Use of the PhastSystem

by Kevin Rice

I. General use and maintanence

The PhastSystem is designed for quick, easy, and reproducible electrophoresis applications for both protein and nucleic acid samples. The system includes pre-cast polyacrylamide gels and buffers in a variety of flavors to accommodate many of your electrophoretic needs. While Phast gels can be invaluable for rapid qualitative analysis, using such small gels does introduce limitations relative to hand-poured gels. First, the resolution of many molecules within a very narrow molecular weight range (DNA sequencing for example) usually requires a longer separation zone. In addition, these very thin gels are inadequate for preparative electrophoresis. Finally, only polyacrylamide gels can be used, precluding the efficient separation of large (>1000 bp) DNA molecules. Despite these limitations, Phast gels are an attractive option for any of the following applications frequently encountered in the Schepartz laboratory:

Protein Analyses – synthetic peptide analysis, recombinant protein expression and purification detection, protein digest analysis
DNA Analyses – PCR fragment analysis, identification of small restriction fragments, analysis of insert manipulation during cloning procedures, synthetic oligonucleotide analysis

Other applications which are not as common in this laboratory and therefore not discussed here are: isoelectric focusing, 2-dimentional protein electrophoresis, transfer for western blotting, and native protein electrophoresis (for more information about applications, see the Amersham Biosciences website *http://www1.amershambiosciences.com/aptrix/upp01077.nsf/Content/Products?OpenDocument&parentid=40314&moduleid=40316*).

The PhastSystem also includes a development chamber most useful for silver-staining gels. Both protein and nucleic acid gels can be silver-stained. Phast gels can also be stained using standard protocols with Coomassie Brilliant Blue, ethidium bromide, and other stains.

Described here is the general procedure for operating the PhastSystem apparatus. Attached is the list of current Phast programs and silver-stain solutions I will not describe method programming here. Should this be necessary, the PhastSystem literature describes that procedure.

A. Gels and Running Buffers

Phast gels are available in homogeneous densities, gradient densities, and isoelectric focusing ranges (not discussed). The buffer within each pre-cast gel is essentially the same (low concentration Tris-OAc). What makes one electrophoretic application unique from the next is the running buffer used. Running buffer is provided soaked into 3% agarose strips that are positioned at either end of the gel. These strips

contain either native or denaturing (with SDS) buffers that subsequently enter the gel matrix giving the gel the desired characteristics. Buffer strips can be regenerated by successive soaks in the appropriate buffer. In addition, strips can be soaked in different buffers to generate buffer environments not provided by Pharmacia. The best combination of Phast gel and buffer strip is not necessarily obvious. Often, one must try different procedures until satisfactory electrophoretic separation is obtained. Much information is available in both the Pharmacia catalog and their website. What you need to get started is provided here.

1. Available Phast Gels:

- Homogeneous 7.5% (Separation Range for SDS PAGE: 30-300 M_r)
- Homogeneous 12.5% (Separation Range for SDS PAGE: 15-250 M_r)
- Homogeneous 20% (Separation Range for SDS PAGE: 2-150 M_r)
- Homogeneous High Density (Separation Range for SDS PAGE: 1-100 M_r)
- Gradient 4-15% (Separation Range for SDS PAGE: 30-300 M_r)
- Gradient 10-15% (Separation Range for SDS PAGE: 10-250 M_r)
- Gradient 8-25% (Separation Range for SDS PAGE: 6-300 M_r)

2. Available Buffer Strips

- SDS (0.2 M Tris, 0.2 M Tricine, 0.55% SDS, pH=8.1)
- Native (0.88 M L-Alanine, 0.25 M Tris, pH=8.8)
- DNA (same as SDS but guaranteed nuclease-free)

B. Sample Preparation

The load volume on a Phast gel can be no more than 4 µL. In that case, the comb for one gel has 6 wells. We also have 8-well combs, but they hold only 1 µL per well. Keep this in mind when preparing your samples, as well as what staining procedure you plan to use. Coomassie stain detects most protein bands at 50-100 ng, while silver stain detects as little as 0.3 ng per band for protein and 20 pg per band for DNA. Remember to keep these facts in mind when preparing marker/ladder samples. Use the same amount of loading buffer (with glycerol or ficoll and dyes) as you would on any other gel. Always prepare at least twice needed volume to allow for slips-of-hand (which happen more often here than with other kinds of gels).

1. Recipes for appropriate loading buffers

<u>Developer:</u> 1 mL 2% formaldehyde (in near fridge) 150 mL 2.5% Na_2CO_3 (stock bottle on bottom shelf)	<u>2% Formaldehyde:</u> 1 mL 37% formaldehyde (in flammables cabinet) 17 mL dH_2O
<u>Background Reducer:</u> 3.7 g Tris-HCl (near balance in hood) 2.5 g sodium thiosulfate (near balance in hood) 100 mL H_2O	<u>0.4% Silver Nitrate:</u> 2 g silver nitrate 498 mL H_2O

C. Apparatus Setup

Chosen gel(s), which are stored in the refrigerator, are removed from their packages by cutting along the dotted lines. If you fail to cut along the lines, you will be demoted back to kindergarten. The front of the package corresponds to the top of the gel. The gel itself is affixed to a piece of plastic. The top of the gel is protected by a thinner piece of plastic that must be carefully removed before use. Often, this protective layer will stick to the package when peeled away from the gel. Once removed, be careful not to scratch the gel. If this happens, discard the gel and get another one as a scratch in the gel will in all likelihood ruin electrophoresis. You can bend back the trapezoidal nub to aid later handling of the gel. Lift up all hinged parts in the PhastSystem separation unit and align each gel with the red outline on the white surface (gel side up). If you are using only one gel, it does not matter which position you choose. Put the chosen buffer strips in the appropriate positions on the clear-plastic removable buffer strip holder, and put the holder on top of the gels, putting the pins in the back of the surface through the holes in the holder. Press down on the strips gently to ensure they make contact with the gel. Lower the hinged parts. Again, press on the electrodes that touch the buffer strips to ensure contact.

Turn the instrument on (button in the back). Press the "SEP temp stand by" button on the console. This will allow you to equilibrate the temperature to 15°, at which most programs run their gels. The readout should give the current temperature as well as the set temp of 15°. If the "(OFF)" is seen, press the "do" button such that "(ON)" shows up on the display.

D. Loading the Sample

Invert the appropriate comb(s) and stabilize upside down with binder clips. Before loading the samples, note that the gel will be running away from you and that the combs you load will be inverted relative to how you load them. Load the wells in such a way that you will be able to read the gel lanes logically once separation is achieved. Load appropriate volume (1 μL or 4 μL) by forming a drop at the end of the pipette tip and then touching it to the well. Note that the outer nubs are not wells. The liquid should be held in place through surface tension. This is the most difficult portion of the procedure and does take some time to master, but you're a scientist and you will get the hang of it. Once the combs are loaded, be gentle with them as a bump or a finger-flick will cause the liquid to spill out of the wells. Invert the combs (liquid should stay in place) and carefully insert into the slotted holder in the separation unit. The comb looks as if it could fit in three logical places. It is not any of those places, but instead, a less obvious slot all the way towards you, right against the outer wall. Be careful not to touch the wells to anything. Also be careful not to press down on the hinged comb holder into which you are inserting the comb. Should this happen, you will prematurely introduce the samples to the gel surface. Close the lid to the separation unit.

E. Running a Program

Once loaded and ready to go, press "SEP start stop." Enter the number of gels (1 or 2) and press "do." Enter the program number (the different programs are on the sheet attached here and posted above the PhastSystem) and press "do." The program will now run automatically. It will not stop until you stop it. Track the progress of the marker dyes

as you would for a hand-poured gel and stop it when the gel has sufficiently run. Stop by pressing "SEP start stop" and then "do" to verify the stopping.

F. Staining

If you are staining your gel with Coomassie or ethidium bromide, treat it as you would any other gel. If you plan to use the development chamber for silver staining, place your gels in the wire brackets within the chamber. Make sure all silver stain solution bottles are full (the locations and recipes for the solutions are in the attached sheet and above the PhastSystem). Staining 1 or 2 gels involves no differences in staining protocol. Press "DEV start stop" and then the number of the development program and then "do." The gel will then be stained automatically.

G. Apparatus Shutdown

Wipe all used components with a wet Kimwipe and then a dry Kimwipe. Clean up the bench area around the PhastSystem or Stacey will hurt you. Shut off apparatus when completed.

H. Phast System Information

Separation (Sep) Programs		**Development (Dev) Programs**	
1	SDS 20A	1	DNA SILVER
2	NATIVE	2	SILVER SDS
3		3	SILVER NATIVE
4		4	
5	SDS 12.5	5	
6		6	
7	SDS 8–25	7	
8	DNA NATIVE	8	
9		9	CLEAN

Silver Solutions		
1	20% TCA (trichlororacetic acid)	(TCA solid in Room 114 fridge)
2	50% Ethanol / 10% HOAc	
3	10% Ethanol / 5% HOAc	(stock bottle on bottom shelf)
4	5% Gluteraldehyde (glutaric dialdehyde)	(stock in flammables cabinet)
5	dH_2O	
6	0.4% Silver nitrate	(in near fridge)
7	Developer	(make fresh)
8	Background Reducer	(make fresh)
9	5-10% Glycerol	

For additional information about the Phastsystem, the user manual is available online as a pdf file:
http://www1.amershambiosciences.com/aptrix/upp00919.nsf/(FileDownload)?OpenAgen&docid=69BE4F0F5FE010BBC1256AB100084A40&file=80132015.pdf