

ISOLATION OF DNA FROM HAIR FOLLICLE page 31b

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Modified from a Edvotec protocol: "PCR-based VNTR Human DNA Typing"

EQUIPMENT:

1.5 mL test tube holders (> 10 holes)
fine tipped permanent marker
1000 μ L micropipettors + sterile tips
200 μ L micropipettors + sterile tips
56°C water bath
floating microfuge tube holder
vortex
boiling water bath
microcentrifuge

SUPPLIES PER STUDENT:

razor blade, 1 per student
microfuge tubes, sterile, 2 per student
lysis buffer*
ice bucket with ice

1. **Pull out several hairs**, keep 3-4 which possess bulbs with a thickened, moist "sheath" at the root.
2. **Trim the bulbs** by cutting off excess shaft (all but 5 mm), place in the bottom of a 1.5 ml centrifuge tube.
3. **Mix and add 150 uL lysis buffer*** to the hair bulbs: The buffer must be thoroughly mixed first by pipetting up and down with a "mochos" prepared pipet tip (**). Before the chelating agent settles, immediately remove and withdraw the 150 μ l and add it to the hair bulbs.
4. Push the bulbs down below the surface of the lysis buffer.
5. **Incubate at 56°C** in a water bath for 15 minutes. Place in a floating tube holder, and incubate in a.
6. **Remove and cool for 30 seconds** at room temp.
7. **Vortex** the tube for 15 seconds.
8. Ensure that the hair sheaths are still submerged in lysis buffer, replace in floating tube holder.
9. **Incubate at 100°C for 10 minutes**: float in a boiling water bath for 10 minutes.
10. **Cool on ice for 2 minutes** by removing the rack and placing on a bed of ice.
11. **Vortex** for 10 seconds.
12. **Spin in a microcentrifuge, top speed for 30 seconds.**
13. **Withdraw 50 uL of supernatant and transfer to a clean 1.5 mL tube.** Do not disturb the pellet. Label with your initials and the date.
14. **Store frozen** until ready for use in PCR.

* Lysis buffer contains 25 mM Tris HCl pH 8.0, 10% chelating agent, 50 μ g/ml of protease K.

** Cut off about 2 mm from the tip of a 1000 uL tip to widen the aperture.