

Isolation of Bovine and Mouse DNA from Buccal and Vaginal Samples Using the BuccalAmp™ DNA Extraction Kit

Isolation of Bovine DNA from Buccal and Vaginal Samples

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Our research focuses on identification of quantitative trait loci affecting economically important traits like milk production and reproduction in commercial dairy cattle. This research requires large numbers of animals from complex pedigrees that are genotyped at microsatellite markers located throughout the genome. We study both males and females in our projects, isolating DNA from semen for males and normally using blood as a reliable source for females. However, collection of blood from cows located on the farm is expensive because a veterinarian usually collects the blood and then the samples must be quickly shipped to the laboratory for processing. Upon arrival, samples would be extracted using either an inexpensive, labor-intensive phenol-chloroform method or quicker, more expensive, solid-phase or spin-column methods.

We elected to try the BuccalAmp™ DNA Extraction Kit to collect samples from cows due to the ease of sample collection, economical method of storage and shipping, and the rapid processing of the samples. We collected buccal and vaginal samples from cows using the Catch-All™ Sample Collection Swabs provided in the kit. Swabs were processed on the same day of collection, while others were kept at room temperature for one week and -20°C for one month.

The kit was easy to use, requiring only a vortex mixer and a hot water bath. After quantitating the extractions through DNA fluorometry, we found no discrepancy in the quality or quantity of DNA obtained from swabs that were processed the same day and those that were stored and processed at a later time. Furthermore, the actual DNA yield surpassed our expectations, with an average DNA concentration of 200 µg/µl. Most importantly, all of these samples repeatedly PCR-amplified using fluorescently-tagged primers (Figure 1). In conclusion, the BuccalAmp Kit was ideal for acquiring vaginal and buccal samples using nonin-

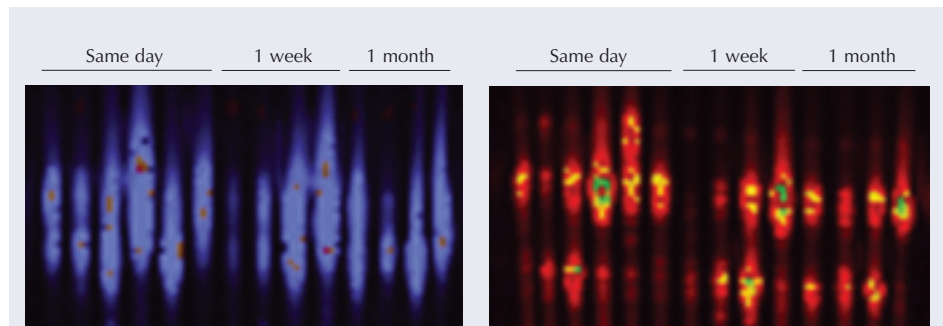


Figure 1. PCR amplification of buccal and vaginal samples. Each 12 µl reaction included 50 ng of template, 1X buffer, 1.5 mM MgCl₂, 200 µM dNTPs, 0.4 µM of each primer, and 0.3 U *Taq* polymerase. PCR products were produced with the following protocol: 95°C for 3 minutes, 94°C for 15 seconds, 56°C for 15 seconds, 72°C for 15 seconds, for 35 cycles, and then 60°C for 30 minutes. An aliquot (1 µl) of each reaction was analyzed on an ABI 377 Automated DNA Sequencer.

vasive methods, providing high quality DNA in a timely fashion.

(Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture.)

Isolation of Mouse DNA from Buccal Samples

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Multiple intestinal neoplasia (*Min*) is an autosomal dominant trait that involves a nonsense mutation in codon 850 of the murine *Apc* gene [murine *Apc* is a homologue to the human *APC* (adenomatous polyposis coli) gene].¹ Because the condition of the *Min* mouse mocks human FAP, the C57BL/6J-*Min*/+ strain is a very important model for finding therapies for the prevention of colon cancer. C57BL/6J females can be crossed with C57BL/6J-*Min*/+ males to produce pups that are either heterozygous for the *Apc* mutation, or normal C57BL/6J. One hundred percent of the C57BL/6J-*Min*/+ mice raised on a high fat diet (AIN) develop adenomas throughout the intestinal tract and most die by the age of 120 days.²

Normal C57BL/6J females (usually 2) are placed in a cage with one C57BL/6J-*Min*/+ male. Plugs will be present up to 12 hours post-copulation. Plugged females are separated and after approximately 19-21 days, parturition occurs.

The pups are weaned at 21-28 days post parturition.

Traditionally, tail snips have been used to genotype for the *Apc* mutation. A 1-2 mm piece of tail is cut with a razor blade and placed into 30 µl of DNA extraction buffer. Then, proteinase K is added and a series of vortexes and water bath incubations occur until finally, PCR-ready DNA is prepared. An allele-specific PCR assay using the primers MAPC MT, MAPC 9, and MAPC 15 is used to genotype the mice. A PCR product of 327 bp is generated in the presence of *Min* alleles, but no product is evident in its absence. MAPC 9 and MAPC 15 together, generate a 618 bp product surrounding the *Min* nonsense mutation. This band is generated in +/+ mice and *Min*/+ mice.³

Recently, DNA extractions were attempted using EPICENTRE's BuccalAmp™ DNA Extraction Kit as an alternative to the use of tail snips. Swabs of the cheek cells of the mice were rotated in the tubes containing the QuickExtract™ DNA Extraction Solution, and DNA extraction was performed following the kit's protocol. The kit provided PCR-ready DNA that was incorporated into the PCR protocol mentioned above. The DNA yield was sufficient, and the samples were run against tail digested samples, on a 4% agarose gel with ethidium bromide (Figure 1).

The BuccalAmp Kit proves more beneficial than tail-snip digestion in two ways. First it saves time in the elimination of tail-tissue digestion. It also provides a less invasive means of sampling DNA from mice, without sacrificing DNA yield.

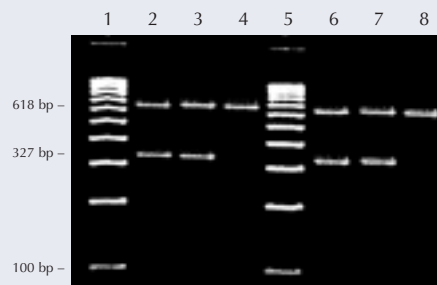


Figure 1. PCR products obtained using the FailSafe™ PCR System and run on a 4% gel with ethidium bromide. Lanes 1 and 5 contain the 100 bp marker. Lanes 2, 3, and 4 are DNA samples collected from the cheek cells of mice. The DNA was extracted using the BuccalAmp™ DNA Extraction Kit. Lanes 6, 7, and 8 are DNA samples collected from tail snips of the same mice. Samples in lanes 2 and 3 and lanes 6 and 7 were positive for the *Apc* gene mutation (*Min/+*). Samples in lanes 4 and 8 did not have the *Apc* gene mutation (+/+).

References

1. Su, L.K. *et al.* (1992) *Science* **256**, 668.
2. Moser, A.R. *et al.* (1990) *Science* **247**, 322.
3. Dietrich, W.F. *et al.* (1993) *Cell* **75**, 631.

www.epicentre.com/buccalamp.asp

BuccalAmp™ DNA Extraction Kits

BQ0901S	1 Kit
BQ0908S	8 Kits
BQ0916S	16 Kits

Contents:

- 15 tubes (1 extraction/tube) of QuickExtract™ DNA Extraction Solution 1.0
- 15 individually-packaged sterile Catch-All™ Swabs

www.epicentre.com/failsafe.asp

FailSafe™ PCR PreMix Selection Kit

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Includes FailSafe™ PCR Enzyme Mix and choice of two FailSafe™ PCR 2X PreMixes.

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Includes FailSafe™ PCR Enzyme Mix and choice of eight FailSafe™ PCR 2X PreMixes.

Science Students Amplify Human Globin Gene DNA from Buccal Samples

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The Derryfield School offers a 10-week biology independent science course for high school seniors as an opportunity to get hands-on scientific experience. Students are expected to write a proposal outlining their interests and expectations for the course. Under the direction of the science teacher, students discuss research plans and specific experiments that can be done in the classroom laboratory. They then design their experiments and proceed through the classical steps of observation, hypothesis, data collection and analysis of results.

Last year, three students, Emily Learner (now at Vassar College), Justin Liu (now at Virginia Military Institute) and Kathryn Karalis (now at Northeastern University) [Figure 1], performed a variety of experiments including the extraction and amplification of human globin gene DNA from buccal samples using the BuccalAmp™ DNA Extraction Kit and the FailSafe™ PCR System.

DNA Extraction

DNA was obtained with the BuccalAmp™ DNA Extraction Kit from EPICENTRE. First, the oral cavity was thoroughly rinsed with water, the inside surface of the cheek was gently brushed with a toothbrush and the oral cavity was rinsed again with water. Samples of buccal epithelial cells were then collected using the sterile Catch-All™ sample collection swab according to directions on the package. The swab was placed directly into a tube containing the QuickExtract™ DNA Extraction Solution and rotated clockwise and counterclockwise 5 times in each direction. Sample tubes were hand vortexed and heated for 30 minutes at 65°C. Samples were hand vortexed again for 15 seconds and heated for 8 minutes at 98°C. This step was repeated and the extracted DNA solutions were stored at -20°C.

DNA Amplification

Amplification primers were purchased from and purified by Integrated DNA Technologies, Inc. Primers were diluted to 1 µM final concentration in the amplification

reaction. Five microliters of extracted DNA were amplified in the presence of the FailSafe™ PCR Enzyme Mix, the FailSafe PCR 2X PreMixes A through K and globin DNA primers in a total volume of 50 µl. PCR cycling conditions were 30 cycles of a three-step cycling program with a denaturing temperature of 98°C for 1 minute, annealing at 55°C for 0.5 minute, and extension at 73°C for 1 minute. Amplified DNA products were examined via electrophoresis using 0.8% agarose in a Tris/Borate/EDTA buffer. Gels were stained with SYBR® Gold and viewed with a Dark Reader System. Digital images were recorded with a Nikon Coolpix 880 (Figure 2).

Conclusion

Emily, Justin, and Kathryn were thrilled with the process of doing science, particularly the detailed work required to accurately perform PCR. This technique lends itself to a number of tasks that students can do and then connect to the classroom discussions. For instance, collecting their own cells and extracting the DNA, preparing PCR tubes, pouring agarose gels and using a thermalcycler are all exciting tasks done in order to visualize DNA that belongs to you, the experimenter.



Figure 1. Student participants (from left) Kathryn Karalis, Emily Leaner, Justin Liu and their science teacher Dr. Jack Sanford.

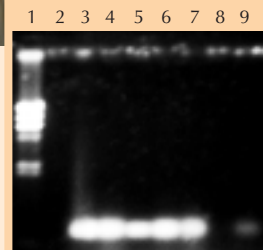


Figure 2. PCR amplification results using the FailSafe™ PCR System. Agarose gels were loaded with 10 µl aliquots from PreMix reactions D through J. Lane 1, *Hind* III lambda digest (1 µg); Lane 2, negative control; Lanes 3-9, PCR products generated in the presence of PreMixes D through J. (Images courtesy of Claudette Knieriem)