Protocol for DNA Isolation

1. **Prepare tissue** weigh up to 25 mg of Tissue (fresh or preserved). Add approximately 5 ml of 0.8% saline and homogenize the same with tissue homogenizer. Pellet down and discard the supernatant. Use the pellet tissue for the further lysis.
2. **Digest tissue** by adding 180 µl of suspension buffer (MS DS0090) and add 20 µl proteinase K solution (20gm/ml) to the tissue. Mix by vortexing and incubate the sample at 55 ^{O}C until the tissue is completely digested. Digestion is completed in 2-4 hours. Vortex briefly after digestion is completed.

   If RNase free DNA is required, add 20 µl of RNase (DS0003) mix and incubate for 2 minute at room temperature (15-25 ^{O}C)
3. **Lyse cell** add 200 µl of lysis solution (C1 DS0010) to the sample. Mix by vortexing thoroughly for 10 second. Incubate at 70 ^{O}C for 10 minute
4. **Binding** add 200 µl of ethanol (96-100%) to the lysate and mix thoroughly by gentle pipetting.
5. **Loading lysate** in Spin column transfer the lysate in to the spin column and centrifuge at 10000 rpm for 1 minute. Discard the flow through liquid and place the column in a same 2 ml collection tube
6. **Prewash** by adding 500 µl of diluted prewash solution (PW DS00011) to the column and centrifuge at 1000 rpm for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.
7. **Wash** add 500 µlof diluted was solution (WS DS0012) to the column and centrifuge at 13000-16000 rpm for 3 minute to dry the column. Discard the flow-through liquid and spin the empty column for 1 minute at same speed. Discard the flow-through liquid and place the column in a new 2 ml uncapped collection tube.
8. **DNA elution** pipette 100µlof elution buffer (ET DS0040) directly into the column without spilling the sides, incubate for 1 minute at room temperature (15-25 ^{O}C) and centrifuge at 10000 rpm for 1 minute to elute the DNA. Repeat the step again with another 100 µl of EB for high yield of DNA.
9. Transfer the eluate to a fresh capped 2 ml collection tube for longer DNA storage.

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