

EPICENTRE Forum

Tools & Techniques for Genomics, Proteomics & RNA Research

The BuccalAmp™ DNA Extraction Kit: A New Tool for Easier, Faster Sample Collection and Extraction of PCR-Ready Genomic DNA from Large Numbers of Samples

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Introduction

In both clinical diagnostics and basic scientific research, fast and reproducible DNA extraction from large numbers of samples is becoming increasingly important. EPICENTRE's one-step BuccalAmp™ DNA Extraction Kit, has become a product of choice for PCR-ready DNA extraction, due to its simplicity, reliability, and high DNA yields.

Genomic DNA extraction of buccal (cheek) cells with the BuccalAmp Kit requires only heating to prepare DNA ready for PCR (Figure 1). Buccal cell sampling with the newly designed foam Catch-All™ Sample Collection Swab is noninvasive and painless, ideal for any segment of the population including children. Moreover, buccal cell samples can be stored and transported for an extended period of time at ambient temperature. These features make BuccalAmp genomic DNA extraction an ideal method for high throughput applications. In this report, we simulate a high throughput environment and demonstrate the efficiency of genomic DNA extraction from a large number of samples for PCR.

Methods

Buccal Cell Sample Collection

Catch-All Sample Collection Swabs were distributed to 96 individuals varying in age. Samples were self-collected, except when young children were assisted by adults. Healthcare professionals were not involved with sample collection.

Each swab containing a buccal cell sample was sent to the laboratory in its

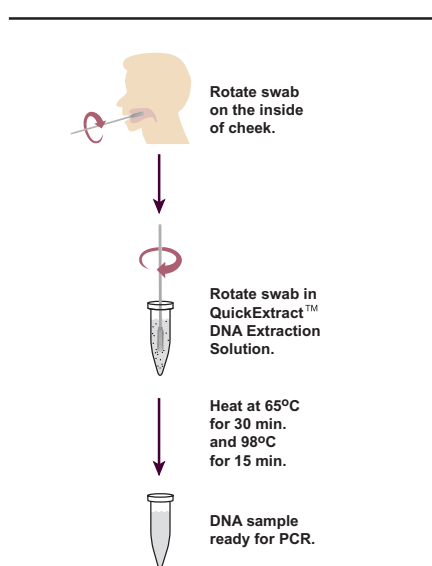


Figure 1. Procedure for obtaining PCR-ready DNA using the BuccalAmp™ DNA Extraction Kit.

original hard plastic collection tube at ambient temperature. Tubes were stored at 4° C for up to 4 weeks before being processed.

Buccal Cell DNA Extraction

Buccal cell DNA was extracted from the swab samples from all 96 individuals according to the standard protocol. Specifically, each sample swab was rotated in 500 µl of QuickExtract™ DNA Extraction Solution. Extraction solutions were then heated in separate wells of a 96 well plate (2 ml per well capacity) at 65° C for 30 minutes followed by 98° C for 15 minutes. The solutions containing extracted DNA were cooled and stored at -20° C until used for PCR amplifications.

β-Globin PCR Amplification

All samples were amplified simultaneously using the FailSafe™ PCR System. The β-globin PCR amplifications were carried out in 50-µl reactions containing 5 µl of

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FailSafe™ PCR System “Never Fail at PCR Again”

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the extraction solution (i.e., 1% of the total volume), 2.5 Units of FailSafe™ PCR Enzyme Mix, and 10 pmoles of each PCR primer in FailSafe™ PCR PreMix A. PCR was performed in a 96-well plate using 30 cycles of 92° C for 40 seconds, 50° C for 40 seconds, and 72° C for 60 seconds. PCR reactions were analyzed by agarose gel electrophoresis.

Multiplex PCR Amplification

Multiplex PCR amplifications using Cooperative Human Linkage Center (CHLC) Markers were performed in 50-µl reactions containing 5 µl of extracted buccal cell DNA, 10 pmoles of each of the 10 primers, and 1.25 Units of FailSafe PCR Enzyme Mix in FailSafe PCR PreMix G. The cycling profile for the multiplex amplification was 30 cycles of 92° C for one minute, 50° C for one minute, and 72° C for one minute.

Results and Discussion

The DNA yield from BuccalAmp DNA Extraction Kit samples typically range from 1-7 µg, which is sufficient for at least 100 PCR amplifications. The human β-globin gene was successfully amplified for all 96 samples as shown by the presence of a 750-bp PCR product on agarose gels (Figure 2). (Note: we have also successfully amplified the β-globin gene using only 1 µl of sample extract.)

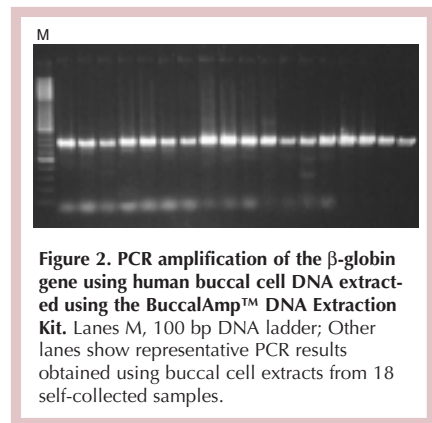


Figure 2. PCR amplification of the β-globin gene using human buccal cell DNA extracted using the BuccalAmp™ DNA Extraction Kit. Lanes M, 100 bp DNA ladder; Other lanes show representative PCR results obtained using buccal cell extracts from 18 self-collected samples.

In the second set of experiments, genomic DNA obtained using the BuccalAmp DNA Extraction Kit was used for genotyping using CHLC primers. CHLC primers amplify a group of highly polymorphic short tandem repeats consisting of tri- and tetra-nucleotide repeats. Four of the sets of CHLC primers used amplify regions of the X chromosome, and the fifth set amplifies a region on the Y chromosome. Multiplex amplifications were performed with these primers using 12 DNA samples. All twelve samples amplified well

with the X chromosome primers which produce products ranging in size from 118 to 299 bp in length (Figure 3). The Y chromosome primers produce products between 205 and 221 bp in length. Products from these primers were seen in samples 2, 4, 5, 7, 8, and 10-12 (Figure 3). As shown, the high quality of the extract DNA resulted in good multiplex amplification using CHLC primers, enabling the genotypes of all samples to be readily differentiated.

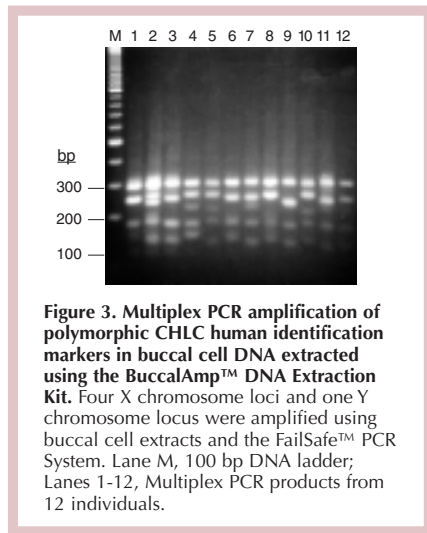


Figure 3. Multiplex PCR amplification of polymorphic CHLC human identification markers in buccal cell DNA extracted using the BuccalAmp™ DNA Extraction Kit. Four X chromosome loci and one Y chromosome locus were amplified using buccal cell extracts and the FailSafe™ PCR System. Lane M, 100 bp DNA ladder; Lanes 1-12, Multiplex PCR products from 12 individuals.

Based on the experiments described above, the BuccalAmp DNA Extraction Kit offers many benefits and advantages over other methods. These include:

Simple and rapid sample processing: The only step required for the DNA extraction process is heating for 45 minutes. No centrifugation or column step is needed. Due to the simplicity of the protocol, many samples can be processed simultaneously, decreasing the time required per sample. After dispersing the buccal cells into the QuickExtract™ DNA Extraction Solution, sample preparation was less than one hour for a 96-well plate and actual hands-on time was only seconds per sample.

No risk for sample cross contamination: The elimination of other steps, such as centrifugation and DNA columns, reduces the possibility of cross contamination and avoids mislabeling of tubes. Reduction in these risks, common for multiple step DNA extraction processes, ensures the accuracy of results.

Simpler and safer sample collection and handling: The Catch-All Swab supplied with the BuccalAmp Kit is made from a soft foam and causes no distress even for children. This results in improved

compliance and cooperation from sample donors.

Self-sampling with reduced sampling cost: Samples can be self-collected by donors with minimal instruction. The fact that the self-collected samples were amplifiable in multiplex PCR demonstrates that BuccalAmp DNA extraction is an excellent alternative to blood draws.

Easy and safer sample storage and transportation: Dry buccal cell samples on the Catch-All Swabs can be stored for an extended period of time and transported at ambient temperature without compromising the genomic DNA. Concerns over sample spillage and deterioration, frequently encountered in blood sample collection, are eliminated.

No blood born hazards: Since there is no blood involved, BuccalAmp DNA Extraction eliminates exposure to any blood borne hazards.

Conclusion

The one-step BuccalAmp DNA Extraction Kit provides an effective and efficient new method to extract genomic DNA for PCR. The simplicity of buccal cell sampling and collection offers convenience for large-scale genomic research or clinical diagnosis and offers a less hazardous alternative to blood sampling. The simplicity of DNA extraction using the kit also facilitates high throughput applications or automated processes.

BuccalAmp™ DNA Extraction Kit

| | |
|-------------|---------|
| BQ0901S-F83 | 1 Kit |
| BQ0908S-F83 | 8 Kits |
| BQ0916S-F83 | 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-F83 | 50 ml |
|-------------|-------|

Bulk solution, sufficient to perform 100 extractions.

Catch-All™ Sample Collection Swabs

| | |
|-------------|-----------|
| QEC091H-F83 | 100 swabs |
|-------------|-----------|

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

Contact EPICENTRE or your local distributor to discuss discounts for larger quantities, special packaging, or standing orders for any of the above items.