Manual of Procedures

Developmental and HyperActive Ras Tumor (DHART) Specialized Program of Research Excellence (SPORE)

Biospecimen Collection, Processing, and Shipment Manual

dhartspore.org

Table of Contents

1.0 ABBREVIATIONS

CPC	Circulating Progenitor Cell
CPT	Cell Preparation Tube
DHART SPORE	Developmental and HyperActive Ras Tumor Specialized Program of Research Excellence
EDTA	Ethylene Diamine Tetra-acetic Acid
IATA	International Air Transport Association
IUGB	Indiana University Genetics Biobank
MNC	Mononuclear Cell
PBMC	Peripheral Blood Mononuclear Cell
PFC	Polychromatic Flow Cytometry
PN	Plexiform Neurofibroma
RBC	Red Blood Cells
RCF	Relative Centrifugal Force
RPM	Revolutions Per Minute

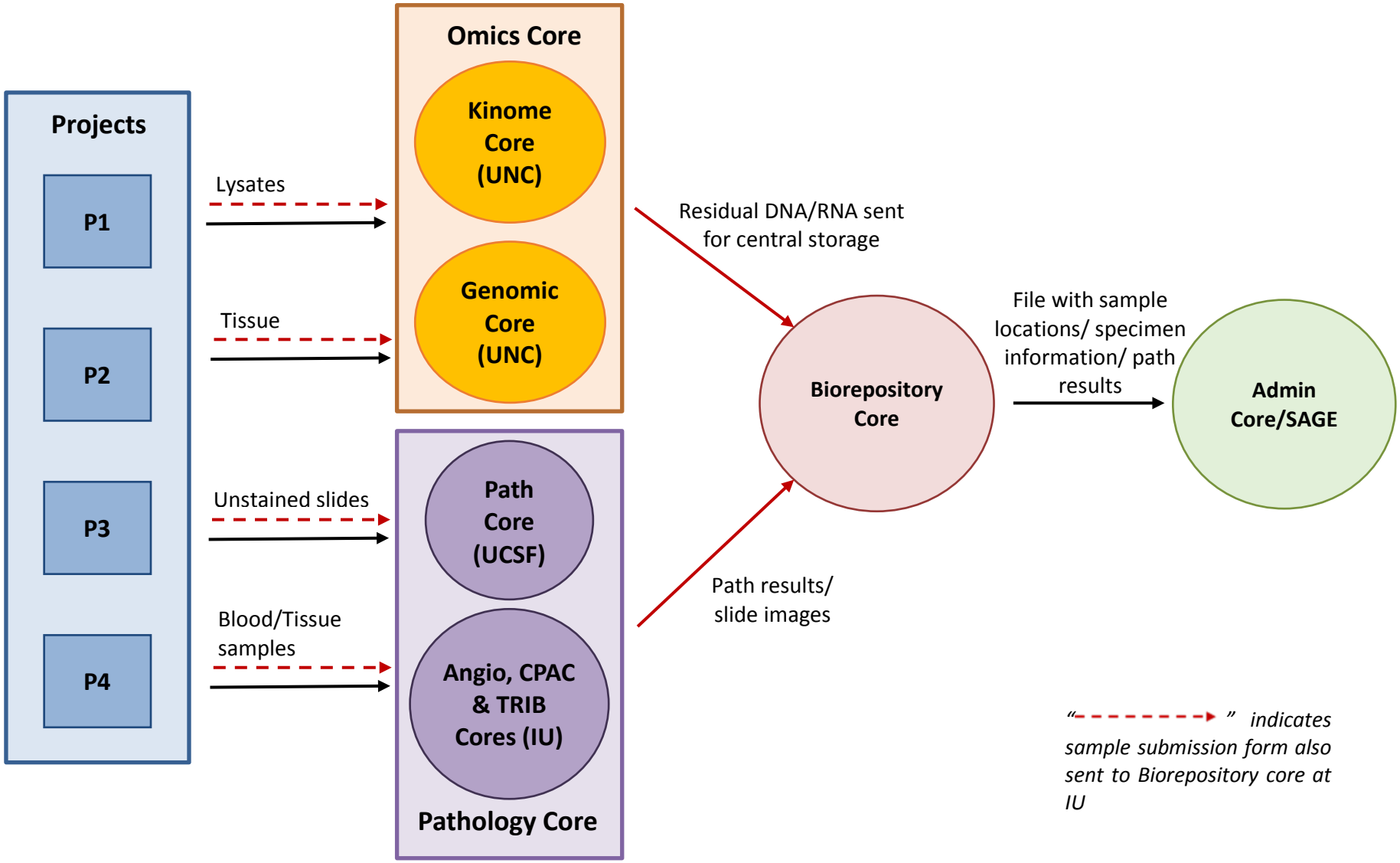
2.0 PURPOSE

The purpose of this manual is to provide DHART SPORE contributing researchers (PIs, study coordinators, and the sample collection and processing teams) with guidelines for collection and submission of biological samples to the DHART SPORE Cores, where specimens will be analysed and catalogued for biobanking. This manual includes detailed instructions for shipping biospecimens to the CPAC, Agio, and Biorepository Cores at Indiana University, the Kinome and Genomic Cores at University of North Carolina, and the Path Core at University of California, San Francisco. This manual includes the instructions for submitting the following samples may be submitted for banking with the DHART SPORE Biorepository:

- Solid human tissue
- Plasma
- Cells for kinome analysis
- Mononuclear cells
- Animal tissue

This manual includes guidelines for snap freezing tissue, uniform collection and fractionation of human blood, aliquoting, labeling, storage prior to shipping, and shipping to the DHART SPORE Cores.

3.0 DHART SPORE Sample Flow Overview



4.0 BIOSPECIMEN/PATHOLOGY CORE

The Biospecimen/Pathology Core will be to serve as the central site to track and store samples and pathological data from the human and mouse biospecimens collected as part of the four SPORE projects. This core will perform pathological review of all samples and will make these data available to SPORE investigators and the broader research community. In addition, the Biospecimen/Pathology Core will maintain an electronic catalog which will link the specimens and tissue to the pathological reports as well as data generated in the projects for each study subject. The project investigators will use this electronic catalog to select the samples needed for analyses. The Biospecimen/Pathology Core includes the Biorepository/Indiana University Genetics Biobank (IUGB), CPAC, TRIB Lab, and Angio Cores at Indiana University, as well as the Path Core at University of California, San Francisco.

4.1 BIOREPOSITORY CORE (IUGB) AT INDIANA UNIVERSITY

4.1.1 Contacts

http://DHARTSPORE.org
dhartbio@iu.edu

Tatiana Foroud, PhD, Biorepository Core Leader
Phone: 317-274-2218
Email: tforoud@iu.edu

Claire Wegel, MPH, CCRP, Project Manager
Phone: 317-278-6158
Fax: 317-278-1100
Email: cwegel@iu.edu

Sample Receiving Address
DHART SPORE Biorepository
IU School of Medicine
Walther Hall – R3 C102
980 W. Walnut Street
Indianapolis, IN 46202

Heather Daniel, Administrative Core Contact
Phone: 317-278-9290
Fax: 317-274-0138
Email: hdaniel@iu.edu

4.1.2 Hours of Operation

Biorepository Core business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Ambient shipments may be sent *Monday through Thursday*. Frozen samples must be shipped *Monday through Wednesday only*. For packing and shipment details of both ambient and frozen samples, please refer to Section 7.0 of this manual.

Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

4.1.3 Holiday Schedules

- Please note that courier services may observe a different set of holidays. Please be sure to verify shipping dates with your courier prior to any holiday.
- **Weekend/holiday delivery must be arranged in advance with DHART SPORE Core staff.**

Date	Holiday
January 1	New Year's Day
3rd Monday in January	Martin Luther King, Jr Day
4th Monday in May	Memorial Day
July 4	Independence Day (observed)
1st Monday in September	Labor Day
4th Thursday in November	Thanksgiving
4th Friday in November	Friday after Thanksgiving
December 25	Christmas Day

Please note that between December 24th and January 2nd, Indiana University will be open Monday through Friday for essential operations **ONLY** and will re-open for normal operations on January 2nd.

Please see: https://DHART SPORE.org/holiday_closures.html for additional information.

4.2 CPAC CORE AT INDIANA UNIVERSITY

4.2.1 Contacts

David Jones, PhD, CPAC Core Director
Phone: 317-278-3054
Email: drjones1@iu.edu

Andi Masters
Phone: 317-274-3053
Email: argrove@iupui.edu

Sample Receiving Address
CPAC Core
Attn: David Jones
699 Riley Hospital Dr., RR 240
Indianapolis, IN 46202

4.2.2 Hours of Operation

CPAC Core business hours are from 7 AM to 5 PM Eastern Time, Monday through Friday.

Frozen samples must be shipped *Monday through Wednesday only*. For packing and shipment details of frozen samples, please refer to Section 7.0 of this manual.

Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

4.3 ANGIO CORE (ANGIO BIOCORE) AT INDIANA UNIVERSITY

4.3.1 Contacts

Karen Pollock, PhD, Angio BioCore Director
Phone: 317-274-8891
Email: kpollok@iu.edu

Emily Sims, Core Manager
Phone: 317-278-7232
Email: ecwillar@iupui.edu

Matthew Repass
Email: mjrepass@iupui.edu

Sample Receiving Address
Angio BioCore
Attn: Emily Sims
980 W. Walnut St., R3-343
Indianapolis, IN 46202

4.3.2 Hours of Operation

Angio BioCore business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Ambient shipments may be sent *Monday through Thursday*. For packing and shipment details of ambient samples, please refer to Section 7.0 of this manual.

Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

4.4 PATH CORE AT UNIVERSITY OF CALIFORNIA SAN FRANCISCO

4.4.1 Contacts

Andrew Horvai, MD, PhD, Path Core Director
Phone: 415-885-7313
Email: Andrew.Horvai@ucsf.edu

Sarah Bowman, Assistant
Phone: 415-514-3668
Email: Sarah.Bowman2@ucsf.edu

Sample Receiving Address
UCSF Medical Center Mission Bay
Attn: Dr. Andrew Horvai
1825 4th St., Room M2354
San Francisco, CA 94158

4.4.2 Hours of Operation

Pathology Core business hours are from 8 AM to 5 PM Pacific Time, Monday through Friday.

Ambient shipments may be sent *Monday through Thursday*. For packing and shipment details of ambient samples, please refer to Section 7.0 of this manual.

Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

4.5 TRANSLATIONAL RESEARCH AND INTEGRATED BIOLOGY (TRIB) LAB AT INDIANA UNIVERSITY

4.5.1 Contacts

Khadijeh (Sholeh) Bijangi-Vishehsaraei, MS, PhD
Phone: 317-278-3035
Email: khbijang@iupui.edu

TRIB Lab
Phone: 317-278-3050

Sample Receiving Address
Translational Research and Integrated Biology
705 Riley Hospital Drive, Room 2641
Indianapolis, IN 46202

4.5.2 Hours of Operation

TRIB Lab business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Frozen samples must be shipped Monday through Wednesday only. For packing and shipment details of frozen samples, please refer to Section 7.0 of this manual.

Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

5.0 OMICS CORE AT UNIVERSITY OF NORTH CAROLINA

The UNC Tissue Procurement Facility (TPF), the Pre-Clinical Genomic Pathology (gPATH) Lab and the Lineberger Comprehensive Cancer Center (LCCC) Bioinformatics Department, referred to collectively as the TPF/gPATH Core for DHART SPORE purposes, have established a successful and consistent team approach to sample intake and tracking, process workflow, and sequencing data analysis over the past 4 years. The TPF is responsible for receipt of the samples, assigning specific tracking information, relabeling the samples with that information and inputting the samples into our electronic database and Lab Information Management System (LIMS). Relabeled and trackable samples are then sent to the gPATH facility where they are processed according to the directives of the specific project. gPATH extracts nucleic acids, QCs those RNAs or DNAs, and makes recommendations on the downstream library preparation process that will maximize the potential success of the library preparation and ultimate sequencing. The gPATH core then prepares NGS libraries for RNASeq and/or DNASeq using an Agilent Bravo automated platform. Sequencing is usually performed using Illumina NextSeq500s in the gPATH core facility, or pooled and transferred to UNC's High Throughput Sequencing Facility (HTSF), if needed. Finally, we have established a direct processing pipeline with LCCC Bioinformatics for capture, alignment and ultimate analysis of the resultant sequencing data. All of these facets and experience will be employed to track and process DHART SPORE samples and, to whatever degree is requested, to analyze subsequent data.

5.1 KINOME CORE (JOHNSON LAB) AT UNIVERSITY OF NORTH CAROLINA

5.1.1 Contacts

Gary Johnson, PhD, Kinome Core Director
Phone: 919-843-3107
Email: glj@med.umnc.edu

Tim Stuhlmiller, PhD, Research Assistant Professor
Email: tjstu@email.unc.edu

Xin Chen, Laboratory Technician
Email: xinchen@email.unc.edu

Sample Receiving Address
UNC Pharmacology
Attn: Tim Stuhlmiller, Johnson Lab
Genetic Medicine Building, Room 4009
120 Mason Farm Rd.
Chapel Hill, NC 27599

5.1.2 Hours of Operation

Kinome Core business hours are from 8 AM to 5 PM Eastern Time, Monday through Friday.

Frozen samples must be shipped *Monday through Wednesday only*. For packing and shipment details of frozen samples, please refer to Section 7.0 of this manual.

Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

5.2 GENOMIC CORE (TPF/GPATH) AT UNIVERSITY OF NORTH CAROLINA

5.2.1 Contacts

Hei Huang, PhD, Genomic Core Manager
Phone: 919-966-2620
Email: mei_huang@med.unc.edu

Tissue Procurement Center Laboratory
Phone: 919-966-2620
Fax: 919-843-9501

Sample Receiving Address
UNC Tissue Procurement Center
Attn: Mei Huang
105 McNider Hall, CB#7304
333 S. Columbia St.
Chapel Hill, NC 27599

5.2.2 Hours of Operation

Genomic Core business hours are from 7:30 AM to 5:30 PM Eastern Time, Monday through Friday.

Frozen samples must be shipped *Monday through Wednesday only*. For packing and shipment details of frozen samples, please refer to Section 7.0 of this manual.

Check for weather or other conditions that may impact the shipping or delivery of the sample(s). Please remember that significant storms anywhere between the origin and destination may delay the shipment, and plan accordingly when possible.

6.0 SPECIMEN COLLECTION AND PROCESSING PROCEDURES

Consistency in sample collection and processing is essential for biomarker studies. All samples are drawn in the same order and then processed in a uniform fashion. **Please read the instructions before collecting any specimens. Have all your supplies and equipment out and prepared prior to drawing blood.**

6.1 Blood and Tissue Samples for the CPAC Core at IU

- CPAC Protocol for Solid Tissue Collection and Storage **(Appendix A)**
- CPAC Protocol for Plasma Collection and Storage **(Appendix B)**

6.2 Blood for the Angio Core at IU

- Blood Collection for Polychromatic Flow Cytometry & Identification of Circulating Progenitor Cells **(Appendix C)**

6.3 Unstained Slides for the Path Core at UCSF

- **Appendix D**

6.4 Blood for the TRIB Laboratory at IU

- **Appendix I**

6.5 Lysates for the Kinome Core at UNC

- Omics Core – Harvesting Cells for MIB (Kinome) Analysis **(Appendix E)**
- Instructions for submitting samples to the Omics Core at UNC **(Appendix G)**
- Omics Core Preferred Volumes **(Appendix H)**

6.6 Tissue for the Genomic Core at UNC

- Protocol for Isolating and Snap Freezing Tumor Samples **(Appendix F)**
- Instructions for submitting samples to the Omics Core at UNC **(Appendix G)**
- Omics Core Preferred Volumes **(Appendix H)**

7.0 PACKAGING AND SHIPPING INSTRUCTIONS

There are strict Federal guidelines for the shipment of human specimens. If you are unsure of the requirements, please visit DHARTSPORE.org to watch the shipment training videos. If you do not have the necessary supplies (such as UN3373 labeling), please contact dhartbio@iu.edu.

7.1 Ambient Shipping Instructions

- **IMPORTANT!** Ambient samples ***must*** be shipped ***Monday through Thursday*** using priority overnight service.
- Please do NOT draw blood for ambient shipments on Fridays.
- Notify the recipient Core and the Biorepository Core of all shipments.

Ambient whole blood tube and tissue shipments are Category B UN3373 and as such must be triple packaged and compliant with IATA Packing Instructions. *See the latest edition of the IATA regulations for complete documentation.*

Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

IATA Packing and Labeling Guidelines

- The primary receptacle (cryovials or blood collection tubes) must be leak proof and must not contain more than 1 L total.
- The secondary packaging must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle and the secondary packaging. The absorbent material should be of sufficient quantity to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest listing the specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible persons (shipper and recipient)
  - ✓ The words "Biological Substance, Category B" UN3373

7.2 Frozen Shipping Instructions

- **IMPORTANT!** Frozen samples ***must*** be shipped ***Monday through Wednesday*** using priority overnight service.
- Please be aware of holidays and inclement weather, and plan your shipments accordingly.
- Notify the recipient Core and the Biorepository Core of all shipments.

Specimens being shipped to the DHART SPORE Biorepository are Category B UN3373 specimens and as such must be triple packaged and compliant with IATA Packing Instructions. *See the latest eEdition of the IATA regulations for complete documentation.*

Triple packaging consists of a primary receptacle(s), a secondary packaging, and a rigid outer packaging. The primary receptacles must be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured, or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.

IATA Packing and Labeling Guidelines

- The primary receptacle (cryovials or blood collection tubes) must be leak proof and must not contain more than 1 L total.
- The secondary packaging (plastic canister or biohazard bag) must be leak proof and if multiple blood tubes are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent direct contact with adjacent blood tubes.
- Absorbent material must be placed between the primary receptacle (cryovials or blood collection tubes) and the secondary packaging. The absorbent material must be of sufficient quantity to absorb the entire contents of the specimens being shipped. Examples of absorbent material are paper towels, absorbent pads, cotton balls, or cellulose wadding.
- A shipping manifest listing the specimens being shipped must be included between the secondary and outer packaging.
- The outer shipping container must display the following labels:
  - ✓ Sender's name and address
  - ✓ Recipient's name and address
  - ✓ Responsible persons (shipper and recipient)
  - ✓ The words "Biological Substance, Category B"
  - ✓ UN3373
  - ✓ Class 9 label including UN 1845, and net weight of dry ice contained

Appendix A: CPAC Protocol for Solid Tissue Collection and Storage

1. Extract tissue from animal.
2. If highly perfused, flush the tissue with saline.
3. Flash freeze in liquid nitrogen.
4. Label a polypropylene tube (screw cap cryogenic vial) with date of tissue collection and animal ID.
5. Place frozen tissue sample in the screw cap cryogenic vial and store in a -80 freezer.
6. Complete the CPAC Submission e-Form (https://dhartspore.org/docs/cpacmanifest.xlsx).
7. Notify the CPAC Core (drjones1@iu.edu) and Biorepository Core (dhartbio@iu.edu) of incoming specimen shipment.
8. Ship frozen on dry ice to the CPAC Core at Indiana University.

Notes: *Store tissue separately*.

Date: 04/07/2016 Provided by David Jones, CPAC Director

Appendix B: CPAC Protocol for Plasma Collection and Storage

1. Collect blood sample in EDTA treated tubes.
2. Mix blood with anticoagulant in tubes by inverting 6-8 times.
3. Allow to set at room temperature for 15 minutes.
4. Centrifuge sample at 3500 rpm for 10 min.
5. Label a polypropylene tube (screw cap cryogenic vial) with date of blood collection and subject ID.
6. Draw off the plasma with a sterile pipette and transfer the plasma into the cryogenic vial.
7. Store plasma in -80 freezer within 2 hours of blood collection.
8. Complete the CPAC Submission e-Form (https://dhartspore.org/docs/cpacmanifest.xlsx).
9. Notify the CPAC Core (drjones1@iu.edu) and Biorepository Core (dhartbio@iu.edu) of incoming specimen shipment.
10. Ship frozen on dry ice to the CPAC Core at Indiana University.

Date: 04/07/2016 Provided by David Jones, CPAC Director

Appendix C: Blood Collection for Polychromatic Flow Cytometry (PFC) & Identification of Circulating Progenitor Cells (CPCs)

1. Two 8 ml sodium heparin CPT tubes and two 6 ml EDTA tubes will be required for this collection. Label each tube with **DATE**, **TIME OF COLLECTION**, and **PATIENT ID NUMBER**.

2. Collect a total of 16 mL of peripheral blood into two 8 ml sodium heparin CPT tubes. These tubes have a red/green closure.

3. Gently invert eight times to mix blood with anticoagulant.

4. **CRITICAL: The sample must be processed within 2 hours of blood collection.**

5. Centrifuge at room temperature for 30 minutes at 1500-1800 xg RCF. Please note, due to the height of these tubes, a swing-bucket rotor centrifuge should be used.

6. The centrifugation will separate the plasma and the mononuclear cell (MNC) fraction above the gel barrier. The red blood cells (RBCs) pass through the gel barrier to the bottom of the tube. To re-suspend the MNCs, **gently** invert the CPT tubes **one** time.

7. Uncap the two EDTA tubes.

8. Transfer the plasma/MNC cell suspension from each CPT tube into separate 6 ml EDTA tubes using a disposable transfer pipette. Do not combine the plasma from the two CPT tubes. Use care to prevent piercing the gel barrier and disturbing the RBC layer. Recap the EDTA tubes.

9. Complete the Angio BioCore DHART SPORE Sample Submission e-Form (https://dhartspore.org/docs/angiomanifest.xlsx) and email a copy to Emily Sims (ecwillar@iupui.edu), Matthew Repass (mjrepass@iupui.edu) and dhartbio@iu.edu.

10. Ship the EDTA tubes according to the Ambient Shipping Instructions (Section 10.1 of this manual). Include a hard copy of the submission form in the shipment.

11. **CRITICAL: The MNCs are stable for 24 hours post collection after centrifugation and MUST be received within those 24 hours.**

Appendix D: Creating slides for the Path Core

1. From a representative paraffin block, cut 5-10 unstained sections (depending on amount of available tissue remaining in block, 10 is preferred), 4 micrometers thick, on charged (+) glass slides.

2. Label the slides with the date and patient ID.

3. Notify the pathology core (Andrew.horvai@ucsf.edu; scott.kogan@uscf.edu and sarah.bowman2@ucsf.edu) Biorepository Core (dhartbio@iu.edu) of the inbound specimen, and also print out the email and enclose with the slides. Please include the following information in the email:
    a. Tumor site
    b. Gross findings
    c. Other comments relevant to the path analysis

4. Package the slides in a plastic slide carrier.

5. Ship Monday to Thursday, overnight express, ambient temperature, in a padded envelope or box with foam to:
    UCSF Medical Center Mission Bay
    Attn: Dr. Andrew Horvai (DHART SPORE)
    1825 4th St., Room M2354
    San Francisco, CA 94158

Appendix E: Harvesting Cells for MIB (Kinome) Analysis

MIB lysis buffer:
50mM Hepes pH 7.5
150mM NaCl
0.5% Triton X-100
1mM EDTA
1mM EGTA
10mM NaF
2.5mM NaVO4
Protease Inhibitor Cocktail (Roche-11873580001, 1 tablet per 25mL)
Phosphatase Inhibitor Cocktail 2 (Sigma-P5726, 250ul per 25mL)
Phosphatase Inhibitor Cocktail 3 (Sigma-P0044, 250ul per 25mL)

Mix all the components together and let it rock at 4C for 30 min to make sure it is well mixed. Filter with a 0.2um filter and use immediately (kept on ice) or store in aliquots at -20C.

1. Wash cells 2 times with cold 1X PBS.
2. Add MIB lysis buffer (0.4ml/150mm plate, made fresh or thawed on ice from the -20C).
3. Dislodge cells using cell lifter and place in a 15mL conical tube.
4. Sonicate cell lysates for 10 seconds; place on ice for 1 minute. (Our sonicator is a Fisher Scientific 550 Sonic Dismembrator with a circumference of the probe tip is ~1cm. It is sonicated on setting 3).
5. Repeat the sonication/ice step 2 more times.
6. Spin down the lysates at 13.3K for 10 min at 4C (we have to break down the volume into 2.0mL eppendorf tubes).
7. Filter the supernatant through a 0.22um filter.
8. Determine the concentration and total volume and freeze immediately at -80C.
9. Complete the DHART SPORE Kinome Assay Request – Sample Submission e-Form (https://dhartspore.org/docs/kinomemanifest.xlsx).

10. Notify the Kinome Core (tjstu@email.unc.edu) and the Biorepository Core (dhartbio@iu.edu) of the incoming sample shipment. Send a copy of the submission form with the email.

11. Ship samples on dry ice in a dry shipper to the Kinome Core at UNC. Include a hard copy of the submission form.

Notes:
The goal is to have no more than 10mLs of total lysate with about 2-5mg of protein. Our standard is to have 2-5mg of protein per sample for MIBs.
Provided by Dr. Johnson's Laboratory, UNC 2016

Appendix F: Protocol for Isolating and Snap Freezing Tumor Samples

Materials:

Fresh tissue (harvest as quickly as possible post mortem)
1.7ml or microcentrifuge tubes or 2ml cryovials
Ice and suitable portable container
Dry ice and suitable portable container
Liquid nitrogen and suitable portable container
Phospho-buffered saline (1% antibiotic preferred)
Surgical instruments
70% ethanol or comparable cleaning agent
Disposable plastic weigh boats

1. Precool PBS and weight boats on ice. Add ~2ml PBS to weigh boat before adding tissue.
2. Label all tubes. Place tubes on dry ice to precool.
3. Dissect tumor tissue-
   a. Care must be taken to harvest tissue as quickly as possible after animal sacrifice.
   b. Instruments should be cleaned between specimens and rinsed in cold PBS.
4. Place tissue in weigh boat/PBS. Rinse as much blood as possible from the specimen. Microdissection tools can help remove blood, if necessary. Blot dry on Kimwipe or paper towel.
5. Place tissue into corresponding microcentrifuge tube/cryovial.
6. Add a small amount (~1ml) of LiN2 (liquid nitrogen) to the tube to snap freeze. Allow LiN2 to evaporate before closing.
7. Maintain the tubes in LiN2 or at -80C.
8. Complete the TPF/gPATH DHART SPORE Sample Submission e-Form (https://dhartspore.org/docs/genomicsmanifest.xlsx).
9. Notify the Genomic Core (mei_huang@med.unc.edu) and the Biorepository Core (dhartbio@iu.edu) of the incoming sample shipment. Send a copy of the submission form with the email.
10. Ship samples on dry ice in dry shipper to the Genomic Core at UNC. Include a hard copy of the submission form.

Appendix G: Instructions for Preparing and Submitting Samples for the DHART-SPORE Project to the Omics Core at UNC (081916)

Section I: Omics Core - Sample Submission and Intake

In order to intake and process DHART SPORE samples in a timely and efficient matter, the Omics core at UNC requests that samples be prepared and submitted in accordance with the following guidelines:

1. All samples of any type should be prepared, packaged and shipped as indicated in Appendix A: TPF/gPATH CORE-PREFERRED VOLUMES AND MEANS OF SUPPLY.

2. Samples (EXCEPT those for proteomics assays) should be sent directly from the DHART SPORE sample processing point to the UNC Tissue Procurement Facility (TPF), c/o Mei Huang. The address is on the sample submission form. Sending samples directly to gPATH will significantly delay processing, as will sending samples for sequencing to the Johnson Lab.

3. The only exception to the arrangement in #2 above is that frozen samples/lysates destined for Proteomics studies in Dr. Gary Johnson's lab will be shipped separately and directly to his lab at the following address:

   UNC Pharmacology
   Attn: Tim Stuhlmiller, Johnson Lab
   Genetic Medicine Building, Room 4009
   120 Mason Farm Rd
   Chapel Hill, NC 27599-7365

   These samples should be processed and packaged according to the protocol supplied by Dr. Johnson's lab.

4. There are separate sample submission forms.

   a. Johnson Lab (DHART SPORE Kinome- Sample Submission e-Form (Re. 081916) Color Code – **Green**, For **Kinome Assays** samples only.

   B. TPF (Tissue Procurement Facility(TPF) /Pre-Clinical Genomic Pathology (gPATH) (Re. 081316) , Color Code – **Orange**, For **RNASeq and DNASeq processes**.

5. If specific quantities of already extracted DNA or RNA are supplied to the TPF, please include the following information (see submission form):

   a) extraction method,

   b) QC method; e.g., UV, Picogreen, Qubit, etc.

These metrics should be selected from the appropriate pull down menu in section 2 of the sample submission form. (Attachment B).

If blood or buffy coats are supplied, include volume in mL.

Please do not use UV absorbance to determine the mass of DNA or RNA if you are submitting it to the TPL/gPATH core for library preps and sequencing, as calculations will be sub-optimal. Please base your calculations on fluorometric assays; e.g., Picogreen, Qubit, Quantas, etc. for such determinations, and please provide 20% extra RNA or DNA, if available.

6. Please supply biopsies and SF tissue in clearly labeled external screw top 2 mL vials.

7. No fewer than 16 samples will be processed via an automated work flow. If less than 16 samples are received for such a process, the Omics core will either: a) store the sample set until additional samples arrive for the specific work flow requested; or, b) if no additional samples are forthcoming for the specific work flow, the samples may be manually processed.

8. Please make sure that each sample/aliquot has a unique identifier that you will be able to track to the original case/patient/time point, etc. (See sample submission sheet, Page 2, #4, Column C). The UNC Tissue Procurement Facility and the gPATH core will assign a new identifier to each sample in order that the sample may be accurately tracked through all Omics core work flows, as well as through LCCC Bioinformatics.

9. Sample transfer process and documentation:

   a. Before samples are transferred to the UNC Tissue Procurement Facility (TPF) or to the Johnson Lab (proteomics) for entry into the processing work flow, please complete the appropriate DHART SPORE Sample Submission e-Form. The submission form will be provided to all DHART SPORE participants. Please check your version as recent updates have been made. The most recent version of the form is also available at the UNC gPATH website. (www.unclineberger/core/gpath)

      Please contact the gPATH lab should you need assistance in completing this form.

   b) Amount of samples needed by process (all quantitation performed by fluorometric means):

      - DNA for Agilent Sureselect$_{xt}$ All Exon Library Prep (Standard Input): 2 ug DNA

      - DNA for Agilent Sureselect$_{xt}$ All Exon Library Prep (Low Input): >250 ng-1000 ng DNA

      - RNA for Illumina Truseq Whole Transcriptome Library Prep: > 1 ug RNA (>100ng/ul)

        - Upon receipt of RNA, fragment distribution will be assessed by Agilent tapestation analysis and RNA integrity number (RIN) observed. Samples with RIN < 7 are not optimal to proceed with this protocol. These lower quality

samples can produce viable libraries using the RNA Access Library Prep protocol (see below). The investigator should be aware of any biases that may result from proceeding through library prep with low quality non-FFPE derived RNA. After such analysis is completed, you will be contacted if any RIN values are lower than 7. At that point, decisions can be made as to the best course of action.

- RNA for Illumina RNA Access Library Prep (Low Input): 50-100 ng RNA (>10 ng/ul)
    - Upon receipt RNA, fragment distribution will be assessed by Agilent tapestation analysis and the percentage of fragments > 200bp (DV200) observed. Adjustments to the library prep starting material will be made based on this quality metric (generally 50-100ng will be used when starting with FFPE derived RNA).
- FFPE sections for DNA or RNA sequencing:
    a. five (5) – ten (10) 10 micron thick sections for each nucleic acid to be isolated. If no macrodissection is required, if possible, please place a quantity of 5, 10 micron thick scrolls per block in a snap cap 1.7 mL microfuge tube. Please supply 2 tubes of 5 scrolls each, if possible.
    b. If macrodissection is required, please provide 5-10 clearly labeled slides with 10 micron thick unstained sections. For each sample, provide an H&E slide with target regions to be dissected clearly circled with a Sharpie.
- Frozen tissue for DNA or RNA sequencing: up to 5-10 mg tissue aliquots (1 specimen/aliquot each for DNA and RNA, if both services are requested)
- Frozen cells for DNA or RNA sequencing: we recommend between 1-5 million cells for each nucleic acid to be isolated.
- Frozen tissues/cell lysate for protein kinome assay: See protocol provided by Johnson Lab.

This sample information is summarized in table form in Appendix A: "Omics Core Preferred Volumes and Means of Supply".

* We can use less than the amounts listed, but if we do the risk of library preparation and/or sequencing failures increase. Please contact gPATH lab (Todd or Gary) to discuss if you are unable to provide the amounts requested.

Section II: INSTRUCTIONS – Completing the Tissue Procurement Facility (TPF)/Pre-Clinical Genomic Pathology (gPATH) (Re. 081316) Sample Submission Form

The submission form includes pull down menus where possible. What follows are instructions on how to complete the sample submission form. Starting in the top left corner with "DHART-SPORE PROJECT # and extending to the end of the document, explanations are provided generally by line item. Submission instructions are essentially the same for the Johnson Lab Kinome Assay request form.

PAGE 1: PROJECT AND DEMOGRAPHIC INFORMATION

1. a.) DHART-SPORE PROJECT#. Please select correct project number from pull down menu.

   b) Please complete all fields, and at a very minimum, the required fields.

   c) The contact designation is very important. This should be the person most knowledgeable about the specific sample submissions included on this form and who can make decisions relative to any changes in processing that may be suggested by extraction and/or QC issues. This is the person the Omics core will contact for information on the samples.

2. **Samples:**

   a) Inside the block, note the date you plan to send the samples to the UNC TPF.

   b) List the sample type and total numbers of each sample type you will be sending. If you are sending already extracted RNA or DNA, from the pulldown menu, please select the extraction and QC method used to process this subject nucleic acid. Please do not use UV absorbance assays; e.g., spectrophotometer, Nanodrop™, etc. to determine the mass of RNA or DNA to send us for library preps and sequencing. Please use a fluorometric assay; e.g., Qubit, Picogreen, Quantas, etc. **For the Kinome Assay submission form, select the lysis method used from the pulldown menu.**

3. **Library Preps**

   a) The TPF/gPATH core can perform standard and low input DNA and RNA library preps. If you know which prep you would like us to perform, please choose it for specific samples listed on page 2. The pull down menus under column H-J allow selection of these and other services.

   b) Please send samples to the UNC TPF address or the Johnson Lab, as appropriate, shown on the left side of page 1 of the sample submission form. Contact Mei Huang if you have a question about the sample submission itself. **For the Johnson Lab Kinome Assay form, contact Tim Stuhlmiller if you have questions.** (See Contact Information, Page 8)

c) On the right side of the bottom of page 1, please provide any additional sample or processing information you feel will be of value to the core labs in processing the samples.

PAGE 2: SAMPLE SUBMISSIONS

a) Column A is used by the TPF/gPATH or Johnson Labs only.

b) In Column B, if there is specific PHI you wish to supply, please do so here and only in this column. The Omics core does not require PHI to track samples.

c) In Column C, the investigator should supply specific unique sample IDs he/she wishes to use to be able to identify samples after processing is complete. These are for investigator use only.

Columns D through K (TPF/gPATH Sample Submission Form) and **Columns D and G through I (Johnson Lab Kinome Sample submission form)** have pull down menus. Please make the appropriate selection.

d) Column D denotes how the sample is to be supplied.

e) Column E allows a tumor or normal tissue designation. **For the Johnson Lab Kinome Sample submission form, Column E asks for entry of concentration information, if appropriate.**

f) Column F allows for the designation of the tumor type. **For the Johnson Lab Kinome Sample submission form, Column F asks for entry of total sample volume/mass, if appropriate.**

g) Column G allows the designation of species from which the sample originates. **For the Johnson Lab Kinome Sample submission form, Column G allows for the selection of tumor or normal tissue type.**

h) Column H is used to select the 1st priority procedure to be applied to the line item sample. **For the Johnson Lab Kinome Sample submission form, Column H allows for the designation of the tumor type.**

i) Column I is used to select the 2nd priority procedure to be applied to the line item sample. **For the Johnson Lab Kinome Sample submission form, Column I allows for the designation of species from which the sample originates.**

j) Column j is used to select the 3rd priority procedure to be applied to the line item sample.

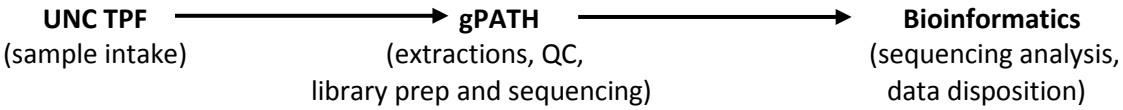
Please note, if multiple procedures are requested for the same sample, the gPATH core will address them sequentially as long as sufficient sample remains.

k) In Column K, you may choose how you would like the sequencing performed if you have a preference. If no selection is made, the default position is "Best Way" which allows the gPATH core to select the most efficient means to sequence a given sample.

If you are submitting more than 36 samples, please use page 3 to continue sample registration.

GENERAL FEEDBACK

After receiving RNA/DNASeq request samples from DHART SPORE participants, the work flow is:



A gPATH representative will try to provide regular status reports at various critical stages of the work flow processes. For example, the first status report will usually come after the completion of the extraction and QC of any nucleic acids involved in the process. Sometimes decisions will need to be made at this point as to the best type of library prep process to employ based on the quantity and/or quality of the nucleic acid.

The second status report usually will come when the samples complete library prep and have been QC'd successfully. After that, you will be advised when the sequencing has been completed and the data passed on to LCCC Bioinformatics.

The time required to complete various aspects of a project vary. gPATH does our best to move samples through the various work flows as quickly as feasible, however, we emphasize accuracy and achieving the goals of the project over speed. We will do our best to keep you informed of the progress of your project samples.

Appendix H: Omics Core Preferred Volumes and Means of Supply

Specimen Type:	For Assay:	Supplied to Omics Core as:	Sample To Be Supplied:	Preferred/Minimum Volume/Concentration for Each Process*
Blood	DNASeq, RNASeq	Buffy Coat	Ext. Thread Cryovials, 2 ml, -80°C	0.5 ML, min. 250ul
Snap Frozen Tissue	DNASeq, RNASeq	Snap Frozen Tissue	Ext. Thread Cryovials, 2 ml, -80°C	10 mg, min. 5 mg
Snap Frozen Cores	DNASeq, RNASeq	Frozen Core	Ext. Thread Cryovials, 2 ml, -80°C	4 x 14 g cores, min. 3x14g cores
Cell Pellets	DNASeq, RNASeq	Snap Frozen Pellet	1.7 ml Snap or Screw Cap Microfuge Tubes, -80°C	$5x10^5$-$1x10^{6**}$
FFPE Scrolls	DNASeq, RNASeq	10 micron scrolls	1.7 ml Snap Cap Microfuge Tubes, Room Temp.	10 scrolls total, 5 scrolls/tube, min. 5 scrolls
FFPE Cores	DNASeq, RNASeq	14 gauge, if possible	1.7 ml Snap Cap Microfuge Tubes, Room Temp.	6 cores, 3 cores/tube, min. 3 cores
FFPE Sections	DNASeq, RNASeq	10 micron sections	Sections mounted on uncharged microscope slides (25mmx75mmx1mm), Room Temp.	10 slides, min. 5 slides

* Process defined as procedure requested; e.g., RNASeq is one process, DNASeq another, etc.

** Possibly can perform process with significantly less cells. Contact the gPATH core (Gary Rosson, gary_rosson@med.unc.edu, or Sofia Dard, sdard@live.unc.edu, ph. 919-962-8324) if you have questions. Please process by trypsinizing cells to gentle release, pellet in neutralized trypsin/media, rinse x 1 with PBS, aspirate completely, flash freeze and store at -80 degrees C. Ship on dry ice.

Appendix I: TRIB Protocol for Plasma Preparation for Cytokine Assays

1. Collect (6mL) peripheral blood into an 8 mL EDTA purple top tube.
2. Spin at 1500 rpm for 5 minutes at 4˚ C.
3. Remove supernatant and aliquot in 1 mL NUNC tubes.
4. Label each tube with: Date, protocol number, and patient ID number.
5. Store samples at -80 C, and ship on dry ice to the address provided below once all samples have been collected for a patient.
6. Complete the TRIB Lab Submission e-Form (https://dhartspore.org/docs/tribmanifest.xlsx).
7. Notify the TRIB Lab (khbijang@iupui.edu) and Biorepository Core (dhartbio@iu.edu) of incoming specimen shipment.
8. Ship frozen on dry ice to the TRIB Lab at Indiana University.