Development of high density (600K) chicken genotyping array

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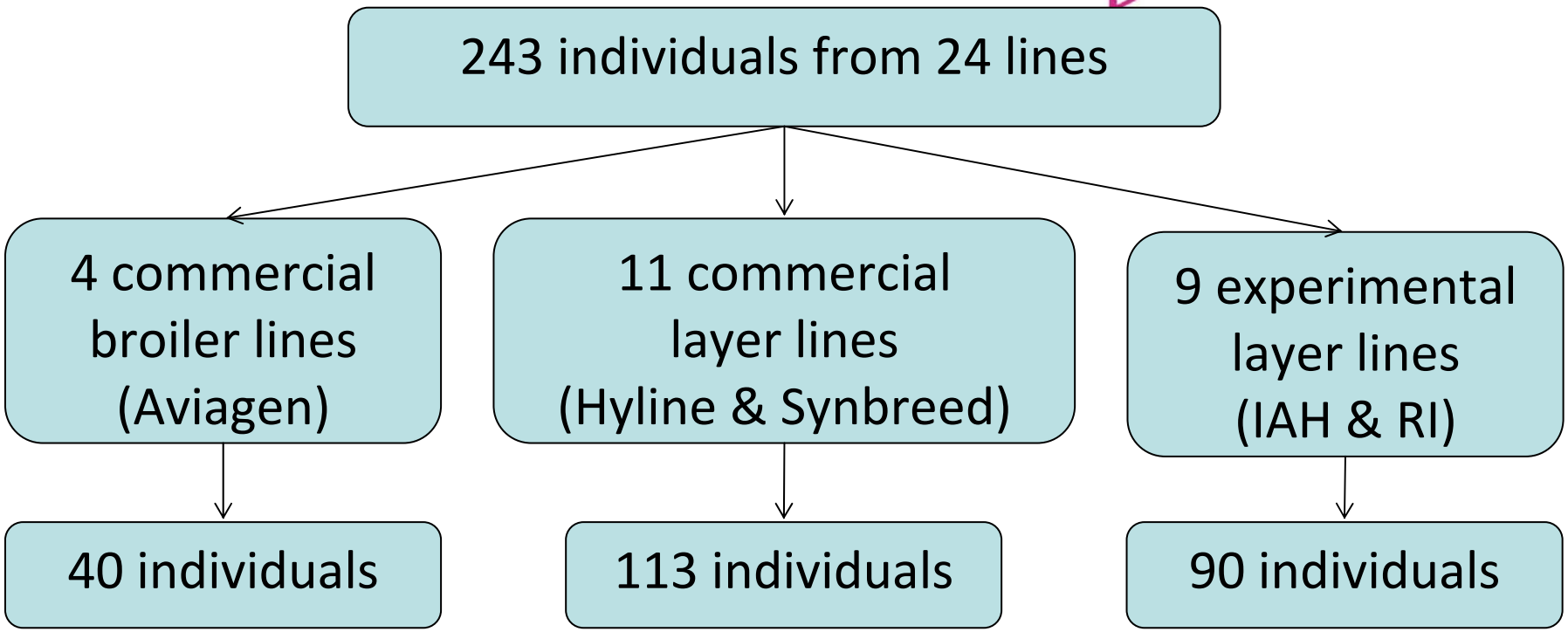
Steps in array development

- **Major steps:**
  - Re-sequencing of many chickens and detection of SNPs
  - Selection of SNPs for validation
  - Validation of selected SNPs
  - Final selection of 600K SNPs for array

Fig: Affymetrix Axiom™ genotyping array

Re-sequencing

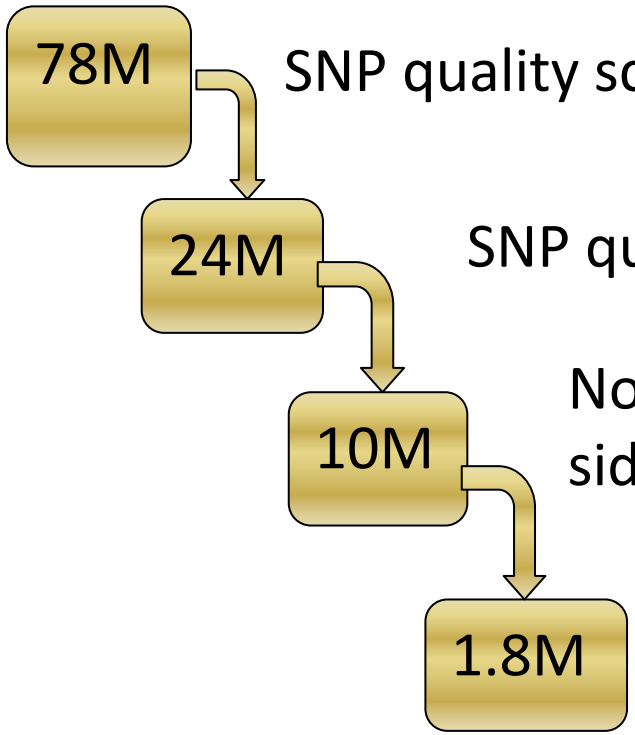


- Illumina high throughput sequencing
- 10-15 samples pooled within line
- Average depth of coverage: 7-17x per line

SNP detection

- Sequence reads were aligned to chicken reference genome (Gallus_gallus-4.0)
- SNP detection performed using Samtools (v .0.1.7a)
- SNP detection done by
  - Within line analyses: 78M SNPs
  - All lines together: 139M SNPs

SNP selection



SNP quality score ≥ 20

SNP quality score ≥ 60

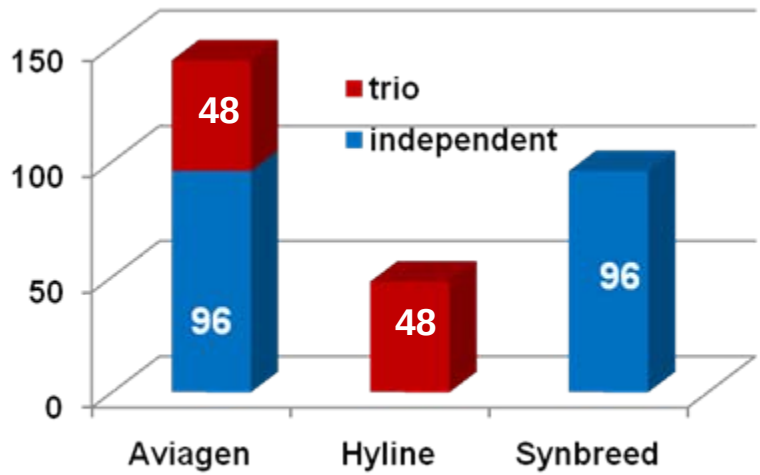
No other SNPs within 10bp on at least one side, uniformly spaced by physical distance

Predicted reproducibility of SNPs, uniformly spaced by genetic distance; 50:50 broiler & layer SNPs

Validation of 1.8M SNPs

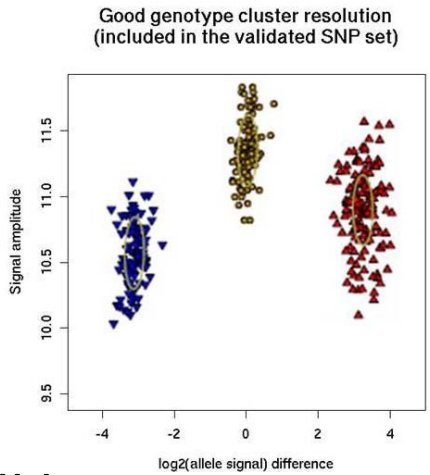
- 1.8M SNPs placed in three Affymetrix arrays for validation

- 288 individuals genotyped:
  - 3 broiler lines
  - 9 layer lines
  - 26 outgroup individuals



SNP validation criteria

- SNP must be polymorphic with ≥3 copies of minor allele
- SNPs must be robust
  - Genotype call rate ≥98%
  - Good cluster separation
  - Reproducibility
- SNPs have stable Mendelian inheritance
- SNPs must meet performance criteria across all lines

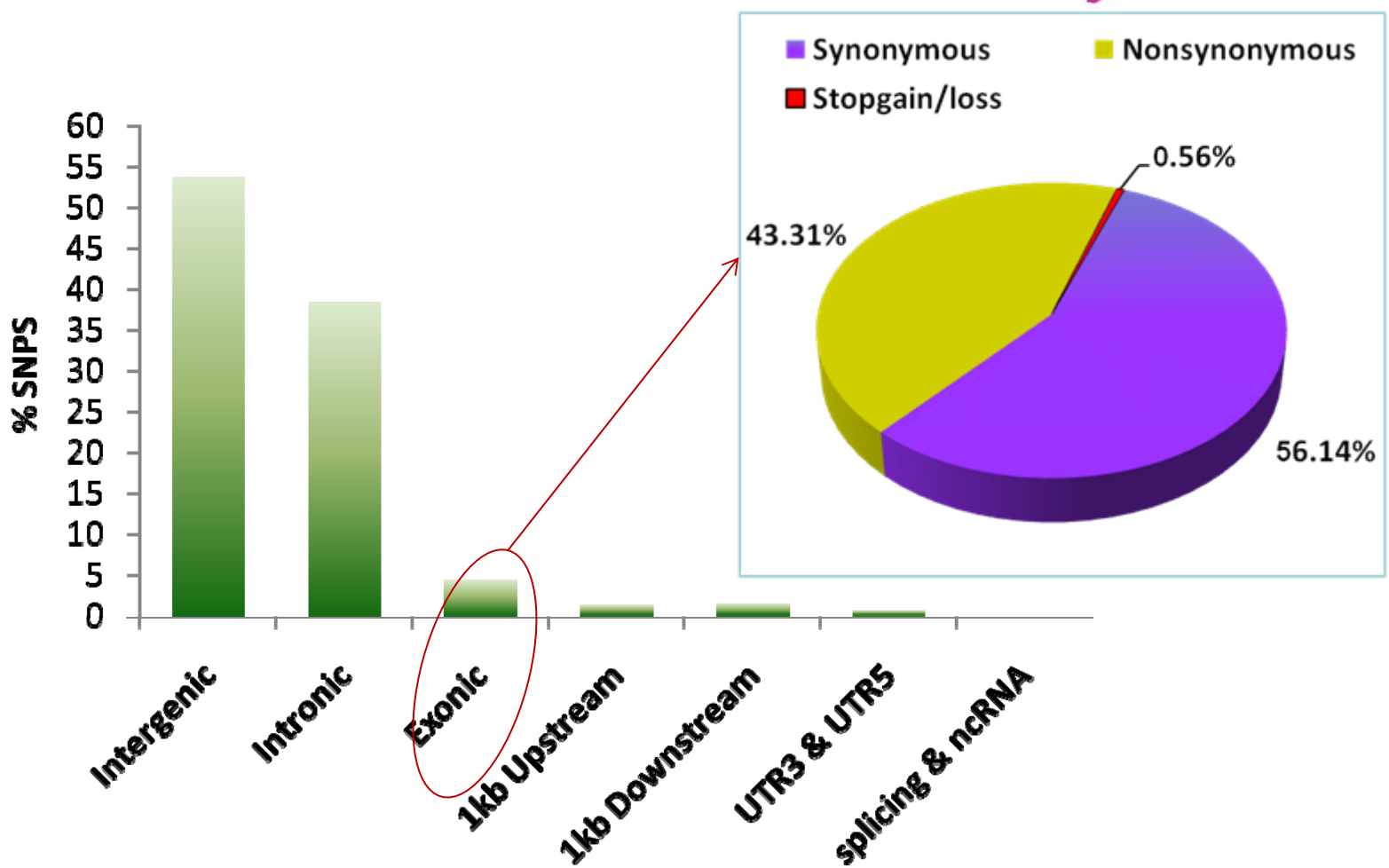


Polymorphic and robust SNPs	**1,187,482**	**65%**
Stable Mendelian inheritance	**1,176,808**	**99%**

Selection of final 600k

- Backbone was created by taking all validated SNPs within exonic gene regions (n=21,534)
- SNPs with extreme breach of HWE (P<0.00001) were removed.
- LD between adjacent pairs of SNPs were calculated (Golden Helix SVS) and the average broiler and layer LDs were used to decide the optimum balance of broiler and layer SNPs (3:2).
- Broiler and layer SNPs were selected to get as even spacing as possible in terms of map distance.

Annotation of 600K SNPs



600K SNP distribution

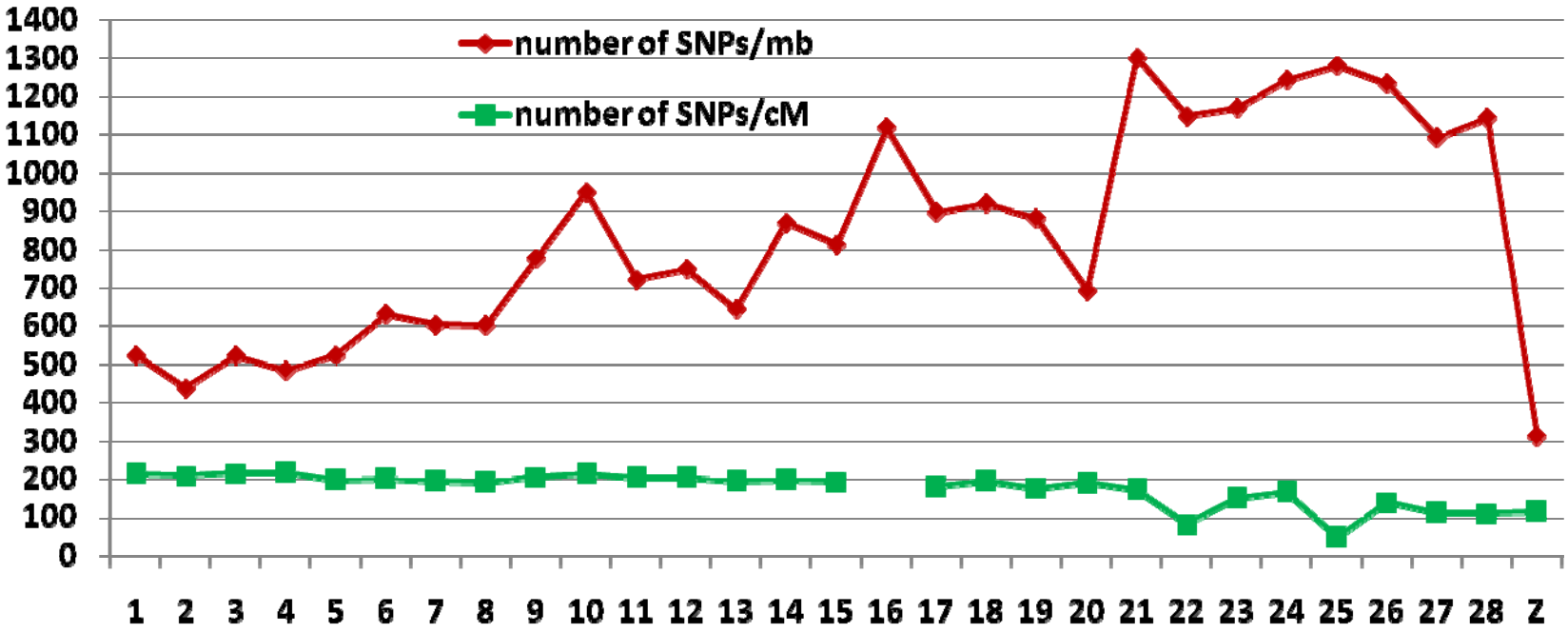
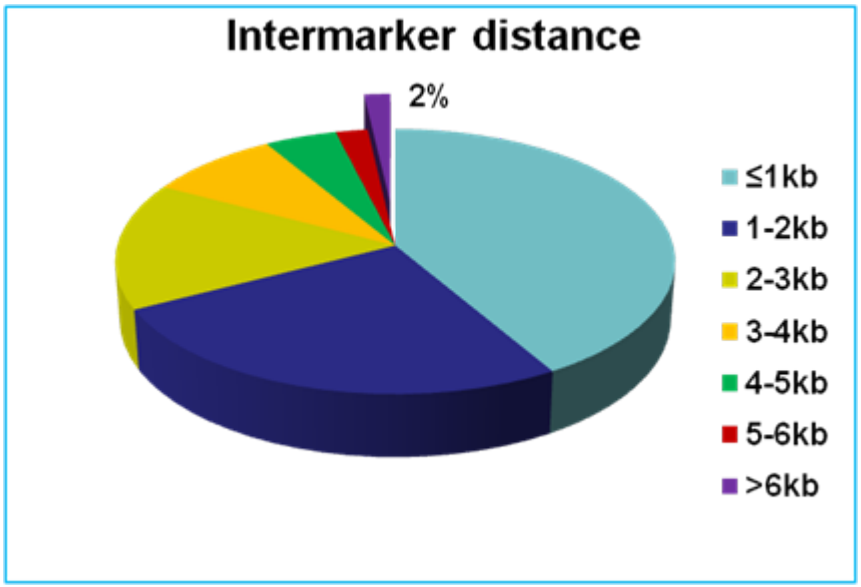
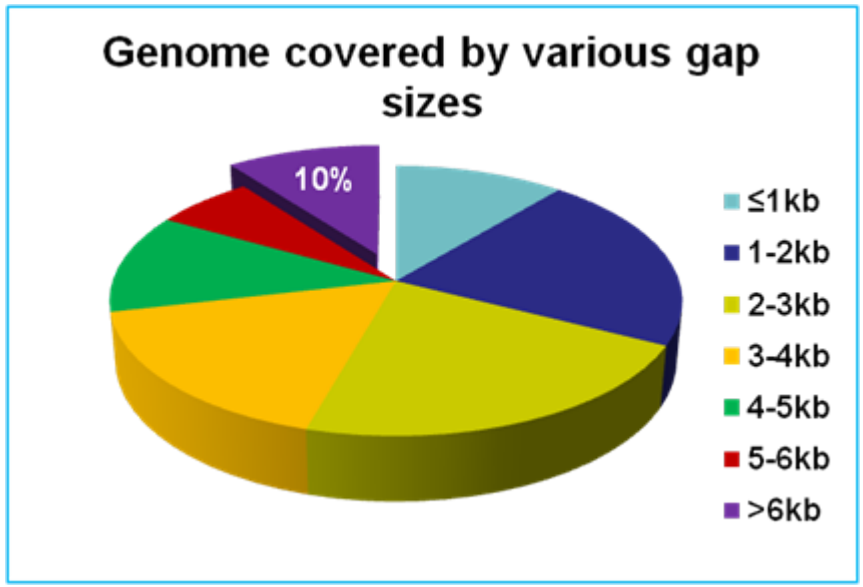


Fig 3: Distribution of SNPs across chromosomes in unit physical (mb) and map distance (cM)

Inter-marker spacing

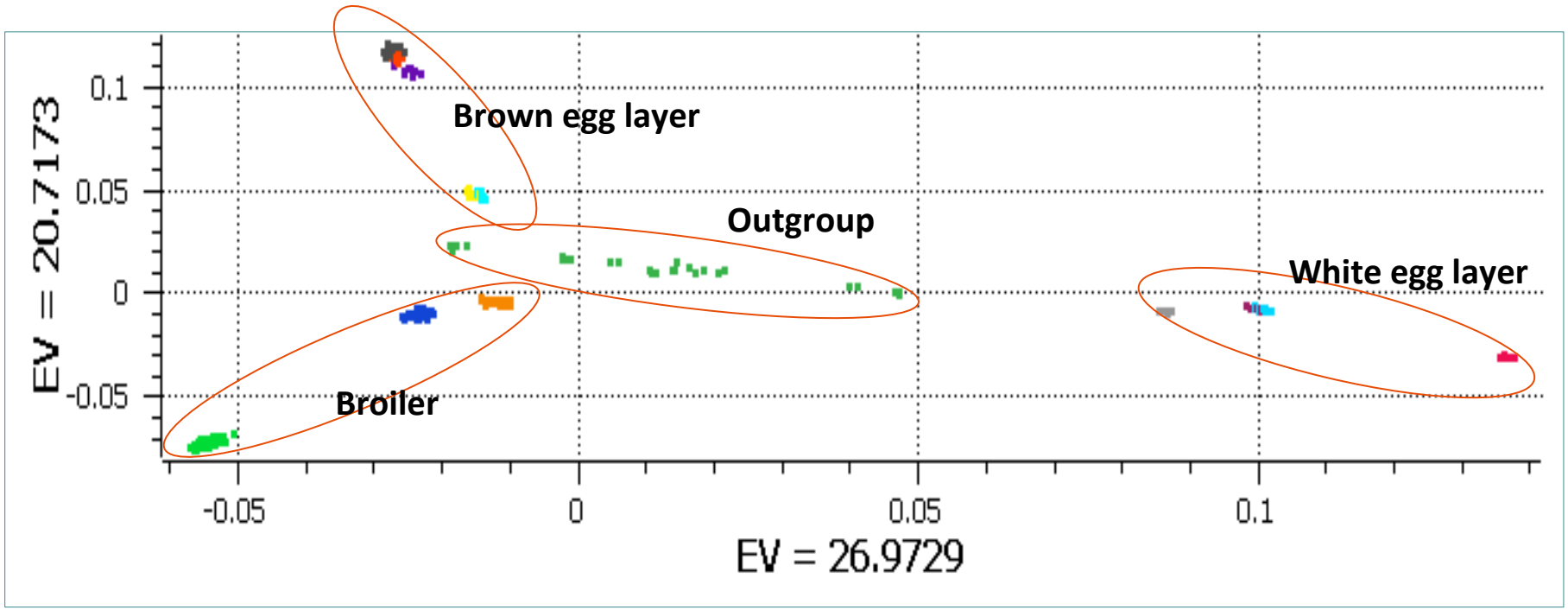




Mean gap size is 1,748 ± 5,274 bases

Principal component analysis

PCA analysis was performed to see if the markers can group the closely related individuals



Uses of high density SNP array

- Genomic selection
- Selection signature analysis
- Genome wide association studies
- For fine mapping of QTLs

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