# eCube Tisseu DNA Mini Kit

# \* Things to do before starting

- 1. Add 1.1 ml ddH<sub>2</sub>O to each Proteinase K tube to make a 10mg/ml stock solution. Store the stock solution at 2-8°C.
- 2. Preheat two dry baths or two water baths before the operation one to 60°C the other 70°C.
- 3. For 50preps, add 8ml ethanol (96-100%) to Wash Buffer 1 and add 40ml ethanol (96-100%) to Wash Buffer 2. For 200 preps, add 32ml ethanol (96-100%) to Wash Buffer 1 and add 160ml ethanol (96-100%) to Wash Buffer 2. For 300 preps, add 45ml ethanol (96-100%) to Wash Buffer 1 and add 200ml ethanol (96-100%) to Wash Buffer 2.
- 4. All centrifuge steps are done at full speed (14,000 rpm or 10,000 x g) in a microcentrifuge.

### Grind the Sample (up to 25mg)



- 1. Add 200ul of XPTG1 Buffer and homogenize the tissue sample more completely with micropestle.
- 2. Add 20ul Proteinase K (10mg/ml) to the sample mixture. Mix thoroughly by vortexing.
- 3. Incubate at 60°C until the tissue is lysed completely (usually in 1hr, depends on the sample types). Vortex every 10 – 15 min during incubation.
- 4. Add 200ul XPTG 2 Buffer to the sample mixture, mix thoroughly by pulse-vortexing and incubate at 70°C for 10 min.
- 5. Add 200ul ethanol (96 100%) to the sample. Mix thoroughly by pulse-vortexing.





- 8. Add 500ul of Wash Buffer 1 to XPTG Column.
  - 9. Centrifuge for 1 min then discard the flow-through
- 10. Add 750ul of Wash Buffer 2 to XPTG Column.
- 11. Centrifuge for 1 min then discard the flow-through
- 12. Centrifuge for 3 min to dry column
- 13. Add 50ul 200ul of Elution Buffer or  $ddH_2O$  (pH 7.0 8.5) to the membrane center of XPTG Column. Stand the column for 3 min.
- 14. Centrifuge for 2 min

15. You can get pure gDNA.

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- 4. Add 200ul XPTG 2 Buffer to the sample mixture, mix thoroughly by pulse-vortexing and incubate at 70°C for 10 min.
- 5. Add 200ul ethanol (96 100%) to the sample. Mix thoroughly by pulse-vortexing.



6. Transfer the sample mixture (including any precipitate) carefully to XPTG Column.



8. Add 500ul of Wash Buffer 1 to XPTG Column.



9. Centrifuge for 1 min then discard the flow-through

7. Centrifuge for 1 min

then discard the flow-through



10. Add **750ul of Wash Buffer 2** to XPTG Column.



11. Centrifuge for 1 min then discard the flow-through

12. Centrifuge for 3 min to dry column



13. Add 50ul - 200ul of Elution Buffer or ddH<sub>2</sub>O (pH 7.0 - 8.5) to the membrane center of XPTG Column, Stand the column for 3 min.



14. Centrifuge for 2 min



15. You can get pure gDNA.



