DNA Pooling as a Low-cost Method to Detect Important Genomic Regions for Difficult Traits in Beef Cattle

Introduction

- Main limiting factor affecting power in the design of GWAS is cost of genotyping and phenotyping
- Genotyping costs range from $100 to $250 per sample using arrays
  - Implies genotyping cost of millions for achieving sufficient power with complex traits
- Reduce cost through DNA pooling?

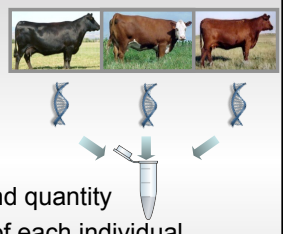
DNA pooling

- Pooling is not a new idea
- Proposed/utilized to reduce genotyping costs for several types of genomic studies (e.g. QTL; Dekkers, 2000)
- More recently for analysis of bead data from Illumina® chips
  - Macgregor et al., 2007 (human)
  - Huang et al., 2010 (bovine)

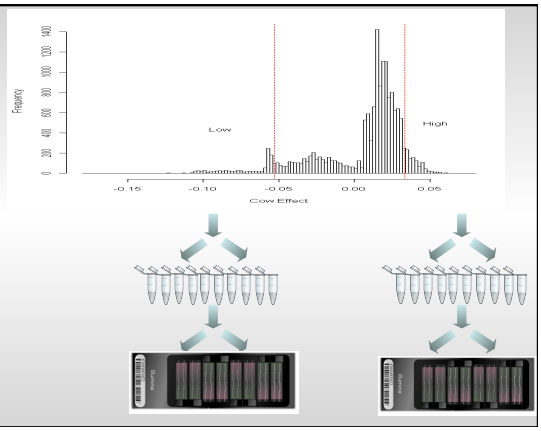
DNA pooling

- Create pools from extremes of phenotypic distributions into pools
  - Estimate allele frequencies between extremes
  - Same concept as case/control
- Larger pools sizes reduce influence of single animals through sampling/mixing
  - Pool size is a balance of phenotyping, array, sample collection and DNA extraction costs

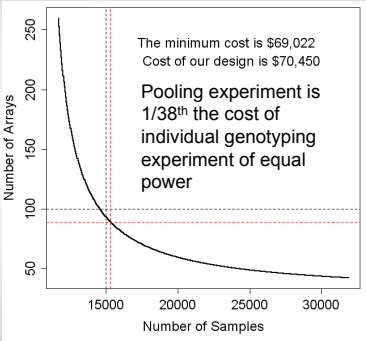
Steps in creating DNA pools



- Extract DNA
  - Tissue
  - Blood
  - FTA cards
- Determine quality and quantity
- Pool equal amount of each individual
- Properly mix pool to ensure consistency of individuals in pool



DNA pooling optimization



Pooling projects at USMARC

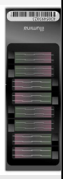
- Pilot – Hereford/Angus bulls
- Propensity to bloat
  - Collaboration with New Zealand
  - Case-control pools, selection study
- Fertility
  - Collaboration with several large US ranches
  - Heifer pregnancy/success after two exposures
- Disease resistance
  - USMARC treatment database
  - Lung lesions on random animals at slaughter
  - Case-control pools

Estimating allele frequencies

- Frequencies are a function of red and green intensities on the chip (Illumina)
- Calculate a pooling allele frequency

PAF = (Red + k) / (Red + Green)

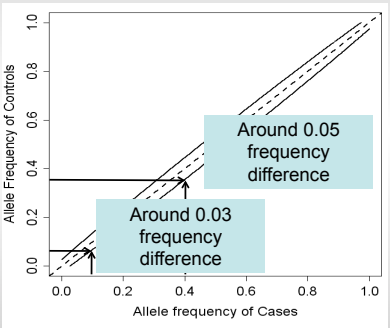
k is a constant to adjust effects of individual chip/ array location (derived across all SNP)



Variance component analysis

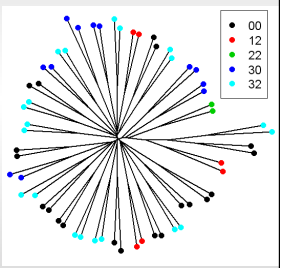
- Using bead level data from chips
  - Calculate pooling allele freq (PAF) from red/green
  - Normalize PAF per Macgregor et al. (2007)
- Sources of variance at multiple levels
  - Technical variance (beads within array, array)
    - Separate estimate for each SNP
  - Pool level
    - Binomial sampling and pool construction (CV^2)
    - Pool at tissue or DNA level

Frequency differences

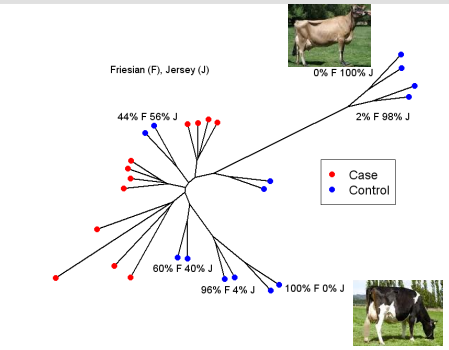


Want case/control to be similar

- Distance diagram based on correlations of PAF among pools
- This example is from a lung lesion pilot project
- Distances consistent between controls and other pools



Cases and unselected controls not always well matched



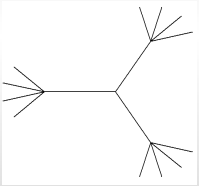
Handling stratification differences

- Adjusting for population hidden population stratification
- Compute correlation among pools using PAF for all SNP
- Spectral decomposition to obtain eigenvalues and eigenvectors
- Use eigenvectors as covariates that correspond to eigenvalues accounting for most of the variance to adjust for hidden population stratification
- Individual SNP have minimal effect on the covariates so analysis Is not circular

Correlation matrix for example

1	.5	.5	.5	0	0	0	0	0	0	0	0
.5	1	.5	.5	0	0	0	0	0	0	0	0
.5	.5	1	.5	0	0	0	0	0	0	0	0
.5	.5	.5	1	0	0	0	0	0	0	0	0
0	0	0	0	1	.5	.5	.5	0	0	0	0
0	0	0	0	.5	1	.5	.5	0	0	0	0
0	0	0	0	.5	.5	1	.5	0	0	0	0
0	0	0	0	.5	.5	.5	1	0	0	0	0
0	0	0	0	0	0	0	0	1	.5	.5	.5
0	0	0	0	0	0	0	0	.5	1	.5	.5
0	0	0	0	0	0	0	0	.5	.5	1	.5
0	0	0	0	0	0	0	0	.5	.5	.5	1

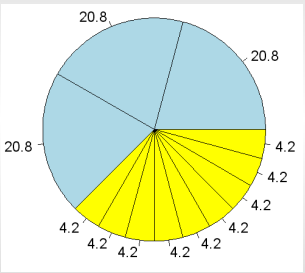
Phylogenetic tree for 3 breeds equally distant from one another



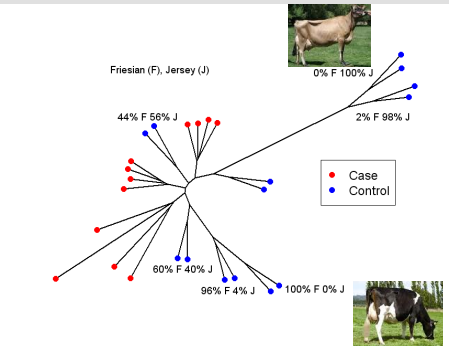
Covariates to adjust for population stratification

0	-.5	0
0	-.5	0
0	-.5	0
0	-.5	0
-.5	0	0
-.5	0	0
-.5	0	0
-.5	0	0
0	0	-.5
0	0	-.5
0	0	-.5
0	0	-.5

Variance accounted for by each Eigen Value

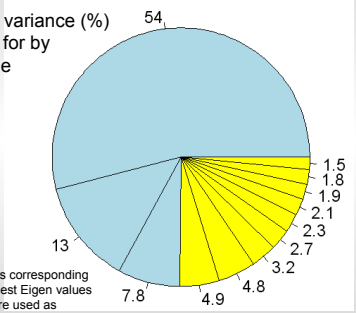


Back to New Zealand Bloat example



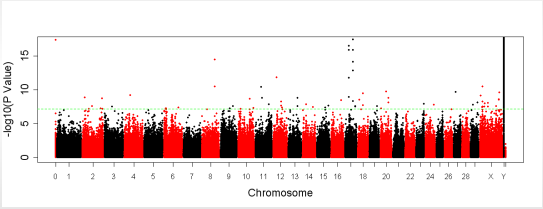
Accounting for Population stratification using Eigen Vectors (like EigenStrat)

Labels are variance (%) accounted for by Eigen Value



Eigen vectors corresponding to the 3 Largest Eigen values (in blue) were used as covariates

Manhattan Plot for New Zealand Industry Bloat Study



Conclusion

- We think pooling can be used to reduce the cost of GWAS
- The mixed model approach does a good job of accounting for technical and biological variation.
  - Should prevent over-interpretation of frequency differences caused by non-genetic sources of variation (sampling, beads, pool construction, hidden population stratification)

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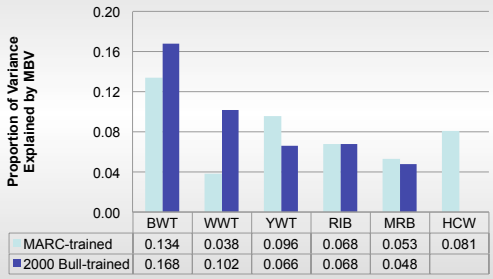
2,000 bull project predictions

- Whole genome selection pilot project with in cooperation with breed associations
  - Over 2,000 industry bulls genotyped with Illumina BovineSNP50
- Formed prediction equations using USMARC GPE data as well as deregressed EPDs from the 2,000+ bulls

Proportion of variance explained



	BWT	WWT	YWT	RIB	MRB	HCW
MARC-trained	0.134	0.038	0.096	0.068	0.053	0.081
2000 Bull-trained	0.168	0.102	0.066	0.068	0.048	

2000 Bulls MBV

- Resulting MBV are being sent to breed associations today
  - Sent by Mark Thallman
- Contact us with any questions

Prediction Equations

- Equations are available at:
  https://www.ars.usda.gov/sp2UserFiles/Place/54380510/2000 Bull Prediction Equations.xlsx

  Link from www.marc.usda.gov ->
  Genetics & Breeding ->
  Documents

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Questions?