DNA Extraction SOP For Animal Tissue

Document NO.:	SOP-SMM-J001
Version NO.:	A0
Effective Date：	2016-4-15

1 Introduction

A phenol-chloroform extraction is a liquid-liquid extraction. A liquid-liquid extraction is a method that separates mixtures of molecules based on the differential solubilities of the individual molecules in two different immiscible liquids. Liquid-liquid extractions are widely used to isolate DNA.

2 Experimental Procedures

A. Pour 1ml lysis buffer.

B. For tissue samples, grind about 200mg with liquid nitrogen into powder and transfer the powder samples into the 2 ml tube contain of 1ml lysis buffer.

C. Incubate the sample at 56℃ in a water incubator for 30~180min. Mix by inversion every 5-10min time period.

D. Centrifuge at 16700×g for 10 minutes after cooling to room temperature.

E. Transfer the supernatant to a new 2.0ml tube, add equal volume of supernatant of Chloroform / isoamyl alcohol(24:1). Gently invert each 3-5min to mixed liquid no obvious boundaries.

F. Transfer the aqueous phase to a new 1.5mL tube; add equal volume of supernatant of isopropyl alcohol. Gently invert each 3-5min to mixed liquid no obvious boundaries.

G. Centrifuge at 16700×g for 10 minutes.

H. Transfer the aqueous phase to a new 1.5mL tube; add 2/3th volume of supernatant of isopropyl alcohol (add $1/10^{th}$ volume of 3M sodium acetate if necessary). Gently mix by inverting at least 3 times and place at -20℃ for 2 hours for precipitation.

I. Centrifuge at 16700×g for 10 minutes and remove the supernatant

J. Wash the RNA pellet with 1 ml 75% cooling ethanol. Re-suspend the pellet and centrifuge at 16700×g for 5 minutes at 4℃ and remove the supernatant

K. Wash the RNA pellet with 500ul 75% cooling ethanol. Re-suspend the pellet and centrifuge at 16700×g for 5 minutes at 4℃ and remove the supernatant.

L. 16700×g for 30~60s, Completely remove the ethanol without disturbing the pellet

M. Air-dry the RNA pellet in the biosafety cabinet

N. Add 25μL~100μL of TE Buffer to dissolve the DNA pellet. If a higher concentration of DNA is desired, 25μL of TE Buffer should be used.

Appendix 1 the list of Reagents and Materials

1-1 Reagents

Reagent Name	**Model**	**Manufacture**
Proteinase K	GPK003001-100mg	GPROAN
Absolute Ethanol	AR 500mL	Sinopharm
RNase A	10mg	FERMENTAS
Guanidinium chloride	GB0242-500g	BBI
SDS	SS0228-1kg	BBI

Appendix 2 the list of Equipment

Equipment Name	**Model**	**Manufacture**
Thermomixer comfort	Thermomixer comfort	Eppendorf
Digital thermostat water bath	HH-8	Jingda
Centrifuge	5427R	Eppendorf
Biological safety cabinets	Airstream	ESCO

----------END----------