Analyst

Electronic Supplementary Information

Design of Molecular beacons: 3' couple quenchers improve fluorogenic properties of a probe in real-time polymerase chain reaction assay

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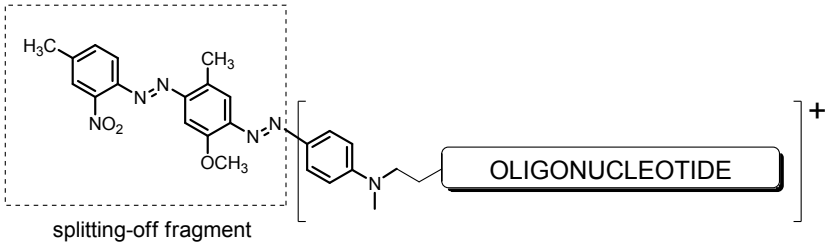
Table S1. Mass-spectral characterization of Molecular beacons.[a]

#	Probe structure, 5'→3' [b]	MALDI MS [c]	
		calculated	found
MB1	**FAM**-gcgggtcattcgaaacgcattcattaccccgc-**BHQ1**	10562	10560
MB2	**FAM**-gcggggtcattcgaaacgcattcattaccccgc-**BHQ1**	10891	10891
MB3	**FAM**-cggggtctcattcgaaacgcattcattaccccg-**BHQ1**	10866	10875
MB4	**FAM**-ccggggctcattcgaaacgcattcattaccccgg-**BHQ1**	11180	11172
MB5	**FAM**-gcggtcattcgaaacgcattcattaccccgc-**BHQ1**	10233	10224
MB6	**FAM**-gccggggctcattcgaaacgcattcattaccccggc-**BHQ1**	11799	11798
MB7	**FAM**-cgccggggctcattcgaaacgcattcattaccccggcg-**BHQ1**	12417	–
MB8	**FAM**-accggggctcattcgaaacgcattcattaccccggt-**BHQ1**	11798	11778
MB9	**FAM**-gcggggtcattcgaaacccattcattaccccgc-**BHQ1**	10851	10839
MB10	**FAM**-gcggggtcattcgaaacacattcattaccccgc-**BHQ1**	10875	10876
MB11	**FAM**-gcggggtcattcgaaactcattcattaccccgc-**BHQ1**	10866	10859
MB12	**FAM**-gcgggg(t^{FAM})cattcgaaacgcattcattaccccgc-**BHQ1**	11440	11446
MB13	**FAM**-gcggggtcattcgaaacgcattcattaccccgc-(**BHQ1**)$_2$	12073	–
MB14	**FAM**-gcggggtcattcgaaacgcattcattaccccgc-**BHQ1-BHQ1**	11445	–

[a] MBs were characterized by MALDI-TOF mass-spectrometry on Bruker Microflex instrument in positive ion mode using a mixture (1:1 v/v) of 2,4,6-dihydroxyacetophenone (40 mg/mL in MeOH) and aqueous diammonium hydrogen citrate (80 mg/mL) as a matrix premixed just before loading the samples onto a plate. MB samples were desalted using Agilent C18 Spec tips (the sample in the tip was washed three times with high purity water and eluted with 50% MeCN).

[b] Structures of modifications see in the main paper.

[c] Average (not monoisotopic) mass; calculated masses are given for structures arising from typical fragmentation of BHQ1-containing oligonucleotides in MALDI mass spectrometer with splitting-off fragment



replaced by OH and with Na^+ added. This type of fragmentation was noted as predominant in our previous paper [Ryazantsev et al. *Anal. Bioanal. Chem.* **404** (2012) 59–68] for BHQ1 containing TaqMan probes. For heavier MB probes in this study the [M–BHQ1 fragment + Na]$^+$ is the only detectable peak in MALDI MS. The most heavy probe, MB7, and probes with two BHQ1 residues, MB13 and MB14, gave no detectable signals. Their identity was confirmed by PAGE and HPLC (data not shown) compared with oligonucleotide probes carrying similar modifications from above mentioned paper.

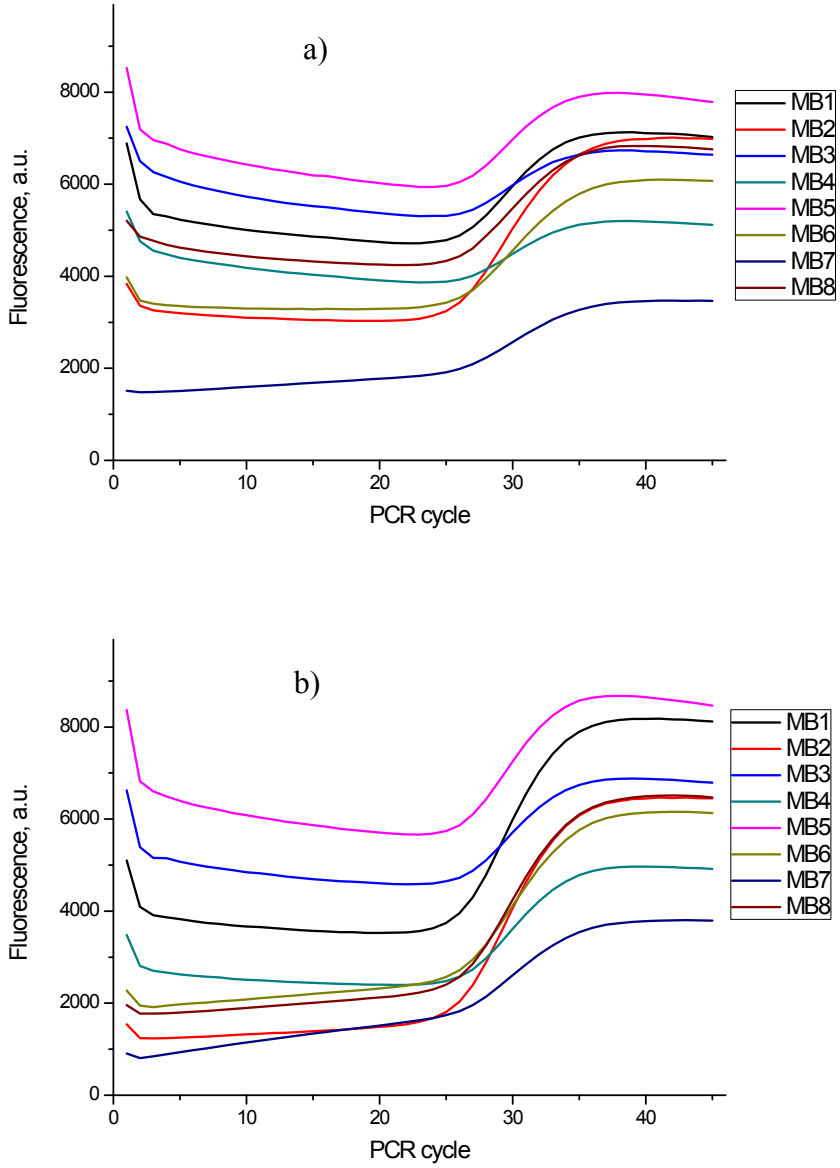


Figure S1. Fluorescence change in the course of rtPCR (45 cycles); probes MB1–MB8; raw data (each mean of two repeats), detection temperature 64ºC (a) and 55ºC (b).

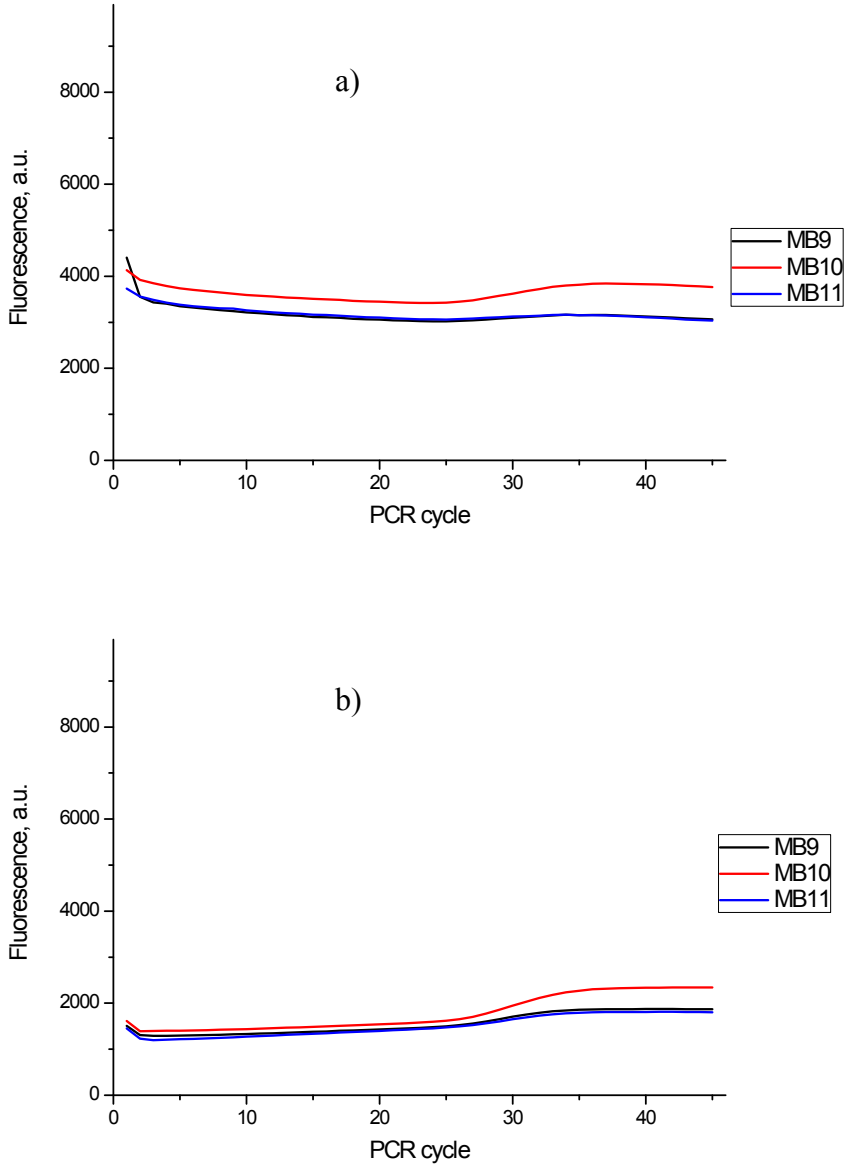


Figure S2. Fluorescence change in the course of rtPCR (45 cycles); probes MB9–MB11; raw data (each mean of two repeats), detection temperature 64ºC (a) and 55ºC (b).

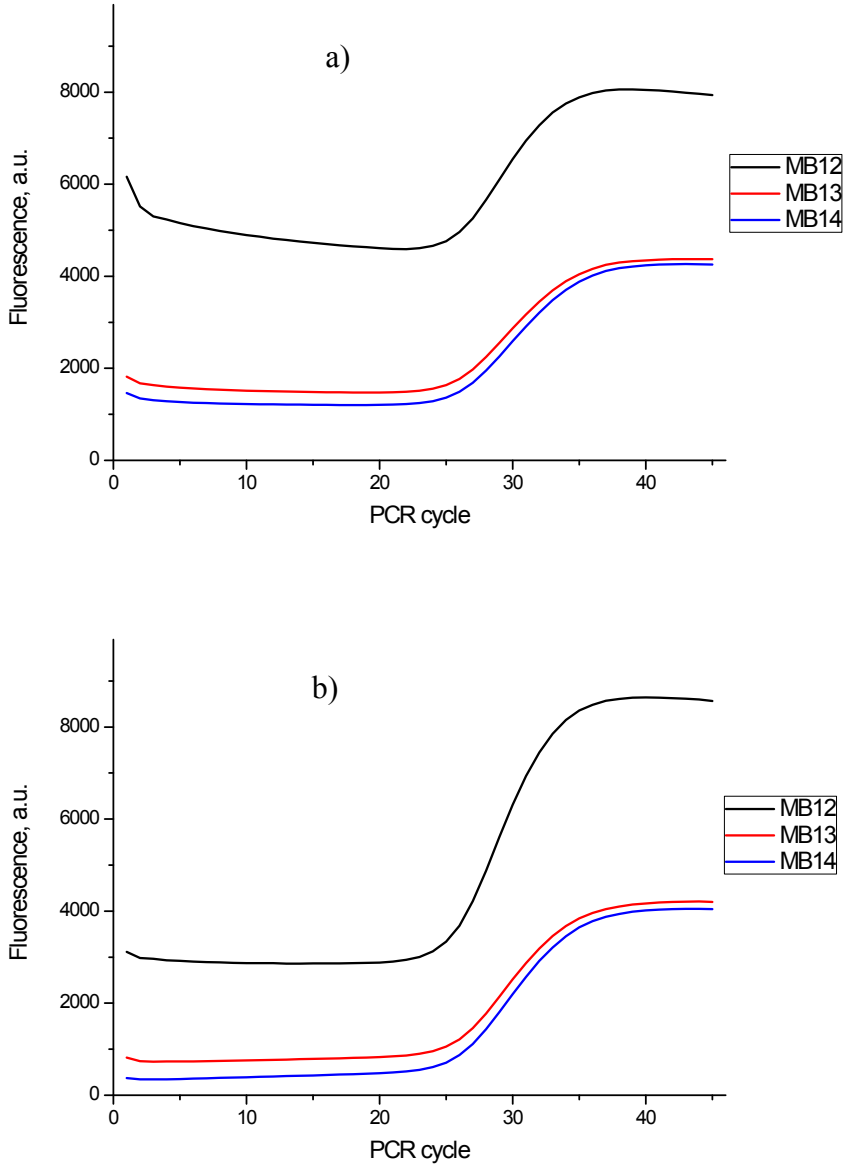


Figure S3. Fluorescence change in the course of rtPCR (45 cycles); probes MB12–MB14; raw data (each mean of two repeats), detection temperature 64ºC (a) and 55ºC (b).

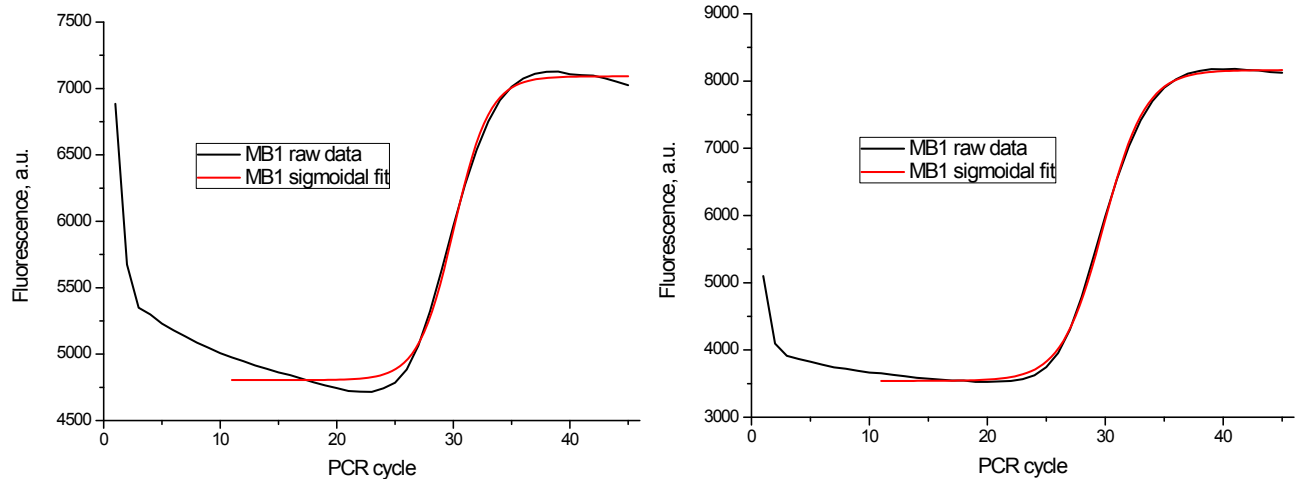


Figure S4. Sigmoidal fit for fluorescence increase in rtPCR (45 cycles) for probe MB1; fluorescence registered 64ºC (left) and 55ºC (right). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

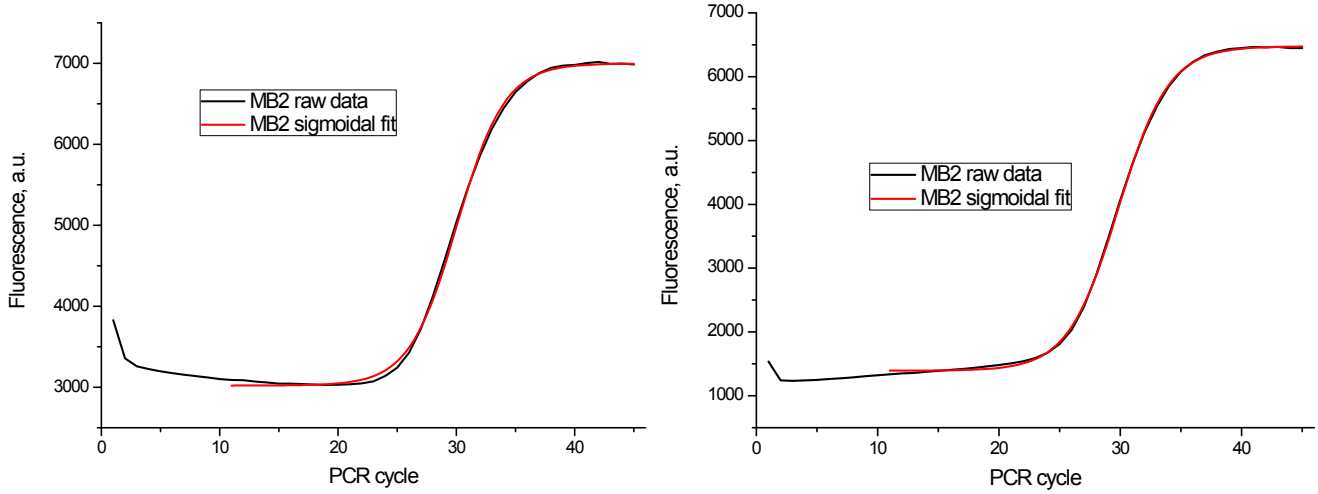


Figure S5. Sigmoidal fit for fluorescence increase in rtPCR (45 cycles) for probe MB2; fluorescence registered 64ºC (left) and 55ºC (right). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

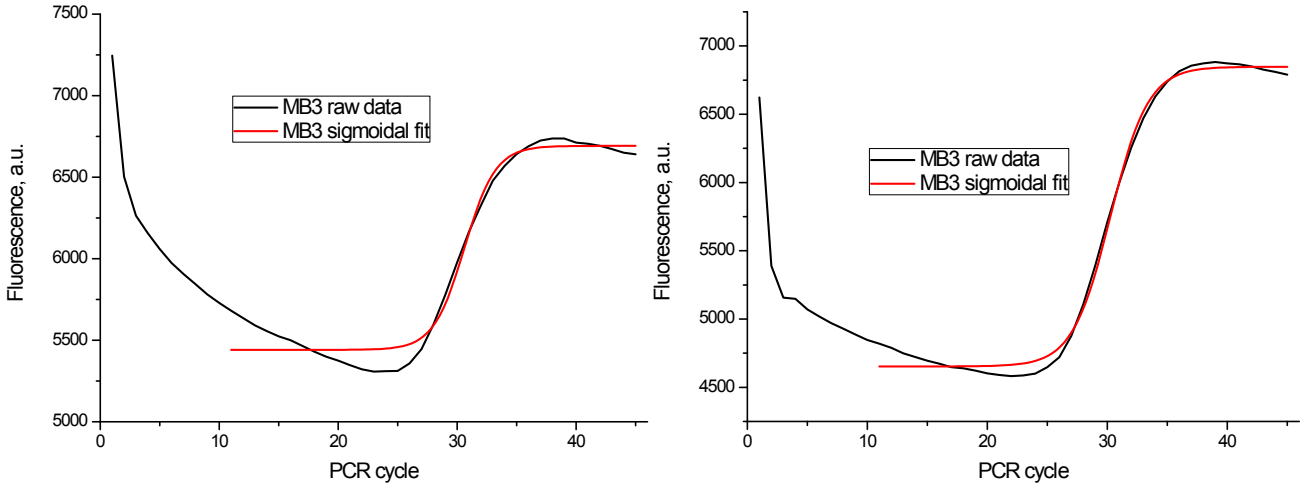


Figure S6. Sigmoidal fit for fluorescence increase in rtPCR (45 cycles) for probe MB3; fluorescence registered 64ºC (left) and 55ºC (right). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

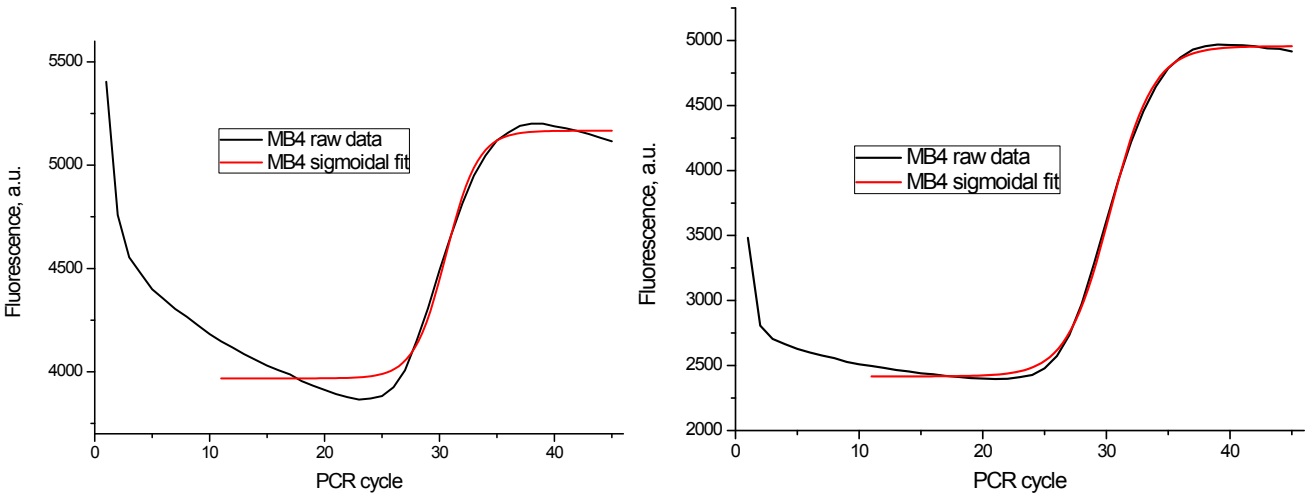


Figure S7. Sigmoidal fit for fluorescence increase in rtPCR (45 cycles) for probe MB4; fluorescence registered 64ºC (left) and 55ºC (right). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

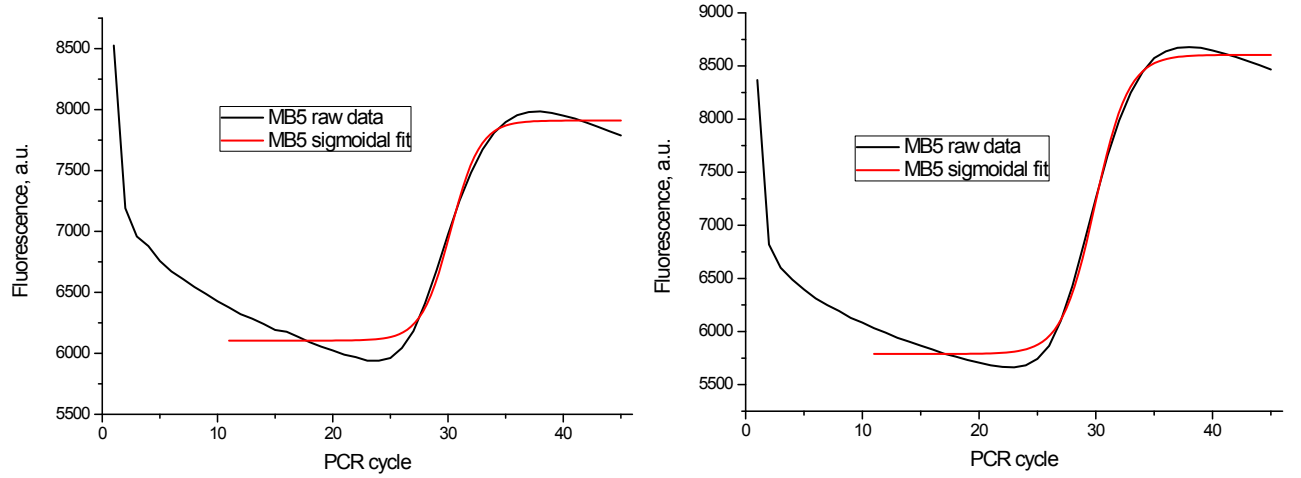


Figure S8. Sigmoidal fit for fluorescence increase in rtPCR (45 cycles) for probe MB5; fluorescence registered 64ºC (left) and 55ºC (right). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

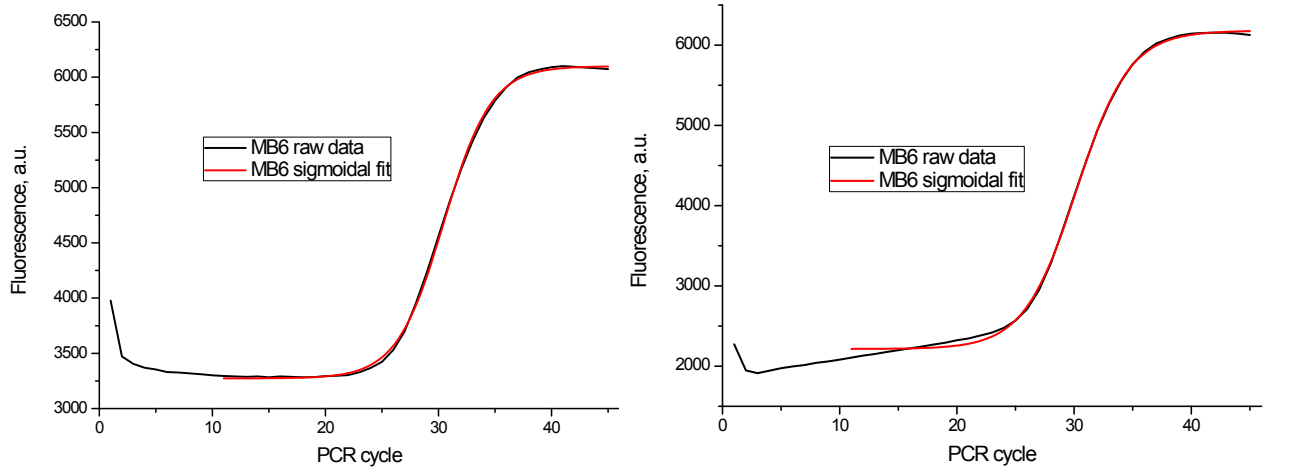


Figure S9. Sigmoidal fit for fluorescence increase in rtPCR (45 cycles) for probe MB6; fluorescence registered 64ºC (left) and 55ºC (right). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

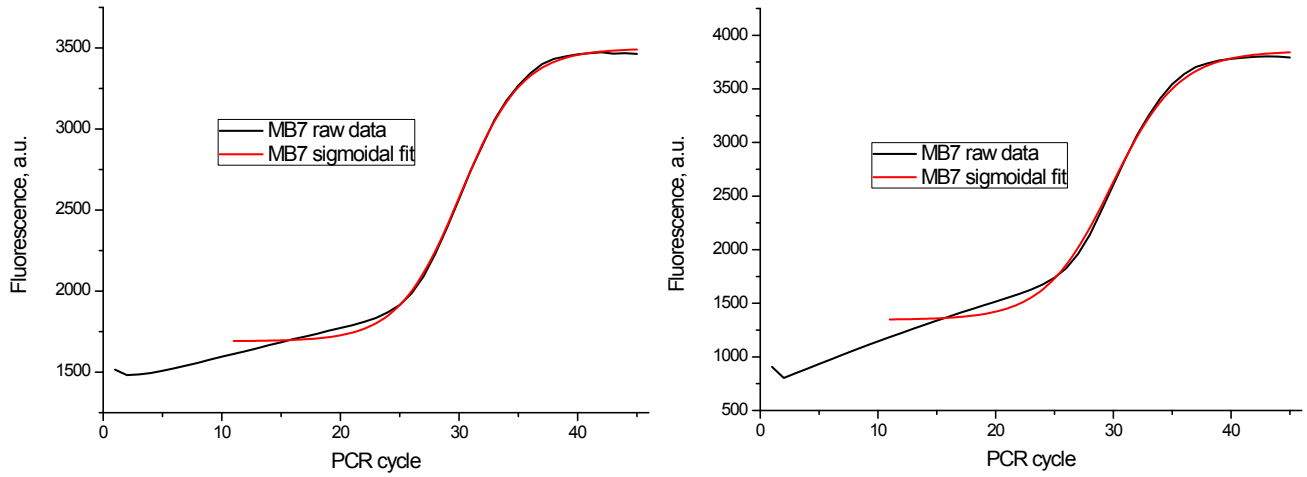


Figure S10. Sigmoidal fit for fluorescence increase in rtPCR (45 cycles) for probe MB7; fluorescence registered 64ºC (left) and 55ºC (right). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

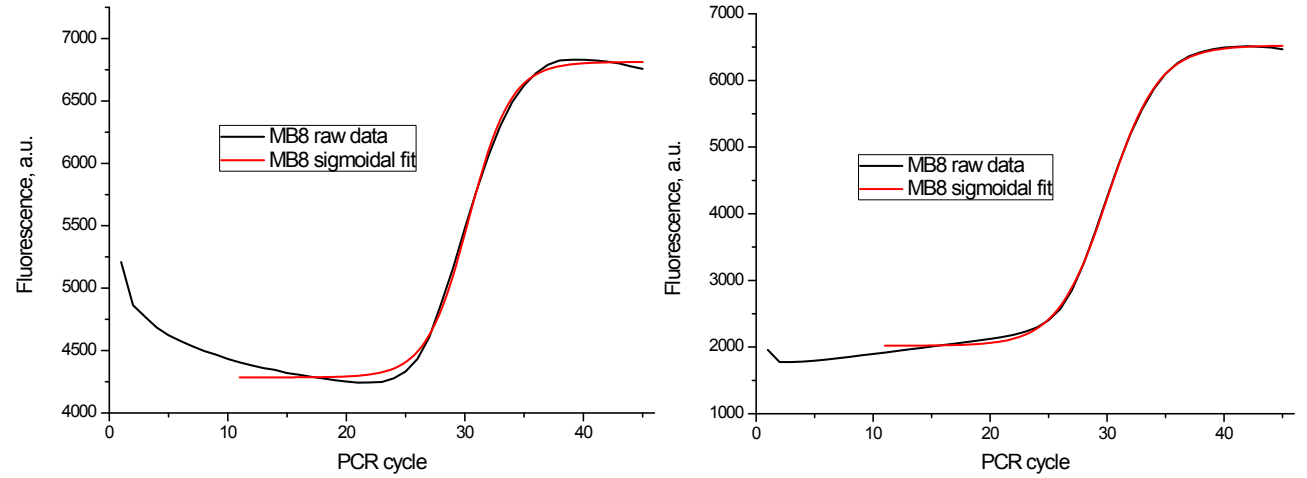


Figure S11. Sigmoidal fit for fluorescence increase in rtPCR (45 cycles) for probe MB8; fluorescence registered 64ºC (left) and 55ºC (right). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

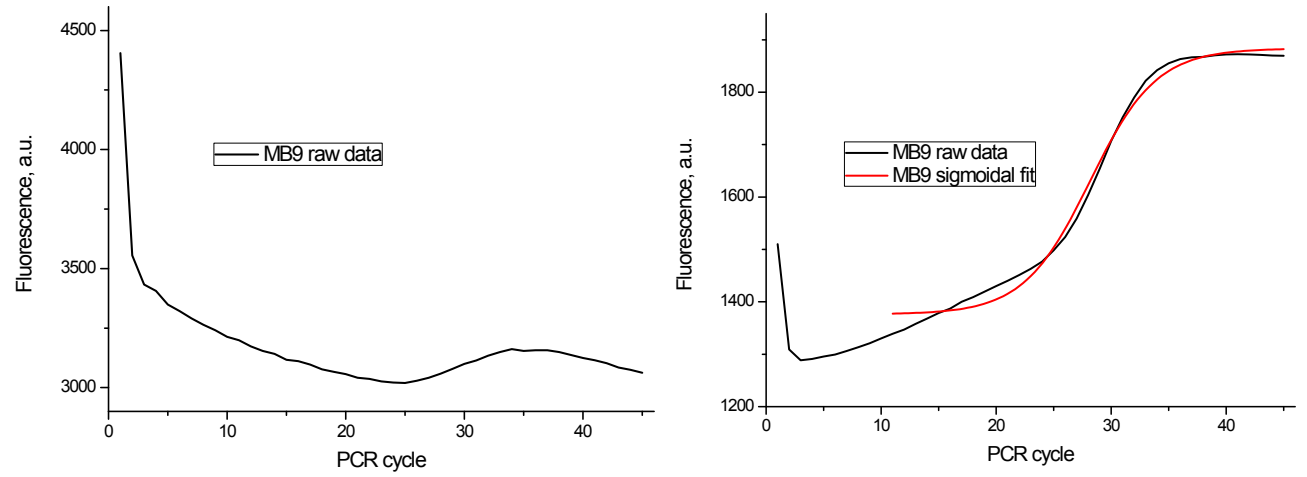


Figure S12. Fluorescence increase in rtPCR (45 cycles) for mismatched probe MB8; fluorescence registered 64ºC (left) and 55ºC (right; + sigmoidal fit). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

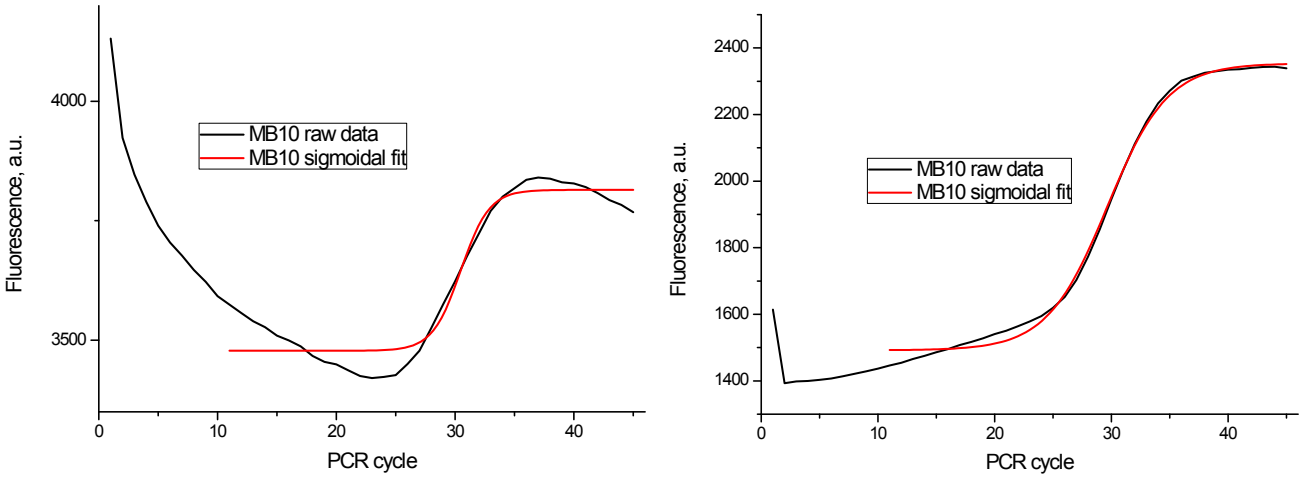


Figure S13. Sigmoidal fit for fluorescence increase in rtPCR (45 cycles) for mismatched probe MB10; fluorescence registered 64ºC (left) and 55ºC (right). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

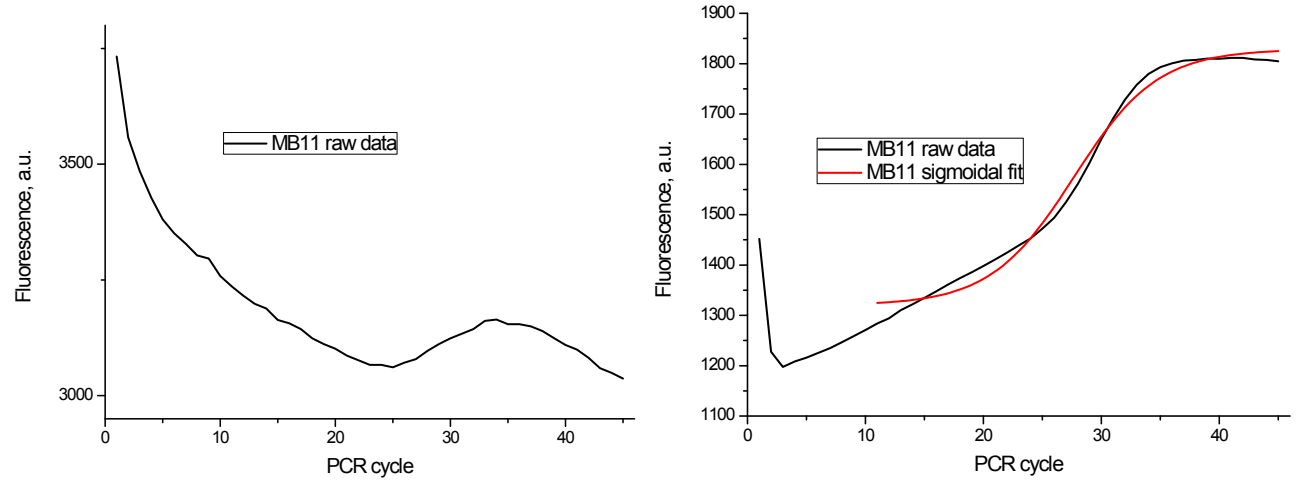


Figure S14. Fluorescence increase in rtPCR (45 cycles) for mismatched probe MB11; fluorescence registered 64ºC (left) and 55ºC (right; + sigmoidal fit). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

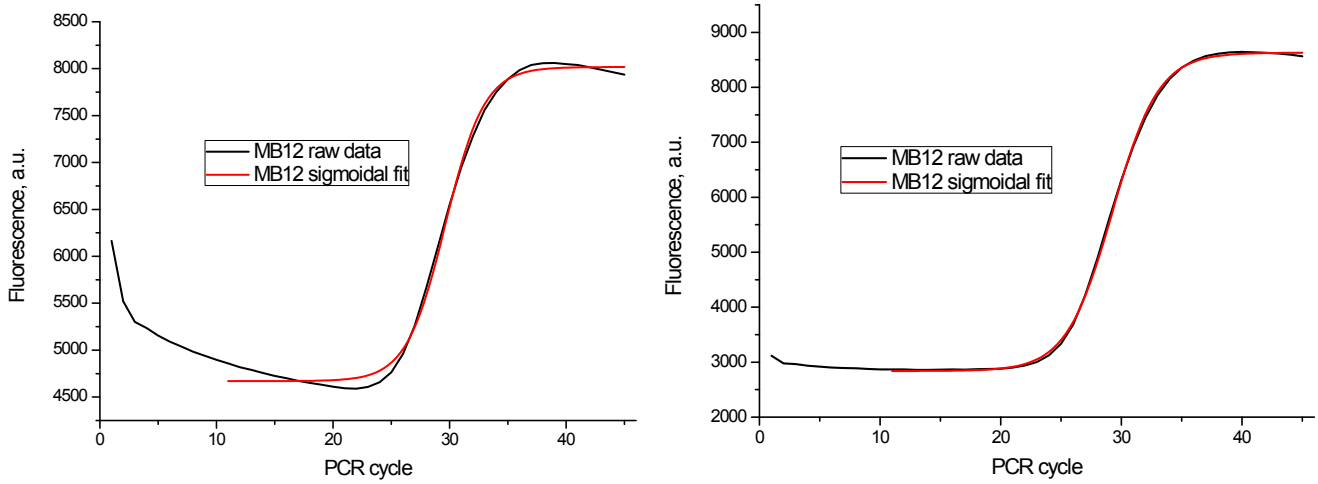


Figure S15. Sigmoidal fit for fluorescence increase in rtPCR (45 cycles) for mismatched probe MB12; fluorescence registered 64ºC (left) and 55ºC (right). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

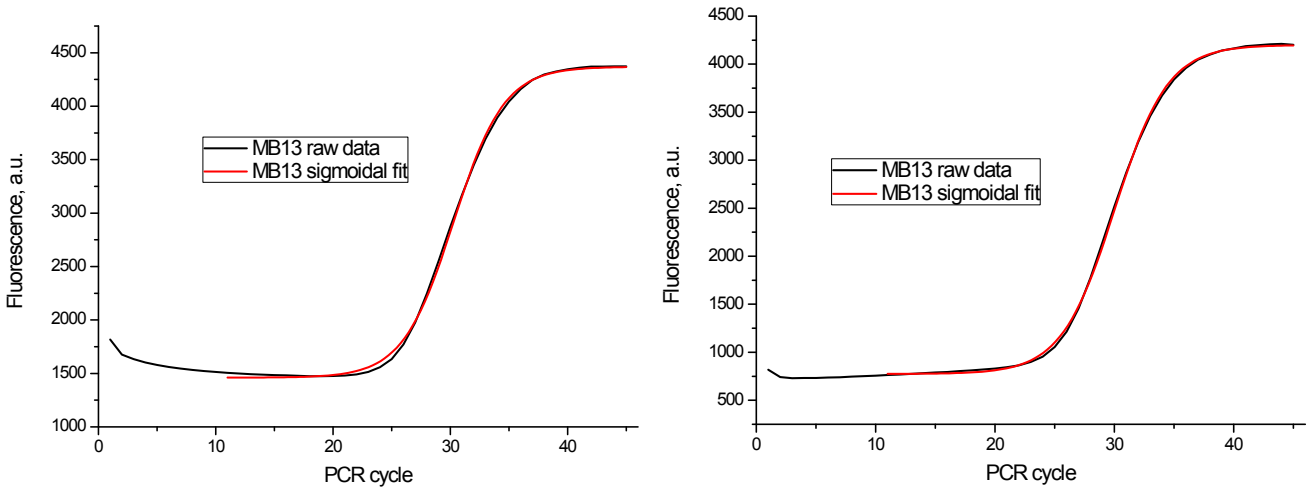


Figure S16. Sigmoidal fit for fluorescence increase in rtPCR (45 cycles) for mismatched probe MB13; fluorescence registered 64ºC (left) and 55ºC (right). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

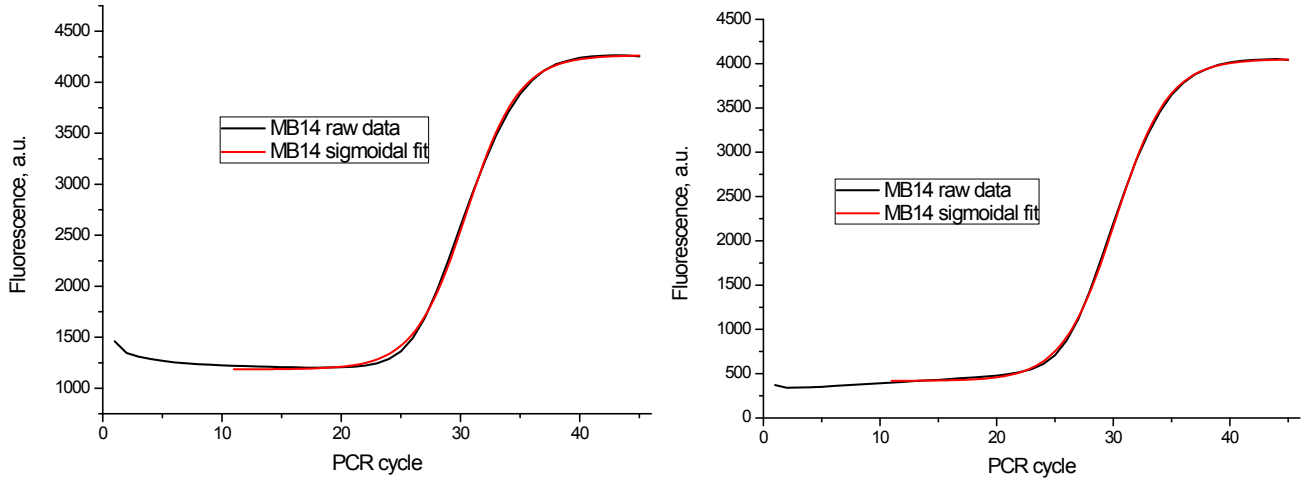


Figure S17. Sigmoidal fit for fluorescence increase in rtPCR (45 cycles) for mismatched probe MB14; fluorescence registered 64ºC (left) and 55ºC (right). The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

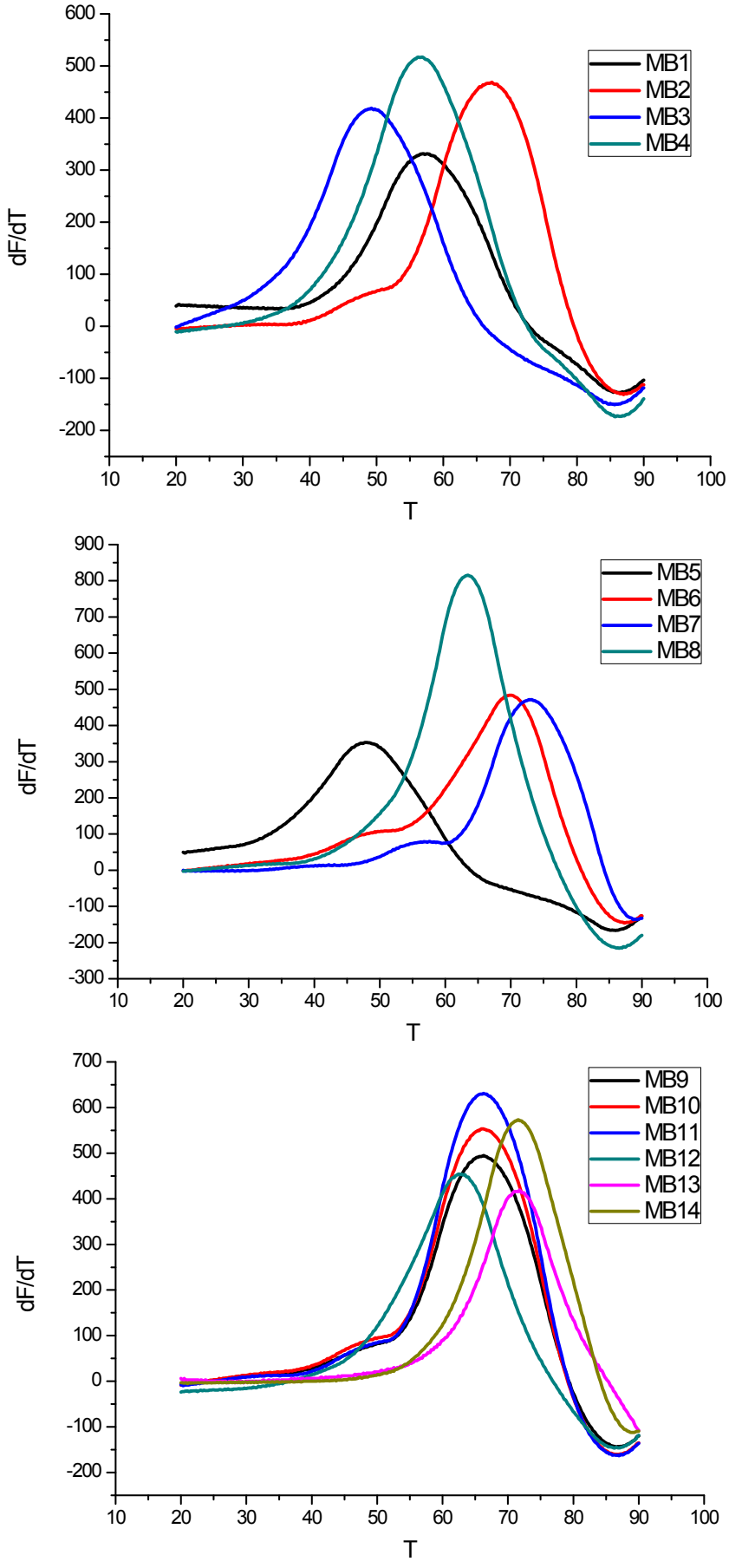
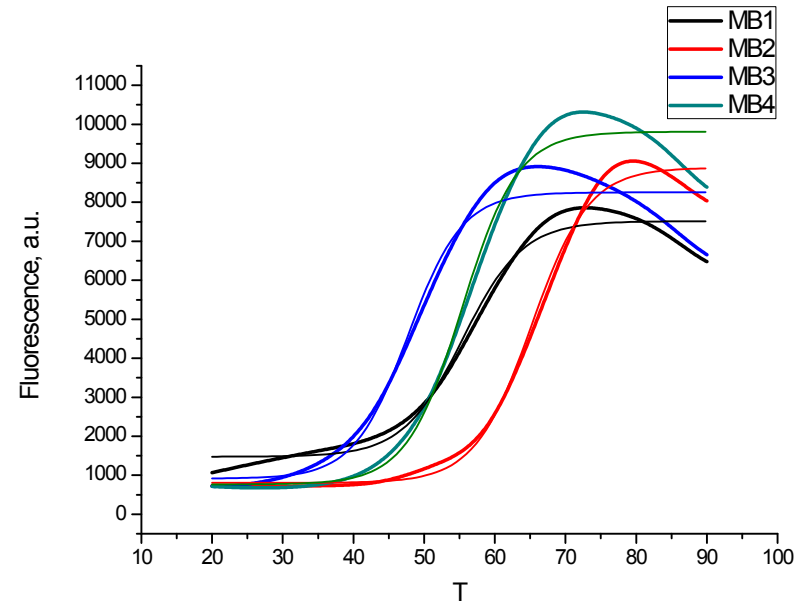
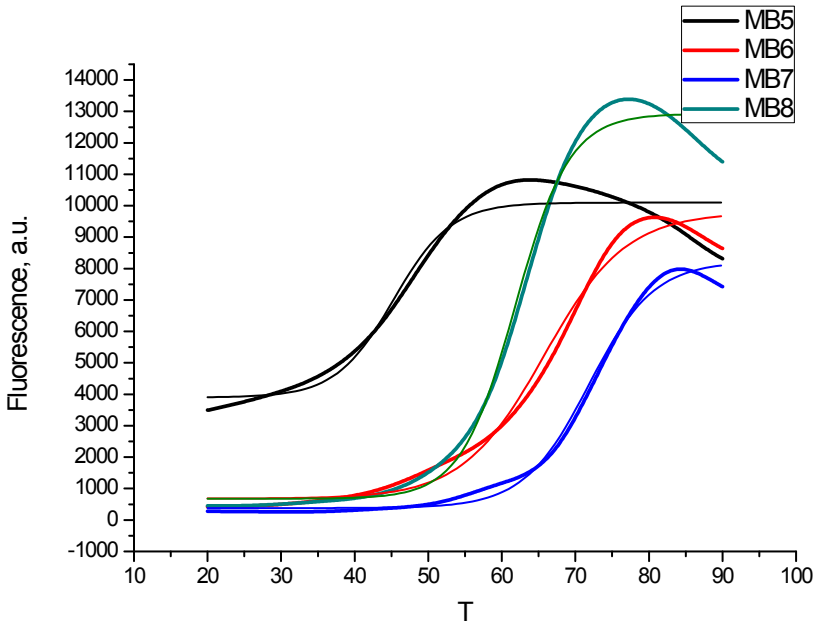
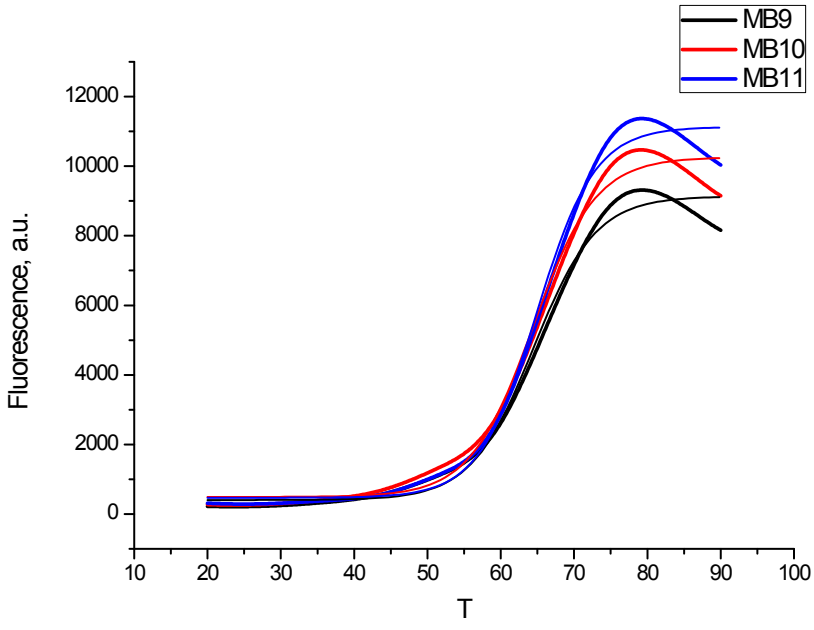


Figure S18. Differential fluorescence melting curves of probes MB1–MB14 (cooling from 90ºC to 20ºC).


MB1
MB2
MB3
MB4
Fluorescence, a.u.
T


MB5
MB6
MB7
MB8
Fluorescence, a.u.
T


MB9
MB10
MB11
Fluorescence, a.u.
T

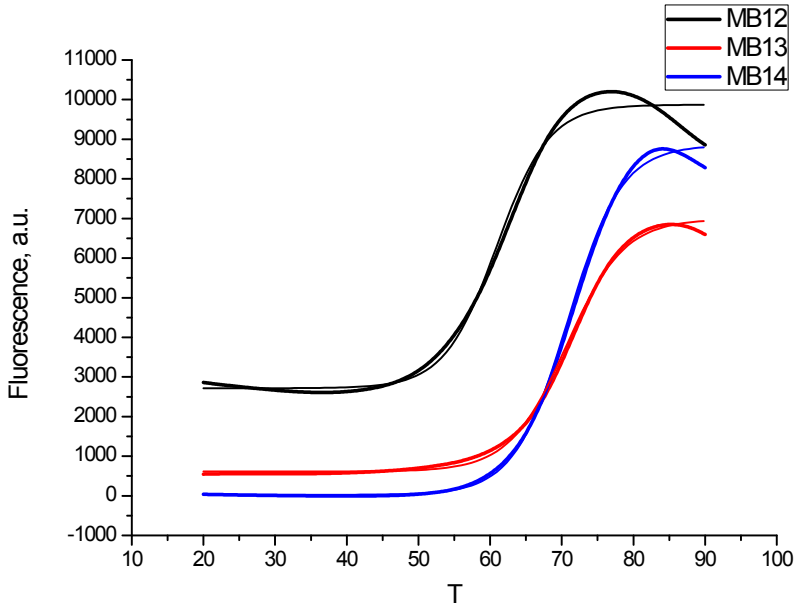


Figure S19. Fluorescence melting curves of probes MB1–MB14 (cooling from 90ºC to 20ºC), raw data and sigmoidal fit. The data analysis was performed using OriginPro 8 software to fit Boltzmann sigmoid.

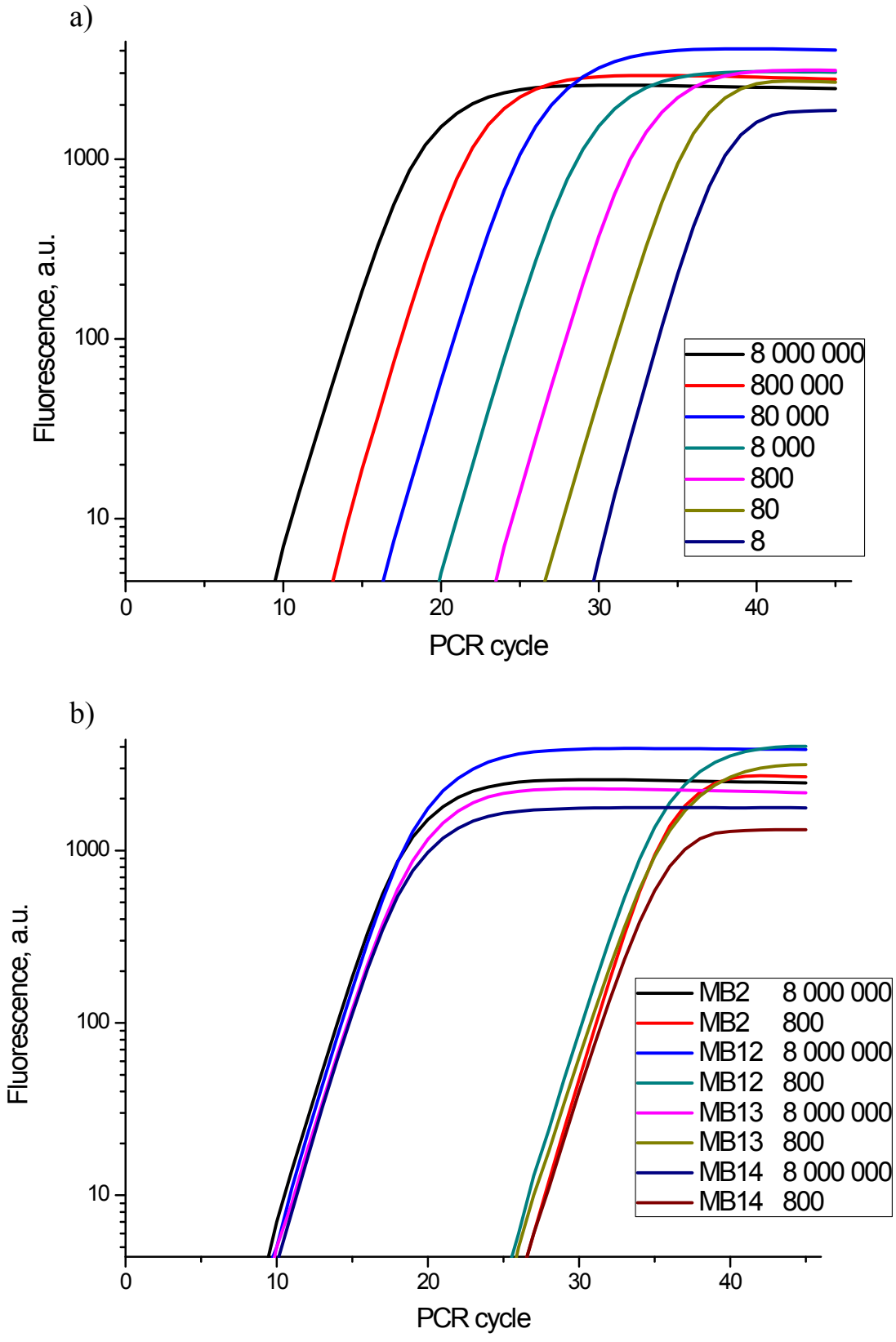


Figure S20. Quantitative real-time PCR a) with fluorogenic probe MB2 and various dilutions of target (8–8 000 000 molecules); b) qrtPCR profiles for probes MB2, MB12, MB13, MB14, and target amounts 8 000 000 and 800 molecules (fluorescence detection at 55ºC). Fluorescence intensity is given in logarithmic scale; the threshold should be selected on linear part of the plots, e.g., at 20–400 a.u.