

## CD™ High-Throughput Protein Crystallization Platform

-----The freeway from cDNA to structure and drug discovery

In order to embrace the advent of the Structural Genomics and Structural Proteomics era, **Creative BioStructure** has established a high throughput platform for automated gene cloning, protein expression, purification, characterization and crystallization named **CD™ High-Throughput Protein Crystallization Platform**. This platform uses modern gene cloning and protein expression techniques in combination with state-of-the-art high throughput protein crystallization equipments to provide a comprehensive solution to mass protein production and crystallization. For novel structure analysis by X-ray diffraction methods, the methionine residues of many proteins will have to be replaced by selenomethionine.

### Manual gene cloning

In contrast with the automated gene cloning strategies employed by other structural genomics platforms that eliminate the use of restriction enzymes [e.g. the *cre-lox* system, the Gateway technology (Invitrogen), In-Fusion™ Technology (Clontech)], we have developed a manual restriction enzyme dependent cloning workflow, thus guaranteeing the precise transfer of open reading frames (ORF) downstream of the promoter of the expression vectors.

### Automated gene expression and protein purification

We use Overnight Express Auto-induction Medium (Novagen) for induction of protein expression in T7 RNA polymerase-based vectors; this medium allows for fully automated growth of cultures to high optical densities without the need for either monitoring growth or induction by IPTG. Deep 96-well microplates are used for small-scale protein expression and purification on the Biomek® FX Laboratory Automation Workstation. We perform small scale protein purification under denaturing and non-denaturing conditions. We use Ni-NTA or glutathione affinity purification for His-tag or GST-tag fusion proteins. All of our procedures take advantage of automated liquid handling technology that can ensure accurate manipulation of all target samples.



Biomek® FX Laboratory Automation Workstation

## High throughput protein crystallization

We rely on a Mosquito crystallization robot and a CRYSTAL FARM system to screen crystallization conditions. Crystallization of proteins is still the bottleneck in structure determination and there is no simple correlation between properties of a protein and the parameters for crystallization of the protein. Consequently, crystallization of proteins requires a broad screening of different crystallization conditions, for which automation in this process has distinct advantages. Mosquito® Liquid handler has great pipetting precision, allows automatic hanging drop setup and automatic sitting drop setup, while CRYSTAL FARM is an automated protein crystallization and imaging system.

To obtain an X-ray quality crystal, each purified protein sample can be tested in hundreds of different conditions. The CRYSTAL FARM provides constant temperature during the crystallization process, checks the test trays for presence of crystals and provides a simple interface for a user to monitor his experiments. WE usually set up a buffer screen consisting of 384 general crystallization conditions (pH, buffer, salt, polyethylene glycol, alcohols, salts) for "hanging drop" vapor diffusion experiments in trays with 96 wells, and the trays are automatically stored at two temperatures, preferably 4°C and 16°C [768 conditions in total]. Tracking of the crystal growth is done by photographing the titer trays. Images are then made available via a web based interface to our staff scientists. CRYSTAL FARM also has image recognition software that automatically finds crystals by means of an ingenious search algorithm that looks for solid particles in the digital images.



Mosquito® Liquid handler

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