

**Supplementary Table 1:** Mutations of the *Neurospora* VS Ribozyme

<b>Symbol of the Mutation<sup>(1)</sup></b>	<b>krel<sup>(2)</sup></b>	<b>Length<sup>(3)</sup></b>	<b>Type<sup>(4)</sup></b>	<b>Location<sup>(5)</sup></b>	<b>Ref. <sup>(6)</sup></b>
Original sequence	1.00000	144	original		
inverse A652	0.00000	144	B	II	1
A652C	0.17000	144	B	II	1
A652G	0.00000	144	B	II	2
A652U	0.24000	144	B	II	1
Δ A652	0.01300	143	B	II	3
Δ A652	0.00000	143	B	II	2
G650C, C773G	1.27000	144	BP	II	2
G653C, C771G	0.09000	144	BP	II	2
G655C, C769G	1.77670	144	BP	II	3
G655C, C769G	1.32000	144	BP	II	2
+650G, +772C	1.10000	136	L	II	1
+653G, +770C	0.00560	136	L	II	1
C769G	0.18000	144	MP	II	2
C771G	0.00000	144	MP	II	2
C773G	0.29000	144	MP	II	2
G650C	0.12000	144	MP	II	2
G653C	0.00000	144	MP	II	2
G655C	0.00000	144	MP	II	2
inverse A718	0.12000	144	B	III	4
Δ A718	0.01500	143	B	III	2
Δ A718	0.14000	143	B	III	4
inverse stem III	0.04000	144	B	III	4
A661U, C662G, G716C, U717A	0.08000	144	BP	III	2
A661U,U717A	0.60000	144	BP	III	4
C658G, G721C	1.34951	144	BP	III	3
C660G, G719C	1.20000	144	BP	III	4
C662G, G716C	0.94000	144	BP	III	2
C662G,G716C	0.60000	144	BP	III	4
C663G,G715C	0.27000	144	BP	III	4
inverse stem III, except the adenine bulge	0.50000	144	BP	III	4
U659A,A720U	1.19000	144	BP	III	2
U659A,A720U	0.80000	144	BP	III	4
+A after C660	0.77000	145	L	III	4
Length 10 stem III	0.00000	151	L	III	4
Length 5 stem III 5	0.00000	141	L	III	4
Length 6 stem III	0.14000	143	L	III	4

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Length 7 stem III	0.85000	145	L	III	4
Length 8 stem III	0.09000	147	L	III	4
Length 9 stem III	0.00370	149	L	III	4
A661U, C662G	0.06000	144	MP	III	2
A720U	0.05000	144	MP	III	2
C662G	0.23000	144	MP	III	2
G716C	0.21000	144	MP	III	2
G716C, U717A	0.02000	144	MP	III	2
U659A	0.00000	144	MP	III	2
C666G,G685C	0.03000	144	BP	IV	4
U670A, C672G, G679C, A681U	0.88000	144	BP	IV	2
Length 2 stem IV	0.01200	132	L	IV	4
Length 4 stem IV	0.61000	136	L	IV	4
Length 6 stem IV	0.49000	140	L	IV	4
G679C, A681U	0.00000	144	MP	IV	2
U670A, C672G	0.54000	144	MP	IV	2
C632A,G697U	0.03000	144	CS	I-V	5
C632G,G697C	0.42600	144	CS	I-V	5
C632U,G697A	0.23000	144	CS	I-V	5
G630A,C699U	0.23800	144	CS	I-V	5
G630C,C699G	0.00600	144	CS	I-V	5
G630U,C699A	0.00100	144	CS	I-V	5
U631A,A698U	0.02400	144	CS	I-V	5
U631C,A698G	0.43500	144	CS	I-V	5
U631G,A698C	0.00700	144	CS	I-V	5
A656C	0.00291	144	CS	J236	3
A656C, G768A	0.00000	144	CS	J236	3
A656C, G768U	0.00194	144	CS	J236	3
A656G	0.06117	144	CS	J236	3
A656G, G768A	0.86408	144	CS	J236	3
A656G, G768U	0.00583	144	CS	J236	3
A656U	0.00194	144	CS	J236	3
A656U, G768A	0.01553	144	CS	J236	3
A656U, G768U	0.00000	144	CS	J236	3
A657U	0.06311	144	CS	J236	3
Δ A656	0.00000	143	CS	J236	3
G768U	0.01553	144	CS	J236	3
Δ A656, A767	0.00000	142	L	J236	3
Δ A767	0.00068	143	L	J236	3

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A764U	0.87379	144	SS	J236	3
A766U	0.05437	144	SS	J236	3
A767U	0.04757	144	SS	J236	3
C765U	0.54369	144	SS	J236	3
G768A	0.57282	144	SS	J236	3
A712G	0.00600	144	CS	J345	4
A712U	0.00500	144	CS	J345	4
C665A	0.01500	144	CS	J345	4
C665G	0.01000	144	CS	J345	2
C665G, G711C	0.01000	144	CS	J345	2
Δ U686	0.00000	143	CS	J345	4
U686A	0.00600	144	CS	J345	4
U710A	0.00200	144	CS	J345	4
U710C	0.03000	144	CS	J345	4
U713A	0.02000	144	CS	J345	4
U714A	0.14000	144	CS	J345	4
G711C	1.00000	144	SS	J345	2
G711U	0.90000	144	SS	J345	4
U664A	0.12000	144	SS	J345	4
A690U, C692G, G704C, U706A	1.48000	144	BP	V	2
A693U, G694, C702G, U703A	0.31000	144	BP	V	2
C687G,G709C	0.00800	144	BP	V	4
U695G, A701C	1.67000	144	BP	V	2
A698C	0.04100	144	CS	V	5
A698G	0.00000	144	CS	V	5
A698U	0.00000	144	CS	V	5
C699A	0.00600	144	CS	V	5
C699G	0.00000	144	CS	V	5
C699U	0.01800	144	CS	V	5
G697A	0.00000	144	CS	V	5
G697C	0.00000	144	CS	V	5
G697U	0.00100	144	CS	V	5
Length 10 stem V	0.03100	146	L	V	4
Length 11 stem V	0.00320	148	L	V	4
Length 12 stem V	0.00000	150	L	V	4
Length 13 stem V	0.00000	152	L	V	4
Length 3 stem V	0.00000	132	L	V	4
Length 5 stem V	0.00000	136	L	V	4
Length 7 stem V	0.04700	140	L	V	4

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A690U, C692G	0.07000	144	MP	V	2
A701C	0.04000	144	MP	V	2
G704C, U706A	0.78000	144	MP	V	2
U695G	0.06000	144	MP	V	2
U696A	0.03000	144	MP	V	5
U696C	0.18300	144	MP	V	5
U696G	0.02400	144	MP	V	5
U705C	0.90000	144	RB	V	4
U708C	0.90000	144	RB	V	4
inverse A725,A726	0.00280	144	B	VI	1
A725C	1.60000	144	B	VI	1
A725G	0.05500	144	B	VI	1
A725U	1.50000	144	B	VI	1
A726C	1.70000	144	B	VI	1
A726G	1.20000	144	B	VI	1
A726U	1.10000	144	B	VI	1
Δ A725	0.17000	143	B	VI	1
Δ A725,A726	0.00710	142	B	VI	1
Δ A725, inverse A726	0.04300	143	B	VI	1
Δ A725,A726C	1.60000	143	B	VI	1
Δ A725,A726G	0.25000	143	B	VI	1
Δ A725,A726U	0.37000	143	B	VI	1
A735U, U737A, A748U, U750A	1.15000	144	BP	VI	2
C723G,G762C	0.84000	144	BP	VI	1
C731A,G754U	0.12000	144	BP	VI	6
C731G,G754C	0.04200	144	BP	VI	1
C731U,G754A	0.30000	144	BP	VI	6
G722C, C723G, G762C, C763G	0.75000	144	BP	VI	2
G722C, C763G	0.81553	144	BP	VI	3
G722C,C763G	0.84000	144	BP	VI	1
G727C, U728A, A759U, C760G	0.94000	144	BP	VI	2
G727C,C760G	0.35000	144	BP	VI	1
G729C,C758G	0.43000	144	BP	VI	1
G732U,U753G,G733U,U752G	0.47000	144	BP	VI	1
U724A, A761U	0.21000	144	BP	VI	1
U728A,A759U	1.30000	144	BP	VI	1
A730C	0.05500	144	CS	VI	1
A730G	0.01100	144	CS	VI	1
A730U	0.03600	144	CS	VI	1

Symbol of the Mutation <sup>(1)</sup>	$k_{rel}$ <sup>(2)</sup>	Length <sup>(3)</sup>	Type <sup>(4)</sup>	Location <sup>(5)</sup>	Ref. <sup>(6)</sup>
A756C	0.00190	144	CS	VI	7
A756C	0.00190	144	CS	VI	1
A756G	0.00270	144	CS	VI	7
A756G	0.00270	144	CS	VI	1
A756U	0.00130	144	CS	VI	7
A756U	0.00130	144	CS	VI	1
substitution of the active site with a A-C basepair	0.00000	142	CS	VI	8
C755A	0.84000	144	CS	VI	1
C755G	0.02000	144	CS	VI	1
C755U	0.17000	144	CS	VI	1
G757A	0.04400	144	CS	VI	1
G757C	0.01500	144	CS	VI	1
G757U	0.01800	144	CS	VI	1
Length 2 stem VI.c	0.46000	128	L	VI	1
Length 4 stem VI.c	0.70000	132	L	VI	1
Length 6 stem VI.c	1.00000	136	L	VI	1
Length 8 stem VI.c	0.97000	140	L	VI	1
A735U, U737A	0.25000	144	MP	VI	2
A748U, U750A	0.28000	144	MP	VI	2
A759U, C760G	0.00000	144	MP	VI	2
C731A	0.01500	144	MP	VI	6
C731G	0.03900	144	MP	VI	6
C731U	0.03900	144	MP	VI	6
G722C, C723G	0.00000	144	MP	VI	2
G727C, U728A	0.00000	144	MP	VI	2
G754A	0.02500	144	MP	VI	6
G754C	0.01200	144	MP	VI	6
G754U	0.02200	144	MP	VI	6
G762C, C763G	0.00000	144	MP	VI	2
U752C	0.80000	144	RB	VI	1
U752C,U753C	0.52000	144	RB	VI	1
U753C	0.42000	144	RB	VI	1

<sup>(1)</sup> **Symbol of the mutation:** We used the conventional descriptions of the mutations. For a point mutation the original nucleotide is listed first, the location (position) of the mutation second, and then the nucleotide to which the original was replaced. So for example the mutation C713A means that cytosine at position 713 is replaced by an adenine. Deletions begin with a  $\Delta$  symbol, followed by the position and the original nucleotide, thus the mutation  $\Delta$  A718 means that the adenine at position 718 is deleted.

If there is more than one mutation in a mutant, then the mutations are comma separated. Some deletions and insertions have textual descriptions see the references for the exact sequence.

<sup>(2)</sup> **Relative enzymatic activity ( $k_{rel}$ ):** The relative activity is the ratio of the enzymatic activity of the mutant and the wild-type ribozyme.

<sup>(3)</sup> **Length:** The length of the ribozyme.

<sup>(4)</sup> **Type of mutation:** Based on structural changes and activity loss, we can discern the following types of mutations:

*Base pair change (BP):* The base pair is either reversed or changed to another base pair. The structure does not change.

*Mutation affecting a single stranded region (SS):* The point mutation affects one of the single stranded regions of the ribozyme, for example the internal loops, the junctions or the hairpins at the end of the stems. The structure does not change.

*Mispair (MP):* The mutation disrupts one of the base pairs, so the pair could not form and the structure of the molecule changes locally (an internal bulge is formed).

*Restoration of a base pair (RB):* A GU wobble pair is changed to one of the canonical base pairs (AU or GC).

*Mutation affecting a critical site (CS):* The mutation affects one of the critical sites of the ribozyme. Structure does not change, but the enzymatic activity is reduced considerably.

*Mutations affecting a bulge (B):* The mutation affects one of the adenine bulges. The mutation can be a deletion, a point mutation or the inverting the bulge (i.e. putting it to the opposite strand).

*Change in length (L):* The mutation is either a deletion or an insertion.

<sup>(5)</sup> **Location of the mutation:** The stem (II, III, IV, V or VI) or junction (J236 or J345) where the mutation occurs.

<sup>(6)</sup> **Reference:** The source of information for the given ribozyme.

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