The genetic basis of size in pet dogs: the study of quantitative genetic variation in an undergraduate laboratory practical

Dr Craig Wilding

School of Natural Sciences and Psychology,

Liverpool John Moores University,

Liverpool.

L3 3AF.

UK.

c.s.wilding@ljmu.ac.uk

+44 (0)151 231 2500

Keywords:

SNP, genotyping, bioinformatics, *Canis familiaris*

Running title:

Association of a SNP in the *IGF-1* gene with size in pet dogs

ABSTRACT

The teaching of quantitative genetic variation in the undergraduate laboratory practical environment can be difficult since, for quantitative phenotypes which are under the control of multiple loci, detection of phenotypic differences caused by individual variants is problematical without large samples, impractical in such classes. Pet dogs provide a clear example of quantitative genetic variation with individual breeds ranging in size from 1-70kg weight yet with little intra-breed variability. In contrast to humans where there are few identified genetic variants known to be involved in the genetically controlled size phenotype, in dogs, seven single nucleotide polymorphisms (SNPs) in six genes have been demonstrated to explain half of the phenotypic variance. In the practical described here, a single G-A SNP (within intron 2 of the *insulin-like growth factor 1* gene) is studied through PCR, sequencing and bioinformatics. Average breed weight of dogs of different genotypes at this SNP show significant differences in size (Median (IQR) of AA=10kg (6-15kg), AG=23.75kg (14-30kg), GG=30kg (24.5-37kg) from our class data) with an estimate of just $\approx N = 16$ dogs needing to be genotyped to demonstrate a significant difference in size between dogs harbouring the two homozygous genotypes. In the practical described herein, from a single laboratory and a single computer session, students are able to see the clear effect of genotype on a quantitative trait. Examination of the variant in the Ensembl browser (www.ensembl.org) allows students to understand the genomic basis of this variant and appreciate the wealth of data and information publicly available in genome browsers.

INTRODUCTION

With the notable exception of the genetics of ear-wax consistency – a trait truly under the control of a single SNP [1] – many genetically controlled traits are not simple monogenic, Mendelian traits. Even for traits such as bitter taste perception, ear lobe shape, tongue rolling etc. which have been used as examples of simple one-locus, two-allele Mendelian traits, as we come to understand the underlying molecular genetic basis of these characteristics it is clear that this is an oversimplification [2]. The genetic basis of quantitative traits is even more complex. Height, weight, IQ, skin colour, blood pressure etc. are examples of quantitative traits but such complex traits are under the control of multiple loci and often the environment. In the constraints of the undergraduate laboratory class, it may be impractical to study a single SNP contributing a minor component of a quantitative trait and to expect to find a measurable effect of that SNP. Failure to detect a measurable difference within the context of an undergraduate practical may impact negatively on understanding and student engagement.

Here I describe a practical focusing on the genetics of a clear quantitative phenotype in pet dogs. Size in dogs is a clear example of genetically controlled trait. Within the tightly controlled constraints of Kennel Club registration, breeds have low within breed variance yet between breeds, sizes can range from the 1.2kg Chihuahua to the Great Dane at over 70 kg. Unlike in humans where size is genetically controlled, but the actual variants underpinning this trait are largely unknown [3], as is the case for many quantitative human traits [4], in dogs a significant component of size has been shown

to be associated with just a small number of polymorphisms [5-10]. This practical studies a single one of these genetic variants previously shown to be significantly associated with size in dogs [10].

This genetic variant was identified through a series of studies which provide important background material and reading for students. Chase *et al.* [11] used QTL mapping of skeletal measurements of Portuguese water dogs, which exhibit striking within-breed size variation, to detect a genomic region on canine chromosome 15 underpinning size variation. Subsequently, Sutter *et al*. [10] further mapped the QTL through sequencing PCR amplicons from across the QTL in a range of dogs and identified a single haplotype of the *insulin-like growth factor 1* (*IGF-1*) gene associated with small size across a range of breeds. One of the single nucleotide polymorphisms within this haplotype (a G-A transition at position 41,221,438 on canine chromosome 15) shows a striking association with breed size [10].

This exercise allows students to undertake sampling from their own pet dogs followed by DNA extraction, PCR amplification of this genetic variant followed by sequencing of the PCR product. It is followed by an extendable computational practical for reading the sample genotype and understanding the genomic basis of the studied variant. It thus combines a range of practical exercises leading students from sample to genotype. Such active learning of genetics techniques will only aid in the students' understanding [12, 13] of some of the complex methodological techniques taught in modern undergraduate genetics courses [14]. This practical has now run successfully for four years in a second-year undergraduate module. The module is available to Biology and Zoology students and whilst focusing on dogs, the learning objectives of the practical can be discussed in

relation to outcomes of relevance to human biology and utilises and teaches practical components of relevance across all spectra of genetics.

Scientists aim for their studies' findings to be replicable [15] and this is particularly true in genetic association studies [16]. Since in this practical, the findings of an important and highly cited study are replicated, the practical thus incorporates modern research into teaching practice, leading students essentially through a procedure to which students can relate, and see the relevance of, and for which the replication of a previous research finding can be directly linked to learning outcomes.

EXPERIMENTAL PROCEDURES

Expected Background Skills

This practical runs in a module which studies how state of the art genetic and genomic tools are used to understand how genes combine with the environment to control organismal phenotypes and disease states. It covers methodologies, practical applications and recent examples of the application of genetics and genomics in the fields of biology, medicine and evolution. Students are expected to have undertaken a first-year module covering transmission, molecular, and population genetics as well as the theory of evolution.

Full equipment lists and methodologies are available as Supporting Information.

Materials

Equipment and software

The practical requires laboratories equipped with facilities for PCR and gel electrophoresis. The costliest outlay is the provision of sufficient micropipettors for large class sizes. Since, as run, students produce only three PCR tubes per pair then a single 96 well PCR machine is sufficient for any normal class size.

Scheduling

It is logical to precede this practical with the genetics of a (presumed) monogenic trait (for instance in this module we also study the genetics of taste perception) and discuss

how this, and many other traits 'advertised' as monogenic are, in fact, polygenic or have environmental components [17].

Part I: Isolation of Genomic DNA and PCR of the IGF-1 gene

DNA sampling is unlikely to be undertaken as freshly as possible since often pet dogs are with families elsewhere in the country. Mouth swabs must therefore be provided with sufficient time for students to arrange sampling and potentially postage. Extracted DNA quality decreases after 3 days from sampling. It is useful to have 'spare' mouth swabs from the dogs of, for example, members of staff for students who have no dogs or are unable to obtain swabs. We have noticed that there is a preponderance of smaller dog breeds owned by students, a trend in line with current dog ownership - Sánchez-Vizcaíno *et al*. [18] report the top 10 most popular dogs in the UK and, of these, only two (German shepherd and Labrador retriever) have high frequencies of the 'large dog allele' at the SNP being studied. Since it is important to sample both large and small dogs in order to have a sample set which can be subsequently tested for significance, then surveying the dog breeds likely to be sampled before the practical is prudent. Having a bank of staff member's dogs which can be used each year will help ensure that sufficient samples are available, and samples from larger dog breeds are particularly valuable here to balance out the expected greater number of small dogs in the student dataset. We have also previously used swabs frozen following collection and this has had no noticeable negative effect on DNA quality. Thus, it is possible to retain a bank of swabs that can be utilised if necessary.

Students are provided with mouthswabs (Sterilin F155CA, Fisher Scientific UK) and instructions to take samples of cheek cells, not drool (Figure 1). DNA is then isolated from swabs using the Gene Jet Whole Blood DNA Purification Kit (ThermoFisher, UK) (alternative extraction kits/techniques may work). Swabs are first swirled in 200µl 1 x PBS in a 1.5 ml Eppendorf tube for 1 minute. The swab tip is cut off (using scissors) and 20 µl proteinase K and 400 µl lysis buffer added. Following a 10 minute 56°C incubation on a hot block the manufacturer's protocol is followed for extraction with DNA eluted in 50 µL of Elution Buffer.

PCR is undertaken with primers SQ5570F and SQ5570R (See Supplementary material of [10] – note that the genomic coordinates of these primers in the paper have changed since this publication due to subsequent revision of the *Canis familiaris* genome assembly [19]):

SQ5570F 5'-ACTAGTTTGGCTGCTTCACTGC-3'

SQ5570R 5'-AGCAGCCATTACCTGTGGTAGA-3'

PCR is done with 1 x Promega Go*Taq* colourless PCR mastermix, 0.2 µM each primer and 4 µl DNA template with cycling conditions of 95°C for 3 minutes then 35 cycles of 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 1 minute and a final extension step of 72°C for 5 minutes. However, this is a robust PCR and any standard polymerase is likely to work.

Each pair of students (working with one extracted DNA template) set up a PCR on their own dog's DNA, and both a positive control (a DNA extract tested previously) and negative control.

Part II: Electrophoresis of PCR products (with optional clean-up)

Due to scheduling demands we allow students to load pre-cast electrophoresis gels with PCR products from previously conducted PCRs. This way, students get to load a gel (one well per student) alongside a ladder and, following 30 minutes of electrophoresis, observe the products under a transilluminator.

It would be feasible to undertake a separate class in which students loaded their own PCR products and subsequently clean up the PCR products for sequencing. However, we have, to date, cleaned up student's products (using GeneJet PCR purification kit, ThermoFisher, UK) and sent for sequencing (using primer SQ5570F) at GATC Biotech (Konstanz, Germany) following the concentration recommendations for LightRun sequencing (multiple other commercial providers have equivalent service).

Part III: Bioinformatic analysis of sequence traces and the genomic context of the studied variant

Sequence traces are returned as .ab1 files which require specific software for viewing. We use the (free) demonstration version of Mutation Surveyor (SoftGenetics) in which traces are compared to a reference file (in this case the sequence of the PCR amplified region from the *Canis familiaris* genome extracted from Ensembl). Mutation Surveyor detects differences between samples and the reference sequence, which for the reference *C. familiaris* genome, is G at the SNP we study. Thus, if dogs are heterozygous AG or homozygous AA at the *IGF1* SNP being studied, Mutation Surveyor will identify mutations. If the dog is homozygous GG then no mutations will be detected. From 110 dogs in four years of running this practical we have seen no other true SNPs within this

642 bp PCR product. Other software could be used. Alternatively, sequence traces can be converted to printable PDFs to provide the students with a permanent record of their dog's sequence trace using software such as FinchTV (http://www.geospiza.com/Products/finchtv.shtml) and SNPs identified without the need for use of computers. (Using FinchTV if the sequence immediately preceding the SNP – TTCGCCAGCC – is searched for, the following base can be interpreted to identify the genotype.) Students extract genotypes from their own dogs and a reference panel of other previously sequenced dogs and input these genotypes into Excel. They are provided with the breed weight for the particular breed (either from the Supplementary Material of Sutter *et al*. [10] or extracted from Kennel Club records). From these data, students can get average breed weights of genotype classes. We do this simply using a simple online R script boxplot drawer (http://shiny.chemgrid.org/boxplotr/) though this can be done in any statistical/graphical package. Tests for statistical significance of size differences can be undertaken in any statistical analysis package.

We also provide students with data extracted from Sutter *et al*. [10] in order for them to recreate Figure 4 of this paper since from this they can identify those dog breeds which are outliers for this SNP (i.e. large dog breeds with a high frequency of the A allele, or small dog breeds with a high frequency of the G allele at this SNP).

Students use Ensembl [20] to further study the sequence and the polymorphism in order to find the location of the sequenced fragment and its genomic context (whether it is intergenic, intronic or a coding variant). For this, the PCR product sequence (see Supporting Information) is entered into the BLAST tool of Ensembl and compared to the

C. familiaris genome assembly. Through zooming in, the location of the SNP can then be seen.

RESULTS

Over four academic years, students have genotyped a total of 110 dogs. On average, approximately 90% of PCRs are successful with failures typically related to poor pipetting skills.

Using primer SQ5570F in sequencing reactions the quality of sequencing traces is invariably excellent. Examples of the three genotypes are shown in Figure 2.

Student and staff dogs genotyped to date have ranged from reported pure breeds, through known cross-breeds, to crosses of unknown breeds. Whilst the practical side is not dependent upon knowledge of breed, the data analysis requires their mass (kg). We have used dog breed weight from American Kennel Club and UK Kennel Club data and, where crosses are known, applied a simple (but not necessarily appropriate!) average. The average breed weight of dogs genotyped to date are shown in Figure 3 and compared to a data set from a single year (*N*=28 dogs). In each of the four years that practical has run there has been a clear and significant difference in average breed weight of the AA and GG genotype classes. The dataset for all 110 dogs genotyped over the four years does show a number of outliers. Some of these are mongrels, whilst others are true breed representatives (e.g. a 7kg dachshund with a GG genotype – dachsunds have a mix of the 'small dog' and 'large dog' haplotypes in the study of Sutter *et al*. [10]).

Once students have seen that there is a difference in average size between genotypes then they study the location and nature of the SNP. Using BLAST on Ensembl to identify

the PCR product sequence, the location of the fragment, identity of the SNP, and details of the gene in which the SNP occurs can be clearly seen and further investigated. The PCR product sequence is within intron 2 of the *IGF-1* gene which has two alternative transcripts. Then, through zooming in the SNP can be identified as being on chromosome 15 at position 41,221,438.

Sample size:

How many samples are needed to detect a difference for a typical class? Based on the data generated over the last four years then the sample size needed to detect a difference can be calculated. Using the sample size calculator at http://epitools.ausvet.com.au/content.php?page=2Means2 and inputting the mean and variances of the AA and GG genotypes and their respective frequencies suggests that a sample size of 8 x AA and 4 x GG would be needed to detect a difference with 80% power and 95% confidence (assuming that the dogs sampled within our class data are representative). Since the frequency of AG heterozygotes is approximately equal to the GG genotype indicates that a minimum sample size of 16 is likely needed.

DISCUSSION

Size in dogs is one of the clearest examples of how artificial selection has produced wide variation in phenotypes [9, 10]. Using association studies, Sutter *et al*. [10] identified a SNP at position 41,221,438 of chromosome 15 as strongly associated. In this exercise students can replicate this association; an important part of any association study. The association is strong despite the lack of clear selection criteria on samples utilised. In each of the four years that this practical has run, the association between genotype and dog size has been clear. However, it is useful to accumulate yearly data to have access to larger sample sizes for analyses. Important points for discussion include the following:

Polygenic nature of size variation

Although this one polymorphism clearly explains a substantial proportion of the variance in the size of dog breeds it clearly does not explain everything. This is a major discussion point concerning these data and pointing students towards clear outliers in the data can help (e.g. Rottweilers are very large dogs with a high frequency of the 'small dog' allele – see Figure 4 of [10]). After considering that size in dogs cannot be a monogenic trait (since this SNP does not completely explain size) the students can be introduced to Rimbault *et al*. [9] which explains how six additional SNPs together with the studied SNP explain 50% of the small size phenotype in dogs. That seven SNPs together explain such a large component of a quantitative trait is a very different situation from that seen in human populations where, even for traits with high heritability, it is unusual to identify the causative variants for a significant proportion e.g. just 5% of the genetic variants

explaining variation in human size are known despite ≈80% heritability of this trait [3, 4]. This shows how useful dogs can be for teaching genetics.

Sample selection criteria

With a sufficient class sample size, mean or median sizes from class data can be compared to the published data from Sutter *et al*. [10]. In our class data (*c.f.* the data of Sutter *et al*. [10]) there is higher variance in size within genotype classes and this can form a discussion point. In [10] dog samples had to satisfy clear selection criteria such as kennel club recognition of the studied dog and three generations of its grandparents. Whilst a number of students had pedigree dogs many were not clear pedigrees and some were cross-breeds (in our data the size of these dogs was roughly estimated as the average of the two parental breeds). This will introduce noise into the dataset. The fact that a clear association can be detected without imposing the rigorous selection criteria of Sutter *et al*. [10] shows the strength of the effect.

Dominance

Students might be encouraged to think about whether there is evidence for dominance/recessivity/co-dominance in the class data. Whilst from single year data (Figure 3) the AG genotype appears to have a size intermediate between the AA and GG genotypes, in the data generated across all classes to date there is no significant difference between median size of AG dogs and of GG dogs – suggestive that the G allele is dominant and this fits with the serum IGF levels depicted in Figure 2 of [10] i.e. the SNP may be (in LD with a SNP) regulating gene expression, and hence protein, levels.

Genomic context of the studied SNP

Through examination of the location of the SNP in the dog genome, it can be seen that the SNP is not exonic but instead located in intron 2 of *IGF-1*. Dependent upon the level of the class, there can be discussion of whether this SNP is in linkage disequilibrium with a causative SNP [21]. Alternatively, if this is too advanced it may be more appropriate to link to the concept that DNA outside coding exons can be important, particularly with respect to regulation of gene expression (e.g see [22]).

Reproducibility

In addition to the genetic component in this exercise, it teaches also an important lesson about the conduct of science. That is, that scientific experimentation should be repeatable and this is particularly true of Genome Wide Association Studies where false positives are likely to be generated [23]. At least in our classes, this has definitely proved to be the case and students have been able to follow a scientific protocol on samples generated by themselves, repeat an important research finding published in an acclaimed journal (*Science*).

Broader relevance

In addition, this practical has clear relevance to subjects outwith genetics. Here, this polymorphism is within (an intron of) the insulin-like growth factor gene – the primary mediator of growth hormone and indeed three of the other SNPs implicated in the size phenotype by Rimbault *et al*. [9] are in the *Insulin Growth Factor Receptor* gene and in

the *Growth Hormone Receptor* gene. How IGF and GH mediate differences in skeletal size [24] will have relevance for physiology and anatomy modules helpfully linking genetics to observable phenotype.

If students have covered the basics of molecular genetics and inheritance, and this practical is preceded by an exercise considering the nature of (non-) monogenic inheritance of phenotypic traits in humans, then this coverage of a polygenically controlled phenotype under strong selection is a logical progression. The use of samples of clear relevance and interest to students, and over which they have ownership, increases both interest and learning. Traditional genetics teaching has frequently involved the use of fruit flies [25, 26] organisms whose tractability and clear visibility of genetically determined phenotypes can make them excellent organisms for genetics teaching. Whilst *Drosophila* is an exceptional species for classical transmission genetics it is not necessarily the most appropriate species for the teaching of the molecular basis of quantitative traits. It is also the case that *Drosophila* are unlikely to be the most popular organisms for students. The use of more familiar animals with more direct relevance to students' experience (i.e. pets) could be expected to increase engagement, enjoyment and learning, since provision of course materials with relevance to students' lives has been demonstrated to increase both motivation and learning [27]. However, in order for such species to be utilisable for genetics teaching the organisms must have easily studied, genetically controlled phenotypic variation, be readily available for sampling, and be easily and safely sampled. The ultimate personal involvement, and hence engagement, would involve sequencing of students' personal genomes. This has indeed been conducted in student classes to good effect [28]. However, ethical issues

pervade such efforts [28-33]. This practical, on pet dogs provides an alternative of clear relevance and interest to students. The study of pets has been undertaken to demonstrable good effect with phenotypic data used in the study of dominance etc. [34, 35] however, to my knowledge, there is no exercise involving molecular genetic testing. Students have indicated that this practical is very popular, dealing as it does with their own pet's samples. Some of the comments from student feedback demonstrate that this practical stimulates their interest and promotes learning. e.g. from end of module questionnaires over 90% of students state that these laboratory and computer practical sessions have contributed to their learning.

Whilst size is an excellent phenotype for the analysis of genetics of a quantitative trait, it would also be possible to develop other simple genetic tests to look at phenotypes. For example the genetics of, and underlying polymorphisms controlling, coat colour in dogs are known [36] and therefore these could be used for genetic testing. Hence, dogs are an excellent study system for students to link molecular genetics to transmission genetics.

ACKNOWLEDGEMENTS

I thank all the students who participated in this practical, especially those bringing in samples from their own dogs and my colleagues who teach on this module.

REFERENCES

1. Yoshiura, K.-I., et al., *A SNP in the ABCC11 gene is the determinant of human earwax type.* Nature Genetics, 2006. **38**(3): p. 324-330.
2. McDonald, J.H. *Myths of human genetics*. Available from: http://udel.edu/~mcdonald/mythintro.html.
3. Yang, J., et al., *Common SNPs explain a large proportion of the heritability for human height.* Nat Genet, 2010. **42**(7): p. 565-569.
4. Frazer, K.A., et al., *Human genetic variation and its contribution to complex traits.* Nat Rev Genet, 2009. **10**(4): p. 241-251.
5. Beale, H. and E.A. Ostrander, *Sizing up dogs.* Current Biology, 2012. **22**(9): p. R315-R316.
6. Boyko, A.R., *The domestic dog: man's best friend in the genomic era.* Genome Biology, 2011. **12**(2): p. 216.
7. Neff, M.W. and J. Rine, *A fetching model organism.* Cell, 2006. **124**(2): p. 229-231.
8. Parker, H.G. and E.A. Ostrander, *Canine genomics and genetics: running with the pack.* PLoS Genetics, 2005. **1**(5): p. e58.
9. Rimbault, M., et al., *Derived variants at six genes explain nearly half of size reduction in dog breeds.* Genome Research, 2013. **23**(12): p. 1985-1995.
10. Sutter, N.B., et al., *A single IGF1 allele is a major determinant of small size in dogs.* Science, 2007. **316**(5821): p. 112-115.
11. Chase, K., et al., *Genetic basis for systems of skeletal quantitative traits: Principal component analysis of the canid skeleton.* Proceedings of the National Academy of Sciences USA, 2002. **99**(15): p. 9930-9935.
12. Sivan, A., et al., *An implementation of active learning and its effect on the quality of student learning.* Innovations in Education & Training International, 2000. **37**(4): p. 381-389.
13. Freeman, S., et al., *Active learning increases student performance in science, engineering, and mathematics.* Proceedings of the National Academy of Sciences, 2014. **111**(23): p. 8410-8415.
14. Redfield, R.J., *"Why Do We Have to Learn This Stuff?"- A New Genetics for 21st Century Students.* PLoS Biol, 2012. **10**(7): p. e1001356.
15. Vaux, D.L., F. Fidler, and G. Cumming, *Replicates and repeats—what is the difference and is it significant?* A brief discussion of statistics and experimental design, 2012. **13**(4): p. 291-296.
16. Chanock, S.J., et al., *Replicating genotype–phenotype associations.* Nature, 2007. **447**(7145): p. 655-660.
17. Learn Genetics. *Observable Human Characteristics*. Available from: http://learn.genetics.utah.edu/content/basics/observable/.
18. Sánchez-Vizcaíno, F., et al., *Demographics of dogs, cats, and rabbits attending veterinary practices in Great Britain as recorded in their electronic health records.* BMC Veterinary Research, 2017. **13**(1): p. 218.
19. Hoeppner, M.P., et al., *An improved canine genome and a comprehensive catalogue of coding genes and non-coding transcripts.* PLoS ONE, 2014. **9**(3): p. e91172.
20. Aken, B.L., et al., *Ensembl 2017.* Nucleic Acids Research, 2017. **45**(D1): p. D635-D642.
21. Hirschhorn, J.N. and M.J. Daly, *Genome-wide association studies for common diseases and complex traits.* Nature Reviews Genetics, 2005. **6**(2): p. 95-108.
22. Cooper, D.N., *Functional intronic polymorphisms: buried treasure awaiting discovery within our genes.* Human Genomics, 2010. **4**(5): p. 284.

23. NCI-NHGRI Working Group on Replication in Association Studies, *Replicating genotype–phenotype associations.* Nature, 2007. **447**(7145): p. 655-660.
24. Giustina, A., G. Mazziotti, and E. Canalis, *Growth hormone, insulin-like growth factors, and the skeleton.* Endocrine Reviews, 2008. **29**(5): p. 535-559.
25. Jennings, B.H., *Drosophila – a versatile model in biology & medicine.* Materials Today, 2011. **14**(5): p. 190-195.
26. Sofer, W. and L. Tompkins, *Drosophila genetics in the classroom.* Genetics, 1994. **136**(1): p. 417-422.
27. Hulleman, C.S. and J.M. Harackiewicz, *Promoting interest and performance in high school science classes.* Science, 2009. **326**(5958): p. 1410-1412.
28. Salari, K., et al., *Evidence that personal genome testing enhances student learning in a course on genomics and personalized medicine.* PLoS ONE, 2013. **8**(7): p. e68853.
29. Garber, K.B., K.M. Hyland, and S. Dasgupta, *Participatory genomic testing as an educational experience.* Trends in Genetics, 2016. **32**(6): p. 317-320.
30. Callier, S.L., *Swabbing students: should universities be allowed to facilitate educational DNA testing?* The American Journal of Bioethics, 2012. **12**(4): p. 32-40.
31. Daley, L.-A.A., et al., *Personal DNA testing in college classrooms: perspectives of students and professors.* Genetic Testing and Molecular Biomarkers, 2013. **17**(6): p. 446-452.
32. Taylor, A.T.S. and J.C. Rogers, *The ethical implications of genetic testing in the classroom.* Biochemistry and Molecular Biology Education, 2011. **39**(4): p. 253-260.
33. Vernez, S.L., et al., *Personal genome testing in medical education: student experiences with genotyping in the classroom.* Genome Medicine, 2013. **5**(3): p. 1-8.
34. Christensen, A.C., *Cats as an aid to teaching genetics.* Genetics, 2000. **155**(3): p. 999-1004.
35. Kalinowski, S.T., et al., *Six classroom exercises to teach natural selection to undergraduate biology students.* CBE-Life Sciences Education, 2013. **12**(3): p. 483-493.
36. Schmutz, S.M. and Y. Melekhovets, *Coat color DNA testing in dogs: theory meets practice.* Molecular and Cellular Probes, 2012. **26**(6): p. 238-242.

Figure 1. Mouth swabs are simple to take from pet dogs. Here, swabbing of a border terrier showing which part of the mouth to swab.

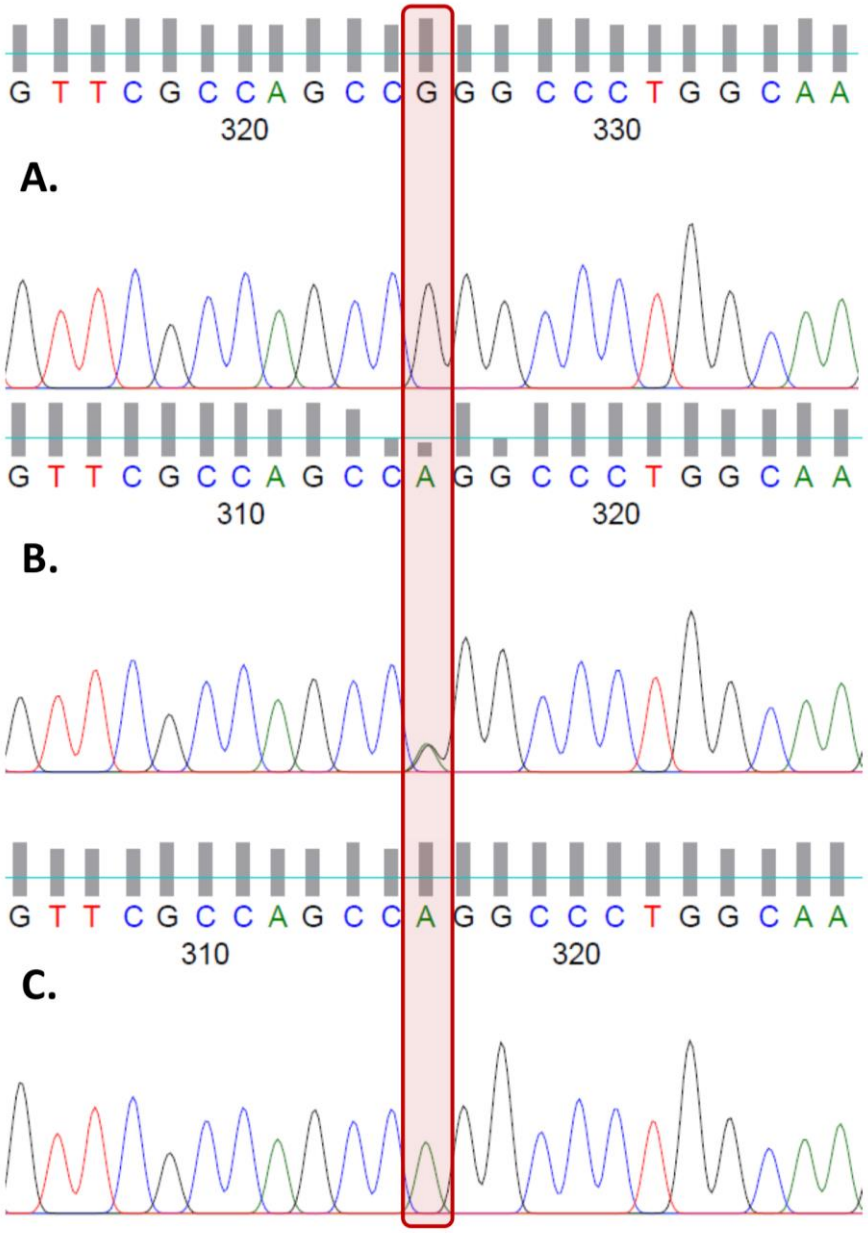

Figure 2. Representative sequence chromatograms from three dogs depicting the three potential genotypes at the SNP on chromosome 15 at position 41,221,438 (only this section of the chromatogram is selected here and aligned across the three representative samples). **A**. GG genotype of Northern Inuit dog. **B**. AG genotype of Labrador (note green A peak and black G peak at the highlighted position). **C**. AA genotype of Yorkshire Terrier.

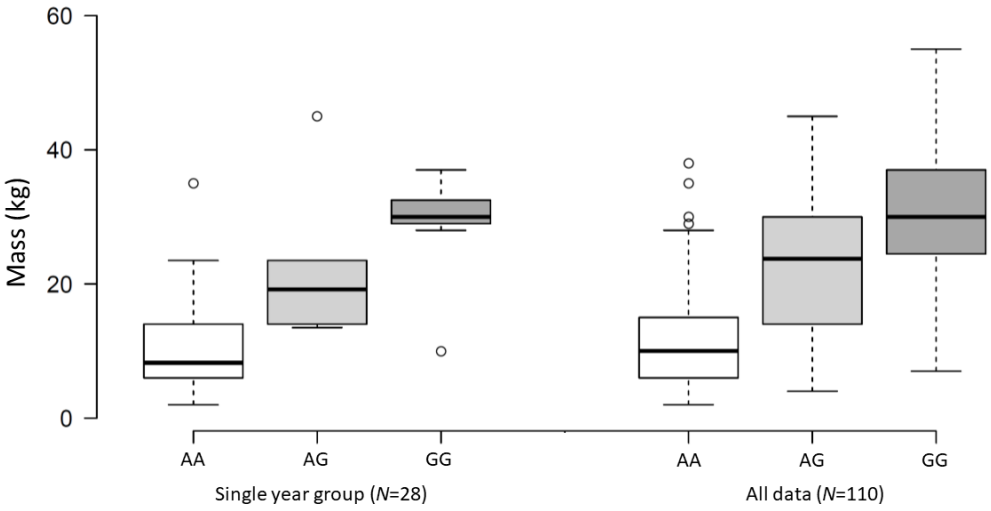

Figure 3. Boxplot of dog size (mass – kg) by genotype from data generated in one single class (AA *N*=14; AG *N*=6; GG *N*=8) and across all four years in which the practical has run (AA *N*=59; AG *N*=24; GG *N*=27). Centre lines show the medians; box limits indicate the 25th and 75th percentiles as determined by R software; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles, outliers are represented by dots. Drawn at http://shiny.chemgrid.org/boxplotr/.