Genomic DNA isolation procedure for blood samples

1. Take at least 50 µL of fresh or frozen blood (200 µL are recommended) and put it into a 1.5 ml microtube (provided).
2. Add 1 volume of **buffer EW** and vortex gently.
3. Centrifuge at 11200g (approx. 12000rpm) for 2 minutes at room temperature.
4. Discard the supernatant with care.
5. Repeat steps 2-4 at least one more time.
   Important: *washing sample at least two times with EW buffer, increases DNA yields.*
6. Add 300µl of **Solution A** and vortex gently.
   Note: *Mix gently before addition if Solution A was stored at 4°C*
7. Incubate at 55°C for 60 minutes. Use of a horizontal shaker (at 100-150rpm) is optional but preferable.
   Optional RNase treatment: *heat sample at 70°C for 5 minutes. Add RNase (not provided) 100 µg/ml (final concentration) and incubate at 37°C for 5 minutes. Finally, continue to step 8.*
8. Cool the samples (from step 6) by incubating approximately 5 minutes on ice or refrigerator.
9. Add 100µl of **Solution B** and vortex gently.
10. Centrifuge at 17000g (approx. 13500rpm) for 10 minutes at room temperature.
11. Carefully pipet the supernatant into a new 1.5 ml microtube (provided), discarding the pellet.
12. Add 40µl of **Solution C** and 400µl of **Solution D.**
13. Shake slightly by inverting the tube several times until a homogenous solution is observed
14. Incubate the tube at room temperature for 10 minutes in a vertical position.
15. Centrifuge at 11200g (12000rpm) for 5 minutes at room temperature.
    Note: *Usually, a little pellet forms.*
16. Discard the supernatant with care.
17. Add 500µl of **Solution E.**
18. Centrifuge at 11200g (12000rpm) for 5 minutes at room temperature.
    Optional: *An additional wash with 70% ethanol can be done to avoid salt excess in the final sample.*
19. Discard the supernatant with care and place the tube (containing the pellet) open and upside down, over a filter paper, for 5-10 minutes.
20. Add 30-50µl of **Solution F** and pipet up and down carefully to re-suspend the genomic DNA.
    Note: *Nuclease-free water can be used but is not recommended for long time storage.*
21. Optional, incubate the tube at 37°C for 30 minutes to help DNA solubilisation
22. Use immediately or store at 4°C (if to be used during the next 48 hours) or at -20°C (for longer storage)