

## Preparing LR White Embedded Tissue with mPrep/s Specimen Capsules

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LR White (LRW) is an acrylic embedding resin with advantages for immuno-labeling, enzyme-chemistry, and cyto-chemistry TEM. Because LRW is hydrophilic, it enables aqueous reagents to diffuse into thin sections without prior etching which can denature antigens. In comparison to epoxies, LRW helps maintain the antigenicity required for immuno-labeling. However, a problem with LRW (and similar acrylics) is that because immuno-labeled tissue is usually not OsO<sub>4</sub> treated to preserve antigenicity, it can be difficult to orient or even find the tissue because some tissues are nearly invisible in resin. In this report, we provide a technique to embed tissue specimens in LRW for TEM using mPrep/s specimen capsules. This solves the tissue orientation and tissue location problem while also minimizing handling and providing rapid preparation.

LRW is a low viscosity resin that must be cured anaerobically. Prior known methods for mPrep/s specimen processing used epoxy resins that have a higher viscosity, and which may be polymerized in air. The low viscosity of LRW, while enabling quick infiltration, presents problems with mPrep/s capsules because resin can leak out when placing mPrep/s capsules into the mPrep silicone bench to seal capsule bottoms. Secondly, the anaerobic polymerization requirement for LRW also presents a problem since mPrep/s capsules do not have an air-tight lid. Herein, we solved both the leakage and sealing problem by modifying an existing method. As shown in Figure 1, mPrep/s capsules were themselves embedded within widely available ‘00’ gelatin capsules, which are often used for LRW preparation. These “gel caps” provide a liquid-tight seal and exclude oxygen during heat polymerization. Moreover, this method enables specimens to be orientated, labeled and processed entirely in mPrep/s capsules.

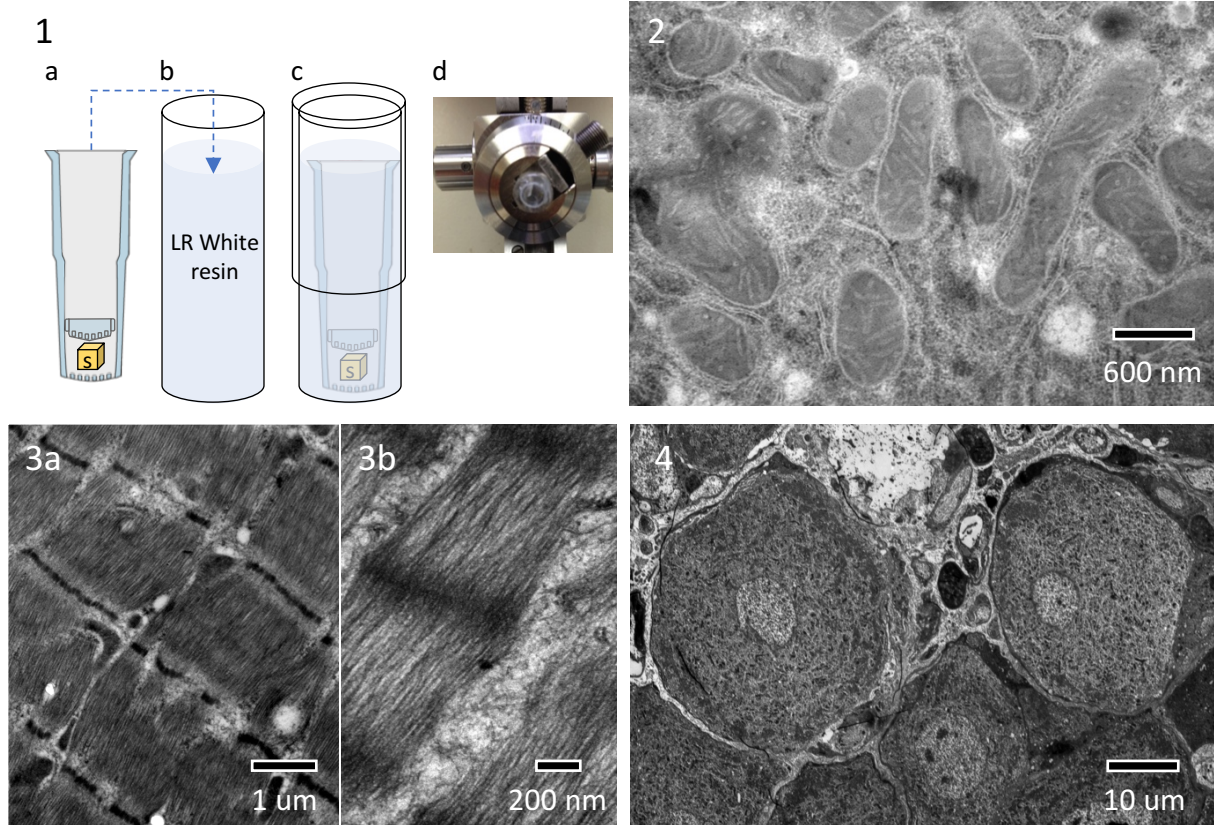
Protocol: Rat tissues were perfusion fixed in Sorensen’s phosphate buffer with 4% paraformaldehyde and 1% glutaraldehyde, while mouse tissue samples were excised and immersed in 4% paraformaldehyde and 0.25% glutaraldehyde. Specimens were then transferred to the EM lab and stored in 1/10 strength fixative at 4 C to maintain antigenicity prior to further processing. Tissue samples were then cut to size (1-2 mm) and oriented in mPrep/s capsules. The mPrep/s capsules were then attached to the ASP-1000 automated specimen processor (ASP). The ASP then automatically exchanged reagents to process specimens through buffer rinses, alcohol dehydration, and LRW infiltration. No OsO<sub>4</sub> post-fixation was used to preserve antigenicity. The total ASP processing time from rinse to resin infiltration was ~30 minutes. After processing, 100% LRW resin was dispensed from the mPrep/s capsules and the capsules were immediately removed from the ASP and placed into gel caps filled with 100% LRW. As the mPrep/s capsules sank into the fresh resin, the specimens were re-infiltrated (Figure 1). Since mPrep/s capsules are just slightly smaller than 00 gel caps, the mPrep/s capsules remain vertically aligned. The mating half of the gel caps were then used to cover the resin filled gel cap bottoms. The covered gel caps with entrapped mPrep/s capsules were then placed in a 60 C oven for overnight polymerization.

Specimens were sectioned directly in the gelatin capsule (holding the mPrep capsule) as shown in Figure 1d. Thin sections (70 nm) were grid stained with 4% uranyl acetate in methanol

followed by Reynolds lead citrate. Micrographs were obtained using a Philips CM120 TEM equipped with a BioSprint camera running AMT Capture Engine V700.

**Results and Discussion:** Tissues were fully embedded and well preserved. Figure 2 shows liver with densely clustered mitochondria with “white” cristae and membranes, because no  $\text{OsO}_4$  post fixation was used. Figure 3 shows well preserved extraocular striated muscle, while Figure 4 shows fully embedded and well preserved dorsal root ganglion nervous tissue.

With this technique, specimens were sectioned directly in gel cap blocks, which in turn held mPrep/s capsules. This protocol enabled tissue orientation to be established after *in situ* aldehyde fixation when the tissue is easily seen and more easily handled than when in resin. This is especially important because non-osmicated tissue can be difficult to find in fluid or polymerized resin. Because the tissue was held in a known centered position in the mPrep/s capsule, this made it easy to find the tissue for sectioning. This therefore lowers the risk of “losing” a nearly invisible tissue sample because it is in an unknown location in a polymerized resin block. Finally, the full reagent protocol following aldehyde fixation required only 30 minutes prior to resin curing.



**Figure 1:** Specimen oriented and entrapped in mPrep/s capsule (a) is placed into 00 size gel cap filled with 100% LRW resin (b). After immersing mPrep capsule in a gel cap, the gel cap is covered with the mating half of the gel cap (c), which is then transferred to a 60 C oven for curing. Gel cap with embedded specimen in mPrep/s capsule is directly clamped in microtome for sectioning (d).

**Figure 2:** Liver (rat) with mitochondria. Membranes are white due to absence of  $\text{OsO}_4$  staining.

**Figure 3:** Extraocular muscle (mouse) at low (a) and higher (b) resolution.

**Figure 4:** Dorsal root ganglion nerve tissue (mouse).

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## Introduction

LR White (LRW) is an acrylic embedding resin with advantages for immuno-labeling, enzyme-chemistry, and cytochemistry for Transmission Electron Microscopy (TEM). Hydrophilic LRW enables aqueous reagents to diffuse into thin sections without prior etching that could denature antigens. In comparison to epoxies, LRW helps maintain antigenicity for immuno-labeling.

## Problems

Tissues in LRW (and similar acrylics) are difficult to orient or even locate in cured and uncured resin because the tissues are unstained since OsO<sub>4</sub> is not used (to preserve antigenicity).

## Solution

We developed a method to use LRW with mPrep capsules and the ASP-1000 automated specimen processor (ASP) to provide:

- Easy specimen location
- Minimal specimen handling
- Always labeled specimens
- Specimen orientation without messy resin handling
- Rapid automated reagent processing in 45 minutes.

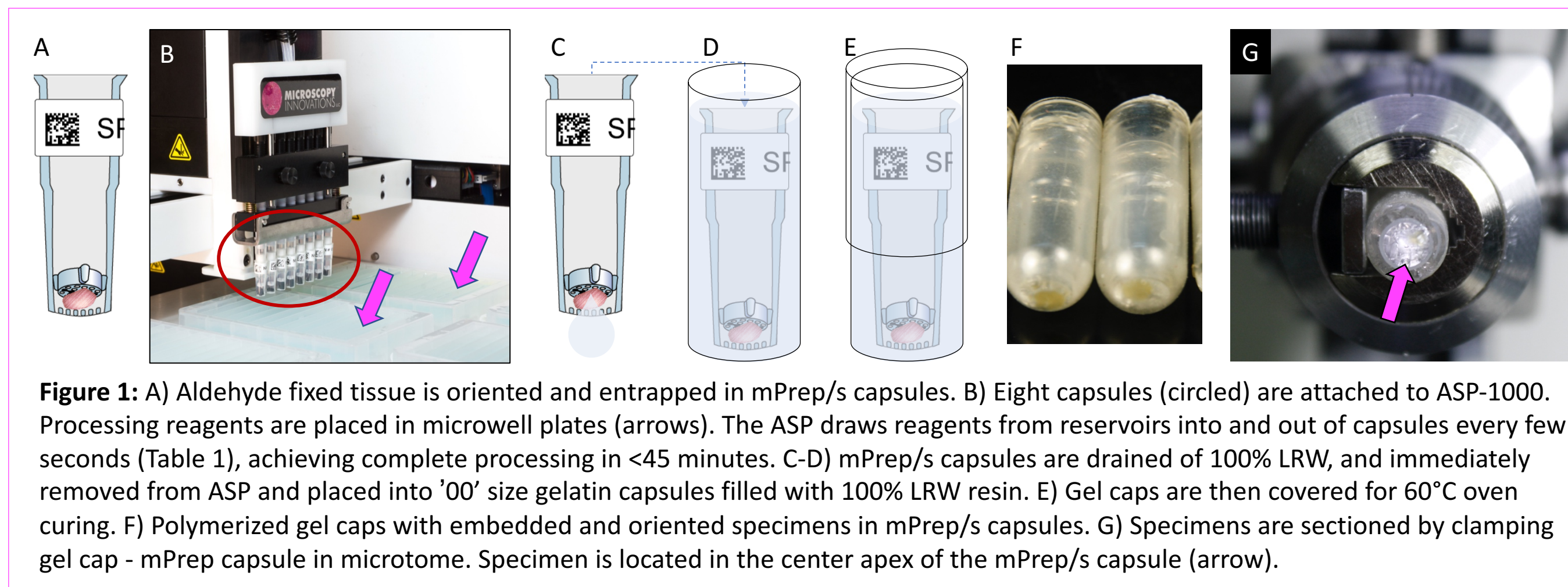
## Experimental

Methods for using mPrep automated processing are well established for epoxy embedding. To adapt these method to LRW processing required:

- Sealing mPrep/s capsules to prevent low viscosity LRW from leaking during polymerization
  - An airtight cover or vessel for anaerobic polymerization.
- Both the leakage and sealing problems were solved by using common '00' gelatin caps with mPrep/s capsules (Figure 1).

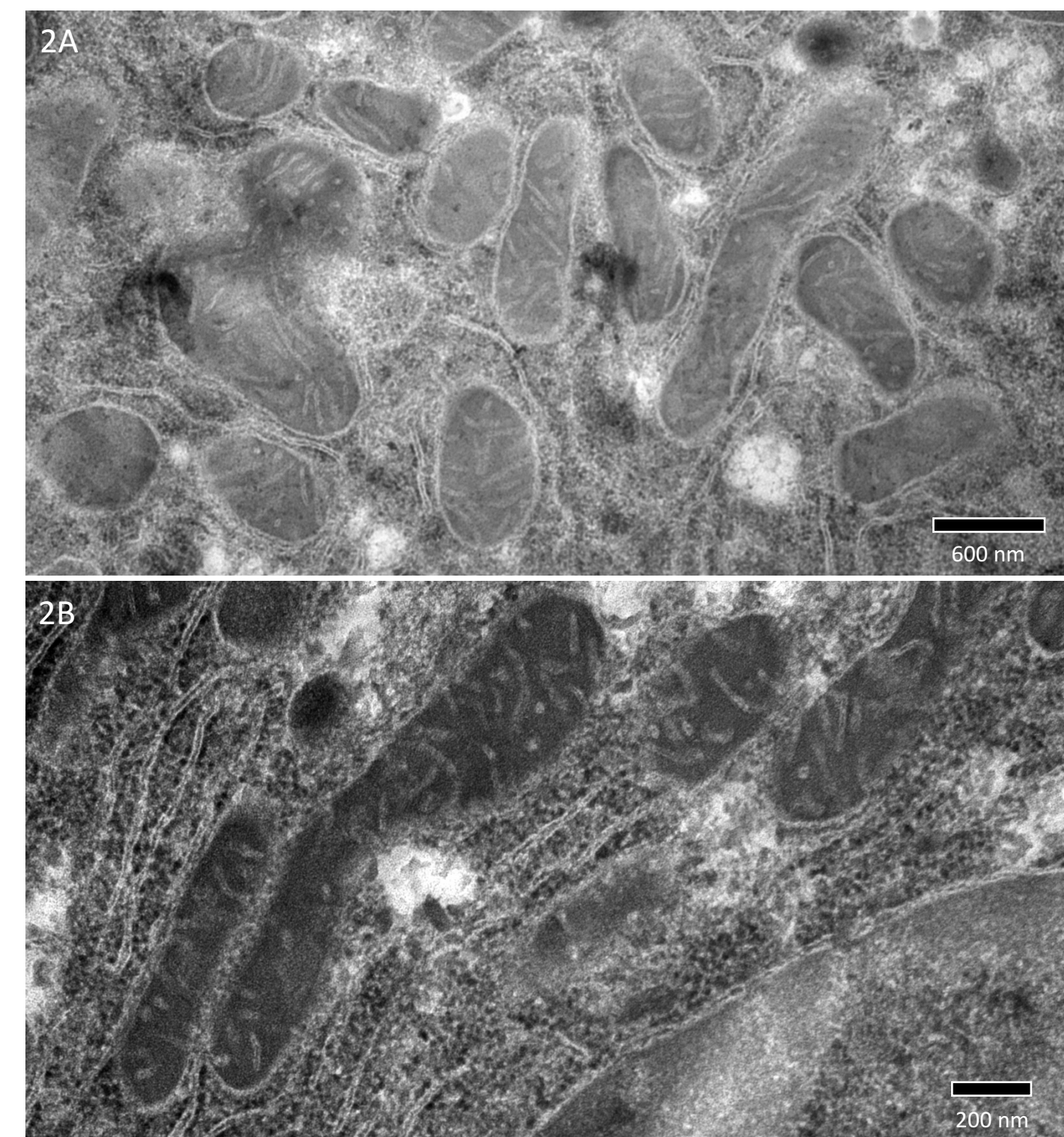
| Step       | Reagent      | Time  | Fluid Exchanges | Pause (sec) |
|------------|--------------|-------|-----------------|-------------|
| 1          | Fix Rinse 1  | 1:00  | 60              | 0           |
| 2          | Fix Rinse 2  | 1:00  | 60              | 0           |
| 3          | Fix Rinse 3  | 1:00  | 60              | 0           |
| 4          | 50% Ethanol  | 1:00  | 60              | 0           |
| 5          | 70% Ethanol  | 1:00  | 60              | 0           |
| 6          | 90% Ethanol  | 1:00  | 60              | 0           |
| 7          | 95% Ethanol  | 1:00  | 60              | 0           |
| 8          | 100% Ethanol | 2:00  | 120             | 0           |
| 9          | 100% Ethanol | 2:00  | 120             | 0           |
| 10         | 25% LRW      | 4:30  | 180             | 0.5         |
| 11         | 50% LRW      | 4:30  | 180             | 0.5         |
| 12         | 75% LRW      | 4:30  | 180             | 0.5         |
| 13         | 90% LRW      | 4:30  | 180             | 0.5         |
| 14         | 100% LRW     | 4:30  | 180             | 0.5         |
| 15         | 100% LRW     | 4:30  | 180             | 0.5         |
| 16         | 100% LRW     | 4:30  | 180             | 0.5         |
| Total Time |              | 42:30 |                 |             |

**Table 1:** Rapid mPrep ASP processing protocol. Note reagents are exchanged as fast as once per second.



## Protocol

1. Rat tissues were perfusion fixed with 4% paraformaldehyde and 1% glutaraldehyde in Sorensen's phosphate buffer.
2. Mouse tissues were excised and immersed in 4% paraformaldehyde and 0.25% glutaraldehyde.
3. Specimens were held in 1/10 strength fixative at 4°C to maintain antigenicity prior to processing.
4. Specimens were cut into 1-2 mm pieces and oriented in capsules using the mPrep/s Workstation (Figure 1A).
5. mPrep/s capsules were attached to ASP (Figure 1B).
6. The ASP exchanged reagents as shown in Table 1. No OsO<sub>4</sub> post-fixation was used to preserve antigenicity. Processing time from rinse to 100% resin infiltration was 42.5 minutes.
7. LRW resin was dispensed from mPrep/s capsules. The capsules were then immediately removed from the ASP and immersed into fresh 100% LRW in '00' gel caps. mPrep/s capsules sank into the fresh resin, filling the capsule and re-immersing the tissue in LRW. The mPrep/s capsules are held vertically aligned in the gel cap (Figure 1C-D).
8. The gel cap was then covered with its mating half (Figure 1E), and transferred to a 60°C oven for overnight polymerization (Figure 1F).
9. Thin sections (70 nm) were cut directly from the gel cap holding the mPrep/s capsule inside (Figure 1G).
10. Grids were stained with 4% uranyl acetate in methanol and Reynolds lead citrate.
11. TEM imaging used a Philips CM120 TEM with a BioSprint camera running AMT Capture Engine V700.

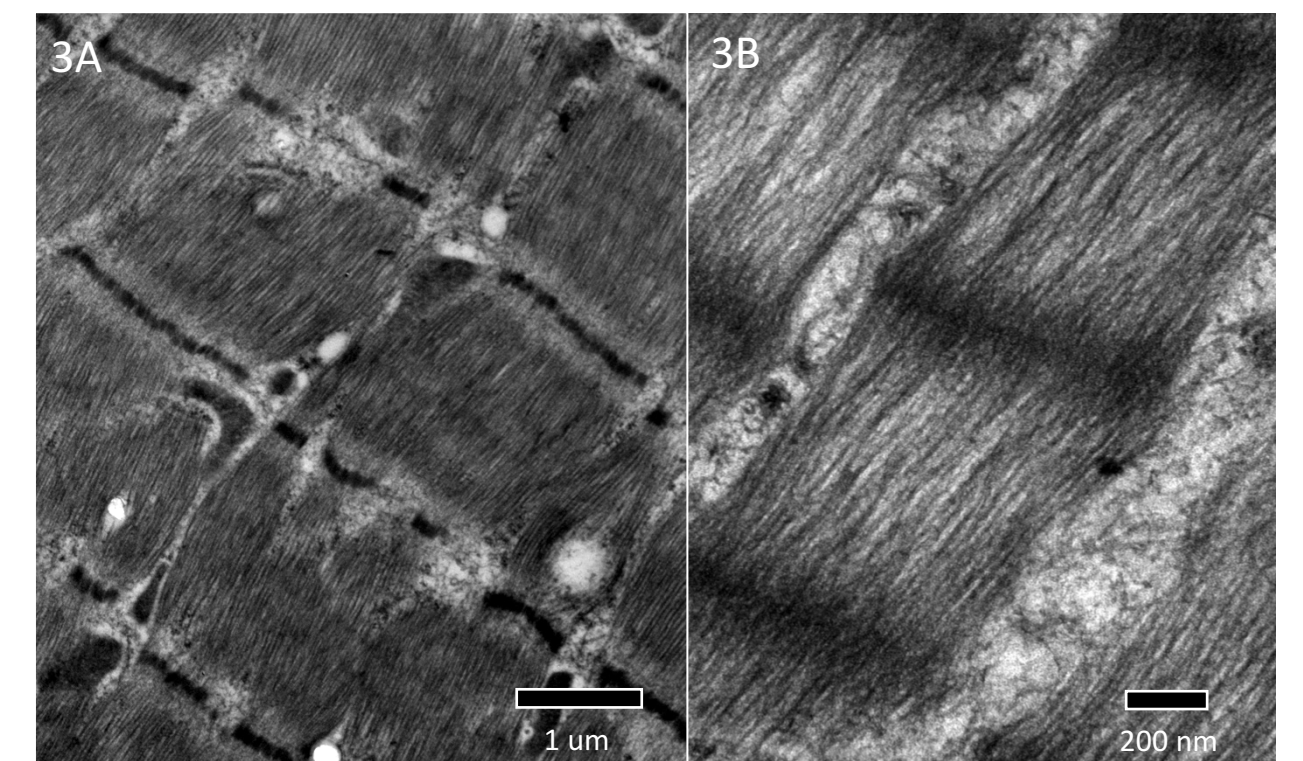


**Figure 2:** Rat liver at low (A) and higher resolution (B). Note abundant mitochondria with well-preserved cristae. White membranes are because tissue was not OsO<sub>4</sub> fixed. B shows part of nucleus.

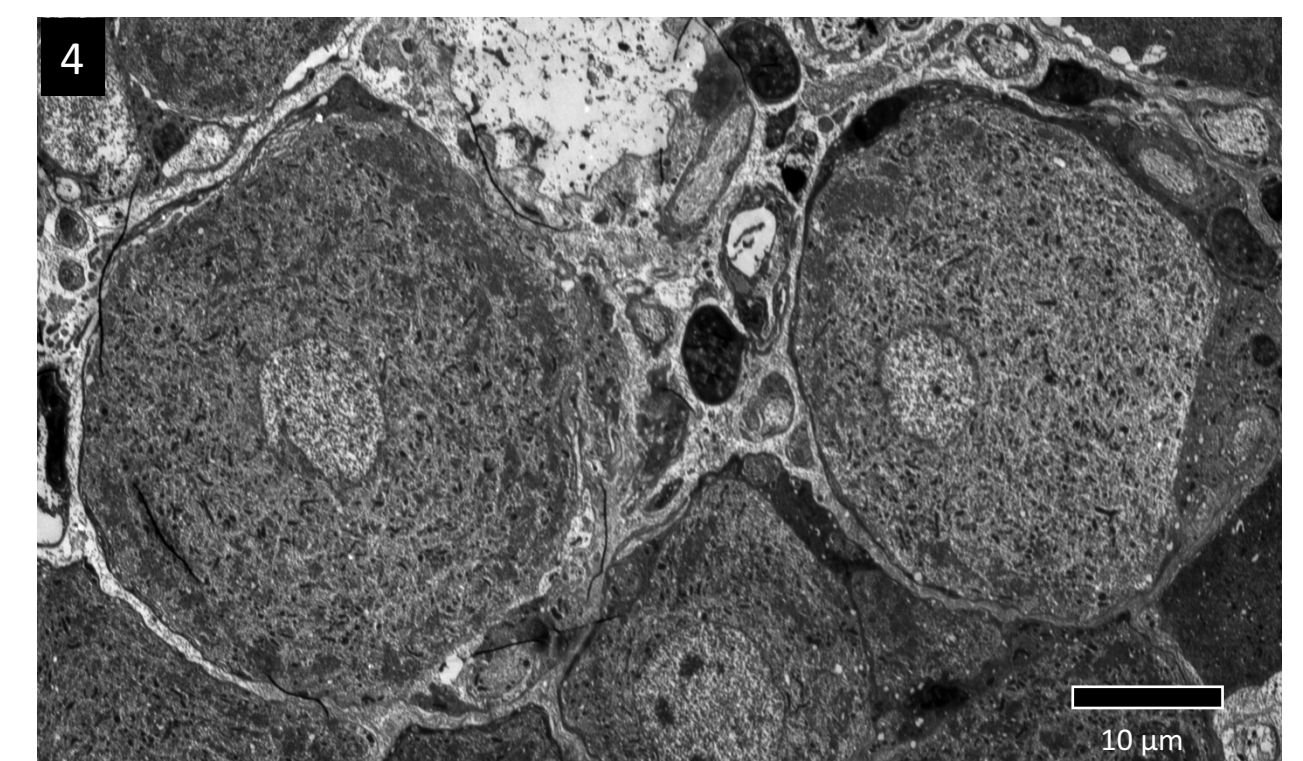
## Results and Discussion

Tissues were fully embedded and well preserved. Rat liver (Figure 2) has densely clustered mitochondria with “white” cristae and membranes because no OsO<sub>4</sub> post fixation was used. Mouse extraocular striated muscle (Figure 3) has clear fibrils and sarcomeres. Dorsal root ganglion shows dense neuronal somas also with “white” membranes (Figure 4). This method provides:

- Easy location of specimens – non-osmicated tissue was easy to find since it was held in the apex of the mPrep/s capsule.
- Tissues were oriented after aldehyde fixation when easily seen and handled, and not in messy resin.
- Minimal specimen handling – Specimens were only touched when inserted into labeled mPrep/s capsules.
- Automated reagent processing protocol required only 45 minutes prior to resin curing.



**Figure 3:** Mouse sarcomeres from extraocular muscle at low (A) and high (B) resolution. Note preservation of all characteristic skeletal muscle features.



**Figure 4:** Mouse neuronal somas from lumbar dorsal root ganglion. Note preservation of nerve cell features.