"Genetic Health of North American Bison"

James N. Derr

June 2015
International Symposium on Bison Health
Saskatoon, Saskatchewan

Texas A&M University College of Veterinary Medicine

Veterinary Pathobiology

Genetics and Genomics

- **Genetics technology is used with North American bison to:**
  - Identify individuals (DNA fingerprinting - forensics)
  - Determine parentage
  - Estimate inbreeding / outbreeding
  - Access levels of genetic diversity / variation
  - Discover the historical lineage of a herd, population or animal
  - Document evidence for and levels of domestic cattle genetic introgression (hybridization)

We have now examined over 50,000 individual bison using these genetic tools (http://vetmed.tamu.edu/dnacore).

Genetics and Genomics

- **Genomics is a game changer.**

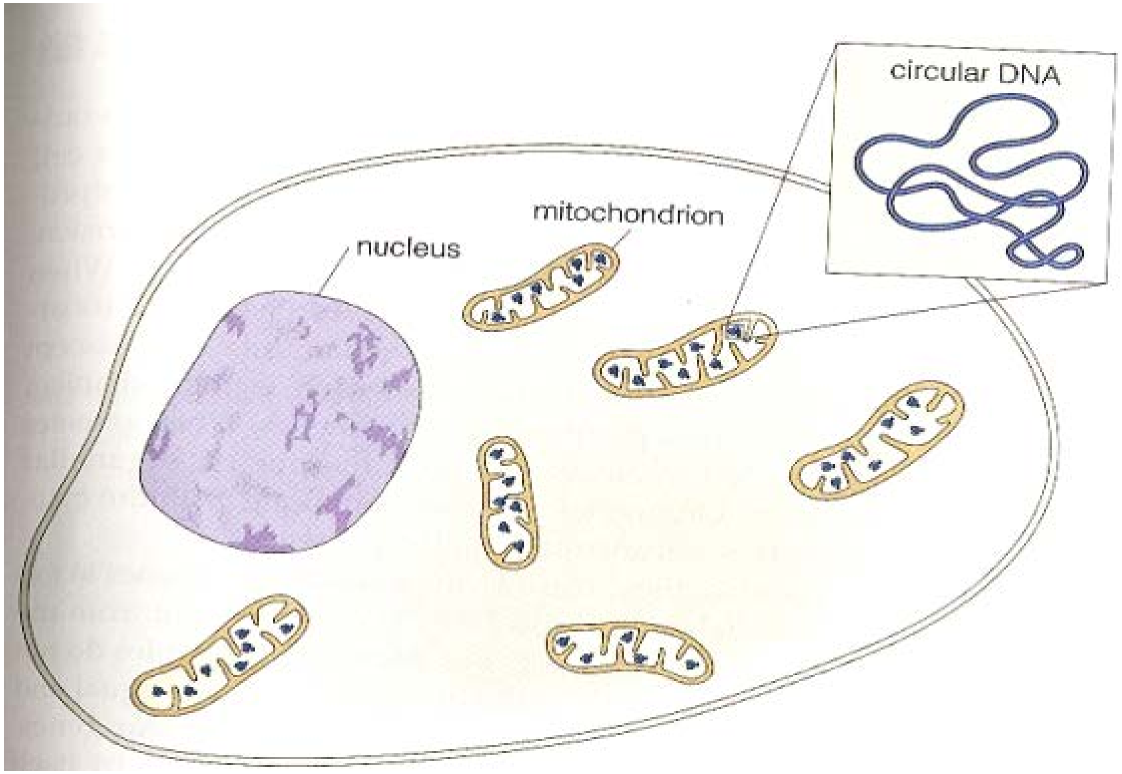
  In practice, it is the determination of the entire DNA sequence of an organism and then fine-scale genetic mapping of the genomic architecture.

Genetics and Genomics

- **Today I am going to focus on bison genomics.**
  - Mitochondrial Genomics
  - Nuclear Genomics

The mitochondria

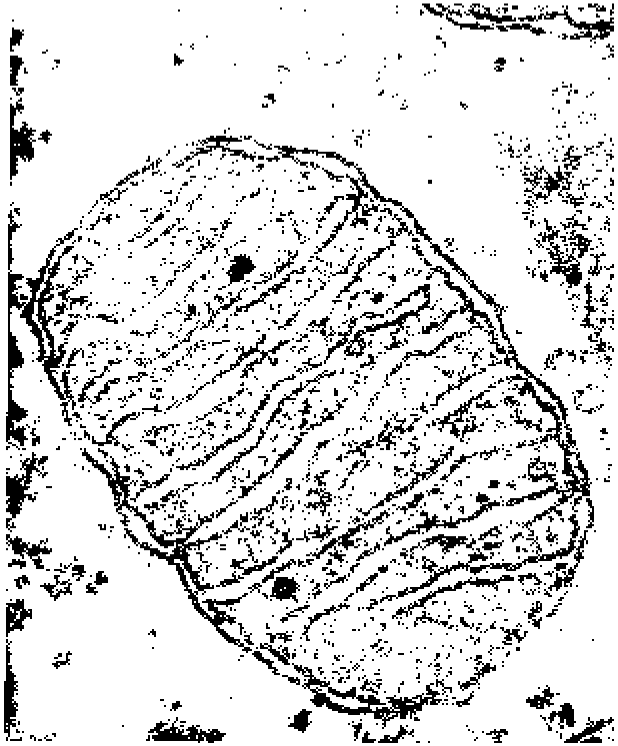
- Tens to hundreds of mitochondria per cell
- 1-15 mtDNA genomes/mitochondria



from Genetics: The Continuity of Life, 1999.

Mitochondrial function

- The "powerhouse" of the cell
- Cellular respiration to make ATP
  - Oxidative phosphorylation
  - Electron transport chain



$(CH_2O)_n + nO_2 \rightarrow nCO_2 + 2nH_2O$

Carbohydrate, Oxygen → Carbon Dioxide, Water

↙

ATP (energy)

The Mitochondrial Genome

- Inherited only through the female lineage.
- Is a haploid genome, no recombination.
- The genome is relatively small (about 16,600 base pairs).
- Required for energy production in the cell.
- High rate of nucleotide substitution.
- DNA sequence is very well characterized.

Mitochondrial DNA Mutations - Human Diseases

- Mitochondrial myopathies – multiple neuromuscular diseases
  - Myoclonic Epilepsy with Ragged Red Fibers (MERRF)
  - Leigh Syndrome
    - seizures, altered states of consciousness, dementia, ventilatory failure
  - Kearns-Sayre Syndrome
    - progressive external ophthalmoplegia, eye pigment disorders, heart disease, cerebellar dysfunction, diabetes, hearing loss, muscle weakness
  - Pearson Syndrome
    - Childhood onset of anemia, dysfunction of pancreas, liver, kidneys (usually fatal)
  - Leber's hereditary optic neuropathy (LHON)
    - degeneration of optic nerve, rapid onset of blindness
    - mostly affects men in their 20s

Mitochondrial DNA and Longevity

- mtDNA somatic deletions accumulate with age
- Especially prevalent in non-dividing cells
- Are mtDNA somatic deletions associated with neurological and muscular function loss?
  - Noted accumulation of partially deleted mtDNAs in heart and brain
  - may be partially causative of degenerative disorders: Parkinson’s, Alzheimer’s, Huntington’s

Potentially associated with different life expectancies observed between bison and cattle

Buffalo Mom

Our First Publication on Bison Mitochondrial DNA

Animal Conservation (1999) **2**, 51–57 Printed in the United Kingdom

Identification of domestic cattle hybrids in wild cattle and bison species: a general approach using mtDNA markers and the parametric bootstrap

Todd J. Ward[1], Joseph P. Bielawski[2], Scott K. Davis[3], Joe W. Templeton[1] and James N. Derr[1]

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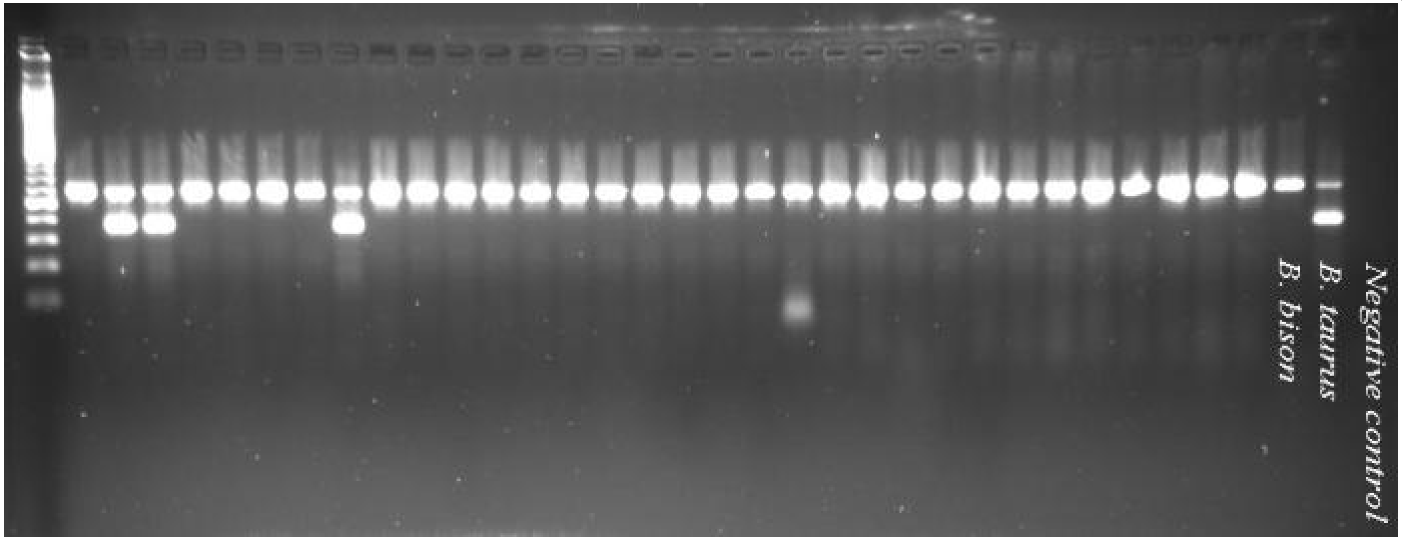
(Received 11 June 1998; accepted 10 September 1998)

Abstract

Many species are currently undergoing reductions in population size due to widespread habitat loss and expanding human activities. Because interspecific hybridization is often a consequence of population decline and fragmentation, identification of individuals or populations with hybrid ancestry is an increasingly important issue in conservation biology. In many wild cattle and bison species, the problem of natural hybridization has been compounded by indiscriminate crossbreeding with domestic cattle for the purpose of improving domesticated stocks. Therefore, a genetic test using the polymerase chain reaction was developed so that wild cattle and bison with domestic cattle mitochondrial DNA (mtDNA) haplotypes could be rapidly identified. Using this genetic test, domestic cattle mtDNA haplotypes were detected in *Bos grunniens* (yak), *Bison bonasus* (European bison), and 6 out of the 15 (40%) *Bison bison* (North American bison) populations tested. In total, 30 out of the 572 (5.2%) North American bison tested, were found to have domestic cattle mtDNA. The hybrid origin of these mtDNA haplotypes was verified in a phylogenetic analysis using the parametric bootstrap. These results are discussed in terms of their implications for the conservation status and future management of wild cattle and bison species.

Diagnostic mtDNA Screen



For the mitochondrial DNA test alone, we have now surveyed over 50,000 individual bison over the last 15 years.

Mitochondrial DNA Results Federal Bison Herds

5,246 bison sampled from US and Canadian federal populations.

All appear free of cattle mtDNA except for a small number of animals (14) at the National Bison Range. The origin of this introgression (was confirmed through DNA sequencing (a few Kansas bison cows).

14 / 5,246 animals = 0.0026%

Mitochondrial DNA Results
State, NGO and Private Bison Herds

Bison sampled from State, NGO and private herds typically show a much different pattern with domestic cattle mtDNA ranging from 0% to 100% across these herds with an overall average of ***about 6%***.

Some examples include:

- A private herd in Texas has 100% cattle mtDNA
- Santa Catalina Island (California) has 47% cattle mtDNA
- Custer State Park (South Dakota) has 20.6% cattle mtDNA
- Antelope Island State Park (Utah) has 0.010% cattle mtDNA
- **Henry Mountains State Park (Utah) has 0.0% cattle mtDNA**

A couple of Texas bison from Caprock Canyons State Park

This publication in 2011 was the first genomics study completed with bison

Mitochondrion 11 (2011) 166–175

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Complete mitochondrial DNA sequence analysis of *Bison bison* and bison–cattle hybrids: Function and phylogeny

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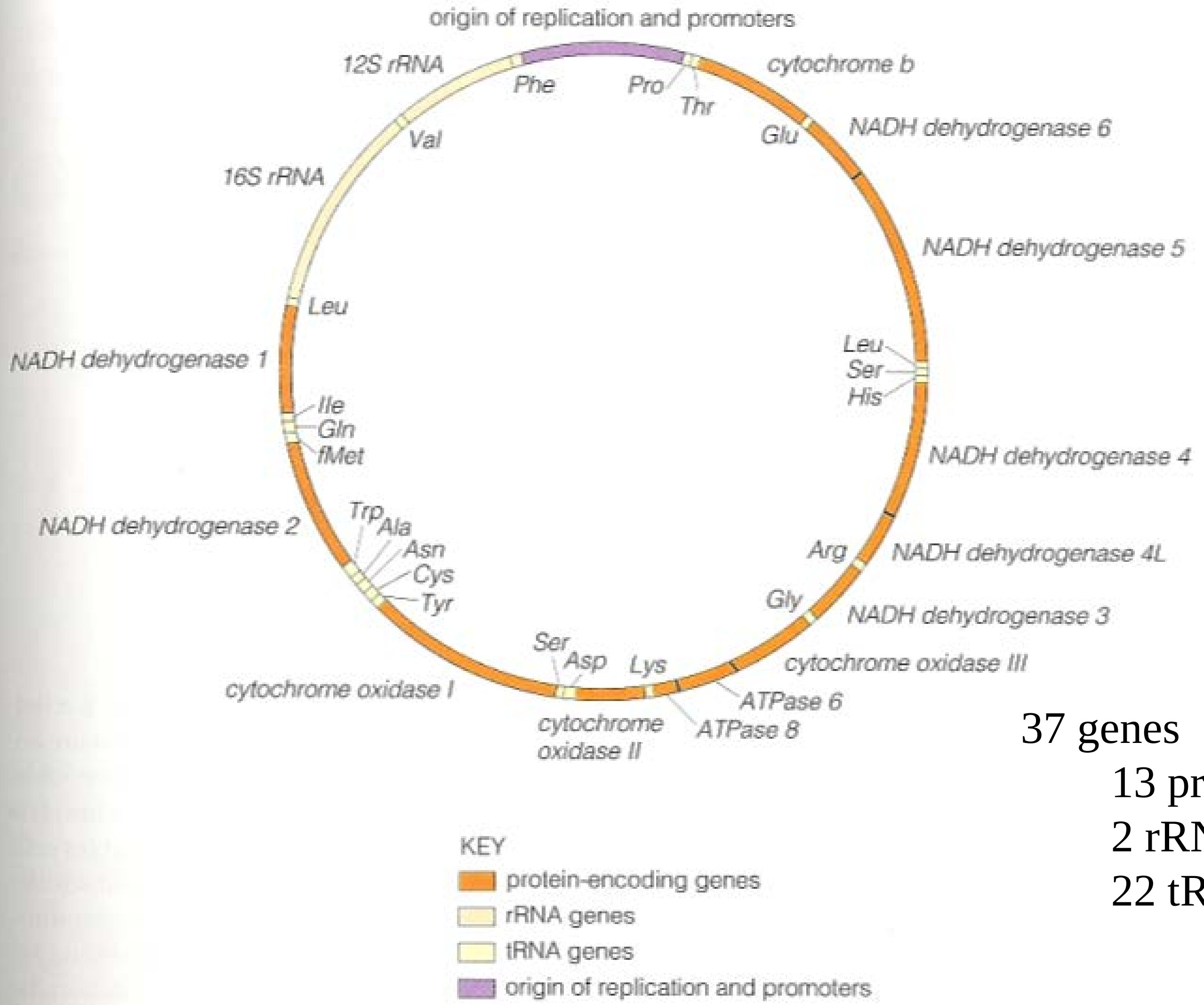
ABSTRACT

Complete mitochondrial DNA (mtDNA) genomes from 43 bison and bison-cattle hybrids were sequenced and compared with other bovids. Selected animals reflect the historical range and current taxonomic structure of bison. This study identified regions of potential nuclear–mitochondrial incompatibilities in hybrids, provided a complete mtDNA phylogenetic tree for this species, and uncovered evidence of bison population substructure. Seventeen bison haplotypes defined by 66 polymorphic sites were discovered, whereas 728 fixed differences and 86 non-synonymous mutations were identified between bison and bison–cattle hybrid sequences. The potential roles of the mtDNA genome in the function of hybrid animals and bison taxonomy are discussed.

Bison Mitochondrial DNA

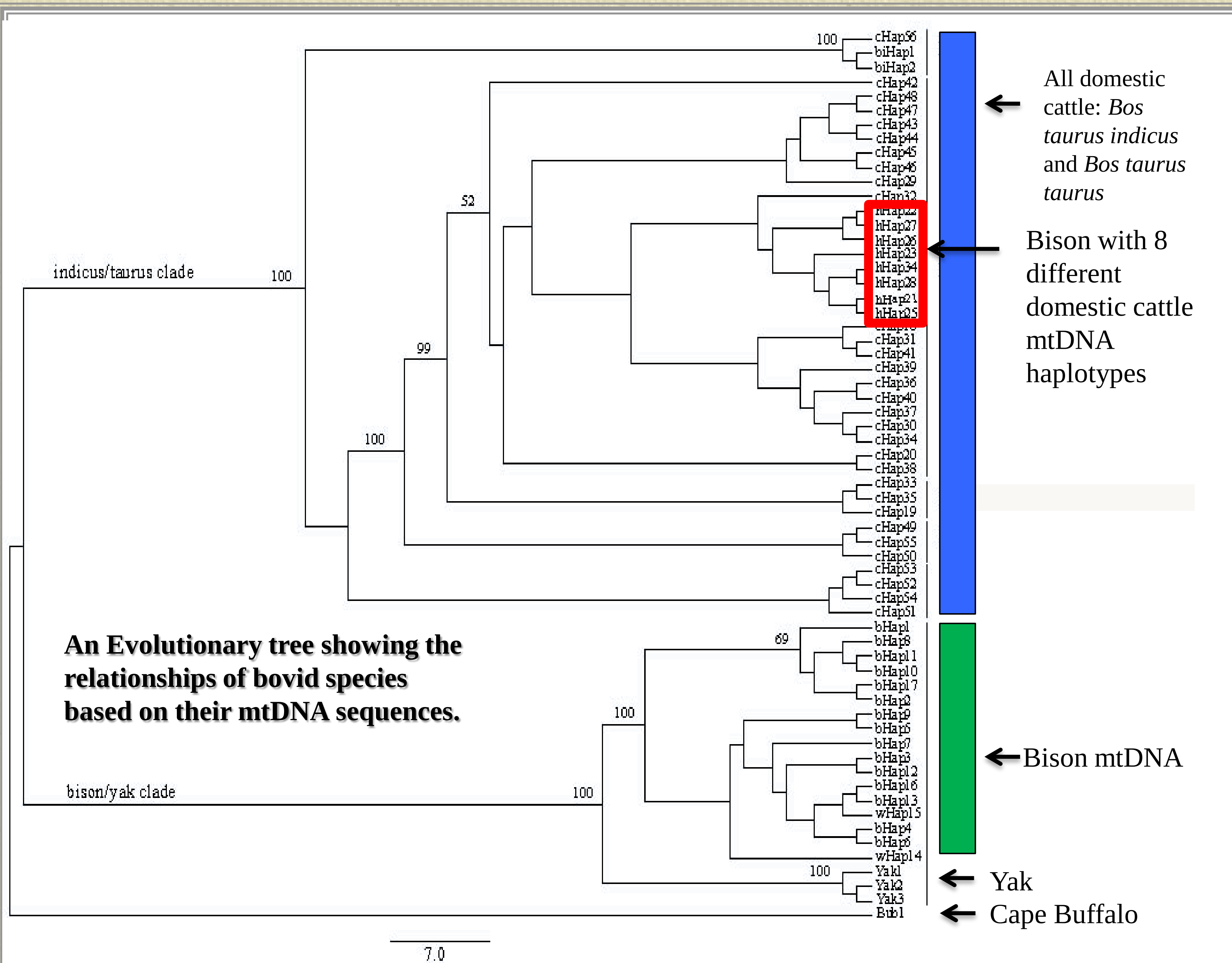


37 genes

- 13 protein-coding
- 2 rRNA
- 22 tRNA

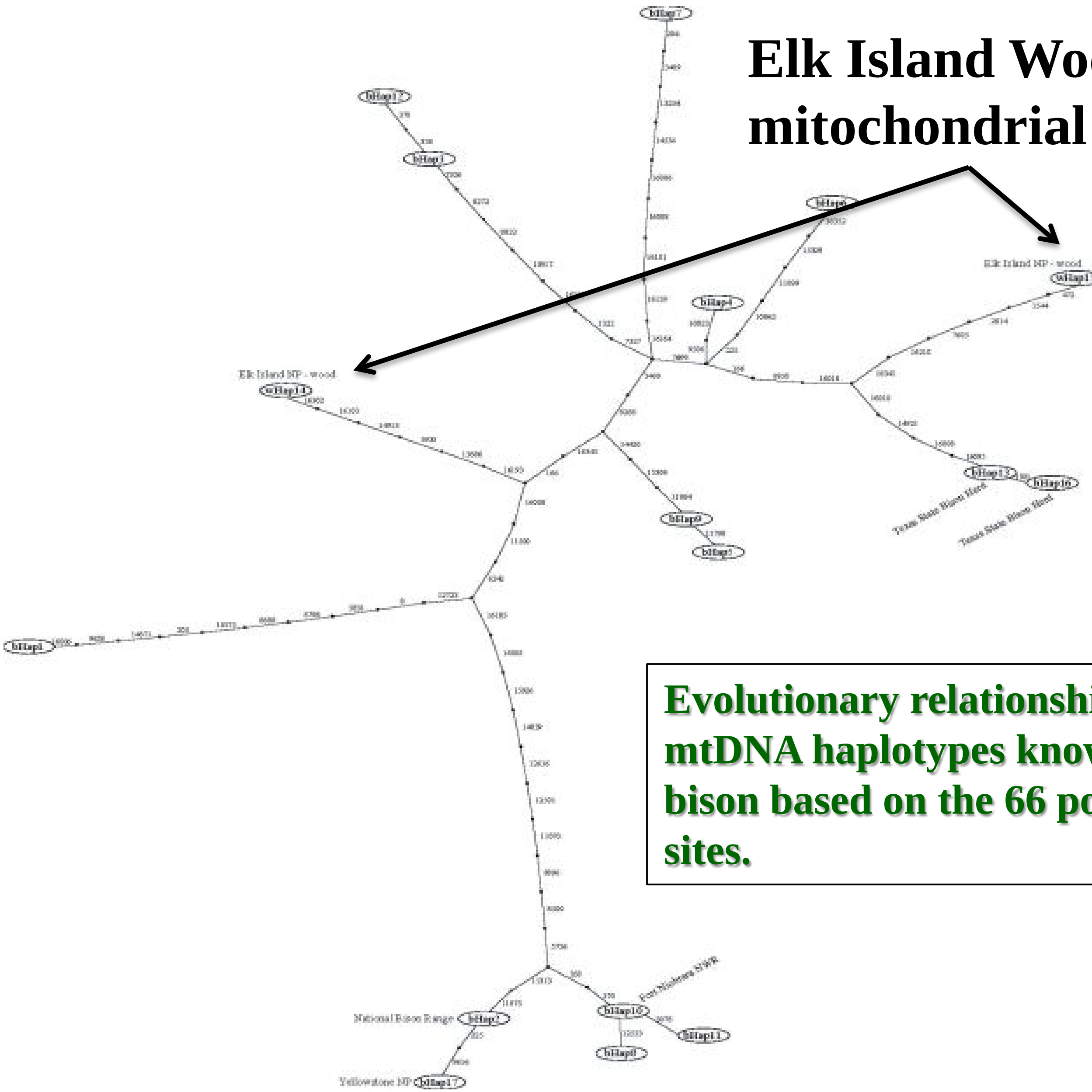
Bison Mitochondrial DNA

- We sequenced the entire mtDNA genomes of 43 bison and bison-cattle hybrids.
- Discovered 17 different bison mtDNA sequences (haplotypes)
- Across all bison types there are 66 mutations in the mtDNA genome. (we also found 426 mutations in domestic cattle)
- There are 728 fixed differences between bison and domestic cattle mtDNA haplotypes. (This divergence between all bison and all cattle haplotypes resulting in an estimated lineage split between these species 1 - 1.4 MYA).
- There are 85 amino acid changing mutations between bison and domestic cattle mtDNA haplotypes.
- We found 8 different domestic cattle mtDNA haplotypes in bison.



An Evolutionary tree showing the relationships of bovid species based on their mtDNA sequences.

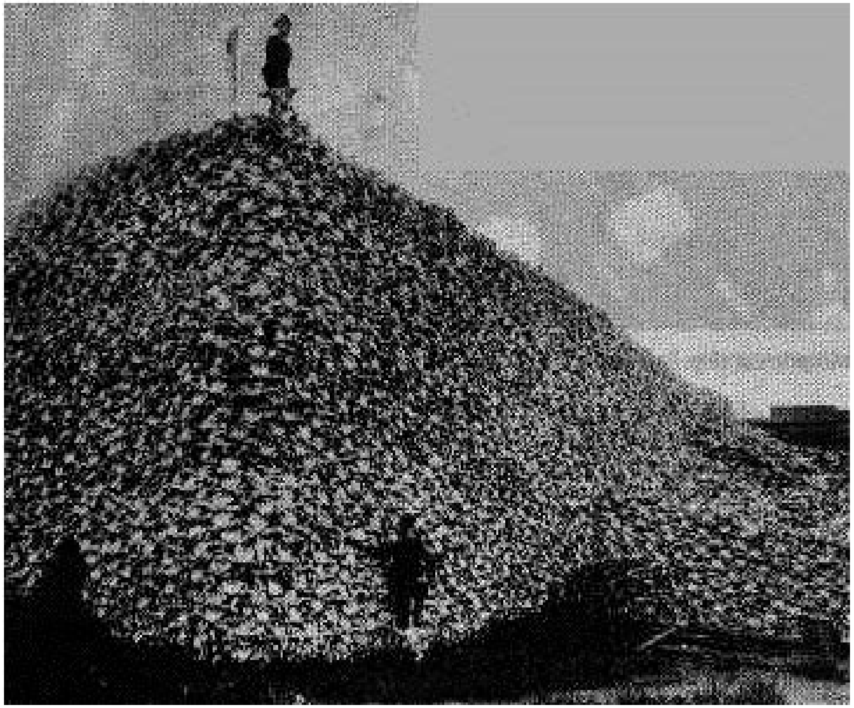
Elk Island Wood Buffalo mitochondrial types.



Evolutionary relationship of the 17 mtDNA haplotypes known from bison based on the 66 polymorphic sites.

With the completion of this study, we now know the amount, type and location of genetic diversity in bison mtDNA and exactly how bison compare with domestic cattle for this important biological trait.

The Bone(s) of Contention……



…"so what if bison have domestic cattle mtDNA….. they look like bison and taste like bison so what is the problem"?

August 2012 in the Journal Conservation Biology

Phenotypic Effects of Cattle Mitochondrial DNA in American Bison

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Abstract: *Hybridization between endangered species and more common species is a significant problem in conservation biology because it may result in extinction or loss of adaptation. The historical reduction in abundance and geographic distribution of the American plains bison* (Bison bison bison) *and their recovery over the last 125 years is well documented. However, introgression from domestic cattle* (Bos taurus) *into the few remaining bison populations that existed in the late 1800s has now been identified in many modern bison herds. We examined the phenotypic effect of this ancestry by comparing weight and height of bison with cattle or bison mitochondrial DNA (mtDNA) from Santa Catalina Island, California (U.S.A.), a nutritionally stressful environment for bison, and of a group of age-matched feedlot bison males in Montana, a nutritionally rich environment. The environmental and nutritional differences between these 2 bison populations were very different and demonstrated the phenotypic effect of domestic cattle mtDNA in bison over a broad range of conditions. For example, the average weight of feedlot males that were 2 years of age was 2.54 times greater than that of males from Santa Catalina Island. In both environments, bison with cattle mtDNA had lower weight compared with bison with bison mtDNA, and on Santa Catalina Island, the height of bison with cattle mtDNA was lower than the height of bison with bison mtDNA. These data support the hypothesis that body size is smaller and height is lower in bison with domestic cattle mtDNA and that genomic integrity is important for the conservation of the American plains bison.*

Keywords: Catalina Island, height, hybridization, introgression, mtDNA, weight

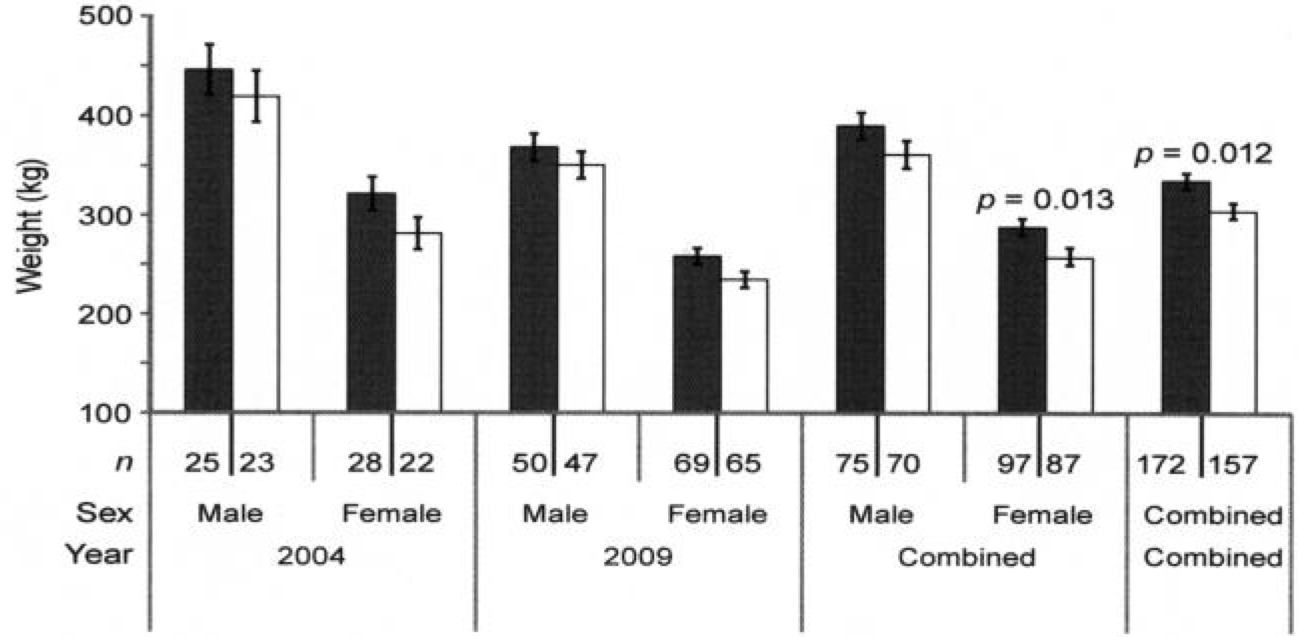
Phenotypic Effects of Cattle Mitochondrial DNA in American Bison

We conducted two separate studies to determine if mitochondrial status of a bison has a biological effect.

- **Nutritionally stressful environment.**
  - Santa Catalina Island
  - 329 animals sampled in 2004 and 2009
  - All animals were weighed and in 2009 all animals were measured for height.
  - 47% of the bison had domestic cattle mtDNA
- **Nutritionally rich environment.**
  - Private feedlot
  - All bull calves
  - 618 animals started the study at weaning and were weighed 8 times over 26 months.
  - 6% of the bison had domestic cattle mtDNA

Mean weight (SE) for male and female bison from Santa Catalina Island with bison mtDNA (shaded bars) or cattle mtDNA (open bars) for samples from 2004 and 2009, for 2004 and 2009 combined, and for combined males and females and years.

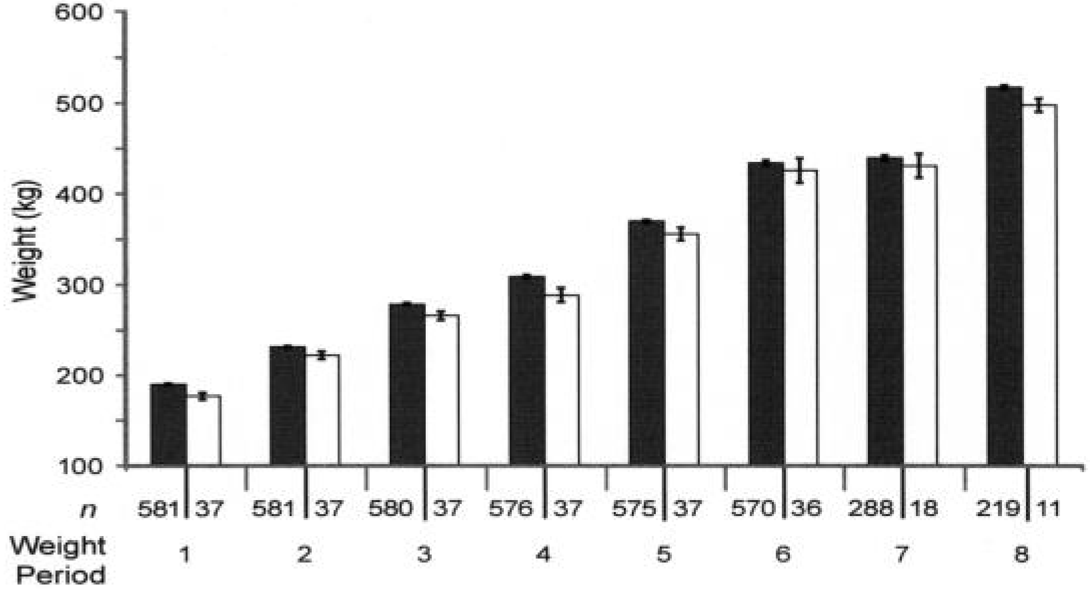
Combined mean difference across both years is 7.8% (25.7 lbs.). Bison with domestic cattle mtDNA were consistently smaller in the harsh nutritional study (Santa Catalina Island).



Mean weight (SE) every 90–120 days for young bison males with bison (shaded bars) or cattle (unshaded bars) mtDNA from a feedlot (n, sample size). All differences were statistically significant ($p < 0.01$).

The mean difference at weaning weight was 7% (28.7 lbs.) and at the final weight was 4% (43 lbs.). Bison with domestic cattle mtDNA were consistently smaller in the high nutritional study (feedlot).

Both studies resulted in the same conclusion, bison with domestic cattle mtDNA are, on average, smaller .



The hybridization experiments conducted by some of the owners of the five foundation herds of the late 1800s, have left a legacy of a small amount of cattle genetics in many of our existing bison herds.

So What if a Bison Herd Has Cattle mtDNA?

First, the ability to identify bison populations with hybrid ancestry allows for responsible conservation decisions regarding animal introductions.

Second, bison in production herds with domestic cattle mtDNA, across different nutrition platforms, on average are *smaller*.

“Sequencing the Nuclear Genome of the North American Bison”

Lauren Dobson

May 11, 2015
Dissertation Defense
Advisor: Dr. J Derr

Bison Nuclear Chromosomes

"Templeton"

The Mitochondria is about this size…

"SEQUENCING THE GENOME OF THE NORTH AMERICAN BISON"

The evolutionary history of a species is written in its genome and can be read from its gene content and its genetic architecture.

GENOMICS HAS THREE MAJOR SUBDIVISIONS

- Structural genomics (a “parts list” of the genes for a species)
- Evolutionary genomics (illuminates the similarities and differences between individuals and species)
- Functional genomics (understanding gene function)

Together, these three approaches contribute to the ultimate goal of understanding the role of every gene.

Objectives of Lauren Dobson's Dissertation.

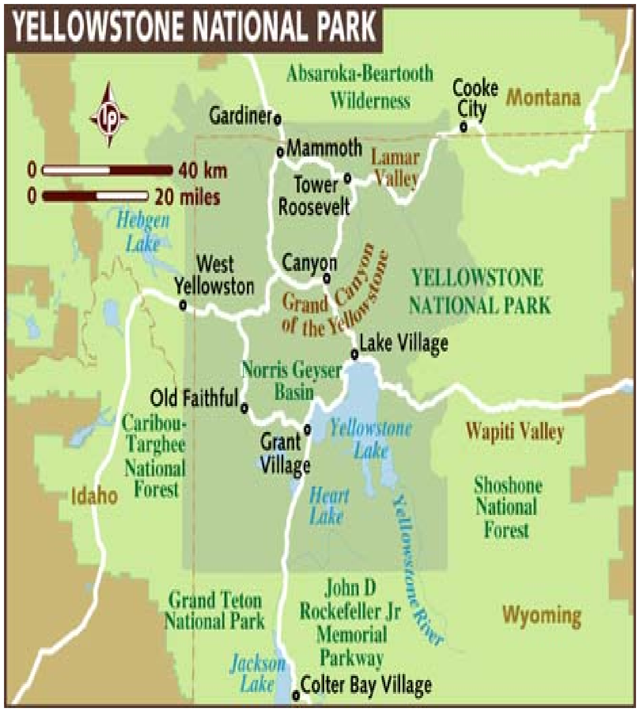
1. Develop an annotated *de novo* bison genome reference sequence assembly of a North American plains bison.
2. Complete a comparative genomic analysis among modern bison, historic bison and domestic cattle.
3. Provide a species level genomic platform to investigate bison genetic traits.

Bison Genome Sequencing Strategy

15 Individual Bison Genomes Were Sequenced

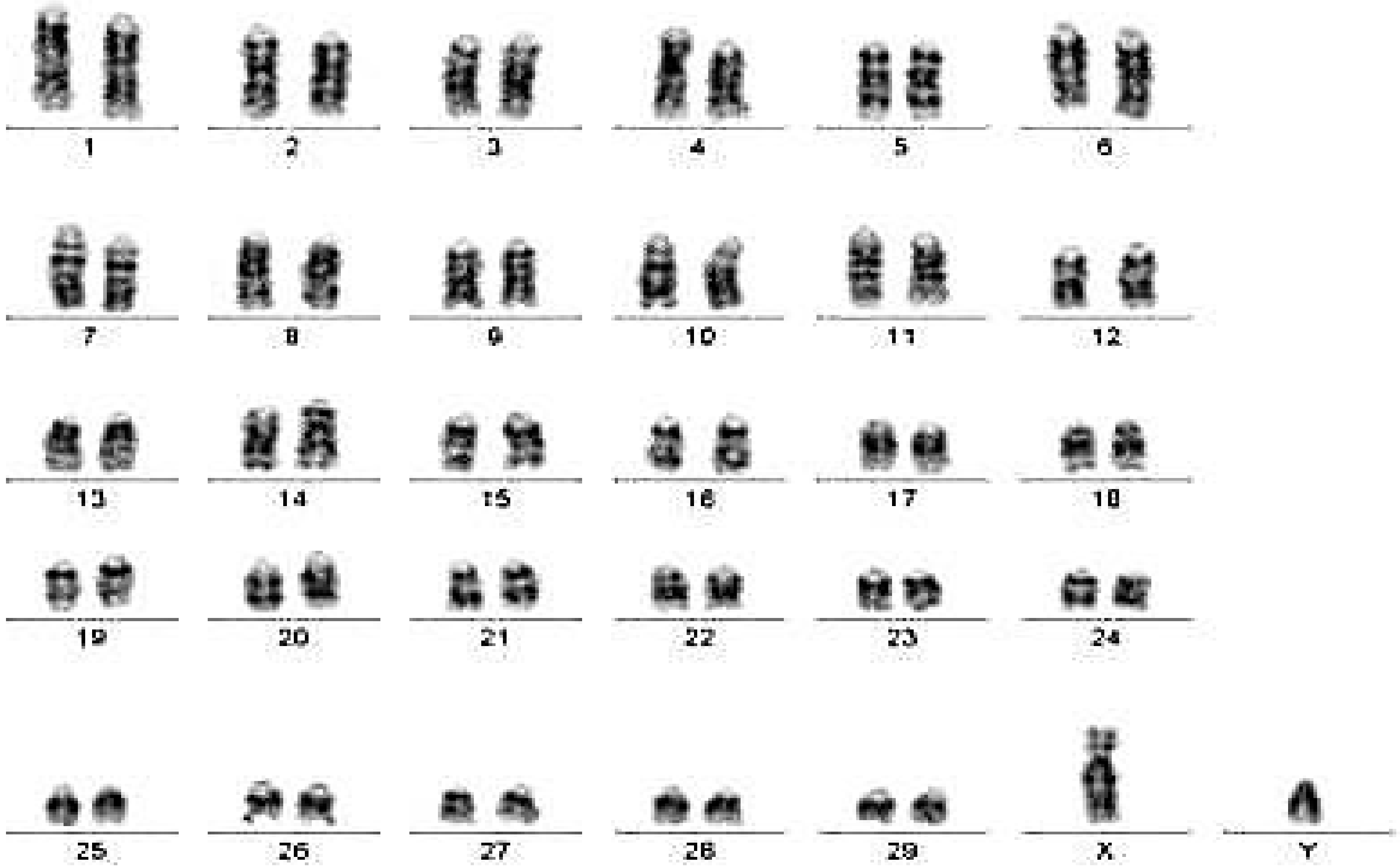
- Templeton: The *de novo* bison reference sequence
- 4 Yellowstone National Park Bison
- 4 Caprock Canyons State Park
  - Charles Goodnight Herd from the 1880s in Texas
- 4 Wood Buffalo
  - Elk Island National Park
- 2 Historical Samples
  - 1856 - Yellowstone area collected by F. V. Hayden (S9)
  - 1886 - Dawson County, MT collected by W. T. Hornaday (S6)

What Animal Is The Reference Bison Genome?

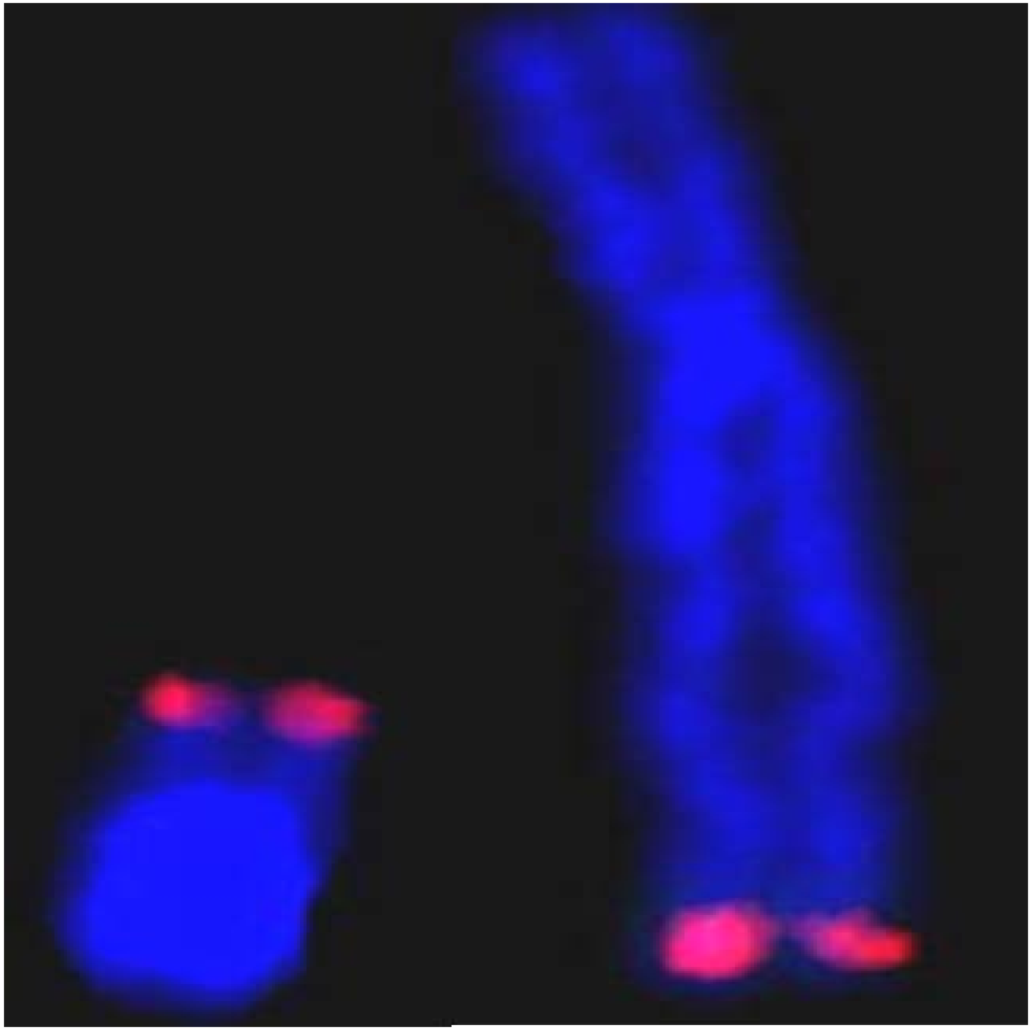


His name is “Templeton”

Templeton's G-banded Karyotypes



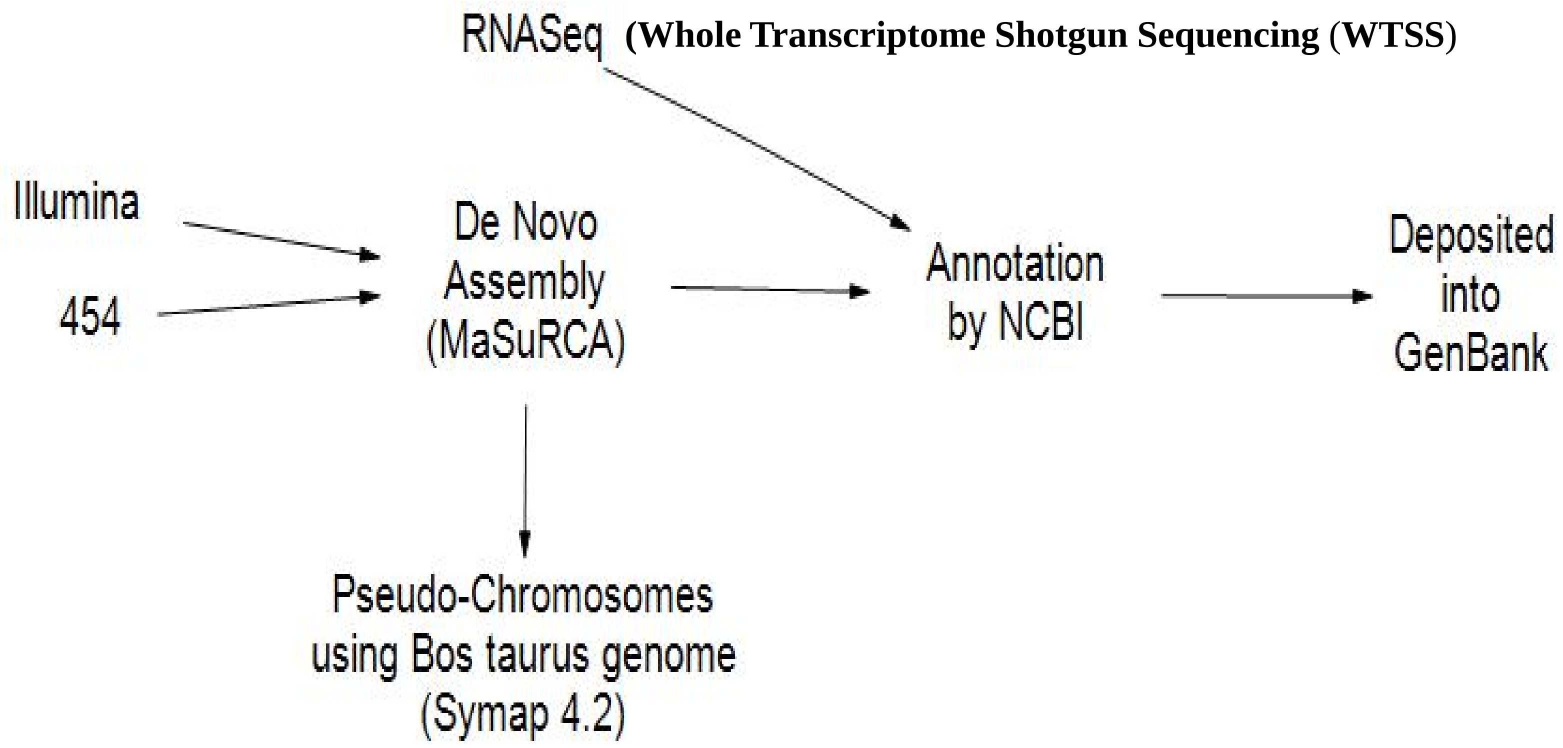
Templeton's X and Y chromosomes by FISH



The American Bison genome was sequenced using a *de novo* assembly method that utilizes hybrid Illumina Inc. and 454 Life Sciences technologies.

Library	Average read length	Number of reads (Millions)	Library mean size (base pairs)	Library standard deviation (base pairs)
Illumina:				
Paired-end	101	1115	300	40
Mate pair	101	85	4,000	800
	101	239	4,500	900
	101	531	6,000	1,000
454:				
Paired-end	398	25.6	15,000	3,500

Total genomic sequencing coverage of 454 and Illumina raw data was approximately 75X.

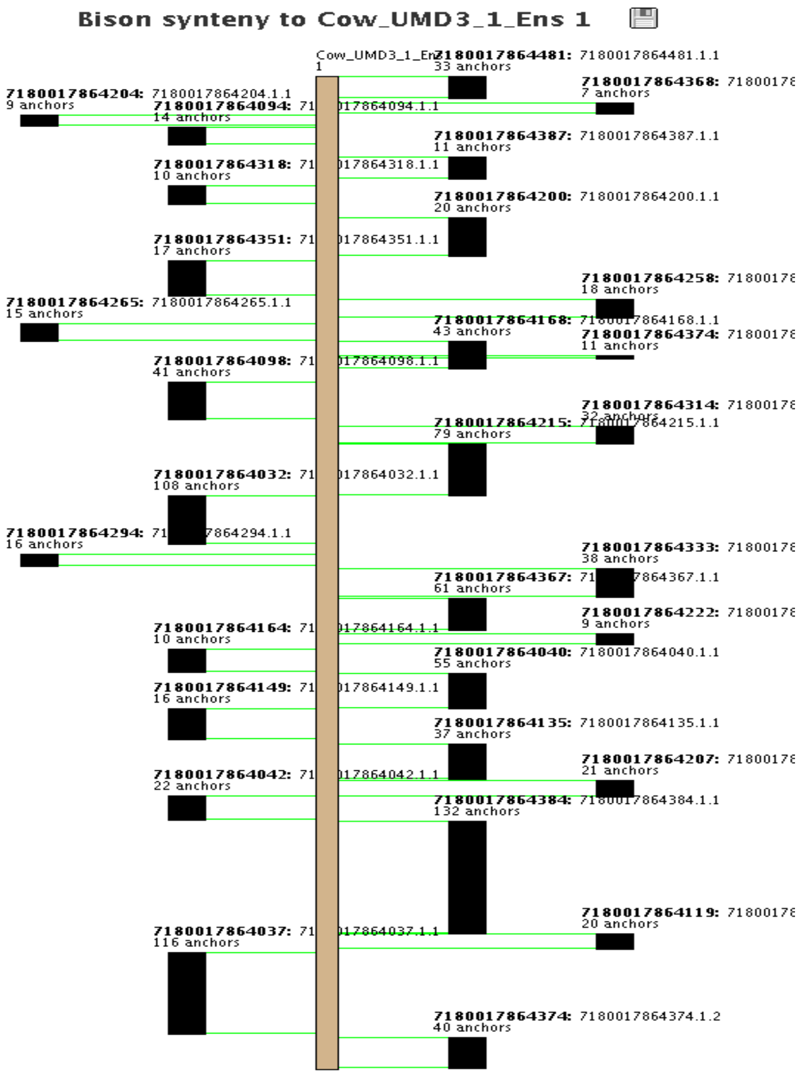


Results

Feature	Bison_UMD1.0
Genes and pseudogenes	26,001
protein-coding	20,782
non-coding	1,677
pseudogenes	3,542
genes with variants	6,158

Bison Sequence Comparison Between Cattle and Human Sequences

Feature	Bison_UMD1.0	Cattle_UMD3.1	HuRef_1	HuRef_2 (GRCh38)
Total sequence length (base pairs)	2,828,031,685	2,670,422,299	2,844,000,504	3,209,286,105
Total number of chromosomes and organelles	31	31	24	25
Genes and pseudogenes	26,001	26,740	39,480	41,722
protein-coding	20,782	19,994	19,691	20,246
non-coding	1,677	3,825	8,555	9,153
pseudogenes	3,542	797	11,234	12,323
genes with variants	6,158	2,581	9,563	14,632
mtDNA size	16,319	16,338		16,569



Bison synteny to Cow_UMD3_1_Ens 1
Cow_UMD3_1_Ens
1
7180017864481: 7180017864481.1.1
33 anchors
7180017864368:
7 anchors
7180017864204: 7180017864204.1.1
9 anchors
7180017864094:
14 anchors
7180017864387: 7180017864387.1.1
11 anchors
7180017864318:
10 anchors
7180017864200: 7180017864200.1.1
20 anchors
7180017864351:
17 anchors
7180017864258:
18 anchors
7180017864265: 7180017864265.1.1
15 anchors
7180017864168:
43 anchors
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11 anchors
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41 anchors
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108 anchors
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16 anchors
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38 anchors
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7180017864222:
9 anchors
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10 anchors
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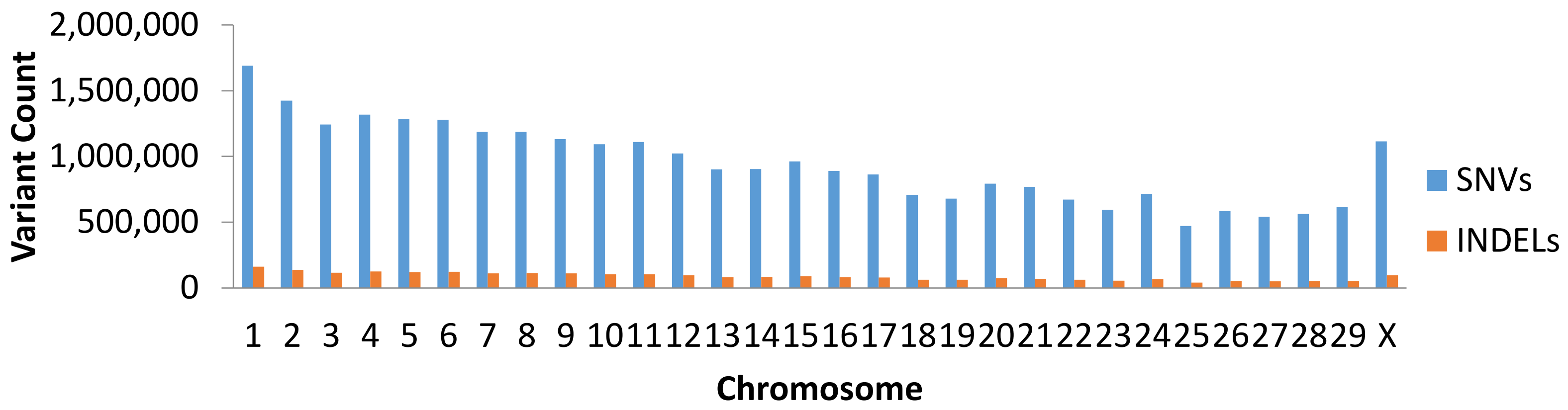
55 anchors
7180017864149:
16 anchors
7180017864135: 7180017864135.1.1
37 anchors
7180017864207:
21 anchors
7180017864042:
22 anchors
7180017864384:
132 anchors
7180017864119:
20 anchors
7180017864037:
116 anchors
7180017864374: 7180017864374.1.2
40 anchors

1.0 to 1.5 mya

Results

98.61% of the raw reads were mapped to the domestic cattle reference

	SNVs	INDELs
Number of variants	28,483,599	2,627,645
Homozygous for variant allele	22,073,944	2,208,623
Heterozygous (one reference one variant)	6,329,185	360,038
Variant rate	1 variant every 93 bases	1 variant every 1,012 bases



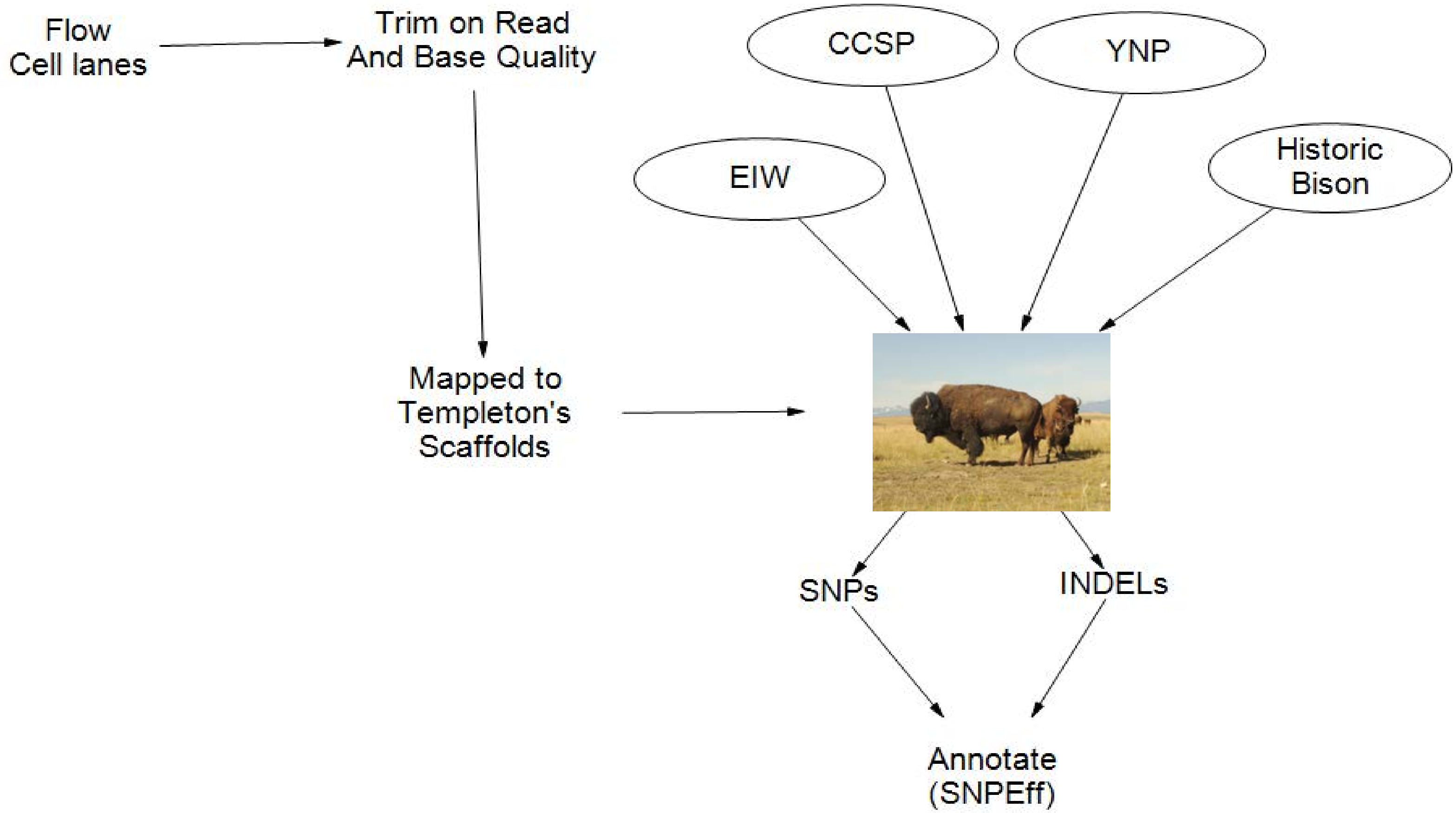
Conclusions

- A 2.82-Gb *de novo* reference assembly of the North American bison genome is complete.
  - With a total of 26,001 genes and pseudogenes
    - 20,782 genes are protein coding genes
- Approximately 30,000,000 new variants were found between the bison and domestic cattle references (1000 bulls project)
- Utilized the bison scaffolds to generate “pseudo-chromosomes”

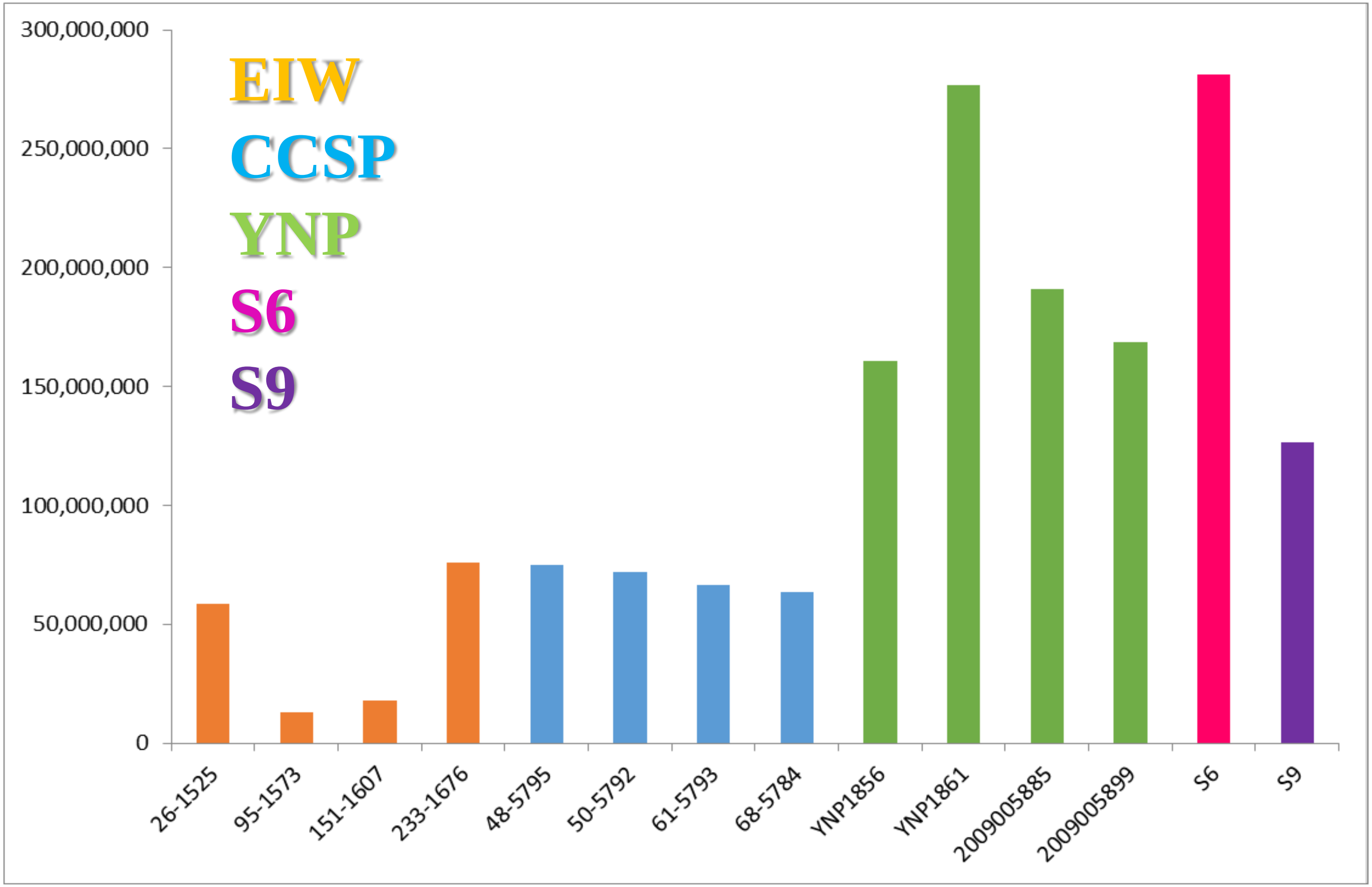
Objectives of Lauren Dobson's Dissertation.

1. Develop an annotated *de novo* bison genome reference sequence assembly of a North American plains bison.
2. Complete a comparative genomic analysis among modern bison, historic bison and domestic cattle.
3. Provide a species level genomic platform to investigate bison genetic traits.

Work Flow for Mapping Addition Bison to Templeton



Total number of raw reads per sample



Population Results

Variant Type	CCSP	EIW	YNP	Templeton	S6	S9
SNP	15,617,914	9,590,819	29,256,990	22,073,944	24,955,527	8,806,184
Homozygous Variant alleles	5,927,794	2,772,582	15,786,097	22,205,662	21,390,382	3,877,017
Heterozygous (one Reference one variant)	299,229	144,539	6,016,703	3,624,586	3,036,493	4,921,122
INSERTION	55,773	22,994	1,085,188	1,233,140	385,125	162,921
DELETION	61,535	24,532	1,210,975	1,394,505	456,563	226,079

Conclusions

- Based on our comparative analyses, the *de novo* bison reference assembly (Templeton) UMD1.0 is of high quality.
- With the completion of this study we have comparative genomic sequences from both subspecies of North American bison (Plains bison and Wood Buffalo), from Northern (YNP) and Southern (CCSP) Plains Bison and from two historic bison samples that pre-date the population bottleneck of the late 1800s.

Bison Genome Applications

Due to the sampling strategy, we have documented the majority of the genetic diversity in this species and compared this diversity with domestic cattle references (1000 bulls project).

Bison Genome Applications

Finally, these bison comparative genomic sequences provide a platform to investigate the underlining genetic components of a number of important traits that involve hybridization, genetic diseases, genetic resistance and susceptibility, fertility, fitness, evolutionary lineages, biodiversity and species level genetic conservation using human and livestock genomes as a guide.

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- Texas A&M University – College of Veterinary Medicine
- Houston Safari Club
- Throlson American Bison Foundation

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  - Steven Olsen
  - David Alt
- USDA Meat and Animal Research Center
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- University of Maryland
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