Single nucleotide polymorphisms in the insulin-like growth factor 1 (IGF1) gene are associated with growth-related traits in farmed Atlantic salmon

H. Y. Tsai*, A. Hamilton†, D. R. Guy† and R. D. Houston*

*The Roslin Institute and Royal (Dick) School of Veterinary Studies, The University of Edinburgh, Midlothian, EH25 9PS, UK. †Landcatch Natural Selection Ltd., 15 Beta Centre, Stirling University Innovation Park, Stirling, FK9 4NF, UK.

Summary

Understanding the genetic basis of variation in traits related to growth and fillet quality in Atlantic salmon is of importance to the aquaculture industry. Several growth-related QTL have been identified via the application of genetic markers. The IGF1 gene is considered a highly conserved and crucial growth-regulating gene in salmonid species. However, the association between polymorphisms in the IGF1 gene and growth-related traits in Atlantic salmon is unknown. Therefore, in this study, regions of the Atlantic salmon IGF1 gene were sequenced, aligned and compared across individuals. Three SNPs were identified in the putative promoter (SNP1, g.5763G>T; GenBank no. AGKD01012745), intron 1 (SNP2, g.7292C>T; GenBank no. AGKD01012745) and intron 3 (SNP3, g.4671A>C; GenBank no. AGKD01133398) regions respectively. These SNPs were genotyped in a population of 4800 commercial Atlantic salmon with data on several weight and fillet traits measured at harvest (at approximately 3 years of age). In a mixed model, association analysis of individual SNPs, SNP1 and SNP3 were both significantly associated with several weight traits (P < 0.05). The estimated additive effect on overall harvest weight was approximately 35 and 110 g for SNPs 1 and 3 respectively. A haplotype analysis confirmed the association between genetic variation in the IGF1 gene with overall body weight (P < 0.05) and fillet component traits (P < 0.05). Our findings suggest the identified nucleotide polymorphisms of the IGF1 gene may either affect farmed Atlantic salmon growth directly or be in population-wide linkage disequilibrium with causal variation, highlighting their possible utility as candidates for marker-assisted selection in the aquaculture industry.

Keywords aquaculture, growth regulation, haplotype, marker-assisted selection, salmonids

Introduction

Atlantic salmon (Salmo salar L.), family Salmonidae, is a key economic species in the aquaculture industry worldwide. From 2008 to 2010, according to the statistics from Fisheries and Aquaculture Division of the Food and Agriculture Organization (FAO), the worldwide production of Atlantic salmon was over 1.4 million tonnes and the estimated value was approximately $7.8 billion (FAO 2010). The genetic improvement of fish through selective breeding for growth-related traits has been applied to many farmed aquatic species, including the salmonid species rainbow trout, Atlantic salmon (Gjedrem et al. 2012) and coho salmon (Neely et al. 2008). In Atlantic salmon, Gjedrem et al. (2012) indicated that genetic improvement through selective breeding resulted in the average growth rate increasing by 5.4% a year. Understanding the quantitative genetic basis of variation in these growth-related traits is of importance for continued improvement and increased rates of genetic gain.

To improve the quality and quantity of fish fillet production, determining the factors controlling muscle growth and development is an important research goal. The growth hormone (GH) system is known to regulate somatic and skeletal growth, cell production, protein synthesis and metabolism process in both mammals and fish (Duan 1997; Tambasco et al. 2003; Moghadam et al. 2007). The insulin-like growth factor (IGF) proteins are key
mediators of GH effects, and IGFs are released from the liver upon stimulation by growth hormone to control development and normal growth (Moriyama et al. 2000). This highly conserved system includes two IGF ligands (IGF1 and IGF2), high-affinity binding proteins (IGFBP-1 to IGFBP-6) and IGF receptors (IGF1 and IGF2 receptors) (Duan 1997; Dupont & Holzenberger 2003). In particular, the highly conserved IGF1 gene can be considered a candidate gene for growth traits in fish due to its well-established role in the GH regulation system (e.g. Duan 1997; Moghadam et al. 2007). The mature IGF1 amino acid sequence in Atlantic salmon is a 70-amino acid basic peptide, with a molecular weight of 7.5 kDa, that connects with three intrachain disulphide bridges.

Genetic variation in the IGF1 gene has been described in Arctic char (Tao & Boulding 2003) and sinipercid species [Siniperca chuatsi (F) × Siniperca scherzeri (M), where they were associated with growth traits (Wang et al. 2013)]. However, the relationship between genetic variation in IGF1 and growth traits in Atlantic salmon is currently unknown. This knowledge could improve our understanding of the regulation of growth in salmonids and lead to marker-assisted selection for favourable alleles in aquaculture breeding programs. Therefore, the aims of this study were to discover SNP variants within the candidate IGF1 gene in Atlantic salmon and to investigate the association of these SNPs with growth and fillet-related traits measured on a large commercial population of Atlantic salmon at harvest.

Materials and methods

Animal sampling and trait measurement

A total of 4800 samples randomly selected from a Landcatch Natural Selection Ltd. population (approximately 60 k individuals) of Atlantic salmon were utilised in this study. The sample comprised 198 full-sibling families, 60 k individuals) of Atlantic salmon were utilised in this study. The sample comprised 198 full-sibling families, which were created in 1999 by crossing 136 sires and 198 dams. This population has previously been described in Penaloza et al. (2013). At approximately 3 years of age, the salmon were harvested from a single commercial marine cage in Shetland. The fish were measured for the following traits: harvest weight (kg), gutted weight (kg), deheaded weight (kg), fillet weight (kg), gutted yield (%), fillet yield (%), fat percentage (as estimated using a Torry Fatmeter, Distell, Ltd.) and fillet colour [assessed visually using the Roche Salmofan scale (Hoffmann-La Roche), ranging from 20 (yellow) to 34 (red)]. Full details of the measurement protocols for these traits are given in the materials and methods sections 2.2.1 to 2.2.2 in Powell et al. (2008). Summary statistics for these traits in this study are given later in Table 3. The sex of the offspring was unknown.

Amplification and sequencing of salmon IGF1 gene

To discover putative SNPs within the IGF1 gene, DNA samples from eight unrelated Atlantic salmon parents were amplified by PCR. This sample of 16 haploid genome copies from the population was used to detect relatively common SNPs [probability approximately 0.97 for detection of a SNP with MAF (minor allele frequency) of 0.2 and approximately 0.81 of detecting a SNP with a MAF of 0.1]. Three sets of primers were designed to sequence from the promoter region to exon 3 of the Atlantic salmon IGF1 gene (Table 1). PCRs were performed in volumes of 20 μl, using 62.5 ng of the DNA sample, 1 μl of buffer and MgCl2, 200 μM of dNTPs, 50 μM each of forward and reverse primer, 0.04 U/μl of FastStart Taq DNA Polymerase (Roche Applied Science) and 8.84 μl of Milli-Q water. The PCR cycling conditions for the primer set were 95 °C for 5 min, 30 cycles of 95 °C for 30 s, Tm for 30 s and 72 °C for 45 s, followed by a final extension at 72 °C for 10 min. Subsequently, PCR products were purifed using the MinElute PCR Purification Kit (Qiagen). The Sanger sequencing of the PCR products was performed in part at Edinburgh Genomics and GATC Biotech AG Ltd.

SNP discovery and genotyping

The sequence of each fragment from each individual fish was aligned to detect putative SNPs using LASERGENE software (DNASTAR, Inc.). The Atlantic salmon IGF1 mRNA sequence was included in the alignment to determine the putative transcribed regions of the gene. The flanking sequence information for the three potential SNP loci detected was provided to LGC Genomics for the design of a Kompetitive

| Fragment amplified region | Amplicon length (bp) | Sequence (5′→3′) | Primer length (bp) | Annealing temperature (°C) |
|---------------------------|----------------------|-------------------|--------------------|--------------------------|
| Promoter-Exon 1           | 743                  | CCAACGCCTATAAACAACCTCATCC | 23                 | 58                       |
|                           |                      | TCCGTCAACCTGCTACCATCC | 22                 | 64                       |
| Exon 2                    | 835                  | GCCATAGGTGCGTAAATCGTGCGT | 24                 | 64                       |
| Exon 3                    | 671                  | CACTTGGCTCTTATCACCATGCTGCCGT | 25                 | 64                       |
|                           |                      | GGGCCCAACTGGTCTTACACATAG | 24                 |                          |
Allele Specific PCR (KASP) assay (LGCGenomics 2013) for SNP genotyping. All 4800 animals with trait data collected were genotyped for the three SNPs.

Statistical analysis

The association between the IGF1 genotypes and each of the measured harvest traits was performed using ASREML 3.0 (Gilmour et al. 2009). First, each SNP genotype was set as the fixed effect in the first analysis model. Second, the predicted haplotypes for each individual were reconstructed using PHASE 2.1 software (Stephens et al. 2001), and for each haplotype, the number of copies (0, 1, 2 copies) was set as the fixed effect. The following animal model was applied:

\[ Y_{ij} = \mu + G_i + A_j + e_{ij} \]

where \( Y_{ij} \) is the trait value measured in the individual \( i \), \( \mu \) is the overall mean value of the trait, \( G_i \) is the fixed effect of the SNP genotype or haplotype, \( A_j \) is the additive effect of the individual based on the pedigree information and \( e_{ij} \) is the residual error. The heritability for each of the harvest traits was estimated using the same model but removing the fixed effect of the SNP and minor allele frequencies respectively and \( e_{ij} \) is the calculated dominant effect.

Table 2 Details of the IGF1 SNPs, the SNP position with respect to the reference gene and genotype frequencies.

| SNP no. | SNP          | GenBank reference sequence ID | Putative region of IGF1 gene | Allele frequencies (n = 4800) |
|---------|--------------|------------------------------|-----------------------------|------------------------------|
| SNP1    | g.5763G>T    | AGKD01012745                 | Promoter                    | 0.61/0.39                    |
| SNP2    | g.7292C>T    | AGKD01012745                 | Intron 1                    | 0.80/0.20                    |
| SNP3    | g.4671A>C    | AGKD01133398                 | Intron 3                    | 0.88/0.12                    |

Table 3 Results of the association analysis including the predicted mean value (and standard error) for each trait for each genotype class, general statistics and heritability of traits (note that results for the non-significant SNP2 are not given).

| Genotype (no. of fish) | SNP1 (g.5763G>T) | SNP3 (g.4671A>C) | Trait | G/G (n = 1697) | G/T (n = 2238) | T/T (n = 669) | C/C (n = 3644) | C/A (n = 1022) | A/A (n = 76) | Mean (SD) | Overall heritability (SE) |
|------------------------|------------------|------------------|-------|----------------|----------------|--------------|----------------|----------------|--------------|-----------|--------------------------|
| HW                     | 2.50 ± 0.03      | 2.51 ± 0.03      | 2.57 ± 0.03* | 2.50 ± 0.03     | 2.55 ± 0.03     | 2.72 ± 0.08** | 2.54 (0.63)    | 0.52 (0.05)    |             |           |                          |
| GW                     | 2.28 ± 0.03      | 2.30 ± 0.02      | 2.37 ± 0.03** | 2.29 ± 0.02     | 2.33 ± 0.03     | 2.46 ± 0.07** | 2.32 (0.58)    | 0.53 (0.05)    |             |           |                          |
| GW                     | 91.7 ± 0.06      | 91.8 ± 0.05      | 91.8 ± 0.08   | 91.7 ± 0.04     | 91.9 ± 0.07     | 91.8 ± 0.24   | 1.00 (0.02)    | 0.04 (0.01)    |             |           |                          |
| GW                     | 1.99 ± 0.02      | 2.01 ± 0.02      | 2.07 ± 0.03** | 2.01 ± 0.02     | 2.03 ± 0.03     | 2.16 ± 0.06** | 6.01 (0.51)    | 0.52 (0.05)    |             |           |                          |
| FW                     | 1.66 ± 0.02      | 1.67 ± 0.02      | 1.71 ± 0.02** | 1.67 ± 0.02     | 1.69 ± 0.02     | 1.83 ± 0.06** | 5.21 (0.43)    | 0.53 (0.05)    |             |           |                          |
| FW                     | 66.2 ± 0.13      | 66.4 ± 0.11      | 66.3 ± 0.19   | 66.3 ± 0.09     | 66.4 ± 0.16     | 66.9 ± 0.54   | 1.03 (0.04)    | 0.05 (0.02)    |             |           |                          |
| FW                     | 12.13 ± 0.19     | 12.07 ± 0.17     | 12.33 ± 0.26  | 12.08 ± 0.16    | 12.21 ± 0.23    | 12.94 ± 0.72  | 41.70 (5.68)   | 0.15 (0.02)    |             |           |                          |
| FC                     | 28.92 ± 0.03     | 28.92 ± 0.02     | 28.96 ± 0.04  | 28.94 ± 0.02    | 28.89 ± 0.03    | 28.94 ± 0.11  | 33.00 (0.75)   | 0.14 (0.03)    |             |           |                          |

HW, harvest weight (kg); GW, gutted weight (kg); DW, deheaded weight (kg); FW, fillet weight (kg); FY, gutted yield (%); FW, fillet yield (%); FP, fat percentage (%); FC, fillet colour [20 (yellow)–34 (red)]. Values in bold represent those that are significant.

*Overall SNP P < 0.1; **Overall SNP P < 0.05.
homzygous categories for the three significant traits (gutted weight, deheaded weight and fillet weight) was approximately 45, 40 and 25 g respectively. SNP3 was significantly associated with harvest weight, gutted weight, deheaded weight and fillet weight, with fish homzygous for the adenine (minor) allele having higher mean trait values and an additive effect of 110, 85, 75 and 80 g respectively (Table 3). There were no significant differences found in SNP2 (and therefore genotypic means are not shown in Table 3). The percentage of additive genetic variation explained by all the SNPs in this study was relatively small, with SNP1 estimated to explain approximately 0.2% of the additive genetic variation in the weight traits and SNP3 estimated to explain <0.1% of the additive genetic variation. This low percentage of additive variation explained may be due to the apparent dominance of the allele associated with lower trait values (Tables 2 and 3) and the low minor allele frequency of the allele associated with higher trait values.

Six haplotypes were observed in the population with Hap1 (TCC) and Hap4 (GCC) being the most frequent types, accounting for 31% and 50% of the haplotypes in the population respectively (Table 4). Hap3 (TAA) was significantly associated with harvest weight, gutted weight, deheaded weight and fillet weight ($P < 0.01$), whereas Hap4 was significantly associated with gutted weight and deheaded weight ($P < 0.05$). The direction of the effect of the haplotypes on the harvest traits was consistent with the predicted effects of the individual SNP genotypes, with the number of copies of Hap 3 associated with higher harvest and fillet weight (Table 5).

### Discussion

There is strong physiological and biochemical evidence that the IGF system, including IGFs, IGF receptors and IGF binding proteins, interacts to regulate the growth of several teleost fish species (Duan 1997). In this study, nucleotide polymorphisms of the Atlantic salmon IGF1 gene were discovered and investigated to assess the association of this genomic variation with growth and fillet-related traits in a commercial population. Two SNPs discovered in the putative promoter and intron 3 respectively showed a significant association with weight traits measured at harvest. Haplotypes constructed from the IGF1 gene variants demonstrated an overall significant association with the harvest weight traits, supporting the role for IGF1 genetic variation in the regulation of weight at harvest and therefore growth in farmed salmon.

The IGF1 gene sequence is highly conserved in salmonid species with sequence identity of between 93% and 98% (Moghadam et al. 2007). Further, the co-location of the IGF1 gene with the MYF5 and MYF6 genes on the genetic maps of rainbow trout (Oncorhynchus mykiss), Atlantic salmon (Salmo salar L.) and Arctic char (Salvelinus alpinus) suggests a conservation of genetic linkage between these genes in salmonids (Moghadam et al. 2007). In the genetic map of Atlantic salmon, the IGF1 gene has been identified on linkage group (LG) 24 alongside one copy of the duplicated MYF6 gene, which is regarded as a regulator of myogenesis and muscle regeneration in a number of organisms (Moghadam et al. 2007). Although some salmonid species (e.g. rainbow trout and Arctic char) have been shown to contain two distinct paralogues for IGF1 as a result of the relatively recent 4R genome duplication event, there is no definitive evidence for distinct paralogues in

#### Table 4
Pattern and frequency of each reconstructed haplotype in the genotyped population. The individual alleles at the haplotypes are given in the order SNP1, SNP2 and SNP3.

| Hap1 | Hap2 | Hap3 | Hap4 | Hap5 | Hap6 |
|------|------|------|------|------|------|
| TCC  | TTC  | TTA  | GCC  | GTC  | GTA  |
| Frequency | 0.307 | 0.033 | 0.048 | 0.495 | 0.043 | 0.074 |

#### Table 5
Summary of the predicted mean value of each phenotypic trait for Hap3 and Hap4 by haplotype copy number.

| Trait | Hap3 (TTA) | Hap4 (GCC) |
|-------|------------|------------|
|       | Haplotype copy no = 0 (n = 4146) | Haplotype copy no = 1 (n = 591) | Haplotype copy no = 2 (n = 18) | Haplotype copy no = 0 (n = 837) | Haplotype copy no = 1 (n = 2187) | Haplotype copy no = 2 (n = 1101) |
| HW    | 2.50 ± 0.02 | 2.57 ± 0.03 | 2.82 ± 0.14** | 2.56 ± 0.03 | 2.51 ± 0.03 | 2.49 ± 0.03* |
| GW    | 2.29 ± 0.02 | 2.36 ± 0.03 | 2.57 ± 0.12** | 2.35 ± 0.03 | 2.30 ± 0.02 | 2.29 ± 0.03** |
| GY    | 91.75 ± 0.04 | 91.94 ± 0.09 | 91.34 ± 0.45* | 91.87 ± 0.08 | 91.84 ± 0.04 | 91.66 ± 0.07 |
| DW    | 2.00 ± 0.02 | 2.07 ± 0.03 | 2.24 ± 0.11** | 2.06 ± 0.03 | 2.01 ± 0.02 | 2.00 ± 0.02** |
| FW    | 1.67 ± 0.02 | 1.70 ± 0.02 | 2.00 ± 0.10** | 1.70 ± 0.02 | 1.67 ± 0.02 | 1.65 ± 0.02* |
| FY    | 66.3 ± 0.09 | 66.33 ± 0.2 | 67.21 ± 1.06 | 66.46 ± 0.17 | 66.37 ± 0.1 | 66.01 ± 0.15* |
| FP    | 12.07 ± 0.15 | 12.36 ± 0.27 | 13.63 ± 1.34 | 12.28 ± 0.24 | 12.07 ± 0.16 | 12.30 ± 0.22 |
| FC    | 28.93 ± 0.02 | 28.89 ± 0.04 | 28.93 ± 0.22 | 28.96 ± 0.03 | 28.92 ± 0.02 | 28.93 ± 0.03 |

HW, harvest weight (kg); GW, gutted weight (kg); DW, deheaded weight (kg); FW, fillet weight (kg); GY, gutted yield (%); FY, fillet yield (%); FP, fat percentage (%); FC, fillet colour (20 [yellow]–34 [red]).

Values in bold represent those that are significant.
*Overall SNP $P < 0.1$; **Overall SNP $P < 0.05$. © 2014 The Authors. Animal Genetics published by John Wiley & Sons Ltd on behalf of Stichting International Foundation for Animal Genetics., 45, 709–715.
Atlantic salmon (Moghadam et al. 2007; Macqueen et al. 2013). Atlantic salmon LG 24 (also denoted chromosome 7 in [http://www.asalbase.org]) has been demonstrated to show homology to both LG 10 (chromosome 9) and LG 22 (chromosome 17; Danzmann et al. 2005). QTL mapping studies have shown that LG24 (Baranski et al. 2010) and LG22 (Boulding et al. 2008; Baranski et al. 2010) harbour loci affecting body weight and fillet-related traits in salmon. Additionally, LG 10 has been shown to contain QTL affecting growth traits (Reid et al. 2005) and harvest condition factor (Houston et al. 2009) in Atlantic salmon. Taken together, these findings suggest that genetic variation on the homeologous salmon LGs 10, 22 and 24 is involved with the body growth and development and raises the possibility that polymorphic variation in IGF1 may play a contributory role.

Interestingly, the G/T SNP identified in the putative promoter of IGF1 gene of Atlantic salmon in the current study (SNP1) was also identified at the same position in a study of Arctic Charr (Salvelinus alpinus L.) (Tao & Boulding 2003). However, no association between the Arctic Charr IGF1 promoter SNP and growth traits was detected (Tao & Boulding 2003). There are several reasons for the discrepancy between the two studies, including the use of different species (Atlantic salmon vs. Arctic Charr), different environments (farmed vs. wild) and different sample sizes (4800 vs. approximately 300 fish). Nonetheless, these findings suggest that the SNP existed before the radiation of salmonids and that the promoter region is highly conserved across at least two different species (Tao & Boulding 2003; Vong et al. 2003), and the findings raise the possibility of locating the same SNP in other species belonging to the Salmonidae genera and assessing its association with growth-related traits.

SNPs located in intronic regions of a targeted gene are usually considered less likely to have a direct function than those in exons or promoter regions. SNP1 (promoter) and SNP3 (intron) were associated with several body weight traits. These SNPs may simply be acting as markers in linkage disequilibrium with causal variants elsewhere in the gene (given that the entirety of the salmon IGF1 gene was not resequenced for SNP detection) or in other linked genes or regulatory regions. However, several studies have suggested that SNPs in intronic regions may regulate gene expression, particularly when the site is close to coding regions (e.g. see De-Santis & Jerry 2007), so the possibility of a causal role for these salmon IGF1 intronic SNPs should not be excluded. Intronic variation in the IGF1 gene has been associated with growth traits in livestock, such as Holstein–Friesian dairy cattle (six SNPs in the intron region of the IGF1 gene) (Mullen et al. 2011) and Nanjiang Huang goat (a SNP in intron 4 of the IGF1 gene) (Zhang et al. 2008). Also, haplotypes within the 5'-untranslated region of the Largemouth seabass (Micropterus salmoides) IGF1 gene have been demonstrated to show a significant association with body weight (Li et al. 2009).

The haplotype analysis based on the discovered IGF1 SNPs supported the results from the individual SNP analysis, with Hap 3 (TTA) and Hap 4 (GCC) showing significant association with harvest weight and its components. Previous studies (e.g. Drysdale et al. 2000; Li et al. 2012) suggested that, compared with individual SNP analysis, the haplotype-based association analysis is more powerful for detecting associations between phenotypic traits and targeted genes by reconstructing the alleles at multiple SNPs into one single fixed factor (haplotype or diplotype) in the analysis model. The rare Hap 3 (4.8% frequency) was associated with substantially higher values for the harvest weight traits, suggesting substantially faster growth of the salmon carrying this haplotype. This is surprising given the selection of this commercial population for improved growth rate for a number of generations. It should be noted that the harvest weight traits measured in the current study showed a very high phenotypic correlation with each other (r ≥ 0.97), and it is therefore unsurprising to find the estimated effect of both the SNP alleles and the haplotypes to be in the same direction for all weight traits (Table 3). It is also noteworthy that there was no significant association detected between the SNPs and the yield-related traits (gutted yield and fillet yield). This may be due to the lower heritability of these traits, which suggests there is less genetic variation for fish proportions than for overall size.

The IGF1 SNP variation described in the current study may have practical application in marker-assisted selection programs, although verification studies in additional salmonid populations would be useful to assess the robustness of its association with performance traits. In the present study, no exonic SNPs were detected in the IGF1 gene, which may reflect a high level of conservation of the coding regions of the IGF1 gene within species. However, it is possible that insufficient numbers (for rare alleles) or genetic diversity (for between-population variation) of Atlantic salmon were resequenced in the current study to detect IGF1 exonic variants. Therefore, future studies could also be aimed at broader surveys of the genetic variability within and between Atlantic salmon populations, given the significant association demonstrated in the current study. It is noteworthy that synonymous SNPs in IGF1 coding regions in crosses of the siniperid species [Siniperca chuatsi (F) × Siniperca scherzeri (M)] have been shown to be associated with growth traits (Wang et al. 2013). Future studies could also examine genetic variation in other components of the IGF system, including the IGF binding protein superfamily (IGFBP) genes, which are known to play a regulatory role in the half-life of circulating IGFs, especially IGF binding protein 5, which has profound effects on fish myoblast differentiation (Duan et al. 2010). The findings of such studies may provide an improved understanding of the IGF regulation of growth in salmon and in fish in general.
Conclusion

The purpose of this study was to identify SNPs in the Atlantic salmon IGF1 gene that may contribute to variation in growth and fillet-related traits in commercial fish populations. The results provide evidence that genetic variation within the IGF1 gene is significantly associated with growth traits in the commercial population of Atlantic salmon. Given the economic importance of these traits to salmon aquaculture, this variation may lead to marker-assisted selection for the faster-growing genotypes.

Acknowledgements

We acknowledge Natalie Lowe, Serap Gonen, Carolina Penalooza and Stephen Bishop for helpful suggestions on the study design and experiments, and staff at Edinburgh Genomics for assistance with the Sanger sequencing. This project was supported by British Biotechnology and Biological Sciences Research Council (BBSRC) Grant Number BB/H022007/1, Roslin Institute’s BBSRC Strategic Funding Grant and by Ministry of Education, Taiwan Government Overseas Studying Funding.

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