

Materials List for:

Markerless Gene Deletion by Floxed Cassette Allelic Exchange Mutagenesis in *Chlamydia trachomatis*

Gabrielle Keb¹, Kenneth A. Fields¹

¹Department of Microbiology, Immunology, and Molecular Genetics, University of Kentucky

Correspondence to: Kenneth A. Fields at ken.fields@uky.edu

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Materials

Name	Company	Catalog Number	Comments
Agarose	KSE Scientific	BMK-A1705	Molecular Biology Grade
Anhydrotetracycline hydrochloride	ACROS Organics	233131000	
CaCl ₂ Buffer			10 mM Tris pH 7.4, 50 mM Calcium Chloride Dihydrate
Calcium Chloride Dihydrate	Sigma	C7902-500G	Suitable for cell culture
Cycloheximide	Sigma	7698-1G	
dam ⁻ dcm ⁻ Competent <i>E. coli</i>	New England BioLabs	C2925H	
DMSO	ATCC	4-X	Sterile filtered cell culture tested
Glutamic acid	Sigma	G8415-100G	L-Glutamic acid
Growth Media #1			RPMI 1640 media supplemented with 10 % (vol/vol) heat-inactivated fetal bovine serum (FBS).
Growth Media #2			RPMI 1640 media supplemented with 10 % (vol/vol) heat-inactivated fetal bovine serum (FBS) and 1 µg/mL cycloheximide
Hanks' Balanced Salt Solution (HBSS) (1x)	Gibco	24020-117	
Heat Inactivated Fetal Bovine Serum Qualified One Shot (FBS)	Gibco	A38402-02	
McCoy Cells	ATCC	CRL-1696	
Monarch Plasmid Miniprep Kit	New England BioLabs	T1010S	Small scale DNA purification
NaH ₂ PO ₄	Sigma	S3139-250G	Sodium phosphate monobasic
Na ₂ HPO ₄	Sigma	S5136-500G	Sodium phosphate dibasic
NEB 10-beta Electrocompetent <i>E. coli</i> Cells	New England BioLabs	C3020K	
NEBuilder HiFi DNA assembly Cloning Kit	New England BioLabs	E5520S	Gibson Assembly Kit
Penicillin G sodium salt	Sigma	P3032-10MU	Bioreagent suitable for cell culture
QIAGEN Plasmid Maxi Kit	QIAGEN	12162	Large scale DNA purification
Q5 Hot Start High-Fidelity DNA Polymerase	New England BioLabs	M0515	Fragment PCR Polymerase
RPMI 1640 Medium (1x)	Gibco	11875-093	Containing 2mM L-glutamine
Sall-HF	New England BioLabs	R3138S	
SbfI-HF	New England BioLabs	R3642S	
Selection Media #1			RPMI 10 % FBS, 1 µg/mL cycloheximide, 500 µg/mL spectinomycin, and 50 ng/mL

			anhydrous tetracycline dissolved in DMSO
Selection Media #2			RMPI 10 % FBS, 1 µg/mL cycloheximide, 500 µg/mL spectinomycin
Selection Media #3			RPMI 10 % FBS, 1 µg/mL cycloheximide, 50 ng/mL aTc, and 0.6 µg/mL penicillin
Sodium Acetate Buffer Solution	Sigma	S7899-100ML	3M
SOC Outgrowth Medium	New England BioLabs	B9020SVIAL	
Spectinomycin dihydrochloride pentahydrate, Cell Culture Grade	Alfa Aesar	J61820	
Sucrose	Sigma	S1888-1KG	Bioreagent suitable for cell culture
Sucrose-Phosphate-Glutamate Buffer (SPG)			37.5g sucrose, 1.25 g Na ₂ HPO ₄ , 0.18 g NaH ₂ PO ₄ , 0.36 glutamic acid for 500 ml tissue culture grade water
Tris	AMRESCO	0497-5KG	Ultrapure grade
Trypsin-EDTA (1x)	Gibco	25200-056	0.25%
Water	Sigma	W3500-500ML	Sterile-filtered, BioReagent, Suitable for cell culture