Genosensor for detecting single-point mutations in dendron chip after blocked recombinase polymerase amplification

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Supplementary Information

Table of content

Table S1. List of used oligonucleotides. (a) single-strand study, (b) study for the discrimination of single-point mutation.

(a)

Target	Use	Sequence 5'-3'
A	Thiol-probe	TTGCTGATTTTCAACATCAGTCTGATAAGCTA-T_{10}-SH
	Amine-probe	GATTTTCAACATCAGTCTGATAAGCTATTTTT-NH_2
	Target	TAGCTTATCAGACTGATGTTGAAAATCAGCAA-DIG
B	Thiol-probe	TTGCTGATTTCAGCTGCTTTTGGGATTCCGTTG-T_{10}-SH
	Amine-probe	GATTTCAGCTGCTTTTGGGATTCCGTTGTTTTT-NH_2
	Target	CAACGGAATCCCAAAAGCAGCTGAAATCAGCAA-DIG
C	Thiol-probe	TTGCTGATTTACCCCTATCACGATTAGCATTAA-T_{10}-SH
	Amine-probe	GATTTACCCCTATCACGATTAGCATTAATTTTT-NH_2
	Target	TTAATGCTAATCGTGATAGGGGTAAATCAGCAA-DIG

(b)

Target	Use	Sequence 5'-3'
PICK3CA	FP-c	TTTGGAGTATTTCATGAAACAAATG
gene	RP	DIG-TGTGTGGAAGATCCAATCCATT
	Blocker	TGAATGATGCACATCATGGTGGCT-23ddC
	Wild-type	NH_2-C6-TTTTTTTTTT-AATGATGCACATCATGGTGGCT
	p.H1047R	NH_2-C6-TTTTTTTTTT-ATGATGCACGTCATGGTGGC
	p.H1047L	NH_2-C6-TTTTTTTTTT-AATGATGCACTTCATGGTGGCT
	p.H1047P	NH_2-C6-TTTTTTTTTT-ATGATGCACCTCATGGTGGC
Control	probe	NH_2-C6-TTTTTTTTTT-GTTGGAGCTGGTGGCGTAGG

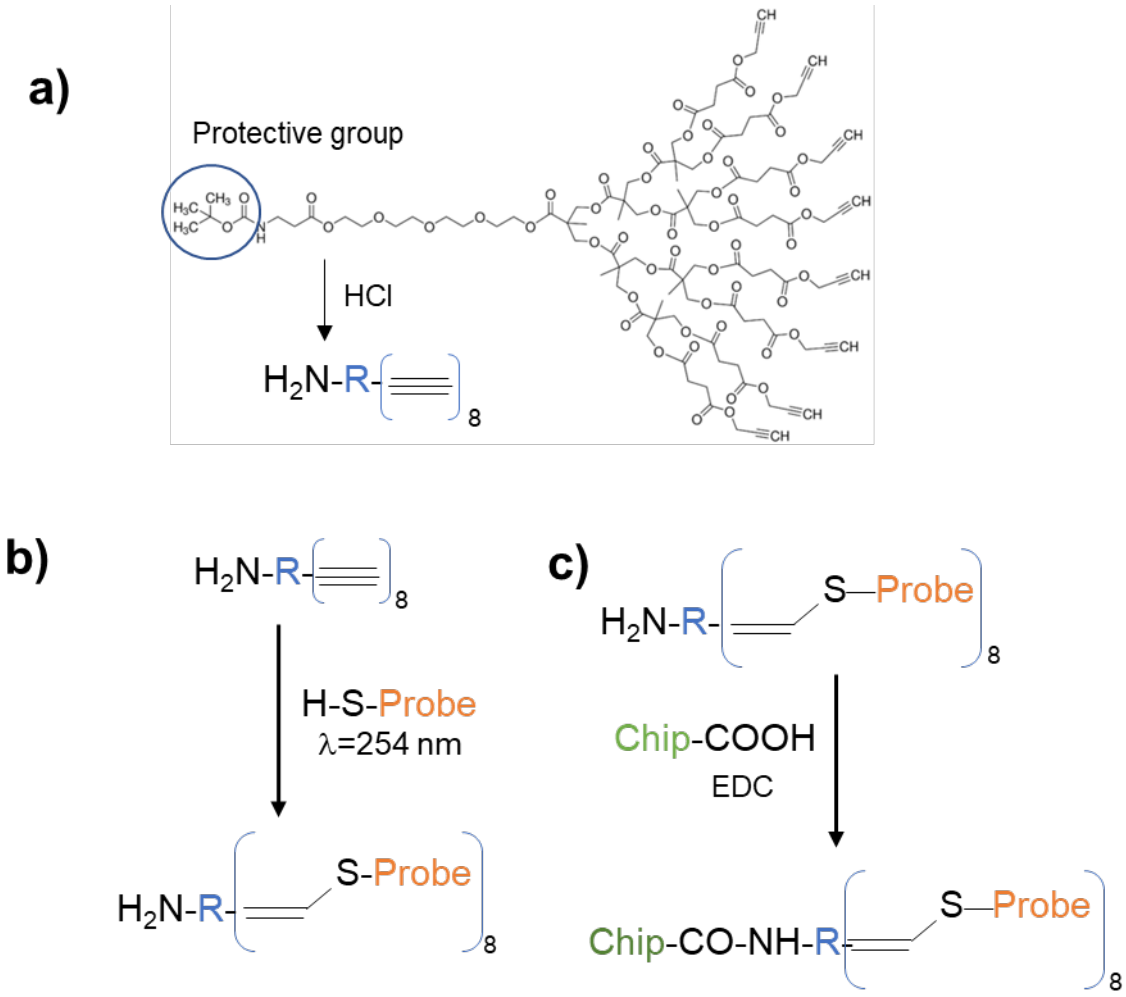


Fig. S1: Dendron-mediated chemistry applied to the DNA-probe immobilization on polycarbonate planar surfaces. **a)** Dendron structure with amine-apex protected by tert-butyloxycarbonyl. The deprotection is a carbamate hydrolysis in acidic conditions. **b)** Conjugation to eight thiol-probes by photoclick-chemistry of thiol-yne **c)** Chip immobilization based on carbodiimide reaction mediated by 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDC); R: dendron, R': probe, R'': activated chip surface.

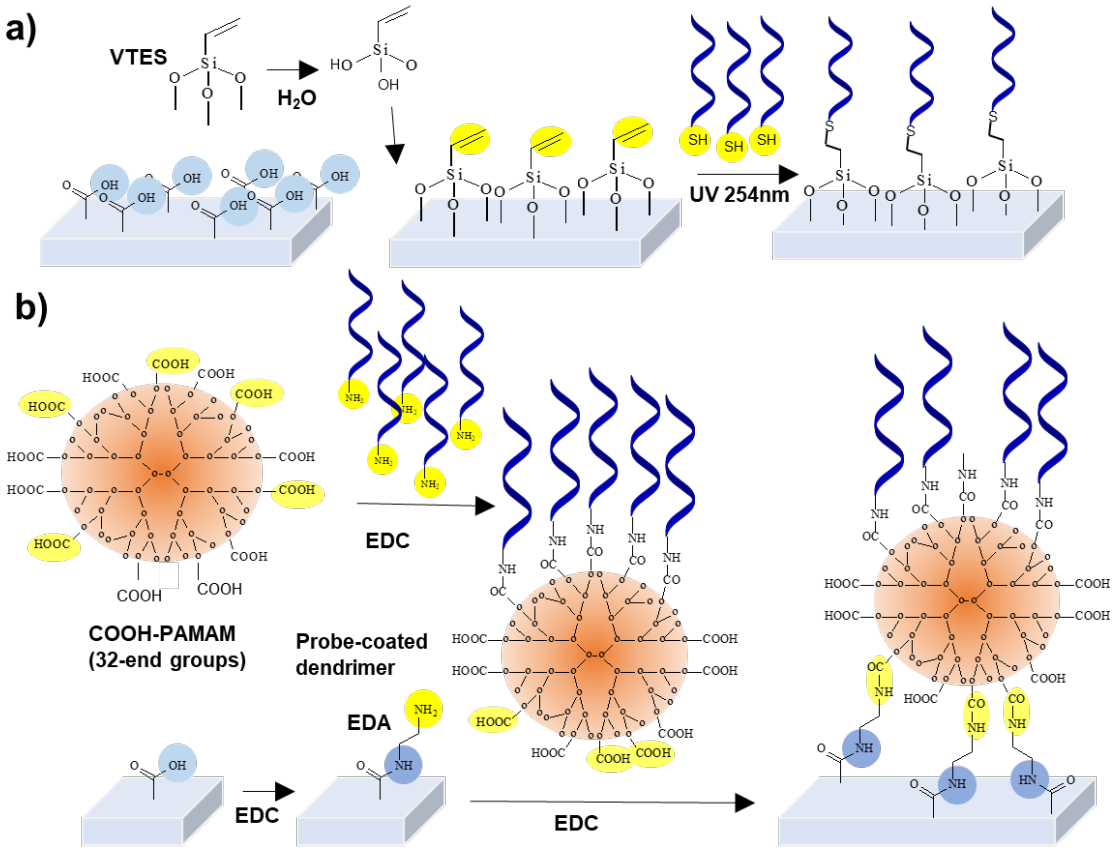


Fig. S2: Reference probe immobilization on polycarbonate surfaces. **a)** VTES-mediated attachment based on the silanization of activated polycarbonate and thiol-ene click chemistry. **b)** Dendrimer-mediated attachment based on carbodiimide chemistry.

VTES chips (Thiol-reaction). Thiol-probes were grafted on activated plastic chips by thiol-ene click chemistry reaction. After photo-activation, a solution of vinyltriethoxysilane (VTES, Sigma Aldrich) at 3% in isopropanol was incubated for 1 h at room temperature. Chips were washed several times with isopropanol, air-dried, and heated at 110 °C for 1 h. Thiol-DNA probe (SH-C_6-T_{10}-ATGATGCACGTCATGGTGGC) in PBS buffer was dispensed on the activated surface (40 nL). Finally, chips were irradiated for 30 s by UV-ozone at 254 nm, washed, and air-dried.

EDA/COOH-PAMAM chips. Amine functionalized dendrimers (COOH-PAMAM dendrimer, generation 3.5, Sigma Aldrich) were immobilized in the active amine-plastic chip. The activation treatment of chips consisted of incubating a solution of ethylenediamine (EDA) at 1% and 1-ethyl-3-(3-dimethyl aminopropyl)carbodiimide (EDC) at 50 mM. The incubation was for 30 min at room temperature. Later, chips were immersed in 70% ethanol solution and dried. Meanwhile, the mixtures of dendrimer (10 nM), NH_2-oligonucleotide probes (200 nM), and EDC (50 mM) were incubated in the printing solution at room temperature for 30 min with end-over rotation. After DNA-dendrimer coupling, solutions were arrayed on EDA-functionalized surfaces (40 nL). Finally, chips were washed and air-dried.

Reference: Martorell, S., Tortajada-Genaro, L. A., González-Martínez, M. A., & Maquieira, A. (2021). Surface coupling of oligo-functionalized dendrimers to detect DNA mutations after blocked isothermal amplification. Microchemical Journal, 106546.

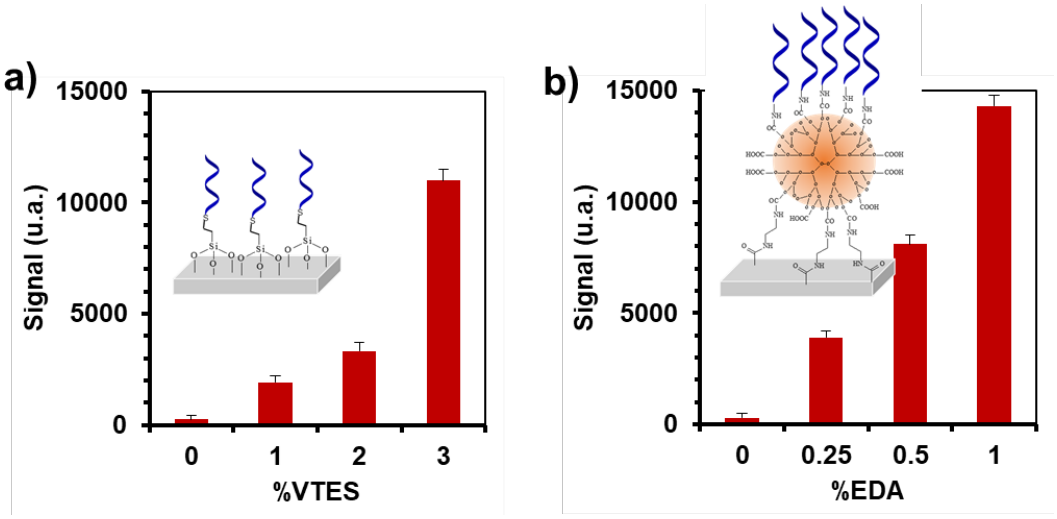
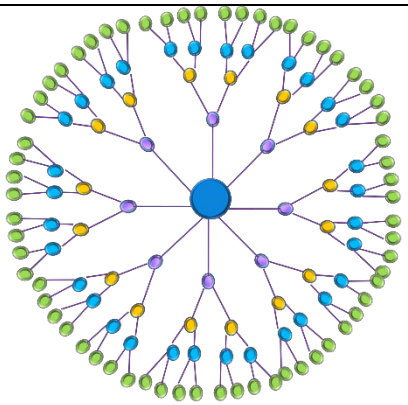
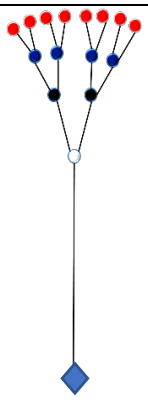


Fig. S3: Spot signal of hybridization assay depending on the crosslinker concentration in reference chips. **a)** VTES-mediated chemistry. **b)** Dendrimer-mediated chemistry.

Table S1. Comparison of dendrimer-mediated and dendron-mediated methods for the immobilization of probes. a) Protocol. b) Performances.

a)	Dendrimer method	Dendron method
Nanomaterial		
Structure		
Width	4.5 nm	2.4 nm
Height	4.5 nm	4.7 nm
Terminal groups	64 groups: -COOH	8 groups: -C≡CH 1 group: -NH_2
Probe-coupling		
Mechanism	Carbodiimide reaction	Photochemistry
Conditions	EDC 50 mM 30 min	Reagent free Light 254 nm, 30 s
Concentrations	Dendrimer 10 nM NH_2-probe 200 nM	Dendron 5 nM HS-probe 50 nM
Chip-coupling		
Mechanism	Carbodiimide reaction through a crosslinker	Direct carbodiimide reaction
Chip activation	Photo-activation Light 254 nm, 10 min NaOH, 30 min, 60 ºC	Photo-activation Light 254 nm, 10 min NaOH, 30 min, 60 ºC
Chip treatment	EDA-functionalized EDA 1%, EDC 50 mM 30 min, 37 ºC	-
Conjugate dispensation	Spotting 40 nL	Spotting 30 nL
Conditions	EDC 50 mM, MES 0.1 M 60 min	EDC 50 mM, MES 0.1 M 60 min

(b)	Dendrimer method	Dendron method
Nanomaterial cost	✓	
Other reagents cost		✓
Probe consumption		✓
Probe coupling		✓
Chip treatment		✓
Chip coupling		✓
Effect on RPA method	✓	✓
Background signal		✓
Analyte signal	✓	✓

✓ Best performing

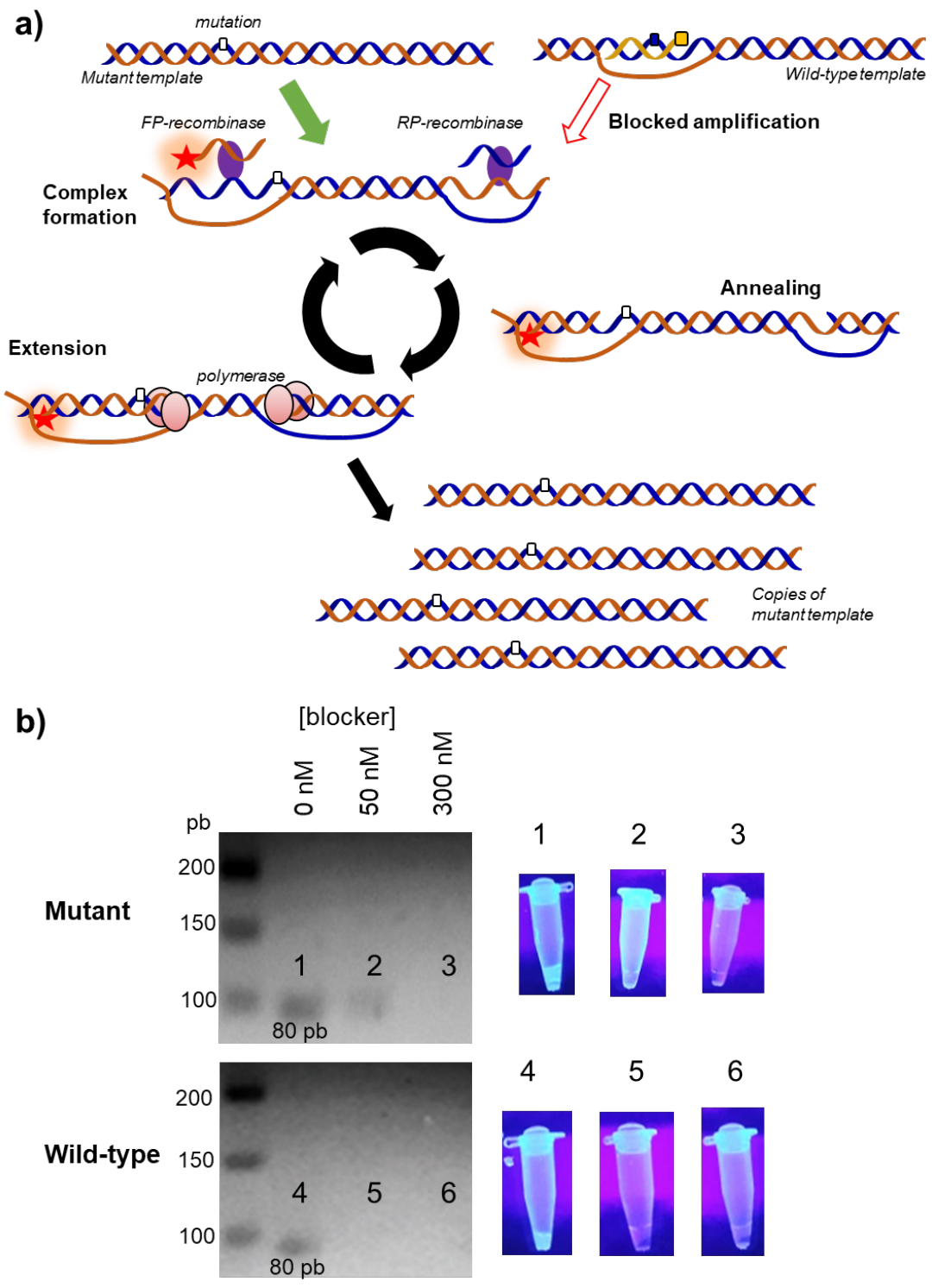


Fig. SI.4: Blocked-RPA. a) Amplification mechanism. b) Gel electrophoresis and direct fluorescent measurement by SYBR safe as a staining agent. Conditions: Agarose 3%, separation at 110 V. Samples: HCT116 cell line (*PIK3CA* gene, c.3140A>G) and SK-N-AS cell line (wild-type).

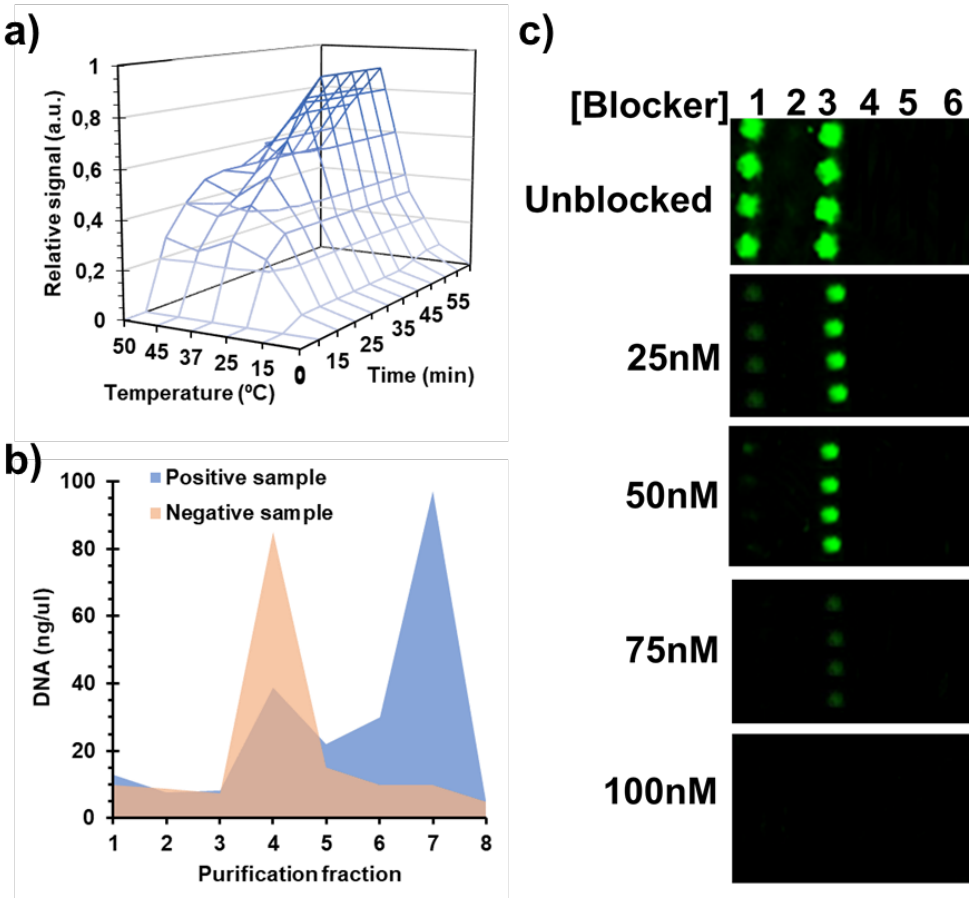


Fig. SI.5: Study of blocked-RPA conditions: **a)** Effect of working temperature and reaction time on the registered signal. **b)** Separation profile of RPA products using a silica-membrane system (Jena Bioscience). DNA concentrations were determined by the NanoDrop instrument (Thermo Scientific). Fraction 4 corresponded to the excess primer (single-strand DNA) and fraction 7 to RPA product (double-strand DNA). **c)** Array images for different amounts of blocking agent (probes: 1. Wild-type (C); 2: mutant 1 (A); 3: mutant 2 (G); 4: mutant 3 (T); 5: negative control 1; 6: negative control 2). Sample: CRC cell line HCT116 with heterozygous H1047R mutation.

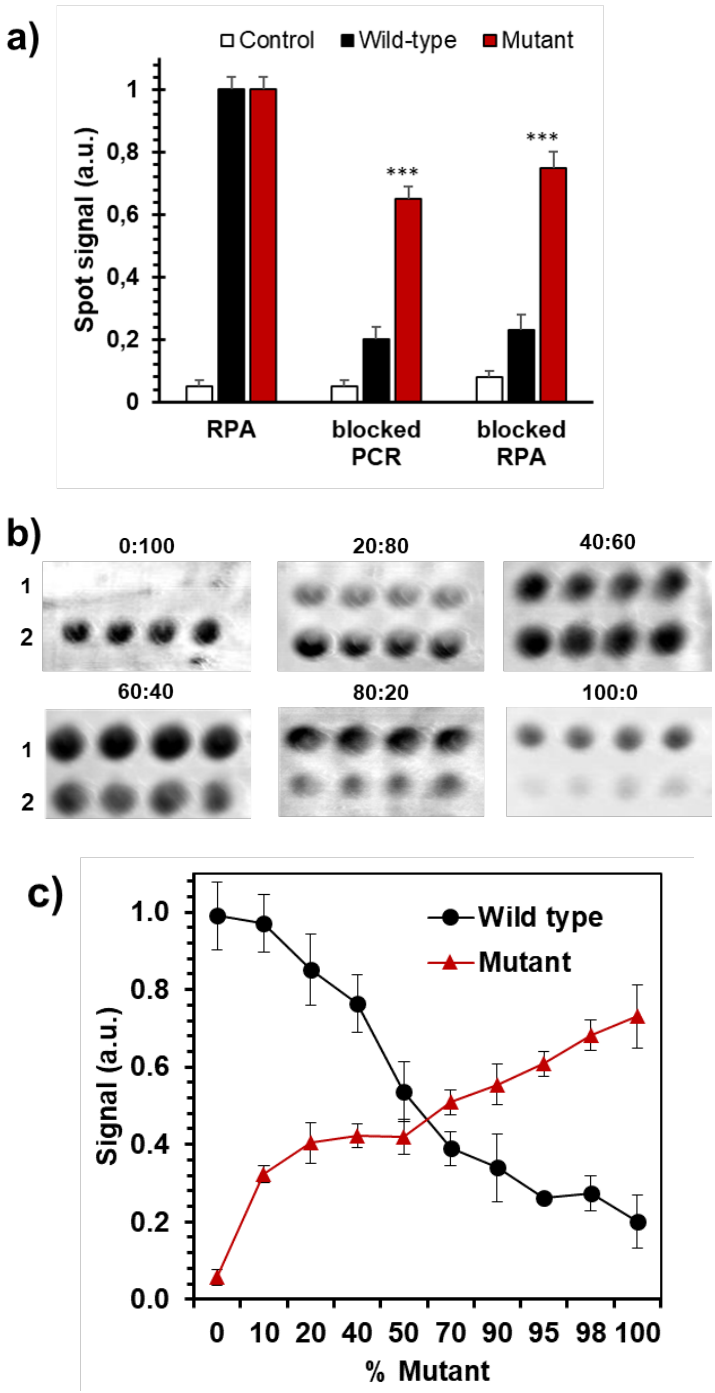


Fig. S6: Mixes of wild-type and mutant genomes. **a)** Comparison of amplification techniques. **b)** Capture-array image of hybridization mixes varying initial percentages (wild-type:mutant). **b)** Spot signal registered for wild-type and mutant probes depending on mutant percentage in the genome mixture.

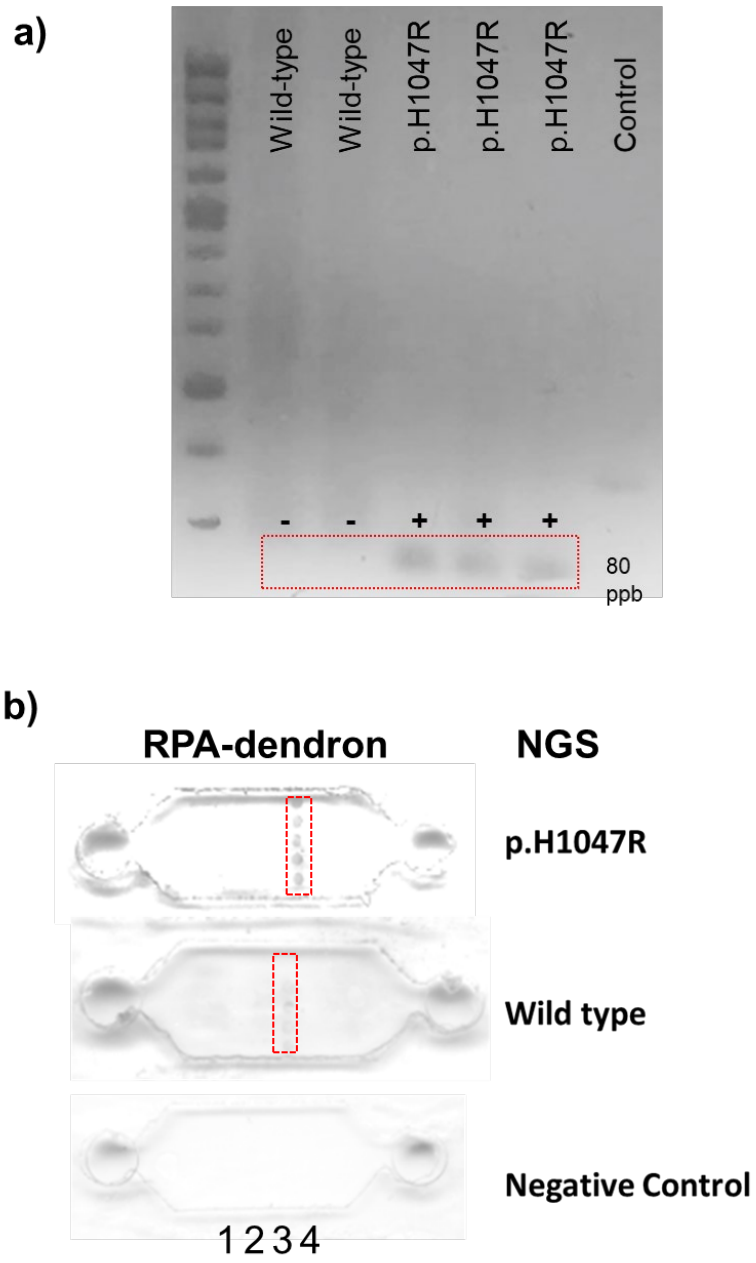


Fig. S7: Analysis of patient samples for genotyping of *PIK3CA* gene based on blocked-RPA and hybridization on dendron-polymer chip. a) Gel electrophoresis: agarose 3%, separation at 110 V. b) Images of chips, compared to the next-generation sequencing (NGS) results. 1: Probe A, 2: Probe C, 3: Probe A, and 4: Probe G.