

DNA Testing in Horses

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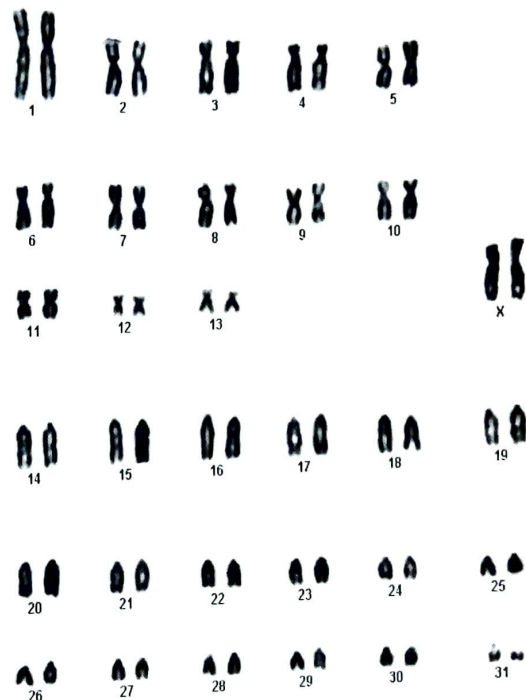
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Once a mysterious and intimidating phrase, DNA testing is now a familiar concept, thanks in no small part to celebrity paternity cases and television shows like *CSI*. And yet most people probably don't know exactly what is being tested when a DNA test is performed. The basis of the test is identical whether the subject is human or animal. Use of DNA for identity or parentage confirmation is now a routine requirement of most horse breed registries which are interested in maintaining the integrity of their stud books. But what exactly is involved in DNA testing?

DNA is the molecule in living things that encodes all the functioning proteins of an organism. Proteins can be structural (muscle for example) or functional (enzymes, antibodies, etc). Each protein is encoded by a gene and although no one knows the exact number of genes it has been estimated to be between 10,000 to 30,000 in humans. A gene is a defined sequence of the four nucleotide building blocks of DNA- A (adenine), C (cytosine), G (guanine) and T (thymidine). In addition to the coding regions of genes there are additional sequences of these nucleotides within and between genes, the function of which is not entirely understood. These long strings of nucleotides, containing genes and nonsense sequences, form DNA molecules, which in turn coil up into the structures known as chromosomes. A human has 23 pairs of chromosomes while a horse has 32 pairs. One pair of chromosomes determines sex, with female mammals having two X chromosomes and males having one X and one Y chromosome. The individual chromosomes in each species can be identified by their size and other features and they are assigned a number, with chromosome 1 being the largest and continuing on to the smallest chromosome in that species- being 22 in the human and 31 in the horse. (Figure 1)

Chromosomes occur in pairs due to the basic tenet of sexual reproduction: the genetic material from a male and female combines to create a new individual. To understand the results of genetic testing, it is important to grasp the concept that each

Figure 1. Giemsa-stained Karyotype of a normal mare (courtesy of Dr. Teri Lear, Gluck Equine Research Center, University of Kentucky)



individual inherits one set of chromosomes from each parent. Hence there are pairs of chromosomes in each cell because one chromosome comes from the father and one from the mother.

Now with these basic concepts in place, what exactly happens when your horse is DNA tested? For identification or parentage testing, we do not test specific genes, rather there are areas in between the genes that contain markers called microsatellites

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or STRs (Simple Tandem Repeats). Using the DNA alphabet of A, C, G and T, an example of an STR would be the CA in a string such as CACACACACACACA. This is a string of 8 repeats. The number of repeats at this spot may vary from horse to horse. In fact a horse may have 8 repeats on one chromosome and 12 repeats at the same spot on the other chromosome of the pair. Such a horse would be heterozygous for this marker. It inherited the 8 repeats from one parent and the 12 markers from the other parent. If both chromosomes in the pair had the same number of repeats at this spot, we would say the horse is homozygous at this marker. That means the sire and dam both contributed the same variant of this marker to the foal. (Figure 2)

Figure 2. Use of Microsatellites in Parentage Verification

SIRE		DAM	
J	_____	L	_____
K	_____	M	_____
POSSIBLE FOALS			
J	_____	K	_____
L	_____	M	_____
J	_____	K	_____
M	_____	L	_____
Number of repeats:		Letter Designation:	
8		J	
9		K	
10		L	
11		M	



Photo by Cally Matherly©

So imagine this process repeated 17 times. A horse DNA test uses 16 regular markers and one marker from the X chromosome for a total of 17 markers. Each marker has a number of defined possibilities or variants as to how many repeats there are. These variants are known as *alleles*. There are multiple alleles for each marker we use in the tests, so when you consider

the number of possible combinations within one marker, and add to that the possible combinations with the other 16 markers and their respective alleles, you can see that the DNA test is a powerful tool for identifying individual horses and determining correct parentage.

DNA is present in nearly all cells of the body, so the lab can use almost any tissue as a source of DNA. The material of choice is hair bulbs because they are easy for the owner to collect and do not require any special storage or expedited shipment. You may wonder why the lab asks for 30-50 pulled hairs when CSI can get a DNA type from a single hair found on a carpet at a murder scene. First, most DNA testing labs are not forensic testing labs. They do not have the equipment, time or specially-trained personnel to devote to these delicate DNA processing techniques. The seemingly large number of hairs requested takes into account that out of 30 hairs, a number will break without yielding a hair bulb. Maybe a third or less of the hairs will have testable bulbs. That leaves 10 usable bulbs and the test requires 3-5 hair bulbs. If the sample has to be rerun to verify an exclusion or resolve some other problem, there may not be sufficient sample.



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A process known as PCR (polymerase chain reaction) is used to amplify the areas of DNA containing the repeat segments used for analysis. These fragments are labeled with a fluorescent dye so they can be "read" by a DNA sequencer machine. The sequencer reads the actual size of the fragments containing the repeats. The sizes will vary depending on how many repeats each fragment contains. The sizes are all internationally standardized and are converted to the letter designations that appear on the horse's DNA report. A single letter listed under the marker name (VHL20, HTG4, etc) means the horse is homozygous for that size fragment. This means it inherited the same size repeat from both the sire and the dam. Two letters under a marker means the horse inherited different size repeats from the sire and the dam. Many people want to know what the DNA type of the horse "means." Think of it as a fingerprint. Just as your fingerprint serves as a means of identification, so does the DNA type. The microsatellite profile has no meaning as far as

Figure 4 shows a parentage exclusion. Note the foal possesses factors not present in the sire or the dam in systems AHT4, ASB2, HMS6 and LEX3.

Very often labs are asked if they can determine what breeds of

horses are present in a horse of unknown ancestry. The horse genetic map was just completed in 2007. Work is underway to investigate breed differences in sequence and markers. This may allow breed-specific markers to be identified that will make it possible to determine what breeds are present in a horse. The dog genetic map has been complete for over 5 years, and yet breed determination has just recently become available for 75 out of about 300 dog breeds. Completion of the horse genetic map will open doors to improving DNA testing, increasing the number of tests for coat color and genetic diseases, developing tests for desirable traits and potentially for breed identification.

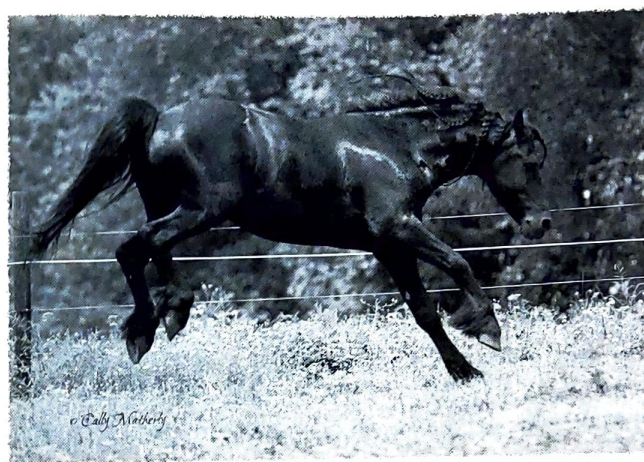


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Figure 3. Parentage Not Excluded

FOAL:		VHL20	HTG4	AHT4	HMS7	ASB2	ASB17	AHT5			
SIRE:	[REDACTED]	R	L	H	NO	KN	FN	JK	AHR	hair1045	
DAM:	[REDACTED]	PR	L	H	LN	K	FS	JK	AHR		
		OR	LM	H	LO	N	NR	JL	AHR		
FOAL:	[REDACTED]	HMS6	ASB23	HTG10	HMS3	LEX33	HTG6	HTG7	HMS2	LEX3	
SIRE:	[REDACTED]	MP	KS	M	NR	GR				P	AHR
DAM:	[REDACTED]	MP	K	M	NQ	LR				MP	AHR
		I.M	IS	MO	PR	GL					

Figure 4. Parentage Exclusion

	ACCNO	BREED	VHL20	HTG4	AHT4	HMS7	ASB2	ASB17	AHT5	RES	RUM535
FOAL:		GT	NO	LN	N	LO	MN	Q	J	RES	rum510
SIRE:		GT	MO	LN	P	LO	NO	Q	J	RES	rum510
DAM:		GT	NO	MN	KP	O	O	Q			

	EED	HMS6	ASB23	HTG10	HMS3	LEX33	HTG6	HTG7	HMS2	LEX3
FOAL:		GT	LP	S	KR	P	KR			HO
SIRE:		GT	OQ	S	OR	P	KR			O
DAM:		GT	LO	S	KO	PQ	R			LO

