

Table 3. Frequency of transfer of pMTL9301 and pMTL9301ΔoriT to *C. difficile* (± SD)

Donor or free DNA used	Recipient	Frequency of transfer/per donor		Frequency of transfer/ per recipient	
		No DNase	+DNase (50 µg/ml)	No DNase	+DNase (50 µg/ml)
CA434 containing pMTL9301	CD37	$3.26 \pm 1.24 \times 10^{-5}$	$3.67 \pm 1.41 \times 10^{-10A}$	$3.32 \pm 4.71 \times 10^{-6}$	$7.53 \pm 4.71 \times 10^{-10A}$
CA434 containing pMTL9301ΔoriT	CD37	$4.68 \pm 1.24 \times 10^{-10B}$	ND	$6.65 \pm 3.48 \times 10^{-10B}$	ND
CA434 containing pMTL9301	630Δerm	$3 \pm 0.47 \times 10^{-5}$	$4.5 \pm 0.47 \times 10^{-5}$	$1.62 \pm 0.04 \times 10^{-5}$	$1.8 \pm 0.04 \times 10^{-5}$
CA434 containing pMTL9301ΔoriT	630Δerm	$1.98 \pm 1.13 \times 10^{-10C}$	$1.67 \pm 1.13 \times 10^{-10C}$	$4.6 \pm 1.5 \times 10^{-9C}$	$6 \pm 1.5 \times 10^{-9C}$
pMTL9301 (4 µg /ml)	CD37	ND	ND	ND	ND
CA434 transformed with pMTL9301 heat killed before mixing with recipient	CD37	ND	ND	ND	ND
HB101 containing pMTL9301	CD37	$4 \pm 2 \times 10^{-10D}$	ND	$5.67 \pm 2.85 \times 10^{-9D}$	ND
HB101	CD37	ND	ND	ND	ND

containing pMTL9301 <i>ΔoriT</i>					
HB101 containing pMTL9301	630 Δ <i>erm</i>	$4.67 \pm 1.1 \times 10^{-10E}$	ND	$4.09 \pm 1.01 \times 10^{-9E}$	ND
HB101 containing pMTL9301 Δ <i>oriT</i>	630 Δ <i>erm</i>	$2.08 \pm (0) \times 10^{-10F}$	ND	$2.32 \pm (0) \times 10^{-9F}$	ND

SD, data were expressed as standard deviation (SD) based on at least three independent experiments.

ND, no transconjugant detected after mating, i.e., a conjugation frequency below the detection limit, ($< 10^{-10}$ transconjugants per donor or recipient)

Note:

^A Transconjugants/ transformants that arose at a frequency of 10^{-10} had 9 to 16 colonies per mating and this was repeated at least 3 times on separate occasions. That the colonies were pMTL9301 containing *C. difficile* strain CD37 was proven as shown in the methods and results sections.

^B Transconjugants / transformants that arose at a frequency of 10^{-10} had 1 to 3 colonies per mating and this was repeated at least 3 times on separate occasions. That the colonies were pMTL9301 Δ *oriT* containing *C. difficile* strain CD37 was proven as shown in the methods and results sections.

^C Transconjugants / transformants that arose at a frequency of 10^{-10} to 10^{-9} had 3 to 12 colonies per mating and this was repeated at least 3 times on separate occasions. That the colonies were pMTL9301 Δ *oriT* containing *C. difficile* strain 630 Δ *erm* was proven as shown in the methods and results sections.

^D Transconjugants / transformants that arose at a frequency of 10^{-10} to 10^{-9} had 1-3 colonies per mating and this was repeated at least 3 times on separate occasions. That the colonies were pMTL9301 Δ *oriT* containing *C. difficile* strain CD37 was proven as shown in the methods and results sections.

^E Transconjugants / transformants that arose at a frequency of 10^{-10} to 10^{-9} had 1-3 colonies per mating and this was repeated at least 3 times on separate occasions. That the colonies were pMTL9301 containing *C. difficile* strain 630 Δ *erm* was proven as shown in the methods and results sections.

^F Transconjugants / transformants that arose at a frequency of 10^{-10} to 10^{-9} had 1-3 colonies per mating and this was repeated at least 3 times on separate occasions. That the colonies were pMTL9301 Δ *oriT* containing *C. difficile* strain 630 Δ *erm* was proven as shown in the methods and results sections.