

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

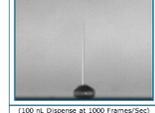


Fully automated system (TECAN) dispenses screens to MRC plates and seals them. Screens are stored in plates "ready to go". Custom matrices (e.g. 2D gradient from 4 corner solutions) are generated for any type of plate.

Rennermaier 96 e1™ Fully Automated High-Throughput Liquid Handler for Protein Crystallography



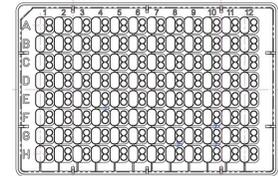
Imnovadyne's Non-Contact Dispense Technology



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. The wells are wide enough and have a lens effect for perfect illumination.
- Easy viewing** The micro-numbering ensures you will never get lost again. 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 100-5 µl drop size. The plates are designed to the 96-well SBS standard for all common holders.
- SBS standard**



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

Initial Screen

Screen	MRC plate
LMB1	Crystal Screen 1 (1-48) (Hampton Research)
LMB2	Crystal Screen 2 (1-48) (Hampton Research)
LMB3	Wizard 1 (1-48) (Emerald Biostructures)
LMB4	Wizard 2 (1-48) (Emerald Biostructures)
LMB5	Ammonium sulfate grid (1-24) (Hampton Research)
LMB6	FLUOROLUX 3.0 (1-48) (Hampton Research)
LMB7	Quick attachment grid screen (24x12) grid (Hampton Research)
LMB8	Hex and Triaxial Research
LMB9	FLUOROLUX (1-24) (Hampton Research)
LMB10	MCR (1-24) (Hampton Research)
LMB11	MasterSet (1-48) (Hampton Research)
LMB12	MasterSet (1-48) (Hampton Research)
LMB13	MasterSet (1-48) (Hampton Research)
LMB14	CrystalScreen Crys. (1-48) (Hampton Research)
LMB15	WizardScreen (1-48) (Emerald Biostructures)
LMB16	WizardScreen 2 (1-48) (Emerald Biostructures)
LMB17	JBS 1 (1-6, 400-24) (Lipid) (Science)
LMB18	JBS 2 (1-6, 400-24) (Lipid) (Science)
LMB19	JBS 3 (1-6, 400-24) (Lipid) (Science)
LMB20	JBS 4 (1-6, 400-24) (Lipid) (Science)
LMB21	JBS 5 (1-6, 400-24) (Lipid) (Science)
LMB22	JBS 6 (1-6, 400-24) (Lipid) (Science)
LMB23	JBS 7 (1-6, 400-24) (Lipid) (Science)
LMB24	JBS 8 (1-6, 400-24) (Lipid) (Science)
LMB25	JBS 9 (1-6, 400-24) (Lipid) (Science)
LMB26	JBS 10 (1-6, 400-24) (Lipid) (Science)
LMB27	JBS 11 (1-6, 400-24) (Lipid) (Science)
LMB28	JBS 12 (1-6, 400-24) (Lipid) (Science)
LMB29	JBS 13 (1-6, 400-24) (Lipid) (Science)
LMB30	JBS 14 (1-6, 400-24) (Lipid) (Science)
LMB31	JBS 15 (1-6, 400-24) (Lipid) (Science)
LMB32	JBS 16 (1-6, 400-24) (Lipid) (Science)
LMB33	JBS 17 (1-6, 400-24) (Lipid) (Science)
LMB34	JBS 18 (1-6, 400-24) (Lipid) (Science)
LMB35	JBS 19 (1-6, 400-24) (Lipid) (Science)
LMB36	JBS 20 (1-6, 400-24) (Lipid) (Science)
LMB37	JBS 21 (1-6, 400-24) (Lipid) (Science)
LMB38	JBS 22 (1-6, 400-24) (Lipid) (Science)
LMB39	JBS 23 (1-6, 400-24) (Lipid) (Science)
LMB40	JBS 24 (1-6, 400-24) (Lipid) (Science)
LMB41	JBS 25 (1-6, 400-24) (Lipid) (Science)
LMB42	JBS 26 (1-6, 400-24) (Lipid) (Science)
LMB43	JBS 27 (1-6, 400-24) (Lipid) (Science)
LMB44	JBS 28 (1-6, 400-24) (Lipid) (Science)
LMB45	JBS 29 (1-6, 400-24) (Lipid) (Science)
LMB46	JBS 30 (1-6, 400-24) (Lipid) (Science)
LMB47	JBS 31 (1-6, 400-24) (Lipid) (Science)
LMB48	JBS 32 (1-6, 400-24) (Lipid) (Science)
LMB49	JBS 33 (1-6, 400-24) (Lipid) (Science)
LMB50	JBS 34 (1-6, 400-24) (Lipid) (Science)
LMB51	JBS 35 (1-6, 400-24) (Lipid) (Science)
LMB52	JBS 36 (1-6, 400-24) (Lipid) (Science)
LMB53	JBS 37 (1-6, 400-24) (Lipid) (Science)
LMB54	JBS 38 (1-6, 400-24) (Lipid) (Science)
LMB55	JBS 39 (1-6, 400-24) (Lipid) (Science)
LMB56	JBS 40 (1-6, 400-24) (Lipid) (Science)
LMB57	JBS 41 (1-6, 400-24) (Lipid) (Science)
LMB58	JBS 42 (1-6, 400-24) (Lipid) (Science)
LMB59	JBS 43 (1-6, 400-24) (Lipid) (Science)
LMB60	JBS 44 (1-6, 400-24) (Lipid) (Science)
LMB61	JBS 45 (1-6, 400-24) (Lipid) (Science)
LMB62	JBS 46 (1-6, 400-24) (Lipid) (Science)
LMB63	JBS 47 (1-6, 400-24) (Lipid) (Science)
LMB64	JBS 48 (1-6, 400-24) (Lipid) (Science)
LMB65	JBS 49 (1-6, 400-24) (Lipid) (Science)
LMB66	JBS 50 (1-6, 400-24) (Lipid) (Science)
LMB67	JBS 51 (1-6, 400-24) (Lipid) (Science)
LMB68	JBS 52 (1-6, 400-24) (Lipid) (Science)
LMB69	JBS 53 (1-6, 400-24) (Lipid) (Science)
LMB70	JBS 54 (1-6, 400-24) (Lipid) (Science)
LMB71	JBS 55 (1-6, 400-24) (Lipid) (Science)
LMB72	JBS 56 (1-6, 400-24) (Lipid) (Science)
LMB73	JBS 57 (1-6, 400-24) (Lipid) (Science)
LMB74	JBS 58 (1-6, 400-24) (Lipid) (Science)
LMB75	JBS 59 (1-6, 400-24) (Lipid) (Science)
LMB76	JBS 60 (1-6, 400-24) (Lipid) (Science)
LMB77	JBS 61 (1-6, 400-24) (Lipid) (Science)
LMB78	JBS 62 (1-6, 400-24) (Lipid) (Science)
LMB79	JBS 63 (1-6, 400-24) (Lipid) (Science)
LMB80	JBS 64 (1-6, 400-24) (Lipid) (Science)
LMB81	JBS 65 (1-6, 400-24) (Lipid) (Science)
LMB82	JBS 66 (1-6, 400-24) (Lipid) (Science)
LMB83	JBS 67 (1-6, 400-24) (Lipid) (Science)
LMB84	JBS 68 (1-6, 400-24) (Lipid) (Science)
LMB85	JBS 69 (1-6, 400-24) (Lipid) (Science)
LMB86	JBS 70 (1-6, 400-24) (Lipid) (Science)
LMB87	JBS 71 (1-6, 400-24) (Lipid) (Science)
LMB88	JBS 72 (1-6, 400-24) (Lipid) (Science)
LMB89	JBS 73 (1-6, 400-24) (Lipid) (Science)
LMB90	JBS 74 (1-6, 400-24) (Lipid) (Science)
LMB91	JBS 75 (1-6, 400-24) (Lipid) (Science)
LMB92	JBS 76 (1-6, 400-24) (Lipid) (Science)
LMB93	JBS 77 (1-6, 400-24) (Lipid) (Science)
LMB94	JBS 78 (1-6, 400-24) (Lipid) (Science)
LMB95	JBS 79 (1-6, 400-24) (Lipid) (Science)
LMB96	JBS 80 (1-6, 400-24) (Lipid) (Science)
LMB97	JBS 81 (1-6, 400-24) (Lipid) (Science)
LMB98	JBS 82 (1-6, 400-24) (Lipid) (Science)
LMB99	JBS 83 (1-6, 400-24) (Lipid) (Science)
LMB100	JBS 84 (1-6, 400-24) (Lipid) (Science)
LMB101	JBS 85 (1-6, 400-24) (Lipid) (Science)
LMB102	JBS 86 (1-6, 400-24) (Lipid) (Science)
LMB103	JBS 87 (1-6, 400-24) (Lipid) (Science)
LMB104	JBS 88 (1-6, 400-24) (Lipid) (Science)
LMB105	JBS 89 (1-6, 400-24) (Lipid) (Science)
LMB106	JBS 90 (1-6, 400-24) (Lipid) (Science)
LMB107	JBS 91 (1-6, 400-24) (Lipid) (Science)
LMB108	JBS 92 (1-6, 400-24) (Lipid) (Science)
LMB109	JBS 93 (1-6, 400-24) (Lipid) (Science)
LMB110	JBS 94 (1-6, 400-24) (Lipid) (Science)
LMB111	JBS 95 (1-6, 400-24) (Lipid) (Science)
LMB112	JBS 96 (1-6, 400-24) (Lipid) (Science)
LMB113	JBS 97 (1-6, 400-24) (Lipid) (Science)
LMB114	JBS 98 (1-6, 400-24) (Lipid) (Science)
LMB115	JBS 99 (1-6, 400-24) (Lipid) (Science)
LMB116	JBS 100 (1-6, 400-24) (Lipid) (Science)

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

Commercial name: Crystal Screen 1
Supplier: Hampton
Tube number: 5

Component Name	Conc.	Unit	pH
MPD	30	% v/v	
sodium citrate tribasic dihydrate	0.2	M	
HEPES sodium salt	0.1	M	7.5

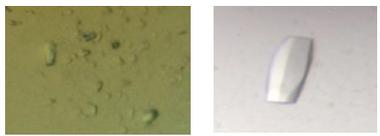
Web interface (Paul Hart, 2007) gives details of condition and protocol for preparation. Data mining is possible using keywords

Multiple constructs: case study



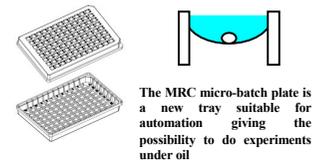
Hit with first construct, optimization hit (46th construct) and structure (Fusinita van den Ent's project, 2007)

Alternative Screen: Morpheus

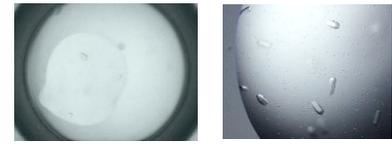


Condition with special mixes of salts and precipitants gave best hit and was successfully optimized (Alex Berndt's project, 2007)

Alternative technique, scale-up



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

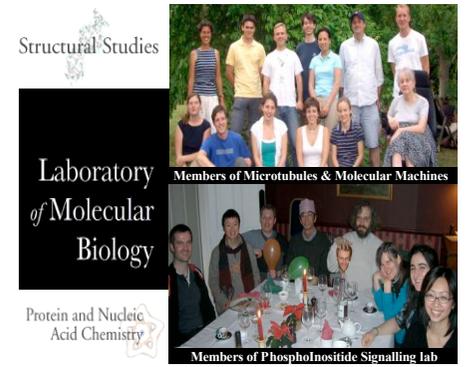


Successful scale-up from MRC plate (200 nl drop) to hanging drop (4 µl) (Divyang Jani's project, 2007)



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