Standard Operating Procedure (SOP) for DNA Extraction from Blood with QIAMP MIDI Kit

I. SCOPE AND PURPOSE

Isolation of large quantities of DNA from blood presents a unique challenge. Blood contains few nucleated cells in a relatively large volume. Qiagen QIAamp Kits are designed to isolate high molecular weight DNA from up to 2 mL of blood or buffy coat. They provide a fast and simple method for the preparation of up to 60 µg of DNA. The purified DNA is ready for use in standard downstream applications such as DNA amplification and microarray.

Any deviation from the Standard Operating Procedure (SOP) will be noted in LabVantage and on the isolation worksheet; the number of the samples affected by the deviation will be noted as well.

II. PROCEDURE

A. Safety Procedure

1. Wear appropriate personal protective equipment (PPE), including lab coats, goggles, face shield, closed toe shoes and gloves.
2. Bloodborne pathogens can be present. Follow universal precautions per BCR-SOP-S009, "Bloodborne Pathogen and Exposure Control Plan." All blood samples are opened behind a face shield.
3. Liquid nitrogen and dry ice are extremely cold and can cause 'burns.' Wear cryogenic gloves designed to withstand extremely cold temperatures when handling samples stored in liquid nitrogen and large quantities of dry ice (see BPC-SAF-004).
4. Liquid nitrogen is an asphyxiant; be sure to use in a well-ventilated area.
5. Buffer AW1 contains guanidine thiocyanate. Buffer AL contains guanidine hydrochloride. PPE must be used when handling this reagent; do not mix these reagents with bleach (produces cyanide gas)

B. Quality Control

1. The incoming samples have a printed label with a 2D barcode and human readable format. The 2D barcode contains the internal LabVantage ID;
2. Working labels are printed from LabVantage and used throughout the extraction process. Labels are printed that match the original parent portion, sub-portion, and newly created DNA portion LabVantage IDs. Four label colors (Blue, Green, Red, and Yellow) are rotated with each sample. Example: Sample A has blue working labels; Sample B has green working labels, etc.
3. Final storage labels are printed for storage in Matrix 2D barcode tubes.
4. Barcode readers are used throughout the process whenever transferring samples from one tube to another.
5. Only one blood sample is opened at a time during the initial transfer of blood into the centrifuge tube.
6. Samples are tracked in LabVantage. Every portion or analyte is tracked with its position in a freezer or refrigerator. When a technologist processes a sample,

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LabVantage displays the user name as having custody of that sample until the sample is checked in to a storage location.

7. DNA analyte stocks are stored as a primary single tube aliquot (when possible) with a smaller secondary aliquot for sample quality control. DNA quality is evaluated for integrity by agarose gel electrophoresis per BCR-SOP-M003, "Gel Electrophoresis with the E-Gel System" and genotypic identity using SNP loci per BCR-SOP-M010, "Tissue Matching by SNP Analysis". DNA is quantified by PicoGreen Assay per BCR-SOP-M017, "PicoGreen DNA Quantification (Manual)". Primary stock aliquots should not be subject to numerous freeze thaw cycles.
8. No aliquot of original specimen, DNA or any other reagent should ever be returned to the original container after sampling.
9. Any deviations from the protocol as written should be documented on extraction worksheets and at the sample level in LabVantage. Protocol deviations that have the potential to compromise analyte quality (pre-analytical variables) should also be documented with an incident report. Pre-analytical variables include, but are not limited to, abnormal sample condition upon arrival (cracked tubes or clotted blood), temperature excursions (in storage freezer or during extraction), abnormal analyte appearance (cloudy or colored analyte elution), and identity failures.
10. The isolation kit is tested against predetermined specifications to ensure consistent product quality.
11. All new lots of reagents are tested in parallel with the one in current use before being put into use; results are recorded in the QC book. All kit components must be quality control tested and used together thereafter. All reagents supplied in a kit must be used only with other reagents in the same kit lot number; reagents with identical lot numbers cannot be used interchangeably between kit lot numbers.
12. At each step in the DNA isolation, the supernatant or pellet that should not contain the DNA is retained until after isolation and quantitation is completed.

C. Specimen Information

1. Type: Blood or buffy coat.
2. Handling Conditions: Follow standard precautions when handling all blood or buffy coats. Samples should be stored in liquid nitrogen vapor phase until isolation can occur.

D. Required Equipment, Supplies and Reagents

1. PPE: Lab coat, gloves, insulated gloves
2. Biological Safety Cabinet or Splash Guard
3. 1.5 mL microcentrifuge tubes (Fisher, #05-408-137)
4. 15 mL Falcon tubes (Fisher, catalog# 14-959-49B)
5. Pipettes, 50 µL – 1,000 µL, single or multichannel
6. Filtered tips for pipettes
7. Absolute ethanol, molecular grade (Sigma, E7023)
8. QIAamp DNA Blood Midi Kit (no substitution, Qiagen, #51185). Do not use Protease supplied with kit.

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Standard Operating Procedure (SOP) for DNA Extraction from Blood with QIAMP MIDI Kit

9. Proteinase K (10mL) (Qiagen, #19133)
10. 70°C water bath
11. 37°C Water bath
12. RNase A, 10 mg/mL (Qiagen , #158924)
13. 1X Phosphate-buffered saline (PBS)(Invitrogen, #10010-031)
14. Tris-EDTA Buffer (100X) (Sigma, T9285)
15. Water nuclease-free (Sigma, # W4502-6X1L)
16. Centrifuges for 15 mL tubes (swinging bucket rotor)
17. Vortex Mixer
18. Capsule Centrifuge
19. Wet and dry ice
20. Insulating trays for dry ice
21. Racks to hold 15mL conical tubes and microtubes
22. Serological pipets for large volumes (5, 10, 25 mL)
23. Pipet-Aid (or equivalent)Colored labels: THT, B, WHT 1x0/5"W0.375"DIA: Blue (Y1439894), Red (Y1439892), Green (Y1439893), Yellow (Y1439895) (Brady)
24. Thermal Transfer Printer Labels (Freezerbondz, # THT-152-492-3)

Notes: It is possible to substitute disposable materials and certain equipment from other vendors as long as they are the equivalent of the item described above.

In the event that a reagent or disposable item either becomes contaminated or is even suspected to be contaminated, it must be discarded.

E. Reagent Preparation (including storage conditions)

1. All reagents will be labeled with name and concentration, date opened (prepared), expiration date, preparer's initials (if applicable), storage conditions, and appropriate hazard labeling.
2. Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96-100%), as indicated on the bottle, to obtain a working solution. For example, add 200 mL EtOH to an unopened bottle of Buffer AW1 to obtain a 298 mL total volume; add 190 mL EtOH to an unopened bottle of Buffer AW2 to obtain a 271 mL total volume. Buffer AW1 and Buffer AW2 are stable for one year at room temperature.
3. 0.1X TE is made by diluting a stock solution of 100X TE. To prepare 1 liter: add 1 mL of 100XTE to 999 mL of deionized water. This reagent may be store at room temperature for up to one year.

F. Genomic DNA Extraction

1. Remove the samples from the storage freezer. Large bloods in EDTA tubes can be thawed at room temperature; buffy coats in cryovial tubes should be thawed quickly in a 37°C water bath. Check frequently for presence of solids and proceed to the next step immediately after thawing.

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Standard Operating Procedure (SOP) for DNA Extraction from Blood with QIAMP MIDI Kit

a) When working with granulocyte samples, begin with the MGL Reference Lab Thawing Procedure (MGL-REF-48 Reference lab Specimen Processing and Banking, Thawing Cryopreserved Cells section). The cell pellets will be resuspended in 2 mL of 1x PBS before proceeding to step 3 (addition of RNase A).

2. Add up to 2 mL of sample (blood or buffy coat) to a 15 mL centrifuge tube. If less than 2 mL of sample is available, bring the volume of the sample up to 2 mL with 1X PBS.
3. Add 4 µL of RNase A and mix briefly by inversion (Note: do not vortex enzymes).
4. Add 200 µL of QIAGEN Proteinase K and mix thoroughly by repeated inversion.
5. Add 2.4 mL of Buffer AL and mix thoroughly by inverting the tube 15 times followed by additional vigorous shaking for at least 1 minute.
6. Incubate in a 70°C water bath for 10 minutes. (Incubation for longer than 10 minutes will not adversely affect sample quality/yield, but should not exceed 1 hour.)
7. Add 2 mL of ethanol (96% – 100%) to the sample and mix by inverting the tube 10 times followed by additional vigorous shaking for 10 seconds.
8. Carefully transfer one half of the solution from step II.F.7. onto the QIAamp Midi column by carefully dispensing the liquid into the column, taking care not to moisten the rim.
9. Close the cap and immediately centrifuge at 3,000 rpm (1610 x g) for 3 minutes (room temperature).
10. Remove the QIAamp Midi column and place the QIAamp Midi column into a new 15 mL centrifuge tube. Load the remainder of the solution from step II.F.7. onto the QIAamp Midi column. Close the cap and centrifuge again at 3,000 rpm (1610 x g) for 3 minutes (room temperature).
11. If necessary, repeat step II.F.10. to obtain a dry column.
12. Remove the QIAamp Midi column and place the QIAamp Midi column into a new, labeled 15 mL centrifuge tube.
13. Add 2 mL of Buffer AW1 to the QIAamp Midi column. Close the cap and centrifuge at 5,000 rpm (4472 x g) for 1 minute (room temperature). Do not discard the flow-through, but do not allow it to come in contact with the column.
14. Carefully, without moistening the rim, add 2 ml Buffer AW2 to the QIAamp Midi column. Close the cap and centrifuge at 5,000 rpm (4472 x g) for 15 min (room temperature).
15. Carefully transfer the QIAamp Midi column to a clean, labeled 15 mL centrifuge tube. Do not allow the flow-through to come in contact with the column during transfer.
16. Pipet 300 µL of 0.1X TE buffer directly onto the membrane of the QIAamp Midi column and close the cap. Incubate at room temperature for 5 minutes and centrifuge at 5,000 rpm (4472 x g) for 2 minutes (room temperature).

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Standard Operating Procedure (SOP) for DNA Extraction from Blood with QIAMP MIDI Kit

17. Transfer the flow-through from the 15 ml conical tube into a labeled 1.5 mL microcentrifuge tube. The label will contain the unique sample identifier of the DNA analyte.

G. Quantification and Normalization of Products – Refer to BCR-SOP-M017, "PicoGreen DNA Quantification (Manual)".

H. Sample Storage – Store the samples in the -80ºC freezer after normalization.

III. REFERENCES

A. QIAamp DNA Midi Blood Handbok June 2012

IV. RELATED PROCEDURES

A. BCR-SOP-M003, "Gel Electrophoresis with the E-Gel System"
B. BCR-SOP-M010, "Tissue Matching by SNP Analysis"
C. BCR-SOP-M017, "PicoGreen DNA Quantification (Manual)"
D. BCR-SOP-S009, "Bloodborne Pathogen and Exposure Control Plan"
E. BPC-SAF-004: "Compressed Gases Hazards and Safety"
F. MGL-REF-48: Reference Lab Processing

V. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version **3**, Effective Date **8/16/2016**

1. Made title not all capitalized
2. Removed normalization calculations

B. Changes made in Version **2**, Effective Date 12/17/2014

1. New format used
2. Minor word and grammatical changes made throughout
3. Removed any reference to TCGA
4. Removed any reference to concentration range
5. Removed the reference to creating an aliquot for SNP and Gel Electrophoresis
6. Removed the reference to pooling samples and re-extracting samples.
7. Updated reagents and vendor catalog numbers
8. Added instruction for processing granulocyte samples.

C. Version 1, Effective Date 9/11/2014 - New

Signatures

Approved By: **Signature on file** **Date:** **Date on file**

Julie Gastier-Foster, PhD, FACMG
Principal Investigator

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Standard Operating Procedure (SOP) for DNA Extraction With QIAamp MINI Kit

I. SCOPE AND PURPOSE

Qiagen QIAamp kits provide a fast, easy, and reliable method to purify total DNA from small quantities of human tissue or blood. The purified DNA is up to 50 kb in size, with fragments of 20 – 30 kb predominating. This length of DNA readily amplifies with high efficiency and is ready for use in standard downstream applications such as sequencing.

Any deviation from this Standard Operating Procedure (SOP) will be noted on the extraction sheet and in LabVantage; the number of the samples affected by the deviation will be noted as well.

II. PROCEDURE

A. Safety procedure

1. Wear appropriate personal protective equipment (PPE) including lab coats, goggles, face shield, closed toe shoes and gloves.
2. Bloodborne pathogens can be present (refer to SOP S009, "Bloodborne Pathogen and Exposure Control Plan"). Use universal precautions. All blood samples are opened behind a face shield.
3. Liquid nitrogen and dry ice are extremely cold and can cause 'burns.' Wear cryogenic gloves designed to withstand extremely cold temperatures when handling samples stored in liquid nitrogen and large quantities of dry ice (see BPC-SAF-004).
4. Liquid nitrogen is an asphyxiant; be sure to use in a well-ventilated area.

1. Buffer AW1 contains guanidine thiocyanate. Buffer AL contains guanidine hydrochloride. PPE must be used when handling this reagent; do not mix these reagents with bleach (produces cyanide gas).

B. Quality Control

1. The incoming samples have a printed label with a 2D barcode and human readable format. The 2D barcode contains the internal LabVantage ID.
2. Working labels are printed from LabVantage and used throughout the extraction process. Labels are printed that match the original parent portion, subportion and newly created DNA analyte LabVantage ID. Four label colors (Blue, Green, Red, and Yellow) are rotated with each sample. Example: Sample A has blue working labels; Sample B has green working labels, etc.
3. Final storage labels are printed for storage in Matrix 2D barcode tubes.
4. Barcode readers are used throughout the process whenever transferring samples from one tube to another.
5. Only one blood sample is opened at a time during the initial placement of the blood into the centrifuge tube.
6. Samples are tracked in LabVantage. Every portion or analyte is tracked with its position in a freezer or refrigerator. When a technologist processes a sample,

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LabVantage displays the user name as having custody of that sample until the sample is checked in to a storage location.

7. DNA analyte stocks are stored as a primary single tube aliquot (when possible) with a smaller secondary aliquot for sample quality control. DNA quality is evaluated for integrity by agarose gel electrophoresis (see SOP M003) and genotypic identity using SNP loci (see SOP M010). DNA is quantified by PicoGreen Assay (see SOP M017, “Picogreen DNA Quantification (Manual)”). Primary stock aliquots should not be subject to numerous freeze thaw cycles.
8. No aliquot of original specimen, DNA or any other reagent should ever be returned to the original container after sampling.
9. Any deviations from the protocol as written should be documented on extraction worksheets and at the sample level in LabVantage. Protocol deviations that have the potential to compromise analyte quality (pre-analytical variables) should also be documented with an incident report. Pre-analytical variables include, but are not limited to, abnormal sample condition upon arrival (cracked tubes or clotted blood), temperature excursions (in storage freezer or during extraction), abnormal analyte appearance (cloudy or colored analyte elution), and identity failures.
10. The isolation kit is tested against predetermined specifications to ensure consistent product quality.
11. All new lots of reagents are tested in parallel with the one in current use before being put into use; results are recorded in the QC book. All kit components must be quality control tested and used together thereafter. All reagents supplied in a kit must be used only with other reagents in the same kit lot number; reagents with identical lot numbers cannot be used interchangeably between kit lot numbers.
12. At each step in the DNA isolation, the supernatant or pellet that should not contain the DNA is retained until after isolation and quantitation is completed.

C. Specimen Information

1. Type: blood, buffy coat, or skin punches. The Qiagen QIAamp Mini Kit is to be used with samples that contain low volume bloods, i.e. ≤ 200 µl or skin biopsy punches. For larger volumes, use SOP M014.
2. Handling Conditions: Follow standard precautions when handling all blood or buffy coats. Samples should be stored in liquid nitrogen vapor phase until isolation can occur.

D. Required Equipment, Supplies and Reagents

1. PPE: Lab coat, gloves, insulated gloves
2. Biological Safety Cabinet or Splash Guard
3. Insulating trays for dry ice (styrofoam or plastic)
4. 1.5 mL microcentrifuge tubes (Fisher, #05-408-137)
5. QIAamp DNA mini kit (Qiagen 51304 or 51306, no substitution)
    a. Do not use the Protease that comes with the kit.
6. Proteinase K (Qiagen, 19133)
7. Micro-Pipettes to cover a range of 0.5 µL – 1,000 µL

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8. Filtered tips for the Micro-Pipettes
9. Pipettor
10. Tris-EDTA Buffer 100X Concentrate (Sigma, #T9285)
11. Ethanol (absolute) (Sigma, E7023)
12. 37°C water bath
13. 56°C water bath
14. Freezer (-80°C)
15. Microcentrifuge
16. Minifuge
17. Vortex
18. Centrifuge for microtubes
19. Racks to hold microcentrifuge tubes
20. Dry Ice
21. Water nuclease-free (Sigma, # W4502-6X1L)
22. 1X Phosphate-buffered saline (PBS)(Invitrogen, #10010-031)
23. Colored labels: THT, B, WHT 1x0/5"W0.375"DIA: Blue (Y1439894), Red (Y1439892), Green (Y1439893), Yellow (Y1439895) (Brady)
24. Thermal Transfer Printer Labels (Freezerbondz, # THT-152-492-3)

It is possible to substitute disposable materials and certain equipment from other vendors as long as they are equivalent to the item described above.

In the case that a reagent or disposable item becomes contaminated or is suspected of being contaminated or reaches its expiration date, it must be discarded.

E. Reagent Preparation (including storage conditions)

1. All reagents will be labeled with name and concentration, date opened (prepared), expiration date, preparer's initials (if applicable), storage conditions, and appropriate hazard labeling.
2. Buffer AW1 and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96-100%), as indicated on the bottle, to obtain a working solution. For example; add 25 mL EtOH to an unopened bottle of Buffer AW1 to obtain a 44 mL total volume; add 30 mL EtOH to an unopened bottle of Buffer AW2 to obtain a 43 mL total volume. Buffer AW1 and Buffer AW2 are stable for 1 year at room temperature.
3. 0.1 X TE is made by diluting a stock solution of 100 X TE. To prepare 1 liter: add 1 mL of 100 X TE to 999 mL of deionized water. This reagent may be store at room temperature for up to one year.

III. PROCEDURE-STEPWISE

A. Initial preparation

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1. If working with low volume blood that has been frozen in liquid nitrogen**,** quick thaw in 37°C water bath before proceeding with lysis. Check frequently for presence of solids and proceed to the next step immediately after thawing.
2. When extracting from skin punches, mince the tissue into smaller pieces to optimize the digestion process. Add 180 µL of ALT buffer to the sample and homogenize in the TissueLyser (MGL-EQP-21: Qiagen Tissuelyser) for 20 seconds at 15 Hz before proceeding directly to DNA Extraction Step 1.
3. When working with buccal swab samples that have been frozen back in freezing media, proceed first with the MGL Reference Lab Thawing Procedure (see MGL-REF-48: Reference Lab Specimen Processing and Banking – Thawing Cryopreserved Cells section). The cell pellet will be resuspended in 200 µL 1x PBS before proceeding to Step 1 of DNA Extraction.

B. DNA Extraction

1. Pipet 20 µL QIAGEN Proteinase K into the bottom of a 1.5 mL microcentrifuge tube.
2. Add up to 200 µL of sample (whole blood, plasma, serum, buffy coat, or homogenized skin punch) to the microcentrifuge tube. If the sample volume is less than 200 µL, add the appropriate volume of PBS to bring the volume up to 200 µL.
3. Add 200 µL Buffer AL to the sample. Mix by pulse-vortexing for 15 seconds.
4. Incubate in a 56°C water bath for 10 min. (Incubation for longer than 10 minutes will not adversely affect sample quality/yield, but should not exceed 1 hour.)
5. Briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.
6. Add 200 µL ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 seconds. After mixing, briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.
7. Carefully apply the mixture from step 6 to the QIAamp Mini spin column without wetting the rim. Close the cap, and centrifuge at 6000 x g for 1 min (room temperature).
8. Place the QIAamp Mini spin column in a clean 2 mL collection tube (provided).
9. Carefully open the QIAamp Mini spin column and add 500 µL Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g for 1 min (room temperature).
10. Place the QIAamp Mini spin column in a clean 2 mL collection tube (provided).
11. Carefully open the QIAamp Mini spin column and add 500 µL Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g) for 3 min (room temperature).
12. Place the QIAamp Mini spin column in a clean, labeled 1.5 mL microcentrifuge tube.
13. Carefully open the QIAamp Mini spin column and add 30 µL of 0.1X TE buffer directly onto the membrane of the QIAamp mini column and close the cap.
14. Incubate at room temperature (15–25°C) for 1 min.
15. Centrifuge at 6,000 x g for 1 min (room temperature).

C. Quantification and Normalization of Samples:

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1. Refer to SOP M017 for DNA quantification and normalization by PicoGreen.

D. Sample Storage

1. Store the samples in a -80ºC freezer.

IV. REFERENCES

E. QIAamp DNA Mini and Blood Mini Handbook 11/2007

V. RELATED PROCEDURES

E. BCR-SOP-M003, "Gel Electrophoresis with the E-Gel System"
F. BCR-SOP-M010, "Tissue Matching by SNP Analysis"
G. BCR-SOP-M017, "PicoGreen DNA Quantification (Manual)"
H. BCR-SOP-S009, "Bloodborne Pathogen and Exposure Control Plan"
I. BPC-SAF-004: "Compressed Gases Hazards and Safety"
J. MGL-EQP-21: Qiagen Tissuelyser
K. MGL-REF-48: Reference Lab Processing

VI. COMPREHENSIVE REVISION HISTORY

A. Changes made in Version 3, Effective Date **8/16/2016**

1. Made title not all capitalized
2. Deviations will also be noted in LV.
   a. Added reference for SAF-004; LN2 and dry ice safety
   b. Added Buffer AL contains guanidine compound; don't mix AL and AW1 with bleach
   c. Added that kits are QC'd and used together thereafter
   d. Added reagents/equipment/supplies: PPE, splash guard, microtubes, racks, labels
   e. Added what must be included on reagent labels
   f. Added reference for Tissuelyser; centrifugations are performed at RT
   g. Added "related procedures"

B. Changes made in Version 2, Effective Date 12/31/2014

1. Updated formatting
2. Removed any reference to TCGA
3. Removed any reference to concentration range
4. Removed the reference to creating an aliquot for SNP and Gel Electrophoresis
5. Removed the reference to pooling samples and re-extracting samples.
6. Updated reagents and vendor catalog numbers
7. Updated DNA extraction steps to reflect Qiagen Mini Kit handbook
8. Added a reagent preparation section.
9. Added instruction for processing buccal cells.

C. Version 1, Effective Date 9/14/2012 - New

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Standard Operating Procedure (SOP) for DNA Extraction With QIAamp MINI Kit

Signatures

Approved By: Signature on file
Julie Gastier-Foster, PhD, FACMG
Principal Investigator

Date: Date on file