final report

Project Code: B.BSC.0014

Prepared by: Mike Goddard[1], Wayne Pitchford[2], Ben Hayes[1], Cynthia Bottema[2], Helen McPartlan[1], Robert herd[3]
[1]Department of Primary Industries Victoria
[2]University of Adelaide
[3]Department of Primary Industries, NSW

Date published: September 2006

PUBLISHED BY
Meat and Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

Map and Identify Genes for Feed Intake and Efficiency of Beef Cattle with Intent to Develop DNA Tests

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Feed conversion efficiency is measured in the beef industry by net feed intake (NFI), which is the amount of feed an animal requires compared to the average animal of the same weight and growth rate. NFI is an important component of the cost of beef production and hence its profitability. There is considerable genetic variation in NFI, but cattle breeders cannot select for this trait because it is too expensive to measure directly. Therefore, the aim of this project was to find genetic markers that could be used to select for NFI. Using lines of cattle selected for high and low NFI and a range of molecular and biochemical approaches, we have discovered over 100 genetic markers that are closely linked to genes affecting NFI. Although these markers could be used to select for NFI in the selection lines, they may not be close enough to the relevant genes to be used in other cattle populations. Therefore, before they are commercialised, their usefulness in other cattle populations needs to be validated and, where necessary, the existing markers should be used to find markers closer to the genes for NFI. This will lead to a panel of commercial DNA markers that cattle breeders can use to select for NFI.

Executive Summary

The largest single cost of beef production is feed. This is true both for feedlot cattle, where feed is purchased, but also for grazing cattle because additional feed requirements require purchase of feed or land. Faster growing and larger cattle will tend to require greater amounts of feed and so part of the feed intake is predictable from weight and growth rate, but there is a part of the feed intake (called net feed intake or NFI) that is not explained in this way. Therefore, NFI must be included in a complete breeding objective for beef cattle. Up to 40% of the variation between cattle in NFI is genetic. It has been demonstrated that improvement in NFI through selection leads to large gains in profitability. However, NFI is a difficult and expensive trait to measure. For feedlot cattle, NFI can be measured by recording the amount of feed eaten and the change in liveweight. However, these tests are expensive. For grazing systems, measuring NFI is very difficult.

An alternative way to select cattle for improved NFI would be to use DNA markers. If the genes carrying mutations affecting NFI could be found, or DNA markers associated with these mutations could be found, cattle could be selected if they carried the DNA marker genotypes associated with improved NFI. The purpose of the project was to map and identify genes for feed intake and efficiency of feed utilisation in beef cattle, in order to develop DNA tests for application by industry.

The Project utilised outputs from previous MLA/MRC projects TR.075 and DAN.075. MLA project TR.075 identified five chromosomal regions that contained genes having significant effects on NFI. MRC project DAN.075 produced phenotyped pedigrees and DNA, which was used within the Project.

The strategy was to use a combination of gene mapping, candidate gene studies and functional studies in two groups of cattle: the Trangie selection line Angus cattle, and the Davies Jersey x Limousin experiment. Using these populations which represent diverse breeds gives us some confidence that DNA markers associated with NFI in both populations will work across the range of breeds used by the beef industry.

The objectives of this project were to

1. Confirm that five chromosome regions containing genes affecting NFI that were previously discovered in the Davies Jersey x Limousin experiment also affect NFI in the Trangie Angus selection lines. This was done using linkage mapping, which exploits recombinations within families.
2. Make use of the very large number of DNA markers now available following the sequencing of the bovine genome to reduce the size (and therefore the number of possible genes) of the chromosome regions affecting NFI. We conducted a powerful genome scan using approximately 10,000 markers spread across the bovine genome in the extremes of the Trangie selection lines. The markers were single nucleotide polymorphisms (SNPs). This experiment detected associations between the SNPs and NFI only if the association existed across the whole population, not just within families. For this to occur, the markers must be very close to the genes affecting NFI, allowing us to greatly narrow existing chromosome regions, as well as detecting new ones.
3. The 10,000 SNPs in the experiment described above were mainly in non-coding DNA. An objective of the current project was also to identify SNPs in candidate genes in the five chromosome regions found to affect NFI in the Davies Jersey x Limousin experiment.

4. A number of functional studies were carried out to confirm that the candidate genes selected were associated with differences in NFI.

Four of the chromosome regions found to affect NFI in Davies Jersey x Limousin experiment clearly affected NFI in the Trangie Angus selection lines. These regions were on chromosomes 1, 6, 8 and 20. There was also suggestive evidence that the remaining chromosome region, on chromosome 11, also affected NFI in the Trangie Angus selection lines.

In the genome scan using the 10,000 genome distributed SNPs, we found 100 SNPs significant at $p<0.001$. At this level of significance, we expect only 10 of these results to occur by chance. This is a surprisingly high number of significant SNPs. If it represented 100 genes affecting NFI, it would imply that most of these genes had a small effect on NFI. However, many of the SNPs are located very close to each other, and therefore, multiple significant SNPs may be associated with a single mutation affecting NFI. By investigating the positions of the SNPs on the genome, we concluded that there were 20 clusters of SNPs on 12 chromosomes that map the position of a gene affecting NFI. Six of these regions confirmed the chromosome regions found to affect NFI in the linkage mapping experiment described above. For these six regions, the interval which must contain the mutation affecting NFI was reduced by a factor of 10, meaning 10 times fewer genes had to be considered as candidates.

In addition to the 10,- 000 genome distributed SNPs, SNPs discovered in candidate genes were also tested for an effect on NFI. In total, 42 genes were screened and 201 SNPs were identified. Three genes contained SNPs with a significant effect on NFI. These genes were adenosine monophosphate kinase (AMPK), uridine monophosphase synthetase (UMPS) and microtubule associated protein 1B (MAP1B). Functional studies showed that the activity of AMPK enzyme appears to be significantly different in high NFI cattle and low NFI cattle; further evidence that there could be a mutation in this gene causing differences in NFI. Proteomic studies revealed differentially expression of several mitochondrial proteins between high and low NFI cattle, including subunits of the mitochondrial oxidative phosphorylation complexes. Enzyme assays also indicated that at least one of mitochondrial oxidative phosphorylation complexes has different levels of activity in high and low NFI cattle.

The results from the project are very promising, and we are confident that a panel of DNA markers can be developed to allow accurate selection for NFI without the need for measuring the trait in all cattle. The benefits of the project will be delivered in a project already underway, in which we will validate all SNP markers in new populations. SNPs will be then selected from the genome scan and candidate genes to generate a panel that explains the maximum genetic variation for NFI across breeds. Industry animals can then be genotyped for the panel of SNPs, and this information can be combined with pedigree information to estimate accurate breeding values for NFI. This will allow the industry to accurately select efficient cattle, thereby increasing profitability. Because this project has used diverse populations representing different breeds, the SNP panel will able to be used across a large proportion of the beef industry in southern Australia.

Contents

1 Background

1.1 Economic importance of Net Feed Intake (NFI)

The largest single cost of beef production is feed. This is obvious when feed is purchased (eg in a feedlot), but is also the case in grazing cattle because additional feed requirements require purchase of feed or land (assuming that the production system already has optimum stocking rate, pastures and fertiliser use).

A breeding objective for the genetic improvement of beef cattle profitability would include

- Growth rate
- Proportion of live weight that is saleable
- Meat quality
- Reproductive rate
- Feed intake

Faster growing and larger cattle will tend to require greater amounts of feed and so part of the feed intake is predictable from weight and growth rate, but there is a part of the feed intake (called net feed intake or NFI) that is not explained in this way. Therefore, NFI must be included in a complete breeding objective for beef cattle.

Across a range of breeding objectives typical of those used for southern Australian beef production systems, inclusion of information on NFI of bulls improved the genetic gain in the breeding objectives from 8 to 38%, with improvement in profit ranging from 9 to 33% (Archer *et al.* 2004).

1.2 Genetic variation in NFI

Experiments at Trangie, NSW, and elsewhere found that there was considerable variation between individuals in NFI and much of this was heritable (h2 = 0.4; Arthur *et al.* 2001b). It is NFI over the whole herd that affects profit and so it is whole herd or life cycle NFI that we wish to improve. However, in practice, we measure NFI over only a short part of the animal's life (eg 70 days post weaning). Therefore, it is important to know the genetic correlation between NFI at different stages of the life cycle (Archer *et al.* 1999).

1.3 Selection experiments for NFI

Beef cattle

In 1993, a research project (MLA Project DAN.075) was started at the Agricultural Research Centre in Trangie, NSW to investigate the potential for genetic improvement in post-weaning feed efficiency as a means of improving whole beef production system efficiency.

Feed intake and efficiency tests were conducted each year using an automated feeding system that delivers and records individual animal feed intake. Feed efficiency was measured immediately post-weaning over a 70-day test and was calculated as NFI. NFI measures whether an animal consumes more or less feed than that predicted for growth and maintenance, with low NFI being superior. In 1994, high feed efficiency (low NFI) and low feed efficiency (high NFI) divergent selection lines were established. The 1993 born and 1994 born animals formed the foundation herd for this study. Starting with the 1993 born animals, the females were allocated to the high efficiency line and the low

efficiency line based on their individual NFI values. The three most efficient bulls born in 1993 were allocated to the high efficiency line and the three least efficient bulls to the low efficiency line. Throughout the experiment, the sole selection criterion for all replacement bulls and heifers in the high and the low efficiency lines was individual NFI. This design was chosen to provide a rapid divergence in NFI between the high and low selection lines. Details of the design of the project have been reported by Arthur *et al.* (1996).

There was significant (P<0.05) divergence between the two selection lines for the selected trait (NFI; Arthur *et al.* 2001a). This translated into an annual realised direct selection response of 0.249 kg/day. Differences in selection line means for the 1999 born animals indicate that after 5 years of selection for NFI (approximately two generations of divergent selection), there was no realised correlated response in either yearling weight or average daily gain (Table 1). This result is consistent with the fact that, theoretically, NFI should be phenotypically independent of test period live weight and growth. Significant (P<0.05) realised correlated responses in feed intake and feed conversion ratio were obtained. Animals in the high efficiency line consumed less feed and had lower values (more efficient) for NFI and feed conversion ratio than those in the low efficiency line.

Table 1. Least squares means (± standard errors) for 1999 born calves, and response to selection in growth and feed efficiency traits after 5 years of selection for net feed intake (from (Arthur *et al.* 2001a).

	Selection line mean		Correlated response[1]	
Trait	High Efficiency	Low efficiency	Total (5 yr)	Per yr
Number of animals	62	73		
Net feed intake (kg/day)	-0.540 ± 0.176[a]	0.707 ± 0.172[b]	1.247	0.249
Yearling weight (kg)	384.3 ± 6.9	380.7 ± 6.7	3.6	0.72
Average daily gain (kg/day)	1.443 ± 0.034	1.400 ± 0.033	0.043	0.009
Feed intake (kg/day)	9.4 ± 0.3[a]	10.6 ± 0.3[b]	1.2	0.24
Feed conversion ratio	6.6 ± 0.2[a]	7.8 ± 0.2[b]	1.2	0.24

[1]Absolute mean difference between high and low efficiency selection lines.
[ab]Selection line means with different superscripts differ significantly (P<0.05).

These results show that selection for NFI results in improvement in post-weaning efficiency of feed utilisation with minimal effect on growth. Given the feed cost of $200 per 1000 kg at Trangie at that time, the divergence of 1.247 kg/day between the lines in 1999, represents savings of $27 in feed costs per animal over a 100 day feeding period.

Mice

To confirm selection for NFI is effective, and to aid gene discovery, mouse studies were conducted in Adelaide in a parallel project to the cattle studies at Trangie. Mouse lines were selected for high or low net feed intake based on a post-weaning test (Hughes *et al.* 1997, Hughes and Pitchford 2004). After seven generations of selection, the lines were approximately 30% different in feed intake with little difference in growth and body composition. There were small differences in a number of other traits.

The conclusion from the selection experiments in both species is that selection for NFI is effective, and that in beef cattle improvement in NFI result in increased profitability. However, direct

measurement of NFI is very expensive, and therefore unlikely to be adopted by industry. An alternative to direct measurement is therefore required to implement selection for NFI in the industry.

1.4 Physiological sources of genetic variation in NFI

Knowledge of the physiological basis for genetic variation in NFI would be useful in understanding how to select for reduced NFI and how this might affect other traits and therefore, overall profit.

In typical beef cattle production systems, the breeding herd accounts for 65-85% of the total feed requirements (Ferrell and Jenkins 1984, Montano-Bermudez *et al.* 1990)) and 65-75% of this is used for maintenance. Primarily, this very large maintenance requirement is because cattle are a large, slowly maturing species with a low annual reproductive rate. Furthermore, only a single product is harvested (meat). Essentially, the 'machinery' of production represented by the breeding cow requires a proportionately higher level of raw 'inputs' to maintain itself than is required to produce the actual 'product', represented by the cow's offspring. The large maintenance requirement is in contrast to other production systems such as pigs or poultry, where the breeding animal has a small intake relative to the total intake of all progeny. Any improvement in the efficiency with which breeding cows maintain body weight will result in an increase in total meat production for a given amount of feed. In addition to the costs of cow maintenance, long-fed cattle for the Japanese market have a large maintenance feed cost because they are close to their mature weight and are fed on a very expensive diet.

Synthesis of results from a number of experiments with Trangie cattle following divergent selection for NFI has revealed many mechanisms are likely to be responsible for explaining the biological basis for variation in NFI (Richardson and Herd 2004).

1.5 Selection criteria to improve NFI

The Trangie experiment demonstrates that direct selection for NFI, based on measuring feed intake, is successful. However, measurement of individual feed intake outside of experimental situations is expensive, difficult and unlikely to be adopted on a large enough scale to have any impact on the industry at large. Therefore, other methods of selecting for NFI are needed.

If the objective were to improve feed conversion efficiency (FCE) of growing steers, selection for growth rate would be enough, because there is a high genetic correlation between growth rate and FCE over the same period. However, the objective is to increase FCE over the whole life cycle and growth rate is not highly correlated with this objective, because increased growth rate is associated with larger mature size and greater maintenance requirements (Herd and Bishop 2000). Therefore, selection for growth rate is not sufficient and by definition, will not improve NFI.

The concentration of the protein insulin-like growth factor-I (IGF-I) in blood has been found to be genetically correlated with NFI and could be used as a selection criterion (Moore *et al.* 2005). However, recent unpublished results show an unfavourable genetic correlation with fertility, so selection for low IGF-I (for lower NFI) may not be desirable as a means to increase profitability. Pitchford (2004) reviewed effects of selection for feed efficiency in a range of other species and raised the concern that negative correlated responses in reproductive performance could be expected.

Therefore, DNA tests for genes affecting NFI directly would be highly desirable because there is no other satisfactory method to improve NFI.

1.6 Mapping genes for NFI

Cattle

Given that net feed efficiency is a difficult trait to select phenotypically, the identification of DNA markers for this trait has become a priority for cattle breeding programs. Towards this end, genomic regions controlling net feed efficiency and related traits (called quantitative trait loci or QTL) have been mapped in the Davies Cattle Gene Mapping Project at the University of Adelaide. The Davies Cattle Gene Mapping Project had two primary goals: i) to study the mode of inheritance of important cattle production traits, and ii) to map the genomic regions (or QTL) controlling these traits. Once it is understood how traits are inherited, then improved selection strategies will be available for producer breeding programs. Mapping the QTL will lead to 1) identification of potential DNA markers for selection and/or 2) the identification of the actual net efficiency genes for study and manipulation.

The ideal design for mapping QTL for a large number of traits is to develop a population with a large amount of genetic variation. A common design is to cross two very different breeds to produce F_1 bulls. In the Davies Cattle Mapping Project, the F_1 bulls were then backcrossed to the two dam breeds to generate as many progeny as feasible for each sire.

The Davies Cattle Gene Mapping Project is part of a worldwide effort to map the cattle genome, and complements the other beef cattle gene mapping programs. However, the Gene Mapping Project had several important features that distinguish the Project from all other beef cattle mapping work. These include the:

- diverse *Bos taurus* breeds (Jersey and Limousin),
- extensive number of phenotypic traits that have been measured (around 300 traits),
- unique herd design (double backcross) which allows testing of QTL by breed interactions,
- large herd size (one of largest in the world with almost 800 gene mapping progeny total),
- additional measurements (fat and protein metabolic traits),
- only herd in two environments (Australia and New Zealand) representing both grain and grass finishing systems, and
- measurement of feedlot efficiency (net feed efficiency of grain finished cattle).

As a result of the Davies Cattle Gene Mapping Project, several QTL affecting feed efficiency were mapped in the Jersey-Limousin backcross progeny (Table 2, from TR.075 final report). One QTL was particularly large and if cattle were selected based on this QTL, the result would a significant reduction in feed intake (14%) with a small effect on cattle size (5%). This QTL, therefore, would improve net feed efficiency by 10%.

Table 2. Net feed intake QTL identified in cattle

QTL	NFI LOD score	NFI size*	DFI LOD score	Mid-wt LOD score	ADG LOD score
1	3.2	98	4.7	3.0	3.9
2	2.2	65	1.6		
3	2.0	57			1.7
4	1.9	50	2.2	1.8	3.4
5	1.7	46	2.2		
6	1.6	50	2.1	2.2	
7	1.6	46	1.5		1.5
8	1.5	51			2.5
9	1.5	46			2.9
10	1.5	45	2.3	2.3	

*Additive size of effect in percentage phenotypic standard deviations (1.55 kg/day in cattle) in family where the QTL had greatest effect, assuming no interaction between the QTL effect and sex or breed.

Some of these QTL are likely to be false positives. QTL mapping involves multiple testing, so stringent P values must be used. Additionally, the QTL detected in this experiment may not be segregating in commercial populations. Therefore, these QTL require verification.

Mice

For most traits mapped in the Davies Cattle Gene Mapping Project, the results could be immediately verified by direct comparison of the QTL data from the progeny raised in Australia and the progeny raised in New Zealand. Unfortunately, as the animals in New Zealand were raised on pasture, NFI and related traits could not be accurately measured in the New Zealand progeny.

Therefore, in addition to mapping QTL for net feed efficiency in cattle, a second approach was taken in a parallel experiment in which mice were used as a model. Mice were selected for high and low net feed intake for seven generations. These lines were then crossed to produce F1 sires and dams that were *inter se* mated to produce F2 progeny. Mapping was conducted in two sire families. (Hughes et al., 1997; Hughes and Pitchford, 2004).

The most significant ten QTL in mice had higher LOD scores than in cattle. This is likely because a more complex model was fitted to the data. Since an F2 design was utilised, dominance was estimated as well as the additive effect on the QTL. Both the additive and dominance components were included in the LOD scores. The more complex model also resulted in a large discrepancy between LOD scores and size of effect of the QTL in mice.

While the mice may not be expected to be a good model for cattle (eg ruminant vs monogastric, single bearing vs litter bearing), four of the QTL for net feed intake were in homologous chromosome regions. These results add to the confidence in the QTL identified.

Table 3. Net feed intake QTL identified in mice

QTL	NFI LOD score	NFI size*	Cattle QTL equivalent **
1	3.5	165	
2	3.2	50	
3	3.1	274	6
4	3.0	203	
5	3.0	28	
6	2.9	199	5
7	2.8	57	1
8	2.5	86	3

*Additive size of effect in percentage phenotypic standard deviations (0.32 g/day in mice) in family where the QTL had greatest effect. Dominance values not reported.
**Homeologous regions between cattle and mouse genomes.

Based on the QTL mapping results for cattle and mice, it was decided that five of the QTL should be further studied by 1) mapping these chromosomes for net feed efficiency in the Trangie Angus selection line families, and 2) identifying candidate genes within these QTL for association studies and fine mapping the QTL in the Davies progeny.

1.7 Purpose of this project

The purpose of the project was to map and identify genes for feed intake and efficiency of feed utilisation in beef cattle for the purpose of developing DNA tests for application by industry.

The Project utilised outputs from MLA/MRC projects TR.075 and DAN.075. MLA project TR.075 identified five chromosomal regions that contained genes having significant effects on NFI. MRC project DAN.075 produced phenotyped pedigrees and DNA, which was used within the Project.

The strategy was to use a combination of gene mapping and candidate gene studies. The gene mapping was carried out using the Trangie selection line Angus cattle. Initially, we used microsatellites to map QTL for NFI on the five chromosomes identified in the Davies Jersey x Limousin experiment. When the ParAllele 10,000 SNP whole genome scan became available, we used this technology to screen for QTL across the whole genome in the Trangie cattle. This powerful experiment allowed us to narrow the chromosome regions containing genes affecting NFI, and to discover new chromosome regions. At the same time, we discovered markers within candidate genes in the chromosome regions, and tested these for effects on NFI in the Davies cattle. They were supported by physiological studies of some of the candidate genes and mechanisms.

2 Project Objectives

1. Five chromosome regions, discovered in MLA project TR.075, will be linkage mapped to determine if they contain genes for NFI that are segregating in the Trangie Angus selection lines.
2. If objectives 1 finds significant quantitative trait loci (QTL), the relevant regions will be fine scaled mapped using linkage disequilibrium mapping to reduce the chromosome region to which the gene has been mapped. This was done using the 10 000 SNP whole genome scan, which as well as narrowing existing QTL regions identified new QTL.
3. Single nucleotide polymorphisms will be identified in candidate genes in the five regions of interest. Both MLA project TR.075 and MRC project DAN.075 cattle will be genotyped for the haplotypes developed.
4. Positional candidate genes from these regions will be selected and tested in association studies for an effect on NFI.
5. If objective 1 yields insufficient significant QTL, additional chromosomes will be linkage mapped. If QTL for NFI are discovered, they will be progressed through objectives 3 and 4.

3 Methodology

3.1 Cattle

3.1.1 Trangie NFI Selection Line herd

In 1993, a research project (MLA Project DAN.075) was started at the Agricultural Research Centre in Trangie, NSW to investigate the potential for genetic improvement in post-weaning feed efficiency as a means of improving whole beef production system efficiency.

Starting with the 1993 born animals, feed intake and efficiency tests were conducted each year using an automated feeding system that delivers and records individual animal feed intake. Feed efficiency was measured immediately post-weaning over a 70-day test and was calculated as NFI. NFI measures whether an animal consumes more or less feed than that predicted for growth and maintenance, with low NFI being superior. In 1994, high feed efficiency (low NFI) and low feed efficiency (high NFI) divergent selection lines were established. The 1993 born and 1994 born animals formed the foundation herd for this study. Starting with the 1993 born animals, the females were allocated to the high efficiency line and the low efficiency line based on their individual NFI values. The three most efficient bulls born in 1993 were allocated to the high efficiency line and the three least efficient bulls to the low efficiency line. Throughout the experiment, the sole selection criterion for all replacement bulls and heifers in the high and the low efficiency lines was individual NFI. This design was chosen to provide a rapid divergence in NFI between the high and low selection lines.

Only 200 animals could be tested in the Trangie feed efficiency testing facility at any one time, and for this project a maximum of 100 males and 100 females were tested per year. Therefore, there was very little selection in the females due to limited numbers. In the males, however, three to six bulls were selected per line each year, depending on the number of females available to be mated. Throughout the project, bulls and heifers were mated at 14 months of age, and bulls were used for only one mating season except for the 1997 and 1998 mating seasons where, for each selection line, one bull from the previous year was used again. Animals from each selection line were grazed together throughout the year, except during mating. Allocation of mates within selection line was completely random, except for the avoidance of half-sib and son-dam matings. All matings were by natural service. The first progeny of selected parents were born in 1995 and the last in 1999. Calves were nursed by their dams until weaning, and the breeding herd was on pasture all year round, with supplementary feed (lucerne hay and wheat) offered during times of limited pasture growth. The animals were brought to the efficiency testing facility a few weeks (generally 4 to 6 weeks) after weaning. Records taken during the test were used to calculate NFI for each animal. Details of the design of the DAN.075 project have been reported by Arthur *et al.* (1996) and establishment of the NFI selection lines by Arthur *et al.* (2001a).

3.1.2 Davies Cattle Gene Mapping Herd

Six large paternal half-sib pedigrees were produced from a reciprocal backcross between the divergent Jersey dairy breed and Limousin beef breed. Three pairs of first cross (F_1=X) brothers were generated, with one from each pair used for mating in either Australia or New Zealand to both pure Jersey (J) and pure Limousin (L) dams, resulting in a total of 784 XJ and XL backcross progeny (range 120-156 progeny/sire). The 366 Australian Limousin-Jersey backcross progeny were born over 3 years (1996-1998) to the 3 F1 sires as follows: 77 born April 1996, 153 born April 1997, and 136 born April 1998. These progeny were grown out on pasture in pre-allocated slaughter groups

until approximately 28 months of age, and then finished on grain concentrates for at least 6 months as part of a feed efficiency trial (age of slaughter 34-40 months). Thus, routinely, the cattle in Australia were kept on grass for 800-900 days and then grain fed for approximately 200 days. [The 418 New Zealand backcross calves were born over 2 years (1996-1997), grown out on pasture without grain concentrates, and slaughtered at 24-28 months of age.] Blood samples from the grandparents, sires, Australian dams and backcross progeny were taken for DNA extraction.

3.2 Phenoytypic measurement

3.2.1 Trangie: Postweaning NFI

At the efficiency testing facility, a pre-test adjustment period of at least 21 days was allowed for the animals to adapt to the feeding system and diet, followed by a 70-day test as recommended by Archer *et al.* (1997). The average age at the start of test was 268 days. During the test, animals had *ad libitum* access to a pelleted ration of approximately 10.5 MJ/kg dry matter and 16% crude protein. Records taken during the test were used to calculate NFI for each animal.

The growth of each animal during the test was modelled by linear regression of weight on time (days), and the regression coefficient represented average daily gain (ADG). The mean weight (MWT) of an animal during the test was computed as the average of the start and end of test weights. Metabolic body weight (MMWT) was calculated as $MWT^{.73}$. Feed intake (FI) was standardised to a concentration of 10 MJ ME/kg dry matter. Feed conversion ratio was calculated as FI divided by ADG. A linear regression model of FI on MMWT and ADG for each animal, with test group and sex included as class variables, was fitted to the data. The regression coefficients from this model were used to obtain expected feed intake of all animals based on ADG and MMWT. NFI was calculated as the actual (measured) FI minus that predicted using the regression equation.

3.2.2 Davies Cattle Gene Mapping Project

To maximise the return on the investment of genotyping, as many traits as possible were measured in the Davies Cattle Gene Mapping progeny. Furthermore, results from Adelaide, Trangie and elsewhere point to possible correlated responses in carcass traits when selecting for net feed intake. Thus, it was important to be able to quantify meat yield and distribution as well as net feed efficiency. Consequently, the animals were trucked to Brisbane (Valley Beef Abattoir, Grantham) for slaughter and boned out similar to the Beef CRC cattle (1996 and 1997 drop). Similar information was collected for the 1998 drop, slaughtered and processed at the T&R Pastoral Abattoir in Murray Bridge, SA. The carcass composition information enabled mapping for genes affecting meat yield and distribution of muscle mass, in addition to feed efficiency.

The traits measured over the lifetime of the progeny fell into the following groups:

1. Pedigree and birth traits (eg. birth weight, linear measurement at birth, gestation length)
2. Growth (live weights every 30-50 days)
3. Skeletal growth (eg. regular live animals linear measurements plus carcass length, pelvic dimensions, bone lengths, bone weights)
4. Puberty traits (eg. age plus weight at puberty by interpolation, dentition and ossification as indicators of physiological traits)

5. Other live animal traits (eg. horns, coat colour, blood enzymes, hormones, metabolites and minerals from slaughter tissues)
6. Temperament traits (eg. exit speed, flight distance, docility score, plasma and urinary cortisol)
7. Food intake/efficiency (plus eating rate) AUSTRALIA ONLY
8. Disease resistance (eg. faecal egg counts, facial eczema score) NEW ZEALAND ONLY
9. Carcass dissection (starting at HCW, including meat distribution and yields)
10. Fat deposition traits (eg. marbling, fat depths (ultrasound and carcass), fat distribution (including fat depots and IMF%)
11. Fat quality traits (eg. fatty acid composition, fat melting point, fat colour)
12. Meat quality traits (eg. tenderness, pH, muscle glycogen, meat colour, compression toughness)
13. Taste traits (eg. cooking loss)
14. Other slaughter measures (eg. organ weights, hide traits, rumen and intestinal weights)

Specifically, for the net feed efficiency traits, the following were determined: mid-weight (kg), weight gain (kg/day), daily feed intake (kg/day), net feed intake (kg/day), maintenance requirement (%feed/mid-weight), gross efficiency (%ADG/kg feed), number of meals (#/day), eating time (minutes/day) and eating rate (g/sec). Principal components were also formed for sub-sets of the traits as these traits were correlated. These traits have all been reported as part of the MLA project TR.075.

3.3 Genotyping

3.3.1 Microsatellites

Davies

Sire-derived alleles were determined for the 784 Australian and New Zealand backcross progeny with a total of 253 informative microsatellite loci (average 185 loci per sire group, range 170-196) spread across the whole genome, excluding the X- and Y-chromosomes (Morris *et al.,* 2001). Average microsatellite marker spacing was <20 cM per F_1 sire family with 3-9 informative markers per sire per chromosome genotyped. Microsatellite markers were genotyped in New Zealand by radioactive PAGE (AgResearch) with each gel independently scored twice.

Trangie

Following results of QTL mapping in the Davies population, five chromosomes were selected for a mapping experiment in the Trangie population. These were chromosomes 1, 6, 8, 11, 20. There were between 7 to 4 microsatellite markers per chromosome. The markers were selected to be evenly spaced along the chromosome. The sire families that were genotyped were selected across the Trangie pedigree, with the main criteria being sufficient numbers of progeny per sire to provide sufficient power to detect QTL of moderate to large effect. 1600 animals were genotyped for each marker.

3.3.2 ParAllele SNPs

Approximately equal numbers of the extreme highest and lowest feed efficiency animals were selected from the Trangie population for SNP genotyping. Care was then taken to ensure that, where possible, animals were in half sib-groups of 2 or more and not exceeding 10, so at least 30

sire groups were sampled. Also where possible, half sibs groups were chosen so that they represented both extremes of the net feed efficiency distribution. A total of 225 animals were selected in this way, and where available, their sire and/or dam DNA was also submitted for SNP genotyping, to increase the accuracy of haplotyping. This resulted in SNP genotypes for 379 animals. The genotyping of 9323 SNPs, randomly distributed across the bovine genome, was performed at ParAllele Bio Science Inc. Of these 9323 SNPs, 8326 were polymorphic (that is, had more than one allele) in our data sample. In an effort to remove genotyping and pedigree errors from the data, we used the pedigree structure of the population to check for Mendelian segregation errors using standard methods. All suspected pedigree and genotyping errors were removed.

3.3.3 Candidate genes

Based on QTL mapping results in the experimental animal data, we concentrated on the QTL for net feed efficiency that mapped to homeologous regions in cattle and mouse. Using available gene mapping data in cattle, we have prepared comparative homology chromosomal maps for human and mouse from 4 cattle QTL map locations. From the available online human genome data, we prepared a list of genes based on their function to be sequenced and genotyped for four cattle chromosomes. Another potential NFI gene list was prepared from the mouse mapping information.

For each candidate gene, primers were designed to amplify the coding regions of the gene and the PCR conditions were optimised to obtain a single genomic product from those primers. The genomic DNA from the 3 F1 mapping sires was amplified and sequenced. Sequence from the three sires were aligned using Sequencher software and any sequence variant (single nucleotide polymorphisms, in/del, etc) was noted. Sequence variants were confirmed by 1) sequencing the sire PCR products in the opposite direction and 2) sequencing PCR products amplified using genomic DNA from the grandparents.

All confirmed, potentially functional DNA variants were genotyped. In addition, wherever possible, two confirmed single nucleotide polymorphisms (SNPs) for each gene were genotyped so that each candidate gene would have 3 possible haplotypes. SNPs were genotyped by amplifying approximately 200 bases flanking the SNP and using primer extension (Applied Biosystems) to discriminate the SNP alleles by fluorescence polarisation.

3.4 Functional studies of candidate genes and pathways

Muscle and liver samples were collected from Angus animals at the abattoir and frozen immediately in liquid nitrogen. The samples were stored at -80^0C until analysed (Fredriksson, *et al* 2005; Brooks and Krahenbuhl, 2000; Rasmussen and Rasmussen, 2000). The samples came from animals in the Angus Elite Progeny Testing Program, a joint venture between Angus Australia and Meat and Livestock Australia (MLA). The animals were derived from the Trangie Angus net feed intake selection lines and measured for net feed efficiency at Tullimba. Samples from the 20 most extreme high and low efficiency animals were used in the assays to ensure the maximum physiological difference.

As intact mitochondria were not required for the enzyme assays conducted (Bourges, *et al*, 2004; Campbell and Fabbraio, 2001; Minchenko, *et al*. 2003) and fresh samples were not available, frozen muscle and liver samples were used to prepare the cytosol and mitochondria. The samples from the high and low efficiency animals were treated identically and any loss in enzyme activity due to freezing should not differ between the samples. The cytosol (for the AMP-activated protein kinase

assay) and the mitochondria (for the complex I, complex II and complex IV assays) were prepared using standard protocols (Bhattacharya, *et al* 1991). The results from these samples were similar to previous reports (Procaccio, *et al* 1999; Bourges, *et al*, 2004). The mitochondrial preparations also were used in the proteomic experiments.

AMP-activated protein kinase (AMPK) assay

In the cell, acetyl-CoA carboxylase and other proteins are phosphorylated by AMPK. However, a synthetic peptide (SAMS) is a specific substrate for AMPK and can be used to measure AMPK activity *in vitro* in the presence of AMP (Davies *et al*. 1989; Hawley *et al*. 1996). In the absence of additional AMP, there is no specific activation and only background activity is observed. Therefore, AMPK activity was determined as the level of phosphorylation measured by using ^{32}P-ATP to label SAMS in the presence and absence of AMP.

Mitochondrial assays

For the complex I assay, the activity of the NADH-ubiquinone oxidoreductase/NADH dehydrogenase was measured spectrophotometrically by following the reduction of 2,6-dichlorophenolindophenol (DCIP) by NADH at 600nm absorbance (Trounce, *et al*. 1996; Madapallimattam, *et al.* 2002). The sensitivity of the assay was verified by using the complex I inhibitor rotenone.

For the complex II assay, the activity of the succinate-ubiquinone oxidoreductase/succinate dehydrogenase was measured spectrophotometrically by following the reduction of 2,6-dichlorophenolindophenol (DCIP) by decylubiquinone (ubiquinone analogue) at 600nm absorbance (Trounce, *et al*. 1996; Madapallimattam, *et al.* 2002). The sensitivity of the assay was verified by using complex II inhibitors (malonate and thenoyltrifluoroacetone).

For the complex IV assay, the activity of the ferrocytochrome c oxygen oxidoreductase/cytochrome c oxidase was measured spectrophotometrically by following the oxidation of reduced cytochrome c at 550nm absorbance (Trounce, *et al*. 1996; Madapallimattam, *et al.* 2002). The sensitivity of the assay was verified by using the complex IV inhibitor potassium cyanide.

Two-dimensional protein gel electrophoresis

To examine whether the concentration of specific proteins in the mitochondria was different between high and low efficiency animals, a more global approach was also taken using proteomics. Proteomics involves using 2D gel electrophoresis to separate proteins based on charge and mass by iso-electrical focusing (Verma*, et al.* 2003; Pedrioli, *et al.* 2004). For the project herein, a differential gel electrophoresis (DIGE) methodology (Albans, *et al* 2003; Alfonso, *et al* 2005) was applied. The steps for each experiment include:

1. Sample preparation and fluorescent labelling
2. First dimensional separation
3. Equilibration
4. Second dimensional separation
5. Scanning
6. Image analysis
7. Protein identification

50 µg (as measured by the Bradford assay) from each sample was used for each 2-D gel. The proteins were dissolved under denaturing conditions (urea and detergents) and labelled with either Cy3 or Cy5 for samples from high and low efficiency animals. The dyes were then swapped for the

samples from high and low efficiency animals in a second experiment. Cy2 is used to label a pool of all samples as a standard.

The proteins are then separated in an electrical field according to their isoelectric points (pI values) using pre-cast immobilised-pH-gradient (IPG) gel strips and carrier ampholytes. The proteins migrated to a position where their net charge is 0. The focusing range was wide (pH 3-11), so resolution was approximately 0.5 pH units.

The gels are then scanned by densitometry to detect differences in quantity of specific proteins between the samples based on the spot intensity. The spots representing proteins that varied by more than 1.5-fold concentration between the samples from high and low efficiency animals were located. These proteins were excised from the gel, digested and identified using mass spectrometry.

3.5 Statistical analysis

3.5.1 Microsatellites

Trangie

The microsatellite data and the NFI phenotypes were analysed in a two-step approach. In the first step, the inheritance of the markers is traced through the complex pedigree that connects all the animals using a Gibbs sampling method. This computer program calculates the probability that any two animals inherited QTL alleles, at a specific point on the chromosome, that are identical by descent. The second step uses these probabilities in a linear model that includes the QTL, polygenic inheritance and fixed effects. A likelihood ratio test was performed to evaluate if the QTL was significant at each point along the chromosome. Let L_0 be the likelihood value for the model under H_0, where the QTL is not included in the model. Then L_1 is the likelihood value for the alternative model, that is, the QTL are included in the model. The test statistic was defined as $-2(\ln L_0 - \ln L_1)$. This statistic is significant at P<0.05 when it exceeds 3.84.

Davies

A full genome scan using microsatellite markers were completed on these 366 calves. There were 3-9 markers typed per chromosome for all 29 autosomes. Only those markers for which the sire was heterozygous were typed in the sire's progeny. In the half-sib design utilised, sires were classified as having the genotype "AB" for each marker. Thus, progeny would either inherit the "A" or "B" allele from the sire and another allele from the dam. Therefore, the potential genotypes of the progeny were AA, AB, BB, AC, or BC where C was any other allele. If the progeny was AB, they were not informative for the analysis as it was not clear which allele they inherited from the sire.

Linkage maps were produced using CRIMAP to confirm the order of the markers and evaluate the data for multiple recombinants. Genotype probabilities were calculated using "QTL Express" (Seaton, et al. 2001) so that at every point (1 cM) of every chromosome, calves were assigned a value of either 0 (=A) or 1 (=B) or somewhere in between depending upon the level of confidence. When markers were uninformative, genotype probabilities were 0.5.

Phenotypes were regressed against the genotype probabilities for every chromosome. Net feed intake was calculated separately for each cohort so cohort was not included as a fixed effect. Breed of dam was a factor in the model and the regression was nested within sire.

3.5.2 ParAllele SNPs

We estimated the effect of each of the 8326 polymorphic SNPs on NFI. The analyses were performed in ASREML (Gilmour et al., 1999) using the following model:

$NFI_{hijkl} = \mu + \text{contempary group}_h + \text{start age}_k + \text{allele1}_i + \text{allele2}_j + \text{animal}_k + e_{ijkl}$

where NFI_{hijkh} is the phenotypic record for net food intake for $animal_k$ with SNP alleles i and j, μ is a fixed effect of the mean, contemporary $group_h$ is defined as "all animals from the same herd||sex||test group||management group subclass" (Arthur *et al*, 2001). Start age_k was the age at which animal k began the NFI test, and was fitted as a covariate. The effects of animal and SNP alleles were treated as either fixed effects or random effects for comparison with the haplotype effects (see below). When the SNPs were fitted as random effects the following (co) variance matrix was used:

$$Var\begin{bmatrix} allele \\ animal \\ e \end{bmatrix} = \begin{bmatrix} \sigma^2_{allele} * I & 0 & 0 \\ 0 & \sigma^2_{animal} * A & 0 \\ 0 & 0 & \sigma^2_e * I \end{bmatrix}$$

where, σ^2_{allele}, σ^2_{animal} and σ^2 are variance components estimated simultaneously with the effects, *I*

e is the identity matrix, and the additive genetic relationship matrix *A* included 4734 animals. Each SNP had two allele effects. When SNPs were fitted as fixed effects, an F test was used to test significance. When SNPs were fitted as random effects, a likelihood ratio test was performed to evaluate if the SNPs had significant effects on NFI. Let L_0 be the likelihood value for the model under H_0, where the haplotype is not included in the model. Then L_1 is the likelihood value for the alternative model, that is, the SNPs are included in the model. The test statistic was defined as $-2(\ln L_0 - \ln L_1)$. This statistic is expected to value a chi square distribution with degrees of freedom 1. We converted the test statistic into a P value using the chi square 1 degree of freedom distribution.

Significance thresholds of decreasing levels of stringency were tested. For example, if the significance threshold was P<0.001, all SNPs with a P values less than 0.001 were considered as significant.

To determine which significance threshold was most appropriate, the false discovery rate (FDR, Weller et al. 1998) was used. The false discovery rate, the proportion of significant SNPs expected to be false positive results (type 1 errors), was calculated as FDR = $m*P_i/n_i$, where m is the number of tests being performed (8326), P_i is a chosen significance threshold, and n_i is the number of SNPs significant in the data set at the i^{th} level of significance. We tested various significant thresholds, and determined that P<0.001 was an acceptable level as 100 SNPs were significant at this level with an acceptable FDR of 0.08. These 100 SNPs were then used in further analyses.

The next step was to determine the location of these 100 SNPs relative to each other on the bovine genome. To do this, we took advantage of both the ongoing bovine genome sequencing project (http://www.hgsc.bcm.tmc.edu/projects/bovine/) and the high degree of synteny between the bovine and human genomes (eg. Gautier *et al*. 2003). From the bovine genome sequencing project, 250 bases of sequence to the left and 250 bases of sequence to the right of each SNP genotyped is available. We used these 500 bases of sequence to determine the contig (bovine genome build 1.1) to which the SNP belonged, using BLASTN. We then took these bovine contigs and positioned them on the sequenced human genome (http://www.ncbi.nlm.nih.gov/genome/guide/human/). This gave a human chromosome physical position for each of our SNPs.

Independently of the physical position information, we determined which of the 100 significant SNPs showed correlations between their alleles, ie linkage disequilbrium. High levels of linkage disequilbrium are indicative of physical linkage. We used the ldmax procedure in the GOLD program (http://bioinformatics.well.ox.ac.uk/~lon/Projects/project-ld.html) to calculate the r^2 measure of linkage disequilbrium (Hill and Robertson 1968) between all possible pairs of the 100 loci. The ldmax procedure uses the algorithm of Slatkin and Excoffier (1995) to calculate r^2 for pairs of loci from genotype data.

The SNP pairs with r^2 levels > 0.25 were extracted from the output. We then grouped the pairs of SNPs into “clusters”, where r^2 between all loci in the cluster was >0.25. The clusters consisted of between 2 and 4 SNPs.

We then compared the physical location of the SNPs from the human genome positions to the clustering results. In 20 cases, the SNPs that were physically located very close on the chromosome (within 1 megabase) also clustered on r^2 values (out of 21 suggested clusters from the r^2 data).

For these 20 “clusters”, we constructed haplotypes of the SNPs within the cluster. To construct haplotypes, the genotypes of the SNPs which were a member of the cluster were extracted from the

data, and the PHASE (Stephens et al., 2001) program was used to determine both the haplotypes in the data and the most likely haplotype pair for each animal.

The effects of the haplotypes on NFI were calculated for each cluster separately. The analyses were performed in ASREML (Gilmour et al., 1999) using the following model:

$NFI_{hijkl} = \mu + \text{contempary group}_h + \text{start age} + \text{haplotype1}_i + \text{haplotype2}_j + \text{animal}_k + e_{ijkl}$

where NFI_{hijkh} is the phenotypic record for net food intake for $animal_k$ with haplotypes i and j (from the PHASE most likely haplotypes) , μ is a fixed effect of the mean, $contemporary\ group_h$ is described above. The effects of animal and SNP alleles were treated as random effects, with the following (co) variance matrix:

$$Var\begin{bmatrix} haplotype \\ animal \\ e \end{bmatrix} = \begin{bmatrix} \sigma^2_{haplotype} * I & 0 & 0 \\ 0 & \sigma^2_{animal} * A & 0 \\ 0 & 0 & \sigma^2_e * I \end{bmatrix}$$

where, $\sigma^2_{haplotype}$ is the variance component associated with the haplotype effects and the other

variance components are defined as above. A likelihood ratio test was performed to evaluate if the haplotypes had significant effects on NFI. Let L_0 be the likelihood value for the model under H_0, where the haplotype is not included in the model. Then L_1 is the likelihood value for the alternative model, that is, the haplotypes are included in the model. The test statistic was defined as $-2(\ln L_0 - \ln L_1)$

.

3.5.3 Candidate genes

In the candidate gene analysis, the candidate genes were placed first positioned on microsatellite linkage maps using CRIMAP. Positions were confirmed from other sources (eg bovine USDA map) where possible. Linkage analysis was performed using these linkage maps and "QTL Express" as described above.

In addition to the linkage analysis, an association (linkage disequilibrium) analysis was carried out in a number of different ways. Initially, all 38 SNPs were coded as factors with 3 levels (e.g. aa, ab, bb) and included in a mixed model across all animals with NFI records. One of the important parameters to estimate is the variance accounted for each SNP. This is a function of both the size of effect (α) and the allele frequency (p) as follows:

Variance = $2p(1-p)\alpha^2$

The advantage of using the mixed model for testing SNPs is that the variance is estimated, indicating that both the size of effect may be useful and the gene frequency intermediate. Both these criteria must be met if the SNP is to be commercially valuable. If the SNP accounts for little variance, it converges to zero and is automatically not included in the final model. However, these variance estimates are poorly estimated with only 3 classes and for a number of the SNPs, there are very unequal numbers across the genotypes. The obvious alternative is to include the SNPs as fixed effects, but this results in a model that is highly parameterized with possibly overestimated size of effects.

It is expected that the primary use of SNP genotype information in the future will be to estimate breeding values. Thus, the genotypes can be coded as covariates with values –1, 0 or 1 representing aa, ab and bb, respectively. Fitting the SNPs in this form either saves a degree of freedom for each SNP (if fixed) or minimizes bias (if random). In addition to examining single SNPs in the two methods outlined, when there were 2 SNPs per gene, haplotypes were formed and tested to see if they were more informative (as expected) than single SNPs.

While fitting mixed models to the Jersey-Limousin data, the opportunity was also taken to estimate heritability to determine the amount of genetic variation in NFI in the population. The population has 3 large half-sib familes (3 sires) and many dams with only single calf records. Thus, the population is less than ideal for parameter estimation. However, the results from the analysis can be used to benchmark the population to larger and better populations (eg Trangie selection lines). The population is also less than ideal for SNP verification because of the low number of half-sib familes. Thus, significant SNPs will have to be verified in additional populations.

4 Results and Discussion

4.1 Microsatellite mapping of QTL on five chromosomes

In the Trangie Angus selection line population, there were clearly significant QTL on BTA 1, 6, 8 and 20 and additional marginally significant QTL on BTA 1, 11 and 20 (Figure 1).

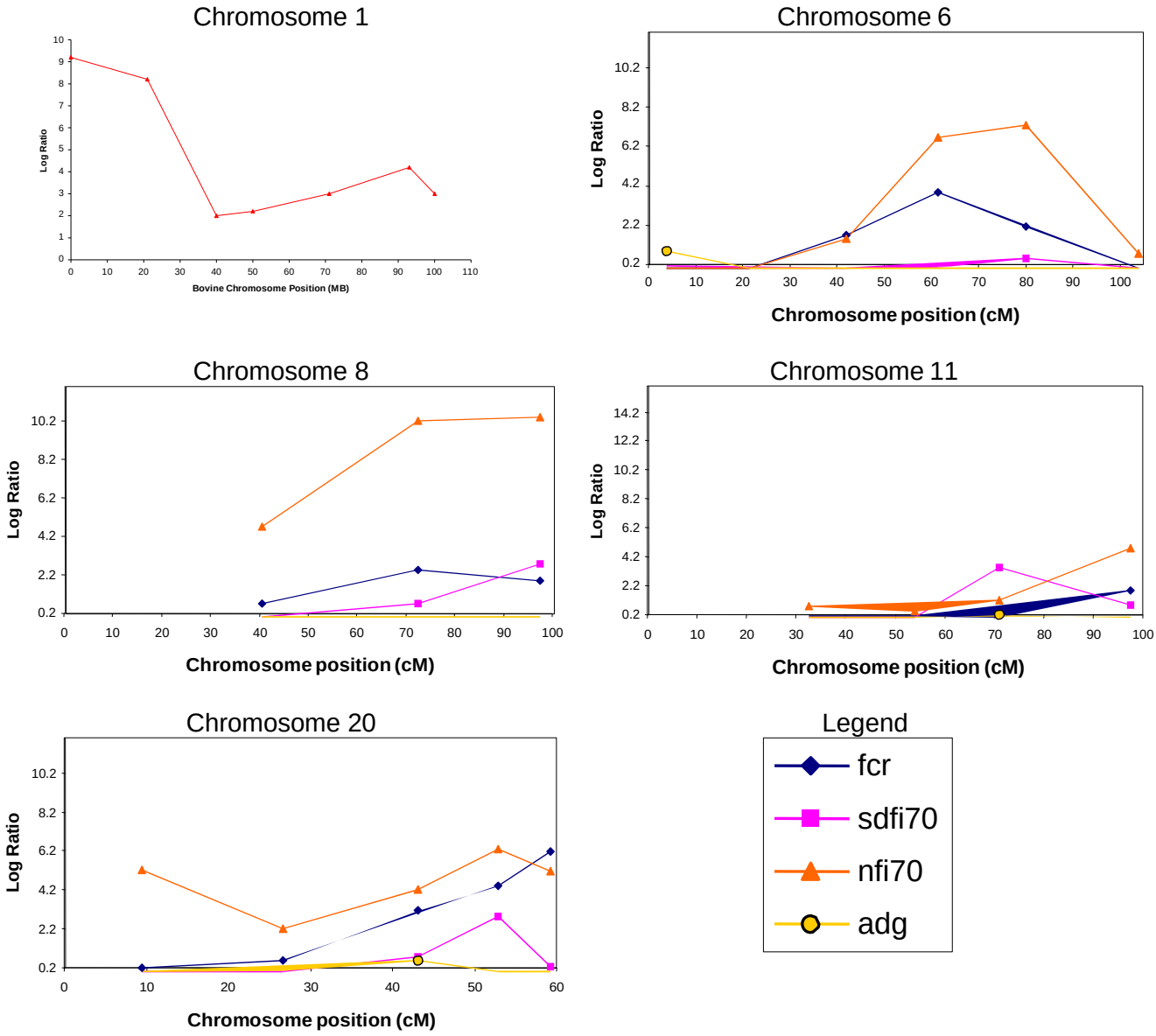


Figure 1. Results of QTL mapping for NFI on chromosomes 1, 6, 8, 11 and 20 in the Trangie Angus selection lines. Log ratio of greater than 3.84 is considered significant.

The clearly significant QTL on BTA 6 and 8 are in similar positions to those found in the Davies herd (Appendix 9.5). Also the marginally significant QTL on BTA 1, 11 and 20 are in similar positions to QTL found in the Davies herd. These could be the same genes as found in the Davies experiment, although we can not be sure of this because of the large standard errors on the position of the QTL in both the Davies and the Trangie cattle.

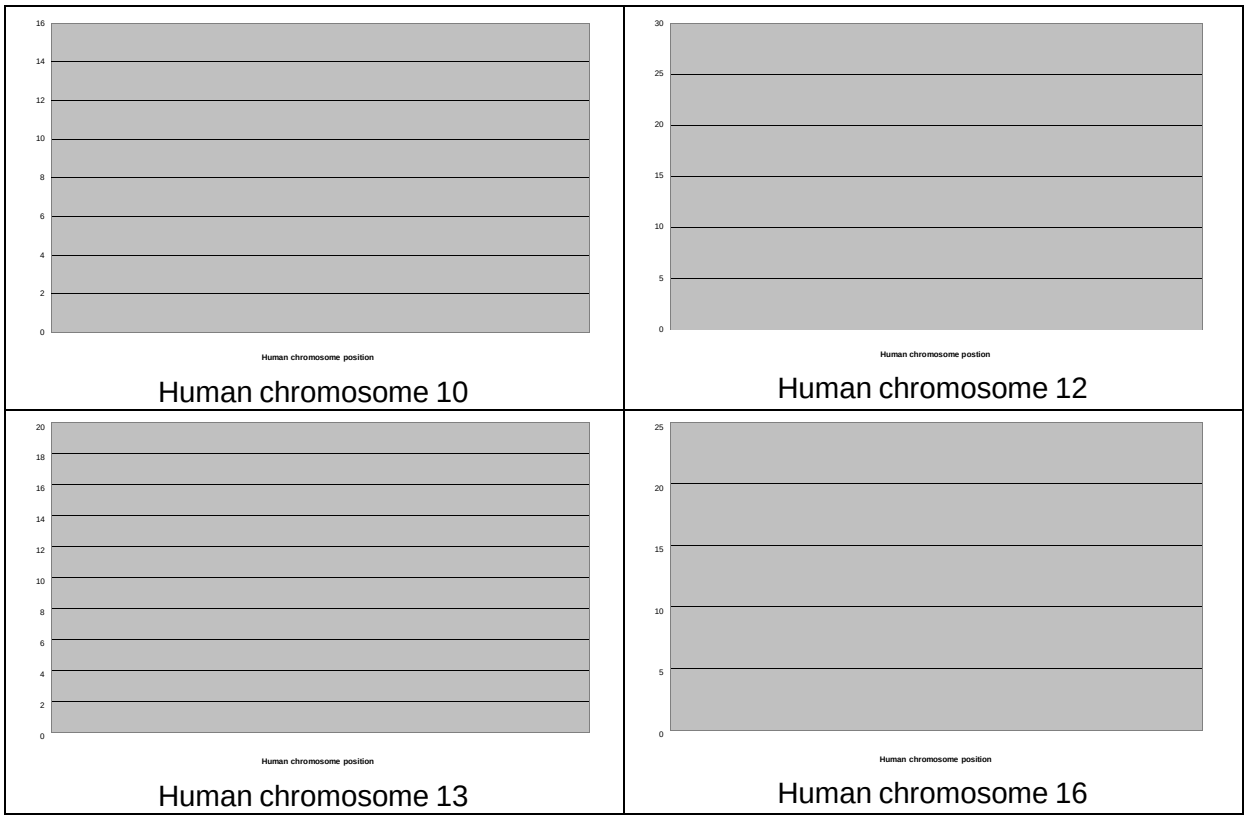
4.2 ParAllele whole genome scan and agreement with linkage mapping results

4.2.1 Analysis of the effect of single SNPs on NFI and location on the genome

There were 100 SNPs significant at P<0.001, when SNPs were fitted as fixed or random effects. The false discovery rate at this level of stringency is 0.08, indicating only 8 of the 100 SNPs are likely to be false positives.

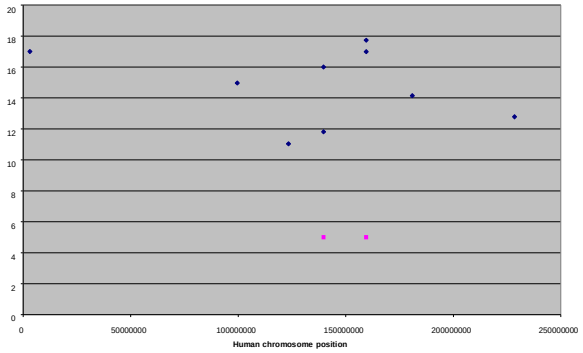
When we investigated the pattern of linkage disequilibrium between these SNPs, 55 pairs of SNPs had an r^2 value of greater than 0.25, indicating the SNPs were likely to be in close proximity to each other. These pairs of SNPs were further grouped into 21 clusters, where the r^2 values between all SNPs in the cluster was greater than 0.25. Of the 100 SNPs, 56 were contained within one of the 21 clusters.

Twenty of the 21 clusters from the r^2 data contained SNPs that had physical locations, when mapped to the human genome, which were within a megabase (Figure 2).

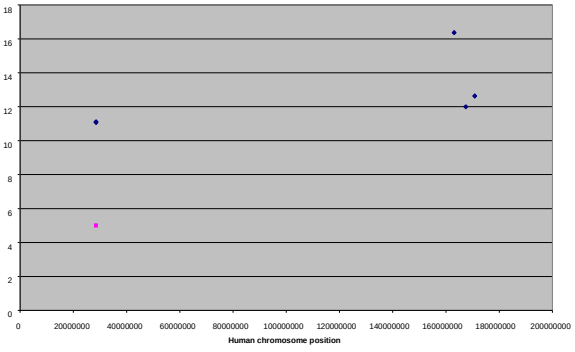


Human chromosome 10 | Human chromosome 12

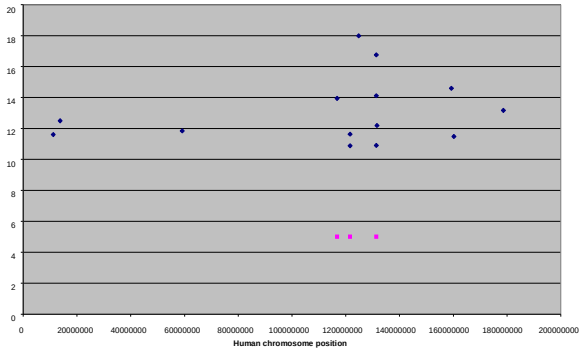
Human chromosome 13 | Human chromosome 16



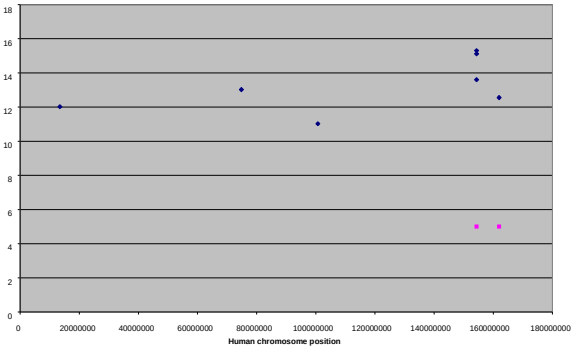
Human chromosome 2



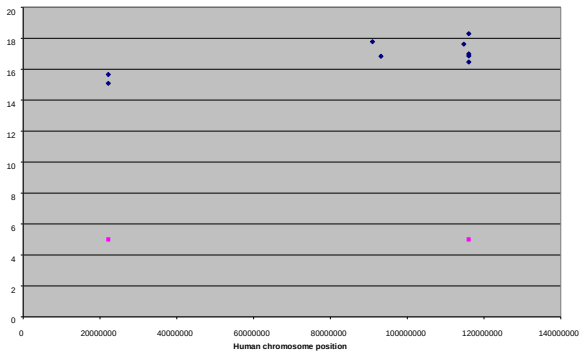
Human chromosome 3



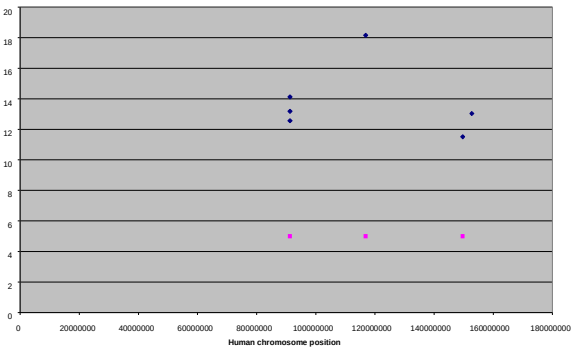
Human chromosome 4



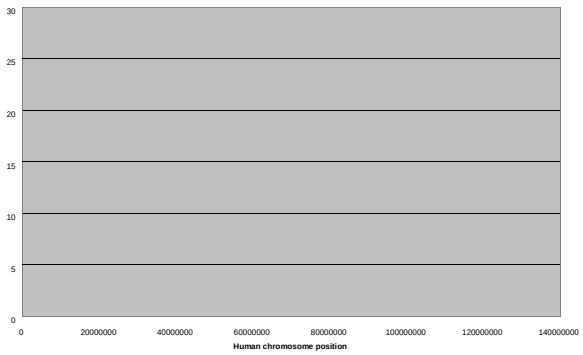
Human chromosome 5



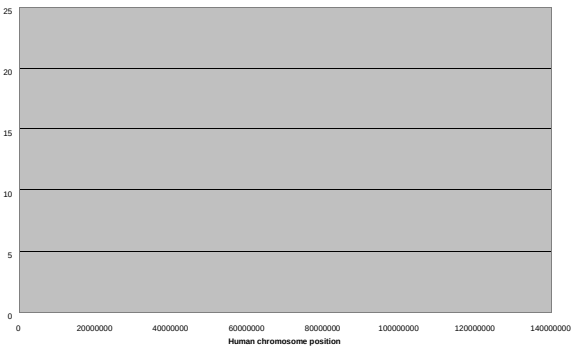
Human chromosome 6



Human chromosome 7



Human chromosome 9



Human chromosome X

Figure 2. Physical position of significant SNPs on the human genome and agreement with SNP "clusters" based on r^2 values of pairs of SNPs. The x-axis of each graph is the physical position across the human chromosome, and the y-axis is the test statistic of significant SNPs (blue triangles). Pairs of adjacent SNPs, which have r^2 values > 0.25, belong to clusters are indicated by pink squares underneath each of the SNPs in the pair.

4.2.2 Analysis of the effect of haplotypes on NFI and location on the genome

When the SNP data within each cluster were sorted into haplotypes, there were between 2 and 8 haplotypes per cluster (Appendix 9.1). The largest favourable effect of a haplotype on NFI was for haplotype 3 of cluster 1 on chromosome 5, with an effect of –0.693 kg, a large effect considering the phenotypes of the animals in the data set for NFI ranged from -4.41 kg to 3.13kg.

As the SNPs we have used in this analysis are randomly distributed across the bovine genome, they are unlikely to be functional, ie the SNP which is actually causing the difference in gene action responsible for differences in NFI (functional mutations are most likely to be within exons, introns (e.g. Andersson and Georges, 2004) promoters and other regulatory sequences (e.g. Hoogendoorn *et al*., 2003). However, the functional mutation(s) will have occurred in an ancestral chromosome segment, copies of which persist in the current generation of animals. These identical by descent (IBD) chromosome segments can be identified in the current population by unique haplotypes of SNP alleles. A haplotype of SNPs is more likely to identify these IBD chromosome segments uniquely than a single SNP. Our results reflect this, as in a number of cases the effect of the haplotypes, as measured by the test statistic, were considerably more significant than the effect of the individual SNPs comprising the haplotypes. For example, the haplotypes of cluster 2 on chromosome 12 have a test statistic of 33.5, while the maximum significance value of an individual SNP within this cluster is 25.2.

4.3 Comparison of Microsatellite mapping of QTL and ParAllele whole genome scan

The results from both the microsatellite mapping and the 10,000 SNP genome wide association test were combined in order to cross validate results and identify a smaller target region for QTL. When analysed separately, a 505MB QTL region over 10 chromosomes was the result (Table 4). When combined, the QTL region decreased to a 60MB region over 5 chromosomes. The QTL mapped by the microsatellites were confirmed for chromosomes 6, 8 11, and 20 (Appendix 9.6).

Table 4: Combined microsatellite and ParAllele QTL results decreases target regions

	ParAllele QTL	Microsatellite QTL
ParAllele QTL	21 (235MB 15 chromosomes)	6 (60MB 5 chromosomes)
Microsatellite QTL		7 (270MB 5 chromosomes)

Cross validation of the results from both experiments can allow us to be confident that six of the seven microsatellite regions contain QTL affecting net feed efficiency. The results of the genome wide association study greatly reduce the number of genes that have to be considered as candidates.

4.4 Effect of SNPs in Candidate genes in the Davies Population

Using comparative mapping between the cattle, human and mouse maps, candidate genes within the net feed intake QTL regions were selected based on their known function (Table 5, Appendix 9.2). Primers were designed for all genes with bovine sequence available such that the coding regions of each gene could be amplified by PCR. Conditions for all primer sets were optimised and the genomic DNA from the three Davies F1 sires was amplified. The PCR products from the 3 sires were sequenced and the DNA sequence data were aligned to determine if any sequence variants were present.

If sequence variants (usually single nucleotide polymorphisms or SNPs) were discovered, they were verified, first by sequencing the PCR product in the opposite direction and then, sequencing the PCR product from the grandparents of the sires to confirm Mendelian inheritance. In total, 42 genes were screened and 201 SNPs were identified.

Table 5. Single nucleotide polymorphism discovery for association tests

BTA	# Genes	# SNPs	# Potentially functional SNPs
1	11	29	8
8	5	17	3
11	4	21	5
20	7	66	11
6	5	20	not determined
16	10	48	not determined
Total	42	201	

Confirmed SNPs were selected as markers for additional linkage analysis in the QTL regions previously identified in the Davies population, as well as association studies in this population. The SNPs markers were chosen such that 3 haplotypes/gene were present and at least 3 - 4 genes were informative in the sire of interest for that QTL (Table 6, Appendix 9.3). Thus, two SNPs per gene were chosen for both linkage analysis and association testing on BTA 1, 8, 11 and 20. The lack of genes mapped on BTA11 resulted in fewer informative genes for this chromosome. The chosen SNP markers were genotyped in the Davies backcross mapping progeny by primer extension. Further, some SNPs were likely to be functional variants. That is, the change in the DNA sequence would be likely to affect the expression or activity of the gene product. All functional SNPs were also genotyped in the Davies gene mapping progeny. The SNP markers were placed on linkage maps using CRIMAP and positions verified where possible from other sources (eg USDA map).

Table 6. Number of genes containing SNPs included in linkage analysis

BTA	# Genes	# Informative Genes[1]			Segregating sire(s)
		361	368	398	
1	7	4	4	5	398
8	4	3	3	4	368, 398
11	4	3	2	0	361
20	7	3	5	4	361, 398

[1]Genes heterozygous for sires 361, 368, and 398

Analysis of the data indicates that the previously observed QTL are still significant (Table 7, Appendices 9.4 - 9.5). Including the SNP genotyping data in linkage analyses gave more statistical support for the previously identified QTL (Table 8).

Table 7. Linkage mapping results across families

BTA	**Segregating sire(s)**	**Old F-value[1]**	**New F-value[2]**	**Old QTL location[1]**	**New QTL location[2]**
1	398	3.4	3.4	104 cM	104 cM
8	368, 398	2.7	2.8	95 cM[3]	95 cM[3]
11	361	3.9	4.1	69 cM	68 cM
20	398	4.5	5.6	53 cM	49 cM

[1]Old = linkage analysis using microsatellite markers only
[2]New = linkage analysis using microsatellite + SNP markers
[3]Note: the putative QTL identified within the family of sire 368 is located at 0 cM.

There is some support for a second QTL on BTA8 segregating in the family of sire 368. For another QTL (BTA20), the statistical support noticeably increased and the map location was refined. There is also some suggestive evidence for QTL on BTA20 segregating in sire 361 and 368 families as well.

In addition to the linkage analysis, the SNPs were examined for association with net feed efficiency using two analyses. The SNPs were fitted as random main effects and haplotypes and coded as factors with 3 classes or additive (covariate) effects. The model also included the fixed effect of breed of dam (Jersey or Limousin). In addition to the SNPs, an animal model to account for polygenic effects was included. However, for both genotype and additive effects, the largest two SNPs were sufficient to prevent the polygenic variance from being estimated.

Most genes that were associated with variation in NFI had similar variance estimates for both methods. The exception was IL12. It is assumed that this is because there is only 6 of one of the homozygous genotypes, and the lack of balance was less severe when fitting as an additive effect. If the actual allelic effect was estimated and based on a simple t-test, only three SNPs (UMPS, MAP1B and AMPK) had a significant effect on NFI. The size of these effects (around 0.3kg/d) shrunk relative to the original estimates from linkage analysis (around 1.0kg/d), but this was expected and is still encouraging. In general, the "within family" linkage analysis was more significant than the "across family" association analyses (results below, Appendix 9.3).

Table 8. Variances and effects for SNPs when fitted as genotype or additive effects on NFI*

Chromosome	**Gene and SNP#**	**Genotype** (kg feed/day)^2	**Additive** (kg feed/day)^2	**Size of additive effect**[b] kg/day
1	PIT1 #1	0.01		
1	UMPS #1 UMPS #2	0.01	0.05 0.13	0.19±0.12 0.32±0.15*
1	SLC2A2 #1 SLC2A2 #2 SLC2A haplotype		0.08	0.24±0.14
1	IL12A #1	0.75	0.10	0.28±0.15
8	CTSB #1 CTSB #2 CTSB haplotype		0.12	0.24±0.25
8	TEK #1 TEK #2	0.01	0.02 0.01	0.07±0.11 0.08±0.09
11	FSHR #2	0.01	0.03	0.12±0.12
11	POMC #1 POMC #2 POMC haplotype	0.004	0.01	0.06±0.08
11	ASS #1	0.03	0.04	0.15±0.12
20	MAP1B, #1	0.08	0.11	0.31±0.13*
20	GHR #1 GHR #2	0.03	0.03 0.02	0.12±0.13 0.10±0.10
20	FST #2	0.05	0.11	0.27±0.18
20	AMPK #1	0.07	0.10	0.29±0.14*

[a]Only non-zero variances presented.
[b]All effects reported as positive but could be selected in either direction. The effect is for a single copy of the allele, so the difference between homozygotes is double this value.
*Based on a simple t-test of significance of difference from zero, $P<0.05$.

Breeding value estimates were calculated from a multiple regression model containing fixed additive effects of the three significant genes (UMPS, MAP1B and AMPK). These were compared with breeding value estimates from the pedigree (animal) model . Only 18% of the variation in EBV was accounted for by the breeding value estimate from SNPs, but more could have been achieved with additional genes (e.g. IL12A, CSTB and FST) added to the model. However, it should be noted that this is not an independent test since the breeding values and gene effects were estimated from the same data. Further analyses should be conducted on additional data sets.

An additional test of significance of the genes is to include them as covariates when undertaking linkage analysis. When fitting these SNPs (UMPS, MAP1B, AMPK) on chromosomes 1 or 20, they basically accounted for the QTL peak on those chromosomes, indicating they could be the genes habouring the mutations affecting NFI, or could be closely linked to, the mutations affecting NFI.

Of the 4 most significant SNPs, only one is within a strong candidate gene (AMPK, Table 9). However, the associated SNP in AMPK gene is not likely to be functional as it is a silent mutation in exon 6. The SNPs in the other three genes are also unlikely to affect net feed efficiency given the known metabolic roles of these genes and the location of the SNPs (Table 10). Therefore, it is more likely that these SNPs are in linkage disequilibrium with the functional DNA variants. Further studies will be necessary to determine if this linkage disequilibrium extends to different cattle populations and breeds so these SNPs can be used for the selection of net feed efficiency.

Based on these results, several strong candidate genes and pathways have been identified (Table 9). The candidates will be further examined by linkage disequilibrium analysis within the Trangie Angus selection lines. Not all candidate genes have been completed sequenced because of GC-rich regions within the genes. Therefore, potentially functional SNPs may yet be discovered.

Table 9. Candidate genes

BTA	Gene	Functional SNPs	Function	Comments
1	cocaine- and amphetamine-regulated transcript (CART)	Nil	Satiety factor	1 intron; GC rich gene = sequencing ongoing
8	Lipoprotein lipase (LPL)	3 amino acid substitutions	fat metabolism	3 other SNPs (flanking, intronic)
11	Proopiomelanocortin (POMC)	3 3'UTR	energy homeostasis, feed intake regulation	7 other SNPs (1 silent, 6 intronic)
11	Interleukin-1beta (IL1B)	3 amino acid substitutions	energy homeostasis	6 other SNPs (flanking, intronic)
20	Adenine monophosphate protein kinase (AMPK)	1 promoter, 1 3'UTR	energy homeostasis, nutrient turnover	6 other SNPs (4 flanking, 2 silent)
20	Growth hormone receptor (GHR)	3 amino acid substitutions, 1 promoter	growth hormone-insulin axis	12 other SNPs (flanking, intronic)
20	Follistatin (FST)	2 amino acid substitutions	myogenesis	6 other SNPs (flanking, intronic)
20	Phosphatidylinositol 3-kinase P-85 -alpha subunit (PI3K)	1 3'UTR	growth hormone-insulin axis	8 other SNPs (2 silent, 6 intronic)

Table 10. Marker genes

BTA	Gene	SNP	Function
1	Interleukin 12A (IL12A)	1 silent	cytotoxic lymphocyte maturation factor
1	Uridine monophosphate synthetase (UMPS)	1 intronic	pyrimidine biosynthesis: orotidine 5'-phosphate = UMP + CO(2)
20	Microtubule-associated protein 1B (MAP1B)	1 silent	Protein associated with cytoskeletal changes during neurite extension

4.5 Functional studies

From the QTL identified for net feed efficiency, a large number of candidate genes were nominated based on comparative mapping to the equivalent regions in the human and mouse genomes. Most of these candidate genes fall within 2 facets of physiology likely to affect feed efficiency; the pathways involved energy metabolism and the pathways involved in growth. The energy metabolic pathways include:

- oxidative phosphorylation (involved in ATP generation),
- malonyl coenzyme A / long chain fatty acid pathway (involved in fatty acid metabolism),
- ubiquitine-proteosome pathway (involved in oxidative stress regulation),
- hexosamine biosynthesis pathway (involved in glucose turnover), and
- leptin-insulin pathway, involved in nutrient sensory feedback.

The growth pathways include:

- growth hormone receptor pathway (involved growth regulation),
- growth hormone / myostatin / insulin-like growth factor 1 pathway (involved in muscle growth),
- insulin / insulin receptor substrate pathway (involved in glucose turnover), and
- mTOR pathway (involved in appetite regulation).

Biochemical and proteomic experiments are being conducted to determine if high and low efficiency animals differ in these pathways and thus, assist in discovering candidate genes. The initial experiments focused on two of these pathways, oxidative phosphorylation and malonyl coenzyme A / long chain fatty acid synthesis.

To examine the malonyl coenzyme A / long chain fatty acid pathway, the activity of a key rate-limiting enzyme in the pathway, AMP-activated protein kinase (AMPK), was studied. AMPK is a crucial regulator of fat metabolism and also a trigger for feed intake. AMPK is primarily activated by changes in the cellular AMP:ATP ratio (Hardie *et al.* 2003). When activated by AMP, AMPK increases ATP concentration by phosphorylating downstream peptide substrates, such as acetyl CoA carboxylase (ACC), which subsequently changes the energy balance.

$$\text{ATP + peptide} \xrightarrow[\text{AMP}]{\text{AMPK}} \text{P-peptide + ADP}$$

The AMPK gene is located within the net feed efficiency QTL on BTA20 in cattle. Hence, AMPK is an obvious candidate gene. Therefore, in addition to investigating the association between net feed efficiency and SNPs within the AMPK gene, the activity of the enzyme was measured in liver

samples from high and low efficiency animals from the Trangie Angus progeny test, based on phenotypes not genotypes. The animals differed in net feed efficiency by up to 6 kg feed/day (Figure 4). Liver samples from the 10 highest and 10 lowest NFI animals were used to make cytoplasmic AMPK enzyme preparations.

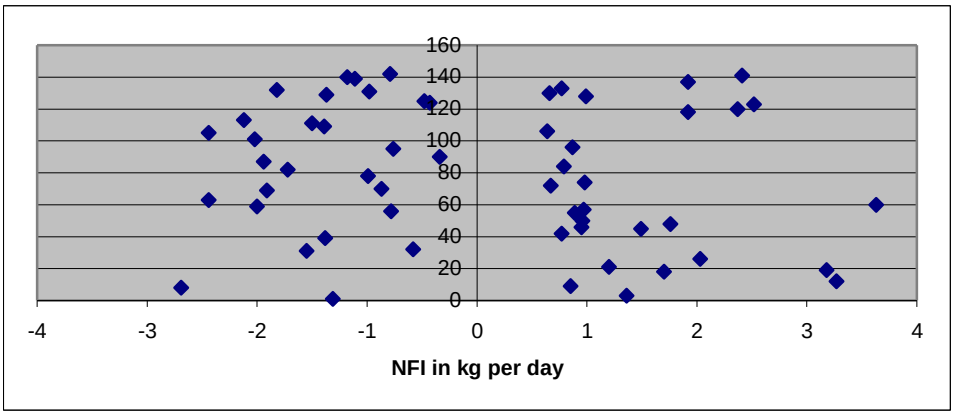


Figure 4. Net feed intake distribution in extreme Trangie Angus test progeny.

The preliminary results indicated that the activity of the enzyme is increased in the highly efficient animals ($p < 0.05$, Figure 5). None of the SNPs discovered in the AMPK gene are likely to be functional DNA variants that could explain this difference observed in activity. Nevertheless, the analysis of the SNP data from the AMPK gene did indicate an association between AMPK and net feed efficiency (see section 4.3).

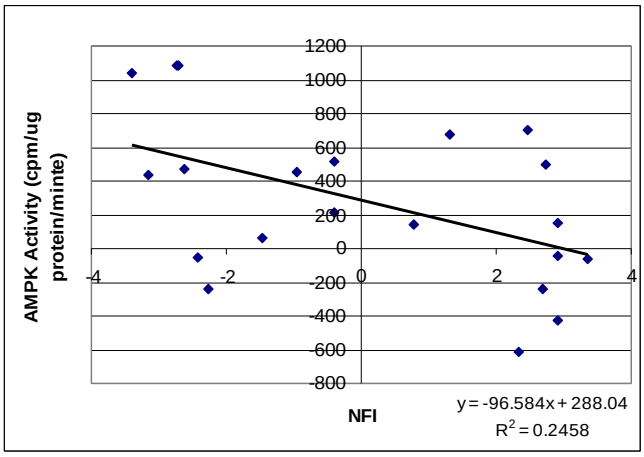


Figure 5. AMPK activity in liver cells of high and low efficiency animals.

The results from the oxidative phosphorylation studies were also promising. The activity of three respiratory enzyme complexes involved in oxidative phosphorylation (complex I, complex II and complex IV) were examined in the liver and muscle mitochondria of these high and low efficiency animals. The data indicated that although there is no difference in the activity of complexes II and IV,

the activity of the rate-limiting complex I does differ by 1.5-fold between liver mitochondria of high and low efficiency animals ($p < 0.02$) (Figure 6). Similar results were obtained from the muscle mitochondria (data not shown).

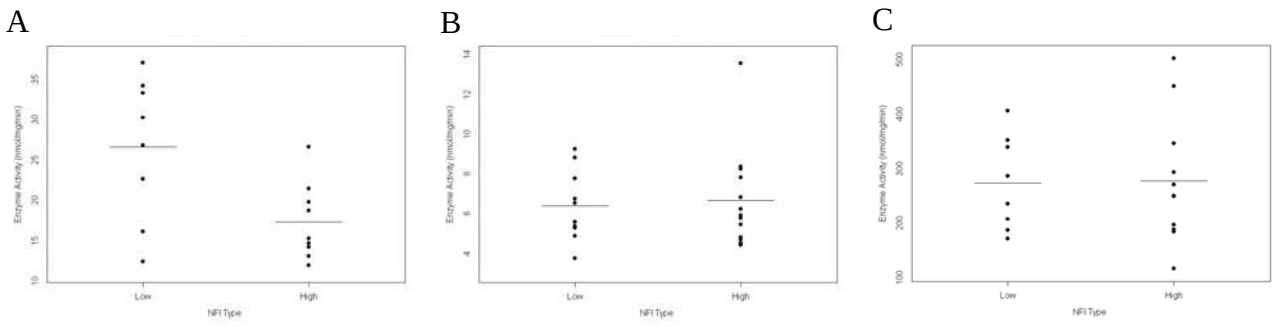


Figure 6. Activity of oxidative phosphoryation complexes in liver mitochondria of high and low efficiency animals. A. Complex I. B. Complex II. C. Complex IV. Bar indicates the mean.

Finally, to have a more global understanding of the potential differences in oxidative phosphorylation between high and low efficiency animals, a proteomic approach was also taken. Mitochondria were isolated from the liver and muscle of high and low efficiency animals and the proteins extracted. The proteins were labelled with either Cy3 or Cy5 depending upon whether the sample was from a high or low efficiency animal. The proteins were then separated by charge and mass using 2D gel electrophoresis. After this iso-electrical focusing, the Cy3 and Cy5 intensities of the protein spots were measured to determine the relative quantity of each protein in the high versus low efficiency animals (Figure 7).

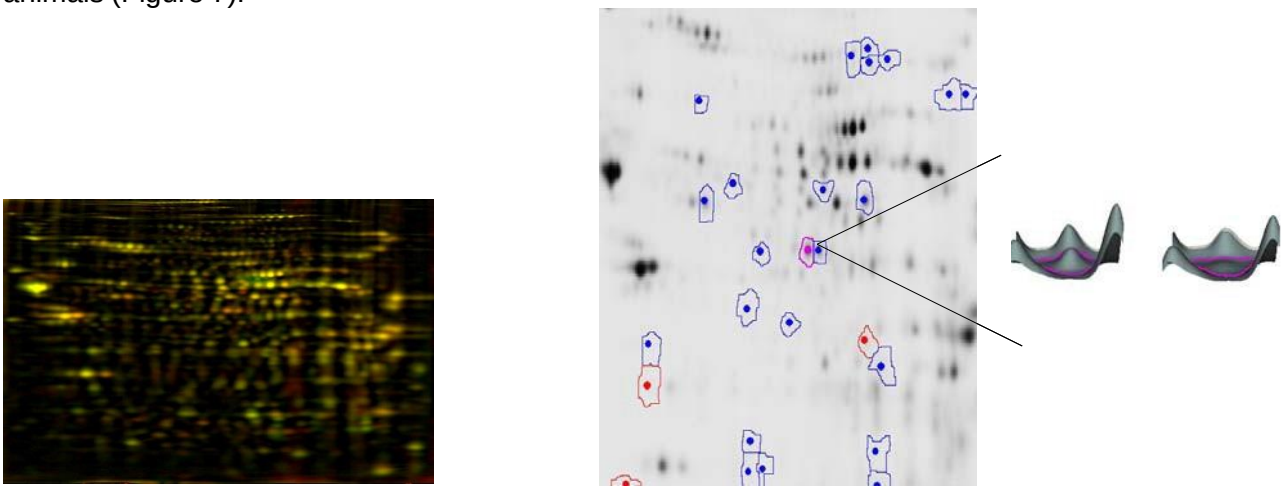


Figure 7. 2D gel of liver mitochondrial proteins from high and low efficiency animals. Example of gel (left) run with Cy3 and Cy5 fluorescently labelled proteins. Example of a protein spot (right) that varies in concentration by more than 1.5-fold, as measured by the difference in signal intensity.

Analysis of the gels indicated that out of the some 550 mitochondrial proteins detected, 15 of the proteins differed in concentration by more than 1.5-fold between the high and low efficiency animals.

Efforts are currently underway to identify all of these proteins, but at least 2 of the proteins are subunits of the oxidative phosphorylation complex I and complex V. In summary, the results thus far indicate that mitochondrial proteins and enzymes within the malonyl coenzyme A / long chain fatty acid pathway are good targets as candidate genes.

5 Success in Achieving Objectives - Section

5.1 Success in Achieving Objectives - Heading

5.1.1 Five chromosome regions, discovered in MLA project TR.075, will be linkage mapped to determine if they contain genes for NFI that are segregating in the Trangie Angus selection lines.

Four of the chromosome regions found to affect NFI in Davies Jersey x Limousin experiment clearly affected NFI in the Trangie Angus selection lines. These regions were on chromosomes 1, 6, 8 and 20. There was also suggestive evidence that the remaining chromosome region, on chromosome 11, also affected NFI in the Trangie Angus selection lines.

5.1.2 Single nucleotide polymorphisms will be identified in candidate genes in the five regions of interest. Both MLA project TR.075 and MRC project DAN.075 cattle will be genotyped for the haplotypes developed.

In addition to the 10,000 genome distributed SNPs, SNPs discovered in candidate genes were also tested for an effect on NFI. In total, 42 genes were screened and 201 SNPs were identified. These SNPs were used for both linkage analysis and association studies (see 5.1.3 and 5.1.4).

Mitochondria have a key role in cell metabolism and energy pathways, and therefore, mitochondrial proteins are candidates for causing differences in NFI. To assist in the decision as to which candidate genes should be targeted, mitochondrial functional studies were undertaken. A proteomics approach indicated that oxidative phosphorylation complexes and other mitochondrial proteins are expressed differently in high and low NFI cattle. Analysis of 2D gels indicated that out of some 550 mitochondrial proteins detected, 15 of the proteins differed in concentration by more than 1.5-fold between high and low efficiency animals. Efforts are currently underway to identify all of these proteins, but at least 2 of the proteins are subunits of the oxidative phosphorylation complex I and complex V. Enzyme assays have confirmed that there are differences in complex I activity between high and low NFI cattle analysed herein.

5.1.3 If objectives 1 and 2 find significant quantitative trait loci (QTL), the relevant regions will be fine scaled mapped using linkage disequilibrium mapping to reduce the chromosome region to which the gene has been mapped.

Significant quantitative trait loci were discovered in objectives 1 and 2. The SNP identified in candidate genes (see 5.1.2) were linkage mapped in the Davies herd to confirm the presence of the QTL.

In order to fine map these regions in Angus, as well as to find new chromosome regions affecting NFI, we made use of the very large number of DNA markers now available following the sequencing of the bovine genome, to reduce the size (and therefore, the number of possible genes) of the chromosome regions affecting NFI. We conducted a powerful genome scan using approximately 10,000 SNP markers spread across the bovine genome in the extremes of the Trangie selection lines. In the genome scan using the 10,000 genome distributed SNPs, we found 100 SNPs significant at $p<0.001$. At this level of significance, we expect only 10 of these results to occur by chance. This is a surprisingly high number of significant SNPs. If it represented 100 genes affecting NFI, it would imply that most of these genes had a small effect on NFI. However, many of the SNPs are located very close to each other, and therefore, multiple significant SNPs may be associated with a single mutation affecting NFI. By investigating the positions of the SNPs on the genome, we concluded that there were 20 clusters of SNPs on 12 chromosomes that map the position of a gene affecting NFI. Six of these regions confirmed the chromosome regions found to affect NFI in the linkage mapping experiment described above. For these six regions, the interval that must contain the mutation affecting NFI was reduced by a factor of 10, meaning 10 times fewer genes had to be considered as candidates.

5.1.4 Positional candidate genes from these regions will be selected and tested in association studies for an effect on NFI.

Of the candidate genes, 22 were tested for association with NFI. Three genes contained SNPs with a significant effect on NFI in the Davies population. These genes were adenosine monophosphate kinase (AMPK), uridine monophosphase synthetase (UMPS) and microtubule associated protein 1B (MAP1B).

In addition, functional studies showed that the activity of adenosine monophosphate kinase (AMPK) enzyme was significantly different between high NFI cattle and low NFI cattle. This provides further evidence that there could be a mutation in this gene causing differences in NFI.

5.1.5 If objective 1 yields insufficient significant QTL, additional chromosomes will be linkage mapped. If QTL for NFI are discovered, they will be progressed through objectives 3 and 4.

Objective one yielded six QTL regions. However, as a result of the genome scan with the 10,000 SNP markers, 14 other QTL regions were also identified. These regions and markers should be considered in choosing the SNP panel that is delivered to industry for improving NFI.

In addition to meeting the objectives of the project, the project also contributed to student training. Madan Naik (PhD, University of Adelaide), Stephen Lee (Honours, University of Adelaide) and Dannielle Hulett (PhD, University of Melbourne) worked exclusively on this project. Madan Naik will complete in December 2006. Stephen Lee graduated in December 2005 and is now Technical Manager with PrimeGro Ltd. Danielle is mid-way through her PhD project.

6 Impact on Meat and Livestock Industry – now & in five years time

6.1 Impact on Meat and Livestock Industry – now & in five years time - Heading

6.1.1 Impact on Meat and Livestock Industry – now & in five years time - Sub Heading

The DNA markers discovered in this project could be used to select cattle for low NFI. This would lead to an increase in the profitability of Australian beef cattle. To assist in the commercialisation of the markers, two provisional patents have been lodged. However, before commercialisation can occur, the markers need to be validated in other cattle populations and, if necessary, better markers discovered. It is planned to carry this out within the CRC for Beef Cattle Genetic Technologies. The steps are as follows:

(i) The ParAllele SNPs that were significantly associated with NFI in the Trangie cattle will be tested in 1000 other cattle.
(ii) SNPs significant in the Davies cattle will be tested in other cattle.
(iii) Candidate genes close to the significant SNPs will be identified based on their known physiological functions.
(iv) SNPs in these candidate genes will be discovered in silico or by sequencing, and tested for an association with NFI.

The use of the Trangie selection line cattle had a benefit and a disadvantage. The experiment using them had more power to detect QTL affecting NFI than alternative experimental designs. However, a disadvantage of this power is that markers located some distance away from the QTL might still be detected as showing a significant effect. Such markers, far away from the QTL, are unlikely to be associated with NFI in other populations. Therefore, it is necessary to validate the markers in other populations and possibly find markers closer to the QTL than the ParAllele markers.

Markers that are successfully validated will be commercialised. Provided this research and commercialisation is successful, in 5 years time the beef industry will have available a panel of genetic markers that can be used to select cattle for decreased NFI. As this technology is used and the genetic change flows through the industry, the cost of beef production will decrease.

7 Conclusions and Recommendations

7.1 Conclusions

7.1.1 Using microsatellites in the Trangie selection line cattle, we have mapped at least 5 genes affecting NFI to broad chromosome regions.

7.1.2 At least 4 of the chromosomes carrying genes affecting NFI in the Davies experiment also carry genes for NFI in the Trangie selection lines. These may be the same genes, but there are many genes affecting NFI and often more than one per chromosome.

7.1.3 We have fine scaled mapped over 20 genes affecting NFI using the ParAllele 10,000 SNP assay. These genes are located on 12 chromosomes and their positions are defined by 100 SNP markers.

7.1.4 Three candidate genes, selected for their role in energy metabolism, have an effect on NFI in the Davies cattle.

7.1.5 Tissues taken from cattle that have low and high NFI appear to differ in their activity of the enzyme AMPK, in the activity of the mitochondrial oxidative phosphorylation complex I and in the concentration of several mitochondrial proteins. These results aid in the selection of candidate genes for further investigation.

7.2 Recommendations

7.2.1 The significant SNPs that have been found should be validated in other cattle. Where necessary, they should be used to find new SNPs closer to the gene causing variation in NFI, which have a consistent effect across breeds. This research should be carried out as part of the CRC for Beef Cattle Genetic technologies. Since there are limited phenotypes for NFI, all possible resources for validation should be explored.

7.2.2 It is anticipated that a panel of markers affecting NFI will be commercialised because many markers will be needed to explain 50% of the genetic variation in NFI.

8 Bibliography

Alban A, David S, Bjorkesten L, Andersson C, Sloge E, Lewis S and Currie I (2003) A novel experimental design for comparative two-dimensional gel analysis: Two-dimensional difference gel electrophoresis incorporating a pooled internal standard. Proteomics. 3: 36-44

Alfonso P, Nunez A, Madoz-Gurpide J, Lombardia L, Sanchez L and Casal I (2005) Proteomic expression analysis of colorectal cancer by two-dimensional differential gel electrophoresis. Proteomics 5: 202-2611.

Archer JA, Arthur PF, Herd RM, Parnell PF, Pitchford WS (1997) Optimum postweaning test for measurement of growth rate, feed intake, and feed efficiency in British breed cattle. *Journal of Animal Science* **75**: 2024-2032.

Archer JA, Barwick SA, Graser HU (2004) Economic evaluation of beef cattle breeding schemes incorporating performance testing of young bulls for feed intake. *Australian Journal of Experimental Agriculture* **44**: 393-404.

Archer JA, Richardson EC, Herd RM, Arthur PF (1999) Potential for selection to improve efficiency of feed use in beef cattle: a review. *Australian Journal of Agricultural Research* **50**: 147-161.

Arthur PF, Archer JA, Herd RM, Melville GJ (2001a) Response to selection for net feed intake in beef cattle. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **14**: 135-138.

Arthur PF, Archer JA, Johnston DJ, Herd RM, Richardson EC, Parnell PF (2001b) Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other postweaning traits in Angus cattle. *Journal of Animal Science* **79**: 2805-2811.

Arthur PF, Herd RM, Wright J, Xu G, Dibley K, Richardson EC (1996) Net feed conversion efficiency and its relationship with other traits in beef cattle. *Proceedings of the Australian Society of Animal Production* **21**: 107-110.

Bourges, I., Ramus, C., Mousson de Camaret, B., Beugnot, R., Remacle, C., Cardol, P., Hofhaus, G. and Issartel, J.-P. (2004) Structural organization of mitochondrial human complex I:role of he ND4 and ND5 mitochondria-encoded subunits and interaction with prohibitin. Biochem. J. 383: 491-499.

Brooks H and Krahenbuhl S, (2000) Development of a new assay for complex I of the respiratory chain *Clinical Chemistry* 46: 345-350

Campbell and Fabbraio (2001) Effect of ovarian hormones on mitochondrial enzyme activity in the fat oxidation pathway of skeletal muscle. *Am J Physiol. Endocrinol Metab* 281: E803-E808.

Davies SP, Carling D, Hardie DG (1989) Tissue distribution of the AMP-activated protein kinase, and lack of activation of cyclic-AMP-dependant protein kinase, studied using a specific and sensitive peptide assay. *European Journal of Biochemistry* **186:** 123-128.

Ferrell CL, Jenkins TG (1984) Energy utilization by mature, non-pregnant, non-lactating cows of different types. *Journal of Animal Science* **58:** 234-243.

Fredriksson K, Radell P, Eriksson L, Hultenby K and Rooyackers O (2005) Effect of prolonged mechanical ventilation on diaphragm muscle mitochondria in piglets. *Acta Anaesthesiol Scand*. 10: 1-7

Hardie DG, Scott JW, Pan DA, Hudson ER (2003) Management of cellular energy by the AMP-activated protein kinase system. *FEBS Letters* **546:** 113-120.

Hawley SA, Davison M, Woods A, Davies SP, Beri RK, Carling D, Hardie DG (1996) Characterization of the AMP-activated protein kinase from rat liver and identification of threonine 172 as the major site at which it phosphorylates AMP-activated protein kinase. *Journal of Biological Chemistry* **271**: 7879-27887.

Herd RM, Bishop SC (2000) Genetic variation in residual feed intake and its association with other production traits in British Hereford cattle. *Livestock Production Science* **63**: 111-119.

Hughes, T.E., Archer, J.A. and Pitchford, W.S. (1997) Response to selection for high and low net feed intake in mice. *Proceedings of the Association for the Advancement of Animal Breeding and Genetics* **12**: 230-233.

Hughes, T.E. and Pitchford, W.S. (2004) Direct response to selection for post-weaning net feed intake in mice, and correlated responses in post-weaning growth, intake, gross digestibility and body composition. *Australian Journal of Experimental Agriculture Beef CRC special edition.* 44:489-500.

Madapallimattam AG, Law L, and Jeejeebhoy KN ((2002) Effect of hypoenergetic feeding on muscle oxidative phosphorylation and mitochondrial complex I-IV activities in rats. *American Journal of Clinical Nutrition* **76**: 1031-1039.

Minchenko J, Williams A and Christodoulou J (2003) Adaptation of a Mitochondrial Complex III Assay for Automation: Examination of Reproducibility and Precision *Clinical Chemistry* 49: 330-332.

Montano-Bermudez M, Nielsen MK, Deutscher GH (1990) Energy requirements for maintenance of crossbred beef cattle with different genetic potential for milk. *Journal of Animal Science* **68**: 2279-2288.

Moore KL, Johnston DJ, Graser HU, Herd RM (2005) Genetic and phenotypic relationships between insulin-like growth factor-I (IGF-I) and net feed intake, fat and growth traits in Angus beef cattle. *Australian Journal of Agricultural Research* **56**: 211-218.

Pedrioli PG, Eng JK, Hubley R, Vogelzang M, Deutsch EW, Raught B, Pratt B, Nilsson E, Angeletti RH, Apweiler R, Cheung K, Costello CE, Hermjakob H, Huang S, Julian RK, Kapp E, McComb ME, Oliver SG, Omenn G, Paton NW, Simpson R, Smith R, Taylor CF, Zhu W, and Aebersold R (2004) A common open representation of mass spectrometry data and its application to proteomics research. *Nature Biotechnology* **22**: 1459-1466.

Pitchford, W.S. (2004) Genetic improvement of feed efficiency of beef cattle: what lessons can be learnt from other species? *Aust. J. Experimental Agriculture. Beef CRC special edition.* 44:371-382.

Procaccio V, Mousson B, Beugnot R, Duborjal H, Feillet F, Puter G, Pignot-Paintrand I, Lombes A, Coo R, Smeets H, Lunardi J and Issartel JP (1999) Nuclear DNA origin of mitochondrial complecI deficiency in fatal infantile lactic acidosis evidenced by transnuclear complementation of cultures fibroblasts. *The journal of clinical investigation.* 104: 83-92.

Rasmussen U and Rasmussen H, (2000) Human quadriceps muscle mitochondria: a functional characterization. Molecular and Cellular Biochemistry. 208: 37-44.

Richardson EC, Herd RM (2004) Biological basis for variation in residual feed intake in beef cattle. 2. Synthesis of results following divergent selection. *Australian Journal of Experimental Agriculture* **44**:431-440.

Seaton, G., Haley, C., Knott, S., Kearsey, M., and Visscher, P. (2001) QTL Express: http://qtl.cap.ed.ac.uk accessed repeatedly during December 2001 and January 2002.

Trounce IA, Kim YL, AS Jun AS, and Wallace DC (1996) Assessment of mitochondrial oxidative phosphorylation in patient muscle biopsies, lymphoblasts, and transmitochondrial cell lines. *Methods of Enzymology* **264**: 484-509.

Verma M, Kagan J, Sidransky D and Srivastava S (2003). Proteomics analysis of cancer-cell mitochondria. *Nature Reviews* **3**: 789-795.

9 Appendices

9.1

Position of clusters, number and frequency of haplotypes with each cluster, and effect of each haplotype on NFI

Human chrom-osome	Cluster	Start point human chromosome	SNP List	Individual SNP test statistic	Haplotype test statistic	Haplotypes	Haplotype alleles	Frequency	Haplotype effects (kg)
10	1	65002671	3987	14.8	25.0	1	111	454	0.635
			4611	13.2		2	121	1	0.000
			2641	12.6		3	122	281	0.294
						4	211	5	-0.272
						5	222	17	-0.657
12	1	64621283	6548	17.7	16.5	1	11	640	-0.239
			5485	14.6		2	22	118	0.239
12	2	103353408	5954	13.9	33.5	1	1111	272	-0.123
			934	11.3		2	1122	288	-0.550
			766	25.1		3	1211	156	-0.089
			5269	25.2		4	1222	1	0.152
						5	2111	1	-0.041
						6	2122	1	0.103
						7	2211	37	0.449
						8	2222	2	0.098
13	1	79722074	6953	17.3	17.4	1	1122	623	0.337
			6951	17.6		2	1121	1	-0.134
			3894	13.7		3	1112	3	0.056
			4904	18.7		4	2221	10	-0.085
						5	2212	2	0.031
						6	2211	119	-0.205
16	1	12965196	2487	16.9	15.7	1	21	404	-0.234
			4688	11.2		2	11	78	0.092
						3	12	276	0.143
2	1	139662007	8814	16.0	14.3	1	21	29	0.100
			7828	11.8		2	22	369	0.121
						3	11	360	-0.221
2	2	159497709	8854	17.7	17.5	1	1221	680	0.325
			7139	17.0		2	2112	78	-0.325
			7138	17.0					
			346	17.0					
3	1	28493054	2403	11.1	11.8	1	21	470	-0.163

			5963	11.1		2	12	288	0.163
3	2	163031401	3271	16.4	23.2	1	112	280	0.389
			1212	12.0		2	122	95	0.223
			3166	12.6		3	121	2	0.019
						4	212	126	0.139
						5	222	235	-0.094
						6	221	20	-0.676
4	1	116761843	4593	13.9	16.9	1	112	669	0.430
			5500	11.6		2	121	9	-0.044
			7963	10.9		3	212	50	-0.008
						4	221	30	-0.379
4	2	131366585	1809	16.8	28.2	1	1221	161	0.569
			1160	14.1		2	1222	8	-0.096
			8116	10.9		3	2111	83	-0.099
			6205	12.2		4	2112	25	-0.281
						5	2121	428	0.198
						6	2122	53	-0.291
5	1	154344482	2748	15.3	27.6	1	1111	539	0.137
			2666	15.1		2	1112	187	0.498
			2665	13.6		3	2221	31	-0.693
			7039	12.6		4	2222	1	0.058
6	1	22167306	6605	15.7	15.7	1	11	685	0.302
			1683	15.1		2	22	73	-0.302
6	2	116037161	1767	18.3	15.9	1	2211	555	0.195
			8867	17.0		2	1122	203	-0.195
			9060	16.9					
			1351	16.5					
7	1	91241662	1671	14.1	13.2	1	112	652	0.199
			5728	13.2		2	122	1	0.076
			8504	12.6		3	221	105	-0.275
7	2	116825885	2936	18.2	21.2	1	11	698	-0.403
			31	18.2		2	22	60	0.403
7	3	149654735	7515	11.5	16.8	1	111	478	0.217
			8750	11.5		2	112	26	0.105
			2697	13.0		3	221	161	0.052
						4	222	93	-0.374
9	1	90366070	7648	12.1	20.8	1	12	100	-0.043

				6763	18.8		2	11	371	0.304
							3	22	264	-0.184
							4	21	23	-0.077
	9	2	115995683	5991	11.4	14.5	1	11	291	-0.282
				4603	11.8		2	12	21	0.146
							3	21	44	0.061
							4	22	402	0.075
	9	3	125856849	8620	11.0	18.7	1	212	376	0.313
				5574	13.0		2	221	72	-0.112
				5156	11.4		3	112	65	-0.096
							4	121	245	-0.106
X		1	76931434	9171	21.9	28.4	1	22	372	-0.025
				8688	13.2		2	21	54	-0.244
							3	12	29	-0.142
							4	11	303	0.411

9.2 Genes with single nucleotide polymorphisms

NAME OF THE GENE (HGNC SYMBOL)	BTA	# SNP FOUND	LOCATION
5-hydroxytryptamine 1E receptor (5-HT-1E) (serotonin receptor) (HTR1F)	1	2	1 functional, 1 intron
5-hydroxytryptamine receptor 3 subunit C. (HTR3C)	1	2	nil
Alpha-HS-glycoprotein (ASHG)	1	3	1 amino acid, 1 silent, 2 intron
Angiotensin receptor 1 (AGTR)	1	2	2 silent
Cytochrome c oxidase copper chaperone: (COX17)	1	nil	nil
D(3) dopamine receptor (DRD3)	1	nil	nil
Eukaryotic initiation factor 4A-II (EIF-4A-II) (EIF4A2)	1	nil	nil
Eukaryotic translation initiation factor 4 gamma (EIF4G)	1	1	1- 3' UTR
G Protein -coupled receptor kinase (GRK7)	1	1	1- 3' UTR
Glucose transporter 2 (GLUT2)	1	7	7 intron or flanking
Glycogenin-1 (GYG1)	1	1	intron
Growth hormone secretagogue receptor type 1 (GH-releasing peptide receptor; ghrelin receptor) (GHSR)	1	nil	nil
Interferon-inducible myxovirus resistance-1 protein gene (Mx1)	1	nil	nil
Interleukin 12 (IL12A)	1	1	1 silent
NADH-Ubiquinone oxidoreductase (SGDH)	1	1	1 intron
NADH-ubiquinone oxidoreductase SGDH subunit, mitochondrial precursor (complex I-SGDH) (NDUFB5)	1	nil	nil
Neprilysin (NLN)	1	2	1-3' UTR
Peroxisomal bifunctional enzyme (PBE) (PBFE) [includes: Enoyl-CoA hydratase; 3,2-trans-enoyl-CoA isomerase; 3-hydroxyacyl-CoA dehydrogenase (EHHADH)	1	nil	nil
Pituitary-specific positive transcription factor 1 (PIT1)	1	5	5 intron or flanking
Purinergic P2Y1 receptor (P2Y1)	1	nil	nil
Somatostatin precursor (SST)	1	1	1 -splice junction
Sorting Nexin 4 (SNX4)	1	8	intron
Uridine monophosphae synthase (UMPS)	1	5	3 UTR, 2 intron
Vacuolar ATP synthase catalytic subunit a, ubiquitous isoform (V-ATPase a subunit 1) (Vacuolar proton pump alpha subunit 1) (V-ATPase 69 KDa subunit 1) (Isoform VA68) (ATP6V1A)	1	nil	nil

9.2. Genes with single nucleotide polymorphisms (continued)

NAME OF THE GENE (HGNC SYMBOL)	BTA	# SNP FOUND	LOCATION
Cathepsin B (CSTB)	8	5	5 intron or flanking
Clusterin (CLU)	8	5	intron
Galactose-1-phosphate uridylyltransferase (EC 2.7.7.12) (Gal-1-P uridylyltransferase) (UDP-glucose--hexose-1-phosphate uridylyltransferase) (GALT)	8	nil	nil
Lipoprotein lipase (LPL)	8	6	3 animo acid, 3 intron or flanking
NADH-ubiquinone oxidoreductase B17 subunit (EC 1.6.5.3) (EC 1.6.99.3) (Complex I-B17) (CI-B17) (NDUFB6)	8	nil	nil
Neurofilament M subunit (NEFM)	8	2	2 intron or flanking
Nociceptin (PNOC)	8	1	1 intron or flanking
Orexigenic neuropeptide QRFP precursor (P518) [Contains: QRF-amide (Pyroglutamylated arginine-phenylalanine-amide peptide)] (OPRS1)	8	nil	nil
Tyrosine kinase, endothelial (TEK)	8	3	3 intron or flanking
Vacuolar ATP synthase subunit B, brain isoform (EC 3.6.3.14) (V-ATPase B2 subunit) (Vacuolar proton pump B isoform 2) (Endomembrane proton pump 58 kDa subunit) (HO57) (ATP6V1B2)	8	nil	nil
Vacuolar ATP synthase subunit G 1 (EC 3.6.3.14) (V-ATPase G subunit 1) (Vacuolar proton pump G subunit 1) (V-ATPase 13 kDa subunit 1) (Vacuolar ATP synthase subunit M16) (ATP6V1G1)	8	nil	nil
Very low-density lipoprotein receptor precursor (VLDL receptor) (VLDLR)	8	nil	nil
Ribonucleoside-diphosphate reductase M2 chain (RRM)	11	6	intron
Argininosuccinate synthase (ASS)	11	3	3 intron or flanking
Aspartate carbamoyltransferase (CAD)	11	0	nil
ATPase, H+ transporting, lysosomal 31kDa, V1 subunit E isoform 2 (ATP6V1E2)	11	nil	nil
ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C isoform 2 (ATP6V1C2)	11	nil	nil
Cytochrome c oxidase subunit VIIa-related protein, mitochondrial precursor (COX7a-related protein) (EB1) (COX7A2L)	11	nil	nil
Follicle stimulating hormone receptor (FSHR)	11	5	2 animo acid, 3 intron or flanking
Growth/differentiation factor 7 precursor (GDF-7)	11	nil	nil
Interleukin 1 beta (IL1B)	11	9	3 animo acid, 6 intron or flanking
MpV17 transgene, murine homolog, glomerulosclerosis (MPV17)	11	nil	nil
Proopiomelanocortin (POMC)	11	10	1 silent, 6 intron, 3 UTR

Trifunctional enzyme alpha subunit, mitochondrial precursor(TP-alpha) (78 kDa gastrin-binding protein) (HADHA)	**11**	**2**	**UTR**

9.2. Genes with single nucleotide polymorphisms (continued)

NAME OF THE GENE (HGNC SYMBOL)	BTA	# SNP FOUND	LOCATION
5-hydroxytryptamine 1a receptor (5-HT-1a) (serotonin receptor) (5- HT-1a) (G-21) (HTR1A)	20	nil	nil
Adenosine monophosphate protein kinase (AMPK)	20	7	4 UTR, 2 silent, 1 intron
Atrial natriuretic peptide receptor type C (NPPC)	20	0	nil
Cocaine- and amphetamine-regulated transcript protein precursor (CART)	20	1	intron
Follistatin (FST)	20	9	1 silent, 8 introns
Growth hormone receptor (GHR)	20	16	3 amino acid, 2 silent, 1 promoter, 8 introns, 2 UTR
HMG-CoA Reductase (HMGCR)	20	0	nil
Insulin gene enhancer protein (ISL1)	20	nil	nil
Integrin alpha 2 (ITGA2)	20	35	35 intron or flanking
Microtubule-associated protein-1B (MAP1B)	20	1	1 silent
mitogen-activated protein kinase kinase kinase 1 (MAPK/ERK kinase kinase 1) (MAP3K1)	20	1	1 UTR
NAD(P) transhydrogenase, mitochondrial precursor (pyridine nucleotide transhydrogenase) (nicotinamide nucleotide transhydrogenase) (NNT)	20	nil	nil
NADH-ubiquinone oxidoreductase 18 kda subunit, mitochondrial precursor (complex I-18 KDa) (CI-18 kda) (complex I- AQDQ) (CI-AQDQ) (NDUFS4)	20	nil	nil
Phosphatidylinositol 3-kinase P-85 -alpha subunit (PI3K)	20	9	2 silent, 1 UTR, 6 intron
Prolactin receptor (PRLR)	20	3	3 intron or flanking
Pro-MCH-like protein 1 (Pro-melanin-concentrating hormone-like protein 1) (PMCHL1)	20	nil	nil
Pro-melanin-concentrating hormone-like 2 (PMCHL2)	20	nil	nil
Prostaglandin E2 receptor, EP4 subtype (PTGER4)	20	0	nil
Transportin (TNPO)	20	2	intron
Alpha –s1-casein (CSN1)	6	8	ND
Bone Sialoprotein (IBSP)	6	2	ND
Epidermal Growth factor (EGF)	6	8	ND
Microsomal triglyceride transfer protein (MTP)	6	1	ND
Osteopontin-k (SPP1)	6	1	ND
6-Phosphofructo-2-kinase (PFKFB)	16	5	ND
complement component 4 binding protein, beta chain (C4BPB)	16	5	ND
Complement factor H (CFH)	16	10	ND
Fibromodulin (FMOD)	16	2	ND
Glutamine synthetase (GLUP)	16	2	ND
Interleukin 10 (IL10)	16	2	ND
Myogenin (MYOG)	16	3	nil
Plasma membrane Ca pumping ATPase (ATP2B)	16	3	ND
Presenilin 2 (PSEN)	16	9	1 -3' UTR
P-selectin (SELP)	16	7	ND

ND = not determined

9.3 Genotyped SNP gene markers

BTA1	POUF1	UMPS	SST	AGTR	AHSG	GLUT2	IL12	Total informative genes	NFI QTL segregating sire(s)
361	Yes	xx	Yes	xx	Yes	Yes	xx	4	398
368	Yes	Yes	Yes	Yes	xx	xx	xx	4	
398	xx	Yes	xx	Yes	Yes	Yes	Yes	5	

BTA 8	Cathepsin	LPL	NOC	TEK				Total informative genes	NFI QTL segregating sire(s)
361	Yes	xx	Yes	Yes				3	368, 398
368	Yes	Yes	Yes	xx				3	
398	Yes	Yes	Yes	Yes				4	

BTA11	FSHR	IL1B	POMC	ASS				Total informative genes	NFI QTL segregating sire(s)
361	Yes	Yes	xx	Yes				3	361
368	xx	Yes	Yes	xx				2	
398	xx	xx	xx	xx				NIL	

BTA 20	MAP1B	GHR	PRLR	PI3K	ITGA2	Follistatin	AMPK	Total informative genes	NFI QTL segregating sire(s)
361	Yes	Yes	xx	xx	Yes	xx	xx	3	398 (361, 368?)
368	xx	xx	Yes	Yes	Yes	Yes	Yes	5	
398	xx	Yes	Yes	xx	xx	Yes	Yes	4	

9.4 Mapping results

BTA1

Trait - RFI			All Sires	361	368	398
Without SNPs	Position (cM)		104.00	53	60	104
	Effect	361	0.03			
		368	-0.01	-0.40	-0.30	1.09
		398	1.00			
	F		3.40	1.15	1.28	8.82
	F-Crit		4.35	6.74	6.59	6.72
With SNPs	Position (cM)		104.00	60.00	60.00	104.00
	Effect	361	-0.01			
		368	0.03	-0.39	-0.25	-1.10
		398	-1.00			
	F		3.43	1.70	1.16	8.98
	F-Crit		4.47	7.23	7.63	7.58

BTA8

Trait - RFI			All Sires	361	368	398
Without SNPs	Position (cM)		73.00	93	0	73
	Effect	361	0.20			
		368	0.06	-0.24	-0.50	0.80
		398	0.85			
	F		2.70	0.63	4.70	4.72
	F-Crit		4.11	6.38	6.01	6.48
With SNPs	Position (cM)		95.00	115.00	0.00	95.00
	Effect	361	0.20			
		368	0.05	-0.25	-0.65	-0.81
		398	-0.86			
	F		2.79	0.64	6.61	5.03
	F-Crit		4.30	7.12	6.87	6.84

BTA11

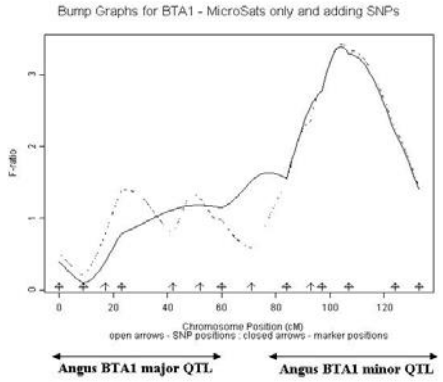
Trait - RFI			All Sires	361	368	398
Without SNPs	Position (cM)		69.00	70	51	4
	Effect	361	-1.16			
		368	0.01	-1.17	0.25	-0.52
		398	-0.36			
	F		3.95	11.63	1.02	1.51
	F-Crit		4.37	6.83	6.24	6.98
With SNPs	Position (cM)		68.00	70.00	49.00	4.00
	Effect	361	-1.15			
		368	-0.13	-1.18	0.70	-0.52
		398	-0.37			
	F		4.10	11.83	4.04	1.51
	F-Crit		4.21	7.29	7.76	6.74

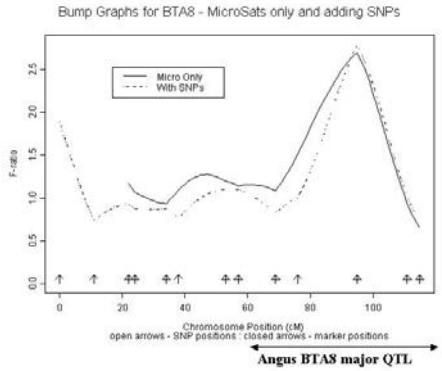
BTA20

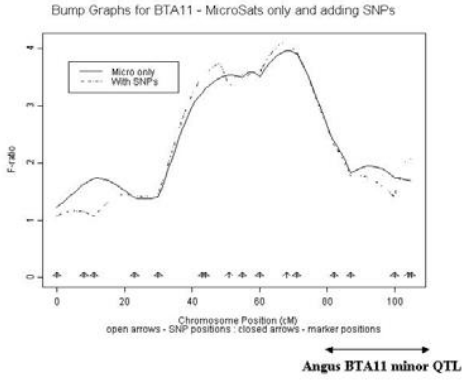
Trait - RFI			All Sires	361	368	398
Without SNPs	Position (cM)		53.00	59	72	52
	Effect	361	0.45			
		368	0.43	0.59	0.55	1.00
		398	1.02			
	F		4.47	3.28	5.56	6.67
	F-Crit		3.93	6.02	6.46	6.27
With SNPs	Position (cM)		49.00	46.00	72.00	49.00
	Effect	361	0.60			
		368	0.48	0.76	0.55	-1.04
		398	-0.99			

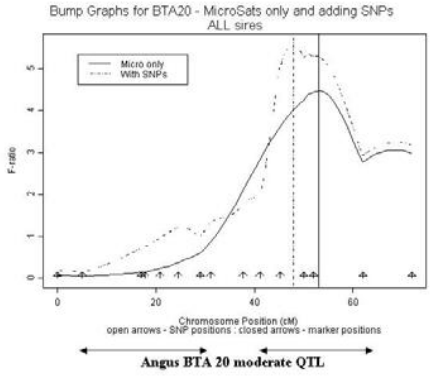
TIAL D	F	5.55	5.53	5.56	8.04
	F-Crit	4.11	6.33	6.80	7.06

9.5 Comparison of Net Feed Efficiency QTL in Jersey-Limousin and Angus



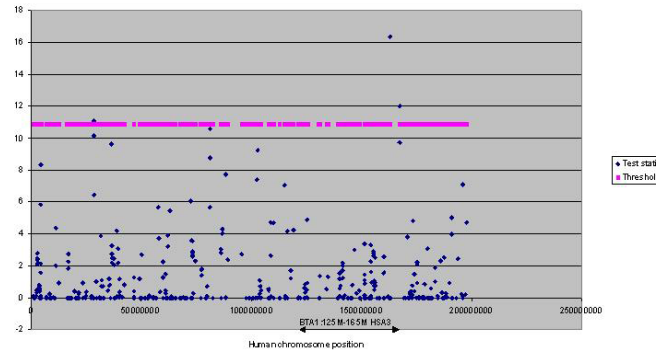




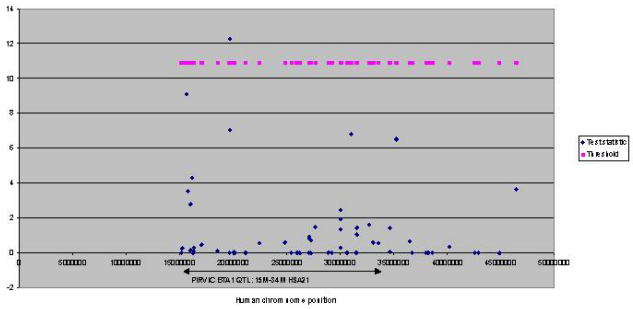


9.6 Comparison of Net Feed Efficiency QTL and ParAllele SNPs

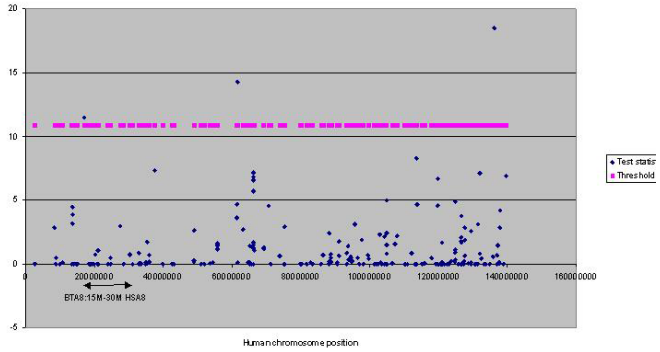
QLT BTA1 = HSA3 (Jersey-Limousin)



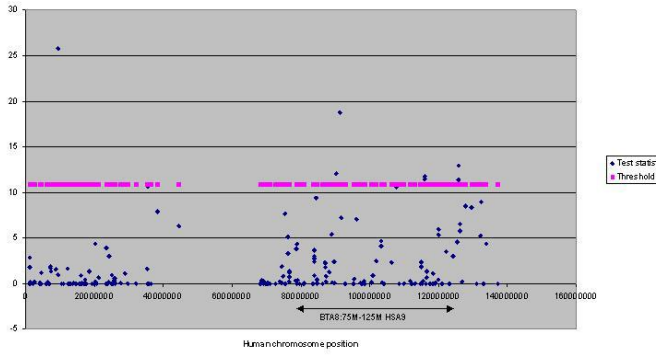
QLT BTA1 = HSA21 (Angus)



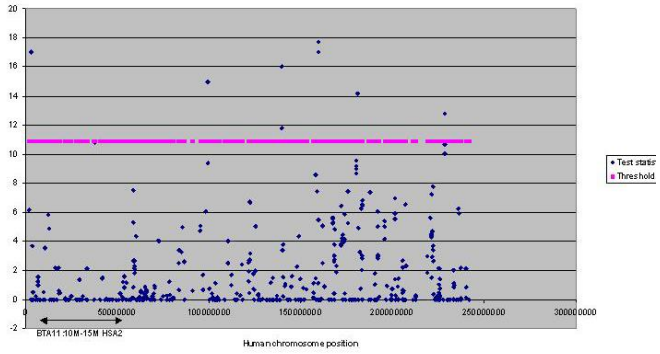
QTL BTA8 = HSA8



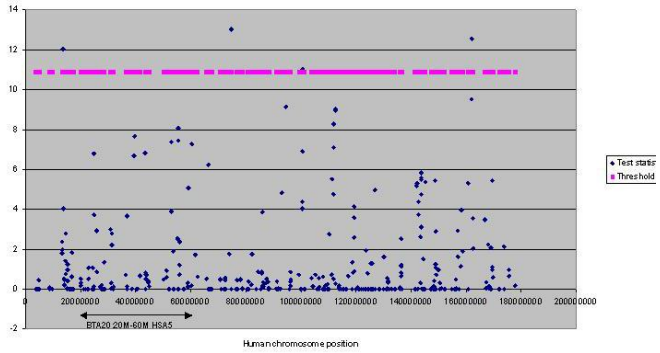
QTL BTA8 = HSA9



QTL BTA11 = HSA2



QTL BTA20 = HSA5



Landscape Page

How to insert a Landscape page

1. Select the text
2. Go to the **File Menu**
3. Then **Page Setup**
4. Under Orientation select **Landscape**
5. Under Apply To select **Selected Text**
6. Click **OK**

[Please **delete** after usage]

MLA highly appreciates your cooperation.

Thank you

Please send your completed
Final Report to MLA at

reports@mla.com.au
With your Administrative Details Report

[Please **delete** after usage]

BLANK PAGE
(TO BE USED OR DELETED ACORDINGLY)