Finding and Maintaining Genetically Pure Bison

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Finding bison without evidence of cattle introgression is often not an easy task. There is no registry or centralized database that you can go to. That leaves only 3 options:

1. Take your chances.

At the moment this is really the only readily available option. Purchase animals, have them tested, and cull those with evidence of introgression. That is how we assembled our herd. Animals with evidence of introgression were raised to slaughter age/weight and sent to the processing plant. They were never bred. If choosing this route, buy from various different herds as you could purchase from a heavily polluted herd. If purchasing bred animals, be aware that if the mother is free of cattle introgression evidence, it does not mean that the calf will be. This we have learned from experience; often the cows will not be introgressed but her calf may be (cattle genes from the bull).

Just because a particular animal is registered or coming from a certified conservation herd also does not mean they are free of cattle genes and/or markers. All registrars at present accept bison hybrids (bison with evidence of cattle introgression); so purchasing a registered bison does not guarantee anything. The same goes for certified conservation herds.

If a seller tells you that the animal has been tested, ask to see the paperwork. I recently watched an online auction where the seller said that the bulls were DNA tested. No other information was provided. What tests were done? Was it even introgression testing? What were the results? Buyers beware without paperwork.

2. Require testing.

Prior to the purchase of an animal, require nDNA and mtDNA introgression testing. Other than purchasing a breeding bull from a reputable seller, I am not sure how receptive the seller would be. We have only done this prior to the purchase of a breeding bull as a condition of purchase.

If you have testing done, understand how to interpret the tests and do not take the sellers word for it. This is not to imply that the seller may be dishonest but rather may not have a good understanding of how to interpret the test. For example, we were told a particular bull did not have any cattle genes (markers) because the report stated "No evidence of Cattle alleles in the

HIGHLIGHTS

- Cattle-free bison can be obtained by:
 - o Buy-test-cull
 - o Test then buy
 - o Buy from pure herd
- Registered bison may contain cattle genes and are not necessarily introgression-free.
- Certified conservation herds may not be cattle-gene free
- Both nuclear and mitochondrial DNA tests must be performed
- Both tests only available through University of California-Davis
- An extended nuclear marker examination with 25 markers is preferred
- Discounted testing through the NBA currently uses only 15 markers
- Maintaining a pure bison herd and diversity is challenging
- Herds with cattle genes can be cleaned with a little effort
- Bulls need to be free of any cattle introgression markers

markers reported". However, what was overlooked was that the Mitochondrial DNA Origin it was "Cattle" (see example 2 below).

Understanding Introgression test results. At present, only the University of California-Davis offers both nuclear and mitochondrial testing [1]. If you go direct to the UC-Davis lab you will get the extended analyses with 25 nuclear microsatellite markers examined. If you go through the National Bison Association (NBA), although a small discount is provided, you will only get 15 microsatellite markers examined. The extended test is obviously preferred as it increases the probability of detecting a hybrid. Why the NBA chooses a deficient test is unknown.

Understanding the introgression test results is rather simple, but often misunderstood. We will use real test results for illustration purposes with identifying data removed.

In Example 1 (test done through the NBA) from a bison breeding bull offered for sale, we see under Analysis Result the statement that "Cattle allele '189' found in BM4307". Clearly this animal has nuclear DNA evidence of introgression, is genetically polluted, and a hybrid. If you remember earlier in the series, this is not the amount of cattle genes, but an event marker that introgression has occurred in the past. It has no bearing or relation to the possible amount of cattle DNA within the animal's genome. Looking below the results, you see a list of the nuclear DNA markers examined. For the BM4307, we see 2 notations, 187/189, which simply means that there are 2 variants of the marker present (remember these markers are repeated many times). In this case, one variant is bison (187) and one is cattle (189). Below the Nuclear DNA Markers is the Mitochondrial DNA Origin which in this case is "Bison". Even though the animal has bison-type mitochondria, this animal is a hybrid with cattle genes.

In Example 2 (test done through the NBA), from a bison breeding bull offered for sale, we see that the Analysis report states "No evidence of Cattle alleles in the markers reported". This animal was reported to us as not having any cattle genes (markers); however, upon inspection of this report we see under Mitochondrial DNA Origin that it states "CATTLE". In this case the animal does not have any nuclear DNA cattle markers but has cattle-type mitochondria indicating that there was a bovine cow in its maternal ancestry. Although no nuclear markers, this bison is still a hybrid with cattle genes.

In <u>Example 3</u> (test done through the NBA), from a bison breeding bull offered for sale, we see that the Analysis report states "No evidence of Cattle alleles in the markers reported" and that the Mitochondrial DNA Origin is "BISON". This animal has no evidence of cattle introgression based on the testing methods employed and can be characterized as "pure" bison (to the best of our knowledge and belief).

From these 3 examples, it is clear that both nuclear and mitochondrial DNA tests must be performed to detect introgression as a hybrid may only have one set of markers.

<u>Example 4</u> is from a calf of a bred female with the extended introgression assay; the mother had no evidence of introgression indicating the bull was a hybrid. As compared to the tests above, the extended panel uses 25 markers to increase the likelihood of detecting cattle introgression. This is evident in the Genetic Markers table that lists 25 loci as oppose to the 15 in the above tests. You will notice the Mitochondrial DNA origin heading has been removed and the mtDNA results are listed within the Genetic Markers table.

To assist in test interpretation, the report has a more thorough description of the results under Analysis Results. These results state that there is evidence of "cattle specific alleles" indicating that the animal has cattle genes and is a hybrid. Although the description in Example 4 is somewhat confusing, new proposed descriptions will be clearer:

In regard to the nuclear DNA markers future descriptions will state:

"No evidence of cattle introgression was detected among nuclear DNA markers reported."

"Evidence of cattle introgression was detected in nuclear markers reported. Cattle alleles are highlighted below in bold and marked with an asterisk."

In regard to Mitochondria future descriptions it will state:

"No evidence of cattle introgression was detected in maternally-inherited mitochondria. Mitochondrial DNA is of bison origin."

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"Evidence of cattle introgression was detected in maternally-inherited mitochondria. Mitochondrial DNA is of cattle origin."

These new descriptions should greatly facilitate test interpretation and limit confusion and misunderstandings. It is assumed that these new descriptions will accompany all reports, including those done through the NBA, but this has not been verified by the author.

3. Purchase from a clean herd.

This is probably the easiest method of insuring that the bison you buy are not hybrids, assuming you can find an introgression-free herd. If you can locate a herd that claims to be "pure", again require paperwork on the individual animal being offered for sale. Be sure that both mtDNA and nDNA tests were performed and that mitochondrial and nuclear introgression markers were not detected. If the animal is a calf and there is paperwork on both the bull and damn, then they should not require paperwork on the individual calf; however, a written guarantee of purity should be provided. If the seller is not prepared to issue a guarantee, then the seller is not sure of the animal's purity and you should perhaps look elsewhere. Remember that a registered bison means nothing in terms of purity and registered bison may be hybrids with evidence of cattle introgression.

Since there are very few verified introgression-free herds and most herds have not been thoroughly tested, your only realistic choices are probably 1 and 2 above, with 2 being unlikely.

Unless the industry makes the decision and a concerted effort to stop breeding hybrids and the propagation of cattle genes, finding bison without evidence of cattle introgression will become harder and the predominate bison will be a hybrid.

Maintaining a Pure Bison Herd

Maintaining a pure bison herd is equally challenging. You do not want to maintain a closed herd because, even though bison have good diversity, inbreeding will almost certainly reduce diversity in addition to causing other problems. Hence, you will need to bring in new stock and, as above, there is no clear-cut method. The only choices are those above; either purchase a tested animal or do test-and-cull on all replacements.

It must be remembered from previous articles that just because an animal does not have any currently employed cattle markers, the animal could still be a hybrid. Previous articles gave examples of several methods whereby the cattle markers, but not the cattle genes, would not be detected. So the term, "introgression-free" or "pure bison" needs to be taken with the understanding that future tests may identify these other animals.

With such in mind, maintaining an introgression-free herd also raises the dilemma of what happens with the development of new tests. Do you go through another test-cull procedure? The markers currently in use are those that are most prevalent and most likely to identify introgression – they are not likely to be the only markers. But this falls under the hypotheticals and "what if's" and should not be considered unless it proves true – we can only do the best we can with the technologies available.

Dealing with an Introgressed Herd

If you have never tested your herd and/or have not assembled a cattle gene-free herd from scratch, then it must be assumed that you have an introgressed herd, i.e., a herd with animals containing cattle genes. What you do with the herd really depends on a few issues:

- 1. Do you care about cattle introgression and genes in your bison herd?
- 2. How large is your herd?
- 3. How much are you willing to spend on dealing with the issue?
- 4. How radical approach are you willing to take?

For the purpose of this discussion, it is assumed that the answer to #1 above is "Yes"; otherwise the discussion has no meaning. It must also be assumed that the introgression rate is approximately 10%, i.e., about 10% of the bison in the herd have cattle introgression markers.

Contrary to claims by many, and as previously noted, you cannot breed-out or get rid of the introgressed cattle genes. You might be able to "hide" the introgression markers, such as breeding a bull with cattle mitochondria without nuclear markers, but the actual cattle genes are still being passed to his offspring. Knowingly doing such and then claiming introgression-free is either deceitful or naïve at best. Newer tests will likely be able to detect these animals.

In herds with less than 100 head, a simple test-cull procedure could be effective and bring about rapid change. The cost to test the entire herd would be \$4000 (\$40/test for the extended panel), which would be easily covered by the sale of culled animals with introgression markers. If having tests done through the NBA, find out if the extended marker panel test can be performed rather than the shortened one. There is no need to have tests performed on your calves as you should be able to determine their status based on the test results of the damn and bull. Of course that assumes you only have one bull or all bulls are introgression free. Remember that a bull with cattle-type mtDNA will pass cattle genes to his offspring without any markers; these animals would be hybrids even though they have no presently detected markers. A less aggressive approach would be to follow the suggestions for larger herds.

In herds with over 100 head, a simple test-cull procedure may not be realistic and could be cost prohibitive. In this case, there are a variety of other options available other than test-cull.

At a minimum, all breeding bulls should be tested and any that have cattle introgression markers culled. Just insuring that your bulls are free from cattle genes is a major step in stopping

the continued dissemination of these genes. Beyond that, it all depends on how aggressive you wish to proceed. Other than test & cull, the sensible approach in a large herd is to separate the herd into a "clean" group and an introgressed group. This can be physical separation or simply by ear tag or other designation. Many large herds already run a breeding herd and a meat herd. Redesignating the meat herd as the introgressed herd and moving animals between these 2 herds as you proceed with slowly testing the herd. This 2-herd approach is a common cattle practice in the control of certain endemic diseases.

Whether you test the entire herd all at once or just a few each time you work them will determine how soon you can achieve an introgression-free herd of pure bison. Making these efforts will make you true stewards of the North American Bison and true Conservation herds.

The only other alternative is to continue what the industry does now: NOTHING. But, when it is finally declared that the North American Bison is genetically extinct and has been replaced by bison-cattle hybrids, will you and the industry stand up and proudly proclaim:

"I helped do that"?

Click here to discuss this topic or add your comments

Articles coming in the series:

None. There are no other articles in the series.

Past Articles in the series:

<u>Understanding Bison-Cattle Introgression</u>
<u>How Introgression is detected</u>
Does it really matter that bison have cattle genes?

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Currently Retired

https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/47298674/?sort=date&direction=descending

References

[1] University of California-Davis. Bison Tests. 2019. Available at:

https://www.vgl.ucdavis.edu/services/dnatyping.php

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BISON HYBRIDIZATION REPORT

Case:
Date Received: 01-May-2018
Print Date: 04-May-2018
Report ID:
Verify report at www.vgl.ucdavis.edu/myvgl/verify.html

Name: Reg:
YOB: Sex: Male Breed: Plains Bison

Sire: Reg:
Dam: Reg:

ANALYSIS RESULT

Cattle allele "189" found in BM4307.

NUCLEAR DNA MARKERS

LOCUS	TYPE	LOCUS	TYPE	LOCUS	TYPE
AGLA17	215	AGLA293	218	BM1314	137
BM4307	187/189	BM4513	132	BM7145	108
BMC3224	176	BMS2270	70	BMS4040	75
CSSM36b	158	CSSM42b	167	RM185	92
RM500	123	<i>SPS113b</i>	130	TGLA227b	72/73

MITOCHONDRIAL DNA ORIGIN

BISON

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Name: Reg:
YOB: Sex: Male Breed: Plains Bison

Sire: Reg:
Dam: Reg:

ANALYSIS RESULT

No evidence of Cattle alleles in the markers reported.

NUCLEAR DNA MARKERS

LOCUS	TYPE	LOCUS	TYPE	LOCUS	TYPE
AGLA17	215	AGLA293	218	BM1314	137
BM4307	185/187	BM4513	132	BM7145	108
BMC3224	176	BMS2270	66/68	BMS4040	75
CSSM36b	158	CSSM42b	167/171	RM185	92
RM500	123	SPS113b	130	TGLA227b	72

MITOCHONDRIAL DNA ORIGIN

CATTLE

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Dam: Reg:

ANALYSIS RESULT

No evidence of Cattle alleles in the markers reported.

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BM4307	185	BM4513	132	BM7145	108
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CSSM36b	158	CSSM42b	169/171	RM185	92
RM500	123	<i>SPS113b</i>	130	TGLA227b	73

MITOCHONDRIAL DNA ORIGIN

BISON

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BISON HYBRIDIZATION REPORT

ROD CHIODINI 3321 JIMMY CREEK RD FOX, AR 72051

EXAMPLE 4

Case: NCB2400

Date Received: 26-Nov-2018

Print Date: 03-Dec-2018

Report ID: 7009-7089-0080-2035

Verify report at www.vgl.ucdavis.edu/myvgl/verify.htm

Name: OBF-88 Reg:

DOB: 05/01/2018 Sex: Female Breed: Plains Bison

ANALYSIS RESULT

Analysis of nuclear DNA provides evidence of cattle specific alleles (highlighted below in bold and marked with an asterisk).

Analysis of mitochondrial DNA provides no evidence of cattle introgression in the lineage of the animal tested.

GENETIC MARKERS

LOCUS	TYPE	LOCUS	TYPE	LOCUS	TYPE
AGLA17	215	AGLA293	218	BL42	225
BM1314	137/ 159 *	BM4307	185	BM4513	132
BM5004	107/123	BM7145	108	BMC3224	176
BMS2270	66/68	BMS4040	75	CSSM36	159
CSSM42	169	FCB193	112/114	FCB304	125/127
ILSTS005	181/187	ILSTS008	187	INRA107	158/160
INRA23	192	<i>MAF209</i>	124	MGTG4B	127
RM185	92	RM500	123	SPS113	138
TGLA227	72			mtDNA	BISON