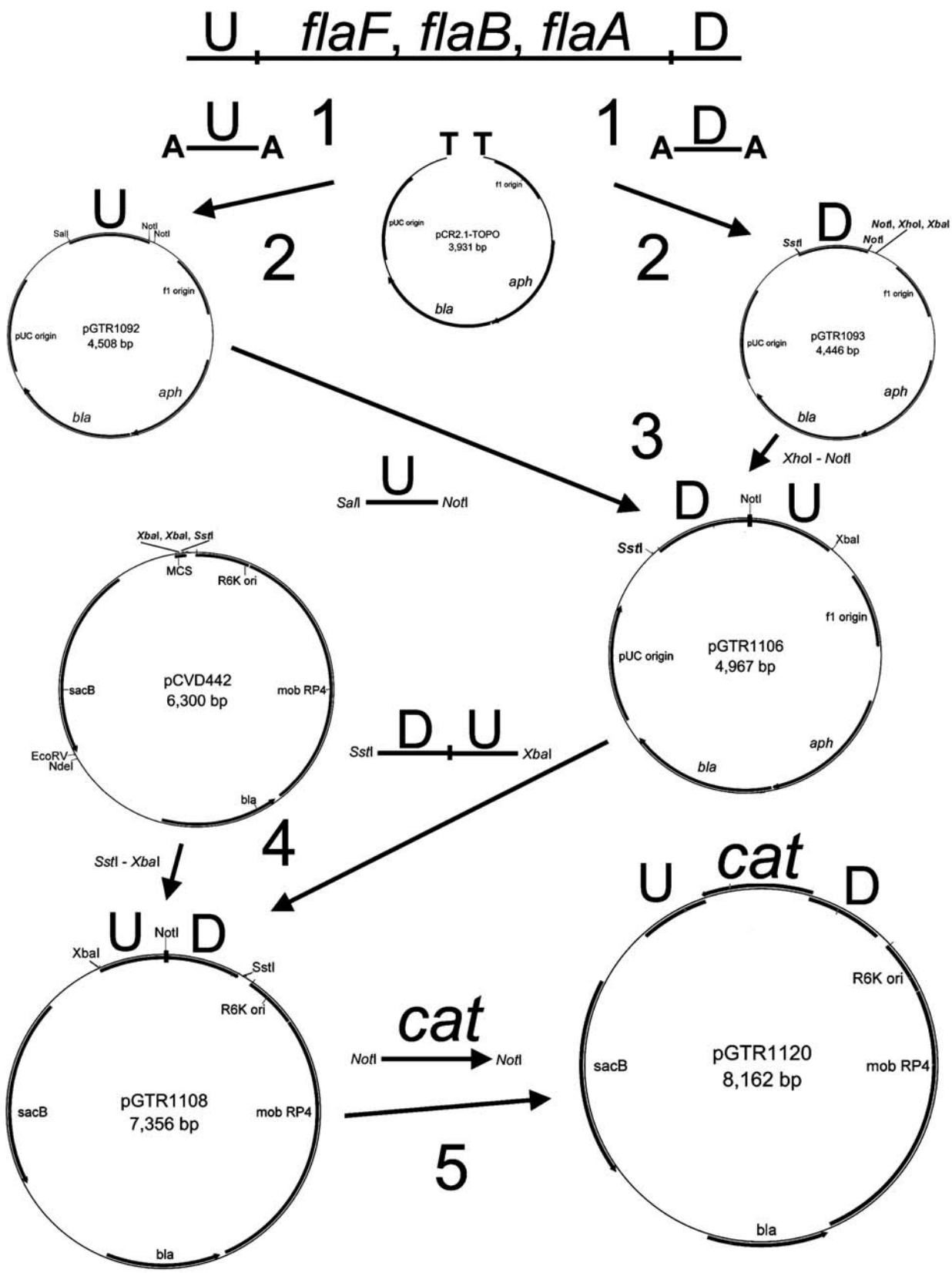
Supplemental Figure 2. Construction of allelic exchange plasmid for deletion of *flaFBA* by TOPO TA cloning and subcloning. The 0.5-kb upstream (U) and downstream (D) sequences flanking the *flaF*, *flaB*, and *flaA* genes were PCR amplified (Step 1) and cloned into pCR2.1-TOPO by TOPO TA cloning (Step 2) yielding plasmids pGTR1092 and pGTR1093, respectively. The upstream flanking sequence from pGTR1092 was excised using *Sal*I and *Not*I and was cloned into pGTR1093 that had been digested with *Xho*I and *Not*I, yielding pGTR1106 (Step 3). The joined upstream and downstream sequences (Δ*flaFBA*) were excised from pGTR1106 on an *Sst*I-*Xba*I fragment and were cloned into pCVD442 that had been digested with *Sst*I and *Xba*I, yielding pGTR1108 (Step 4). A chloramphenicol resistance gene (*cat*) on a *Not*I fragment was inserted into the *Not*I site between the upstream and downstream sequences of pGTR1108, yielding pGTR1120 encoding Δ*flaFBA*::*cat* (Step 5). pGTR1120 was subsequently used to recombine the Δ*flaFBA*::*cat* mutation into the *V. vulnificus* CMCP6 genome by the two-step *sacB*-assisted allelic exchange method.


U flaF, flaB, flaA D
A U A 1
1 A D A
T T
f1 origin
pUC origin
pCR2.1-TOPO
3,931 bp
aph
bla
2
2
U
SalI
NotI
NotI
f1 origin
pUC origin
pGTR1092
4,508 bp
aph
bla
D
NotI, XhoI, XbaI
SstI
NotI
f1 origin
pUC origin
pGTR1093
4,446 bp
aph
bla
3
XhoI - NotI
SalI U NotI
D NotI U
XbaI, XbaI, SstI
MCS
R6K ori
sacB
pCVD442
6,300 bp
mob RP4
EcoRV
NdeI
bla
SstI
XbaI
f1 origin
pUC origin
pGTR1106
4,967 bp
aph
bla
SstI D U XbaI
4
SstI - XbaI
U NotI D
XbaI
SstI
R6K ori
sacB
pGTR1108
7,356 bp
mob RP4
bla
cat
NotI NotI
5
U cat D
R6K ori
sacB
pGTR1120
8,162 bp
mob RP4
bla