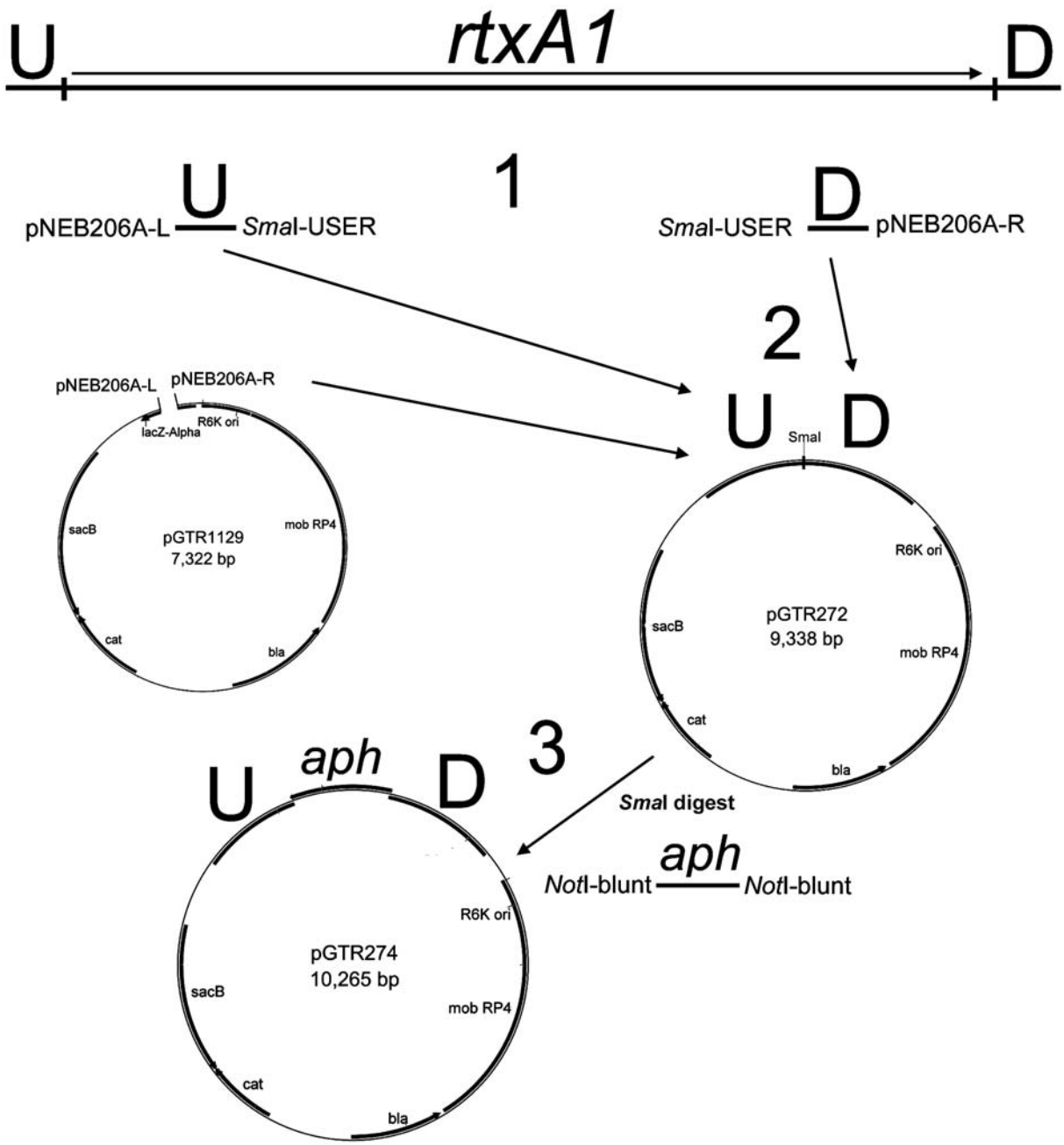
**Supplemental Figure 3. Construction of allelic exchange plasmid for deletion of *rtxA1* by three part USER friendly cloning.** The 1.0-kb upstream (U) and downstream (D) sequences flanking the *rtxA1* gene were PCR amplified (Step 1). The outside ends of the fragments were amplified with oligonucleotides that contained DNA sequences that were complementary to the left and right USER friendly sequences of pNEN206A, respectively. The inside ends of the fragments were amplified with oligonucleotides that contained a *Sma*I-USER sequence. The USER friendly allelic exchange cloning vector pGTR1129 was treated with *Xba*I and Nt.BbvCI to create the 8-bp pNEB206A-left and pNEB206A-right USER friendly cloning sites, and the upstream and downstream fragments were treated with the USER enzyme mix to create the 8-bp overhangs for USER friendly cloning. The upstream fragment, downstream fragment, and pGTR1129 were ligated in a three part ligation (Step 2), yielding pGTR272 encoding Δ*rtxA1*. pGTR272 was linearized between the *rtxA1* upstream and downstream sequences by *Sma*I digestion, and an *aph* kanamycin resistance gene cassette on a *Not*I fragment made blunt-ended with Klenow was ligated into the *Sma*I site, yielding pGTR274 encoding Δ*rtxA1*::*aph*. pGTR274 was subsequently used to recombine the Δ*rtxA1*::aph mutation into the *V. vulnificus* CMCP6 genome by the two-step *sacB*-assisted allelic exchange method.


U
rtxA1
D
1
U
pNEB206A-L
SmaI-USER
SmaI-USER
D
pNEB206A-R
2
pNEB206A-L
pNEB206A-R
lacZ-Alpha
R6K ori
sacB
pGTR1129
7,322 bp
mob RP4
cat
bla
U
SmaI
D
R6K ori
sacB
pGTR272
9,338 bp
mob RP4
cat
bla
3
U
aph
D
SmaI digest
aph
NotI-blunt
NotI-blunt
R6K ori
pGTR274
10,265 bp
sacB
mob RP4
cat
bla