

Materials List for:

Split Green Fluorescent Protein System to Visualize Effectors Delivered from Bacteria During Infection

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URL: <https://www.jove.com/video/57719>

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Materials

Name	Company	Catalog Number	Comments
Arabidopsis transgenic lines			Park, E., Lee, H. Y., Woo, J., Choi, D. & Dinesh-Kumar, S. P. Spatiotemporal Monitoring of Pseudomonas syringae Effectors via Type III Secretion Using Split Fluorescent Protein Fragments. Plant Cell. 29 (7), 1571-1584 (2017)
CYTO-sfGFP1-10	ABRC	CS69831	
NU-sfGFP1-10	ABRC	CS69832	
PT-sfGFP1-10	ABRC	CS69833	
MT-sfGFP1-10	ABRC	CS69834	
PX-sfGFP1-10	ABRC	CS69835	
ER-sfGFP1-10	ABRC	CS69836	
GO-sfGFP1-10	ABRC	CS69837	
PM-sfGFP1-10	ABRC	CS69838	
Organelle-targeted sfGFP1-10OPT plasmid			Park, E., Lee, H. Y., Woo, J., Choi, D. & Dinesh-Kumar, S. P. Spatiotemporal Monitoring of Pseudomonas syringae Effectors via Type III Secretion Using Split Fluorescent Protein Fragments. Plant Cell. 29 (7), 1571-1584 (2017)
CYTO-sfGFP1-10	Addgene	97387	
NU-sfGFP1-10	Addgene	97388	
PT-sfGFP1-10	Addgene	97389	
MT-sfGFP1-10	Addgene	97390	
PX-sfGFP1-10	Addgene	97391	
ER-sfGFP1-10	Addgene	97392	
GO-sfGFP1-10	Addgene	97393	
PM-sfGFP1-10	Addgene	97394	
ER-sfCherry1-10	Addgene	97403	
ER-sfYFP1-10	Addgene	97404	
CYTO-sfCFP1-10	Addgene	97405	
sfGFP11-tagged Gateway compatible vector for T3SS-based effector delivery system	Park, E., Lee, H. Y., Woo, J., Choi, D. & Dinesh-Kumar, S. P. Spatiotemporal Monitoring of Pseudomonas syringae Effectors		

	via Type III Secretion Using Split Fluorescent Protein Fragments. Plant Cell. 29 (7), 1571-1584 (2017)		
pBK-GW-1-2	Addgene	98250	pAvrRpm1:GW:HA-sfGFP11:AvrRpm1t; Resistant to Kanamycin (25 ug/ml)
pBK-GW-1-4	Addgene	98251	pAvrRpm1:GW:HA-2xsfGFP11:AvrRpm1t; Resistant to Kanamycin (25 ug/ml)
pBK-GW-2-2	Addgene	98252	pAvrRpm1:AvrRPM1sp:GW:HA-sfGFP11:AvrRpm1t; Resistant to Kanamycin (25 ug/ml)
pBK-GW-2-4	Addgene	98253	pAvrRpm1:AvrRPM1sp:GW:HA-2xsfGFP11:AvrRpm1t; Resistant to Kanamycin (25 ug/ml)
pBG-GW-1-2	Addgene	98254	pAvrRpm1:GW:HA-sfGFP11:AvrRpm1t; Resistant to Gentamycin (25 ug/ml)
pBG-GW-1-4	Addgene	98255	pAvrRpm1:GW:HA-2xsfGFP11:AvrRpm1t; Resistant to Gentamycin (25 ug/ml)
pBG-GW-2-2	Addgene	98256	pAvrRpm1:AvrRPM1sp:GW:HA-sfGFP11:AvrRpm1t; Resistant to Gentamycin (25 ug/ml)
pBG-GW-2-4	Addgene	98257	pAvrRpm1:AvrRPM1sp:GW:HA-2xsfGFP11:AvrRpm1t; Resistant to Gentamycin (25 ug/ml)
Bacterial strains			
<i>Agrobacterium tumefaciens</i> GV3101			Csaba Koncz and Jeff Schell, The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by a novel type of Agrobacterium binary vector. Mol Gen Genet. 204,383-396 (1986); Resistant to gentamycin (50 ug/ml) and rifampicin (50 ug/ml)
<i>Pseudomonas syringae</i> pv. <i>Tomato</i> CUCPB5500			Kvitko, B. H. et al. Deletions in the repertoire of <i>Pseudomonas syringae</i> pv. <i>tomato</i> DC3000 type III secretion effector genes reveal functional overlap among effectors. PLoS Pathog. 5 (4) (2009).; Resistant to rifampicin (100 ug/ml)
Media components			
Plant germination media			
Murashige & Skoog medium including vitamins	Duchefa Biochemie	M0222	Store at 4 °C.
Sucrose	Duchefa Biochemie	S0809	
Phytigel	Sigma-Aldrich	P8169	
LB media			
Tryptone	BD Bioscience	211705	
Yeast extract	BD Bioscience	212750	
NaCl	Duchefa Biochemie	S0520	
			Add 10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl to water. For solid media, add 15 g/L micro agar. Autoclave. Allow solution to cool to 55 °C, and add antibiotic if needed.

Micro agar	Duchefa Biochemie	M1002	
King's B media			10 g/L protease peptone #2, 1.5 g/L anhydrous K ₂ HPO ₄ , 15 g/L of agar to water. Autoclave. Cool down to 55 °C and add sterile 15 ml/L glycerol, 5 ml/L MgSO ₄ to the medium. Add antibiotics if needed.
Protease peptone	BD Bioscience	212120	
Anhydrous K ₂ HPO ₄	Sigma-Aldrich	1551128 USP	
Glycerol	Duchefa Biochemie	G1345	
MgSO ₄	Sigma-Aldrich	M7506	
Bacto Agar	BD Bioscience	214010	
Mannitol-Glutamate (MG) liquid media			Add 10 g/L of mannitol, 2 g/L of L-glutamic acid, 0.5 g/L of KH ₂ PO ₄ , 0.2 g/L of NaCl, and 0.2 g/L of MgSO ₄ to water. Adjust to pH 7
Mannitol	Duchefa Biochemie	M0803	
L-glutamic acid	Duchefa Biochemie	G0707	
KH ₂ PO ₄	Sigma-Aldrich	NIST200B	
Infiltration buffer			10 mM MES (2-(N-morpholino)-ethane sulfonic acid), 10 mM MgCl ₂ , 150 μM acetosyringone. pH 5.6; Prepare a fresh buffer before use.
MES	Duchefa Biochemie	M1503	Prepare 100 mM (pH 5.6) stock in water. Filter sterilize.
MgCl ₂	Sigma-Aldrich	M8266	Prepare 100 mM stock in water. Autoclave.
Acetosyringone	Sigma-Aldrich	D134406	Prepare 150 mM stock in DMSO.
Confocal microscope equipments/ materials			
710 laser scanning confocal system	Carl Zeiss		
Axio observer Z1 inverted microscope	Carl Zeiss		
Propidium iodide	ThermoFisher	P1304MP	