

Supplementary Information

A FRET-based assay for characterization of alternative splicing events using peptide nucleic acid fluorescence *in situ* hybridization.

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A	ROI	ROI 1	ROI 2	ROI 3	ROI 4	ROI 5	ROI 6	ROI 7	ROI 8	ROI 9	ROI 10
	FRET	108.99	132.97	152.51	93.66	122.97	157.98	79.98	104.76	81.81	0.00
	FRETeff	0.37	0.38	0.50	0.35	0.43	0.59	0.52	0.49	0.53	0.00
	A (ch1)	1395.09	1736.34	1815.65	1330.34	1486.02	1821.07	759.41	896.07	772.48	0.00
	B (ch2)	584.64	707.44	711.71	534.06	608.13	686.60	331.96	429.43	337.88	1.17
	C (ch3)	296.65	346.26	307.70	265.62	287.83	266.38	152.39	215.63	154.70	6.16
B	ROI	ROI 1	ROI 2	ROI 3	ROI 4	ROI 5	ROI 6	ROI 7	ROI 8		
	FRET	87.75	82.35	57.97	72.95	72.29	72.94	73.27	88.65		
	FRETeff	0.07	0.07	0.05	0.05	0.05	0.05	0.05	0.07		
	A (ch1)	1679.70	1470.21	1571.84	1795.93	2030.80	2048.29	1989.47	1681.55		
	B (ch2)	1378.88	1213.82	1268.97	1414.40	1533.58	1550.17	1561.17	1303.71		
	C (ch3)	1318.40	1155.83	1237.51	1356.61	1458.49	1475.61	1505.38	1214.57		
C	ROI	ROI 1	ROI 2	ROI 3	ROI 4	ROI 5	ROI 6	ROI 7	ROI 8		
	FRET	167.95	131.95	175.88	234.93	140.00	158.50	151.74	212.55		
	FRETeff	0.74	0.52	0.80	0.73	0.75	0.99	0.85	0.93		
	A (ch1)	2337.68	2006.16	2618.89	2686.84	1926.50	2050.87	1910.66	2114.46		
	B (ch2)	757.80	683.89	811.35	887.16	627.13	646.73	628.51	764.67		
	C (ch3)	225.72	253.61	219.69	225.76	187.37	159.71	177.51	228.53		
D	ROI	ROI 1	ROI 2	ROI 3	ROI 4	ROI 5	ROI 6	ROI 7			
	FRET	0.00	0.00	0.00	0.00	64.51	0.00	0.00			
	FRETeff	0.00	0.00	0.00	0.00	0.06	0.00	0.00			
	A (ch1)	2466.05	2082.23	1886.69	1411.73	1719.72	1768.88	1423.56			
	B (ch2)	2058.19	1504.80	1402.78	1265.97	1452.93	1101.37	1298.98			
	C (ch3)	1923.55	1212.02	1276.53	1227.11	1161.87	1123.41	1215.56			

Figure S.1.- Values of FRET, FRET efficiency and excitation channels of the representative laser scanning microscope FRET reports derived from e1A/e2D PNA probes detecting FSI isoform of *LY6G5B* gene. After manual ROIs selection, the software generated a report with FRET values information, and FRET efficiency in percent of each ROI selected, correcting the optical crosstalk and the background effect as reference with Channel A (A,ch1) that is the excitation with excitation wavelength of donor and detection with parameter settings of donor (donor channel), with Channel B (B, ch2) that is the excitation with excitation wavelength of donor and detection with parameter settings of acceptor (FRET channel) and Channel C (C, ch3) is the excitation with excitation wavelength of acceptor and detection with parameter settings of acceptor (acceptor channel). (A) Values of the different cytoplasmic ROIs selected in the e1A-Cy5/e2D-Cy3 FRET report shown in Figure 4.A; (B) Values derived from the cytoplasmic negative e1A-Cy5/e2Dsense-Cy3 FRET report shown in Figure 4.B; (C) Values of the different nucleolar ROIs selected in the e1A-Cy5/e2D-Cy3 FRET report shown in Figure 5.A; and (D) Values derived from the nucleolar negative e1A-Cy5/e2Dsense-Cy3 FRET report shown in Figure 5.B.

A	ROI	ROI 1	ROI 2	ROI 3	ROI 4	ROI 5	ROI 6	ROI 7	ROI 8	ROI 9
	FRET	799.30	653.35	765.46	418.41	339.84	548.05	834.61	664.56	0.00
	FRETEff	0.30	0.28	0.30	0.38	0.32	0.35	0.31	0.29	0.00
	A (ch1)	2906.08	2563.27	2766.04	1373.07	1210.19	1671.33	2932.55	2553.01	0.00
	B (ch2)	2972.96	2530.79	2816.38	1332.70	1199.01	1803.25	3041.88	2516.12	0.00
	C (ch3)	2686.08	2313.52	2531.46	1111.39	1054.19	1551.94	2729.86	2278.56	0.46
B	ROI	ROI 1	ROI 2	ROI 3	ROI 4	ROI 5	ROI 6	ROI 7	ROI 8	
	FRET	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
	FRETEff	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	
	A (ch1)	2043.20	2311.34	2213.07	2380.95	1754.1	1953.39	2256.88	0.00	
	B (ch2)	1081.75	1166.72	1121.36	1194.30	942.40	1026.81	1117.60	0.00	
	C (ch3)	1271.69	1326.78	1315.47	1424.57	1075.7	1200.26	1271.95	0.00	
C	ROI	ROI 1	ROI 2	ROI 3	ROI 4	ROI 5	ROI 6			
	FRET	183.96	174.47	282.05	222.41	167.18	217.41			
	FRETEff	0.15	0.14	0.22	0.17	0.14	0.22			
	A (ch1)	2062.36	2236.55	2425.31	2395.52	1873.54	1549.20			
	B (ch2)	1784.24	1857.25	2088.16	2032.66	1663.91	1464.65			
	C (ch3)	1213.86	1213.33	1279.17	1314.95	1186.78	1000.13			
D	ROI	ROI 1	ROI 2	ROI 3	ROI 4	ROI 5	ROI 6	ROI 7	ROI 8	ROI 9
	FRET	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	FRETEff	0.00	0.01	0.00	0.00	0.00	0.00	0.03	0.00	0.00
	A (ch1)	3678.12	3046.66	2820.83	3125.01	2934.49	3178.58	3389.92	3634.56	3385.23
	B (ch2)	1436.73	1201.29	984.94	1191.34	1065.86	1164.26	1265.81	1448.63	1279.39
	C (ch3)	910.13	838.23	769.45	742.74	717.16	662.46	709.04	829.07	870.86
		ROI 10	ROI 11	ROI 12	ROI 13	ROI 14				
		0.00	0.00	0.00	0.00	0.00				
		0.00	0.00	0.00	0.00	0.00				
		3430.84	3283.72	3058.75	3101.22	3566.87				
		1254.73	1192.23	1158.65	1160.54	1351.50				
		719.17	671.89	819.28	857.35	915.53				

Figure S.2.- Values of FRET, FRET efficiency and excitation channels of the representative laser scanning microscope FRET reports derived from e1A/i1D PNA probes detecting IRI isoform of *LY6G5B* gene. After manual ROIs selection, the software generated a report with FRET values information, and FRET efficiency in percent of each ROI selected, correcting the optical crosstalk and the background effect as reference with Channel A (A,ch1), Channel B (B, ch2) and Channel C (C, ch3). (A) Values of the different cytoplasmic ROIs selected in the e1A-Cy5/i1D-Cy3 FRET report shown in Figure 6.A; (B) Values derived from the cytoplasmic negative e1A-Cy5/i1Dsense-Cy3 FRET report shown in Figure 6.B; (C) Values of the different nucleolar ROIs selected in the e1A-Cy5/i1D-Cy3 FRET report shown in Figure 7.A; and (D) Values derived from the nucleolar negative e1A-Cy5/i1Dsense-Cy3 FRET report shown in Figure 7.B.