

Automated Microbial Cultivation and Adaptive Evolution using Microbial Microdroplet Culture System (MMC)

Xingjin Jian^{*1,2}, Xiaojie Guo^{*1,2,3}, Jia Wang⁴, Zheng Lin Tan^{1,2,6}, Xin-hui Xing^{1,2,5}, Liyan Wang³, Chong Zhang^{1,2,5}

¹Department of Chemical Engineering, Institute of Biochemical Engineering, Tsinghua University ²Key Laboratory of Industrial Biocatalysis, Ministry of Education, Tsinghua University ³Luoyang TMAXTREE Biotechnology Co., Ltd. ⁴Biochemical Engineering Research Group, School of Chemical Engineering and Technology, Xi'an Jiaotong University ⁵Center for Synthetic & Systems Biology, Tsinghua University ⁶School of Life Science and Technology, Tokyo Institute of Technology

* These authors contributed equally

Corresponding Authors

Liyan Wang

wangliyan@biobreeding.com

Chong Zhang

chongzhang@tsinghua.edu.cn

Citation

Jian, X., Guo, X., Wang, J., Tan, Z.L., Xing, X.h., Wang, L., Zhang, C. Automated Microbial Cultivation and Adaptive Evolution using Microbial Microdroplet Culture System (MMC). *J. Vis. Exp.* (180), e62800, doi:10.3791/62800 (2022).

Date Published

February 18, 2022

DOI

10.3791/62800

URL

jove.com/video/62800

Materials

Name	Company	Catalog Number	Comments
0.22 μm PVDF filter membrane	Merck Millipore Ltd.	SLGPR33RB	Sterilize the MMC oil
4 °C refrigerator	Haier	BCD-289BSW	For reagent storage
Agar	Becton, Dickinson and Company	214010	For solid plate preparation
CaCl ₂ ·2H ₂ O	Sinopharm Chemical Reagent Beijing Co., Ltd.	20011160	Component of the special medium for MeSV2.2.
Clean bench	Beijing Donglian Har Instrument Manufacture Co., Ltd.	DL-CJ-INDII	For aseptic operation and UV sterilization
CoCl ₂ ·6H ₂ O	Sinopharm Chemical Reagent Beijing Co., Ltd.	10007216	Component of the special medium for MeSV2.2.
Computer	Lenovo	E450	Software installation and MMC control
Constant temperature incubator	Shanghai qixin scientific instrument co., LTD	LRH 250	For the microbial cultivation using solid medium
CuSO ₄ ·5H ₂ O	Sinopharm Chemical Reagent Beijing Co., Ltd.	10008218	Component of the special medium for MeSV2.2.
Electronic balance	OHAUS	AR 3130	For reagent weighing
EP tube	Thermo Fisher	1.5 mL	For droplet collection
FeCl ₃ ·6H ₂ O	Sinopharm Chemical Reagent Beijing Co., Ltd.	10011928	Component of the special medium for MeSV2.2.
Freezing Tube	Thermo Fisher	2.0 mL	For strain preservation
Gluconate	Sigma-Aldrich	S2054	Component of the special medium for MeSV2.2.
Glycerol	GENERAL-REAGENT	G66258A	For strain preservation
High-Pressure Steam Sterilization Pot	SANYO Electric	MLS3020	For autoclaved sterilization
isopropyl-β-d-thiogalactopyranoside (IPTG)	Biotopped	420322	Component of the special medium for MeSV2.2.

Kanamycin sulfate	Solarbio	K8020	Component of the special medium for MeSV2.2.
KH ₂ PO ₄	MACKLIN	P815661	Component of the special medium for MeSV2.2.
Methanol	MACKLIN	M813895	Component of the special medium for MeSV2.2.
MgSO ₄ ·7H ₂ O	BIOBYING	1305715	Component of the special medium for MeSV2.2.
Microbial Microdroplet Culture System (MMC)	Luoyang TMAXTREE Biotechnology Co., Ltd.	MMC-I	Performing growth curve determination and adaptive evolution. Please refer to http://www.tmaxtree.com/en/index.php?v=news&id=110
Microfluidic chip	Luoyang TMAXTREE Biotechnology Co., Ltd.	MMC-ALE-OD	For various droplet operations. Please refer to http://www.tmaxtree.com/en/
MMC oil	Luoyang TMAXTREE Biotechnology Co., Ltd.	MMC-M/S-OD	The oil phase for droplet microfluidics. Please refer to http://www.tmaxtree.com/en/
MnCl ₂	Sinopharm Chemical Reagent Beijing Co., Ltd.	20026118	Component of the special medium for MeSV2.2.
NaCl	GENERAL-REAGENT	G81793J	Component of the LB medium
Na ₂ HPO ₄ ·12H ₂ O	GENERAL-REAGENT	G10267B	Component of the special medium for MeSV2.2.
NH ₄ Cl	Sinopharm Chemical Reagent Beijing Co., Ltd.	10001518	Component of the special medium for MeSV2.2.
Petri dish	Corning Incorporated	90 mm	For the preparation of solid medium
Pipette	eppendorf	2.5 μL, 10 μL, 100μL, 1000μL	For liquid handling
Quick connector A	Luoyang TMAXTREE Biotechnology Co., Ltd.	—	For the connection of each joint. Please refer to http://www.tmaxtree.com/en/
Reagent bottle	Luoyang TMAXTREE Biotechnology Co., Ltd.	MMC-PCB	Sampling and storage of bacteria solution and reagents. Please refer to http://www.tmaxtree.com/en/
Shake flask	Union-Biotech	50 mL	For microbial cultivation
Shaking incubator	Shanghai Sukun Industrial Co., Ltd.	SKY-210 2B	For the microbial cultivation in shake flask
Streptomycin sulfate	Solarbio	S8290	Component of the special medium for MeSV2.2.
Syringe	JIANGSU ZHIYU MEDICAL INSTRUMENT CO., LTD	10 mL	Draw liquid and inject it into the reagent bottle
Syringe needle	OUBEL Hardware Store	22G	Inner diameter is 0.41 mm and outer diameter is 0.71 mm.
Tryptone	Oxoid Ltd.	LP0042	Component of the LB medium
Ultra low temperature refrigerator	SANYO Ultra-low	MDF-U4086S	For strain preservation (-80 °C)
UV–Vis spectrophotometer	General Electric Company	Ultrospec 3100 pro	For the measurement of OD values
Vitamin B1	Solarbio	SV8080	Component of the special medium for MeSV2.2.
Yeast extract	Oxoid Ltd.	LP0021	Component of the LB medium
ZnSO ₄ ·7H ₂ O	Sinopharm Chemical Reagent Beijing Co., Ltd.	10024018	Component of the special medium for MeSV2.2.