

Supplementary Material: Determination of Genetic Variations of Toll-Like Receptor (TLR) 2, 4 and 6 with Next Generation Sequencing in Native Cattle Breeds of Anatolia and Holstein Friesian

Nuket Bilgen, Bengi Cinar Kul, Victoria Offord, Dirk Werling and Okan Ertugrul

Table S1. Oligonucleotide sequences were used in amplification.

Primer Name	Primer Sequence
TLR2_cow_F1	TCGTTTCCCTTACAGACACGCTTCA
TLR2_cow_R1.1.1	GATCCTAATTCCTGACCGACGTACC
TLR2_cow_F1.1.2	CCAGGTACGTCGGTCAGGAATTAGG
TLR2_cow_R1.1.2	TCATTTATAGAGGTTCAAAGGGCGTG
TLR2_cow_F1.1.3	GGCTTCACGCCCTTTGAACCTC
TLR2_cow_R1.1	AAAACAATGGAATAATAGCCCCAAAGG
TLR2_cow_F1.2	AGGTCTTTAATGTCTGGAAATGTCTTAGC
TLR2_cow_R1	GAAGACAGCATCATCCTAGGCCCTCAG
TLR2_cow_F2	TCTGTGGGTTCTGTGTCTATTGTCTGC
TLR2_cow_R2.1-2	TAAACTTTAGTGTTAAGGCAACCTCGC
TLR2_cow_F2.2	TCTCTTACTTTTTTCGATGATCCAGTGG
TLR2_cow_R2	GACATAGGTGATCTCATTGTTGGACAGG
TLR2_cow_F3	GTGGTCTACCTGTGTTAGTGTGTTGG
TLR2_cow_R3.1	TGTAAAGGACAGGAAGTCACAGGAGC
TLR2_cow_F3.2	TGCCTCCTTCTTACCCGTGTTATCAG
TLR2_cow_R3	TCTCTCGTTTCCTCTTCTGAACCTGC
TLR4_cow_F1.1-2	AAACGAAAGCAGAAAGCCACAGTTCC
TLR4_cow_R1.1.1	ACATAGCCCAGATGTGGAACAATAGG
TLR4_cow_F1.1.2	CCACTTAGGAATTACATGGGAACCTTAGG
TLR4_cow_R1.1	GCAGGAGAGACATAGAGAGGAGGACAG
TLR4_cow_F1.2	ATGGACTTGACTATTGCTTTGGAAACC
TLR4_cow_R1.2.1	GGACATTAAAGGCAAATACAGGGAGTG
TLR4_cow_F1.2.2	TTGCCTCATATAAAGCTCCTCACTCC
TLR4_cow_R1	GAAGACATTTGCTATCAAGTGCTGTGG
TLR4_cow_F2	CTCTAGATGGGGAGCTGATGGGAGTC
TLR4_cow_R2.1	GAGACCTGAAGAAGGGAGATAGCTTGC
TLR4_cow_F2.2	CAGAAAGCCTGATTCATGAGGTCTCC
TLR4_cow_R2	TAGAGAAGCAGAGGATGAAGGTGAAGG
TLR4_cow_F3	ATATTCACATGGCTTGTTGACAACAGG
TLR4_cow_R3.1	GTCAGTTCTGTGAAGATGTCAGGGAGC
TLR4_cow_F3.2	TTCCATGGCATCTTTACTGGCTTAGTC
TLR4_cow_R3	GCCTTCAGGACAGTCATAACGTACTAGG
TLR6_cow_F1	TTAAGGGTTTCCAAACACTGTCCAGG
TLR6_cow_R1.1	AGTTCATAATGGCACCATTTCGCTCTG
TLR6_cow_F1.2	GCATTGTGGAATAATCATCAACTGC
TLR6_cow_R1	ACTGAGCTATCTGTTTGTGATGTGAAGC
TLR6_cow_F2	GCAGTTTTTGTGAGCAGATTTCTGACC
TLR6_cow_R2.1	AATTTCCCATCCATTCTAGTTTGTGAG
TLR6_cow_F2.2-2	TTATAACCAGATTAGGCGCTCTAAAACC
TLR6_cow_R2.2-2	CACCAGTTAGCAGTGGACAAGCACAC

TLR6_cow_F3	AATCCACCTAGACTCAAGCCATCCAG
TLR6_cow_R3.1	AGAAACCCGTGAGATGTTTGTGATCC
TLR6_cow_F3.2	TTTAAACGAACCTCATCTCAGGCACG
TLR6_cow_R3	TCTTCACATGTCCCCTCAGATCTCTC
TLR6_cow_F4	GGGCATCTCTGTGAAAGGGTAAGTCC
TLR6_cow_R4.1	AGCCAAATGTGGAACAAAAGTGGTG
TLR6_cow_F4.2	TTTCTAGACCTACTCATGTTGCAAATGG
TLR6_cow_R4.2	TACAGAAATGGGCTAATTTGGGATGG
TLR6_cow_F4.3-2	TCTCCATTTCAAATGTGAAACTACAAGG
TLR6_cow_R4.3-2	TCAGTCCTAGCAGAGAAATAGATGAATCC
TLR2_H326QF	AGGCATCCTTACAGGCTGAG
TLR2_H326QR	CGGAAGTTGCATATTCCACAG

Table S2. PCR conditions and chemicals.

Chemical	Amount	Company
Buffer 5X contains MgCl ₂	1X	Thermo Scientific
dNTP	200 nmol	Thermo Scientific
Polymerase	1 U	Thermo Scientific (Phire II)
DMSO	5%	Thermo Scientific
Forward primer	5 pmol	
Reverse primer	5 pmol	
DNA	~50 ng	
Total	Add water to 25 µL	

Table S3. Determined haplotypes in *TLR2*, *TLR4* and *TLR6* gene regions.

	<i>TLR2</i>	<i>TLR4</i>	<i>TLR6</i>		<i>TLR2</i>	<i>TLR4</i>	<i>TLR6</i>
AB1	1–2	1–1	7–95	SAR1	1–2	1–1	5–18
AB2	1–2	2–3	5–21	SAR2	2–28	1–18	11–43
AB4	7–23	1–7	26–8	SAR3	1–7	1–7	28–92
AB5	2–28	1–7	40–14	SAR4	7–28	3–7	36–85
AB6	2–23	1–7	52–74	SAR5	1–2	1–3	69–75
AB7	1–2	1–7	11–51	SAR6	7–24	3–5	56–64
AB8	2–28	22–24	45–83	SAR7	17–33	1–3	61–69
AB9	6–24	1–9	35–7	SAR8	2–22	3–7	63–91
AB10	1–2	3–7	11–43	SAR9	9–22	3–7	88–98
AB11	2–25	3–7	39–81	SAR10	17–36	10–12	76–86
AB12	2–4	7–9	49–81	SAR11	2–24	7–9	46–92
AB13	1–2	3–7	11–51	SAR12	1–7	11–15	65–97
AB14	2–24	1–1	50–82	SAR14	2–26	1–1	27–79
AB15	2–28	1–3	48–82	SAR15	8–21	1–1	59–69
AB16	1–2	1–2	12–42	SAR16	1–7	5–9	66–16
AB17	2–23	1–1	11–51	SAR17	19–33	1–1	20–73
AB18	1–2	1–7	21–38	SAR18	9–26	1–7	24–93
AB19	2–3	1–9	2–32	SAR19	2–2	8–19	25–67
AB20	1–2	7–9	31–9	SAR20	2–3	1–3	10–17
AB21	2–23	1–1	34–71	SAR21	2–29	1–7	37–96
EAR1	12–31	7–9	5–19	TG1	1–7	1–1	3–3
EAR2	1–7	3–7	56–73	TG2	18–34	3–7	6–22
EAR3	6–22	1–9	11–43	TG3	15–3	1–2	8–1
EAR4	2–4	1–2	11–44	TG4	14–3	1–21	11–41
EAR5	2–4	3–7	5–54	TG5	15–3	1–1	23–9

EAR6	6–26	17–25	11–43	TG6	1–2	7–9	11–43
EAR7	17–33	7–9	72–29	TG7	11–7	7–9	4–34
EAR8	4–7	7–9	4–62	TG8	1–7	1–1	5–15
EAR9	16–35	7–9	11–43	TG9	13–32	1–9	11–43
EAR10	2–27	1–7	11–43	TG10	2–4	4–13	11–94
EAR11	6–26	1–2	33–84	HOL4	1–2	1–7	11–43
EAR12	4–7	7–9	5–55	HOL45	1–2	1–1	11–47
EAR13	12–35	6–17	78–87	HOL80	1–2	16–23	13–48
EAR14	5–1	2–3	5–54	HOL97	1–2	1–1	11–43
EAR15	6–22	6–17	11–43	HOL99	1–2	7–9	9–58
EAR16	4–7	1–9	11–44	HOL115	1–1	1–1	4–6
EAR17	1–2	1–7	5–53	HOL150	1–1	14–23	11–43
EAR18	1–2	1–9	68–75	HOL157	1–6	1–1	9–57
EAR19	1–7	1–1	9–57	HOL160	1–1	1–3	11–43
EAR20	6–24	7–9	77–89	HOL161	<i>Excluded</i>		
