

Materials List for:

Preparation, Imaging, and Quantification of Bacterial Surface Motility Assays

Nydia Morales-Soto^{1,2}, Morgen E. Anyan¹, Anne E. Mattingly¹, Chinedu S. Madukoma¹, Cameron W. Harvey³, Mark Alber³, Eric Déziel⁴, Daniel B. Kearns⁵, Joshua D. Shrout^{1,2,6}

¹Department of Civil and Environmental Engineering and Earth Sciences, University of Notre Dame

²Eck Institute for Global Health, University of Notre Dame

³Department of Applied and Computational Mathematics and Statistics, University of Notre Dame

⁴INRS-Institut Armand-Frappier

⁵Department of Biology, Indiana University

⁶Department of Biological Sciences, University of Notre Dame

Correspondence to: Joshua D. Shrout at joshua.shrout@nd.edu

URL: <https://www.jove.com/video/52338>

DOI: [doi:10.3791/52338](https://doi.org/10.3791/52338)

Materials

Name	Company	Catalog Number	Comments
Reagents^a			
FAB Minimal Media:			Prepare every ~4 weeks. Top to 1 L with nanopure H ₂ O.
(NH ₄) ₂ SO ₄	Sigma	A4418	2 g. Not used in <i>P. aeruginosa</i> tendril formation studies.
Na ₂ HPO ₄ x 7H ₂ O	Sigma-Aldrich	S9390	9 g
KH ₂ PO ₄	Sigma	P5655	3 g
NaCl	BDH	BDH8014	3 g
MgCl ₂ x 6H ₂ O solution (198 g/L)	Fisher Scientific	M33	1 ml
CaCl ₂ x 2H ₂ O solution (14 g/L)	Fisher Scientific	C79	1 ml
Trace metal solution (see below)	n/a	n/a	1 ml
Trace Metal Solution:			Top to 1 L with nanopure H ₂ O. Maintain in a glass bottle, stirring and covered with foil.
CaSO ₄ x 2H ₂ O	Sigma-Aldrich	255548	200 mg
MnSO ₄ x H ₂ O	Sigma-Aldrich	M7634	20 mg
CuSO ₄ x 5H ₂ O	Fisher Scientific	C493	20 mg
ZnSO ₄ x 7H ₂ O	Sigma-Aldrich	Z4750	20 mg
CoSO ₄ x 7H ₂ O	Sigma-Aldrich	C6768	10 mg
NaMoO ₄ x 2H ₂ O	Sigma	S6646	10 mg
H ₃ BO ₃	Fisher Scientific	A74	5 mg
FeSO ₄ x 7H ₂ O	Sigma-Aldrich	F7002	200 mg
CTT Media:			Prepare as needed. Top to 100 ml with nanopure H ₂ O.
Tris-HCl, 1 M solution (adjust to pH 8.0)	Amresco	0234	1 ml. Prepare a 1 M stock solution in nano pure H ₂ O. Adjust pH to 8.0 and filter sterilize (0.2 μm pore).
K ₂ HPO ₄ , 1 M solution (adjust to pH 7.6)	Sigma-Aldrich	P3786	0.1 ml. Prepare a 1 M stock solution in nano pure H ₂ O. Adjust pH to 7.6 and filter sterilize (0.2 μm pore).

MgSO ₄ solution	Fisher Scientific	M65	0.8 ml. Prepare a 1 M stock solution in nano pure H ₂ O. Filter sterilize (0.2 µm pore).
Casitone	BD Diagnostics	225930	1 g
Additional Reagents:			
LB Broth, Lennox	BD Diagnostics	240230	2% (wt/vol)
D-(+)-Glucose	Sigma-Aldrich	G5767	30 mM for overnight broth cultures; 12 mM for swarm media. Prepare a 1.2 M filter sterilized stock solution in nano pure H ₂ O. Add to media after autoclaving.
Casamino acids (CAA)	Amresco	J851	0.10% (wt/vol). Recommended for <i>P. aeruginosa</i> tendrill formation studies. Add to media prior to autoclaving.
Agar, Noble	Sigma-Aldrich	A5431	0.45% (wt/vol). Preferred Noble agar for <i>P. aeruginosa</i> surface motility studies. Add to media prior to autoclaving.
Agar, Noble	Affymetrix	10907	1.50% (wt/vol). Used in <i>M. xanthus</i> surface motility studies. Not recommended for <i>P. aeruginosa</i> motility studies. Add to media prior to autoclaving.
Agar, Granulated	Fisher Scientific	BP1423	0.60% (wt/vol)
Higgins Waterproof Black India Ink	Higgins	HIG44201	0.50% (vol/vol). Mix ink with inoculum to test swarm media surface moisture.
SYTO® 64 Red Fluorescent Nucleic Acid Stain	Invitrogen	S-11346	Use 4 µl (for <i>P. aeruginosa</i>) or 8 µl (for <i>M. xanthus</i>) of SYTO® 64 per 100 ml of molten agar (added after autoclaving).
Relevant Materials and Equipment			
Petri dish, sterile, 150 mm x 15 mm (Dia. x H)	VWR	25384-326	
Petri dish, sterile, 100 mm x 15 mm (Dia. x H)	VWR	25384-342	
Petri dish, sterile, 60 mm x 15 mm (Dia. x H)	VWR	25384-092	
In-Vivo Xstream	Bruker		Use for the macroscopic imaging of surface motility studies. http://www.bruker.com/products/preclinical-imaging/opticalx-ray-imaging/in-vivo-xtreme/overview.html
Bruker MI software	Bruker		http://www.bruker.com/fileadmin/user_upload/8-PDF-Docs/PreclinicalImaging/Brochures/MI-software-brochure.pdf
ImageJ software	NIH		http://imagej.nih.gov/ij/
^a See MSDS of reagents for handling and disposal information.			