



## Genomic Discover of Bovine Respiratory Disease Traits Using Commercial Data and DNA Pooling

U.S. Meat Animal Research Center



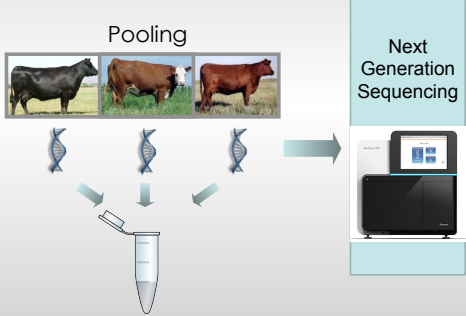
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### DNA Pooling Rationale

- Running the Bovine HD array on 96 animals costs \$14,880. Running the Bovine HD array on a pool of 96 animals costs \$155. There is tremendous savings in genotyping costs but do you achieve the same goal. No.
- Pooling can be used to estimate GWAS effects (additive) but individual genotyping or bar coding is required for estimating EBV and making selection decisions.

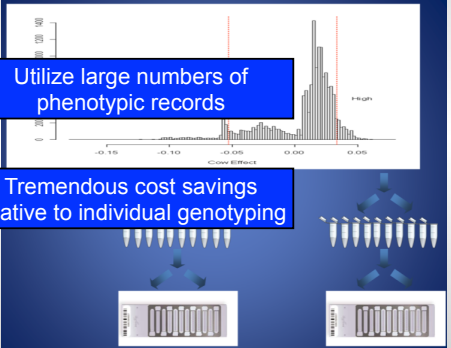
### GWAS pooling study

- Tools for overcoming challenges



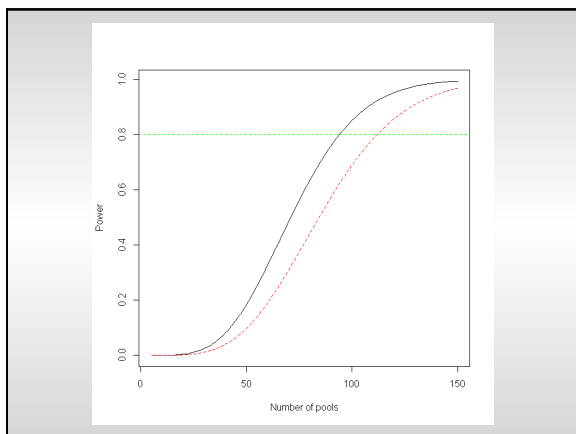


### DNA pooling



Utilize large numbers of phenotypic records

Tremendous cost savings relative to individual genotyping



### Cost

- 112 pools of 96 animals (10,952 animals total) to achieve power of 80 % with pooling
- 9,120 animals to achieve power of 80 % with individual genotyping
- Cost of Bovine HD genotypes plus DNA extraction is \$38,860 for pooling and \$1,431,840 for individual genotyping.

### Pooling studies at USMARC

- Lung lesions in cattle and swine (Schuyler, Lexington, Crete)
- General Disease (BRDC, foot rot, pink eye)
- BVDV (in collaboration with Eduardo Casas)
- Bloat (New Zealand collaboration)
- Pregnant vs not pregnant in cattle
- BRDC in industry feedlot cattle (NIFA grant)
- Failure of passive transfer in piglets
- Color stability and meat quality traits in swine.

### Methods

- DNA samples pooled and sent to Neogen (GeneSeek) for genotyping with the Illumina Bovine HD Bead array (770K SNP).
- Statistical analysis
  - $PAF = X / (X + Y)$
  - PAF is distributed as multivariate normal with a mean of  $X\beta$  and a variance of  $A\sigma^2$ .
  - $A$  is the genome-wide covariance among pools across SNP
  - $\sigma^2$  is an SNP specific scaling factor estimated using REML.

### Statistical Analysis

- Bonferroni correction to 5 % genome-wide
  - Simple M, Effective number of tests was 343,000
  - Nominal P-value was  $1.49 \times 10^{-7}$  (.05 / 343,000)
- False discovery rate of 5 %
  - $5.47 \times 10^{-6}$
- Results
  - Adjusting for population stratification
  - Also tested without stratification

### Objective

- Identify SNP associated with severe lung lesions in fed beef cattle.

### Methods

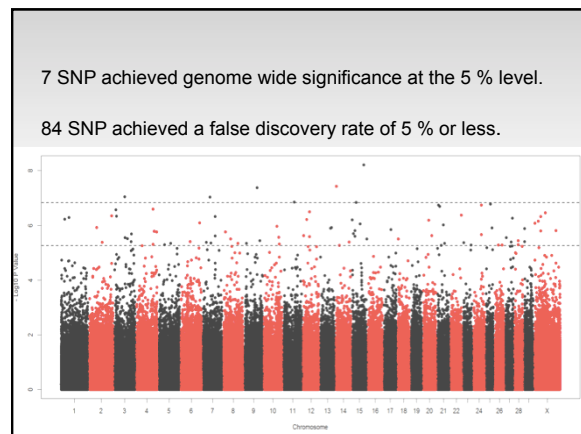
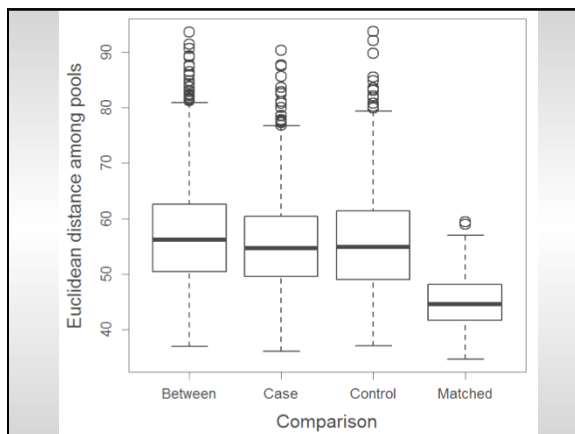
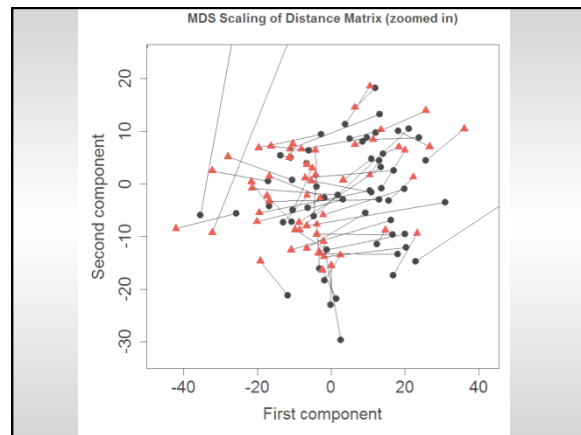
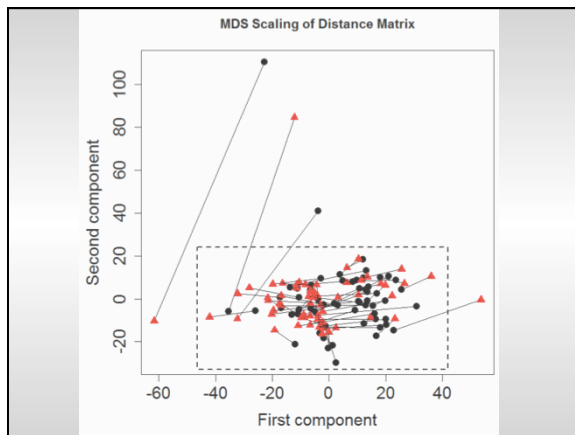
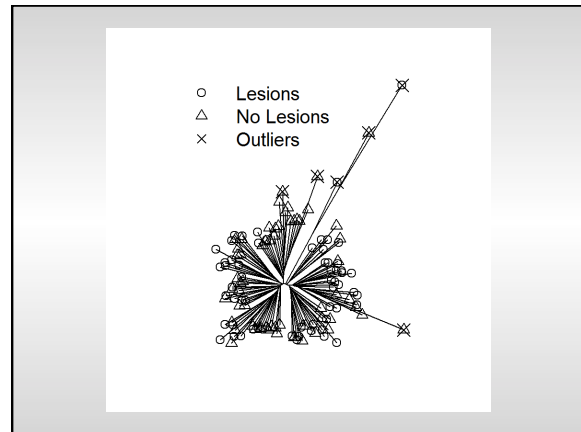
- 11,520 lungs were sampled from a central Nebraska beef processing plant with a throughput of 2,500 cattle per 8 h shift.
- On average 900 lungs were sampled per day.
- The majority of the lungs came from cattle raised without antibiotics.
- Case – Control Definitions
  - Half (5,760) had severe lung lesions (Case)
  - Half (5,760) had mild or no lesions (Control).

### Methods

- Lungs were scored as severe if they had greater than 50 % of lung tissue affected with lesions associated with BRDC including pleural adhesion to the thoracic cavity.
- Sampling variation in lung lesions– Lesion (L), Normal (N); green for sample and red for don't sample.
  - LLLLLLLLLLNNNNNNNNNNLLLLLLLLLLNNNN
  - LLNLNNLLNLNNNNLLNLNNLLLLNNLLNLLNLN
  - NNNNNNNNNNLNNNNNNNNNNNNNNNNLN

## Methods

- Small cores of lung tissue were placed in 120 pools
  - 60 case (with lesions) pools
  - 60 control (without lesions) pools
- Each pool consisted of cores of 96 lungs collected within about a two hour period.
- DNA was extracted from each pool using a standard salt extraction procedure.



### Conclusion

- Function of genes near significant SNP
  - Tissue repair and regeneration
  - Tumor suppression
  - Control of organ size
  - Immunity
- We reduced the cost of genotyping by exploiting sample pooling of extreme animals.
- Identified SNP that might be useful to reduce BRDC and reliance on antibiotics.

### Conclusion

- Chain sampling method approximates contemporary group effects
- Doesn't directly result in an input for national cattle evaluation like whole genome selection approaches using individual genotyping

### Conclusion

- May provide inputs to weighted WGS approaches as BRDC recording becomes more prominent

### Future work - CSU collaboration

- Funded NIFA grant
- Working with a set of feedlots (Nebraska, Colorado, Kansas) to obtain treatment records
- Also collecting DNA samples at feedlots or at plants
- Trying to focus sampling on high BRDC incidence pens/groups

### Feedlot BRDC grant

- Hoping to obtain 7,500 case samples (treated for BRDC) and matched controls from the same pens
- Planning to form approximately 50 case and 50 control pools and analyze with the Illumina BovineHD

### Feedlot BRDC grant

- Remaining 2,500 cases and matched controls will be used for targeted sequencing effort
  - Focus on regions from array
  - May target whole exome depending on cost
  - Will serve as fine mapping and validation of array markers

### BRDC pooling

- Hoping that lung and feedlot project will produce complimentary results
- We see this as an initial parameterization of whole genome selection models for BRDC susceptibility in national cattle evaluations

### Acknowledgements

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Participating feedlots and plants

### Questions

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