1-Phenylethynylpyrene (PEPy) as a novel blue-emitting dye for qPCR

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Supporting Information

Oligonucleotide PEPy-G2z - HPLC traces and MS spectrum

Oligonucleotide PEPy-G3z - HPLC traces and MS spectrum

Oligonucleotide PEPy-G4z - HPLC traces and MS spectrum

Oligonucleotide AMCA-P4z - HPLC traces and MS spectrum

Oligonucleotide AMCA-P8z - HPLC traces and MS spectrum

Oligonucleotide AMCA-G1z - HPLC traces and MS spectrum

Oligonucleotide AMCA-G2z - HPLC traces and MS spectrum

Oligonucleotide AMCA-G3z - HPLC traces and MS spectrum

Oligonucleotide AMCA-G4z - HPLC traces and MS spectrum

Secondary structure of P4z probes optimized by Mfold. Raw and normalized data of qPCR

Secondary structure of P8z probes optimized by Mfold. Raw and normalized data of qPCR

Secondary structure of G1z probes optimized by Mfold. Raw and normalized data of qPCR

Secondary structure of G2z probes optimized by Mfold. Raw and normalized data of qPCR

Secondary structure of G3z probes optimized by Mfold. Raw and normalized data of qPCR

Secondary structure of G4z probes optimized by Mfold. Raw and normalized data of qPCR

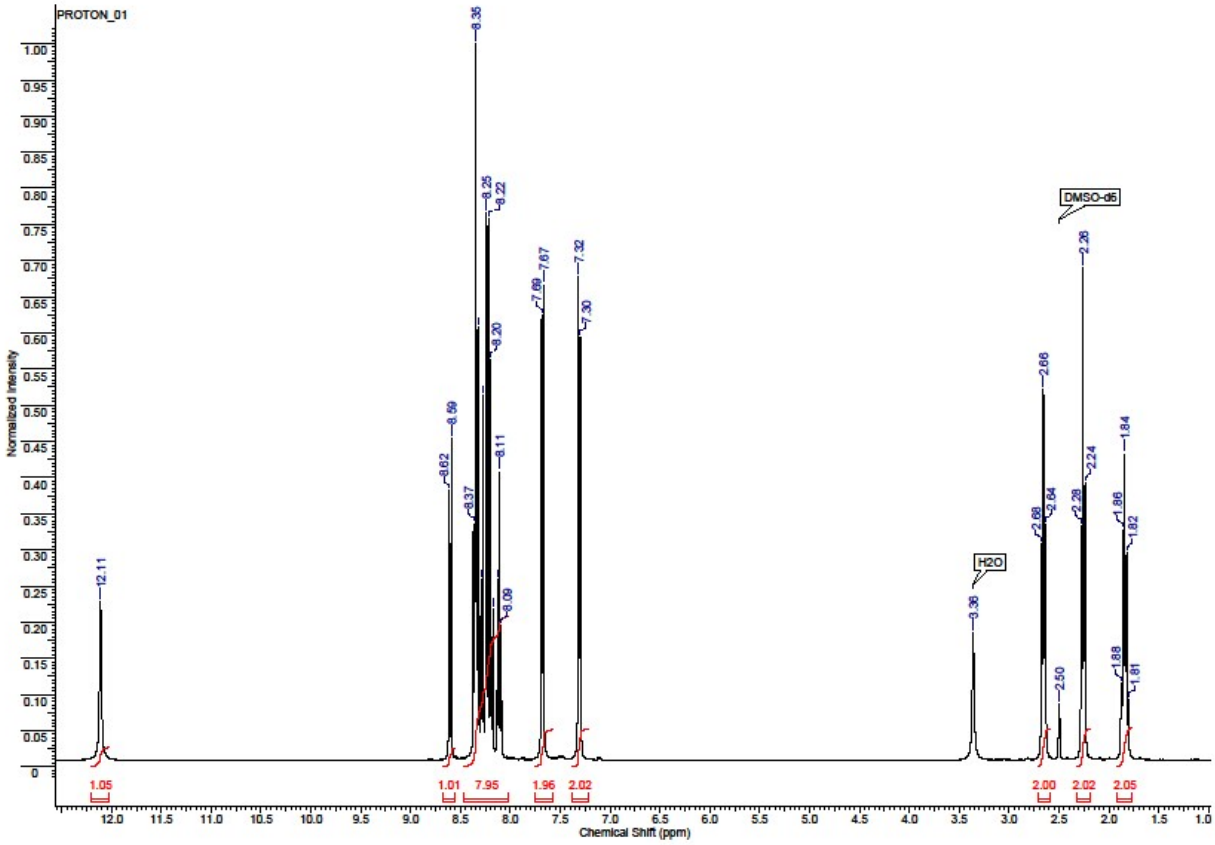
Melting curves for PEPy-labeled P8z, P4z, G1z, G2z, G3z, G4z probes.

qPCR data for cross-talk studies between "blue" and "green" channels.

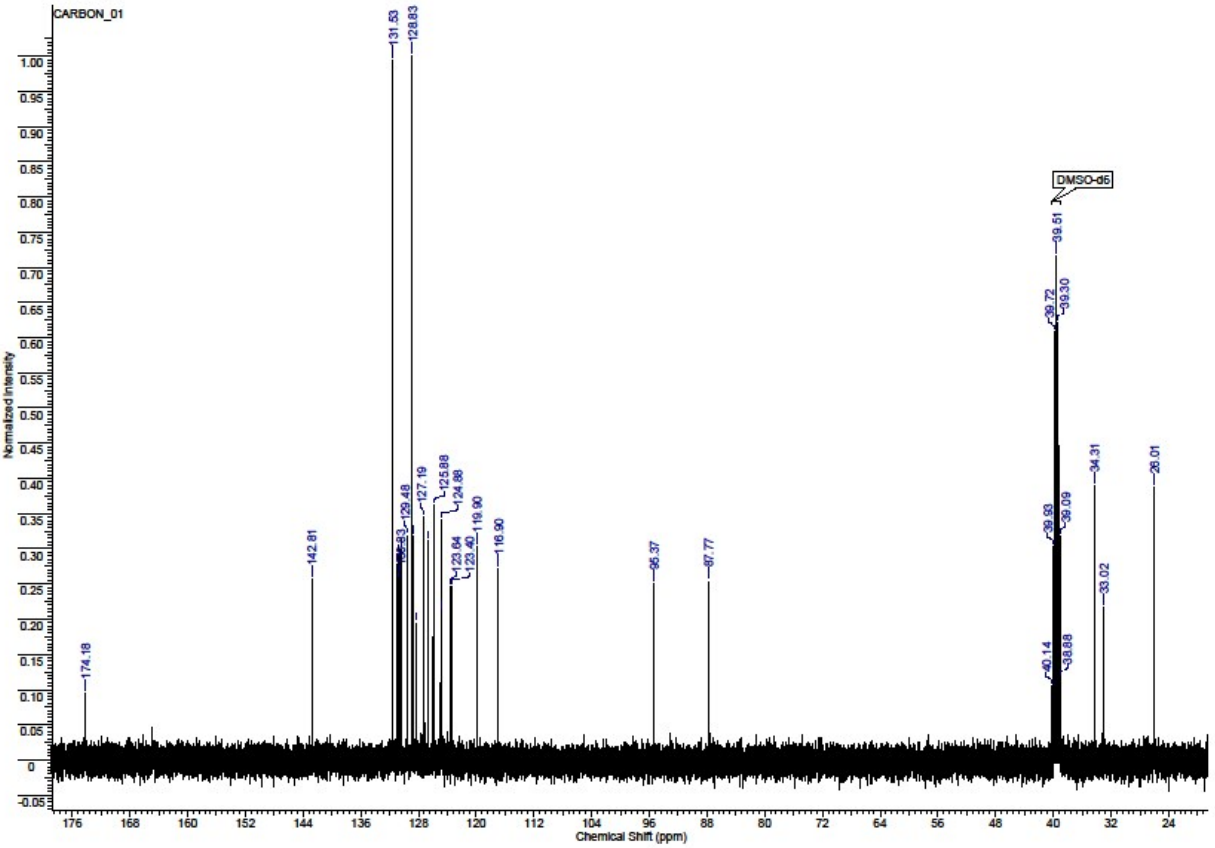
Table S4. qPCR data for AMCA, Alexa 350 and PEPy dual labeled probes.

Table S5. Normalized fluorescence data for AMCA, Alexa 350 and PEPy dual labeled probes in end-point qPCR.

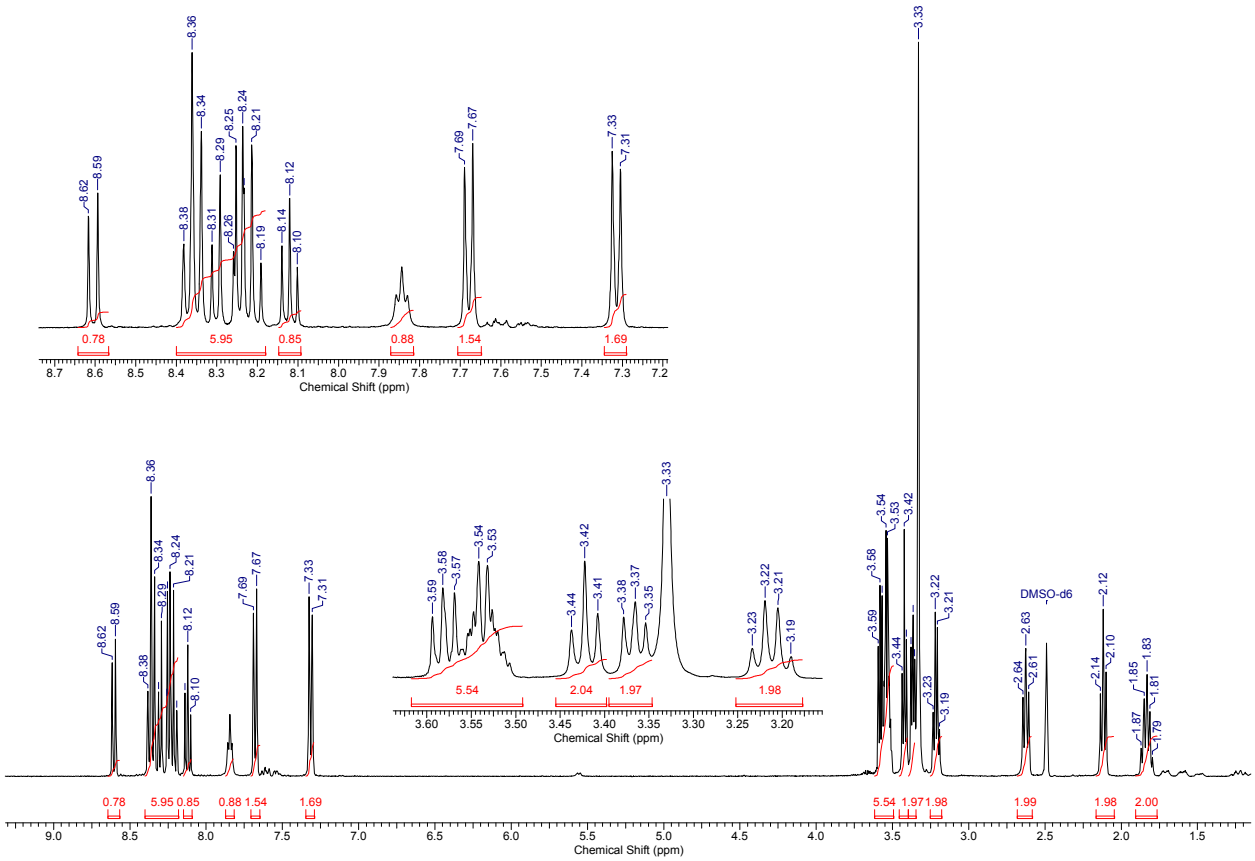
^{1}H NMR of 3



^{13}C NMR of 3



^{1}H NMR of 5



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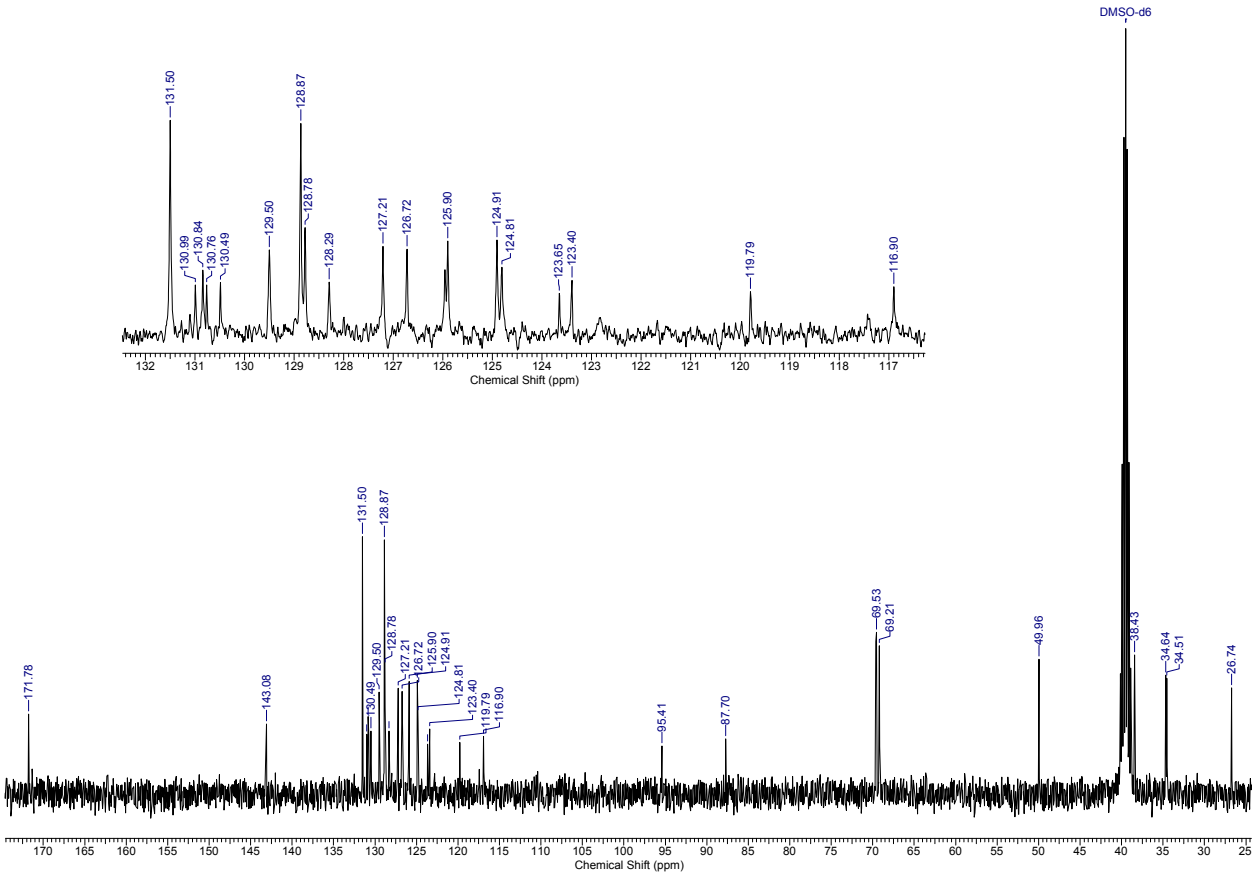


Table S1. Quantum yields (Φ_f) of fluorescence of AMCA- or PEPy-modified oligonucleotides and their duplexes with fully matched and mismatched complementary DNA.[a]

Oligonucleotides **6** and **7** (5′→3′) and their duplexes with targets **8**	X = AMCA			X = PEPy		
	ON	Φ_f, %	Brightness, $L \cdot M^{-1}cm^{-1}$	ON	Φ_f, %	Brightness, $L \cdot M^{-1}cm^{-1}$
X-AACCCAACTGAAGCAGCA	6A	58±4	11000±800	7A	63±4	28000±1800
X-TACCCAACTGAAGCAGCA	6T	52±3	9900±700	7T	47±3	21000±1700
X-CACCCAACTGAAGCAGCA	6C	55±3	10500±700	7C	51±3	23000±1600
X-GACCCAACTGAAGCAGCA	6G	61±4	11600±800	7G	52±3	23000±1600
X-AACCCAACTGAAGCAGCA ACAAACATA**A**TGGGTTGACTTCGTCGT	6A/8A	45±3	8600±600	7A/8A	53±3	23700±1600
X-TACCCAACTGAAGCAGCA ACAAACATA**A**TGGGTTGACTTCGTCGT	6T/8A	48±3	9100±600	7T/8A	51±3	22800±1600
X-CACCCAACTGAAGCAGCA ACAAACATA**A**TGGGTTGACTTCGTCGT	6C/8A	46±3	8700±600	7C/8A	40±3	17900±1200
X-GACCCAACTGAAGCAGCA ACAAACATA**A**TGGGTTGACTTCGTCGT	6G/8A	46±3	8700±600	7G/8A	46±3	20600±1400
X-AACCCAACTGAAGCAGCA ACAAACATA**T**TGGGTTGACTTCGTCGT	6A/8T	49±3	9300±600	7A/8T	59±3	26400±1800
X-TACCCAACTGAAGCAGCA ACAAACATA**T**TGGGTTGACTTCGTCGT	6T/8T	52±3	9900±700	7T/8T	48±3	21500±1500
X-CACCCAACTGAAGCAGCA ACAAACATA**T**TGGGTTGACTTCGTCGT	6C/8T	51±3	9700±700	7C/8T	40±3	17900±1200
X-GACCCAACTGAAGCAGCA ACAAACATA**T**TGGGTTGACTTCGTCGT	6G/8T	50±3	9500±600	7G/8T	37±2	16500±1100
X-AACCCAACTGAAGCAGCA ACAAACATA**C**TGGGTTGACTTCGTCGT	6A/8C	49±3	9300±600	7A/8C	53±3	23700±1600
X-TACCCAACTGAAGCAGCA ACAAACATA**C**TGGGTTGACTTCGTCGT	6T/8C	50±3	9500±600	7T/8C	39±3	17400±1200
X-CACCCAACTGAAGCAGCA ACAAACATA**C**TGGGTTGACTTCGTCGT	6C/8C	45±3	8600±600	7C/8C	50±3	22400±1500
X-GACCCAACTGAAGCAGCA ACAAACATA**C**TGGGTTGACTTCGTCGT	6G/8C	43±3	8200±600	7G/8C	28±2	12500±900
X-AACCCAACTGAAGCAGCA ACAAACATA**G**TGGGTTGACTTCGTCGT	6A/8G	48±3	9100±600	7A/8G	51±3	22800±1600
X-TACCCAACTGAAGCAGCA ACAAACATA**G**TGGGTTGACTTCGTCGT	6T/8G	53±3	10100±700	7T/8G	36±2	16100±1100
X-CACCCAACTGAAGCAGCA ACAAACATA**G**TGGGTTGACTTCGTCGT	6C/8G	51±3	9700±700	7C/8G	22±2	9800±700
X-GACCCAACTGAAGCAGCA ACAAACATA**A**TGGGTTGACTTCGTCGT	6G/8G	49±3	9300±650	7G/8G	43±3	19200±1300

[a] Mismatched nucleosides are highlighted in red. Measurements were taken for 3.0×10^{-7} M solutions in hybridization buffer (1x PBS, pH 7.0) at 20°C. Excitation wavelength 365 nm, fluorescence maxima are 442 nm (AMCA), 399 nm, 420 nm (PEPy); for the technique of Φ_f calculation see Experimental part.

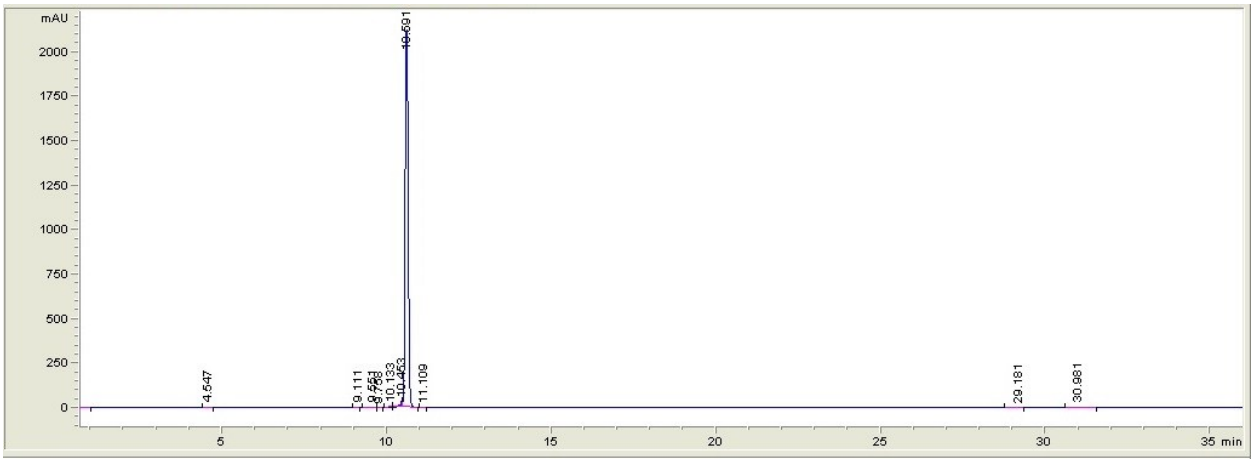
Table S2. Labeled oligonucleotides and Taqman probes, used in the study. MS data

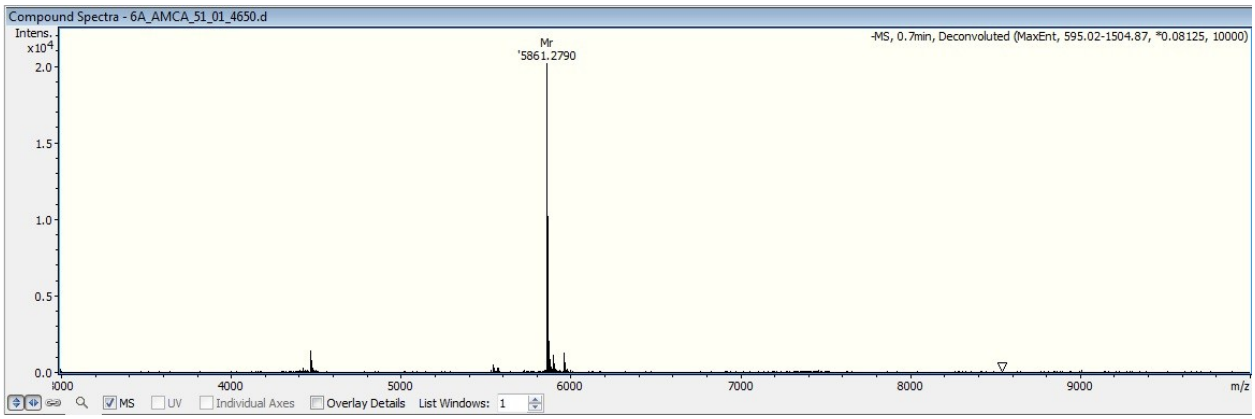
N	Name	Oligonucleotide, 5'-3'	ESI-MS (calcd/ observed)
1	**6A**	AMCA-AACCCAACTGAAGCAGCA	5867 / 5861
2	**6T**	AMCA-TACCCAACTGAAGCAGCA	5858 / 5852
3	**6C**	AMCA-CACCCAACTGAAGCAGCA	5843 / 5837
4	**6G**	AMCA-GACCCAACTGAAGCAGCA	5883 / 5877
5	**7A**	PEPy-AACCCAACTGAAGCAGCA	6286 / 6282
6	**7T**	PEPy-TACCCAACTGAAGCAGCA	6277 / 6273
7	**7C**	PEPy-CACCCAACTGAAGCAGCA	6262 / 6258
8	**7G**	PEPy-GACCCAACTGAAGCAGCA	6302 / 6298
9	**9.1**	PEPy-AAACATATAATACGG	5408 / 5405
10	**9.2**	AMCA-AAACATATAATACGG	4990 / 4984
11	**9.3**	PEPy-TTTTTTTTTTAAACATATAATACGG	8450 / 8446
12	**9.4**	AMCA-TTTTTTTTTTAAACATATAATACGG	8032 / 8025
13	**9D**	CCGTATTATATGTTT-DABCYL	4978 / 4975
14	**PEPy-P4z**	PEPy-CAC TCT GAC TAC TAC CTT TAA ACA GAG CG-Dabcyl	10165 / 10159
15	**PEPy-P8z**	PEPy-GGA CTG CAG TCR TTG CTG TTG AAC-Dabcyl	8743 / 8738 8759 / 8754
16	**PEPy-G1z**	PEPy-CTG AGC TTT AGT YAA GGC AAA TAA TGC TCA G-Dabcyl	10902 / 10896 10917 / 10911
17	**PEPy-G2z**	PEPy-CAG CGT CTA GTG ATC CCG TTA TTG GC-Dabcyl	9312 / 9307
18	**PEPy-G3z**	PEPy-TAC CCA ACT GAA GCA GCA ACA GGG TA-Dabcyl	9341 / 9336
19	**PEPy-G4z**	PEPy-CTG AAC YTG TCG GCC ATC CTT TGG TT-Dabcyl	9263 / 9258 9278 / 9273
20	**AMCA-P4z**	AMCA-CAC TCT GAC TAC TAC CTT TAA ACA GAG CG-Dabcyl	9747 / 9738
21	**AMCA-P8z**	AMCA-GGA CTG CAG TCR TTG CTG TTG AAC-Dabcyl	8325 / 8317 8341 / 8333
22	**AMCA-G1z**	AMCA-CTG AGC TTT AGT YAA GGC AAA TAA TGC TCA G-Dabcyl	10484 / 10475 10499 / 10490
23	**AMCA-G2z**	AMCA-CAG CGT CTA GTG ATC CCG TTA TTG GC-Dabcyl	8894 /8886
24	**AMCA-G3z**	AMCA-TAC CCA ACT GAA GCA GCA ACA GGG TA-Dabcyl	8923 / 8914
25	**AMCA-G4z**	AMCA-CTG AAC YTG TCG GCC ATC CTT TGG TT-Dabcyl	8845 / 8837 8860 / 8852

Table S3. Sequences of primers used in the study.

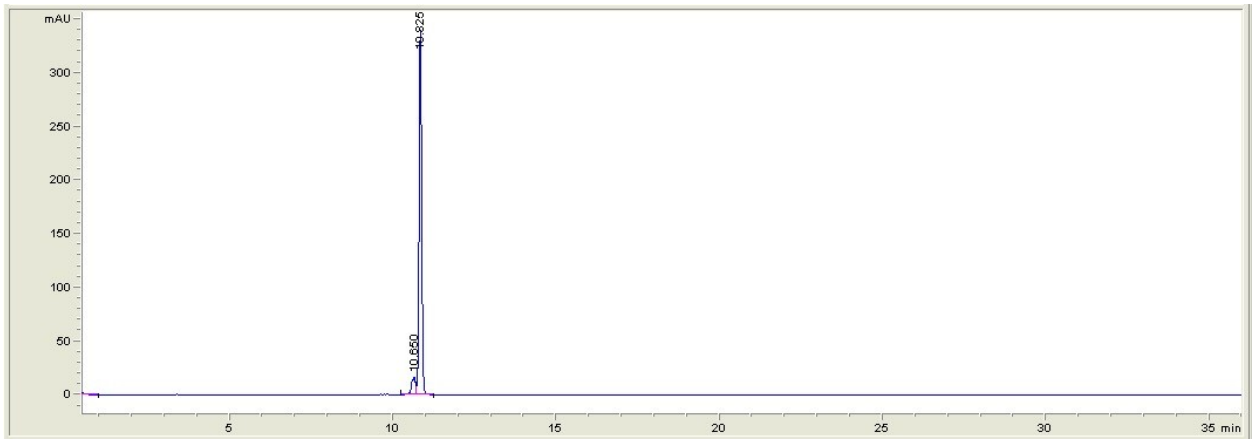
Primer		Target gene	Location (GenBank accession number)
VP4 for	TAAAATGGCTTCGCTCATTTATAGACA		
VP4 rev	GGTTATAAATGGTCCGAAATATCATATA		
VP7 for	GGCTTTAAAAGAGAGAATTTCCGTCTGG		
VP7 rev	TCAACTACAGTATAAAATACTTGCCACCA		
4P385	GAGAATAAGCAGTTTAACGTAGA	P[4]	379-401 (M32559)
2T1	CTATTGTTAGAGGTTAGAGTC	P[4]	494-474 (M32559)
1G86	GATATCAATCATTCTACTCAAC	G1	87-108 (M21843)
1G310	GATTTGAGTACTTGCTTCAGT	G1	327-307 (M21843)
9T3P	GTCCAGTTGCAGTGTAGC	G3	501-484 (AF161823)
3G276	CCTAACTTCGACTTTATGTT	G3	276-295 (AF161823)
9T2	GTT AGA AAT GAT TCT CCA CT	G2	281-262 (U73974)
2G170	CTC TGA TAT CAC CAT TTG TG	G2	173-192 (U73974)
9T4	GGG TCG ATG GAA AAT TCT	G4	423-440 (D86284)
4G319	CCA ACT CAA ATT AGT GAC AC	G4	707-726 (D86284)
8P214	CCT TAT CAR CCT ACT ACA TTT AC	P8	210-232 (M21014)
1-T1m	GTA TCT ACT GGA TTA ACG TGC	P8	336-356 (M21014)

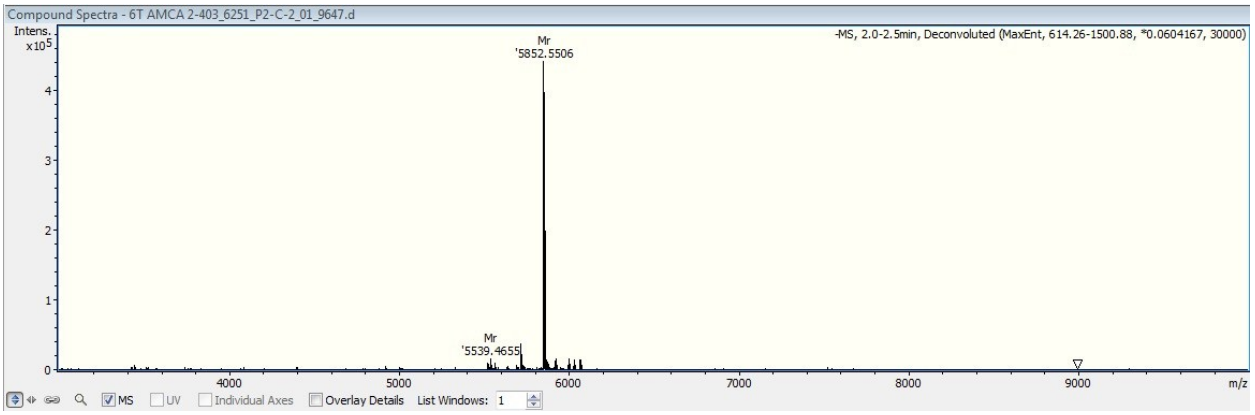
Oligonucleotide 6A - HPLC traces and MS spectrum.



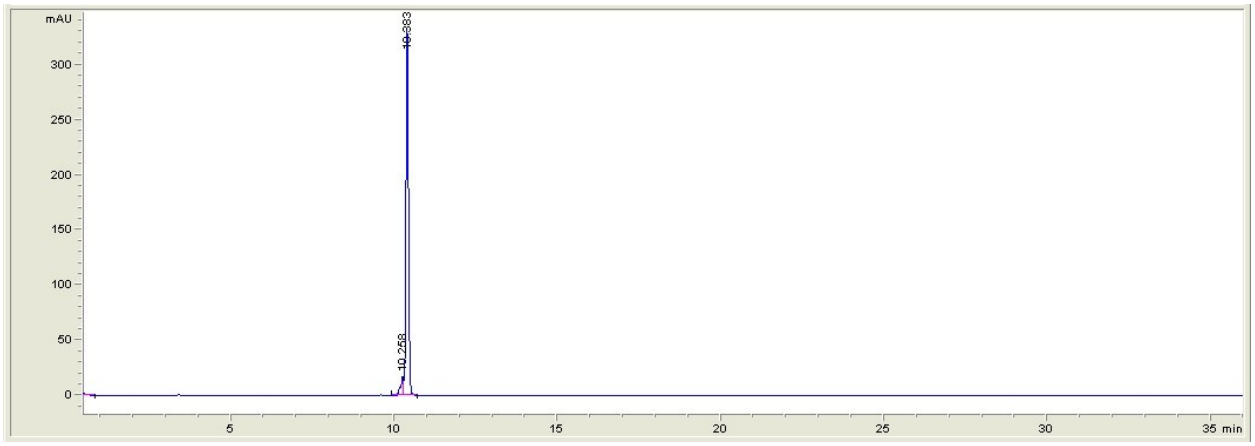


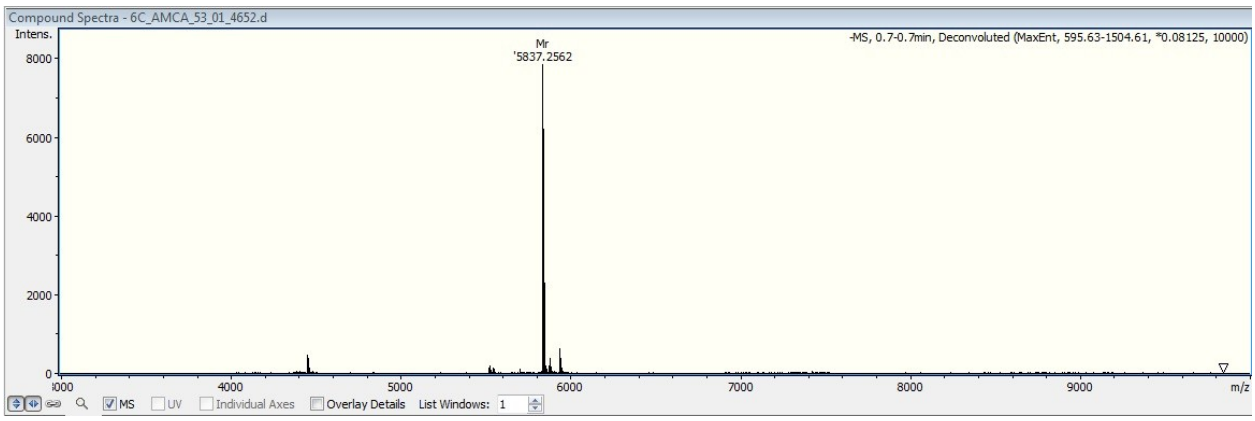
Oligonucleotide 6T - HPLC traces and MS spectrum



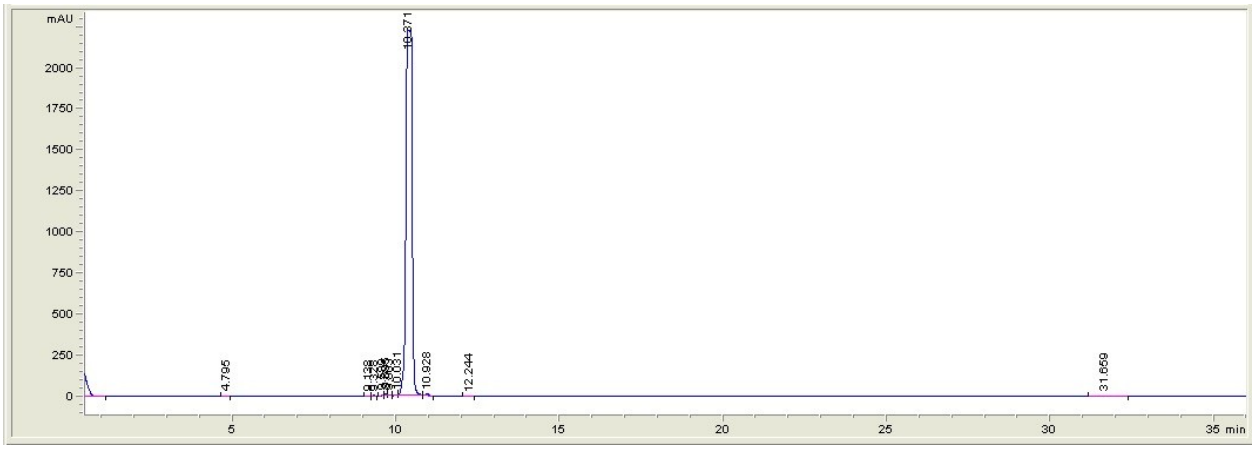


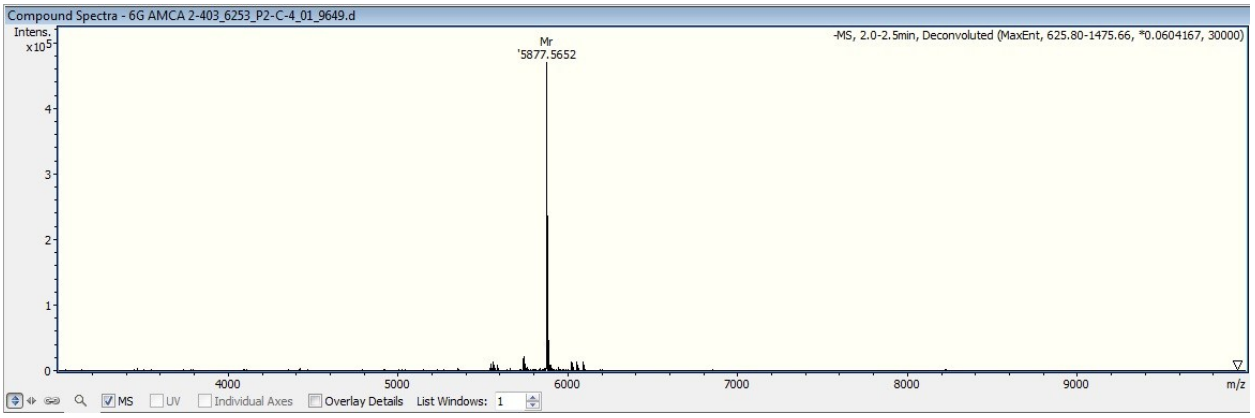
Oligonucleotide 6C - HPLC traces and MS spectrum



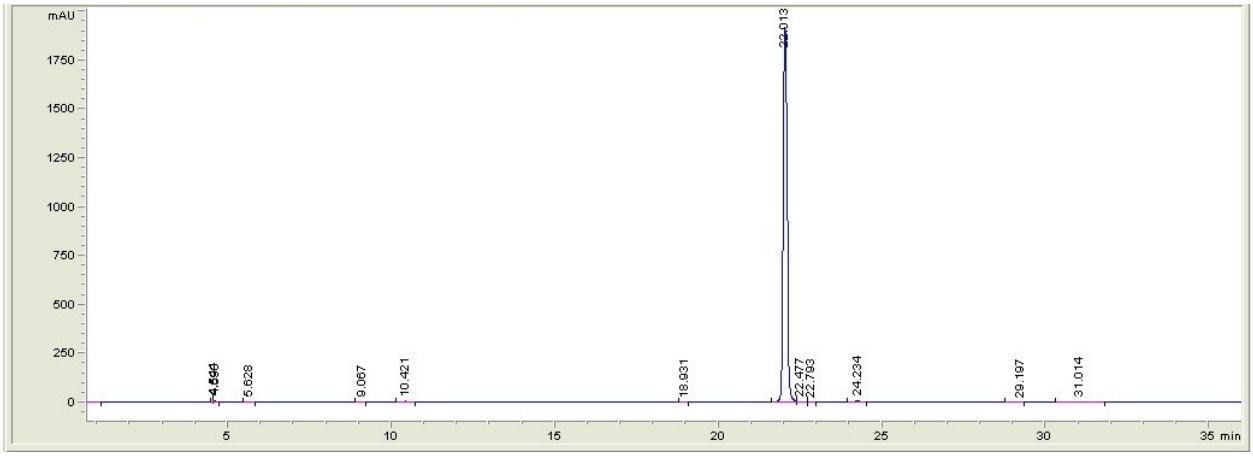


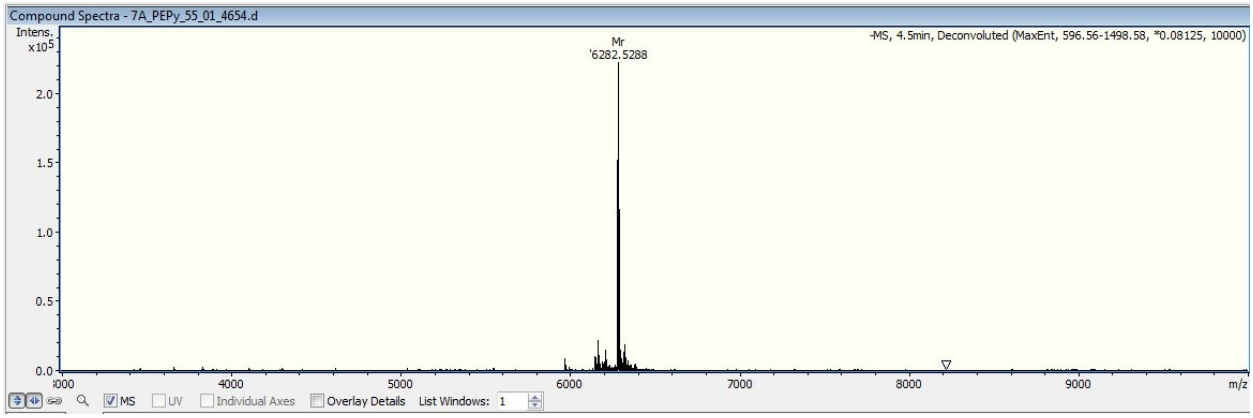
Oligonucleotide 6G - HPLC traces and MS spectrum



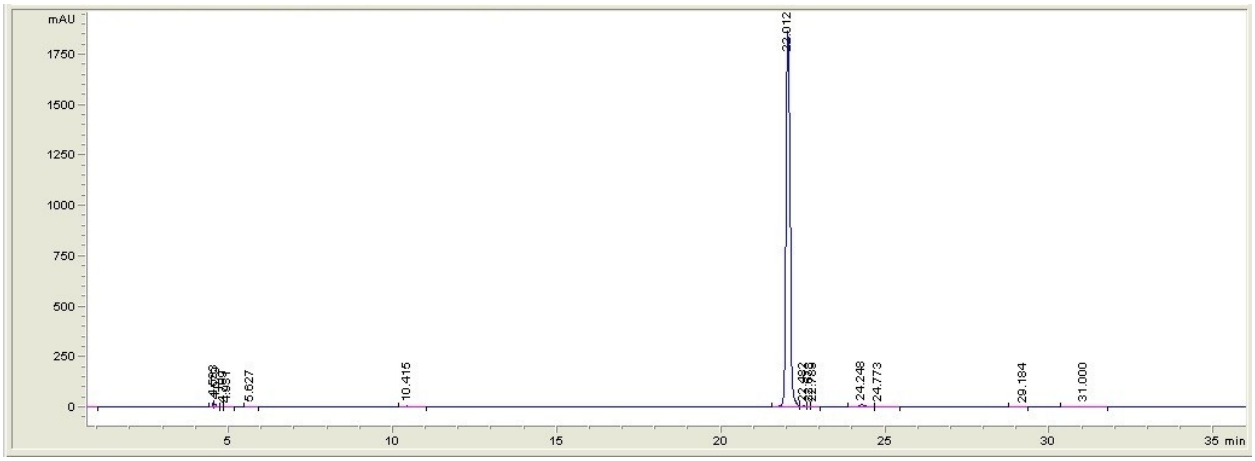


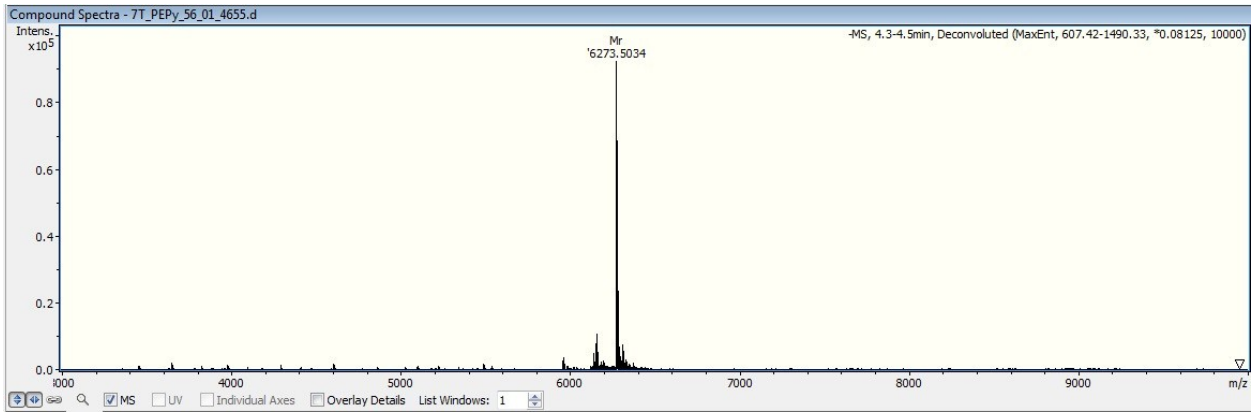
Oligonucleotide 7A - HPLC traces and MS spectrum



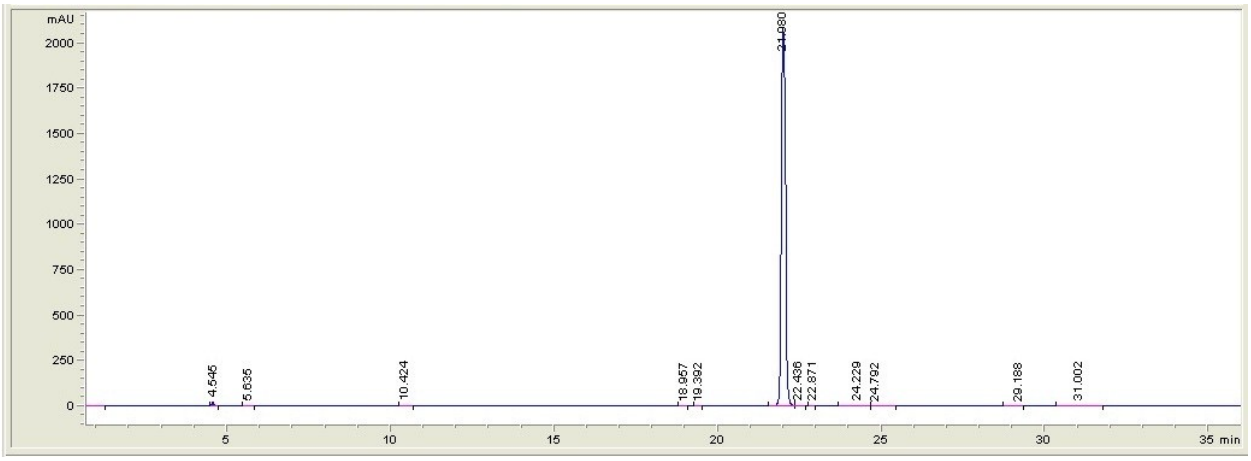


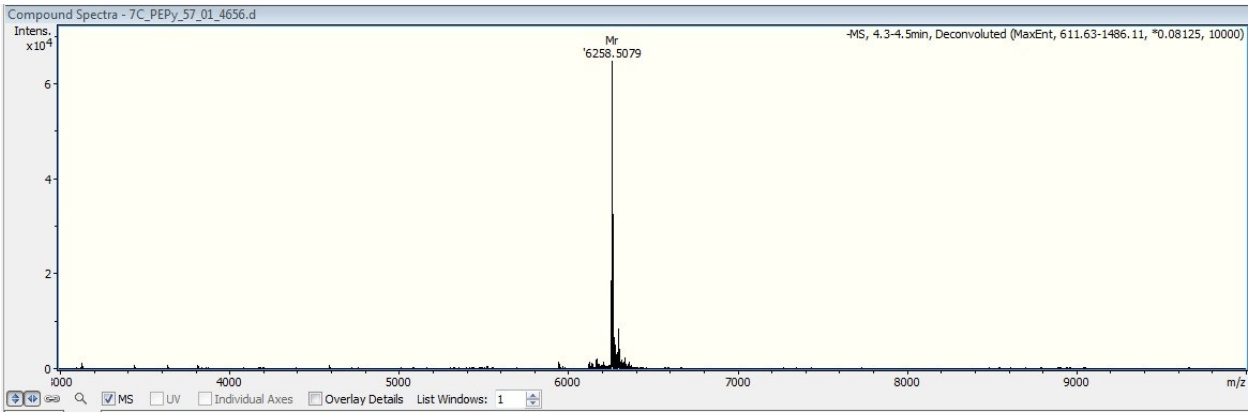
Oligonucleotide 7T - HPLC traces and MS spectrum



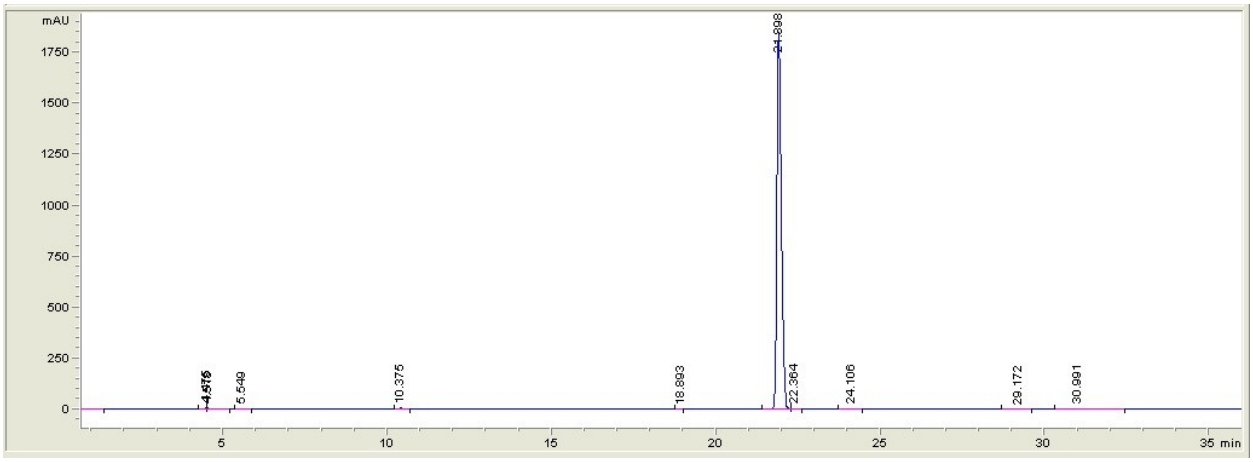


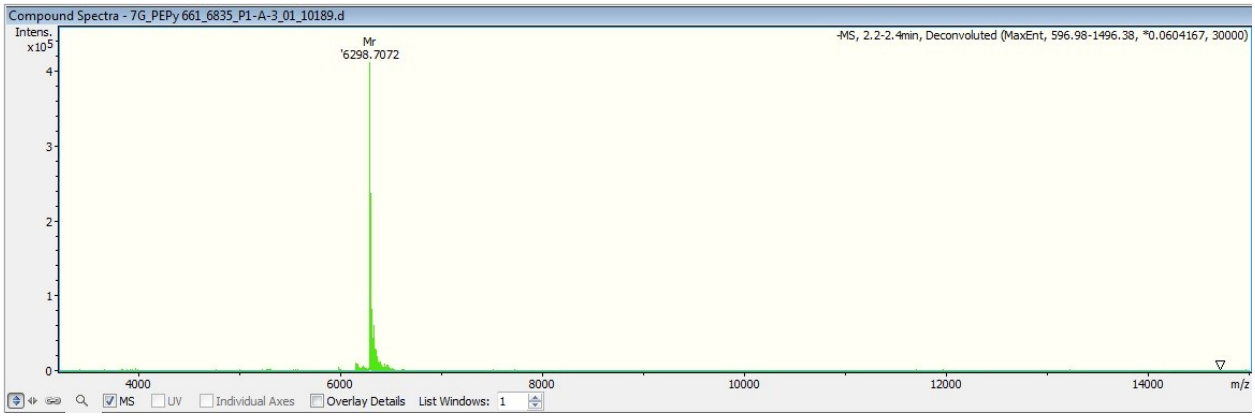
Oligonucleotide 7C - HPLC traces and MS spectrum



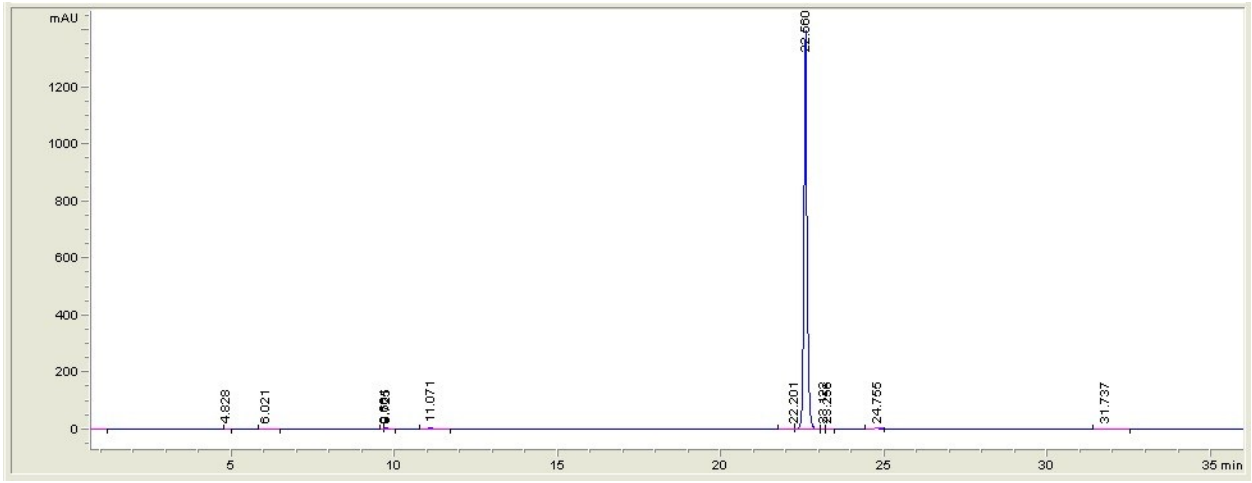


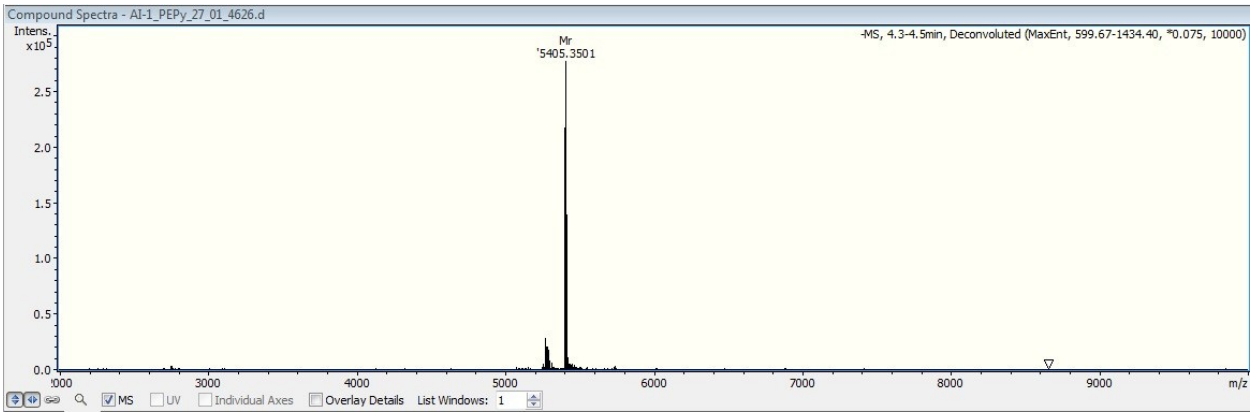
Oligonucleotide 7G - HPLC traces and MS spectrum



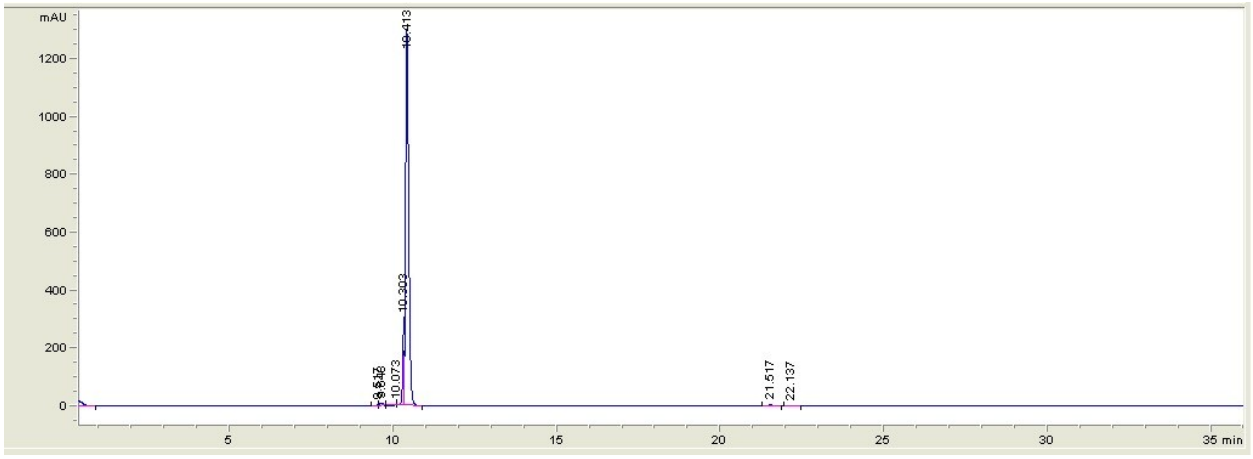


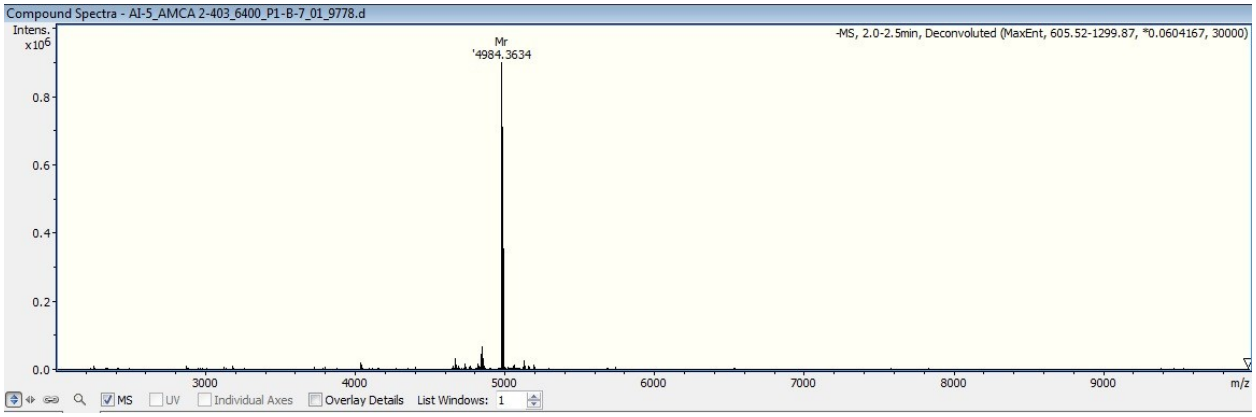
Oligonucleotide 9.1 - HPLC traces and MS spectrum



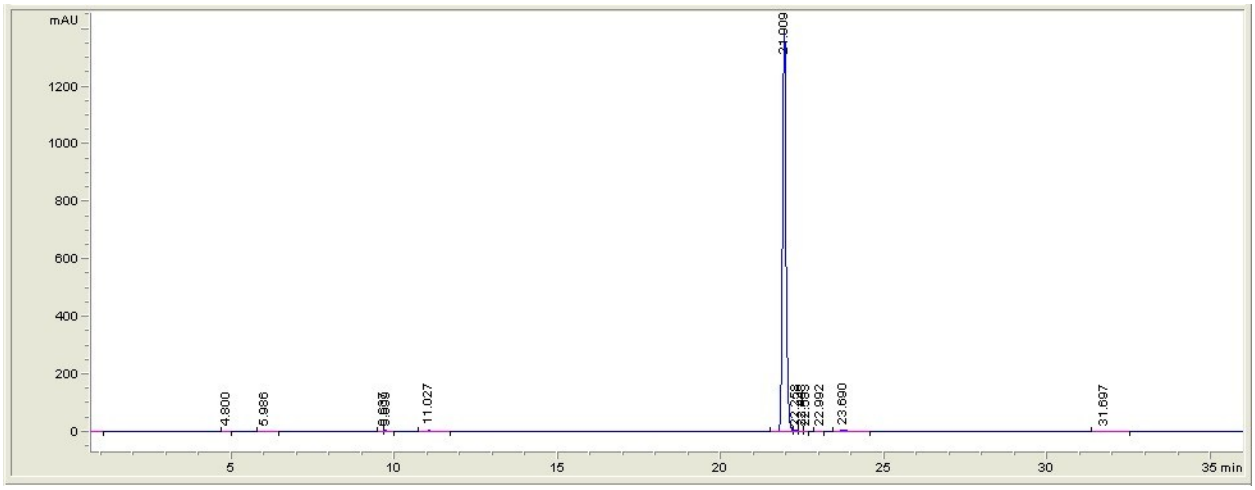


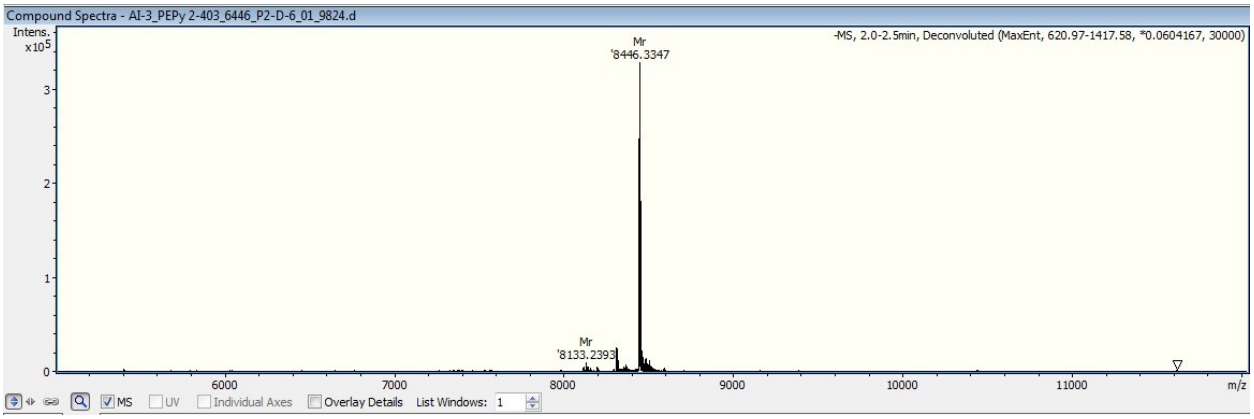
Oligonucleotide 9.2 - HPLC traces and MS spectrum



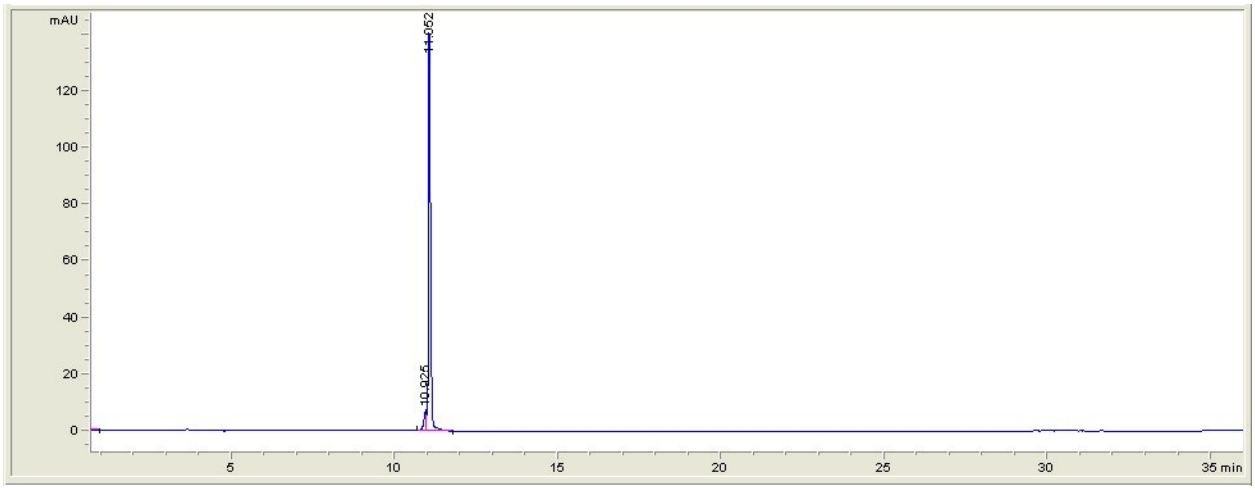


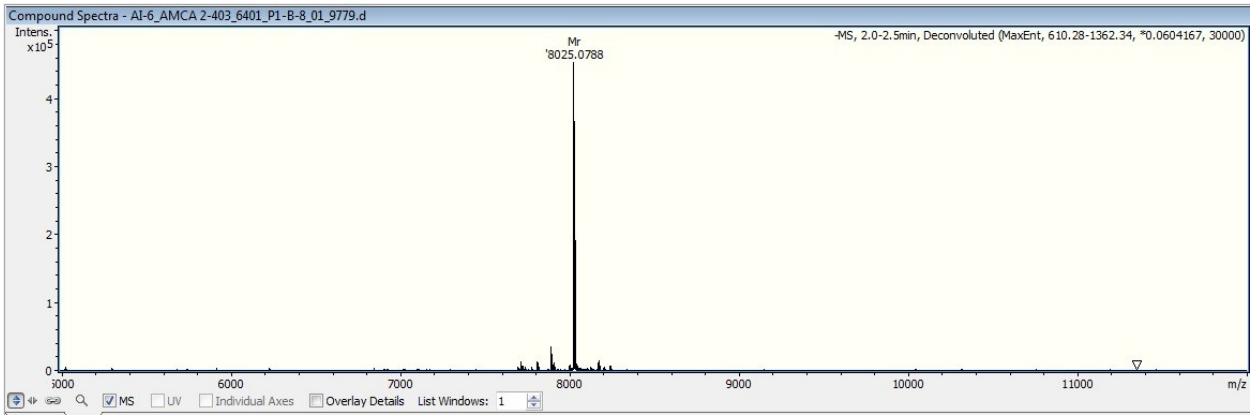
Oligonucleotide 9.3 - HPLC traces and MS spectrum



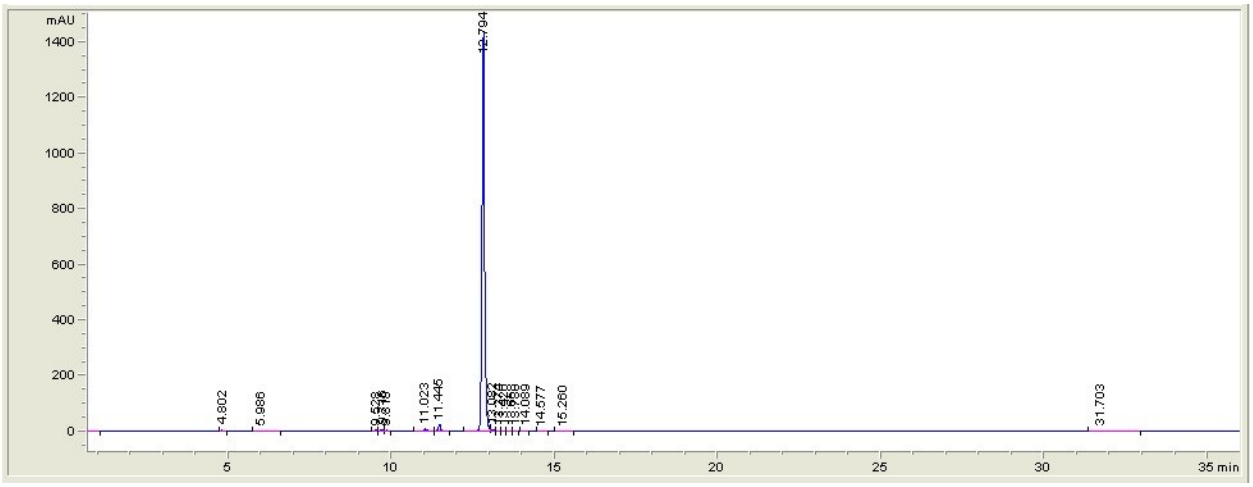


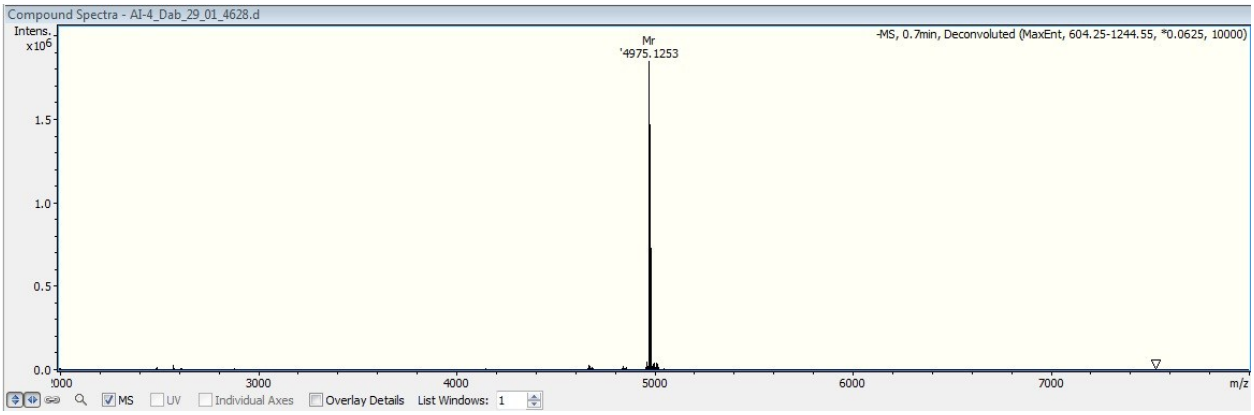
Oligonucleotide 9.4 - HPLC traces and MS spectrum



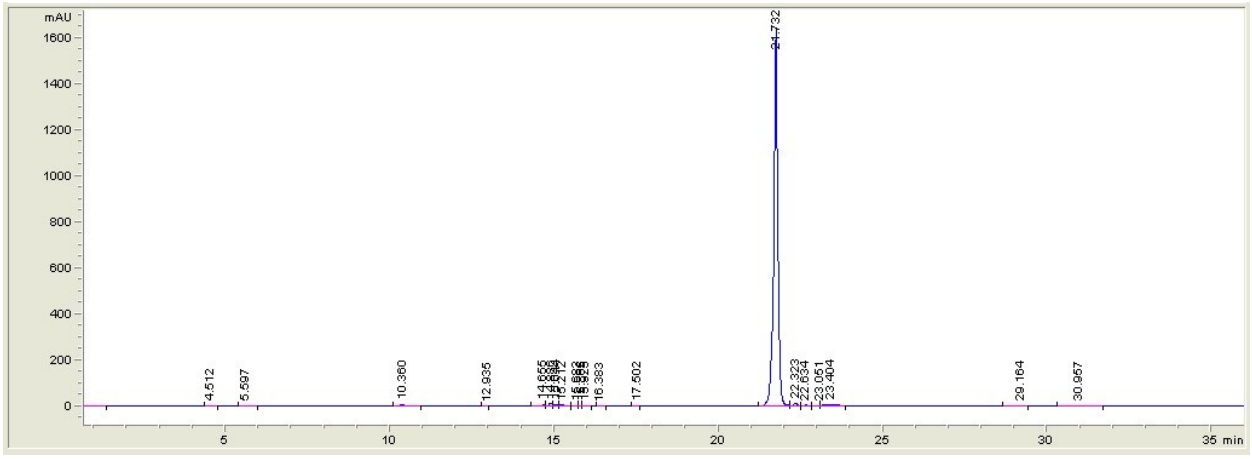


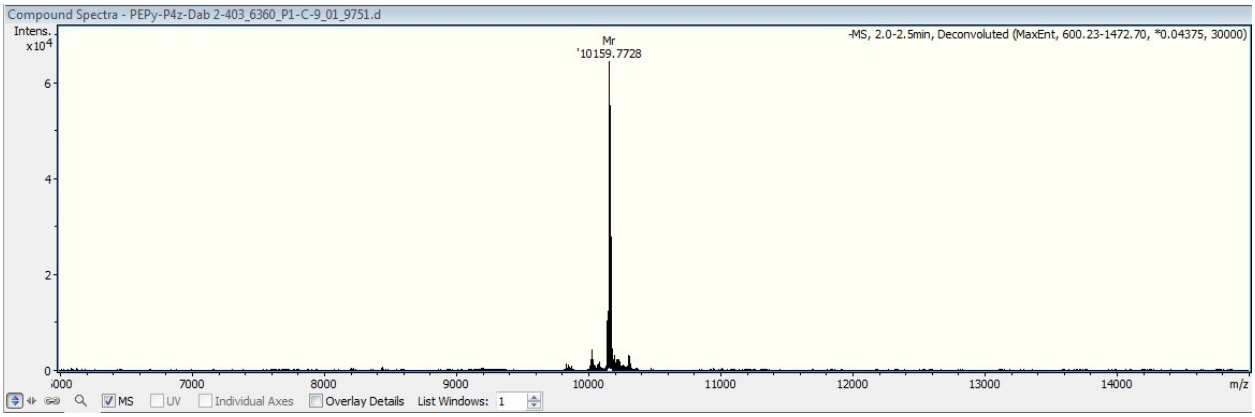
Oligonucleotide 9D - HPLC traces and MS spectrum



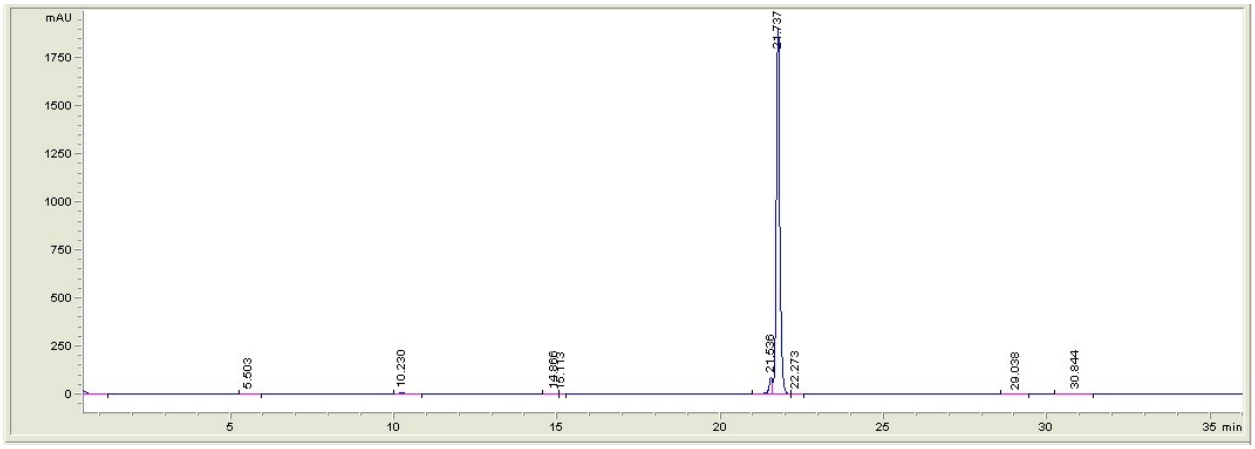


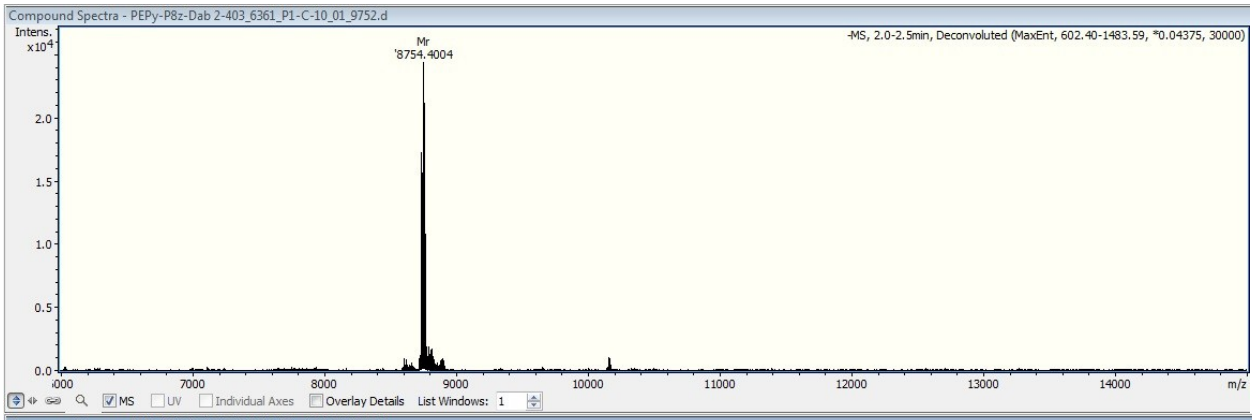
Oligonucleotide PEPy-P4z - HPLC traces and MS spectrum

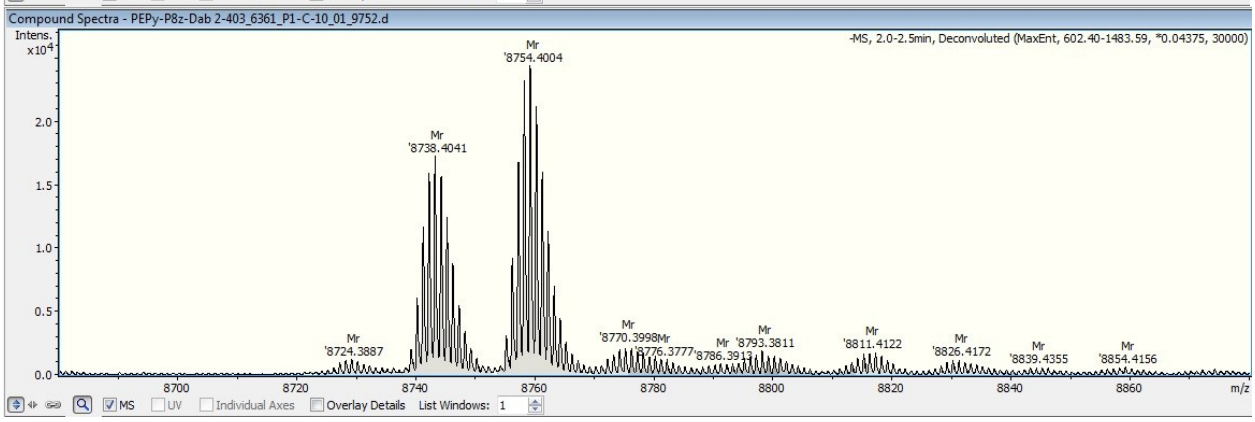




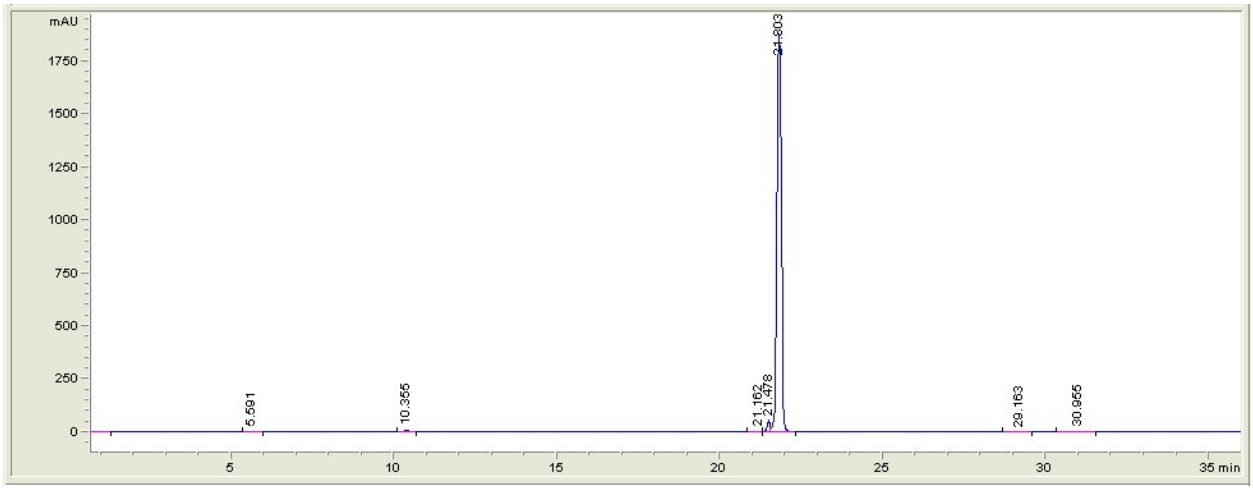
Oligonucleotide PEPy-P8z - HPLC traces and MS spectrum

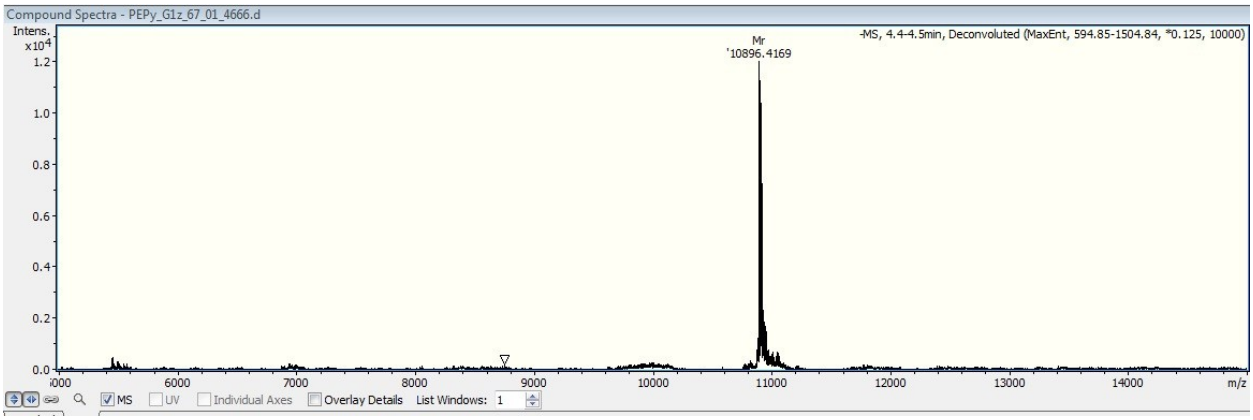


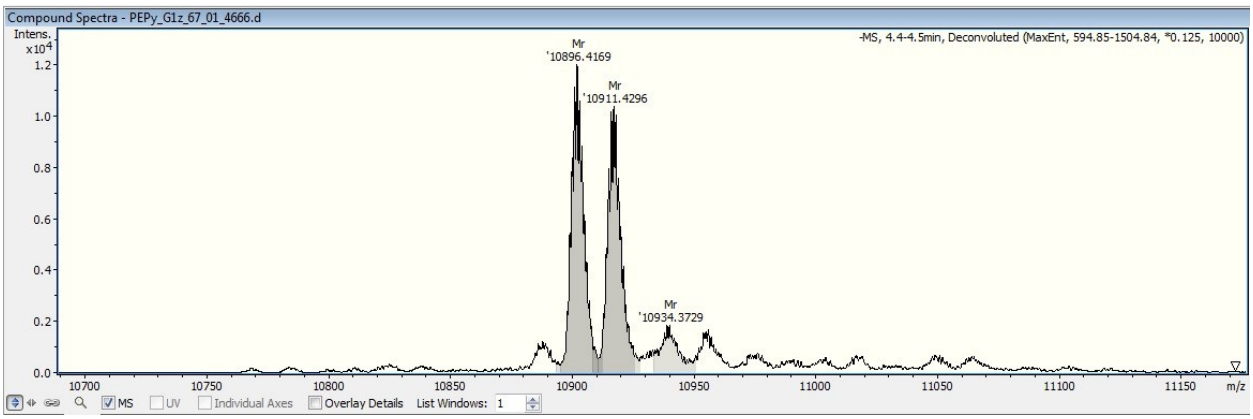




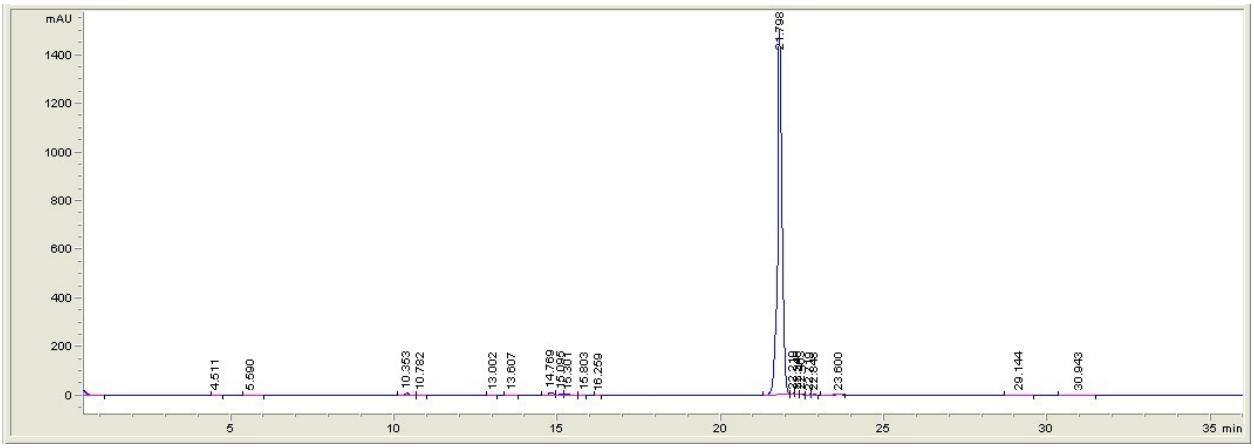
Oligonucleotide PEPy-G1z - HPLC traces and MS spectrum

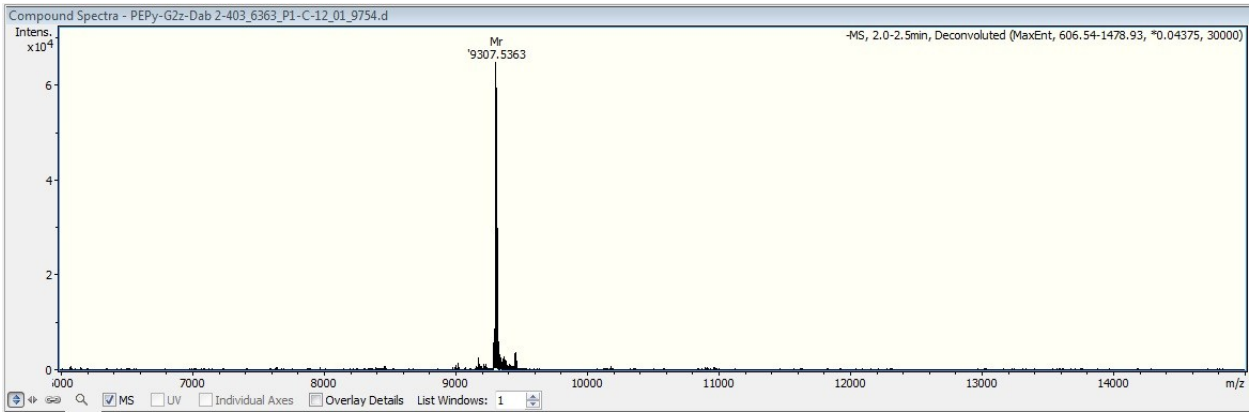




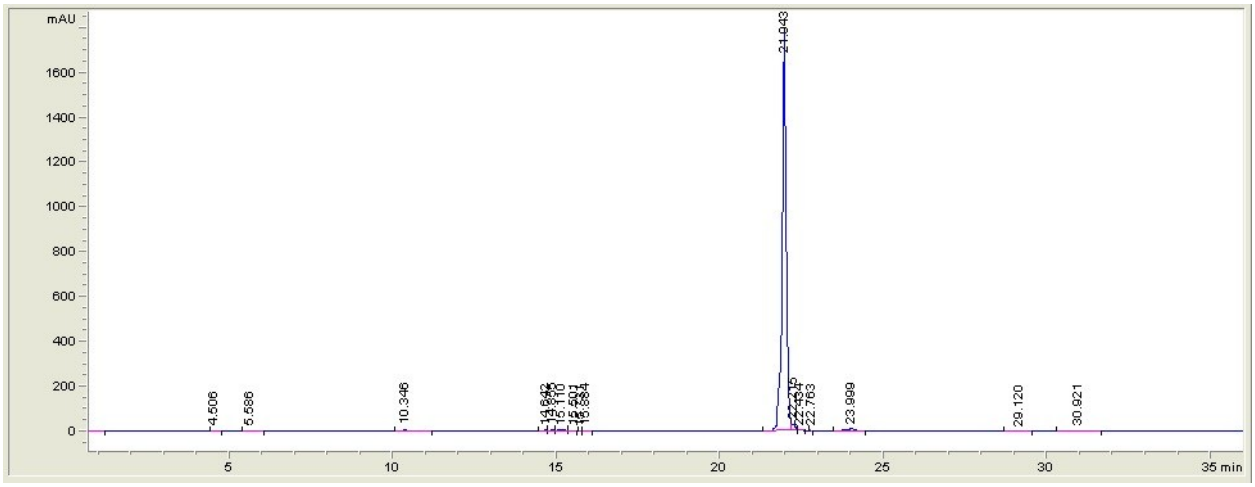


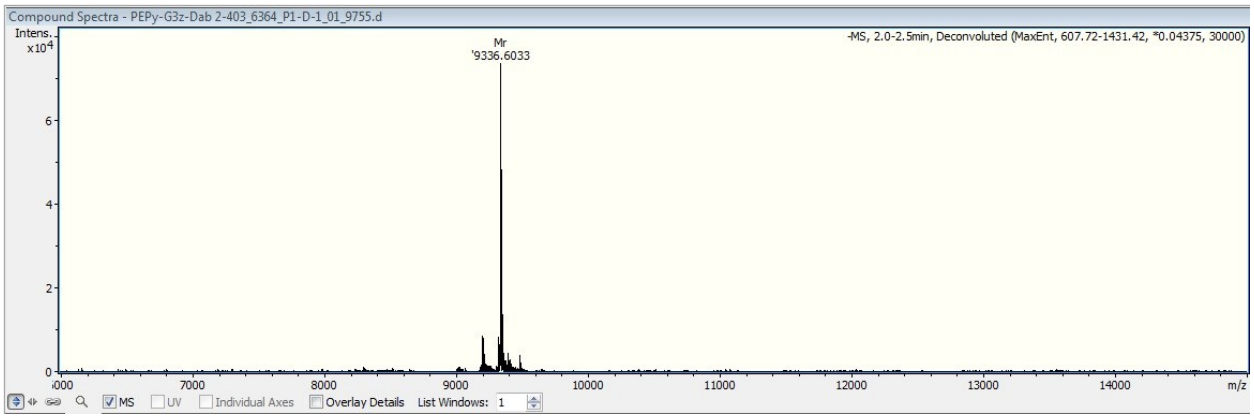
Oligonucleotide PEPy-G2z - HPLC traces and MS spectrum



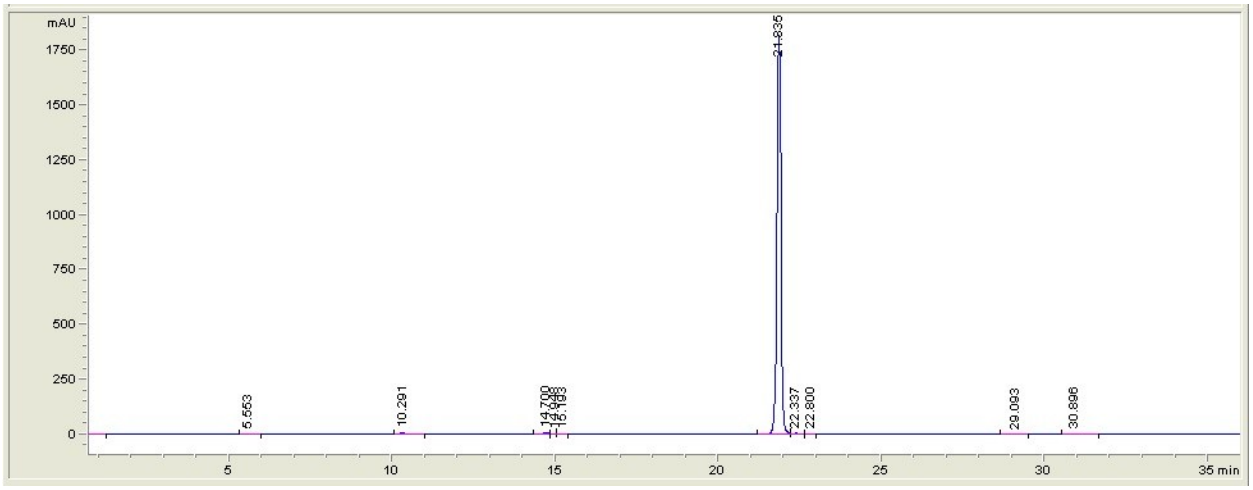


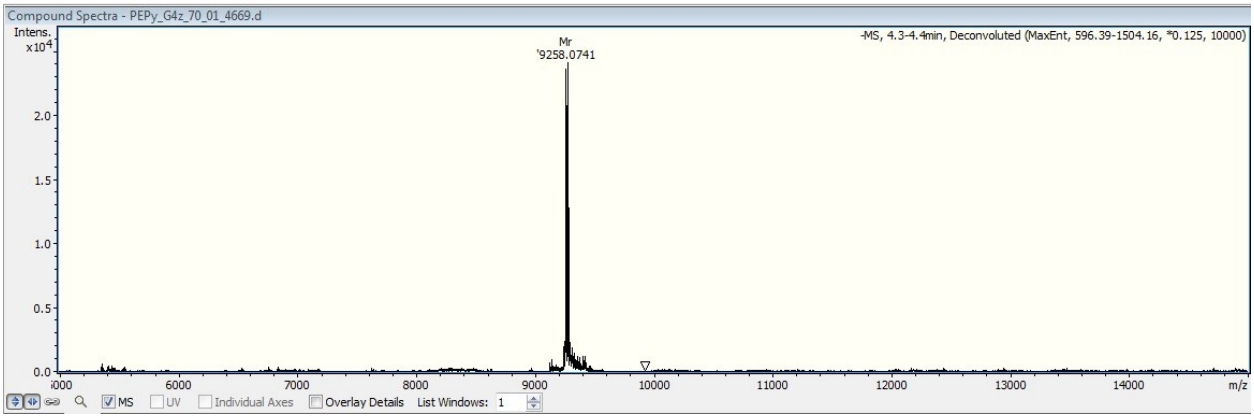
Oligonucleotide PEPy-G3z - HPLC traces and MS spectrum

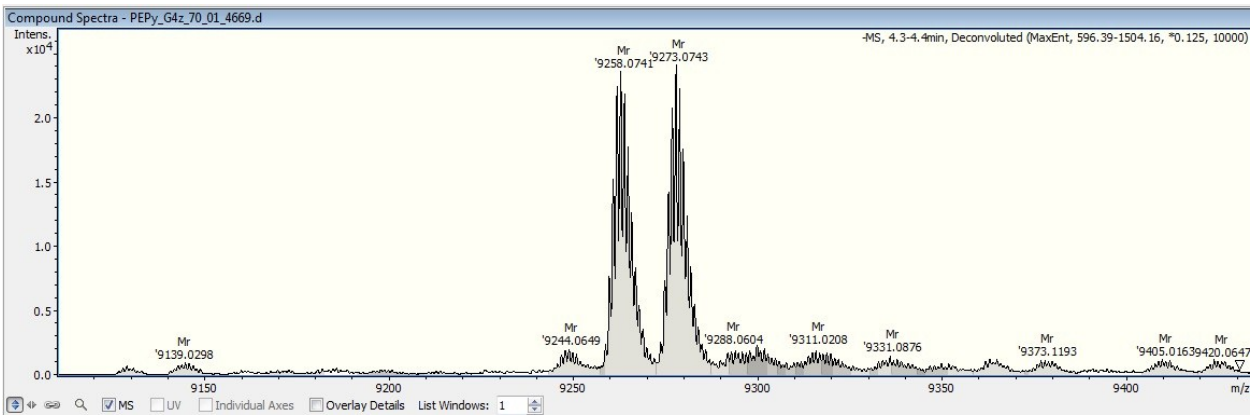




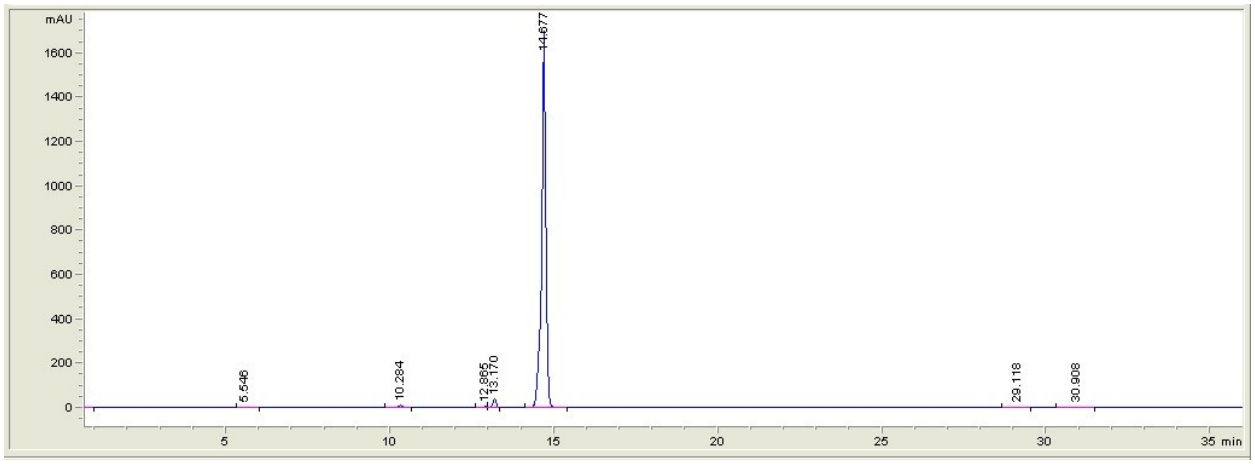
Oligonucleotide PEPy-G4z - HPLC traces and MS spectrum

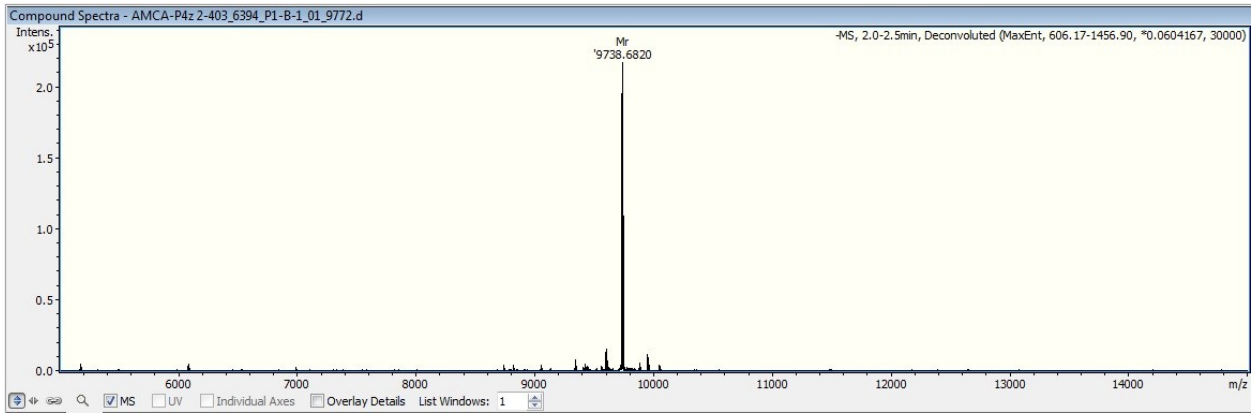




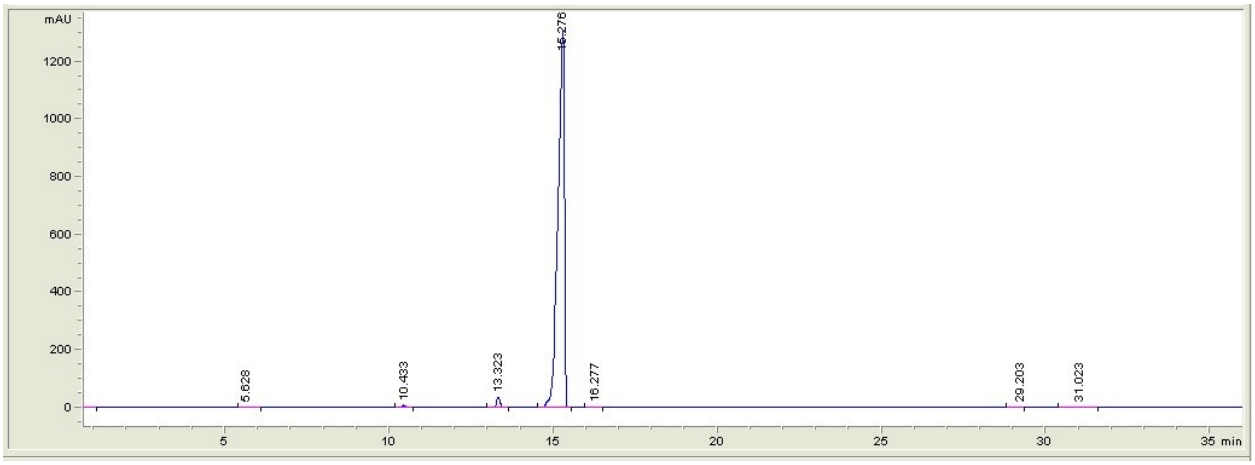


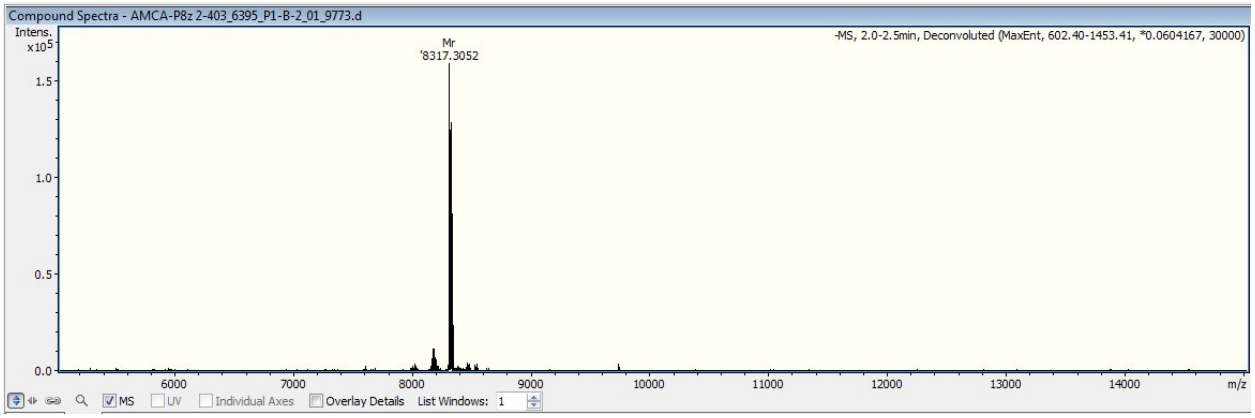
Oligonucleotide AMCA-P4z - HPLC traces and MS spectrum

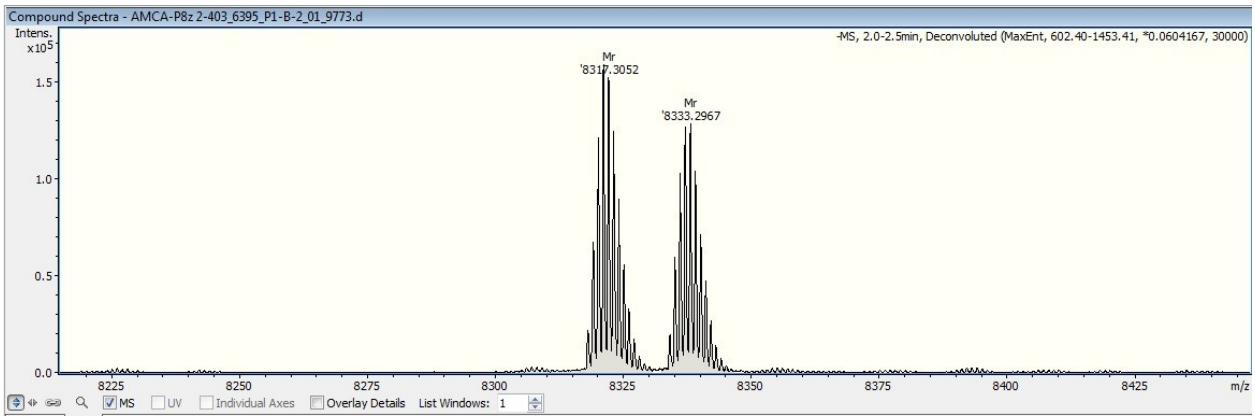




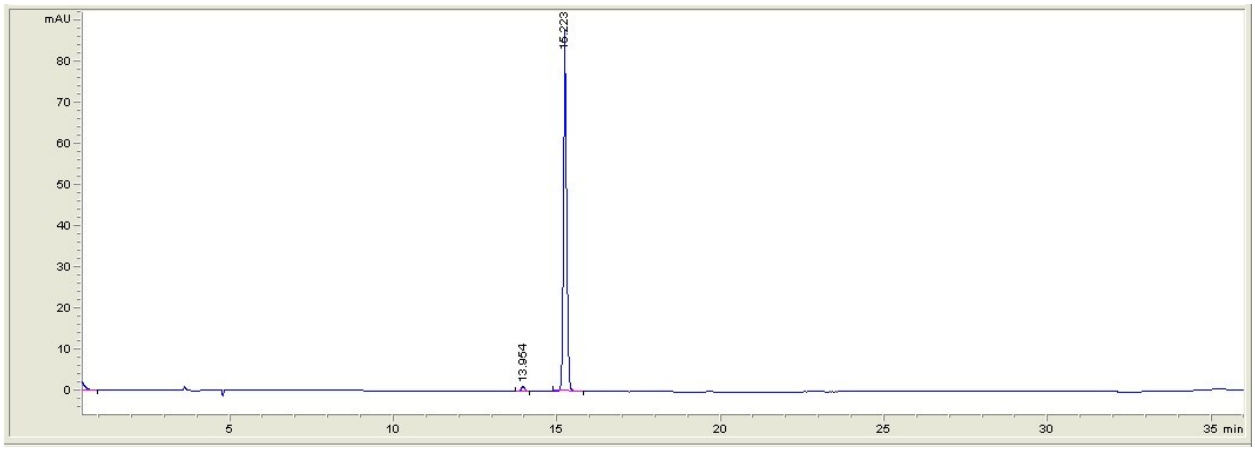
Oligonucleotide AMCA-P8z - HPLC traces and MS spectrum

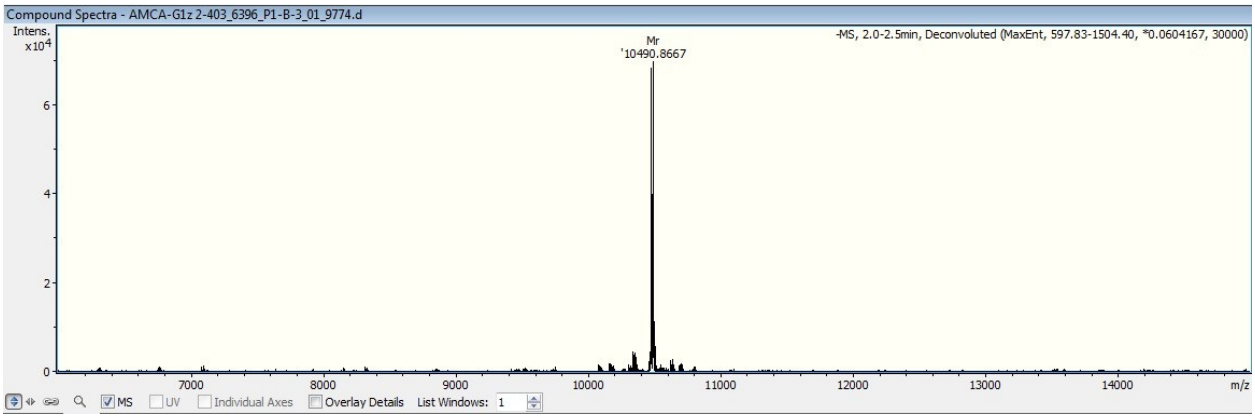


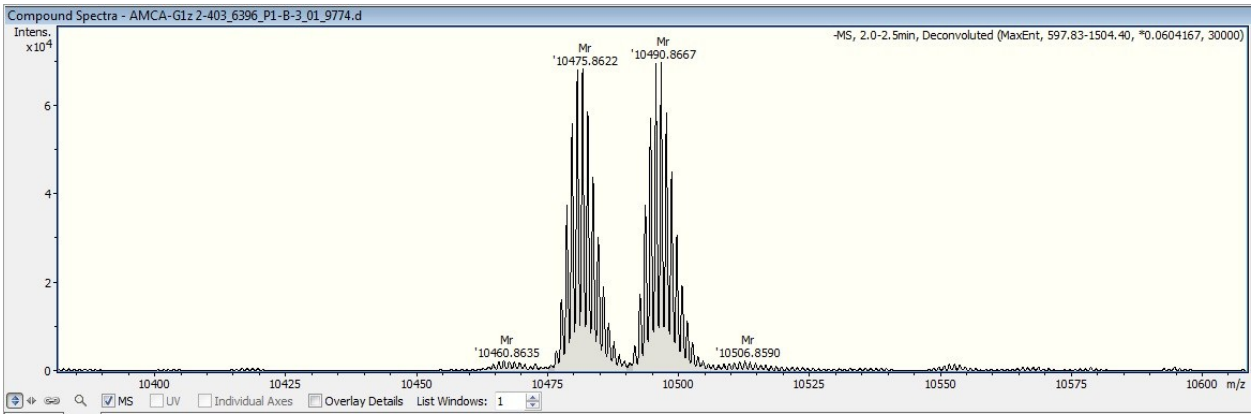




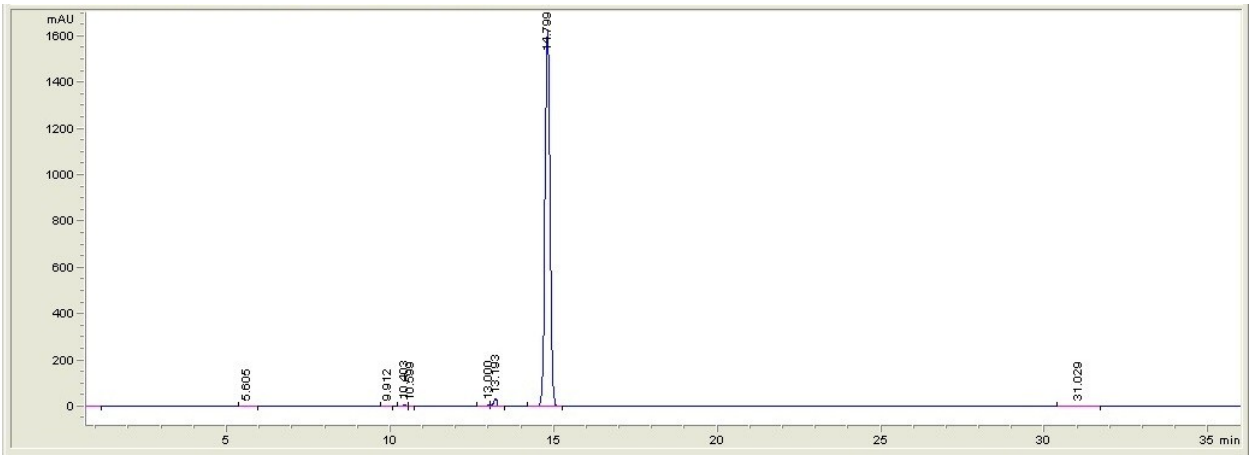
Oligonucleotide AMCA-G1z - HPLC traces and MS spectrum

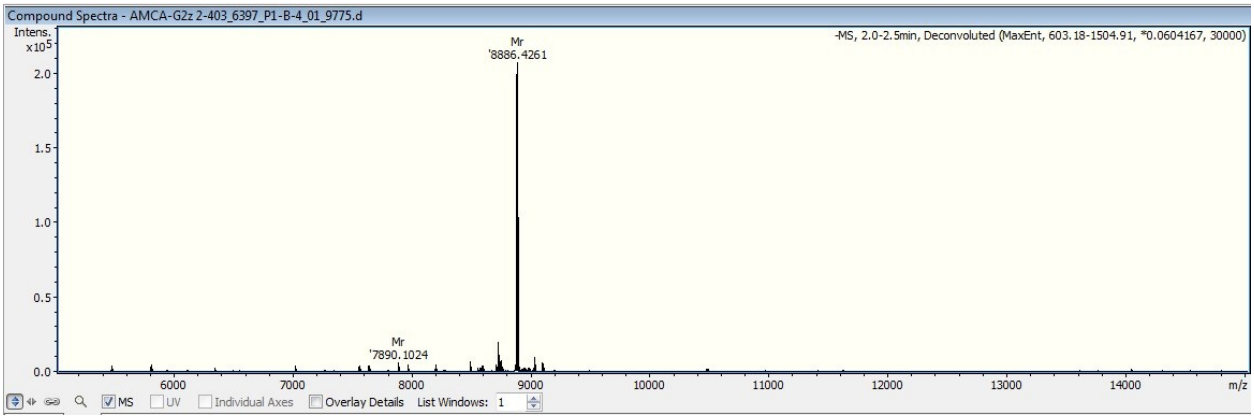




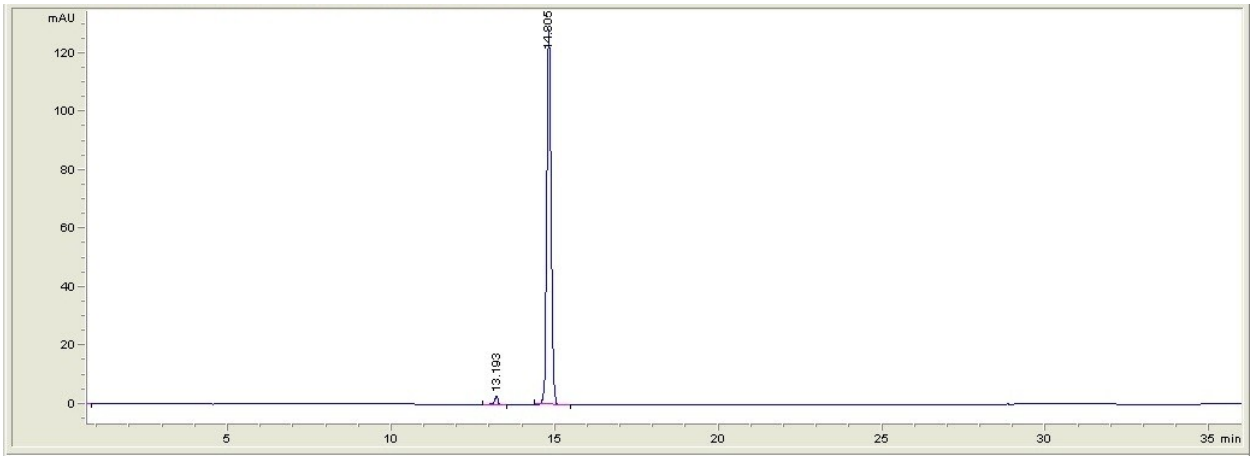


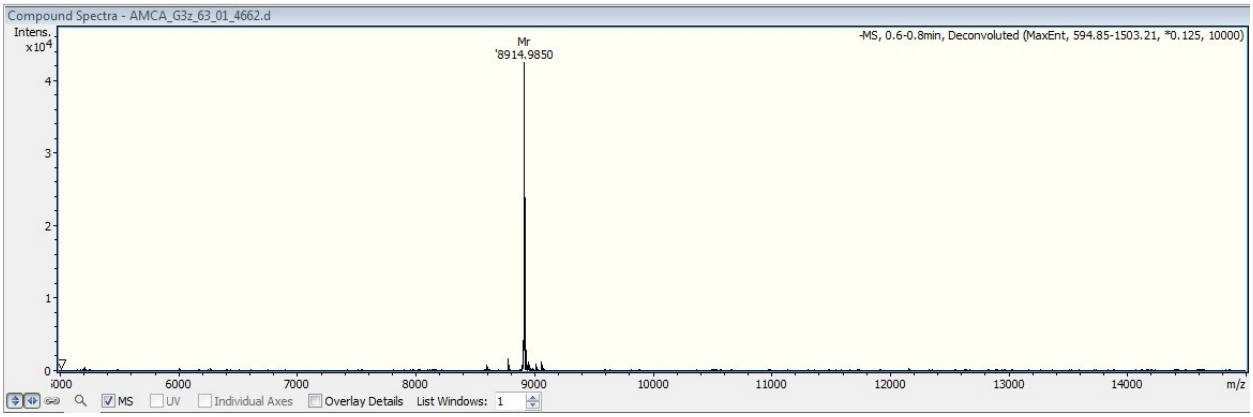
Oligonucleotide AMCA-G2z - HPLC traces and MS spectrum



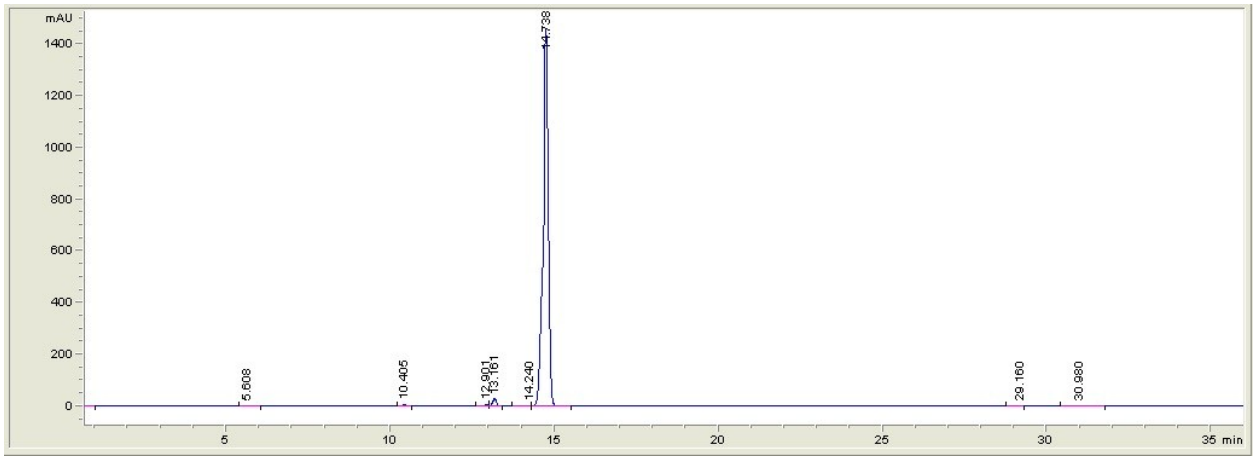


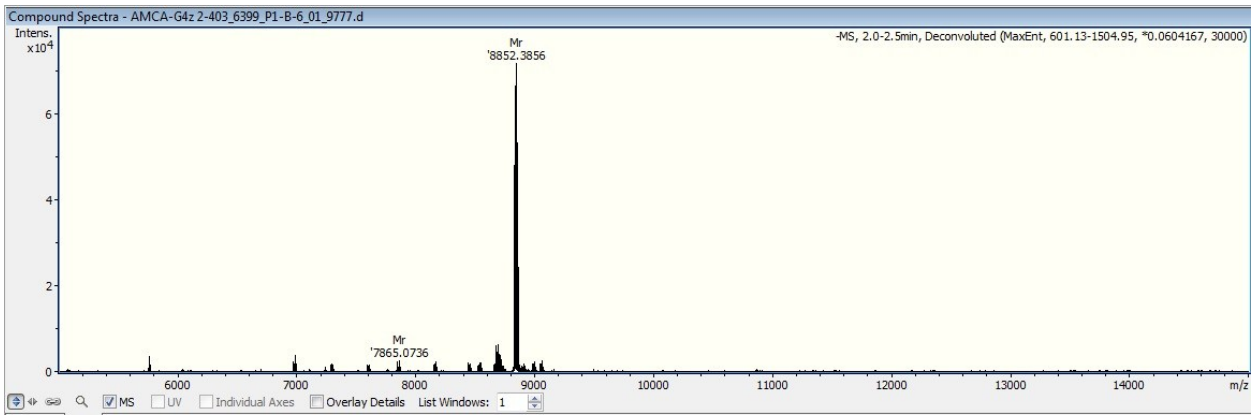
Oligonucleotide AMCA-G3z - HPLC traces and MS spectrum

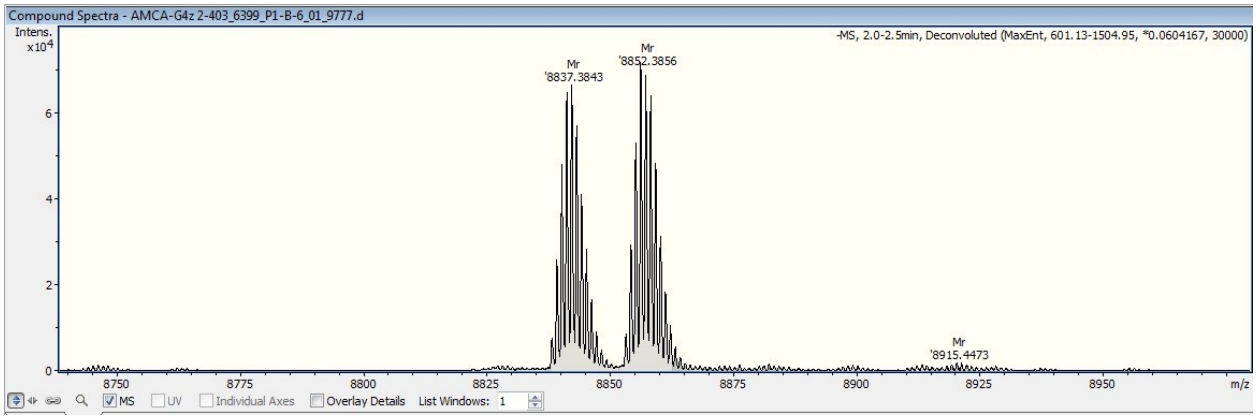




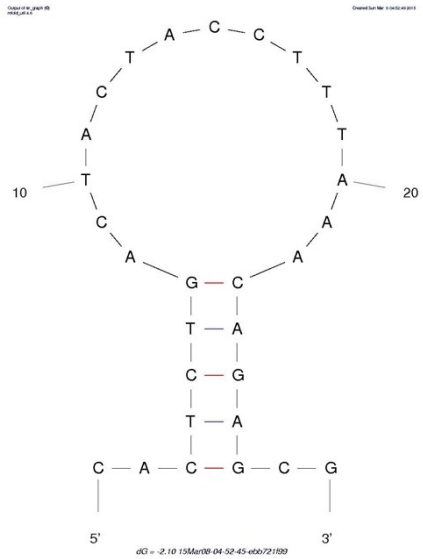
Oligonucleotide AMCA-G4z - HPLC traces and MS spectrum



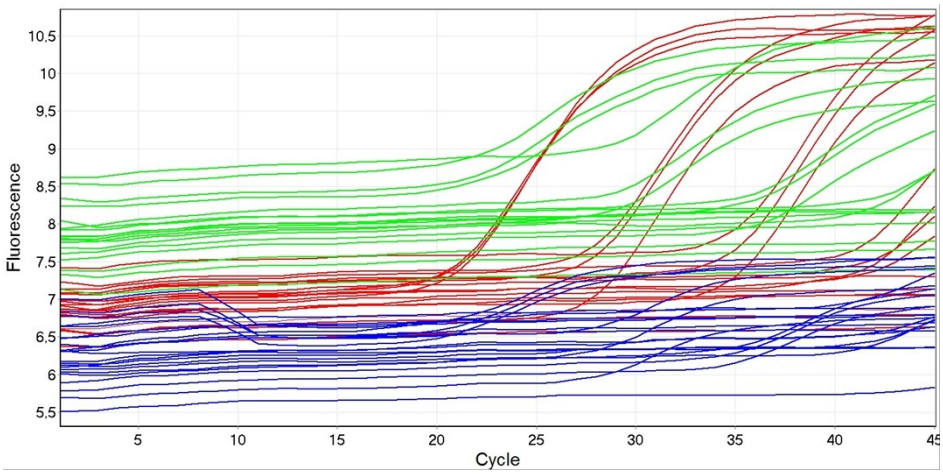




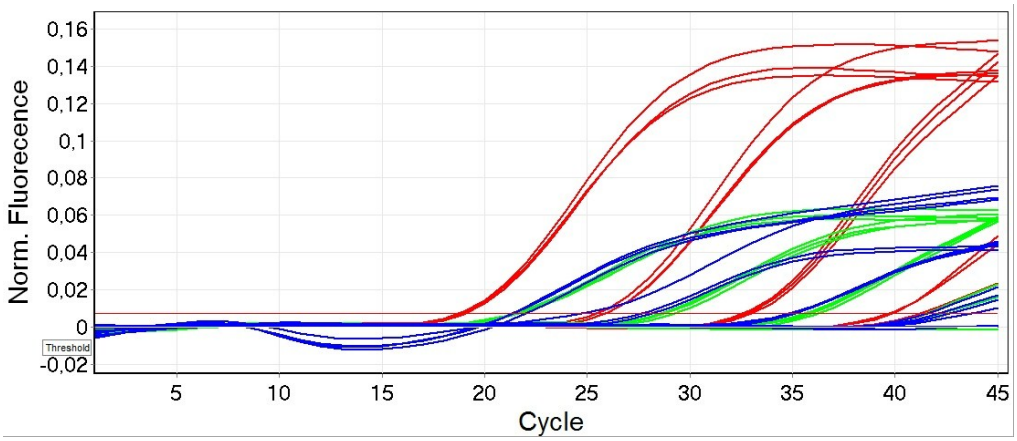
Secondary structure of P4z probes optimized by Mfold. Raw and normalized data of qPCR.



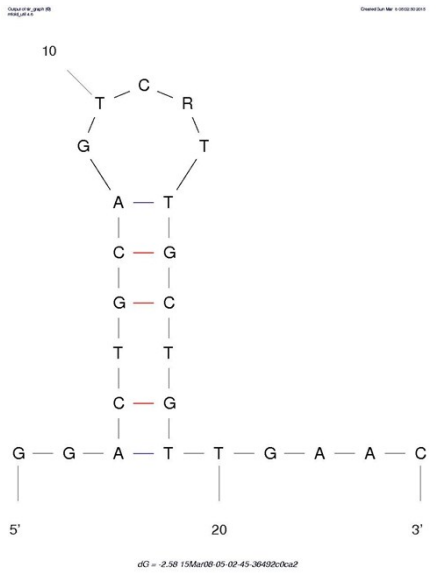
qPCR - raw data



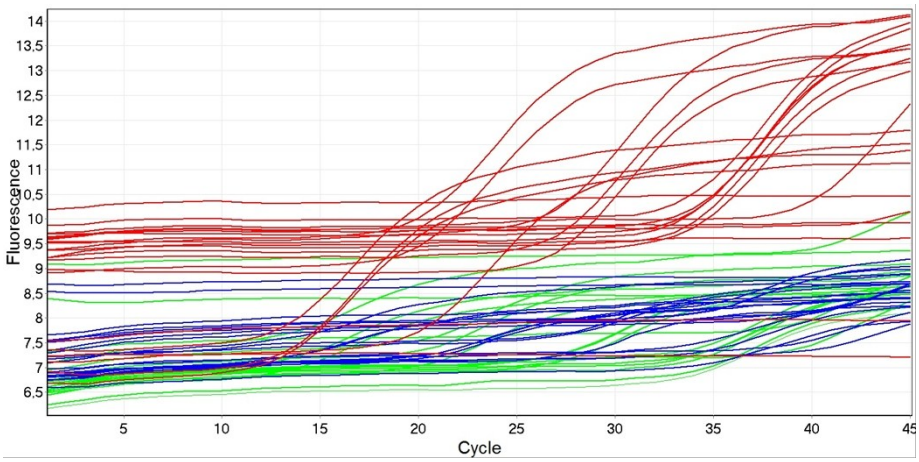
qPCR - normalized data



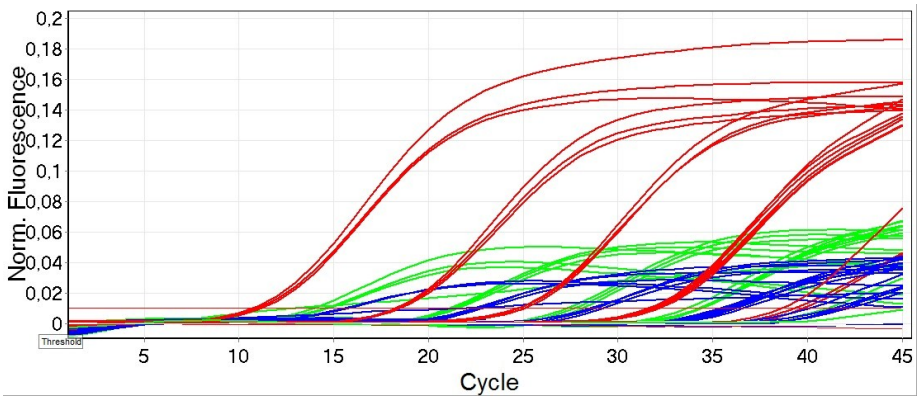
Secondary structure of P8z probes optimized by Mfold. Raw and normalized data of qPCR



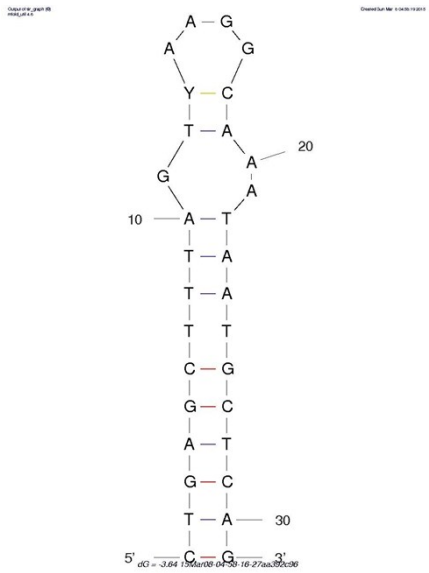
qPCR - raw data



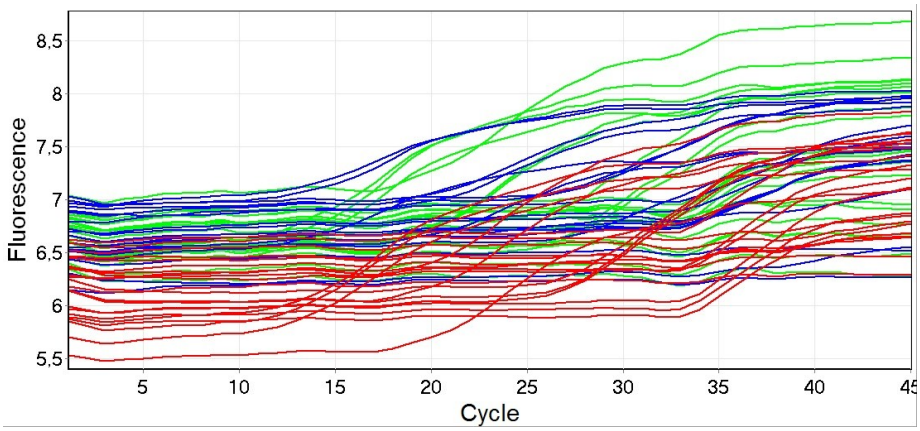
qPCR - normalized data



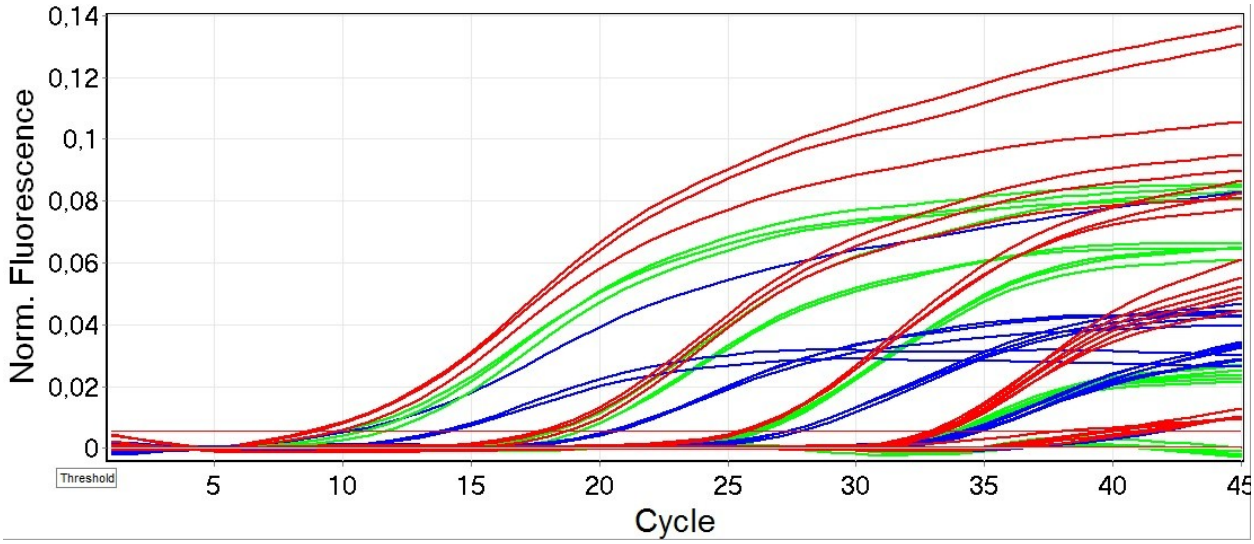
Secondary structure of G1z probes optimized by Mfold. Raw and normalized data of qPCR



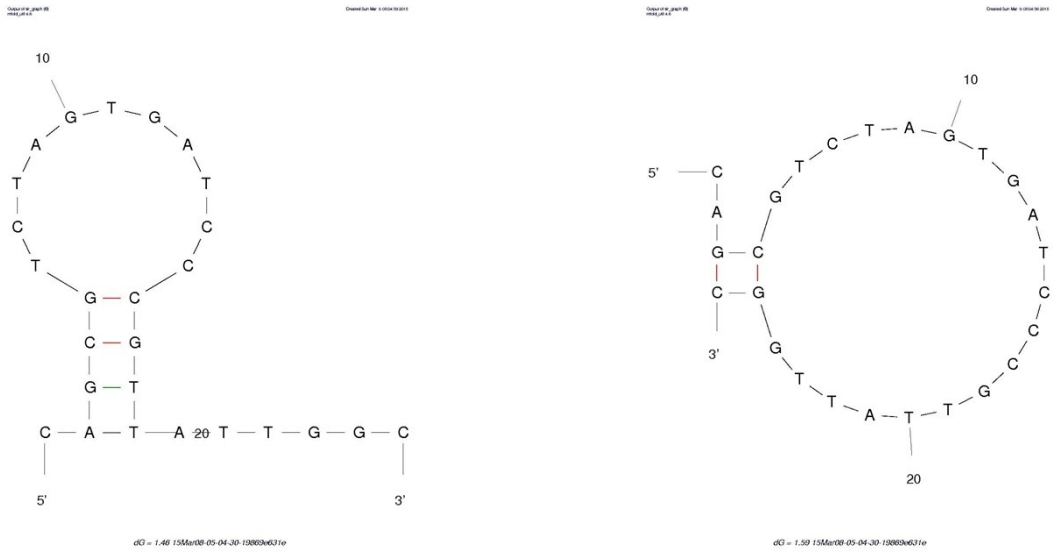
qPCR - raw data



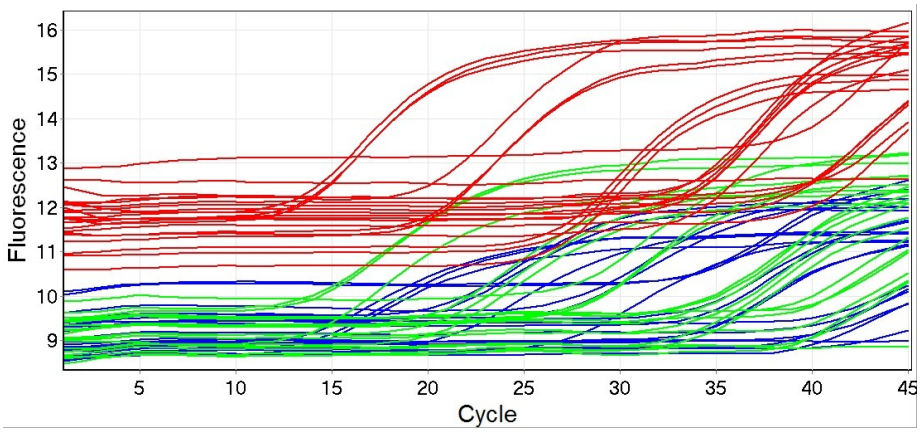
qPCR - normalized data



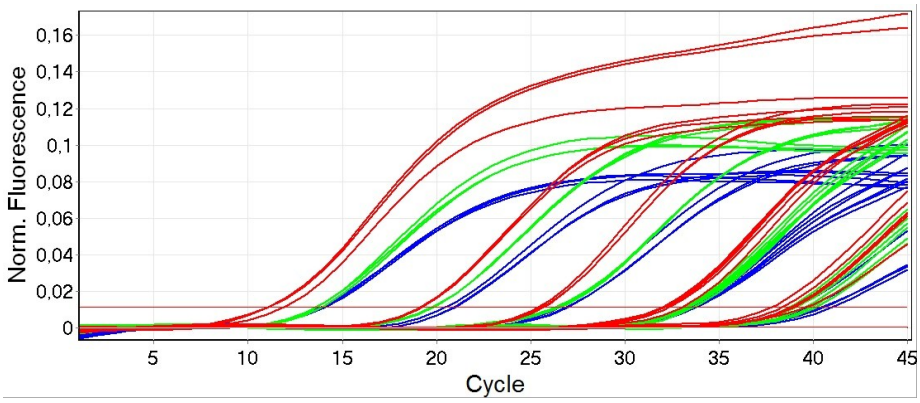
Secondary structure of G2z probes optimized by Mfold. Raw and normalized data of qPCR



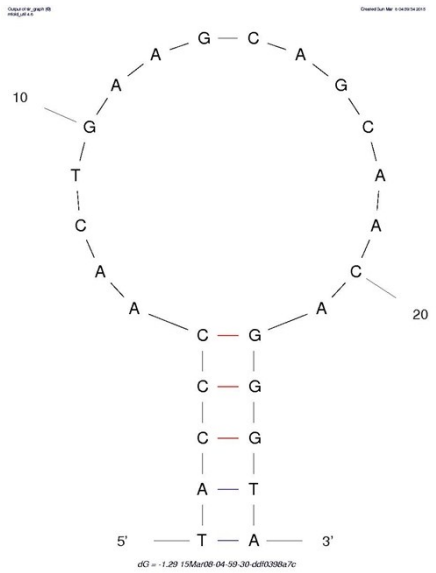
qPCR - raw data



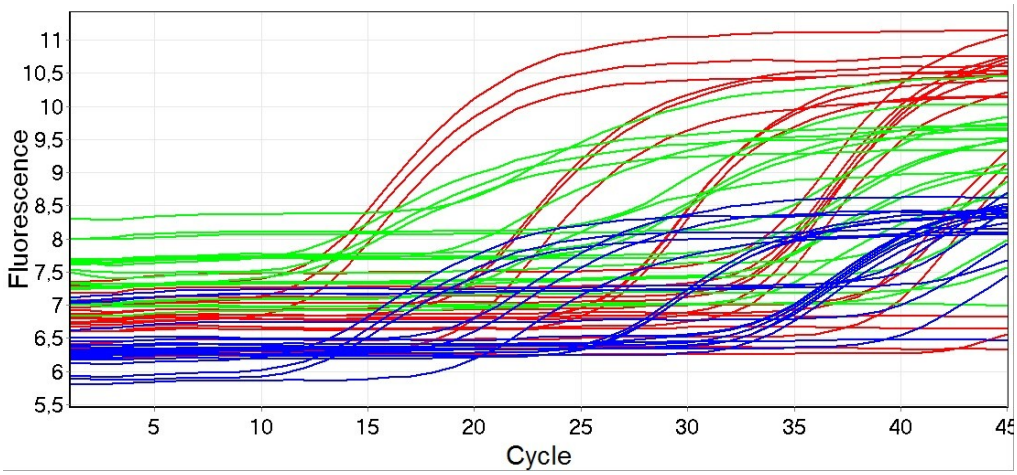
qPCR - normalized data



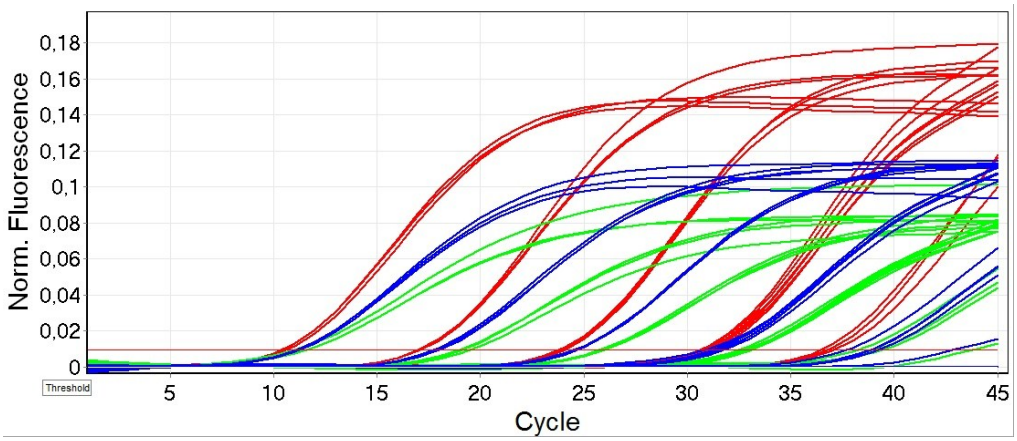
Secondary structure of G3z probes optimized by Mfold. Raw and normalized data of qPCR



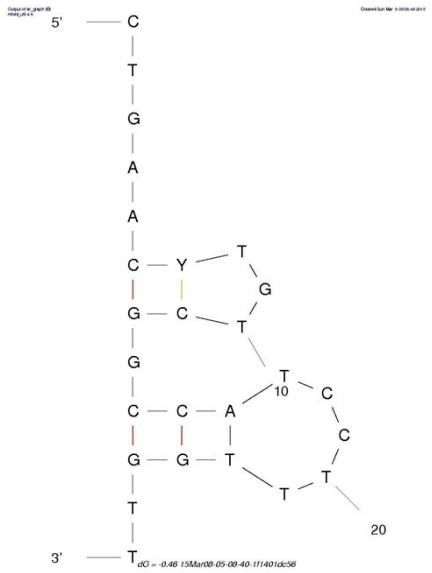
qPCR - raw data



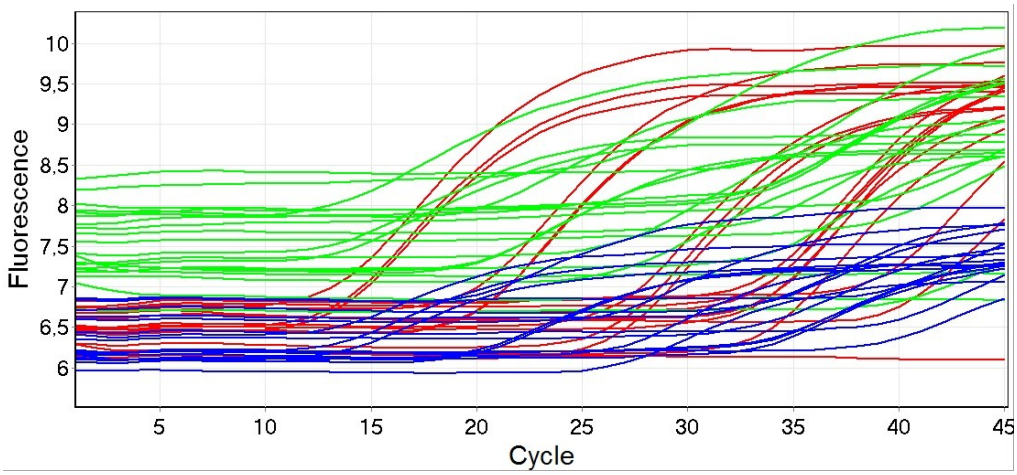
qPCR - normalized data



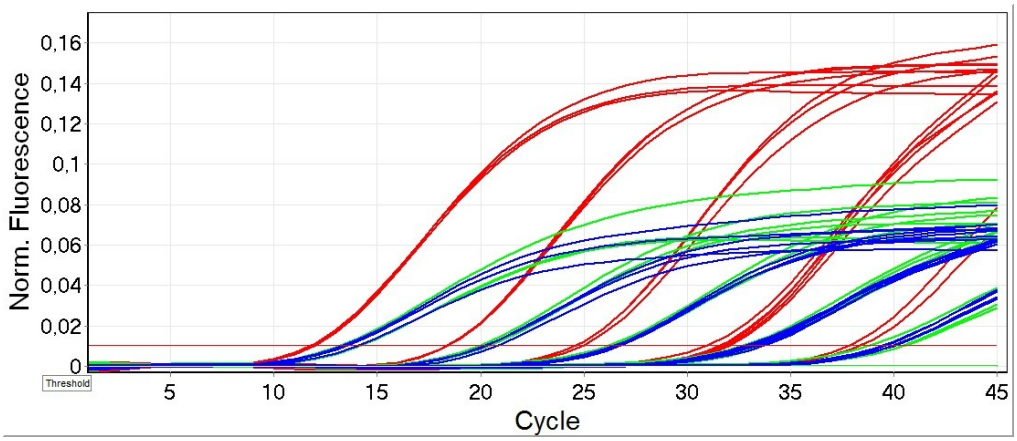
Secondary structure of G4z probes optimized by Mfold. Raw and normalized data of qPCR



qPCR - raw data



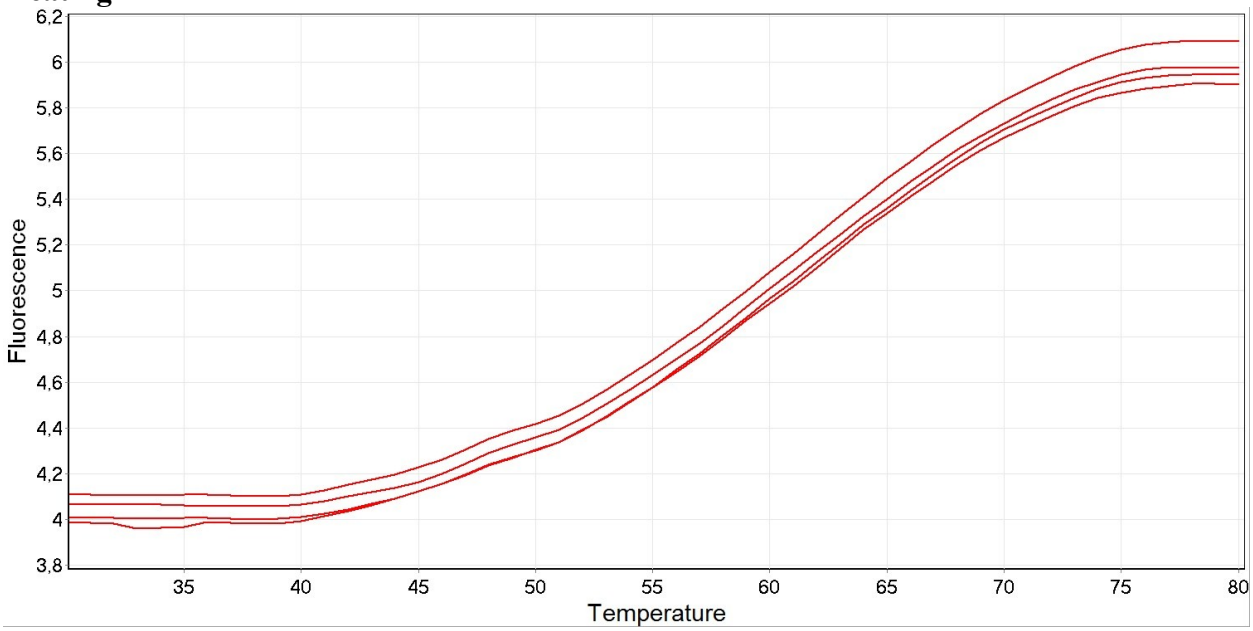
qPCR - normalized data



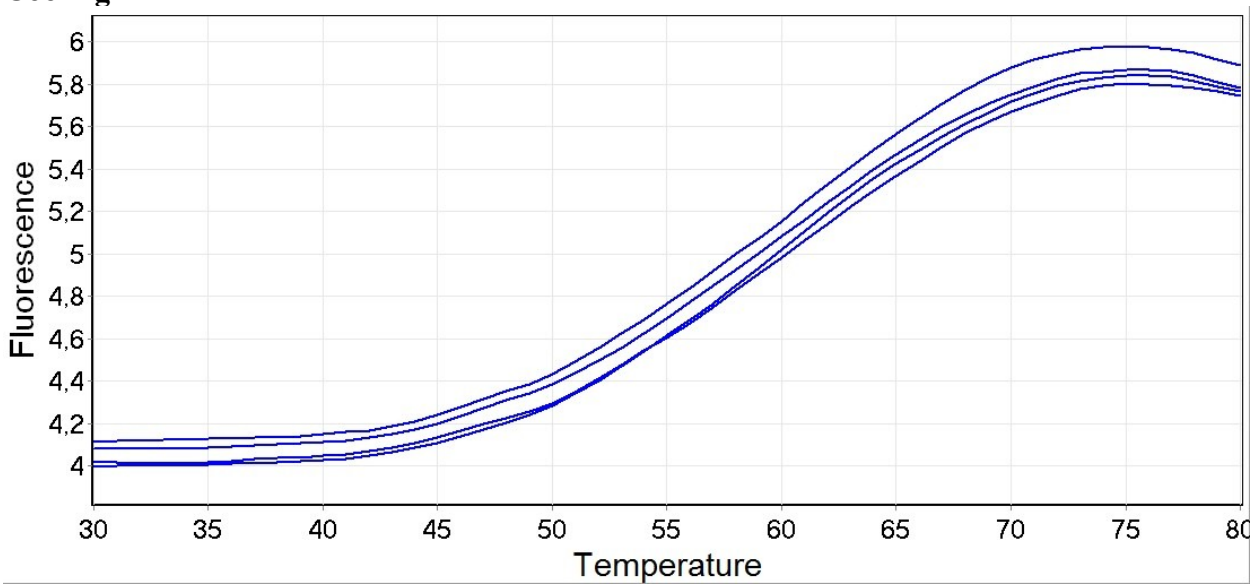
Melting curves for PEPy-labeled P8z, P4z, G1z, G2z, G3z, G4z probes.

PEPy-P8z

Heating

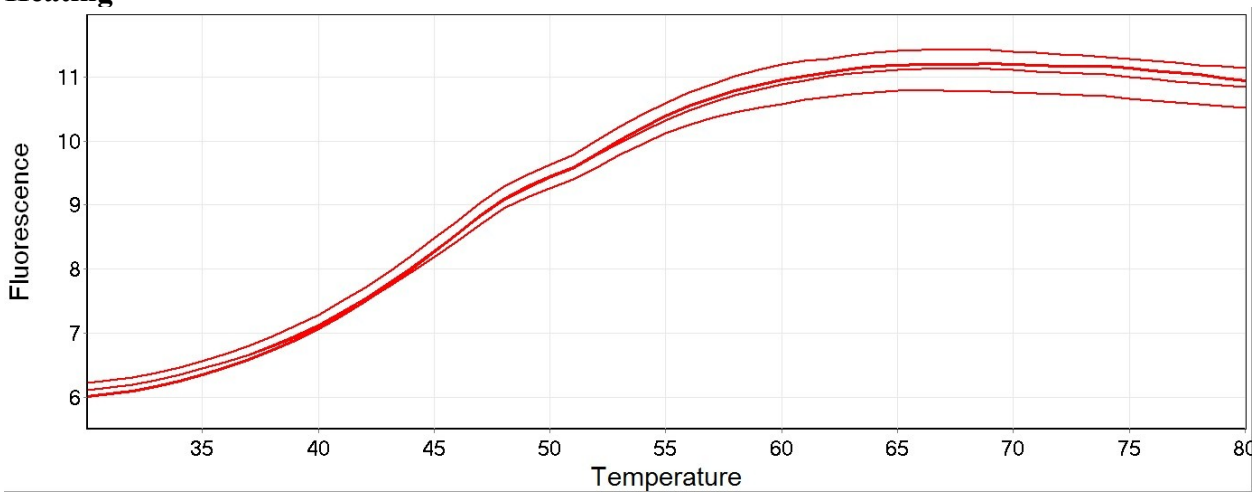


Cooling

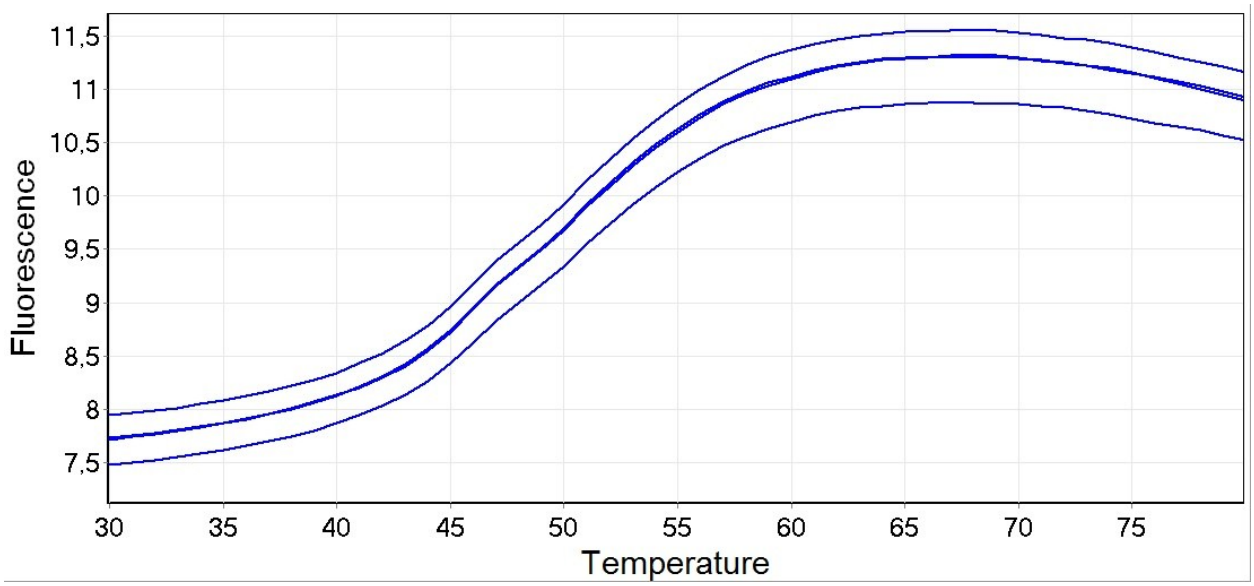


PEPy-P4z

Heating

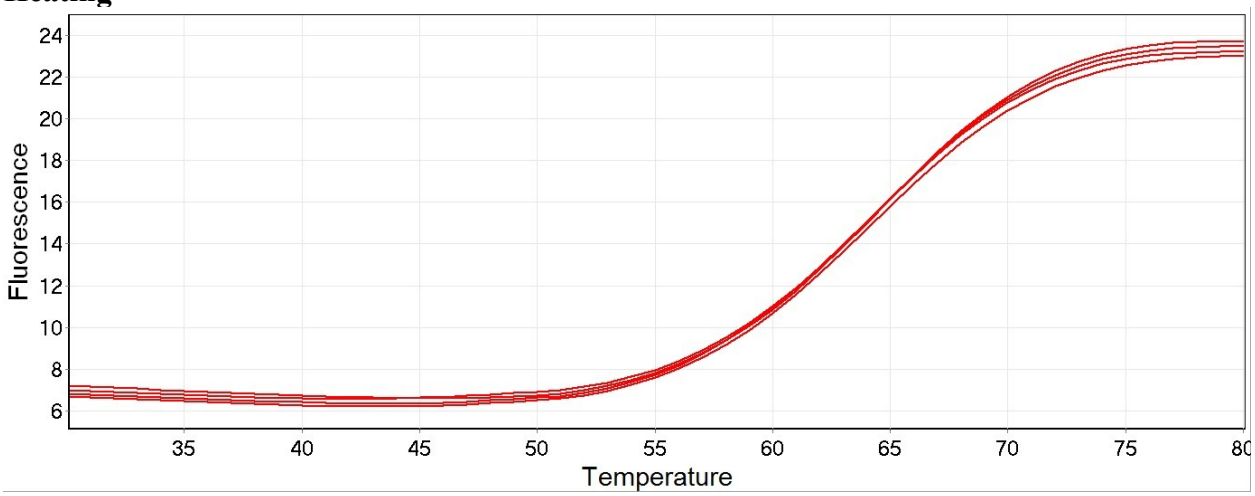


Cooling

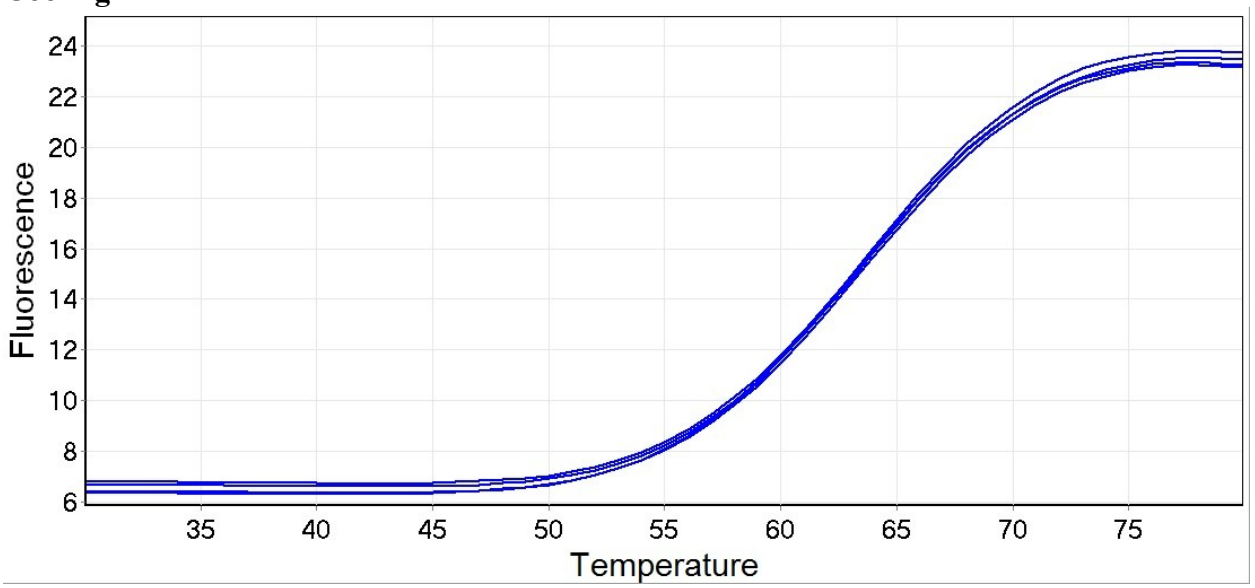


PEPy-G1z

Heating

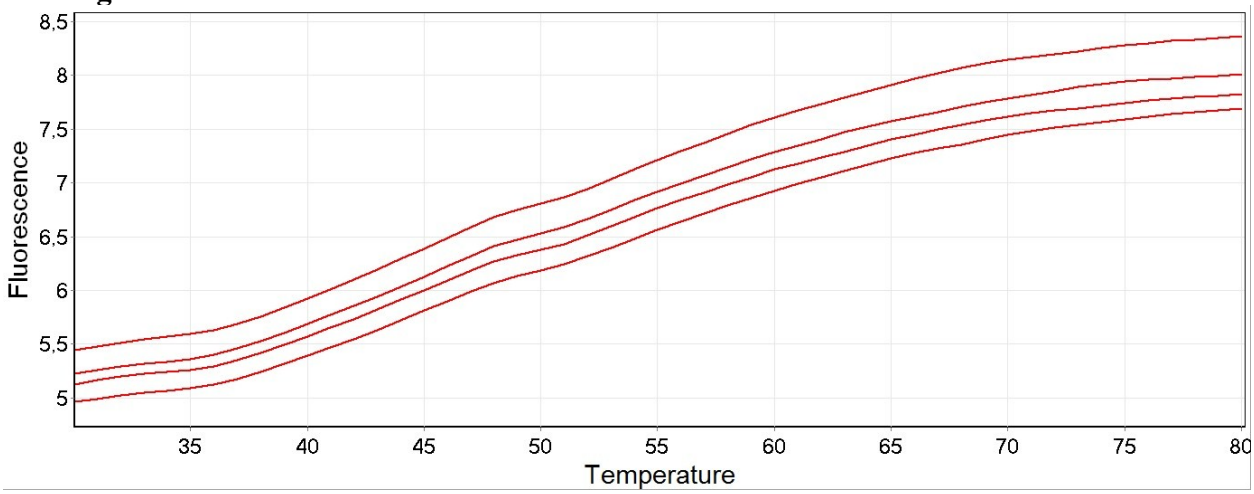


Cooling

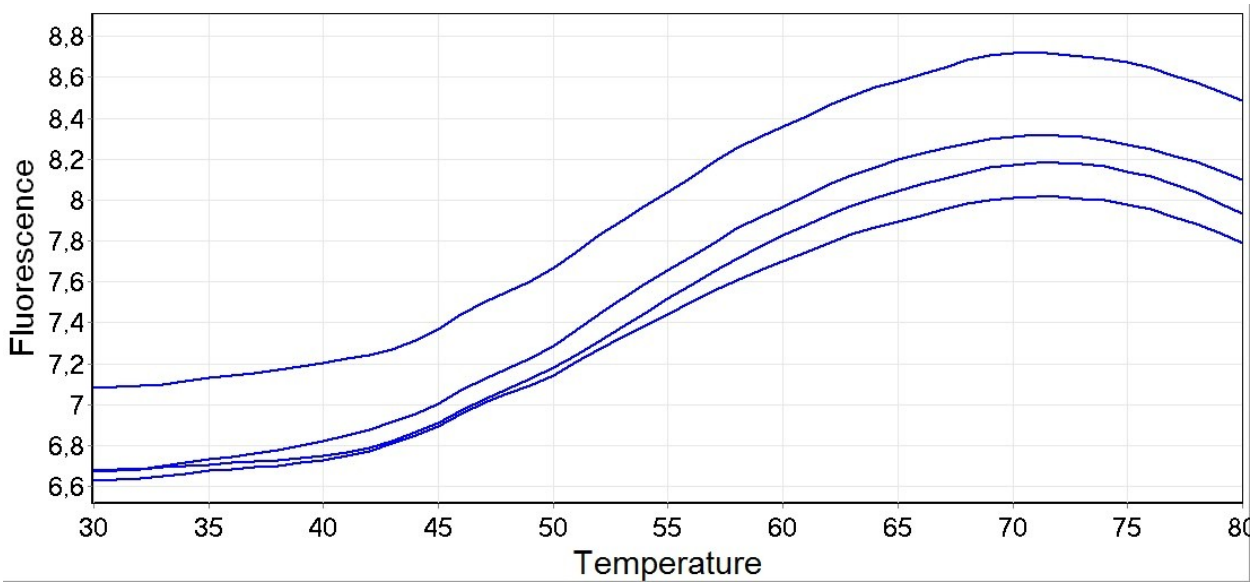


PEPy-G2z

Heating

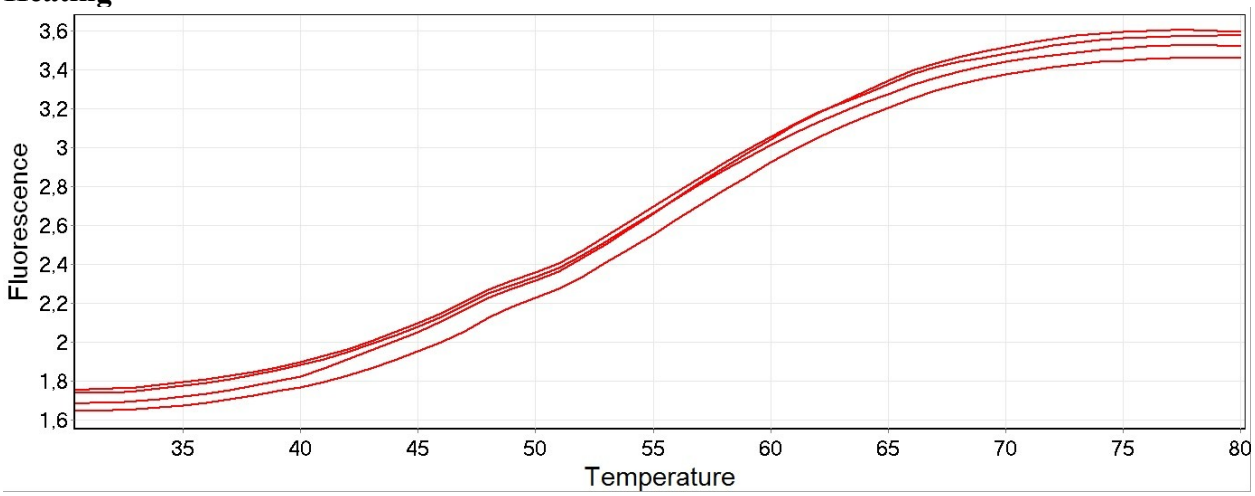


Cooling

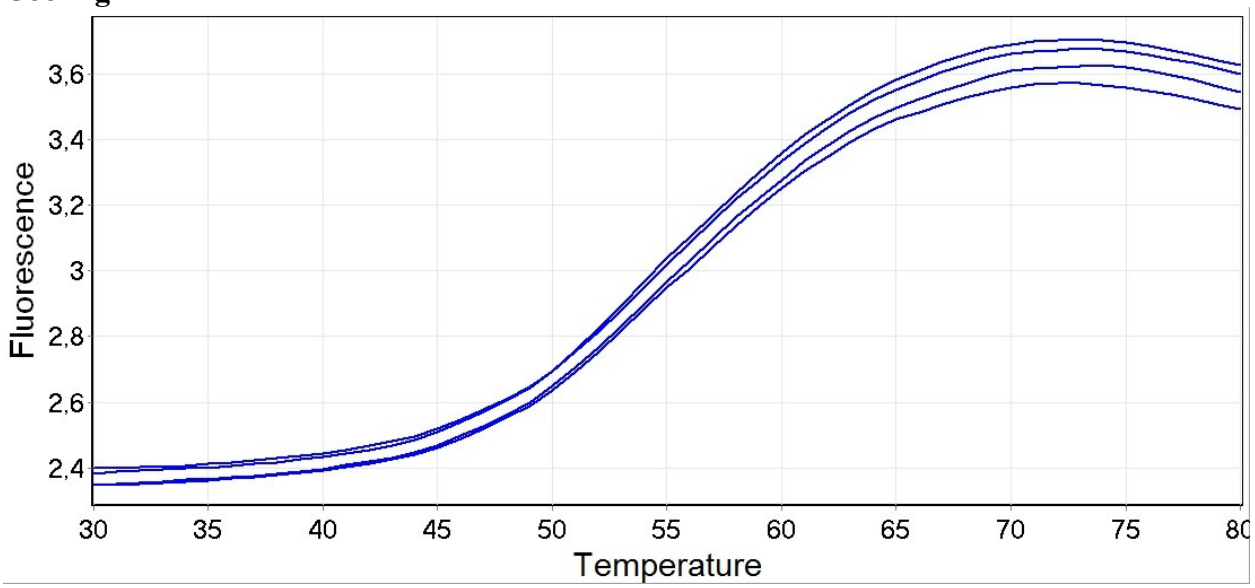


PEPy-G3z

Heating

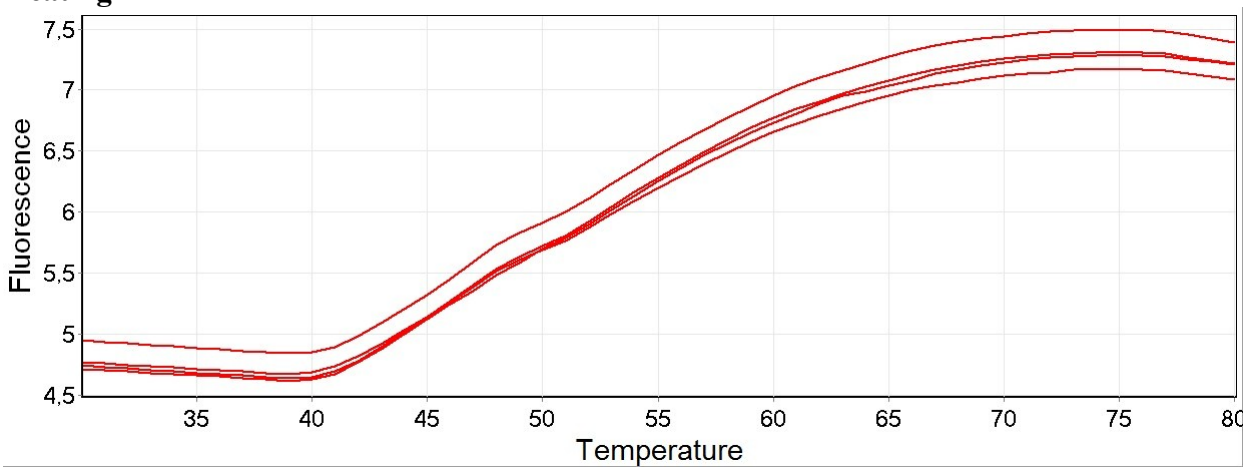


Cooling

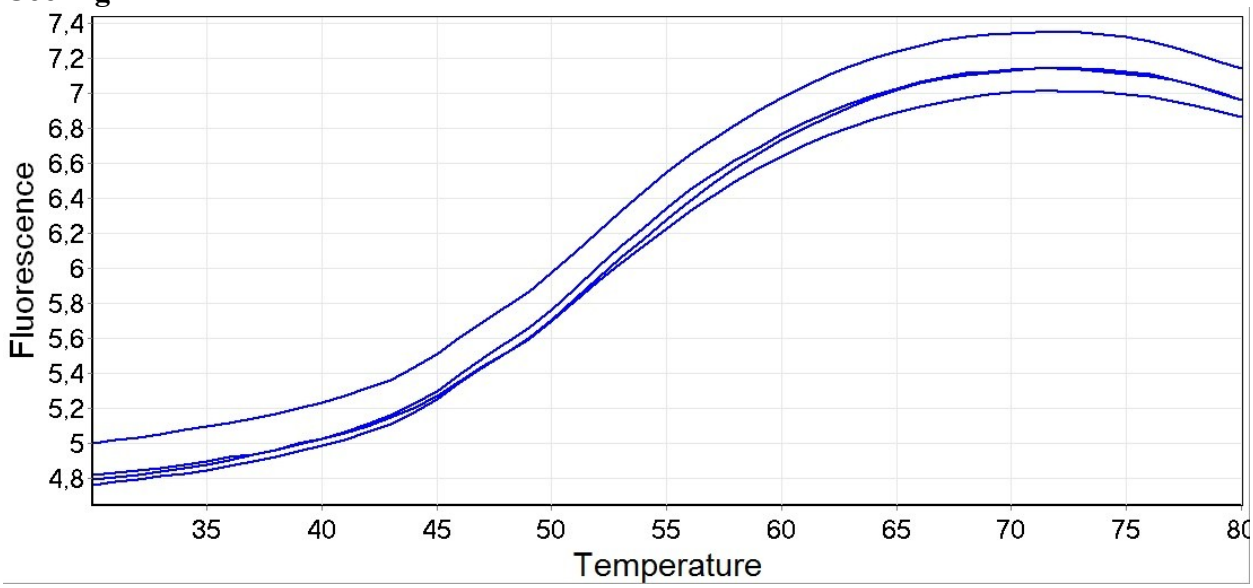


PEPy-G4z

Heating



Cooling



qPCR data for cross-talk studies between “blue” and “green” channels.

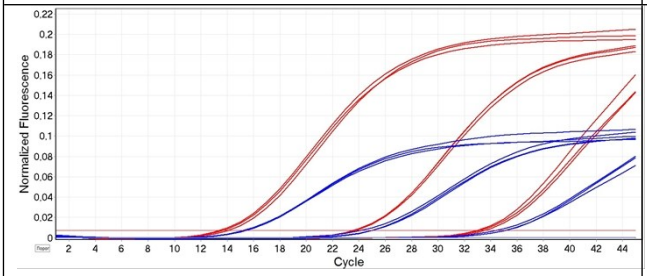
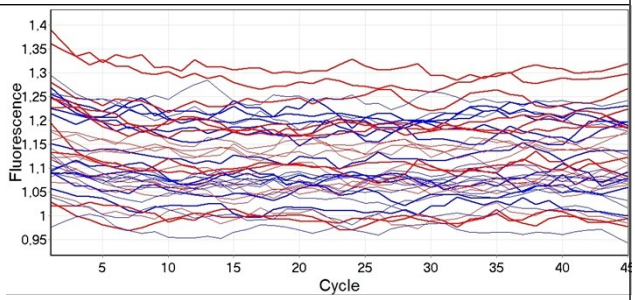
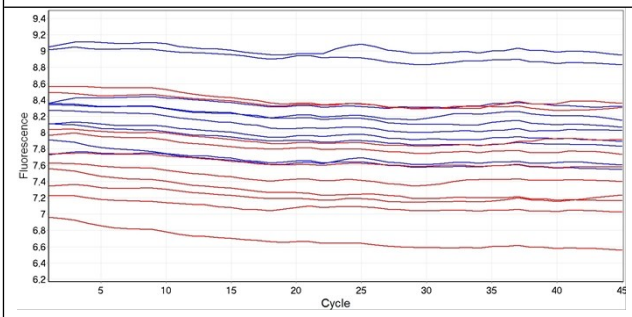
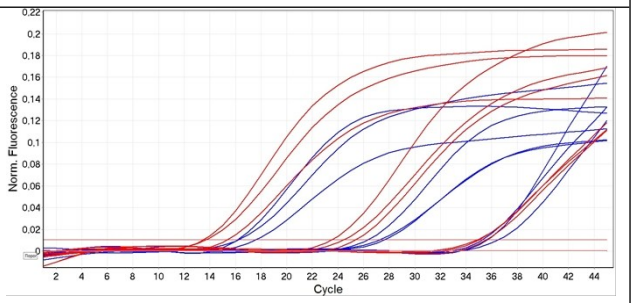
Blue channel	Green channel
	
Pepy-G4z (red), AMCA-G4z (blue)	**Pepy-G4z (red), AMCA-G4z (blue)**
	
FAM-G4z (red), FAM-G2z (blue)	**FAM-G4z (red), FAM-G2z (blue)**

Table S4. qPCR data for AMCA, Alexa 350 and PEPy dual labeled probes.

Probe	Probe sequence, 5′-3′	qPCR data					
		Target DNA, copies/ reaction	Cq (PEPy)	Cq (AMCA)	Cq (Alexa350)	Δ Cq (Ct_{AMCA}-Ct_{PEPy})	Δ Cq ($Ct_{Alexa350}$-Ct_{PEPy})
P4z	**Dye**-CAC TCT GAC TAC TAC CTT TAA ACA GAG CG-**Dabcyl**	$1.0\cdot10^{10}$	18.9±0.4	21.6±0.3	21.6±0.2	2.7±0.4	2.7±0.3
		$1.0\cdot10^{8}$	25.8±0.3	28.9±0.2	26.9±0.3	3.1±0.3	1.1±0.3
		$1.0\cdot10^{6}$	32.8±0.2	35.9±0.3	35.0±0.4	3.1±0.3	2.2±0.4
		$1.0\cdot10^{4}$	40.5±0.3	41.9±0.3	42.5±0.3	1.4±0.3	2.0±0.3
		$1.0\cdot10^{2}$	-	-	-	-	-
P8z	**Dye**-GGA CTG CAG TCR TTG CTG TTG AAC-**Dabcyl**	$1.0\cdot10^{10}$	11.0±0.4	15.7±0.4	14.4±0.4	4.7±0.3	3.4±0.4
		$1.0\cdot10^{8}$	18.4±0.3	22.6±0.3	21.3±0.3	4.2±0.3	2.9±0.3
		$1.0\cdot10^{6}$	24.8±0.4	28.9±0.2	27.7±0.4	4.1±0.4	2.9±0.4
		$1.0\cdot10^{4}$	31.9±0.3	36.0±0.3	34.2±0.3	4.1±0.3	2.3±0.3
		$1.0\cdot10^{2}$	38.7±0.4	41.5±0.3	40.1±0.3	3.8±0.4	1.4±0.4
G1z	**Dye**-CTG AGC TTT AGT YAA GGC AAA TAA TGC TCA G-**Dabcyl**	$1.0\cdot10^{10}$	10.6±0.3	14.5±0.3	12.0±0.3	3.9±0.3	1.4±0.3
		$1.0\cdot10^{8}$	19.2±0.2	21.9±0.3	19.9±0.3	2.7±0.3	0.7±0.3
		$1.0\cdot10^{6}$	26.7±0.3	28.9±0.3	27.1±0.3	2.2±0.3	0.4±0.3
		$1.0\cdot10^{4}$	34.1±0.3	35.9±0.3	35.0±0.3	1.8±0.3	0.9±0.3
		$1.0\cdot10^{2}$	43.3±0.3	44.4±0.3	>45	1.1±0.3	>1.7
G2z	**Dye**-CAG CGT CTA GTG ATC CCG TTA TTG GC-**Dabcyl**	$1.0\cdot10^{10}$	11.5±0.3	14.1±0.3	13.8±0.3	2.6±0.3	2.3±0.2
		$1.0\cdot10^{8}$	19.3±0.3	20.9±0.3	19.9±0.3	1.6±0.3	0.6±0.3
		$1.0\cdot10^{6}$	25.6±0.3	26.9±0.3	26.5±0.3	1.3±0.3	0.9±0.3
		$1.0\cdot10^{4}$	32.3±0.3	33.7±0.3	33.4±0.3	1.4±0.3	1.1±0.3
		$1.0\cdot10^{2}$	38.8±0.3	41.0±0.3	39.5±0.3	2.2±0.3	0.7±0.3
G3z	**Dye**-TAC CCA ACT GAA GCA GCA ACA GGG TA-**Dabcyl**	$1.0\cdot10^{10}$	10.5±0.3	11.4±0.3	11.1±0.3	0.9±0.3	0.8±0.3
		$1.0\cdot10^{8}$	17.1±0.3	19.2±0.3	17.9±0.3	0.8±0.3	0.8±0.3
		$1.0\cdot10^{6}$	24.0±0.3	25.9±0.3	24.6±0.3	1.9±0.3	0.6±0.3
		$1.0\cdot10^{4}$	30.9±0.3	32.9±0.3	31.4±0.3	0.5±0.3	0.5±0.3
		$1.0\cdot10^{2}$	36.8±0.3	40.2±0.3	39.5±0.3	3.4±0.3	2.7±0.3
G4z	**Dye**-CTG AAC YTG TCG GCC ATC CTT TGG TT-**Dabcyl**	$1.0\cdot10^{10}$	12.7±0.3	15.0±0.3	14.8±0.5	2.3±0.5	2.1±0.3
		$1.0\cdot10^{8}$	19.2±0.3	21.3±0.3	21.5±0.3	2.1±0.3	2.3±0.3
		$1.0\cdot10^{6}$	25.6±0.3	27.6±0.3	27.7±0.3	2.0±0.3	2.1±0.3
		$1.0\cdot10^{4}$	32.6±0.3	34.7±0.3	34.5±0.3	2.1±0.3	1.9±0.3
		$1.0\cdot10^{2}$	39.1±0.3	41.3±0.3	41.0±0.3	2.2±0.3	1.9±0.3

Table S5. Normalized fluorescence data for AMCA, Alexa 350 and PEPy dual labeled probes in end-point qPCR.

Probe	Probe sequence, 5′-3′	Fluorescence data	
		Dye	Increase of normalized fluorescence
P4z	**Dye**-CAC TCT GAC TAC TAC CTT TAA ACA GAG CG-**Dabcyl**	PEPy	0.14±0.01
		Alexa 350	0.06±0.01
		AMCA	0.05±0.01
P8z	**Dye**-GGA CTG CAG TCR TTG CTG TTG AAC-**Dabcyl**	PEPy	0.15±0.01
		Alexa 350	0.06±0.01
		AMCA	0.04±0.01
G1z	**Dye**-CTG AGC TTT AGT YAA GGC AAA TAA TGC TCA G-**Dabcyl**	PEPy	0.10±0.01
		Alexa 350	0.08±0.01
		AMCA	0.04±0.01
G2z	**Dye**-CAG CGT CTA GTG ATC CCG TTA TTG GC-**Dabcyl**	PEPy	0.13±0.01
		Alexa 350	0.12±0.01
		AMCA	0.09±0.01
G3z	**Dye**-TAC CCA ACT GAA GCA GCA ACA GGG TA-**Dabcyl**	PEPy	0.16±0.01
		Alexa 350	0.08±0.01
		AMCA	1.5±0.01
G4z	**Dye**-CTG AAC YTG TCG GCC ATC CTT TGG TT-**Dabcyl**	PEPy	19.5±0.01
		Alexa 350	24.5±0.01
		AMCA	1.3±0.01