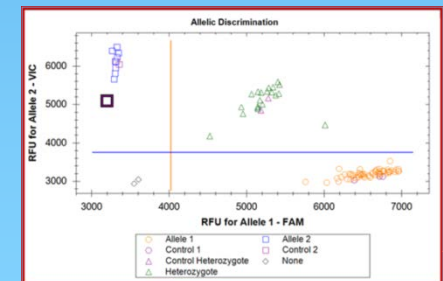
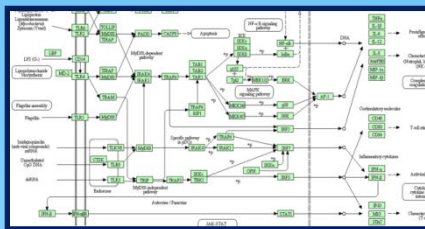
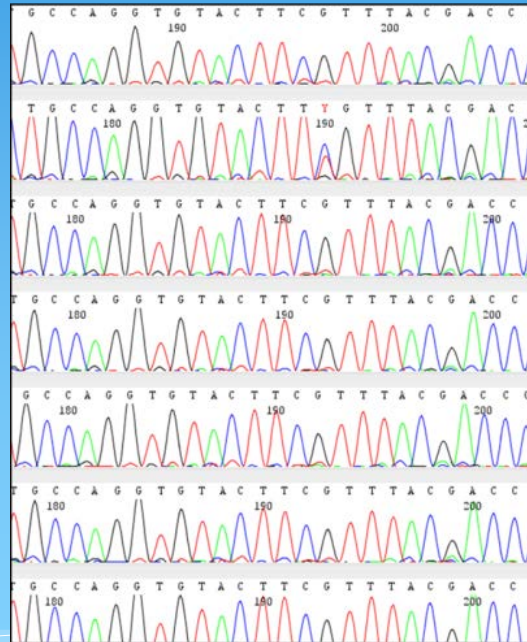


Breeding sheep for parasite resistance: What traits to measure? Phenotypes and Genotypes



Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

**Animal Production and Health Laboratory,
IAEA Laboratories, Seibersdorf**

Why breeding sheep for parasite resistance?

- Helminthiasis (disease caused by gastro-intestinal nematodes) is the most important livestock diseases worldwide
- Use of anthelmintic drugs is increasingly regarded as unsustainable due to emergence of multiple drug resistant parasites
- Integrated parasite management strategies – Breeding sheep for parasite resistance is an important long term component objective to reduce the dependence on drugs for control



Why breeding sheep for parasite resistance?

- Acquisition and expression of immunity against GI nematodes is genetically controlled and varies between breeds and between individuals within breeds

Between breed variation

Red Maasai vs Dorper

Barbados Black Belly vs INRA composites

Garole vs Deccani

Santa Ines vs Suffolk

Ile de France vs Poll Dorset

Within breed variation

- Genetic resistance to parasites is a heritable trait
- Scope for selection of sheep for nematode resistance



Genetic components of traits

Genetic components related to inheritance of parasite resistance characteristics include

Breed

Heritability

Genotype X Environment interaction

Correlations with other traits of economic importance

Host-parasite interaction



Focus of Genetic Selection and Improvement

Selection focus

Resistance - Tolerance/Resilience

Resistance – Ability of the host to resist infection

Tolerance - Host is infected by pathogen but suffers little with adverse effect

Selection should be focused on reducing the transmission of infection (i.e. resistance) rather than reducing the clinical signs (resilience/tolerance)



What traits to measure?

Identification of the most appropriate indicator trait (phenotype) for parasite resistance is difficult

Measures of resistance

Fecal egg count, Worm burden, Worm size and Fecundity

Measures of immune response

Eosinophilia, Antibodies such as IgA (CarLA), IgG and IgM

Measures of impact of infection

Anemia (PCV, FAMACHA), gastrin, pepsinogen or fructosamine concentrations

Measures of resilience

Growth rate, Treatment frequency



What traits to measure? Fecal Egg Count (FEC)

Fecal egg count (FEC)

- Most within breed studies of genetic resistance use FEC as indicator trait
- Extensive within breed variation and significant heritability reported in small ruminants

Sheep

$h^2 = (0.149)$ Avikalin

$h^2 = (0.24)$ Muzafarnagri

$h^2 = (0.4-0.5)$ Katahdin

Goats

$h^2 = 0.13$ (Galla and East African)

$h^2 = 0.11-0.16$ (Jamunapari)

$h^2 = 0.37$ (Creole, French West Indies)



What traits to measure? Packed Cell Volume (PCV)

- Packed cell volume is a measure of RBCs and indicates the level of anemia in animals
- PCV has been used to evaluate within breed variations and one of the useful indicators for parasite resistance/resilience in sheep/goat
- Heritability estimates vary between 0.12 to 0.31 (e.g. Santa Ines sheep, composite of Dorset, Romney and Finn sheep)



What traits to measure?

FAMACHA

- FAMACHA system was initially introduced to manage haemonchosis in sheep and goats using targeted selective treatment (Vanwyk and Bath, 2002)
- FAMACHA scoring is based on the correlation between level of anemia and the colour of eye mucous membrane
- FAMACHA scoring is a practical and relatively easily obtained phenotype
- Riley and Vanwyk (2011) proposed genetic evaluations based on FAMACHA scoring combined with simple penalties
- Predicted breeding values based on FAMACHA scores can help to improve resistance and/or resilience with the ultimate objective of producing animals that survives and produce without deworming

What traits to measure? IgA (CarLA)

- Immunoglobulin IgA – Isotype closely associated with intestinal mucosal responses
- Prevents larvae from establishing in the gut and resulting in rapid expulsion
- Commercial saliva test available (CarLA) www.carlasalivatest.com

CarLA – *T. colubriformis* L3 carbohydrate surface antigen

- IgA antibody response to CarLA challenge
- Positively associated with resistance to parasites: High CarLA animals have low FEC and improved growth

Phenotype ontology – An issue for consideration?

QTL_symbol	Trait_name
FECGEN	Fecal egg count
FOC	Fecal oocyst count
HFEC_1	Haemonchus contortus FEC1
HFEC_2	Haemonchus contortus FEC2
IGE_2	Immunoglobulin E nematode challenge 2
LATRICH_2	Abomasal Trichostrongylus sp adults and larvae challenge 2
LSITRICH_2	Small Intestine Trichostrongylus sp adults and larvae challenge
NFEC	Nematodirus FEC
NFEC_1	Nematodirus FEC1 (August)
NFEC_2	Nematodirus FEC2 (September)
NFEC_3	Nematodirus FEC3 (October)
NFEC_AVE	Nematodirus FEC Average
SFEC	Strongyle FEC
SFEC_3	Strongyle FEC3
SFEC_AVE	Strongyle FEC average
TC_IGG_2	Trichostrongylus colubriformis serum IgG challenge 2
TFEC_1	Trichostrongylus colubriformis FEC1
TFEC_2	Trichostrongylus colubriformis FEC2

(Animal QTL db,
<http://www.animalgenome.org/cgi-bin/QTLdb/index>)



Genetic markers for selection

Genetic markers

QTLs associated with parasite resistance

MAS (Marker assisted selection (QTLs),
candidate gene markers

Genomic selection

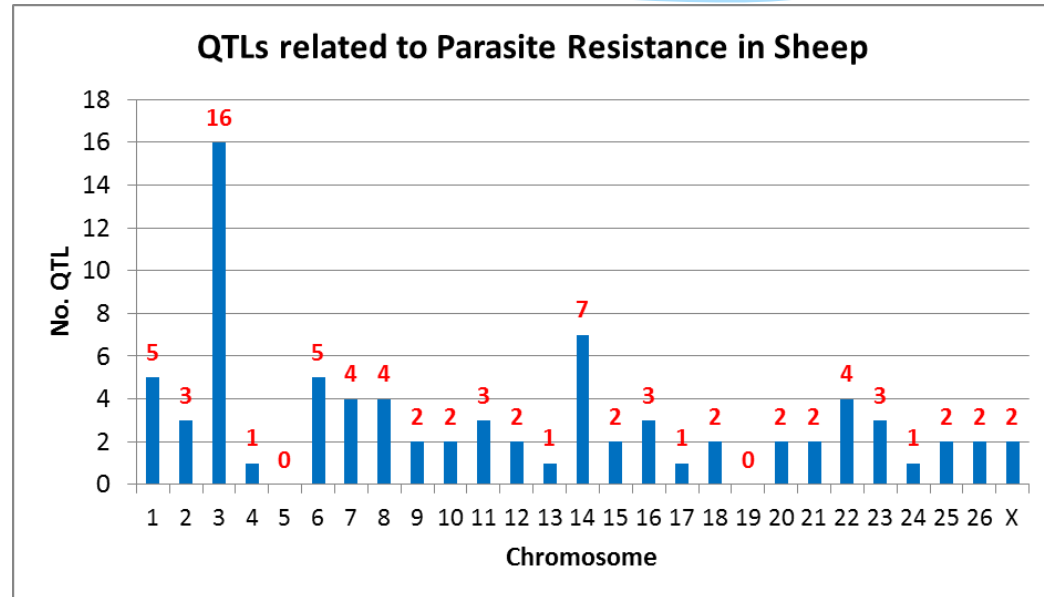


QTLs for parasite resistance in sheep

(Animal QTL db, <http://www.animalgenome.org/cgi-bin/QTLdb/index>)

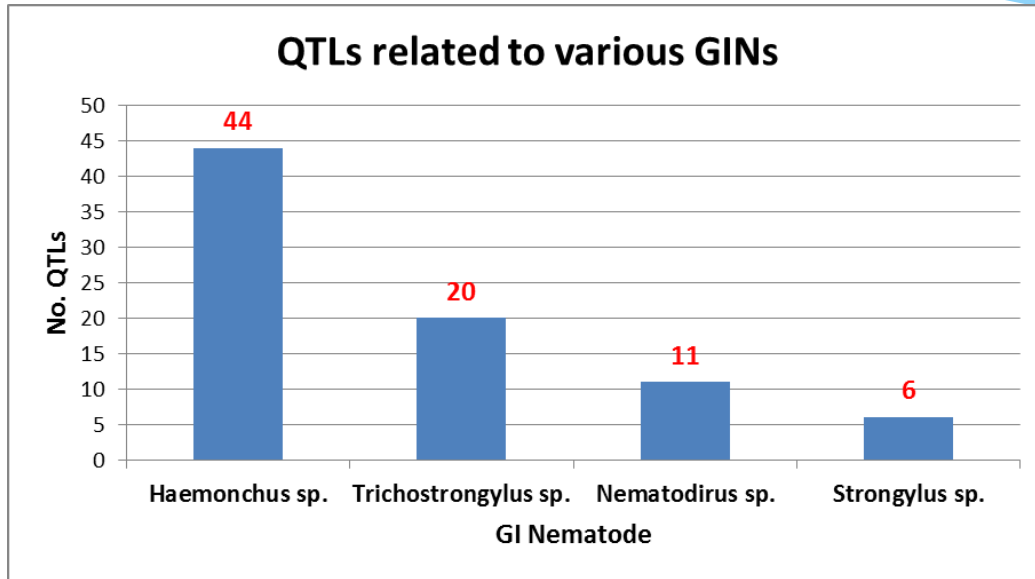
Sheep QTLs

- 753 QTLs from 86 studies in sheep for various economic traits
- 81 QTLs related to parasite resistance
- Chromosome 3 with 16 QTLs followed by Chromosome 14 with 7 QTLs
- QTLs related to parasite resistance are w.r.t. Haemonchus, Trichostrongyles, Strongyles and Nematodirus)



QTLs related to various GINs in sheep

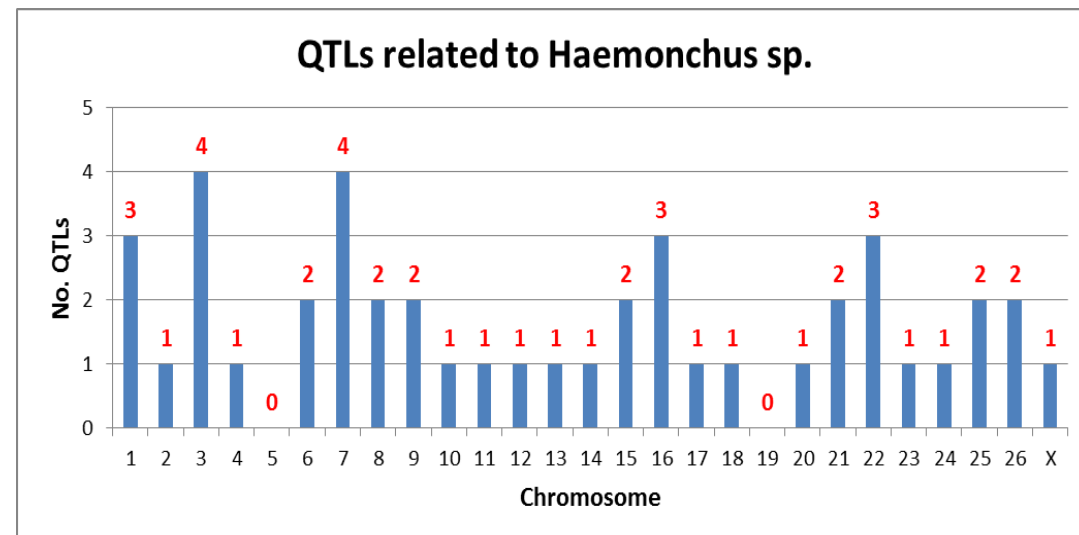
(Animal QTL db, <http://www.animalgenome.org/cgi-bin/QTLdb/index>)



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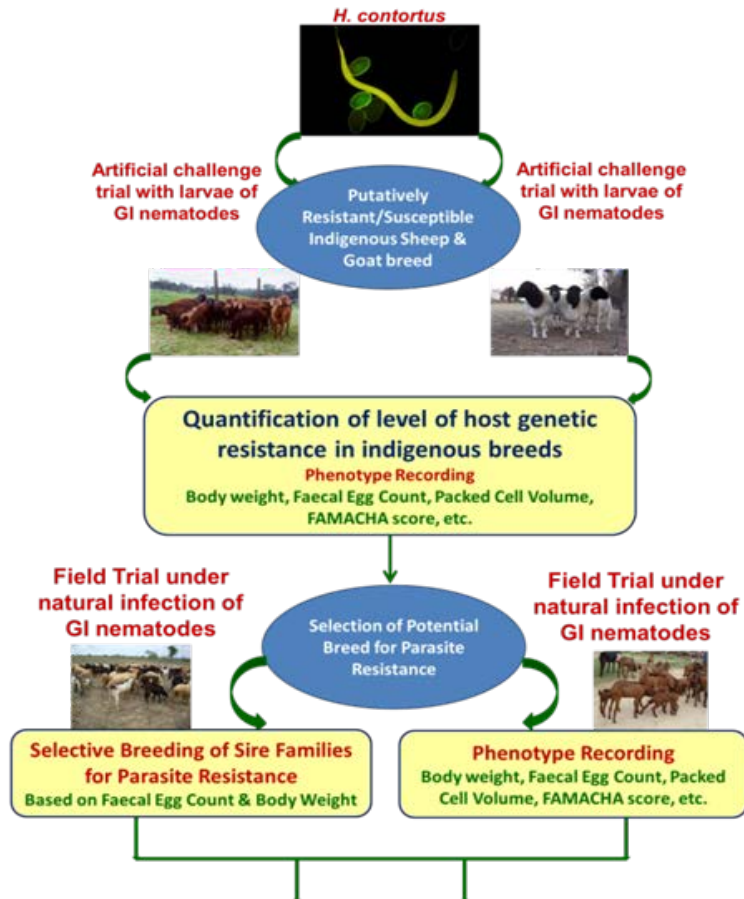
**QTLs for HFEC = 44
(Haemonchus Fecal
Egg count)**

- 4 QTLs – Chr 3, 7
- 3 QTLs = Chr 1, 16, 22



Candidate Gene Markers

Conceptual Outline of Coordinated Research Project



Candidate genes involved in innate and adaptive immune pathways

Genome Wide Association Study (GWAS)

- Genotyping field samples with 50K SNP chip to assess whole genome variation
- Detection of Selection Signature within sheep & goat genome for parasite resistance
- Whole genome association with phenotype

Candidate Gene Study

- Targeted re-sequencing of candidate genes involved in immune pathways, pathogen recognition receptors, adaptive immune response, etc.
- Detection of functional allelic variants
- Association of allelic variants with phenotype



Candidate genes re-sequenced for SNP discovery in Sheep

- ~80 candidate genes were re-sequenced in a panel of eight unrelated sheep
- Candidate genes
 - Pattern recognition receptors
 - Toll like receptors
 - NOD like receptors
 - RIG I like receptors
 - C type Lectin binding receptors
 - Cytokine genes (e.g. Interleukins, Interferons)
 - Ovine Histocompatibility genes



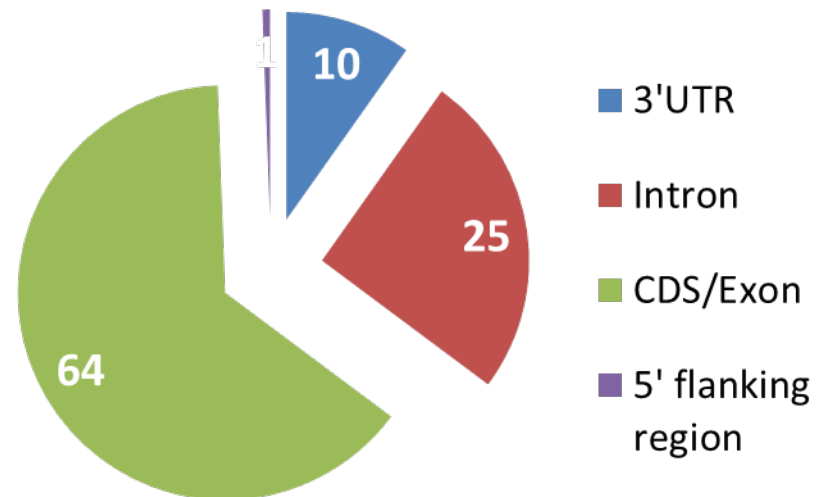
SNPs identified in Sheep



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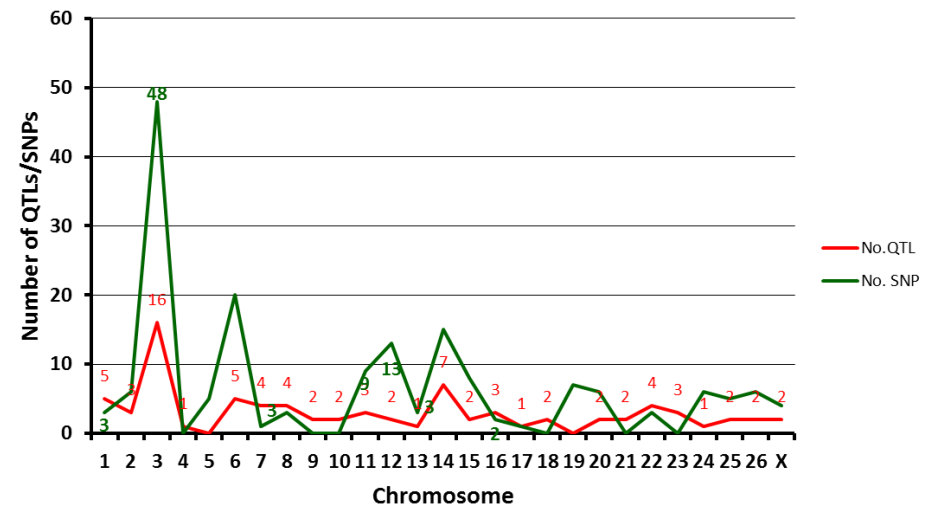
- 208 SNPs were identified
- 174 KASP SNP assays were developed
- No. SNPs per candidate gene varied from 1 to 9
- Among CDS/Exon SNPs, 51 were non-synonymous and 61 were synonymous

Genic Region	No. SNPs	% SNPs
3'UTR	17	10
Intron	44	25
CDS/Exon	112	64
5' flanking region	1	1

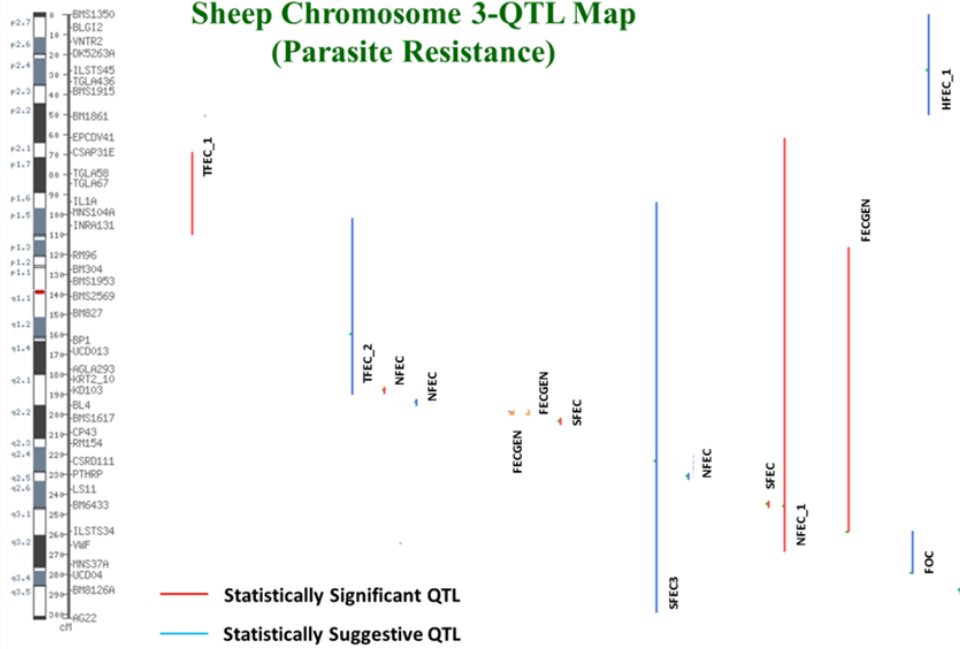


Distribution of SNPs in Sheep genome

Chromosome	No. SNPs	Chromosome	No. SNPs
1	3	15	8
2	6	16	2
3	48	17	1
4	0	18	0
5	5	19	7
6	20	20	6
7	1	21	0
8	3	22	3
9	0	23	0
10	0	24	6
11	9	25	5
12	13	26	6
13	3	X	4
14	15		



Sheep Chromosome 3-QTL Map (Parasite Resistance)



- Significant number of the identified SNPs (48) are located in chromosome 3
- Many are involved in different immune pathways

(Animal QTL db, <http://www.animalgenome.org/cgi-in/QTLdb/index>)

Candidate Gene Study

Goat

- **Candidate genes involved in innate and adaptive immune pathways**
- ~72 candidate genes were re-sequenced in a panel of eight unrelated goats
- Candidate genes
 - Pattern recognition receptors
 - Toll like receptors
 - NOD like receptors
 - RIG I like receptors
 - C type Lectin binding receptors
 - Cytokine genes (e.g. Interleukins, Interferons)
 - Caprine histocompatibility genes



SNPs identified in Goat

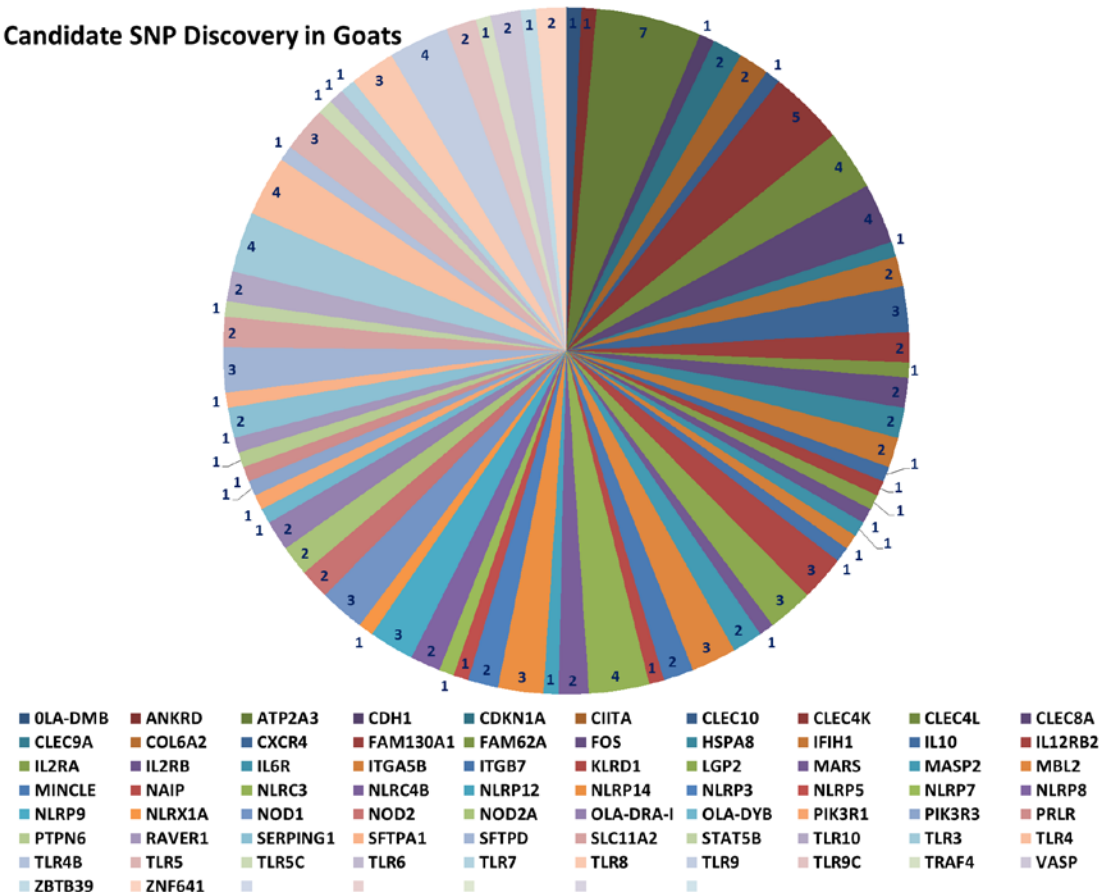
- 187 SNPs were identified
- 141 KASP SNP assays were developed
- No. SNPs per candidate gene varied from 1 to 7



IAEA

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Candidate SNP Discovery in Goats



Status of genotyping sheep samples from RCHs

Country	Species	Samples arrived @ IAEA	Samples genotyped	No. SNP per sample	No. genotypes generated	Genotyping in progress
Brazil	Sheep	721	679	157	106603	42
Argentina	Sheep	1234	895	157	140515	339
Iran	Sheep	309	309	157	48513	0
Indonesia	Sheep	203	152	157	23864	51
Ethiopia	Sheep	423	113	157	17741	310
Burkina	Sheep	225	140	157	21980	85
Total	Sheep	3115	2288		359216	827

Additional samples from Austria, Bulgaria, India, Sri Lanka, Iraq, Peru and Pakistan have been genotyped

Status of genotyping goat samples from RCHs

Country	Species	Samples arrived @ IAEA	Samples genotyped	No. SNP per sample	No. genotypes generated	Genotyping failure
China	Goat	288	280	141	39480	8
Sri Lanka	Goat	567	518	141	73038	49
Bangladesh	Goat	233	227	141	32007	6
Nigeria	Goat	306	84	141	11844	222
Total	Goat	1394	1109		156369	

Additional samples from Myanmar, Austria, India and Pakistan have been genotyped

Genotyping Method Employed for Candidate gene SNPs

KASP Genotyping



↓ Design allele-specific primers (nucleotides 1–17)



↓ PCR with **ASP1** or **ASP2**
+ conserved primer (CON)



Competitive allele specific PCR (KASP Genotyping)



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KBioscience



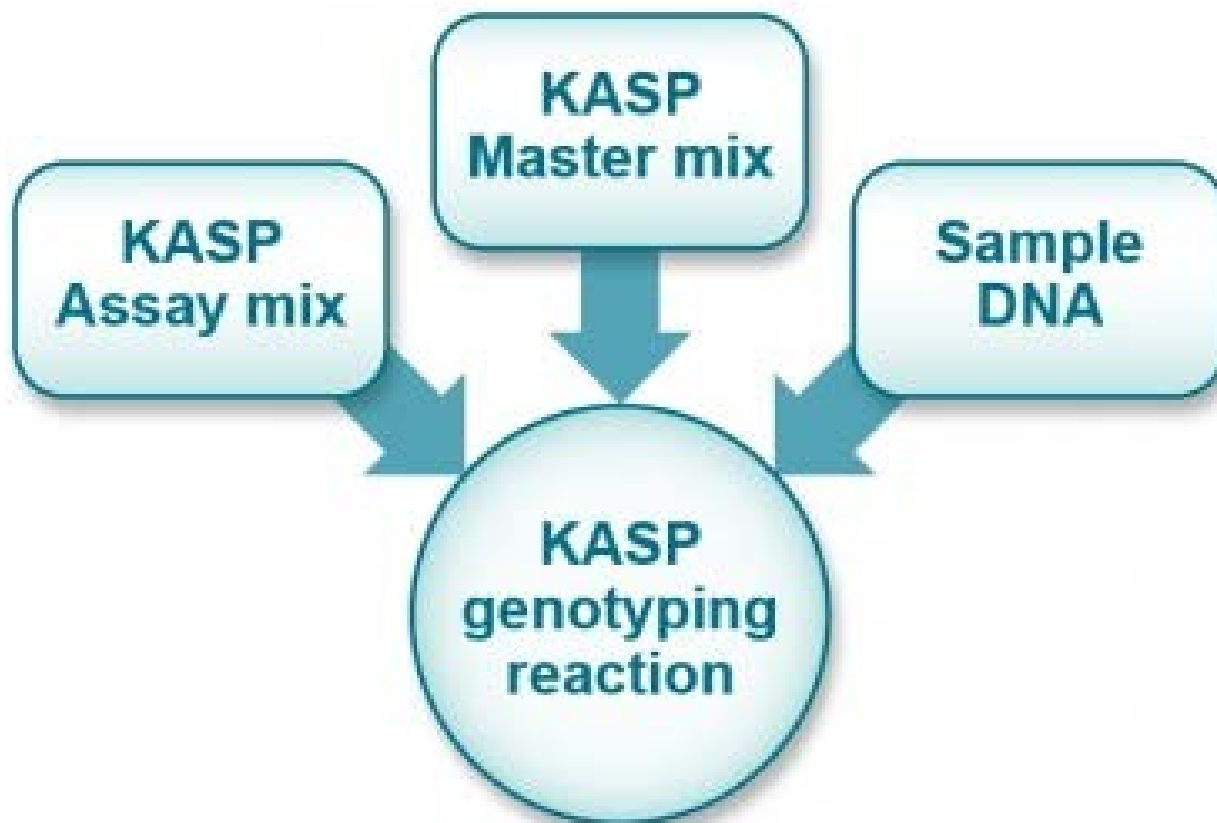
KASP for SNP Genotyping

- PCR-based KASP genotyping assay is a homogeneous, fluorescence (FRET) based assay that enables bi-allelic discrimination of known SNPs and InDels.
- KASP genotyping chemistry requires no labeling of the target-specific primers/probes

KASP Genotyping



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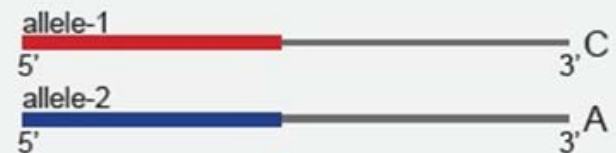
KASP Assay mix



- Two allele-specific primers (one for each SNP allele). Each primer contains a unique unlabelled tail sequence at the 5' end.
- One common (reverse) primer.

A) Primer mix

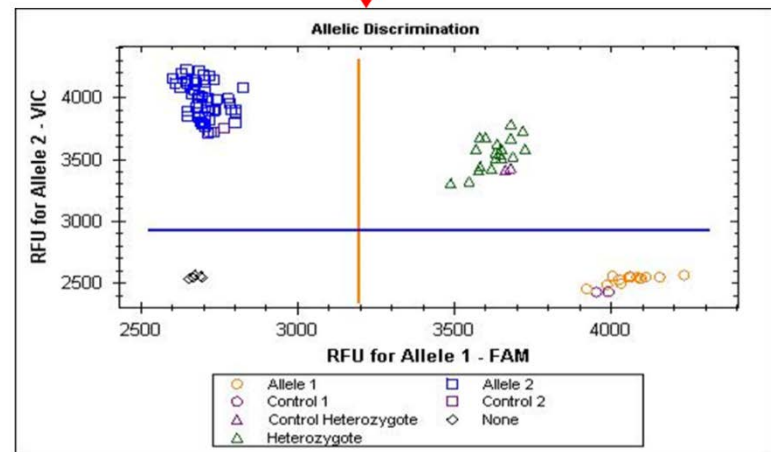
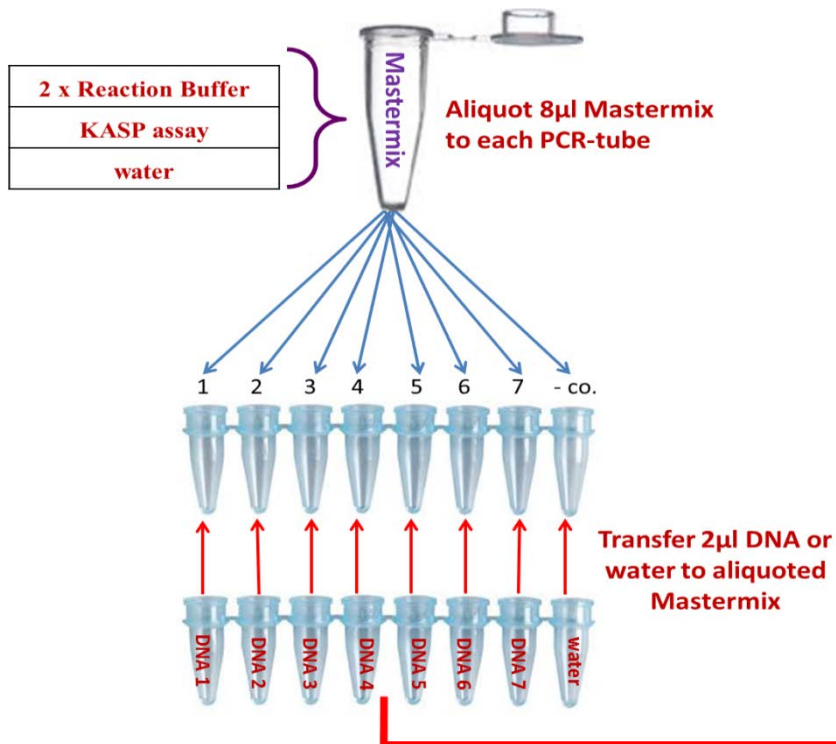
Allele specific forward primers:



Reverse primer:



Work Flow of KASP Genotyping

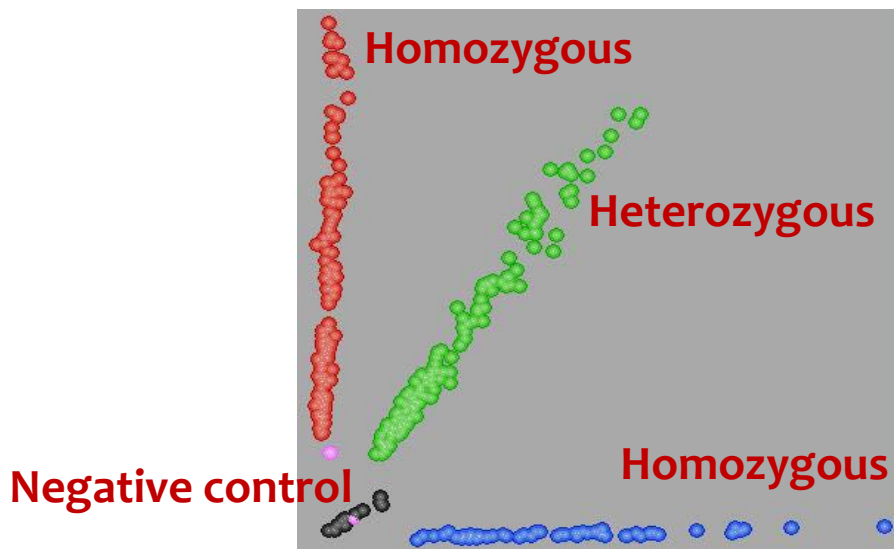


Graphical viewing of genotyping data

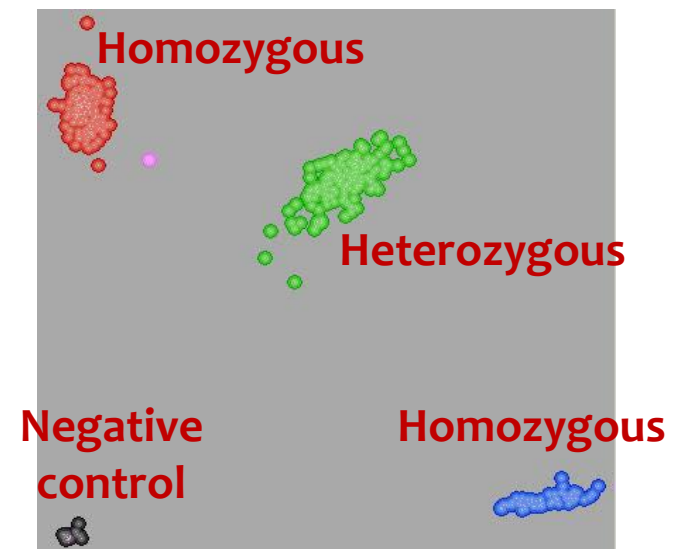


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- The fluorescence data of each sample from both the dyes attached to SNP alleles can be plotted to visualize the cluster of genotypes.
- Can be visualized in Kluster Caller or Allele Discrimination module incorporated in real time PCR systems



Without Passive reference dye (ROX)



With Passive reference dye (ROX)

Genome wide Association Study

- Custom designed 60K SNP array from Affymetrix will be used for genome wide SNP typing
- First set of 96 samples (48 samples from the tail of phenotypes; low and high FEC) from Argentina has been genotyped
- 384 additional samples will be processed with representation from indigenous breeds of different countries



Thank You

