Johnes Disease Project
Update

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Bovine DNA Archive

- ## Genomics Approach

  Phenotype

  – Collect and store DNA samples from JD case animals:
  2000 Johne's positive cows identified using ELISA
 on milk sample screening, followed by serum confirmation

  Genotype

  – 777k SNP panel for the affected Johne's animals

  – Existing LIC population data (23,000+) used as control data

Delivering genomic technology

- Semen – 80%[+] of cows in NZ AI
  - LIC has 80% market share
  - Genomic technology was introduced in 2007
  - 40%[+] of semen used is DNA proven

- Genotyping today
  - 200,000 animals/yr parentage tested
  - Possibility of adding a Johne's disease marker
  - Expected to be less efficient than breeding

- Screening test for farmers
  - Could be developed by refining current system

Method of Identification of JD-case Animals

JOHNE'S DISEASE
RESEARCH CONSORTIUM
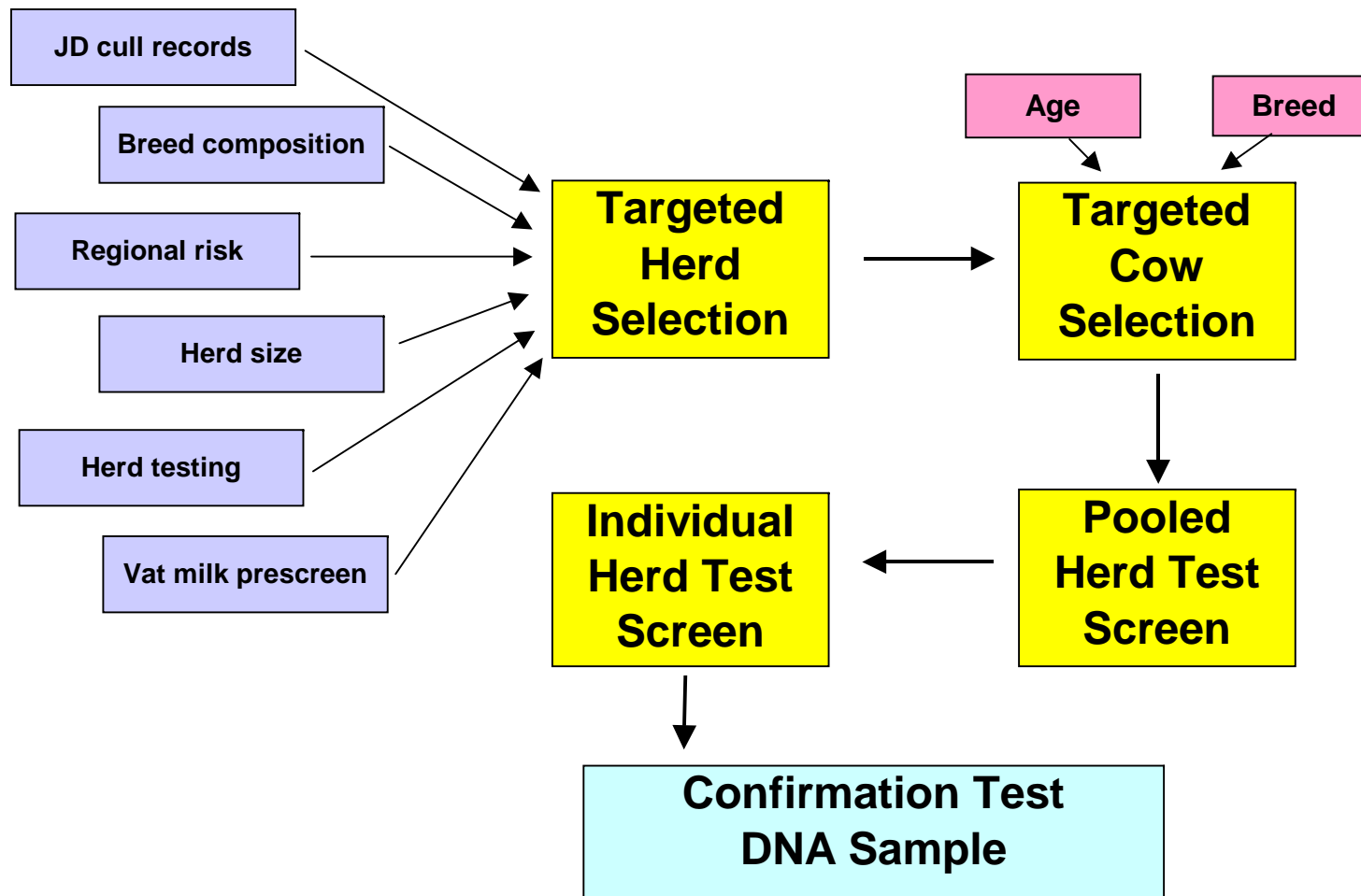
- Vat milk pre-screen is a valuable tool.

- An efficient method to screen wider herd population if needed including 'low-risk' regions, smaller herds etc

- Individual samples pooled (10:1)
  – If pool shows reactor, individual samples analysed

- ~60-70% fewer tests required than if individual testing

- Pool $\rightarrow$ individual testing is much more robust with little impact of raising cut-off on total number of kits needed

- Allows targeting of more advanced JD cows for the genomics study

LIC

- ## Year 1
  - milk positive cows: blood sample + faecal culture
- ## Year 2
  - milk positive cows: blood sample only
  - milk negative cows: blood sample + faecal culture

	Milk Samples	Blood samples		Faecal Culture	
	Positive	*Positive*	*Negative*	*Positive*	*Negative*
Year 1	287	250	37	216	71
Year 2	*Positive* 1587	1357	230		
	Negative 101	3	98	24	70

- Prevalence rates
  - Positive: S/P ratio > 0.1   prevalence = 3%
  - Suspect: S/P ratio >0.05 prevalence = 1.5 – 2%
  - Check: S/P ratio >0.04 prevalence = 0.5%

- Used the remaining herds from the original data extract + repeat herds

- Selected 390 for vat test screen

- 40 herds to test on above criteria + 20 repeat herds + 5 new requests
  - From 33 herds, 160 milk positive cows identified so far

Samples for Genotyping

- Extracted DNA from1650 samples from blood positive cows

- The genotyping phase of the project commenced early

- 1440 samples sent for genotyping Nov 2010
    - Genotypes received Dec 2010

Johnes-positive Samples by Breed

Breed	Number
Other Cross	41
Holstein Friesian	258
Jersey	660
Ayrshire	1
KiwiCross	364

Johnes-positive Samples by Age

Age	Number
15	1
13	5
12	9
11	14
10	34
9	76
8	127
7	162
6	193
5	233
4	253
3	155
2	62

Johnes-positive Samples by Region

| Region	Number
Northland	40
Waikato	272
Bay of Plenty	34
Taranaki	322
Wellington/Hawkes Bay	130
South Island	526

Farmers perception

- **How farmers view the herd test screening approach**
  - Industry-good research project
  - Tool to reduce JD in herd that they would pay for

- **Demand for this service**
  - Small at present - 25 of 350 herds repeat tested
  - But new requests from 'word of mouth'
  - Vets expressing interest for use for clients with JD problem

- **Cost**
  - happy to pay the equivalent of 1-2 first calving heifers due to cost of raising replacements (~$2000)
  - Short-term ~ 3 years

Genotypes

- 1324 Johnes-positive HD genotypes

- High Density panel (HD)
  - Illumina (777k SNPs)
  - Released July 2010
  - Used within LIC genomics programme
  - May allow cross-breed analysis

QC Processing

- **Sample exclusion criteria:**
  - Call rate < 87%
  - Heterozygous Rate <20% or >40%

- **Any animals failing parentage (sire) retained**

- **711955 SNPs retained after QC**
  - MAF > 2%
  - Autosomal
 - Known location

Call rates

LIC

Method of Analysis
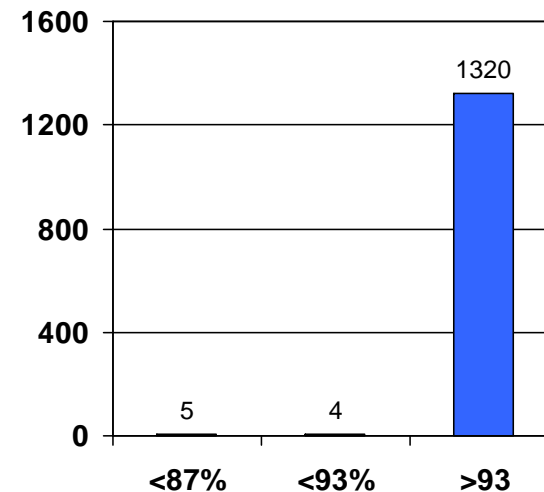
- Test for SNP association with susceptibility to Johnes disease

  - compare allele frequencies:

    infected cows vs population (Wellcome Trust approach)

- Estimate population allele frequency from existing LIC population data

  - 23,000 genotypes imputed to HD

  - Imputation of HD genotypes from 50k has an accuracy of 99.5%

Challenge: To choose appropriate control population

Detecting SNPs of interest
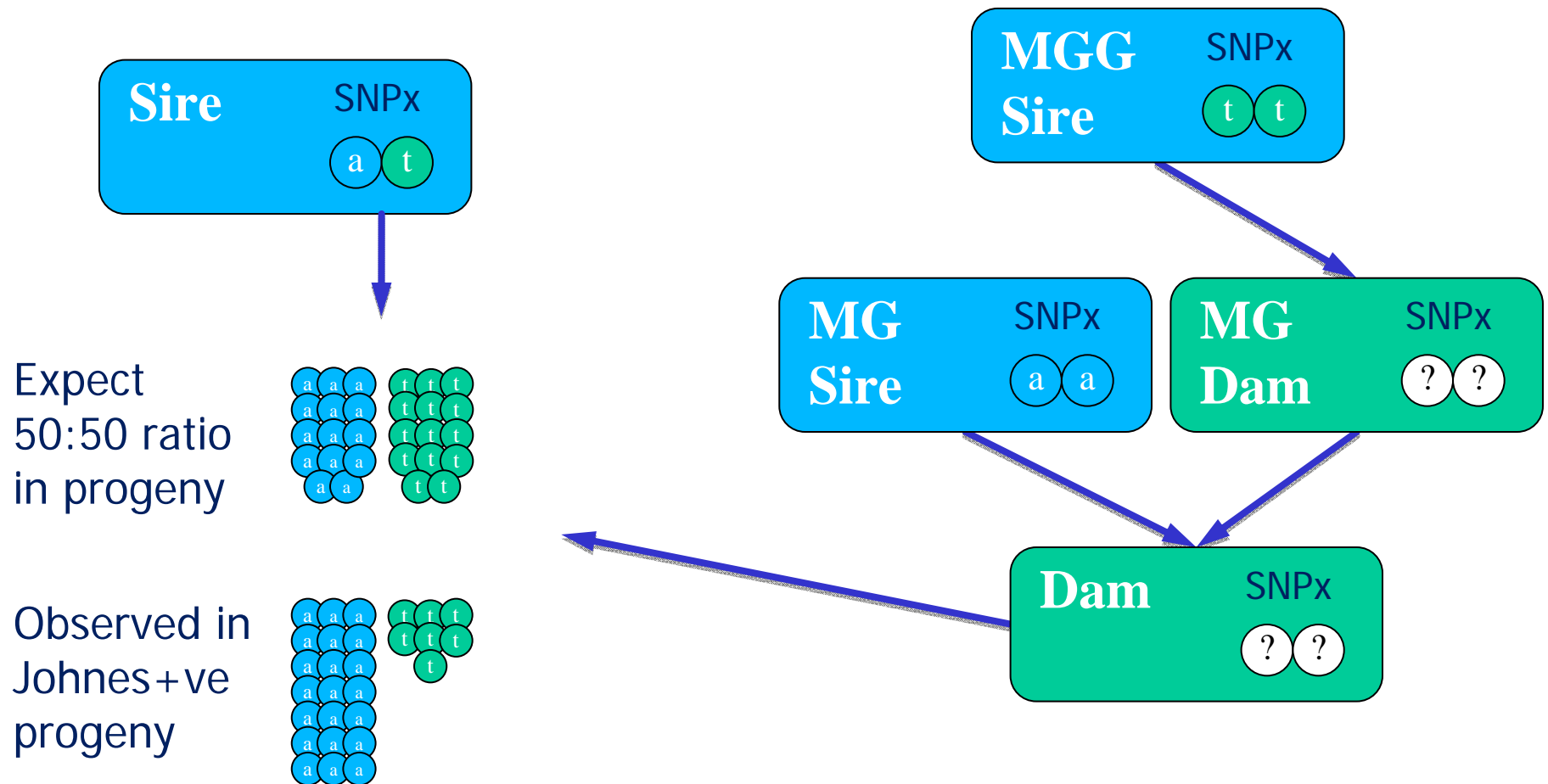
Johnes-positive Genotypes by Sires

+ve Daughters	Count of Sires
>49	3
20-49	7
10-19	21
5-9	23
<5	282

+ve Daughters	Count of MG Sires
>49	0
20-49	10
10-19	20
5-9	25
<5	345

- 336 sires with Johnes +ve daughters
- 10 sires accounted for 32.3% of the positive daughters
- 9 of top 10 sires have HD genotypes

- 400 Maternal grand-sires with Johnes +ve daughters
- 10 sires accounted for 23.7% of the positive daughters
- 3 of top 5 MG sires have HD genotypes

Preliminary Analysis

- Analysis of chromosome 20
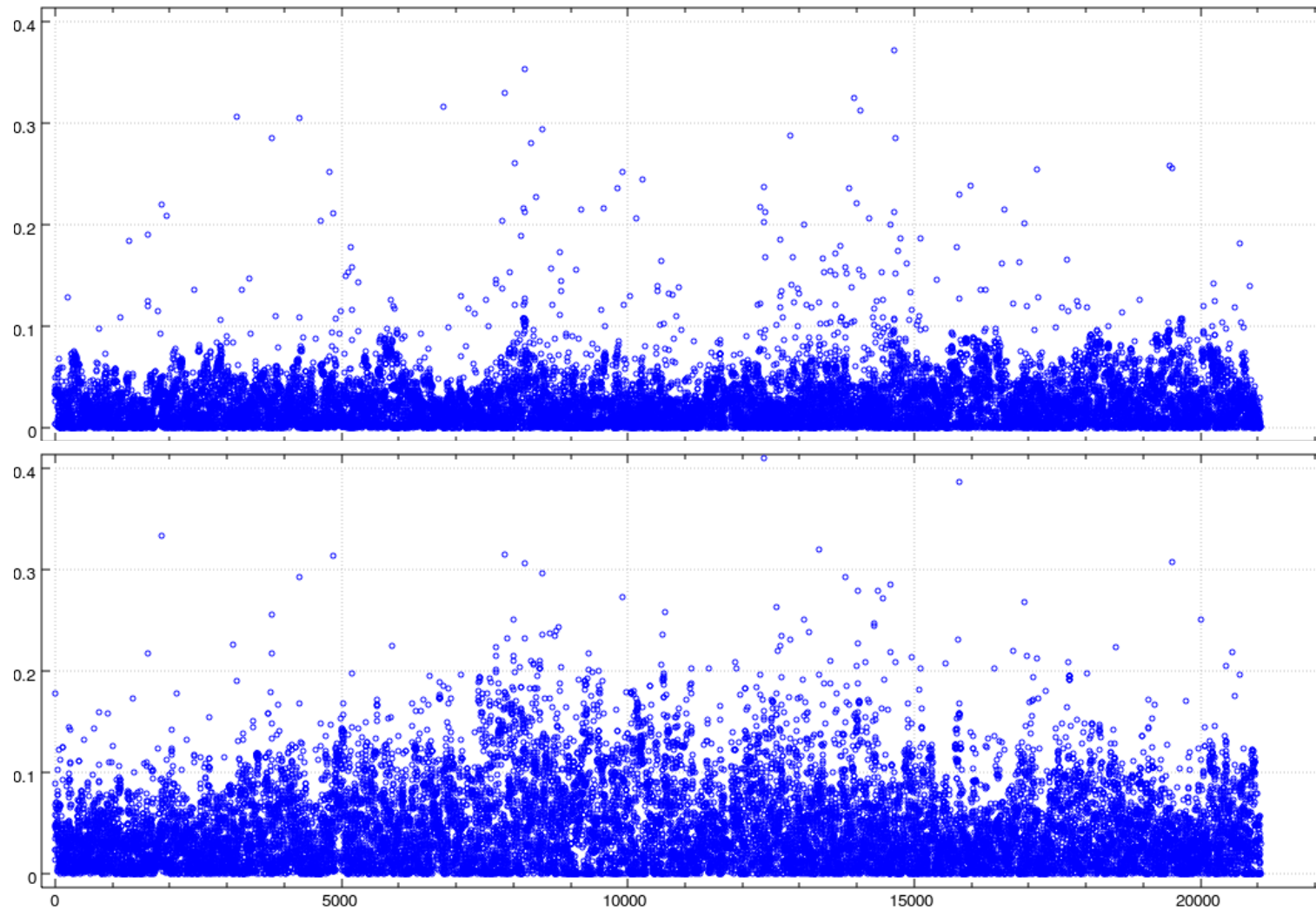- Using existing LIC HD genotypes as Control popln

Breed	HD Control	Case (Johnes+ve)
Other Cross	0	41
Holstein Friesian	999	258
Jersey	447	660
Ayrshire	0	1
KiwiCross	11	364
Total	1457	1324

Chromosome 20
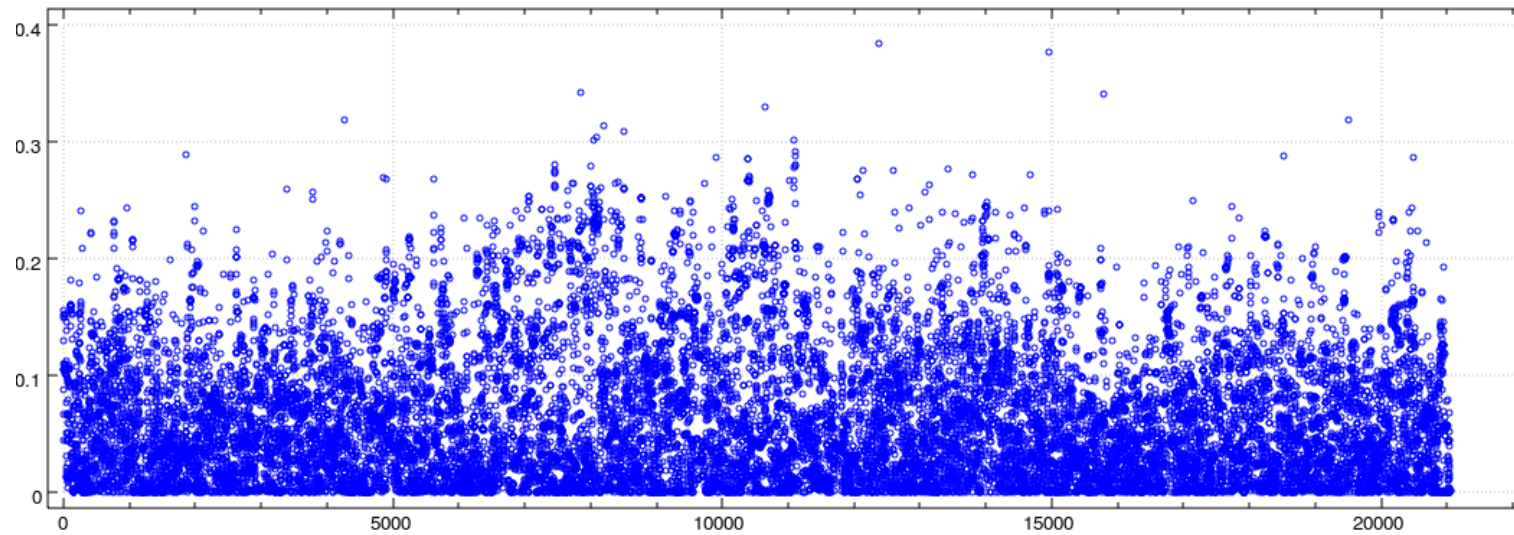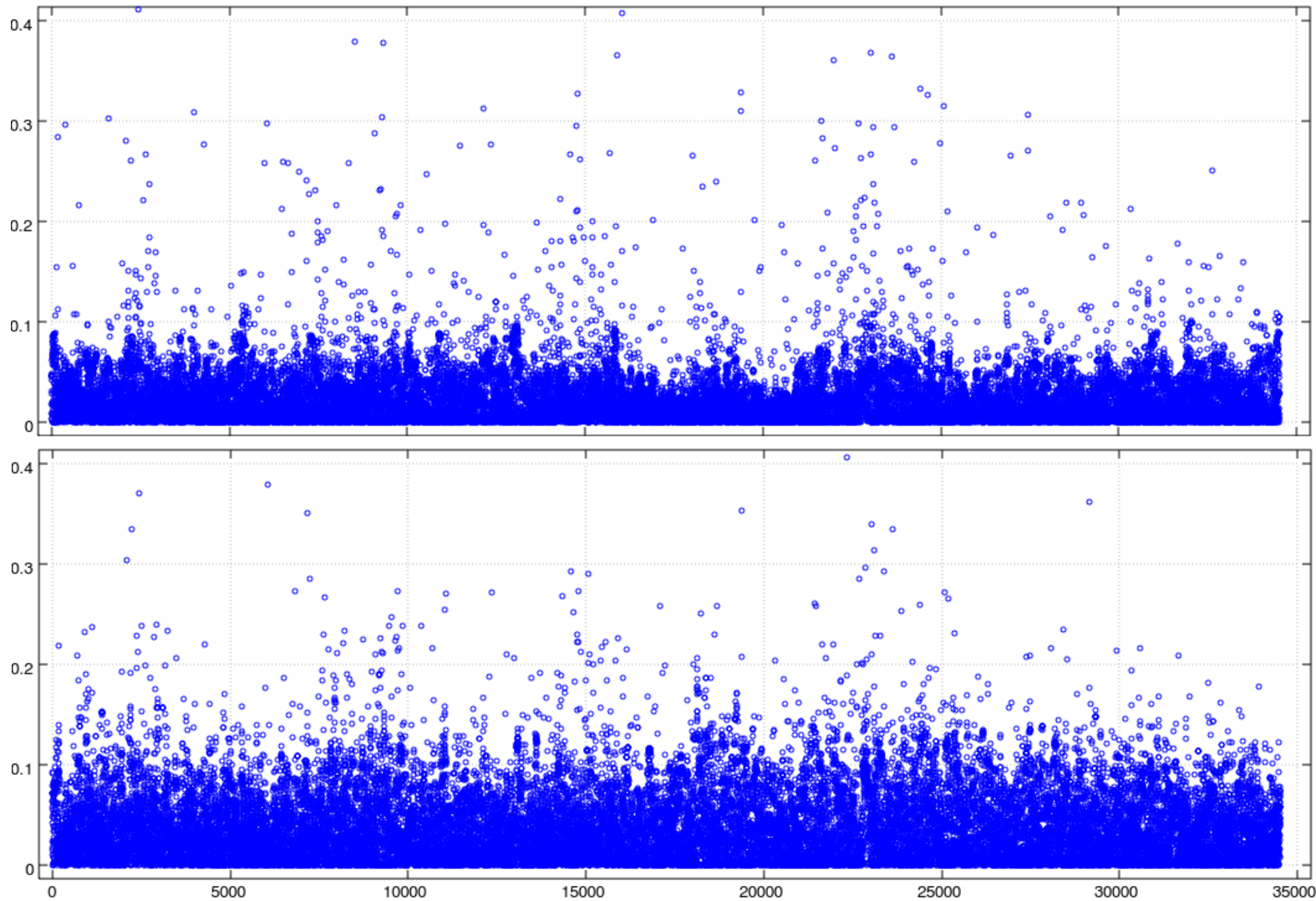
All
Breeds

Chromosome 20

Jersey

Holstein
Friesian

Chromosome 6

Jersey

Holstein
Friesian

Where to from here?

- Collect and genotype remaining Johnes +ve samples

- Add additional 20,000 imputed genotypes to control population

- Examine for evidence of stratification
  - Breed/Sires/Age

- Refine genomic analysis

Summary

- Sample collection near completion

- Genotyping of ~70% of the samples has been completed
  - Ahead of schedule
  - Rest of samples in the next couple of months (HD)

- Initial analysis of the data undertaken
  - Refinement of the control population

- Outcomes can be transferred via semen and genotyping tests