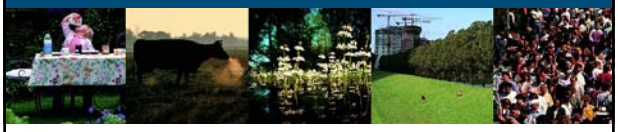
SNP discovery and analysis of selective sweeps using massive parallel short-read sequencing

Martien Groenen



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Research objectives

- Changes in the genome of farm animals and wild life species due to:
  - Speciation
  - Breed formation
  - Selection

SNP chips

Direct sequence comparisons

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SNP discovery: two scenario's

- Reference genome available
  - Pigs
  - Chicken
- No reference genome available
  - Turkey
  - Duck
  - Great tit
  - Tilapia
  - Goat
  - Salmon (planned)

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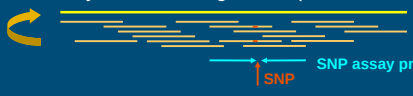
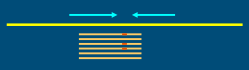
1: Without reference genome: 3 strategies used

(1) Assembly of reference genome (RRLs of 3000 bp fragments)



Turkey
Tilapia
Duck
Great tit

(2) Combination of and Illumina 454 reads



Pig and chicken (missing chromosomes)

(3) Long paired end Illumina reads covering complete fragment

Goat
Duck

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2: Reference genome available

- Pigs
  - Only 100,000 SNPs available before start of project and only a few with minor allele frequency (MAF)
  - 400,000 high quality SNPs + MAF
  - 60K Ilumina iSelect BeadChip
- Chicken
  - 3 million SNPs but no MAF
  - 350,000 high quality SNPs + MAF
  - 60K Ilumina iSelect BeadChip

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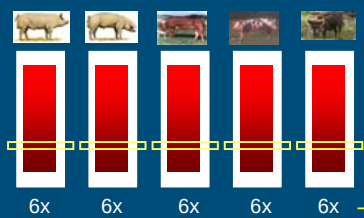
1: Reference genome available



- 5 Reduced representation libraries (RRL)
- Restriction digest of DNA

150-250 bp

6x 6x 6x 6x 6x → Variation within breeds

30x → Highly reliable SNP identification and overall MAF

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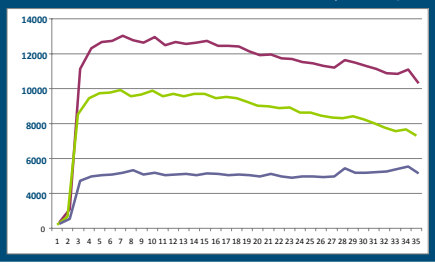
Align to reference genome and identify SNPs using MAQ

Estimated 10 % of genome covered; Total 400 million 36 bp reads

Distribution of SNPs across GA reads

- Total: 400,000 SNPs identified
  - 333,000 minor allele seen ≥ 3 times
  - 67,000 minor allele seen 2 times + sequence Q minor allele= 40



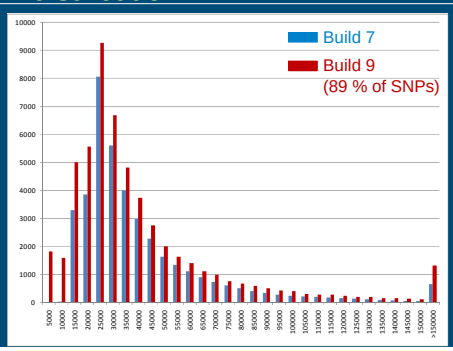
All porcine SNPs currently available

SNP source	Number
HQ Solexa SNPs WU*	333,000
454 sequencing MARC	110,000
7K iSelect chip AU-RI	5,500
dbSNP	17,700
INRA (Sanger seq)	61,700
Cambridge University	14,900
Total No SNPs	543,000
Total Unique SNPs	**510,000**

*Plus additional 58,000 SNPs where minor allele seen twice

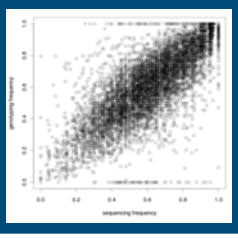
- TOTAL #SNPs submitted for the chip: 72,000
  - 70 % mapped on build 7
  - 7 % predicted
  - 23 % unmapped

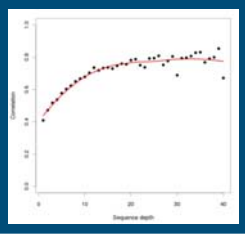
SNP distribution



Porcine 60K BeadChip: Excellent performance

- Final total number of SNPs on the chip: 62,163
- Number of SNPs with MAF>0.05 in at least 1 breed: 59K
- Conversion rate overall: 94.8% (Illumina GA SNPs: 96 %)
- Good correlation between allele count based on GA sequencing and Geotyping



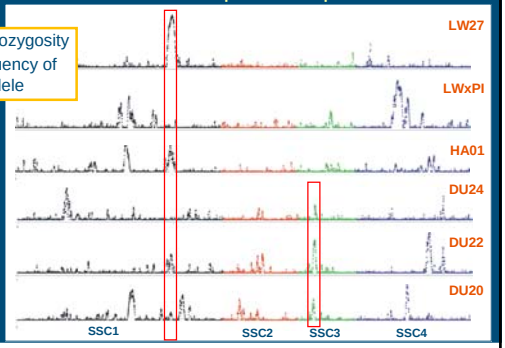


The porcine HapMap project

~1700 Individuals from European and Asian breeds, Wild boar from across Europe and Asia and museum samples

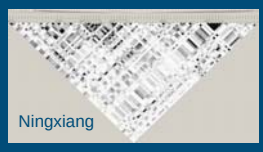
Identification of selective sweeps: Examples on SSC1-4

- Low heterozygosity
- High frequency of derived allele



Use of the 60K chip: European vs Chinese breeds

- Biased towards common variants
- Biased towards SNPs in European breeds
- SNP density of the 60K chip too low for Chinese breeds



Amaral et al. (2008) Genetics 179: 569

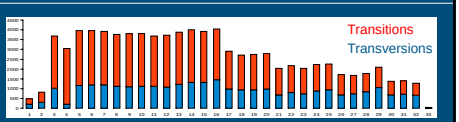
From SNP typing to resequencing

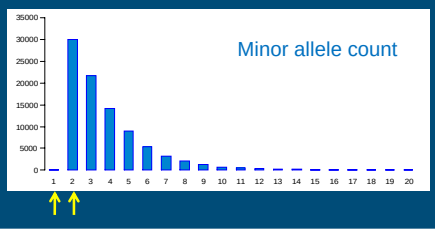
(1) Site frequency spectrum analysis based on RRL sequence data

- RRL cover 5-10 % of the genome
- Coverage differs between breeds
- 4 commercial white breeds + Wild Boar
- Data from pools
- Within 500 Kb windows estimate:
  - Watterson's estimator: $\theta = f(S, n)$.
  - Tajima's estimator: $\pi = f(S, n, freqs.)$.
  - Tajima's D: $D = (\pi - \theta) / sd$.
  - Fst measures population differentiation

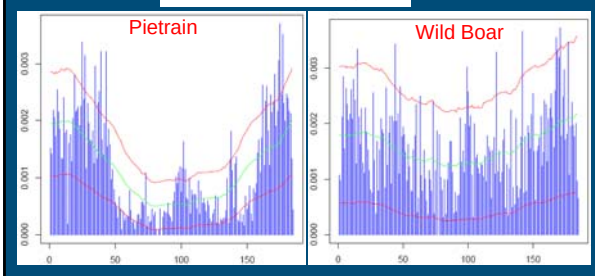
Reanalyse data per breed

SNP distributions





Variation of nucleotide diversity



Suidae tree

~ 1 MY
~ 2 MY
~ 4 MY
~ 10 MY
~ 50 MY

Sus scrofa
Sus barbatus
Sus cebrifons
Sus celebensis
Sus verrucosus
Potomochoerus l.
Potomochoerus
Phacochoerus a
Babyrous
a
Pecari tajacu

Species	Average heter.
Sus scrofa	23 %
Sus barbatus	3 %
Sus celebensis	2 %
Sus cebrifons	0.8 %
Sus verrucosus	0.6 %
Phacochoerus	0.8 %
Potomochoerus l.	0.7 %
Potomochoerus p.	0.9 %
Babyrousa b.	0.9 %

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Sequencing of Sus Verrucosus (Java warty pig)


~2 My

- 8x on Illumina GA
- 200, 500 and 3000 bp libraries

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Swine genome sequencing consortium

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