# High-throughput protein crystallization at the MRC-LMB


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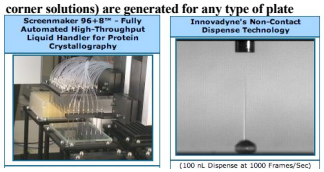
**Laboratory of Molecular Biology (LMB) scientists can undertake a standard initial protein crystallization screen on a robotic nanolitre system with each new sample (1). A standard initial screen is made in *MRC sitting drop plates* with a wide variety of commercially available crystallization reagents. The system is high throughput. Since 2002 more than 30,000 *MRC plates* have been set up for initial screening only. The system is fast and reliable. This is crucial when a target is especially difficult to crystallize and many rounds of screening are required to test new constructs. Setting up plates is easy and all LMB groups involved in structure determination operate independently. Constant developments offer alternatives and new tools. For instance, the *MRC micro-batch plate*, in-house custom *Morpheus screens* and a new Web based tool (2) have been integrated recently to the system.**

## Robotics

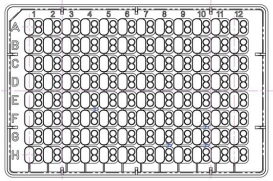
**Fully automated system (TECAN) dispenses screens to MRC plates and seals them. Screens are stored in plates "ready to go" Custom matrices (e.g. 2D gradient from 4 corner solutions) are generated for any type of plate**




**Nano-dispenser is used for setting up full initial screen (16 screens) in one hour with 200 µl of protein. Other protocols are also available (e.g. micro-batch, seeding)**

## MRC Plate

| | |
|---|---|
| Easy crystal retrieval | Raised wide wells make the crystal mounting especially easy. |
| Easy viewing | The wells are wide conical and have a lens effect for perfect illumination.<br><br>The micro-numbering ensures you will never get lost again.<br><br>The optically superior polymer is even UV transmissible! |
| Better sealing | Wide partition walls between the wells give plenty of area for good sealing with tape. |
| Wide range of volumes | Typical volumes are 50-100 µl of reservoir and 10nl-5µl drop size. |
| SBS standard | The plates are designed to the 96-well SBS standard for all common holders. |



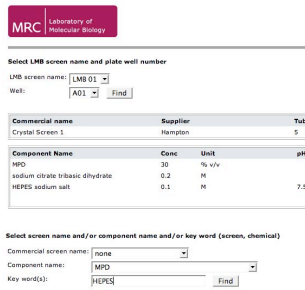
**The MRC plate and its advantages (Jan Löwe, 2006)**

## Initial Screen

| | | MRC plates |
|---|---|---|
| LMB1 | Crystal Screen 1 (1-48) (Hampton Research)<br>Crystal Screen 2 (1-48) (Hampton Research) | LMB1 2730<br>LMB2 2520 |
| LMB2 | Wizard 1 (1-48) (Emerald BioStructures)<br>Wizard 2 (1-48) (Emerald BioStructures) | LMB3 2205 |
| LMB3 | Ammonium sulfate grid (1-24) (Hampton Research)<br>PEG8000/LiCl (1-24) (Hampton Research)<br>Quik phosphate grid screen (Na/K PI. grid) (Hampton Research)<br>NaCl grid (Hampton Research)<br>PEG6000 (1-24) (Hampton Research) | LMB4 2465<br>LMB5 2364<br>LMB6 1930 |
| LMB4 | MPD (1-24) (Hampton Research)<br>MembFac (1-48) (Hampton Research) | LMB7 1921<br>LMB8 2063 |
| LMB5 | PEG-Ion screen (1-48) (Hampton Research)<br>Natrix (1-48) (Hampton Research) | LMB9 2048<br>LMB10 1937 |
| LMB6 | CrystalScreen Lite (1-48) (Hampton Research)<br>CrystalScreen Cryo (1-48) (Hampton Research) | LMB11 1759<br>LMB12 1892 |
| LMB7 | WizardCryo 1 (1-48) (Emerald BioStructures)<br>WizardCryo 2 (1-48) (Emerald BioStructures) | LMB13 1345<br>LMB14 1331 |
| LMB8 | JBS1 (PEG 400-3K) (JenaBioScience)<br>JBS2 (PEG 4K) (JenaBioScience)<br>JBS3 (PEG 4K +) (JenaBioScience)<br>JBS4 (PEG 6K-8K) (JenaBioScience) | LMB15 1501 |
| LMB9 | JBS 5 (PEG 8K-20K) (JenaBioScience)<br>JBS 6 (Ammoniumsulfate) (JenaBioScience)<br>JBS 7 (MPD) (JenaBioScience)<br>JBS 8 (MPD/alcohols) (JenaBioScience<br>JBS9 (alcohols/salt) (JenaBioScience)<br>JBS10 (salt) (JenaBioScience). | |
| LMB10 | Clear Strategy Screen 1 pH 4.5 (1-24) Molecular Dimensions Limited | |
| LMB11 | Clear Strategy Screen 1 pH 5.5 (1-24) Molecular Dimensions Limited<br>Clear Strategy Screen 1 pH 6.5 (1-24) Molecular Dimensions Limited<br>Clear Strategy Screen 1 pH 7.5 (1-24) Molecular Dimensions Limited<br>Clear Strategy Screen 1 pH 8.5 (1-24) Molecular Dimensions Limited<br>Clear Strategy Screen 2 pH 4.5 (1-24) Molecular Dimensions Limited | |
| LMB12 | Clear Strategy Screen 2 pH 5.5 (1-24) Molecular Dimensions Limited<br>Clear Strategy Screen 2 pH 6.5 (1-24) Molecular Dimensions Limited<br>Clear Strategy Screen 2 pH 7.5 (1-24) Molecular Dimensions Limited<br>Clear Strategy Screen 2 pH 8.5 (1-24) Molecular Dimensions Limited | |
| LMB13 | Index (1-96) (Hampton Research) | |
| LMB14 | SaltRX (1-96) (Hampton Research) | |
| LMB15 | MemStart (1-48) (Molecular Dimensions Limited)<br>MemSys (1-48) (Molecular Dimensions Limited) | |
| LMB16 | JCSG+ (Qiagen / Nextal) | |

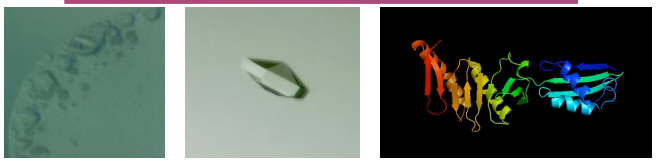
**Initial screen: 42 commercial kits composing 16 LMB screens. Table: MRC plates set up in a span of 5 years** (LMB16 was integrated only recently)

## LMB Screen Database



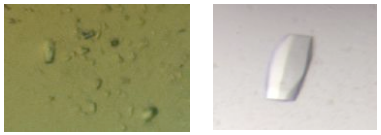

**Web interface (Paul Hart, 2007) gives details of condition and protocol for preparation. Data mining is possible using keywords**

## Multiple constructs: case study



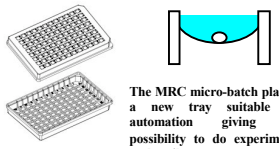
**Hit with first construct, optimization hit (46th construct) and structure (Fusinita van den Ent's project, 2007)**

## Alternative Screen: Morpheus

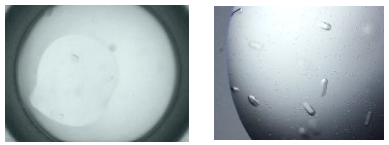


**Condition with special mixes of salts and precipitants gave best hit and was successfully optimized (Alex Berndt's project, 2007)**

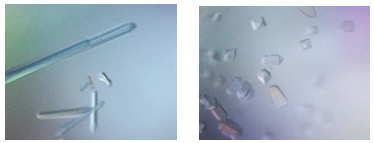
## Alternative technique, scale-up



**The MRC micro-batch plate is a new tray suitable for automation giving the possibility to do experiments under oil**



**Successful scale-up from MRC plate (200 nl drop) to hanging drop (4 µl ) (Divyang Jani's project, 2007)**



**Crystals in 4 µl sitting drops (Catejan Neubauer's project, 2007)**


(1) D. Stock, O. Perisic, J. Löwe "Robotic nanolitre protein crystallisation at the LMB", Prog. Biophys. Mol. Biol. 88 (2005) 311-327

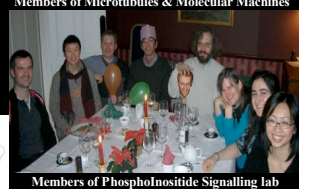
(2) http://www2.mrc-lmb.cam.ac.uk/screens.html


Structural Studies

Laboratory of Molecular Biology

Protein and Nucleic Acid Chemistry

**Members of Microtubules & Molecular Machines**



**Members of PhosphoInositide Signalling lab**

**Structural Studies and PNAC divisions have participated actively in the development of the system which is used independently by all LMB groups (33 in total)**