef](#)]
15. Hughes, J.F.; Rozen, S. Genomics and Genetics of Human and Primate Y Chromosomes. *Annu. Rev. Genom. Hum. Genet.* **2012**, *13*, 83–108. [[CrossRef](#)]
16. Chang, T.C.; Yang, Y.; Retzel, E.F.; Liu, W.S. Male-specific region of the bovine Y chromosome is gene rich with a high transcriptomic activity in testis development. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 12373–12378. [[CrossRef](#)]
17. Jobling, M.A. Copy number variation on the human Y chromosome. *Cytogenet. Genome Res.* **2008**, *123*, 253–262. [[CrossRef](#)]
18. Michelizzi, V.N.; Dodson, M.V.; Pan, Z.; Amaral, M.E.J.; Michal, J.J.; Mclean, D.J.; Womack, J.E.; Jiang, Z. Water Buffalo Genome Science Comes of Age. *Int. J. Biol. Sci.* **2010**, *6*, 333–349. [[CrossRef](#)]
19. Fischer, H.; Ulbrich, F. Chromosomes of the Murrah buffalo and its crossbreds with the Asiatic swamp buffalo (*Bubalus bubalis*). *Z. Tierz. Züchtungsbiol.* **1967**, *84*, 110–114. [[CrossRef](#)]
20. Iannuzzi, L. Standard karyotype of the river buffalo (*Bubalus bubalis* L., 2n = 50). Report of the committee for the standardization of banded karyotypes of the river buffalo. *Cytogenet. Cell Genet.* **1994**, *67*. [[CrossRef](#)]
21. Li, W.; Bickhart, D.M.; Ramunno, L.; Iamartino, D.; Williams, J.L.; Liu, G.E. Genomic structural differences between cattle and River Buffalo identified through comparative genomic and transcriptomic analysis. *Data Brief* **2018**, *19*, 236–239. [[CrossRef](#)] [[PubMed](#)]
22. Liu, S.; Kang, X.; Catacchio, C.R.; Liu, M.; Fang, L.; Schroeder, S.G.; Li, W.; Rosen, B.D.; Iamartino, D.; Iannuzzi, L.; et al. Computational detection and experimental validation of segmental duplications and associated copy number variations in water buffalo (*Bubalus bubalis*). *Funct. Integr. Genom.* **2019**, *19*, 409–419. [[CrossRef](#)] [[PubMed](#)]
23. Zhang, X.; Han, H.; Zhang, T.; Sun, T.; Xi, Y.; Chen, N.; Huang, Y.; Dang, R.; Lan, X.; Chen, H. HSFY and ZNF280BY show copy number variations within 17 water buffalo populations. *Anim. Genet.* **2016**, *48*, 221–224. [[CrossRef](#)] [[PubMed](#)]
24. Fortes, M.R.S.; Reverter, A.; Hawken, R.J.; Bolormaa, S.; Lehnert, S.A. Candidate Genes Associated with Testicular Development, Sperm Quality, and Hormone Levels of Inhibin, Luteinizing Hormone, and Insulin-Like Growth Factor 1 in Brahman Bulls1. *Biol. Reprod.* **2012**, *87*. [[CrossRef](#)]
25. Paria, N.; Raudsepp, T.; Wilkerson, A.J.P.; O’Brien, P.C.M.; Ferguson-Smith, M.A.; Love, C.C.; Arnold, C.; Rakestraw, P.; Murphy, W.J.; Chowdhary, B.P. A Gene Catalogue of the Euchromatic Male-Specific Region of the Horse Y Chromosome: Comparison with Human and Other Mammals. *PLoS ONE* **2011**, *6*, e21374. [[CrossRef](#)]
26. Turner, M.E.; Martin, C.; Martins, A.S.; Dunmire, J.; Farkas, J.; Ely, D.L.; Milsted, A. Genomic and expression analysis of multiple Sry loci from a single *Rattus norvegicus* Y chromosome. *BMC Genet.* **2007**, *8*, 11. [[CrossRef](#)]
27. Wilkerson, A.J.P.; Raudsepp, T.; Graves, T.; Albracht, D.; Warren, W.; Chowdhary, B.P.; Skow, L.C.; Murphy, W.J. Gene discovery and comparative analysis of X-degenerate genes from the domestic cat Y chromosome. *Genomics* **2008**, *92*, 329–338. [[CrossRef](#)]
28. Geraldès, A.; Ferrand, N. A 7-bp insertion in the 3′ untranslated region suggests the duplication and concerted evolution of the rabbit SRY gene. *Genet. Sel. Evol.* **2006**, *38*, 1–8. [[CrossRef](#)]
29. Liu, Y.; Qin, X.; Song, X.-Z.H.; Jiang, H.; Shen, Y.; Durbin, K.J.; Lien, S.; Kent, M.P.; Sodeland, M.; Ren, Y.; et al. Bos taurus genome assembly. *BMC Genom.* **2009**, *10*, 180. [[CrossRef](#)]

30. Liu, W.S.; Wang, A.; Yang, Y.; Chang, T.C.; Landrito, E.; Yasue, H. Molecular characterization of the DDX3Y gene and its homologs in cattle. *Cytogenet. Genome Res.* **2009**, *126*, 318–328. [[CrossRef](#)]
31. Wu, Y.; Zhang, W.X.; Zuo, F.; Zhang, G.W. Comparison of mRNA expression from Y-chromosome X-degenerate region genes in taurine cattle, yaks and interspecific hybrid bulls. *Anim. Genet.* **2019**, *50*, 740–743. [[CrossRef](#)] [[PubMed](#)]
32. Skinner, B.M.; Sargent, C.A.; Churcher, C.; Hunt, T.; Herrero, J.; Loveland, J.E.; Dunn, M.; Louzada, S.; Fu, B.; Chow, W. The pig X and Y Chromosomes: Structure, sequence, and evolution. *Genome Res.* **2016**, *26*, 130–139. [[CrossRef](#)] [[PubMed](#)]
33. Chang, T.-C.; Klabnik, J.L.; Liu, W.-S. Regional selection acting on the OFD1 gene family. *PLoS ONE* **2011**, *6*, e26195. [[CrossRef](#)] [[PubMed](#)]
34. Keller, L.C.; Romijn, E.P.; Zamora, I.; Yates, J.R.; Marshall, W.F. Proteomic Analysis of Isolated Chlamydomonas Centrioles Reveals Orthologs of Ciliary-Disease Genes. *Curr. Biol.* **2005**, *15*, 1090–1098. [[CrossRef](#)] [[PubMed](#)]
35. Xu, S. *Y-SNPs Scanning and Multi-Copy Genes Identification in Water Buffalo*; Northwest A&F University: Yangling, China, 2014. (In Chinese)
36. Sambrook, J.; Russell, D. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, USA, 2001.
37. Liu, G.E.; Hou, Y.; Zhu, B.; Cardone, M.F.; Jiang, L.; Cellamare, A.; Mitra, A.; Alexander, L.J.; Coutinho, L.L.; Dell'Aquila, M.E. Analysis of copy number variations among diverse cattle breeds. *Genome Res.* **2010**, *20*, 693–703. [[CrossRef](#)]
38. Bickhart, D.M.; Hou, Y.; Schroeder, S.G.; Alkan, C.; Cardone, M.F.; Matukumalli, L.K.; Song, J.; Schnabel, R.D.; Ventura, M.; Taylor, J.F. Copy number variation of individual cattle genomes using next-generation sequencing. *Genome Res.* **2012**, *22*, 778–790. [[CrossRef](#)]
39. Yue, X.P.; Chang, T.C.; Dejarnette, J.M.; Marshall, C.E.; Lei, C.Z.; Liu, W.S. Copy number variation of PRAMEY across breeds and its association with male fertility in Holstein sires. *J. Dairy Sci.* **2013**, *96*, 8024–8034. [[CrossRef](#)]
40. Yue, X.P.; Dechow, C.; Chang, T.C.; Dejarnette, J.M.; Marshall, C.E.; Lei, C.Z.; Liu, W.S. Copy number variations of the extensively amplified Y-linked genes, HSFY and ZNF280BY, in cattle and their association with male reproductive traits in Holstein bulls. *BMC Genom.* **2014**, *15*, 113. [[CrossRef](#)]



© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).