

Fetching SNPs: A Dog Genotyping Laboratory for Undergraduate Biology—Student Manual

Introduction

Recently the dog, *Canis lupus familiaris*, has emerged as a model organism for studying human genetic disease. The breeding of dogs over thousands of years has led to the fixation of many desired traits including the size and shape of the dog, length and color of their coat, even complex hunting behaviors like retrieving or pointing. Unfortunately, inbreeding within dog breeds also increases homozygosity including many recessive disease loci. Some of these canine genetic diseases are very similar to human genetic disorders (1, 2). Dogs have a degenerative spinal cord condition similar to Lou Gehrig's disease, for example, and both disorders may be due to mutations in the same gene, called SOD1 (3). Genetic studies in dogs is much easier than in humans: it is easier to identify parental alleles due to their homozygosity, and we can control their breeding in ways that is unethical and problematic in humans. The mapping and studying of genetic diseases in dogs may therefore lead to a wealth of information regarding these shared disorders. After the sequencing of the dog genome in 2005 (4), several studies have identified genetic regions that are associated with particular dog traits (5).

Our class will be examining several of these genetic markers linked to traits using a genotyping method called Derived Cleaved Amplified Polymorphic Sequences, or dCAPS (6). This method will allow us to identify the genotype of 7 Single Nucleotide Polymorphisms (SNPs) in the dog genome within a sample of dogs that our class will collect. These markers are referred to as Dog SNPs 1-7 in this exercise, or DS1-7 for short. We will explore several different questions relating to how the genotype of the dogs, as defined by which alleles of each SNP they carry in their DNA, relates to their expressed phenotype, defined by their visual traits. We will also learn the practical applications for genotyping as a way to identify individuals and to identify parent-offspring relationships. The entire project will be split up into several labs and will cover a handful of methods we have discussed in class including DNA extraction, PCR, endonuclease restriction digests, and gel electrophoresis.

Before the laboratory portion can begin, the class must collect DNA samples from dogs. Students can sample from their own pet or seek out interesting breeds. Because several of the markers are associated with size, you might want to find a few samples from both large breeds and small breeds. Your instructor will ask you if you know of a potential candidate and the class will decide how many dogs and which dogs should be used for the project. Your instructor will also present the class with one or more scenarios involving genotyping and ask that you formulate hypotheses.

After you have finished the project and analyzed the results, you will prepare a short "genetic report" to give to one of the volunteer dog owners. This report will summarize the methods and conclusions you reached at the end of the lab.

Lab 1: Genomic DNA purification from buccal swab using the QIAamp DNA Mini Kit

After buccal (cheek cell) swabs have been obtained, your instructor will distribute the samples for the class. Follow the steps below to purify genomic DNA from each of your designated samples. These steps were adapted from the QIAamp DNA Mini kit from Qiagen(7). Dogs will be labeled by letters, so label your tubes according to which samples you are handling.

1. Obtain a buccal swab in a 1.5 ml microcentrifuge tube and pipette 400 μ l 1 X PBS to the sample.

Lyse cells

2. Add 20 μ l QIAGEN Protease stock solution followed by 400 μ l of Buffer AL to the sample. Mix immediately by vortexing for 15 s.
3. Incubate in the 56°C water bath for 10 min.

Precipitate DNA to collect on spin column

4. Briefly centrifuge to remove drops from inside the lid, then add 400 μ l of 100% ethanol to the sample, and mix again by vortexing.
5. Briefly centrifuge to remove drops from inside the lid. Carefully pipette 700 μ l of the mixture from step 4 to the QIAamp Mini spin column (placed in a 2 ml collection tube). Close the cap, and centrifuge at 6000 x g for 1 min. During centrifugation steps, cap each spin column to avoid aerosol formation. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided in kit), and discard the tube containing the filtrate.
6. Repeat step 5 by applying up to 700 μ l of the remaining mixture from step 4 to the QIAamp Mini spin column.

Wash DNA on spin column

7. Carefully open the QIAamp Mini spin column and add 500 μ l Buffer AW1, without wetting the rim. Close the cap and centrifuge at 6000 x g for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.
8. Carefully open the QIAamp Mini spin column and add 500 μ l Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g) for 3 min.
9. Place the QIAamp Mini spin column in a clean 1.5 ml collection tube (not provided by kit)

and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min. This step helps to eliminate the chance of possible Buffer AW2 carryover.

Sample elution and collection

10. Place the QIAamp Mini spin column in a clean 1.5 ml microfuge tube labeled with the dog ID# on the top of the cap and your initials on the side of the tube. Discard the collection tube containing the filtrate. Carefully open the QIAamp Mini spin column and add 150 μ l Buffer AE. Incubate at room temperature for 1 min, and then centrifuge at 6000 x g for 1 min.

Check the quantity and quality of sample

Your instructor may ask you to check your sample(s) using a spectrophotometer. Determine the concentration and absorbance ratios (A_{260} / A_{280}) according to your instructor. Write the concentration on the side of your microfuge tube and record the values in your notebook. Your instructor will also tell you how to store your DNA for the next lab.

According to the QIAamp manual, one buccal swab will yield 0.5–3.5 μ g of genomic DNA in 150 μ l of buffer (3–23 ng/ μ l). Expected absorbance ratios from this kit are between 1.7 and 1.9. If your absorbance ratios are different, you may have protein or wash buffer contaminating your sample. Consult with your instructor and classmates regarding which samples are acceptable for using in Lab 2.

Lab 2: PCR of SNP markers

We will be running a genotyping reaction for each dog and two control PCR reactions for each SNP marker. The no template control reaction will tell us whether there is contamination of dog DNA or even human DNA in the reaction. The genotyping reactions will give us a product that will be cut by a restriction enzyme for only one allele of the SNP. This way we will be able to determine which allele or alleles are present in each of the dog DNA samples by whether it shows either the large, small, or both large and small alleles after digestion. But how will we know if a large allele is a result of not having a restriction site in the PCR product or whether our restriction enzyme is bad? That is the reason for including a positive control reaction. This reaction will produce a PCR product that will contain the restriction site regardless of which allele is present in the dog. This control therefore serves two purposes: 1. To make sure that the restriction digest worked, and 2. To create a band on our genotyping gel so that we can compare our genotyping reaction to a cut version of the allele. It is only necessary to have a single no-template control and a single positive control reaction for each SNP marker, even if you are genotyping many dogs. Choose the dog sample that had the highest quality genomic DNA for both the positive and negative controls.

Shown below is the recipe for making the PCR reactions for one SNP marker. Your instructor will tell you which SNP markers your group will be responsible for and how many dog DNA samples you will be running.

Table 1, QIAGEN PCR Master Mix recipe for 25 µl reactions.

Positive Control Rxn (µl)	No template Rxn (µl)	Genotyping Rxn (µl)	Genotyping Rxn Combined Mix for N dogs	Ingredient
5	0	5	-	Genomic Template (10 ng/µl)
12.5	12.5	12.5	12.5(N+1*)	Taq PCR Master Mix
6.5	11.5	6.5	6.5(N+1*)	Water
-	0.5	0.5	0.5(N+1*)	Forward Genotyping Primer (10 µM)
0.5	-	-	-	Forward Positive Control Primer (10 µM)
0.5	0.5	0.5	0.5(N+1*)	Reverse Primer (10 µM)

* Ensure there is at least 10% extra Reaction Mix for pipetting error. For example, if the number of dogs, N, is between 10 and 20, multiply the component volumes by N+2 to ensure there is enough for all of your reactions.

For each DS SNP marker:

1. Mix together the ingredients for a Positive control reaction into a PCR tube. Your instructor will tell you which dog sample to use for the template. Label tube with DS# P, for example: "DS1-P"
2. Mix together the ingredients for a Negative control reaction. Use the same dog sample you used for the positive control reaction. Label the tube DS# NC (for Dog SNP number Negative Control), for example "DS1-NC"
3. Aliquot 5 μ l of Dog DNA for each of your genotyping reactions into its own PCR tube, label with the Dog SNP number (DS#) and dog letter ID. For example "DS1-A"
4. Add 20 μ l of Genotyping Combined Rxn Mix to each genotyping reaction.

An example of a PCR setup for genotyping 5 dogs (A-E) with 2 SNP markers (DS1 and DS2):

DS1 SNP Marker

Tube	label
1	DS1-PC
2	DS1-NC
3	DS1-A
4	DS1-B
5	DS1-C
6	DS1-D
7	DS1-E

DS2 SNP Marker

Tube	label
8	DS2-PC
9	DS2-NC
10	DS2-A
11	DS2-B
12	DS2-C
13	DS2-D
14	DS2-E

After you put together the PCR reactions, set them on ice until the class is ready to place all of the reactions in the thermocycler.

The PCR program for the thermocycler is as follows:

Step 1	96°C	3'	Initial Melt
Step 2	94°C	30"	Cycle Melt
Step 3	55°C	30"	Anneal

Step 4	68°C	30"	Elongation
Step 5	Go to Step 2 for 35 cycles		
Step 6	68°C	3'	Final elongation
Step 7	4°C	<input type="checkbox"/>	Hold

Lab Question 1: *Why might we choose to have a lower annealing temperature for our dCAPS primers than a standard PCR reaction? Hint: draw the duplex DNA strand that is created when our primers will anneal to the template strand.*

Lab 2.5: RECOMMENDED STEP - Confirm PCR amplification

Your instructor may ask you to run a gel of the PCR reactions before moving on to the restriction digest. Follow instructions given by your instructor to make a 1% agarose gel in TBE. Load 5 μ l of your PCR reactions along with a 100 bp marker. After running your gel for 30-60 minutes at 100V, visualize your samples.

1. Confirm that you can see PCR products for the positive control reaction and your genotyping reactions. If any PCR products are not visible, notify the instructor.
2. Check the size of your products and record all band sizes in your notebook. You can check expected size of the uncut PCR reactions in Table 3, Dog Genotyping Results
3. Confirm that there is an absence of bands in the no-template negative control reaction.
4. It may be necessary to re-run the PCR using different parameters or with more or less DNA as template, depending on how successful your PCR reactions were. Your instructor will help you determine the necessary changes, if any.

Lab 3: Restriction digest of dCAPS PCR products

After confirming the PCR reactions worked, we will run a restriction digest that will let us distinguish between the two alleles of each SNP.

Pre-lab preparation

1. Refer to Table 3 to identify the restriction enzyme(s) your group will use for your assigned SNP markers.
2. Each enzyme has different optimal conditions and recipes for digestion. You should read about these conditions for your enzymes using the NEB catalog, the NEB website (www.NEB.com), or the NEB mobile App. Specifically, we must know which NEB buffer to use, whether we need to add BSA or SAM, and the incubation temperature.

Table 2, General Recipe for Digests.

10 μ l	PCR product
2 μ l	10x NEB Enzyme Buffer (See NEB catalog)
2 μ l	10x BSA or SAM (if recommended)
5 Units	Restriction enzyme
up to 20 μ l	Water

3. Write down your specific recipe for each enzyme/SNP combo for your group in your lab notebook.

In-lab procedure

You must set up your reactions according to your specific recipe. Don't forget to digest the positive control (DS1-P, DS2-P for example) PCR reactions.

Example enzymatic digest set up for two DS markers:

DS1 SNP Marker

- | | |
|------|-------|
| Tube | label |
| 1 | DS1-P |
| 2 | DS1-A |
| 3 | DS1-B |
| 4 | DS1-C |
| 5 | DS1-D |
| 6 | DS1-E |

DS2 SNP Marker

Tube	label
7	DS2-P
8	DS2-A
9	DS2-B
10	DS2-C
11	DS2-D
12	DS2-E

Note: you do not need to digest the no-template negative control reactions. If you have already run the negative control samples on the gel described in lab 2.5, and confirmed an absence of product, those control samples are no longer needed. Alternatively, you can run the undigested negative control samples in the genotyping gel described later.

Incubate your digestion reactions for 2 hours at the appropriate temperature. See the NEB catalog or website for information on your enzyme

Lab Question 2: *We are setting up these reactions for a complete digestion (no cleavable product remains after our incubation). Why is this important for our experiment? How would it hinder our analysis if only 50% of cleavable products were digested?*

During the digestion incubation, set up your gel apparatus and pour your genotyping gel as described in the next section.

Genotyping electrophoresis gel and analysis of dCAPS markers

In order to distinguish between the cut and uncut products, which are only different by ~20bp, we will need to run a high percentage (2.5%) agarose gel. Below are instructions for making 100ml of agarose gel in TBE buffer. Scale up or down according to the volume of your gel trays and the number of gels you will need.

100 ml	1x TBE Buffer
2.5 g	Agarose (Hi Res if available)
	Ethidium bromide
	Gel Loading Dye, Orange (5x)

1. Mix the TBE buffer and agarose in a 250 ml Erlenmeyer flask.
2. Microwave for approximately 1 minute, or just before boiling. Swirl to mix.
3. Allow to cool to the touch before adding Ethidium bromide to a final concentration of 0.5 μ g / ml.
4. Pour the gel and insert the appropriate size comb.
5. When the gel has solidified, load the gel in the following in order:

Lane	5x loading dye Vol (µl)	Sample Vol (µl)	Sample
1	-	5	100 bp Marker
2	3	12.5	Positive control cut (digested from lab 3)
3	1.5	5	Uncut PCR from dogA (undigested PCR from lab 2)
4	3	12.5	Cut PCR from dogA (digested from lab 3)
5	3	12.5	Cut PCR from dogB (digested from lab 3)
6	3	12.5	Cut PCR from dogC (digested from lab 3)
...			...etc.
N	1.5	5	No template control (undigested PCR from lab 2)

- Repeat this order for each SNP marker your group is responsible for.
- Run the gels at 80 Volts for 1 hour, or until the orange dye front has reached near the end of the gel (~90%). Do not let the dye migrate off the gel, as you could lose your products.
- Turn off the electrophoresis power and remove your gel from the tray.
- Visualize with UV light and photograph your results. Each student should place a copy of the photo in his or her notebook for analysis. Give your instructor a copy to share with the rest of the class.

The entire class will now use the gel images to genotype each dog at every SNP marker.

Table 3, Trait Associations for DS markers and Allele Sizes.

DS#	Enzyme	SNP	Trait Associations	Uncut Allele		Cut Allele	
				Allele	Size	Allele	Size
1	BamHI	C/T	C - Low Weight, Short height	T	162	C	142
2	BamHI	C/G	C - Long coat	G	220	C	200
3	EcoRI	C/T	A - Low Snout ratio	T	152	C	129
4	EcoRI	C/T	A - High Snout ratio	T	119	C	105
5	PstI	G/A	A - High Weight, Tall height	A	134	G	114
6	PstI	G/A	A - Low Snout ratio	G	136	A	110
7	Sall	C/T	T - Low Weight	T	186	G	166

Recreate Table 4 below in your lab notebook; adding notes for any reactions that did not work or you are unable to resolve completely. Your instructor will show the gel images for the entire class on an overhead projector. Expand the table for the number of dog samples your class is analyzing. After you have determined the genotype at each locus, discuss your results with your classmates to check your work.

Table 4, Dog Genotyping Results

SNP	Positive control		Uncut		DogA		DogB	
	Exp	Obs	Exp	Obs	Bands present	Geno-type	Bands present	Geno-type
DS1	142		162					
DS2	200		220					
DS3	129		152					
DS4	105		119					
DS5	114		134					
DS6	110		136					
DS7	166		186					

Analysis and Lab Report of Genotype Data

Once you have determined the genotype at each locus, you can evaluate your initial hypotheses and answer any questions your instructor has proposed. You should write your conclusions to these in your lab notebook using proper scientific language.

In addition each student will also write a short “genetic report” to give to one of the dog volunteers. They will be interested to find out what genetic markers their dog has, and what we have learned about them. Write this report using language for a non-expert. Use the following structure:

Introduce the question. Explain the questions our class tried to answer. You can make this specific to the dog. For example, if the volunteer was a Great Dane you might tell them that you were interested if they had all of the known markers for large size.

Describe the methods we used to genotype their dog. Remember to explain methods like PCR as best you can to a lay audience. This should be a concise but descriptive paragraph. Tell them the results for their dog and what their genotype might tell us. Did their dog’s sample help to confirm our hypothesis? Were there any surprising results from their sample?

Finally, thank them for their participation. You should also tell them that these markers are not associated with any known disease and that these results do not raise health concerns.

Supplemental dCAPS design protocol

1. Open the DogSNP.xls document (modified from Supplemental Table 4 from Jones, 2008) in Excel and familiarize yourself with the information in the file. We will be using the Bio437 Name column (DS_# for Dog SNP #) to keep track of the different SNPs rather than the actual name (column B). LG (column C) refers to the Linkage Group or chromosome that the SNP is on. SNP (column D) refers to the polymorphism, DS_1 for example has SNP A/G, meaning that this SNP is either an A or a G. The phenotypic trait that each SNP is associated with is shown under Trait (column E). The allele associated with the trait and the direction of association is listed under allele (column F). For further information on these SNPs, see the Jones 2008 paper in the Dog Genotyping Folder. The sequence that surrounds the SNP is listed under sequence (column G). After this are several empty fields that you will fill in, more on these later.
2. To design the dCAPS diagnostic PCR primers we will be using the website:
<http://helix.wustl.edu/dcaps/dcaps.html>

This website will search through all of the known restriction endonuclease recognition sites and will find those that will selectively digest our amplified products. However, this website requires a specific format for the input. Depending on the source of your SNP data, you may need to convert your sequences.

The helix format is below. I have indicated the SNP nucleotide in **bold**, however this is only for clarity as the helix website will ignore formatting. Importantly, each SNP sequence includes up to 30 nucleotides flanking upstream and downstream:

Wild Type:

TTATGTTACTTAGAGAAAAATGAAAA**A**TATTCTTGAAAAGAGGCCCAATAAAGT

Mutant:

TTATGTTACTTAGAGAAAAATGAAAA**G**TATTCTTGAAAAGAGGCCCAATAAAGT

This format must be less than 60 total characters and the only difference between Wild Type and Mutant must be the SNP. Get rid of any other non-[ATCG] characters such as “[”, “/” or “]” that may be in your sequence.

Please note: although helix calls one haplotype “Wild Type” and the other “Mutant,” we will just use the first letter given in the sequence of the SNP as the “Wild Type” and the second

as the "Mutant." In the case above, "A" is "Wild Type" and "G" is "Mutant". You will see how we will keep track of this from the helix output file.

3. When you are done formatting all of your sequences, open up the <http://helix.wustl.edu/dcaps/dcaps.html> website.
4. Enter in the formatted sequences of your SNPs into the Wild Type and Mutant fields.
5. Enter 0 into the "How many mismatches in the primer?" field and click submit. This should open a new tab or window with the output information. Using 0 will tell us whether the standard CAPs (i.e. not derived) method can be used on this SNP, allowing us to use a non-mutagenic primer.
6. Go back to the input page and enter 1 into the "How many mismatches in the primer?" field and click submit.
7. Go back to the input page and enter 2 into the "How many mismatches in the primer?" field and click submit.
8. You can now look at all three output pages to determine which primer and enzyme to use.

To determine which enzyme and primer to use, there are no hard and fast rules. However, we would like to limit (1) the number of enzymes we will use, and have to buy, and (2) the number of mutations introduced by each SNP primer. Limiting the number of enzymes is more important than limiting the number of mutations, so if you find a common enzyme that will cut using two mutations we will prefer this to an uncommon enzyme that we don't have in the lab that will cut with 0 or 1 mutations. Consult with your partner and your instructor or TA if you need help.

9. Once you have decided which primer and enzyme you will use for the SNP, record all of the information in a text file or spreadsheet.
 - a. Copy the dCAPS primer you choose and paste it into the xls file. If it is a Forward primer, paste it into the forward primer column and enter "forward" into the Cuts (primer) cell (column L).
 - b. Enter the enzyme you chose with this primer and enter it into the Enzyme field (column J).
 - c. Enter the SNP that will be cut using this primer and enzyme into the Cuts (SNP) field (column K).

10. Repeat the above steps for each one of your assigned SNPs. Please make sure you record everything you do in your notebook, making sure to reference any saved files in your folder.
11. Design the second primer that we will use to amplify the SNP. Copy the entire template sequence into the Primer3 website <http://frodo.wi.mit.edu/>
 - a. Remove the SNP brackets and replace the SNP with a single nucleotide.
12. Find a primer that will amplify a 100-200 bp region along with the mutating dCAPS primer. There are several ways to do this:
 - a. You can enter one primer sequence in, and have the program find a second primer that will amplify a given length. Note that you will not be able to enter in the dCAPS primer in Primer3, as it will not allow you to have mismatches in the primer sequence. You can, however, replace the mismatch nucleotides of the dCAPS primer with nucleotides that exactly match the template sequence.
 - b. You can use brackets in the template sequence to force the program to include that region. If you bracket from the SNP to 100 bp away in the direction you need (if your dCAPS primer is reverse, and you need a forward primer: bracket 100 bp to the left) the program will design a primer that is at least 100 bp away from your dCAPS primer.
13. Once you have designed the second primer, enter that sequence into your TextWrangler file and the DogSNPs.xls file in column H or I.
14. Calculate the size of the PCR product prior to digestion and enter it into the Size 1 field.
15. Calculate the size of the successfully digested SNP and enter that into Size 2 field.
16. Recheck everything! Make sure that your primers will indeed amplify both SNPs. Check whether only one will be cut with the enzyme that you entered in the Enzyme field. Make sure everything is entered correctly.

Table 5, Primer Sequences and Melting Temperatures for DS SNP Markers

F: forward primer, P: positive control forward primer, R: reverse primer used with either forward primer or positive control primer. The bold nucleotides in primer sequence are mutagenic.

Primer Name	Sequence	Tm
DS1-F	GCCTTGCCTAAATGTAGT GGATC	55
DS1-P	GCCTTGCCTAAATGTAGT GGATCC	57
DS1-R	AAACCCAGAAGGGGAGAAGA	56
DS2-F	AAAGCCTTGGAACTCGGAT	54
DS2-P	AAAGCCTTGGAACTCGGAT C	55
DS2-R	GGAAAGGGTGGACTGTGAGA	57
DS3-F	GAATTACAGTAACCACAGTGGAGAATT	55
DS3-P	GAATTACAGTAACCACAGTGGAGAAT TC	56
DS3-R	CATCCTGGCCAACAGAACT	55
DS4-F	GGGGCCATCAGAGGAATT	55
DS4-P	GGGGCCATCAGAGGAAT TC	56
DS4-R	CTGGGTGGGAGAAGAGACTG	57
DS5-F	GGAAGTGTATCACAGAGTCTGCA	56
DS5-P	GGAAGTGTATCACAGAGTCTG CAG	57
DS5-R	CCGAGTGGCTCTATTTTTCC	53
DS6-F	AAAACACTCTAGAGAAGCTAGTTCTG C	58
DS6-P	AAAACACTCTAGAGAAGCTAGTTCTG C A	59
DS6-R	GGGAAGCTTTTCTCCTACAGC	56
DS7-F	TTAAAGCAGCTACATTTGTG T CGA	55
DS7-P	TTAAAGCAGCTACATTTGTG T CGA C	56
DS7-R	CTGACTCCTGCTCCCATCTC	57

Table 6, SNP Sequence in Dog Genome for DS Markers

LG: Linkage Group (Chromosome) location of SNP marker. SNP is in brackets.

DS SNP Marker	LG	Genomic sequence of SNP
DS1	10	GCCTTGCCTAAATGTAGTCGATA[C/T]ATAAAAACATAAGAATCATATTTA
DS2	25	AAAGCCTTGGAACTCGGGT[C/G]CCATGCACAATTCTTCCTTTATTCCTGAA
DS3	1	GAATTACAGTAACCACAGTGGAGA ACT [C/T]TGAAACCAGATTTGTTTACCC
DS4	9	GGGGCCATCAGAGGAAAT[C/T]GGGAAGGGAGGCTCTCCATAGACCATCTCT
DS5	10	GGAAGTGTATCACAGAGTCTGAA[G/A]GCTGGACTTTTCAGACAGTCATCCT

DS6	17	AAAAC TCACTCTAGAGAAGCTAGTTCTCC[G/A]GAAGTAAATCTCCAGTTTG
DS7	6	TTAAAGCAGCTACATTTGTGGTGA[C/T]TTGTGTTATATAGTGAGAGAAAAG

References

1. J. M. Howell *et al.*, Use of the dog model for Duchenne muscular dystrophy in gene therapy trials. *Neuromuscul. Disord.* **7**, 325–328 (1997).
2. G. M. Acland *et al.*, Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet* **28**, 92–95 (2001).
3. T. Awano *et al.*, Genome-wide association analysis reveals a SOD1 mutation in canine degenerative myelopathy that resembles amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA* **106**, 2794–2799 (2009).
4. K. Lindblad-Toh *et al.*, Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature* **438**, 803–819 (2005).
5. P. Jones *et al.*, Single-nucleotide-polymorphism-based association mapping of dog stereotypes. *Genetics* **179**, 1033–1044 (2008).
6. M. M. Neff, J. D. Neff, J. Chory, A. E. Pepper, dCAPS, a simple technique for the genetic analysis of single nucleotide polymorphisms: experimental applications in *Arabidopsis thaliana* genetics. *Plant J* **14**, 387–392 (1998).
7. *QIAamp DNA Mini and Blood Mini Handbook* (Qiagen, ed. 3, 2012), pp. 36–38.