

our collaborator Dr. Rice that relied on the harsh detergent sodium dodecyl sulfate, and later the more amenable sodium dodecanoate, and high temperatures in order to maximally solubilize the sample[6, 29].

Results. This research is fully described in Goecker et al. [1]. To summarize: the reference protocol, while resulting in a high level of solubilization also resulted in a high level of deamidation and lower level of peptide identifications. We therefore tried two approaches to maximize the amount of proteomic information obtained and therefore maximizing the amount of genetic information that could be obtained through detection of genetically variant peptides. The first was to use gentle chemistry in processing the sample. By using shorter trypsin digestion time, and lower temperatures, we observed less solubilization but we also saw much greater levels of peptide identifications. The other advance we tried was a dramatic increase in the level of reductants. By increasing DTT levels from the 25 mM level up to the 100 mM level we observed a dramatic increase in GVP detection. Together the sensitivity (TP/(TP+FN)) of GVP detection increased from 11.4% to 33.6%, an almost 3 fold increase[1]. The total identified GVPs increased from 45 to 127 for the optimized processing method, the number of unique peptides from 1585 ± 162 to 2703 ± 230 ($p = 5 \times 10^{-13}$) and the average number of genetically variant peptides detected increased from 20 ± 5 to 73 ± 5 ($p = 1 \times 10^{-13}$). The RMP increased from a maximum of 1 in 1380 and a median value of 1 in 24 for the original processing method to up to 1 in 624 million from a single hair with a median value of 1 in 1.1 million after chemical processing optimization ($p = 4 \times 10^{-7}$). Likewise, median RMPs for the African samples increased from 1 in 5.1×10^1 to 1 in 1.5×10^8 , and European samples increased from 1 in 1.3×10^1 to 1 in 2.2×10^3 . These increases in RMP also meant that the likelihood of ancestral background increased as well. A likelihood ratio (LR) defined as the RMP calculated from the African population divided by the RMP calculated from the European population. With optimization and increased GVP detection, the likelihood ratio for European samples (eq. 2) decreased by 0.94 ± 0.39 orders of magnitude ($p = 1 \times 10^{-4}$), while the African samples increased by 3.90 ± 0.32 orders of magnitude ($p = 5 \times 10^{-4}$). The GVP profiles from African subjects were therefore considerably less frequent in European populations than in African ones and *vice versa*.

