INTEGRATED DNA TECHNOLOGIES

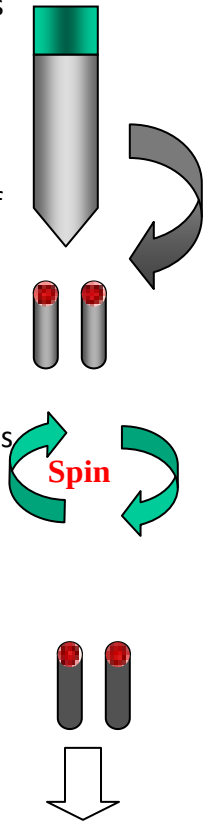
"Spit Prep"
Cheek Cell mtDNA Isolation and Purification

What you will do: This is a nearly fool-proof non-invasive method for obtaining cells from any individual and extracting, isolating, and purifying DNA from those cells that can be used for nearly any PCR or DNA sequencing experiment.

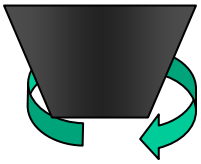
What you will need: The "equipment" list is very simple and inexpensive.

- 8 oz Styrofoam cups
- 2 mL or 1.5 mL microcentrifuge tubes
- 15 mL centrifuge tubes
- Bulb type transfer pipettes (plastic disposable, 3 mL)
- Bio-Rad Chelex-100 Molecular Biology Grade Resin
- HPLC grade water
- Microcentrifuge
- Heat Block or water bath

1. Take 10 mL of sterile HPLC-grade water in a 15 mL tube and a Styrofoam drinking cup.
2. Vigorously swish water in mouth for 30–60 seconds. The water will collect cells from the lining of the mouth.
3. Spit water into cup and pour back into 15 mL tube.
4. Cap tube and let stand for 15–20 minutes. The cells will settle to the bottom of the tube.
5. Using a bulb pipette, transfer cells from the 15 mL tube to two 2.0 mL microcentrifuge tubes.
6. Cap the 2.0 mL tubes and spin at full speed in the microcentrifuge for 5 minutes. This will pellet the cells at the bottom of the tubes.
7. Pour off as much of the liquid as possible leaving just the cells.
8. Add 100 µL of Chelex resin to each tube and resuspend the cells.
9. Incubate the suspension at 100°C for 10 minutes. The heat will break open the cells and release all of the DNA and proteins.



10. Place the tubes on ice for 3 minutes. This will allow the Chelex to bind with everything except the DNA.

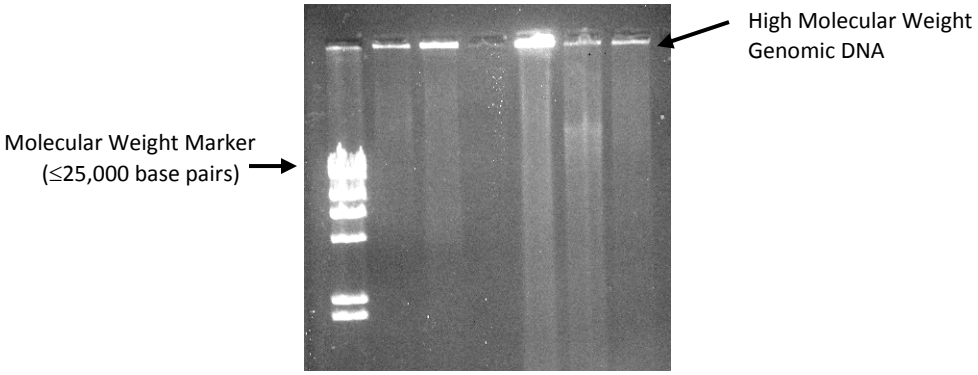


11. Spin the tubes for 5 minutes at full speed in the microcentrifuge. The Chelex-bound material will be forced to the bottom of the tube and the liquid above the pellet will contain DNA only.

12. Transfer the liquid to a clean 1.5 mL tube. This is your own DNA. (If you have them, you may want to use 0.5 mL or 0.2 mL microcentrifuge tubes for storage)

Checking your DNA preps: You will not be able to see the DNA from this prep unless you run an agarose gel. Prepare a 1.0% agarose gel. Take 5 μL of the DNA prep plus 5 μL of water and 2 μL of tracking dye (see below for dye recipe). Load the entire 12 μL on the gel and run at 80 v for one hour.

The photograph below shows DNA from several individuals using this protocol.



6X Tracking Dye: 0.25% (w/v)[#] bromophenol blue
0.25% (w/v) xylene cyanol
30% (v/v) glycerol*
*store at 4°C. If you substitute 15% Ficoll Type 400, the buffer can be stored at room temperature.

10% (w/v) Chelex solution: 1 g Chelex per 10 mL sterile water. Note: the Chelex resin will settle out very quickly so it is best to invert the tube frequently when pipetting.

[#](w/v) stands for weight to volume and (v/v) stands for volume to volume.