



## Purification of Total DNA from Human Tissue

### 1.0 Purpose

The purpose of this procedure is to establish guidelines to isolate Genomic DNA from human tissue from nPOD cases.

### 2.0 Application/Scope

This procedure shall be applied to all DNA extraction procedures performed in the nPOD OPPC.

### 3.0 Definitions

nPOD: Network for Pancreatic Organ Donors with Diabetes  
OPPC: Organ Procurement and Pathology Core  
DNA: Deoxyribonucleic acid

### 4.0 Associated SOPs

4.1 nPOD1 Shipping and Handling  
4.2 nPOD3 Organ Processing and Histopathology

### 5.0 Responsibilities

5.1 nPOD Lab Technician  
5.1.1 Extract DNA following procedures outlined in this SOP.

### 6.0 Materials and Equipment

6.1 Qiagen DNA Kit  
6.2 Centrifuge  
6.3 Spectrophotometer  
6.4 Pipettes

### 8.0 Procedure

8.1 Cut 25 mg of tissue and place in a 1.5 ml microcentrifuge tube  
8.2 Add 180 ul Buffer ATL  
8.3 Add 20 ul proteinase K and mix thoroughly by vortexing  
8.4 Incubate at 56°C in a water bath until tissue is completely lysed (overnight)  
8.5 After incubation: vortex for 15 s and add 200 ul Buffer AL to the sample  
8.6 Mix thoroughly by vortexing and add 200 ul 100% Ethanol and mix again

- 8.7 Pipette the mixture into the DNAeasy Mini spin column placed in a 2 ml collection tube
- 8.8 Centrifuge at 6000 x g for 1 min. Discard flow-through
- 8.9 Place the DNAeasy Mini spin column in a new 2 ml collection tube and add 500 ul Buffer AW1
- 8.10 Centrifuge for 1 min at 6000 x g. Discard flow-through and collection tube
- 8.11 Place the DNAeasy column in a new 2 ml collection tube and add 500 ul Buffer AW2
- 8.12 Centrifuge for 3 min at 20000 x g to dry the DNAeasy membrane
- 8.13 Discard flow-through and collection tube
- 8.14 Place DNAeasy Mini spin column in a clean 1.5 ml microcentrifuge tube and pipette 200 µl Sterile H2O directly onto the DNAeasy membrane
- 8.15 Incubate at room temperature for 1 min and then centrifuge for 1 min at 6000 x g to elute
- 8.16 For maximum DNA yield, repeat elution once as described in step 15
- 8.17 Quantify DNA 260/280nm and store at -20°C.

<b>JDRF nPOD Standard Operating Procedures</b>	
nPOD10	Title: Purification of Total DNA from Animal Tissues
Signature:	
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