Multiple gene deletion of Mannheimia succiniciproducens using mutant lox sequences

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A markerless gene deletion method was developed to generate genetically modified *Mannheimia succiniciproducens*, which is a rumen bacterium producing succinic acid as a major metabolite. Cre recombinase was transiently expressed using a temperature sensitive plasmid to excise the antibiotic marker between the mutant *lox* sites. As a demonstration of the method, *ldhA* gene and *oadGAB* operon was sequentially deleted without leaving an antibiotic marker inside the genome of *M. succiniciproducens*.