Bison Genomics Project Update

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General Manager
Canadian Bison Association Convention
November 24, 2019

January 1st: Delta Genomics sold its genetic testing business and assets to Neogen Canada

- Provide Canadian industries and researchers with access to same testing services as our US, Australian, European, South American, and Chinese counterparts
- Able to reduce pricing for a number of services on Day 1

- Majority of Delta Genomics team was retained through the transition
    - Have grown from 8 employees to 14 employees
- Neogen Canada has headquartered in Edmonton area

Shout Out to the Project Funders

Alberta Agriculture and Forestry

Agriculture and Agri-Food Canada

Mitacs Accelerate

CGEn
Canada's Genomics Enterprise

Introduction to DNA

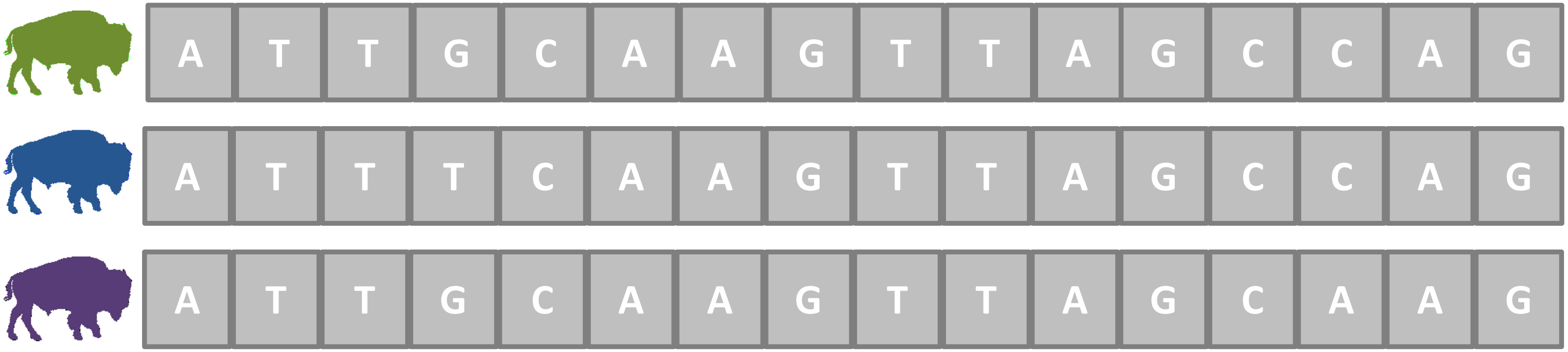
- Made up of units called Base Pairs

- Four types of Base Pairs
  - A, T, G, C

- Mammals have around 3 billion Base Pairs

SNPs = **Small Nucleotide Polymorphisms**

(small changes in the DNA)

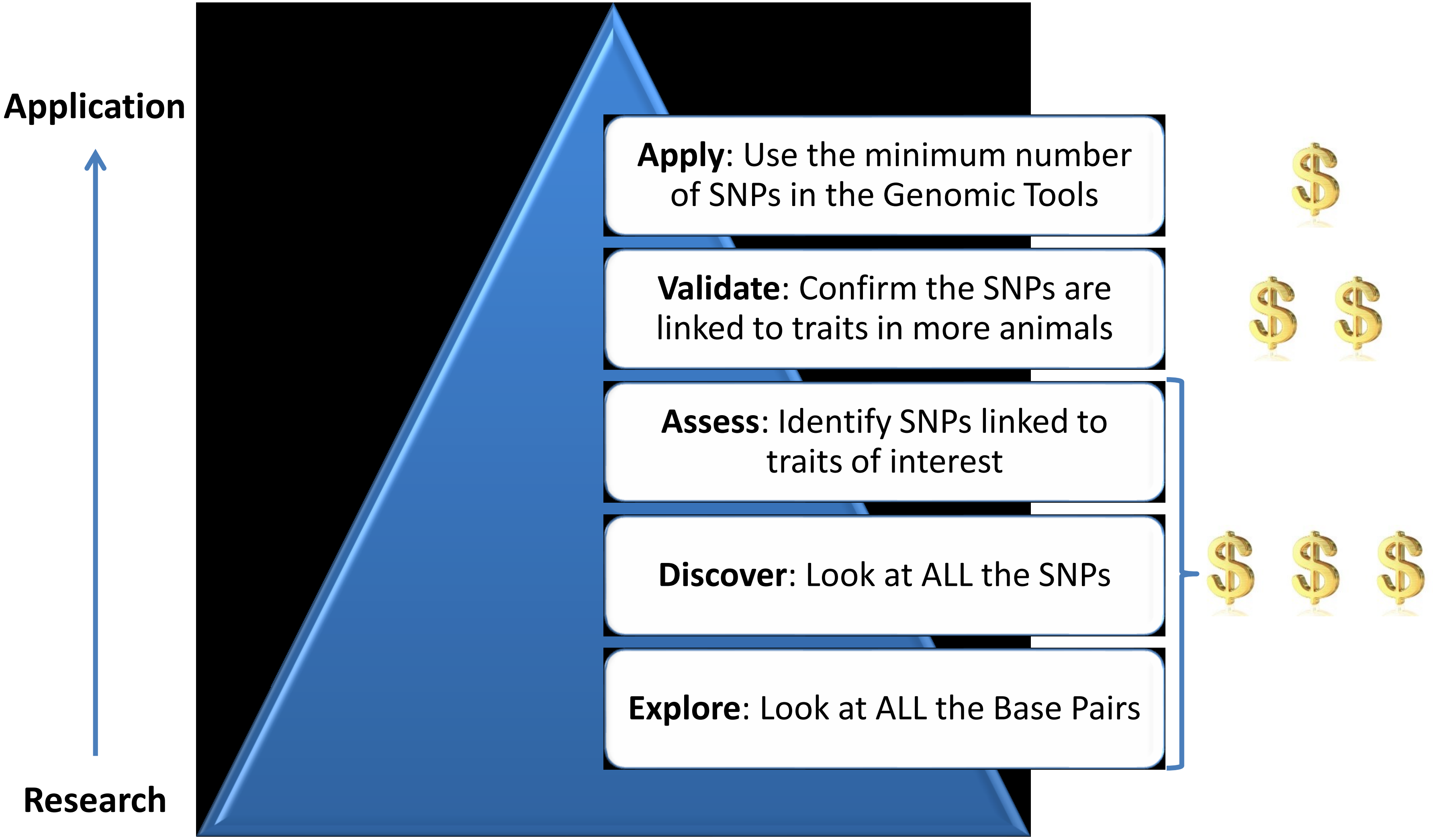


A	T	T	G	C	A	A	G	T	T	A	G	C	C	A	G
A	T	T	T	C	A	A	G	T	T	A	G	C	C	A	G
A	T	T	G	C	A	A	G	T	T	A	G	C	A	A	G

SNPs show us the differences in the DNA

Through research we can link SNPs to traits and features we want

The Process: Research to Application



Application

Research

Apply: Use the minimum number of SNPs in the Genomic Tools

Validate: Confirm the SNPs are linked to traits in more animals

Assess: Identify SNPs linked to traits of interest

Discover: Look at ALL the SNPs

Explore: Look at ALL the Base Pairs

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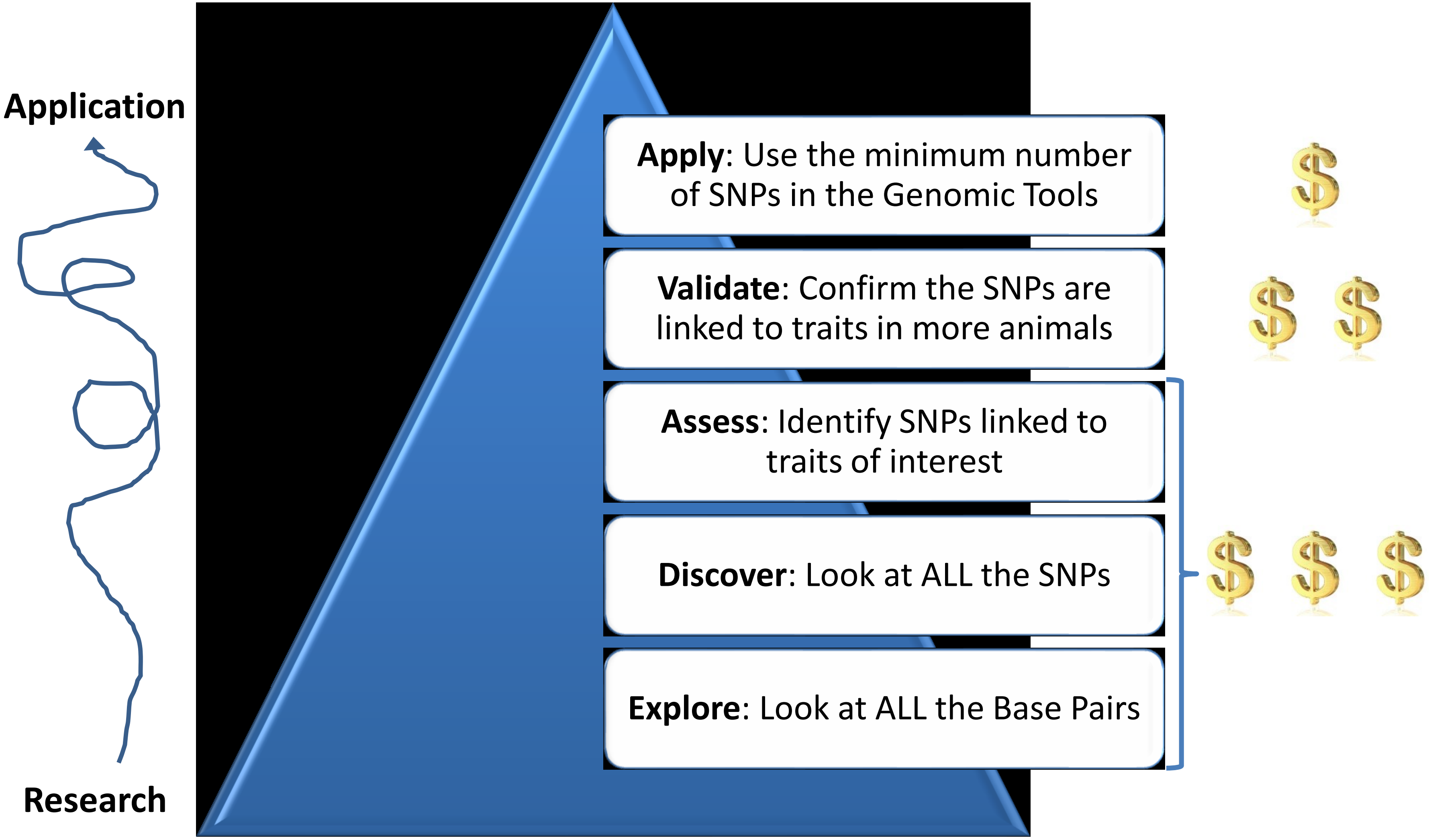

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Genomics Project Objectives

The Genomics Project has 3 Objectives

1. SNP Parentage Test
2. Cattle Introgression Measurements
3. Wood-Plains Subspecies Differentiation

Project Methodology

☑ 1
- Whole Genome Sequencing of 27 bison

☑ 2
- Share Whole Genome Sequences with Dr. Derr's group at Texas A&M

☑ 3
- Discovery of SNPs for the 3 objectives

☑ 4
- Validation genotyping of 480 bison where we already know the answer

5
- Final edits and launch of the Genomic Tools

1. Whole Genome Sequencing ☑

Look at all 3 billion base pairs in 27 bison from the project

Four main groups of bison were selected for sequencing based on Subspecies and Time Period:

Wood Bison Historic (1892-1937) • Smithsonian – 2 • CMN – 4	**Plains Bison Historic (1871-1912)** • CMN - 8
Wood Bison Modern (2010) • Elk Island – 2 + 1	**Plains Bison Modern** *Dr. Derr's Research Group* • Elk Island – 2 • Caprock Canyons – 2 • Wind Cave – 2 • Santa Catalina – 2 • Yellowstone – 2

CGEn
Canada's Genomics Enterprise

1. Whole Genome Sequencing – a tangent

- CanSeq150 is an initiative to sequence 150 of Canada's most iconic species
- Wood Bison male from Elk Island National Park has been included

Why we're sequencing the genomes of Canada's iconic species

By sequencing the genomes of other species, we can better understand our place in natural history.

theconversation.com

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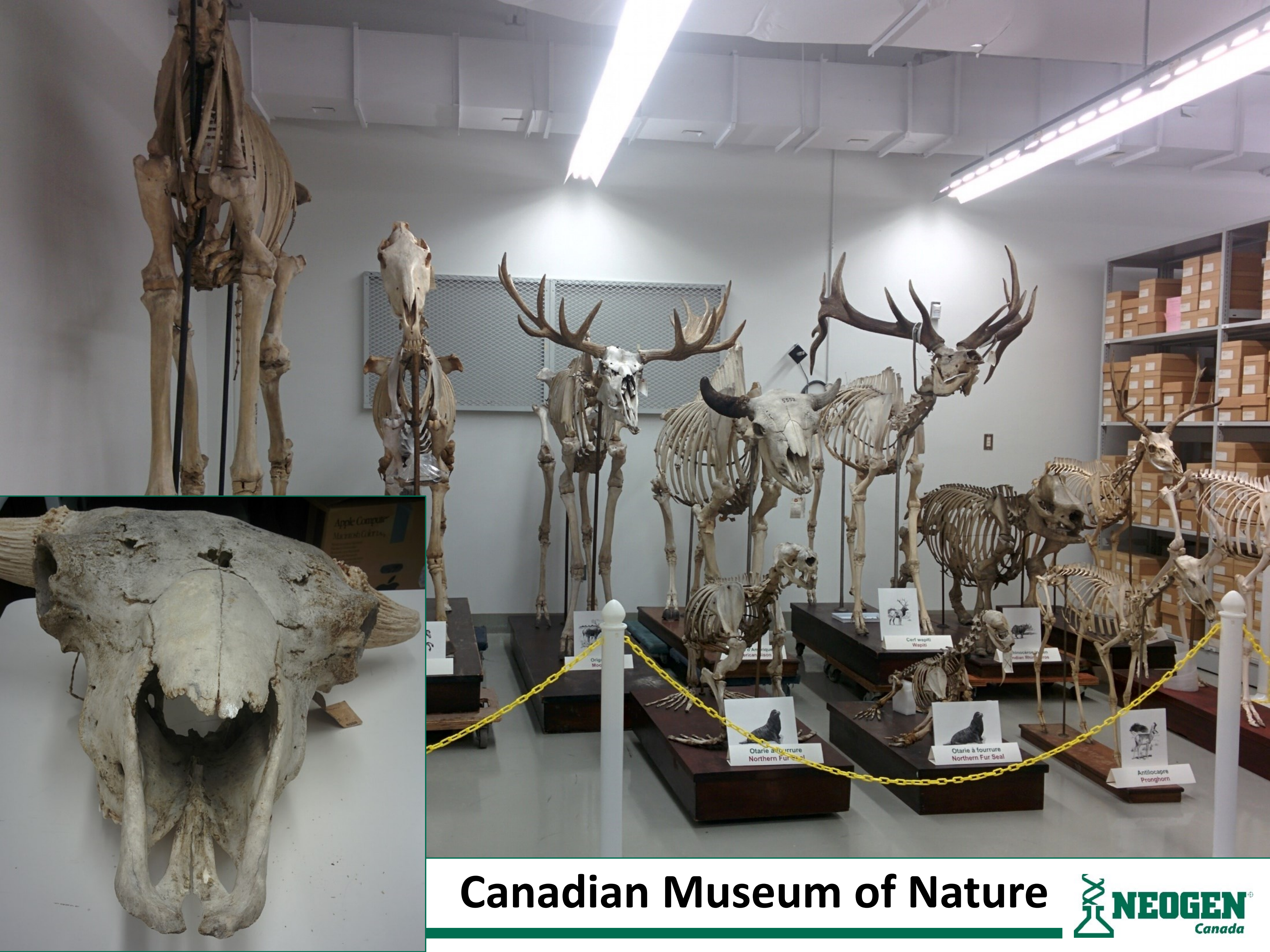
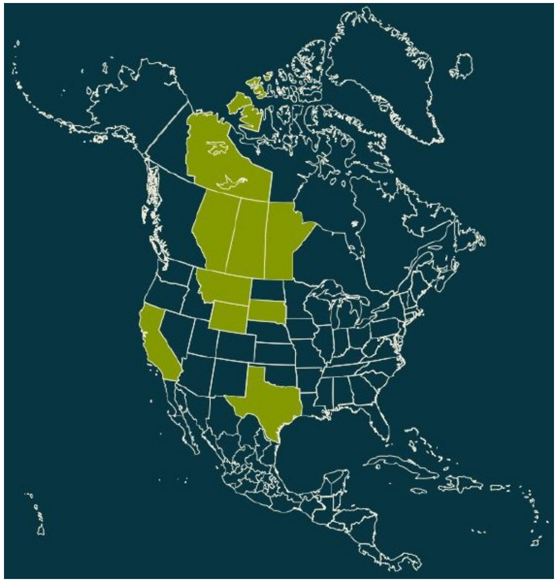


Canadian Museum of Nature

2. Sharing Whole Genome Data ☑



14 Whole Genome Sequences

- Elk Island Wood Bison – 4
- Caprock Canyons Plains Bison – 4
- Yellowstone National Park – 4
- Historic Greater Yellowstone Area – 2

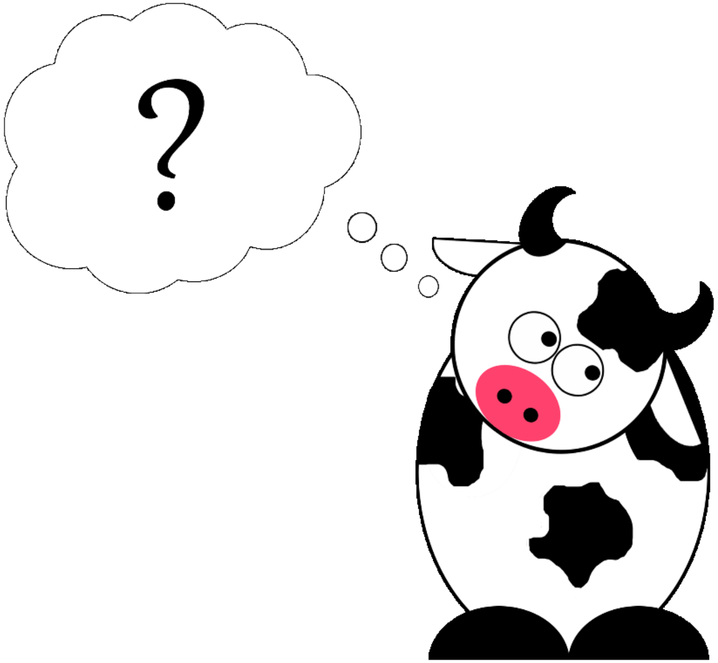
1 Publicly Available Genome

- Templeton from Yellowstone (Reference Genome)

2. More Data Sharing

73 Bovine GGP-50K Genotypes o

- Antelope Island
- Caprock Canyons
- 777 Ranch
- Elk Island Plains
- Elk Island Wood
- Fort Niobrara
- Henry Mountains
- National Bison Ranch
- Turner – Vermejo
- Wichita Mountains
- Yellowstone



2 Wood Bison Whole Genomes from Mackenzie Sanctuary

- In progress

Time to do Something with all this Data

3. SNP Discovery & Selection ☑

- From Whole Genome Sequence data:
  - ~54 million SNPs detected in project bison
  - Beef cattle have ~30 million SNPs
- MAF (Minor Allele Frequency)
  - Every SNP gets an MAF
  - Higher MAF = More variation at that SNP
- SNP Spacing
  - When SNPs are too close together, they get “stuck” together
  - Spacing measures in base pairs (bp)

3. SNP Discovery & Selection ☑

Objective	Number of SNPs	How the SNPs were Selected
Parentage	2,955	• Looked for SNPs with variation within all bison genomes in the project • MAF > 0.45 • Spacing: 1,000,000 bp minimum

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Wood vs Plains	17,026	• Looked for SNPs with very little variation in Plains bison and with variation in Wood bison; MAF > 0.10 • Looked for SNPs with very little variation in Wood bison and with variation in Plains bison; MAF > 0.10

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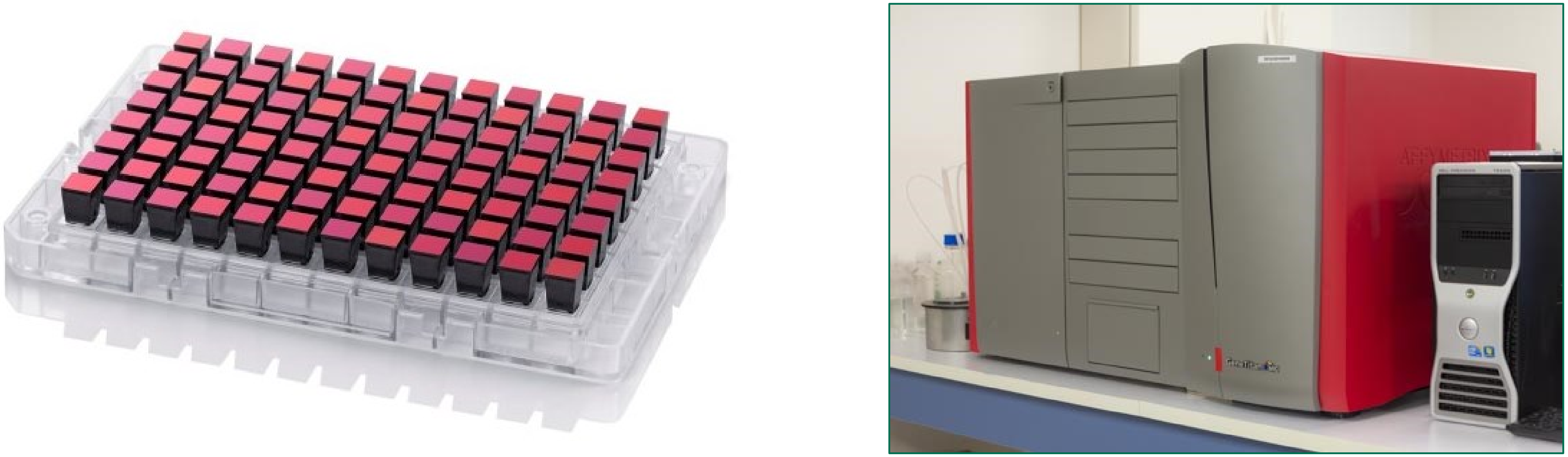
Cattle Controls	314	• Previously vetted SNPs from GGP-50K

3. SNP Discovery & Selection ☑

A Custom Genotyping Assay was designed and built with 83,039 SNPs



4. Validation Genotyping ☑

- Received 793 samples from CBA members, parks, research institutes
  - 17 sources of samples
  - Average number of samples: 27
  - Maximum number of samples: 120
  - Minimum number of samples: 1

- Genotyped 480 animals with the Custom Genotyping Assay of 83,039 SNPs

- 461 animals (96%) passed genotyping
  - Call Rate above 95% (standard threshold)

Validation Genotyping Results Summary

Objective	Number of SNPs BEFORE	Number of SNPs AFTER
Parentage	2,955	~400
Cattle Introgression	62,744	~10,000
Wood vs Plains	17,026	~3,300
Cattle Controls	314	314
Totals	83,039	~13,000

Reducing the Final SNP Number

- Removed SNPs that worked poorly on custom array
- Double checked spacing requirements
- Ran analysis with less SNPs each time until results started to change

Validation Genotyping Parentage Results

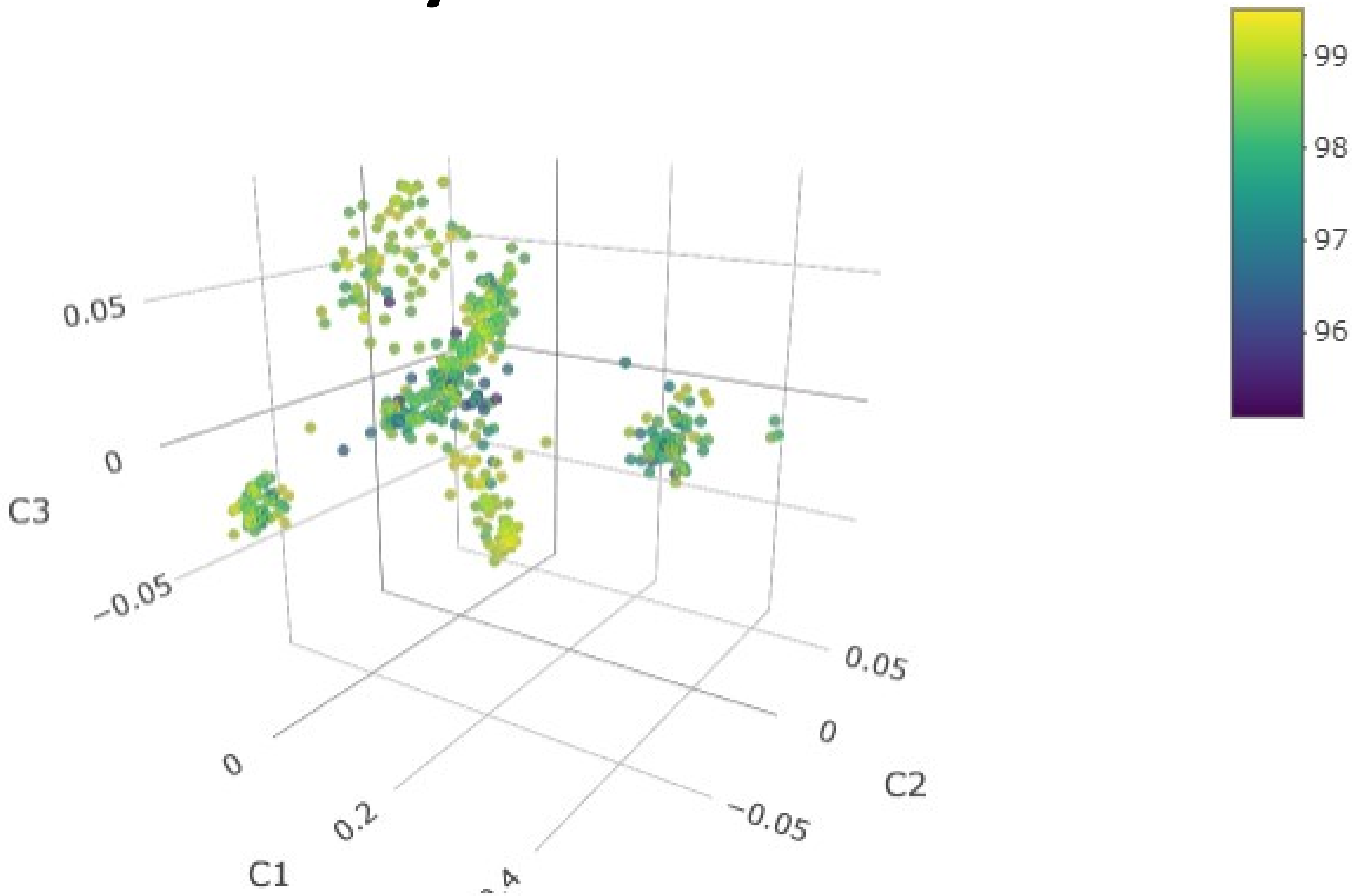
Parentage

- All known parent-offspring pairs verified with the SNP test at 0.00% discordance
- 11 pairs tested previously with microsatellites (STR)

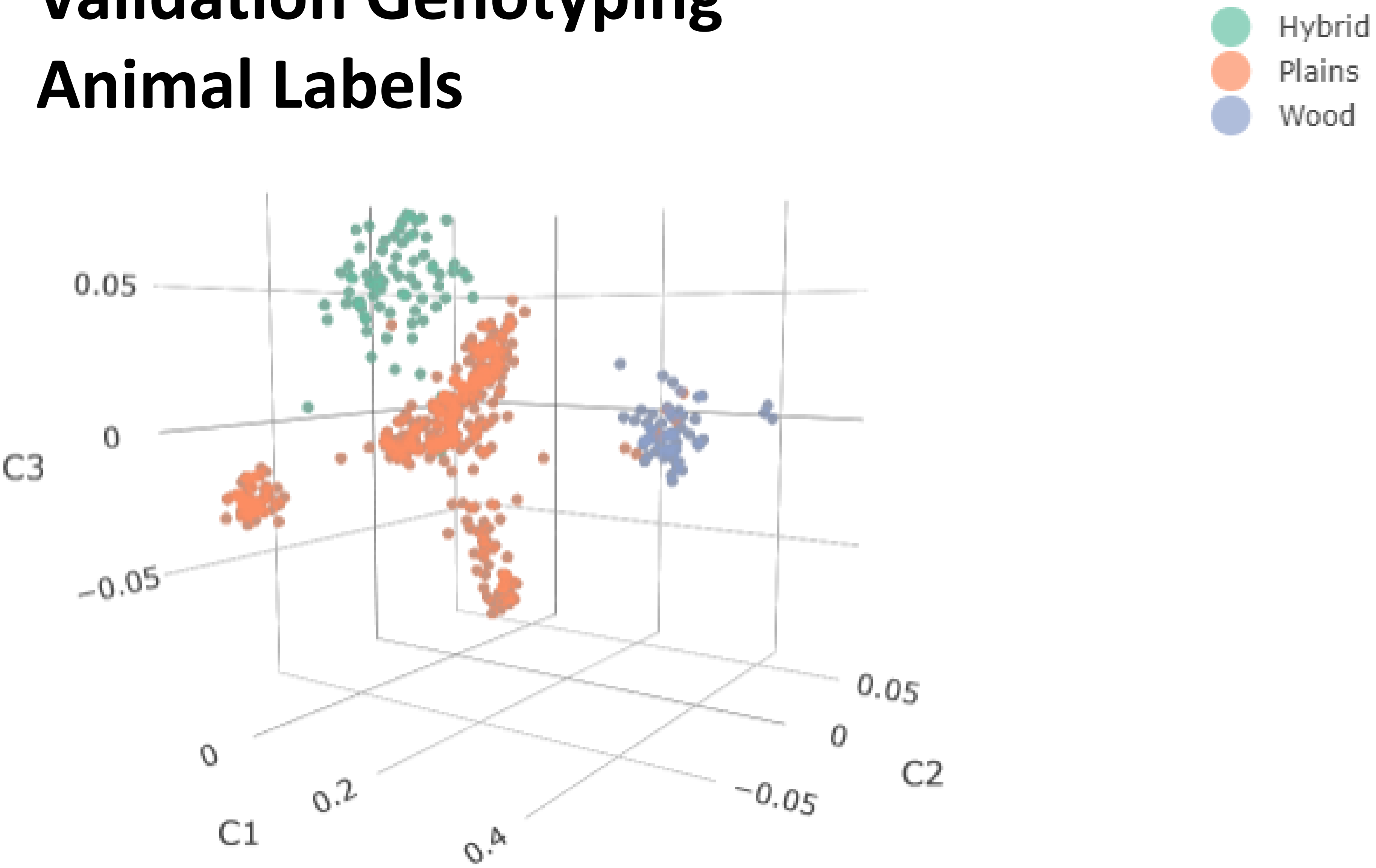
Future Consideration: would be nice to have more known parent-offspring pairs or trios

Photo Source: Pinterest

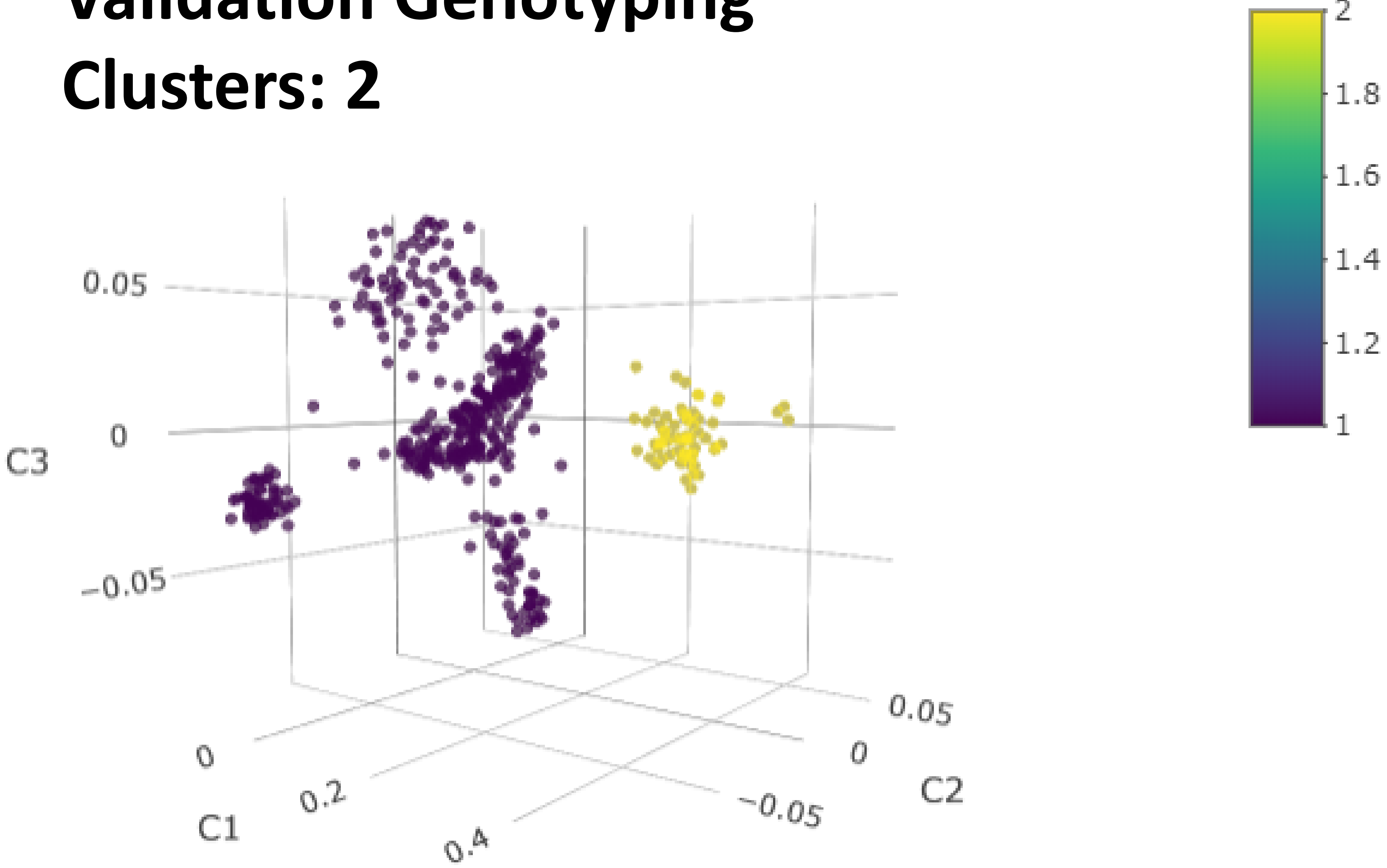
Validation Genotyping Results Summary



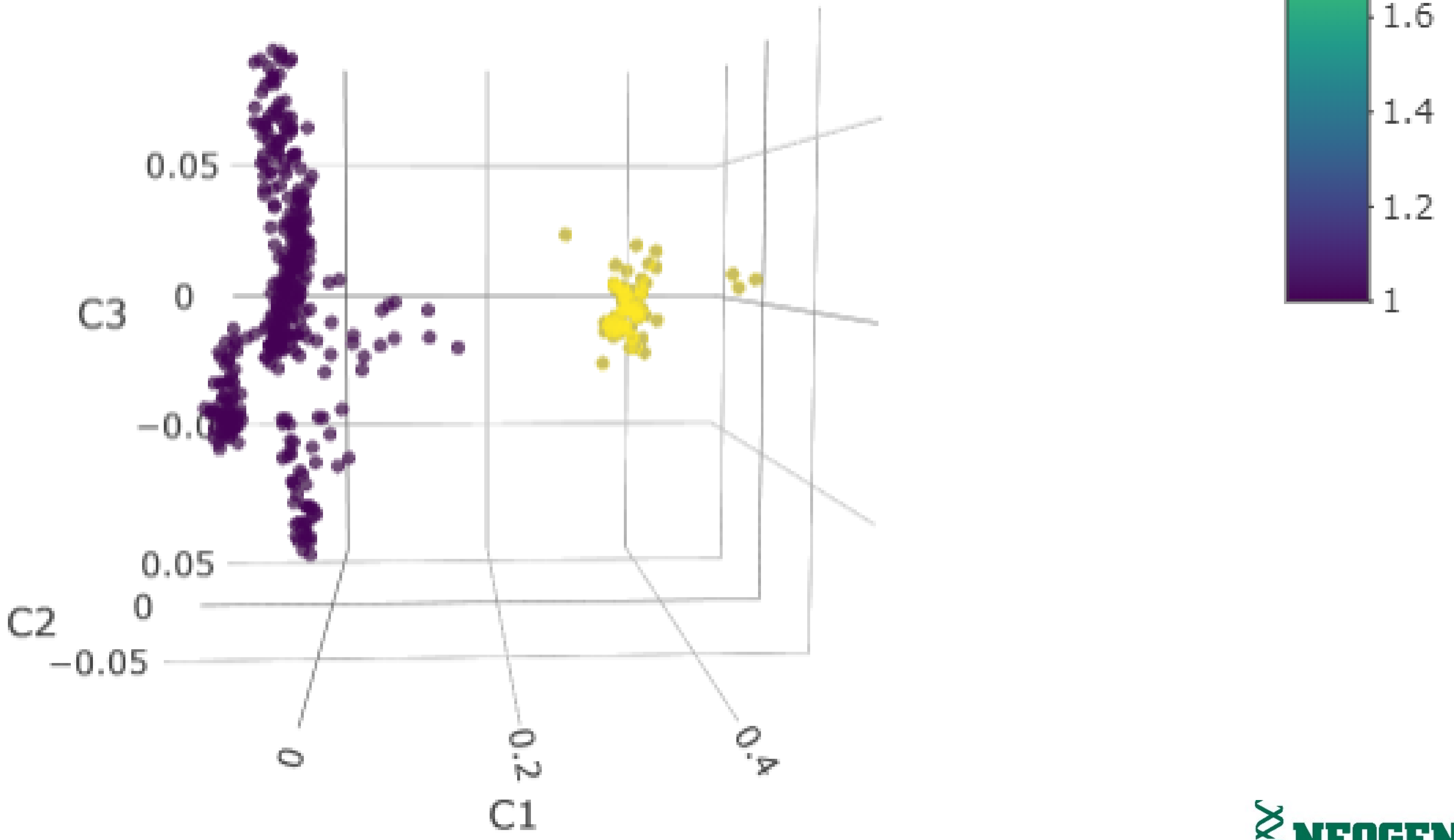
Validation Genotyping Animal Labels



Validation Genotyping
Clusters: 2



Validation Genotyping Clusters: 2 (Different View)



Validation Genotyping Cattle Introgression

- Animals with Cattle Introgression: 325
- Animals without Cattle Introgression: 136

- Maximum Cattle Score: 4.5%
- Minimum Cattle Score: 0.0%

Photo Source: npr.org

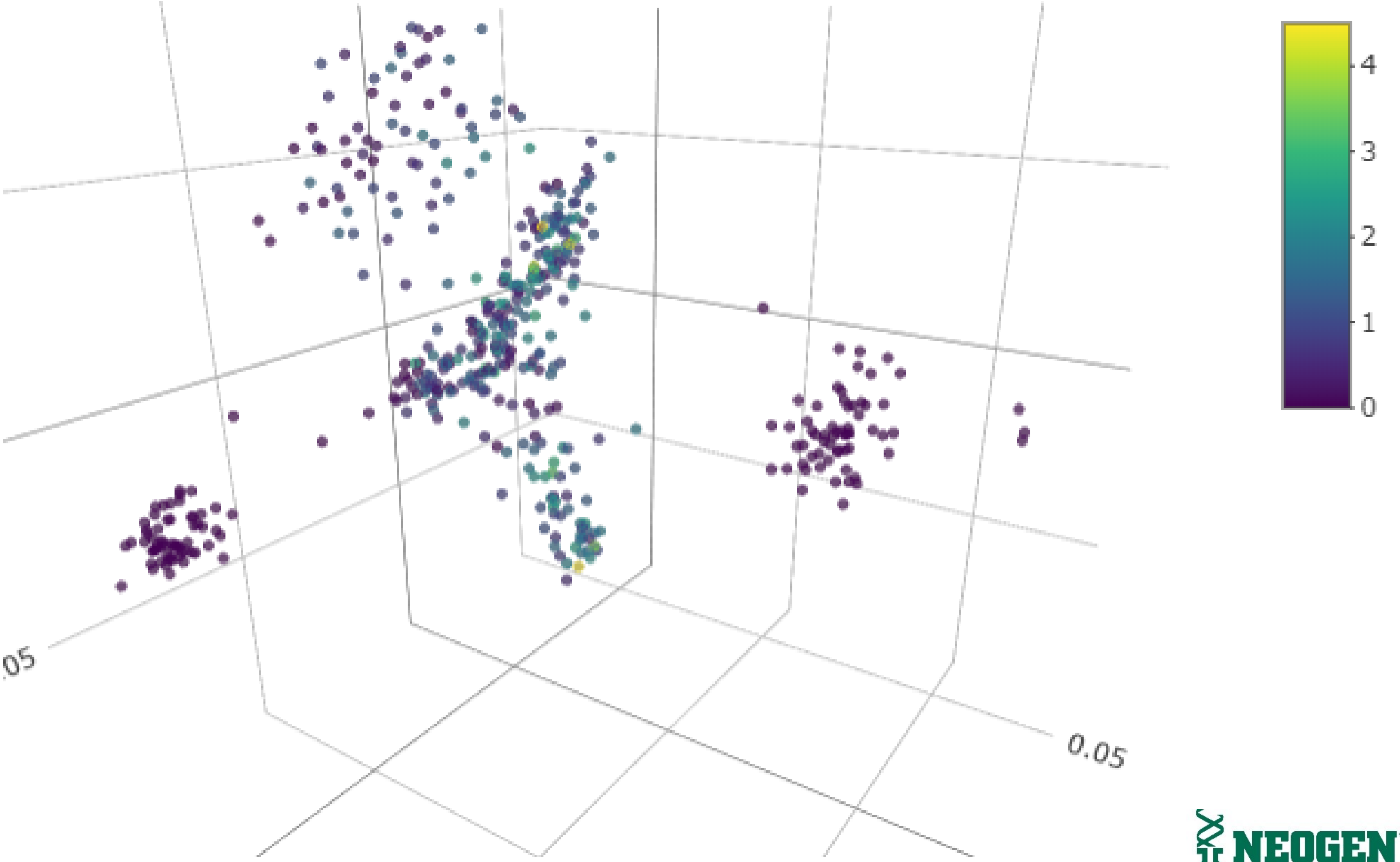
Validation Genotyping
Cattle Introgression

- Animals with Cattle Introgression: 325
- Animals without Cattle Introgression: 136

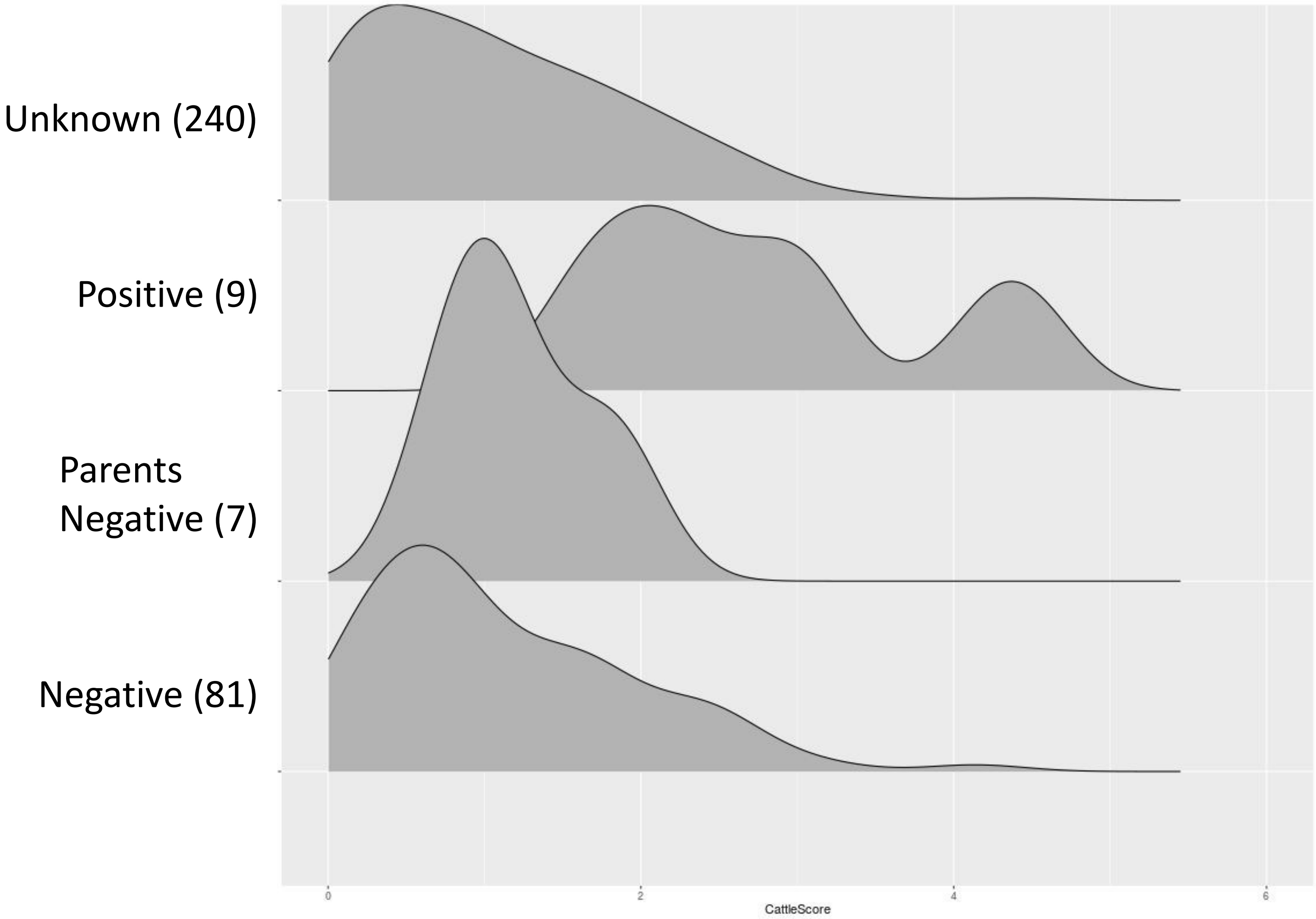
- Maximum Cattle Score: 4.5%
- Minimum Cattle Score: 0.0%

- This project provides a Cattle Score
- This project does not tell us the significance of that Cattle Score (what does it mean?)

Validation Genotyping Cattle Introgression



Validation Genotyping
Cattle Introgression



Validation Genotyping Wood vs Plains: Round 1

- Number of 100% Plains: 40
- Number of 100% Wood: 25

- Number of >50% Plains: 398
- Number of >90% Plains: 271
- Number of >95% Plains: 80

- Number of >50% Wood: 63
- Number of >90% Wood: 61
- Number of >95% Wood: 55

Problem with Round 1

- Plains bison with American genetics were showing up with 5-20% Wood bison genetics
- Did not make sense with known historical movements of animals and commercial availability of Wood bison

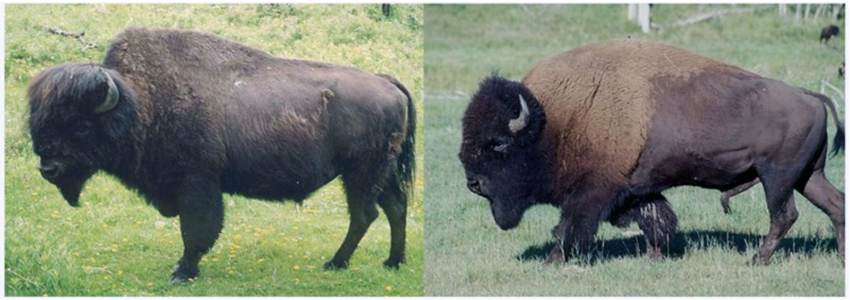
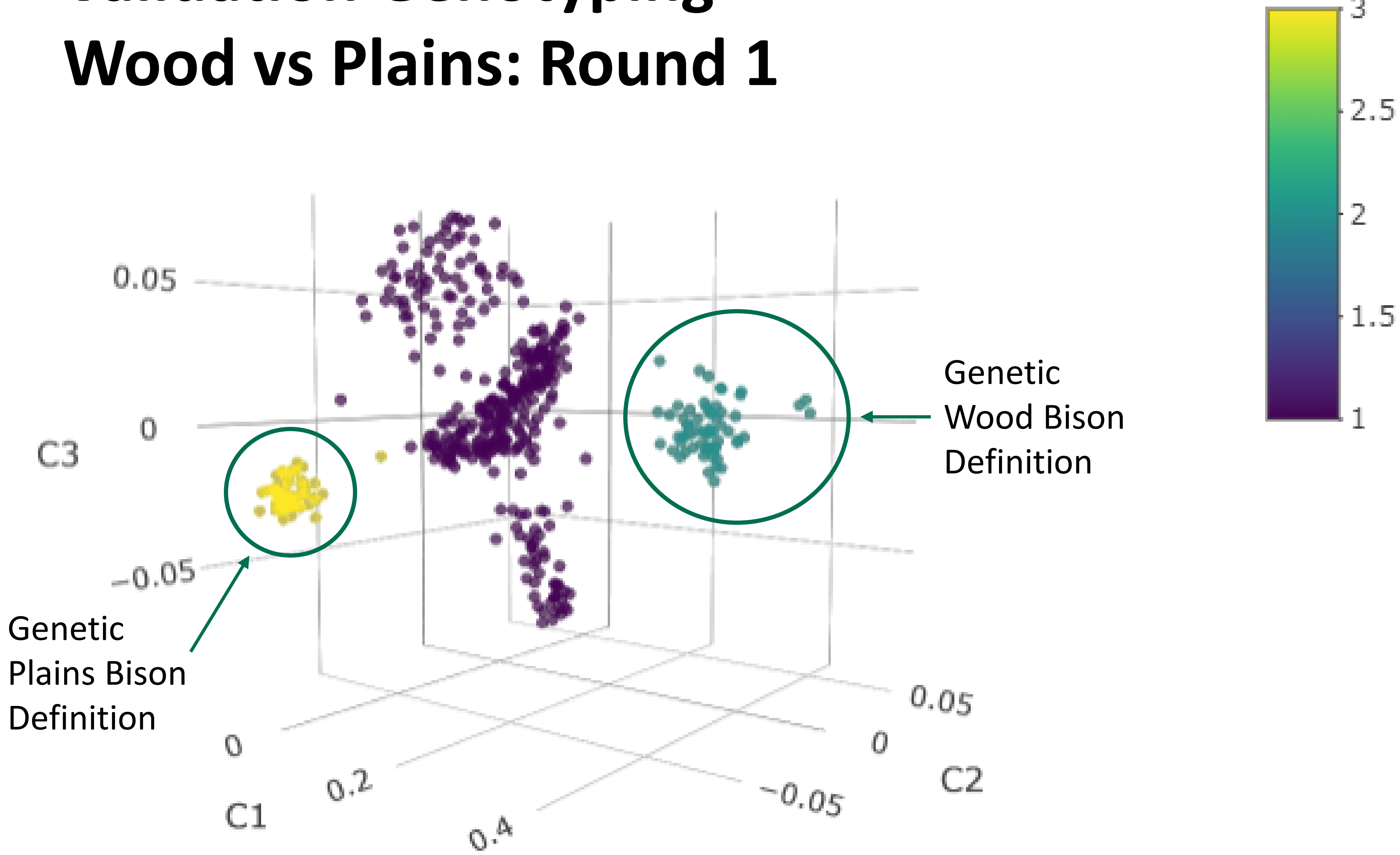


Photo Source: canada.ca

Validation Genotyping
Wood vs Plains: Round 1



Genetic Wood Bison Definition

Genetic Plains Bison Definition

Validation Genotyping Wood vs Plains: Round 2

- Number of 100% Plains: 162
- Number of 100% Wood: 25

- Number of >50% Plains: 399
- Number of >90% Plains: 374
- Number of >95% Plains: 363

- Number of >50% Wood: 62
- Number of >90% Wood: 61
- Number of >95% Wood: 53

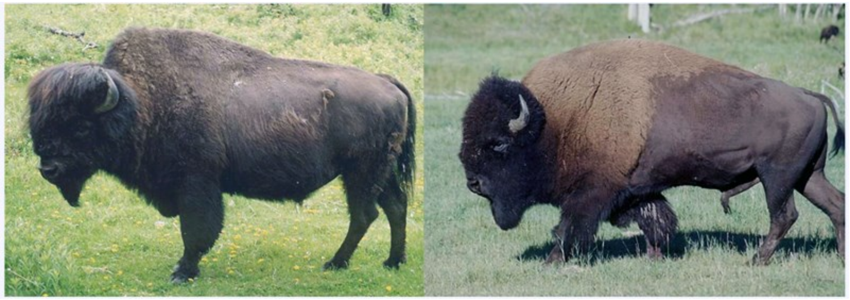
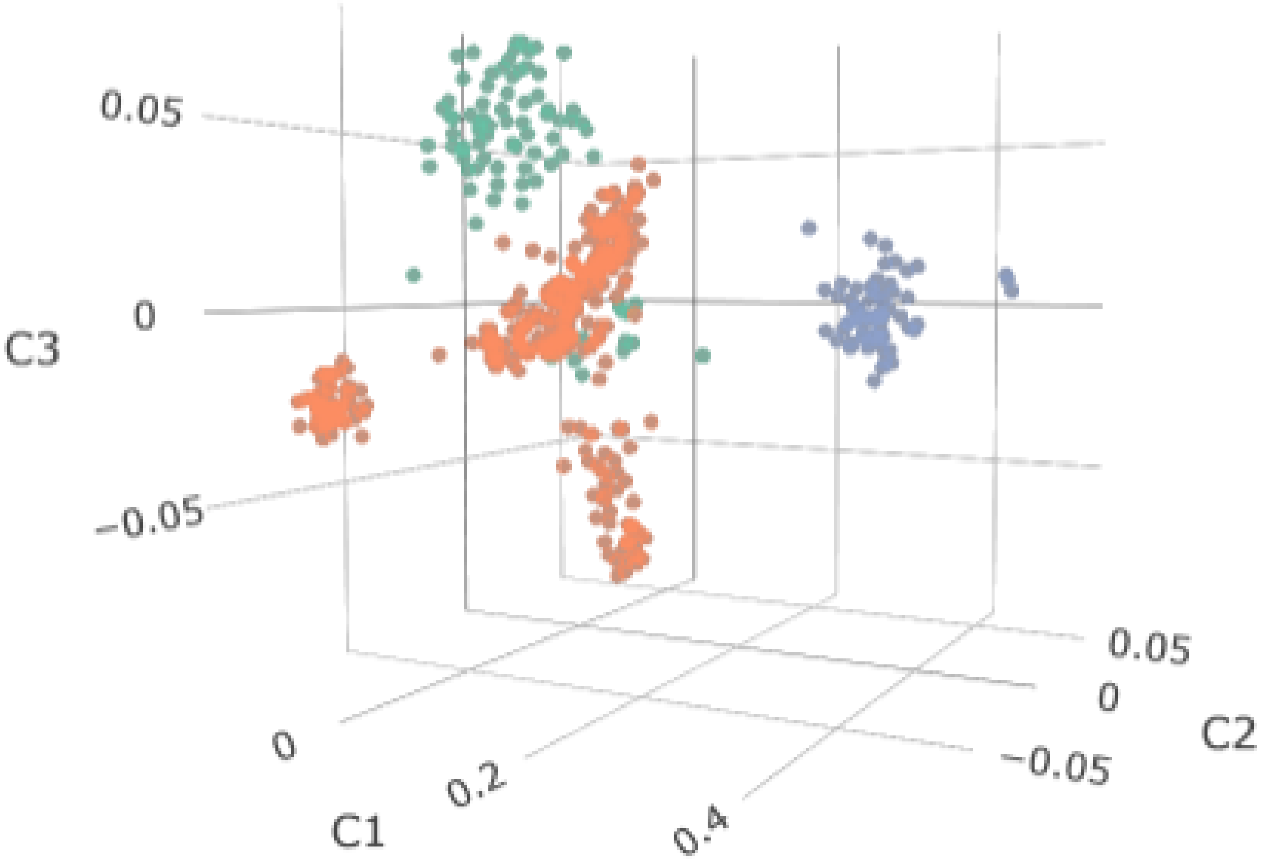


Photo Source: canada.ca

Validation Genotyping
Wood vs Plains: Round 2



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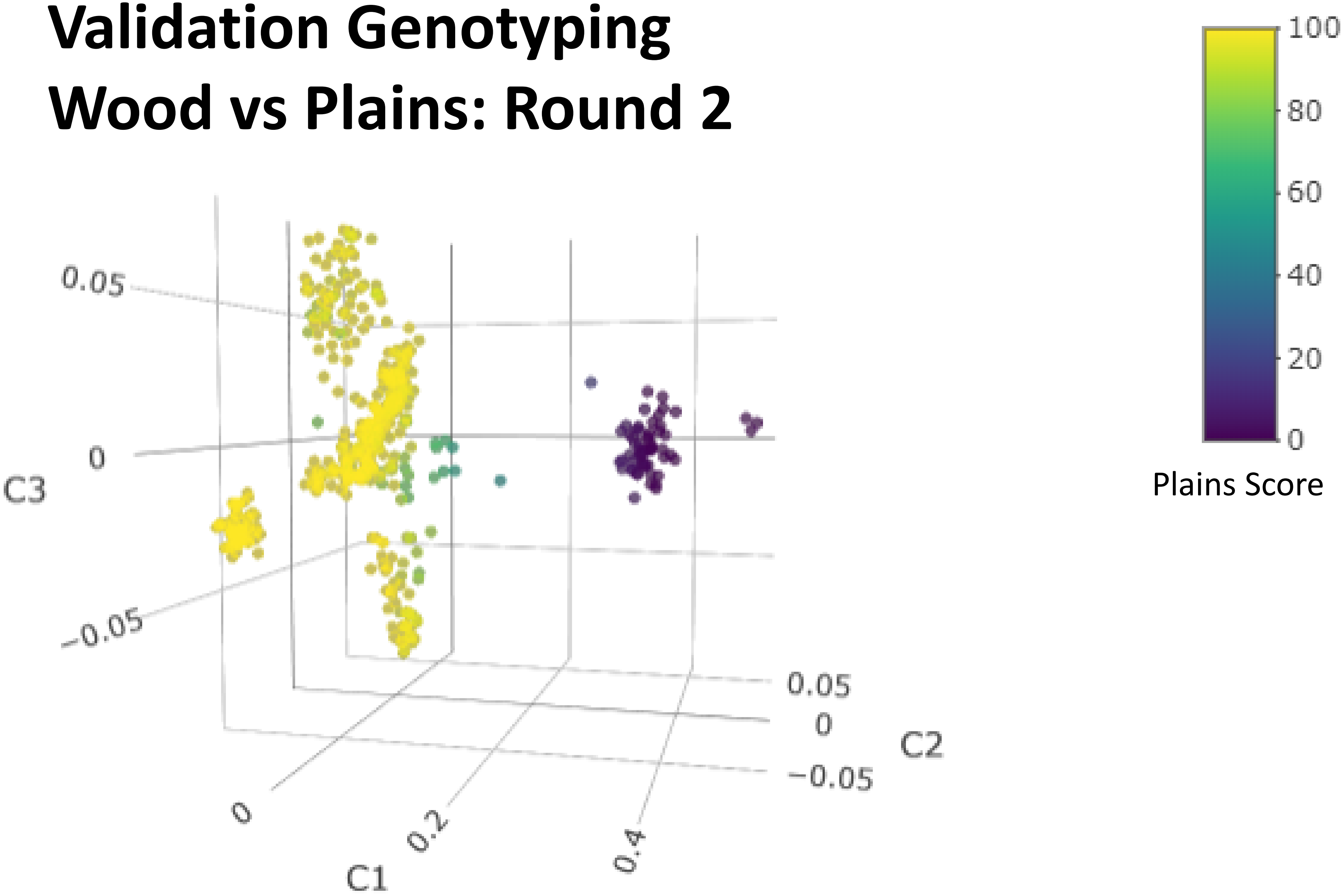

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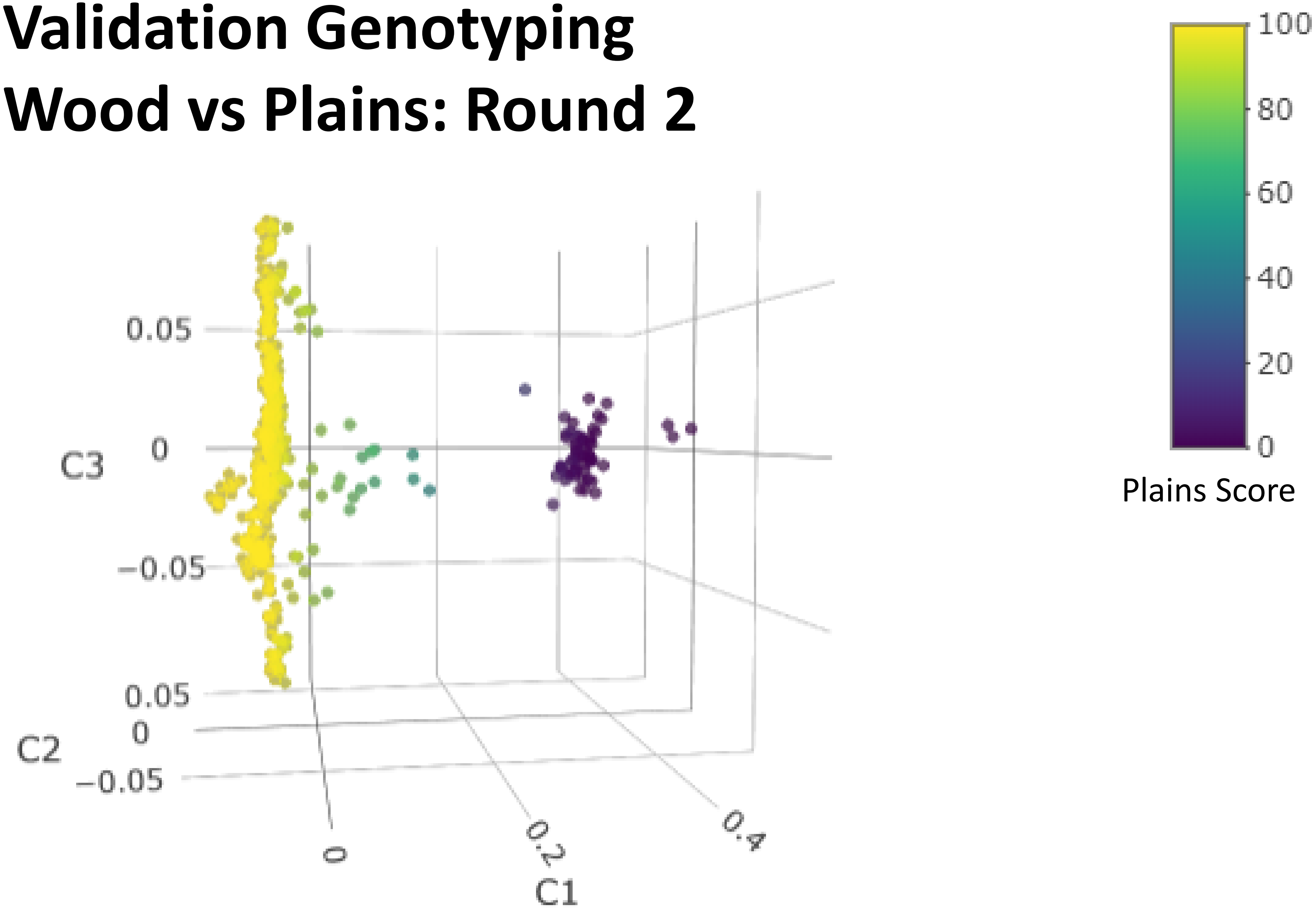
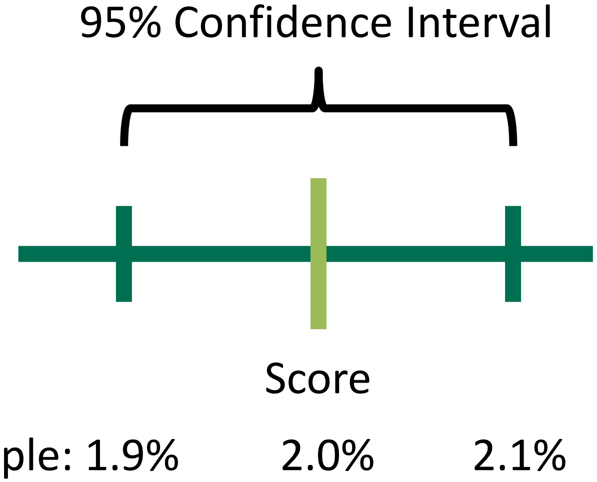


Validation Genotyping Considerations: Statistical Error

Math isn’t perfect, but it can tell us how far off we are

95% Confidence Intervals

	Average	Minimum	Maximum
Cattle Introgression	0.21%	0.00%	0.74%
Wood vs Plains	2.57%	0.00%	5.55%


95% Confidence Interval

Score

Example: 1.9% 2.0% 2.1%

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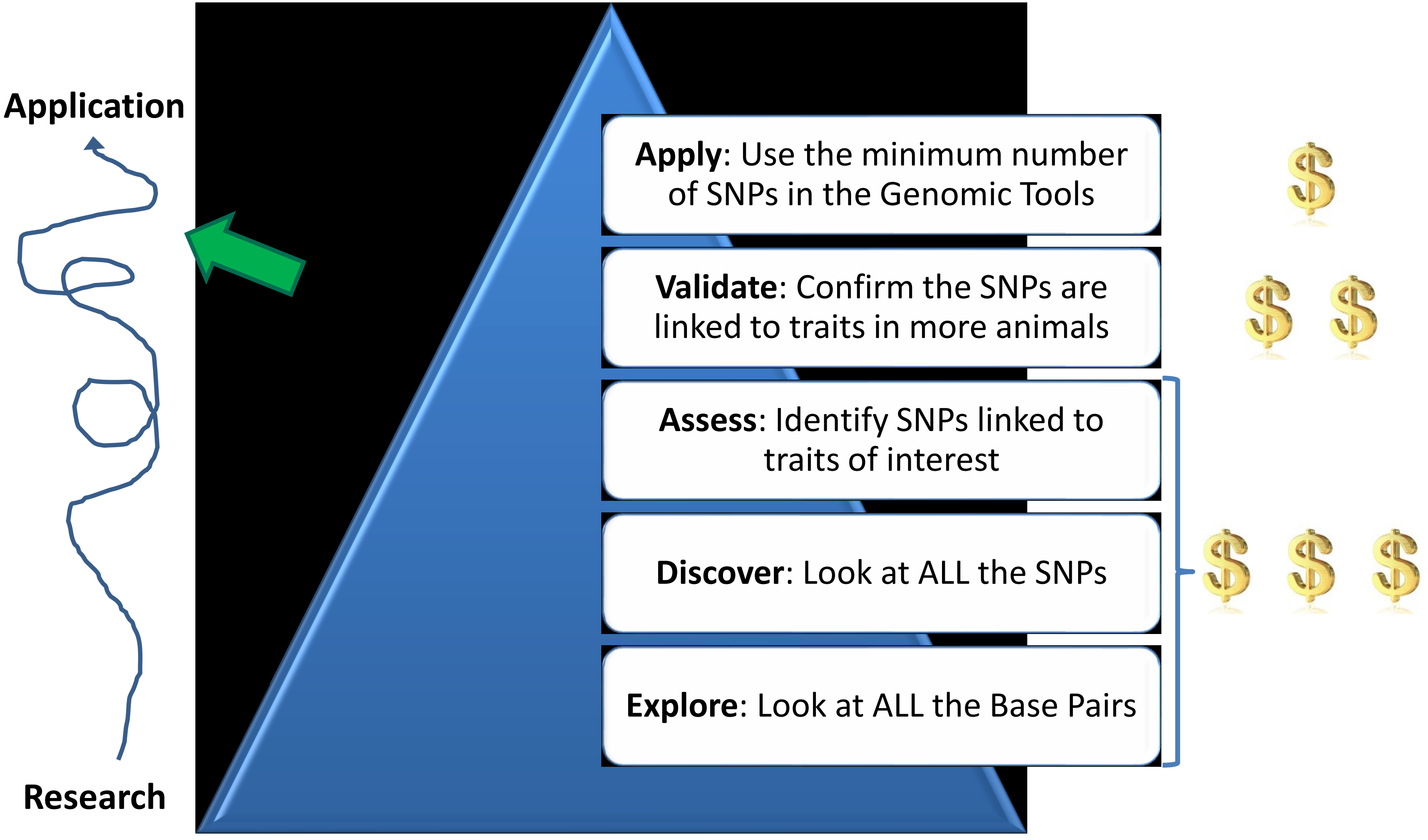
Photo Source: NASA

The Process: Research to Application



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Apply: Use the minimum number of SNPs in the Genomic Tools

Validate: Confirm the SNPs are linked to traits in more animals

Assess: Identify SNPs linked to traits of interest

Discover: Look at ALL the SNPs

Explore: Look at ALL the Base Pairs

Photo Source: imgflip.com

Genomics Project: Next Steps

1. Start developing a product for SNP Parentage

2. Further validation of the Cattle and Plains Scores
    - Verification of subspecies and cattle introgression labels from validation genotyping
    - Consideration of another project to increase sample numbers

What Can You Do?

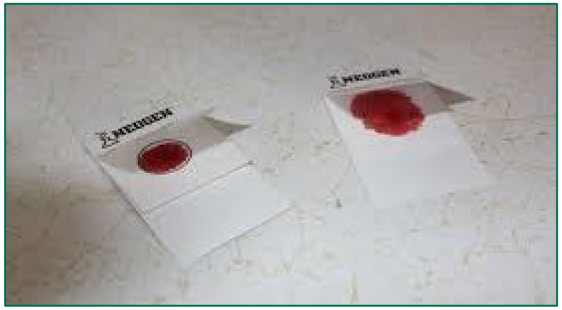
Collect samples from your animals

Sample Collector Options:

($4 loose hair charge will be implemented in Canada June 1st, 2020)

Hair Cards

Blood Cards



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ORDERS :
NeogenCanada@neogen.com
780/492-2538

Overall Summary

- We are a lot further along than we were 2 years ago in terms of using genomic tools
- Moving forward through the research process with care and leveraging what we have learned

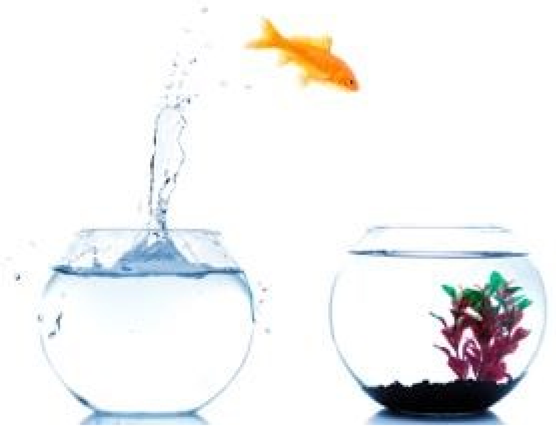

Photo Source: oppb.com.au

THANK-YOU!