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A few general comments about the genetic variability analysis based upon DNA microsatellites compared to blood typing. The DNA systems are more variable than blood typing.

### Genetic Analysis of the Pryor Mountains Wild horse Range, MT

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August 22, 2013

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#### METHODS

A total of 48 samples were received by Texas A&M University, Equine Genetics Lab on February 21, 2013. DNA was extracted from the samples and tested for variation at 12 equine microsatellite (msat) systems. These were AHTA, AHTB, ASBT, ASBT2, AVS2, HARS, AVS2, AVS4, WIC10, LEX3, and VML2. These systems were tested using an automated DNA sequencer to separate Polymerase Chain Reaction (PCR) products.

A variety of genetic variability measures were calculated from the gene marker data. The measures were observed heterozygosity (Ho) which is the actual number of loci heterozygous per individual, expected heterozygosity (He) which is the predicted number of heterozygous loci based upon gene frequencies, effective number of alleles (Ne) which is a measure of marker

The following is a report of the genetic analysis of the Pryor Mountains Wild horse Range, MT.

A few general comments about the genetic variability analysis based upon DNA microsatellites compared to blood typing. The DNA systems are more variable than blood typing systems, thus variation levels will be higher. Variation at microsatellite loci is strongly influenced by allelic diversity and changes in variation will be seen in allelic measures more quickly than at heterozygosity, which is why more allelic diversity measures are calculated. For mean values, there are a greater proportion of rare domestic breeds included in the estimates than for blood typing so relative values for the measures are lower compared to the feral horse values. As well, feral values are relatively higher because the majority of herds tested are of mixed ancestry which results in a relatively greater increase in heterozygosity values based upon the microsatellite data. There are no specific variants related to breed type so similarity is based upon the total data set.

## **METHODS**

A total of 45 samples were received by Texas A&M University, Equine Genetics Lab on February 21, 2013. DNA was extracted from the samples and tested for variation at 12 equine microsatellite (mSat) systems. These were *AHT4*, *AHT5*, *ASB2*, *ASB17*, *ASB23*, *HMS3*, *HMS6*, *HMS7*, *HTG4*, *HTG10*, *LEX33*, and *VHL20*. These systems were tested using an automated DNA sequencer to separate Polymerase Chain Reaction (PCR) products.

A variety of genetic variability measures were calculated from the gene marker data. The measures were observed heterozygosity (*Ho*) which is the actual number of loci heterozygous per individual; expected heterozygosity (*He*), which is the predicted number of heterozygous loci based upon gene frequencies; effective number of alleles (*Ae*) which is a measure of marker

system diversity; total number of variants (*TNV*); mean number of alleles per locus (*MNA*); the number of rare alleles observed which are alleles that occur with a frequency of 0.05 or less (*RA*); the percent of rare alleles (*%RA*); and estimated inbreeding level (*Fis*) which is calculated as  $1-Ho/He$ .

Genetic markers also can provide information about ancestry in some cases. Genetic resemblance to domestic horse breeds was calculated using Rogers' genetic similarity coefficient, *S*. This resemblance was summarized by use of a restricted maximum likelihood (RML) procedure.

## RESULTS AND DISCUSSION

Variants present and allele frequencies are given in Table 1. No variants were observed which have not been seen in horse breeds. Table 2 gives the values for the genetic variability measures of the Pryor Mountains Wild horse Range herd. Also shown in Table 2 are values from a representative group of domestic horse breeds. The breeds were selected to cover the range of variability measures in domestic horse populations. Mean values for feral herds (based upon data from 126 herds) and mean values for domestic breeds (based upon 80 domestic horse populations) also are shown.

Mean genetic similarity of the Pryor Mountains Wild horse Range herd to domestic horse breed types are shown in Table 3. A dendrogram of relationship of the Pryor Mountains Wild horse Range herd to a standard set of domestic breeds is shown in Figure 1.

**Genetic Variants:** A total of 75 variants were seen in the Pryor Mountains Wild horse Range herd which is slightly above the mean for feral herds and slightly below the mean for domestic breeds. Of these, 15 had frequencies below 0.05 which is below the average percentage

of variants at risk of future loss. Allelic diversity as represented by  $A_e$  is somewhat higher than the average for feral herds as is  $MNA$ .

**Genetic Variation:** Both observed heterozygosity and expected heterozygosity in the Pryor Mountains Wild horse Range herd is above the feral mean.  $H_e$  is slightly higher than  $H_o$  which could indicate the very beginning of evidence of inbreeding. However, the difference at this point is not statistically significant.

**Genetic Similarity:** Overall similarity of the Pryor Mountains Wild horse Range herd to domestic breeds was low for feral herds. Highest mean genetic similarity of the Pryor Mountains Wild horse Range herd was with Light Racing and Riding breeds, followed by the Old World Iberian breeds. As seen in Fig. 1, the Pryor Mountains Wild horse Range herd clusters within a group of Iberian horses. Previous studies had indicated Iberian ancestry for this herd but cluster results have not always supported the qualitative indications. As with most trees involving feral herds, the tree is somewhat distorted.

## SUMMARY

Genetic variability of this herd in general is on the high side but compared to past sampling of this herd, variability levels for all measures has been in decline. This is likely due to the population size that has been maintained in recent years. Overall, the herd is showing evidence of Spanish heritage that is stronger than seen recently, although the known mixed ancestry is still apparent. This is possibly due to the efforts to remove horses derived from introductions.

## RECOMMENDATIONS

Current variability levels are high enough that no immediate action is needed. However, there has been a general trend for a decline in variations levels of the herd. If the trend continues

the variability levels of the herd could drop below the feral average within the next five to ten years. The best way to maintain current levels would be to increase population size if range conditions allow.



**Table 1.** Allele frequencies of genetic variants observed in Pryor Mountains Wild horse Range feral horse herd.

<b>VHL20</b>															
<b>I</b>	<b>J</b>	<b>K</b>	<b>L</b>	<b>M</b>	<b>N</b>	<b>O</b>	<b>P</b>	<b>Q</b>	<b>R</b>	<b>S</b>					
0.111	0.144	0.033	0.000	0.357	0.133	0.067	0.022	0.011	0.122	0.000					
<b>HTG4</b>															
<b>I</b>	<b>J</b>	<b>K</b>	<b>L</b>	<b>M</b>	<b>N</b>	<b>O</b>	<b>P</b>	<b>Q</b>	<b>R</b>						
0.000	0.000	0.278	0.000	0.488	0.000	0.067	0.167	0.000	0.000						
<b>AHT4</b>															
<b>H</b>	<b>I</b>	<b>J</b>	<b>K</b>	<b>L</b>	<b>M</b>	<b>N</b>	<b>O</b>	<b>P</b>	<b>Q</b>	<b>R</b>					
0.200	0.011	0.244	0.289	0.056	0.011	0.011	0.089	0.089	0.000	0.000					
<b>HMS7</b>															
<b>I</b>	<b>J</b>	<b>K</b>	<b>L</b>	<b>M</b>	<b>N</b>	<b>O</b>	<b>P</b>	<b>Q</b>	<b>R</b>						
0.000	0.000	0.000	0.633	0.056	0.022	0.289	0.000	0.000	0.000						
<b>AHT5</b>															
<b>I</b>	<b>J</b>	<b>K</b>	<b>L</b>	<b>M</b>	<b>N</b>	<b>O</b>	<b>P</b>	<b>Q</b>	<b>R</b>						
0.000	0.133	0.533	0.133	0.000	0.179	0.022	0.000	0.000	0.000						
<b>HMS6</b>															
<b>I</b>	<b>J</b>	<b>K</b>	<b>L</b>	<b>M</b>	<b>N</b>	<b>O</b>	<b>P</b>	<b>Q</b>	<b>R</b>						
0.000	0.000	0.011	0.200	0.144	0.056	0.189	0.400	0.000	0.000						
<b>ASB2</b>															
<b>B</b>	<b>I</b>	<b>J</b>	<b>K</b>	<b>L</b>	<b>M</b>	<b>N</b>	<b>O</b>	<b>P</b>	<b>Q</b>	<b>R</b>					
0.056	0.000	0.000	0.433	0.000	0.000	0.144	0.067	0.000	0.300	0.000					
<b>HTG10</b>															
<b>H</b>	<b>I</b>	<b>J</b>	<b>K</b>	<b>L</b>	<b>M</b>	<b>N</b>	<b>O</b>	<b>P</b>	<b>Q</b>	<b>R</b>	<b>S</b>	<b>T</b>			
0.000	0.100	0.000	0.033	0.033	0.144	0.133	0.156	0.000	0.268	0.133	0.000	0.000			
<b>HMS3</b>															
<b>H</b>	<b>I</b>	<b>J</b>	<b>K</b>	<b>L</b>	<b>M</b>	<b>N</b>	<b>O</b>	<b>P</b>	<b>Q</b>	<b>R</b>	<b>S</b>				
0.000	0.022	0.000	0.000	0.000	0.056	0.133	0.200	0.467	0.000	0.122	0.000				
<b>ASB17</b>															
<b>D</b>	<b>F</b>	<b>G</b>	<b>H</b>	<b>I</b>	<b>J</b>	<b>K</b>	<b>L</b>	<b>M</b>	<b>N</b>	<b>O</b>	<b>P</b>	<b>Q</b>	<b>R</b>	<b>S</b>	<b>T</b>
0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.300	0.167	0.000	0.000	0.178	0.277	0.011	0.000
<b>ASB23</b>															
<b>G</b>	<b>H</b>	<b>I</b>	<b>J</b>	<b>K</b>	<b>L</b>	<b>M</b>	<b>N</b>	<b>O</b>	<b>P</b>	<b>Q</b>	<b>R</b>	<b>S</b>	<b>T</b>	<b>U</b>	<b>V</b>
0.000	0.156	0.056	0.089	0.000	0.156	0.000	0.000	0.000	0.000	0.000	0.000	0.267	0.000	0.276	0.000
<b>LEX33</b>															
<b>F</b>	<b>G</b>	<b>K</b>	<b>L</b>	<b>M</b>	<b>N</b>	<b>O</b>	<b>P</b>	<b>Q</b>	<b>R</b>	<b>S</b>	<b>T</b>				
0.000	0.011	0.111	0.267	0.033	0.000	0.300	0.000	0.178	0.100	0.000	0.000				

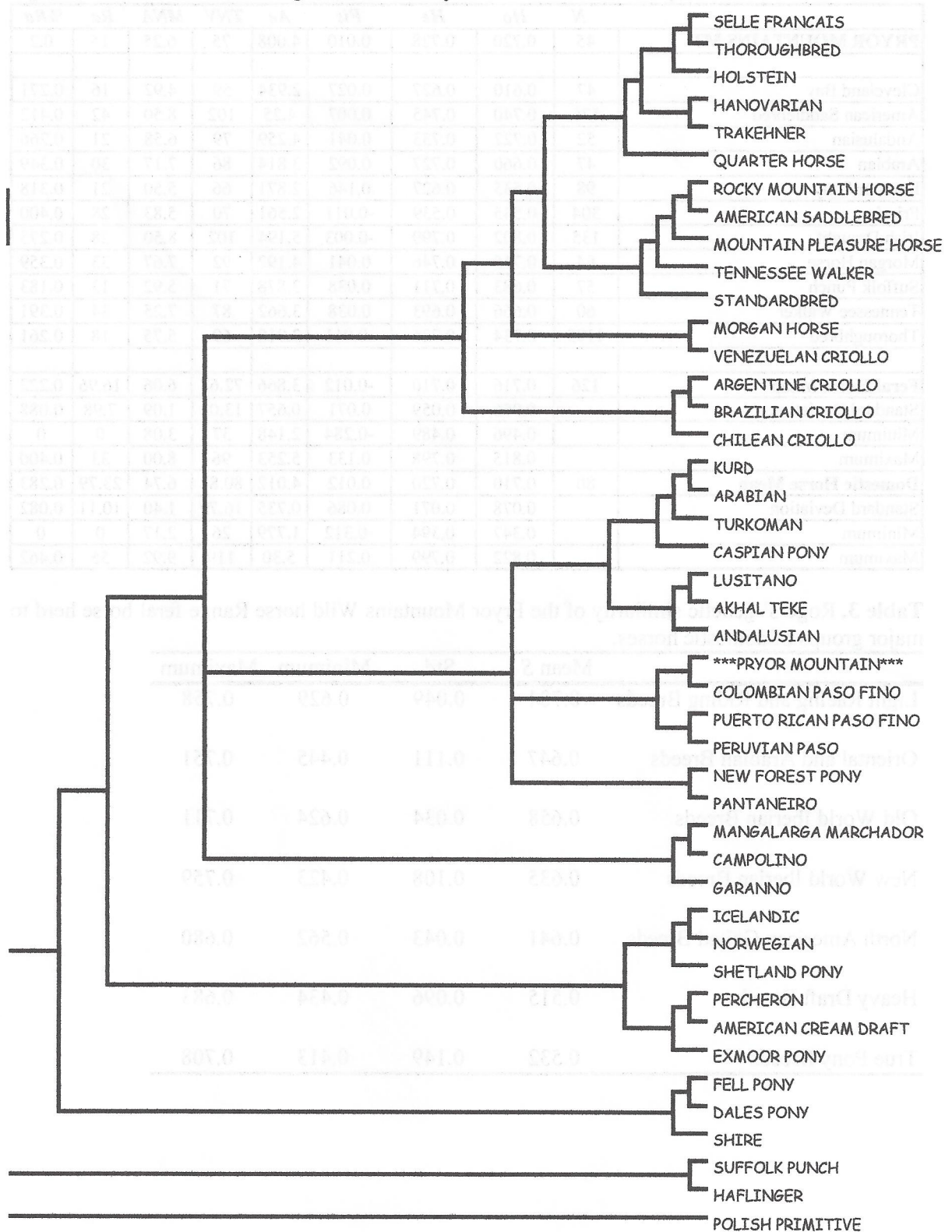
**Table 2.** Genetic variability measures.

	<i>N</i>	<i>Ho</i>	<i>He</i>	<i>Fis</i>	<i>Ae</i>	<i>TNV</i>	<i>MNA</i>	<i>Ra</i>	<i>%Ra</i>
<b>PRYOR MOUNTAINS MT</b>	45	0.720	0.728	0.010	4.008	75	6.25	15	0.2
Cleveland Bay	47	0.610	0.627	0.027	2.934	59	4.92	16	0.271
American Saddlebred	576	0.740	0.745	0.007	4.25	102	8.50	42	0.412
Andalusian	52	0.722	0.753	0.041	4.259	79	6.58	21	0.266
Arabian	47	0.660	0.727	0.092	3.814	86	7.17	30	0.349
Exmoor Pony	98	0.535	0.627	0.146	2.871	66	5.50	21	0.318
Friesian	304	0.545	0.539	-0.011	2.561	70	5.83	28	0.400
Irish Draught	135	0.802	0.799	-0.003	5.194	102	8.50	28	0.275
Morgan Horse	64	0.715	0.746	0.041	4.192	92	7.67	33	0.359
Suffolk Punch	57	0.683	0.711	0.038	3.878	71	5.92	13	0.183
Tennessee Walker	60	0.666	0.693	0.038	3.662	87	7.25	34	0.391
Thoroughbred	1195	0.734	0.726	-0.011	3.918	69	5.75	18	0.261
<b>Feral Horse Mean</b>	126	0.716	0.710	-0.012	3.866	72.68	6.06	16.96	0.222
Standard Deviation		0.056	0.059	0.071	0.657	13.02	1.09	7.98	0.088
Minimum		0.496	0.489	-0.284	2.148	37	3.08	0	0
Maximum		0.815	0.798	0.133	5.253	96	8.00	33	0.400
<b>Domestic Horse Mean</b>	80	0.710	0.720	0.012	4.012	80.88	6.74	23.79	0.283
Standard Deviation		0.078	0.071	0.086	0.735	16.79	1.40	10.11	0.082
Minimum		0.347	0.394	-0.312	1.779	26	2.17	0	0
Maximum		0.822	0.799	0.211	5.30	119	9.92	55	0.462

**Table 3.** Rogers' genetic similarity of the Pryor Mountains Wild horse Range feral horse herd to major groups of domestic horses.

	Mean <i>S</i>	Std	Minimum	Maximum
Light Racing and Riding Breeds	0.704	0.049	0.629	0.758
Oriental and Arabian Breeds	0.647	0.111	0.445	0.751
Old World Iberian Breeds	0.658	0.034	0.624	0.711
New World Iberian Breeds	0.635	0.108	0.423	0.759
North American Gaited Breeds	0.641	0.043	0.562	0.680
Heavy Draft Breeds	0.515	0.096	0.434	0.683
True Pony Breeds	0.532	0.149	0.413	0.708

Figure 1. Partial RML tree of genetic similarity to domestic horse breeds.





Appendix 1. DNA data for the Pryor Mountains Wild horse Range, MT herd.

AID	VHL20	HTG4	AHT4	HMS7	HTG6	AHT5	HMS6	ASB2	HTG10	HTG7	HMS3	HMS2	ASB17	ASB23	LEX33	LEX3
63824	IM	KM	JK	LL	**	KL	MP	NO	NQ	**	OP	**	RR	UU	LR	HP
63825	IJ	OP	HK	LL	**	KK	LP	KQ	MR	**	OR	**	MM	SS	LR	HH
63826	II	KM	JK	LO	**	JK	NP	KQ	QR	**	OP	**	KM	UU	LO	FH
63827	NO	KP	KO	LO	**	JK	PP	KN	QR	**	OP	**	RR	SU	KO	HP
63828	JO	KM	HK	LL	**	JK	PP	BK	LO	**	OP	**	RR	IU	KL	LP
63829	MR	KM	JK	LL	**	KK	LL	BO	NO	**	NP	**	KR	HS	LL	FH
63830	IP	KP	HM	LO	**	KK	LP	KQ	LM	**	MM	**	MR	HU	OO	HH
63831	MN	MP	JP	LL	**	KN	LP	KN	OR	**	NR	**	NR	LS	MQ	HP
63832	MR	KP	JJ	LO	**	KN	OP	NO	OR	**	NP	**	MN	HS	KR	HM
63833	MM	MP	HP	LL	**	KK	MP	KQ	IQ	**	PP	**	KQ	LS	QQ	HL
63834	KM	KM	HJ	LL	**	KL	MO	OQ	KQ	**	PR	**	MR	SU	LR	HP
63835	MN	MP	JL	LL	**	KN	MM	QQ	IM	**	NN	**	MN	JL	LO	HL
63836	NR	MM	KL	LM	**	KK	PP	QQ	KQ	**	IP	**	QQ	LU	KQ	HM
63837	MR	MM	JK	LL	**	KK	MP	BQ	KQ	**	PP	**	QR	LU	KR	HH
63838	IM	KM	JK	OO	**	JK	NP	NQ	IR	**	OP	**	KM	UU	LO	HP
63839	JN	KP	IK	MO	**	KO	NP	KQ	OQ	**	IO	**	QQ	HU	OQ	HP
63840	MM	MM	HJ	LL	**	LL	LP	NQ	QQ	**	PR	**	RR	LU	LL	PP
63841	MO	MM	HK	LO	**	JL	LN	KN	QQ	**	OR	**	MR	LS	LM	PP
63842	MN	KK	KO	LO	**	KO	OP	KN	OQ	**	OP	**	MQ	HU	LO	HH
63843	NR	MM	JP	LO	**	KN	LM	KQ	IM	**	NP	**	MQ	HL	LR	FL
63844	RR	MM	OP	OO	**	KN	LP	KQ	MN	**	NP	**	MQ	LS	OR	LL
63845	NO	MM	KL	LL	**	KK	OO	KK	OQ	**	PP	**	RR	UU	LL	PP
63846	IM	MP	HL	LM	**	KK	LM	KQ	IL	**	NP	**	QR	JS	OQ	HH
63847	MM	MP	HL	LL	**	KK	MP	KQ	IM	**	PR	**	KQ	JS	QQ	HH
63848	JM	KM	KO	LO	**	KN	KO	QQ	OR	**	PP	**	MR	HJ	MQ	PP
63849	KM	MP	KK	LL	**	KK	OP	KK	QQ	**	PP	**	RR	SU	LQ	PP
63850	MQ	KM	JK	LO	**	KL	NP	KK	IM	**	OP	**	MR	SS	OO	PP
63851	MO	KM	JK	LL	**	KL	LP	KO	NQ	**	NP	**	MR	SU	LL	HP
63852	MR	KM	JP	LL	**	JK	LP	BK	MN	**	PP	**	MM	HU	LO	HH
63853	IJ	MM	HO	OO	**	JK	OO	KK	QR	**	OP	**	NQ	HS	LO	FF
63854	MM	KP	JJ	LO	**	KK	LM	BN	RR	**	OO	**	NR	HJ	KK	FF
63855	NO	MP	HH	LO	**	LL	MP	KK	MR	**	MP	**	MM	IU	OQ	HH
63856	JN	KM	KO	LO	**	JN	PP	KN	IN	**	OP	**	QR	LU	OQ	LL
63857	MN	MM	PP	LL	**	KK	LP	KK	NO	**	NP	**	MN	LL	QR	LL
63858	KM	KK	KO	LL	**	KL	LO	NQ	QR	**	OP	**	MN	HU	LL	HH
63859	JM	OP	JK	NN	**	NN	OP	KQ	NO	**	NO	**	KS	IL	KO	HH
63860	MP	KM	KN	LL	**	NN	LO	KN	OO	**	OR	**	NN	HJ	OO	HH
63861	NR	MO	JK	MO	**	KN	LM	OQ	OQ	**	PR	**	MQ	LS	OR	HH
63862	JR	MM	HK	MO	**	JK	PP	KK	MO	**	PP	**	MM	SS	LO	HL
63863	JM	MO	KO	LO	**	KL	OP	KQ	NQ	**	PP	**	NQ	IS	OQ	FL
63864	JJ	MM	HJ	LO	**	JN	OP	KK	MN	**	PR	**	MN	JS	OO	HH
63865	MM	OP	KP	LO	**	KN	PP	KQ	QQ	**	NP	**	NN	IU	KQ	FL
63866	JJ	KO	JJ	LL	**	JN	OO	KQ	NN	**	RR	**	MN	HU	KO	MM
63867	MR	KM	HH	LL	**	JN	OP	KQ	IQ	**	OP	**	QR	HJ	GO	HH
63868	II	KK	HH	OO	**	KL	LM	NQ	MM	**	MM	**	MN	SS	OQ	PP