



# High-Throughput Protein Crystallography with the Corning Protein Crystallography Plate on the Beckman Coulter Multimek 96 Automated 96-Channel Pipettor

Phillip Bradford, Dr. Rongbao Li, Jennifer Fiedler and Dr. Thomas Fletcher III (Southern Research Institute), Edwin Dario and Tom Harrison (Beckman Coulter), Dr. Ma Sha and Gerald Campbell, Jr. (Corning)

## Abstract

One way to develop new avenues to utilize High Throughput technology is to adapt existing platforms to use innovative designs in labware. The Corning Protein Crystallography Plate allows crystallographers to scale up protein crystallography experiments to a 96-well format (actually 192 wells). Adapting the Beckman Coulter Multimek 96 Automated 96-channel pipettor to use this plate moves the experiment into the realm of High Throughput. Simultaneously pipetting all 96 wells significantly reduces the time required to conduct sitting-drop vapor diffusion crystallization experiments, thus allowing a significant increase in the number of protein crystallization experiments that can be attained. The software extant with the Multimek does not allow for pipetting into a 192-well plate. However, modification of an existing labware definition does allow it. This study describes the steps necessary to modify the Beckman Coulter Multimek 96 to achieve proper aspiration and dispensing parameters, and the methodology used to conduct the experiment.

## Introduction

With the increasing focus on Proteomics and Structural Genomics in the modern drug discovery laboratory, the need for high throughput methods of protein crystallization is increasing daily. Structural Genomics refers to the process of producing large numbers of proteins, crystallizing those proteins by a high-throughput manner and then determining their structures. This process has made high-throughput protein crystallization an integral part of drug discovery, providing structural and functional information about proteins that can be used as drug screening targets. Structural determination is often indispensable for correctly determining the biological function of a protein. Binding sites can be identified and drugs designed to fit those sites to block their function in the disease pathway.

Sitting-drop protein crystallization experiments have previously been painstaking, protracted methods involving multiple pipetting steps with single- and/or multi-channel pipettors. To meet the demands of high-throughput protein crystallization for drug discovery, a means needed to be developed to increase throughput above and beyond that possible with manual methods. This high throughput methodology would ideally be developed using existing labware and liquid handling platforms, eliminating the need for expensive, dedicated crystallization equipment.

By adapting our existing 96-channel liquid handling platform to use the Corning Crystallography Plate, the value and versatility of the equipment has been improved, and the overall throughput has been increased well beyond that allowed by traditional manual methods of crystallization. While the manual techniques average 60 drops/hour<sup>(1)</sup>, the high-throughput method using the Multimek averages 96 drops every two minutes, or 2880 drops/hour. This is a 48-fold increase in throughput, which allows crystallographers to produce a much larger array of crystals from which to choose for structural and functional information.

The software changes necessary for the adaptation of the Multimek required only a few minutes to accomplish. However, the trial and error process to perfect the method occupied several hours over the course of the day. Once the Multimek is dispensing the correct volumes to the proper area, no adjustments should be necessary and the method is ready to run.

## Materials and Methods

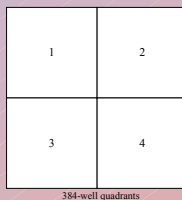
### Corning® 96 Well Protein Crystallization Plate

The Corning Crystallization Plate (CCP) was designed for the sitting-drop vapor diffusion format and to conform to the standard SBS 96-well plate footprint. The CCP is a merged well design, consisting of 96 large reservoir wells with adjacent smaller protein wells, and is constructed of an optically clear polymer to allow precise crystal visualization. The use of commercially available crystal screens in deep well storage blocks and the CCP allows all 96 wells to be simultaneously prepared on the Multimek.

### Beckman Coulter Multimek 96

An automated 96-channel pipettor, the Multimek allows simultaneous liquid transfers to all wells of a standard 96-well microplate. Definitions for various labware used on the Multimek platform are stored in the *resource.ini* file within the Multimek software. These define the parameters of each specific piece of labware, i.e., 96-well microplate, 384-well microplate, reservoir, etc. Edwin Dario and Tom Harrison of Beckman Coulter, Inc. modified the standard 384-well microplate definition to allow pipetting into 192 wells. The 384-well microplate is defined with the same standard SBS 96-well footprint, with each well being divided into four quadrants, numbered 1-2, left to right, top and 3-4, left to right, bottom. By setting the Y-offset to 0, all four quadrants are accessed on the same Y-axis, thus quadrants 1 and 3 have identical Y-axis values, as do quadrants 2 and 4. This, in effect, defined the plate as having 192 wells. The complete parameters for defining the Corning Crystallography Plate are:

Long name = Corning Flat PCP 192  
ShortName = PCP192  
PictureName = Shallowg.bmp  
\*Height = 14.3  
Area = 9.11  
Wells = 384  
Xoffset = 3  
Yoffset = 0  
TouchOffset = 2.4  
TouchDepth = 1.0  
Bottom = 4.0  
Description = Corning Flat PCP192  
Configuration = 2  
Enabled = 1

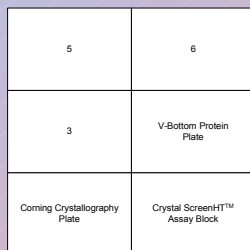


\*Note: Two different dispensing heights are used for this plate. For quadrants 1 and 3, Height = 7.5 and for quadrants 2 and 4, Height = 14.3<sup>(1)</sup>.

After trial and error it was found that a few minor changes in the labware definitions enhanced accuracy and droplet placement on the CCP. The Xoffset value was changed from 3 to 2.7. This moved the pipet tips to the middle of the smaller wells when dispensing. Every other value was left unchanged in the *resource.ini* file. The height for dispensing into the small protein well (quadrant 2 or 4) was changed from 14.3 to 14.5 via the Method Editor. The height of the large reservoir for the mother liquor (quadrant 1 or 3) was unchanged. Due to the high viscosity of a number of solvents used for crystallization, aspiration speed was set at 0.5% and dispensing speed at 0.25% to facilitate proper droplet placement. The tip touch feature was enabled to help mix the two droplets and ensure the droplets remained in the center of the small protein well.



Beckman Coulter Multimek96



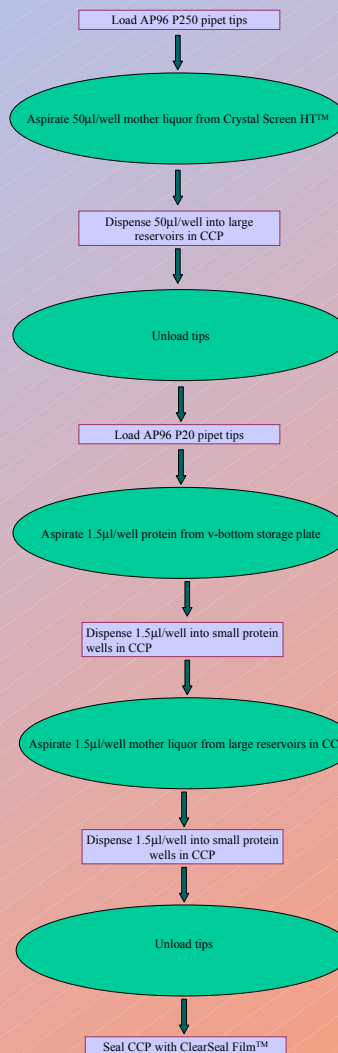
Multimek96 Deck Layout



Southern Research Institute's Beckman Coulter ORCA System

### Crystal Screen HT™ and ClearSeal Film™

A high throughput reagent kit from Hampton Research consisting of an array of trial crystallization reagents in varying concentrations of salt, pH and precipitants, was used to provide the mother liquors for the crystallization experiment. ClearSeal Film from Hampton Research is an optically clear sealing tape used to seal the CCP after adding the protein and mother liquors.



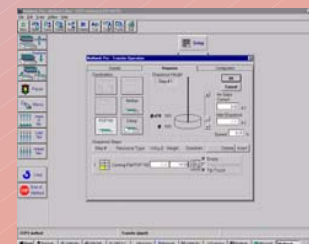
Corning® 96 Well Protein Crystallization Plate

### Sitting-Drop Protein Crystallization Method

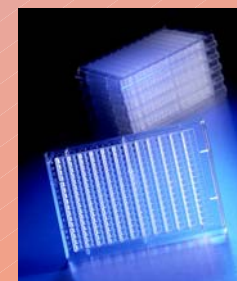
Typically, 50-100µl of solvent ("mother liquor") is placed in each large reservoir well, a 1-2µl drop of protein is placed in each small protein well, and 1-2µl of the mother liquor is transferred into the protein drop. The plate is then sealed with an optically clear sealing tape. Vapor diffusion across the concentration gradient of solvent in the two wells then initiates crystal growth.

### HTS Sitting-Drop Protein Crystallization Method

A Multimek method was created to aspirate 50µl/well of the mother liquor from the Crystal Screen HT™ assay block and dispense this into each large reservoir well of the CCP using Beckman AP96 P250 (250µl capacity) pipet tips. A separate method was created to use the Beckman AP96 P20 (20µl capacity) pipet tips to aspirate 1.5µl/well protein from a Corning Costar v-bottom storage plate and dispense this into the middle of each protein well, then aspirate 1.5µl/well mother liquor from the large CCP reservoirs and dispense this into the protein drops in the small protein wells. The CCP was then sealed manually with Hampton Research ClearSeal Film™.



Multimek Method Editor Dispense Configuration



Corning 384-well Crystallography Plate

### Future plans

In protein crystallization experiments, the most costly element is the protein itself. Reduction in scale of crystallization experiments can help to reduce the total amount of protein required and thus reduce cost while increasing the total number of experiments possible with a given amount of protein. Corning has introduced a 384 well CCP that promises to further increase throughput in crystallization experiments. Our plans include adaptation of the Multimek platform to use this new plate. This should be possible with either the 96 or 384 well dispense head, and should allow increased efficiency with scarce protein sources.

### References

1. Dario, E. (2002) Defining the Corning 96-Well Crystallization Plate for Use on the Multimek 96-Channel Pipettor. Beckman Coulter T3 Update 6, 10.
2. Jancarik, J. et al. (1991) Sparse matrix sampling: a screening method for crystallization of proteins. *J. Appl. Cryst.* 24, 409-411.
3. Villaseñor, A. et al. (2002). Fast Drops: A High-Throughput Approach for Setting Up Protein Crystal Screens. *BioTechniques* 32, 184-188.

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