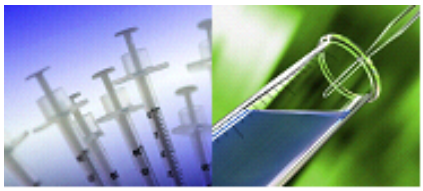

nPOD
Network for Pancreatic Organ Donors with Diabetes

DNA EXTRACTION

1 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to outline procedures for extracting high quality genomic DNA from nPOD samples.

2 SCOPE

This SOP will be applied to the extraction of DNA from fresh or snap frozen tissue, OCT-embedded frozen blocks, and formalin-fixed, paraffin-embedded blocks.

3 RESPONSIBILITIES

3.1 Managers and supervisors are responsible for making sure that technicians are properly trained and equipment and facility are maintained in good working order.
3.2 Laboratory personnel are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs. They are responsible for following clinical laboratory and tissue banking best practices.

4 EQUIPMENT and MATERIALS

The materials, equipment and forms listed in the following list are recommendations only and alternative products as suitable may be substituted for the site specific task or procedure.

QIAamp DNA Mini Kit (Qiagen, Cat. No. 51304)
Microcentrifuge, vortex or mixer, hybridization oven at 56°C
Low TE Buffer (10 mM Tris-HCl, 0.1 mM EDTA), pH 8.0, DNase/RNase- free (FisherScientific Cat. No. 50843207)
100 % Ethanol (molecular biology grade)
Xylene
Absorbent towels
Spectrophotometer for DNA concentration
Mini-gel electrophoresis chamber, agarose, TAE buffer, ethidium bromide, DNA molecular weight marker, DNA loading buffer (Fisher Scientific), gel photography system

5 SAFETY

5.1 Use universal safety precautions when handling human samples and personal protective equipment (e.g., face mask with shield, gloves, lab coat or apron).
5.2 Ethidium bromide is a carcinogen. Take universal precautions when handling and dispose of properly according to MSDS.

6 PROCEDURE

6.1 <u>DNA Extraction from Tissue</u>

6.1.1 Place tissue in a 1.5 ml microcentrifuge tube.
6.1.1.1 Use about 10 mg spleen or 25 mg pancreas.
6.1.1.2 Mince further with sterile scissors or scalpel to aid digestion.
6.1.2 Add 180 ul Buffer ATL.
6.1.3 Add 20 ul proteinase K and mix thoroughly by vortexing.
6.1.4 Incubate at 56°C in a hybridization chamber with moderate rotation to mix until tissue is digested.
6.1.4.1 Check level of digestion at 2 hours. If digestion is incomplete, continue up to overnight.
6.1.5 Vortex for about 15 seconds and add 200 ul Buffer AL to the sample.
6.1.6 Mix thoroughly by vortexing and add 200 ul 100% ethanol and mix again.
6.1.7 Pipette the mixture into the DNAeasy Mini spin column placed in a 2 ml collection tube.
6.1.8 Centrifuge at 6000 x g for 1 minute. Discard flow-through.
6.1.9 Place the DNAeasy Mini spin column in a new 2 ml collection tube and add 500 ul Buffer AW1 to column.
6.1.10 Centrifuge for 1 minute at 6000 x g. Discard flow-through and collection tube.
6.1.11 Place the DNAeasy column in a new 2 ml collection tube and add 500 ul Buffer AW2.
6.1.12 Centrifuge for 3 minutes at 20,000 x g to dry the DNAeasy membrane.
6.1.13 Discard second flow-through and collection tube.
6.1.14 Place DNAeasy Mini spin column in a clean 1.5 ml microcentrifuge tube and pipette 200 ul low TE buffer directly onto the DNAeasy membrane.
6.1.15 Incubate at room temperature for 3 minutes and then centrifuge for 1 minute at 6000 x g to elute DNA.

6.2 DNA Extraction from OCT Blocks
6.2.1 Section several thick sections of tissue under DNase-free conditions and place in microfuge tube. Continue as for tissue described above.

6.3 DNA Extraction from Formalin-fixed Paraffin-embedded Tissue Sections
6.3.1 Dissect a small amount of tissue with paraffin from block (about 0.3 x 0.3 x 0.3 cm^3). Cut the tissue into 5-6 pieces and place into a microcentrifuge tube.
6.3.2 Add 1200 ul of xylene and incubate at room temperature for 2 minutes.
6.3.3 Centrifuge at full speed 20,000 x g for 5 minutes.
6.3.4 Aspirate off the xylene and place all small tissue chunks on absorbent towels to get rid of extra xylene.
6.3.5 Weigh the tissue.
6.3.5.1 About 15-25 mg of tissue is ideal for two preparations.
6.3.6 Finely mince the tissue with sterile scissors or scalpel to aid in digestion.
6.3.7 Place all minced tissue in microcentrifuge tube and repeat step 6.3.2.
6.3.8 Aspirate off the xylene and wash the tissue in 1200 ul of 100% ethanol. Spin at 20,000 x g for 5 minutes and then remove ethanol. Repeat.
6.3.9 Incubate the open microcentrifuge tube at 37°C for 15 minutes until the ethanol has evaporated.
6.3.10 Re-suspend the tissue pellet in 180 ul ATL buffer.
6.3.11 Add 20 ul of proteinase K, mix by vortexing, and incubate in the hybridization oven with rotation at 56°C overnight until completely digested. Vortex occasionally during incubation.
6.3.12 Continue as for tissue described above.

6.4 Quantification and Quality Analysis

6.4.1 Determine DNA quantity using a spectrophotometer by measuring optical density at A260 nm wavelength. Determine the A260/A280 nm wavelength ratio, which should be 1.8 – 2.0 for high quality DNA preparations.

6.4.2 Save all raw data. Import into the DNA Calculator Excel file to show A260/A280 ratio, stock DNA concentration (ng/ul), extraction volume (ul), and total DNA yield (ng).

6.4.3 Analyze extracted DNA quality by 0.8% agarose gel electrophoresis. See SOP DNA Gel Electrophoresis.

6.4.3.1 Expected results should show a large smear of high molecular weight DNA between 15 and 50 kb. Photograph gel and save to raw data folder.

6.4.3.2 Note: The length of DNA purified from tissue in a paraffin block is usually < 650 base pairs.

6.5 Adjust DNA concentration to end-user specifications using low TE buffer and aliquot stock to avoid multiple thaw/freeze cycles.

6.5.1 Expected yield from 10 mg spleen is 5-30 ug DNA.

6.6 Freeze DNA and store at -20°C (those to be shipped for lab analyses within 6 months) or -80°C (long term storage).

7 REFERENCES

7.1 SOP 27 Linear Array Genotyping System for HLA
7.2 SOP 28 MODY Analysis
7.3 SOP 57 Case Processing
7.4 SOP 80 DNA Gel Electrophoresis

8 REVISION HISTORY

Version	Date	Revision

Prepared by	Li Zhang		
Approved by	Martha Campbell-Thompson		
	Name	Signature	Date