



Rapid Extraction of PCR-Ready DNA from Buccal Cells Using the BuccalAmp™ DNA Extraction Kit

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Introduction

The new BuccalAmp™ DNA Extraction Kit is a single-tube system that provides

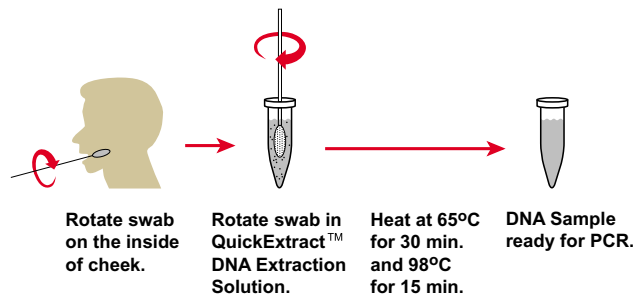


Figure 1. Procedure for obtaining PCR-ready genomic DNA using the BuccalAmp™ DNA Extraction Kit.

an extremely easy and rapid method for extraction of PCR-ready genomic DNA from humans or small animals. The use of buccal (cheek) samples eliminates the time and discomfort required for sample collection by blood draws. Buccal cells are first adsorbed onto individually packaged sterile Catch-All™ Sample Collection Swabs - a soft foam swab on a flexible plastic handle (Figure 2). Catch-All Swabs are easy and safe to use, even for pediatric sampling, and their porous foam captures more sample than standard buccal brushes. The sample is collected simply by rotating the swab on the inside of the cheek. Then the swab is returned to the hard-pack plastic cylinder for storage until processed.

Sample processing is simple (Figure 1). The swab with sample is rotated in the QuickExtract™ DNA Extraction Solution and briefly heated to obtain high yields

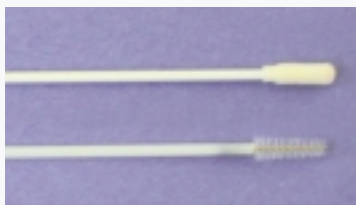


Figure 2. Catch-All™ Sample Collection Swabs (upper) are gentle and effective - even for pediatric sampling. The porous swab improves DNA yields by absorbing more sample than standard buccal brushes (lower). Catch-All Swabs are supplied individually in sterile hard plastic cylinders.

of PCR-ready genomic DNA. The method requires no organic solvents or even centrifugation, permitting rapid extraction of both individual samples, as well as multiple samples for high throughput processing.

Here we demonstrate the high yields of buccal cell DNA extracted from individuals in a family consisting of both children and adults. We also show that the samples can be processed immediately or stored at room temperature for later processing, thus permit-

ting sample collection at remote sites and shipment to a processing lab. Finally, we demonstrate that the genomic DNA obtained is suitable for use for PCR amplification of even difficult sequences, including both highly repetitive and GC-rich sequences, using the FailSafe™ PCR System.

Methods

Buccal samples were collected and DNA was extracted from 9 members of an extended family. Five of the samples were obtained at remote sites. Briefly, each buccal cell sample from these individuals was air dried onto the Catch-All Swab, and placed in the hard plastic cylinder in which it was supplied. All samples were shipped or stored at room temperature for one week before DNA extraction. Two of the samples were collected from children ages 5 and 7. DNA was extracted from all nine samples by standard kit protocol as summarized in Figure 1. The DNA recovered was quantified by fluorimetry.

Results

The genomic DNA concentrations obtained for each 500-µl sample of QuickExtract DNA Extraction Solution ranged from 3 to 14 ng/µl (Table 1). Thus, total DNA yield per individual varied from 1.5 to 7.0 µg of genomic DNA, about two-fold higher than with other methods. The variation in yield of extracted DNA between individuals is normal and varies depending on each individual's sloughing of buccal cells, as well as sampling technique and other

Table 1. DNA yields obtained from 9 individuals using the BuccalAmp™ DNA Extraction Kit

| Sample | DNA conc. (ng/µl) | Total yield (µg) |
|--------|-------------------|------------------|
| 1# | 14 ng/µl | 7.0 µg |
| 2# | 9 | 4.5 |
| 3# | 13 | 6.5 |
| 4# | 12 | 6.0 |
| 5# | 7 | 3.5 |
| 6 | 7 | 3.5 |
| 7* | 3 | 1.5 |
| 8* | 10 | 5.0 |
| 9 | 4 | 2.0 |

*pediatric samples
#samples collected at remote sites

variables. Yields of DNA extracted from remote-site samples, as well as from pediatric samples, were comparable to yields obtained from adult samples extracted immediately (Table 1).

Five microliters of QuickExtract Solution containing buccal cell DNA from each of the 9 individuals (1% of the total buccal cell DNA extracted from each) was used in PCR amplification reactions with the FailSafe PCR System (see p. 15-16). The 5-µl aliquots provided ample DNA to examine differences in polymorphic repeat sequences between these related individuals. The repeats varied in length from 180 to 320 basepairs. The PCR products were easily detected and distinguished by size, as seen in Figure 3. The extracted pediatric DNA, as well as the DNA obtained by remote-site sampling, amplified as efficiently as the adult samples extracted immediately.

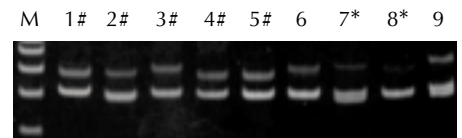


Figure 3. PCR of polymorphic repeat sequences in DNA from buccal samples from 9 individuals obtained using the BuccalAmp™ DNA Extraction Kit. DNA was amplified using the FailSafe™ PCR System. Lane M, 100 bp marker.
remote-site collected sample
* pediatric sample

The buccal DNA from all nine individuals was also amplified with primers to

the tumor necrosis factor (TNF) gene.¹ This GC-rich 740-bp PCR product was amplified with the FailSafe PCR System using FailSafe PCR PreMix F for all of the samples tested, including pediatric DNA as well as remote-site collected DNA (Figure 4).

M 1 2 3 4 5 6 7 8 9 10

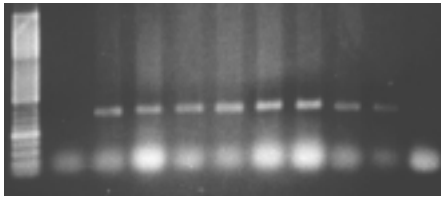


Figure 4. FailSafe™ DNA amplification of TNF-beta from BuccalAmp™ extracted, genomic DNA.

Lane 1, negative control; Lane M, 100 bp ladder. Lanes 2-10, TNF PCR products from individuals 1-9.

Discussion

High yields of PCR-ready DNA were obtained from buccal cells collected with the Catch-All Sample Collection Swabs and extracted with the BuccalAmp DNA Extraction Kit reagents. Buccal DNA yields from samples collected at remote sites produced comparable yields to samples extracted immediately, demonstrating the effectiveness of the Catch-All Swabs for remote-site collection when transported in their hard plastic cylinders. The soft, porous Catch-All Swabs were also shown to be effective for collecting buccal cells from children.

The simple DNA extraction protocol requires minimal hands-on processing time, allowing simultaneous processing of a large number of samples. The resulting DNA is sufficient for more than 100 amplification reactions.

References

1. Moffatt, M. *et al.* (1999) *Thorax*, **54**:757-761.

BuccalAmp™ DNA Extraction Kit

| | |
|---------|---------|
| BQ0901S | 1 Kit |
| BQ0908S | 8 Kits |
| BQ0916S | 16 Kits |

Contents:

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

QuickExtract™ DNA Extraction Solution 1.0

QE09050 50 ml
Bulk solution, sufficient to perform 100 extractions.

Catch-All™ Sample Collection Swabs

QEC091H 100 swabs
100 individually-packaged swabs in sterile hard-pack plastic cylinders.

High Fidelity PCR Amplification of DNA up to 40 Kb Using the MasterAmp™ Extra-Long PCR Kit

The FailSafe™ PCR System (page 15-16) is ideal for consistent and accurate amplification of any template up to about 20 Kb, whatever its sequence and without need for "hot start" techniques. However, for sequences up to 40 Kb, the MasterAmp™ Extra-Long PCR Kit enables consistent and accurate amplification. This kit efficiently amplifies regions up to at least 40 Kb from lambda DNA, 30 Kb from *E. coli* DNA and 28 Kb from human DNA. "Hot start" techniques are typically not required when using the MasterAmp Extra-Long Kit.

The MasterPure™ Extra-Long DNA Polymerase contained in the kit combines MasterAmp™ Taq DNA Polymerase with a proprietary 3' – 5' proofreading enzyme to achieve PCR fidelity at least three times better than Taq DNA Polymerase alone. The kit includes MasterAmp Extra-Long DNA Polymerase and nine

different Extra-Long PCR 2X PreMixes for convenient and fast PCR set-up. The nine Extra-Long PCR PreMixes each contain buffer, dNTPS and differing amounts of both Mg²⁺ and MasterAmp™ PCR Enhancer (with betaine*). Once the optimal PreMix is identified for a particular template/primer combination, consistent amplification of the template will be achieved using the same PreMix.

* Patents issued and pending.

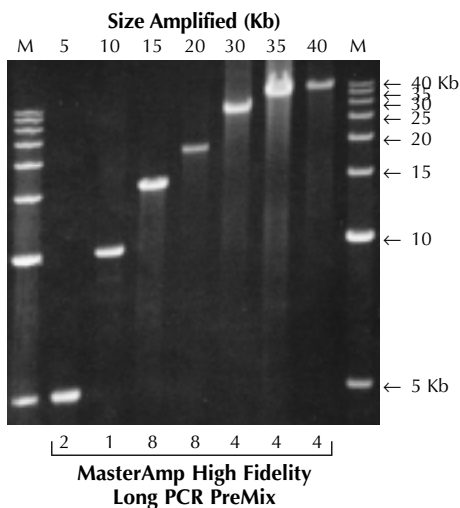


Figure 1. Amplification of 5, 10, 15, 20, 30, 35, and 40 Kb sequences from lambda DNA.

One nanogram of lambda DNA was used to amplify 5, 10, 15, 20, 30, 35, and 40 Kb sequences. Lane M, 5 Kb DNA ladder. Results were analyzed on a 0.5% agarose gel run at 30 V for 20 hours.

MasterAmp™ Extra-Long PCR Kit

MHF9220 50 Reactions

Contents:

MasterAmp™ Extra-Long PCR PreMixes 1-9
MasterAmp™ Extra-Long DNA Polymerase Mix
Control Lambda DNA/Primers

Individual Extra-Long PCR 2X PreMixes

| | |
|--|------|
| MasterAmp™ Extra-Long PCR 2X PreMix 1 | |
| MHF925A | 5 ml |
| MasterAmp™ Extra-Long PCR 2X PreMix 2 | |
| MHF925B | 5 ml |
| MasterAmp™ Extra-Long PCR 2X PreMix 3 | |
| MHF925C | 5 ml |
| MasterAmp™ Extra-Long PCR 2X PreMix 4 | |
| MHF925D | 5 ml |
| MasterAmp™ Extra-Long PCR 2X PreMix 5 | |
| MHF925E | 5 ml |
| MasterAmp™ Extra-Long PCR 2X PreMix 6 | |
| MHF925F | 5 ml |
| MasterAmp™ Extra-Long PCR 2X PreMix 7 | |
| MHF925G | 5 ml |
| MasterAmp™ Extra-Long PCR 2X PreMix 8 | |
| MHF925H | 5 ml |
| MasterAmp™ Extra-Long PCR 2X PreMix 9 | |
| MHF925I | 5 ml |

MasterAmp™ Extra-Long DNA Polymerase Mix

| | |
|---------|---------|
| QU92125 | 125 U |
| QU92500 | 500 U |
| QU9201K | 1,000 U |