

Constructing Mutants in Serotype 1 *Streptococcus pneumoniae* strain 519/43

 Vanessa S. Terra¹, Charles D. Plumptre², Emma C. Wall³, Jeremy S. Brown², Brendan W. Wren¹
¹Faculty of Infections and Tropical Diseases, Department of Infection Biology, London School of Hygiene and Tropical Medicine ²Division of Medicine, University College London ³Division of Infection and Immunity, University College London UCL

Corresponding Author

Vanessa S. Terra
vanessa.terra@lshtm.ac.uk

Citation

Terra, V.S., Plumptre, C.D., Wall, E.C., Brown, J.S., Wren, B.W. Constructing Mutants in Serotype 1 *Streptococcus pneumoniae* strain 519/43. *J. Vis. Exp.* (), e61594, doi:10.3791/61594 (2020).

Date Published

September 11, 2020

DOI

10.3791/61594

URL

jove.com/video/61594

Materials

Name	Company	Catalog Number	Comments
AccuPrime Pfx DNA polymerase	Invitrogen	12344024	Used for amplification of the fragments
Ampicillin sodium salt	Sigma Aldrich	A9518	Used for bacterial selection on stage 1(pSD1)
Blood Agar Base	Oxoid	CM0055	Used to plate <i>S. pneumoniae</i> transformants
Bovine Serum Albumine	sigma	55470	used for <i>S. pneumoniae</i> Transformation
Brain Heart Infusion	Oxoid	CM1135	used to grow <i>S. pneumoniae</i> cells
Calcium Chloride CaCl ₂	Sigma	449709	used for <i>S. pneumoniae</i> Transformation
Competence stimulating peptide 1	AnaSpec	AS-63779	used for <i>S. pneumoniae</i> Transformation
Luria Broth Agar	Gibco	22700025	used for plating and selection of pSD1 and pSD2
Luria Broth Base (Miller's formulation)	Gibco	12795027	used for plating and selection of pSD1 and pSD2
Monarch Gel Extraction Kit	NEB	T1020S	Used to extract the bands from the DNA gel
Monarch Plasmid Miniprep Kit	NEB	T1010S	Used to extract plasmid from the cells
pGEM T-easy	Promega	A1360	used as suicide plasmid
S.O.C.	Invitrogen	15544034	used for recovery of cells after transformation
Sodium Hydroxide (NaOH)	Sigma	S0899	used for <i>S.pneumoniae</i> Transformation
Spectinomycin Hydrochloride	SigmaAldrich	PHR1426	Used for bacterial selection
Subcloning Efficiency DH5α Competent Cells	Invitrogen	18265017	used for the creation of pSD1 and pSD2