

# Breaking Laser Capture Microdissection Sample Size Road Blocks with Simple Western

## Introduction

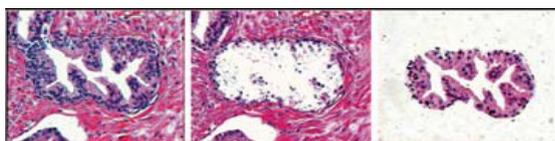
Laser capture microdissection (LCM) is a powerful tool to identify and isolate a pure sample of the cell type you're interested in. But, proteomic studies with LCM samples are really restricted by the small amounts of tissue collected with each capture since there just isn't much to work with. You often have to use the entire sample captured for traditional Western blot analysis and that only leaves you with one data point! So, researchers often use 2D Electrophoresis and mass spectrometry instead to max out the amount of data they can generate<sup>1</sup>. Both of these methods have their own limitations when it comes to ease-of-use and reproducibility though. Immunohistochemistry is also used to analyze LCM samples as it's a more accessible technique, but it really doesn't give you a lot of info either.

Simple Western is an automated capillary-based immunoassay that changes the proteomic research game. You only need 1-10  $\mu$ L of LCM sample for each data point, so you'll get more data points for each sample you collect. Not to mention the sensitivity that comes with it will even let you analyze proteins you couldn't previously do with traditional Western blot. And it's all wrapped up in a simple workflow that minimizes your hands-on time. Simple Western is a sensitive, easy-to-use analytical tool that ups the ante on protein analysis with LCM samples.

In this application note, we'll show you two examples of how Simple Western changed what researchers could do with their precious LCM samples for the better.

## LCM basics

Tissues are made up of a complex mixture of different cell types that can respond differently to external stimuli, potentially biasing your analysis. Laser-capture microdissection helps you avoid this bias by isolating a specific cell type so you'll have a homogeneous sample to analyze (**Figure 1**).



**FIGURE 1.** Human prostate gland tissue purified using laser capture microdissection<sup>3</sup>. Tissue section prior to LCM (left), tissue after removal of cells of interest (middle) and purified LCM sample (right) shown.

After staining, tissues are visualized on a stage of a specially designed microscope, and a laser is used to cut out specific sections of the tissue based on cell morphology<sup>2</sup>. Two kinds of lasers, infrared and ultraviolet, are used to cut the tissue and the purified cells are collected and then transferred to a microcentrifuge tube for genomic or proteomic analysis.

There are a lot of different options when it comes to laser capturing your samples. Pick the LCM method that is most suited for your tissue or cell type. Just make sure your method, from fixing and staining the tissue to capturing and collecting your cells is compatible with extracting protein from the LCM sample.

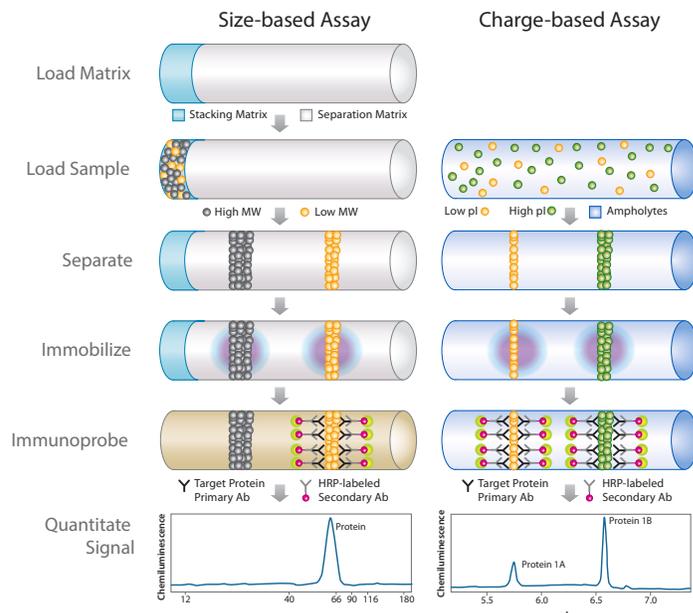
## Simple Western Basics

Simple Western is a fully automated immunoassay that takes place in a 5 cm x 100 µm ID capillary. It comes in two flavors – size or charge. Size-based separation gives you the molecular weight (MW) and specificity you'd get with a SDS-PAGE and Western blot, only with a lot less hassle. Charge-based separation is similar to an IEF Western where proteins separate in a pH gradient to the pI where they have neutral net charge.

Samples and either separation matrix or ampholyte gradients (depending on your separation mode) are vacuum-loaded into a capillary, voltage is applied to separate proteins and then a proprietary UV-induced capture chemistry immobilizes the protein to the capillary wall. Uncaptured material is then washed away so your primary antibody can enter the capillary and specifically bind to your protein of interest. Next steps include an HRP-labeled secondary and luminol/peroxide that generates a quantitative chemiluminescent signal that is visualized in an electropherogram or lane view using Compass for Simple Western software (**Figure 2**).

Since phosphorylation events will cause the isoelectric point of a protein to change, charge separation is especially powerful for studying post-translational modifications. You can separate proteins based on phosphorylation state and immunoprobe with a pan antibody, which means you don't have to run samples multiple times in order to probe with different phospho-specific antibodies anymore. You also won't have to worry about phospho-specific antibodies having different affinities if you want to compare the level of phosphorylation. And you can uncover new phosphorylation events you couldn't see before because there simply wasn't a phospho-specific antibody available for that isoform.

Size-based separation on Wes, Sally Sue, and Peggy Sue, only requires 5 µL of denatured sample that contains your lysate, 1X Fluorescent Standard, DTT, and Sample Buffer for each data point. Wes is a single-cycle instrument that gives you 1 data point per 5 µL of sample for a total of 13 or 25 data points per run in a mere 3 hours. Sally Sue and Peggy Sue are multi-cycle systems (8 cycles x 12 capillaries/cycle) that give you an extra bang for your buck with 8 data points per 5 µL of sample for a total of 96 data points overnight.



**FIGURE 2.** Simple Western technology. Size-based assay (left) and charge-based assay (right).

For charge-based separation on Peggy Sue and NP1000 systems, you only need 10 µL of native sample that contains your lysate. Both systems are multi-cycle instruments (8 cycles x 12 capillaries/cycle) giving you up to 8 data points per sample in 11-19 hours.

An additional bonus? It only takes about 30 minutes of hands-on time to set up an experiment! Simple Western systems do everything for you, freeing up hours that you can now spend on more important things.

## Simple Western Size for analysis of diabetes and exercise research

Dr. Charles Stuart's lab at the department of Internal Medicine at East Tennessee State University Quillen College of Medicine studies the effects of exercise training on diabetes. They use LCM to collect purified samples of myosin heavy and light chain fiber types from vastus lateralis skeletal muscle biopsies to look for various changes in fiber composition. LCM samples were routinely analyzed using traditional Western blot, but data collection was really slow since it took more than a day to collect LCM samples and then another day to immunoprobe one data point for each sample.

The Stuart lab uses the ArcturusR LCM System that cuts samples with UV and captures protein with IR. Unfixed, 10  $\mu\text{m}$  frozen tissue is first sectioned onto an Arcturus LCM slide where they are stored at  $-80^\circ\text{C}$  until staining. Tissues are acclimated at room temperature for five minutes before fixing for five minutes in  $-20^\circ\text{C}$  acetone followed by five minutes of air drying. Samples are then stained using a Vector Laboratories peroxidase kit to visualize the different fiber types. After staining, tissue is rinsed for two minutes in water followed by desiccation in a vacuum desiccator for 10 to 15 minutes to help tissue capture.

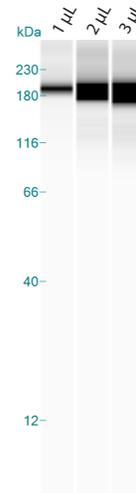
Approximately 2  $\text{mm}^2$  of tissue is isolated and then lysed with 20  $\mu\text{L}$  of RIPA Buffer (ProteinSimple, PN 040-483) containing protease inhibitors. Samples are homogenized with a syringe 10 times, sonicated five times, or hand homogenized. Lysate is then pre-cleared at 17,000  $\times g$  for 20 minutes to remove insoluble tissue.

The Stuart lab then uses 1-4  $\mu\text{L}$  of lysate depending on the protein of interest to prepare a 5  $\mu\text{L}$  sample that includes 1X Fluorescent Standard and DTT for size-based analysis on Wes (**Figure 3**). Because they can use such a small sample size for analysis, the Stuart lab gets anywhere from five to 10X more data per LCM sample than they used to with traditional Western blot. And they do it using default assay conditions with the 12-230 kDa Separation Matrix!

The lab is now taking things a step further, continuing assay optimization with the 66 – 440 kDa Separation Matrix. Not only is the sizing range more suited to their needs, but they can now get data without any of the hassles associated with transferring a high molecular weight protein on a Western blot.

### Simple Western Charge detects differences between normal and mutant aberrant crypt foci

LCM is also commonly used to differentiate between tissues with different somatic mutations. Dr. David Rosenberg at the Center for Molecular Medicine at the University of Connecticut Health Center used LCM to isolate aberrant crypt foci (ACF) with KRAS and BRAF mutations associated with colorectal cancer<sup>4</sup>. Before implementing Simple Western in their research,



**FIGURE 3.** Simple Western Size on Wes detects myosin heavy chain 7 (MHC7) with 1, 2 and 3  $\mu\text{L}$  of LCM sample. Similar experiments with traditional Western blot require 20  $\mu\text{L}$  of LCM sample.

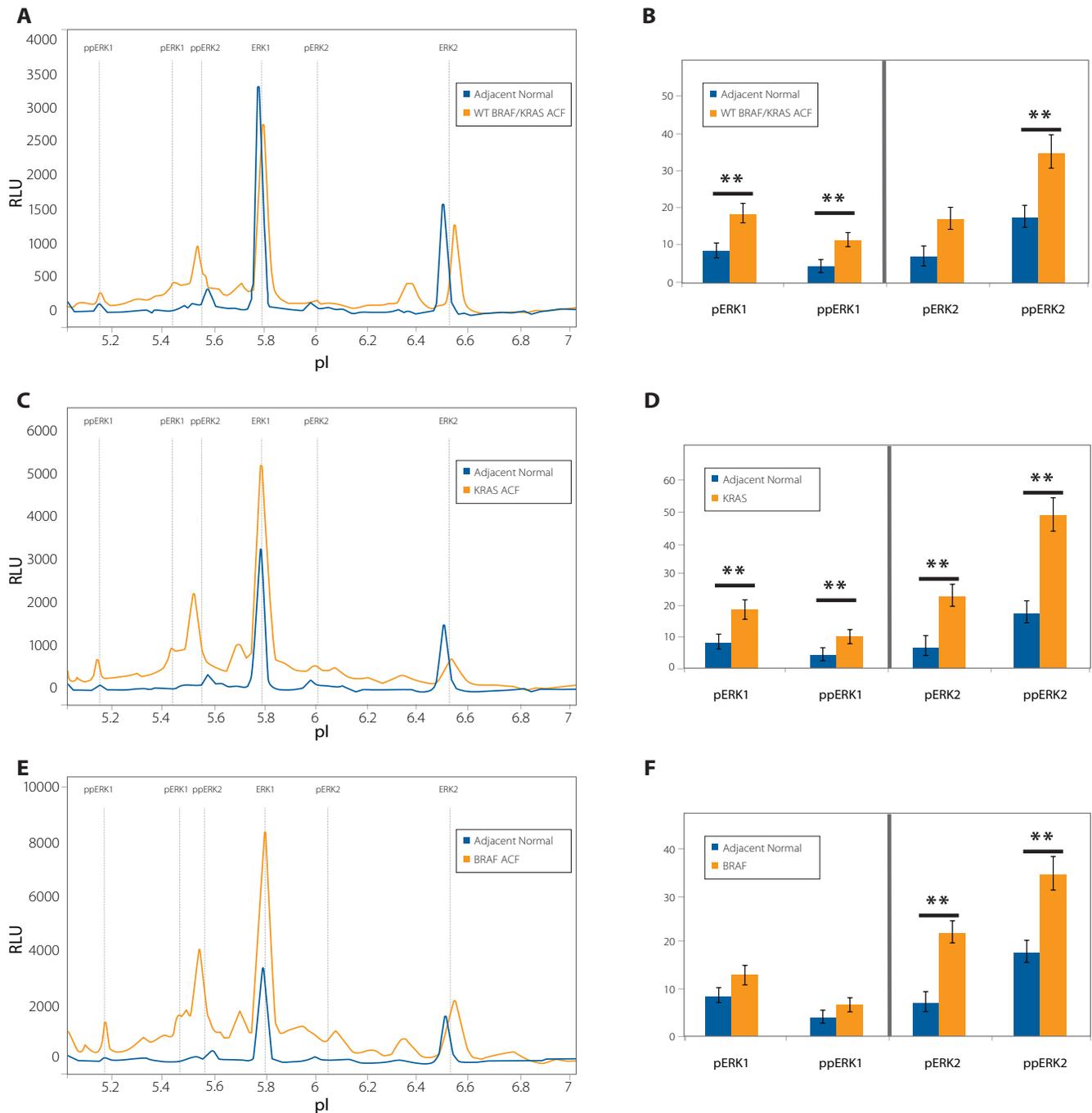
proteomic analysis was limited to routine histological and immunohistological analysis due to the small sample size.

ACF biopsies were sectioned using a Leica CM1900 cryostat and 12  $\mu\text{m}$  sections were collected on Arcturus PEN membrane glass slides for LCM purification. At least 1  $\text{mm}^2$  of tissue was collected from each sample using a Veritas microdissection instrument with a combined UV-cutting and IR transfer method to avoid compromising tissue quality. Adjacent normal crypts and LCM samples from normal biopsy samples were captured too. The captured LCM samples were stored no more than 24 hours at  $-80^\circ\text{C}$  before tissue lysis with 10  $\mu\text{L}$  of Bicine/CHAP Lysis Buffer (ProteinSimple PN 040-486) containing protease and phosphatase inhibitors directly. Samples were incubated on ice for 10 minutes, spun at 6,600 rpm, and then sat on ice for another 10 minutes. Tubes were spun at 10,000  $\times g$  for 10 minutes at  $4^\circ\text{C}$  to remove the cellular debris and supernatants transferred to a low-retention microcentrifuge tube and stored at  $-80^\circ\text{C}$ . Total protein concentrations were determined using the microBCA Assay (ThermoFisher Scientific).

Samples were separated using Simple Western Charge and probed for total ERK1/2. 0.16  $\text{mg}/\text{mL}$  of sample was mixed at a 1:4 ratio with G2 premix, pH 5-8 and pI Marker Ladder 3 for a final concentration of just 0.04  $\mu\text{g}/\text{mL}$  total protein. Sample peaks observed with the pan-ERK antibody were further identified using phospho- and isoform-specific ERK antibodies.

ERK1 and ERK2 phosphorylation of normal ACF samples were compared to WT BRAF/KRAS ACF, KRAS, and BRAF tissue. Increased levels of pERK1 and ppERK1 were observed in WT BRAF/KRAS and KRAS tissue only while pERK1, ppERK1, pERK2, and ppERK2 levels were increased in all of the tissue samples (**Figure 4**).

Simple Western Charge gave Dr. David Rosenberg a method that increased the informative potential of a single biopsy specimen that can ultimately enable them to finally understand disease progression in their ACF samples at a molecular level.



**FIGURE 4.** ERK1/2 detected in aberrant crypt foci (ACF)<sup>4</sup>. WT KRAS/BRAF (A and B), KRAS mutant (C and D) and BRAF (E and F) LCM samples were compared to adjacent normal tissue (purple) using Simple Western Charge. Phospho-ERK1 levels were only elevated in WT BRAF/KRAS and KRAS mutants while phospho-ERK2 levels were increased in all ACF samples.

## Conclusion

Laser capture microdissection gives you purified samples so you can avoid bias when investigating the molecular mechanisms for a host of diseases and pathways. But until now, you couldn't do much in the way of proteomic research on a per-sample basis with existing techniques because they use most, if not all, of the limited amount of sample you get with LCM. Simple Western Size and Charge changes all that. They only need 5-10  $\mu$ l of sample to measure protein abundance and/or post-translational modifications, and they do it in a capillary. The whole process is completely automated, and only needs about 30 minutes of hands-on time!

Researchers at East Tennessee State University and the University of Connecticut Health Center used Simple Western to study LCM samples derived from human muscle and human ACF. Because of the small sample size needed for analysis, the Stuart lab at East Tennessee State University now gets anywhere from five to 10 data points per LCM sample with Simple Western Size compared to the one data point they used to get with traditional Western blot. Simple Western Charge gave the University of Connecticut a method that increased the informative potential of a single biopsy specimen that can ultimately enable them to finally understand disease progression in their ACF samples at a molecular level. So if you want to get more out of your research with LCM samples, we've got a Simple Western solution for you!

## References

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