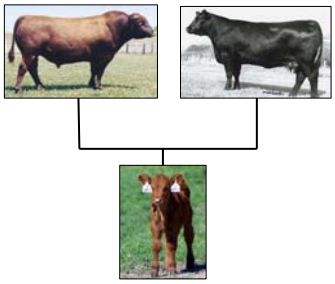
Parentage-based DNA traceback in beef and dairy cattle



Mike Heaton, Ph.D.

USDA, ARS, U.S. Meat Animal Research Center, Clay Center, Nebraska

Updated 7-15-2008

Why is animal ID needed?

From a production perspective:

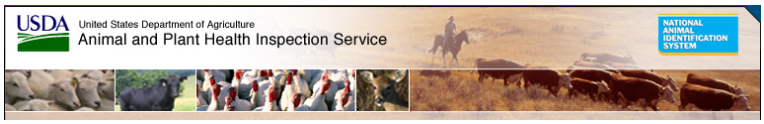
- **Track carcasses at slaughter for beef improvement programs**
- **Verify source and age for branded beef programs**

- **Identify superior sires in multi-sire mating systems**
- **Identify mis-mothering cases**

- **Verify artificial insemination records**
- **Solve rustling cases**
- **Verify claims of tissue-residue violations at slaughter**

Why is animal ID needed?

From an animal health perspective:



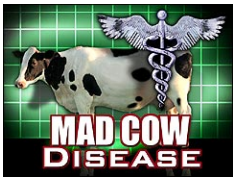
"…to help protect American animal agriculture from foreign or domestic disease threats." APHIS

Goal: to traceback within 48 hours of discovery.

Desired outcome: reduced financial and social impact of disease outbreaks

Examples:

- **brucellosis and tuberculosis eradication programs**
- **foreign animal disease - BSE traceback**



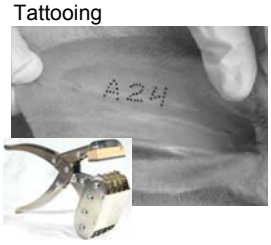
Our goal:

to support DNA-based disease traceback in cattle.

Ways to physically label individual cattle

Branding

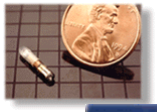
Tattooing



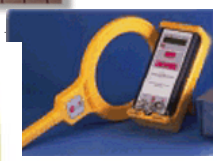
Tags and passports

Ways to physically label individual cattle

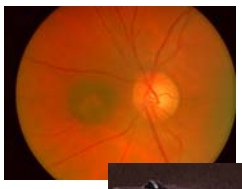
Radio frequency tags/implants



(RFID)



Retinal scans



Problems:

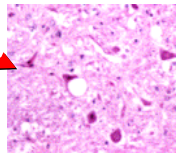
- **Physical labels are moved or removed at some point during beef processing**
- **The fidelity of labeling may be disputed**

Disputes arise even when animal identification systems are in place

Example: 2003 Washington State BSE case

Holstein downer cow processed at Moses Lake, WA on December 9th

BSE



The dispute

“There's some confusion about the paperwork….”

Which of the 9 downer cattle slaughtered that day had the BSE-infected brain?

“DNA testing by the best experts available could compare samples from the mad cow and its offspring or parents.”

Dr. Brian Evans
Chief Veterinary Officer
Canadian Food Inspection Agency

The resolution

USDA’s Chief Veterinarian
Dr. Ron DeHaven of APHIS

December 31, 2003

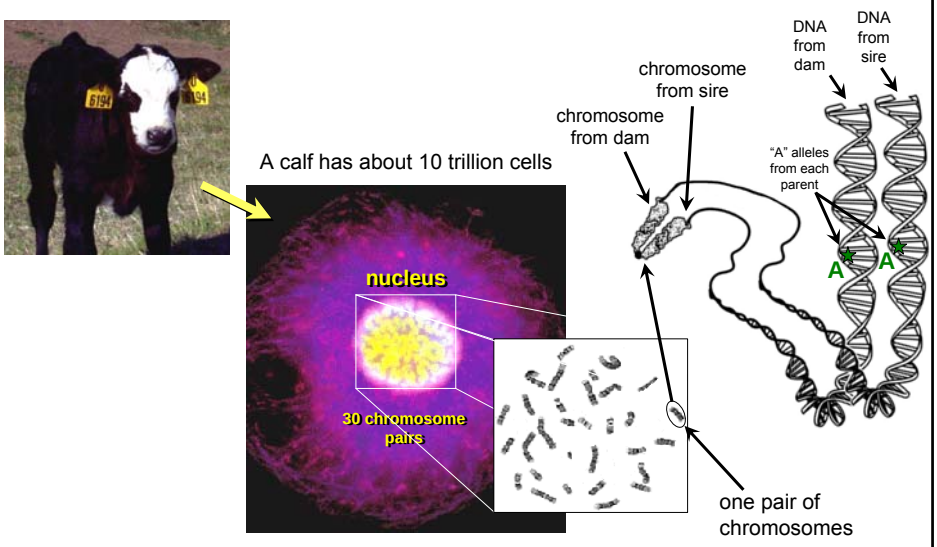
“…we are sending multiple samples to two laboratories -- one in Canada and one in the United States.”

“… the U.S. laboratory is in Nebraska, …. [and] It's a USDA laboratory that has that expertise.“

January 6, 2004

"We now have DNA evidence that allows us to verify with a high degree of certainty, the [Canadian] birthplace of the BSE-infected cow.”

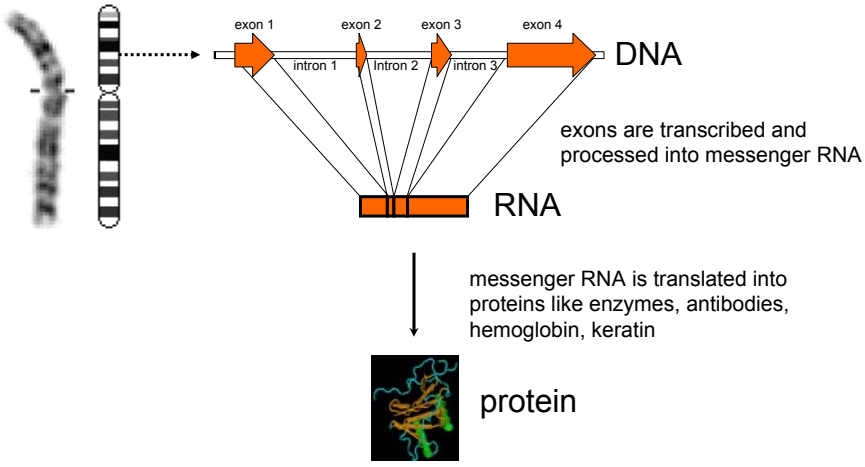
DNA is the label never removed from beef



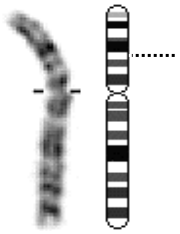
A chromosome has genes and other DNA

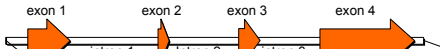
- A chromosome may contain more than 1000 genes.
- Genes comprise about 1 to 3 % of the genome.

physical map of a gene



Genes are encoded by sequences of DNA





nucleotides: A, C, G, T

```
   1 tcgataaaac cctccaatgg aaaagacatt gggaggtttc ggtaaaagcc tactgcattt
  61 gcaagtaact gggagaggct gtttactgtg cagcctccat tcacacggca ggaatacctg
 121 aatagctgga ccacaggtga gcaaactcac cttctccact tgtccccctg gaaagaagaa
 181 atcaaaagga aatctctctc tccagtgttc aaaatcgacc gccaatggct atctgtagca
 241 tttctctgtt ctagaacaca actgagaagg aaaccttctg cagggttgga attgagctta
 301 ggcgtatcta caggagccac acgtgcttga acaaattcct gggcggactt gacaggaatc
 361 tcaacagctt ggcaagcaag gtaagctgtg ctatggcaac agcctgcctt ccaatggatg
 421 gcagtactac ttcttaaaat attcctcctt atgactagca gtacactttt aatcagcttg
 481 ttcattcatt tgacaaattc tatttgttga gactctgtgc gtgttgctta taggtactgt
 541 tctaggcact ggggatacat ttcctgctct cacagaatgt tgtaatgggt cagtgagaaa
 601 agcaaacaag catacagcat gatgtctagt agtgataagt gctatgaaga aaaataaagg
 661 ggtaaagagc ataggggtca agagggagag ggctgatata gtagctggcg acatcatcta
 721 agcagctatt tgaattaagc aagagagttg gccacatcac taacttggaa ggaacattcc
 781 aggcagaggg aacagctgta caaaggccct aaggtagaaa taacttcatg tgttcaaaga
 841 tcaacaggag gcccagtgtg gctggatctg agtaagtgag gggcttttag gaggagacca
 901 cacagggcct atggattctg ccccacgcat ggtgggaaac ttccacaggg ttctgagcag
 961 ggcagagact accacctgat ttatgtgatt ttaaatgatc attctggctt ctgtgtggta
1021 aacagactga gtaggggtaa gcacagaagt aagatggctt gttgggaagc taccacagta
1081 ctccagctgc agatgatggt gggtgttgca gagtaagagt ggcagacatt gttggatttt
1141 ggctatgatc aaaaatcga
```

At what level does *most* of the genetic diversity occur in cattle?

Most of the genetic diversity in cattle occurs at the nucleotide level

DNA sequence of maternal chromosome: 5′-A-T-C-G-A-T-T-3′

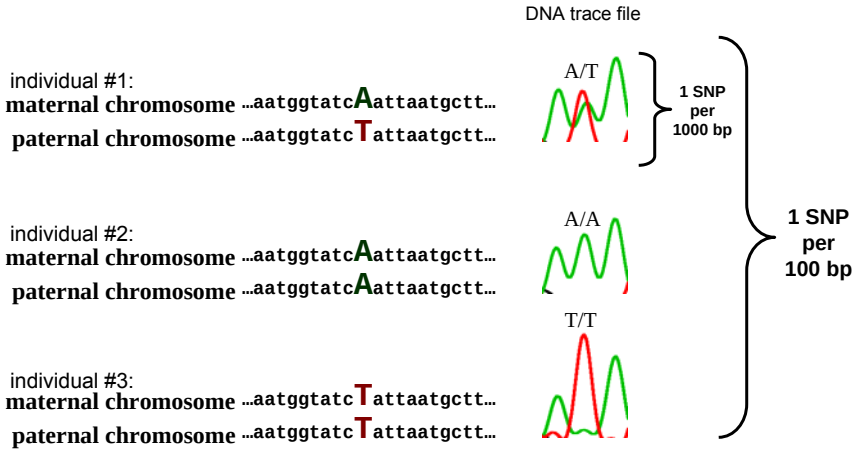
DNA sequence of paternal chromosome: 5′-A-T-C-T-A-T-T-3′

SNP

- Single nucleotide polymorphisms (SNPs) occur, on average, once in every 100 nucleotides in a diverse group of 96 U.S. cattle
  - Heaton et al., Mamm. Genome, 2002.

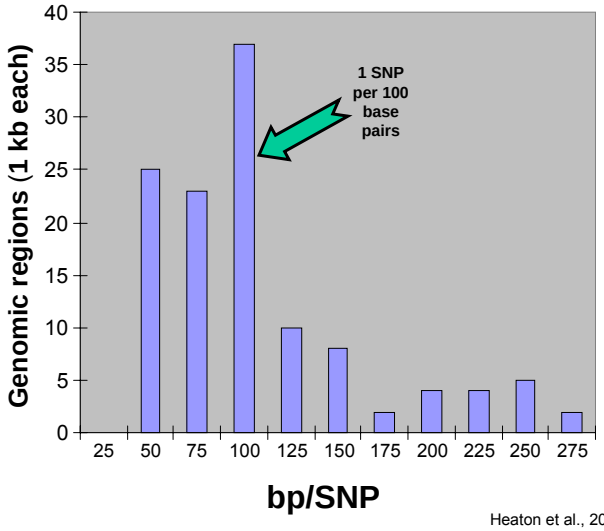
What are SNPs?

SNPs are sites in the genome where two different nucleotides may be observed



SNP density distribution per 1 kb genomic region

(122 parentage markers tested, 216 beef and dairy cattle sequenced)



Heaton et al., 2006, unpublished results

Properties of SNPs

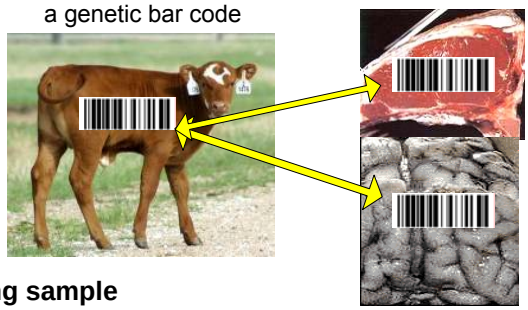
- **Abundant (approximately 30 million sites in cattle)**
- **Stable (low mutation reversal rate)**
- **Amenable to high-throughput automatic scoring**
- **Low cost per SNP genotype**
- **Many genotyping platforms available**
- **SNP scoring is comparable between labs & platforms**

Bottom line: SNP markers are the new "gold standard" for genetic analysis

Ways to use DNA for traceback

- **DNA fingerprinting (sample matching)**
  - **comparing genotypes between samples**
  - **resolves disputes if samples were collected at the point of origin *before* a disease outbreak occurred.**

a genetic bar code



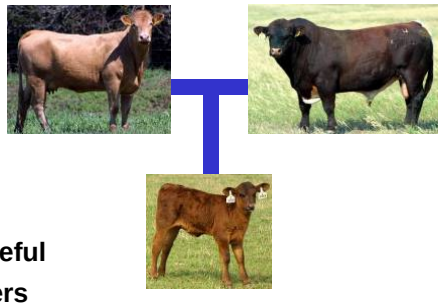
- **Advantages:**
  - **high degree of power**
  - **all genotypes useful**
- **Disadvantages:**
  - **requires a preexisting sample**
- ***Example:*** **20 SNPs were sufficient for verifying sample tracking in dairy beef processing** (Heaton et al. J Am Vet Med Assoc. 2005).

Ways to use DNA for traceback

- **Parentage analysis**
  - determining whether alleles are shared between parents and offspring
  - may confirm the origin of a diseased animal if tissues from a parent are available.

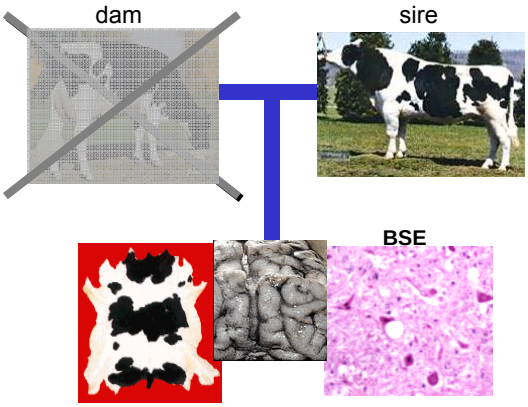
- **Advantages:**
  - preexisting sample of "case" not needed

- **Disadvantages:**
  - not all genotypes useful
  - requires more markers
  - requires more samples



Sometimes parentage testing is the last resort for DNA-based traceback

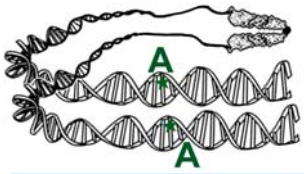
- **Worst case scenario: only one parent available**
  - 2003 Washington State BSE case



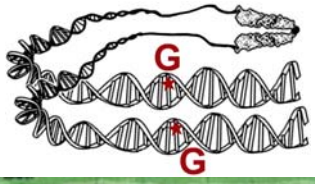
offspring - BSE case

Using SNP markers for parentage

The offspring must share an allele with *each* parent



dam



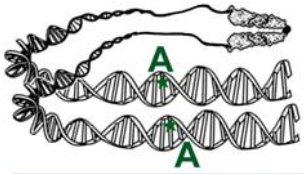
bull

calf A,A

excluded sire G,G

Using SNP markers for parentage

The offspring must share an allele with *each* parent



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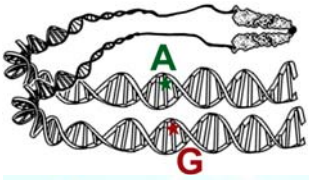


bull

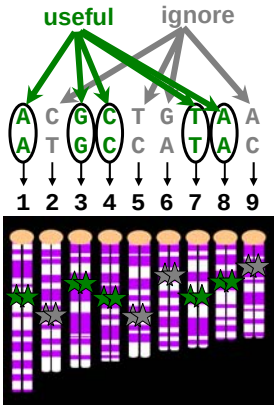
calf A,A

possible sire A,G

Accurate sire determination requires many DNA markers
(example: 9 markers on 9 chromosome pairs)

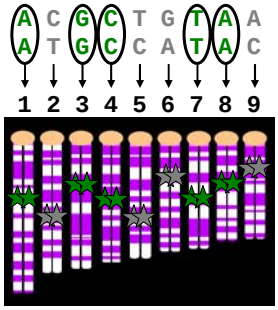
When comparing only sires and offspring (i.e. dams not used)…..

- the calf's homozygous DNA markers are the most useful
- the calf's heterozygous markers are ignored because every sire will share an allele with the calf for those markers



Comparing the calf to the sire: elimination

Calf 1

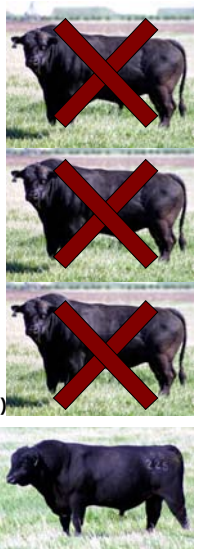


Bull 1 G G * * * * * * * * * *
1
(eliminated)

Bull 2 G T G T * * * * *
A T G T * * * * *
1 2 3 4
(eliminated)

Bull 3 G T G C C A C * *
A T G T C A C * *
1 2 3 4 5 6 7
(eliminated)

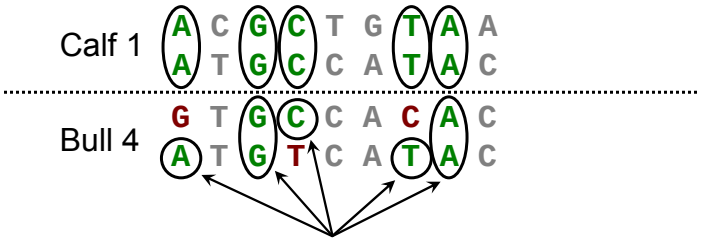
Bull 4 G T G C C A C A C
A T G T C A T A C
1 2 3 4 5 6 7 8 9
(not eliminated)



Comparing the calf to a possible sire

Calf 1

Bull 4



Alleles shared between animals at the homozygous sites of the calf

(note: the calf's heterozygous sites [grey text] are ignored because every bull shares an allele with the this calf at those sites)

The ideal parentage SNP markers

- **have both alleles in equal proportions in all breeds**
  - **balanced alleles provide more power**
- **are evenly spaced throughout the genome**
  - **more independent inheritance provides more power**
- **can be accurately scored**
  - **one wrong genotype can confound a traceback dispute**
- **are publicly available**
  - **encourages standardization and fair competition**

The ideal marker is frequent in all breeds

A collaborative effort was undertaken to assemble many beef and dairy breeds for testing (screening) allele frequency

96 diverse sires from 19 beef breeds (Drs. Heaton and Laegreid; ARS, MARC)

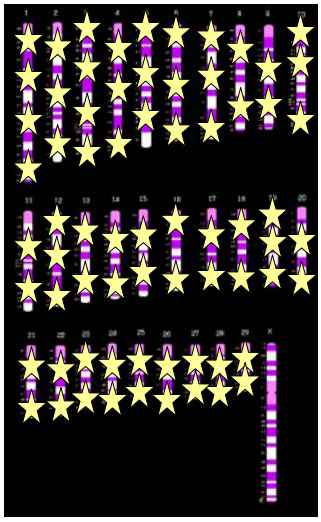
464 cross-bred Canadian beef cattle containing germplasm primarily from Angus, Charolais, Hereford, Simmental, Galloway, and other breeds (Dr. Moore, University of Alberta)

120 prominent sires from 4 dairy breeds (Drs. Van Tassell and Sonstegard; ARS, BARC)

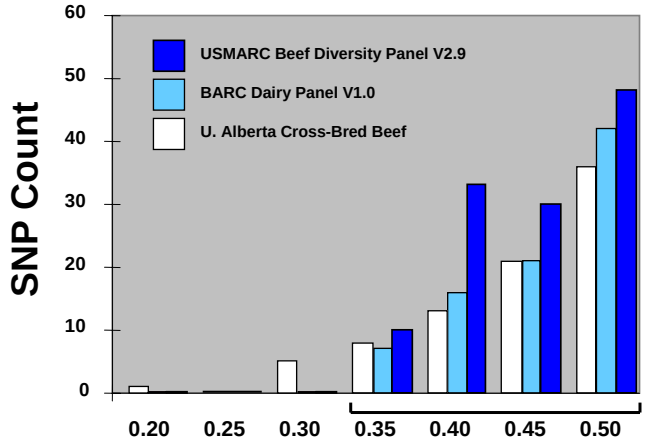
More than 4000 candidate SNPs, mostly from the Bovine Genome Project, were genotyped to select those with best minor allele frequencies (Drs. Heaton, McKay, Moore, and Murdock; MARC and U. Alberta)

The ideal markers are evenly distributed across the genome

- **We used the MARC composite map to select regions for markers**
- **Developed by Dr. Warren Snelling at MARC, this map contains positions of thousands of DNA markers.**
- **We chose SNP candidates in chromosomal regions that were approximately 20 to 50 cM apart**
- **X and Y chromosomes not used**



Distribution of minor allele frequencies for 121 parentage SNPs in US and Canadian cattle



Collaborators:
Drs. Bennett and Keele, MARC
Dr. Moore, U. Alberta.

The ideal parentage marker scores accurately

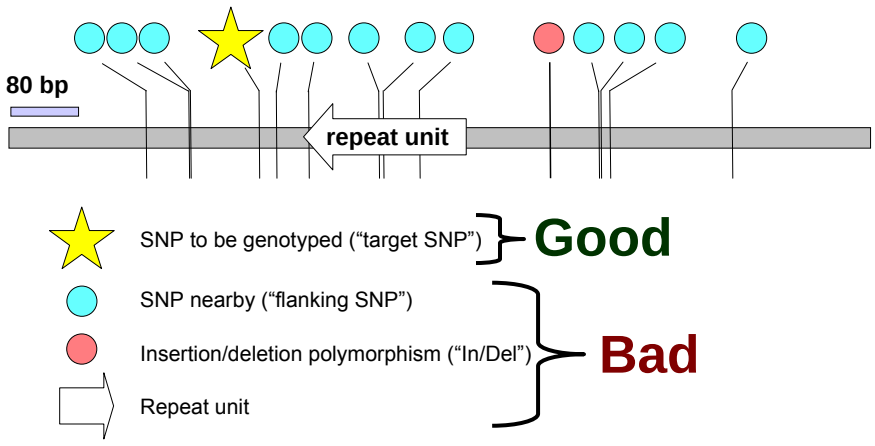
- **One wrong genotype may exclude the *true* parent.**
- **In disease traceback this causes delays, confusion, and loss of credibility.**

Accurate scoring requires that SNPs:

- are in unique genomic regions (non-repetitive)
- are in regions with well characterized flanking SNPs
- have no major insertion/deletions nearby

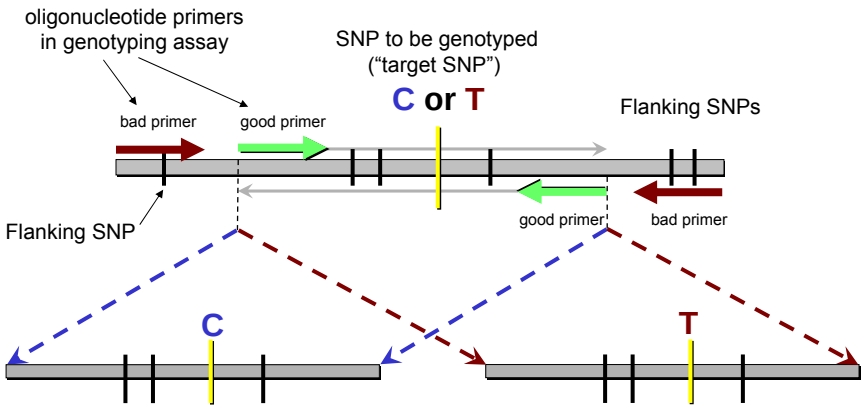
The nucleotide diversity in a typical region of the bovine genome

1 SNP occurs every 100 bp when comparing the 96 diverse beef sires from 19 breeds



The consequence of 1 SNP every 80 bp

- **Wrong genotype assigned to some animals**
  - **Because some oligonucleotide primers do not bind correctly**



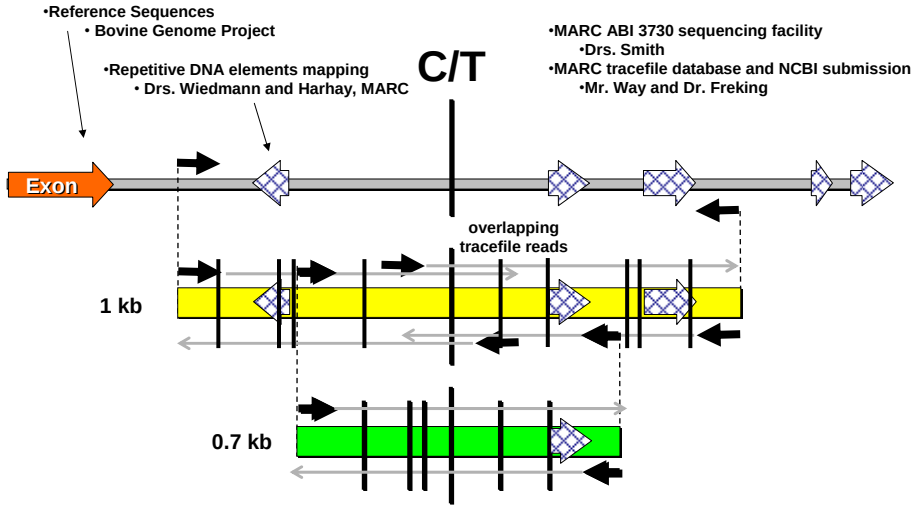
Aim: accurate amplification of both maternal and paternal alleles

Bottom line: hidden SNPs may cause the wrong genotypes to be scored

- Increases costs
- Decreases throughput
- Frustrates customers *and* genotyping companies
- Decreases platform flexibility
- Decreases competition in the genotyping markets

Solution: determine the flanking DNA sequence of parentage SNPs in populations to be genotyped

DNA sequencing strategy: nested PCR



Produce six or seven tracefile reads over each parentage SNP

Parentage SNP population sequencing results

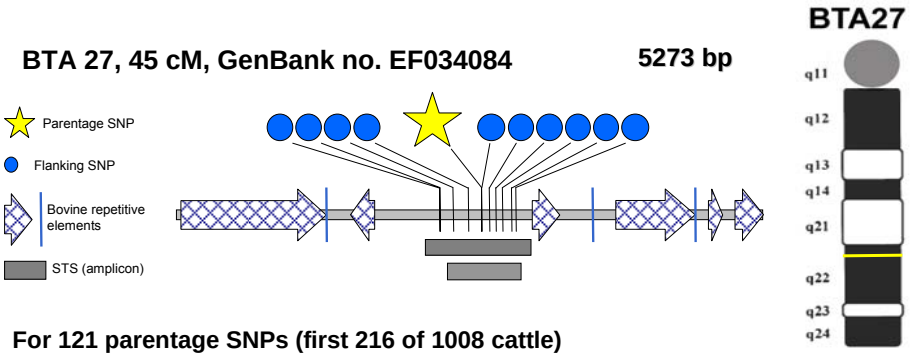
- **121 different regions with parentage SNPs analyzed**
  - **USDA Bovine SNP Set for Parentage-Based Traceback (UPT_BTA_2007.121.V1)**
- **42 breeds with 24 diverse animals from each breed (1008 total cattle)**

Angus	Gelbvieh	Piedmontese
Ankole-Watusi	Guernsey	Pinzgauer
Ayrshire	Hereford	Red Angus
Beefmaster	Highland	Red Poll
Belgian Blue	Holstein	Romagnola
Blonde D'Aquitaine	Jersey	Salers
Brahman	Limousin	Santa Gertrudis
Brahmousin	Maine-Anjou	Senepol
Brangus	Marchigiana	Shorthorn
Braunvieh	Mini-Hereford	Simmental
Brown Swiss	Mini-Zebu	Tarentaise
Charolais	Montbeliard	Texas Longhorn
Chianina	Murray Grey	Tuli
Corriente	Nelore	Wagyu

Collaborators:
Dr. Neibergs, WSU
Dr. Chase, ARS, STARS
Dr. Bob Bohlender
Mr. Goode, Goode Cattle

Recent additions:
Indu-Brazil
Dexter

Physical map of one region with a parentage SNP



For 121 parentage SNPs (first 216 of 1008 cattle)

0.425 average minor allele frequency beef
0.436 average minor allele frequency dairy
1646 adjacent SNPs
259 overlapping amplicons sequenced
137k tracefiles deposited at GenBank
89 Mb of sequence
2004 bovine repetitive elements
258 exons
183 CDSs

- Average of 9.8 kb annotated per file (1.2 Mb total)

- Collaborators in depositing GenBank annotation
- Dr. Anjanette Johnston
  GenBank Submissions Staff
- Dr. Ilene Karsch Mizrachi
  GenBank Coordinator

Immediate public access requested

- During marker development a number of companies wanted immediate access to the results.
  - Some companies needed to design tests as fast as we could generate the marker information
- How to provide fair public access?
- GenBank provides access but *not* easily

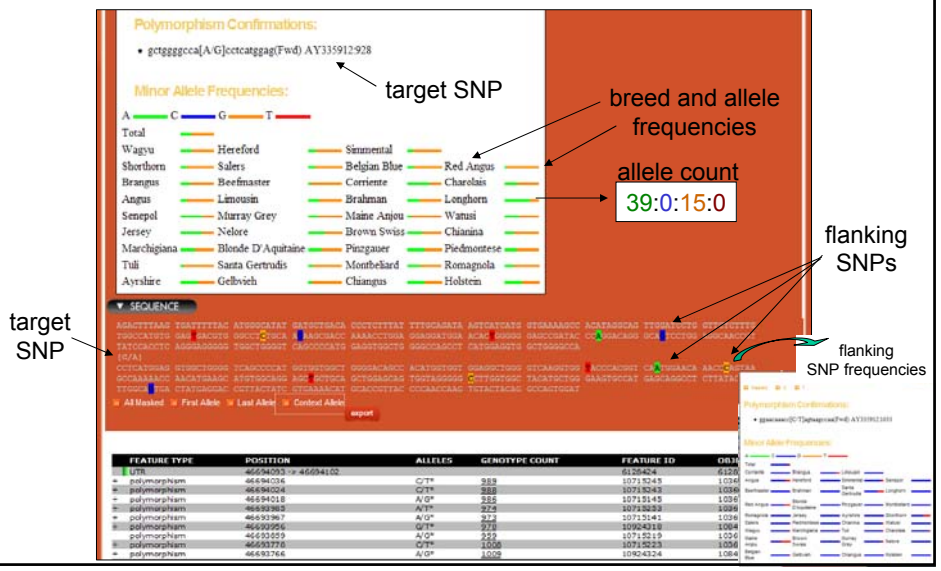
Public access to detailed SNP information

http://cgemm.louisville.edu/usmarc/MARC_web_page/traceback.html

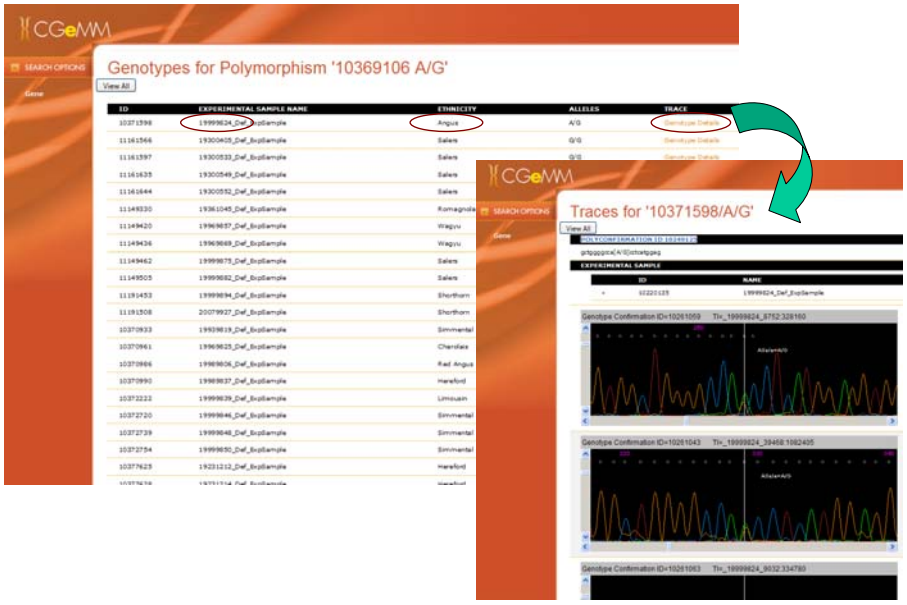
Collaborator:
Dr. Ted Kalbfleisch,
U. of Louisville

- **We first started with a "static" page for downloading key summary information**
  - **UPT_BTA_2007.121.V1**
- **Next, a set of interactive pages were developed that access relational data base information.**

Public internet access to flanking SNPs and allele frequencies by breed



Individual animal genotypes linked to tracefiles



Where are we now?

- **Many of the 121 USDA Parentage-Based Traceback SNPs**
  - **are on the USDA-Illumina Bov50k SNP chip**
  - **have been adapted by companies into their parentage tests**
  - **have been used in additional peer-review research publications**
- **Database development and uploading of detailed SNP information is ongoing at U. of Louisville and MARC**
- **The 121 USDA Parentage SNPs are continually being sequenced and tested in new breeds to evaluate their utility in the event that an emergency DNA-based traceback is needed.**
  - **The results are being made available as they are produced**
- **This set of SNPs currently represents the best group of well-characterized parentage SNPs available.**

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