Genetic Analysis of the Pryor Mountains HMA, MT

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The following is a report of the genetic analysis of the Pryor Mountains HMA, 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 that 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 105 samples were received by Texas A&M University, Equine Genetics Lab on October 7, 2009. DNA was extracted from the samples and tested for variation at 12 equine microsatellite (mSat) systems. These were *AHT*4, *AHT*5 *ASB*2, *ASB*17, *ASB*23, *HMS*3, *HMS*6, *HMS*7, *HTG*4, *HTG*10, *LEX*33, and *VHL*20. 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 HMA 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 HMA herd to domestic horse breed types are shown in Table 3. A dendrogram of relationship of the Pryor Mountains HMA herd to a standard set of domestic breeds is shown in Figure 1.

Genetic Variants: A total of 79 variants were seen in the Pryor Mountains HMA herd which is just above the mean for feral herds and slightly below the mean for domestic breeds. Of these, 15 had frequencies below 0.05and this percentage of variants at risk of future loss is slightly above average. Allelic diversity as represented by *Ae* also is just above the average for feral herds as is *MNA.*

Genetic Variation: Genetic variation, as indicated by heterozygosity, in the Pryor Mountains HMA herd is well above the feral mean. *Ho* is slightly lower than *He* but the difference is so small the population could be considered in genetic equilibrium.

Genetic Similarity: Overall similarity of the Pryor Mountains HMA herd to domestic breeds was about average for feral herds. Highest mean genetic similarity of the Pryor Mountains HMA herd was with the Light Racing and Riding breeds followed by the Old World Iberian horses. As seen in Fig. 1, the Pryor Mountains HMA herd fits on the outside of the darft horse and pony cluster and in between the Light Riding Horse and Iberian horse clusters. I think this is consistent with what we have seen with past analysis of blood type data. The Pryor herd clearly has some mixed blood ancestry but shows strong evidence of old Spanish blood as well.

COMPARISON TO PAST SAMPLING PERIODS

The Pryor Mountains herd has been sampled several times in the past with DNA results available for the years 1994, 1997 and 2001, however, the 1990s samples were typed with a set of markers that had only 9 loci in common with the typing from the 2000s samples so the results are based upon those 9 loci. Genetic variability of this herd at these three times was very consistent. There was almost no difference in either heterozygosity or allelic diversity between any of the years. 2009 does show a difference in both types of measures, although the difference is not large (Table 4). Heterozygosity levels are higher in 2009 while the total number of variants, *MNA* and the percentage of rare alleles are lower. *Ae* is higher which indicates an evening out of allele frequencies. I think the changes in variation show the effects of the removal of horses that were known to have ancestry from outside the Pryor Mountain HMA. This has reduced the number of alleles and since many of the introduced alleles would have low frequencies in the herd the rare allele percentage has declined. However, effective variation has

increased as shown by the *H* and *Ae* increases. The herd now appears to be in genetic equilibrium. There is no evidence of a bottleneck as if this were the case *Ho* would be greater than *He*. At least to this point, the Pryor Mountain herd appears to be maintaining good levels of variability. The number of animals on the range has been kept at fairly consistent levels since 2001 and this is consistent with the variability levels and the targeted removals mentioned above. The number of foals also has remained consistent. There also are efforts to even the sex ratio of breeding age animals which can increase effective population size.

SUMMARY

Genetic variability of this herd overall remains above the feral horse means values for all measures. However, comparison the past sample periods indicate some loss of allelic diversity which is probably due to the effort to remove animals with a known introduced ancestry. Genetic similarity results suggest a herd with mixed ancestry that includes Spanish blood.

RECOMMENDATIONS

Current variability levels are high enough that no action is needed at this point, however, it is important that the population size of the herd be maintained at the level of a minimum of 120 breeding aged animals. The population size at the time of this sampling was above that and if it is now lower, it should be restored to that level within the next five years. Considering the significance of this herd to the wild horse program and to the Colonial Spanish horses of the USA, as much variation as possible should be preserved without introduction of horses into the herd in the future.

Table 1. Allele frequencies of genetic variants observed in Pryor Mountains HMA feral horse herd.

VHL20															
I	J	K	L	M	N	O	P	Q	R	S					
0.209	0.223	0.029	0.000	0.262	0.073	0.073	0.029	0.015	0.087	0.000					
HTG4															
I	J	K	L	M	N	O	P	Q	R						
0.000	0.000	0.282	0.019	0.417	0.010	0.068	0.204	0.000	0.000						
AHT4															
H	I	J	K	L	M	N	O	P	Q	R					
0.222	0.039	0.194	0.209	0.034	0.010	0.083	0.131	0.078	0.000	0.000					
HMS7															
I	J	K	L	M	N	O	P	Q	R						
0.000	0.000	0.000	0.505	0.136	0.044	0.315	0.000	0.000	0.000						
AHT5															
I	J	K	L	M	N	O	P	Q	R						
0.000	0.155	0.443	0.189	0.000	0.194	0.019	0.000	0.000	0.000						
HMS6															
I	J	K	L	M	N	O	P	Q	R						
0.000	0.000	0.005	0.286	0.117	0.068	0.194	0.330	0.000	0.000						
ASB2															
B	I	J	K	L	M	N	O	P	Q	R					
0.097	0.000	0.000	0.330	0.000	0.010	0.170	0.107	0.000	0.286	0.000					
HTG10															
H	I	J	K	L	M	N	O	P	Q	R	S	T			
0.000	0.068	0.000	0.083	0.102	0.170	0.126	0.204	0.000	0.184	0.063	0.000	0.000			
HMS3															
H	I	J	K	L	M	N	O	P	Q	R	S				
0.000	0.034	0.000	0.000	0.000	0.092	0.121	0.141	0.428	0.000	0.184	0.000				
ASB17															
D	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.000	0.374	0.209	0.000	0.000	0.087	0.238	0.039	0.000
ASB23															
G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
0.000	0.150	0.141	0.058	0.010	0.117	0.000	0.000	0.000	0.000	0.000	0.000	0.247	0.000	0.277	0.000
LEX33															
F	G	K	L	M	N	O	P	Q	R	S	T				
0.000	0.068	0.102	0.248	0.068	0.000	0.276	0.000	0.180	0.058	0.000	0.000				

Table 2. Genetic variability measures.

	N	*Ho*	*He*	*Fis*	*Ae*	*TNV*	*MNA*	*Ra*	*%Ra*
PRYOR MOUNTAINS M	**105**	**0.757**	**0.762**	**0.007**	**4.51**	**79**	**6.58**	**15**	**0.190**
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
Feral Horse Mean	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
Domestic Horse Mean	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

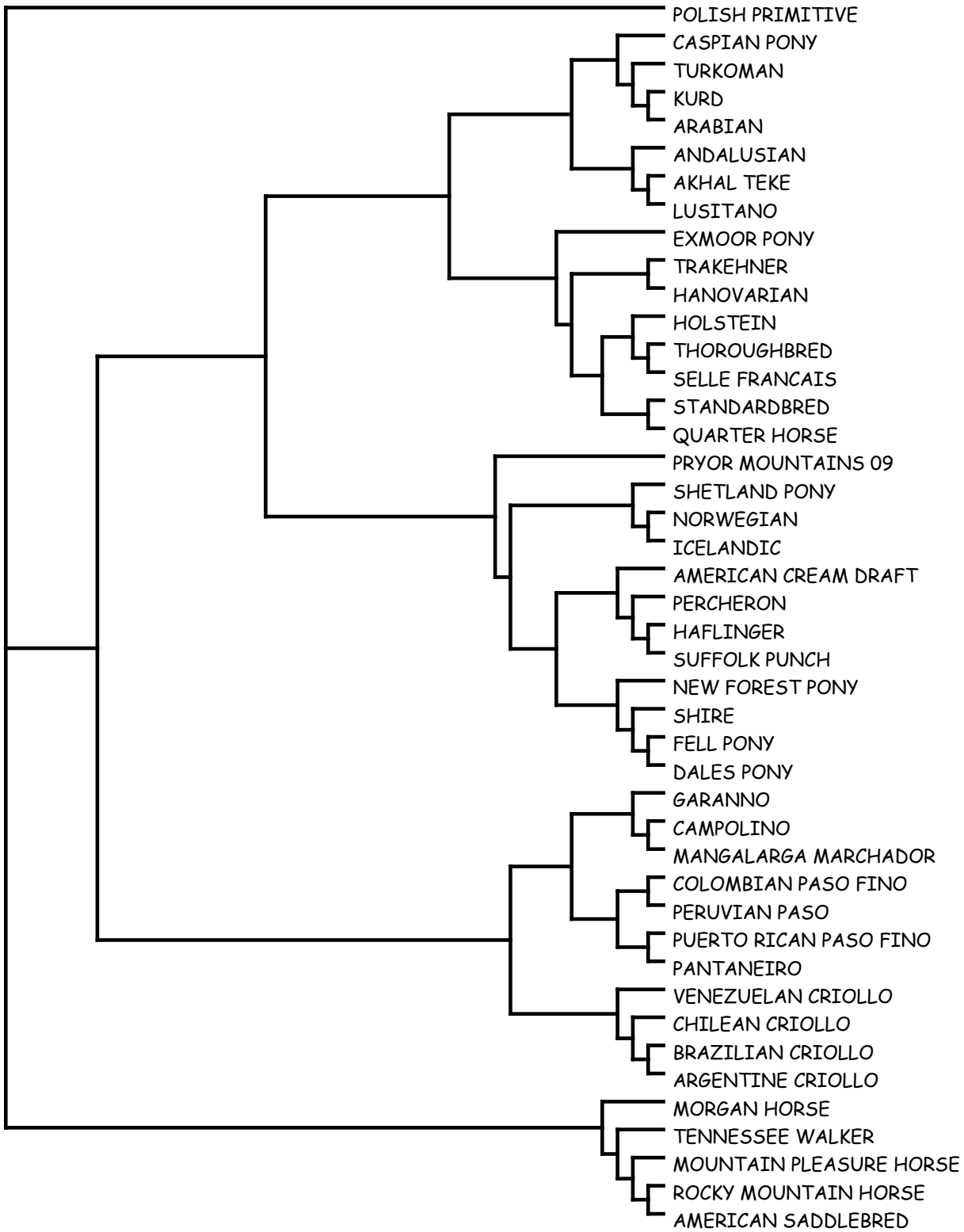
Table 3. Rogers' genetic similarity of the Pryor Mountains HMA feral horse herd to major groups of domestic horses.

	Mean *S*	Std	Minimum	Maximum
Light Racing and Riding Breeds	0.762	0.026	0.728	0.793
Oriental and Arabian Breeds	0.745	0.043	0.672	0.791
Old World Iberian Breeds	0.756	0.015	0.741	0.778
New World Iberian Breeds	0.742	0.031	0.689	0.778
North American Gaited Breeds	0.738	0.018	0.722	0.762
Heavy Draft Breeds	0.707	0.041	0.654	0.780
True Pony Breeds	0.711	0.045	0.664	0.759

Table 4. Genetic variability measures of the Pryor Mountain herd over time.

YEAR	*N*	*TNV*	*MNA*	*Ae*	*%RA*	*Ho*	*He*	*Fis*
1994	122	64	7.11	4.05	0.391	0.668	0.735	0.091
1997	86	64	7.11	3.89	0.391	0.650	0.728	0.107
2001	209	66	7.33	3.89	0.333	0.665	0.724	0.082
2009	103	59	6.56	4.43	0.220	0.742	0.755	0.016

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



Appendix 1. DNA data for the Pryor Mountains HMA, MT herd.

AID	ID	AHT4	AHT5	ASB17	ASB2	ASB23	HMS3	HMS6	HMS7	HTG10	HTG4	LEX3	LEX33	VHL20
42132	2009-17	HK	JJ	MM	BK	SS	PP	PP	LM	MN	KM	HL	KQ	IR
42133	2000-15	JP	KN	MR	BN	HI	OR	PP	MO	MO	KP	HP	LO	JM
42134	1993-18	JJ	LL	KN	**	JK	**	NN	LL	**	KM	**	**	MM
42135	2009-15	HN	KL	MN	NQ	HS	MM	LL	OO	MM	KK	PP	KL	II
42136	1970-15	KK	JK	MR	KK	SU	PP	NO	LL	IQ	KM	PP	LM	KO
42137	2009-31	KO	KL	MN	NQ	HU	OP	LO	LL	QR	KK	HH	LL	KM
42138	2007-27	KP	KL	MN	KO	SU	NP	LN	LO	KQ	KM	HP	LL	IM
42139	2006-14	KP	KK	MR	OQ	HS	NP	LO	LL	NQ	KM	HP	LL	KM
42140	2009-32	JP	LN	NR	BK	HS	PR	LP	OO	KR	KM	PP	LL	JM
42141	1996-29	JP	KL	MR	BO	HU	NP	LM	LL	KN	KM	HH	LR	MR
42142	2003-15	HN	KK	MM	KK	IS	MP	MP	MO	OO	PP	HH	LQ	II
42143	2002-16	JJ	NN	NR	KN	KS	PR	LP	LM	LR	MP	PP	MQ	JM
42144	2005-27	JK	KL	MR	BK	HS	OP	MN	LO	NQ	KM	HP	MR	IR
42145	2008-16	JO	LN	KN	NQ	KU	PP	LO	LO	LR	MP	FH	KO	JM
42146	2005-17	HK	KL	KM	QQ	SS	PR	MN	LO	MQ	MP	HP	MQ	IM
42147	2009-05	HL	KK	NR	OQ	HJ	MN	MP	LM	IO	PP	MM	LO	MN
42148	2008-21	KP	KK	MM	KQ	LS	PP	MP	LO	KQ	MM	HH	MQ	IN
42149	2006-04	HO	KK	QR	KO	HS	MN	LP	LM	LO	MP	HM	GO	IM
42150	2009-16	JL	KN	MN	QQ	JL	NN	MM	LL	IM	MP	HL	LO	MN
42151	2001-28	JP	KL	MM	QQ	HL	NN	LM	LO	MN	MM	FH	OR	MR
42152	2006-22	IJ	NO	MM	KN	IS	IO	NP	MM	OQ	KM	HP	OQ	MN
42153	2007-23	KO	KL	MM	QQ	HL	NP	LP	LL	KO	KM	FM	LO	JM
42154	2006-21	IK	KO	MQ	KN	UU	IP	NP	MO	OR	KP	PP	KO	IJ
42155	2008-27	HI	KN	MM	NQ	IU	IP	NO	LM	LO	KK	PP	LQ	NO
42156	2009-34	IK	KO	QQ	KQ	HU	IO	NP	MO	OQ	KP	HP	OQ	JN
42157	2008-15	HN	KL	MM	KQ	SS	MM	LP	OO	LM	KP	HH	OQ	II
42158	200734	MN	KK	MR	KK	SU	MM	MP	OO	LM	KM	HP	OQ	II
42159	2003-26	HH	JL	MN	KN	SS	MO	LP	OO	MQ	KK	HP	LQ	IM
42160	2007-26	HP	JL	NR	NQ	SU	OP	LL	LO	KQ	KM	FH	OQ	MM
42161	2001-40	KO	KL	RR	QQ	SU	NP	LP	LO	OQ	MM	LP	MO	QR
42162	2007-19	HK	KL	KM	KK	HS	MP	LN	LO	LQ	KM	HP	LQ	IQ
42163	1995-13	HN	KL	MM	KQ	HS	MP	LM	OO	LM	KP	HH	OQ	II
42164	2009-06	HM	KK	MR	NQ	SU	PP	MP	OO	MM	MP	HH	GO	IP
42165	2007-20	HP	KK	MM	KK	LS	NP	LO	OO	MO	MP	FH	OO	IJ
42166	2007-03	HK	KL	RR	KK	IS	PP	OP	LO	KQ	KP	HP	LQ	KN
42167	2007-33	JK	KK	MR	KQ	SU	PP	NP	LL	LQ	KM	PP	LL	OO
42168	2009-03	HJ	LL	RR	NQ	LU	PR	LP	LL	QQ	MM	PP	LL	MM
42169	2006-23	HK	JJ	MR	BK	IS	NP	PP	LL	OO	KM	HL	KL	JJ
42170	2007-07	HO	KL	MR	NQ	IU	PR	NO	LL	LQ	KM	PP	LL	IO
42171	2002-09	HI	KN	MS	KQ	IS	MP	LP	OO	LM	PP	HH	LQ	IJ
42172	2007-30	KO	KL	MN	BQ	LU	OP	OP	MO	MO	MM	PP	OQ	MR
42173	2008-23	KO	KL	MN	BQ	LU	OO	LP	LM	KM	KO	PP	LO	JR
42174	2008-24	KO	JK	MQ	BQ	UU	NP	LP	LM	KK	LM	PP	OQ	MR
42175	2005-30	HI	KK	MS	KQ	IS	MO	LP	OO	MO	MP	HH	OQ	II
42176	2005-20	JO	KL	MR	BO	UU	PP	LP	LL	MN	LM	MM	LL	MM
42177	2006-20	KP	KK	NR	KO	HS	NP	MP	LO	NQ	KM	PP	MR	IM
42178	2006-03	JK	KN	KQ	OQ	HI	OP	MP	LO	MO	KK	PP	LR	JM
42179	2007-28	HP	LL	MR	BN	SU	NR	MN	LL	NQ	MM	PP	LR	MO
42180	2007-09	JN	KK	MS	OQ	HS	MN	LM	MO	OO	KP	HH	GO	IM
42181	2007-12	JP	KL	MN	QQ	HL	NP	LP	LO	NO	MM	HH	QR	NR
42182	2009-23	HK	JK	NR	KN	SU	PP	LP	LL	QR	MP	HH	MO	MN
42183	2008-09	JK	JL	RR	BK	UU	PP	LP	LO	MQ	MM	LM	LL	MO
42184	2005-23	HO	JK	NR	NN	SU	PP	LP	LL	RR	KP	HP	KO	JN
42185	2001-31	KK	JK	MR	KK	SS	OP	NP	LO	IQ	KM	HH	MO	MO
42186	2007-05	KO	JJ	MQ	NQ	UU	PP	OP	LM	LL	LM	PP	LO	JJ
42187	2006-16	HH	JK	NR	NQ	LU	PP	KP	LO	OQ	MM	PP	LO	IJ
42188	2009-14	HH	LL	MM	KK	IU	OP	MP	OO	KL	MM	HM	OQ	NO
42189	2002-07	HH	JK	MN	KO	IL	PR	PP	LL	OO	MN	HP	LQ	II
42190	2008-10	HN	KK	MR	KK	HI	OP	NP	LO	MR	MP	HH	GL	IN
42191	2008-11	KL	KK	MN	KO	JU	PR	PP	LL	OQ	MN	PP	OQ	IO
42192	2001-02	HN	LL	MR	KK	HU	MO	MN	MO	LR	MP	HH	LO	IO
42193	2005-34	KL	KN	MR	KK	IU	PP	OP	LL	OQ	KM	PP	LO	NO
42194	2002-09	HI	KN	MS	KQ	IS	MP	LP	OO	LM	PP	HH	LQ	IJ
42195	2008-28	JK	KN	MQ	OO	HS	NR	LP	OO	NO	KK	PP	GO	MM
42196	2007-25	HN	KK	MS	MQ	SS	MP	LM	LM	LO	PP	HH	LQ	IJ
42197	1993-11	JK	KN	MM	OQ	SU	PR	LP	LO	NR	KO	HP	OR	JM
42198	1991-04	IN	KN	MS	KQ	SS	MP	LM	MO	LO	KP	HH	LQ	II
42199	2005-31	JO	KK	NR	KN	SU	PR	OP	LL	MR	KM	HH	OQ	KR
42200	2002-10	LO	KN	MQ	BO	IL	NP	OP	LL	KM	KP	HM	QR	MN
42201	2009-19	JO	KK	KM	BQ	II	OP	OO	LO	MM	KP	HM	LR	JM
42202	2008-07	KL	JK	NQ	KO	LL	IN	LP	LM	KO	PP	HL	LR	NR
42203	2001-23	NO	LL	MM	BN	HS	PR	LP	MM	OQ	MM	FP	LM	IM
42204	2008-25	JK	JN	NR	KO	II	PR	LL	LM	MQ	KM	LP	LM	OR
42205	2003-29	HO	KN	NR	KQ	LU	IP	OP	LM	KO	LM	PP	KQ	KR
42206	2007-31	HO	KK	NN	QQ	LS	IP	OP	LL	KR	MM	PP	OQ	KR
42207	2008-29	JK	KK	MM	BO	IS	PP	LO	OO	MM	MM	HM	OR	JM
42208	2006-27	HJ	JL	KM	BN	IS	PP	OP	LM	KL	MP	FL	OQ	JM
42209	2006-17	HJ	KL	MR	BK	HU	PP	MP	MO	NO	MP	FF	GQ	JM
42210	2007-29	JO	JK	KQ	KN	UU	PR	LO	MO	LL	MM	FF	KQ	JJ
42211	2008-26	KP	NO	MM	KN	HU	OP	LL	LO	NQ	MM	FF	KO	JJ
42212	2007-16	LO	KK	MR	KK	LU	PR	LP	LO	KQ	KM	MM	KQ	IQ
42213	2009-12	JJ	JN	MN	KQ	HU	RR	OO	LL	NN	KO	MM	KO	JJ
42214	2007-17	JN	KN	NN	KK	JL	RR	OP	LN	NN	KO	HH	KO	JJ
42215	2008-02	HH	NN	QR	KO	HU	RR	LO	LL	IM	MM	PP	G	JP
42216	2009-11	HO	KN	QR	KM	JU	OR	PP	LL	IQ	MM	HH	GO	IR
42217	2001-03	HO	JN	QR	NO	HU	PR	LM	LN	MN	MM	NP	GK	JP
42218	2009-04	HO	NN	RR	KO	HU	PR	MO	LN	IM	MM	PP	GK	PP
42219	2007-01	HO	JN	RR	KN	HU	RR	LP	LL	IM	MM	NP	GK	JR
42220	2008-03	HO	JN	KR	KQ	JU	OP	OO	LL	IQ	KM	HP	GO	MR
42221	2007-02	HO	JK	QR	KQ	HH	OR	OO	LL	MQ	KM	HH	GO	MR
42222	2009-02	JN	KN	NQ	BK	IS	OP	LO	LL	NO	KO	PP	OO	JM
42223	2008-12	JK	KL	NR	KN	LL	RR	LL	LL	NN	MM	FM	KM	JM
42224	2007-13	JK	JK	NN	KN	LU	RR	LL	LL	IN	KM	MM	MO	JM
42225	2001-13	KN	JN	NQ	BN	IJ	OO	OO	LL	NO	KO	HH	OQ	JP
42226	2005-10	JJ	JK	NR	QQ	SU	PR	LL	LL	IN	KM	PP	KM	MM
42227	2004-27	JP	JK	MN	KN	JL	OP	LO	LO	IQ	PP	HH	OQ	JO
42228	2009-33	JN	NN	KN	NQ	JU	PR	OO	NO	IQ	OP	HH	LO	JM
42229	2006-05	JJ	JN	MN	NN	JL	OP	LO	LO	IQ	KP	HH	OO	JJ
42230	2009-28	KP	JN	NS	NQ	UU	PR	LO	LO	IO	KO	PP	LO	JO
42231	2008-13	KP	JK	NN	KQ	JU	PR	LO	NO	OO	OP	HH	LO	IO
42232	2007-04	NO	JL	NQ	OQ	IU	OP	MP	MO	OQ	MO	LN	LQ	JM
42233	2002-24	KK	LN	KN	QQ	IU	NR	PP	LN	NO	KO	HL	KO	IM
42234	2008-31	JK	KN	MN	NQ	IJ	NO	OP	LL	OQ	KO	HH	KM	MM
42235	2006-30	KN	LN	NR	QQ	IU	NR	OP	LN	OQ	KO	HL	KO	IM
42236	2009-37	JK	NN	KS	KQ	IL	NO	OP	NN	NO	OP	HH	KO	JM