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*Supplement of*

## **Eight Y chromosome genes show copy number variations in horses**

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1 **Table S1.** primer information for eight horse Y chromosome genes

Gene		Primer F	Primer R	Tm (°C)	Length (bp)	Accession number
<i>beta actin</i>		CACGCCTTTTGCTCACTGTGC	ACGGGCTTTGTACACGAG	63-65.6	203	NC_009156
<i>ETSTY1</i>	Equus Testis-specific transcript on Y 1	GACGGACGACCTTGTTGTTTT	CTAGTGGCGAGTCCTTTTGG	63	234	EU687549
<i>ETSTY4</i>	Equus Testis-specific transcript on Y 4	GCGTCTGTGCAGATGTGTCT	GCTCATGCAGTCAAACAGGA	65	175	EU687552
<i>ETSTY5</i>	Equus Testis-specific transcript on Y 5	CAAAACCAAGAGGAGGACCA	CTCCAGAGGCAGGTACTTCG	65	210	EU687553
<i>EIF1AY</i>	Translation initiation factor 1A Y	GATCGTGGCCTTCTGACATT	TTATTTTGGGCATGGTGGT	63	187	ET052957
<i>SRY</i>	Sex determining region Y	CCAACGCTTTATCTTCGCA	CCATTTCTCTTGTTTCACTCC	63	203	NM_001081810
<i>ETY4</i>	Equus transcript Y4	TGGGGATATTGGCTTAGCTG	CTGGGAGCACGTCTGTATCA	65.6	180	EU687558
<i>UBE1Y</i>	Ubiquitin activating enzyme Y	TGGCCAACCTCACGCTGATCCAA	CTTCTCCACTCACCTACTTGGG	65.6	210	EU687568
<i>YIR2</i>	Inverted repeat 2 Y	AGGGTTGGGCTAAGTCACCT	ACCTTGGATCCAGACTCACG	65.6	170	EU687570

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3

4 **Table S2** TA cloning results for *EIF1AY* gene

	2	3	3	5	5	6	7	7	7	8	9	10	10	11	12	12	14	17	17	17	19	20	22	23	23	24	24	25	26	26	26	27	30	30	30	32	33	35	36	36	36	39	40	42	43	43	44							
reference sequence	A	T	T	T	T	A	T	T	A	T	G	A	T	G	C	A	T	G	T	T	A	C	T	A	T	T	T	T	C	T	C	T	A	T	T	T	C	A	A	A	A	G	A	C	A									
BS1 TA1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.					
BS1 TA2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
BS1 TA3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
BS1 TA4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
BS1 TA5	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			
BS1 TA6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
BS1 TA7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
BS1 TA8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
BS1 TA9	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
BS1 TA10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
BS1 TA11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
BS1 TA12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
BS1 TA13	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
BS1 TA14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
BS1 TA15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Q TA1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Q TA2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Q TA3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Q TA4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Q TA5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Q TA6	..... G .....
Q TA7	..... C .....
Q TA8	.....
Q TA9	. C .....
Q TA10	.....
Q TA11	.....
HN3 TA1	.....
HN3 TA2	.....
HN3 TA3	.....
HN3 TA4	.....
HN3 TA5	..... A . . . . . G . . . . . T . . . T .
HN3 TA6	..... C . . . . . C . . . . .
HN3 TA7	.....
HN3 TA8	..... C . . . . .
HN3 TA9	.....
HN3 TA10	.....
HN3 TA11	.....
HN3 TA12	..... T . . . . .
HN3 TA13	.....
HN3 TA14	.....
BS24 TA1	..... A . . . . .
BS24 TA2	.....
BS24 TA3	..... T . . . . .
BS24 TA4	.....
BS24 TA5	..... C . . . . .
BS24 TA6	.....
BS24 TA7	.....
BS24 TA8	.....
BS24 TA9	..... C . . . . .
BS24 TA10	.....
BS24 TA11	..... A . . . . . G . . . . .
BS24 TA12	.....
BS24 TA13	..... G . . . . . T . . . . .
DB21 TA1	.....
DB21 TA2	.....
DB21 TA3	..... T . . . . .
DB21 TA4	.....
DB21 TA5	.....

DB21 TA6	..... C .....
DB21 TA7	.....
DB21 TA8	.....
DB21 TA9	.....
DB21 TA10	..... C .....
DB21 TA11	..... T .....
DB21 TA12	.....
DB21 TA13	.....
DB21 TA14	..... C ..... C .....
YJ26 TA1	..... C .....
YJ26 TA2	..... C .....
YJ26 TA3	..... C .....
YJ26 TA4	..... C ..... C .....
YJ26 TA5	..... C ..... G .....
YJ26 TA6	..... C C ..... A .....
YJ26 TA7	..... C .....
YJ26 TA8	..... C ..... C .....
YJ26 TA9	..... C .....
YJ26 TA10	..... C ..... A .....
YJ26 TA11	..... C ..... A .....
YJ26 TA12	..... C .....
YJ26 TA13	..... C .....
YJ26 TA14	..... C .....
YJ28 TA1	..... C .....
YJ28 TA2	..... C .....
YJ28 TA3	..... C .....
YJ28 TA4	..... C ..... G .....
YJ28 TA5	..... C .....
YJ28 TA6	..... C ..... C . T .....
YJ28 TA7	..... C .....
YJ28 TA8	..... C . T .....
YJ28 TA9	..... C .....
YJ28 TA10	..... A C ..... G .....
YJ28 TA11	..... C .....
YJ28 TA12	..... C .....
YJ28 TA13	.....
BS38 TA1	..... C .....
BS38 TA2	..... C .....

BS38 TA3	..... C .....
BS38 TA4	..... C ..... C .....
BS38 TA5	..... C .....
BS38 TA6	..... C .....
BS38 TA7	..... C .....
BS38 TA8	G..... C .....
BS38 TA9	..... C .....
BS38 TA10	..... C .....
BS38 TA11	..... C .....
BS38 TA12	..... C ..... C .....
BS38 TA13	..... C .....
BS38 TA14	..... C ..... G ..... T .....

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**Table S3:** Multiple pair-wise comparisons of median copy number (MCN) of *UBE1Y* gene

	BLK	BS	CD	CKY	DB	GU	GZ	HN	HSK	NQ	MY	MG	YJ	YS
BLK	—													
BS	nsd	—												
CD	*	nsd	—											
CKY	**	nsd	nsd	—										
DB	nsd	nsd	nsd	*	—									
GU	nsd	nsd	*	*	nsd	—								
GZ	nsd	***	***	***	***	nsd	—							
HN	nsd	nsd	**	nsd	nsd	nsd	***	—						
HSK	nsd	nsd	**	nsd	nsd	nsd	***	nsd	—					
NQ	*	nsd	nsd	nsd	nsd	nsd	***	nsd	nsd	—				
MY	*	nsd	nsd	nsd	nsd	nsd	***	nsd	nsd	nsd	—			
MG	***	**	*	nsd	**	*	***	***	**	nsd	nsd	—		
YJ	nsd	nsd	nsd	nsd	nsd	nsd	***	nsd	nsd	nsd	nsd	**	—	
YS	**	nsd	nsd	nsd	nsd	nsd	***	nsd	nsd	nsd	nsd	nsd	nsd	—

8 Note: nsd: no significant difference; \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001. The full name of each breed is  
9 listed in Table 1.

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11  
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13  
14  
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17 **Table S4:** Multiple pair-wise comparisons of median copy number (MCN) of *SRY* gene

	BLK	BS	CD	CKY	DB	GU	GZ	HN	HSK	NQ	MY	MG	YJ	YS
BLK	—													
BS	nsd	—												
CD	nsd	nsd	—											
CKY	nsd	nsd	nsd	—										
DB	nsd	nsd	nsd	nsd	—									
GU	nsd	nsd	nsd	nsd	nsd	—								
GZ	nsd	nsd	nsd	nsd	nsd	nsd	—							
HN	nsd	nsd	nsd	nsd	nsd	nsd	nsd	—						
HSK	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	—					
NQ	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	—				
MY	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	—			
MG	nsd	nsd	*	*	*	nsd	**	*	nsd	nsd	nsd	—		
YJ	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	—	
YS	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	**	nsd	—

18 Note: nsd: no significant difference; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ . The full name of each breed is  
 19 listed in Table 1.

20

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22 **Table S5:** Multiple pair-wise comparisons of median copy number (MCN) of *EIF1AY* gene

	BLK	BS	CD	CKY	DB	GU	GZ	HN	HSK	NQ	MY	MG	YJ	YS
BLK	—													
BS	nsd	—												
CD	***	**	—											
CKY	*	*	nsd	—										
DB	nsd	nsd	nsd	nsd	—									
GU	nsd	nsd	*	nsd	nsd	—								
GZ	nsd	nsd	***	**	nsd	nsd	—							
HN	nsd	nsd	**	*	nsd	nsd	nsd	—						
HSK	*	nsd	nsd	nsd	nsd	nsd	*	nsd	—					
NQ	*	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	—				
MY	nsd	nsd	*	nsd	nsd	nsd	nsd	nsd	nsd	nsd	—			
MG	***	***	*	**	**	**	***	***	*	*	**	—		
YJ	nsd	nsd	***	***	nsd	nsd	nsd	nsd	**	*	nsd	***	—	
YS	nsd	nsd	***	**	nsd	nsd	nsd	nsd	*	nsd	nsd	***	nsd	—

23 Note: nsd: no significant difference; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ . The full name of each breed is  
 24 listed in Table 1.

25

26 **Table S6:** Multiple pair-wise comparisons of median copy number (MCN) of *ETSTY1* gene

	BLK	BS	CD	CKY	DB	GU	GZ	HN	HSK	NQ	MY	MG	YJ	YS
BLK	—													
BS	nsd	—												
CD	***	**	—											
CKY	nsd	nsd	nsd	—										
DB	nsd	nsd	**	nsd	—									
GU	nsd	nsd	nsd	nsd	nsd	—								
GZ	**	*	nsd	nsd	*	nsd	—							
HN	nsd	nsd	nsd	nsd	nsd	nsd	nsd	—						
HSK	nsd	nsd	**	*	nsd	nsd	**	nsd	—					
NQ	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	—				
MY	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	—			
MG	nsd	nsd	***	**	nsd	nsd	***	nsd	nsd	nsd	nsd	—		
YJ	nsd	nsd	***	**	nsd	nsd	***	nsd	nsd	nsd	nsd	nsd	—	
YS	nsd	nsd	***	**	nsd	nsd	***	*	nsd	nsd	nsd	nsd	nsd	—

27 Note: nsd: no significant difference; \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001. The full name of each breed is  
 28 listed in Table 1.

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31 **Table S7:** Multiple pair-wise comparisons of median copy number (MCN) of *ETSTY4* gene

	BLK	BS	CD	CKY	DB	GU	GZ	HN	HSK	NQ	MY	MG	YJ	YS
BLK	—													
BS	nsd	—												
CD	nsd	nsd	—											
CKY	**	*	*	—										
DB	*	nsd	nsd	nsd	—									
GU	nsd	nsd	nsd	nsd	nsd	—								
GZ	***	***	***	***	***	*	—							
HN	nsd	nsd	nsd	*	*	nsd	***	—						
HSK	nsd	nsd	nsd	nsd	nsd	nsd	***	nsd	—					
NQ	nsd	nsd	nsd	nsd	nsd	nsd	**	nsd	nsd	—				
MY	nsd	nsd	nsd	nsd	nsd	nsd	**	nsd	nsd	nsd	—			
MG	*	nsd	nsd	nsd	nsd	nsd	***	*	nsd	nsd	nsd	—		
YJ	nsd	nsd	nsd	nsd	nsd	nsd	***	nsd	nsd	nsd	nsd	nsd	—	
YS	nsd	nsd	nsd	nsd	nsd	nsd	***	nsd	nsd	nsd	nsd	nsd	nsd	—

32 Note: nsd: no significant difference; \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001. The full name of each breed is  
 33 listed in Table 1.

34

35 **Table S8:** Multiple pair-wise comparisons of median copy number (MCN) of *ETSTY5* gene.

	BLK	BS	CD	CKY	DB	GU	GZ	HN	HSK	NQ	MY	MG	YJ	YS
BLK	—													
BS	nsd	—												
CD	***	***	—											
CKY	**	**	***	—										
DB	**	***	***	nsd	—									
GU	nsd	nsd	nsd	nsd	nsd	—								
GZ	***	***	nsd	***	***	nsd	—							
HN	**	***	***	*	nsd	nsd	***	—						
HSK	**	***	**	*	nsd	nsd	**	nsd	—					
NQ	nsd	nsd	**	nsd	nsd	nsd	**	nsd	nsd	—				
MY	*	***	*	*	nsd	nsd	*	nsd	nsd	nsd	—			
MG	nsd	nsd	***	nsd	nsd	nsd	***	**	**	nsd	*	—		
YJ	***	***	**	***	*	nsd	***	nsd	nsd	nsd	nsd	***	—	
YS	*	*	***	nsd	nsd	nsd	***	*	*	nsd	nsd	nsd	***	—

36 Note: nsd: no significant difference; \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001. The full name of each breed is  
 37 listed in Table 1.

38

39

40 **Table S9:** Multiple pair-wise comparisons of median copy number (MCN) of *ETY4* gene

	BLK	BS	CD	CKY	DB	GU	GZ	HN	HSK	NQ	MY	MG	YJ	YS
BLK	—													
BS	nsd	—												
CD	nsd	nsd	—											
CKY	*	**	***	—										
DB	nsd	nsd	nsd	nsd	—									
GU	nsd	nsd	nsd	nsd	nsd	—								
GZ	nsd	*	nsd	***	*	nsd	—							
HN	nsd	nsd	nsd	*	nsd	nsd	**	—						
HSK	nsd	nsd	nsd	nsd	nsd	nsd	nsd	nsd	—					
NQ	*	nsd	*	nsd	nsd	nsd	**	nsd	nsd	—				
MY	nsd	nsd	*	nsd	nsd	nsd	**	nsd	nsd	nsd	—			
MG	***	**	***	nsd	*	nsd	***	**	nsd	nsd	nsd	—		
YJ	nsd	nsd	nsd	**	nsd	nsd	nsd	nsd	nsd	*	nsd	***	—	
YS	nsd	nsd	nsd	*	nsd	nsd	**	nsd	nsd	nsd	nsd	**	nsd	—

41 Note: nsd: no significant difference; \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001. The full name of each breed is  
 42 listed in Table 1.

43



44 **Table S10:** Multiple pair-wise comparisons of median copy number (MCN) of *YIR2* gene

	BLK	BS	CD	CKY	DB	GU	GZ	HN	HSK	NQ	MY	MG	YJ	YS
BLK	—													
BS	nsd	—												
CD	nsd	nsd	—											
CKY	nsd	nsd	*	—										
DB	nsd	nsd	nsd	*	—									
GU	nsd	nsd	nsd	*	*	—								
GZ	*	***	nsd	***	*	nsd	—							
HN	nsd	nsd	*	nsd	**	*	***	—						
HSK	nsd	nsd	nsd	nsd	*	*	***	nsd	—					
NQ	nsd	nsd	nsd	nsd	nsd	nsd	*	nsd	nsd	—				
MY	nsd	nsd	nsd	nsd	nsd	nsd	**	nsd	nsd	nsd	—			
MG	nsd	nsd	nsd	nsd	nsd	*	***	nsd	nsd	nsd	nsd	—		
YJ	nsd	nsd	nsd	nsd	nsd	*	**	nsd	nsd	nsd	nsd	nsd	—	
YS	nsd	**	nsd	***	nsd	nsd	*	**	**	*	**	**	nsd	—

45 Note: nsd: no significant difference; \* P < 0.05; \*\* P < 0.01; \*\*\* P < 0.001. The full name of each breed is  
 46 listed in Table 1.