Nonsense-mediated mRNA decay in the *ADAMTS13* gene caused by a 29-nucleotide deletion

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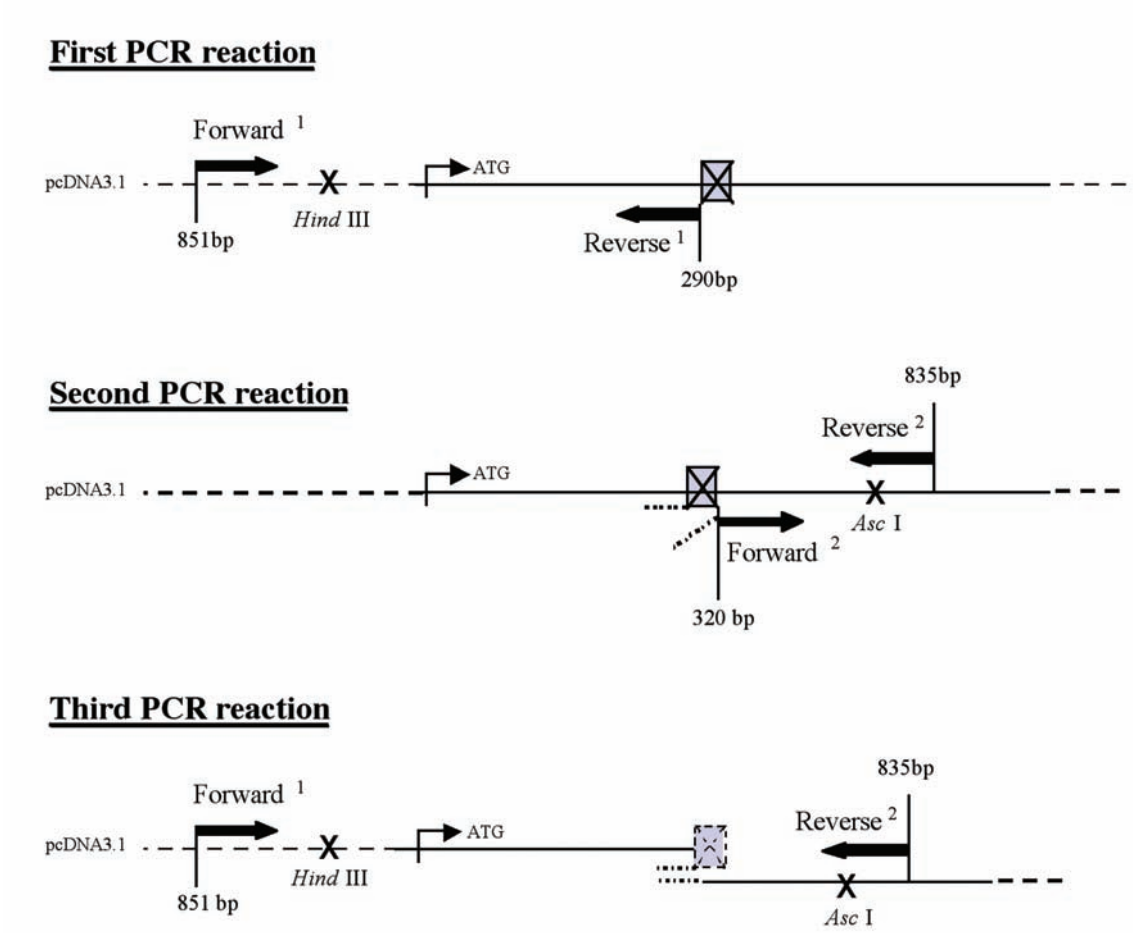
Construction and cloning of the ADAMTS13-29del expression vector

The deletion of 29 nucleotides identified in exon 3 was inserted into the *ADAMTS13* cDNA by overlapping PCR. For the first and second PCR reactions, the ADAMTS13-WT expression vector was used as a template. The upstream primer extended from position 851 to 873 in the T7 promoter/priming site of the pcDNA™3.1/V5-His expression vector (5'GCTTATCGAAATTAATACGACTC3'). The downstream primer began at position 290 located behind the 29 bp deletion in exon 3 of ADAMTS13-WT cDNA (5'TGGTGAGCCTGGAAGACATCGG3'). This PCR fragment contains an endogenous *Hind*III restriction site, which was used for ligation into the ADAMTS-WT expression vector (*Supplementary Figure S1*). For the second PCR reaction, the upstream primer extended from position 320 to 341 within exon 3. In addition, an extension of 20 bases was included at 5' of this primer (position 290 to 271) which overlaps with the 20 nucleotides located at 5' of the 29 bp deletion mutation in exon 3 of *ADAMTS13* cDNA (5'gatgtcttccaggctcaccaACCTCAACATCGGGGCAGAAC3'). The downstream primer extended from position 834 to 808 in exon 7 (5'CCGTCCTGCGCTGAGCAGGCTCAGCAG3'). This PCR product contains an endogenous *Asc*I restriction site for ligation into the ADAMTS13-WT expression vector (Figure 1). For the third PCR reaction, the products obtained from the first and second reactions were used as templates, and the upstream primer from reaction 1 and downstream primer from reaction 2 were used to generate the overlapping PCR fragment containing the 29 bp deletion (*Supplementary Figure S1*). The presence of the 29 bp deletion was confirmed by sequence analysis. The product of the overlapping PCR was purified, digested with *Hind*III and *Asc*I, and ligated into the ADAMTS13-WT expression vector which had been previously digested with the same enzymes. The result of the cloning reaction was subsequently sequenced, confirming the presence of the 29 bp deletion.



Supplementary Figure S1. Schematic representation of the introduction of the 29 bp deletion mutation into pcDNA™3.1ADAMTS13-WT by overlapping PCR reaction. The arrows (⟶) show the direction of the primers and the box (☒) represents the 29 bp deletion mutation.