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Mutant generation in Streptococcus mitis strain B6

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We use this protocol and it's working

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Abstract

This protocol is the methodology that we have successfully employed to generate and confirm insertion deletion mutants in *Streptococcus mitis* strain B6. Attached to the protocol is a file that includes primers for the MonX mutation

Attachments

DOCX

B6 mutagenesis of Mo...

14KB

Materials

Table 1: Primers Used in This Study

	A	B	C	D
	Target	**Name**	**Sequence 5' to 3'**	**Location (accession no.)**
	Spec	S1	CGATTTTCGTTCGTGAATAC	5418–5399 (KM009065)
		S2	TATGCAAGGGTTTATTGTTTTC	4265–4286 (KM009065)
	pJet	P1	CGACTCACTATAGGGAGAGCGGC	310-332 (EF694056.1)
		P2	AAGAACATCGATTTTCCATGGCAG	405-428 (EF694056.1)

Safety warnings

Appropriate biosafety procedures need to be followed

Streptococcus mitis B6 mutagenesis protocol

1 **Creation of a plasmid construct**

1.1 The regions upstream and downstream of the region to be deleted were amplified using primers 1 and 2, and 3 and 4, respectively. These primers were designed to contain appropriate overhangs to allow In-fusion with pJET 1.2/blunt and the antibiotic resistance cassette.

1.2 The spectinomycin resistance cassette (*aad*9) was amplified using primers S1 and S2

1.3 PCR products were then purified with a Qiagen PCR Purification Kit

1.4 The three fragments for each mutant construct were cloned into pJET 1.2/Blunt (Thermo Fisher Scientific) using In-Fusion Snap Assembly (Takara) and transformed into Escherichia coli stellar competent cells.

1.5 Transformants were selected on LB agar plates supplemented with ampicillin (100 µg/ml) and incubated at 37^{o}C overnight

1.6 The resulting colonies were confirmed as ampicillin resistant by streaking on a new LB agar plate supplemented with ampicillin (100 µg/ml).

1.7 Transformants were screened by colony PCR using pJET 1.2/blunt Fwd and Rev primers.

1.8 For transformants giving an appropriate PCR product, a 5 mL LB culture supplemented with ampicillin (100 µg/ml) was grown overnight at 37 °C with shaking at 200 rpm.

1.9 The plasmid was then purified using Qiagen Miniprep Kit and confirmed by sequencing

2 **Transformation of *S. mitis* B6**

2.1 Strains were growth at 37°C in C+Y pH8 [1] – starting at a low inoculum i.e. from a plate or diluting from a culture 1:100 (starting optical density at 600nm [OD_{600}] = 0.03 to 0.05).

2.2 When the culture was close to OD_{600} =0.1, 950 µl of C+Y pH 8.0 medium was added to a 1.5 ml tube with 10 µl of 100 mM CaCl2, 2 µl of competence stimulating peptide (CSP) (EMRKPDGALFNLFRRR - 1 mg/ml), and 150 ng of DNA. A no DNA control tube is included to account for potential contamination. These tubes were prewarmed in a waterbath to 37°C.

2.3 When the culture reached an OD_{600} of 0.10 to 0.12, 50 µl of culture was added to the prewarmed tubes.

2.4 Tubes were incubated in a waterbath at 37 °C for 2 hr.

2.5 Reactions were pelleted by centrifugation and resuspended in approximately 100 µl of media. This was plated on selective Tryptic Soy Agar (TSA) plates spread with 5000 U catalase (Worthington Biochemical Corporation).

2.6 Plates were incubated at 37oC in 5% CO2 overnight and then patched onto selective plates.

3 **Confirmation of putative transformants**

3.1 Putative transformants were grown in tryptic soy broth and DNA prepared as previously described [2].

3.2 The mutations were confirmed by PCR and sequencing (using primers S1 and S2) or genome sequencing.

Protocol references

1. LACKS S, HOTCHKISS RD. A study of the genetic material determining an enzyme in Pneumococcus. Biochim Biophys Acta. 1960;39:508-18. doi: 10.1016/0006-3002(60)90205-5. PubMed PMID: 14413322.

2. Gaytán MO, Singh AK, Woodiga SA, Patel SA, An SS, Vera-Ponce de León A, et al. A novel sialic acid-binding adhesin present in multiple species contributes to the pathogenesis of Infective endocarditis. PLoS Pathog. 2021;17(1):e1009222. Epub 20210119. doi: 10.1371/journal.ppat.1009222. PubMed PMID: 33465168; PubMed Central PMCID: PMCPMC7846122.