



## **CORE 2: PROTOCOL**

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The Blood and Tissue Procurement Core Facility at UNC is located within the BioSpecimen Processing (BSP) Facility, with laboratory space in the Hooker Research Laboratory Building (3-213). The Blood and Tissue Procurement Core is a Shared Core Facility that performs DNA isolation from clinical specimens for genotyping studies.

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### **A. STANDARD PROTOCOL FOR SPECIMEN HANDLING BY THE BLOOD AND TISSUE PROCUREMENT CORE**

#### **I. Overview of Specimen Collection and Transport to the Blood and Tissue Procurement Core Located within the BioSpecimen Processing Facility at UNC and the Blood and Tissue Procurement Core Facilities at LSU:**

Blood and tissue samples will be collected by the Consortium Nurses at the time of the in home interview of study subjects using specimen collection kits prepared by Core 1 at UNC and at LSU. The components of the blood and tissue specimen collection portion of the interview kit will include:

- Three yellow topped vacutainers (8.5 ml glass tubes),
- One red topped vacutainer (10 ml glass tube),
- One lavender topped vacutainer (6 ml plastic tube),
- Two screw cap cryovials for adipose tissue (3.6 ml cryovials for Luer-lock hub and needle storage),
- Two screw cap conical tubes (15 ml) for urine (one containing 20 mg of crystalline ascorbic acid and one with no preservative added),
- One small manila envelope for toenail clippings,
- One red topped 15 ml Vacutainer tube, 15 gauge needle, and Luer-lock hub for collection of fat aspiration specimen,
- One screw cap tube with 3.7 ml of sterile normal saline solution containing BHT (10µl of 0.5% BHT in ethanol), for flushing needle and hub used in the fat aspiration, and
- One 100 ml Kendall specimen jar and one 44ml. bottle of Scope mouthwash (LSU only) for collection of a buccal mouth rinse specimen from those patients that refuse or are not eligible for a blood draw.

NOTE: UNC Subjects that either did not have blood drawn, or for some other reason DNA was not obtained, will be re-contacted at the end of the UNC portion of the study to seek consent for providing a mouth rinse sample. If the study subject consents, a specimen jar, Scope mouthwash, and all other appropriate packaging and mailing materials will be sent to them for a home collection.

The three yellow-topped tubes (ACD solution) will be processed to provide plasma, DNA, and immortalized white blood cells. The red-topped tube (no additives) will be processed to provide serum. The lavender-topped tube (EDTA) will be processed to provide plasma and packed red blood cells. The buccal mouth rinse will be processed to provide DNA. The yellow-topped tubes are collected, transported, and stored at ambient temperature in Styrofoam containers inside insulated lunch boxes prior to processing. The mouth rinse and toenail specimens will be transported and stored at ambient temperature prior to processing. The lavender-topped tubes, red-topped tubes, adipose tissue specimens and urine specimens are collected, transported and stored on ice, or at 4°C, prior to processing.

## **II. Collection, transport and short-term storage of specimens:**

**1. Yellow-topped vacutainers (three, 8.5 ml glass tubes).** The yellow-topped vacutainers will be collected, transported and stored at ambient temperature until delivered to the BioSpecimen Processing Facility (BSP) at UNC. The processing of the yellow-topped vacutainers optimally will be performed within 24 hrs of collection. The Consortium Nurse will log the specimens into the Specimen Tracking System at the Consortium Office upon returning to the Consortium Central Office at LSU or UNC.

a. LSU: After log-in, the specimens will be placed into an approved shipping container and shipped at ambient temperature to the BioSpecimen Processing Facility at UNC by FedEx, or other overnight carrier. It is important that the specimen make the evening shipment on the day of collection.

b. UNC: After log-in at the UNC field office or the UNC BSP facility, the specimens will be stored at ambient temperature overnight. The tubes will be transported from the Consortium Central Office to the BSP by a contract courier service each morning.

c. BSP at UNC: Upon receipt of the three yellow topped vacutainers from LSU, by overnight carrier, a UNC nurse, or from the UNC Consortium Central Office, by courier, a BSP technician will log the specimens into the Specimen Tracking System:  
-Two of the tubes will remain in the BSP for processing for DNA and plasma.  
-One of the tubes will be transported by the BSP Technician to the Tissue Culture Facility (TCF), for processing to immortalized Peripheral Blood Mononuclear Cells (PBMC) and packed red blood cells (RBC). If sample is delivered from the BSP to the TCF by 2 PM, the immortalization protocol will begin that day.

**2. Red topped vacutainer (one, 10 ml glass tube, foil wrapped).** The red-topped vacutainer will be collected, transported and stored on ice/at 4°C until processing at LSU or UNC. The processing of the red-topped vacutainers optimally will be performed within 24 hrs of collection. The Consortium Nurse will log the specimens into the Specimen Tracking System at the Consortium Office upon returning to the Consortium Central Office at LSU or UNC.

a. LSU: After log-in, the specimens will be stored on ice/at 4°C until transported to the Blood and Tissue Procurement Core Laboratory at LSU for processing to serum. The specimens will be logged into the specimen tracking system upon receipt in the Blood and Tissue Procurement Core Laboratory and stored at 4°C until processing.

b. UNC: After log-in, the specimens will be stored on ice/at 4°C until transport. The tubes will be transported on ice/at 4°C directly to the BSP by a consortium nurse, or will be transported from the Consortium Central Office to the BSP by a contract courier service. The specimen will be logged into the specimen tracking system upon receipt in the BSP and stored on ice/at 4°C until processing.

**3. Lavender-topped vacutainer (one, 6 ml glass tube, foil wrapped).** The lavender-topped vacutainer will be collected, transported and stored on ice/at 4°C until processing at LSU or UNC. The processing of the lavender-topped vacutainers optimally will be performed within 24 hours of collection. The Consortium Nurse will log the specimens into the Specimen Tracking System at the Consortium Office upon returning to the Consortium Central Office at LSU or UNC.

a. LSU: After log-in, the specimens will be stored at 4°C until transported on ice to the Blood and Tissue Procurement Core Laboratory at LSU for processing for plasma and packed RBC. The specimen will be logged into the specimen tracking system upon receipt in the Blood and Tissue Procurement Core Laboratory and stored at 4°C until processing.

b. UNC: After log-in, the specimens will be stored at 4°C until transported to lab. The tubes will be transported on ice directly to the BSP by a consortium nurse or will be transported from the Consortium Central Office to the BSP by a contract courier service. The specimen will be logged into the specimen tracking system upon receipt in the BSP and stored at 4°C until processing.

**4. Adipose Tissue Aspirates** will be collected in two cryovials (3.6 ml, foil wrapped). The adipose tissue aspirate specimens will be collected, transported and stored on ice/4°C until logged in at LSU or UNC. The Consortium Nurse will log the specimens into the Specimen Tracking System at the Consortium Office upon returning to the Consortium Central Office at LSU or UNC.

a. LSU: After log-in, the cryovials will be stored at 4°C until transported on ice to the Blood and Tissue Procurement Core Laboratory at LSU. The specimens will be logged into the specimen tracking system upon receipt in the Blood and Tissue Procurement Core Laboratory and stored at -80°C until batch shipped to the BSP at UNC.

b. UNC: After log-in, the cryovials will be stored at 4°C until transported to lab. The cryovials will be transported on ice directly to the BSP by a consortium nurse, or will be

transported from the Consortium Central Office to the BSP by a contract courier service. The specimens will be logged into the specimen tracking system upon receipt in the TPF and stored at -80°C until transfer to Project 3.

**5. Urine Specimen in Screw-Cap Centrifuge Tube (two, 15 ml plastic, foil wrapped).**

Urine specimens will be collected into a specimen cup, and 10 mls aliquoted into each of two separate 15 ml conical tubes, one tube containing 20mg of a preservative (ascorbic acid in crystalline form). The Consortium Nurse will repeatedly invert the capped urine tube containing ascorbic acid until the ascorbic acid crystals are dissolved fully. The urine specimen tubes will be placed on ice, and transported and stored on ice/at 4°C, until logged in at LSU or UNC. The Consortium Nurse will log the specimens into the Specimen Tracking System at the Consortium Office upon returning to the Consortium Central Office at LSU or UNC.

a. LSU: After log-in, the conical tubes will be inverted again to ensure dissolution of ascorbic acid crystals, and stored at 4°C until transported on ice to the Blood and Tissue Procurement Core Laboratory at LSU. The specimens will be logged into the specimen tracking system upon receipt in the Blood and Tissue Procurement Core Laboratory and stored at -20°C.

b. UNC: After log-in, the conical tubes will be stored at 4°C overnight. The conical tubes will be transported on ice directly to the BSP by a consortium nurse or will be transported from the Consortium Central Office to the BSP by a contract courier service. Upon receipt in the TPF, the specimen will be inverted again to ensure dissolution of ascorbic acid crystals, logged into the specimen tracking system and stored at -20°C.

**6. Toenail Specimen in a cryovial (2.0 ml).** The toenail specimen will be collected, transported and stored at ambient temperature until storage at LSU or UNC. The Consortium Nurse will log the specimens into the Specimen Tracking System at the Consortium Office upon returning to the Consortium Central Office at LSU or UNC. Alternatively, the toenail specimen will be mailed directly to the BSP by the study subject after harvesting by a podiatrist.

a. LSU: After log in, the cryovial will be stored at ambient temperature until transported to the Blood and Tissue Procurement Core Laboratory at LSU. The specimen will be logged into the specimen tracking system upon receipt in the Blood and Tissue Procurement Core Laboratory and stored at ambient temperature until shipped to UNC.

b. UNC: After log-in, the cryovial will be stored at ambient temperature overnight. The cryovial will be transported at ambient temperature directly to the BSP by a consortium nurse or will be transported from the Consortium Central Office to the BSP by a contract courier service, or mailed later directly by the subject, or the toenail specimen will arrive in the mail. The specimen will be logged into the specimen tracking system upon

receipt in the BSP, transferred from the brown envelope to a cryovial, and stored at ambient temperature.

7. LSU prospective **Scope Mouth rinse (one, 100 ml plastic Kendall Specimen Jar).**

The specimen jars containing a mouthrinse specimen if collected, will be transported and stored at ambient temperature until delivered to the BioSpecimen Processing Facility (BSP) at UNC. The initial processing of the mouth rinse sample optimally will be performed within 24 hrs of collection.

a. LSU: The specimen will be placed into an approved shipping container and shipped at ambient temperature to the BSP at UNC by FedEx, or other overnight carrier. It is important that the specimen make the evening shipment on the day of collection.

b. BSP at UNC: Upon receipt of the specimen jar from LSU by overnight carrier, or by carrier after shipment by study participant in NC or LA, the BSP Technician will enter the sample into the Specimen Tracking System as follows:

- 1.) Log in to SPT
- 2.) Add
  - a. **Leave the date blank!**
  - b. Enter the subject ID, Processing lab = LSUfield, Location = LSUFO
  - c. Next, add from template dropdown = mouth rinse LSU
  - d. Save and Print label
- 3.) Update the location to LSUFIELD
- 4.) Checkin to LSU Field Office
- 5.) Enter the processing (collection) date and time that are on the handwritten label
  - a. Enter the volume as mls (optional at this point)
- 6.) Now treat it like any other sample you receive by mail from LSU
  - a. Check into TPF
  - b. Process-Centrifuge, etc

After log in, the mouth rinse will be transferred to a 50ml Autopure tube, centrifuged and the cell pellet frozen at -80°C until DNA extraction is performed on the Autopure Instrument.

### **III. Specimen Handling at the Blood and Tissue Procurement Core Facilities at UNC and LSU:**

#### **A. Overview:**

Processing of the yellow-topped vacutainers, or mouth rinses, for preparation of DNA, plasma, immortalized WBCs and packed RBCs will be performed at the BSP and TCF at UNC. Consequently, yellow-topped vacutainers, or mouth rinse specimen jars, collected in Louisiana will be shipped to UNC by overnight courier for processing in the BSP. Processing of red-topped vacutainers for preparation of serum specimens, and of

lavender-topped vacutainers for preparation of plasma and packed RBC specimens, will be performed in the Tissue Procurement Core Laboratories at LSU and in the BSP at UNC. The specimens prepared at LSU from processing the red-topped and lavender-topped vacutainers will be stored at  $-80^{\circ}\text{C}$ , and batch shipped on dry ice to UNC for long-term storage. The urine and adipose tissue specimens will be collected at the Blood and Tissue Procurement Core Laboratories at LSU and at UNC, stored at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ , respectively, and the specimens collected at LSU batch shipped on dry ice, to UNC for archiving. The toenail specimens will be collected at the Blood and Tissue Procurement Core Laboratories at LSU and at UNC, stored at ambient temperature, and specimens collected at LSU batch shipped at ambient temperature to UNC.

## **B. Processing and long-term storage of specimens:**

### **1. Yellow-topped vacutainers (three tubes: two for preparation of DNA and plasma, one for preparation of immortalized WBCs).**

The yellow-topped vacutainers will be received by overnight carrier, or by local courier, at the BSP at UNC, logged into the Specimen Tracking System, two vacutainers will be retained in the BSP for processing, and one vacutainer transported to the TCF for processing.

#### **a. BSP lab: Processing of DNA and plasma.**

Two 8.5 ml yellow-topped (ACD) vacutainer tubes received at ambient temperature will be processed to provide purified DNA and plasma specimens. Upon log-in upon receipt, bar-coded labels for the intermediate and final products are printed. DNA is extracted from the cell pellet recovered from the tubes by centrifugation and plasma removal. DNA is isolated from the cell pellet by one of three approaches that all utilize a modification of the PureGene chemistry employed in the automated DNA Purification Instrument present within the Shared Core Laboratory. Independent of the DNA extraction protocol utilized, the specimens are processed manually through the cell lysis step, and are maintained for at least 1 day before proceeding into any of the three DNA extraction protocols. The three protocols are: 1) the default protocol that will be used when 1-7 study subject samples are to be processed simultaneously by manual preparation of the cell pellets and lysates, with automated extraction of DNA on the AutoPure Instrument; 2) when more than 8 study subject specimens are to be processed simultaneously, DNA extractions will be performed by totally automated processing on the AutoPure instrument; or 3) when indicated, DNA will be extracted from individual specimens by an entirely manual technique.

The contents of the two yellow-topped tubes are mixed by inverting and are transferred into two 50 ml conical tubes. The 50 ml tubes are centrifuged at  $3250g$  for 10 min. at  $15^{\circ}\text{C}$ . The Plasma (supernatant) is removed with a sterile pipet, and aliquoted in equal volumes into 6 cryovials (i.e. if 3 ml of plasma is obtained, each of the six 2.0 ml cryovials will have 0.5 ml added) for long-term storage. The Plasma specimens are labeled with study subject and sample-type specific bar coded labels, and the specimens logged into the specimen tracking system in preparation for long-term storage. The finished Plasma specimens are stored at  $-80^{\circ}\text{C}$  until distributed to

the individual research projects for analysis. The Packed RBC pellet containing the mononuclear white blood cells is processed to provide the purified DNA specimens. The packed cell pellet is processed using PurGene DNA extraction chemistry either manually, or by automated methods, as described above. If samples are processed manually, the technician will follow the automated protocol as closely as possible to ensure consistency in processing techniques and yields. Purified DNA specimens will be aliquoted in cryovials in preparation for archiving as: seven aliquots of 5.0 µg (or 0.5 µg) of DNA, seven cryovials containing 10 µg (or 1.0 µg) of DNA, and one cryovial (stock sample) containing the remainder of the recovered DNA. The fifteen specimens of purified DNA are barcode labeled with study subject and sample-type specific bar coded labels, and the specimens logged into the specimen tracking system in preparation for long-term storage. The finished DNA specimens will be stored at –80°C until distributed to the individual research projects for analysis

b. Immortalization of WBCs and preparation of packed RBCs at the TCF at UNC

One 8.5 ml yellow-topped (ACD) vacutainer tube received at the BSP, and transported to the TCF at ambient temperature, will be processed to produce immortalized peripheral blood mononuclear cells (PBMC) and recovered packed Red Blood Cells (RBC). The content of the yellow-topped tube is mixed by inverting, PBS is added to bring the volume to a 1:2 dilution, and the contents mixed thoroughly. The specimen is slowly and carefully under-layered with 10 ml of Ficoll, and centrifuged at 400 x g for 30-40 minutes. The lymphocyte region of the Ficoll gradient is transferred to a 50 ml conical tube, PBS added to give a final volume of 30-45 ml, and the mixture centrifuged at 100 x g for 10 minutes. The WBC cell pellet is washed twice with PBS (30 ml), the cells re-suspended in 10 ml of PBS, counted and aliquoted for transformation (5-10 x 10<sup>6</sup> cells/specimen). If excess lymphocytes are recovered, extra PBMC are cryopreserved for additional transformation attempts, if required. The cell aliquot is recovered by centrifugation, the cell pellet re-suspended in 3.0 ml EBV supernatant and the cell suspension transferred to a T-25 flask. The cell/EBV mixtures are incubated overnight at 37°C in a 5% CO<sub>2</sub> incubator, and the following day transformation media is added to the cell suspension (7 ml of RPMI + 15% FBS + Cyclosporin 50 µg/ml) for expansion of the transformed cell population. When the transformed culture has expanded to a sufficient number, 6 cryovials of immortalized lymphocyte continuous lines (LCL) are cryopreserved in 1.0 ml of freezing media (3-5 x 10<sup>6</sup> cells/ml of RPMI-1640 + 15% FBS + 10% DMSO). One of these cryovials will be recovered for performance testing after two-three weeks in LN<sub>2</sub>. At the completion of the performance test, cells in the hold back flasks, and cells in the performance test flask(s), will be combined, expanded as appropriate to ~45 ml, and transferred to the BSP facility where DNA will be extracted and aliquoted (as described in section III.B.7). All samples will be processed at the TCF according to the above protocol, and will be appropriately marked as such for sample tracking purposes. The immortalized WBC will be stored in LN<sub>2</sub> until distributed to individual research projects, or for subsequent expansion by the Tissue Culture Facility (TCF). The packed red blood cell (RBC) pellet from the Ficoll gradient is recovered, and two (2) x 1.5 ml aliquots are transferred to cryovials (2 x 2.0 ml) and stored at –80°C.



## **2. Red topped vacutainers: (preparation of serum).**

The refrigerated foil-wrapped red-topped vacutainer will be received at the Blood and Tissue Procurement Core at LSU and the BSP at UNC, logged into the Specimen Tracking System, and the blood specimen processed for serum. Ideally, the specimen will be processed within 24 hr of collection. The vacutainer will be centrifuged for 10 min. at 3250g in a swinging bucket rotor to separate serum from coagulated blood. With a sterile pipette, the serum fraction is removed and aliquoted in equal volumes into 10 cryovials (i.e. if 5 ml of serum is obtained then each of the ten 2.0 ml cryovials will contain 0.5 ml) that are pre-labeled with patient and sample-type specific bar coded labels, and the aliquoted specimens logged into the specimen tracking system. Specimens processed at UNC will be placed directly into long-term storage in the Consortium freezers at  $-80^{\circ}\text{C}$ . Specimens collected and processed at LSU will be stored at  $-80^{\circ}\text{C}$  at LSU, and batch shipped on dry ice to the BSP at UNC for long-term storage. The Serum specimens are stored at  $-80^{\circ}\text{C}$  until distributed to the individual research projects for analysis.

## **3. Lavender-topped vacutainer (preparation of Plasma and packed RBC pellet).**

The refrigerated, foil-wrapped, lavender-topped vacutainer will be received at the Blood and Tissue Procurement Core at LSU and the BSP at UNC, logged into the Specimen Tracking System, and the blood specimen processed for Plasma and packed RBCs. Ideally, the specimen will be processed within 24 hr of collection. The vacutainer tube will be centrifuged at 3250g for 10min. using a swinging bucket rotor to allow separation of plasma and RBCs. After centrifugation of the vacutainer, the plasma fraction is removed with a sterile disposable pipette and aliquoted in equal volumes into six 2.0 ml cryovials (i.e. if 3.0 ml of plasma is obtained then each of the six 2.0 ml cryovials will have 0.5 ml) for long term storage. With a new sterile disposable pipette, the packed red blood cell pellet is removed, and divided equally into 2 cryovials. Specimens processed at UNC will be placed directly into long-term storage in the Consortium freezers, and specimens collected at LSU will be stored at  $-80^{\circ}\text{C}$  at LSU, and later batch shipped on dry ice to the BSP at UNC, for long-term storage. The plasma and packed RBC specimens are stored at  $-80^{\circ}\text{C}$  until distributed to the individual research projects for analysis.

## **4. Urine (two 15 ml conical, screw capped centrifuge tubes).**

The refrigerated, foil-wrapped, conical centrifuge tubes containing urine will be received at the Blood and Tissue Procurement Core at LSU and the BSP at UNC, and logged into the Specimen Tracking System. Ideally, the specimen will be processed within 24 hr of collection. The conical tube with ascorbic acid preservative will be inverted and mixed to ensure that the ascorbic acid is completely dissolved. Specimens processed at UNC will be placed directly into long-term storage in the Consortium freezers ( $-20^{\circ}\text{C}$ ), specimens collected at LSU will be stored at  $-20^{\circ}\text{C}$  at LSU, and batch shipped on dry

ice to the TPF at UNC for long-term storage. The urine specimens are stored at  $-20^{\circ}\text{C}$  until distributed to the individual research projects for analysis.

### **5. Adipose Tissue (two 3.6 ml cryovials).**

Two refrigerated, foil-wrapped, cryovials containing the adipose tissue specimen will be received at the Blood and Tissue Procurement Core at LSU and the TPF at UNC. One cryovial (3.6 ml cryovial) will contain the needle used in the aspiration and the other cryovial (3.6 ml cryovial) will contain the Luer-lock hub that contains additional adipose tissue. The two specimens will be logged into the Specimen Tracking System, and the adipose tissue specimen will be frozen at  $-80^{\circ}\text{C}$ . Ideally, the specimen will be received by the core facilities within 24 hr of collection. At both LSU and UNC, the cryovials containing the adipose tissue aspirate, labeled with study subject and sample-type specific bar coded labels, is logged into the specimen tracking system and stored in a freezer at  $-80^{\circ}\text{C}$ . Specimens processed at UNC will be placed directly into long-term storage in the Consortium freezers ( $-80^{\circ}\text{C}$ ), specimens collected at LSU will be stored at  $-80^{\circ}\text{C}$  at LSU, and batch shipped on dry ice to the BSP at UNC for long-term storage. The adipose tissue aspirate specimens are stored at  $-80^{\circ}\text{C}$  until transfer to the Project 3.

### **6. Toenails (one 2.0 ml cryovial).**

The cryovial containing toenails will be received at the Blood and Tissue Procurement Core at LSU and the BSP at UNC, logged into the Specimen Tracking System, and the toenails stored at ambient temperature. At both LSU and UNC, the cryovial containing the toenails that was pre-labeled with study subject and sample-type specific bar coded labels, is logged into the specimen tracking system and stored at ambient temperature. Specimens processed at UNC will be placed directly into long-term storage, specimens collected at LSU will be stored at LSU, and batch shipped at ambient temperature to the BSP at UNC for long-term storage. The toenail specimens are stored at ambient temperature until distributed to the individual research projects for analysis.

### **7. Genomic DNA from immortalized WBCs.**

Immortalized cells will be placed in culture in a T75 flask in the TCF and flask picked up by the Blood and Tissue Procurement Core technician when the culture has achieved appropriate cell density. The culture media will be centrifuged at 3000g for 3 minutes, the supernatant of culture media decanted, and the pellet or immortalized PBMCs stored at  $-80^{\circ}\text{C}$ . Cell pellets will be accumulated until samples from eight study participants have been collected, the cell pellets will be thawed at  $37^{\circ}\text{C}$ , and the cells processed for DNA on the AutoPure Instrument with the Cultured Cell Pellet program. Purified DNA will be aliquoted at 100ng/ul in 18 cryovials: 9 cryovials will be archived in one freezer box, 9 cryovials in a second freezer box, and a stock tube containing the remaining DNA will be stored in a third box of stock DNA specimens. These will be stored at  $-80^{\circ}\text{C}$  until distributed to individual research projects.

## **8. Mouth rinse specimen (one specimen containing ~50mls of mouthrinse) for preparation of DNA.**

The mouth rinse specimen jar will be received by overnight carrier at the BSP at UNC, logged into the Specimen Tracking System, the cell pellet collected in a 50-ml tube and cells frozen at -80°C until DNA extraction.

### **a. BSP lab: Preparation of Pellet and Extraction of DNA.**

One 100 ml specimen jar containing a mouth rinse specimen received at ambient temperature will be processed by centrifugation to a cell pellet in a 50 ml tube, and the pellet frozen at -80°C until DNA extraction is performed on the cell pellet. Upon log-in at the time of receipt, bar-coded labels for the intermediate and final products are printed. DNA is isolated from the cell pellet using automated extraction of DNA on the AutoPure Instrument in batches of eight, or manually using a protocol and reagents comparable to the Puregene instrument.

### **C. Summary of specimens to be collected by nurses:**

- Yellow Top Tube (8.5 ml) 3 X 8.5 ml
- Purple Top (6 ml) 1 X 6 ml
- Red Top (10 ml) 1 X 10 ml
- 41.5 ml total

Urine: in 15 ml tubes, 1 with preservative 2 X 10 ml

Fat: in two 3.6 ml tubes, 1 with preservative, 1 in Luer-lock hub 2 X 3.6 ml

Toenails: in envelope or 2 ml cryovials 1 X 2.0 ml

Optionally a mouth rinse sample will be collected in a 100ml specimen jar if no yellow top blood tubes are collected.

### **D. Schedule for batch shipments from LSU to UNC:**

Batch shipping of samples per subject:

- Six - 2.0 ml cryovials lavender top plasma
- Two - 2.0 ml cryovials lavender top packed red blood cell
- Ten - 2.0 ml cryovials red top serum
- Two - 15 ml conicals containing urine (one with ascorbic acid)
- Two - 3.6 ml cryovials containing adipose tissue, one containing the aspiration needle one containing the Luer-lock hub
- One - 2.0 ml cryovial containing toenails

Ship to:  
Patricia Basta 919-966-7738  
Michael J. Hooker Research Center  
UNC-Chapel Hill  
Room 3-213, BSP Facility  
CB# 7406  
Chapel Hill, NC 27599-7304  
Proposed Shipping Dates by Federal Express: the first Tuesday of every month

#### **IV. Protocols for PCaP Specimen Handling at LSU**

##### **A. Principle**

The collection of consistent human blood samples for DNA, serum, plasma, packed RBCs; adipose tissue; urine; toenails, and potentially mouth rinse specimens at the LSU and UNC sites requires that collection, storage, transport and handling of the biological specimens of the study subjects adhere to rigid guidelines to achieve optimal results.

##### **B. Materials List:**

- Gloves
- Red top 10 ml glass vacutainer tube (1), no additive, no clot activator with uncoated interior (Fisher Scientific, cat. # 0 2685 112).
- Yellow top 8.5 ml glass vacutainer tubes (3), additive acid citrate, dextrose solution (Fisher Scientific)
- Lavender top 6 ml glass vacutainer tube (1), additive EDTA (Fisher Scientific, cat. # 02 683 99d)
- NUNC cryovials, 3.6 ml (2) for adipose tissue (Fisher Scientific (cat. # 05 669 59)
- 100 ml Kendall specimen jar
- 44ml bottle of Scope mouthwash
- Centrifuge with swinging bucket rotor (Beckman Allegra 6R )centrifuge or equivalent
- 10 ml Sterile disposable serological Costar pipette (cat# 07 200 11)
- 5 ml Sterile disposable serological Costar pipette (cat# 07 200)
- Nalgene polypropylene cryovials, 2 ml (cat# 03 337 7D)
- Brady brand labels (cat# PTL 76-461)

##### **C. Procedures**

- Scan specimens into the specimen tracking system as instructed by the SPT instruction guide.
- Follow the SPT instruction guide “Process Wizard” using the update?/process? wizards to make labels for 24 specimens according to the schema.

	TPF 043xxx
	CAPxxx
	0300BL
	04/21/2004
	YELLOWTOP

- Place the adipose specimens in the -80°C freezer .
- Place the two urine specimens (one with preservative, one without) in the -80°C freezer
- Place the cryovial containing the toenails in storage at room temperature.
- Put the 10 ml red topped vacutainer and the 6 ml lavender topped vacutainer in the centrifuge to spin at 3250g for 10 minutes at 4°C.
- Put labels on aliquot tubes while waiting for the centrifuge to finish, organize tubes according to study subject.
- Take red topped and lavender topped tubes out of the centrifuge
- Withdraw the plasma from the lavender top tube with a 5 ml. pipette, aliquot equal volumes of plasma into 6 cryovial tubes with study subject and specimen specific labels, record volume, store at -80°C.
  - With a new 5 ml. Pipette, withdraw the red blood cell pellet and divide the volume equally into 2 cryovial tubes with study subject and specimen specific labels, record volume, store at -80°C.
- Withdraw the serum from the red top tube with a 5 ml pipette, aliquot equal volumes into 10 cryovial tubes with study subject and specimen specific labels, record volume, and store at -80°C.

## V. Protocols for PCaP Specimen Handling at UNC

### A. Principle

The collection of consistent human blood samples for DNA, serum, plasma, packed RBCs; adipose tissue; urine; toenails, and potentially mouth rinse specimens requires at the LSU and UNC sites requires that collection, storage, transport and handling of the biological specimens of the study subjects adhere to rigid guidelines to achieve optimal results.

### B. Materials List:

- Gloves
- Red top 10 ml glass vacutainer tube (1), no additive, no clot activator with uncoated interior (Fisher Scientific, cat. # 0 2685 112).
- Yellow top 8.5 ml glass vacutainer tubes (3), additive acid citrate, dextrose solution (Fisher Scientific)
- Lavender top 6 ml glass vacutainer tube (1), additive EDTA (Fisher Scientific, cat. # 02 683 99d)
- NUNC cryovials, 3.6 ml (2) for adipose tissue (Fisher Scientific (cat. # 05 669 59)

- 100 ml Kendall specimen jar
- 44ml bottle of Scope mouthwash
- Centrifuge with swinging bucket rotor (Beckman Allegra 6R )centrifuge or equivalent
- 10 ml Sterile disposable serological Costar pipette (cat# 07 200 11)
- 5 ml Sterile disposable serological Costar pipette (cat# 07 200)
- Nalgene polypropylene cryovials, 2 ml (cat# 03 337 7D)
- Brady brand labels (cat# PTL 76-461)

### C. Procedures

- Scan specimens into the specimen tracking system as instructed by the SPT instruction guide.
- Follow the SPT instruction guide “Process Wizard” using the update?/process? wizards to make labels for 24 specimens according to the schema.

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- Place the adipose specimens in the -80°C freezer.
- Place the two urine specimens (one with preservative, one without) in the -80°C freezer.
- Place the cryovial containing the toenails storage at room temperature.
- Invert the two yellow tops gently 5 times.
- Pour the contents of the two yellow-topped tubes into two separate 50 ml tubes;
- Put the two 50 ml tubes, the 10 ml red topped vacutainer and the 6 ml lavender topped vacutainer in the centrifuge to spin at 3250g for 10 minutes at 4°C.
- Put labels on aliquot tubes while waiting for the centrifuge to finish, organize tubes according to study subject.
- Take two 50 ml tubes, the red topped Vacutainer and the lavender topped vacutainer out of the centrifuge
- Process the two 50 ml tubes for isolation of DNA (see below)
- Withdraw the plasma from the lavender top tube with a 5 ml. pipette, aliquot equal volumes of plasma into 6 cryovial tubes with study subject and specimen specific labels, record volume, store at -80°C.
  - With a new 5 ml Pipette, withdraw the red blood cell pellet and divide the volume equally into 2 cryovial tubes with study subject and specimen specific labels, record volume, store at -80°C.
- Withdraw the serum from the red top tube with a 5 ml pipette, aliquot equal volumes into 10 cryovial tubes with study subject and specimen specific labels, record volume, and store at -80°C.

- If collected, transfer the contents of the specimen jar to a 50ml tube and centrifuge to collect the cell pellet. Freeze pellet in the tube at -80°C until processing to DNA either using an automated method on the Autopure, in batches of 8, or a manual method. Both methods use the Puregene high-salt DNA extraction chemistry.

## **1. Manual Technique for DNA Isolation from Yellow-Top Blood Collection Tube**

### **Isolation and Lysis of WBCs**

- Remove 50 ml tubes from centrifuge.
- With a new 5 ml pipette withdraw the plasma from the two 50 ml tubes, aliquot equal volumes into the 6 cryovials with study subject and specimen specific labels, record volume and store at -80°C
- Add 35 ml RBC Lysis Solution to the pellets, invert 20X by hand or put on a Nutator to mix, and let sit at room temperature for 6.5 minutes.
- Spin 50 ml tubes at 3000g for 2 minutes.
- Pour off supernatant of RBC lysis solution.
- Vortex pellet
- Add 10 ml WBC Lysis Solution with a 10 ml pipette; reflux slurry up-and-down in pipette 5X, or vortex for 20 seconds, to initiate cell lysis.
- Let sit at room temperature (if clumped, incubate in a 37°C water bath.) to complete cell lysis; can sit overnight at this step.

### **Protein Precipitation**

- Add 3.33 ml of Protein Precipitation Solution to the lysed WBC pellet
- Vortex 20 seconds at high speed
- Spin at 2000g for 2 minutes.
- Set up 50 ml conical tubes with 10 mls of 100% Isopropanol

### **DNA Precipitation**

- Pour supernatant of protein precipitation step into the Isopropanol
- Invert 50X and look for a white, thread like precipitate
- Spin at 3000g for 2 minutes.
- Pour off supernatant, drain
- Add 10 mls of 70% Ethanol, invert 10X
- Spin at 2000g for 3 minutes.
- Carefully pour off ethanol, be careful to NOT disturb pellet
- Drain tube and air dry (10 to 60 minutes)

## **DNA Hydration**

- Add 1 ml of DNA Hydration Solution to each 50 ml conical and rehydrate DNA at 55°C for 1 hour to overnight, can be nutated overnight (or longer) at room temperature.
- Combine rehydrated DNA in one conical tube in a total of 2 ml.
- Store rehydrated DNA at 4°C until optical density reading and gel electrophoresis evaluation of integrity are performed.

## **2. Manual/Automated Technique of DNA Extraction from Yellow-Top Blood Collection Tube**

- Remove 50 ml tubes from centrifuge.
- With a new 5 ml pipette withdraw the plasma from the two 50 ml tubes, aliquot equal volumes into the 6 cryovials with study subject and specimen specific labels, record volume and store at -80°C
- Add 35 ml RBC Lysis Solution to the pellets, invert 20X by hand or put on a Nutator to mix, and let sit at room temperature for 6.5 minutes.
- Spin 50 ml tubes at 3000g for 2 minutes.
- Pour off supernatant of RBC lysis solution.
- Vortex pellet
- Add 10 ml WBC Lysis Solution with a 10 ml pipette; reflux slurry up-and-down in pipette 5X, or vortex for 20 seconds, to initiate cell lysis.
- Let sit at room temperature (if clumped, incubate in a 37°C water bath.) to complete cell lysis; can sit overnight at this step.
- After completion of cell lysis, place samples on the AutoPure Instrument to perform automated DNA extraction
- Log onto AutoPure LS
- Enter rack set-up menu.
- Choose Cell Lysate-DNA protocol. (The automated cell lysate protocol will be identical to the steps after the room temperature incubation in cell lysis buffer.)
- Choose correct sample volume (5.1-10 ml).
- Choose number of tubes (8 or 16).
- Choose re-hydration volume (1 ml for PCaP samples).
- Enter run name (run# and date)
- Enter rack number
- Type or scan specimen ID or BSP # in first pink-top tube
- Scan input (pink-topped cell lysis tubes) and output (blue-topped tubes).
- Save rack information
- Place rack on machine
- Press "Start Run"
- After run has finished, remove Output Tubes and incubate at 55°C overnight (alternatively, incubate at 65°C for 1 hour) followed by an overnight incubation at room temperature (RT).
- Evaluate DNA solution after overnight incubation, if DNA is not in solution more rehydration buffer may be added followed by additional incubations at 55°C/65°C and/or room temperature



- Combine rehydrated DNA into one conical for a total of 2.0 ml of rehydrated DNA.
- Room temperature incubation may be performed on rotator set on a speed of 25-30.
- Store rehydrated DNA at 4°C until optical density reading and gel electrophoresis evaluation of integrity are performed.

### **3. Completely automated DNA extraction from Yellow-Top Blood Collection Tube**

- Take 50 ml conicals out of centrifuge.
- With a new 5 ml pipette withdraw the plasma from the two 50 ml conical tubes, aliquot equal volumes into the 6 plasma cryovial tubes, record volume and store at -80°C
- Log on to AutoPure LS
- Enter rack set-up menu.
- Choose either cell lysate DNA preparation protocol.
- Choose sample volume 5.1-10 mls.
- Choose number of tubes (8 or 16).
- Choose re-hydration volume (1.0 ml for PCaP samples).
- Enter run name (run# and date)
- Enter rack number
- Scan input (pink-topped tubes with RBC) and output (blue-topped tubes). Type or scan subject ID or BSP # in first pink-top tubes window
- Place rack on machine
- Press Start Run
- When run is finished remove tubes from machine, briefly vortex vigorously, and store at RT from 1-7 days.
- After incubation at RT to solubilize DNA, place samples back on the AutoPure as described above and run the Cell Lysis DNA extraction protocol.
- After run has finished, remove Output tubes, and incubate tubes at 55°C overnight (alternatively, incubate at 65°C for 1 hour, followed by overnight incubation at room temperature (RT)).
- Evaluate DNA solution after overnight incubation, if DNA is not in solution more rehydration buffer may be added, followed by additional incubations at 55°C/65°C and/or room temperature
- Combine rehydrated DNA in one conical tube, for a total of 2 ml of rehydrated DNA.

### **4. DNA Isolation From Immortalized WBC**

- Fresh cultures of WBCs will be obtained from the TCF in T75 flasks
- Transfer WBCs in media to 50 ml tubes
- Log on to AutoPure LS
- Enter rack set-up menu
- Choose appropriate fields and protocol for extraction from immortalized cells in media
- The automated extraction protocol entails the following steps that can be performed manually, if necessary:
  - o Cells in media will be centrifuged for 4 minutes at 4°C to pellet the cells

- o Decant the media into a waste container
- o Dispense 1.0 ml (0-10 million cell protocol), 3.0 ml (10-20 million cell protocol), 6.0 ml (20-50 million cell protocol) or 12.0 ml (50-150 million cell protocol) of Cell Lysis Solution into each input tube.
- o Mix samples vigorously for 2 minutes to lyse the cells.
- o Incubate the samples for 15 minutes at room temperature.
- o Centrifuge samples at 3,000g for 2 minutes to collect all liquid at the bottom of the tube.
- o Dispense 2.0 ml (20-50 million cell protocol) of Protein Precipitation Solution to each sample.
- o Mix the samples vigorously for 2 minutes to precipitate proteins.
- o Centrifuge the samples at 3,000g for 15 minutes to pellet the proteins.
- o During centrifugation, 6.0 ml (20-50 million cell protocol) of Isopropanol is dispensed into the output tubes. Pour the supernatant that contains the DNA- into the output tubes containing Isopropanol
- o Rotate the output tubes gently 50 times to precipitate the DNA.
- o Centrifuge the samples at 3,000g for 5 minutes to pellet the DNA.
- o Pour the Isopropanol supernatant into waste and drain the output tubes for 1 minute
- o Dispense 6.0 ml (20-50 million cell protocol) of 70% Ethanol into output tubes
- o Centrifuge the samples at 3,000g, pour the ethanol supernatant to waste and drain the output tubes for 1 minute to evaporate any remaining alcohol
- o Dispense the set volume of DNA Hydration Solution into the output tubes, and incubate at 65°C for 1-2 hours, and at room temperature overnight
- o Store at 4°C until optical density reading and gel electrophoresis evaluation.

#### **4. DNA Isolation from Mouth rinse cell pellets**

##### Manual Method

##### Lysis

- Thaw cell pellets at 37°C.
- Add 2.0 ml Cell Lysis Buffer and 10µl RNase A to all the samples. (Note: Cell Lysis Solution containing RNase A is made up fresh as a batch in the amount needed for the number of tubes to be processed that day).
- Incubate sample for 15 minutes at room temperature. (Note: preparation can be kept for up to two years at this step). If after 15 minutes there are still clumps, break them up if possible with a pipette prior to adding the proteinase K.
- Add 10 µl of Proteinase K Solution (20 mg/ml) to the cell lysate and invert 50 times to mix.
- Incubate lysate for 10 minutes at 65°C.

##### Protein Precipitation

- Add 660 µl Protein Precipitation Solution to the cell lysate.
- Vortex samples at high speed for 10 seconds to mix the Protein Precipitation Solution uniformly with the lysate.
- Place in an ice bath for 10 minutes to ensure a tight pellet in the following centrifugation step.

- Centrifuge at 3,000 x g for 10 minutes at 4°C. The precipitated proteins should form a tight pellet. Repeat centrifugation if pellet is loose.
- To ensure the protein pellets remain tight, keep samples on ice while transferring supernatant into an output tube containing the isopropanol.

#### DNA Precipitation

- Pour the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 15 or 50 ml output tube containing 2.0 ml 100% isopropanol (2-propanol) and 13.3µl Glycogen Solution (20 mg/ml).
- Mix the sample by inverting gently 50 times.
- Centrifuge at 3,000 x g for 5 minutes at 4°C. The DNA may, or may not, be visible as a small white pellet, depending on yield.
- Pour off the supernatant and drain tube briefly on clean absorbent paper. Add 1.0 ml 70% Ethanol and shake the tube gently to wash the DNA pellet.
- Centrifuge at 3,000 x g for 2 minutes. Carefully pour off the Ethanol as DNA pellet may be loose.
- Invert and drain the tube on clean absorbent paper and allow to air dry 1 minute.

#### DNA Hydration

- Add 500-2000 µl of DNA Hydration Solution (based on the size of the initial cell pellet and the final DNA pellet).
- Rehydrate DNA by incubating at 65°C for 1 hour followed by overnight incubation at room temperature (RT).
- Evaluate DNA solution after overnight incubation; if DNA is not in solution more rehydration buffer may be added, followed by additional incubation at 65°C and/or RT
- RT incubation may be performed on rotator set on a speed of 25-30.
- After hydration, combine any samples that had been split due to a large volume at the initial cell pelleting step.
- For storage, vortex sample briefly, pulse spin, and transfer to a 1.5 ml tube.
- Place DNA at 4°C prior to quantitation and short term storage. For long-term storage, store at -80°C.

#### Autopure Automated Method

- Remove frozen pellets in batches of 8 from -80°C freezer. Thaw cell pellets at 37°C.
- Add 13.3 µl of glycogen to OutPut Qubes
- Log-on to AutoPure LS
- Place Proteinase K reagent on machine
- Enter rack set-up menu.
- Choose either “mouthwash” protocol or “extended spin” (check name) protocol.
- Choose samples volume 10 or 40 mls (note: the “mouthwash” protocol only has a 10 ml choice, but this can handle anything from pellets to a 10 ml volume). Choice of protocol depends on sample volume (anything over 10 mls must be performed using the “extend spin” protocol). If the highest throughput is needed, (for pellets -10ml volumes) the “mouthwash” protocol should be used.
- Choose number of tubes (8 or16).

- Choose re-hydration volume (200-1000µl for saline rinses or 500-2000 µl for Scope rinses). Large-volume initial sample require larger the hydration volumes.
- Enter run name
- Enter rack number
- Scan input and output qubes
- Place rack on machine
- Press start run
- The automated extraction protocol entails the following steps that can be performed manually, if necessary:
  - Dispense 1.0 ml Cell Lysis Solution (containing 5µl RNase A @ 4 mg/ml) to the cell pellet.
  - Mix samples gently and incubate the samples for 15 minutes at room temperature.
  - Dispense 10 µl Proteinase K solution into each input tube to complete lysis.
  - Mix sample vigorously for 2 minutes, followed by a 10 minute room temperature incubation to complete lysis.
  - Dispense 0.33ml of Protein Precipitation Solution to each sample.
  - Mix the samples vigorously for 2 minutes to precipitate proteins.
  - Centrifuge the samples at 3,000g for 10 minutes to pellet the proteins.
  - During centrifugation, 1.0 ml of isopropanol is dispensed into the output tubes.
  - Pour the supernatant that contains the DNA into the output tubes containing isopropanol.
  - Rotate the output tubes gently 50 times to precipitate the DNA.
  - Centrifuge the samples at 3,000g for 5 minutes to pellet the DNA.
  - Pour the isopropanol supernatant into waste and drain the output tubes for 1 minute
  - Dispense 1.0 ml of 70% Ethanol into the output tubes
  - Centrifuge the samples at 3,000 x g, for 1 minute at 10°C to pellet the DNA. Pour the ethanol supernatant to waste and drain the output tubes for 1 minute to evaporate any remaining alcohol
  - Dispense the set volume of DNA Hydration Solution (1.0 ml) into the output tubes, and incubate at 65°C for 1-2 hours, and at room temperature overnight
  - Store at 4°C until optical density reading and gel electrophoresis are performed.
  - Aliquot appropriately (same as for DNA extracted from blood) and freeze at -80°C.

## **VI. Storage Protocol for PCaP Specimens**

### **A. Overview of Long Term Storage for Specimens at the BioSpecimen Processing Facility at UNC**

Study participant specimens, with the exception of urine, will be stored in freezer containers with 81 wells. Consequently, samples of a single type of specimen from 81 randomized patients will be stored as a set, with the specimen from the same patient appearing in the same well in storage boxes for each sample type. This format will facilitate distribution of matched sets of specimens to consortium investigators, and facilitate linkage to the consortium data base. DNA extracted from immortalized PBMC will be stored in boxes with 81 wells, with nine specimens from a single study participant occupying all nine wells of a 9 row, in each of two matched boxes. The single DNA stock samples from immortalized PBMCs will be stored in sequential wells in the order processed.

After specimen processing, purified DNA and plasma from the yellow-top tubes, plasma and packed RBCs from the lavender-top tube, serum from the red-top tube, adipose tissue, urine with and without ascorbic acid, and toenails will be archived for long-term storage until specimen sets are requested by consortium, or outside investigators. Long-term storage boxes will be labeled with an **alphabetical** symbol that will represent a set of eighty-one subjects, as well as a **numerical** symbol that will represent the specimen contents (i.e. the very first box will be labeled A1 that will represent DNA specimens (5 µg/tube) of study subjects 1-81. The box numbers will correspond to Table 1 below. A typical freezer rack set-up is illustrated in Table 2 below. The inventory boxes will be placed directly in the -80°C and -20°C freezers. When placed in the respective freezers, the boxes will be placed into metal racking systems. Odd numbered boxes will be placed in racks with the number 1 (eg. 1-1, 1-2, 1-3, 1-4). Even numbered boxes will be placed in racks with the number 2 (eg. 2-1, 2-2, 2-3, and 2-4). The long-term storage freezers (-80°C) will be placed in two different buildings at UNC to minimize the risk of loss of specimens due to power outage. For specimen types for which there are multiple samples (i.e., 5.0 µg of DNA for which there will be seven identical boxes, plasma, serum, RBC, etc ), the boxes (sets A-S only) will be distributed between the two freezers by designation of 1 or 2 on the rack. For sets T, the end of the study, both sets of boxes will be stored in one freezer only.

## **B. Long term storage of DNA from Yellow-top Tubes or Mouth Rinse Samples**

After the yellow-top tubes have been received and processed, the DNA will be distributed as follows: seven cryovials containing 5.0 µg (or 0.5µg) of DNA, seven cryovials containing 10 µg (or 1.0 µg) of DNA, and one cryovial containing the remaining DNA recovered (stock). After aliquoting the DNA into the fifteen tubes, the specimens will be ready for long term storage in the PCaP designated freezer at the UNC BioSpecimen Processing Facility. The 5.0 µg (or 0.5µg) DNA cryovials will be placed in boxes labeled with an alphabetical symbol, designating subject set (in increments of 81 study subjects), and numbers from 1-7 designating 5.0 µg of DNA (i.e. A1, A2...A7). The 10 µg (or 1.0 µg) DNA cryovials will be placed in boxes labeled with an alphabetical symbol, designating subject set, and numbers 8-14 designating 10µg of DNA (i.e. A8, A9...A14). The cryovial containing the remaining stock DNA will be placed in a box

labeled with an alphabetical symbol, designating subject set, and number 15 designating the remaining stock DNA (i.e. A15). Inside each of the fifteen boxes the cryovial from a specific study subject will be placed in the same grid position, starting with the top left corner and ending with the bottom right corner. If for any reason a specimen is absent, the grid corresponding position designated for that specimen will be left open. The boxes will be placed in their appropriate freezer racks in the -80°C freezers.

### **C. Long term storage of DNA (I-DNA) from Immortalized PBMC**

After receipt from the TCF and processing, the resulting DNA will be aliquoted in to nineteen cryovials, eighteen containing 100ng/ml and the nineteenth with the remaining stock DNA. Nine of the cryovials containing 100 ng/ml will be placed in one row of an 81 well box and the other nine will be placed in a second box in an identical manner. The remaining “stock” DNA will be in an 81 well box. The box and position (and rack positions for those samples archived at UNC) locations of all samples will be recorded in the SPT for future usage. The DNA isolated from the Immortalized B-Cells are stored in the PCaP freezers as follows. The storage boxes are labeled with a number 1,2,3,4 followed by either A or B for the set. There are 18 aliquots for one sample of IDNA. The first 9 will aliquots will be placed in the box labeled as the A set and other 9 will go in second box labeled as the B set. Therefore, the boxes are labeled as 1-A, 1-B, 2-A, 2-B, etc. After labeling, the boxes will be placed in racks labeled as follows. The rack labeling will follow the pattern I-1A, I-1B, I-2A, I-2B, etc. All the set A boxes will be placed in a rack labeled I-# A and all the set B boxes will go in a rack labeled I-#B. Stock I-DNA boxes are labeled starting as follows IDNA-1, IDNA-2. These boxes are placed in the 40<sup>th</sup> position of regular racks of set B. The stock boxes are labeled as 2-5, 2-10, and 2-15. Stock I-DNA boxes will be archived at UNC in PCaP freezers# 2 and 3, all other I-DNA aliquots will be archived at Roswell Park under the direction of Dr. Gary Smith.

### **D. Long term storage of Plasma from Yellow-top tube**

After the yellow-top tubes have been received, processed, and the plasma equally distributed into 6 x 2 ml cryovials, the six tubes will be ready for long term storage in the PCaP designated freezer at the UNC BSP. The cryovials containing plasma will be placed in boxes labeled with an alphabetical symbol, designating study subject set, and numbers 16-21 designating plasma from yellow-top tubes (i.e. A16, A17...A21). Each of the six boxes will receive one cryovial of plasma from each study subject. Inside each of the six boxes the cryovial will be placed in the same grid position, starting with the top left corner and ending with the bottom right corner. If for any reason a specimen is absent the grid position corresponding to that specimen will be left open. The boxes will be placed in the appropriate rack in the -80°C freezer.

### **E. Long term storage of Serum from Red-top tube**

After the red top tube has been processed, and the serum equally distributed in 10 cryovials of approximately 2.0 ml, the serum specimens will be ready for long term storage in the PCaP designated freezer at the UNC Bio-Specimen Processing Facility. The samples processed at LSU will be stored at -80°C and batch shipped on dry ice to UNC BSP according to the shipping calendar. The specimens processed at UNC will go directly into long-term storage. The cryovials containing serum will be placed in boxes labeled with an alphabetical symbol, designating study subject set, and numbers 22-31 designating serum (i.e. A22, A23...A31). Each of the ten boxes will receive one cryovial of serum. Inside each of the ten boxes, the cryovials from a specific study subject will be placed in the same grid position, starting with the top left corner and ending with the bottom right corner. If for any reason a specimen is absent, the corresponding grid position belonging to that specimen will be left open. The boxes will be placed in the appropriate rack in the -80°C freezer.

### **F. Long term storage of Plasma from Lavender-top tube**

After the lavender-top tube has been processed, and the plasma equally distributed into 6 cryovials of approximately 2.0 ml, the plasma specimens will be ready for long term storage in the PCaP designated freezer at the UNC Bio-Specimen Processing Facility. The samples processed at LSU will be stored at -80 °C and batch shipped on dry ice to the BSP at UNC according to the shipping calendar. The specimens processed at UNC will go directly into long-term storage. The cryovials of plasma will be placed in boxes labeled with an alphabetical symbol, designating study subject set, and numbers 32-37 designating plasma from lavender-top tubes (i.e. A32, A33...A37). Each of the six boxes will receive one cryovial of plasma. Inside each of the ten boxes, the cryovial from a specific study subject will be placed in the same grid position, starting with the top left corner and ending with the bottom right corner. If for any reason a specimen is absent, the corresponding grid position belonging to that specimen will be left open. The boxes will be placed in the appropriate rack in the -80°C freezer.

### **G. Long term storage of packed Red Blood Cells from Lavender-top tube**

After the lavender-top tube has been processed, and the red blood cells equally distributed in to 2 x 2.0 ml cryovials, the packed RBC specimens will be ready for long term storage in the PCaP designated freezer at the UNC BSP. The samples processed at LSU will be stored at -80 °C and batch shipped on dry ice to UNC BSP facility according to the shipping calendar. The specimens processed at UNC will go directly into long-term storage. The cryovials of packed RBCs will be placed in boxes labeled with an alphabetical symbol, designating study subject set, and numbers 38 and 39 designating red blood cells (i.e. A38 and A39). Each of the boxes will receive one cryovial of red blood cells. Inside each of the boxes the cryovial of a specific study subject will be placed in the same grid position, starting with the top left corner and

ending with the bottom right corner. If for any reason a specimen is absent, the corresponding grid position will be left open. The boxes will be placed in the appropriate rack in the -80°C freezer.

#### **H. Long term storage of Adipose tissue**

After the adipose tissue has been received at the BSP at UNC in 2 x 3.6 ml cryovials, the adipose tissue specimens will be ready for storage in the PCaP designated freezer at the UNC BSP until transported to Project 3. The samples received at LSU will be stored at -80°C and batch shipped on dry ice to the BSP at UNC according to the shipping calendar. The specimens received at UNC will go directly into storage. The cryovials of adipose tissue will be placed in boxes labeled with an alphabetical symbol, designating study subject set, and numbers 40 and 41 designating adipose tissue in the needle (A40) and in the Luer-lock hub (A41). Inside each of the boxes the cryovial will be placed in the same grid position, starting with the top left corner and ending with the bottom right corner. If for any reason a specimen is absent the corresponding grid position belonging to that specimen will be left open. The boxes will be placed in the appropriate -80°C freezer.

#### **I. Long term storage of Urine samples**

After the urine samples from Louisiana or North Carolina have been received at the BSP at UNC in 15 ml conical tubes, they will be placed in long-term storage in the -20°C freezer. The samples received at LSU will be stored at -20°C at LSU and batch shipped to the BSP at UNC according to the shipping calendar. The specimens received at UNC will go directly into storage. Conical tubes will be stored one subject per 3X6 Birtran bag, with barcoded labels for each sample on the outside of the bag. The 3X6 bags will then be placed into a larger 16X16 bag. There will be two bags per set of subjects (eg. A-1, A-2, B-1,B-2) . The specimens will be placed in the -20°C freezer in the BSP facility.

#### **J. Long term storage of Toenail clippings**

After the toenail clippings from Louisiana and North Carolina have been received at the BSP at UNC in 2 ml cryovials, they will be ready for storage in the PCaP designated area at the UNC BioSpecimen Procurement Facility. The samples received at LSU will be stored at ambient temperature and batch shipped to the BSP at UNC according to the shipping calendar. The specimens received at UNC will go directly into storage. The cryovials containing toenail specimens will be placed in boxes labeled with an alphabetical symbol, designating study subject set, and numbers 1-42, designating toe nail clippings (i.e. A42). Inside each of the boxes the cryovials will be placed in the corresponding grid position, starting with the top left corner and ending with the bottom right corner. If for any reason a toenail specimen is absent, the grid position belonging to that specimen will be left open.



## **K. Long-term Storage of Immortalized WBC and Packed RBC.**

Long term storage of immortalized PBMCs and Packed RBCs isolated during purification of the PBMC fraction will be handled by the TCF in LCCC.

## **L. Long-term Storage of DNA isolated from Mouth rinses.**

The fifteen DNA aliquots prepared from mouth wash specimens collected at the time of in-home visits in LA will be archived in the same cell of the 15 boxes of a set (i.e., currently set U). The fifteen aliquots represent 14 matched specimens of 100  $\mu$ l of DNA at a concentration of 10  $\mu$ g/ml; the fifteenth aliquot is the remainder of the DNA specimen in a single tube. If both a whole blood specimen in (Yellow-top tube) and a mouth wash specimen are received for a study participant, the DNA specimens prepared from the blood sample will be archived in the boxes 1-15, and the DNA specimens prepared from the mouth wash sample will be archived in a set of boxes labeled Duplicate DNA Specimens. The aliquots with even numbers will be placed in one row of one box of "Duplicate DNA Specimens" and the odd numbered aliquots will be placed in a single row in a second box.

The fifteen aliquots of DNA extracted from mouth wash specimens collected retrospectively from study participants in NC and LA for whom DNA specimens were not archived successfully, will be placed into the appropriate empty cells in boxes 1-15 of sets A-T. The DNA aliquots will be placed in the appropriate set of boxes in the corresponding cells where the plasma, serum and RBC samples are archived. However, because the box labeled 9 from sets A-T has been sent to Roswell Park Cancer Institute for processing of the DNA specimens for genotyping analyses, the specimens for box 9 will be archived in order of processing in a set of boxes labeled Specimen 9, and the boxes forwarded to Roswell Park Cancer Institute, as filled, for processing for genotyping analysis. For study participants that were contacted for retrospective mouth wash samples because of low yields of DNA from inadequate blood specimens, the DNA specimens prepared from the mouth wash samples will be placed into the appropriate cells within boxes 1-15 of sets A-T, and the pre-archived DNA specimens with low DNA concentrations will be removed and be placed in a single row in each of two boxes labeled Duplicate DNAs: the even numbered DNA specimens from a single study participant will be archived in one box, and the odd numbered DNA specimens archived in the same wells of the second box.

## **VII. PCaP Consortium Freezers**

The BioSpecimen Processing Facility at 3-213 Hooker Research Center will contain PCaP Freezer 1, a ThermoElectron -80°C freezer, and one -20°C freezer. PCaP

Freezers 2 and 3 will be located in B01 of the Lineberger Comprehensive Cancer Center.

**Description: ThermoElectron Forma -80 °C freezer**

Model: 906

Serial # Freezer 1: 806020-731

Serial # Freezer 2: 806021-750

Serial# Freezer 3: 811853-2193

Note: once new freezer is ordered MOP will need to be updated

**-20 Kenmore Freezer**

Model:

Serial #:438066048

**VIII. Specimen Retrieval and Requests**

**A. Procedures for Requesting Specimens**

Requests for archived specimens of DNA, serum, plasma, RBCs, adipose tissue, urine and/or toenail clippings will be processed through the Consortium Executive Committee. Specimens requested for Projects of the PCaP Consortium will be distributed by the BSP after approval by the Director of Core 2. Requests for specimens by investigators outside of the Consortium will be forwarded to the Consortium Executive Committee for evaluation, and distributed only after receiving approval. The specimens will be logged-out through the specimen tracking system. The specimen tracking system will identify location, date of departure, and the quantity released to individual investigators. The Consortium will not accept return of unused samples due to the potential for contamination of the specimen.

**B. Tables and Forms for Specimen Handling by the Blood and Tissue Procurement Core**

**Table 1. Tissue Procurement Lab Storage**

<b>Sample</b>	<b>Amount</b>	<b>Box ID *</b>	<b>Storage Temp</b>	<b>Tubes</b>	<b>Box Type</b>	<b>Total samples</b>
5µg DNA	N/A	1-7	-80°C	2.0 ml cryovial	2"	7
10µg DNA	N/A	8-14	-80°C	2.0 ml cryovial	2"	7
Stock DNA	N/A	15	-80°C	2.0 ml cryovial	2"	1
Plasma (Yellow)	N/A	16-21	-80°C	2.0 ml cryovial	2"	6
Serum (Red)	N/A	22-31	-80°C	2.0 ml cryovial	2"	10
Plasma (Lavender)	N/A	32-37	-80°C	2.0 ml cryovial	2"	6
RBC (Lavender)	N/A	38-39	-80°C	2.0 ml cryovial	2"	2
Fat aspirate in Saline	N/A	40	-80°C	3.6 ml cryovial	3"	1
Fat aspirate with Luer-lock	N/A	41	-80°C	3.6 ml cryovial	3"	1
Toe nails	N/A	42	-80°C	Ambient	2"	1
Urine with preservative	15 ml		-20°C	15 ml conical tube	Bitran bags	1
Urine	15 ml		-20°C	15 ml conical tube	Birtran bags	1
Immortalize WBC			-80°C	2.0 ml cryovial	2"	3

*\*The alphabetic identification will indicate subject set.*

**Table 2 Typical freezer rack setup**

<b>Drawer</b>	<b>BOX</b>			
<b>1-1</b>	A-1 DNA (5ug)	A-3 DNA (5ug)	A-5 DNA (5ug)	A-7 DNA (5ug)
<b>1-2</b>	A-9 DNA (10ug)	A-11 DNA (10ug)	A-13 DNA (10ug)	A-15 stock DNA
<b>1-3</b>	A-17 YT PLS	A-19 YT PLS	A-21 YT PLS	A-23 RT SER
<b>1-4</b>	A-25 RT SER	A-27 RT SER	A-29 RT SER	A-31 RT SER
<b>1-5</b>	A-33 LT PLS	A-35 LT PLS	A-37 LT PLS	A-39 LT RBC

Duplicate rack for freezer 2

<b>Drawer</b>	<b>BOX</b>			
<b>2-1</b>	A-2 (5ug)	A-4 (5ug)	A-6 (5ug)	A-8 (10ug)
<b>2-2</b>	A-10 (10ug)	A-12 (10ug)	A-14 (10ug)	A-16 YT PLS
<b>2-3</b>	A-18 YT PLS	A-20 YT PLS	A-22 RT SER	A-24 RT SER
<b>2-4</b>	A-26 RT SER	A-28 RT SER	A-30 RT SER	A-32 LT PLS
<b>2-5</b>	A-34 LT PLS	A-36 LT PLS	A-38 LT RBC	BLANK (IDNA STOCK)

**Table 3. Supplies:**

Item	Vendor	Catalog#	Qty	Cost	Contact
Red top plastic 10 ml	Fisher Scientific	02 685 112	100	20.64	Fishersci.com
Yellow top glass 6 ml	Fisher Scientific	02 684 29	100	29.74	Fishersci.com
Lavender top plastic 6 ml	Fisher Scientific	02 683 99d	100	24.29	Fishersci.com
NUNC cryovial 3.6 ml	Fisher Scientific	05 669 59	100	30.38	Fishersci.com
25 ml Stripette serological	Fisher Scientific	07 200 14	200	164.73	Fishersci.com
10 ml Stripette serological	Fisher Scientific	07 200 11	500	188.73	Fishersci.com
5 ml Stripette serological	Fisher Scientific	07 200 8	500	178.27	Fishersci.com
Costar microcentrifuge tube, 1.7 ml	Fisher Scientific	07 200 534	500	38.28	Fishersci.com
Nalgene cryovials, 2 ml	Fisher Scientific	03 337 7D	500	189.49	Fishersci.com
50 ml conicals 30x115mm	Fisher Scientific	14 959 49A	500	239.20	Fishersci.com
15 ml conicals 17x120mm	Fisher Scientific	14 959 70C	500	176.50	Fishersci.com
Brady Brand Labels	R.H. Hughes	PTL 76-461	2500	187.90	877.774.8443
45 mm storage boxes	Sarstedt	95.064.981	10	30.30	866.727.7838
84 mm storage boxes	Sarstedt	95.064.951	10	34.70	866.727.7838
Gentra DNA extraction kit (no RNase)	Gentra Systems	D40K	Kit	684.00	800.866.3039

**Table 4. Order Request Form**  
**PCaP REQUEST FOR ORDER**

To: Jennifer Liesegang

Email: [jennifer\\_liesegang@med.unc.edu](mailto:jennifer_liesegang@med.unc.edu)

Fax: 919.966.9439

<b>Ordered by:</b>		<b>Date:</b>
<b>Dept./ PCaP Core:</b>		
<b>Phone:</b>	<b>Fax:</b>	
<b>Approved by:</b>		

**DELIVER TO:**

<b>Name:</b>		
<b>Company/Dept:</b>		
<b>Address:</b>		
<b>Phone:</b>	<b>Fax:</b>	

**PURCHASE FROM: (VENDOR)**

<b>Company Name:</b>		
<b>Contact Name:</b>		
<b>Address:</b>		
<b>Phone:</b>	<b>Fax:</b>	

QUANTITY	ITEM DESCRIPTION	ITEM NUMBER	UNIT PRICE	TOTAL PRICE

NOTE: Remember to attach price quote.  
 COMMENTS:

**Table 5. Laboratory Log Sheet**

<b>PCaP Lab Log Sheet</b>										
<b>PCaP ID #</b>	<b>Lab ID #</b>	<b>Date Drawn</b>	<b>Vacutainer Yellow # of Tubes</b>	<b>Vacutainer Lavender</b>	<b>Vacutainer Red</b>	<b>Fat</b>	<b>Urine # of Tubes</b>	<b>Toenails</b>	<b>Today's Date</b>	<b>Logged By</b>

**Form 1. Specimen Requisition Form**

**PCaP Specimen Requisition Form**

1. Provide a brief description of the laboratory assay(s) to be performed and the data that will be generated from the samples.
2. Provide a brief justification for the type and quantity of sample(s) requested for each study subject.
3. Note the total number of study subjects from whom you are requesting samples.
4. If you requesting samples from a selected subset of the PCaP study subjects, list the specific characteristics that will be used to define the subset(s) for your study (e.g., race, tumor characteristics).  
-Indicate the number of participants you wish to include in each subset (or 'all available', as appropriate).
- 5 NOTE: PCaP provides the following data for each study subject:
  1. unique PCaP identifier
  2. age at diagnosis
  3. race
  4. state of residence
  5. Gleason score (tumor grade) at diagnosis
  6. PSA at diagnosis
  7. clinical stage
  8. date of interview
  9. time of biologic sample collection

\*Following successful transfer of Analysis Data to the PCaP Central Database further arrangements for additional data transfer/analysis approved by the PCaP Management Committee as a part of an Ancillary Study Proposal, OR included in the original statement of work of a PCaP Project (1-9), will be made.

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For Office Use Only

PCaP Core, Project or Ancillary Study # \_\_\_\_\_

release of specimens Yes\_\_\_ No\_\_\_

Date of MC review: \_\_\_\_\_

MC Approval for



## PCaP Specimen Requisition Form

Investigator: \_\_\_\_\_ Location/Shipping Address: \_\_\_\_\_  
 Signature \_\_\_\_\_  
 Contact/Phone \_\_\_\_\_

Project/Study Name: \_\_\_\_\_

Sample Requested	*Aliquot Size Available	Number of Aliquots Requested	Date Requested	Initials of Requester	Number of Aliquots Distributed	Initials Core 2 Staff	Date Distributed	Location Box ID	Shipping Temp	Storage Temp
Serum from Red top	0.4 ml									-80°C
Plasma from Lavender top	0.4 ml									-80°C
RBC from Lavender top	1.0 ml									-80°C
Plasma from Yellow top	0.8 ml									-80°C
Urine	10 ml									-20°C
Urine with preservative	10 ml									-20°C
Adipose in saline/needle	NA									-80°C
Adipose in Luer-lock	NA									-80°C
DNA 100µl (1 or 10 ng)	NA									-80°C
DNA 50µl (0.5 or 5 ng)	NA									-80°C
Sample Requested	*Aliquot Size Available	Number of Aliquots Requested	Date Requested	Initials of Requester	Number of Aliquots Distributed	Initials Core 2 Staff	Date Distributed	Location Box ID	Shipping Temp	Storage Temp
Immortalized B-Cell Culture (cryopreserved or growing recommend)	1 ml vial OR 25 cm <sup>2</sup> culture	32 Pick up in flask	9/8/05	c.godfrey						
Diagnostic Biopsy Tissue	4 µm slide									Rm Temp
Prostatectomy Tissue Microarray (TMA)	4 µm slide									Rm Temp
Toe Nails	NA									Ambient

Shipped by: \_\_\_\_\_ Date: \_\_\_\_\_ Shipping: \_\_\_\_\_

*\*Investigators: note that this is only an average per aliquot. Samples may have more or less of product.*

## II. Specimen Quality Control of Specimen Processing

### A. Quantitation of DNA by Optical Density Reading

#### 1. Principle

The concentration of DNA in the isolate is obtained by optical density reading. The 260/280 ratio indicates the level of protein contamination in the nucleic acid sample. In this analysis a ratio of 2.0 is ideal, but 1.8 and higher is acceptable.

#### 2. Materials

- Prepared DNA samples
- 96 well plate that is UV transparent and DNase/RNase free for Molecular Device readings
- or 2.5µl pipette for NanoDrop readings
- Sterile tips for pipetting
- Holding boats if using a multi channel pipette
- Sterile water

#### 3. Procedure

##### a. Using the Molecular Devices Spectrophotometer

Samples are placed in 96 well plates. The total volume for each well should total 100 µl. Duplicate optical density readings of 1:20 and 1:40 dilutions will be performed. A template is established so that two wells in sequential rows are duplicate 1:20 dilutions, and the following two sequential rows are duplicate 1:40 dilution samples of the same DNA specimen (e.g. A1, B1 are the 1:20 dilution of sample X and C1, D1 are the 1:40 dilution of sample X).

Part 1: Preparing the solutions for evaluation on the Molecular Devices spectrophotometer (Molecular Devices Spectra Max 384).

- Centrifuge rehydrated DNA sample to reduce condensation on sides of tubes
- Place 5 µl of DNA in the first and second well in the designated column and row.
- Place 2.5 µl of DNA in the third and fourth well in the designated column and row.
- Continue in this manner until plate is full (22 subjects per plate using the above template) or all samples have been added to plate. The plate does not have to be full to be analyzed.
- Using a multi channel pipette, place 95 µl of sterile water in the first and second rows, designated as the 1:20 dilution, making sure to draw up and down several times to ensure mixture of DNA and water for an accurate reading.
- Using a multi channel pipette, place 97.5 µl of sterile water into the third and fourth rows, designated as the 1:40 dilution, making sure to draw up and down several times to ensure mixture of DNA and water for an accurate reading.
- Place the plate on the machine.

### Part 2: Using the Molecular Devices spectrophotometer

- On the computer desktop, choose the Soft Max Pro program
- Once the program opens, go to file and choose 'open', and choose the appropriate .ppr file template.
- Enter in the appropriate concentrations and subject ID into the template
- Save your information and click on 'read'
- Print readings and save to computer for study records.

### b. Using the NanoDrop Spectrophotometer

- Centrifuge rehydrated DNA samples to reduce condensation on the sides of tubes.
- Open the NanoDrop program on the computers desktop
- Log in as the PCaP user and choose Nucleic Acid.
- Clean the lower and upper measurement pedestal with deionized water and a kim-wipe
- Place a 2.0 µl drop of deionized water on the lower measurement pedestal and lower the sampling arm. The computer program will then prompt the user to click the 'OK' icon on the screen.
- Clean water from both pedestals with kim-wipe
- Next, place 2.0 µl of Pure Gene hydration solution on lower pedestal and lower the sampling arm.
- Type 'blank' in the appropriate window and click the 'blank' icon on the computer screen.
- After reading of the blank is complete, lift sampling arm and clean both pedestals with a kim-wipe.
- Place 2.0 µl of DNA solution onto the lower measurement pedestal and lower the sampling arm.
- Type the lab identifier in to the appropriate window and click the 'measure' icon on the computer screen.
- After all samples have been measured save data to the PCaP OD file on the M drive.

## **B. Gel Electrophoresis of DNA Specimens to Determine Integrity.**

### 1. Principle

Gel electrophoresis is an analytical technique used to separate DNA based upon size of the DNA fragment. Analysis of the pattern of DNA mobility in agarose gels provides an assessment of the quantity and quality of the isolated DNA specimen.

### 2. Materials

- Agarose: molecular biology grade, DNase and RNase free
- Gel apparatus
- Ethidium Bromide 10mg/ml (gel dye)

- Bromphenol blue (loading buffer)
- 1X TAE
- Ficoll
- Xylene cyanol
- 10% Sodium Dodecyl Sulfate solution
- Hind III generated molecular weight markers

### 3. Instructions:

Numerous stock reagents need to be made in preparation for gel electrophoretic analyses.

Making 0.5M EDTA solution:

- Add 186.12 gm of EDTA (with a FW of 372.24) to a graduated cylinder
- QS with distilled water to 1000 ml
- Add up to 40 gm of NaOH until pH reads 8.0
- Mix well on stir plate
- Check pH to ensure pH of 8.0

Making 10 X TAE (Tris- Acetate- EDTA) solutions:

- Add 24.2 gm Tris Crystallized Free Base to 500 ml graduated cylinder
- Next add 10 ml 0.5M EDTA solution with pH 8
- Add up to 500 ml of distilled water
- Add 5.71 ml of Glacial Acetic Acid
- Mix well on stir plate
- Check pH to ensure pH of 8
- Recipe can be doubled to make 500 ml of 20xTAE

Making 1X TAE solution:

- Dilute 10xTAE or 20xTAE to make 1xTAE
- Add 50 ml of 10X TAE
- QS with distilled water to 500 ml
- Mix well

Making Bromophenol Blue

- Add 12.5 gm of Ficoll 400 to 50 ml beaker
- Next add 0.5 gm of Bromophenol Blue
- Add 0.5 ml 10% xylene cyanol
- Add 10 ml 0.5M EDTA
- 2.5 ml 10% Sodium Dodecyl Sulfate solution
- QS to 50 ml with distilled water
- Mix well. Store at room temperature until used.

Making Hind III marker for gel

- Add 40  $\mu$ l Hind III 500ug/ $\mu$ l

- Add 166  $\mu$ l Bromphenol Blue
- Add 794  $\mu$ l 1xTAE
- Freeze at -20 in cryovials until used

#### Making 0.5% Agarose for gel:

- In a microwave-safe beaker add 0.5 gm of agarose
- Add 125 ml 1X TAE
- Place in microwave for 90 seconds at full power. Be sure agarose is completely in solution.
- Agarose may be stored in bottles and melted again in the microwave
- After the gel is poured, the gel may be stored at 4°C.
- Prepare the running plates for addition of molten agarose such that the agarose gel does not escape while solidifying between the plates.
- Place the well-forming comb, or combs, in the plate spaced far enough apart that two rows of samples may be run simultaneously.
- Pour the agarose solution that contains ethidium bromide/propidium bromide into the plate and let the gel solidify. The gel will look opaque and will be cool to the touch when properly set.

#### Preparing DNA

- While the gel is solidifying prepare the samples. The easiest way to do this is to use a V-bottom 96 well plate.
- Add 3  $\mu$ l of prepared un-cut DNA
- Add 4  $\mu$ l of Bromphenol Blue
- Add 8  $\mu$ l of distilled H<sub>2</sub>O
- Mix each specimen well with a multi-channel pipette.
- Once the gel has solidified, remove the comb, or combs. Place the gel plate in the running box.
- Fill the gel box with 1X TAE buffer until it covers the surface of the gel and fills the wells made by the combs.
- Add 10  $\mu$ l Ethidium Bromide to the 1xTAE solution in the box. Mix the EtBr well into the solution.
- Place 10  $\mu$ l of the pre-made Hind III marker solution in to the first and last well of each row, by slowly delivering the marker into the well taking care not to puncture the bottom of the well. A small amount of solution will escape the well into the TAE liquid.
- The 15  $\mu$ l of .solution of sample should then be delivered one sample per well very carefully so as not to puncture the well.
- Repeat this procedure for each specimen to be analyzed. Note the sample ID of the specimen in each well to allow proper annotation of the photographic image of the gel.
- Once all of the wells have been filled, replace the 'charge interrupt lid' on the gel apparatus and connect the red cable to the positive electrode on the gel electrophoresis apparatus and the black cable to the negative electrode on the gel box.

- Make sure the gel is positioned in the box such that the samples will run from the negative pole to the positive pole (RUN TO RED).
- Turn the power supply on and run at 75 volts for approximately two hours. Be careful not to touch any part of the electrophoresis apparatus during the run while the power is on.
- Once the electrophoretic run is complete, turn off the power supply, disconnect the leads, remove the 'charge interrupt' lid, and wearing gloves, remove the gel from the gel box and place the gel in the trans-illuminator for photography.
- Dispose of gel, gloves and other ethidium bromide contaminated items in hazardous waste containers.

#### D. CORE 2 CONTACT INFORMATION:

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