

Materials List for

Isolation and Identification of Waterborne Antibiotic-Resistant Bacteria and Molecular Characterization of their Antibiotic Resistance Genes

Devika Ghadigaonkar¹, Archana Rath¹

¹Antimicrobial Research (AMR) Lab, Department of Biotechnology, University of Mumbai

Corresponding Author

Archana Rath
drarath@mu.ac.in

Citation

Ghadigaonkar, D., Rath, A. Isolation and Identification of Waterborne Antibiotic-Resistant Bacteria and Molecular Characterization of their Antibiotic Resistance Genes. *J. Vis. Exp.* (193), e63934, doi:10.3791/63934 (2023).

Date Published

March 3, 2023

DOI

10.3791/63934

URL

jove.com/video/63934

Materials

Name	Company	Catalog Number	Comments
100 bp DNA ladder	HiMedia	MBT049-50LN	For estimation of size of the amplicons
2x PCR Taq mastermix	HiMedia	MBT061-50R	For making PCR reaction mixture
37 °C Incubator	GS-192, Gayatri Scientific	NA	For incubation of bacteria
6x Gel Loading Buffer	HiMedia	ML015-1ML	Loading and Tracking dye which helps to weigh down the DNA sample and track the progress of electrophoresis
Agarose powder	HiMedia	MB229-50G	For resolving amplicons during Agarose Gel Electrophoresis (AGE)
Ampicillin antibiotic disc	HiMedia	SD002	For performing AST
Autoclave	Equitron	NA	Required for sterilization of media, glass plates, test tubes, etc
Bioanalyzer 2100	Agilent Technologies	NA	To check the quality and quantity of the amplified library
Bisafety B2 Cabinet	IMSET	IMSET BSC-Class II Type B2	Used for microbiological work like bacterial culturing, AST etc.
Cefotaxime antibiotic disc	HiMedia	SD295E-5VL	For performing AST
Cefotaxime antibiotic powder	HiMedia	TC352-5G	For preparation of antibiotic stock solution required during isolation of antibiotic resistant bacteria
Ceftriaxone antibiotic disc	HiMedia	SD065	For performing AST
Centrifuge Minispin	Eppendorf	Minispin Plus-5453	Used to pellet the debris during crude DNA preparation
Chloramphenicol antibiotic disc	HiMedia	SD006-5x50DS	For performing AST
Ciprofloxacin antibiotic disc	HiMedia	SD060-5x50DS	For performing AST
Ciprofloxacin antibiotic powder	HiMedia	TC447-5G	For preparation of antibiotic stock solution required during isolation of antibiotic resistant bacteria

Colorimeter	Quest	NA	For checking the OD of culture suspensions
Comprehensive Antibiotic Resistance Database (CARD) database			functional annotation of ARGs; https://card.mcmaster.ca/
Cooling Shaker Incubator	BTL41 Allied Scientific	NA	For incubation of media plates for culturing bacteria
Deep Freezer (-40 °C)	Haier	DW40L, Haier Biomedicals	For storage of glycerol stocks
DNA Library Prep Kit	NEB Next Ultra DNA Library Prep Kit for Illumina	NA	Paired-end sequencing library preparation
EDTA	HiMedia	GRM1195-100G	For preparation of Gel running buffer for Agarose Gel Electrophoresis (AGE)
Electrophoresis Apparatus	TechResource	15 cm gel casting tray	For making the agarose gel and carrying out electrophoresis
Electrophoresis Power pack with electrodes	Genei	NA	For running the AGE
Erythromycin antibiotic disc	HiMedia	SD222-5VL	For performing AST
Erythromycin antibiotic powder	HiMedia	CMS528-1G	For preparation of antibiotic stock solution required during isolation of antibiotic resistant bacteria
Erythromycin antibiotic powder	HiMedia	TC024-5G	For preparation of antibiotic stock solution required during isolation of antibiotic resistant bacteria
<i>Escherichia coli</i> ATCC 25922	HiMedia	0335X-1	Used as a control while performing AST
Ethidium Bromide	HiMedia	MB071-1G	Intercalating agent and visualization of DNA after electrophoresis under Gel Documentation System
Fluorometer	Qubit 2.0	NA	For determining concentration of extracted metagenomic DNA
Gel Documentation System	BioRad		Used for visualizing PCR amplicons after electrophoresis
Gentamicin antibiotic disc	HiMedia	SD170-5x50DS	For performing AST
Glacial Acetic Acid	HiMedia	AS119-500ML	For preparation of Gel running buffer for Agarose Gel Electrophoresis (AGE)
Glycerol	HiMedia	GRM1027-500ML	For making glycerol stocks
Imipenem antibiotic disc	HiMedia	SD073	For performing AST
Kaiju Database	NA	NA	For taxonomical classification of reads; https://kaiju.binf.ku.dk/
Kanamycin antibiotic disc	HiMedia	SD017-5x50DS	For performing AST
Kanamycin antibiotic powder	HiMedia	MB105-5G	For preparation of antibiotic stock solution required during isolation of antibiotic resistant bacteria
Levofloxacin antibiotic disc	HiMedia	SD216-5VL	For performing AST
Luria Bertani broth	HiMedia	M1245-500G	For enrichment of cultures
McFarland Standards	HiMedia	R092-1No	To compare density of culture suspension
Molecular Biology water	HiMedia	TCL018-500ML	For making PCR reaction mixture
Mueller-Hinton Agar (MHA)	HiMedia	M173-500G	For performing Antibiotic Susceptibility Testing (AST)
Neomycin antibiotic disc	HiMedia	SD731-5x50DS	For performing AST
PCR Gradient Thermal Cycler	Eppendorf	Mastercycler Nexus Gradient 230V/50-60 Hz	Used for performing PCR for amplification of 16S rRNA region and various Antibiotic Resistance genes
Primers	Xcelris	NA	For PCR amplification

R2A Agar, Modified	HiMedia	M1743	For preparation of media plates for isolation of total and antibiotic resistant (AR) bacterial load
Scaffold generation	CLC Genomics Workbench 6.0	NA	For generation of scaffolds
Sequencer	Illumina platform (2 x 150 bp chemistry)	NA	Sequencing of amplified library
Sodium Chloride	HiMedia	TC046-500G	For preparation of 0.85% saline for serially diluting the water sample
Soil DNA isolation Kit	Xcelgen	NA	For extraction of whole metagenomic DNA from the filtered water sample
<i>Staphylococcus aureus</i> subsp. aureus ATCC 29213	HiMedia	0365P	Used as a control while performing AST
Taxonomical Classification	Kaiju ioinformatics tool	NA	For classification of reads into different taxonomic groups from phylum to genus level
The Comprehensive Antibiotic Resistance Database (CARD)	NA	NA	For functional annotation of ARGs
Tigecycline antibiotic disc	HiMedia	SD278	For performing AST
Trimethoprim antibiotic disc	HiMedia	SD039-5x50DS	For performing AST
Tris base	HiMedia	TC072-500G	For preparation of Gel running buffer for Agarose Gel Electrophoresis (AGE)
Vancomycin antibiotic powder	HiMedia	CMS217	For preparation of antibiotic stock solution required during isolation of antibiotic resistant bacteria
Weighing Balance	Mettler Toledo	ME204 Mettler Toledo	Used for weighing media powders, reagent powders etc.
NA - Not Applicable			