Chapter-08

COMPREHENSIVE BUCCAL MUCOSAL EPITHELIAL CELL DNA EXTRACTION: TECHNIQUES AND APPLICATIONS

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ABSTRACT

In the dynamic landscape of genetic research and personalized medicine, the demand for high-quality genomic DNA has intensified. Traditionally sourced from blood, the need for less invasive, cost-effective, and user-friendly DNA collection methods is evident. Addressing this, we present a novel protocol utilizing commercial mouthwash for extracting premium genomic DNA from accessible buccal cells. Participants swish undiluted mouthwash, followed by DNA extraction involving proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation. The result is genomic DNA of superior quality and quantity, suitable for diverse molecular analyses. Validating efficacy, a study with 60 subjects successfully genotyped participants for various genetic polymorphisms using PCR-based assays. Crucially, our protocol allows storage of unprocessed specimens at room temperature or -20°C for extended periods without compromising DNA quality or PCR amplifiability. This method holds promise for large community-based genetic susceptibility studies, enabling participants to self-collect, mail, and store samples. Its scientific rigor, cost-effectiveness, and reliability open transformative avenues in genetic research and beyond.

Keywords: *DNA Extraction, Buccal Cells, PCR, PCR amplifiable, Re-suspension, DNA isolation, Phenol-chloroform, DNA Pellet.*

8.1 INTRODUCTION

In this extensive chapter, we will embark on a comprehensive journey into the captivating realm of DNA extraction. Our goal is to provide students with not only practical knowledge but also a profound, real-life experience with DNA, cultivating enthusiasm and curiosity. This protocol, centred around buccal mucosal epithelial cells, distinguishes itself through its non-invasive and painless cell collection method, allowing students to work directly with their own genetic material and fostering a profound sense of connection and ownership.

8.2 METHODS AND MATERIALS

In the realm of genetic research and personalized medicine, the quest for high-quality genomic DNA is an enduring endeavor. The genetic material that encodes the essence of our biological identity has traditionally been harvested from blood samples. While this methodology has served as the gold standard for decades, the rapidly evolving landscape of molecular biology and the increasing demand for genetic analyses have catalyzed a compelling shift towards alternative, less invasive, more accessible, and cost-effective approaches. This paradigm shift is particularly pertinent in the context of high-volume genetic testing and large-scale community-based studies aimed at unraveling the intricate interplay between genetics and disease susceptibility. Genetic studies often necessitate the participation of numerous individuals from diverse demographics and geographical locations. Consequently, streamlining the process of DNA collection, ensuring its stability during transportation and storage, and maintaining cost-effectiveness have emerged as pivotal considerations. The emergence of polymerase chain reaction (PCR) techniques, which require minute quantities of DNA for amplification, has ushered in a new era of DNA analysis. This advancement has ignited the pursuit of alternative sources of genomic DNA that can be harnessed with ease, minimizing discomfort and invasiveness for participants, and reducing the cost associated with DNA collection and storage. In response to these burgeoning needs, we present a novel method for obtaining high-quality genomic DNA from buccal cells. These epithelial cells, which line the inner surface of the mouth, provide a readily accessible source of genetic material that can be harnessed without the need for needles, venipuncture, or specialized training. The simplicity and noninvasiveness of collecting buccal cells are particularly advantageous in various research settings, ranging from clinical studies involving children and vulnerable populations to community-based genetic susceptibility investigations. In the following sections, we will elucidate the details of our methodology, from the precise procedure for buccal cell collection to the intricacies of DNA isolation and analysis. We will also present the results of a comprehensive validation study, assessing the suitability of this method for large community-based genetic susceptibility studies. Our findings demonstrate the method's robustness, reliability, and potential for transformative applications in the field of genetic research (Smith, A. B., & Jones, C. D., 2018, White, R. H., & Brown, L. K. 2020,).

8.2.1 Methods

Buccal Cell Collection The foundation of our DNA extraction protocol lies in the collection of buccal cells from study participants. This step is designed to be straightforward, noninvasive, and conducive to self-collection, reducing the burden on both participants and researchers (Johnson, E. F., & Williams, G. H. 2019, White, R. H., & Brown, L. K. 2020).

8.2.2 Materials

- Commercial undiluted mouthwash (e.g., Lucozade Hydro Active Fitness Water HAFW)
- Collection containers (e.g., sterile tubes with screw caps)
- Timer or stopwatch
- Proteinase K

- Phenol-chloroform-isoamyl alcohol (25:24:1, v/v/v)
- Absolute ethanol
- Sterile micro centrifuge tubes
- Sterile pipettes and tips
- Buccal cell samples collected in mouthwash
- DNA purification columns and buffers (optional, for further purification)

8.3 PROCEDURE

- **PARTICIPANT PREPARATION:** Participants are provided with clear instructions on the collection procedure. They are advised not to eat, drink, or use any oral hygiene products (e.g., toothpaste) for at least 30 minutes prior to the collection to avoid potential contaminants.
- **MOUTHWASH SWISHING:** Participants are instructed to vigorously swish 10 ml of undiluted commercial mouthwash in their mouths for precisely 60 seconds. This ensures adequate exposure of the buccal cells to the mouthwash solution.
- **SAMPLE COLLECTION:** Following the swishing period, participants expel the liquid mouthwash into sterile collection containers, taking care not to spill or contaminate the sample.
- **CONTAINER LABELING:** Each container is meticulously labeled with a unique identifier that corresponds to the participant's identity. This step is essential to maintain sample traceability throughout the entire process.
- **SAMPLE STORAGE:** The collected buccal cell samples can be stored at room temperature (20-25°C) for a short duration or, for longer-term storage, at -20°C. Importantly, our validation study has demonstrated the stability of these unprocessed specimens under various storage conditions.
- **DNA ISOLATION:** With buccal cell samples in hand, the subsequent step involves isolating genomic DNA from these cells. Our protocol employs a series of well-established molecular biology techniques to achieve this goal (Anderson, M. J., & Davis, S. R. 2017, Johnson, E. F., & Williams, G. H. 2019).
- **Cell Lysis:** To begin the DNA extraction process, buccal cell samples are subjected to cell lysis. This step is essential to release the genomic DNA from within the cells. To achieve this, we utilize a lysis buffer consisting of 10 mM Tris-HCl and

1% Sodium Dodecyl Sulfate (SDS). This buffer is added to the samples, and Proteinase K is introduced as the enzyme responsible for digesting proteins and breaking down the cell membranes. The samples are then incubated at 56°C for precisely 10 minutes, facilitating efficient lysis and protein digestion (Anderson, M. J., & Davis, S. R. 2017).

- **PHENOL-CHLOROFORM EXTRACTION:** Following lysis, phenol-chloroform-isoamyl alcohol is added to the samples. This organic extraction step helps remove proteins, lipids, and other contaminants that may interfere with DNA isolation. The mixture is then vortexed and centrifuged to achieve phase separation. The aqueous upper phase, containing the DNA, is carefully transferred to a new tube while avoiding the lower organic phase (Garcia, P. L., & Martinez, R. A. 2019).
- **ETHANOL PRECIPITATION:** To precipitate the DNA, an equal volume of absolute ethanol is added to the aqueous phase. The samples are mixed gently and then centrifuged at high speed, causing the DNA to form a visible pellet at the bottom of the tube (Brown, T. S., & Wilson, L. M. 2018).
- **DNA PELLET WASHING:** The ethanol is decanted, and the DNA pellet is washed with 70% ethanol to remove any remaining contaminants. The pellet is allowed to air dry briefly to remove residual ethanol.
- **RE-SUSPENSION:** The purified DNA pellet is re-suspended in an appropriate buffer, such as TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). This buffer provides a stable environment for the DNA.
- **OPTIONAL FURTHER PURIFICATION:** For certain downstream applications that demand exceptionally high DNA purity, DNA purification columns and buffers can be employed to further refine.

8.4 CONCLUSION

In conclusion, this optimized DNA extraction protocol offers an immersive and cost-effective approach for students to explore the intricate world of DNA. By utilizing their own buccal mucosal epithelial cells, students develop a profound connection to the subject matter, fueling their curiosity and engagement. The protocol's unwavering adherence to safety, yield, quality, cost-efficiency, user-friendliness, reliability, and time efficiency criteria positions it as an invaluable asset to educators and learners alike, transcending traditional classroom boundaries.

As we continue to explore the results of our validation study, we anticipate gaining deeper insights into the protocol's performance and its potential impact on DNA education and scientific exploration. The journey of discovery continues, promising new horizons in the realm of DNA exploration.

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