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High Molecular Weight DNA from Tails--Jones Rapid Method

NOTES: *When pipetting genomic DNA samples, only use pipette tips from which the tips have been snipped off. This will prevent shearing the DNA and keep the average size in excess of 80-100 kb.*

1. Cut approx. 1.0-1.5 cm from the tip of the tails and place in Eppendorf tubes. Add 700 µl of Lysis Buffer to each tube.

2. Add 20 µl of freshly prepared 20 mg/ml Proteinase K (in sterile water) to each tube.

3. Rock or shake the tubes gently overnight @ 55-65° C.

4. Pellet the hair and debris by spinning the tubes for 2 min @ max speed.

5. Transfer supernatant (recover prox 650 µl) to clean 1.5 ml Epp. tubes.

6. Add 650 µl of phenol/chloroform/iso-amyl alcohol mix (25:24:1) to each tube and mix well by hand. (make sure organic is buffered to neutral pH to avoid trapping of the DNA at the interface)

7. Spin in microfuge at 13000 rpm for 3 min. Recover aqueous (top) phase to fresh tube, leaving behind interface and organic layer.

8. Add one-tenth volume (70 ul) of 3M Na-acetate. (make sure NaOAC is above pH6 to avoid precip. EDTA) Add equal volume (700 µl) of absolute ethanol (room temperature) to each tube and precipitate the DNA by gentle inversion.

8. Spin down the DNA in microfuge -30 seconds on high.

9. Rinse once in 80% EtOH and let air dry, or dry briefly in Savant.

10. Add 100 µl of TE and dissolve overnight at room temp.
Store the tubes @ 4° C until ready to be analyzed.

Lysis Buffer:

		for 100 ml:
50 mM	Tris pH 7.5	5 ml of 1.0 M Tris pH 7.5
50 mM	EDTA pH 8.0	10 ml of 0.5 M EDTA pH 8.0
100 mM	NaCl	2 ml of 5.0 M NaCl
5 mM	DTT	0.5 ml of 1.0 M DTT
0.5 mM	Spermidine	50 µl of 1.0 M Spermidine
1% SDS.		10 ml of 10% SDS

For PCR, replace 1% SDS final with 1% Triton X-100 (V/V) final.
(1ml in 100ml)