PCR*opsis*™ Reagent Buccal mediates direct PCR amplification of buccal sample, even when diluted 1 / 10 million

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Abstract:
Sample collection with buccal swabs provides a convenient and non-invasive means of collecting DNA for genetic testing, law enforcement, and many other applications. These samples usually undergo a multi-step DNA extraction procedure before desired gene targets are amplified. Here we present a fast, easy, and cost effective alternative to DNA extraction using PCR*opsis*™ Reagent Buccal. Specimen material bound to a dry buccal swab is first eluted in this reagent through vortexing and a 10-minute heating step is then applied to simultaneously lyse cells, sequester PCR inhibitors, and make the DNA accessible to the polymerase. The resulting sample is ready for a variety of downstream PCR applications. This approach was used with a buccal specimen diluted up to 10 million fold. The undiluted sample and all tested dilutions processes with PCR*opsis*™ Reagent Buccal resulted in dose-dependent amplification of human glyceraldehyde 3-phosphate dehydrogenase (hGAPDH). In contrast, the undiluted sample not processed with PCR*opsis*™ Reagent Buccal was not amplified. This indicates that the reagent is capable of mediating direct PCR from samples containing high and low concentrations of target DNA.

Results:

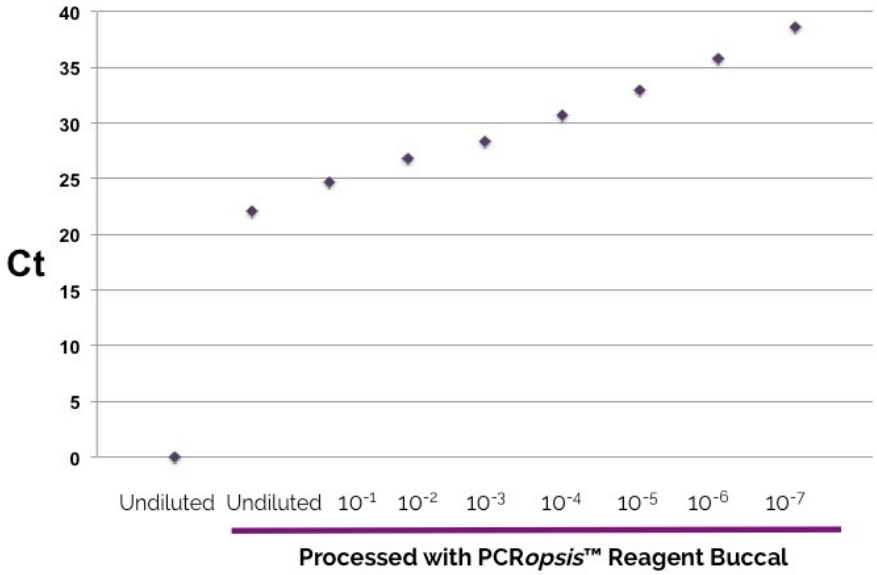

Figure 1. Reagent Buccal facilitates elution and extraction-free amplification of buccal cells dried onto a synthetic swab and diluted up to 1 / 10 million. Buccal cells were eluted from the swab, diluted 1/10, and processed with Reagent Buccal. qPCR was performed using hGAPDH primers. Study was performed in duplicates and average Ct readings shown.

Conclusions & Discussion:

- Reagent Buccal + qPCR resulted in amplification of all tested dilutions of buccal cells (initial cell concentration was not determined). We observed dilution dependent amplification of hGAPDH.

- Reagent Buccal facilitated qPCR amplification of the undiluted buccal sample. This indicates the reagent is able to counteract PCR inhibitors from samples containing high numbers of buccal cells and residual saliva.

- Reagent Buccal facilitated qPCR amplification of buccal samples diluted up to 10 million fold. This indicates the reagent can be used to detect low abundant cells or DNA from buccal samples.

- Repeat studies with diverse donors and expanded dilution series are warranted to completely assess the ability of Reagent Buccal to mediate direct PCR from buccal samples.

Methods:

Materials:

- Synthetic buccal swab
- IDT: hGAPDH primers
- Entopsis:
    - PCR*opsis*™ Reagent Buccal
    - PCR*opsis*™ 5x PCR Master Mix
- Stellar Scientific: Thin-walled PCR tubes
- Chai: Open qPCR Thermocycler

1. Prepare swabs with dried sample:
    a. Rub the inner cheek region of a person using a buccal swab twice on each side of the mouth
    b. Let swab dry at room temperature in a biosafety cabinet for 1 hour
2. Elute material from swabs:
    a. Add 200 µL of PCR*opsis*™ Buccal or nuclease-free water (control) to a 1.5 mL tube
    b. Submerge a dried, sample-containing swab into the reagent
    c. Vortex for ~30 seconds, 4 times
3. Sample dilution:
    a. Perform 1 / 10 serial dilutions of eluted buccal sample in PCR*opsis*™ Buccal in thin-walled PCR tubes
4. Sample processing:
    a. Heat diluted samples for 10 minutes at 95˚C
    b. Let cool at room temperature for ~10 seconds before continuing
5. Perform qPCR:
    a. Add 5 µL of heated sample to 15 µL of qPCR mix
        i. PCR*opsis*™ 5x PCR Master Mix: 4 µL
        ii. Forward primer: 1 µL
        iii. Reverse primer: 1 µL
        iv. Lumniprobe (100x): 0.2 µL
        v. Nuclease-Free Water: 8.8 µl
        vi. Heated sample: 5 µL
    b. Run samples on qPCR Thermocycler for 40 cycles
        i. Initiate denaturation: 10 minutes,
            1. 95˚C 30 seconds,
            2. 55˚C 30 seconds,
            3. 72˚C 30 seconds
        ii. Hold: 4˚C