

Case Study

Saliva as an alternative source of high yield canine genomic DNA for genotyping studies

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About the study

The objective of the study was to compare the yield and quality of DNA obtained from matched buccal swab, blood and Oragene®•ANIMAL/saliva samples. In addition, the performance of the samples was assessed for use on PCR-based downstream applications.

Samples were collected from 15 dogs with matched saliva and buccal swab samples from 12 of the dogs and matched saliva, blood and buccal swab samples from 3 of the dogs. Oragene•ANIMAL/saliva samples were collected by study authors or dog owners using the Oragene•ANIMAL kit while buccal swabs were collected using the Isohelix T swabs. Blood was collected in EDTA tubes by a licensed veterinarian or veterinary technician.

Saliva was purified using the Oragene•ANIMAL reagent, blood was purified using the Qiagen DNeasy blood and tissue kit and buccal swabs were pooled and purified using the Qiagen QIAmp DNA mini kit.

The DNA was then used for PCR of a 1.1-kb genomic DNA fragment and SNP genotyping by PCR-RFLP.

Main challenges

Successful implementation of studies that use canine samples is dependent on the quality of the samples that can be obtained and the number of samples the researcher has access to. It can be difficult to maximize the number of samples collected when blood is the required sample source. As a result, non-invasive DNA collection methods are preferred in order to increase study participation and compliance.

Collection methods considered

The quality and quantity of DNA from blood, buccal swabs and Oragene•ANIMAL/saliva samples were assessed.

“We demonstrate that DNA yields from canine saliva are higher than those from blood or buccal swabs. The quality of DNA extracted from saliva is sufficient for successful amplification of a 1.1-kb fragment and for accurate SNP genotyping by PCR-RFLP. We conclude that saliva presents a non-invasive alternative source of high quantities of canine genomic DNA suitable for genotyping studies.”

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Results summary

The comparison demonstrated that the yield obtained with Oragene•ANIMAL/saliva samples was significantly higher than that obtained from the matched blood ($p=0.0198$) and buccal swab ($p=0.0008$) samples that were collected. The quality of DNA from the blood and Oragene•ANIMAL/saliva samples was also greater as determined by electrophoretic analysis. In addition, a 1.1-kb PCR fragment was successfully amplified using the paired DNA samples and genotyping by PCR-RFLP yielded identical results. The researcher concluded that Oragene•ANIMAL/saliva samples present a non-invasive alternative source of high quantities of canine genomic DNA that is suitable for genotyping studies.

The following table is an excerpt from the researchers publication: Saliva as an alternative source of high yield canine genomic DNA for genotyping studies. *BMC Research Notes* 2009, 2:219, Katherine Mitsouras and Erica A Faulhaber.

Table 1 - Comparison of the total DNA yields by collection method

	Total DNA Yield (μg)			Fold	Fold
	Oragene® •ANIMAL	Buccal Swabs	Whole Blood	Difference (Oragene® •ANIMAL / Buccal)	Difference (Oragene® •ANIMAL / Blood)
Dog 1	27.68	1.32	3.04	20.97	9.10
Dog 2	22.28	1.26	2.18	17.68	10.22
Dog 3	19.13	1.10		17.47	
Dog 4	20.63	3.38		6.11	
Dog 5	12.30	1.37		9.01	
Dog 6	8.62	0.57		15.12	
Dog 7	67.55	0.63		107.22	
Dog 8	6.25	1.22		5.14	
Dog 9	6.49	1.13		5.74	
Dog 10	16.80	0.39	2.01	43.08	8.34
Dog 11	13.13	0.50		26.52	
Dog 12	4.74	0.39		12.15	
Dog 13	4.02	0.63		6.37	
Dog 14	34.30	0.50		69.29	
Dog 15	17.50	0.30		58.33	
Average				28.01	9.22
P-value	0.0008 ¹	0.0198 ²			

¹ comparison of total DNA yields obtained from Oragene®•ANIMAL kit and buccal swabs by paired t-test

² comparison of total DNA yields obtained from Oragene®•ANIMAL kit and whole blood by paired t-test

“Comparison of DNA yields from matched saliva, blood and buccal swab samples showed that yields from saliva were significantly higher than those from blood ($p=0.0198$) or buccal swabs ($p=0.0008$).”

Katherine Mitsouras, PhD



DNA sample collection using the Oragene•ANIMAL kit

Why is Oragene•ANIMAL the preferred collection method?

The DNA quality and performance is comparable to that of genomic DNA from blood, and Oragene•ANIMAL/saliva has the added significant advantage that it is non-invasive (unlike blood), and stable at room temperature (unlike swabs that are susceptible to bacterial contamination). These features allow for the non-invasive collection of samples that can be easily shipped and stored without sacrificing DNA quality. Additionally, it is much easier for dog owners to correctly collect the sample, resulting in a more reliable sample. Buccal swabs collected by owners gave poor yields and low quality DNA.

