DNA Extraction Protocol

1. Thaw blood sample (1-40 µl blood in 500 µl SET buffer) and mix
2. Add 13 µl of SDS (20%) and 15 µl proteinase K (20 mg/ml)
   * mix well and spin down
3. Put samples on water bath (55° C) overnight (mix samples after one hour may increase yield of DNA, especially if blood forms clots)
4. (You may need to split sample here instead of before beginning if it was too thick originally)
5. Spin down, add 50 µl NaCl (5M)
   * mix and spin down
6. Add 500 µl phenol
   * mix well so that blood/buffer and phenol makes a homogenous blend
   * let sample rest for 40-60 min on bench (in hood)
7. Mix again and spin at 10,000 rpm for 15 minutes
8. Label a set of new tubes (2 ml) and add 500 µl chloroform/isamylalcohol
9. Use 1000 µl pipette and pre-cut tips and move supernatant to new tubes
   * Discard top layer of proteins
   * Throw old tubes containing the phenol phase in container labeled ‘fenolslask’
10. Mix and spin at 10,000 rpm for 15 mins
11. Label a new set of tubes (1.5 ml) and add 50 µl of NaAc (3M)
12. Use the 1000 µl pipette and pre-cut tips and move the supernatant to the new tubes
13. Add 2 volumes (1000 µl) of ice cold 99 % ethanol
14. Mix well until you can see a fluff of precipitated DNA
15. Put in -20° C freezer for at least 1 hr (overnight)
16. Spin at 10,000 rpm for 10 minutes (place tubes with labelling facing out from centre)
17. Locate the DNA pellet and remove the liquid phase with a Pasteur pipette
18. Add 500 µl of ice cold 70% ethanol
19. Repeat steps 15 and 16
20. Dry the DNA pellet in the vacuum centrifuge for 8-10 minutes
21. Solve the pellet in approximately 50 µl 1 x TE buffer (10-200 µl depending on amount of DNA)
22. Put samples in refrigerator until ready for DNA-quantification

SET buffer: 0.15 M NaCl, 0.05 M Tris, 0.001 M EDTA, 0.2% SDS pH 8.0