

New Automated Systems for Size-Fractionation of Protein Samples



Rosemary O'Brien, Ezra Abrams, Simran Singh, Todd Barbera, Chris Boles
Sage Science, Inc., 500 Cummings Center, Suite 3150, Beverly, MA 01915



Abstract

Sage Science has developed two new systems for automated preparative electrophoresis of protein samples. In both systems, the user simply loads samples into precast gel cassettes, programs the desired collection ranges into instrument software, and starts the run. At run completion, the user removes the desired protein fraction(s) from membrane-bounded elution modules within the cassette. The fractionated samples are recovered in SDS gel buffer -- no gel extraction is required. Instrument software controls of timing of protein fractionation using input from on-board optical detection units, which monitor the progress of fluorescently-labeled molecular weight markers during electrophoresis.

The two systems differ significantly in fractionation strategy. The first system is based on Sage's existing BluePippin electrophoresis instrument for nucleic acid fractionation, but features precast gel cassettes developed specifically for protein fractionation. The protein BluePippin cassettes are useful for collecting one or two targeted protein fractions from a sample.

The second system, the Sage ELF system (for Electrophoretic Lateral Fractionator), is a completely new instrument/cassette system designed to fractionate a protein sample into 12 contiguous size fractions.

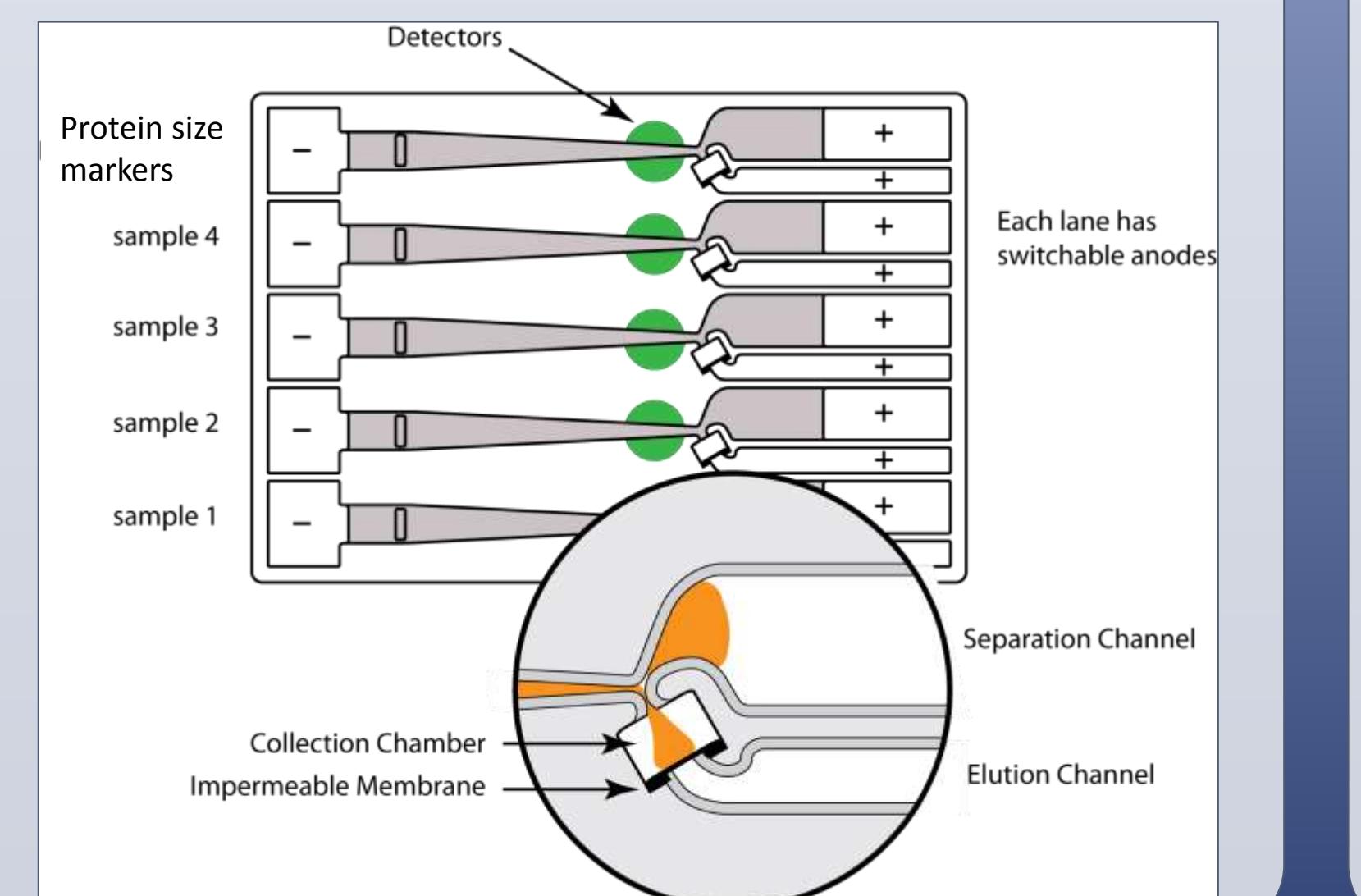
In both systems, SDS agarose gels are used as the separation matrix, and fractionated proteins recovered in SDS gel buffer. 5% agarose gels are used for proteins 18 to 80 kD in size, and 3% agarose gels are used for sample 50-200 kb in size. The width of the size collection window can be varied by the user, with a minimum window of about 20 kD.

The new Sage systems should provide increased reproducibility and ease-of-use for procedures that require gel isolation of proteins, such as top-down proteomics studies and other protein mass spectrometry methods.

The Sage BluePippin: Targeted Size-Fractionation

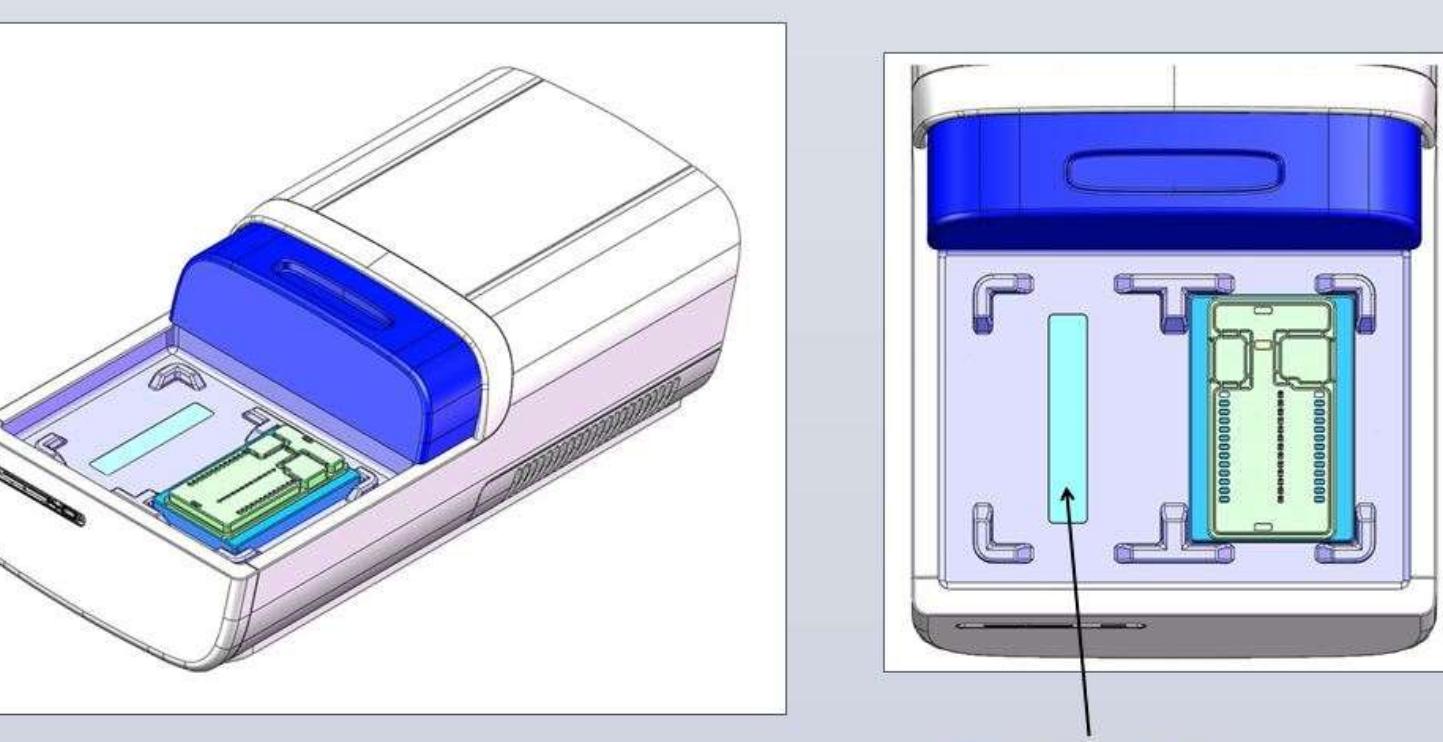
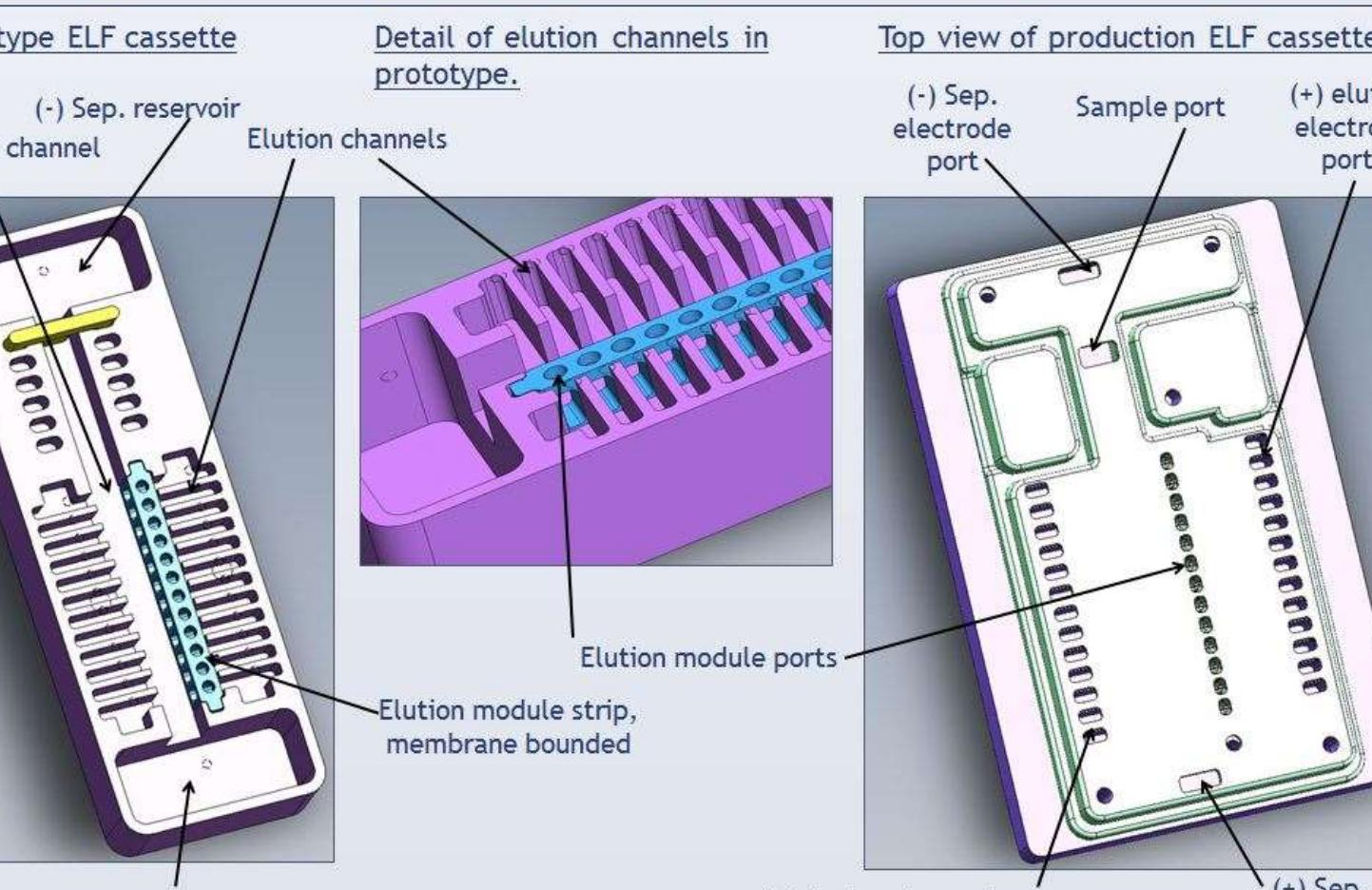


The BluePippin is an enhanced version of the Sage's original Pippin Prep instrument for DNA size fractionation. The system includes disposable pre-cast SDS agarose gel cassettes with five physically isolated lanes. Each lane has a tapered separation channel that branches into two channels with positive electrodes at their termini. Through independent control of the positive electrodes, proteins exiting the separation channel can be directed left or right at the branch point. The selected protein fraction is collected in liquid buffer within a membrane-delimited elution module. As in the original, the BluePippin instrument contains a single board PC, a fluorescence detection unit, and an electrophoresis power supply. Timing of fraction collection is determined using the mobility of fluorescein-labeled reference markers loaded in one of the five lanes.



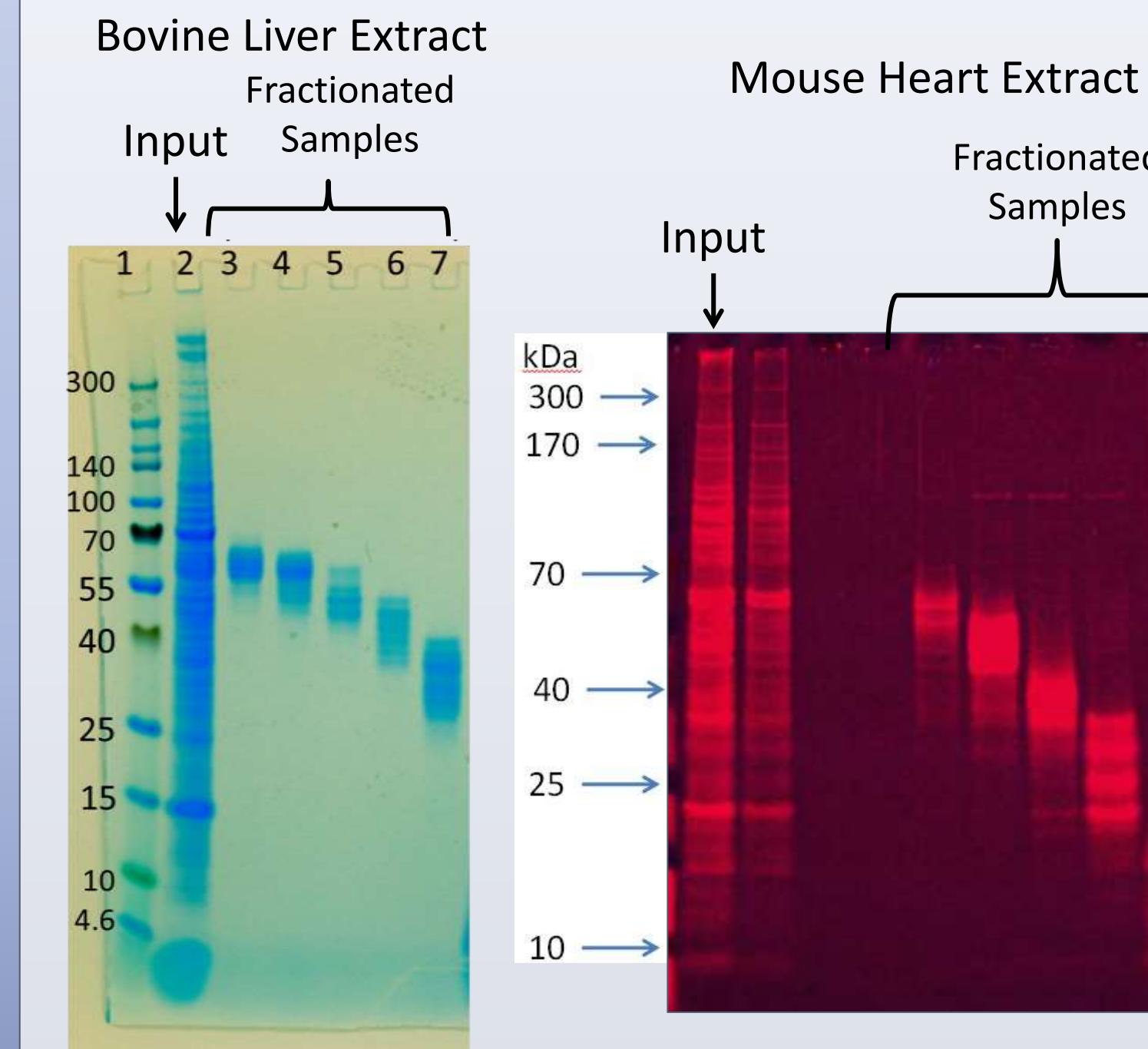
The Sage ELF System: Multifractionation

The system utilizes precast SDS agarose cassettes that are processed in two automated steps. In the first step, proteins are electrophoretically separated in an agarose channel. In the second step, proteins are electroeluted sideways out of the separation channel into a linear array of 12 membrane-bounded elution modules that are located alongside the lower two-thirds of the separation channel. The fractionation range can be adjusted through selection of gel concentration and run time. The instrument uses a CCD camera to track movement of standard electrophoresis tracking dyes through the cassette and determine run times. The first ELF instrument will accommodate 2 samples per run with manual cassette loading and unloading. Follow-on instruments will feature automated sample and cassette handling for higher-throughput sample processing.



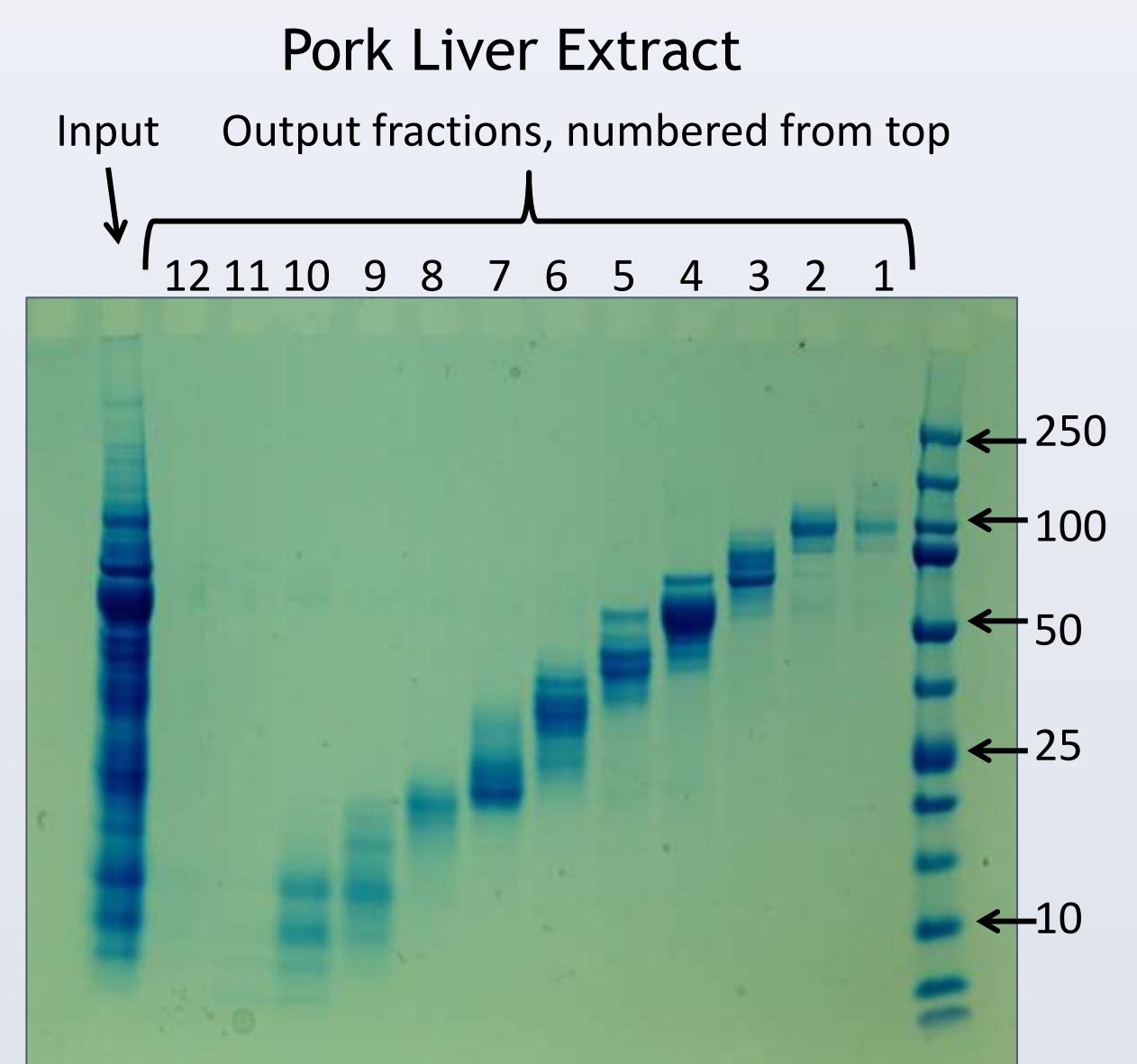
Example of BluePippin Fractionation

Crude SDS tissue extracts were separated on 5% SDS agarose BluePippin cassettes. Each sample lane of the BluePippin received 50 µg of total extract ("Input"). The figures show analytical SDS/PAGE gel of the collected fractions from the BluePippin ("Fractionated Samples" lanes).



Example of ELF System Fractionation

A crude pork liver extract was fractionated using an ELF prototype cassette containing a 5% SDS agarose separation gel. The figure shows SDS PAGE analysis of the input and ELF fractions produced.



Conclusions

The Sage BluePippin and ELF systems are attractive, complementary tools for preparation of protein samples for mass spectrometry. The BluePippin system is useful for applications where a single targeted fraction containing a protein of interest is needed. The ELF system is useful for broader proteomic studies including top-down proteomics and GeLC-MS.

More Information:

General: info@sagescience.com
Sales: rosemary.obrien@sagescience.com
Technical: chris.boles@sagescience.com

www.sagescience.com