

Supplementary Information

Table S1. Oligos used in domain deletion mutagenesis of Foxp3

Common Primers*

RV5 tcaagccctttgtacaccctaag 1545B ataagaatgcgccgccagggcagggttgagcactt

Domain Specific Primers in 1st PCR

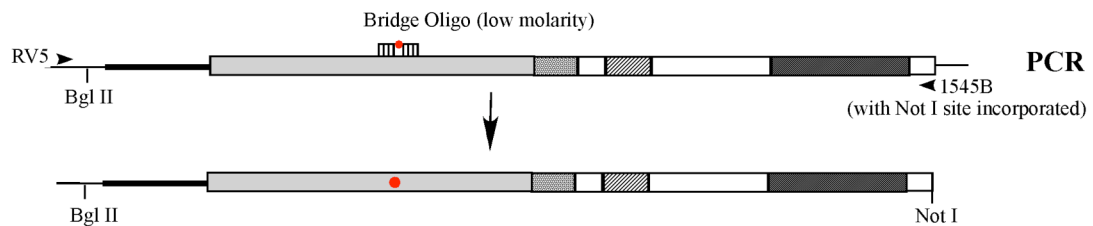
Domain	5' Fragment Backward Primer	3' Fragment Forward Primer
N-terminal	NTB cattgggttcttgcagaggcaggct	NTF ggagtctgcaagtggcctggttg
Zinc Finger	ZincB attgccagcagtggttagga	ZincF ctctggatgagaaggcaag
Leucine Zip	ZipB cacttctcttgaggagca	ZipF ggccaaggctccatctgtggcctcaatg
Forkhead	FkhB gtggtacttgaagttagtccatg	FkhF cccaacaagtgtccaatccctg

Bridge Oligos in 2nd PCR

Domain	Bridge Oligo
N-terminal	NtDel tgctctgacaagaaccaatgggagtctgcaagtggctggt
Zinc Finger	ZincDel tcctaccactgctggcaaatctctggatgagaaaggcaag
Leucine Zip	ZipDel tgctcctccagagagaagtggccaaggctccatctgtggcct
Forkhead	FkhDel catggactacttcaagtaccaccaacaagtgtccaatccct

* Primer RV5 was used as the forward primer for all 5' fragments in 1st PCR, and forward amplification primer in the 2nd PCR. Primer 1545B was used as the backward primer for all 3' fragments in the 1st PCR, and the backward amplification primer in the 2nd PCR.

A.



B.

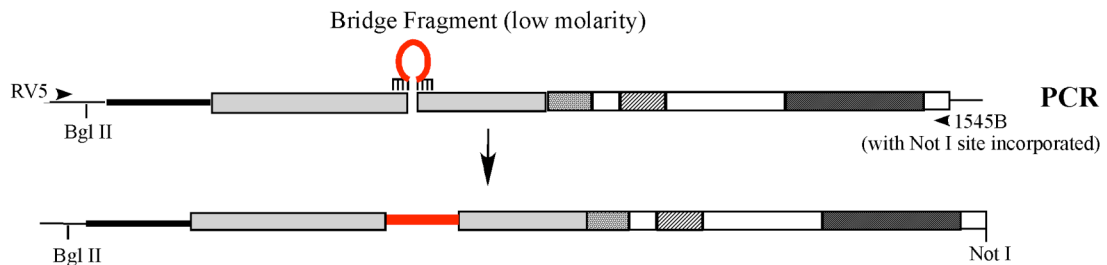


Figure S1. Bridge-mediated mutagenesis can be used for point mutation (A) or insertion mutation (B). For point mutation, the bridge oligo contains the replacement nucleotide(s) (represented by the red dot) surrounded by DNA sequences 5' and 3' to the nucleotide(s) to be replaced. For insertion mutagenesis, the bridge fragment contains the insertion DNA fragment with sequences attached to its ends that anneal to the 5' and 3' fragments.

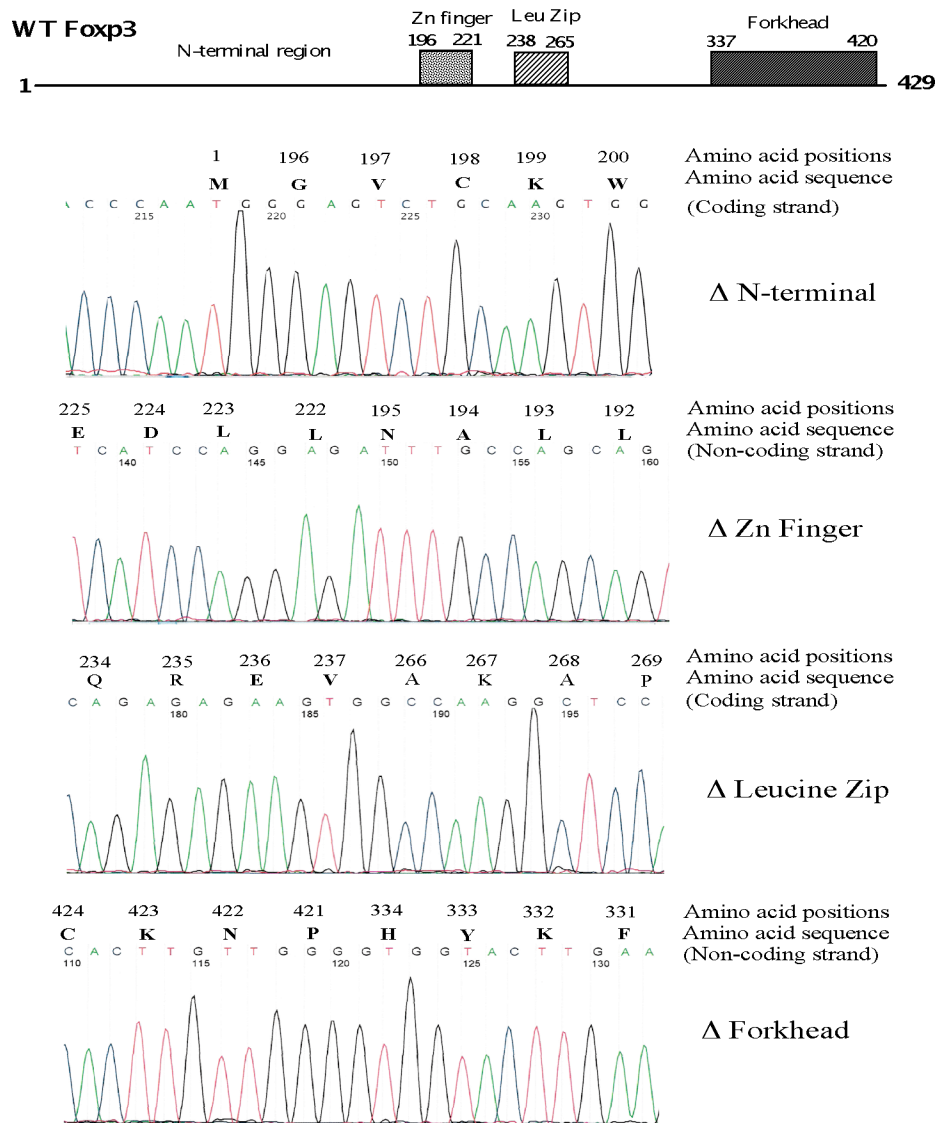


Figure S2. Junction sequences of Foxp3 deletion mutants. A schematic diagram of the structure of murine Foxp3 protein is shown at the top. The amino acid positions of boundaries of each domain are indicated. Results of DNA sequences at the deletion sites of the domain deletion mutants are shown. Based on the positions of the sequencing primers, either the coding or non-coding strands of the mutants are sequenced. The numbers of the amino acid positions correspond to those in the wild type Foxp3 protein.