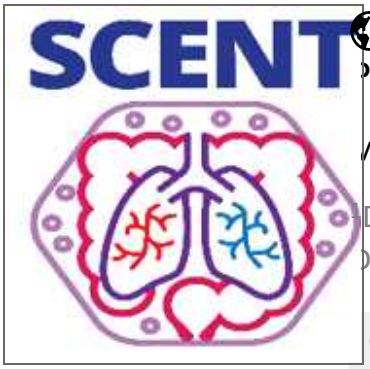

U54 SCENT Pediatric Colonoscopy Tissue Collection Procedure V.2

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Cellular Senescence Network (SenNet) Method Development Community

VERSION 2

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ABSTRACT

This document outlines the required criteria and acquisition for pediatric colonoscopy colon specimens collected at Duke University Hospital through the Biorepository and Precision Pathology Center (BRPC) in the Department of Pathology.

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

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PROTOCOL integer ID: 86610

Inclusion and Exclusion Criteria

1 Inclusion Criteria:

• Healthy patients age 5-18 years of age presenting for outpatient colonoscopy.

• Screening may wish to focus on the following indications: abdominal pain, constipation, diarrhea, bloating/gas, patients with normal labs and/or imaging.

Exclusion Criteria:

• 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 within 10 cm of these lesions.

Collection Protocol

2 1. U54 CRC will coordinate with GI team, to screen for colonoscopy cases where normal tissue may be available.

2. Scheduled in advance, U54 CRC or BRPC team member will approach patient's in-person for consent.

3. If patient/parents' consent, U54 CRC will coordinate with the GI Physician on procedure timing

and biopsy procurement.

4. Upon day of procedure, Receive maximum 8 forceps biopsy fragments, 3-4 from the mid-ascending colon (“right colon”) and 3-4 from the mid-descending colon (or the sigmoid colon as alternate site) (“left colon”).

5. Process the biopsy fragments into the following samples. Keep samples from right colon and left colon separate.

a. FFPE – 1 biopsy per block
b. Snap frozen:
    i. 1 snap freeze in cryovial as normal
    ii. 1 will be put into freezing media
        1. Wash tissue with PBS to remove the blood and fecal matter.
        2. Place biopsy fragment into cryopreserved tubes with 1mL freezing media.
        3. Cells should be frozen slowly at 1°C/min. Put tubes in the freezing container (e.g., Mr. Frosty) with isopropyl alcohol and place them overnight in a -80'C freezer.
        4. Next day, transfer tubes to liquid nitrogen.

6. Route FFPE samples to Research Histology Lab to:

a. Create an H&E from each FFPE sample and route to pathologist for review.
b. After review, create 2 tissue scrolls from the tissue blocks and coordinate RNA and DNA extraction.
c. Once RNA and DNA extraction is complete, the frozen LN2 samples will be shipped to lab for
scRNAseq