Supplemental Data

HIGHLY EFFICIENT AND MORE GENERAL *CIS*- AND *TRANS*-SPLICING INTEINS THROUGH SEQUENTIAL DIRECTED EVOLUTION

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Table of contents

Figure S1

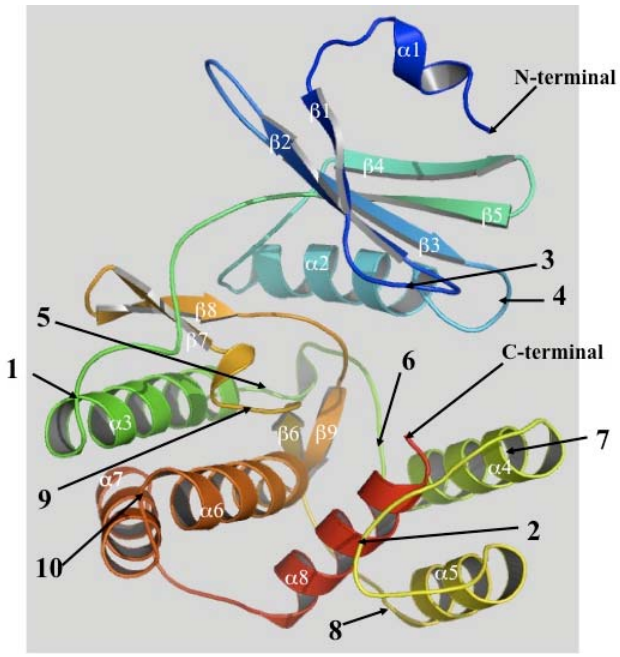

FIGURE S1: Structural presentation of selected insertion sites in the KanR protein. The ribbon structure is adapted from the crystal structure (32) of APH(3')-IIa, which is homologous to the APH(3')-I KanR protein used in this study. The selected insertion sites are numbered 1 through 10 and marked with arrows.

Figure S2

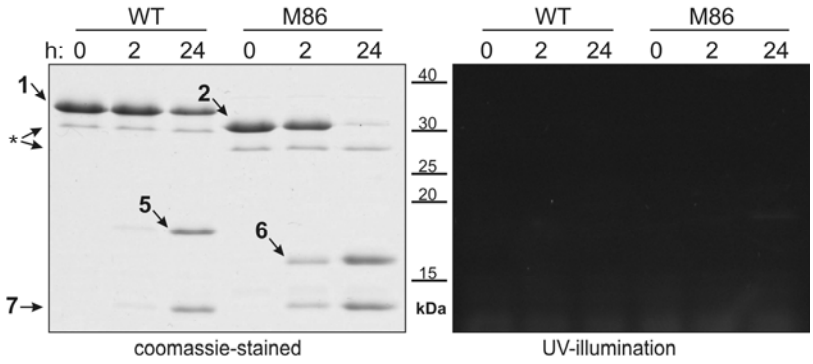

FIGURE S2: C-terminal cleavage activity of inteins with mutated N-terminal splice junction. The peptide containing an alanine at the position of the catalytic Cys1 (pep**3**, 75 µM) was incubated with constructs **1** (wild-type, 15 µM) and **2** (M86 mutant, 15 µM) for 24 h at 25°C. Aliquots were withdrawn at the indicated time points and the reaction mixtures were separated by SDS-PAGE and analyzed using Coomassie Brilliant Blue staining (left) and UV-illumination (right). See Fig. 3 for the numbering of the proteins and for the calculated molecular weights. The asterisk denotes contaminant impurity protein bands.

Table S1: Contributions of individual mutations to the splicing efficiency at the insertion site 1 in the KanR protein.

Mutant Inteins	Mutations[a]			Splicing Efficiency (%) At Insertion Site 1
WT				<5
M1	K20R	D24G	I58T	73
M1-1	K20R			8
M3-1		D24G		11
M3-2			I58T	30
M1-2	K20R	D24G		24
M3		D24G	I58T	36

[a] All mutations are relative to the wild-type sequence of the *Ssp* DnaB mini-intein.