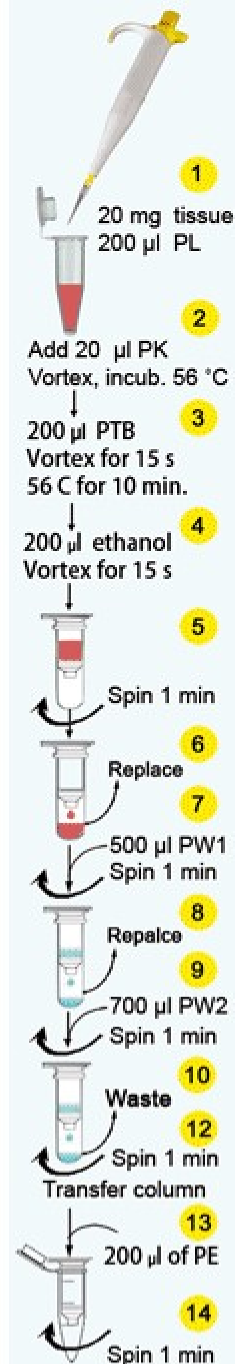
Before starting

1. Add 10 ml of absolute ethanol to the PW1 (only at the first use).
2. Add 48 ml of absolute ethanol to the PW2 (only at the first use).
3. Add Proteinase K (PK) solution to the lyophilized powder of proteinase K and store at -20 ℃ until usage (only at the first use).
4. Check PW1, PL and PTB for salt precipitation. Redissolve any preciptation at 50 ℃.
5. Preheat the solution of PE to 56 °C before starting the extraction process to enhance DNA extraction yield.

Tissue DNA Extraction Protocol

1. Transfer 20 mg of tissue (10 mg for liver or spleen) to a 1.5 ml tube and add 200 µl of PL solution. Cutting the tissue into the small pieces increases the yield of genomic DNA and reduce lysis incubation time.
2. Add 20 µl of Proteinase K and mix them well by vortexing and incubate at 56 ℃ until complete lysis (vortex occasianally). Lysis time varies depending on the tissue type.
3. After lysis of tissue, add 200 µl of PTB solution and vortex for 15 seconds and incubate at 56 ℃ for 10 minutes.
4. Add 200 µl of absolute ethanol and mix by puls-vortxing (15 s).
5. Carefully trasfer lysate to the spin column. A quick spin before lysate transfer would be prefered if there was any debries in the mixture. Spin column for 1 min at 13,000 rpm.
6. Replace the collection tube with a new one.
7. Add 500 µl of PW1 into the column and spin for 1 min at 13,000 rpm.
8. Replace the collection tube with a new one.
9. Add 700 µl of PW2 into the column and spin for 1 min at 13,000 rpm.
10. Pour off the flow-through of collection tube.
11. Repeat step 8 and 9 with 500 µl of PW2 (optional)
12. Spin for 1 min at 13,000 rpm to remove the remaining of the wash buffer. Transfer the spin column to a new 1.5 ml microtube.
13. Add 200 µl of preheated PE, wait 3 min at room temprature. If you want more concentration add less PE (100 µl).
14. Spin for 1 min at 13,000 rpm to elute DNA from the column. Store DNA solution at -20 °C.



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