



## Identification of Female Specific Genes in the W Chromosome that are Expressed during Gonadal Differentiation in the Chicken

Harikrishna Reddy Rallabandi<sup>1</sup>, Hyeon Yang<sup>2</sup>, Yong Jin Jo<sup>3</sup>, Hwi Cheul Lee<sup>2</sup>,  
Sung June Byun<sup>4</sup> and Bo Ram Lee<sup>2†</sup>

<sup>1</sup>Postdoctoral Researcher, Animal Biotechnology Division, National Institute of Animal Science, Rural Development Administration, Wanju-gun 55365, Republic of Korea

<sup>2</sup>Researcher, Animal Biotechnology Division, National Institute of Animal Science, Rural Development Administration, Wanju-gun 55365, Republic of Korea

<sup>3</sup>Action Officer, Animal Biotechnology Division, National Institute of Animal Science, Rural Development Administration, Wanju-gun 55365, Republic of Korea

<sup>4</sup>Senior Researcher, Animal Biotechnology Division, National Institute of Animal Science, Rural Development Administration, Wanju-gun 55365, Republic of Korea

**ABSTRACT** Avian sex determination system involves the male ZZ and female ZW chromosomes. However, very few studies are reported the expression, functional role and importance of genes on the W chromosome because of its small and highly heterochromatic genomic regions. Recent studies demonstrated that the W chromosome may have critical roles in physiology, sex determination and subsequent sexual differentiation in chickens. Therefore, gene annotation, including describing the expression and function of genes in the chicken W chromosome, is needed. In this study, we have searched the W chromosome of chickens and selected a total of 36 genes to evaluate their specific expression in the testis and ovary at various developmental stages such as embryonic day 6 (E6), hatch and adult. Interestingly, out of 36 genes in chicken W chromosome, we have found seven female-specific expression at E6.5 day, indicating that they are functionally related to female chicken gonadal differentiation. In addition, we have identified the stage specific gene expression from the sex specific genes. Furthermore, we analyzed the relative location of genes in the chicken W chromosome. Collectively, these results will contribute molecular insights into the sexual determination, differentiation and female development based on the W chromosome.

(Key words: chicken, W chromosome, gonadal differentiation, female specific expression)

## INTRODUCTION

In avian species, the sex determination system is similar to mammal genetic system, but sex chromosomes are different. Further, the mechanisms of sex-determination are still controversial and described by two hypotheses (Chue and Smith, 2011). To date, many studies have centered on understanding the functional roles of the Z chromosome in sex determination and sexual phenotypes in a chicken model, and Smith and colleagues demonstrated that the Z-linked gene DMRT1, which is a strong sex-determinant, is required for male sex determination in chickens, supporting the Z-dosage model (Smith et al., 2009). Furthermore, the avian sex-determination system has two potential mechanisms: the presence

of the W chromosome triggers femaleness, or the presence of two Z chromosomes confers maleness (Smith and Sinclair, 2004).

The W chromosome is a type of sex chromosome that exists primarily in birds, insects, fishes, reptiles, crustaceans and silkworms (Matsubara et al., 2006). The W chromosome passes through the ovum of females and determines the sex of the offspring, unlike the XY system in mammals (Bachtrog et al., 2011). In this system, males have two Z sex chromosomes, whereas females have Z and W sex chromosomes. Whereas the Z chromosome carries many genes and is larger, the W chromosome carries only a few genes (Graves, 2014). Moreover, their functional properties of the Y chromosome in the XY system and the W chromosome in the ZW system are

<sup>†</sup> To whom correspondence should be addressed : mir88@korea.kr

completely different (Mank, 2012). However, comprehensive investigation of the W chromosome has yet to be undertaken in birds due to the presence of smaller and highly heterochromatic genomic regions in the W chromosome.

Recently, multiple roles for the W chromosome in key sex-specific evolutionary processes and sex determination were reported (Moghadam et al., 2012). In avian species, the W chromosome plays important roles in female fitness traits, sex determination and, subsequently, sex differentiation during embryonic gonadal development (Moghadam et al., 2012; Ayers et al., 2013b). In the silkworm, the W chromosome has a dominant role in female sex determination, suggesting the existence of a dominant feminizing gene in this chromosome (Kiuchi et al., 2014).

In chicken embryos, gonad formation starts from Hamilton and Hamburger (HH) stage 18 (E3), and morphological differentiation initiates at HH stage 29 (E6) and fully developed gonads are seen at HH stage 36 (E10.5) (Hamburger and Hamilton, 1951). This is the critical stage where the gonad-specific gene expression governs the sex determination of the offspring; as previously reported, the early DMRT1 gene on the Z chromosome plays a key role in testes formation (Smith et al., 2009). However, the female-specific gonadal genes are not precisely defined (Chue and Smith, 2011). In this study, we performed a comprehensive analysis and review of genes in the W chromosome to reveal their detailed characteristics and functionality. Furthermore, we found a set of female-specific genes expressed during gonadal differentiation in chickens. Finally, these results further provide molecular insights into sex determination and gonadal sexual differentiation with respect to the avian W chromosome.

## MATERIALS AND METHODS

### 1. Experimental Animals and Animal Care

The care and experimental use of White Leghorn (WL) chickens was approved by the Institutional Animal Care and Use Committee (IACUC) of National Institute of Animal Science (NIAS-2019-407), Korea. All procedures, including chicken maintenance, feeding, reproduction, treatment, and sample collection, followed the standard operating protocols of Animal Biotechnology Division at the National Institute of

Animal Science.

### 2. PCR-based Sexing and Sample Collection

White Leghorn eggs were incubated with intermittent rocking at 37~38°C under 60~70% relative humidity until sample collection. Adult testis and ovary tissues were collected from 24 to 30-week-old chickens, and embryonic male and female gonad tissues were collected from 6-day-old and hatched chicks after sexing. To determine the sex of the embryos, a small hole was made on the pointed end of 2.5 days incubated eggs, and 2 µL of blood was drawn and boiled. The punctured egg was sealed with Parafilm, and laid down with the pointed end towards the bottom and incubated until day 6 (HH29). For direct genetic sexing, the DNA samples were briefly heated at 95°C for 10 min, followed by 5 cycles at 94°C for 5 min and 55°C for 5 min. The genetic sex of the embryos was determined by PCR amplification of W chromosome-specific repeat sequences from the prepared template (sexing F: 5-AGA ATG AGA AAC TGT GCA AAA CAG-3, sexing R: 5-CTA TCA GAT CCA GAA TAT CTT CTG C-3). Polymerase chain reactions were performed with an iCycler thermal cycler (Bio-Rad, Hercules, CA). The conditions were denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s and 72°C for 30 s, and the final step was 5 min at 72°C.

### 3. Search on Annotation of Total Genes Available in Chicken W Chromosome

To identify genes in the chicken W chromosome, we compared the genomes of avian model organisms: Japanese quail (*Coturnix japonica*) and turkey (*Meleagris gallopavo*). For further understanding, we performed cross validation of the recent version of the W chromosomes from avian models, and we found that chicken W chromosomes are small in size compared to those of other birds (Table 1). Then, coding sequences (CDS) (n=36) were predicted for the chicken (*Gallus gallus*) W chromosome along with start and end positions. To annotate the predicted genes (n=36 genes), related gene symbols and gene IDs were searched to identify the chromosomal location on the latest version of chicken genome assembly, GRCg6a (GCF\_000002315.5), found in the NCBI public database. In addition, the UCSC genome browser

**Table 1.** The National Center for Biotechnology Information (NCBI) reference sequence assembly and accession information of W chromosomes in avian species

Species	Common name	Reference sequence assembly accession <sup>1</sup>	Annotation release	Reference sequence accession	Size (Mb)
<i>Gallus gallus</i>	Chicken	GCF_000002315.5	103	NC_006126.4	5.16
<i>Coturnix japonica</i>	Japanese quail	GCF_001577835.1	100	NC_029546.1	12.12
<i>Meleagris gallopavo</i>	Turkey	GCF_000146605.2	102	NC_015042.2	26.06

<sup>1</sup> Contributors: International Chicken Genome Consortium (Chicken); McDonnell Genome Institute - Washington University School of Medicine (Japanese quail); Turkey Genome Consortium (Turkey).

(<http://genome.ucsc.edu/cgi-bin/hgGateway?org=chicken>) was used to determine the gene locations of the W chromosome genes (Supplemental Table S1).

#### 4. cDNA Synthesis and RT-PCR

Total RNA from the test samples was isolated using the Trizol Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNA quality was checked by agarose gel electrophoresis, and the RNA quantity was determined by spectrophotometry readings at 260 nm (Lee et al., 2007). cDNA was synthesized from the RNA using a Superscript III First-Strand Synthesis System (Invitrogen). The cDNA was serially diluted five-fold and quantitatively normalized for PCR amplification.

To examine tissue-specific expression, reverse transcription-polymerase chain reactions (RT-PCRs) were performed with the prepared cDNA as previously reported (Rengaraj et al., 2011). Primers for 36 genes were tested in this study; the primers were designed using Primer 3 software and sequences from the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>) (Untergasser et al., 2012). Each test sample was run in triplicate.

## RESULTS

### 1. Identification and Annotation of Genes in the Chicken W Chromosome

In the avian sex determination system, both Z and W chromosomes have indispensable roles. In chickens, the W chromosome is shorter in size than the Z chromosome and is important for female development. Initially, the latest assem-

blies of the W chromosome of the avian model organisms Japanese quail (12.12 Mb) and turkey (26.06 Mb) were extracted, and in comparing the size of the three avian models, the chicken W chromosome was smaller (5.16 Mb) (Table 1). Based on the available sequence, total 36 genes in the chicken W chromosome were predicted with properties such as strand specification (+ and -), start and end positions, gene ID and putative gene symbols. Most of these transcripts were not annotated, and their chromosomal location was unidentified. Furthermore, *in silico* analysis using public databases NCBI Entrez and UCSC Genome Browser uncovered the chromosomal location of the predicted 36 genes. Among 36 genes analyzed, four genes were uncharacterized: LOC107055427, LOC100857682, LOC107055438 and LOC107055441. All genes that exhibited sex-specific expression patterns were annotated with putative names (Supplemental Table S1).

### 2. Female Specific Genes are Exclusively Expressed on W Chromosome

RT-PCR was used as a molecular screening method to explore the expression of sex-biased genes from the chicken W chromosome at day E6, hatching day and 24-week ovary and testes samples. The screening results showed nine female-specific genes (Fig. 1). The set of nine female-specific genes were exclusively expressed in a dosage-dependent manner in various developmental stages of the chicken. The initial stage of differentiation occurs at day E6 gonad, and 7 femalespecific genes such as NIPBLL, MIER3L, LOC100859467 (RSA1), RPL17L, LOC100859602, LOC107055424 (ZNF-532L) and LOC107055441, were expressed at this phase. In the second

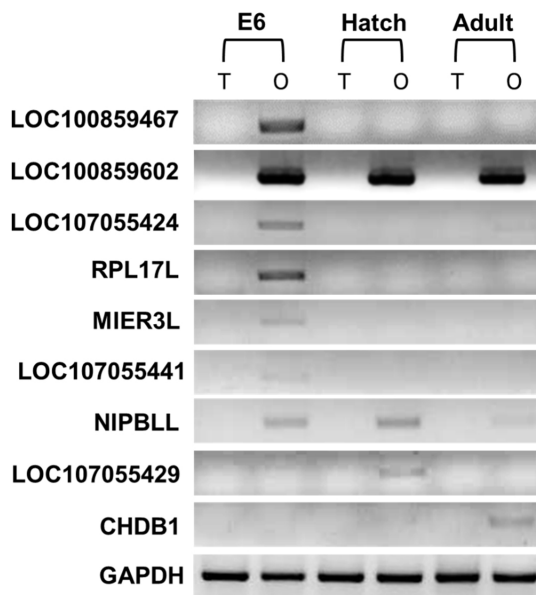
development stage, during hatching, LOC100859602, NIPBLL, and LOC107055429 were expressed in female samples. Finally, after 24 weeks, adult ovaries expressed LOC100859602, NIPBLL and CHDB1 in a dose-dependent manner. LOC100859602 was constantly expressed in all three developmental stages (Fig. 1).

## DISCUSSION

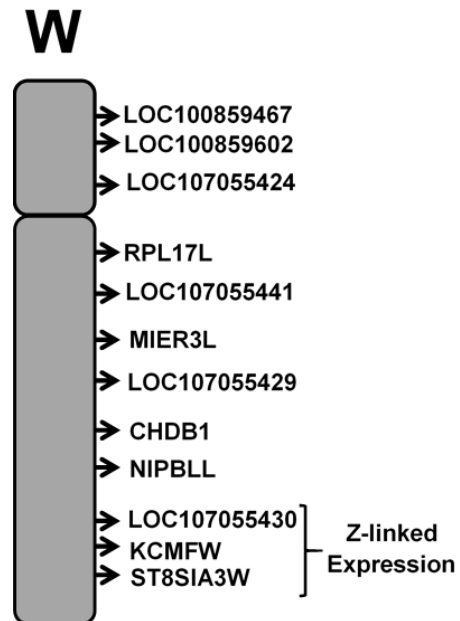
The avian sex determination chromosomal (ZW) system differs from the usual mammal XY chromosomal system. In contrast to mammals, males are homogametic (ZZ) in birds, and females are heterogametes (ZW) (Smith and Sinclair, 2004). In avian species, sex determination and gonad differentiation starts during early embryonic stages (E6). Sex determination in chickens is beyond the gonads and is cell autonomous, and previous studies have shown that the Z-linked DMRT1 gene is explicitly involved in male development in a dose-dependent manner (Smith et al., 2009). However, chromosome W is involved in female development, and no precise information is available about the genetic

factors governing female gonad development and sex determination. In this study, we identified all coding genes in the W chromosome of white Leghorn chickens using RT-PCR and annotated the genes using *in silico* techniques and the tool UCSC genome browser (Fig. 2). Furthermore, we identified a set of genes in chicken W chromosome related to all developmental stages from gonad differentiation to adult reproductive system functioning in female chicken.

CHDB1 was the first gene identified on the W chromosome of non-ratite birds, including chickens (Ellergren Hans, 1996). The chicken W chromosome is smaller in size and volume (5.16 Mb) than the W chromosome of other non-ratite avian models, and it is also smaller than the chicken sex chromosome Z (Table 1). The W chromosome with accession number NC\_006126 has 5160035 base pairs consisting of 36 coding genes that have putative functions (Table 1). The molecular screening analysis performed with RT-PCR affirmed that there were sex-specific genes out of 36 chromosome W-related genes, and they were found in both



**Fig. 1.** Identification of female-specific gene expression from a total of 36 genes available on the chicken W chromosome. RT-PCR was performed to examine the female-specific expression using the prepared testis (T) and ovary (O) at various developmental stages, such as embryonic day 6 (E6), hatch and adult.



**Fig. 2.** Gene locations of female specific and Z-linked genes selected from a total of 36 genes based on the chicken W chromosome. Schematic diagram of chicken W chromosomes showing representative chromosomal locations of female specific and Z-linked genes in W chromosome involved in sexual differentiation and germline development. The location of genes is indicated based on an ensemble browser illustration.

male and female samples. As suggested in previous studies, avian sex chromosomes have an ancestral relationship with autosomes; hence, out of 36 predicted genes, only a few genes (female=9) showed sex specificity, while the rest were expressed ubiquitously in both sexes with putative physiological functions (Fridolfsson et al., 1998). In this study, we analyzed the W chromosome from three major stages of the chicken development life cycle. Initial samples were derived from the early stages of development, the HH29 phase (day E6), in which gonadal differentiation is initiated, and gonads differentiate into male and female sexes. Screening results identified seven genes responsible for female sex development. In earlier transcriptome studies, KL Ayers et al. (2013) reported that RPL17L, MIER3L, and LOC107055424 (ZNF-532L), which are W-linked genes, were expressed in the mesoderm of day E4.5 gonads, and the data were confirmed by RNA-Seq. (Ayers et al., 2013a). Additionally, other studies related to the avian W chromosome Maghadam et al. mentioned RPL17L and hnRNPCL as femalespecific genes (Maghadam et al., 2012). In our results, we observed similar expression profiles in day E6 gonads. Additionally, we observed LOC107055441, an uncharacterized gene with a female-specific gonad expression pattern, which indicates the robustness of our study and the significance of the genes in early stage sex differentiation. Furthermore, the day E4.5 and day E6 expression profiles show that the dominant expression of these genes in dimorphic gonads predetermines sex. Along with female genes, three genes related to masculinity were also identified on W chromosome.

In the second developmental stage, during birth or on the day of hatching, LOC107055429 (E3 ubiquitin-protein ligase NEDD4-like) and LOC427025 (NIPBLL) were expressed specifically in female chicks, and no genes related to maleness were found on chromosome W during the hatching stage. In the final stage of the chicken life cycle, the gene LOC374195 (CHDB1) was found to be exclusively expressed in 24-week-old adult female ovaries and was the first gene identified in the avian W chromosome (Ellergen Hans, 1996). Fridolfsson et al. (1998) reported that CHDB1 is a female-specific gene that evolved from autosomes. This indicates the requirement of sex-linked genes in the development of female

morphological characteristics and the reproductive system in later stages. In addition to prior studies, we have shown stage-specific genes that govern femininity throughout the chicken life cycle. Interestingly, the W chromosome in the female gene LOC1008859602 (annotated as Zinc finger SWIM domain containing protein 6-like (ZSWIM6-like)) and the gene Nipped-B homolog-like (NIPBLL) were expressed in all the developmental stages of the chick, which signifies a role for these genes in sex determination and for maintaining the sex-associated physiological characteristics throughout bird life. In comparison with prior studies, our molecular screening results enabled more precise annotation of the gonad-specific gene expression. To the best of our knowledge, this is the first report explicitly showing the expression profiles of chicken W chromosome genes pertinent to each stage of chicken development, observed the temporal expression of W chromosome genes in all major developmental stages right from the gonadal differentiation to adult. Further, this study provides solid evidence for the role of the W chromosome in female chicken development. Since avian sex is determined by factors beyond the gonads and is cell autonomous, there is a need to confirm the ontology of these genes. We assume that further detailed studies on germ and somatic cells will reveal precise information about expression and maintenance of gender related characteristics by sex-biased genes.

## CONCLUSION

The molecular screening of the chicken W chromosome revealed female-specific genes in chicken. Stage-wise analysis of bimorphic gonads and ovaries from hatching and adult chicks emphasized the role of those genes required for advancement of particular developmental stage such as initiation of gonadal differentiation and direct genetics of sex determination on birth and matured adult. To conclude, our study is in agreement with previous studies and found additional female-specific marker genes in relevance to the chicken W chromosome. Further studies are required to understand the molecular function and ontologies of these genes, which will help to better understand their role in sex determination in

avian models.

## ACKNOWLEDGMENTS

This work was carried out with the support of Basic Science Research Program (Grant No. NRF-2017R1D1A1B 03029512) through the National Research Foundation (NRF) of Korea grant funded by the Ministry of Education and the support (Grant No. PJ0145102019) of the National Institute of Animal Sciences, Rural Development Administration (RDA), Republic of Korea. The authors declare no conflicts of interest.

## ORCID

Harikrishna Reddy Rallabandi <https://orcid.org/0000-0001-7757-9119>  
 Hyeon Yang <https://orcid.org/0000-0003-4162-4410>  
 Yong Jin Jo <https://orcid.org/0000-0001-9081-8874>  
 Hwi Cheul Lee <https://orcid.org/0000-0002-7644-2839>  
 Sung June Byun <https://orcid.org/0000-0001-6909-1025>  
 Bo Ram Lee <https://orcid.org/0000-0002-0537-6205>

## REFERENCES

- Ayers KL, Davidson NM, Demiyah D, Roeszler KN, Grutzner F, Sinclair AH, Oshlack A, Smith CA 2013a RNA sequencing reveals sexually dimorphic gene expression before gonadal differentiation in chicken and allows comprehensive annotation of the W-chromosome. *Genome Biology* 14(3):R26.
- Ayers KL, Sinclair AH, Smith CA 2013b The molecular genetics of ovarian differentiation in the avian model. *Sex Dev* 7(1-3):80-94.
- Bachtrog D, Kirkpatrick M, Mank JE, McDaniel SF, Pires JC, Rice WR, Valenzuela N 2011 Are all sex chromosomes created equal? *Trends in Genetics* 27(9):350-357.
- Berlin S, Tomaras D, Charlesworth B 2007 Low mitochondrial variability in birds may indicate Hill-Robertson effects on the W chromosome. *Heredity* 99(4):389-396.
- Chue J, Smith CA 2011 Sex determination and sexual differentiation in the avian model. *FEBS J* 278(7):1027-1034.
- Ellergren Hans 1996 First gene on the avian W chromosome (CHD) provides a tag for universal sexing of non-ratite birds. *Proceedings of the Royal Society B* 263(1377):1635-1641.
- Fridolfsson AK, Cheng H, Copeland NG, Jenkins NA, Liu HC, Raudsepp T, Woodage T, Chowdhary B, Halverson J, Ellegren H 1998 Evolution of the avian sex chromosomes from an ancestral pair of autosomes. *Proceedings of the National Academy of Sciences of the United States of America* 95(14):8147-8152.
- Graves JAM 2014 Avian sex, sex chromosomes, and dosage compensation in the age of genomics. *Chromosome Research* 22(1):45-57.
- Hamburger V, Hamilton HL 1951 A series of normal stages in the development of the chick embryo. *J Morphol* 88(1):49-92.
- Kiuchi T, Koga H, Kawamoto M, Shoji K, Sakai H, Arai Y, Ishihara G, Kawaoka S, Sugano S, Shimada T, Suzuki Y, Suzuki MG, Katsuma S 2014 A single female-specific piRNA is the primary determiner of sex in the silkworm. *Nature* 509(7502):633-636.
- Lee BR, Kim H, Park TS, Moon S, Cho S, Park T, Lim JM, Han JY 2007 A set of stage-specific gene transcripts identified in EK stage X and HH stage 3 chick embryos. *BMC Developmental Biology* 7(1):60.
- Mank JE 2012 Small but mighty: the evolutionary dynamics of W and Y sex chromosomes. *Chromosome Research* 20(1):21-33.
- Matsubara K, Tarui H, Toriba M, Yamada K, Nishida-Umehara C, Agata K, Matsuda Y 2006 Evidence for different origin of sex chromosomes in snakes, birds, and mammals and step-wise differentiation of snake sex chromosomes. *Proceedings of the National Academy of Sciences* 103(48):18190-18195.
- Moghadam HK, Pointer MA, Wright AE, Berlin S, Mank JE 2012 W chromosome expression responds to female-specific selection. *Proceedings of the National Academy of Sciences of the United States of America* 109(21):8207-8211.
- Rengaraj D, Lee BR, Lee SI, Seo HW, Han JY 2011 Expre-

- ssion patterns and miRNA regulation of DNA methyltransferases in chicken primordial germ cells. *Plos One* 6(5):e19524.
- Smith CA, Roeszler KN, Ohnesorg T, Cummins DM, Farlie PG, Doran TJ, Sinclair AH 2009 The avian Z-linked gene DMRT1 is required for male sex determination in the chicken. *Nature* 461(7261):267-271.
- Smith CA, Sinclair AH 2004 Sex determination: insights from the chicken. *Bioessays* 26(2):120-132.
- Solari AJ, Fechheimer NS, Bitgood JJ 1988 Pairing of ZW gonosomes and the localized recombination nodule in two Z-autosome translocations in *Gallus domesticus*. *Cytogenet* 48(3):130-136.
- Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG 2012 Primer3--new capabilities and interfaces. *Nucleic Acids Res* 40(15):e115.
- 
- Received Oct. 29, 2019, Revised Nov. 26, 2019, Accepted Nov. 29, 2019

**Supplemental Table S1.** Genomic information of total 36 genes available on chicken W chromosome and primers used for the gene expression analysis

No.	Chromosome	Start	Stop	Strand	GeneID	Gene symbol	Gene name	Refseq mRNA accession no	Refseq protein accession no	Protein length	F primer (RT-PCR)	R primer (RT-PCR)	Product size
1	W	13086	74398	-	100859467	LOC100859467	ras GTPase-activating protein 1-like	XM_015300408.1	XP_015155894.1	414	ATGTCGGAGC AAGGAACCTGG	CAAAGCGACG TCCACCAATG	548
2	W	99066	172630	+	427134	UBE2R2L	ubiquitin-conjugating enzyme E2 R2-like	XM_424727.5	XP_424727.3	238	CGGCTACTTCA AGGCTACA	CACGACTCCT CAITGGCCAGA	552
3	W	192483	241426	-	407091	UBAP2	ubiquitin associated protein 2	NM_001277104.1	NP_001264033.1	1099	CAGCCTTGGC CTGAGTGTAA	GGAGTCGAG GCAGTGTCA A	567
4	W	589579	917322	-	100859602	LOC100859602	zinc finger SWIM domain-containing protein 6-like	XM_015300409.1	XP_015155895.1	772	TGCAAAGCGT CTCTGCTGTA	AACAGTTTCC GGCTCCGATT	565
5	W	1442318	1504348	+	431564	ATP5A1W	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle	XM_429118.5	XP_429118.3	553	GCTCAGTTGGT GAAGAGGCT	AGCTTCATGG TACCTGCCAC	536
6	W	1511476	1710555	+	100858742	LOC100858742	chromosome Z open reading frame, human C18orf25 pseudogene	XM_015300411.1	XP_015155897.1	354	GAGAGCGATA AGGCAACGGA	CTGAGGTACT GCAGTTGCCA	585
7	W	1769135	1812821	+	425347	LOC425347	zinc finger protein 532-like	XM_015300414.1	XP_015155900.1	517	CTGCCAGATG CTGCTTCCTA	TATTCCCAGG TTTGGTGGCC	559
8	W	1845590	1847326	+	107055424	LOC107055424	zinc finger protein 532-like	XM_015300415.1	XP_015155901.1	167	CAACCTCTGCT CTATCGCCA	TCATTACCT TTCCAIGTTC CTTCC	401
9	W	1953096	1954928	-	100857334	RPL17L	60S ribosomal protein L17-like	XM_003643344.3	XP_003643392.2	146	GGCCACCAAG TACCTGAAGG	TTACTCCCGA GCCATCAGC	427
10	W	2000273	2003732	+	107049046	LOC107049046	60S ribosomal protein L17	XM_015300416.1	XP_015155902.1	213	ACCTACCAA GTGTGAGTGC	TCATCTCAAT GTGGCAGGG G	504
11	W	2095868	2103906	+	107055427	LOC107055427	uncharacterized LOC107055427	XM_015300418.1	XP_015155904.1	233	TGAGACGCAG GAACAATGCT	CAGGGGATCT TGGCCAACAA	570
12	W	2255017	2281254	+	107055428	SMAD7L	mothers against decapentaplegic homolog 7-like	XM_015300419.1	XP_015155905.1	388	GGAGGGTGAA CTGAAGGCTC	CAAGGAGGG CTCTTGGACA G	556
13	W	2631470	2638494	-	107055429	LOC107055429	E3 ubiquitin-protein ligase NEDD4-like	XM_015300420.1	XP_015155906.1	202	ACCTGAAAGCC AAATGGGTCT	CCTTCAGCAT TTTCAACTGC CA	544



Supplemental Table S1. Continued

No.	Chromosome	Start	Stop	Strand	GeneID	Gene symbol	Gene name	Refseq mRNA accession no	Refseq protein accession no	Protein length	F primer (RT-PCR)	R primer (RT-PCR)	Product size
14	W	2720821	2942316	-	107055431	LOC107055431	E3 ubiquitin-protein ligase NEDD4-like	XM_015300422.1	XP_015155908.1	663	TGCCAGTCCTC AGGAGCTAT	AGGAGAGCT GTAGGGTGAG G	553
15	W	2951669	2960297	+	107055430	LOC107055430	endogenous retrovirus group K member 11 Pol protein-like	XM_015300421.1	XP_015155907.1	357	ATGGAITGTGA CGCATGTGGT	CTATAAGCGG CCACGACTGT	574
16	W	3065668	3086087	-	426516	HNRNPKL	heterogeneous nuclear ribonucleoprotein K-like	NM_001031385.1	NP_001026556.1	427	CAAAATGGCAA ACGTCCTGCA	GGGCCCCGTCC TTTAAATTGGA	543
17	W	3151299	3287050	-	107049174	LOC107049174	Golgi phosphoprotein 3-like	XM_015300432.1	XP_015155918.1	293	CTCAGGATTA CGTGGCTGCA	GCCGTFACCT CTTTGTGGCT	551
18	W	3303786	3305258	-	100857682	LOC100857682	uncharacterized	XM_015300431.1	XP_015155917.1	443	GTGGGGAAA GACCTTACGG	CAAACACAGGA GTCCAGCCAT	591
19	W	3399794	3402362	-	107055436	LOC107055436	endogenous retrovirus group K member 8 Pol protein-like	XM_015300435.1	XP_015155921.1	634	GACGCGGAT GGAAGTCTAG	TTATGGAGGC TGCACACTGG	527
20	W	3408001	3408486	-	107055434	LOC107055434	ribonuclease H-like	XM_015300433.1	XP_015155919.1	161	CGGAGAAAGAG CCCCAATCAG	CCAGCGTGC TGCTTCATCA	438
21	W	3431953	3485141	+	107055435	LOC107055435	vasculin-like	XM_015300434.1	XP_015155920.1	332	TTTGCTCCAGC CTGGCTTAA	GCTTGTGCCG GTTCATTTT	600
22	W	3609318	3611707	-	107055438	LOC107055438	uncharacterized	XM_015300437.1	XP_015155923.1	217	CTTCTCCCCAT GTCTGCCAG	TGAGGGACCA TGTCAAAAGGC	541
23	W	3638690	3789295	+	426615	MIER3L	mesoderm induction early response protein 3-like	XM_015300438.1	XP_015155924.1	602	GTTTCTCTGGT TCCCTCCCG	TGGCCGAGGA AAGAAAGTCTG	538
24	W	3847684	3849033	+	107055437	LOC107055437	endogenous retrovirus group K member 18 Pol protein-like	XM_015300436.1	XP_015155922.1	449	AACTCTCCGC ACCTTGGAAAG	AGCTGTACAC ACACAGGCTC	523
25	W	3906265	3971384	-	769000	SMAD2W	SMAD family member 2-W	XM_001232180.4	XP_001232181.2	467	AGCAGAGCTG TCTCCAGTA	TGCTTGTAC TGTTTGCCCG	563
26	W	3996042	3998479	-	107055441	LOC107055441	uncharacterized	XM_015300445.1	XP_015155931.1	248	ACATGCTGGC TCGAGTTTGA	AACGTTCTAT GAGGCTGCC	529
27	W	4069695	4084145	-	776262	ST8SIA3W	ST8 alpha-N-acetylneuraminidase 2,8-sialyltransferase 3-W	XM_001235792.4	XP_001235793.3	432	TTACAACGTTT GCGCTGTGG	CCCAAAGGGC CAGAAATCCAT	502

Supplemental Table S1. Continued

No.	Chromosome	Start	Stop	Strand	GeneID	Gene symbol	Gene name	Refseq mRNA accession no	Refseq protein accession no	Protein length	F primer (RT-PCR)	R primer (RT-PCR)	Product size
28	W	4175497	4224565	-	431003	LOC431003	E3 ubiquitin-protein ligase KCMF1-like	XM_015300446.1	XP_015155932.1	329	TGTGCAGCAATT ACCTGGAGG	ACTGACGCTC TGCTTCTGAC	583
29	W	4303233	4416452	-	374014	SPIN1W	spindlin 1-W	NM_204191.1	NP_989522.1	262	ACGATCCAGA GCTGATGCAG	TTCACCTGGT TCCCGTTCG	592
30	W	4624865	4626568	-	107055442	LOC107055442	endogenous retrovirus group K member 18 Pol protein-like	XM_015300452.1	XP_015155938.1	567	ACCCCGGTGC AGTCAITTC	GCAGGCAATG TGAAGGCCAA A	512
31	W	4671567	4677341	-	107055443	LOC107055443	endogenous retrovirus group K member 25 Pol protein-like	XM_015300453.1	XP_015155939.1	246	GCCCTTACCA ACTGGGGTAC	AATCACTGCA TGGGACCAGG	588
32	W	4686614	4703657	-	107055444	RPTC15L	activated RNA polymerase II transcriptional coactivator p15-like	XM_015300454.1	XP_015155940.1	126	TGTGTCTTCAA GCTCATCTGCA	GCTGGTTCCA CTGTCTTCCA	314
33	W	4706658	4711173	-	107055445	LOC107055445	endogenous retrovirus group K member 25 Pol protein-like	XM_015300456.1	XP_015155942.1	246	GCCCTTACCA ACTGGGGTAC	AATCACTGCA TGGGACCAGG	588
34	W	4746744	4852709	+	427010	ZFRL1	zinc finger RNA-binding protein-like 1	XM_004938654.2	XP_004938711.2	1085	GCCTGGTTCG GCATGTACT	CACITGTCTG GTTTGCTGGG	558
35	W	4900482	5016478	+	374195	CHDB1	chromodomain helicase DNA binding protein 1	XM_015300460.1	XP_015155946.1	1777	TTGGCATCTGC TGACACTGT	GTCGCCACTG AACTTCTGGA	544
36	W	5058571	5156723	-	427025	NIPBL	Nipped-B homolog-like	NM_001257348.1	NP_001244277.1	2769	CTCCAACCTCC CACCATGAC	ACACAATGCT GAACAACCCG	520