# SNP Genotyping For Fine mapping Eggshell Quality Traits in Chicken

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# MTT Agrifood Research Finland


- leading research institute in the agriculture, food and environment sectors in Finland
- operates in 14 different locations around the country
- operates under the Ministry of Agriculture and Forestry
- 800 professionals, 300 of whom work in research

# SNP Genotyping For Fine mapping Eggshell Quality Traits in Chicken

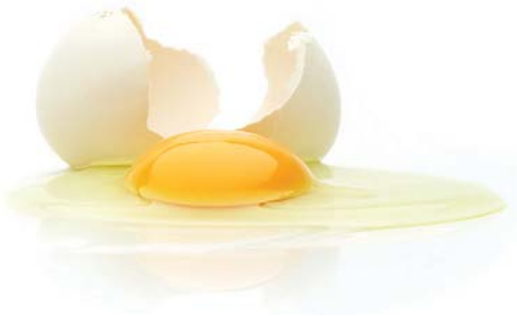
BACKGROUND

- EU project: SABRE (http://www.sabre-eu.eu/)
  - SABRE = Cutting Edge Genomics for Sustainable Animal Breeding
  - WP7 product safety
    - task 7.1 Fine mapping of QTL in a resource population

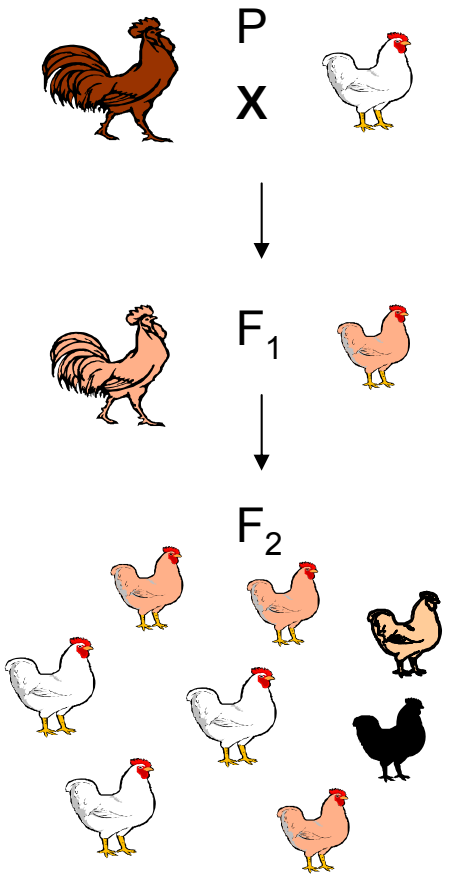
- Partners
  - MTT Agrifood Research Finland
  - Lohmann Tierzucht GmbH, Germany
  - The Roslin Institute, Scotland
  - INRA, France

# BACKGROUND

- egg quality traits are poorly studied
- only some QTLs for eggshell quality
- poor eggshell quality
  - → cracked or broken eggs
  - → a route for pathogen contamination
  - → losses may be up to 10 % of total production

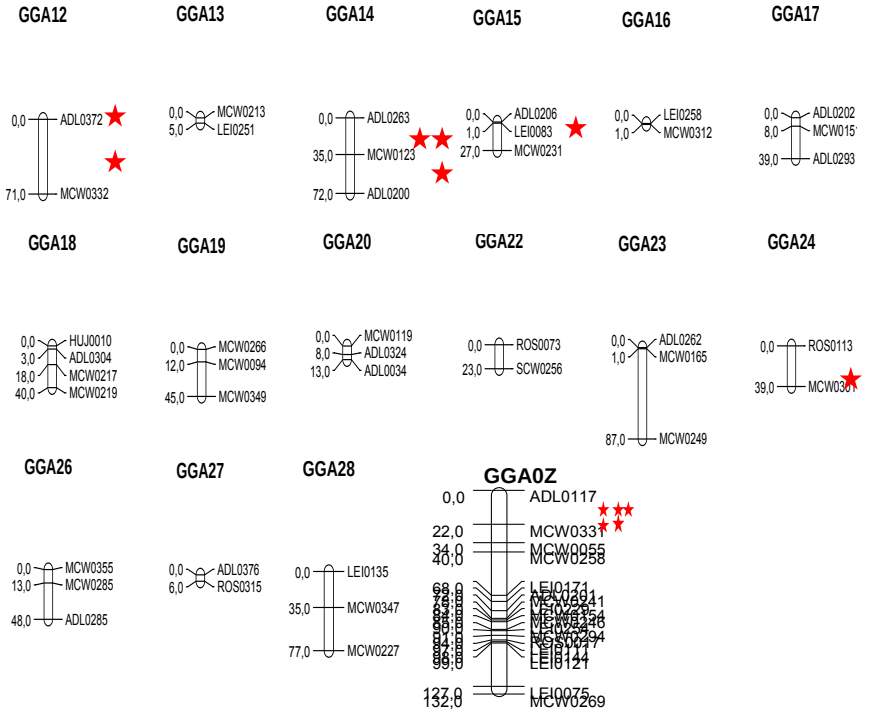


# GENOME SCAN WITH MICROSATELLITES




- mapping population (1791 individuals)
  - 162 microsatellites, 26 chromosomes
- Parent generation
  - reciprocal line cross
  - RIR x White Rock
  - phenotypes for egg quality
- $F_1$ generation
  - phenotypes for egg quality
  - for producing $F_2$ generation
- $F_2$ generation
  - phenotypes for egg quality

# QTL REGIONS FROM GENOME SCAN



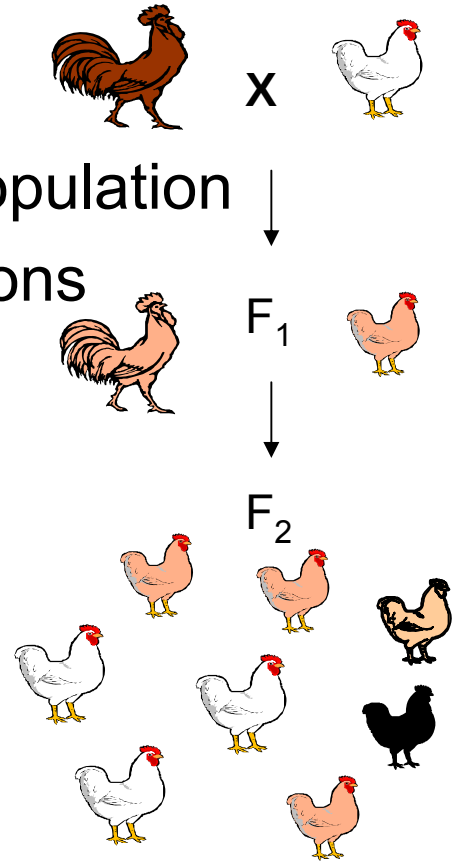

- QTL (Quantitative Trait Loci) analyses were done using line cross model
- five interesting QTL regions from five different chromosomes

- 230 genes specifically expressed in the shell gland
- 28 are located within the QTL regions found in this study

# FINE MAPPING OF QTL REGIONS

- 1599 $F_2$ hens
  - from 16 different families
  - parents included to the fine mapping population

  → totally 1791 individuals, three generations



# SNP GENOTYPING

- BeadXpress Reader
  - Veracode application: genotyping with Goldengate assay kit
  - GoldenGate oligo pools (OPAs)
  - 48-, 96-, 144-, 192-, and 384- SNP plex
  - one OPA set → 480 individuals (5x96 plates)

- fine mapping in two different laboratories with same individuals
    - MTT: one OPA, two QTL regions (chromosomes 14 and Z)
    - The Roslin institute: one OPA, three QTL regions

# DESINGING THE OPA

- validated SNPs were available from public databases (www.ncbi.nlm.nih.gov/sites/entrez)
- totally 449 SNPs available for the two QTL regions (chr 14; 9Mb and Z; 43Mb)
- OPA was designed with online Assay Design Tool (www.illumina.com/)

# DESINGING THE OPA

- Chr 14
  - 202 SNPs, 181 SNPs passed the quality restriction (SNP score > 0.7)
- Chr Z
  - 247 SNPs, 203 SNPs passed the quality restriction (SNP score > 0.7)

→ Custom OPA for 384 chicken SNPs
  - 5 OPA sets, totally 2400 individuals

# GENOTYPING

- DNA was extracted from the blood samples using salting out procedure (phenol – chloroform)
  - 250 ng of DNA needed per individual
- 2 – 3 plates per week
  - about 1,5 months to get the system work correctly
  - remote sessions with Illumina's experts has been useful
- 19 plates genotyped, ~1800 individuals

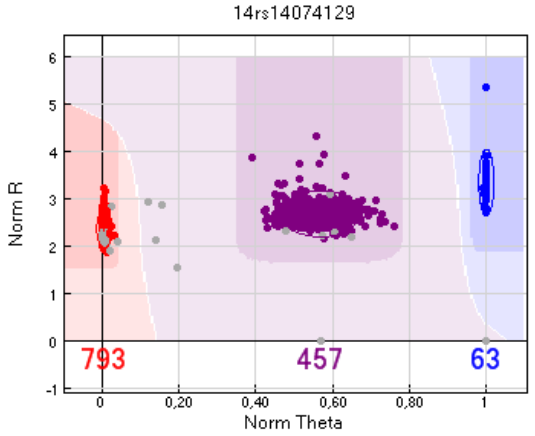
- genotypes analysed with BeadStudio Genotyping Module
  - manual checking
  - remote sessions with Illumina's experts has been useful

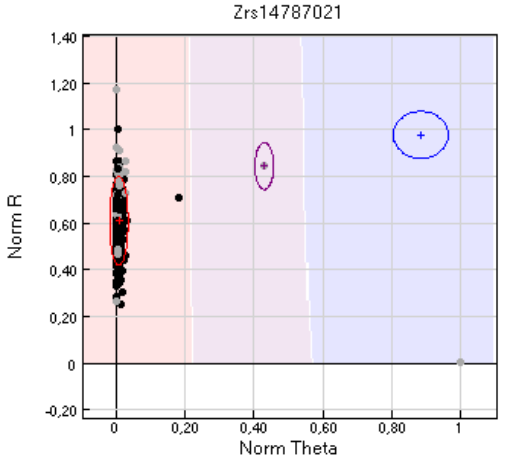
# RESULTS

- 331 successfully genotyped SNPs

out of them

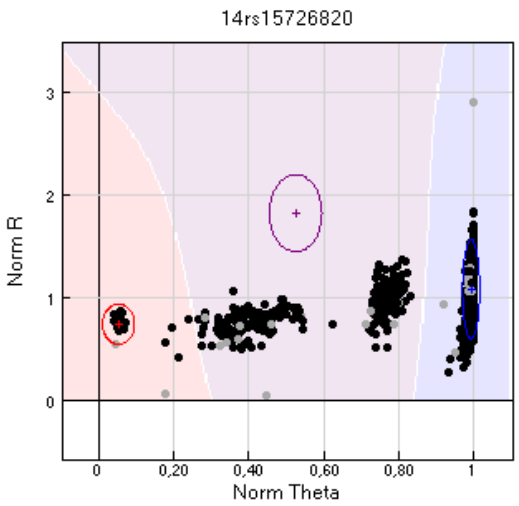
- 120 were monomorphic
  - 33 from chr 14
  - 87 from chr Z
    - hens: ZW, roosters:ZZ

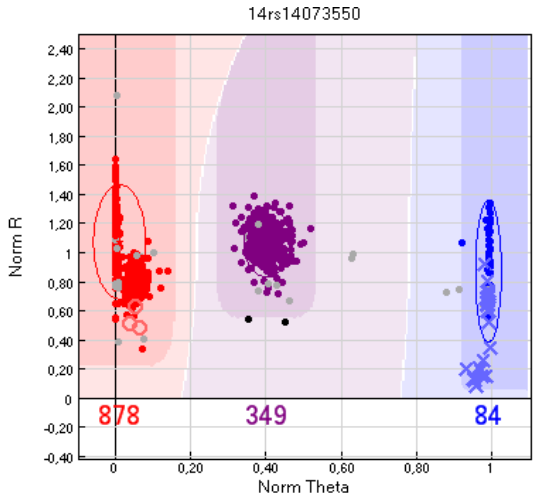


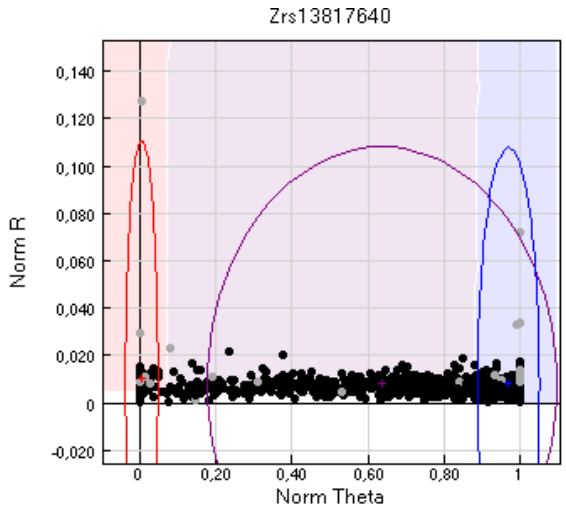



# RESULTS

- 53 SNPs failed
  - multiple clusters (12), heritability errors (10), poor signals (31)




- 5 individuals were in wrong families

# RESULTS

- MAF < 0.05
  - 24 SNPs
    - 13 from Chr14
    - 11 from ChrZ

- In summary: out of 384 SNPs
  - 211 were in good quality
  - 120 were monomorphic
  - 53 failed

# ISSUES

- 1 OPA set was from different synthesizing lot
  - created genotyping problems
  - can not be clustered with genotypes from other 4 OPA sets
- 1 plate worked poorly

→ 3 different genotyping project
  - One with genotypes from 4 OPA sets, one with genotypes from 1 OPA set and one plate separately

- family structure helps genotyping and SNP clustering
- replicates are useful
  - 3 to 5 per plate
  - costly
- technical reasons caused some errors
  - BeadXpress reader stopped during the run

# NEXT

- association analyses using different models
- genotype commercial chicken population using the same OPA we have already used and test the marker effects
  - 500 individuals
  - DNA extraction from feather sheaths using lysis
    - DNA quality
  - preliminary results show that DNA lysis works

# Thank You for Your Attention!