

## An inexpensive resource for the proteinresearch community

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The Penn State Protein Ladder after protein electrophoresis and staining with Coomassie Blue dye. Individual proteins are shown together with the combined protein ladder on the right. Credit: Song Tan, Penn State



Labs can easily make their own protein ladders—molecular rulers for estimating the sizes of proteins—for less than a penny per experiment using the newly developed, license-free "Penn State Protein Ladder system." A research team of undergraduate students led by Song Tan, Verne M. Willaman Professor of Molecular Biology at Penn State, developed the ladders to be easily used in two of the most common experiments in protein research, gel electrophoresis and Western blots—which researchers use to separate or detect proteins based on their size.

A paper describing the research appears Aug. 18 in the journal *Scientific Reports*. The paper was co-authored by Ryan T. Santilli, John E. Williamson III, Yoshitaka Shibata, Rosalie P. Sowers and Andrew N. Fleischman, all of whom were Penn State undergraduate students.

"It has been exciting and rewarding to develop this project from just an idea to tools that can serve science," said Williamson.

Protein ladders provide size standards that can be compared to proteins of interest when run through a gel that is similar to a stiff gelatin. This process—gel electrophoresis—uses an electric field to separate proteins based on their size because smaller proteins move more quickly and therefore further through the gel. The proteins and <u>ladder</u> standards appear as small bands on the gel when stained and imaged, allowing the researchers to compare their sizes.

"Any lab working with proteins uses these ladders on a daily basis and the cost for commercially available ladders adds up—averaging about \$1 per experiment," said Tan. "Our Penn State Protein Ladders can be easily made for a tiny fraction of that cost using source material that we make available to nonprofit academic researchers through the Addgene and DNASU plasmid repositories."



The Penn State Protein Ladder is composed of nine proteins that range in molecular weight and provide standard bands in increments from 10 to 100 kilodaltons (kD). Each <u>protein</u> is encoded on an individual plasmid—a circular form of DNA—enabling production at very high levels in the bacteria E. coli. Using standard laboratory protocols, researchers can grow E. coli cells containing the plasmids and then isolate the proteins using a purification tag designed into each protein. Fifty milliliters (ml) of cells produce enough of each individual protein for 20,000 experiments.

"Researchers can pick and choose amongst the nine individual proteins to design a ladder optimized for their research project," said Tan. "They might not need the entire set, or they might want to individually control the intensity of individual bands on a gel. This customization is easy to accomplish with the Penn State Protein Ladder system."

For even more efficient production, the ladders also are available as two co-expression plasmids to produce either the set of 10, 30, 50, 100 kD proteins or the set of 20, 40, 60, 80 kD proteins. All the ladder proteins can be stained using dyes such as Coomassie Blue and are also directly detectable in Western blots, an analytical method using antibodies to detect proteins.

"We are thrilled to share these reagents with the global scientific community," said Santilli.

Undergraduate students working with Tan previously developed the Penn State DNA ladder, which allows researchers to produce their own DNA ladders for about a penny per experiment.

"Inexpensive and easy-to-use laboratory reagents like these help to democratize science by making it accessible to more labs and schools around the world," said Tan. "The Penn State DNA ladders have been



requested by almost 500 labs on every continent except for Antarctica. We hope that the Penn State Protein Ladders will be similarly useful to the research community."

**More information:** Ryan T. Santilli et al, The Penn State Protein Ladder system for inexpensive protein molecular weight markers, *Scientific Reports* (2021). DOI: 10.1038/s41598-021-96051-x

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