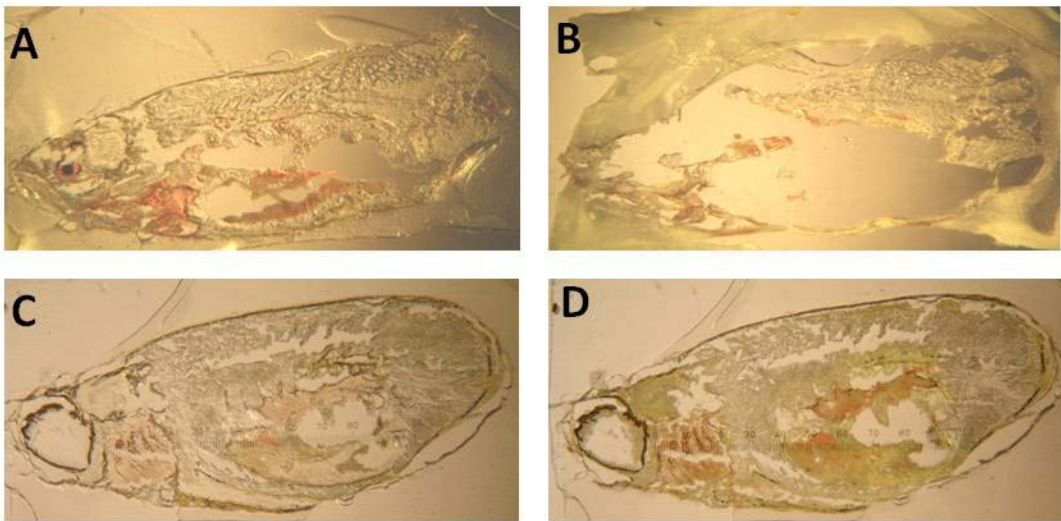
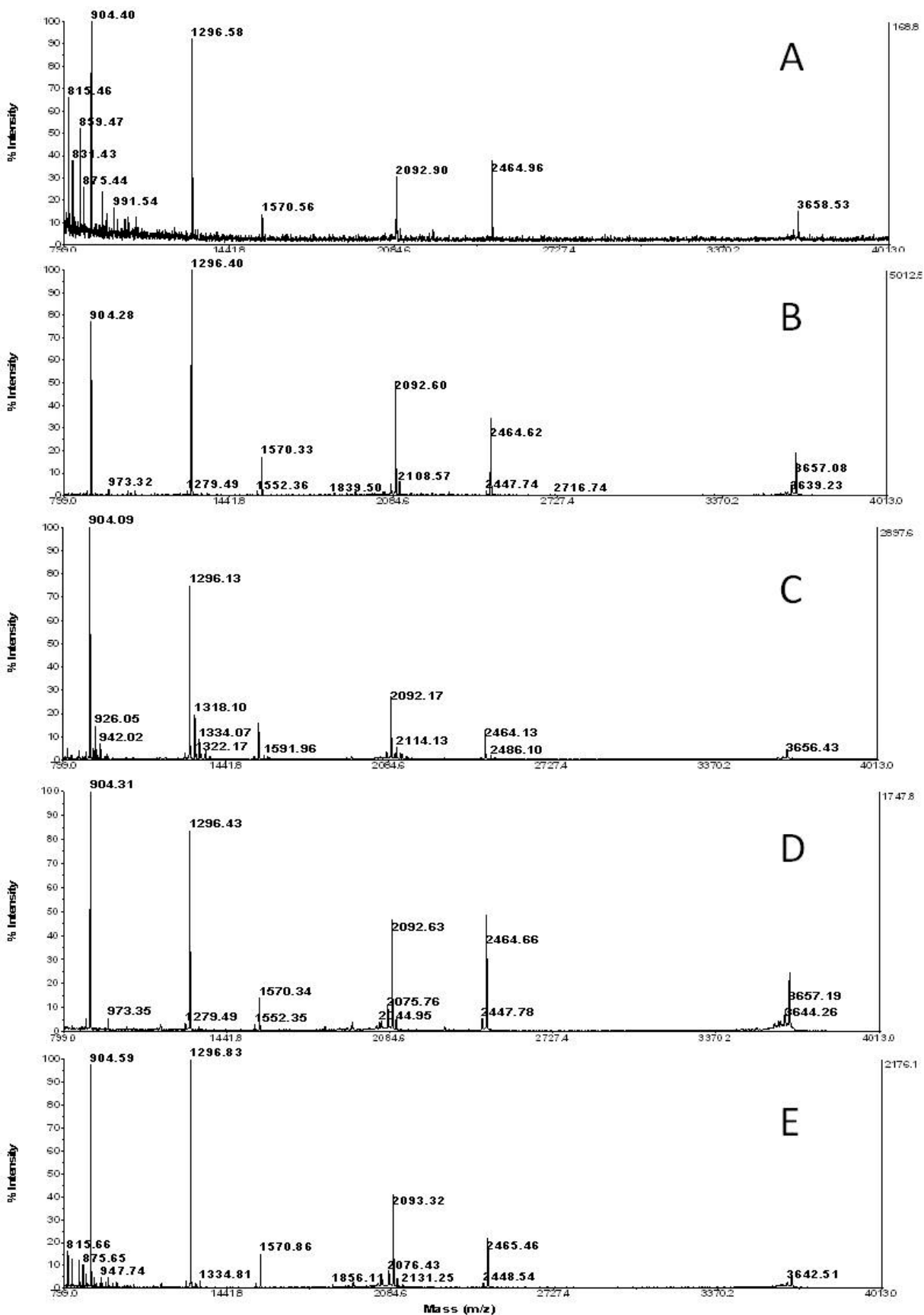


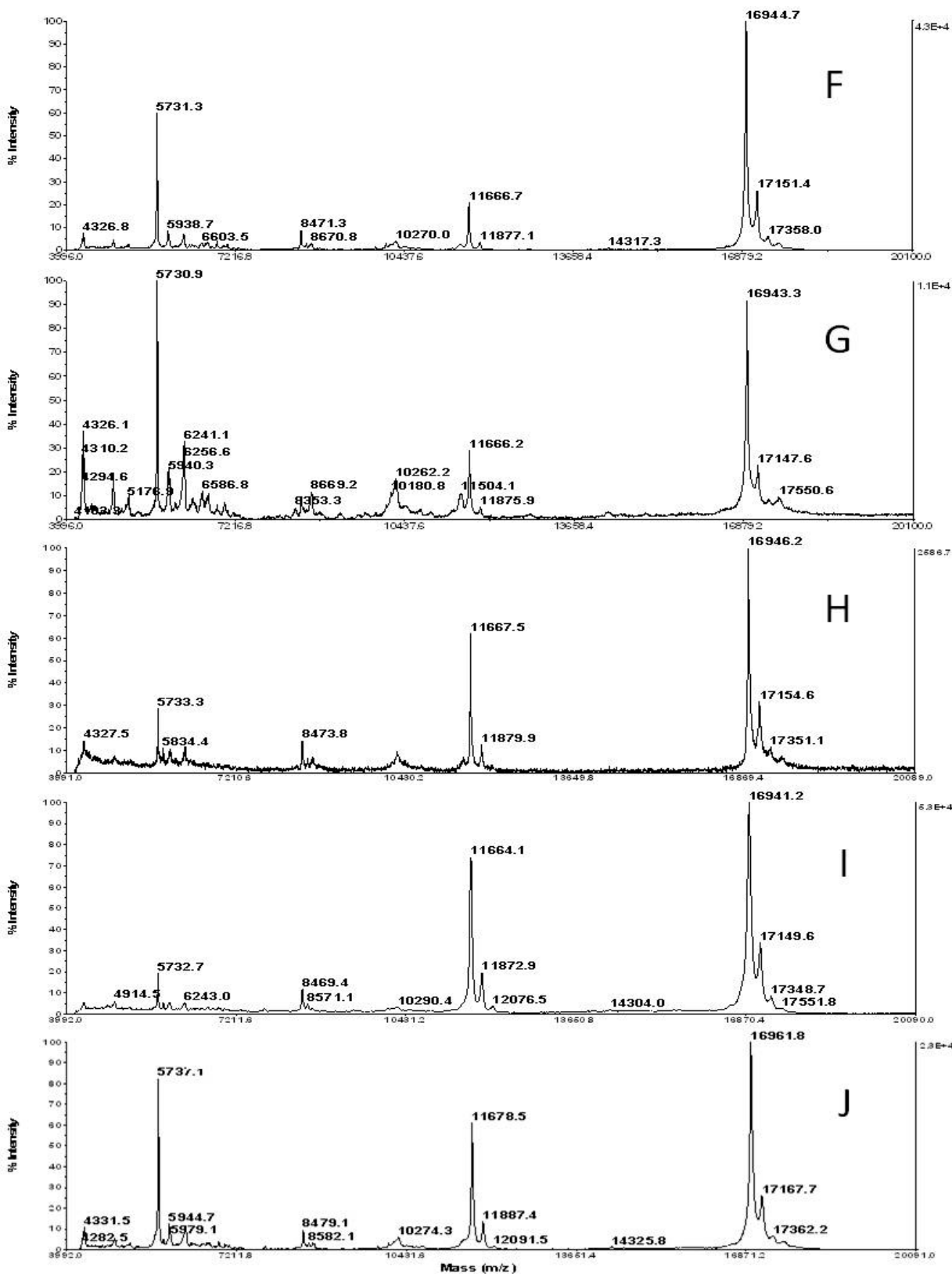
## Supplemental Data Section

**Supplemental Figure 1.** We have documented the destructive nature of the multi-step rinse<sup>1</sup> on zebrafish cryosections. Though the multi-step rinse may be effective for single organ tissues, the technique is not adaptable to whole-body zebrafish cryosections that contain multiple areas of discontinuous tissues and air spaces. The following photos demonstrate the effect of the multi-step rinse versus the effect of the recommended chloroform rinse method:<sup>2</sup> (A) pre multi-step rinse; (B) post multi-step rinse; (C) pre chloroform rinse; (D) post chloroform rinse.

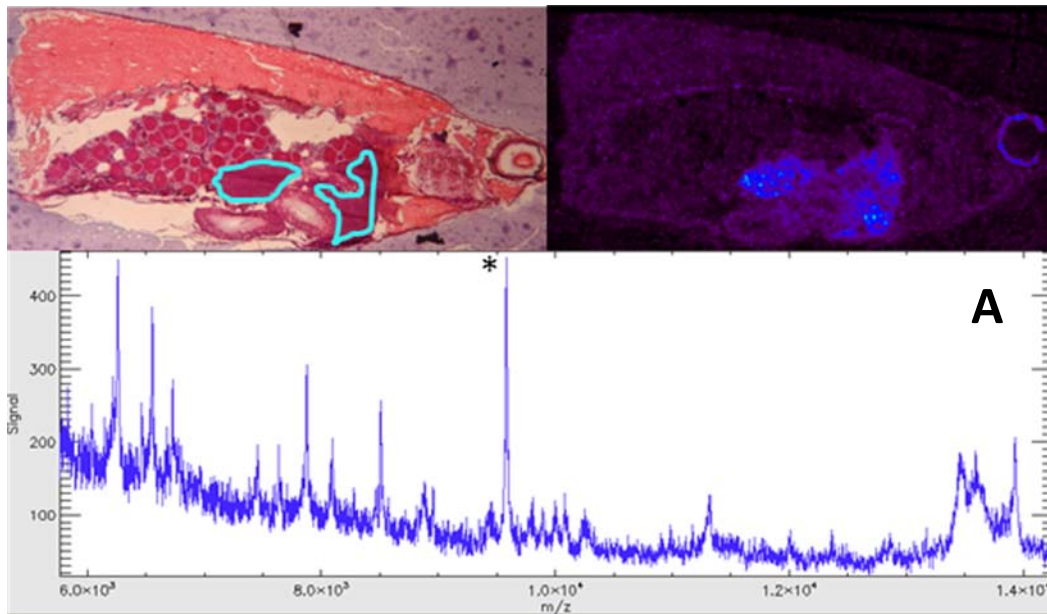


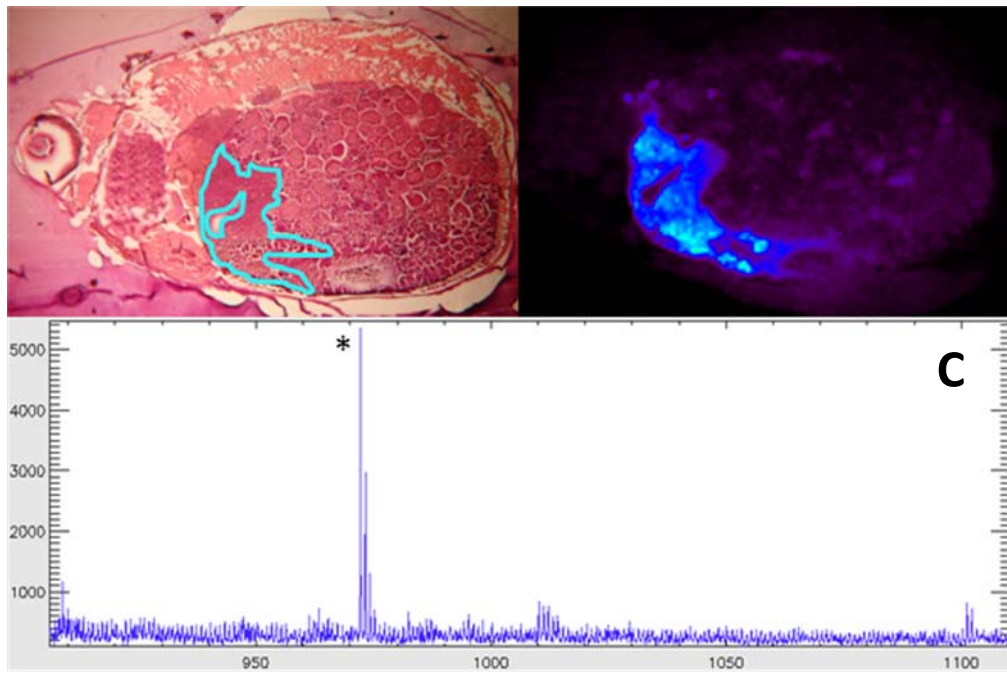
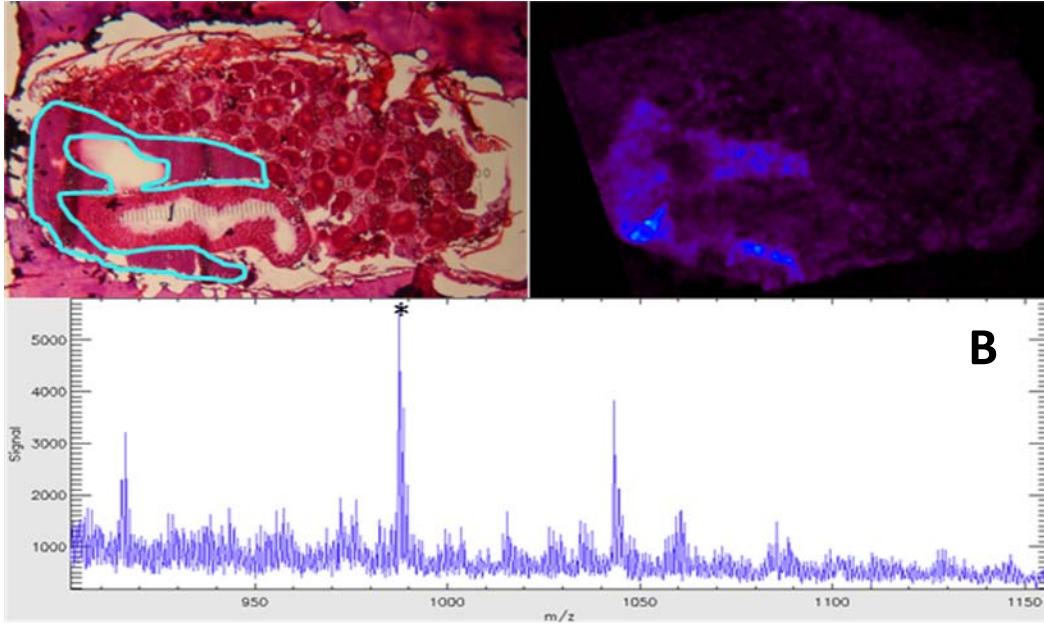
**Supplemental Figure 2.** Representative spectra representing the additional media types that did not suppress peptide or protein ions; media sections spotted with peptide/protein standards mixed with saturated matrix in 50% ACN, 0.1% TFA: (A) 5% gelatin media with CHCA; (B) 10% gelatin media with CHCA; (C) 2% CMC+5% gelatin media with CHCA; (D) 0.6 M sucrose+5% gelatin media with CHCA; (E) 1% agarose+5% gelatin media with CHCA; (F) 5% gelatin media with SPA; (G) 10% gelatin media with SPA; (H) 2% CMC+5% gelatin media with SPA; (I) 0.6 M sucrose+5% gelatin media with SPA; (J) 1% agarose+5% gelatin media with SPA.





**Supplemental Figure 3.** Representative stained sections and MS images of liver-specific masses (blue outline) from zebrafish sprayed with different matrices. MS/MS peptide identification was not attempted, though the AmP matrix additive improved low mass signal and peak resolution: (A) 5 mg/ml SPA without baseline correction, asterisk represents liver-specific mass: 9584  $m/z$ ; (B) 5 mg/ml CHCA, asterisk represents liver-specific mass: 987.44  $m/z$ ; (C) 5 mg/ml CHCA + 6mM ammonium phosphate monobasic, asterisk represents liver-specific mass: 972.22  $m/z$ .





**Supplemental References**

1. Yang J, Caprioli RM. Matrix sublimation/recrystallization for imaging proteins by mass spectrometry at high spatial resolution. *Anal Chem.* 2011;83:5728-5734.
2. Lemaire R, Wisztorski M, Desmons A, et al. MALDI-MS direct tissue analysis of proteins: Improving signal sensitivity using organic treatments. *Anal Chem.* 2006;78:7145-7153.