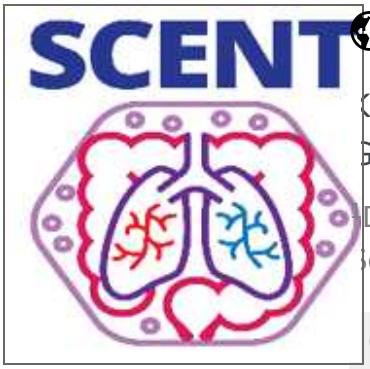

U54 SCENT Adult Colonoscopy Tissue Collection Procedure

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Cellular Senescence Network (SenNet) Method Development Community

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Protocol status: Working We use this protocol and it's working

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ABSTRACT

This protocol outlines the inclusion and exclusion criteria as well as collection and processing of adult colon tissue at Duke University

MATERIALS

SUPPLY REQUIREMENTS

Tissue acquisition supplies:
- Forceps (Jumbo, large and standard)
- tweezers, needle, razor blade
- Petri dish
- PBS for washing in the petri dish
- Two 1.8 ml microfuge tubes (empty), labeled
- Liquid Nitrogen*** and dowar
- -80 Freezer
- Media (Bambanker and recovery)
- One Microfuge tube with freezing media from Dr. Roper's lab
- One microfuge tube with Bambanker cell freezing media
- 4 jars with 10% buffered formalin, labeled
- 2 cryoboxes
- Mr. Frosty

Blood acquisition supplies:
- 3 ACD tubes (provided by SSCRS until GI can obtain tubes)
- One 10mL purple EDTA tube
- Butterfly needle and vacutainer, as needed
- IV vacutainer adapter

PROCESSING TIPS:

1) Work quickly and minimize handling during all tissue processing steps.

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2) Wash tissues in a clean glass petri dish with cold PBS upon harvest and absorb excess blood using a laboratory wipe.
3) Each snap-frozen biopsy will be placed in a separate cryovial (labelled).
4) To flash freeze, either submerge the cryovial in liquid nitrogen or a liquid-nitrogen cooled bath (e.g. isopentane) or place the tube deep in a bucket of dry ice. Wait at least 2–3 minutes for the tissue to freeze all the way through, and transfer the tube containing the tissue to vapor phase liquid nitrogen for long-term storage.
5) For frozen samples, place the samples on dry ice (or keep in N2) until placing the sample in -80C freezer for long term storage.
6) Tissues should be stored long-term in a cryovial in liquid nitrogen for best results. Tissues can be stored short-term (1–2 days) at -80°C if needed. Samples will need to be transferred to Karen Abramson (or Garman lab for liquid nitrogen freezing) – both these labs are in Carmichael.
7) Once removed from liquid nitrogen, tissues should be maintained at -80°C or on dry ice until use.
8) Transfer samples on dry ice when needed.

HISTOLOGICAL PROCESSING (FORMALIN) BY BRPC:

Needed Supplies:
• Forceps, tweezers, and alcohol pads
• Labeled tissue cassettes (labeled with pencil)
• Formalin in glass jars (room temperature)
1. After the endoscopist has isolated the biopsies, place the biopsies in the pencil-labeled tissue cassette.
2. Place the tissue cassette in one of the jars of formalin. (Make note of the time the sample is placed in formalin.)
3. Transfer the sample to the BRPC within 24 hours.****

24 Hours****
The sample needs to be transferred out of the formalin into ethanol or other reagent (by the BRPC team) within 24 hours or the sample will over-fix.

General Guidelines

1

1. Biobanking will proceed through the Duke GI Tissue Repository (PRO00001662), established to screen patients presenting for upper endoscopy and colonoscopy in Duke GI. The Duke GI Tissue Repository already partners with BRPC for sample processing and discernment.

2. Weekly screening of the endoscopy schedule will identify patient in three age groups presenting for screening colonoscopy. Cases will be reviewed with Dr. Garman for eligibility and flagged for samples collection for SCENT. Dr. Roper will act as a backup reviewer as needed.

3. Informed consent for participation in the GI Tissue Repository will be obtained electronically using REDCap. Paper ICF will be obtained as needed.

4. SCENT Colonoscopy Adult Enrollment: Three different patient age groups are of interest. Enrolling patients across the age span is a major focus of this project:

- Young <35 years
- Middle Aged: 35-55 years
- Older: > 55

5. Tissue Samples will be used for:

- I. Formalin fixed samples:
- Immunohistochemistry (FFPE) – formalin fixation
- Visium spatial transcriptomics (FFPE) – formalin fixation
- II. Snap frozen for single nuclei ATAC seq and RNA work
- III. Media for Mint-ChIP
- IV. Ice-cold media for organoid generation

6. Biobanking Protocol for Blood: Blood samples will be banked for SASPS and cytokines using yellow-top ACD tubes (three) and purple top tubes (one).

Inclusion Criteria

2 Healthy patients age 18-90 presenting for outpatient colonoscopy.

CRC screening may wish to focus on the following indications:

- Colorectal cancer screening
- Iron deficiency anemia
- GI bleeding (prior or active) (without recent transfusion)
- Diarrhea without evidence of colitis
- Abdominal pain
- Constipation
- Evaluation for abnormal imaging
- Finding young patients (<45) who do not have exclusion criteria may be challenging, so pay particular attention to young patients on the endoscopy schedule.

Exclusion Criteria

3

- Systemic Illness such as diabetes is a relative exclusion but may be hard to avoid
- Autoimmune disease such as SLE (lupus) or rheumatoid arthritis
- Active infectious disease such as HIV, HBV, active COVID, active (not treated) HCV
- Obesity (BMI > 35) is a relative exclusion
- BMI < 18.5
- Any chemotherapy or radiation for any condition, including cancer treatment in any organ (patients may be included with untreated or surgically only treated cancer in another organ)

- Metastatic malignancy
- Whole blood transfusion in the past 48 hours
- Inflammatory bowel disease, microscopic colitis, and infectious colitis
- Known inherited conditions with increased risk of colon cancer such as Lynch Syndrome, FAP, DNA repair defects etc
- Use of blood thinners other than a baby aspirin (due to increased bleeding risk)
- Underlying liver disease with cirrhosis or coagulopathy (due to increased bleeding risk)
- Note: while diverticulosis and colon polyps are not exclusions, we will not collect samples withing 10 cm of these lesions.

After enrollment and sample collection, Dr. Garman and Dr. Roper will review any clinical findings from the endoscopy. New diagnosis of colitis, advanced adenoma, or colorectal cancer will result in exclusion from SCENT (patient can remain in the GI Tissue Repository).

Blood Collection

4 Subjects will be asked to provide blood samples for storage and analysis. Blood is obtained for research purposes either before (often at the time that the IV is placed) or after the procedure. Blood may be drawn from the GI endoscopy prep bay, the recovery bay, or the phlebotomy station in the adjacent GI clinic. For the Duke Tissue Repository, up to 35 mL of blood may be drawn (we increased total blood amount for this protocol). For this protocol, whole blood will be collected in purple-top (EDTA) tubes and yellow-top ACD tubes (for isolating plasma).

Note that the blood these samples will be drawn during the subject's clinic visit or prior to endoscopic procedures; however, if necessary the blood draw can be drawn at another time that is convenient to the subject and research staff (ie: a separate scheduled visit).

Blood will be taken by research staff and held for SSCRS to pickup in room 2351. SSCRS will process and store the blood samples.

Yellow top ACD (three 8.5mL tubes)
Note that it is important to fill the tube all the way. These tubes are not processed but will be delivered to SSCRS via courier.

Purple top tube (K2EDTA -One 10mL purple)
Sample is inverted after collection to mix the blood and the EDTA. Note that it is important to fill the tube. These tubes are not processed but will be delivered to SSCRS via courier.

Scheduling SSCRS pickup (refer to SSCRS SOP)
1. 24 hours prior to sample collection:
   a. Send meeting invite to surgerysubstrate@duke.edu, Amanda Mandy, Anna Diocareza, Mari Kopping and POD-Repository-calendar
   b. Confirm correct procedure date
   c. Time should be the anticipated blood draw time

d. Location “Clinic 2J- room 2351”

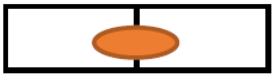
2. Following blood samples collection:
a. Label yellow and purple top tubes with the GI repository study ID.
b. Complete the SSCRS transport form
c. Copy the transport form for GI repository records
d. Place the GI repository label (F1) on the transport form
e. Place tubes and transport form in biohazard lab bag
f. Page 970-0705 with the following:
i. GI-U54; clinic 2J room 2351; (call back #)

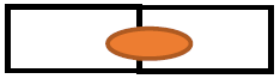
Tissue Collection

5 Tissue samples will be collected during colonoscopy – 10 research biopsies of the normal colon will be obtained in accordance with Pro00001662.

Collect using jumbo forceps (endoscopy provider to determine forceps size)

Sample Splitting Note:
Tissue samples will be split in half (when biopsies are elongated, they will be split into two halves down the middle into two shorter halves, not two long halves). We will need two CRCs to help stabilize the sample and cut it (see the line in the middle of the boxes below as an example)





We will focus on collecting from two colonic locations from eligible subjects: the mid- ascending colon(“right colon”) and the mid-descending colon (or the sigmoid colon as alternate site) (“left colon”). Goal will be to collect from both the right and left colon as there are regional variations in the type of polyps that form in these locations). Locations may be adjusted depending on presence of diverticula / polyps – ideally right and left colon.

Biopsy site Location (Total of 10 biopsies):
5 biopsies from the right colon (specifics below)
5 biopsies from the left colon (specifics below)

Tissue Sample Processing

6 RIGHT COLON (mid-ascending colon).
• Collect using Jumbo forceps (endoscopy provider to determine forceps size)
• Make sure formalin jars (provided by BRPC) and cryovials are labelled prior to processing

o Split biopsies will be labeled using an alphanumerical order

 Example: R1A and R1B is the same biopsy. R1A is ½ of the biopsy and R1B is the other ½.

This is referred to as a paired sample. The second paired biopsy will be labeled as R2A and

R2B.

• Place four of the biopsies in a petri dish for washing in PBS (see below for processing

o choose the 2 larger biopsies to split (butterflied)

• The fifth biopsy is for Dr. Roper's lab (see below for processing)

Two Biopsies for splitting

• Transfer the two largest biopsies into another petri dish for splitting:

o One CRC will hold each biopsy stable with forceps/needle

o A second CRC will cut in half with a razor blade or scalpel

• ½ of each paired biopsy sample (R1A and R2A) will go into 10% buffered formalin for FFPE (each right colon biopsy will go into separate formalin jars)

o Label each jar with the GI repository subject ID and accordingly as R1A or R2A

o Place both R1A and R2A in biohazard lab bag

o Copy the transport form and place it in the bag

o Place the GI repository sticker (A1) on the transport form

o Deliver to BRPC for processing

• ½ of each paired biopsy sample (R1B and R2B) will go into Liquid nitrogen for single nuclear ATAC seq. (each right colon biopsy will go into separate cryovials)

o Prior to collection: Label each jar with the GI repository subject ID and accordingly as R1B or R2B

o Cryovials will be dropped into a dewar of liquid nitrogen

o Copy transport form and deliver with samples

o Place the GI repository sticker (C1) on the transport form

o Deliver dewar/samples to BRPC

• Clean instruments under running water per BRPC/pathology standard process

Two Biopsies (mid-ascending colon – proximal transverse colon if unable to obtain ascending)

• Label cryovial with GI repository subject ID # and sticker (C2)

• Collected and will also be washed in PBS with samples above

• Place the two biopsies into one cryovial filled with 1mL Bambanker Cell Freezing Medium for cryopreservation.

• Place cryovial in Mr. Frosty to slowly freeze for 24 hours

• Email CRC to pick up sample the next day or on Monday (if collected on Friday)

One Biopsy (mid-ascending right colon – proximal transverse colon if unable to obtain ascending)

• Place biopsy (not washed in PBS) into cryo media provided by Dr. Roper's lab for colonic organoids and Dr. Roper

• Label cryovial with GI repository sticker (A2)

• Contact Ghada Mohamed 646-675-1869 for pick up

LEFT COLON (mid-descending colon).
• Collect using Jumbo forceps (endoscopy provider to determine forceps size)
• Make sure formalin jars (provided by BRPC) and cryovials are labelled prior to processing
  o Split biopsies will be labeled using an alphanumerical order
    Example: L1A and L1B is the same biopsy. L1A is ½ of the biopsy and L1B is the other ½. This is
    referred to as a paired sample. The second paired biopsy will be labeled as L2A and L2B.
• Place four of the biopsies in a petri dish for washing in PBS (see below for processing
  o Choose the 2 larger biopsies to split (butterflied)
• The fifth biopsy is for Dr. Roper's lab (see below for processing)

Two Biopsies for splitting
• Transfer the two largest biopsies into another petri dish for splitting:
  o One CRC will hold each biopsy stable with forceps/needle
  o A second CRC will cut in half with a razor blade or scalpel
• ½ of each paired biopsy sample (L1A and L2A) will go into 10% buffered formalin for FFPE (each left colon biopsy will go into separate formalin jars)
  o Label each jar with the GI repository subject ID and accordingly as L1A or L2A
  o Place both L1A and L2A in biohazard lab bag
  o Copy the transport form and place it in the bag
  o Place the GI repository sticker (B1) on the transport form
  o Deliver to BRPC for processing
• ½ of each paired biopsy sample (L1B and L2B) will go into Liquid nitrogen for single nuclear ATAC seq. (each left colon biopsy will go into separate cryovial)
  o Prior to collection: Label each jar with the GI repository subject ID and accordingly as L1B or L2B
  o Cryovials will be dropped into a dewar of liquid nitrogen
  o Place GI repository sticker (D1) on the transport form
  o Deliver dewar/samples with transport form to BRPC
• Clean instruments under running water per BRPC/pathology standard process

Two Biopsies (descending colon – sigmoid if unable to obtain descending)
• Label cryovial with GI repository subject ID # and sticker (D2)
• Collected and will also be washed in PBS with samples above
• Place the two biopsies into one cryovial filled with 1mL Bambanker Cell Freezing Medium for cryopreservation.
• Place cryovial in Mr. Frosty to slowly freeze for 24 hours
• Email CRC to pick up sample the next day or on Monday (if collected on Friday)
• Copy of transport form to be retained for these samples

One Biopsy (mid-descending left colon)
• Place biopsy (not washed in PBS) into cryo media provided by Dr. Roper's lab for colonic

organoids
and Dr. Roper
• Label cryovial with GI repository sticker (A2)
• Contact Ghada Mohamed 646-675-1869 for pick up

Special Note: If unable to use JUMBO forceps, protocol will be altered as follows and NO splitting will be attempted:

Right colon: Ascending colon or proximal transverse:
• One large biopsy in formalin for spatial
• One large biopsy from adjacent tissue in cryovial and snap frozen in liquid nitrogen
• Two large biopies cryopreserved in Bambanker Cell Freezing Media
• One large biopsy for Roper lab into media for organoids

Left colon: Descending colon or sigmoid colon:
• One large biopsy in formalin for spatial
• One large biopsy from adjacent tissue in cryovial and snap frozen in liquid nitrogen
• Two large biopies cryopreserved in Bambanker Cell Freezing Media
• One large biopsy for Roper lab into media for organoids

GI REPOSITORY LABELING (ALL tubes will be labelled with GI repository ID #)
A1: Placed on the transport form given to BRPC on day of collection.
    • Two formalin jars for right colon samples (2)
A2: Placed on the cryovial with Roper's media
    • One cryovial with right colon samples (2)
B1: Placed on the transport form given to BRPC on day of collection.
    • Two formalin jars for left colon samples (2)
B2: Placed on the cryovial with Roper's media
    • One cryovial with right colon samples (2)
C1: Placed on the transport form given to BRPC on day of collection.
    • One cryovial in liquid nitrogen for right colon samples (2)
C2: Placed on the cryovial with bambanker media
    • One cryovial with right colon samples (2)
D1: Placed on the transport form given to BRPC on day of collection.
    • One cryovial in liquid nitrogen for left colon samples (2)
D2: Placed on the cryovial with bambanker media
    • One cryovial with left colon samples (2)
F1: Placed on the transport form given to SSCRS on day of collection
    • 3 ACD and 1 purple top blood tubes
F2: Placed on the GI repository sample acquisition form per normal practice

Data Collection

7 Data that will be collected from Maestro Care includes, but is not limited to, MRN, age, sex, BMI, medical history, history of colonic polyps/adenoma based on colonoscopy and pathology data (previous colonoscopies and current colonoscopy). Data will be stored in REDCap.

Each GI repository sample acquisition label will have the sample intake form and the sample check out REDCap form completed.

Sample Distribution

8

• FFPE (formalin) - to BRPC on day of collection
• Cryopreserved paired – transported to BRPC on day of collection in liquid nitrogen
• Bambanker media – transport to BRPC the day AFTER the procedure on dry ice
• Organoid samples - to Roper lab ASAP after banking
• Blood tubes:
o Yellow-top ACD and purple top tubes go to SSCRS for processing same. Page SSCRS for pick
up