

# Laboratory Focus

BY KEVIN SEELEY, PhD AND YARDENAH BRICKMAN, PhD

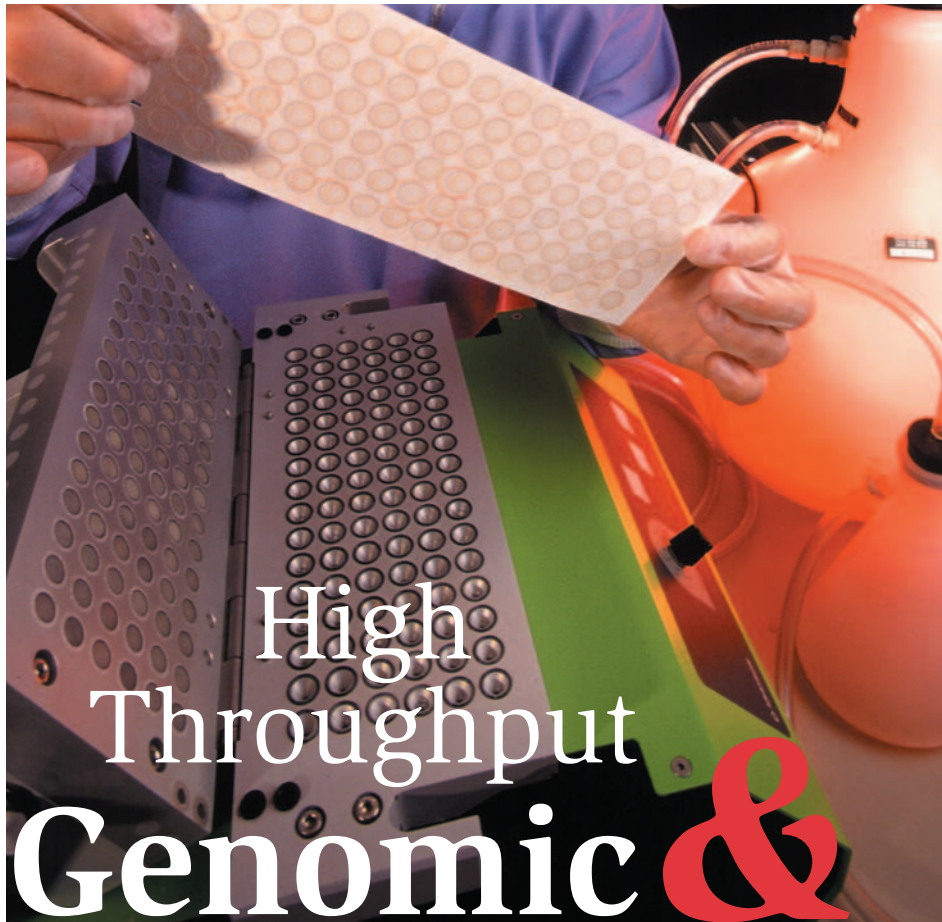


Photo: Keith Weller

## High Throughput Genomic & Proteomic Sample Preparation

Historically, most filtration devices were designed to process individual samples when only a few precious samples at a time were needed. Changes in methods have led to a number of high throughput research projects in which a process must be automated in order to accommodate the demand for parallel processing and screening. Certain design features that are part of the filtration devices used in the genomic data generation explosion are not conducive to protein isolation and purification processes.

To address this need, multiwell plates have been modified to accommodate the requirements of proteomic researchers, and to enable those researchers to catch up with the quest for understanding the proteomes of numerous organisms. These added features, which will be discussed below, also benefit genomic isolation processes.

Pall Life Sciences, a division of Pall Corp. (East Hills, NY), created a family of microfiltration and ultrafiltration (UF) devices that are capable of processing multiple samples simultaneously. Particulate-laden samples are clarified using an open prefilter over a more restrictive microporous membrane, which combines the benefits of fast flow with the ability to more completely clarify particulate-laden solutions.

The filters in the AcroPrep™ 96 plates are low in biomolecule binding and are designed to limit weeping and resist chemical degradation. They are compatible with the guidelines established for automated plate-handling systems so that the speed is not diminished due to personnel limitations. Finally, a proprietary design used to seal the membrane also allows the integral seal of each well with either single or multiple membrane layers.

High-performance UF membranes with well-defined pore structures have a long and successful history of separating molecules according to size. The primary advantage to separating molecules using mechanical, rather than chemical, means is that sample concentration and recovery can be achieved without additional buffers or salts.

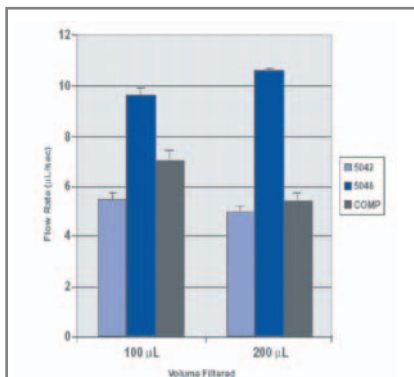
Beyond high throughput sample clarification, ultrafiltration can be used to rapidly concentrate and recover biomolecules in either vacuum or centrifugal modes using tightly sealed ultrafiltration membranes.

### Genomic Sample Preparation

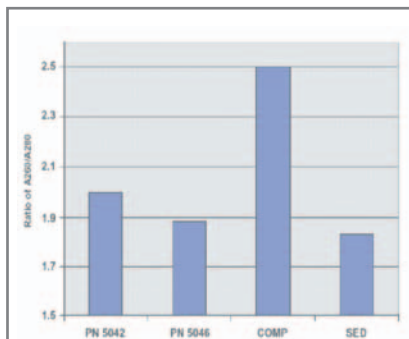
**Plasmid Lysate Filtration:** The most problematic step in the purification of plasmid DNA is the clarification of the sample once the cells are lysed, and the lysate has been treated with salts to precipitate the detergent. Sedimentation of cellular debris has several limitations that create roadblocks to many applications where small-scale, high throughput processing is required.

It is very difficult to sediment the fluffy pellet in a multiwell plate while still recovering supernatant. Other problems with sedimentation include long spin times, rotor capacity for single-tube processing and handling issues associated with the use of microfuge tubes. Perhaps the greatest problem is that sedimentation allows the fluffy pellets to trap a large portion of the desired plasmid DNA, and pellets are notoriously difficult to 'wash' without fragmenting the plasmid samples.

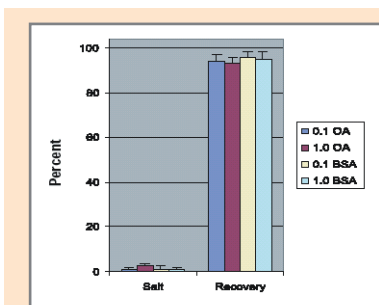
The use of filtration for the clarification of plasmid purification lysates enables a rapid and effective alternative to centrifugal sedimentation. However, the filter can quickly become clogged with viscous debris. The use of a prefilter not only resists clogging, but also shortens filtration times and allows greater flow rates to be maintained at higher loads (Fig. 1). In addition, using a prefiltration configuration to process the lysate allowed the highest  $A_{260}$  recovery with ratios equivalent to sedimentation (Fig. 2). Typically,  $A_{260}/A_{280}$  ratios of 1.9 indicate a more pure DNA



**Figure 1. Flow Rate Comparisons at Two Sample Volumes** Filtration times were measured for 100-µl and 200-µl lysate samples. Average flow rates were calculated for the AcroPrep 96 filter plate with Bio-Inert membrane (PN 5042), AcroPrep 96 filter plate with Glass Fiber over Bio-Inert membrane (PN 5046) and Competitor hydrophilic plate containing a floating prefilter (COMP). Error bars indicate standard error (n=8).

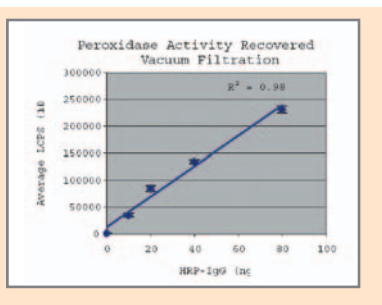


**Figure 2. Clarification Effectiveness as a Measure of Absorbance** The UV absorbance of the supernatant or filtrate was measured using a UV spectrophotometer at 260 and 280 nm. The plot shows the ratios of  $A_{260}$  over  $A_{280}$  readings for AcroPrep 96 filter plate with Bio-Inert membrane (PN 5042), AcroPrep 96 filter plate with Glass Fiber over Bio-Inert membrane (PN 5046), Competitor plate (COMP) and Sedimentation (SED). Pall's prefiltration plate had the highest recovery of DNA and a ratio closest to sedimentation.



**Figure 3. Desalting Protein Solutions**

Protein samples (300 ml) were added to either a 10K for ovalbumin (OA) or a 30K AcroPrep UF plate for bovine serum albumin (BSA). The plates were centrifuged at 2000xg for 40 minutes. Following centrifugation, the proteins were recovered in 300 ml buffer by pipetting up and down 10 times. Protein concentration was quantitated using UV spectrometric analysis, and salt concentrations were quantitated using conductivity.



**Figure 4. Maintenance of Biological Activity**

Protein samples (100 ml) containing horseradish peroxidase conjugated IgG (10 to 80 ng total protein) were added to a 100K AcroPrep UF plate. The plates were vacuum filtered at 25 inches Hg for 10 minutes, then incubated for five minutes in 100 ml 1X PBS prior to recovery by pipetting up and down 10 times. Enzyme activity was activated using 50 ml chemiluminescent substrate and counted on a MicroBeta® multilabel counter (PerkinElmer Life and Analytical Sciences, Boston, MA). Error bars indicate standard error from two independent experiments.

sample, so there is an argument for the need for prefiltration to achieve effective clarification.

Agarose gel electrophoresis of the samples confirms that the amount of DNA recovered (data not shown) was highest for the prefiltration configuration, further demonstrating that the AcroPrep multiple-layer sealing technology allows greater flexibility in addressing hard-to-filter applications.

## Proteomic Sample Preparation

**Desalting Biomolecules:** Biomolecule purification involves a complex series of steps in which molecules are selectively separated using size or a variety of other biochemical properties. Many steps require desalting and

buffer exchange as well as sample concentration to prepare the biomolecule sample for the next step in purification.

Desalting/buffer exchange can be a critical step in which yields and biological activity can be significantly reduced. Desalting can be accomplished using methods such as gel filtration, size-exclusion HPLC and dialysis, as well as ultrafiltration. Compared to the use of non-membrane-based processes, filtration using ultrafiltration has a number of technical advantages resulting from simplicity, ability to automate and consistency.

The efficiency of AcroPrep 96 UF filter plates to remove salts and other small molecules is not at the expense of sample recovery.

The recovery of ovalbumin proteins (OA, 45 kDa) using a 10K MWCO membrane at two different concentrations was greater than 93%, with desalting exceeding 97% (Fig. 3). Using BSA (66 kDa) and a 30K MWCO membrane, we observed protein recoveries of greater than 94% at two different concentrations (0.1 and 1.0 milligrams per millilitre), with greater than 98% salt removal. Rapid desalting using this automation-friendly plate is equally effective with centrifugal or vacuum filtration.


**Retention of Biological Activity:** Mechanical separation by size using the AcroPrep 96 UF plate results in a gentle and consistent method for the desalting and recovery of biomolecules. In independent experiments, conjugated antibodies were concentrated and recovered following filtration. The activity of the conjugated enzyme was measured to determine if the recovery correlated to the enzyme function. Enzyme activities gave a high correlation over dilute concentrations ranging from 10 to 80 nanograms total protein (Fig. 4). A high correlation indicates low protein binding as well as gentle handling, allowing the maintenance of biological activity.

## Summary

The use of integrally sealed prefilters for particulate-laden samples not only improves filtration times, but also prevents fouling of the more restrictive microfiltration membranes, ultimately improving the quality of the clarified solution. The use of ultrafiltration technologies in a high throughput mode for protein desalting and concentration allows the use of automation to address the growing requirements for the parallel recovery of active biomolecules.



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*Pall Canada for five years. Brickman completed her PhD at the University of Melbourne (Melbourne, Australia) in 1996. She then managed a neurological research laboratory at the Alfred Hospital in Melbourne before returning to Canada in 1998. Upon return she worked on a post-doctoral research project with funding from the Medical Research Council (now CIHR). Brickman provides assistance and expertise for a wide range of laboratory and pilot-scale applications, including HTS, membrane chromatography, nucleic acid affinity membranes and HPLC. *