

Supplementary data - table 1. Triplexes with TINA in the TFO strand
 Tm data for parallel triplexes with one to four TINAs in the TFO strand, base mismatches in the target strand and D-256 as AD strand. Tm for parallel triplexes and parallel duplexes was determined using 1.0 μM of each strand in sodium acetate buffer (50 mM NaOAc, 100 mM NaCl and 10 mM MgCl₂) at pH 5.0. Tm for antiparallel duplexes was determined using 0.5 μM of each strand in sodium phosphate buffer (50 mM NaH₂PO₄/Na₂HPO₄, 100 mM NaCl and 0.1 mM EDTA) at pH 7.0. All dissociation curves were run with a ramp rate of 3°C/minute. X symbolises TINA.

Oligo name	Target sequence	D-200	D-201	D-202	D-203	D-204	D-205	D-206	D-222	D-213	D-214	D-215	D-218	D-207	D-208	D-209	D-210	D-211	D-212	D-224	Control			
TFO sequence	All triplexes consist of a target sequence (columns) with or without base mismatch + an antiparallel duplex (AD) sequence (D-256: 5'-GTATCCCTCCCTTCTTTCCCTCCCT 3') + a triplex forming oligonucleotide (TFO) sequence (rows). AD D-484 is an antiparallel duplex consisting of D-484 and a target sequence. PD D-284 is a parallel duplex (PD) consisting of a target and a TFO sequence without the AD sequence.	5'-ATT0495-AGGGAAAAGAGGGAGGATAC 3'	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	ATT0495-AGGGAAAAGAGGGAGGATAC	Setup and run control D-200 + D-256 + D-284		
		D-284	5'-ATT0590-TCCCTTTTCCTCCCTCCCT 3'	73.51	65.92	65.44	66.14	61.42	63.16	61.12	58.36	60.90	61.03	61.42	62.85	61.72	60.14	61.59	65.76	65.28	65.19		52.72	
		D-285	ATT0590-TCCCTTTTCCTCCCTCCCT	75.03	67.34	66.81	67.44	63.55	65.16	63.66	62.58	63.40	64.65	63.86	65.52	64.09	63.91	63.73	67.64	67.25	66.89		57.91	73.59
		D-286	ATT0590-TCCCTTTTCCTCCCTCCCT	74.07	67.86	67.07	67.90	62.34	64.16	62.37	59.76	61.58	62.08	62.08	63.96	62.49	61.43	62.29	66.6	66.06	65.81		53.89	73.56
		D-287	ATT0590-TCCCTTTTCCTCCCTCCCT	74.18	68.66	67.72	68.86	63.23	64.52	62.71	59.47	61.43	61.66	61.90	63.64	62.41	60.78	62.39	66.89	66.14	66.11		54.16	73.51
		D-288	ATT0590-TCCCTTTTCCTCCCTCCCT	74.18	68.92	67.68	68.85	68.74	64.86	64.92	59.3	61.78	61.23	61.90	63.69	62.69	60.57	62.69	67.43	66.60	66.71		54.71	73.58
		D-289	ATT0590-TCCCTTTTCCTCCCTCCCT	75.61	71.19	69.97	71.47	66.92	66.17	66.80	61.52	63.52	63.52	63.67	65.31	64.57	63.19	64.72	69.51	68.66	68.94		58.73	73.71
		D-290	ATT0590-TCCCTTTTCCTCCCTCCCT	78.16	67.83	69.70	67.80	69.32	68.85	69.67	64.11	66.20	66.08	66.11	68.23	67.70	66.27	68.05	72.34	71.86	72.01		60.07	73.55
		D-291	ATT0590-TCCCTTTTCCTCCCTCCCT	78.39	68.47	70.50	68.49	71.88	70.82	71.88	64.26	66.28	65.89	66.24	68.04	67.81	66.03	68.17	72.61	72.22	72.05		59.34	73.58
		D-292	ATT0590-TCCCTTTTCCTCCCTCCCT	79.46	69.59	72.31	69.62	72.43	71.34	72.47	66.31	67.77	67.07	67.8	69.46	68.62	67.04	69.06	73.77	73.44	73.36		61.81	73.59
		D-293	ATT0590-TCCCTTTTCCTCCCTCCCT	78.92	69.70	72.75	69.66	69.13	69.35	68.90	69.08	67.11	66.64	67.14	67.76	67.25	65.43	68.04	72.74	72.22	72.25		65.88	73.49
		D-294	ATT0590-TCCCTTTTCCTCCCTCCCT	78.26	69.60	69.70	69.64	66.79	69.68	66.32	69.03	70.65	68.30	71.40	68.65	65.88	64.34	66.35	71.69	70.98	70.98		68.13	73.60
		D-295	ATT0590-TCCCTTTTCCTCCCTCCCT	79.31	71.38	71.34	71.39	67.31	70.38	67.08	65.00	72.64	70.47	72.89	73.04	68.32	65.84	68.93	72.75	72.36	72.36		62.96	73.56
		D-296	ATT0590-TCCCTTTTCCTCCCTCCCT	78.84	71.23	70.96	71.16	67.28	69.51	67.04	63.06	69.37	66.78	68.93	71.98	71.27	67.39	72.70	72.00	71.18	71.60		63.37	73.58
		D-297	ATT0590-TCCCTTTTCCTCCCTCCCT	78.29	70.96	70.66	71.11	66.86	69.18	66.49	61.78	64.64	64.72	64.65	67.79	73.85	70.26	74.09	72.49	71.03	72.17		62.31	73.47
		D-298	ATT0590-TCCCTTTTCCTCCCTCCCT	78.31	71.37	71.13	71.43	67.38	69.41	67.14	63.58	64.00	64.66	64.29	67.47	74.87	71.53	75.00	70.39	70.03	70.18		61.49	73.51
		D-299	ATT0590-TCCCTTTTCCTCCCTCCCT	78.15	71.25	70.70	71.06	67.33	69.32	67.19	63.77	65.97	64.66	65.61	68.11	74.41	70.37	75.03	71.20	72.14	71.86		62.38	73.66
		D-300	ATT0590-TCCCTTTTCCTCCCTCCCT	79.09	72.19	71.89	72.14	68.43	70.35	68.31	65.25	65.46	66.32	65.97	67.26	67.72	64.96	68.96	75.75	74.59	75.21		58.29	73.57
		D-301	ATT0590-TCCCTTTTCCTCCCTCCCT	76.97	69.37	68.99	69.60	65.62	67.48	65.48	61.34	62.28	62.92	62.90	64.88	63.68	61.63	64.02	74.39	73.22	74.14		54.72	73.62
		D-302	ATT0590-TCCCTTTTCCTCCCTCCCT	77.64	69.12	68.64	69.23	65.68	67.39	65.09	61.13	62.72	62.87	63.14	64.55	63.51	61.98	63.43	74.07	73.21	73.93		54.09	73.74
		D-303	ATT0590-TCCCTTTTCCTCCCTCCCT	77.94	69.84	69.25	69.87	66.22	67.96	65.49	62.20	63.75	64.32	63.86	65.53	64.31	63.31	64.26	72.02	71.35	72.08		55.64	73.61
		D-304	ATT0590-TCCCTTTTCCTCCCTCCCT	78.36	70.77	70.37	70.94	67.26	68.91	66.80	63.72	64.95	65.27	65.10	66.60	65.45	64.51	65.28	70.73	69.82	70.41		58.11	73.60
		D-305	ATT0590-TCCCTTTTCCTCCCTCCCT	75.66	65.49	65.90	65.72	64.00	66.14	63.66	69.32	74.95	73.84	75.54	73.18	67.80	63.49	67.97	68.74	67.63	67.98		68.19	73.48
D-306	ATT0590-TCCCTTTTCCTCCCTCCCT	78.51	69.80	70.11	69.72	67.22	69.91	67.25	71.64	73.46	71.49	73.79	74.45	72.55	69.29	73.41	73.13	72.76	72.76	71.94	73.58			
D-307	ATT0590-TCCCTTTTCCTCCCTCCCT	80.22	71.53	74.41	71.36	71.01	71.19	70.99	72.08	72.23	72.00	72.35	73.82	74.52	72.31	74.97	75.18	75.16	74.82	71.91	73.60			
D-308	ATT0590-TCCCTTTTCCTCCCTCCCT	80.00	71.50	74.39	71.37	70.86	70.95	70.76	71.12	68.71	68.63	68.98	70.85	76.17	74.29	75.93	75.49	74.88	75.24	72.01	73.56			
D-309A	ATT0590-TCCCTTTTCCTCCCTCCCT	81.13	72.31	75.04	72.2	74.49	73.62	74.77	68.45	69.83	69.39	70.10	72.84	76.88	75.71	77.03	76.58	76.14	76.24	68.37	73.44			
D-309B	ATT0590-TCCCTTTTCCTCCCTCCCT	80.13	70.77	73.85	70.77	73.92	72.73	74.13	67.40	68.63	68.16	69.01	71.95	77.17	75.73	77.11	74.62	74.77	74.55	67.56	73.50			
D-310	ATT0590-TCCCTTTTCCTCCCTCCCT	80.31	71.67	73.33	71.52	73.59	72.76	73.73	67.08	68.34	67.75	68.46	71.54	77.35	76.03	77.38	75.01	75.24	75.00	66.29	73.47			
D-311	ATT0590-TCCCTTTTCCTCCCTCCCT	80.61	72.07	74.08	72.16	74.13	73.40	73.34	67.93	70.00	68.80	69.80	72.61	77.64	76.00	77.66	76.27	77.06	76.56	67.02	73.40			
D-312	ATT0590-TCCCTTTTCCTCCCTCCCT	80.54	72.26	73.81	72.14	72.09	71.83	72.56	67.54	70.25	68.83	69.88	72.81	77.57	75.89	77.49	76.35	76.85	76.40	67.34	73.53			
D-313	ATT0590-TCCCTTTTCCTCCCTCCCT	81.98	73.59	75.24	73.52	73.20	72.38	73.43	68.38	70.09	69.93	70.47	71.81	74.14	71.15	74.67	79.49	78.67	78.84	63.55	73.47			
D-314	ATT0590-TCCCTTTTCCTCCCTCCCT	79.75	76.15	75.20	76.32	71.00	70.92	71.00	66.49	67.75	67.95	67.90	71.03	68.60	72.00	76.97	76.14	76.62	62.74	73.54				
D-229	ATT0590-TCCCTTTTCCTCCCTCCCT	79.98	75.78	74.53	75.87	70.26	70.68	70.44	66.53	68.07	68.30	68.30	69.83	69.97	68.48	70.36	77.47	76.71	77.33	63.28	73.47			
D-230	ATT0590-TCCCTTTTCCTCCCTCCCT	79.14	74.02	72.91	74.09	68.45	69.86	68.18	65.52	67.13	67.13	67.48	68.69	68.21	66.43	68.59	76.79	75.76	76.47	61.05	73.40			
D-231	ATT0590-TCCCTTTTCCTCCCTCCCT	79.49	74.07	72.50	74.09	67.91	69.44	67.8	65.25	66.95	66.95	66.98	68.42	67.98	66.45	68.11	75.93	75.46	76.88	61.02	73.47			
D-232	ATT0590-TCCCTTTTCCTCCCTCCCT	78.49	73.90	72.96	74.02	67.69	69.10	67.34	64.06	65.46	65.69	65.58	66.98	66.17	64.64	65.93	74.62	74.08	74.43	58.84	73.49			
D-233	ATT0590-TCCCTTTTCCTCCCTCCCT	78.98	74.21	73.04	74.21	68.90	67.35	64.03	65.43	65.66	65.66	66.67	66.18	65.02	66.30	73.10	72.64	73.15	59.15	73.50				
D-234	ATT0590-TCCCTTTTCCTCCCTCCCT	79.20	73.14	72.32	73.09	68.21	69.42	67.62	64.43	65.02	65.84	65.37	66.78	65.75	64.72	65.81	73.06	72.39	72.97	57.65	73.36			
D-235	ATT0590-TCCCTTTTCCTCCCTCCCT	79.81	74.15	73.41	74.03	68.93	70.82	68.61	65.35	66.26	66.85	66.42	67.82	66.83	65.84	66.81	72.01	71.21	71.80	60.45	73.33			
D-236	ATT0590-TCCCTTTTCCTCCCTCCCT	80.95	73.36	72.93	73.48	70.31	72.19	69.84	66.89	67.62	68.17	67.69	69.30	68.08	67.21	68.15	73.34	72.52	72.87	62.53	73.42			
D-315	ATT0590-TCCCTTTTCCTCCCTCCCT	78.80	69.47	72.64	69.48	69.79	69.76	68.89	71.68	73.15	73.07	73.96	73.66	69.88	67.14	70.44	73.45	73.17	72.85	69.91	73.56			
D-316	ATT0590-TCCCTTTTCCTCCCTCCCT	80.34	71.33	74.05	71.12	73.72	72.71	74.12	70.03	74.30	73.90	75.09	75.33	72.34	70.41	72.52	75.1	75.15	74.78	67.65	73.57			
D-317	ATT0590-TCCCTTTTCCTCCCTCCCT	80.53	71.69	73.43	71.57	72.95	72.37	73.54	69.12	74.30	73.42	74.75	75.55	72.26	70.10	72.44	75.27	75.30	74.96	66.43	73.61			
D-318	ATT0590-TCCCTTTTCCTCCCTCCCT	80.45	71.92	73.28	71.72	71.20	71.42	68.98	74.09	73.17	74.72	75.49	72.											

Supplementary data - table 2. Triplexes with TINA in the AD strand

Tm data for parallel triplexes with one or two TINAs in the AD strand, base mismatches in the target strand and D-284 as TFO strand. Tm was determined using 1.0 μM of each strand in sodium acetate buffer (50 mM NaOAc, 100 mM NaCl and 10 mM MgCl₂) at pH 5.0.

All dissociation curves were run with a ramp rate of 3°C/minute. X symbolises TINA.

Oligo name	Target sequence	D-200	D-201	D-202	D-203	D-204	D-205	D-206	D-222	D-213	D-214	D-215	D-218	D-207	D-208	D-209	D-210	D-211	D-212	D-224	Control			
AD sequence	All triplexes consist of a target sequence (columns) with or without a base mismatch + an antiparallel duplex (AD) sequence with or without TINA (rows) + a triplex forming oligonucleotide (TFO) sequence (D-284: 5' ATTO590-TCCCTTTTCTCCCTCCCT 3').	5' ATTO495-AGGGAAAAGAAAGGGAGGGATAAC 3'	ATTO495-AGGCAAAAAGAAAGGGAGGGATAAC	ATTO495-AGGAAAAAAGAAAGGGAGGGATAAC	ATTO495-AGGTAATAAGAAAGGGAGGGATAAC	ATTO495-AGGGAAACAAGAAAGGGAGGGATAAC	ATTO495-AGGGAAACAAGAAAGGGAGGGATAAC	ATTO495-AGGGAAATAAGAAAGGGAGGGATAAC	ATTO495-AGGGAAAAAGCAGGGAGGGATAAC	ATTO495-AGGGAAAAAGCAGGGAGGGATAAC	ATTO495-AGGGAAAAAGGAGGGAGGGATAAC	ATTO495-AGGGAAAAAGTAGGGAGGGATAAC	ATTO495-AGGGAAAAAGAGGGAGGGATAAC	ATTO495-AGGGAAAAAGAAAGGGAGGGATAAC	ATTO495-AGGGAAAAAGAAAGGGAGGGATAAC	ATTO495-AGGGAAAAAGAAAGGGAGGGATAAC	ATTO495-AGGGAAAAAGAAAGGGAGGGATAAC	ATTO495-AGGGAAAAAGAAAGGGAGGGATAAC	ATTO495-AGGGAAAAAGAAAGGGAGGGATAAC	ATTO495-AGGGAAAAAGAAAGGGAGGGATAAC	ATTO495-AGGGAAAAAGAAAGGGAGGGATAAC	Setup and run control D-200 + D-256 + D-284		
		D-256	5' GTTATCCCTCCCTTCTTTTCCCT 3'	73.51	65.92	65.44	66.14	61.42	63.16	61.12	58.36	60.9	61.03	61.42	62.85	61.72	60.14	61.59	65.76	65.28	65.19		52.72	
		D-445	GTTATCCCTCCCTTCTTTTCCCT X	77.07	69.67	69.47	69.65	66.56	68.22	67.18	67.78	67.66	67.02	70.66	70.80	70.18	67.01	67.96	67.38	69.03	65.70		61.57	73.51
		D-446	GTTATCCCTCCCTTCTTTTCCCT X CT	75.01	69.35	68.88	69.83	64.39	66.99	64.47	64.78	64.42	64.54	68.34	68.06	67.81	64.11	65.14	64.53	66.08	63.30		58.32	73.46
		D-447	GTTATCCCTCCCTTCTTTTCCCT X TTCCCT	77.62	68.59	68.8	68.56	69.06	68.77	69.57	66.84	65.78	67.20	71.65	71.22	71.25	66.14	66.38	66.06	67.72	64.27		61.25	73.56
		D-448	GTTATCCCTCCCTTCTTTTCCCT X TTTTCCCT	76.85	67.88	67.96	67.85	66.53	66.82	66.58	65.39	64.33	65.64	70.02	68.27	66.02	68.75	68.27	69.54	67.97	65.94		63.86	73.62
		D-449	GTTATCCCTCC X CTTCTTTTCCCT	78.63	71.07	70.95	71.37	67.09	68.99	66.73	73.14	71.86	73.94	71.33	71.22	70.50	65.91	66.17	66.52	67.63	64.41		60.27	73.56
		D-450	GTTATCCC X TCCCTTCTTTTCCCT	80.00	72.77	73.02	73.02	69.03	70.96	68.85	68.50	67.24	68.77	74.41	73.30	74.37	68.00	68.07	68.29	69.36	66.28		60.99	73.50
		D-451	GTTAT X CCCTCCCTTCTTTTCCCT	77.00	69.18	68.76	69.3	65.51	66.93	65.03	65.27	64.19	65.19	69.15	68.55	68.83	64.30	64.71	64.59	65.92	62.85		57.99	73.59
		D-452	GT X TATCCCTCCCTTCTTTTCCCT	74.33	66.45	65.86	66.57	61.62	63.52	61.24	62.16	60.38	61.98	67.60	67.13	67.72	61.01	61.09	61.28	63.14	58.42		53.19	73.59
		D-498	GTTATCCCTCCCT X TCTTTTCCCT	75.29	65.78	66.34	65.64	64.81	65.52	64.92	67.64	65.43	67.94	69.06	68.52	68.18	70.22	68.91	70.81	69.98	66.04		64.04	73.26
		D-499	GTTATCCCTCCC X TTCTTTTCCCT	78.54	69.77	70.89	69.59	69.47	69.71	69.83	75.08	73.86	75.22	73.36	73.31	72.88	70.63	69.33	70.66	71.92	69.86		70.10	73.24
		D-500	GTTATCCCTCC X CTTCTTTTCCCT	80.22	71.84	73.03	71.72	71.96	71.61	72.36	76.31	75.11	76.34	74.32	74.34	73.88	69.12	69.32	69.71	71.30	67.84		66.65	73.36
		D-501	GTTATCCCTC X CTTCTTTTCCCT	79.66	72.38	72.86	72.38	69.05	72.06	72.77	75.32	73.64	75.13	73.52	73.92	73.03	69.24	69.27	69.62	70.81	68.00		65.16	73.27
		D-502	GTTATCCCT X CCCTTCTTTTCCCT	80.61	73.41	73.69	73.12	72.16	71.84	72.43	73.70	72.31	73.62	73.73	73.8	73.56	70.60	70.72	70.94	72.35	69.32		65.55	73.38
		D-503	GTTATCC X CTCCCTTCTTTTCCCT	79.53	74.76	74.10	75.05	70.18	72.38	70.36	69.38	68.70	69.53	73.97	73.13	73.60	69.32	69.35	69.70	70.30	68.05		64.30	73.48

Supplementary data - table 3. Triplexes with TINA in the AD and the TFO strand
 Tm data for parallel triplexes with one or two TINAs in the AD strand, one or two TINAs in the TFO strand and D-200 as target strand. Triplex Tm was determined using 1.0 µM of each strand in sodium acetate buffer (50 mM NaOAc, 100 mM NaCl and 10 mM MgCl₂) at pH 5.0. All dissociation curves were run with a ramp rate of 3°C/minute. X symbolises TINA.

Oligo name	AD sequence	D-256	D-445	D-446	D-447	D-448	D-449	D-450	D-451	D-452	D-498	D-499	D-500	D-501	D-502	D-503
TFO sequence	All triplexes consist of a target sequence (D-200: 5' ATTO495-AGGGAAAAGAAGGGAGGGATAAC) + an antiparallel duplex (AD) sequence with or without TINA (columns) + a triplex forming oligonucleotide (TFO) sequence with or without TINA (rows).	5' GTTATCCCTCCCTCTTTCCTTCCCT 3'	GTTATCCCTCCCTCTTTCCTTCCCTX	GTTATCCCTCCCTCTTTCCTTCCCT	GTTATCCCTCCCTCTTTCCTTCCCT	GTTATCCCTCCCTCTTTCCTTCCCT	GTTATCCCTCCCTCTTTCCTTCCCT	GTTATCCCTCCCTCTTTCCTTCCCT	GTTATCCCTCCCTCTTTCCTTCCCT	GTTATCCCTCCCTCTTTCCTTCCCT	GTTATCCCTCCCTCTTTCCTTCCCT	GTTATCCCTCCCTCTTTCCTTCCCT	GTTATCCCTCCCTCTTTCCTTCCCT	GTTATCCCTCCCTCTTTCCTTCCCT	GTTATCCCTCCCTCTTTCCTTCCCT	GTTATCCCTCCCTCTTTCCTTCCCT
LC D-284	5' ATTO590-TCCCTTTTCTTCCCTCCCT 3'	73.51	77.15	75.09	77.64	76.70	78.65	79.92	77.07	74.45	75.61	78.65	80.37	79.79	80.73	79.63
LC D-285	ATTO590-XTCCCTTTTCTTCCCTCCCT	75.03	77.55	77.03	79.66	78.34	80.37	81.52	78.55	76.03	77.43	80.58	82.15	81.60	82.44	81.29
LC D-286	ATTO590-TXCCCTTTTCTTCCCTCCCT	74.07	77.26	75.93	78.56	77.69	79.76	80.96	77.79	75.3	76.58	79.92	81.53	80.84	81.76	80.59
LC D-287	ATTO590-TCXCCTTTTCTTCCCTCCCT	74.18	77.59	74.00	77.62	77.10	79.15	80.36	77.48	74.85	75.77	79.09	80.81	80.01	80.84	78.61
LC D-288	ATTO590-TCCXCCTTTTCTTCCCTCCCT	74.18	77.75	73.03	76.78	76.67	78.62	79.91	77.22	74.75	74.93	78.38	79.87	79.48	79.95	77.55
LC D-289	ATTO590-TCCCXTTTTCTTCCCTCCCT	75.61	79.19	76.36	77.05	77.14	79.25	80.79	78.22	76.20	75.30	78.5	79.98	79.25	78.97	80.56
LC D-290	ATTO590-TCCCTXTTTTCTTCCCTCCCT	78.16	81.26	78.58	77.42	78.71	80.91	82.39	80.43	78.69	76.49	79.57	80.93	79.40	76.42	82.39
LC D-291	ATTO590-TCCCTTXXTTTCTTCCCTCCCT	78.39	81.50	78.75	72.82	78.96	81.02	82.53	80.74	78.92	76.38	80.04	80.61	76.08	81.05	82.62
LC D-292	ATTO590-TCCCTTTTXXCTTCCCTCCCT	79.46	82.37	79.95	78.58	79.89	81.63	83.21	81.70	80.00	77.29	79.87	76.6	80.23	82.12	83.40
LC D-293	ATTO590-TCCCTTTTXXCTTCCCTCCCT	78.92	81.16	79.57	79.38	78.11	80.96	82.58	80.94	79.36	75.26	75.04	80.28	80.58	81.68	83.00
LC D-294	ATTO590-TCCCTTTTXXCTTCCCTCCCT	78.26	80.43	79.29	79.21	74.38	80.48	82.08	80.38	78.74	72.87	79.27	81.38	80.11	81.68	82.99
LC D-295	ATTO590-TCCCTTTTCTXXTCCCTCCCT	79.31	81.51	80.25	80.95	79.13	81.23	82.93	81.50	79.78	72.21	79.31	81.73	81.44	82.63	83.66
LC D-296	ATTO590-TCCCTTTTCTTXXCCTCCCT	78.84	80.83	79.84	81.03	79.28	79.96	82.47	81.28	79.40	76.05	75.03	80.70	81.09	82.43	83.49
LC D-297	ATTO590-TCCCTTTTCTTXXCCTCCCT	78.29	80.17	79.31	80.75	78.96	76.76	82.01	80.70	78.77	77.36	79.84	77.97	80.49	82.07	82.99
LC D-298	ATTO590-TCCCTTTTCTTCCXCTCCCT	78.31	80.41	79.37	80.96	79.66	80.68	81.90	80.73	78.88	77.51	80.88	81.44	79.19	81.83	83.01
LC D-299	ATTO590-TCCCTTTTCTTCCXCTCCCT	78.15	80.32	79.25	80.71	79.23	81.66	80.08	80.51	78.49	77.61	80.29	82.28	81.68	79.39	82.77
LC D-300	ATTO590-TCCCTTTTCTTCCCTXXCCT	79.09	81.71	80.70	81.85	80.46	82.16	76.83	81.64	79.89	78.53	81.49	82.94	83.01	83.14	82.77
LC D-301	ATTO590-TCCCTTTTCTTCCCTXXCCT	76.97	79.88	78.84	80.76	79.42	81.47	81.85	79.14	77.79	77.39	80.32	82.19	81.99	83.17	80.74
LC D-302	ATTO590-TCCCTTTTCTTCCCTXXCT	77.64	80.19	79.10	81.18	79.80	81.47	82.5	78.61	77.72	77.79	80.72	82.31	82.10	83.20	83.19
LC D-303	ATTO590-TCCCTTTTCTTCCCTXXCT	77.94	80.87	79.81	81.59	80.25	81.77	82.71	79.06	77.87	78.27	81.02	82.55	82.45	83.54	84.12
LC D-304	ATTO590-TCCCTTTTCTTCCCTXXCT	78.36	81.31	80.16	81.92	80.62	82.10	82.75	79.99	77.74	78.71	81.30	82.93	82.57	83.71	83.94
	Setup and run control D-200 + D-256 + D-284		73.50	73.48	73.44	73.56	73.50	73.52	73.55	73.59	73.56	73.53	73.56	73.47	73.59	73.56
LC D-284	5' ATTO590-TCCCTTTTCTTCCCTCCCT 3'	73.51	77.08	75.22	77.71	76.90	78.75	80.22	76.97	74.39	75.91	78.72	80.48	79.77	80.67	79.6
LC D-305	ATTO590-TCCCTTTTCTTXXCCTCCCT	75.66	78.38	77.13	76.69	73.13	77.13	79.29	77.38	75.96	69.62	74.34	77.88	77.28	79.47	80.67
LC D-306	ATTO590-TCCCTTTTCTTXXCCTCCCT	78.51	81.89	80.19	79.30	75.43	78.28	81.54	80.47	79.10	72.73	73.67	79.16	79.19	80.96	82.86
LC D-307	ATTO590-TCCCTTTTCTTXXCCTCCCT	80.22	83.86	81.44	81.04	79.12	79.99	82.97	81.96	80.72	74.96	70.61	79.63	81.08	82.14	84.13
LC D-308	ATTO590-TCCCTTTTCTTXXCCTCCCT	80.00	83.48	81.35	80.98	79.09	77.38	82.67	81.84	80.46	76.59	76.00	77.26	80.52	81.89	84.04
LC D-309A	ATTO590-TCCCTTTTCTTXXCCTCCCT	81.13	84.89	82.15	81.09	80.66	78.48	83.70	82.79	81.51	78.34	80.52	74.11	80.85	82.82	84.96
LC D-309B	ATTO590-TCCCTTTTCTTXXCCTCCCT	80.13	83.72	81.24	80.15	80.37	81.20	82.49	81.93	80.57	77.74	81.12	77.32	79.12	81.95	84.01
LC D-310	ATTO590-TCCCTTTTCTTXXCCTCCCT	80.31	84.06	81.29	75.78	80.25	81.45	82.86	82.02	80.84	77.82	81.72	81.45	75.09	82.09	84.44
LC D-311	ATTO590-TCCCTTTTCTTXXCCTCCCT	80.61	84.75	81.71	76.14	80.51	82.78	81.78	82.47	81.20	78.51	81.62	82.74	78.28	80.44	84.54
LC D-312	ATTO590-TCCCTTTTCTTXXCCTCCCT	80.54	84.34	81.70	80.60	80.24	82.77	81.75	82.38	80.90	78.21	81.37	83.01	81.58	75.61	84.54
LC D-313	ATTO590-TCCCTTTTCTTXXCCTCCCT	81.98	85.85	83.34	81.93	81.67	83.40	79.67	83.45	82.59	79.55	82.48	83.84	82.96	80.11	84.93
LC D-314	ATTO590-TCCCXTTTTCTTCCCTXXCCT	79.75	83.81	81.23	81.64	80.42	82.18	77.65	82.04	80.46	78.19	81.26	82.80	82.60	82.24	83.51
LC D-229	ATTO590-TCCCXTTTTCTTCCCTXXCCT	79.98	84.16	81.67	81.95	80.75	82.84	83.58	81.90	80.79	78.46	81.53	83.30	82.72	83.27	83.07
LC D-230	ATTO590-TCCXCCTTTTCTTCCCTXXCCT	79.14	83.11	77.26	81.67	80.44	82.35	83.13	81.41	79.91	78.17	81.12	83.00	82.75	83.81	79.87
LC D-231	ATTO590-TCCXCCTTTTCTTCCCTXXCT	79.49	83.42	77.17	81.91	80.71	82.27	83.73	80.82	79.84	78.57	81.41	82.95	82.89	83.69	82.83
LC D-232	ATTO590-TCCXCCTTTTCTTCCCTXXCT	78.49	81.63	78.48	82.14	80.8	82.44	83.66	80.46	79.28	78.55	81.36	83.32	83.13	84.08	83.55
LC D-233	ATTO590-TCCXCCTTTTCTTCCCTXXCT	78.98	81.87	78.85	82.38	81.13	82.69	83.93	80.49	79.09	78.70	81.66	83.47	83.02	84.43	84.53
LC D-234	ATTO590-TCCXCCTTTTCTTCCCTXXCT	79.20	80.66	81.18	83.47	81.95	83.58	84.72	81.41	79.84	79.63	82.34	84.25	83.94	85.26	86.04
LC D-235	ATTO590-TCCXCCTTTTCTTCCCTXXCT	79.81	80.98	81.53	83.77	82.35	83.72	84.82	82.15	79.76	79.96	82.81	84.52	84.18	85.10	85.46
LC D-236	ATTO590-TCCXCCTTTTCTTCCCTXXCT	80.95	81.43	82.22	84.23	82.86	84.24	85.25	83.10	80.94	80.79	83.28	84.87	84.68	85.79	85.85
	Setup and run control D-200 + D-256 + D-284		73.44	73.52	73.65	73.56	73.45	73.59	73.56	73.56	73.59	73.50	73.59	73.62	73.56	73.50

Supplementary data - table 4. TFOs with different length and number of TINAs

T_m data for parallel triplexes with different TFO lengths and zero to three TINAs, base mismatches in the target strand and D-256 as AD strand. T_m for parallel triplexes and parallel duplexes was determined using 1.0 μM of each strand in sodium acetate buffer (50 mM NaOAc, 100 mM NaCl and 10 mM MgCl₂) at pH 4.5. For each run a setup control was included. This control consisted of D-256, D-284 and D-200 to D-206 in sodium acetate buffer at pH 5.0 and are comparable with data from supplementary data table 1. X symbolises TINA. All dissociation curves were run with a ramp rate of 3°C/minute.

Oligo name	Target sequence	D-200	D-201	D-202	D-203	D-204	D-205	D-206	
TFO sequence	All triplexes consist of a target sequence (columns) with or without base mismatch + an antiparallel duplex (AD) sequence (D-256: 5' GTTATCCCTCCCTTCTTTCCCT 3') + a triplex forming oligonucleotide (TFO) sequence with different length and number of TINAs (rows).	5' ATTO495-AGGGAAAAGAAAGGGAGGGATAAC 3'	ATTO495-AGGC AAAAGAAAGGGAGGGATAAC	ATTO495-AGG A AAAAGAAAGGGAGGGATAAC	ATTO495-AGG T AAAAGAAAGGGAGGGATAAC	ATTO495-AGGGAA C AGAAGGGAGGGATAAC	ATTO495-AGGGAA G AGAAGGGAGGGATAAC	ATTO495-AGGGAA T AGAAGGGAGGGATAAC	
LC D-284	ATTO590-TCCCTTTTCTTCCCTCCCT	83.87	75.55	75.23	75.44	72.50	74.23	72.08	
LC D-407	ATTO590-TCCCTTTTCTTCCCTC	77.73	65.77	64.04	65.92	62.04	63.75	61.08	
LC D-408	ATTO590-TCCCTTTTCTTCC	67.86	51.05	45.68	51.20	48.50	52.11	49.43	
LC D-409	ATTO590-TCCCTTTTCT	57.87	38.95	38.51	39.10	39.54	38.43	38.78	
LC D-464	ATTO590-TCCCTTTTC	50.82							
LC D-461	ATTO590-TCCCTTTT	39.90							
LC D-304	ATTO590-TCCCTTTTCTTCCCTCCCT X	86.73	78.32	78.47	78.08	75.41	77.28	75.33	
LC D-589	ATTO590-TCCCTTTTCTTCCCTC X	82.84	71.71	71.01	71.57	68.85	71.06	68.27	
LC D-588	ATTO590-TCCCTTTTCTTCC X	74.12	60.94	57.22	61.23	53.70	56.44	52.47	
LC D-467	ATTO590-TCCCTTTTCT X	64.48	46.41			44.97	46.53	41.35	42.37
LC D-465	ATTO590-TCCCTTTTC X	58.49				39.95	40.06		
LC D-462	ATTO590-TCCCTTTT X	50.27				40.13	39.85	39.55	
LC D-459	ATTO590-TCCCTTT X	47.83				42.37	42.36	42.53	
LC D-236	ATTO590- X TCCCTTTTCTTCCCTCCCT X	88.31	79.84	80.02	79.75	77.49	79.16	77.64	
LC D-587	ATTO590- X TCCCTTTTCTTCCCTC X	85.29	74.59	74.33	74.42	71.65	73.75	71.18	
LC D-586	ATTO590- X TCCCTTTTCTTCC X	77.70	63.17	61.54	63.57	57.67	60.23	58.61	
LC D-585	ATTO590- X TCCCTTTTCT X	68.84	49.92	46.19	50.89	49.68	52.59	49.66	
LC D-514	ATTO590- X TCCCTTTTC X	66.49	41.14			41.64	48.30	47.34	
LC D-512	ATTO590- X TCCCTTTT X	64.45				51.24	46.01		
LC D-509	ATTO590- X TCCCTTT X	64.30				54.77	51.77	59.11	
LC D-584	ATTO590- X TCCCTTTTCT X TCCCTCCCT X	88.36	78.36	79.61	78.29	77.79	79.65	77.66	
LC D-583	ATTO590- X TCCCTTTTCT X TCCCTCCC X	88.84	79.34	80.32	79.25	78.53	80.23	78.39	
LC D-582	ATTO590- X TCCCTTTTCT X TCCCTC X	86.90	75.31	79.73	75.23	76.77	76.71	76.68	
LC D-581	ATTO590- X TCCCTTTTCT X TCCC X	84.57	70.54	74.90	70.61	76.41	74.87	76.32	
LC D-580	ATTO590- X TCCCTTTTCT X TCTC X	81.79	64.17	65.63	64.44	69.71	67.28	69.04	
LC D-470	ATTO590- X TCCCTTTTCT X	76.27	56.98	59.79	56.27				
LC D-515	ATTO590- X TCCCTTTTCT X	70.51	43.90	45.22	44.40	52.71	53.24	55.65	
LC D-513	ATTO590- X TCCCCTTTT X	64.17	42.96	48.19	43.34	51.89	45.99		
LC D-579	ATTO590- X TCCCCTTTT X	58.91	40.61	42.08	40.52	53.55	48.22	55.02	
Control	Setup and run control D-256 + D-284 + D-200 to D-206 at pH 5.0	73.43	65.84	64.92	65.68	61.08	62.87	60.76	

Supplementary data - table 5. LightCycler ramp rate and influence on Tm determinations

Tm data for parallel triplexes from table 1 determined using 1.0 μM of each strand in sodium acetate buffer (50 mM NaOAc, 100 mM NaCl and 10 mM MgCl₂) at pH 5.0. X symbolises TINA. Shattered boxes symbolises that Tm could not be determined. The basic LightCycler programs are described below.

Basic LightCycler2.0 dissociation program

- 1) Heating to 90°C at a ramp rate of 0.2°C/second and hold for 15 seconds.
- 2) Cooling to 37°C at a ramp rate of 0.2°C/second and hold for Y minutes before
- 3) Heating to 90°C at a ramp rate of 0.05°C/second and continuous measurements or heating with a ramp rate of 0.05°C/second with hold for Z seconds before single measurements (one measurement per °C).

Basic LightCycler2.0 annealing program

- 1) Heating to 90°C at a ramp rate of 0.2°C/second and hold for Y minutes.
- 2) Cooling to 37°C at a ramp rate of 0.05°C/second and continuous measurements or cooling with a ramp rate of 0.05°C/second with hold for Z seconds before single measurements (one measurement per °C).

LightCycler program type ramp rate (°C/minute)	Dissociation				Annealing			
	3	1	0.5	0.2	3	1	0.5	0.2
Y (minutes)	5	30	30	30	5	10	10	10
Z (seconds)	none	40	100	280	none	40	100	280

TFO sequence	LightCycler2.0 program. Influence of ramp rate on determination of Tm. All triplexes consist of D-200 (5' ATTO495-AGGGAAAAGAAGGGAGGGATAAC 3') as target sequence with D-256 (5' GTTATCCCTCCCTTCTTTCCCT 3') as antiparallel duplex strand (AD) and different triplex forming oligonucleotides (TFOs)	Dissociation ramp rate 3°C/minute	Dissociation ramp rate	Dissociation ramp rate	Dissociation ramp rate	Annealing ramp rate	Annealing ramp rate	Annealing ramp rate	Annealing ramp rate
			1°C/minute	0.5°C/minute	0.2°C/minute	3°C/minute	1°C/minute	0.5°C/minute	0.2°C/minute
LC D-284	ATTO590-TCCCTTTTCTTCCCTCCCT	73.49	71.23	70.81	70.43	68.31	69.48	69.80	69.80
LC D-293	ATTO590-TCCCTTTT X CTTCCCTCCCT	78.71	76.11	74.89	73.37	66.74	68.37	69.51	70.69
LC D-294	ATTO590-TCCCTTTT CX TTCCCTCCCT	78.24	75.32	74.13	72.61	66.36	68.14	69.29	70.23
LC D-295	ATTO590-TCCCTTTT CTX TCCCTCCCT	79.18	76.55	75.21	73.69	66.76	68.60	69.74	70.89
LC D-297	ATTO590-TCCCTTTT CTTCX CTCCCT	78.12	75.00	73.8	72.39	66.78	68.83	69.86	70.66
LC D-299	ATTO590-TCCCTTTT CTTCCX CTCCCT	77.87	74.89	73.58	72.50	67.38	69.40	70.32	71.12
LC D-300	ATTO590-TCCCTTTT CTTCCCTX CCCT	78.95	76.08	74.93	73.44	67.85	69.86	70.89	71.84
LC D-301	ATTO590-TCCCTTTT CTTCCCTC XCT	76.61	73.72	72.72	71.83	67.73	69.51	70.38	70.78
LC D-304	ATTO590-TCCCTTTT CTTCCCTCCCTX	78.20	75.21	74.74	73.15	69.11	70.89	71.77	72.38
LC D-305	ATTO590-TCCCTTTT CXTX TCCCTCCCT	75.46	72.62	71.47	70.22	60.82	66.42	66.53	68.02
LC D-307	ATTO590-TCCCTTTT XCTT XCCCTCCCT	80.32	77.82	76.73	75.10	62.08	66.87	67.10	69.20
LC D-309A	ATTO590-TCCCTTTT XCTT CXCTCCCT	81.05	78.58	77.49	76.08	63.16	67.22	67.79	69.78
LC D-310	ATTO590-TCCCTTT XTTCTT CCXCTCCCT	80.34	77.71	76.52	75.00	62.23	66.53	67.79	69.32
LC D-313	ATTO590-TCCCT XTTTCTT CCCTXCCT	81.80	79.12	78.17	76.41	64.48	67.56	68.60	70.46
LC D-231	ATTO590-TCC XCTTTTCTT CCCTCCXCT	79.31	76.36	75.21	73.69	67.63	69.29	70.43	71.50
LC D-236	ATTO590- X TCCCTTTTCTTCCCTCCCT X	80.96	78.13	76.87	75.44	69.93	71.58	72.50	73.22
LC D-341	ATTO590-TCCCTTTTCTTCCCTCC CTX	76.97	73.88	72.94	72.04	68.56	70.23	70.86	71.24
LC D-343	ATTO590-TCCCTTTTCTTCCCT CXCTX	80.16	77.55	76.16	74.55	68.21	70.35	71.21	72.19
LC D-344	ATTO590-TCCCTTTTCTTCCCT XCCCTX	81.83	79.12	77.93	76.31	67.51	69.63	70.66	72.19
LC D-346	ATTO590-TCCCTTTTCTTCC XCT CCCT X	82.34	79.65	78.48	76.86	67.60	69.78	70.78	72.19
LC D-347	ATTO590-TCCCTTTTCTT CXCT CCCT X	81.71	78.91	77.67	76.16	67.05	69.09	70.09	71.38
LC D-237	ATTO590-TCCCTTTT XCTT CCCTCCCT X	82.21	79.55	78.36	76.66	67.16	69.20	70.17	71.81
LC D-426	ATTO590-TCCCTTTT CXTX TCCCTCCCT	73.46	70.65	69.46	68.02				
LC D-428	ATTO590-TCCCTTTT XCTX TCXCTCCCT	80.57	78.25	77.32	75.93				
LC D-254	ATTO590-TCCCTTTT XCTT CXCTC XCT	83.23	80.69	79.79	78.14		67.37	66.42	67.59
LC D-435	ATTO590-TCCCTTT XCTT CXCTCC CTX	84.61	82.32	81.27	79.76	64.61	66.91	67.33	68.97
LC D-584	ATTO590- X TCCCTTTTCT X TCCCTCCCT X	83.64	80.97	79.88	78.02	68.06	69.63	70.55	71.70
LC D-438	ATTO590- X TCCCTT XTTCTT CXCTCC CTX	86.91	84.46	83.37	82.04	65.96	68.02	68.83	70.20

Supplementary data - table 6. Influence of preincubation time before dissociation curve acquisition

T_m data for parallel triplexes from table 1 determined using 1.0 μM of each strand in sodium acetate buffer (50 mM NaOAc, 100 mM NaCl and 10 mM MgCl₂) at pH 5.0. X symbolises TINA.

Basic LightCycler2.0 dissociation program

- 1) Heating to 90°C at a ramp rate of 0.2°C/second and hold for 15 seconds.
- 2) Cooling to 37°C at a ramp rate of 0.2°C/second and hold for Y minutes before
- 3) Heating to 90°C at a ramp rate of 0.05°C/second and continuous measurements or heating with a ramp rate of 0.05°C/second with hold for Z seconds before single measurements (one measurement per °C).

LightCycler program type ramp rate (°C/minute)	Dissociation							
	3	3	3	3	0.2	0.2	0.2	0.2
Y (minutes)	5	30	60	1440	5	30	60	1440
Z (seconds)	none	none	none	none	280	280	280	280

TFO sequence	LightCycler2.0 program. Influence of preincubation time on determination of T _m . All triplexes consist of D-200 (5' ATTO495-AGGGAAAAGAAGGGAGGGATAAC 3') as target sequence with D-256 (5' GTTATCCCTCCCTTCTTTCCCT 3') as antiparallel duplex strand (AD) and different triplex forming oligonucleotides (TFOs)	Hold for 5 minutes (ramp rate 3°C/minute)	Hold for 30 minutes (ramp rate 3°C/minute)		Hold for 24 hours (ramp rate 3°C/minute)	Hold for 5 minutes (ramp rate 0.2°C/minute)	Hold for 30 minutes (ramp rate 0.2°C/minute)		Hold for 24 hours (ramp rate 0.2°C/minute)
LC D-284	ATTO590-TCCCTTTTCTTCCCTCCCT	73.49	73.20	73.28	73.21	70.23	70.43	70.38	69.94
LC D-293	ATTO590-TCCCTTTT X CTTCCCTCCCT	78.71	78.38	78.39	78.46	72.94	73.37	73.14	72.59
LC D-294	ATTO590-TCCCTTTT C XTTCCCTCCCT	78.24	77.72	77.86	77.85	72.32	72.61	72.67	71.99
LC D-295	ATTO590-TCCCTTTTCT X TCCCTCCCT	79.18	78.77	78.96	79.04	73.14	73.69	73.55	72.82
LC D-297	ATTO590-TCCCTTTTCTT C XCCTCCCT	78.12	77.61	77.88	77.77	72.21	72.39	72.36	71.79
LC D-299	ATTO590-TCCCTTTTCTTCCC X TCCCT	77.87	77.38	77.57	77.65	72.09	72.50	72.36	71.90
LC D-300	ATTO590-TCCCTTTTCTTCCCT X CCCT	78.95	78.56	78.51	78.71	73.18	73.44	73.46	72.82
LC D-301	ATTO590-TCCCTTTTCTTCCCT C XCCT	76.61	76.18	76.24	76.50	71.68	71.83	71.77	71.21
LC D-304	ATTO590-TCCCTTTTCTTCCCTCCCT X	78.20	77.72	77.74	77.94	72.87	73.15	73.14	72.59
LC D-305	ATTO590-TCCCTTTT C X T X TCCCTCCCT	75.46	75.17	75.35	75.43	69.65	70.22	70.17	69.37
LC D-307	ATTO590-TCCCTTTT X CTT X CCCTCCCT	80.32	79.81	79.99	80.01	74.09	75.10	74.88	73.40
LC D-309A	ATTO590-TCCCTTT X TCTT C XCCCTCCCT	81.05	80.59	80.82	80.75	75.23	76.08	75.84	74.55
LC D-310	ATTO590-TCCCTT X TTCTTCC X CTCCCT	80.34	79.85	80.10	79.90	74.07	75.00	74.58	73.40
LC D-313	ATTO590-TCCCT X TTTCTTCCCT X CCCT	81.80	81.37	81.56	81.66	75.68	76.41	76.35	74.98
LC D-231	ATTO590-TCC X CTTTTCTTCCCTCC X CT	79.31	78.60	79.01	78.95	73.13	73.69	73.54	73.00
LC D-236	ATTO590- X TCCCTTTTCTTCCCTCCCT X	80.96	80.64	80.81	80.90	75.00	75.44	75.27	74.52
LC D-341	ATTO590-TCCCTTTTCTTCCCTCCC X T X	76.97	76.52	76.80	76.92	71.68	72.04	71.96	71.29
LC D-343	ATTO590-TCCCTTTTCTTCCCT C XCCCT X	80.16	79.86	80.08	80.09	73.77	74.55	74.24	73.64
LC D-344	ATTO590-TCCCTTTTCTTCCCT X CCCT X	81.83	81.49	81.65	81.72	75.55	76.31	76.05	75.21
LC D-346	ATTO590-TCCCTTTTCTTCC X CTCCCT X	82.34	82.20	82.12	82.22	76.01	76.86	76.28	75.64
LC D-347	ATTO590-TCCCTTTTCTT C XCCCTCCCT X	81.71	81.47	81.58	81.62	75.27	76.16	75.70	74.78
LC D-237	ATTO590-TCCCTTTT X CTTCCCTCCCT X	82.21	81.87	82.00	81.99	76.04	76.66	76.51	75.70
LC D-426	ATTO590-TCCCTTTT C X T X TCCCTCCCT	73.46	72.99	73.17	73.06	67.23	68.02	67.91	66.49
LC D-428	ATTO590-TCCCTTTT X CT X T C XCCCTCCCT	80.57	80.24	80.27	80.19	75.47	75.93	75.81	75.01
LC D-254	ATTO590-TCCCTTTT X CTT C XCCCT C XCCCT	83.23	82.76	82.86	82.80	77.67	78.14	78.21	77.32
LC D-435	ATTO590-TCCCTTTT X TCTT C XCCCTCCC X T	84.61	84.41	84.53	84.54	79.62	79.76	79.92	78.93
LC D-584	ATTO590- X TCCCTTTTCT X TCCCTCCCT X	83.64	83.42	83.54	83.38	77.10	78.02	77.69	76.71
LC D-438	ATTO590- X TCCCTT X TTCTT C XCCCTCCC X T	86.91	86.49	86.51	86.67	81.78	82.04	82.19	81.12

Supplementary data - table 7. Influence of pH on Tm determinations

Tm data for parallel triplexes from table 1 determined using 1.0 µM of each strand in sodium acetate buffer (50 mM NaOAc, 100 mM NaCl and 10 mM MgCl₂) at pH 5.0, 5.5 or 6.0. X symbolises TINA. Shattered boxes symbolises that Tm could not be determined. Melting curves for Tms marked in bold are shown as examples of melting curves at the end of supplementary data.

Basic LightCycler2.0 dissociation program

- 1) Heating to 90°C at a ramp rate of 0.2°C/second and hold for 15 seconds.
- 2) Cooling to 37°C at a ramp rate of 0.2°C/second and hold for Y minutes before
- 3) Heating to 90°C at a ramp rate of 0.05°C/second and continuous measurements or heating with a ramp rate of 0.05°C/second with hold for Z seconds before single measurements (one measurement per °C).

Basic LightCycler2.0 annealing program

- 1) Heating to 90°C at a ramp rate of 0.2°C/second and hold for Y minutes.
- 2) Cooling to 37°C at a ramp rate of 0.05°C/second and continuous measurements or cooling with a ramp rate of 0.05°C/second with hold for Z seconds before single measurements (one measurement per °C).

LightCycler program type	Dissociation						Annealing					
ramp rate (°C/minute)	3	3	3	0.2	0.2	0.2	3	3	3	0.2	0.2	0.2
Y (minutes)	5	5	5	30	30	30	5	5	5	10	10	10
Z (seconds)	none	none	none	280	280	280	none	none	none	280	280	280
pH	5.0	5.5	6.0	5.0	5.5	6.0	5.0	5.5	6.0	5.0	5.5	6.0

TFO sequence	LightCycler2.0 program. Influence of pH on determination of Tm. All triplexes consist of D-200 (5' ATTO495-AGGGAAAAGAGGGAGGGATAAC 3') as target sequence with D-256 (5' GTTATCCCTCCCTTCTTTCCCT 3') as antiparallel duplex strand (AD) and different triplex forming oligonucleotides (TFOs)	Dissociation			Dissociation			Annealing			Annealing		
		pH 5.0 (dissociation ramp rate 3°C/minute)	pH 5.5 (dissociation ramp rate 3°C/minute)	pH 6.0 (dissociation ramp rate 3°C/minute)	pH 5.0 (dissociation ramp rate 0.2°C/minute)	pH 5.5 (dissociation ramp rate 0.2°C/minute)	pH 6.0 (dissociation ramp rate 0.2°C/minute)	pH 5.0 (annealing ramp rate 3°C/minute)	pH 5.5 (annealing ramp rate 3°C/minute)	pH 6.0 (annealing ramp rate 3°C/minute)	pH 5.0 (annealing ramp rate 0.2°C/minute)	pH 5.5 (annealing ramp rate 0.2°C/minute)	pH 6.0 (annealing ramp rate 0.2°C/minute)
LC D-284	ATTO590-TCCCTTTTCTTCCCTCCCT	73.49	60.29	43.69	70.43	57.28	38.08	68.31	55.39		69.80	56.98	37.43
LC D-293	ATTO590-TCCCTTTT X CTTCCCTCCCT	78.71	67.40	53.08	73.37	62.48	47.34	66.74	53.51		70.69	60.71	40.68
LC D-294	ATTO590-TCCCTTTT CX TTCCCTCCCT	78.24	66.55	51.57	72.61	61.21	45.94	66.36	52.32		70.23	59.39	40.74
LC D-295	ATTO590-TCCCTTTTCT X TCCCTCCCT	79.18	67.70	52.84	73.69	62.51	47.16	66.76	54.08		70.89	61.16	42.80
LC D-297	ATTO590-TCCCTTTTCTT CX CTCCCT	78.12	65.71	50.72	72.39	61.02	44.94	66.78	57.17		70.66	60.44	41.47
LC D-299	ATTO590-TCCCTTTTCTTCC CX TCCCT	77.87	65.69	50.07	72.50	60.83	44.54	67.38	58.76		71.12	60.62	42.40
LC D-300	ATTO590-TCCCTTTTCTTCCCT X CCCT	78.95	67.33	51.85	73.44	62.89	46.34	67.85	60.43		71.84	62.79	44.52
LC D-301	ATTO590-TCCCTTTTCTTCCCT CX CT	76.61	64.51	48.83	71.83	60.44	43.21	67.73	59.08		70.78	60.26	41.87
LC D-304	ATTO590-TCCCTTTTCTTCCCT CX CT	78.20	65.76	49.61	73.15	62.38	44.81	69.11	61.93		72.38	62.37	43.99
LC D-305	ATTO590-TCCCTTTT CX TCCCTCCCT	75.46	64.41	49.19	70.22	58.40	43.46	60.82	50.60		68.02	54.05	
LC D-307	ATTO590-TCCCTTTT X CTTCCCTCCCT	80.32	70.01	57.46	75.10	65.09	51.53	62.08	52.51		69.20	61.38	45.78
LC D-309A	ATTO590-TCCCTTTT X TCTTCCCTCCCT	81.05	70.88	57.86	76.08	66.06	51.80	63.16	61.61		69.78	63.55	46.24
LC D-310	ATTO590-TCCCTTTTCTTCC X CTCCCT	80.34	69.91	56.07	75.00	64.71	50.52	62.23	62.47		69.32	62.46	45.12
LC D-313	ATTO590-TCCCT X TTTCTTCCCT X CCCT	81.80	71.85	58.75	76.41	67.37	53.48	64.48	64.67		70.46	66.45	50.75
LC D-231	ATTO590-TCC X TTTTCTTCCCTCC X CT	79.31	68.35	53.64	73.69	63.93	48.68	67.63	64.03		71.50	63.79	46.25
LC D-236	ATTO590- X TCCCTTTTCTTCCCTCCCT X	80.96	70.10	52.11	75.44	68.39	49.94	69.93	68.21		73.22	68.23	50.15
LC D-341	ATTO590-TCCCTTTTCTTCCCTCC CX T X	76.97	64.56	47.94	72.04	60.83	42.84	68.56	60.08		71.24	60.38	42.13
LC D-343	ATTO590-TCCCTTTTCTTCCCTC X CT X	80.16	68.62	52.91	74.55	64.22	47.01	68.21	62.28		72.19	63.91	45.58
LC D-344	ATTO590-TCCCTTTTCTTCCCT X CCCT X	81.83	71.11	56.18	76.31	66.35	50.32	67.51	62.37		72.19	65.54	47.43
LC D-346	ATTO590-TCCCTTTTCTTCC X CTCCCT X	82.34	71.95	56.69	76.86	67.03	50.72	67.60	59.69		72.19	65.18	46.98
LC D-347	ATTO590-TCCCTTTTCTT CX CTCCCT X	81.71	70.93	55.72	76.16	65.87	49.61	67.05	58.64		71.38	64.64	46.44
LC D-237	ATTO590-TCCCTTTT X CTTCCCTCCCT X	82.21	72.02	58.31	76.66	67.61	52.33	67.16	62.47		71.81	66.26	47.04
LC D-426	ATTO590-TCCCTTTT CX T X TCCCTCCCT	73.46	63.47		68.02	57.01	43.58						
LC D-428	ATTO590-TCCCTTTT X CT X TCCCTCCCT	80.57	72.73		75.93	67.23	54.89					59.17	
LC D-254	ATTO590-TCCCTTTT X CTTCCCTC X CT	83.23	75.67		78.14	70.14	57.37				67.59	61.38	49.22
LC D-435	ATTO590-TCCCTTTT X TCTTCCCTCC CX T	84.61	76.25	62.70	79.76	71.00	57.41	64.61	57.87		68.97	66.45	50.23
LC D-584	ATTO590- X TCCCTTTTCT X TCCCTCCCT X	83.64	72.95	56.95	78.02	68.29	51.01	68.06	64.19		71.70	67.89	49.99
LC D-438	ATTO590- X TCCCTTTTCTTCCCTCC CX T	86.91	79.04	65.30	82.04	73.43	59.59	65.96	63.24		70.20	68.44	52.74

Supplementary data - table 8. Influence of buffer and pH on ΔTm of antiparallel duplexes and parallel triplexes

Parallel triplexes consisting of LC D-256 (5' GTTATCCCTCCCTTCTTTCCCT 3') as antiparallel duplex strand, LC D-284 (5' ATTO590-TCCCTTTTCTCCCTCCCT 3') as triplex forming oligonucleotide (TFO) and different target strands (LC D-200 to LC D-224) using 1.0 μM of each oligonucleotide in sodium acetate buffer (50 mM NaOAc, 100 mM NaCl and 10 mM MgCl₂) at pH 5.0, 5.5 or 6.0.

Antiparallel duplexes consisting of LC D-484 (5' GTTATCCCTCCCTTCTTTCCCT-ATTO590 3') as antiparallel duplex strand and different target strands (LC D-200 to LC D-224) using 0.5 μM of each oligonucleotide in either sodium acetate buffer (50 mM NaOAc, 100 mM NaCl and 10 mM MgCl₂) at pH 5.0, 5.5 or 6.0 or phosphate buffer (50 mM NaH₂PO₄/Na₂HPO₄, 100 mM NaCl and 0.1 mM EDTA) at pH 5.5, 6.0 and 7.0.

base mismatches are marked in bold. The lower temperature limit of the LightCycler2.0 is 37°C.

Basic LightCycler2.0 dissociation program

- 1) Heating to 90°C at a ramp rate of 0.2°C/second and hold for 15 seconds.
- 2) Cooling to 37°C at a ramp rate of 0.2°C/second and hold for Y minutes (Y either 5 minutes or 30 minutes) before
- 3) Heating to 90°C at a ramp rate of 0.05°C/second and continuous measurements or heating with a ramp rate of 0.05°C/second with hold for 280 seconds (Z) before single measurements (one measurement per °C).

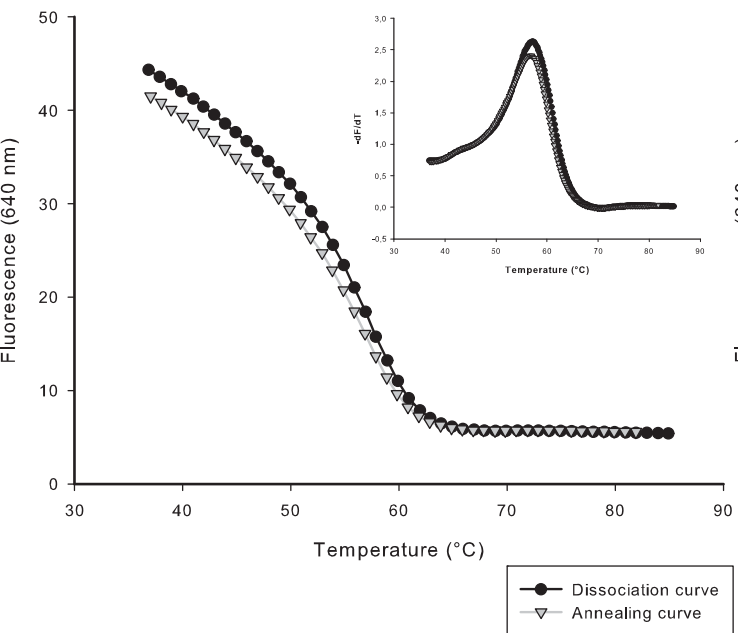
Basic LightCycler2.0 annealing program

- 1) Heating to 90°C at a ramp rate of 0.2°C/second and hold for Y minutes (Y either 5 minutes or 10 minutes).
- 2) Cooling to 37°C at a ramp rate of 0.05°C/second and continuous measurements or cooling with a ramp rate of 0.05°C/second with hold for 280 seconds (Z) before single measurements (one measurement per °C).

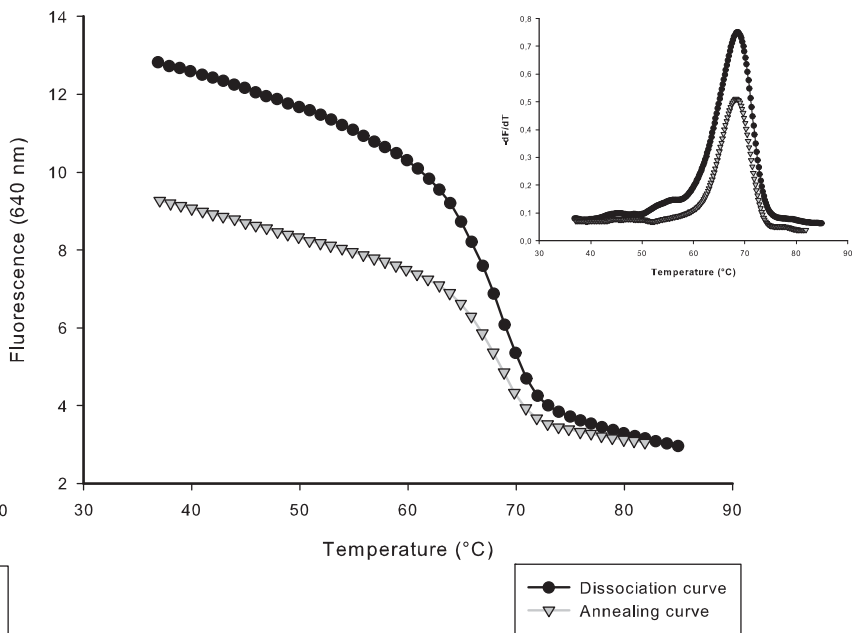
Parallel triplex (PT) or antiparallel duplex (AD)		PT		AD		PT		AD		AD		PT		AD		AD		AD		
pH		5.0		5.0		5.5		5.5		5.5		6.0		6.0		6.0		7.0		
Buffer type		Acetate		Acetate		Acetate		Acetate		Phosphate		Acetate		Acetate		Phosphate		Phosphate		
Dissociation 3°C/minute (Y = 5 minutes, Z = none)																				
LC D-200	ATTO495-AGGGAAAAGAAGGGAGGGATAAC	73.09	70.24	59.60	71.96	68.51	43.00	72.48	69.42	70.17										
LC D-201	ATTO495-AGGGAAAAGAAGGGAGGGATAAC	65.28	64.20	51.87	65.81	61.94	<37.00	66.25	62.39	63.26										
LC D-202	ATTO495-AGGG AAA AGAAGGGAGGGATAAC	64.68	65.89	51.30	66.83	63.33	<37.00	67.03	63.39	63.67										
LC D-203	ATTO495-AGGG T AAAAGAAGGGAGGGATAAC	65.39	63.54	52.11	65.74	61.06	<37.00	66.40	62.13	63.39										
LC D-204	ATTO495-AGGGAA C AGAAGGGAGGGATAAC	61.08	61.70	46.00	64.02	60.16	<37.00	64.90	61.25	62.38										
LC D-205	ATTO495-AGGGAA G AGAAGGGAGGGATAAC	62.89	68.32	48.34	69.45	62.67	<37.00	69.67	63.51	64.64										
LC D-206	ATTO495-AGGGAA T AGAAGGGAGGGATAAC	60.72	64.05	44.95	66.21	62.07	<37.00	66.50	62.98	64.02										
LC D-222	ATTO495-AGGGAAA A AAGGGAGGGATAAC	58.40	65.26	44.00	65.95	62.09	<37.00	65.44	61.65	61.24										
LC D-213	ATTO495-AGGGAAA A CAGGGAGGGATAAC	60.66	63.18	44.36	64.90	61.18	<37.00	65.77	61.90	63.73										
LC D-214	ATTO495-AGGGAAA A GAGGGAGGGATAAC	61.01	65.95	45.04	67.93	63.91	<37.00	68.65	64.75	66.15										
LC D-215	ATTO495-AGGGAAA A G T AGGGAGGGATAAC	60.99	63.74	45.26	65.73	61.56	<37.00	66.42	62.86	64.58										
LC D-218	ATTO495-AGGGAAA A GAGGGAGGGATAAC	62.80	65.32	47.59	67.37	63.60	<37.00	68.09	64.66	65.38										
LC D-207	ATTO495-AGGGAAA A GA C GAGGGATAAC	61.86	63.84	47.63	64.67	61.46	<37.00	64.86	61.18	60.66										
LC D-208	ATTO495-AGGGAAA A GA A GAGGGATAAC	60.09	65.00	46.02	65.57	62.41	<37.00	65.26	62.42	61.73										
LC D-209	ATTO495-AGGGAAA A GA T GAGGGATAAC	61.65	62.11	47.51	64.17	59.96	<37.00	64.66	60.65	61.79										
LC D-210	ATTO495-AGGGAAA A GA G CGGATAAC	66.17	63.30	54.34	64.63	60.63	<37.00	64.86	60.43	60.09										
LC D-211	ATTO495-AGGGAAA A GA A GGGAAGGATAAC	65.32	64.42	53.06	65.15	61.58	<37.00	65.06	61.27	61.27										
LC D-212	ATTO495-AGGGAAA A GA T GGATAAC	65.14	62.45	53.12	64.10	59.56	<37.00	64.92	60.33	61.37										
LC D-224	ATTO495-AGGGAAA A AGGGAGGGATAAC	52.90	60.90	39.97	62.25	57.73	<37.00	62.65	57.78	58.73										
Annealing 3°C/minute (Y = 5 minutes, Z = none)																				
LC D-200	ATTO495-AGGGAAAAGAAGGGAGGGATAAC	68.49	69.75	55.28	71.25	68.01	<37.00	71.88	69.13	70.01										
LC D-201	ATTO495-AGGGAAAAGAAGGGAGGGATAAC	61.40	63.58	47.51	65.23	61.36	<37.00	65.61	61.99	62.89										
LC D-202	ATTO495-AGGG AAA AGAAGGGAGGGATAAC	61.64	65.17	47.45	66.60	62.67	<37.00	66.57	62.80	63.21										
LC D-203	ATTO495-AGGG T AAAAGAAGGGAGGGATAAC	61.22	63.02	47.88	65.17	60.91	<37.00	66.06	61.74	63.11										
LC D-204	ATTO495-AGGGAA C AGAAGGGAGGGATAAC	57.44	61.24	43.27	63.73	59.87	<37.00	64.83	60.58	62.33										
LC D-205	ATTO495-AGGGAA G AGAAGGGAGGGATAAC	59.06	66.67	44.83	68.16	62.67	<37.00	68.76	63.39	64.39										
LC D-206	ATTO495-AGGGAA T AGAAGGGAGGGATAAC	59.15	63.64	42.68	65.51	61.64	<37.00	66.14	62.77	63.64										
LC D-222	ATTO495-AGGGAAA A AAGGGAGGGATAAC	57.06	64.66	43.25	65.46	61.47	<37.00	64.89	61.24	61.13										
LC D-213	ATTO495-AGGGAAA A CAGGGAGGGATAAC	59.33	62.65	42.67	64.43	60.93	<37.00	65.34	61.70	63.18										
LC D-214	ATTO495-AGGGAAA A GAGGGAGGGATAAC	59.65	65.34	43.41	67.36	63.60	<37.00	68.13	64.26	65.77										
LC D-215	ATTO495-AGGGAAA A G T AGGGAGGGATAAC	59.73	63.15	43.33	65.20	61.33	<37.00	66.00	62.35	64.06										
LC D-218	ATTO495-AGGGAAA A GAGGGAGGGATAAC	61.25	64.92	45.57	66.79	63.07	<37.00	67.51	64.32	65.12										
LC D-207	ATTO495-AGGGAAA A GA C GAGGGATAAC	60.31	63.31	45.75	64.19	60.90	<37.00	64.62	60.70	60.24										
LC D-208	ATTO495-AGGGAAA A GA A GAGGGATAAC	58.99	64.59	44.01	65.28	61.84	<37.00	65.08	61.88	61.53										
LC D-209	ATTO495-AGGGAAA A GA T GAGGGATAAC	59.99	61.59	46.17	63.52	59.53	<37.00	64.32	60.30	61.21										
LC D-210	ATTO495-AGGGAAA A GA G CGGATAAC	62.28	62.63	51.95	63.90	60.02	<37.00	64.17	59.85	59.65										
LC D-211	ATTO495-AGGGAAA A GA A GGGAAGGATAAC	62.84	63.78	50.88	64.69	61.05	<37.00	64.81	60.90	60.79										
LC D-212	ATTO495-AGGGAAA A GA T GGATAAC	61.67	61.81	50.88	63.60	59.16	<37.00	64.29	59.73	60.87										
LC D-224	ATTO495-AGGGAAA A AGGGAGGGATAAC	51.93	60.48	<37.00	61.84	57.29	<37.00	62.04	57.37	58.48										
Dissociation 0,2°C/minute (Y = 30 minutes, Z = 280 seconds)																				
LC D-200	ATTO495-AGGGAAAAGAAGGGAGGGATAAC	70.35	69.97	56.58	71.65	68.38	38.67	72.12	69.56	70.34										
LC D-201	ATTO495-AGGGAAAAGAAGGGAGGGATAAC	62.77	63.89	48.09	65.48	61.65	<37.00	66.01	62.25	63.24										
LC D-202	ATTO495-AGGG AAA AGAAGGGAGGGATAAC	62.96	65.48	47.81	66.69	63.05	<37.00	66.98	63.14	63.64										
LC D-203	ATTO495-AGGG T AAAAGAAGGGAGGGATAAC	62.77	63.14	48.00	65.29	60.90	<37.00	66.10	62.22	63.09										
LC D-204	ATTO495-AGGGAA C AGAAGGGAGGGATAAC	58.78	61.32	44.01	63.44	59.86	<37.00	64.49	61.17	62.48										
LC D-205	ATTO495-AGGGAA G AGAAGGGAGGGATAAC	60.21	67.20	46.66	68.60	62.57	<37.00	69.95	63.53	64.41										
LC D-206	ATTO495-AGGGAA T AGAAGGGAGGGATAAC	59.60	63.36	43.69	65.28	61.96	<37.00	66.46	62.66	63.85										
LC D-222	ATTO495-AGGGAAA A AAGGGAGGGATAAC	57.26	64.41	43.36	65.54	61.81	<37.00	64.93	61.55	61.20										
LC D-213	ATTO495-AGGGAAA A CAGGGAGGGATAAC	59.68	62.86	43.01	64.51	60.87	<37.00	65.45	61.65	63.33										
LC D-214	ATTO495-AGGGAAA A GAGGGAGGGATAAC	59.96	65.35	43.76	67.26	63.95	<37.00	68.35	64.54	65.95										
LC D-215	ATTO495-AGGGAAA A G T AGGGAGGGATAAC	59.87	63.23	44.07	65.35	61.27	<37.00	66.22	62.58	64.36										
LC D-218	ATTO495-AGGGAAA A GAGGGAGGGATAAC	61.34	64.90	46.07	67.02	63.37	<37.00	67.82	64.67	65.42										
LC D-207	ATTO495-AGGGAAA A GA C GAGGGATAAC	60.15	63.52	46.02	64.64	61.37	<37.00	64.92	61.27	60.99										
LC D-208	ATTO495-AGGGAAA A GA A GAGGGATAAC	58.66	64.73	44.82	65.85	62.68	<37.00	65.39	62.68	62.30										
LC D-209	ATTO495-AGGGAAA A GA T GAGGGATAAC	60.43	62.11	46.40	63.89	59.96	<37.00	64.45	60.87	61.93										
LC D-210	ATTO495-AGGGAAA A GA G CGGATAAC	63.88	62.57	52.52	64.06	60.30	<37.00	64.49	60.12	59.87										
LC D-211	ATTO495-AGGGAAA A GA A GGGAAGGATAAC	64.07	64.13	52.05	64.84	61.14	<37.00	64.99	61.00	61.00										
LC D-212	ATTO495-AGGGAAA A GA T GGATAAC	63.29	61.78	51.82	63.90	59.34	<37.00	64.41	60.04	61.08										
LC D-224	ATTO495-AGGGAAA A AGGGAGGGATAAC	51.89	60.30	< 37.00	61.78	57.34	<37.00	62.30	57.72	58.59										
Annealing 0,2°C/minute (Y = 10 minutes, Z = 280 seconds)																				
LC D-200	ATTO495-AGGGAAAAGAAGGGAGGGATAAC	70.26	70.15	55.99	72.02	68.70	<37.00	72.39	69.78	70.81										
LC D-201	ATTO495-AGGGAAAAGAAGGGAGGGATAAC	62.34	63.92	47.36	65.50	62.25	<37.00	66.06	62.62	63.64										
LC D-202	ATTO495-AGGG AAA AGAAGGGAGGGATAAC	62.34	65.60	47.14	66.71	63.46	<37.00	66.99	63.64	63.92										
LC D-203	ATTO495-AGGG T AAAAGAAGGGAGGGATAAC	62.43	63.36	47.36	65.32	61.59	<37.00	66.12	62.59											

Examples of dissociation and annealing melting curves (from supplementary data table 6)

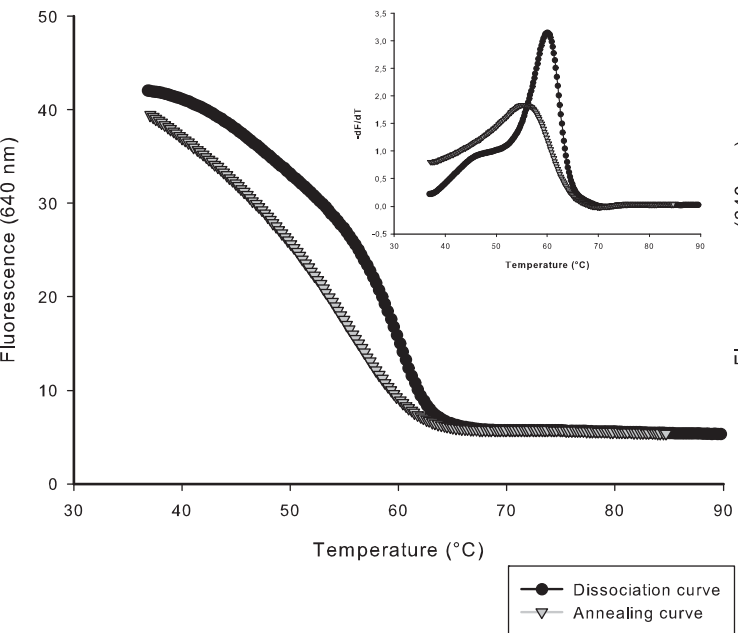
LC D-284: Ramp rate of 0.2°C/minute at pH 5.5



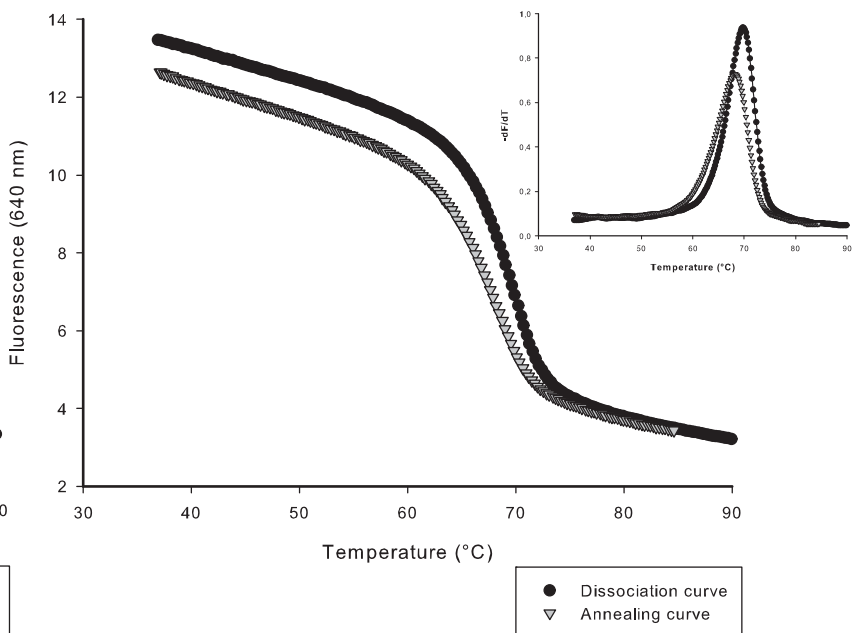
LC D-236: Ramp rate of 0.2°C/minute at pH 5.5



LC D-284: Ramp rate of 3°C/minute at pH 5.5



LC D-236: Ramp rate of 3°C/minute at pH 5.5



Parallel triplexes consisting of LC D-200 as target strand with LC D-256 as antiparallel duplex strand and either LC D-284 (DNA oligonucleotide) or LC D-236 (oligonucleotide with 5' and 3' placed TINAs) as triplex forming oligo (TFO) strands. Melting curves collected using 1.0 μ M of each oligonucleotide in sodium acetate buffer at pH 5.5 (50 mM NaOAc, 100 mM NaCl and 10 mM MgCl₂). At a ramp rate of 0.2°C/minute no hysteresis is observed for TFOs with an without TINA at pH 5.5. At a ramp rate of 3°C/minute parallel shifted dissociation curves with hysteresis are observed for both DNA oligonucleotides and oligonucleotides with TINA modifications. Examples are taken from supplementary data table 7.