Supporting Information

Discrimination of the *V600E* Mutation in *BRAF* by Rolling Circle Amplification and Förster Resonance Energy Transfer

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Table S1. Oligonucleotides used for validation of the padlock probe sensitivity in dependence on the single-strand target length.

	Characteristics of the selected oligonucleotide sequences and primers
Probe function	Sequence (5′-3')
MT ssDNA 22 nt	TCTAGCTACAGAGAAATCTCGA
MT ssDNA 60 nt	GACCTCACAGTAAAAATAGGTGATTTTGGTCTAGCTACAGAGAAATCTCGATGGAGTGGG
MT ssDNA 97 nt	CTTTACTTACTACACCTCAGATATATTTCTTCATGAAGACCTCACAGTAAAAATAGGTGATTTTGGTCTAGCTACAG AGAAATCTCGATGGAGTGGG

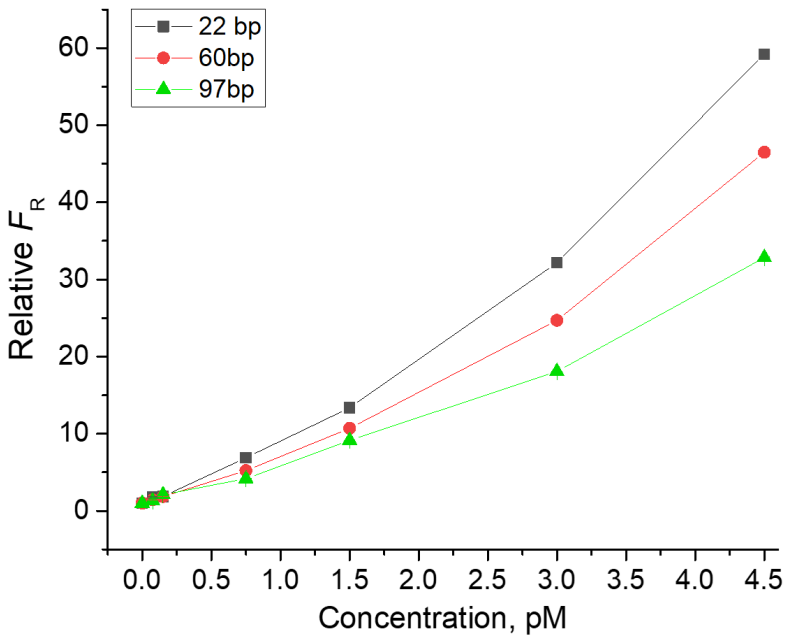

Figure S1. Validation of the padlock probe sensitivity in dependence of the target length. The assay was performed with MT padlock and ssDNA MT target with sequence lengths of 22, 60, and 97 nt. It was shown that an increasing target length slightly influenced RCA production. Nevertheless, even for the longest target, high sensitivity was observed. Concentrations correspond to target in a 10 µL sample. Concentrations in the total assay volume (150 µL) were 15-fold lower.

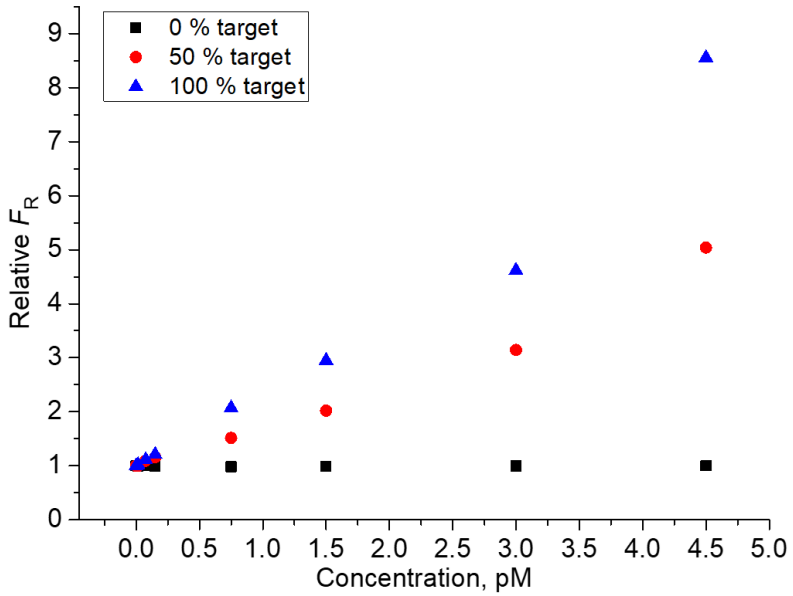

Figure S2. High specificity of the padlock probe complementary to the WT target. 0 % target: 100 % MT and absence of WT; 50 % target: mixture of 50% WT and 50% MT; 100 %: 100 % WT and absence of MT. Concentrations correspond to target (WT + MT) in a 10 µL sample. Concentrations in the total assay volume (150 µL) were 15-fold lower.

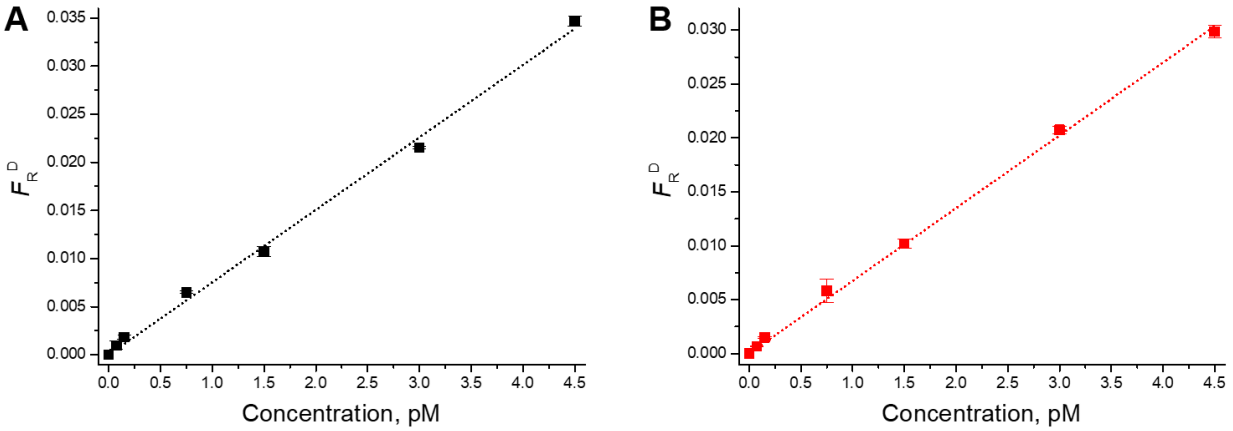

Figure S3. Calibration curves of WT (**A**) and MT (**B**) targets used for duplexed assays. Concentrations correspond to target in 10 µL sample. For all samples, assays were prepared in duplicates (*n* = 2).

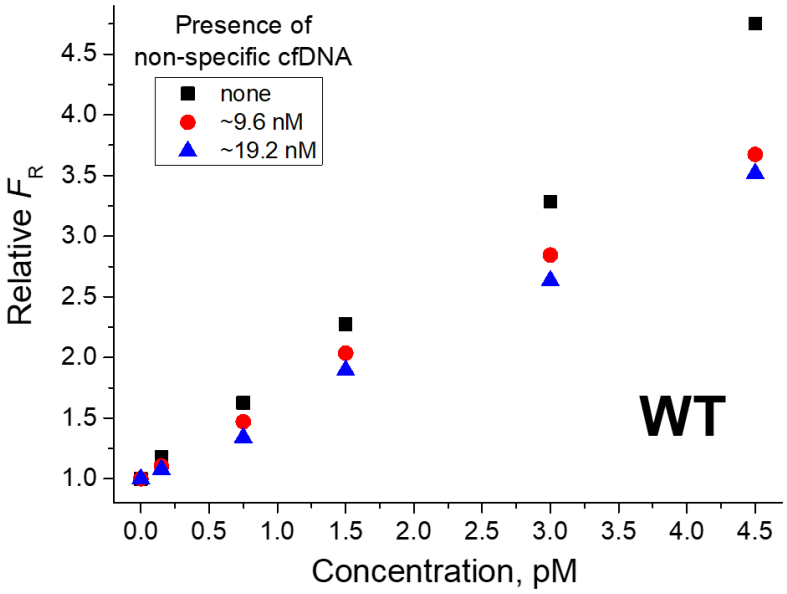

Figure S4. Very high concentrations of non-specific DNA extracted from plasma samples of healthy individuals can slightly influence the target quantification. The WT target calibration curves compare samples without extracted DNA (black squares) and with 4.7 (red circles) and 9.4 ng (blue triangles). Target concentrations are shown for the 10 µL sample (concentrations in the total assay volume of 150µL were 15-fold lower).