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Mouth cell collection device for newborn mice

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Abstract

For efficient and accurate genotyping of transgenic and knockout mice, the ability to reduce pain and suffering and to obtain DNA early in life are critical. We have developed a novel method to sample buccal cells from neonatal mice to obtain DNA. Our mouse mouth cell collection process includes an oral speculum and collection device which enables rapid extraction of enough DNA for up to 50 PCRs from each buccal sampling. This cell collection device fills a clear need for buccal sampling from neonatal mice, greatly facilitating research in mouse models of human disease. Eliminating the pain, distress, and death caused by invasive and mutilating procedures lessens the potential for confounding variables between control and experimental animals. In conclusion, our mouse mouth cell collection process can be applied to very small animals for which there exists no current device.
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Introduction

Obtaining cells from newborn rodents is critical for investigations involving transgenic and knockout mice. Common practices used to acquire cells for DNA genotyping involve total or partial amputation of a neonatal mouse's toe, ear or tail. These practices are both invasive and mutilating, and toe clipping generally has been banned. Furthermore, in response to these practices, mice exhibit responses to pain that may include one or more specific behaviors, such as vocalizing, biting, and an avoidance response to tail clipping, showing that they experience significant pain.

Non-invasive and painless buccal cell isolation methods involving saliva, mouthwash, treated filter paper, cytobrush, and foam or cotton-tip swabs are available for collecting buccal cells from humans for diagnostic analyses [1–3]. These methods, however, are extremely difficult, if not impossible, to adapt for the small size of the newborn mouse. For example, the filter paper on the Bode Buccal DNA collector is about the width of a neonatal mouse head [1]. The Oragene saliva collection device requires 1 ml of saliva sample to extract sufficient DNA for processing, or approximately the total weight of the 1 g neonatal mouse (DNA Genotek Inc.). Traditional cotton swabs are much too large to collect buccal cells from a day of life (DOL) 1 mouse and would have the potential to suffocate the mouse if forced into the mouth. Although comparison of cytobrush, mouthwash and treated card for obtaining human buccal cells found that the cytobrush was the best method for human sampling [3], these approaches cannot be adapted directly to mice, especially DOL1 mice. A serrated pipette tip used to collect human cells for RNA isolation is too abrasive for the fragile DOL1 mouse cheek [2]. Mouse pups require methods of handling that minimize pain and stress, since they are very fragile and can die easily during execution of the experimental protocol.

An improvement in the welfare of the research animals being studied reduces confounding variables for the

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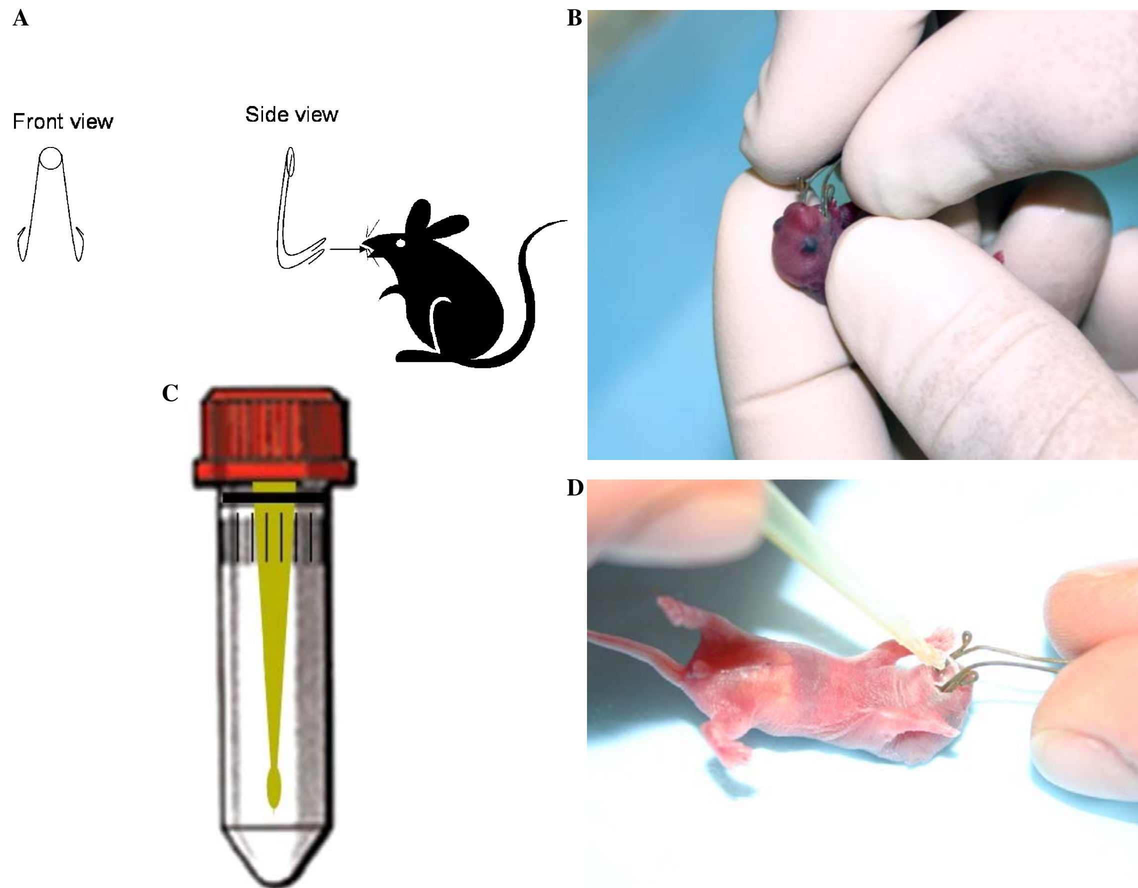

Fig. 1. Devices used for mouse pup buccal cell collection. (A) The oral speculum provides gentle pressure to open the pup's mouth after it is inserted. (B) This photograph shows the speculum after insertion into the oral cavity of a DOL1 neonatal mouse to hold the mouth in an open position. (C) The buccal cell collection device includes a small scoop at the end of a 3 cm probe attached to the lid of a 1.5 ml centrifuge tube. (D) This photograph shows the buccal cell collection device inserted into the mouth held open by the speculum (right). The small scoop collector is extending from the 3 cm handle (left).

scientific results obtained [4]. Protocols have been developed to address minimally invasive mouse buccal cell sampling. An oral rinse has been used as a buccal cell source from mice as young as 2.5–3 weeks old (weanlings) [4,5]. This method involves potential sample loss due to swallowing, and requires two rounds of PCR in order to obtain a gel-visible genotype. Another method for sampling and extraction of mouse DNA used a cotton swab adapted for adult, but not neonatal, mice [6]. A common toothpick, though more appropriate in size, lacks a reservoir for collecting adequate buccal cells from the newborn mouse for subsequent analyses. Kits involving buccal brush or swab (Epicentre), mouse saliva (10 μl; Sigma), and buccal cells applied on a card with a swab (Whatman) are examples of commercial products available for adult mice. However, the youngest mouse description in these protocols is 1 month old.

To date, known, standardized, relatively non-invasive methods for buccal cell sampling from newborn mice are not available from commercial sources or in the published literature. Therefore, there is a need for a less invasive, non-mutilating approach to obtain cells from neonatal mice for experimental procedures that require buccal cell sampling, for example, for DNA extraction and genotyping.

We have developed a novel method to sample buccal cells from tiny neonatal mice to obtain DNA. This mouse mouth cell collection process includes a small oral speculum device that we developed to open the newborn mouse mouth gently for easier insertion of the buccal cell collector. Scraping the check with the cell collector is painless and minimizes stress as seen by lack of overall body movement and struggle and can generate enough DNA for at least 50 PCR amplification reactions. The cells obtained from this procedure could also be used for RNA and protein extraction and for generating cell lines [7]. Veterinarians look for the least harmful and least invasive method for procedures on an animal, and our device has been approved by the director of the Department of Laboratory Animal Medicine at UCLA. Our mouse mouth cell collection device fills a clear need for buccal sampling from neonatal mice and has the potential to replace all existing methods of obtaining cells from newborn mice.

Materials and methods

Animal model

Gyk KO mice were obtained from W.J. Craigen at Baylor College of Medicine [8]. They were bred and housed under an Animal Research Committee (ARC) approved protocol at the University of California, Los Angeles (UCLA). All experiments were performed according to a UCLA ARC approved protocol. DOL1 neonatal mice were used for mouth cell sampling.

Genotyping

Primers for genotyping were neo-F2 (5′-gcgcatcgccttctatcgcc-3′) and *Gyk*R (5′-gttcaagactccacacaccaacc-3′), which amplified the neomycin-*Gyk* junction fragment and primers for the normal allele, were *Gyk*F (5′-gatgccatgaatcgcgactgt-3′) and *Gyk*R [9]. We developed sex-typing primers to detect the X and Y chromosomes: primer #1 (5′-ccgctgccaaattctttgg-3′) and primer #2 (5′-tgaagcttttggctttgag-3′). A female produces one band: 340 bp band whereas a male produces two bands: 301 and 340 bp.

Mouth cell collection process

We developed an oral speculum (Fig. 1A) that facilitates insertion and manipulation of the buccal cell collection device (Fig. 1B). The speculum is inserted into the mouse pup's mouth, and exerts gentle pressure to open the mouth and maintain it in the position during the collection procedure. The cell collector consists of a 1.5 ml screwcap microcentrifuge tube with a 3 cm probe fused to the lid (Fig. 1B). The tip of the probe has a miniature spoon-shaped scoop and acts as a collection reservoir for buccal cells scraped from the mouse cheek. Scraping the buccal mucosa on the inside of the mouse cheek removes a large number of cells. The cap with the attached probe is sufficiently large to facilitate manipulation in the oral cavity of the neonatal mouse. For the collection procedure, a probe with integrated scoop was used to lightly rub 3–5 mm of the buccal mucosa in each neonatal mouse cheek pouch twice. The collection instrument was removed from each mouse mouth concave side up and placed back in the microcentrifuge tube containing 1 ml of normal (0.9%) NaCl. The mouth cell collection device was used to seal the tube and was vortexed to suspend the cells in the saline.

DNA extraction

Each cell suspension was centrifuged at 14,000 rpm (15,800*g*) for 10 s and the supernatant discarded. Fifty microliter of lysis solution (25 mM NaOH, 0.2 mM EDTA) was added to each cell pellet and vortexed briefly. The resuspended pellet was heated at 98 °C for 20 min, vortexed briefly, and placed on ice. Fifty microliter of 40 mM Tris–Cl (pH 5.0) was added to neutralize the lysis solution and yielded a total volume of 100 μl.

PCR

Two microliter of neutralized lysis extract was taken as an aliquot for PCR (total PCR volume 25 μl). Genotyping PCR for *Gyk* genotyping was performed according to our previously published protocol [9]. Sex-typing PCR was performed as follows: 95° for 3 min; then 30 cycles of 94° for 30 s, 60° for 1 min, and 72° for 3 min; and finally an extension at 72° for 5 min.

Results

For efficient and accurate genotyping of transgenic and knockout mice, the ability to reduce pain and suffering and to obtain DNA early in life are critical. In many cases as in the murine model for human glycerol kinase deficiency (GKD), the knockout mouse may be the only non-human mammalian model available. *Gyk* knockout mice require immediate genotyping, because they die at DOL3–4. We are able to obtain sufficient DNA from buccal scrapings of mouse pup's as young as DOL1 to genotype and sex-type them for use in investigations before the knockout mice die (Table 1).

We were able to extract enough DNA for up to 50 PCRs from each buccal sampling. Fig. 2 shows stained buccal cells obtained from the mouth cell remover. Fig. 3A shows sex-typing PCR results from amplified DNA obtained from male and female neonatal mouse pups. This DNA was diluted to various concentrations and successfully amplified as well (Fig. 3B) showing signal from <1 μl of a 1:10 dilution of the 1 μl PCR amplification. These results show that at least 50 amplification reactions can be obtained from a single buccal collection in a newborn mouse pup. Genotyping PCR was performed to distinguish wild type, carrier and knockout DOL1 neonatal mice (Figs. 4A and B). These results were confirmed by tail biopsies (data not shown). We have been able to successfully replicate these procedures for 50 mouse pups.

Discussion

We have improved cell retrieval from tiny (1.3 g average weight) newborn mice with our mouse mouth cell collection process that includes an oral speculum and collection device. The collection device is a sterile, disposable apparatus designed to collect significant amounts of buccal cells

Table 1
DNA obtained from mouse pups and pup weights

DNA	Average	Minimum	Maximum
Concentrations (ng/μl)	10	5	15
Total DNA (μg) in total volume 100 μl	1.0	0.5	1.5
Pup weight (g)	1.30 ± 0.20	0.89	1.66

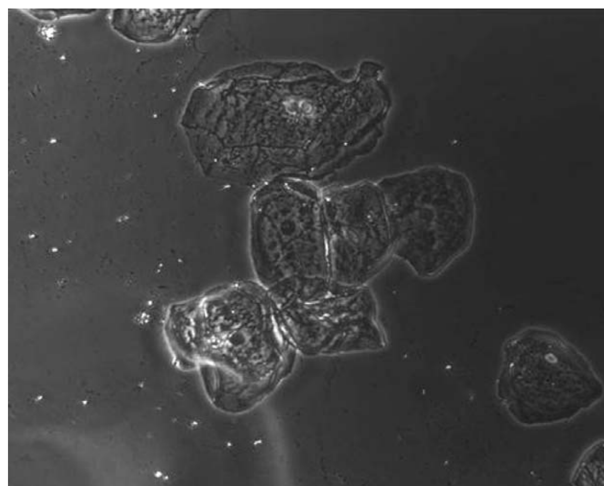

Fig. 2. Buccal cells from DOL1 neonatal mouse.

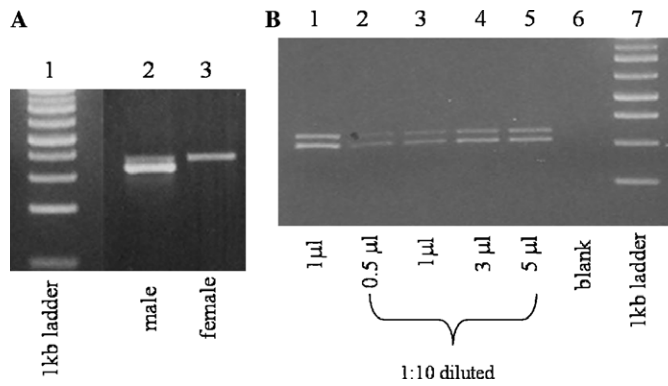

Fig. 3. Sex-typing PCR of DOL1 neonatal mice. (A) Male (lane 2) and female (lane 3). (B) Dilutions of male mouse DNA show sensitivity to <1 µl of a 1:10 dilution of the neutralized lysis extract, showing ample DNA for >50 PCR sex-typing amplifications.

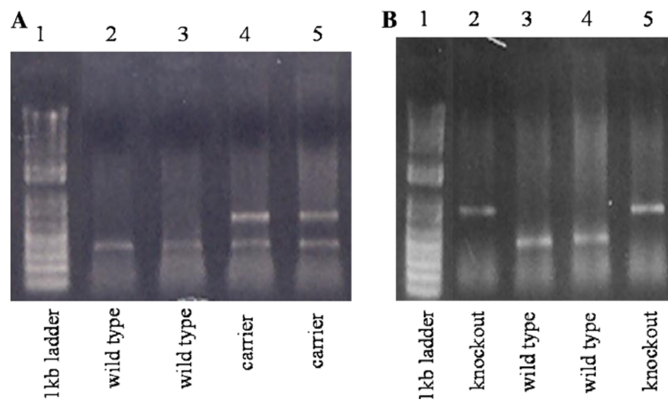

Fig. 4. Genotyping PCR of *Gyk* knockout, carrier and wild type mice. (A) Genotyping PCR of *Gyk* wild type and carrier mice. (B) Genotyping PCR of knockout and wild type mice.

from neonatal mice without eliciting a pain response as evidenced by a lack of significant contortions or back and forth body movements. The mouse mouth cell collection device yields a high concentration of buccal cells for DNA extraction, since significant DNA can be extracted for at least 50 PCRs. RNA and protein extractions, and cell culturing could also be performed on these cells [7].

Gentle cheek scraping to replace painful ear or tail clipping would greatly facilitate research in mouse models of human disease. Eliminating the pain, distress, and death caused by invasive and mutilating procedures lessens the potential for generating confounding variables between control and experimental animals. Most importantly, this mouse mouth cell collection process can be applied to very small animals for which exists no suitable, current device.

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