

Scope Mouthwash Buccal DNA Protocol

Materials:

- 10 ml Scope original mint mouthwash
- 50 ml and 15 ml sterile conical centrifuge tubes
- Puregene DNA Isolation Kit for Tissue (or other Gentra kit with Pro K and RNase A, or can use our own lab stock enzymes-adjust volumes for concentration differences)
- Glycogen (DNase-free) 20 mg/ml
- Isopropanol (2-propanol)
- 70% Ethanol
- 55o C water bath
- 37o C water bath

Collection of sample:

Wait 1 hour after eating, drinking, or brushing teeth before collecting buccal sample. Swish 10 ml of Scope original mouthwash in mouth for about 20 seconds, making sure to cover area on the inside of cheeks. Use fingers to rub teeth gently on inside of lip or cheek to aid in collection of cells. Spit mouthwash into 50 ml sterile conical centrifuge tube. Sample may be stored at RT up to 1-2 weeks.

Processing of sample:

1. Spin tube at 2000g for 10 min. Immediately pour off supernatant, leaving about 100 ul residual liquid. Vortex vigorously for 5 sec. to resuspend cells.
2. Add 3 ml Cell Lysis Solution and vortex 5 sec. at medium speed. (Note: Sample can be stored at this point at RT or 4o C for up to 2 years.)
3. Add 15 ul Pro K (20 mg/ml), mix, and incubate 1 hour at 55o C.
4. Add 15 ul RNase A (4 mg/ml), invert 25 X to mix, and incubate 15 min. at 37o C.
5. Cool sample on ice. Add 1 ml Protein Precipitation Solution. Vortex at high speed for 20 sec.
6. Place in ice bath for 10 min.
7. Spin at 2000g for 15 min. The precipitated proteins should form a tight, green pellet.
8. While sample is spinning, add 3 ml isopropanol and 15 ul glycogen solution (20 mg/ml) to a 15 ml cent. tube, and mix.
9. Immediately after spinning, pour DNA solution from 50 ml tube into 15 ml conical tube (leaving behind the protein pellet) and mix by inverting gently 50 times. Keep the tube at RT for at least 5 min.
10. Centrifuge at 2000g for 10 min. The DNA may or may not be visible as a small white pellet, depending on yield.
11. Pour off the supernatant and drain tube briefly on clean Accuwipe. Add 3 ml 70% ethanol and invert the tube several times to wash the DNA pellet.
12. Centrifuge at 2000g for 3 min. Carefully pour off ethanol. Invert and drain the tube on a clean Accuwipe and allow to air dry 10-15 min.
13. Add 200 ul of DNA Hydration Solution (200 ul will give a conc. of 0.1 ug/ul if the total yield is 20 ug of DNA)
14. Allow DNA to rehydrate by incubating at 65o C for 1 hour and/or overnight at RT. Mix tube periodically (or place on rotator) to aid in dispersing the DNA.
15. For storage, sample should be vortexed, centrifuged briefly, and transferred to a 1.5 ml tube. Store at 4o C, or for long-term storage at -20 or -80o C.

Approximate yield is estimated at 4-40 ug per sample, with a mean of 16 ug. To check concentration using the spectrophotometer, make a dilution of approx. 1:10 in Hydration Solution, and use the Hydration Solution to blank the spec.

To use lab stock enzymes:

- If using a Proteinase K stock at 10 mg/ml, use 30 ul per sample.
- If using an RNase A stock at 10 mg/ml, use 6 ul per sample.