1. Schedule an appointment for the following by emailing the laboratory

strlhistology@uthscsa.edu

* + Drop off project
	+ Embed project
	+ Discuss project details
	+ To use gross station to label molds, cassettes, etc.
1. A **complete STRL Histology** research requisition slip must accompany all research projects.

***All research request forms must have the following:***

* + **Investigators** Full Name – last name, first name
	+ Project ID# - PID #, PO# for Invoice billing (not expired) or billing contact information – name of individual, email, phone #, and physical address
	+ Department ID# or Billing Address for Invoice
	+ Fund Source ID#
	+ Number of blocks
	+ ID of blocks – a complete list of specimen ID’s that matches the writing on the cassettes is required.
	+ Description of work to be done
	+ Phone number/email address of individual who will pick up completed project.
1. **Samples for paraffin block processing:**

***Investigator is responsible for the following:***

Tissues should be fixed and placed in labeled cassettes

*Fixed tissue:* We accept tissue which has been fixed in a variety of fixatives. Once fixation is complete, please transfer samples to 70% Ethanol.

* **To prepare cassettes:**
1. **Fix tissue as desired:**Allow ~1 mm/hour for the fixative to penetrate your tissues and a volume of 10-20 times fixative volume to tissue.
2. **Label cassettes:**When labelling cassettes, use only a #2 hard lead pencil for cassettes, never a pen or Sharpie marker. Solvents used in processing can remove the ink from many "permanent" Sharpie markers.
3. **Ensure small pieces will not be lost:** To prevent small tissues from being lost during processing, place in biopsy cassettes or used biopsy sponges. The tissue processor uses vacuum to facilitate infiltration which can remove tissue from the cassette.
4. **Place in cassettes in desired orientation:** When tissue is placed into cassettes the tissue surface that is place down in the cassette will be placed down for embedding and this is the surface that is sectioned first. Please list any special embedding requests on submission form.
	* Refrain from overcrowding. Tissue that is compressed in the cassette will not adequately fix, infiltrate, section, or stain.
	* Specimens should be cut thin enough to allow adequate fixative penetration.
	* Do not allow tissue to touch all sides of the cassette or become smashed in the lid.
	* The thickness between 0.2 and 0.5 cm (approximately the size of a nickel) is a good guide to use when trimming samples.
	* Specifying the type of tissue and any special instructions for embedding on the request form.
	* Bisecting large pieces of tissue before placing into the cassette for processing. No thicker than 3mm.
5. **Place specimens in spill proof container with a tight-fitting lid.**Be sure that all cassettes are completely submerged in 70% Ethanol, so samples do not dry out. Transfer your project to a laboratory beaker if you wish to keep the container, you brought your project in.
6. **Containers NOT acceptable for submission** - zip lock bags, broken glass containers, or small mouth containers.
7. **Label the transport container:** Please include the name of the PI, name of the Researcher (submitter), solution, and date.
8. **Provide samples and submission form to the Histology laboratory.** A separate sheet can be used to list the specimen identification. Ensuring that the number of cassettes matches the number written on the requisition form; the researcher will be contacted before cassettes are processed if this does not match.
9. **Samples for frozen blocks**:

***Investigator is response for the following:***

* **Labeling** tubes, bags, or cryomolds with researcher’s specimen identification numbers.
* **Supplying** their own dry ice and insulated transport box.
* **Provide samples and submission form to the Histology laboratory.** A separate sheet can be used to list the specimen identification. Ensuring that the number of samples match the number written on the requisition form; the researcher will be contacted before work is started if this does not match.
1. **Turnaround time for routine research projects**
	* Routine TAT is 14-28 working days.
	* If special testing (complex special stains, immunohistochemistry, decal, etc.) is needed project may take longer.
	* Projects are completed on a first come first serve basis.
	* An email will be sent to the investigator when the project is complete.
	* Pick up times for completed projects are Monday- Friday 12:00 pm to 1:00 pm.
2. **Project Cost**
	* Cost of each individual category or task is noted on the request form
	* Cost for tech time assistance for cutting specific areas of a block will be $25/hour additional fee.
	* Cost for items not categorized on the request form will need to be discussed before project is submitted to the laboratory for processing.

Helpful Hints, Terms, & Definitions

Cassette- Small Tissues

* **Make sure to select either a slatted (large specimen) or biopsy (small specimen)**

* P**roper way to sandwich a small specimen in a cassette:**

Sponge

Cassette

Tissue

Sponge

**Tissue height can only be as tall as the cassette is deep (0.5 cm)**

Embedding & Orientation

**Bottom of cassette**– the bottom of the cassette is the side opposite of the cassette lid.

**Bottom of mold**—the bottom of the mold is the side facing down when embedding, and which is cut first on the microtome.

**Cassette**—the cassette is a plastic container that is used to hold identified specimens. It has two parts: the cassette body and the cassette lid. Both parts have small holes which allow for the *fixative* and *holding solution* to associate with the tissue inside the cassette.
**Note:** we require that all specimens be submitted in labelled cassettes (use pencil) and that each cassette label is listed on the accompanying requisition form.

**Inked margins**—inked tissue indicates where cutting is to be performed first.

**Lumen**– the central cavity of a tubular or other hollow structure in an organism or cell.

**On edge**—embedding any tissue with a wall “on edge” makes all the layers of the tissue’s wall microscopically apparent upon cutting; examples of tissues routinely embedded on edge include cysts, gallbladder, specimens with epithelial layers (e.g. skin), and colonoscopic, endoscopic, & cervical biopsies.

**On end**—embedding any tissue with a lumen “on end” makes “all layers of the mucosa, submucosa, and external muscle layers” apparent microscopically upon cutting; this embedding *orientation* is routinely applied to tissues with a *lumen* such as veins, the appendix, and other bodily tubes.

**Top of cassette**—the top of the *cassette* is the side that has the cassette lid.

**Top of mold**—the top of the mold is the side of the mold holding the *cassette* while embedding and is closest to the embedder; it is the side opposite of where cutting will first be performed.

**Swiss rolls**– refers to arranging a colon in the shape of a Swiss roll, i.e. rolling the material around in circles on itself. Embedding colon in the shape of a Swiss roll puts the outer, middle, and inner parts of the colon *on edge*.

Cutting & Sectioning

**One glass slide with one section**: **One glass slide with two sections:**

$3.45 $4.45

**Levels on multiple slides:**

 Section #1 25µ Section #2 25µ Section #3 25µ Section #4 25µ Section #5

Cutting/Sectioning

**Cutting**– a microtome is used to cut sections from a *FFPE* tissue block. Typical *section* thickness is 3-5µm. For contrast, a typical piece of paper is 100µm thick.  Cutting is often referred to as *sectioning*.

**Levels**– refers to the method of gathering *sections* from multiple transections of a tissue specimen: a section is collected from near the surface of the block, after which a much thicker cross-segment of the block is cut away and discarded (e.g. 50µm) and then another section is collected. This process can be repeated iteratively until the block has been exhausted of tissue.

**Sample**—used as a synonym for “block,” “tissue block,” “FFPE block,” or “specimen,” the term is used when referring to any given tissue piece, whether paraffin embedded or not.

**Section**– a “section” refers to one full-face slice off of a FFPE block from a microtome.
**Note** that multiple sections can be placed on one *slide*, if applicable.

**Serial section/ribbon**—a string of sections is known as a serial section or tissue ribbon. This is how sections come off the microtome, which are then separated into individual sections and placed on a *slide*.

Frozen Specimens: Preparation & Embedding

**Cryomolds**—cryomolds used to embed frozen specimens in *OCT*. We use three different mold sizes to accommodate different tissue sizes: astandard size (25mm x 20mm x 5mm), an intermediate size (15mm x 15mm x 5mm) and a deep size (surface size is slightly larger than the standard, and depth is for particularly thick pieces of tissue).

**Cryoprocessing**– refers to the process of preparing frozen tissue for embedding and cutting. As freezing tissue functions as a (physical) “*fixative*,” so to speak, the tissue itself does not need to be placed into a chemical fixative. Frozen tissue can be submitted  either fresh, wrapped in saline moistened gauze, on wet ice or in a 30% sucrose holding solution.

**OCT Compound**– stands for “Optimal Cutting Temperature” compound, this admixture of water-soluble glycols and resins is used to embed tissue specimens prior to *frozen sectioning* on a microtome-cryostat.