

Materials List for:

From a Natural Product to Its Biosynthetic Gene Cluster: A Demonstration Using Polyketomycin from *Streptomyces diastatochromogenes* Tü6028

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Materials

Name	Company	Catalog Number	Comments
agar	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	5210.4	
agarose	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	6352.4	
D-mannitol	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	4175.1	
glucose	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	6780.1	
LB	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	X964.3	
malt extract	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	X976.2	
MgCl ₂	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	2189.1	
Peptone	Carl Roth GmbH + Co. KG, Karlsruhe, Germany		
soy flour	W.Schoenemberger GmbH, Magstadt, Germany		Hensec-Vollsoja
tryptic soy broth	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	X938.3	Caso-Bouillon
yeast extract	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	2363.2	
Solvents			
Acetic acid	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	3738.5	
Acetone	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	9372.2	
Acetonitrile	Avantor Performance Materials B.V., Deventer, The Netherlands	JT-9012-03	
Dichlorofrom	Fisher Scientific GmbH, Schwerte, Germany	1530754	
DMSO (Dimethyl sulfoxide)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	4720.1	
DMSO-d ₆ (Dimethyl sulfoxide-d ₆) 99.9 atom% D	ARMAR Chemicals, Döttingen, Switzerland	15200.204	
Ethyl acetate	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	6784.4	
Hydrochloric acid	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	6331.4	

Methanol	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	7342.1	
Enzymes			
restriction enzymes	New England Biolabs GmbH, Frankfurt am Main, Germany		
polymerase	New England Biolabs GmbH, Frankfurt am Main, Germany		
DNA Polymerase I Large (Klenow) Fragment	Promega GmbH, Mannheim, Germany		
Antibiotics			
apramycin	AppliChem GmbH, Darmstadt, Germany	A7682.0005	
fosfomycin	Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany	P5396-50G	
kanamycin	Carl Roth GmbH + Co. KG, Karlsruhe, Germany	T832.2	
Plasmid/Vectors			
pKC1132	Bierman <i>et al.</i> 1992		
Primer			
pokPI_for	TGATGGTGCCGCTGGCCATGG	Primer to amplify fragment containing pokPI gene	
pokPI_rev	AGCGTTCACTGTTCCGCCCGAC		
Bacterial strains			
<i>Bacillus subtilis</i> COHN ATCC6051		Gram-positive test strain	
<i>Escherichia coli</i> ET12567 pUZ8002	MacNeil <i>et al.</i> 1992	strain for conjugation	
<i>Escherichia coli</i> XL1 Blue	Agilent Technologies, Santa Clara, USA	Gram-negative test strain + cloning host	
<i>Streptomyces diastatochromogenes</i> Tü6028	Paululat <i>et al.</i> , 1999	Polyketomycin producer	
Online services			
antismash (Antibiotics and Secondary Metabolite Analysis Shell)	http://antismash.secondarymetabolites.org/	Detection of secondary metabolite gene cluster	
BLAST (Basic Local Alignment Search Tool)	http://blast.ncbi.nlm.nih.gov/Blast.cgi	finds regions of similarity between biological sequences	
GenDB	https://www.uni-giessen.de/fbz/fb08/Inst/bioinformatik/software/genedb	Annotation of ORFs	
MiBIG (Minimum Information about a Biosynthetic Gene cluster)	http://mibig.secondarymetabolites.org/	Database of biosynthetic gene clusters	
NaPDoS	http://napdos.ucsd.edu/	Detection of secondary metabolite gene cluster	
NCBI Prokaryotic Genome Annotation Pipeline	http://www.ncbi.nlm.nih.gov/genome/annotation_prok/	Annotation of ORFs	
NRPSpredictor	http://nrps.informatik.uni-tuebingen.de/	Detection of NRPS domains	
Prokka (rapid prokaryotic genome annotation)	http://www.vicbioinformatics.com/software.prokka.shtml	Annotation of ORFs	
RAST (rapid annotation using subsystems technology)	http://rast.nmpdr.org/	Annotation of ORFs	
Other programs			

Chem Station Rev. A.09.03	Agilent Technologies, Waldbronn, Germany	Handling program for HPLC	
Clone Manager Suite 7	Scientific and Educational Software, Cary, USA	Designing Cloning Experiment	
Newbler v2.8	Roche Diagnostics	Alignment of sequencing reads	
Machines			
Centrifuge Avanti J-6000, Rotor JA-10	Beckman Coulter GmbH, Krefeld, Germany		
HPLC/MS	Agilent Technologies, Waldbronn, Germany		
_Autosampler: G1313A	Agilent Technologies, Waldbronn, Germany		
_Pre-column: XBridge C18 (20 mm x 4.6 mm; Particle size: 3.5 µm)	Agilent Technologies, Waldbronn, Germany		
_Column:Xbridge C18 (100 mm x 4.6 mm; Particle size: 3.5 µm)	Agilent Technologies, Waldbronn, Germany		
_semi-prep Pre-Column: Zorbax B-C18 (9.4 x 150 mm; Particle size: 5 µm)	Agilent Technologies, Waldbronn, Germany		
_semi-prep Column: Zorbax B-C18 (9.4 x 20 mm; Particle size: 5 µm)	Agilent Technologies, Waldbronn, Germany		
_Degasser: G1322A	Agilent Technologies, Waldbronn, Germany		
_Quarternary pump: G1311A	Agilent Technologies, Waldbronn, Germany		
_Diode array detector (DAD)G1315B (λ = 254 nm and 400 nm)	Agilent Technologies, Waldbronn, Germany		
_Quadrupole mass detector (MSD) G1946D(2-3000 m/z)	Agilent Technologies, Waldbronn, Germany		
rotary evaporator			
_heating bath Hei-VAP Value/G3	Heidolph Instruments GmbH & Co.KG, Schwabach, Germany		
_vacuum pump system SC 920 G	KNF Global Strategies AG, Sursee, Switzerland		
Other material			
Sephadex LH20	GE Healthcare,		
Chromafil PVDF-45/15MS (pore size 0.45 µm; filter Ø15 mm)	MACHEREY-NAGEL GmbH & Co. KG, Düren, Germany		
SPE column Oasis HLB 20 35 cc (6g)	Waters GmbH, Eschborn, Germany		
<i>E. coli</i>	Carl Roth GmbH + Co. KG, Karlsruhe, Germany		Medium: LB, Composition: LB, Amount to 1 L H ₂ O: 20 g
<i>Bacillus</i>	Carl Roth GmbH + Co. KG, Karlsruhe, Germany		Medium: LB, Composition: LB, Amount to 1 L H ₂ O: 20 g
<i>Streptomyces</i> sp.	Carl Roth GmbH + Co. KG, Karlsruhe, Germany		Medium: TSB, Composition: CASO Boullion, Amount to 1 L H ₂ O: 30 g
fungus	Carl Roth GmbH + Co. KG, Karlsruhe, Germany		Medium: YPD, Composition: Yeast extract, Amount to 1 L H ₂ O: 10 g
fungus	Carl Roth GmbH + Co. KG, Karlsruhe, Germany		Medium: YPD, Composition: Peptone, Amount to 1 L H ₂ O: 20 g
fungus	Carl Roth GmbH + Co. KG, Karlsruhe, Germany		Medium: YPD, Composition: Glucose, Amount to 1 L H ₂ O: 20 g

for agar plates add 2% agar