The Fleischman Lab

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Title	**Isolation of Genomic DNA from Peripheral Blood**	
Introduction	This DNA extraction method utilizes sodium dodecyl sulfate (SDS) to efficiently lyse cells and nuclei. Protein is removed by digestion with proteinase K. Whole blood samples need to be first processed with ammonium chloride potassium (ACK) buffer to lyse erythrocytes. DNA is precipitated with isopropanol and washed with ethanol. DNA isolated from WBCs can be used for PCR amplification and other purposes. This protocol includes instructions for mouse blood, but other cells (e.g. frozen ACK pellets from patient samples, cell lines) may be used as well.	
Materials	1. 1x lysis buffer (0.1M Tris pH8, 0.2M NaCl, 5mM EDTA, 0.4% SDS) 2. Proteinase K 3. 100% Isopropanol 4. 70% Ethanol 5. 1x ACK 6. 1x PBS 7. TE Buffer (0.001M EDTA pH8, 0.01M Tris pH8) 8. Table top centrifuge 9. Heating plate set at 56°C 10. 1.5ml Eppendorf 11. 100mM EDTA	
Protocol	**A. Blood Processing**	*Notes*
1.	Collect 30-50µl mouse blood into 20µl EDTA.	*Mix well to prevent clogging.*
2.	Add 1ml ACK blood on ice for 10-15 minutes and centrifuge at 1000g for 5 minutes. Aspirate supernatant.	
3.	Resuspend pellet in 1ml PBS and centrifuge at 1000g for 5 minutes. Aspirate supernatant.	
	B. DNA Isolation	
5.	Add 400µl lysis buffer and 20µl proteinase K. Vortex, and leave in heating plate at 56°C for 2 hours or overnight.	
6.	Add 500µl of 100% isopropanol and invert several times to precipitate out DNA. Spin at 12000g for 3 minutes. Aspirate supernatant.	
7.	Add 500µl of 70% ethanol. Spin at 12000g for 3 minutes. Aspirate supernatant.	
8.	Allow DNA to air dry for at least 10 minutes.	*Dry well to prevent ethanol contamination.*
9.	Resuspend with 80µl TE buffer and incubate at 56°C for 1 hour. Sample can be stored in -20.	