

Split hybridisation probes for electrochemical typing of single-nucleotide polymorphisms

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Supplementary Material Available:

Table S-1. List of primer pairs and PCR conditions used for mutational screening of *TP53*.

Figure S-1. Sequencing electropherograms for heterozygous, homozygous mutant and wild-type samples.

Figure S-2. 169 bp sequence amplified from exon 7 of *TP53*.

Figure S-3. Calculated secondary structure of the *TP53* amplified target.

Figure S-4. Influence of the auxiliary oligonucleotide Aux on the heterogeneous hybridisation of the amplicon at variable capture probe surface coverages.

Figure S-5. Analysis of the homozygous mutant target by means of the split probes assay at 24-26°C.

Table S-1. Primers pairs and PCR conditions used for mutational screening of *TP53*.

Product location	Primer sequence (5' to 3')*
Exon 4	ATCTACAGTCCCCCTTGCCG (+)
	GCAACTGACCGTGCAAGTCA (-)
Exon 5	TTCCTCTTCCTGCAGTACTC (+)
	ACCCTGGGCAACCAGCCCTGT (-)
Exon 6	ACAGGGCTGGTTGCCCAGGGT (+)
	AGTTGCAAACCAGACCTCAG (-)
Exon 7	AAGGCGCACTGGCCTCATCTT (+)
	CCGAAATGTGATGAGAGGT (-)
Exon 8	TATCCTGAGTAGTGGTAATC (+)
	AAGTGAATCTGAGGCATAAC (-)
Exon 9	GCAGTTATGCCTCAGATTAC (+)
	AAGACTTAGTACCTGAAGGGT (-)
Exon 10	CTCTGTTGCTGCAGATCC (+)
	GCTGAGGTCACTCACCTGG (-)
Exon 11	GCTTCTGTCTCCTACAGCC (+)
	CAACAAGAAGTGGAGAATGTC (-)

*(+) sense primer, (-) antisense primer

PCR was performed in a final volume of 30 μ L containing 100 ng of genomic DNA, 20 pmol of each primer, 200 μ M of dNTP, 0.75 units of *Taq* Polymerase, 15 mM of Tris-HCl (pH 8.0), 50 mM of KCl and 2 mM of MgCl₂. Number of cycles (a cycle being: 30" at 94.0°C, 30" at 60.0°C, 30" at 72.0°C): 35.

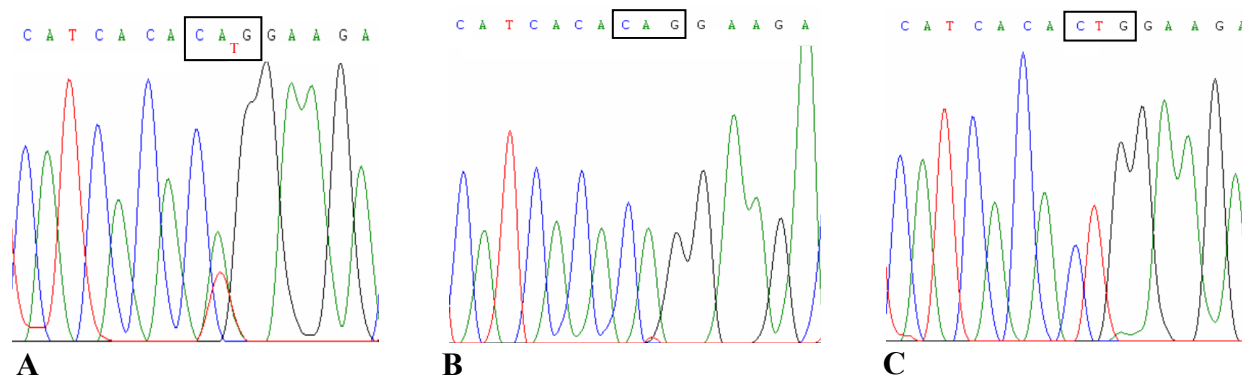


Figure S-1. Portions of DNA sequencing electropherograms. A) heterozygous sample (mother, SFS26); B) homozygous mutant sample (son, SFS28); C) wild-type sample (daughter, SFS27). Sequencing of the PCR products was performed on a 3100 Genetic Analyzer (Applied Biosystems) equipped with the Sequencing Analysis software. The reliability of the results was ensured by sequencing both DNA strands (GenBank: U94788.1). Each fluorescence peak trace reflects the identity of a dye-labelled dideoxynucleotide, as reported on top of each graph. The rectangular boxes are used to indicate the bases of codon 257.

5'-AAGGCGCACTGGCCTCATCTTGGGCCTGTGTTATCTCCTAGGTTGGCTCTGACTGTACCACCATCC
ACTACA ACTACATGTGTAACAGTTCCTGCATGGGCGGCATGAACCGGAGGCCCATCCTCACCATCAT
CACACCTGGGAAGACTCCAGGTCAGGAGCCACTTGCCA-3'

Figure S-2. Wild-type amplicon from exon 7 of *TP53* (169 bp). Underlined sequences: sense and antisense primer, respectively. Annealing temperature: 63.4°C; other PCR conditions as in Table S-1. Codon 257 is highlighted in grey italics with the site of mutation underlined.

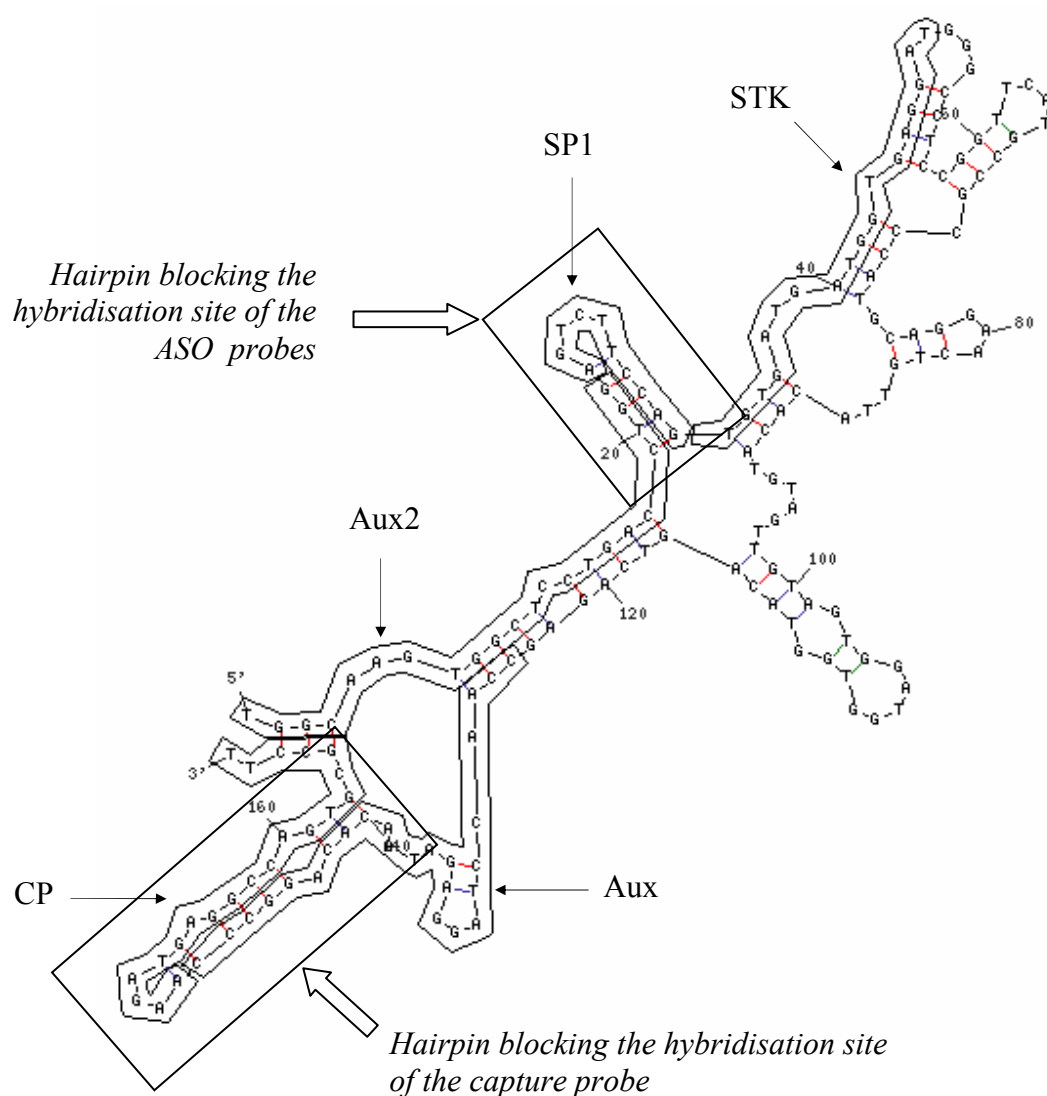


Figure S-3. Calculated secondary structure of the *TP53* amplified target and relative position of capture probe (CP), stacking probe (STK), ASO probe (SP1) and auxiliary oligos (Aux and Aux2). Software: Mfold web server; folding conditions: $[Na^+] = 0.82$ M; $[Mg^{2+}] = 0.0$ M; temperature = $23.0^\circ C$; correction = polymer.

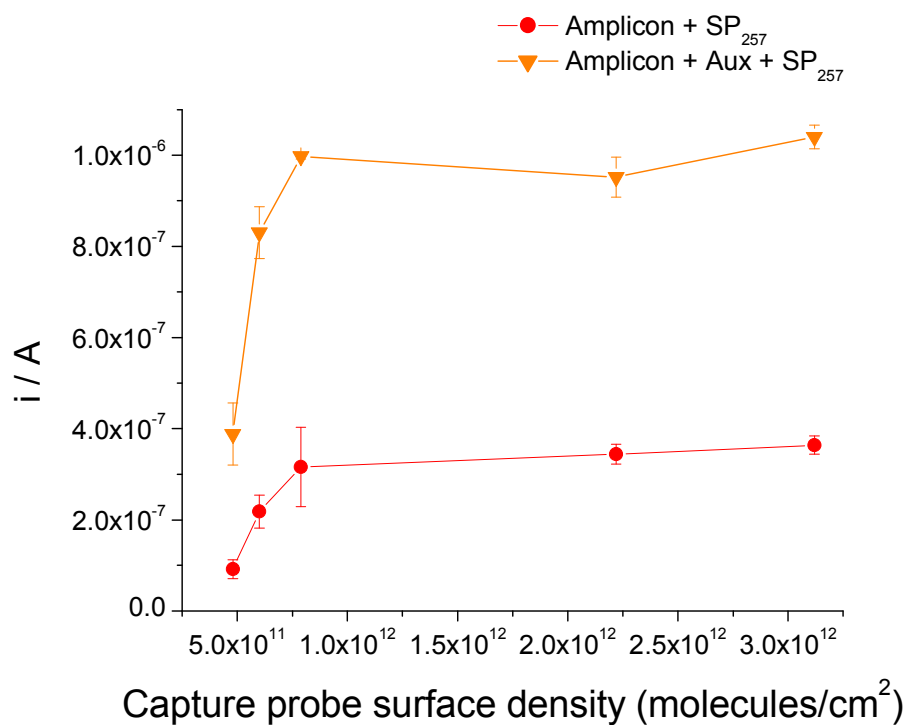


Figure S-4. Influence of the auxiliary oligonucleotide Aux on the heterogeneous hybridisation of the amplicon at variable capture probe surface coverages. The PCR products (10 nM) were analysed in the absence and presence of Aux (100 nM) and using SP₂₅₇ (100 nM) as the biotinylated reporter sequence. Capture probe densities were estimated by chronocoulometric measurement (Steel, A.B., Herne, T.M., Tarlov, M.J., 1998. Anal. Chem. 70, 4670-4677) of the surface excess of the [Ru(NH₃)₆]³⁺ complex (25 μM in 1 mM Tris buffer, pH 7.4). Other details as described in the Experimental section.

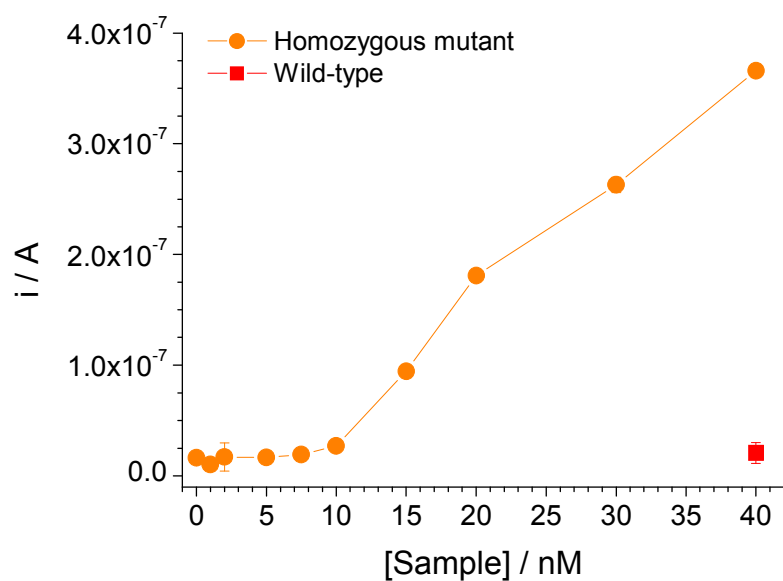


Figure S-5. Analysis of both wild-type and homozygous mutant amplicons by means of the split probes assay (STK/SP2/Aux3) at 24-26°C. Other details as described in the Experimental section.