Bench philosophy (5): Polyacrylamide gel electrophoresis without stacking gel

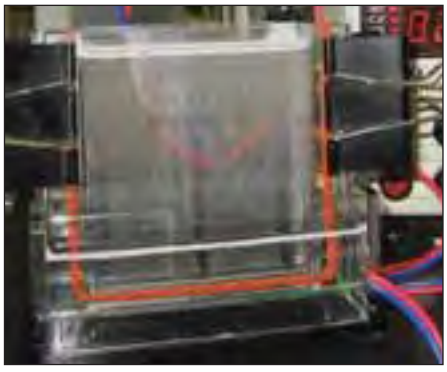
Secret Desires of Men of Methods

What motivates people who dedicate their time to developing methods? Is it the desire to help one's colleagues? Is it the urge to play? Sometimes the true driving forces appear a little different and odd. "The Experimenter" author, Hubert Rehm, analyses the motives of three method men who work in protein gel electrophoresis, protein determination and tissue homogenisation.

Most people have hidden compulsions. Some simply must check three times if they really have switched off the stove; others have to overtake every cyclist appearing in front of their bike. I feel compelled to brush-up on the "Experimenter, Protein Biochemistry/Proteomics" every other year. I yearn to sit in the dry air of the Max Planck Institute for Immunobiology – they have the best scientific library in Freiburg – and flick through the methods journals plus the *Journal of Biological Chemistry*, *PNAS* and *Cell*. My particular favourite is *Analytical Biochemistry*. This is usually a frustrating job as there are few useful inventions. Most are variations of existing methods and of doubtful value.

Hidden treasures

Sometimes, however, you detect experimental treasures. Would you believe in an improvement to the good old Lämmli-gel electrophoresis? After all, the Lämmli system has been in use since 1970, that's nigh on 37 years. There *are* better systems, says South Korean researcher Taeho Ahn in a Paper in *Analytical Biochemistry* (291, 300-303). According to Ahn, a simple change of buffers will eliminate the need for a stacking gel. This does not compromise separation and is somewhat better than the Lämmli system because the separating gel is longer. In addition, you save solution and time.



Almost 40 years old, but still works: SDS-PAGE with Lämmli-buffer system

What goes into Ahn's gel? You need acrylamide stock solution, running buffer, separating gel buffer and sample buffer. Running buffer and sample buffer are identical to the ones used in the Lämmli system. Ahn's secret is in the separating gel buffer. It is comprised of 76 mM Tris-HCl and 0.1 M of each of the amino acids Serine, Glycine and Asparagine. The buffer's pH value is 7.4. Note: The buffer contains no SDS. This means that Ahn's gel is also useful for native gel electrophoresis, just omit SDS from the sample and running buffers. There is no problem with a separating gel free of SDS. The SDS from sample and running buffer runs faster than the proteins. Therefore, they always stay in a SDS containing medium.

This is not the end of the Ahn gel advantages. Because its pH is 7.4 and not 8.8 as with the Lämmli system; gels are more stable – hydrolysation of acrylamide is significantly slower – and Ahn's gels may be stored for up to half a year at 4°C. Finally, electrophoresis in an Ahn gel is not sensitive to sample volume, NaCl concentrations up to 0.5 M and up to 2% (w/v) Chaps or Triton. Of course, it is possible to stain or blot the proteins after the run.

When I read Ahn's paper, I did not believe his claims. I have often experienced method inventors who are so in love with their pet that they are blind to its weaknesses and tend to overestimate its strengths. Nevertheless, I published it in a recent edition of "The Experimenter" and in our sister journal *Laborjournal* and waited for the reactions of my readers. Reactions came. Günter Fritz from the University of Zürich wrote:

"*We have been using the new recipe since the article (of Ahn et al.) appeared in Analytical Biochemistry. All in all, I will not use Lämmli any more. The "new" gels are faster to prepare, run quite beautifully (I used to produce "smilies" because of salt etc.), there is more separating space and you may produce higher gels (up to 18% acrylamide) for the separation of very small proteins. In addition, you do not have to mess around with the slimy stacking gel when you try to stain or blot the separating gel.*

There may be a small disadvantage. I often overload my gels to see minor components and I have the impression that Lämmli gels are a bit more tolerant to overload."

Invented the famous "Lämmli-buffer": Ulrich Lämmli

Achieving Fame

What was Taeho Ahn's motive to play around with buffers, salts and amino acids for months or years just to spare the world stacking gels? Probably the fact that Ulrich Lämmli is – due to his 1970 *Nature* paper – one of the most cited authors of all times. Ahn may have aspired to jump on the band wagon. However, a look into the ISI databank recorded only six citations since 2001. How unjust life can be!

A similar motive to Ahn may have been the catalyst in Barry Starcher's work. He tried to displace citation record man Oliv-

Godfather of protein determination, Oliver Lowry

er Lowry, by developing a new protein determination method. As you know there are dozens of methods to determine protein, the most commonly used are the Lowry method and the Coomassie reaction. All have serious disadvantages, e.g. the reagents are not specific for proteins, the protein to be determined has to be in solution and different proteins react with different sensitivity (variation in specific staining). A microgram BSA gives other readings compared to a microgram haemoglobin. These differences may amount to up to one order of magnitude. Protein determination sometimes degenerates to a lottery.

Again in *Analytical Biochemistry* (292, 125-129) Starcher presents a protein determination which determines soluble as well as insoluble proteins (i.e. the protein of tissue samples). He claims his method is more sensitive than using the Coomassie test and has the additional advantage of staining different proteins with the same intensity. Starcher's test seems to be authentic protein determination.

However, Starcher's test is not really a new idea. Rather he tries to solve the old problem of variation in specific staining with the old reaction of amino acids with ninhydrin. Starcher hydrolyses the sample (protein solution or tissue) in microfuge tubes with 0.5 ml 6 N HCl at 100 °C for 24 hours. The result is a soup of amino acids. The soup is dried in a speed-vac and the residue is resolved in water. An aliquot is then placed onto a microtiter plate and ninhydrin reagent is added. After 10 minutes swimming in hot water the microplate reader gives you the result.

Starcher has stabilised the ninhydrin reagent, which usually decomposes in a few hours. Under Starcher's conditions, it is stable for a few days.

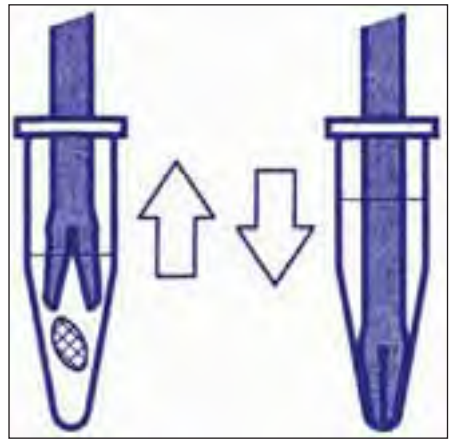
However, even with this improvement, the test is a tedious affair. It takes more than 24 hours and you have to hydrolyse, to speed-vac, to pipette, to heat and to centrifuge. No wonder then that since its publication, Barry Starcher's paper has received only 34 citations. Oliver Lowry's *Journal of Biological Chemistry* paper from 1951 is still the most cited life science publication.

Introduced a new protein determination method, Barry Starcher

Old wine in new bottles

Harald Jockusch, Professor of Developmental Biology at the University of Bielefeld, read about Starcher's test in "The Experimenter" and felt obliged to comment. "*I have never heard of Mr. Starcher but his test – ninhydrin reaction after hydrolysation of the proteins – seemed familiar. The beautiful red-purple you measure at 570 nm – a robust and sensitive method that is independent of the protein species (except for extremely proline-rich polypeptides). I remember this test because I did it myself more than 37 years ago! I used it to determine denaturation kinetics but I used alkaline hydrolysation of the proteins and not acid hydrolysation (BBRC 24, 577-583). (...) The take home message: If somebody were to systematically screen literature from the 1960's and older, many useful and "new" techniques would be discovered avoiding tedious lab work and expensive chemicals...*"

I have to admit SDS gel electrophoresis and protein determination have little sex appeal on the surface. However, in *Analytical Biochemistry* you sometimes find articles with a distinct pornographic quality. You will find a particularly suggestive and distasteful example in issue 294, 185-186. The title: *Homogenisation of tissue samples using a split pestle*. The four Japanese authors pretend to describe a method to improve the efficiency of tissue homogenisation for automatic sample preparation. They use a plastic pestle and microtubes serve as a mortar.



New split pestle for better tissue homogenisation. Or what do you have in mind?

For homogenisation, they repeatedly drive the pestle in and out and in and out...

Apart from the tedious pestle/mortar process and its problematic (in)decency, the combination of small tissue samples and big buffer volumes, "sample escape" becomes a problem. The sample fears being crushed by the pestle so it parries the pestle in an elegant downward move then an upward move and vice versa. The authors of the paper in *Anal. Biochem.* (note, even the name of the journal is morally problematic!) solve this problem with a slit in the polypropylene pestle (see picture). Of course, it is immediately apparent that this modification lends the method a definite pornographic tone. The advantages the slit allegedly offers do not disguise this fact. Thus with the up and down of the pestle in the conical end of the microtube, the slit opens and closes and crushes the piece of tissue. More importantly, the sample cannot escape; it remains in the slit like in a pair of pliers. The authors show that with the slit pestle they are able to isolate twice as much RNA from mouse liver than with a normal pestle, which just grinds the tissue between pestle and microtube wall. With samples of less than 50 mg, conventional pestles are completely ineffective whereas the slit pestle still yields RNA.

This may or may not be the case. I have not tried and I am reluctant to guess at the motives – conscious or unconscious – that have inspired the authors in their work.

Hubert Rehm

Fancy composing an installment of "Bench Philosophy"?

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