



With Reproducibility Proven, New Study May Engender 2D Gel Standards, Protocols, References

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An effort to prove the utility of 2D-gel technology has moved into its second phase: creating guidelines and tools for users of the technology.

The project, which kicked off late last year, is a follow-up to a study conducted in 2007 by the Human Proteome Organization's Industry Advisory Board that set out to prove the reproducibility of 2D gels across different laboratories. Now, phase 2, which involves 15 labs worldwide, will try to create standards, protocols, and references, organizers of the effort told *ProteoMonitor* this week.

Phase 1, which involved five labs, "was really a differential analysis. Phase 2 is not a differential analysis," said Hans Voshol, a senior research investigator and group leader at the Novartis Institutes for BioMedical Research, and who is responsible for directing analysis of results from both phases.

"It's really a technology-based experiment [to answer]: 'How can we make [2D gel] technology accessible to everybody, and what tools do we need to provide?'" he said.

Any standards that may come out of phase 2 can then be made into a commercial product, and a website may be set up "so that people can align their images," he added.

The plan is to have the 15 labs analyze and present their data at HUPO's annual conference in Toronto in September. By the end of the year, a draft article of the findings would be submitted for publication in a journal, according to Kumar Bala, marketing manager at Bio-Rad Laboratories, which is coordinating the study with the Novartis Institutes for BioMedical Research and Nonlinear Dynamics.

In addition to supplying reagents to study participants, Bio-Rad plans to conduct clinics to help researchers run reproducible gels using the approach created from the second phase of the study, Bala said. Meanwhile, Nonlinear Dynamics is developing a free application to benchmark 2D-gel analysis against a reference image from the approach. And the Novartis Institutes for BioMedical Research is making available to the public domain sample-preparation and gel-running protocols proven to be reproducible.

When phase 2 ends, Bio-Rad also will evaluate whether it can develop a marketable protein test standard, Bala said.

The IAB is no longer officially involved with the study anymore, but the effort has the "unofficial blessing" of the board, Will Dracup, CEO of Nonlinear Dynamics, said in an e-mail.

Phase 2 began in September when organizers started shipping HeLa cell lysates created by

CilBiotech in Belgium to participants. As part of the study, each participating lab will run the HeLa cell standard by 2D PAGE following a prescribed protocol created by study organizers. The labs will then capture their 2D gel images and upload their analysis to a site created by Fixing Proteomics — a campaign spearheaded by Nonlinear Dynamics to improve proteomics work and restore credibility to the field [See [PM 09/25/08](#)] — which will then further analyze the data and compare the results.

Though each lab is expected to return its results by mid-April, Bala said that labs that are interested in being part of the phase 2 study can still join.

About 10 of the labs have already submitted their results, he said, and some insight is being gleaned into the kinds of common mistakes that may be made by researchers doing 2D work. For example, he said, those who sonicate their samples tend to get a "much higher extraction of proteins from the sample."

Voshol also said that while phase 1 indicated that 1D separation was robust — contrary to the perception that separation is the main bottleneck in 2D gel reproducibility — some phase 2 researchers have experienced problems with their 1D work.

"It's mostly streaking in the first dimension, which is in many cases due to what they do with the electrodes," he said. "So I think the isoelectric focusing is still open to improvement."

One of the goals of the overall study is to identify sources of variations and mistakes and determine ways to overcome them. Indeed, Dracup at Nonlinear Dynamics said that "less optimal results" will prove as beneficial to researchers as the best results. By sharing data and results as the 15 labs try to produce a set of 2D gel standards, "we can help you see what your lab needs to do to reach this position, or if it has already," he said.

The participants in the study are: Ben Herbert, University of Technology in Sydney; Toni Posch, Aran Paulus, and Katrina Academia, Bio-Rad; Aisling Robinson and Michael Dunn, University College, Dublin; Alexander Archakov and Stanislav Melnik, the Russian Academy of Medical Sciences; Jose Bermudez, the University of Santiago de Compostela; Francesc Canals, Vall d'Hebron University, Barcelona; Peter James, Lund University; Glen Kemp and Kevin Emani, North East Proteome Analysis Facility, Newcastle Upon Tyne, UK; Jun Wheeler and Iolanda Vandrell, National Institute for Biological Standards and Control, Hertfordshire, UK; Amrita Cheerna, Georgetown University; Wayne Chadwick, National Institute on Aging; Aldrin Gomes, University of California, Davis; Melissa Dondej, University of California, Los Angeles; Philip Andrews, University of Michigan; and Jim Malone, Washington University

Andrews and Dun also participated in the first study.

Go Forth and Reproduce

The current project follows a study conducted two years ago by the IAB and Fixing Proteomics essentially to restore the credibility of 2D gels as a relevant technology for proteomics research [See [PM 11/15/07](#)].

One reason that they chose to focus on 2D gels was that the technology is "challenged more frequently than LC-MS," Voshol said. "Nobody will challenge mass spectrometry as a useful tool in proteomics, but people do challenge 2D gels. That's probably why the community is more motivated to prove that what they're doing makes sense."

The first study set out to prove that 2D gel experiments were, in fact, reproducible, despite the widely held view that they aren't. Though the ability to reproduce experimental results is one of the basic tenets of science, many if not most proteomics experiments still fail that critical test.

Indeed, one of the core missions of the Fixing Proteomics campaign is to get people in the field to focus on reproducibility in their experiments in order raise the overall credibility of the field.

"Fixing Proteomics would go as far as saying that if your results cannot be reproduced then they should not be published," the campaign says on its website.

The phase 1 2D gel study showed that as long as a robust protocol was followed, 2D results could be duplicated, at least among the five labs that participated in it.

The labs were asked to look at two samples, one of untreated *H. influenza* and the other a sample treated with actinonin. The key question was whether the five groups could come up with the same differences when analyzing the datasets.

According to a presentation made at last summer's HUPO conference, an analysis of the results from the five labs concluded that "protein-expression analysis based on 2D PAGE is reproducible across labs (and highly reproducible within a lab). ...With some constraints, experimental procedures appear to be remarkably robust."

The main sources of variability, it added, are user manipulation and interpretation of images and sample preparation. Voshol and David Bramwell at Nonlinear Dynamics are in the process of preparing a paper describing the phase 1 study and its results for publication.

Now, as he gets ready for the second phase of the 2D gel initiative, Voshol said that "ultimately, my goal would be for everybody who wants to do 2D gels ... to get these samples.

"Ten or 15 years ago when I started, it was very difficult to judge what you were doing, because in the beginning you didn't know what to expect. If you know what to expect, then you can see quickly whether you are on the right track or not. ... And I think that's what I'm looking for – [to] give the people a standard, a reference image and a tool maybe to align their reference images with," he said.



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