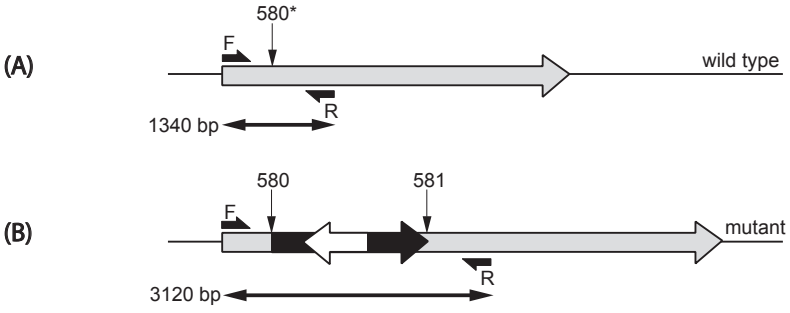
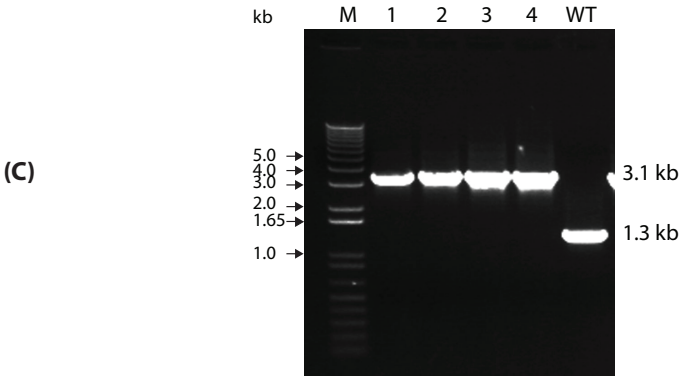

(A)
580*
F
wild type
R
1340 bp
(B)
580
581
F
mutant
R
3120 bp


(C)
kb
M
1
2
3
4
WT
5.0
4.0
3.0
2.0
1.65
1.0
3.1 kb
1.3 kb

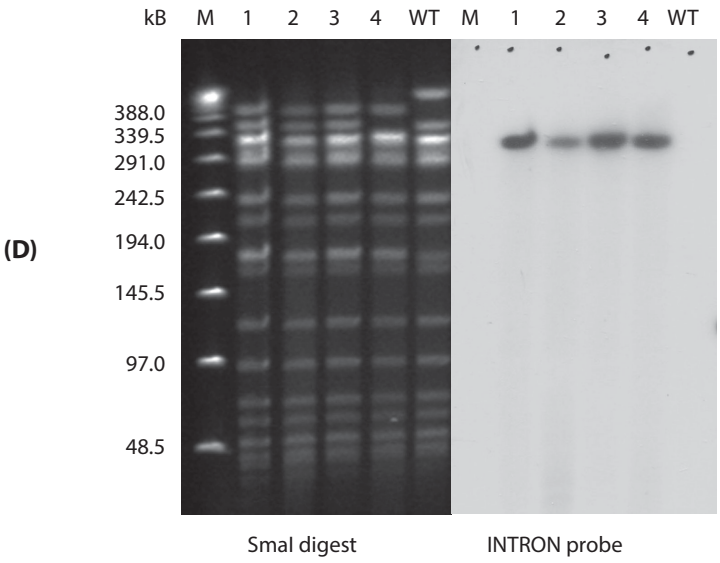

(D)
kB
M
1
2
3
4
WT
M
1
2
3
4
WT
388.0
339.5
291.0
242.5
194.0
145.5
97.0
48.5
Smal digest
INTRON probe

Figure S1. Analysis of the botulinum toxin mutant clones and the wild type *C. botulinum* strain Hall A-*hyper*.

(**A** and **B**) Schematic presentation of the wild type (A) and mutated (B) botulinum neurotoxin genes. The ClosTron insertion site is shown with a vertical arrow an asterisk. The locations of PCR primers F (LC/F) and R (LC/R) are shown with horizontal arrows on either side of the intron insertion site, and sizes of the expected size PCR products are indicated.

(**C**) PCR products of four randomly selected putative mutant clones (lanes 1 - 4) and wild-type strain Hall A-*hyper* (lane 5, wt). M, 1 kb Plus DNA ladder (Life Technologies); wt, wild type.

(**D**) Pulsed-field gel electrophoresis (PFGE) and Southern hybridization analysis of the toxin mutant clones and the wild type *C. botulinum* strain Hall A-*hyper*. Left panel, PFGE of *Sma*I digested chromosomal DNA; Right panel, Southern hybridization with the group II intron (ClosTron) probe specific to the erythromycin gene. Lanes 1 – 4, randomly selected putative mutant clones, lane 5, wild type strain Hall A-*hyper* (wt). M, lambda ladder, PFGE marker (New England Biolabs).