iSpinach: a fluorogenic RNA aptamer optimized for *in vitro* applications

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Supplementary Tables

Table S1. Folding efficiencies of Spinach2, Broccoli and iSpinach as a function of temperature in the presence of sodium or potassium. Values are expressed in percentage of folded molecule and are the mean of three independent experiments and error bars correspond to ± 1 standard error.

	25	°C	37	°C	45 °C								
	К+	Na ⁺	к*	Na ⁺	к+	Na ⁺							
Spinach 2.0	61.0 ± 12.2	n.m.	27.0 ± 4.7	n.m.	2.3± 1.4	n.m.							
Broccoli	17.7 ± 3.2	n.m.	15.5 ± 4.8	n.m.	9.9 ± 2.6	n.m.							
iSpinach	69.0 ± 4.0	59.6±8.1	53.7 ± 3.7	23.6±3.4	37.4±3.2	6.4±1.5							

n.m. : not measurable

Salt	Round	PCR droplets occupancy (%)	λ value ^a	Fusion efficiency (%)	Number of analyzed droplets	Number of sorted droplets
Sodium	S1	32	0.38	92	1,392,500	9,028
	S2	12	0.12	75	250,950	2,526
	S3	15	0.16	85	776,251	11,278
	S4	16	0.17	90	879,000	7,896
Potassium	S1	26	0.30	70	738,875	22,730
	S2	23	0.26	87	397,950	4,514
	S 3	17	0.18	80	1,526,750	28,071
	S4	18	0.19	75	225,750	2,589

 Table S2. Experimental parameters.

^a Calculated as described in (1).

a Position	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	2 43	3 44	4 4	54	64	7 48	8 49	9 50) 5	15	25	3 5	64 5	5	56	57	58	59	60	61	62	63	64	65
b Region					P1								J1	-2							P2						Qı	uarte	et		Τ								P3								Τ		ι	_3	
SpiSel	G	Α	С	G	С	G	Α	С	U	G	Α	Α	U	G	Α	Α	Α	U	G	G	U	G	Α	Α	G	G	Α	C	: 6	6 (G (βl	JC	: C	A		G (G	U	G	U	G	G	С	U	G	С	U	U	С	G
Clone68			U									G						С						G																	\top						U	С	\square		
Clone34	х	С						U				С												G																A							\square	\square			
Clone49		х			U																						U																								
Clone56		х	х															С																				(С			Α						С			
Clone46	х	х	х	х												U																				4	4														
Clone70			U															С																						Α									С	U	
Clone41			U															С																		4	4														
Clone84			U								G										Α																						Α				U				
Clone79		х	U															С																							С										
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Clone67																		С																													U				
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Clone63			U									х	х		х	х	х																			1	۹.														
Clone50								Α					х		х																										\perp	\square									
Clone39				Α				U										С																														С			
Clone50								Α					х		х																										\perp	\square									
Clone64		G										G			х	х																									\downarrow	\square						\square			
Clone22		G	U									G				U																					1	4			\downarrow							\square			
Clone23			U									U						С						G																											

Table S3-1. Sequences of isolated mutants. Black crosses represent deletions.

^a Positions numbered according to SpiSel sequence

^b Regions labeled according to the model proposed in (2)

a Position	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	. 92	2 93	94	4 95	596	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113		
b Region									Р3													Qu	arte	et							P2	2					J1	-2			P1									
SpiSel	G	С	Α	G	U	G	С	Α	G	С	U	U	G	U	U	G	Α	G	U	Α	G	Α	G	U	G	U	G	A	G	i C	: U	С	С	G	U	Α	Α	С	U	Α	G	U	С	G	С	G	U	С		
Clone68											С																	G	Τ													\square				\square	\square			
Clone34									Α																			G	Τ					Α		U	G	U								\square				
Clone49																													Г					Α																
Clone56																																																		
Clone46																																		Α														Α		
Clone70						Α					С																									G									U					
Clone41						Α																																U												
Clone84						Α																															G	U			U									
Clone79							U				С																																							
Clone62					С		U				С																				G														U					
Clone24											С																							Α				х					U		U					
Clone77						Α																												Α		G				G			U							
Clone86																																				G						С								
Clone51											С																																U		U					
Clone73									С																									Α					x									U		
Clone78				С							С																							Α			G													
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Clone67																																																		
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Clone39									Α																																			Α						
Clone50							U				С																						U	Α																
Clone64											С																								С								U					U		
Clone22																																		Α											U					
Clone23							U		Α		С	С																															Α							

Table S3-2. Sequences of isolated mutants. Black crosses represent deletions.

^a Positions numbered according to SpiSel sequence

^b Regions labeled according to the model proposed in (2)

Supplementary Figures



Figure S1. Fluorescence of isolated mutants. Aptamer-coding genes were PCR amplified and DNA *in vitro* transcribed in the presence of DFHBI. The co-transcriptional fluorescence increase was monitored at 37°C. Relative fluorescence was calculated by dividing the fluorescence increase rate of the analyzed clone by the one of SpiSel.



Figure S2. Mutations isolated from the *in vitro* evolution process and summary of the changes used to convert Spinach 1.1 into iSpinach. **A.** The mutations isolated for the screening and identified through sequence alignments (Table S3) are shown in green and mapped onto the secondary structure of the original Spinach 1.1. **B.** Conversion of Spinach 1.1 into iSpinach. The mutations originally identified from the screening process are shown in green, the residue rationally transplanted is squared in cyan and the nucleotides rationally deleted are shown in red.



Figure S3. Effect of the temperature on RNA/DFHBI complex dissociation. Arrehnius plots are shown for DFHBI in complex with Spinach2 (left) or iSpinach (right) in the presence of potassium (squares) or sodium (triangles).



Figure S4. Excitation and emission spectra of DFHBI in complex with Spinach2 or iSpinach in the presence of sodium or potassium. Excitations spectra (dashed lines) were determined by recording the fluorescence emitted at 500 nm following excitation at a wavelength ranging from 350 nm to 400 nm with a step of 1 nm. Emission spectra (continuous line) were determined by exciting the sample at 400 nm and recording the fluorescence emitted from 475 nm to 600 nm with a step of 1 nm.

References

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