Advancing Western Blotting: Improving the Sensitivity of Protein Detection with Smaller Well Engineering

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Western Blotting is one of the most fundamental laboratory techniques used for identifying specific proteins and semi-quantifying protein amounts. As powerful and developed as the procedure has become, one of the challenges facing the current methods of western blotting is sample amount. Protein and antibody sample constitute the majority of western blotting experimentation cost, and often a large amount of sample must be used to detect low-abundance proteins. This is a problem especially with protein samples that are expensive, rare, or otherwise difficult to obtain. In order to address this problem, this work aimed to improve the sensitivity of western blots and allow any improvement to be applicable to large-scale research. Gel electrophoresis combs were constructed by hand to have well sizes smaller than those of today's standard size combs. Then, western blots were conducted with common antibodies using both the standard combs and the new combs. The results consistently demonstrated a 2 fold increase in the intensity of the protein bands, suggesting that the newly engineered combs with smaller width wells have a strong potential to increase the sensitivity of the western blotting procedure. Furthermore, the findings suggest that combs with smaller wells can reduce not only protein requirement, but also antibody requirement. The significance of the results is that an increase in the sensitivity of western blotting equates to a decrease in protein and antibody requirements, reduced total experimental cost, and a higher probability of detecting previously undetectable low abundance proteins. The impact of this research includes areas where western blotting plays a vital role, such as signal transduction, vaccine development, and proteomics.

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