

Materials List for

# Isolation, Characterization, and Total DNA Extraction to Identify Endophytic Fungi in Mycoheterotrophic Plants

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## Materials

Name	Company	Catalog Number	Comments
Adhesive tape			(from any company, for adhesive tape mount in micromorphological analyses)
Ampicillin	Sigma-Aldrich	A5354	(for installation of plant fragments; other antibiotics may be used - check step 2.2.1)
Autoclave			(from any company, for materials sterilization in many steps)
Bacteriological agar	Sigma-Aldrich	A1296	(for many steps)
C1, C2, C3, C4, C5, and C6 solutions	Qiagen	12888-50	(purchased with DNeasy PowerSoil kit)
Centrifuge	Merck/Eppendorf	5810 G	(for total DNA extraction from fungal isolates)
Centrifuge tubes	Merck	CLS430828	(for samples collection)
Chloroform	Sigma-Aldrich	C2432	(for total DNA extraction from fungal isolates)
Congo red	Supelco	75768	(for hyphae staining)
Cryotubes	Merck	BR114831	(for many steps)
Ethanol	Supelco	100983	It will be necessary to carry out the appropriate dilutions (for many steps)
Ethylenediaminetetraacetic acid (EDTA)	Sigma-Aldrich	3609	(for total DNA extraction from fungal isolates)
Filter paper	Merck	WHA10010155	(for many steps)
Glass test tubes	Merck	CLS7082516	(for cryopreservation in unhulled rice grains)
Glass wool	Supelco	20411	(for cryopreservation in unhulled rice grains)
Glucose	Sigma-Aldrich	G8270	Or dextrose (for cryopreservation in vermiculite)
Glycerol	Sigma-Aldrich	G5516	Or glycerin (for cryopreservation in vermiculite, for preparing LPCB)
Isopropanol	Sigma-Aldrich	563935	(for total DNA extraction from fungal isolates)

Lactic acid	Sigma-Aldrich	252476	(for preparing LPCB - hyphae staining)
Lactophenol blue solution (LPCB)	Sigma-Aldrich	61335	(for hyphae staining)
Laminar flow hood			(class I, from any company, for many steps)
Light microscope			(from any company, for hyphae observation)
MB Spin Columns	Qiagen	12888-50	(purchased with DNeasy PowerSoil kit)
Methyl blue (cotton blue)	Sigma-Aldrich	M5528	(for preparing LPCB - hyphae staining)
Microcentrifuge tube (1.5 mL)	Merck	HS4323	(for total DNA extraction from fungal isolates)
Microcentrifuge tube (2 mL)	Merck	BR780546	(for many steps)
Mineral oil			(for preservation of fungal isolates)
Paper bags			Average size 150 mm x 200 mm (for samples collection)
Petri dish (Glass, 120 mm x 20 mm)	Merck/Pyrex	SLW1480/10D	(autoclavable, for fungi slide culture, prefer higher ones)
Petri dish (Glass, 50 mm x 17 mm)	Merck/Aldrich	Z740618	(for purification of fungal isolates); alternatively: polystyrene petri dishes (sterile, $\gamma$ -irradiated, non-autoclavable)
Petri dish (Glass, 80 mm x 15 mm)	Merck/Brand	BR455732	(for installation of plant fragments); alternatively: polystyrene petri dishes (sterile, $\gamma$ -irradiated, non-autoclavable)
Phenol	Sigma-Aldrich	P1037	(for total DNA extraction from fungal isolates, for preparing LPCB)
Porcelain mortar	Sigma-Aldrich	Z247464	(for total DNA extraction from fungal isolates)
Porcelain pestle	Sigma-Aldrich	Z247502	(for total DNA extraction from fungal isolates)
Potato dextrose agar (PDA)	Millipore	P2182	(for many steps)
PowerBead tubes	Qiagen	12888-50	(purchased with DNeasy PowerSoil kit)
Rapid mounting medium (Entellan)	Sigma-Aldrich	1.0796	(for fungi slide culture)
Silica gel	Supelco	717185	(for cryopreservation in unhulled rice grains)
Sodium chloride (NaCl)	Sigma-Aldrich	S9888	(for total DNA extraction from fungal isolates)
Sodium dodecyl sulfate (SDS)	Sigma-Aldrich	L3771	Lauryl sulfate sodium salt (for total DNA extraction from fungal isolates)
Sodium hypochlorite (w/ 2% active chlorine)			(commercial product, for superficial desinfestation)
Soil DNA extraction kit (DNeasy PowerSoil kit)	Qiagen	12888-50	(for total DNA extraction from plant organs)
Spectrophotometer - Nanodrop 2000/2000c	ThermoFisher Scientific	ND2000CLAPTOP	(for total DNA extraction from plant organs)
Stereomicroscope			(=dissecting microscope, from any company, for macromorphological analyses)
Tetracycline	Sigma-Aldrich	T7660	(for installation of plant fragments)
Thermoblock	Merck/Eppendorf	EP5362000035	(or from other companies)
Tissue homogenizer and cell lyzer	SPEX SamplePrep		2010 Geno/Grinder - Automated Tissue Homogenizer and Cell Lyzer

			(for total DNA extraction from plant organs)
Toluidine blue O	Sigma-Aldrich/Harleco	364-M	(for hyphae staining)
Trehalose	Sigma-Aldrich	T9531	(for cryopreservation in vermiculite)
Tris Base Solution (Tris)	Sigma-Aldrich	T1699	(for total DNA extraction from fungal isolates)
Unhulled rice grains			(for cryopreservation)
U-shaped glass rod			(or an adaptation - check step 5.4.1, for fungi slide culture)
Vermiculite			Fine granulometry (for cryopreservation in vermiculite)
Vortexer	Sigma-Aldrich/BenchMixer	BMSBV1000	(for total DNA extraction from fungal isolates)
Yeast extract	Sigma-Aldrich	Y1625	(for cryopreservation in vermiculite)