

Materials List for:

Live Cell Fluorescence Microscopy to Observe Essential Processes During Microbial Cell Growth

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Materials

Name	Company	Catalog Number	Comments
Bacterial Strains			
<i>Agrobacterium tumefaciens</i> C58	ATCC	33970	Watson B, Currier TC, Gordon MP, Chilton MD, Nester EW. 1975. Plasmid required for virulence of <i>Agrobacterium tumefaciens</i> . J Bacteriol 123:255-264.
<i>Agrobacterium tumefaciens</i> C58ΔtetRA::mini-Tn7T-GM-Ptac-ctrA ΔctrA			Figueroa-Cuilan W, Daniel JJ, Howell M, Sulaiman A, Brown PJB. 2016. Mini-Tn7 insertion in an artificial attTn7 site enables depletion of the essential master regulator CtrA in the phytopathogen <i>Agrobacterium tumefaciens</i> . Appl Environ Microbiol. 82:5015-5025.
Media Components			
ATGN Minimal Medium			To 1 L of sterilized water add 50 ml 20X Buffer, 50 ml 20X Salts, 12.5 ml 40% glucose. For plates, add 15 g Bacto Agar to 1 L of water and autoclave. Cool to 55 °C and add 50 ml 20X Buffer, 50 ml 20X Salts, 12.5 ml 40% glucose.
20X AT Buffer			Add 214 g/L KH ₂ PO ₄ to water and adjust pH to 7.0 with sodium hydroxide. Autoclave.
NaOH	Fisher BioReagents	BP359	
KH ₂ PO ₄	Fisher Chemical	P288	
20X AT Salts			Add 40 g/L (NH ₄) ₂ SO ₄ , 3.2 g/L MgSO ₄ •7H ₂ O, 0.2 g/L CaCl ₂ •2H ₂ O, and 0.024 g/L MnSO ₄ •H ₂ O to water. Autoclave.
(NH ₄) ₂ SO ₄	Fisher Chemical	A701	
MgSO ₄ •7H ₂ O	Fisher BioReagents	BP213	
CaCl ₂ •2H ₂ O	Fisher BioReagents	BP510	
MnSO ₄ •H ₂ O	Fisher Chemical	M114	
Glucose	Fisher Chemical	D16	Prepare 40% stock in water. Filter sterilize.
Bacto Agar	Fisher BioReagents	BP1423	Add 15 g to 1 L of water when preparing plates.
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Optional Media Additives			
Kanamycin	GoldBio	K-120	Prepare as a 100 mg/ml stock solution in water and filter sterilize. Use at final concentration of 200 µg/ml.
IPTG	GoldBio	I2481C5	Prepare as a 1 M stock solution in water and filter sterilize. Use at final concentration of 1 mM as needed for induction.
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Microscopy Materials			
Microscope Slides	Fisherbrand	12-550D	25 X 75 X 1.0 mm. Clean with Sparkle glass cleaner.
Microscope Cover Glass	Fisherbrand	12-541-B	22 X 22 mm. No. 1.5. Clean with Sparkle glass cleaner.
Sparkle Glass Cleaner	Home Depot	203261385	Ammonia and alcohol free.
Ultra Pure Agarose	Invitrogen	16500-100	Add to water, PBS, or media to a final concentration of 1 - 1.5%. Melt in microwave and place on 70 C
PBS	Fisher BioReagents	BP399500	10X solution to be diluted to 1X with sterile water.
Parafilm	Bemis	PM-999	Laboratory film used as gasket in agarose pad preparation.
VALAP			Add equal weights of lanolin, parafin wax, and petroleum jelly to a conical tube. Heat tube in 70 °C dry, bead or water bath to melt and mix. Apply VALAP while still molten.
Lanolin Butter	SAAQIN	SQ-LAB-R1	
Petroleum Jelly	Target Corp.	06-17644	
Paraffin Wax	Crafty Candles	263012	
Name	Company	Catalog Number	Comments
Target-specific dyes			
DMSO	Fisher BioReagents	BP231-1	Use to dilute stock solutions of dyes as needed.
FDAAs (NADA, HADA, TADA)			FDAAs can be synthesized or acquired through agreement with Mike VanNieuwenhze (Indiana University). Prepare 100 mM stock solution in DMSO. Use at a final concentration of 5 mM.
DAPI	ThermoFisher Scientific	62247	Prepare 1 mg/ml stock solution in DMSO. Use at final concentration of 1 µg/ml.
SYTOX Orange Nucleic Acid Stain	Invitrogen	S11368	Stock concentration is 5 mM in DMSO. Use at final concentration of 5 µM.
FM4-64	Invitrogen	T3166	Prepare 8 mg/ml stock solution in DMSO. Use at final concentration of 8 µg/ml.
Name	Company	Catalog Number	Comments
Equipment			
Dry bath	Sheldon Manufacturing, Inc.	52120-200	
Metallic thermal beads	Lab Armor	42370-002	

Epifluorescence microscope equipped with an EMCCD camera			Nikon Eclipse TiE equipped with a QImaging Rolera em-c2 1K electron-multiplying charge-coupled-device (EMCCD) camera is used in this work.
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