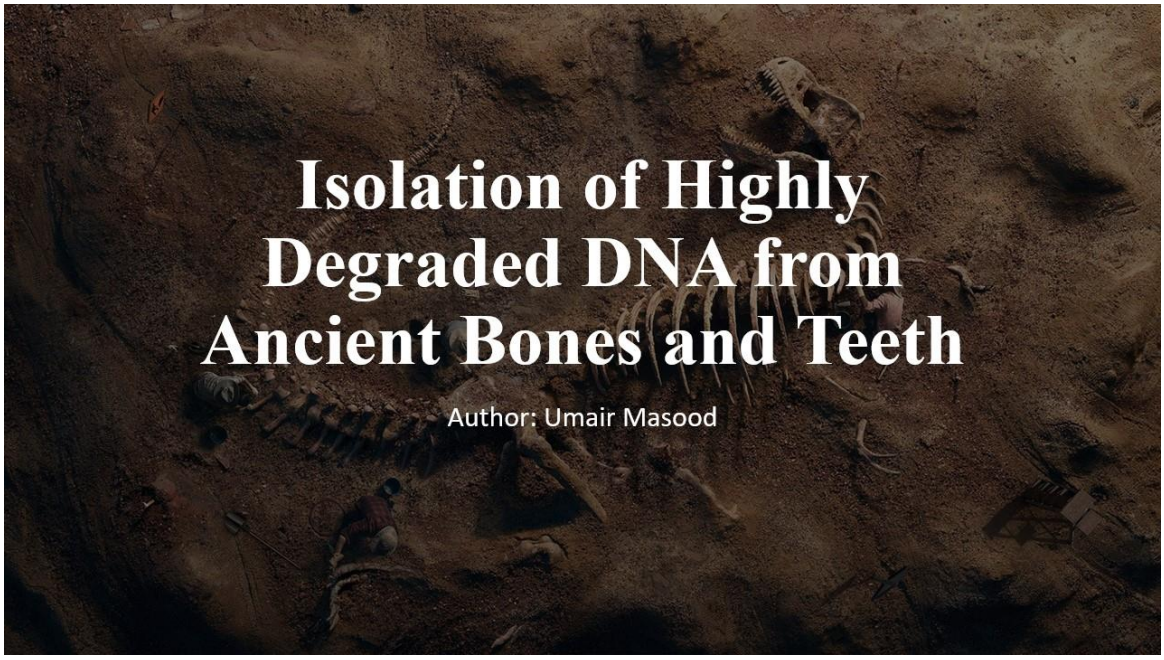

[Isolation of highly degraded DNA from ancient bones and teeth]

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Isolation of highly degraded DNA from ancient bones and teeth:

Buffer Preparation:

- Isolation Buffer(10ml):

H2O	745 µL
EDTA (0.5M)	9 mL
Proteinase K (10mg\mL)	250 µL
Tween-20	5 µL

Restricting Buffer:

- Restricting Buffer(50mL):

GuHCl (5 M)	23.88 g
Water	30 mL.
isopropanol	20 mL
Tween-20.	25 µL

sample preparation:

- Gather 10–150 mg powdered (grind) sample in a 2 mL tube.
- put-on 1 mL of extraction buffer and Mix gently by vertexing
- Incubate at 37°C for 16-24h

DNA restriction:

1. For each sample along control, shift approximately 10 mL of restriction buffer to a tagged 15 mL tube, put-on (400 µL 3M) sodium acetate.
2. Centrifuge samples for 2 min
3. shift supernatant to the 15 mL tube carrying a restriction buffer. Mix well by shaking.
4. stream the restriction buffer mixture into the reservoir of the spin-column assembly, cover the 50 mL tube with a screw cap
5. Centrifuge at 400g for 4min
6. spin tubes 90°
7. centrifuge again at 400g for 2min
8. Discard the screw cap from the 50 mL tube, and shift the spin-column assembly to a clean 2 mL tube and discard the extension reservoir.
9. Close and tagged the spin-column cap
10. complete a dry spin at 3000g for 1min using benchtop centrifuge
11. Remove any flow-through.
12. put-on 750 µL PE buffer to each column. centrifuge at 3000 for 30s and Remove any flow-through
13. Repeat step 13
14. Complete a dry spin at 16,000 g for 1min

15. Shift the column to a new 1.5 mL tube
16. put-on 50 µL TET buffer immediately on to the silica membrane. keep sit for 5 min
17. Centrifuge at maximum speed for 1 min
18. Repeat steps 16 and 17 by shifting the eluate back on to the silica membrane
19. shift the (final DNA extract) to a new 1.5 mL tube and stored at -20°C