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Bacterial strains and Plasmids

Strain	Genotype
Escherichia coli DH5α	Φ8dlacZ Δ M15, recA1, endA1, gyr A96, thi-1, hsd17 (r_k^-, m_k^-) supE44, relA1,deoR, (LacZYA-argF)U19
Helper strain -HB101:: pRK 2013 (Clontech)	
Agrobacterium tumefaciens (EHA105)	A virulent strain carrying pMP90 Ti-plasmid conferring resistance against gentamicin and rifampicin as chromosomal selection

Plasmid	pBI121 (Clontech), pSB 8, pSB8ß

Media and Solutions

YEP:	1% Yeast Extract
	1% Bacto Peptone
	0.5% NaCl
Luria Broth (LB):	25 g/l
LB agar:	32 g/l
Micropropagation medium (MM):	MS medium
	0.1 mg/l IAA, pH 5.6-5.8
Callus induction medium (CIM):	Gamborg's B-5 medium
	0.4 mg/l NAA, pH 5.6-5.8
Shoot induction medium (SIM):	Gamborg's B-5 medium
	0.1-0.4 mg/l NAA, pH 5.6-5.8
Linsmaier and Skoog medium:	LS medium
TE (pH 8.0):	10 mM Tris-HCl (pH 8.0)
	1 mM EDTA (pH 8.0)
20X SSC:	175.3 g NaCl
	88.2 g Trisodium citrate
	pH 7.0

6X Endo-R:	30% Ficoll 400
	60 mM EDTA (pH 8.0)
	0.6% SDS
High Salt TE:	10 mM Tris (pH 8.0)
	1 mM EDTA
Fixative:	0.3% paraformaldehyde
	10 mM MES pH5.6
	0.3 M Mannitol
	50 mM NaH_2PO_4 (pH 7.0)
20X SSC (1 liter):	175.3 g Sodium Chloride
	88.2g Sodium Citrate, pH 7.0
10X MOPS Buffer:	200 mM 3-[*N*-morpholino] propanesulfonic acid (MOPS)
	50 mM sodium acetate
	10 mM EDTA
	final pH of 6.5–7.0 with NaOH
RNA loading dye:	95% formamide
	0.025% SDS
	0.025% bromophenol blue
	0.025% xylene cyanol FF
	0.025% ethidium bromide
2X Extraction Buffer:	50 mM Tris (pH 6.8)
	1% 2-mercaptoethanol
	1 mM PMSF
	1 mM EDTA
4X Stacking Buffer:	0.5 M Tris HCl (pH 6.8)
8X Resolving Buffer:	3 M Tris HCl (pH 8.8)

12.5% polyacrylamide gel: (40 ml)	Acrylamide (30:0.8) - 16.68 ml
	4X buffer (pH 8.8) - 10 ml
	MilliQ - 12.92 ml
	SDS (20%) - 200 µl
	APS (10%) - 200 µl
	TEMED - 15 µl
Reservoir Buffer (10X):	0.25 M Tris (pH 8.3)
	1.92 M Glycine
	1% SDS
Towbin's Buffer:	25 mM Tris
	190 mM Glycine
	20% Methanol
TBS:	10 mM Tris-HCl, pH 8.0
	100 mM Tris, pH 8.0
	150 mM NaCl
TBST:	TBS, 0.05% Tween 20
Blocking Solution:	TBS + 5% Fat free dry milk
AP Buffer:	100 mM Tris-Cl, pH 9.5
	100 mM NaCl
	50 mM $MgCl_2$
AP Colour Development Solution:	10 ml AP Buffer
	66 µl NBT (50 mg/ml, 70% DMF)
	33 µl BCIP (50 mg/ml DMF)
X-gal:	20 mg/ml (in DMF)
IPTG:	200 mg/ml in H_2O
Antibiotics:	Kanamycin 100 mg/ml of water
	Rifampicin 50 mg/ml of methanol
	Cefotaxime 250 mg/ml of water
	Paromomycin sulfate 50mg/l of water

List of primers:

Genes	**Primer sequence**
*nptII*F *nptII*R	5' - ATGATTGAACAAGATGGATTGCACGCAGG -3' 5'- GAAGAACTCGTCAAGAAGGCGATA -3'
*AmA1*F *AmA1*R	5'-CACCATGGCGGGATTACCAGTG-3' 5'-CAAGGAAGAACCCTCTTGTTTCC-3'
*TubRT*F *TubRT*R	5'- AGGACCCTTGTGTTTGGTGTTAA- 3' 5'- CCCACTCATCGTTGCAGAAA-3'
*GAPDHRT*F *GAPDHRT*R	5'- AAGAAAACAAAAGCACGGCACTA-3' 5'- AAGTGGAAAAAGGATTCGGTGTAT-3'
*Actin*F *Actin*R	5'-CTCCCCTAATGAGTGTGATGTGAT-3' 5'- GAGCCCCATGAGAACATTACCA-3'
*GUS*F *GUS*R	5'-TGGTAATTACCGACGAAAACGGC-3' 5'-ACGCGTGGTTACAGTCTTGCG-3'
*AmA1RT*F *AmA1RT*R	5'-GGGAATGATCCTCGCGAAA-3' 5'- AAAATCATGCACATCCGACCTA-3'
*AmA1UTRRT*F *AmA1UTRRT*R	5'- GAGATAATAGAATTGGGATCCAACAAC-3' 5'- CCAAAGAGACGACTTACAACGTTTT-3'

List of chemicals:

Type	Material	Source
Antibiotics	Ampicillin, Kanamycin, Rifampicin, Spectinomycin, Geneticin	Sigma
Disposable filters	PVDF 0.45 µm filter unit	Millipore
Enzymes	Commonly used restriction enzymes	NEB
	Taq DNA Polymerase	Clontech, Finnezym
	T4 DNA Ligase	NEB
	RNase	BioBasic, Amersham
Dyes	Ethidium Bromide, Xylene cyanol Methylene Blue, Coomassie Brilliant Blue	Amersham
Culture media components	Tryptone, Yeast Extract, Agar, MS salts	Difco, Invitrogen, Sigma
Locally available chemicals	Isopropanol, iso-amyl alcohol**,** $CaCl_2$, NaCl, NaOH, Glucose, Methanol, $MgCl_2$, KOH, Potassium acetate, Chloroform, Glycerol, Acetic acid, NaH_2PO_4, Na_2HPO_4, $MgSO_4$, HCl, H_2SO_4, Glycine, KCl, Sucrose, Pot. Dichromate, Sodium hypochlorite, Mercuric chloride, tri-Sodium citrate, Formaldehyde.	Qualigens and Merck
Foreign chemicals	DEPC, HEPES, IPTG, MOPS, Sephadex G-50, EDTA, CTAB, Acrylamide, Bis-Acrylamide, TEMED, Spermine, Spermidine, Polyvinyl Polypyrollidine, Triton-X-100, X-gal	Amersham, Sigma, Ambion,