SOP#107: Genomic DNA Extraction and Quantification

1. PURPOSE:

This protocol describes genomic DNA extraction using QIAGEN's QIAamp DNA Mini and Blood Mini kits and quantification using NanoDrop 2000c. More information on DNA extraction using QIAamp DNA Mini and Blood Mini kits can be found in "QIAamp DNA Mini and Blood Mini kits handbook".

2. MATERIALS/REAGENTS/EQUIPMENT

2.1 Reagents and supplies

Material	Vendor	Cat#
RNAse A	Qiagen	1007885
Qiagen® Protease	Qiagen	1017784
Buffer AL	Qiagen	1014600
Buffer AW1	Qiagen	1014797
Buffer AW2	Qiagen	1014577
QIAmp Mini Spin Column	Qiagen	1011706
2 mL Collection Tubes	Qiagen	1016810
Ethanol	varies	N/A
Kimwipes	Kimtech	varies
P1000 pipetteman and filter tips	varies	varies
P200 pipetteman and filter tips	varies	varies
P20 pipetteman and filter tips	varies	varies
1.5 ml eppendorf tubes (presterilized)	varies	varies
Eppendorf tube racks for 1.5 ml tubes	varies	varies
Marker pen	varies	N/A

Buffer AL, protease, AW1, AW2, mini spin columns and AE are part of the QIAmp mini DNA kit (Cat #51106)
Complete AW1 buffer is made by adding 130 ml of ethanol to the AW1 bottle provided in the kit
Complete AW2 buffer is made by adding 160 ml of ethanol to the AW2 bottle provided in the kit

2.2 Equipment

- Eppendorf microfuge, model 5415D
- Scientific Industries Vortex Genie-2
- Eppendorf thermomixer
- Thermo scientific NanoDrop 2000c
- Tissue culture hood, certified and prepared for sterile technique

3. EXPERIMENTAL PROCEDURES

NOTE: steps 3.1.1 to 3.1.6 need to be performed in tissue culture hood

3.1. DNA Extraction

3.1.1. Lysing Cells

3.1.1.1. Remove frozen whole blood samples from -80ºC freezer to thaw at room temperature.

3.1.1.2. Turn thermomixer on at 56ºC and record log numbers for each reagent in the sample log sheet for the study.

NOTE: DNA yield reaches a maximum after lysis for 10 min at 56°C. Longer incubation times up to 1 hour have no effect on yield or quality of the purified DNA.

3.1.1.3. Label 1.5 ml eppendorf tubes with sample names.

NOTE: Batch size of 16 or 24. Takes approximately 10-30 minutes to thaw depending on tube size and blood volume

3.1.1.4. Prepare 3 sets of collection tubes in racks.

3.1.1.4.1. Prepare 3 times as many collection tubes as samples and an equal number of QIAmp Mini Spin Columns and 1.5 ml eppendorf tubes. Label mini spin columns and eppendorf tubes with sample IDs.

3.1.1.5. Mix thawed tubes by inverting 6-8 times.

3.1.1.6. Add 20 µl of Qiagen® Protease to all eppendorf tubes.

3.1.1.4. Add 200 µl of sample to its corresponding eppendorf tubes.

3.1.1.5. Add 4 µl of RNAse A to all eppendorf tubes.

3.1.1.6. Add 200 µl of AL lysis buffer to all eppendorf tubes. Mix samples for 15 seconds using Vortex Genie-2. Briefly spin eppendorf tubes in microfuge by holding down the "short" button for 7 seconds

3.1.1.8. Place samples in eppendorf thermomixer for 10 minutes at 56 °C and 900 rpm.

3.1.2. Washing Cell Lysate

3.1.2.1. Remove samples from thermomixer.

3.1.2.2. Briefly spin eppendorf tubes in microfuge by holding down the "short" button for 7 seconds. Add 200 µl of ethanol to samples, vortex for 15 seconds, then spin briefly again.

3.1.2.3. Carefully transfer cell lysate to corresponding QIAmp Mini Spin Columns. Pipette towards the center of the spin column. Take care not to touch and wet the rim of the spin columns.

3.1.2.4. Spin columns in eppendorf microfuge for 1 minute at 8,000 rpm.

NOTE: If the lysate has not completely passed through the column after centrifugation, centrifuge again at 13,200 rpm for another 30 seconds until the QIAamp Mini spin column is empty.

Note: When preparing DNA from buffy coat or lymphocytes, centrifugation at full speed is recommended to avoid clogging.

3.1.2.5. Transfer spin columns to clean collection tubes and discard old collection tubes with filtrate.

3.1.2.6. Wash columns by adding 500 µl of Buffer AW1. Pipette towards the center of the spin column. Take care not to touch and wet the rim of the spin columns. Spin at 8,000 rpm in eppendorf microfuge for 1 minute. Transfer the column to a clean collection tube and discard the old collection tubes with the filtrate.

3.1.2.7. Wash columns by adding 500 µl of Buffer AW2. Spin at maximum speed (13,2000 rpm) in eppendorf microfuge for 3 minutes. Transfer the column to a clean collection tube and discard the old collection tubes with the filtrate.

3.1.2.8. Transfer spin columns to clean collection tubes and discard old collection tubes

with filtrate. Spin columns down for an additional minute at maximum speed (13,200 rpm).

3.1.3. Collecting DNA

3.1.3.1. Transfer spin columns to clean, labeled 1.5 ml eppendorf tubes.

3.1.3.2. Add 50 µl Buffer AE to spin columns.

NOTE: It is vital that when you pipette the Buffer AE that you pipette directly onto the spin column membrane, avoiding the sides of the tube. Pipetting down the sides could greatly decrease your final DNA purity and affect downstream applications.

3.1.3.3 Let columns stand for 5 minutes.

3.1.3.4. Spin columns down for 1 minute at 8,000 rpm.

3.1.3.4.1. Angle eppendorf tube caps along the centrifuge holes to prevent caps from breaking. Do not overcrowd the centrifuge, repeat this step if necessary.

3.2. DNA Quantification

3.2.1. NanoDrop 2000c

3.2.1. Open the ND 2000 program.

3.2.2. Select Nucleic Acids. Click OK.

3.2.3. Load 2 µl of AE buffer onto the NanoDrop 2000c. Click Blank.

3.2.4. Load 2 µl of sample onto NanoDrop 2000c. Record sample ID, then click measure.

3.2.4.1. After every measurement, wipe down the pedestal and arm of the NanoDrop 2000c with a wet Kimwipe.

3.2.5. Click on Reports, then Export and export the data as an .xml file.

3.2.6. Store DNA at -80 ºC.

REVISION HISTORY

DATE	AUTHOR	REVISION #	REVISION REASON
June 12, 2015	Colin Huang	A	Initial Release
August 3, 2017	Peter Huang	B	Minor wording changes
July 17, 2018	Jue Lin	C	Minor wording changes