

Materials List for:

Genetic Manipulation of the Plant Pathogen *Ustilago maydis* to Study Fungal Biology and Plant Microbe Interactions

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Materials

Name	Company	Catalog Number	Comments
Aminobenzoic acid (Free Acid)	Sigma Aldrich	A-9878	
Bacto agar	BD	214010	alternatively use local supplier
Bacto peptone	BD	211677	alternatively use local supplier
Bacto yeast extract	BD	212750	alternatively use local supplier
CaCl ₂ *2H ₂ O	Grüssing GmbH	10234	alternatively use local supplier
Ca-Pantothenat (Hemi-Ca. salt)	Sigma Aldrich	P-2250	
Carboxin	Sigma Aldrich	45371	
Casamino acids	BD	223050	
Cholinchlorid	Sigma Aldrich	C-1879	
Citric acid	ChemSolute	24,321,000	alternatively use local supplier
CuSO ₄ *5H ₂ O	Fluka	61240	alternatively use local supplier
D(+)-Sucrose	Roth	4621.1	alternatively use local supplier
DNA degr. free acid	Sigma-Aldrich	D-3159	
EDTA	Sigma Aldrich	E4378	
FeCl ₃ *6H ₂ O	Grüssing GmbH	10288	alternatively use local supplier
Geneticin (G418) disulfate salt	Sigma Aldrich	A1720	
Trichoderma lysing enzymes	Sigma Aldrich	L1412	
D(+)-Glucose	Caelo	2580	alternatively use local supplier
Glycerin	Fisher Chemical	G065015	alternatively use local supplier
H ₃ BO ₃	AppliChem	A2940	Dangerous substance. Please check manufacturer's safety instructions.
Heparin sodium salt	Sigma Aldrich	H3393-50KU	
Hygromycin B-solution	Roth	1287.2	Dangerous substance.
KCl	VWR	26764298	alternatively use local supplier
KH ₂ PO ₄	AppliChem	A3620	alternatively use local supplier
MgSO ₄ waterfree	Merck	7487-88-9	Water free is critical. Alternatively use local supplier
MnCl ₂ *4H ₂ O	AppliChem	A2087	alternatively use local supplier
myo-Inositol	Sigma Aldrich	I-5125	
Na ₂ -EDTA*2H ₂ O	AppliChem	A2937	alternatively use local supplier

Na ₂ MoO ₄ *2H ₂ O	Roth	0274.2	alternatively use local supplier
Na ₂ SO ₄	Grüssing GmbH	12174	alternatively use local supplier
NaCl	Fisher Chemical	S316060	alternatively use local supplier
NaOH	ChemSolute	13,751,000	alternatively use local supplier
NH ₄ NO ₃	Roth	K299.1	alternatively use local supplier
Nicotinic acid (Free acid)	Sigma Aldrich	N-4126	
Nourseothricin dihydrogen sulfate	Werner BioAgents	5,001,000	
Nutrient broth	Difco		local suppliers
Phenol:Chloroform:Isoamyl alcohol (25:24:1) pH 6.7	Sigma Aldrich	P3803	Dangerous substance. Please check manufacturer's safety instructions.
polyethylene glycol (PEG)	Sigma Aldrich	P-3640	
Potassium acetate	AppliChem	121479	alternatively use local supplier
Pyridoxin (monohydrochlorid)	Sigma Aldrich	P-9755	
Riboflavin	Sigma Aldrich	R4500	
RNaseA	Sigma Aldrich	R5503	
SDS	Roth	Cn30.3	alternatively use local supplier
small syringe	BD	300300	alternatively use local supplier
sterile filter, 22 µm	VWR	28145-477	alternatively use local supplier
Sorbitol	Roth	6213.1	alternatively use local supplier
Thiamin-hydrochloride	Serva	36020.02	alternatively use local supplier
tri-Na-Citrate	Fisher Chemical	S332060	alternatively use local supplier
Tris- (hydroxymethyl) aminomethane	VWR	103156X	alternatively use local supplier
Tris hydrochloride	Roth	9090.4	alternatively use local supplier
Triton X-100	Serva	37240	alternatively use local supplier
ZnCl ₂	Fluka	96470	alternatively use local supplier
Composition of solutions/ preparation of material			Composition of solutions
Carboxin			Stock: 5 mg/ml in methanol, final concentration: 2 µg/ml
CM plates			0.25 % (w/v) Casamino acids, 0.1 % (w/v) Yeast Extract, 1.0 % (v/v) Holliday vitamin solution, 6.25 % (v/v); Holliday salt solution, 0.05 % (w/v) DNA degr. free acid, 0.15 % (w/v) NH ₄ NO ₃ , 2.0 % (w/v) Bacto Agar; adjust to pH 7.0 using 5 M NaOH; after autoclaving add 1 % glucose
Geneticin (G418)			Stock: 50 mg/ml in H ₂ O, final concentration: 500 µg/ml
HCl-washed glass beads (0.35-0.45 mm)			Cover glass beads with concentrated HCl (25 %, 7.8 M) and incubate for 60 min. Sway several times. Decant HCl (keep decanted liquid) and wash glass beads with 3 M HCl (keep decanted liquid). Wash glass beads several times with double distilled H ₂ O until the pH is 7 (the liquid should not be yellow-green anymore). Aliquot the glass beads and dry them at 180 °C. The

			decanted HCl has to be neutralized before disposal.
Heparin			Stock: 15 mg/ml
Holliday salt solution			16.0 ‰ (w/v) KH ₂ PO ₄ , 4.0 ‰ (w/v) Na ₂ SO ₄ , 8.0 ‰ (w/v) KCl, 1.32 ‰ (w/v) CaCl ₂ *2H ₂ O, 8.0 ‰ (v/v) trace elements, 2.0 ‰ (w/v) MgSO ₄ ; sterile filtrate
Holliday vitamin solution			0.1‰ (w/v) Thiamin, 0.05‰ (w/v) Riboflavin, 0.05‰ (w/v) Pyridoxin, 0.2‰ (w/v) Ca-Pantothenat, (0.05‰ (w/v) Aminobenzoic acid, 0.2‰ (w/v) Nicotinic acid, 0.2‰ (w/v) Cholinchlorid, 1.0‰ (w/v) myo-Inositol; may be stored at -20 °C
Hygromycin			Stock: 50 mg/ml in PBS, final concentration: 200 µg/ml
Nourseothricin			Stock: 200 mg/ml in H ₂ O, final concentration: 150 µg/ml
NSY-glycerol-medium			0.8 % (w/v) Nutrient Broth, 0.1 % (w/v) Yeast Extract, 0.5 % (w/v) Sucrose, 80.0 % (v/v) 87% Glycerin (f.c. 69.6%)
RegLight			1.0% (w/v) Yeast Extract 0.4 % (w/v) Bacto Peptone, 0.4 % (w/v) Sucrose, 18.22 % (w/v) Sorbitol, 1.5 % (w/v) Agar
SCS, pH 5.8			Solution 1: 20 mM tri-Na-citrate, 1 M Sorbitol; colution 2: 20 mM Citric acid, 1 M Sorbitol, add solution 2 into solution 1 until pH 5.8 is reached; autoclave
STC, pH 8			1 M Sorbitol, 10 mM Tris-HCl pH 7.5, 100 mM CaCl ₂ ; filter sterile
STC/PEG			40 % (v/v) PEG in STC-buffer
TE buffer, pH 8			1.31 mM Tris-Base, 8.69 mM Tris-HCl, 10 mM Na ₂ -EDTA*2H ₂ O
TE/RNase			10 µg/ml RNaseA in TE buffer
Trace elements			0.06‰ (w/v) H ₃ BO ₃ , 0.14‰ (w/v) MnCl*4H ₂ O, 0.4 ‰ (w/v) ZnCl ₂ , 0.4 ‰ (w/v) Na ₂ MoO ₄ *H ₂ O:2, 0.1 ‰ (w/v) FeCl ₃ *6H ₂ O, 0.04‰ (w/v) CuSO ₄ *5H ₂ O
Trichoderma lysing enzymes solution			12.5 mg/ml SCS; filter sterile; prepare shortly before use
Tris-HCl pH 7.5			806 mM Tris-HCl, 194 mM Tris-Base; check the pH and if necessary adjust with HCl; autoclave
Usti-lysis buffer 1, pH 8			10 mM Tris-HCl (pH 8.0), 10 mM NaCl, 1% (w/v) SDS, 2% (v/v) TritonX-100, 1 mM EDTA. Do not measure pH using pH meter.
Usti-lysis buffer 2			mix Usti lysis buffer 1 with 1x TE in a 1:1 ratio
YEPS-Light medium			1.0% (w/v) Yeast Extract, 0.4% (w/v) Bacto Peptone, 0.4% (w/v)

			Sucrose, for plates: 1.5% (w/v) Bacto Agar
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