Sectioning tissues for an actual zipseq run

1. The quality of zipcoding/imaging is highly dependent on the quality of the slices (thinness, evenness and integrity). That being said some tissues may be difficult to apply this to. Others may need optimization of cutting parameters. For example, B16 tumors, when large, become necrotic in the center and disintegrate upon sectioning so they are unsuitable for this. We have better luck catching the tumors at a smaller stage. This protocol is done on the mouse lymph node as an exemplar.
2. Following your institutional guidelines, harvest inguinal lymph node. Remove as much connective tissue as possible from the surface and keep in cold PBS
3. Using your favorite live tissue slicer (we use either a leica vibratome VT1000) or a precisionary instruments VF-310-0Z (https://tinyurl.com/y3dbbscq) compresstome make sections of desired thickness and orientation. These instructions assume you are using the Precisionary Instruments compresstome.

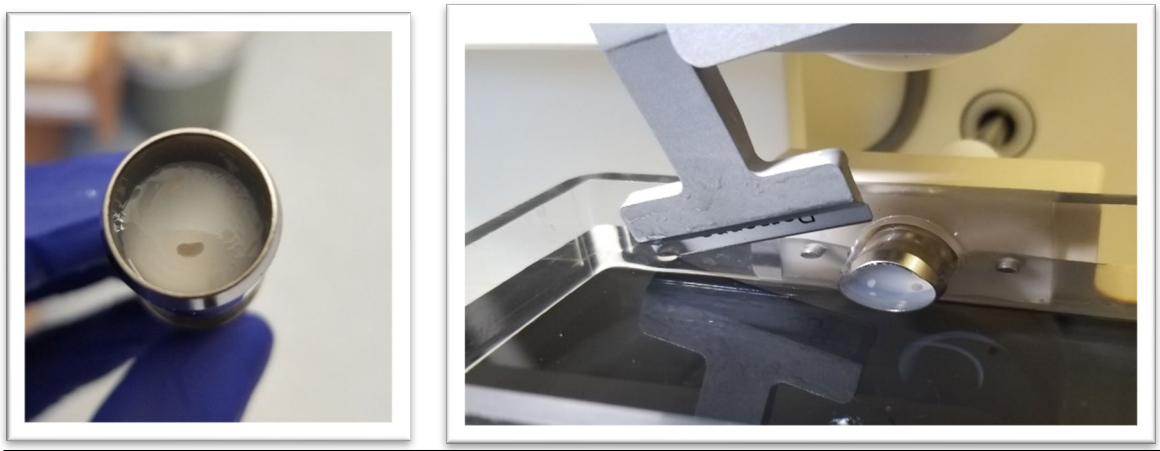

Fig. 1: (A) Inguinal lymph node embedded in 2% agarose. (B) sample tube in the compresstome with PBS in the sample chamber

- Choice of agarose % will depend on tissue, however I've found 1.8-2% agarose (non-low melt) to work quite well for lymph nodes and tumors. Microwave to melt, cool to about 32C. Dab away excess moisture on your tissue, then quickly immerse your tissue into the still liquid agarose, then use a cooling metal block to quickly cast the agarose resulting in an agarose-embedded tissue (Fig. 1A).
- Insert the sample holder tube into the compresstome as seen in Fig. 1B
- Begin sectioning, sample thickness is ideally <150 microns. Oscillation frequency and advance speed are highly sample dependent. For lymph node on the compresstome, an advance speed of 2 and frequency of 5 works well.
- Sectioning should result in discs of agarose with tissue sections embedded as seen in Fig. 2A. Having the agarose stuck to the tissue is actually a good thing as we will see in a bit. Using a glass slide, fish out the disc and use a biopsy punch to cut out a circle around the tissue section (Fig 2B). Using forceps, carefully place into blocking solution (10ug/mL ssDNA, and at least 2% BSA or FCS) (Fig. 3A).

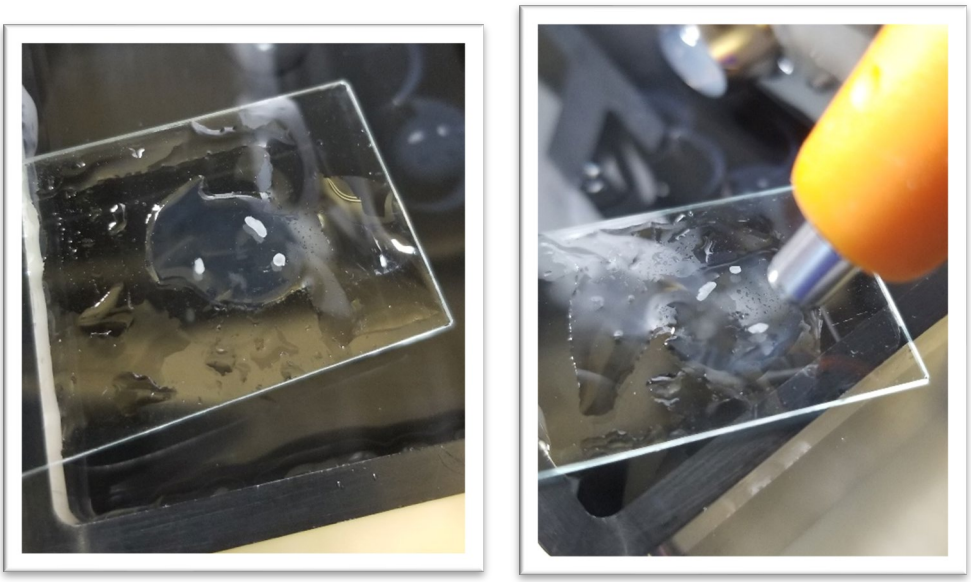

Fig. 2 (A) an example of a 150um thick slice of agarose plug with lymph node sections (3) embedded. (B) using a biopsy punch to punch out a circle of agarose with tissue encased.

- Doing so will actually result in agarose sticking to the tissue section (see picture below) which can be punched out via biopsy punch and affixed to a chambered slide using adhesive, without applying adhesive to the tissue itself. Going from here, we are ready to stain with antibody-caged DNA conjugate, imaging, and zipcoding. Please refer to the next document in the series.

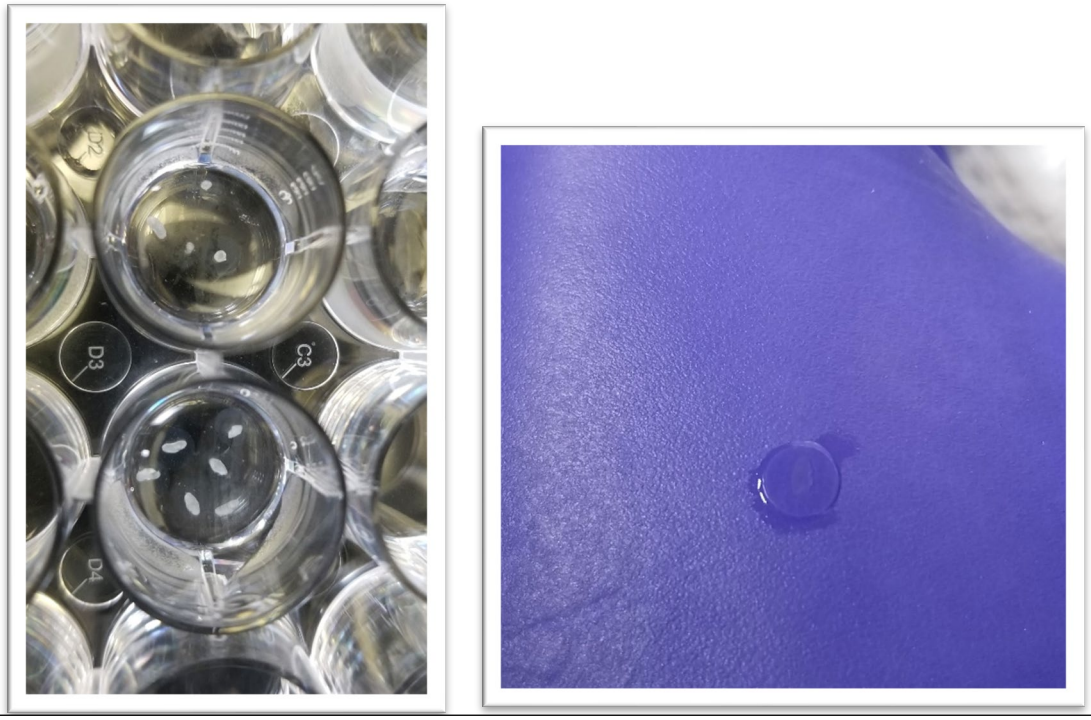

Fig. 3 (A) Discs of lymph node sections sitting in blocking buffer. (B) an example of an agarose disc with a barely visible tissue section in the middle resting on my hand.