ISOLATION OF BUCCAL CELL DNA

9 January 2001, 13 Feb 01, 3 Jan 03, 2 Jan 04, 14 Feb 06, 16Feb09, 31Dec09, 1Feb11
modified from the protocol of Sonia Wallman
David B. Fankhauser, Professor of Biology and Chemistry
http://biology.clc.uc.edu/fankhauser/Labs/Genetics/Buccal_DNA_isolation/DNA_from_buccal_cells.htm

EQUIPMENT:

5mL pipettor + sterile tips
1000 µL micropipettor + sterile tips
200 µL micropipettor + sterile tips
clinical centrifuge, balance
1.5 mL test tube holder (> 10 holes)
boiling water bath with microtube float
microcentrifuge

SUPPLIES *PER STUDENT*:

1	15 mL sterile centrifuge tubes with:
	10 mL 0.9% saline aliquot
2	sterile 1.5 mL Eppendorf tube
	fine tip lab pen
1	beverage straw
0.5 mL	10 % Chelex resin beads
	(in sterile DNA grade dH_2O)

1. **HARVEST BUCCAL CELLS:** Label a **15 mL polypropylene centrifuge test tube**which contains 10 mL of sterile 0.9% NaCl.
2. Label two 1.5ml Eppendorf tubes: A) with your student number and name.
   B) with your student #, name, date, "buccal DNA"
3. Pour the 10 mL of saline solution into your mouth and vigorously swish for 10 seconds.
4. Using a beverage straw, deliver the saline wash back into the labeled 15 mL centrifuge tube.
5. Place your centrifuge tube in a balanced array with other tubes in the clinical centrifuge.
6. **SPIN DOWN CELLS:** Centrifuge at 2000 x g for 10 minutes. (Top speed, setting number 7 on the tabletop clinical centrifuge.)
7. **DISCARD THE SUPERNATANT** by decanting into the sink with running water, taking care not to disturb pelleted cheek cells at the bottom of the tube.
8. Set micropipettor to 500 µL. Snip off 2 mm from tip to enlarge the aperture.
9. **ADD CHELEX BEADS:** Resuspend Chelex resin beads by shaking nd pipetting the beads in and out of the micropipettor. Before resin settles, pipet 500 µL of Chelex to the 15 mL tube containing your cheek cell pellet. Save the pipet tip.
10. **RESUSPEND CHEEK CELLS WITH CHELEX** by pipetting in and out several times. Examine to confirm that no visible clumps of pellet remain.
11. Pipet in and out again to resuspend cells and Chelex, then (same pipet), transfer 500 µL of the suspension to the 1.5 mL Eppendorf tube (A) labeled with your name and number.
12. **BOIL FOR 10 MINUTES**: Place your sample, along with others, into a floating 1.5 mL test tube holder and place in a boiling water bath for 10 min.
13. **CHILL ON ICE:** Transfer all samples to crushed ice.
14. **SPIN DOWN CHELEX:** Place your chilled sample, along with others, in a balanced array in a microcentrifuge and spin for 30 to 60 seconds to pack the precipitate.
15. **SAVE 200 µL CLEAR SUPERNATANT:** Use a fresh pipet tip to transfer 200 µL of the clear supernatant to a clean 1.5 mL Eppendorf tube (B) labeled with your number, name, date, and "cheek DNA." Take care not to pick up any of the cheek cell debris or resin from the bottom of the tube. (An A260 of 1 corresponds to a concentration of 50 ug/ml for 2x DNA.)
16. Store your sample for a few minutes or hours on crushed ice or for days at -20°C until you are ready to proceed to Set up and run PCR reaction.

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DNA EXTRACTION USING DNAzol® DIRECT

1 February 2011

This is an alternative means of isolating your DNA. It is new for us this year. We will see how it goes! Thanks to Drs Kossenjans and Preston for the suggestion. We will try both DNA preparations for PCR to compare their usefulness.

1) Label a sterile microfuge tube as in step 2above: your initials, your number, the date and "DNAzol"
2) Place 0.4 mL of DNAzol® DIRECT solution in the tube, set in rack.
3) Swab the cheek several swipes with a sterile cotton swab.
4) Swish the cotton swab several times in the DNAzol® DIRECT solution.
5) Heat the tube at 90°C for 10 minutes. Place on ice, store frozen. Use 2-5 uL for PCR.