final report

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SheepGenomics Parasite Subprogram

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Executive summary

The Parasite Subprogram combined the sheep resource flocks from CSIRO Livestock Industries, Sydney University and the University of New England with skilled animal geneticists and animal and molecular biologists from the 3 organisations, Monash University and PIRVic. The Subprogram focussed on 5 major activities:

1. Confirmation of 3 worm resistance QTL for the generation of DNA markers;
2. A suite of at least 5 confirmed candidate genes for worm resistance in sheep;
3. Alternative(s) to faecal egg counts as diagnostic measures of worm resistance;
4. Serum biomarkers as indicators of parasite resistance
5. Establishment of gene characterisation technologies to enable gene modulation strategies in a second-round Sheep Genomics

Results from the subprogram are presented in some detail in the body of the text, but the priorities for future (combined strategy) research (section 6) are summarised below from the proceedings of an HRIP workshop at MLA in May 2010. These were:

1. Genetic Markers

Focus on *in-silico* GWS genetics which can be validated and readily implemented. The result would be prioritisation of SNP for inclusion on the 5K array if M4's OAR3 parasite trait haplotype stands up within the INF data. Similarly complete a concordance analysis of FMFS linkage results with those obtained within the ISGC HapMap data. This would seek to overlay 'selection signature' based results with the 'within family' results already in hand from FMFS. Again, the result would be SNP which look interesting for key traits (parasites, carcass and poll) and which could be included on the 5K array.

2. Meta-Analysis for Integrated Genomics

The targets should be for new therapeutics/drugs. This should be conducted to look at genes/regulators which work on both genome/genotypic values and more importantly PHENOTYPIC measures especially in defined infections in clean backgrounds. To approach this, existing data sets in HRIP projects need to be completed and linked with global domain data sets in sheep and other species.

3. Protein biomarkers- validate and establish utility for parasites and disease.

Two integrated approaches are needed- (blood/ serum/plasma) markers prior to infection (marking/ weaning) and markers post-infection. Studies can be conducted on existing samples and in specific trials to examine straight utility (existing samples in ROs, FMFS and CRC) and gather information of robustness and timing (pen/pasture controlled trial using deliberate trickle and natural pick-up)

A combined strategy is envisaged using current priority markers from several projects including a range of (up to the 15) innate immune mediators that have flexible sampling times, grehlin, KCNJ15, DUOX, trefoils CARLA (NZ). Note that these could be used as predictive, associative or indicative markers. Lead up work is needed to assess/ generate assays for proteins or peptides (eg Grehlin/KCNJ15) and to examine the utility of "signature" peptides/ proteins in Mass-specs approaches using non-depleted serum or whole blood. Bio-assays (antibody) would work on whole or freeze-thawed blood.

We need to get (both):

1. The biomarker panel validated in samples with clean /defined infections and preferably worm burden data as well as FEC data for assessing the “best case scenario” that we can expect from combining all markers.
2. Establish how the sensitivity and specificity of biomarker data performs in flocks with mixed/unknown infections both from timing and nature of infections (as the worst-case scenario under field conditions)
3. Note added by DE. Advise that negotiations should be commenced for CARLA studies in Australia as this may be useful in areas of relatively endemic parasitism (ie relatively stable seasonal parasite challenge). Sheep CRC may have samples?

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1. Industry context at the outset of SheepGENOMICS- Gastrointestinal parasitism and the sheep Industry

Parasitism with 3 main gastrointestinal nematodes (GIN), *Haemonchus contortus*, (Barber's pole), *Ostertagia (Teladorsagia) circumcincta* (small brown stomach worm) and *Trichostrongylus colubriformis* (black scour worm) are the principal animal health constraints for the Australian Sheep Industry, costing around $370m annually in control and lost productivity or 8.7% of its total value in 2006 (Holmes & Sackett, 2006). In fact between GIN, flies and lice, the total cost was more than 15% of the value of the Industry. Integrated parasite management (IPM) schemes such as Drenchplan, Wormkill and Wormbuster programs which were developed to minimise the infective doses of parasites and their effects on production, relied on strategic and effective anthelmintic treatment as the cornerstone of control measures. With the development of widespread anthelmintic resistance in the New England region (*Haemonchus)* and WA (*Ostertagia*) (see issues of "*Turning the Worm*"), another component of IPM programs, the breeding of parasite resistant sheep, became a top priority. In this context, sheep breeders were encouraged to submit worm egg count trait data in Industry databases such as SheepGenetics Australia, MerinoPlan and LambPlan. Concomitantly, several research organisations (CSIRO, USyd, UNE, DAWA-Rylington) undertook genetic selection based on parasite resistance in sheep flocks based on quantitative weaning and hogget faecal (worm) egg counts (fec/wec) with substantial progress in genetic mapping and EBVs. Animals from these genetically-selected resource flocks were crucial to the strategic plan of the parasite subprogram.

In this context there were a number of key targets to which the sheepGENOMICS parasite program was directed:

- development of genetic, genomic and protein markers for resistance traits as expressed by faecal (worm) egg counts (wec/fec) and other measures after infection;
- assessment of alternatives to fec as more accurate phenotypic measures of parasite burdens and resistance; and,
- development of therapeutic approaches to manipulate or augment resistance mechanisms.

The Industry imperative for parasite markers (as delineated by Alex Ball) was for ...

"An indirect estimate (marker or phenotypic or genomic parameter) of worm resistance that:

- can be taken at a young age,
- can be used in all environments and
- covers all worm species and
- doesn't require infection".

OR, paraphrased for the HRIP subprogram was:

"what is the best (combination of) measures for worm resistance that can be supplied to Industry"?

2. Major targeted outputs from the parasite Subprogram

a. establishment of a complementary genetic and genomic pipeline of technologies and resources directed towards gene discovery, gene/protein expression and gene function in sheep genetically-selected for parasite resistance and susceptibility;

b. QTL, DNA and genomic markers for parasite resistance traits;

c. identification of the key biomarkers of parasite resistance in serum of Merino sheep; and,

d. development of modulatory treatments that enhance the induction and maintenance of parasite resistance.

3. Structure and strategic focus of the Program

The subprogram utilised genetically selected resource flocks to focus on genetic, genomic and proteomic discovery in sheep both before and following worm infection by:

a) identifying QTL and markers for key parasite resistance traits including fec, liveweight gain and haematocrit to enable selection of animals at an early age;

b) identifying key genes and their functional pathways involved in the induction of parasite resistance at the various stages of larval establishment, worm growth and fecundity and rejection of adult worms after infection of sheep;

c) identifying the key serum and mucosal biomarkers differentiating resistant from susceptible sheep; and,

d) identifying therapeutic means of manipulating innate or acquired resistance.

These activities are summarised in Figures 1 & 2.

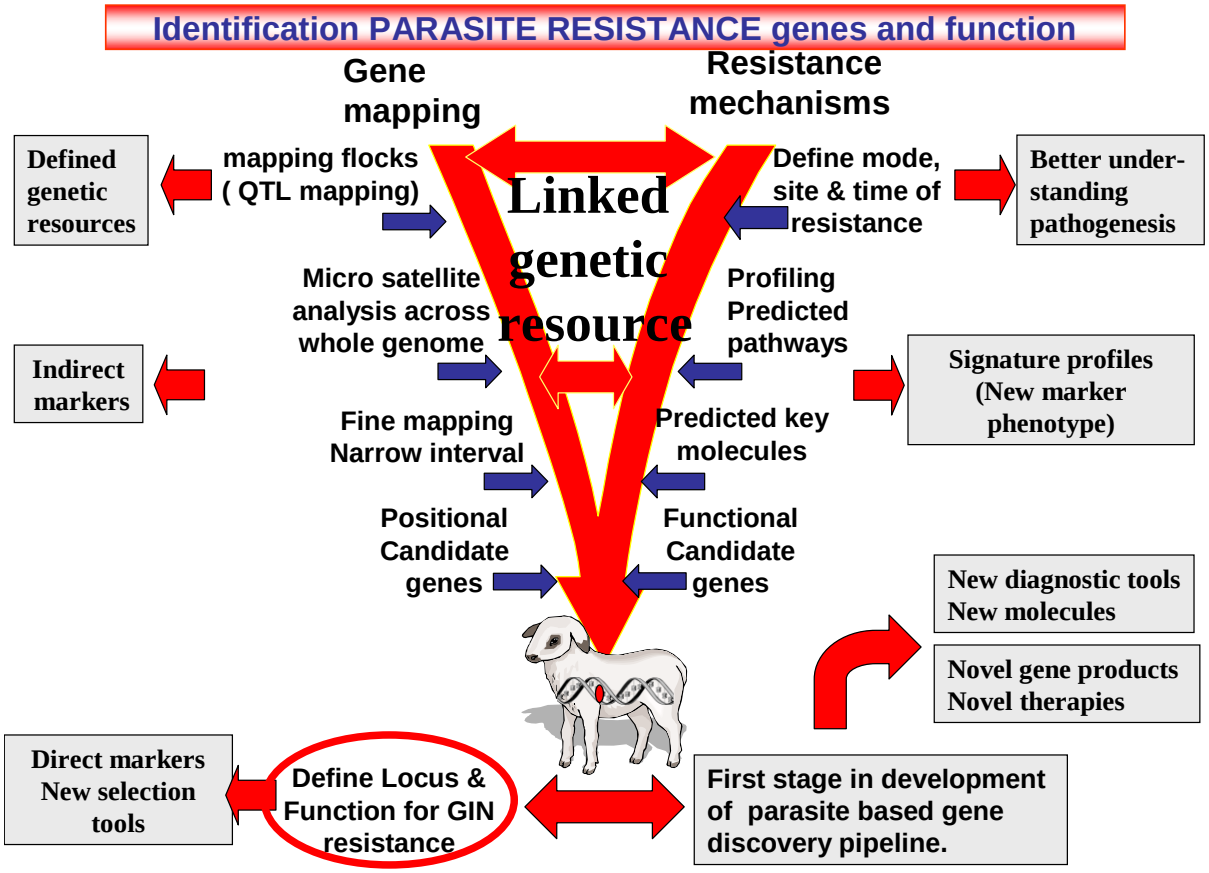

Figure 1. The original strategic plan of the parasite program involving convergent genetic, genomic and proteomic approaches based on analysis of parasite resistance after infection of genetically-selected and selectively-bred sheep resources. It was anticipated that mutual confirmation from genetic and genomic approaches would give greater certainty to outcomes and provide a mechanistic foundation for intervention.

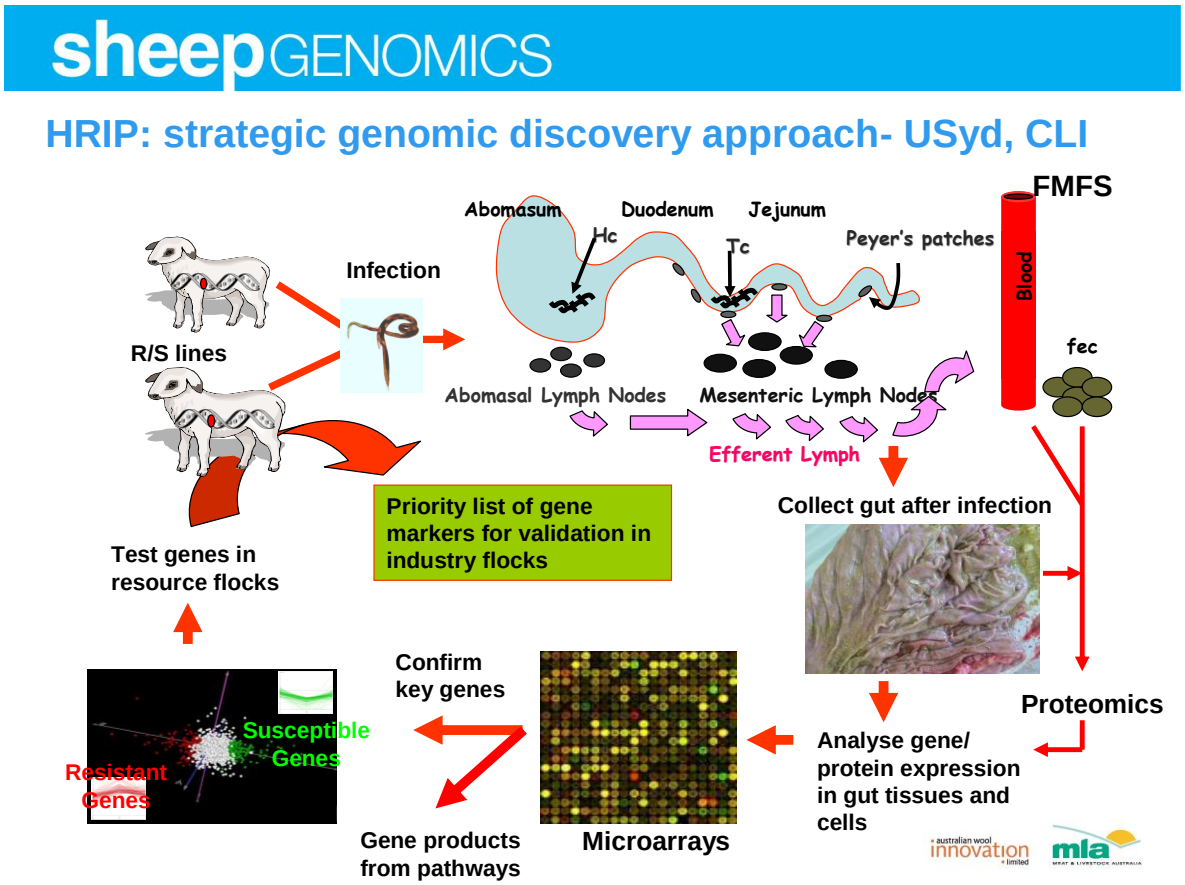

Figure 2. The strategic approach to analyse sequential gene expression in resource flocks following parasite infection and attempt integration and confirmation with Falkiner (FMFS) results using "Industry-relevant" samples from faeces and blood

Strategically, several decisions regarding the operational plan were made to facilitate research with different parasites, each with different host-parasite biology. *Haemonchus* (Hc) and *Trichostrongylus* (Tc) spp., which colonise abomasum and small intestine, respectively, were used in single (innate resistance) and multiple infections (acquired resistance) and using trickle (continuous) and bolus infections. It was also important to establish methodologies for the analysis of gene expression (quantitative, spatial, temporal), and assessment of gene function *in vitro* and *in vivo* as well as methods for high-throughput screening of gene products. This was achieved over the first 18 months of the subprogram due to a lack of human expertise together with reduced numbers of breeding stock in resource flocks to provide experimental animals.

- A collaborative team of researchers with high credibility in parasite genetics and biological research was established (see below).

- A tissue bank of ovine gut tissues and sera was acquired following infections with Hc and Tc in sheep flocks bred for resistance/ susceptibility to Hc (HSF) and Tc (TSF) (at CLI- SG205,6) as well as from offspring from a specific breeding flock from high EBV (fec) Coopworth sires x susceptible Merino (TSF) ewes (at USyd- SG204). The tissue and serum bank was augmented by samples following sequential infection of 5200 progeny of the FMFS mapping flock with Tc and Hc parasites. It should be appreciated that the logistical demands and ethical considerations of the sampling of resource flocks and FMFS dictated that single dose infections with identical protocols were used in the FMFS infections and for the primary infections at CLI (SG205). These are compromises for the experimental "continuous" or "trickle" infections used

in the selection indices for genetic selection and QTL mapping in the resource flocks and in data emanating from “natural” field infections in Industry and in the Sheep CRC.

- Reagents for detection of all elements of ovine mucosal tissue were amassed. These included a panel of 30 monoclonal antibodies and staining techniques to identify the majority of cells in ovine gut tissues and blood as well as enabling possible fractionation and collection from cellular suspensions or blood (SG203).
- Initial microarray analyses were conducted using the Dairy CRC “immune array” (SG205-6) where 120 “candidate” genes representing ovine mucosal immunity, inflammation, cytokines and intestinal growth and repair were included in the array. Subsequently Affymetrix bovine gene expression arrays were deemed suitable for sheep by Core-tech (Tellam et.al, MLA milestone report FG520) as a “global” gene expression tool and robust methods for quantification of candidate genes in gut tissues and blood leucocytes were developed using MAS, RMA (random mixed association) and GC-RMA analyses within the SG biostatistical team (Cedric Gondro was the principal link for HRIP).
- To examine whether differential antibody responses where phenotypic indicators of parasite resistance, Tc17 kDa, ESA1 (or gp30) and *H.contortus* antigens Hc24 kDa, gut antigen 1(GA1) and metalloproteinase 1B (MEP1B) antigens were cloned and expressed. Isotypic immune responses from resource flock animals were assayed after infection with both parasites (SG219).
- An Hc larval establishment assay was developed for assay of gut and serum products (SG222, 232) and an ovine intestinal cell culture system was investigated for high throughput screening.
- A genetics project team was established and for the first time, genotyping results from resource flocks at USyd (ITT, RxM), CLI (RxM, TSF, HSF) and UNE (Golden Ram) were pooled to identify 3 principal QTL regions for parasite resistance (SG226-8).
- A analytical team of biostatisticians was established through Core-tech program.

4. The participants in the Parasite Subprogram

At various (and not always contiguous) times, the following research organisations and personnel were involved in the subprogram.

Research Organisation	Researcher	Technical staff	Postdoctoral Fellows	PhD Scholars & students*
University of Sydney	Prof Herman Raadsma A/Prof David Emery Prof Nick Sangster Dr Ken Beh	Mr Luke Chappel Dr Renee Rawson	Dr Anne Lehnert Dr Tony Rowe Dr Natasha Ellis Dr Sayet Katsis	Nathan Quigley* Verity Ambler* Kristy Mann* Paul Partland* Ms Findy Au*
University of New England	Prof Julius van der Werf Dr Karen Marshall	Ms Lisa Mascord	Dr SH Lee	
Monash University	Dr David Peidrafita	Dr Garry Barcham	Dr Joanna Kemp	Nick Robinson Cecelia

Research Organisation	Researcher	Technical staff	Postdoctoral Fellows	PhD Scholars & students*
		Ms Jane Lydall		Rodriguez*
CSIRO LI	Dr Ross Windon Dr Peter Hunt Dr Laurie Piper Dr Aaron Ingham Dr Kritaya Kongsuwan Dr Sonia Dominik Dr Sandra Eady	Ms Moira Menzies Mr Callam Mack Mr David Callan Ms Jody McNally Mr Peter Josh	Dr Nick Andronicos	Catherine Ryan* Marcus Keil*
PIRVIC	Dr Matt McDonaugh	Mr Steve Binos		

5. Project area summaries and outputs

5.1 Genetic markers (SG226-8)

For the identification of 3 major QTL involved in worm resistance, microsatellite marker analysis was conducted on 5 resource flocks with historical background mapping research namely: Golden Ram (UNE), TSF/HSF and Romney x Merino selection flock (CLI), Indonesian Thin tail (ITT) x Merino backcross (75% Mer, USyd). A comparison of designated QTL regions were tabulated with selection criteria of;

- occurrence in more than 1 resource flock,
- LOD score >1.5 and
- “robustness” in statistical methodology,.

These data and criteria identified 2 QTL on Chr 1 and 3, and a possible 3rd on Chr 6 with measurable and significant effects.

A comparison of QTL studies in NZ where Hc has limited presence (before any effects of climate change), found that only QTL´s are on Chr 3q (around IFN-g) and Chr 20 (DQA-1) were reasonably strongly supported. The HRIP QTL on Chr 3 was also in the 3q region (Fig 3). Catapult (NZ) has released a “Fectest” based on DQA-1 genotyping but the veracity for Australia has yet to be determined. Indeed the utility of a “stand-alone” parasite genotyping test in an era of WGS is questionable.

Further fine-mapping using microsatellite markers was superseded by the development of the 60K SNP panel through the ISGC and around 1500 SNP markers relevant to the HRIP QTL regions were selected from the panel. DNA from 2200 animals (on 24 plates) were sent to John Hopkins University and assayed by Illumina in early 2009. The financial crisis in Nov-Dec 2008 reduced the AUD by 30% and the number of plates which could be analysed within budget from 24 to 16 plates. Data was provided to SG biostatisticians in May 2009 (owned by SG). Concomitantly, subsets of the data from each contributing resource flock families was provided to the respective contributing Institution (CLI, USyd, UNE) for individual analysis. Of the 1536 SNPS attempted, data was released from 1380 SNPs and the illumine analysis deemed 1286 of “high quality” while 1257 were accepted by SG (Cedric’s report). The overall data was therefore considered of “high quality” and the association analysis was performed on phenotypic indicators including fec/wec (Hc/Tc) and haematocrit (Hc only) by PirVic (Ben Hayes).

Whole genome scanning was also considered as a discovery technology for novel QTL with the distinct intention to integrate the HRIP SNP analysis with the Coretech FMFS genotyping data (since progeny from the "Golden Ram" progeny and a CLI RxM sire [P4] were also present at FMFS and were subjected to the parasite challenge and phenotyping).

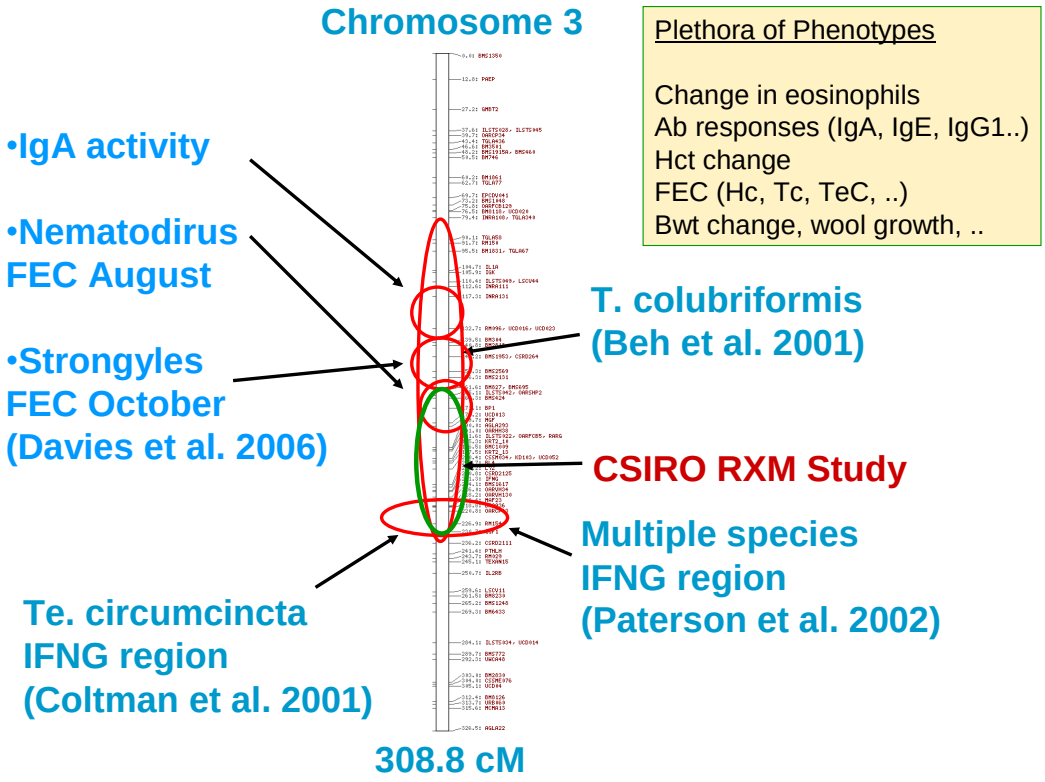

Figure 3. The current evidence for a parasite resistance QTL on Ovine chromosome 3 (from Peter Hunt)

5.2 Genomic analyses (SG204-6, 215; extended to SG229, 235-6)

Three linked projects examined differential gene expression following worm infection. The significant differentially-expressed (DE) genes were subjected to ontological ("pathway or cluster") analysis to identify pathways which could provide metabolic products that could inform biomarker analysis as well as modulatory strategies (eg ref 7).

- In SG215 and Hc infection, mRNA expression levels of 3 genes were identified (DQA2, IGHE and EBP2). These were cross-validated in the Res/Susc WBC samples for SG204-CLI resource flocks where the DQA2 gene transcript was expressed at higher levels in the resistant line of sheep. However there was a large variation in the level of this gene between individuals, making it an unreliable biomarker candidate. DQA2 mRNA expression levels may be a useful marker in unchallenged animals. The IGHE and EBP2 gene transcript levels are not consistently different between the resistant and susceptible lines.
- In SG204, nine priority genes were identified and subjected to sequential analysis in FG3 where samples were collected weekly during a primary infection. DE genes with "down-flat-flat" or "up-flat-flat" expression profiles (ie consistently up or down-regulated over an extended time for Industry), were selected for validation in SG215. Four top candidates were identified (Ghrelin- chr22, KCNJ15-chr1, SETBP1-chr24,

PON3-chr7). It is noted that only one of these was present on the candidate QTL regions (chrs 1 and 3).

- The upstream region of each of the 4 priority candidates was located in an attempt to define the potential promoter regions. These regions were compared to each other using the MEME program to define any regions of conserved sequence, indicative of a common regulator. Three different motifs were found in each of the 4 upstream sequences. Each of these regions was also searched for conserved transcription factor binding sites using the TF-SEARCH. Bioinformatic analysis of the promoter region of our 4 priority candidates (ghrelin, KCNJ15, PON3 and SETBP1) revealed 3 conserved sections of sequence present in the upstream region of each of these genes. Such conserved sites may indicate that the genes are under control of a global regulator.
- In SG222, the development of an *invitro* larval establishment bioassay (ref 11) also indicated from genomic analyses that Galectin 15 was expressed markedly higher in tissues from *Haemonchus*-resistant sheep and reflected in protein levels in the assay media. This may potentially be an effective marker for the resistance status of sheep (but of course will not be elevated except in currently challenged sheep). This marker was kept in mind when proteomic results were examined.

The genomic initiatives were halted when SG2 funding was not forthcoming and HRIP efforts concentrated on genetic (SNP) markers and biomarkers in serum.

5.3 Proteomic analyses and biomarkers (SG207, 219, 231)

1. Antibody markers

- Despite exhaustive analysis of ovine antibody responses against worm extracts or a panel of individual or combined recombinant antigens from both Tc and Hc, no responses could differentiate resistant from susceptible sheep. Antibody titres of IgG, IgA and IgE isotypes increased faster in response to infection in resistant animals, but the levels did not presage resistant genotypes. This was not surprising as single or multiple antigens identified using differential reactivity of reagents from immune/naïve or res/susc animals could not be used successfully in vaccines. However, Tc CARLA was not examined as a marker, and a discovery approach was used as the technical expertise in proteomics matured.

2. Discovery approach using MUDPIT analysis of depleted serum (see below)

6. Outputs and Strategies/ recommendations for further work

(Summarised from the May 2010 workshop at MLA)

6.1. Industry felt need, issues and the value proposition for diagnostic tools that provide an alternatives to Worm Egg Count (WEC)

Noted from Alex Ball with IFN flock and CRC data

- Sire ASBV's scores for WEC range from -70 to +200 and across breeds and populations, heritability for the trait, even in 'poor' environments remains consistently in the range 0.3-0.3.5 for terminal sire breeds and Merinos respectively.

- In the ram-lamb enterprise, Sheep CRC data shows worm effects reduce dressing percentage around 4% as well as impacting other production traits and by-products, thus the cost to industry is not insignificant.
- For ram and semen sales, FEC data is adding around $200 per ram ($15-30 per ram sold for terminal and maternal sires). Commercial WEC assays are on average $5 each!! (lowest around $3.80).
- Reluctance and decline in the number of breeders challenging young sires with nematodes in case of adverse findings and poor ASBV ranking. However, the market considers WEC to be an important production trait and stud rams with good ASBV ranking can realise a premium of $150-200/ram as well as positively impacting on semen sales from such rams.
- The SG database holds WEC testing information from 200 flocks (with epg >300 in south and >500 in north) representing 30,000-50,000 sheep. Another 50-80 flocks currently have worm challenges below threshold levels of detection for recording.
- Currently SG have DNA samples from 100 sires (target 7000?-can we confirm this over what range of breeds/bloodlines and how long to get this- NZ has 7000 sires in their genome wide selection training set so they can make good predictions for a range of breeds) in 5 categories of rams.
- Management of gastrointestinal parasites as a result of climatic factors has shifted to a more atypical basis with catastrophic events occurring every 3-5 years followed by periods of little or no detectable infection. The risk is little or no immunity is built by animals through non-lethal trickle infections in successive drops of lambs and consequently when infection occurs it is becoming lethal. Huge worm problems occurred at Walgett in Autumn 2010 after drought break- dying of Hc and epg up to 30,000 with Tc (incredible!). Where did they come from and what about IPM, even if sheep were totally naive?-(This is maybe not enough to waste selection pressure on if it is a one in 20 account-like blowflies– selection for resistance takes along time and diverts selection pressure from other $$ objectives). This is an argument for manipulation of immunity rather than the curative approach used in the area.
- The aim will be to phenotype at a younger age +/- worm exposure where post worm exposure the r^2 with worm burden (WB) has to be better than FEC (ie r>0.7 and <$5 and easier- a big ask)

A biomarker pre challenge has to make ACCURATE predictions for a whole range of challenge scenarios – and the proportion of variation accounted in WB (preferred) or possibly FEC for needs to be quantified against/within existing or new data samples. Some new data may also be forthcoming from existing samples.

6.2. Current and future work: *Genomics*

Differential gene expression associated with parasite resistance in different genetic lines of sheep is shown to be heritable. Two lead biomarkers identified using expression arrays: grehlin gene is located on OAR 19, but its receptor is located on OAR1. Grehlin is a secreted hormone peptide associated with appetite stimulation and elevated protein levels have been validated and detected in whole blood and faeces associated with susceptible animals; KCNJ15 is a cellular marker located on OAR 1 (commonly contains QTL in mapping studies) and likely functions as a potassium ion channel. Markers show differential expression in naïve animals and associated with resistant phenotypes and difference in innate immunity to parasite infection.

- Top priority genes identified were grehlin (Antibody assay reagents disappeared, but fits ¾ criteria for industry diagnostic), redox genes (DUOX1) up in Res early, KCNJ15 (on cells assoc with K+ channels, so needs whole blood assay) has a 6x differential at time 0.
- Time course analyses show no consistent patterns post-infection, but large differences at day 0 for possible innate marker. Proportion of phenotypic variation accounted for by biomarker is unknown (see above)
- In FG3 (mRNA time course in TRF), noted grehlin, C1QG, KLF11 (the KLF family contain transcription factors for grehlin and DUOX) fitted the “down-flat-flat” or “up-flat’flat” sequential expression pattern sought to enable more flexible sampling. Noted that grehlin is high in Sus animals and declines post infection, while gene expression levels are low in Res animals but increases after challenge. Less “spikey” responses on pasture than pens (related to feed/bleed times?)
- Gene expression profiles between R and S lines known to differ for MHCII gene DQA2 associated with Th2 cells (antigen presenting cells and important in the development of acquired immunity and antibody expression). Interesting to speculate whether MHCII serotypes/ gene variants at the locus correlate with SNP polymorphisms.

Recommendations for future confirmation/validation for markers of Res/Sus

Short to Intermediate Goals

- Top genes for moving forward are :
    - For time 0: grehlin/ KCNJ15
    - Post-infection: redox DUOX, trefoil proteins, KCNJ15
- Develop assay for KCNJ15 and repeat generation of ghrelin reagents (maybe choose MAbs to cover grehlin splice variants that may have other functions; or Ghrelin mass spec assay available to cover splice variants in the protein)
- Determine expression from birth / through weaning and onto solid feed (pasture and animal house) to 6 weeks post-weaning.
- Compare outbred (CRC) to R and S lines. This could be done on selection flocks or measured in samples from marking in the CRC IFN flock (advantage of known sires with EBVs on progeny linked to fec data). The CRC flock in different environments may indicate a pan-disease marker. OJD bleeds should also be available
- Measure in existing samples (in ROs, in projects SG204/205; note grehlin is an acetylated protein and maybe labile in blood samples if collection method is not appropriate) with phenotype and genotype (FNFS samples, also linked to fec data?). It would be most advantageous to have samples with guaranteed no prior exposure to confirm initial findings and determine the accuracy of the biomarker-ie proportion of variation post infection accounted for by having prior information not just FEC but preferably additional worm burden data
- Combine with top leads from SGP231 proteomic outcomes (innate factors)

***Assess CARLA from NZ as Hc marker and do the whole lot together to estimate the effect of combining multiple prediction markers.

Best leads seem to be from time 0, but variations from birth, marking, weaning and post-weaning on pasture need to be confirmed and can be done quickly in existing samples or samples collected specifically with next lambing (July-Sept). There are also some additional post infection candidates (DUOX, TFF, CXCL13, WARS) but pre-infection markers were the initial priority in SG1.

Longer term work

- Ovine cell culture system developed (well done Nick!) could be used to examine modulators (including MAbs to grehlin etc) on worm infectivity- like what was proposed for David Piedrafita. Could also assess/confirm current known inflammatory actives in the LMI such as PAF and LTC4 (at physiological levels). (No nerve/MMC input without more complex systems)
- Characterisation of DUOX / Redox response- up in every worm infection, consistent with previous work and increased gut muscle mass after worms (current CLI PhD).
- Characterisation of ghrelin (current CLI PhD project -Mass spec assays developed to look at role of splice variants)
- Regulatory networks (Reverter)
  - Pathways / co-expression clusters
  - Shared regulator of DUOX and ghrelin identified
- eQTL??? what do we mean-
  - integrating expression data with QTL/SNP data is an immediate strategy this can be done from existing resources and clarify what the integrated networks are (as Herman suggested from SG204/SG227)
  - QTL where a micro-array is the phenotype- ie genes controlling gene expression - is a huge job and not yielded much in cattle or humans or mice- this would need possible 800 microarrays to be done in a mapping flock! It could be possible to select extremes of phenotype and do the work with fewer animals, or a pooling strategy? But we would need to do multiple tissues and possibly multiple time points.
  - Using whole transcriptome analyse of existing samples at critical times maybe a better approach to enrich genome sequence data –this can be done more cost effectively now with next gen sequencing-they are talking about a whole transcriptome for $150!! (cost us $50,000/run 5 years ago) this would require well defined samples phenotypes and tissues as was collected in SG204/5 (needs to be planned as strategic effort)

6.3 Current and future work: Genes for resistance to Gastro-Intestinal Nematodes and Whole Genome Selection

We are cross-talking on two main things:

- targeted QTL fine mapping on OAR 1 and OAR 3 in special resource mapping populations, and
- whole genome selection- all SNP in large populations/families ie CRC FMFS IFN

This was a result from the OAR 1 OAR 3 fine mapping combined UNE CSIRO USYD analysis and validated in the FMFS.

- Fine-mapping resource flocks confirmed and refined QTL on OAR 1 and possibly 2 QTL on OAR 3
- Few significant SNPS in fine-mapped QTL but in some cases highly significant and may be flock-specific, remembering the limited sires sampled. The proportion of variance explained by SNP with largest F-value from the combined fine mapping flock was validated in FMFS where it was best for Hc fec2 in FMFS: 1.0% (~ 3% of genetic variance). Proportion of variance explained by SNP with largest F-value for Tc fec2 in FMFS: 0.6% (~ 2.5% of genetic variance); OR for the biologists, the likelihood that challenged progeny will exceed 1000 eggs/g and need to be drenched.
- Small effects need combinations of many QTL /many SNPs to explain a significant proportion of heritable fec and also note worm burden is the real objective/ target not FEC as an endpoint (even though reasonable progress made with FEC selection indices (Alex))
- Effects are small from the Whole Genome Selection, better for HC than TC.- all SNP combined have moderate Predictive genomic EBV based on genome have low correlations (0.05-0.25 in Merinos and 0.36-0.43 in strong wools)- this is still better than the 1% of variance accounted for by single SNP effects
- Some SNPs located on FMFS sire MQ (Tfec, Chr5) and M4 (Hfec, Chr3). –this still was QTL and needs to be checked in each flock separately. This still requires many SNPs to cover the confidence interval- using family specific QTL can only be of use eif they are of very large effect and can be rapidly amplified in commercial flocks. Recommendation that a FEC haplotype analysis of key sire M4 using his progeny in the INF be completed. Sonja would be the person to carry this out in consultation with John. The result would be prioritisation of SNP for inclusion on the 5K array if M4's OAR3 parasite trait haplotype stands up within the INF data (particularly as this sire's progeny are widespread in Industry (Alex)).
- Noted that single SNP analyses had high FDRs and needs care in interpretation, when taking each SNP into a separate analysis/effect
- On the lack of correlation between QTL linkage data and (single) SNP analysis, John Henshall suggested that this may occur because: not sure this is true-genome wide analysis showed no regions of very strong association across all animals analysed – QTL analysis showed a significant QTL covered by many SNPs on OAR 1 in one particular sire. This QTL was not seen in other sires and in genome wide analysis reasons maybe:
    - It is a rare allele (so dam alleles not apparent)
    - Epigenetic affect or heterosis!!
    - How to interpret LA and LD and analyse together. Need more meioses not just SNP markers. Sire families not large enough to detect effects.

****Do we have better markers than current (associative) tests like Fec and PCV?? Phenotypic r^2 FEC –worm burden =0.7, r PCV& EOS to FEC is 0.6-0.8. Neither gene markers nor biomarkers can probably get close to this currently. Predictive genomic EBV based on genome have low correlations (0.05-0.25 in Merinos and 0.36-0.43 in strong wools)- this is still better than the 1% of variance accounted for by single SNP effects.

NOTE: under a 30% heritability FEC and a 0.4 genetic correlation with MBV (derived from all SNP) the molecular co-heritability is about 0.2 (SQRT 0.3 (heritability FEC)* sqrt 1 (heritability marker) * 0.4 genetic relationship = 0.2 so(0.2/0.3); so 67% of heritability of FEC accounted for by GWS marker panel ---contrast this with 3% of heritability accounted for by a single SNP on either OAR 1 or OAR 3! This needs checking but does suggest that GWS using all markers works reasonably well in some populations. The trialling and test populations need to be larger and there is merit in completing genome scans in the resource populations used for fine mapping and adding this to the pool as well as perhaps recovering or updating/samples from older genetic experiments where family and FEC data is available.

We probably need more and better phenotypes and more SNPs! The GWS did suggest show additional regions of interest -there are additional resource populations other than CRC/ IFN- see above. It is also possible to quickly use the SNP chip to genotype the resistance flocks (TRF/TSF) to define whether resistance and susceptibility are opposites (different mutations in the same gene) or controlled by different genes/pathways. By comparing HSF and TSF we could also see if the SNP are conserved or indicative of multiple pathways. If RES and SUS are different genes then selection could be approached not only by selecting for resistance but also by selecting against susceptibility. (*Ad nauseum* we are informed by genomic selection advocates that this isn't important but it rings of some practical sense, fitting in with culling worm factories by fec). If the response isn't conserved, then there would be a great benefit in identifying multiple SNP that explain more of the phenotypic variance. In any case, this is a relatively straight forward exercise and the results could easily be added to the parentage plus chip that is under discussion.

James is keen on a concordant (international) exercise on existing data sets (incl HRIP) to attempt to identify resistance independent of breed or parasite, rather like the original intention of the Jill's international HRIP meta-analysis. However, this is simply a selective sweep on different breeds but they have no known genetic performance for resistance (only on spec) like the meta analysis that was done in SG227 using all known QTL and combined in a single analysis.

- This aims to detect signatures of selection using global sample of sheep breeds. Worked previously with Poll and myostatin and based on selection in a challenge environment over many, many millennia. But these are single gene effects with a well defined and known phenotype (ie horns or not). For parasite resistance we don't know if the breeds are resistant or susceptible and doing this on spec can lead to spurious over-interpretation of the data confounded with other breed characteristics- ie hair vs wool.
- Furthermore, from all the genome scans we know there are unlikely genes of very large effect like single gene effects of note for parasite resistance-if they do exist they are extremely rare and we/others haven't seen them over decades of genetic analyses- even though it may be argued that we have not looked broadly enough except for Soay, Scottish Blackface, golden Ram, TSF.
    - This has some added incentive with the HapMap enterprise where 3500 (global) animals have been SNP genotyped through the ISGC.
    - Additional resources with DNA are the CRC-IFN flocks, CLI flocks, Gulf coast native x American Suffolk (USDA) and probably ITT, and Garole? INRA and ILRI maybe other sources, but appropriate phenotypes are unlikely to be available as they will not be measured and have not been measured under a single environment.

  - Problems of challenge models and parasite load (raised with HRIP meta-analysis) and the IP issues that plagued us earlier. Note that ISGC results are public domain!
  - Recommendation to complete a concordance analysis of FMFS linkage results with those obtained within the ISGC HapMap data. This would seek to overlay 'selection signature' based results with the 'within family' results already in hand from FMFS. Again, the result would be SNP which look interesting for key traits (parasites, carcass and poll) and which we want on the 5K array. The proposed activity would see the possible inclusion of SNP onto the 5K product which would in turn provide validation results. JK perform this analysis, with help from Sonja.
  - EC framework7 3SR initiative- $3m for chips and WGS with ISGC for parasite resistance- we should be linked in.
  - There is the view that SNP data should give information for biology- CARLA saliva test was raised here. This is complicated unless genomics can be linked with genetics and physiological pathways which was proposed by Herman & Peter.
  - Still presumes that somewhere there is a gene of major effect,- see comment above or better than we have found to date OR integrated pathway that involves a number of genes

- The draft reference genome sequence for sheep should be available in 3 months from combined ISGC and Bejing Genomics Institute (BGI)(James). This will enable location/mapping of all priority genes and regulatory elements. Herman and Peter suggested that the biology/ mechanistic information could be boosted using known genes mapped to Chr 3 (important in many studies for worm res) or anywhere on the genome if genome-wide mapping was completed. In an integrated genomics analysis, this should be able to source and add-value from existing phenotyped resources and examine function related to SNP/QTL positioning, history and extrapolated function to get to modulatory opportunities and protocols.

6.4. Current and future work: Proteomics- Identification of Plasma Biomarkers for detection of GIN resistance and susceptibility.

Reasonably successful depletion used both IgY and hexapeptide depletion approaches and plasma from FG1 (Tc infection) in SG205.

- At time 0; 9 proteins were identified as having statistically different expression levels between Res and Sus via 1 and 2 tailed t-tests (NOTE: when doing 1000s of fragments you can just get 50/1000 by chance .NOTE very high false discovery rate!)
- At 28d after primary Tc infection, 9 proteins were identified as having statistically different expression levels between Res and Sus via 1 and 2 tailed t-tests
- At 14d after second Tc infection, 8 proteins were identified as having statistically different expression levels between Res and Sus via 1 and 2 tailed t-tests
- 11 considered as potential stand-alone peptide biomarkers of resistance or susceptibility to parasitic infection and worthy of further attention
  - 11 with a mean ratio trend (Fpr <0.05) higher in one genotype group across all 3 time points

- Full results and trends summarised in Matt's table below between R (A) vs S (B)
- ceruloplasmin (ferroxidase, CP-2), and complement C4 at least worth following as C4 has previous form. However, these many be indictors of inflammatory potential.

Functional Group	Protein	Expression		
		t0	t15.1	t15.2
Wound Healing				
	Fibrinogen	Up B***	Up A**	Up A**
	Fibronectin^	Up B**	Up A	Up B**
	a-2 macroglobulin	Up B	Up A	
	Plasminogen	Up A**		
	Prothrombin (thromin)	Up A	Up B	
	Vitronectin			Up B
Compliment Pathway				
	Complement C4	Up B**	Up B***	Up A***
	Complement C4a (cleavage product)	Up B		
	Complement C3	Up A		Up A
	Complement C3a (cleavage product)		Up A	
	Mannose-binding lectin			Up B
	C1 inhibitor (Serpin Clade G)		Up B	
Transport				
	Albumin	Up B***	Up A	Up A**
	Clusterin (apolipoprotein J)	Up A	Up A	Up A
	Apolipoprotein E	Up B	Up B	Up B**
	Ceruloplasmin	Up B**	Up A**	Up A***
	Haemoglobin-alpha globin chain		Up A**	
	Haemoglobin-beta globin chain		Up A	
	Vitamin D-Binding Protein		Up B	
Inflammation				
	Alpha-1-antitrypsin (Serpin Clade A)			Up B
Immune Response to Pathogen				
	Immunoglobulin kappa light chain	Up A		
	Immunoglobulin lambda light chain		Up B	
	Ig mu chain		Up B**	
	Beta-adrenergic receptor kinase 2		Up A	
Lipid Absorption/metabolism				
	Apolipoprotein A-IV	Up B**		
	Apolipoprotein A-I, apoA-1		Up B	
	Paraoxonase 1			Up A**
	Apolipoprotein C-III	Up A		
Gene Transcription				
	Meiosis-specific nuclear structural protein 1	Up B		
	DNA primase small subunit	Up B		
Structural	Actin			Up A
	Netrin-1	Up B		
	43 kD AChR-associated protein of the synapse			Up A
	Nebulin			Up B

** confirmed 2/3 analyses

*** 3/3 analyses

Confirmation/ validation

- Original HRIP plan envisaged use of remaining samples in SG205

How critical is the collection/storage procedure to the outcome (eg haemolysis?) and any proposed depletion variations between samples

- For these proteins, many assays (human clinical pathology) are available for some, while others may need reagent development like grehlin
- Alternate assays are Mass-spec
- New machine (QQQ- triple-Q) uses non-depleted serum
- Needs to be rapid and cost-competitive

6.5. Future research: *Combined strategies*

6.5.1. Genetic Markers

Focus on *in-silico* GWS genetics which can be validated and readily implemented. The use of a genome wide scan shows much greater utility than single SNP/Single QTL approach, so the effort should be directed at procuring more resources where FEC and WB has been measured in a genetic population or controlled experiment. These resources exist and should be tabulated and checked. GWS is largely a numbers game with links between "training" populations and test sets, so if we get 0.25-0.4 in accuracy of EBV prediction we have probably done well. This can't be done easily by single SNP analysis on a SNP by SNP basis. Merit however in short term goal of completing a FEC haplotype analysis of key sire M4 using his progeny in the INF to identify rare QTL's. The result would be prioritisation of SNP for inclusion on the 5K array if M4's OAR3 parasite trait haplotype stands up within the INF data. Similarly complete a concordance analysis of FMFS linkage results with those obtained within the ISGC HapMap data. This would seek to overlay 'selection signature' based results with the 'within family' results already in hand from FMFS. Again, the result would be SNP which look interesting for key traits (parasites, carcass and poll) and which we want on the 5K array.

6.5.2. Meta-Analysis for Integrated Genomics

The targets should be for new therapeutics/drugs. This should be conducted to look at genes/regulators which work on both genome/genotypic values and more importantly PHENOTYPIC measures especially in defined infections in clean backgrounds. In the latter, we see and can reliably envisage much stronger signatures of effects (going for effect to maximise opportunity). From the integrated analysis, we should be able to assess possibilities to derive new targets for therapeutics and drug targets. To approach this, existing data sets in HRIP projects need to be completed and linked with global domain data sets in sheep and other species.

5.3. Protein biomarkers- validate and establish utility for parasites and disease

Two integrated approaches are needed- (blood/ serum/plasma) markers prior to infection (marking/ weaning) and markers post-infection. Studies can be conducted on existing samples and in specific trials to examine straight utility (existing samples in ROs, FMFS and CRC) and gather information of robustness and timing (pen/pasture controlled trial using deliberate trickle and natural pick-up)

A combined strategy is envisaged using current priority markers from several projects including a range of (up to the 15?) innate immune mediators (Matt) that have flexible sampling times, grehlin, KCNJ15, DUOX, trefoils? (Aaron), CARLA (NZ). Note that these could be used as predictive, associative or indicative markers.Lead up work is needed to assess/ generate assays for proteins or peptides (eg Grehlin/KCNJ15) and to examine the utility of "signature" peptides/ proteins in Mass-specs approaches using non-depleted serum or whole blood. Bio-assays (antibody) would work on whole or freeze-thawed blood.

We need to get (both):

1. The biomarker panel validated in samples with clean /defined infections and preferably worm burden data as well as FEC data for assessing the “best case scenario” that we can expect from combining all markers.
2. Establish how the sensitivity and specificity of biomarker data performs in flocks with mixed/unknown infections both from timing and nature of infections (as the worst-case scenario under field conditions)

Current plasma samples include HRIP resource flocks, FMFS whole blood (all frozen) and CRC-IFN progeny. It is obvious that ALL samples should have phenotypic data (fec etc).

Future trials and sampling should examine;

- the optimal times for sampling to gather sensitivity and specificity data for the test,
- to define the minimum numbers of proteins for maximum robustness and discrimination in a range of enterprises and environments AND,
- to examine whether any are predictive/associative markers for comprehensive disease resistance (eg OJD/footrot/ fleecerot) or production related traits (with meat and wool).

7. Publications

A. Refereed journals

1. Rowe A., Yun K., Emery DL & Sangster N. 2008. *Haemonchus contortus*: development of a two-step, differential-display PCR to detect differential gene expression in nematodes from immune and naive sheep. Exptal Parasitol.119 (2): 207-216.
2. Rowe A., McMaster K., Emery DL & Sangster N. (2008). *Haemonchus contortus* infection in sheep: parasite fecundity correlates with worm size and host lymphocyte counts. Vet Parasitol.153,(3-4): 285-293.
3. Rowe A., Gondro C., Emery DL & Sangster N. (2008). Genomic analyses of *Haemonchus contortus* infection in sheep: abomasal fistulation and two *Haemonchus* strains do not substantially confound host gene expression in microarrays. Vet Parasitol. 2008,. 154 (1-2): 71-81.
4. Rowe A., Gondro C., Emery DL & Sangster N. (2009). Sequential microarray to identify timing of molecular responses to *Haemonchus contortus* infection in sheep. Int J. Parasitol., (epub jan 13, 2009).
5. Moira Menzies, Antonio Reverter, Nick Andronicos, Peter Hunt, Ross Windon and Aaron Ingham* Nematode challenge induces differential expression of oxidant, antioxidant and mucous genes down the longitudinal axis of the sheep gut. Parasite Immunol. (submitted)
6. Reverter A, Ingham A, Dalrymple BP. Mining tissue specificity, gene connectivity and disease association to reveal a set of genes that modify the action of disease causing genes. BioData Min. 2008 Sep 19;1(1):8.
7. Ingham A, Reverter A, Windon R, Hunt P, Menzies M. Gastrointestinal nematode challenge induces some conserved gene expression changes in the gut mucosa of genetically resistant sheep. Int J Parasitol. 2008 Mar;38(3-4):431-42.
8. Kijas JW, Menzies M, Ingham A. Sequence diversity and rates of molecular evolution between sheep and cattle genes. *Anim Genet. 2006 Apr;37(2):171-4.*
9. Menzies M., Ingham A. Identification and expression of Toll-like receptors 1-10 in selected bovine and ovine tissues. *Vet Immunol Immunopathol. 2006 Jan 15;109(1-2):23-30.*
10. *Kiel M*, *Josh P*, *Jones A*, *Windon R*, *Hunt P*, *Kongsuwan K*. Identification of immuno-reactive proteins from a sheep gastrointestinal nematode, Trichostrongylus colubriformis, using two-dimensional electrophoresis and mass spectrometry. Int. J. Parasitology, 2007, 13:1419-29.
11. JM Kemp, NA Robinson, E N T Meeusen, D M Piedrafita. The relationship between the rapid rejection of *Haemonchus contortus* larvae with cells and mediators in abomasal tissues in immune sheep. International Journal for Parasitology (in press, 2009).

B. Conference presentations

1. Shivashankar H Nagaraj, Antonio Reverter, Moira Menzies, Nick Andronicos, Aaron Ingham. On the expression profile of candidate genes conferring resistance to gastro-intestinal nematodes in sheep Accepted for AAABG (2009).

2. S Nagaraj, A Ingham, A Reverter, (2008) Promoter sequence analysis of differentially expressed genes in sheep following a nematode parasite challenge Abstract for GIW conference (Nov 2008).
3. Hutton Oddy[1], Brian Dalrymple[2], John McEwan[3], James Kijas[2], Ben Hayes[5], Julius van der Werf[1], David Emery[4], Phil Hynd[6], Terry Longhurst[7], Troy Fischer[8], Duncan Ferguson[7] , Rob Forage[7,8], Noelle Cockett[9] and Frank Nicholas[4] . SheepGenomics and the International SheepGenomics Consortium (AAABG, 2009)
4. D.L. Emery and K.J. Beh (2005) Genetic and Biological Approaches to Modulate Nematode Resistance Mechanisms in Sheep , AAABG, 16, 385-388.
5. J. Wang-Holmes*, P Josh*, L Cadogan* K Kongsuwan* (2005).Novel Diagnosis of Gastrointestinal Nematode Resistance in Sheep. (Combio, 2006)
6. S Binos, C Buck, M McDonagh, N.Andronicos* and R.Windon. (2008) Proteomic comparison of a subproteome of plasma proteins potentially involved in host-parasitic interaction in sheep selected for resistance and/or susceptibility to Trichostrongylus colubriformis. EMOP.
7. H. N. Kadarmideen, N. Andronicos, N. S. Watson-Haigh. Systems Genetics Analysis Reveals Gene Modules And Heritable Biomarkers for Sheep Intestinal Parasite Resistance (AAABG, 2009).
8. K. Marshall, S. H. Lee and J. H. J. Van der Werf. Information Sources in Linkage Disequilibrium – Linkage Mapping Using Half-Sib Designs (AAABG, 2006).
9. Hunt, P. AWI/MLA SheepGenomics Program: Host Resistance to Internal Parasites (HRIP) sub-program. (WAAVP, Christchurch, 2005)
10. A.Ingham*, M Menzies, N Andronicos, T Reverter, SH-Nagaraj. The Search for Master Immune regulators Abstract for Immunity-Infection Conference. Gold Coast June 2009.

Appendix X Asset Register

Table 1 HRIP asset register (adapted from combine SheepGENOMICS asset register)

Parasites	Parasite DNA markers	26	SG201	Markers for low faecal egg count	CLI	25,757	21,037	55%	45%	y	N/A	N/A
Parasites	Management	6	SG202	HRIP Subprogram Leader	CLI	40,134	-	100%	0%	y	N/A	N/A
Parasites	Parasite DNA markers	26	SG203	Resistance to nematode infection	UMEL	186,750	562,875	25%	75%	y	N/A	Enabling technology (see SG222)
Parasites	Candidates	27	SG204	Genes for H contortus II	USYD	765,300	794,999	49%	51%	Library		Blood and tissue samples; unused SG semen. Blood used for proteomic analyses in SG231
Parasites	Candidates	27	SG205	Genes for Nematodes I	CLI	657,750	539,709	55%	45%	Database	Library	Plasma and tissues in SG213 freezer. Candidate gene lists (see SG229)
Parasites	Parasite DNA markers	26	SG206	Candidate Genes for Nematodes II	CLI	372,783	305,004	55%	45%	y	N/A	Enabling technology (see SG235)
Parasites	Candidates	27	SG207	Proteins for resistance to nematodes	CLI	75,000	228,695	25%	75%	y	N/A	Enabling technology (see SG219)

Parasites	Management	6	**SG208**	AWI Ancillary Expenses for HRIP Subprogram	AWI	14,910	-	100%	0%	y	N/A	N/A
Parasites	Management	6	**SG209**	HRIP Subprogram Leader 2	USYD	344,000	-	100%	0%	y	N/A	N/A
Parasites	FMFS phenotype database	12	**SG210**	Worms for Falkiner	CLI	77,400	-	100%	0%	y	N/A	N/A (see SG223)
Parasites	FMFS phenotype database	12	**SG211**	FEC & service provision for FMFS	CLI	104,249	-	100%	0%	y	N/A	N/A (see SG223)
Parasites	Management	6	**SG212**	Expenses for HRIP SAC	AWI	19,936	-	100%	0%	y	N/A	N/A
Parasites	Candidates	27	**SG213**	Purchase of -80C Freezer	AWI	10,170	-	100%	0%	Equipment		-80 freezer
Parasites	FMFS phenotype database	12	**SG214**	Processing blood samples for FMFS	AWI	65,953	-	100%	0%	Library		Blood samples from FMFS challenge trials (frozen USyd @ -20C)
Parasites	Candidates	27	**SG215**	GINTIP	USYD	199,780	35,850	85%	15%	y	N/A	N/A (See SG225)

Parasites	Candidates	27	**SG217**	Metabolic basis of resilience to intestinal parasites in sheep	AGRES	-	-	100%	0%	y	N/A	N/A
Parasites	Candidates	27	**SG219**	Novel diagnosis of resistance to nematode infections	CLI	150,000	121,284	56%	45%	Markers (Bio)	Reagents	Cloned sheep cytokine genes IL-4,-10,-13 and trefoil peptide + antisera; Haemonchus proteins 24kDa and GA1 & antisera. Markers TBD
Parasites			**SG220**	HRIP Meta Analysis	UMEL	-	-	100%	0%	y	N/A	N/A
Parasites	Management	6	**SG221**	HRIP SubProgram Leader Expenses	USYD	52,776	-	100%	0%	y	N/A	N/A
Parasites	Assays	28	**SG222**	Development of in vitro assays	MASH	187,250	187,250	50%	50%	Assay	Know How	In vitro larval establishment assay. Works with Haemonchus but not Trichostrongylus. Probably made public domain through publication
Parasites	FMFS phenotype database	12	**SG223**	Faecal Egg Phenotyping from FMFS	AWI	57,890	-	100%	0%	Database		Parasite phenotypes in FMFS database (See SG543)

Parasites	FMFS phenotype database	12	**SG224**	Supply of Larvae	USYD	26,677	-	100%	0%	y	N/A	N/A (see SG223)
Parasites	FMFS phenotype database	12	**SG225**	Processing of blood samples from FMFS	AWI	36,321	-	100%	0%	Library		Frozen blood samples (USyd @ -80C)
Parasites	Parasite DNA markers	26	**SG226**	Identification of marker haplotypes for resistance to Haemonchus contortus I	CLI	242,000	195,397	55%	45%	Markers (DNA)		DNA markers for 2 QTL, cross checked in SG227 & SG228
Parasites	Parasite DNA markers	26	**SG227**	Identification of marker haplotypes for resistance to Haemonchus contortus II	USYD	300,000	307,560	49%	51%	Markers (DNA)		DNA markers for 2 QTL, cross checked in SG226 & SG228
Parasites	Parasite DNA markers	26	**SG228**	Identification of marker haplotypes for resistance to Haemonchus contortus III	UNE	250,000	86,336	74%	26%	Markers (DNA)		DNA markers for 2 QTL, cross checked in SG226 & SG227
Parasites	Candidates	27	**SG229**	Gene expression markers associated with gastrointestinal nematode infections	CLI	400,000	333,867	55%	45%	Markers (Bio)	Reagents	Plasma and tissues in SG213 freezer; anti-grehlin antiserum. Markers TBD

Parasites	Miscellaneous	28	**SG230**	Discovering virulence determinants of H.contortus for diagnosis of sheep resistance	USYD	50,000	12,150	80%	20%	Reagents		6 strains of Haemonchus of proven differential virulence. Stored at USyd Vet Sci
Parasites	Candidates	27	**SG231**	Proteomics? ON HOLD	PIRV	135,000	-	100%	0%	Markers (Bio)		Suite of circulating biomarkers TBD
Parasites	Candidates	27	**SG232**	SG203 extension ON HOLD	MASH	140,000	-	100%	0%	y	N/A	N/A
Parasites	Management	6	**SG233**	CMA expenses	USYD	47,783	-	100%	0%	y	N/A	N/A
Parasites	Candidates	27	**SG234**	Scoping of the parasite proteomics - Mark Wilkins	AWI	7,063	-	100%	0%	y	N/A	N/A
Parasites	Parasite DNA markers	26	**SG235**	SG206 extension (Aaron Ingham)	CLI	200,000	166,790	55%	45%	Markers (DNA)	Reagents	Primers & sequences for 120 candidate R/S genes. Many others in milestone reports. DNA markers and biomarkers TBD
Parasites	Candidates	27	**SG236**	SG215 extension (To Rowe)	USYD	90,000				x	N/A	