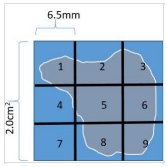

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Biospecimen collection and processing 2.0

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1 Works for me dx.doi.org/10.17504/protocols.io.bszynf7w

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SUBMIT TO PLOS ONE

ABSTRACT

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This is a description of the steps that the Washington University in St. Louis HTAN center has developed to collect surgical resection biospecimens, for molecular and genomic characterization. There are two separate protocols because the technique has evolved and better methods for tumor sampling have been developed. This is the second sample protocol and employs an innovative grid system for sampling.

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PROTOCOL CITATION

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GUIDELINES

Pathology standards of practice take absolute precedence and should be respected at all times by research staff. This protocol must be performed with the supervision of either a board certified pathologist or board certified licensed pathology assistant. Details of this protocol are specific to the tumor samples and characterization methods used by Washington University and will need to be empirically determined for use with any other tumor tissue and other facility.

Approval from internal institutional review boards must be obtained as well as signed informed consent from each patient before proceeding.

MATERIALS TEXT

Gloves
Safety goggles
Storage tubes with Dulbecco's Minimal Essential Medium (DMEM)
1.5 to 2.0ml Cryovials preferably with internal threads for flash freezing tissue in liquid nitrogen
10% Neutral Buffered Formalin (formalin)
24 well tissue culture plate, or similar plate
Dissecting Instruments, including forceps, scalpel blades with handle.
Surface to place tissue on and perform dissection, e.g. self healing cutting board, dedicated pathology cutting board, etc.
Tissue punches
Dewar to transport and store liquid nitrogen
Cryo-molds
OCT compound, clear
Container with a metal block surrounded by dry ice

SAFETY WARNINGS

This protocol involves the handling of un-fixed human tissue and universal precautions must be adhered to at all times.
Procedures must be performed in approved lab space dedicated to the handling of human tissues.
Dry ice should be handled with care and used in a well ventilated area.
Liquid Nitrogen should be used in a well ventilated area and placed in to a proper approved dewar, special cryo-handling gloves and face shield should also be employed.
Formalin is a toxic chemical and should handled with caution in a certified chemical fume hood.
Dissecting tools and scalpels are sharp and should be handled with care and disposed of in properly designated waste containers

BEFORE STARTING

Make sure all equipment is ready:
Ice and tubes for storing fresh samples
Liquid Nitrogen for flash freezing
Dry ice for OCT
Formalin for fixation of tissue
Dissecting instruments and scalpels
Ruler and scale to measure and weigh tissue
camera to document

1 Record time that the tumor sample arrived in the pathology gross lab

2 Examine the gross tumor sample with a Pathologist or Pathology Assistant

3 Once the tumor mass has been identified, photograph the tumor in situ

4 Request that the Pathologist remove a tumor piece that is ideally 2.0 cm^2 x 0.5cm thick.

5 Request Pathologist orient the specimen by noting pertinent anatomical directions, i.e. anterior, posterior, lateral, medial

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6 Photograph specimen with orientation markings, be sure there is no identifiable PHI, i.e. date.

7 Place specimen on cutting board and measuring with a ruler, cut the tumor sample in a grid style fashion to obtain anywhere from 2 to 9 pieces of equally sized subdivisions of tissue. Each subdivision should be 0.65 cm^2.
When squares are completely cut, photograph with a ruler to later annotate and subsequently measure the spatial relationships among the pieces.

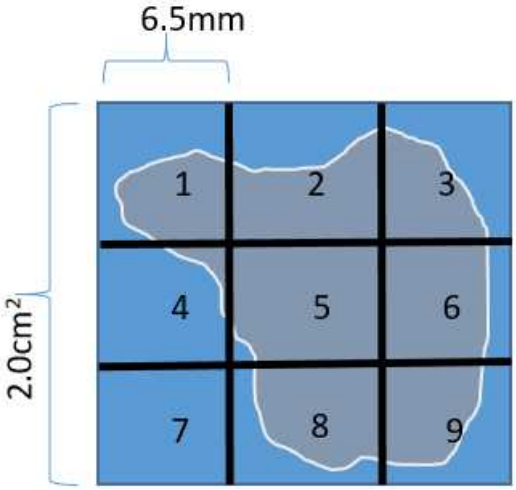
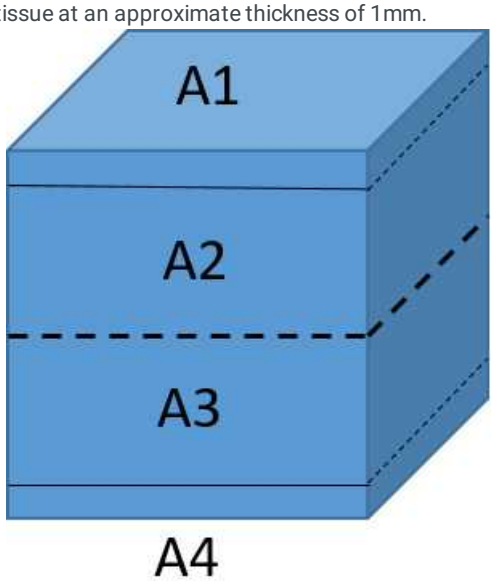


Figure 1

8 **For each grid square:**
turn the tissue square on it's side between the teeth of a pair of forceps and with a fresh scalpel blade begin slicing the tissue at an approximate thickness of 1mm.



9 Place the superior slice (A1) in to one well of a 24 well tissue culture dish with 10% Formalin, label appropriately

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10 Place the next slice (A2) in to a cryo mold, fill with OCT, and place on to a metal block surrounded by dry ice to freeze. Record the time.

11 Place the next slice (A3) in to either DMEM or Flash freeze in liquid nitrogen. This will depend on the characterization assay to be performed using that slice.
Record the time.

12 Place the last slice (A4) which is the inferior slice in to one well of a 24 well tissue culture dish with 10% formalin, label appropriately.

13 Repeat the above steps 8-12 for each tumor subdivision.

14 All the above steps assume a tumor slice that is uniform in size at about 2.0 cm^2 x 0.5 cm. Tumors may vary in size and priorities will have to assessed in order to determine what samples can be taken and for what downstream processes can be done. Be prepared to improvise!

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