Supplementary Material

Table S1:

KCl				$(NH_4)_2SO_4$			
pH	k_{rev} (min^{-1})	k_{for} (min^{-1})	K_{SER} (min^{-1})	**pH**	k_{rev} (min^{-1})	k_{for} (min^{-1})	K_{SER} (min^{-1})
4.29	-	-	-	**4.87**	-	-	-
4.81	0.0045(1)	0.0016(1)	0.0037(1)	**5.37**	0.019(1)	0.0087(4)	0.0051(1)
5.36	0.017(1)	0.0043(1)	0.015(1)	**5.88**	0.043(1)	0.042(2)	0.0125(2)
5.90	0.051(1)	0.0127(6)	0.046(1)	**5.88**	0.040(1)	0.024(1)	0.0133(2)
5.90	0.056(1)	0.0117(5)	0.047(1)	**5.88**	0.048(1)	0.035(1)	0.0142(2)
5.90	0.050(1)	0.013(1)	0.044(1)	**6.10**	0.057(2)	0.054(2)	0.0201(2)
5.90[1]	0.057(2)	0.013(1)	0.050(1)	**6.36**	0.086(3)	0.064(4)	0.0391(7)
5.90[2]	0.055(6)	0.010(2)	0.051(1)	**6.23**	0.075(2)	0.065(3)	0.0289(4)
6.14	0.079(4)	0.020(2)	0.066(2)	**6.50**	0.101(4)	0.085(6)	0.045(1)
6.31	0.112(2)	0.024(1)	0.096(1)	**6.69**	0.112(5)	0.100(7)	0.067(1)
6.40	0.130(3)	0.031(2)	0.115(2)	**7.08**	0.151(4)	0.145(7)	0.089(1)
6.66	0.153(5)	0.042(3)	0.137(3)	**7.51**	0.169(5)	0.207(10)	0.120(1)
6.88	0.204(7)	0.049(3)	0.196(5)				
7.33	0.326(10)	0.096(6)	0.373(7)				
7.75	0.383(18)	0.123(11)	0.474(13)				

[1] sample script for this measurement can be found in the supplementary material

[2] heterogeneous intron preparation with 18 % correctly terminated introns

Table S1: Kinetic rates determined at varying pH values. The values were calculated with Dynafit 3 according to the model proposed in Fig. 4A. Numbers in brackets represent standard 1σ errors of the last shown digit.

Figure S1:

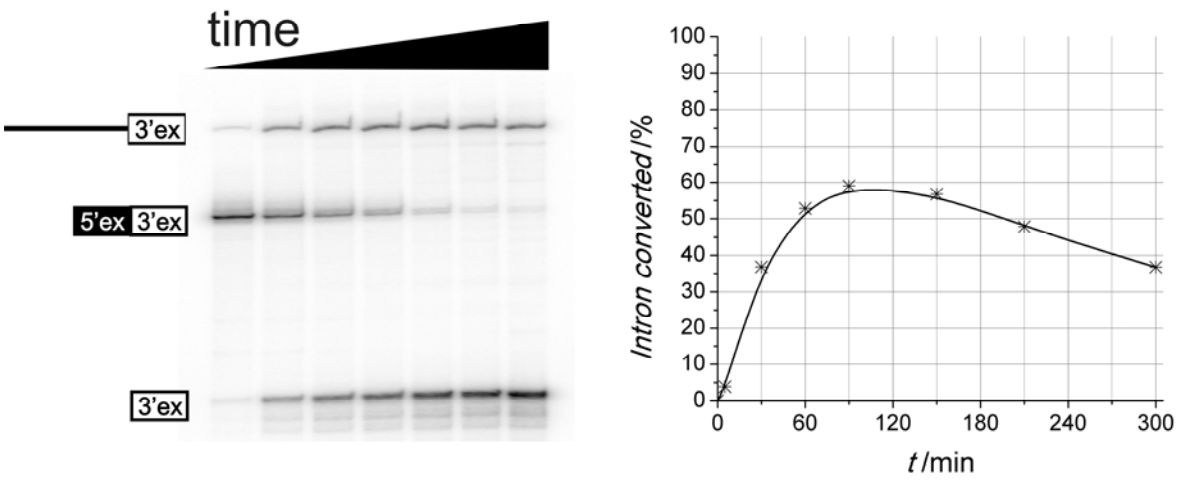

Figure S1: 200 nM 3'-end labeled RNA substrate RS2 was reacted with 100 nM linear ai5γ intron. Reaction products were separated by denaturing PAGE (left). The amount of converted intron (right) was calculated from the product fractions, which were quantified with Imagequant.

Figure S2:

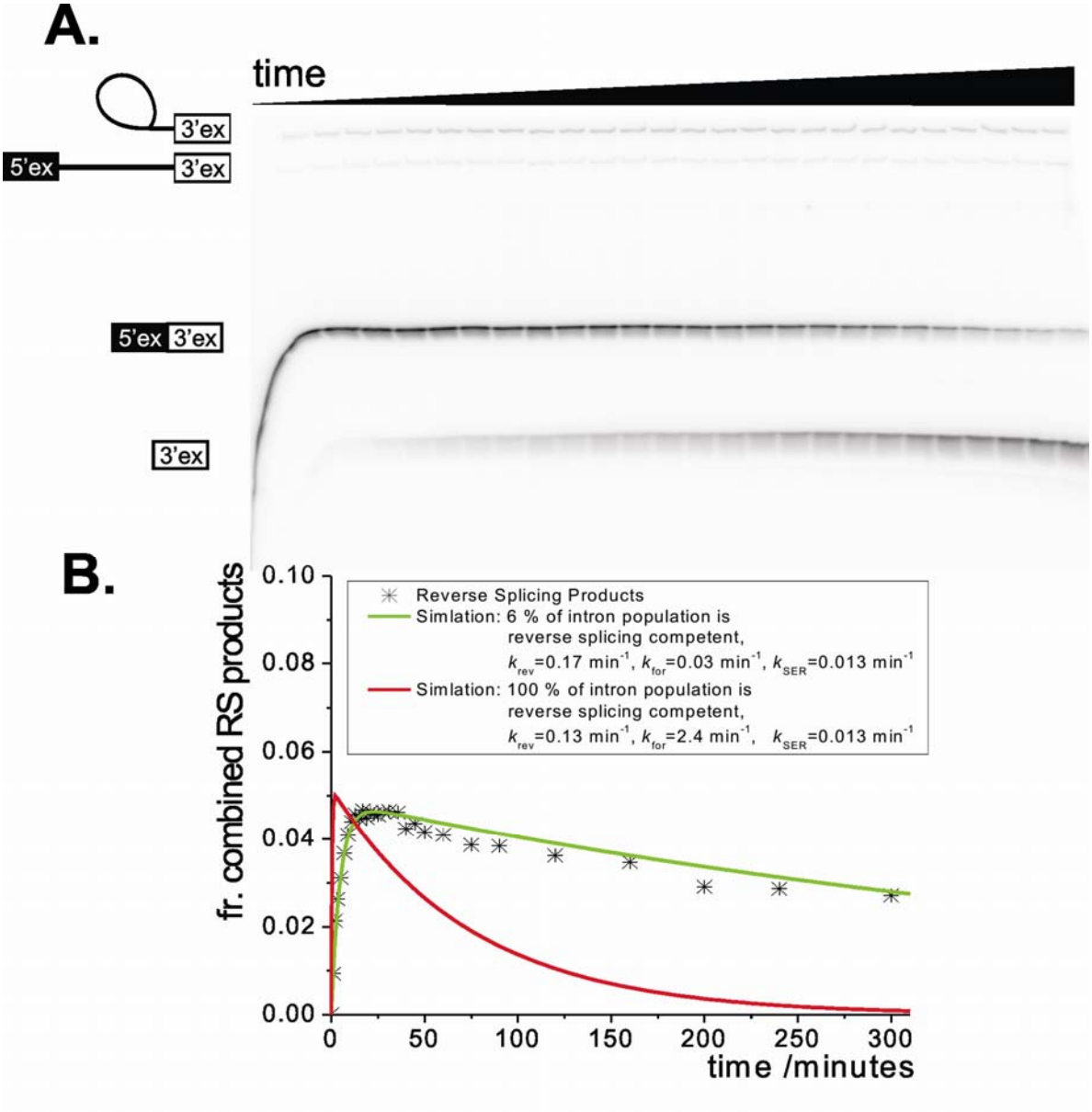

Figure S2: (**A**) Gel showing reverse splicing by lariat intron. Timepoints were taken between 0 and 300 min. (**B**) Experimental data from the gel above and simulation of two different models. The green line shows the simulation of a model in which the transesterification rate constants are in the same order of magnitude as those determined for the linear intron, 6 % of the lariat intron molecules are capable of performing reverse splicing and all molecules can catalyze SER. The SER rate constant used for both models was experimentally determined. The red line simulates a model in which all intron molecules can perform reverse splicing and SER. The forward splicing rate constant was taken from an earlier work (Dème E, Nolte A, Jacquier A. *Biochemistry* **1999**, *38*, 3157-3167) and the reverse splicing rate was calculated such that the observed maximum of 5 % combined reverse splicing products are formed.

Figure S3:

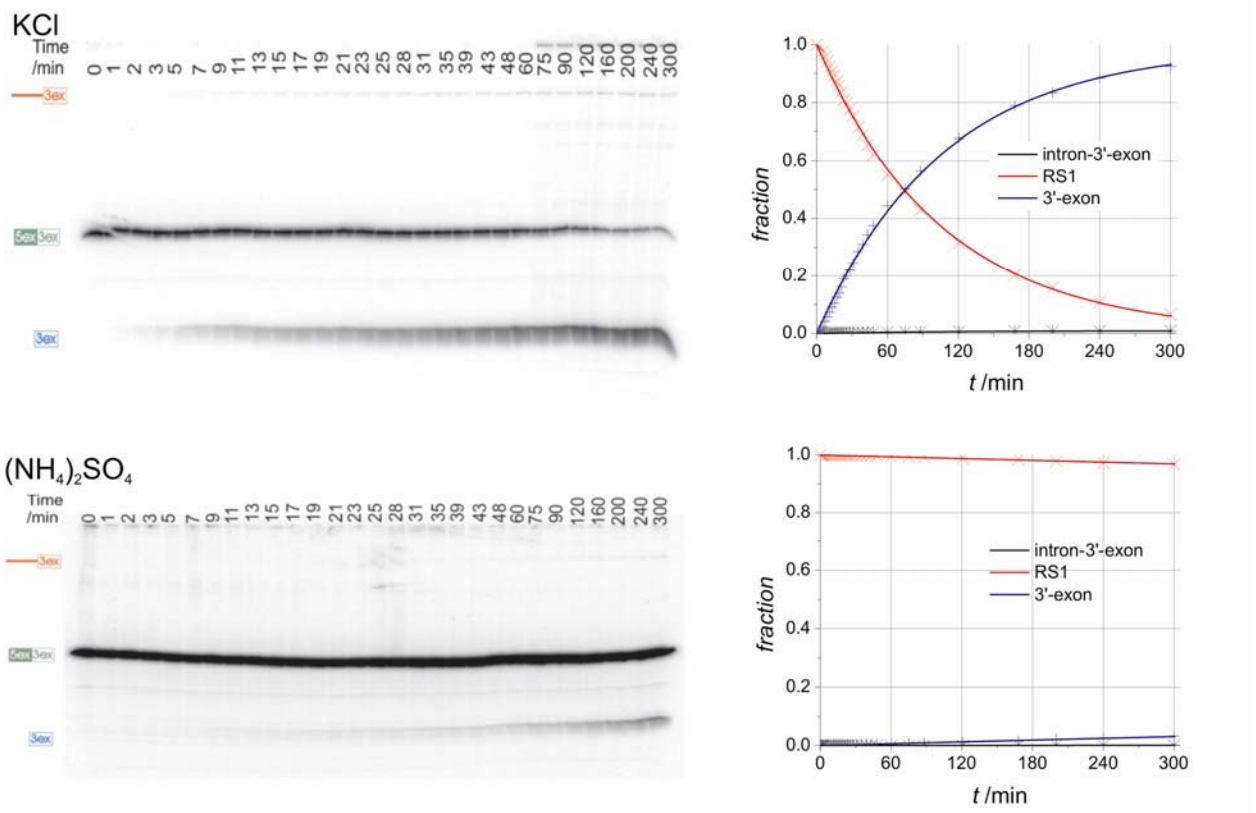

Figure S3: The ai5γ-ΔG588,ΔA589 mutant shows drastically decreased reactivity compared to the wild type intron. The effect is more severe in the $(NH_4)_2SO_4$ (bottom) than in the KCl (top) buffer system.

Figure S4:

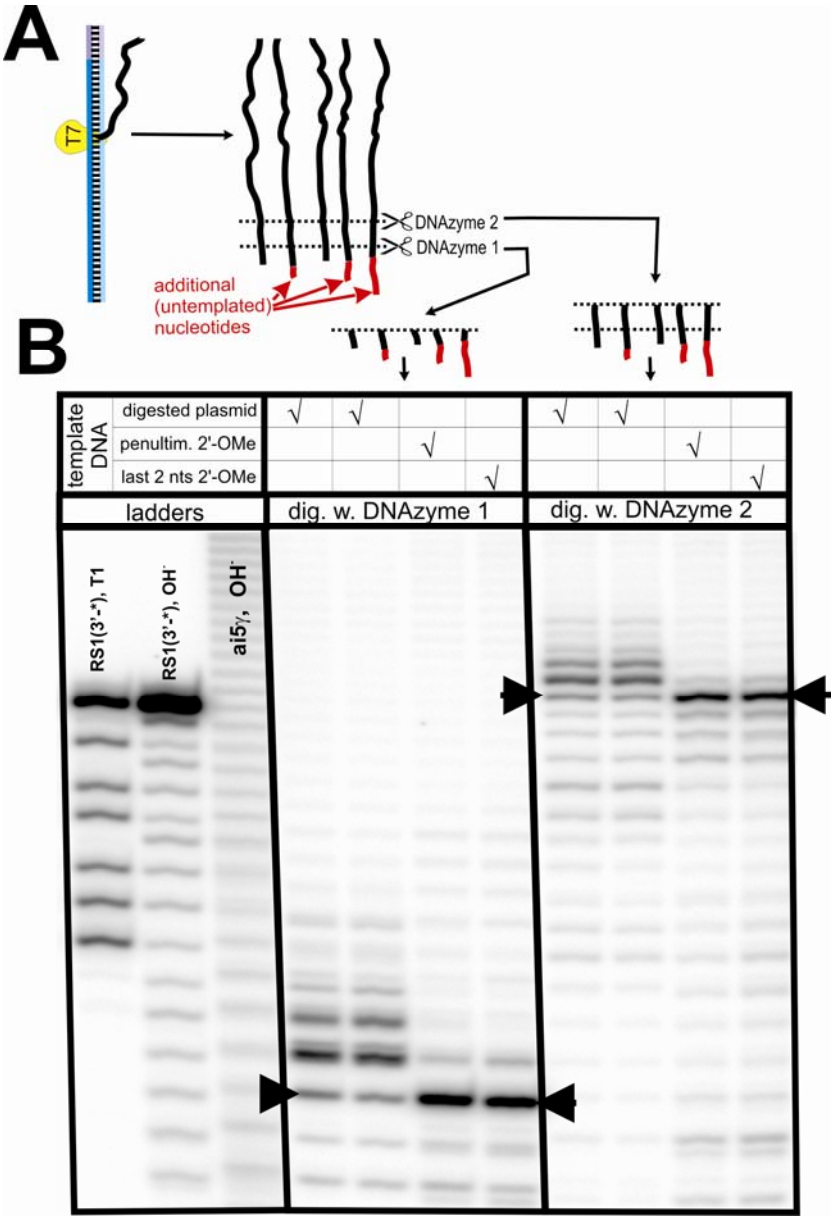

Figure S4: Determination of the 3'-end heterogeneity of the transcribed intron. (**A**) Template DNA is depicted by blue lines, T7 RNA polymerase (yellow sphere) and transcribedRNA (solid black line) are indicated. Additional nucleotides at the 3'-end of the transcripts are shown as short red lines. Cleavage sites of the two DNAymes (scissors) are indicated by dottetd lines. (**B**) Different batches of ai5γ that have been transcribed from digested plasmid or from 2'-OMe modified DNA templates were 3'-end labeled and cleaved close to the 3'-end with two different "10-23" family DNAzymes. Fractions belonging to correctly sized transcripts are marked by arrows. Ladders (left) were obtained by RNase T1 and OH^- digestion of 3'-end labeled substrate RS1 and linear ai5γ intron, respectively.

Example script for Dynafit 3

Script_file_MR99.txt

```
[task]
 data = progress
 task = fit

[mechanism]
 I_E1E2 <==> IE2_E1 : ki kl
 I_E1E2 ---> I_E1 + E2 : kh
 Iaa_E1E2 ---> Iaa_E1 + E2 : kh

[constants]
 ki = 0.057 ?
 kl = 0.0126 ?
 kh = 0.050 ?

[concentrations]
I_E1E2=0.96
Iaa_E1E2=0.04
IE2_E1=0
I_E1=0
E2=0

;[responses] ; needed for
DynaFit 4
[progress]
;[data] ; needed for DynaFit 4

 directory ./../MR99

 file MR99-RNA34-3-I3ex.txt
  response IE2_E1=1

 file MR99-RNA34-3-5ex3ex.txt
  response I_E1E2=1
  response Iaa_E1E2=1

 file MR99-RNA34-3-3ex.txt
  response E2=1

[output]
 directory
./../MR99/ResultsRNA34-3

[end]
```

File MR99-RNA34-3-I3ex.txt

0	1.83883E-4
1	0.08699
3	0.17072
7	0.29
12	0.32561
18	0.36404
30	0.4045
45	0.37498
60	0.36234
90	0.31489
135	0.21617
180	0.19073
240	0.14649
300	0.09355
360	0.0886

File MR99-RNA34-3-5ex3ex.txt

0	0.99968
1	0.86946
3	0.70908
7	0.46395
12	0.33058
18	0.21427
30	0.10716
45	0.05523
60	0.04464
90	0.03192
135	0.02322
180	0.01742
240	0.01197
300	0.01269
360	0.00894

File MR99-RNA34-3-3ex.txt

0	1.39482E-4
1	0.04355
3	0.12021
7	0.24605
12	0.34381
18	0.42169
30	0.48834
45	0.56979
60	0.59302
90	0.65319
135	0.76061
180	0.79185
240	0.84153
300	0.89376
360	0.90246