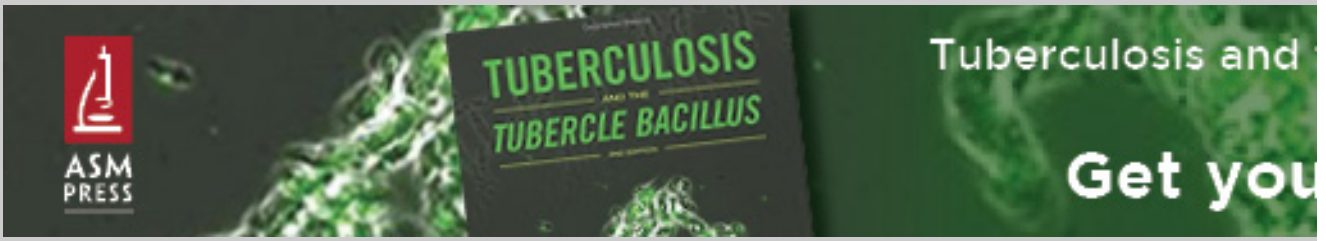

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New Vector for Efficient Allelic Replacement in Naturally Nontransformable, Low-GC-Content, Gram-Positive Bacteria†

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ABSTRACT

A shuttle vector designated pMAD was constructed for quickly generating gene inactivation mutants in naturally nontransformable gram-positive bacteria. This vector allows, on X-Gal (5-bromo-4-chloro-3-indolyl- -D-galactopyranoside) plates, a quick colorimetric blue-white discrimination of bacteria which have lost the plasmid, greatly facilitating clone identification during mutagenesis. The plasmid was used in *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus* to efficiently construct mutants with or without an associated antibiotic resistance gene.

FOOTNOTES

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† This paper is dedicated to the memory of Maryvonne Arnaud, who died on 1 February 2004.

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