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A single-step transconjugation system for gene deletion in *Aggregatibacter actinomycetemcomitans*

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ABSTRACT

Aggregatibacter (*A.*) *actinomycetemcomitans* is a periodontopathogenic bacterium causing aggressive periodontitis. Here we describe a single-step transconjugation system as novel and easily applicable protocol for site-specific genetic manipulation of *A. actinomycetemcomitans*. Deletion of PgaC, which is involved in the synthesis of biofilm matrix, led to a reduced biofilm formation.

Aggregatibacter (*A.*) *actinomycetemcomitans* is a Gram-negative capnophilic bacterium. Its primary ecologic niche is the oral cavity of humans and old-world primates (Lin et al., 2007; Yue et al., 2007). The capability of *A. actinomycetemcomitans* to form tenacious biofilms is an important virulence trait that promotes persistence and progression of infection (Fives-Taylor et al., 1999; Henderson et al., 2010). A major component of the cell-surrounding matrix in these biofilms is polymeric β-1,6-*N*-acetyl-D-glucosamine (PGA), functioning as an intercellular adhesin (Kaplan et al., 2004). In *A. actinomycetemcomitans*, PGA production is mediated by enzymes encoded in the operon *pgaABCD* and the expression of all four genes is required for PGA biosynthesis (Izano et al., 2008).

Elucidation of the actual function of a gene product in a cellular context often requires the genetic modification of the target organism but efficient strategies for gene manipulations in microorganisms that are outside the focus of industrial applications are lacking (Dong and Zhang, 2014). Several protocols for mutagenesis of *A. actinomycetemcomitans* depend on insertional (Kolodrubetz et al., 1995) or transposon-mediated (Thomson et al., 1999) mutagenesis bearing the risk of unwanted downstream effects on gene expression. The generation of markerless gene deletions in *A. actinomycetemcomitans* has been described using the *loxP*/Cre system (Cheng et al., 2014). However, with this system one copy of a *loxP* site remains in the genome. We adapted the single-step transconjugation system described by Oswald et al. (Oswald et al., 1999) for genomic deletions in *A. actinomycetemcomitans*. This system is well established and frequently applied in the related swine pathogen *Actinobacillus* (*A.*) *pleuropneumoniae* (Oswald et al., 1999; Baltes et al., 2003; Buettner et al., 2008; Buettner et al., 2009) and enables the generation of unmarked and non-polar isogenic deletions. Importantly, such mutants are not rated as genetically modified organisms, a fact of utmost importance concerning laboratory safety requirements and applicability as attenuated live vaccines. A similar approach using a suicide vector for the generation of deletion mutants was recently described for *A. actinomycetemcomitans* (Juarez-Rodriguez et al., 2013). In contrast to the latter system which relies on transformation, our system depends on transconjugation of the vector.

As a model gene for testing the applicability of the transconjugation system for *A. actinomycetemcomitans* we chose *pgaC*, encoding an *N*-glycosyltransferase involved in biofilm formation (Izano et al., 2008).

Genomic fragments of 976 bps and 915 bps located largely upstream of the *pgaC* (Acc.-No. ACX81587.1 in *A. actinomycetemcomitans* strain D11S-1) start codon and largely downstream of the *pgaC* stop codon, respectively, were PCR amplified from *A. actinomycetemcomitans* DSM 11123 using the primer sets oPgaC_1/oPgaC_2 and oPgaC_3/oPgaC_4 and cloned into pUC18. The upstream and downstream fragments were then cloned adjacently and the resulting product contained a truncated version of *pgaC* lacking 1140 bp between position 59 and 1199 of the *pgaC* open reading frame. The obtained fragment containing Δ*pgaC* was subsequently cloned into the transconjugation plasmid pEMOC2 (Baltes et al., 2003) (Fig. 1, Table 1) harboring a broad host mobilization system *mob*RP4 (Simon et al., 1983) for conjugal mobility. Furthermore pEMOC2 contains the *A. pleuropneumoniae* specific *omlA* promoter to drive the expression of the chloramphenicol resistance gene and of the suicide gene *sacB* of *Bacillus subtilis*, encoding the levansucrase.

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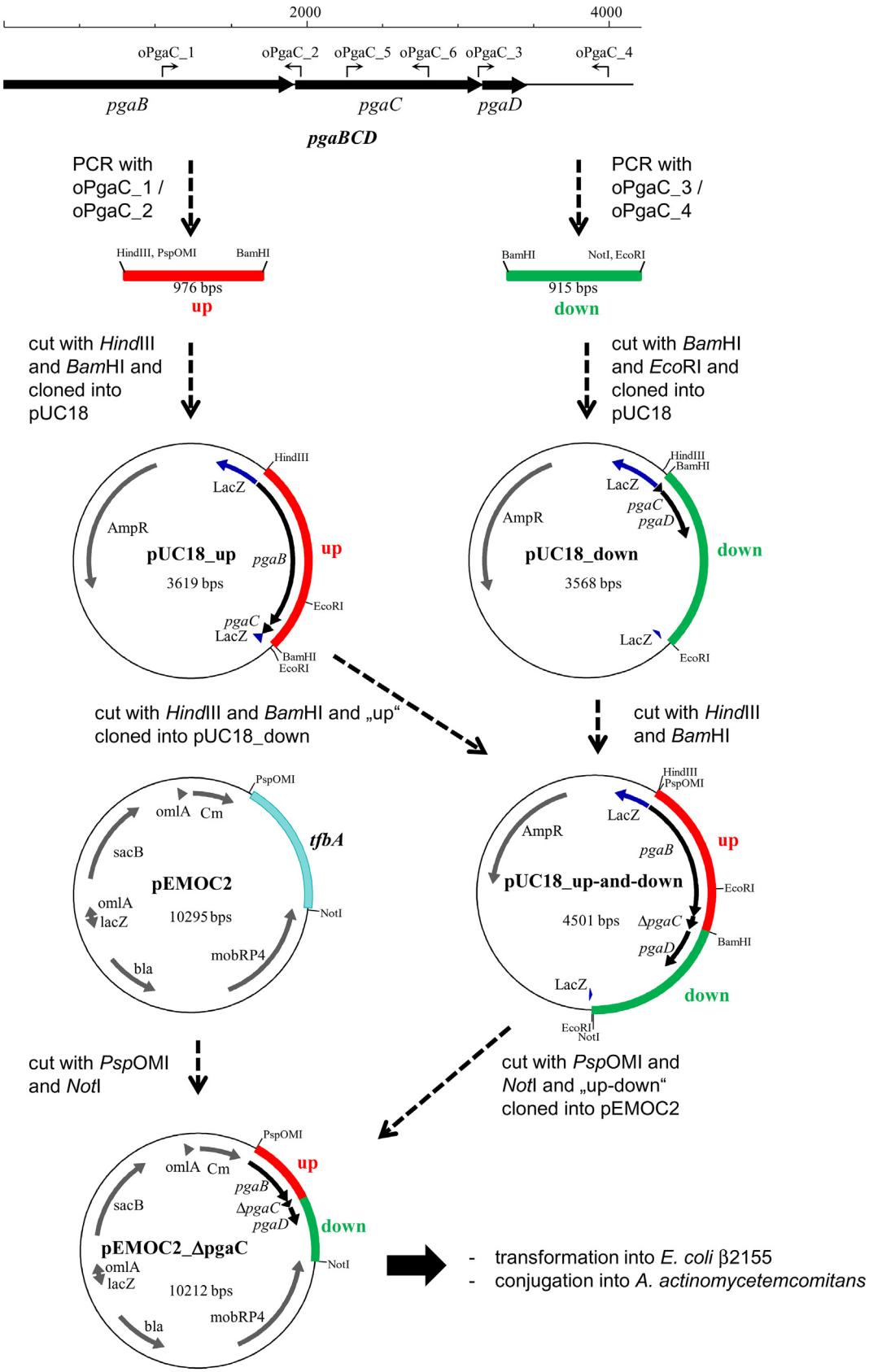

Fig. 1. Scheme showing the cloning of a truncated *pgaC* gene into the pEMOC2 vector. Upstream and downstream regions flanking the *pgaC* gene were fused. These regions facilitate homologous recombination in *A. actinomycetemcomitans* leading to cointegration of pEMOC2 vector sequence into the genomic DNA. Cointegrates can be selected by cultivation on chloramphenicol-containing agar. A second homologous recombination can lead to removal of the intact *pgaC* gene and clears the vector sequence from the genome. Selection of mutants is achieved by cultivation in the presence of sucrose.