Detection of TP53 mutations by Sanger sequencing

(IARC protocol, 2019 update)

PCR conditions

PCR primers and conditions for amplifying genomic DNA sequences within exons 2-11 of human TP53 gene are summarized in the following tables. Depending on the quality of your DNA template, you may use primer pairs that amplify large (good DNA quality) or small (poor DNA quality) fragments (see **Table 1**). Nucleotides highlighted in yellow have been described as site of polymorphisms that may affect PCR in certain populations (see **Table 2** for other sets of primers).

Table 1: Original IARC primers

IARC code	Primer pairs (5' → 3')	Direction	Region amplified	Product length	PCR program	PCR mix
P-559	tctcatgctggatccccact	F	Exons 2-3	344 bp	A or B	1
P-E3Ri	agtcagaggaccaggtcctc	R				
P-329	tgctcttttcacccatctac	F	Exon 4	353 bp	B	1
P-330	atacggccaggcattgaagt	R				
P-326	tgaggacctggtcctctgac	F	Exon 4	413 bp	B	1
P-327	agaggaatcccaaagttcca	R				
P-312	ttcaactctgtctccttcct	F	Exon 5	248 bp	B	1
P-271	cagccctgtcgtctctccag	R				
P-239	gcctctgattcctcactgat	F	Exon 6	181 bp	B	1
P-240	ttaacccctcctcccagaga	R				
P-236	tgttcacttgtgccctgact	F	Exons 5-6	467 bp	B	1
P-240	ttaacccctcctcccagaga	R				
P-333	cttgccacaggtctccccaa	F	Exon 7	237 bp	C	2
P-313	aggggtcagaggcaagcaga	R				
P-237	aggcgcactggcctcatctt	F	Exon 7	177 bp	B	1
P-238	tgtgcagggtggcaagtggc	R				
P-316	ttccttactgcctcttgctt	F	Exon 8	231 bp	B	1
P-319	aggcataactgcacccttgg	R				
P-314	ttgggagtagatggagcct	F	Exons 8-9	445 bp	B	1
P-315	agtgttagactggaaacttt	R				
9F	gacaagaagcggtggag	F	Exon 9	215	E	1
9R	cggcattttgagtgttagac	R				
P-E10Li	caattgtaacttgaaccatc	F	Exon 10	260 bp	D	1
P-562	ggatgagaatggaatcctat	R				
P-E11Le	agaccctctcactcatgtga	F	Exon 11	245 bp	B	1
P-E11Re	tgacgcacacctattgcaag	R				

Table 2: Primers from Haque MM et al., 2018

Amplicon	Primer	Annealing Temp
Exon 1F	CACAGCTCTGGCTTGCAGA	63.2°C
Exon 1R	AGCGATTTTCCCGAGCTGA	
Exon 2F	AGCTGTCTCAGACACTGGCA	63.2°C
Exon 2R	GAGCAGAAAGTCAGTCCCATG	
Exon 3+4-P1-F	AGACCTATGGAAACTGTGAGTGGA	58-51Touch Down
Exon 3+4-P1-R	GAAGCCTAAGGGTGAAGAGGA	
Exon 3+4-P2-F$^{\pm}$	AGACCTATGGAAACTGTGAGTGGA	68°C
Exon 3+4-P2-R$^{\pm}$	AGGAAGCCAAAGGGTGAAGAGG	
Exon 5+6F	CGCTAGTGGGTTGCAGGA	63.2°C
Exon 5+6R	CACTGACAACCACCCTTAAC	
Exon 7-P1-F	CTGCTTGCCACAGGTCTC	63.2°C
Exon 7-P1-R	TGGATGGGTAGTAGTATGGAAG	
Exon 7-P2-F$^{\pm}$	AGAATGGCGTGAACCTGGGC	66°C
Exon 7-P2-R$^{\pm}$	TCCATCTACTCCCAACCACC	
Exon 8+9F	GTTGGGAGTAGATGGAGCCT	63.2°C
Exon 8+9R	GGCATTTTGAGTGTTAGACTG	
Exon 10F	CTCAGGTACTGTGTATATACTTAC	57.8°C
Exon 10R	ATACACTGAGGCAAGAAT	
Exon 11F	TCCCGTTGTCCCAGCCTT	57.8°C
Exon 11R	TAACCCTTAACTGCAAGAACAT	

PCR mix

1. GoTaq Hot Start Polymerase (Promega)

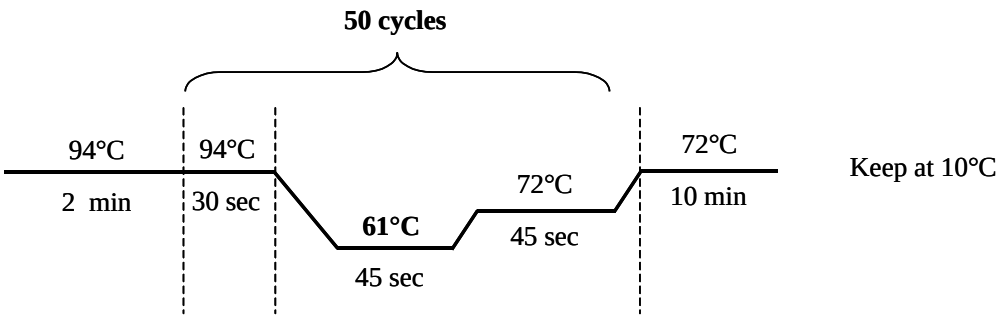
Components	Volume/reaction	Final concentration
- 5X PCR buffer without MgCl2	4 µl	1X
- 25mM MgCl2	1.2 µl	1.5mM
- dNTP mix (5mM each)	0.8 µl	0.2mM each
- Primer, forward 10µM	0.8 µl	0.4µM
- Primer, reverse 10 µM	0.8 µl	0.4µM
- GoTaq DNA polymerase (5U/ul)	0.1 µl	0.5 U
- Template DNA	50 ng	
- Water, molecular biology grade	Qsp 20 µl	

2. HotStarTaq (Qiagen)

Components	Volume/reaction	Final concentration
- 10X PCR buffer containing 15 mM MgCl2	2 µl	1X
- 5X Q-Solution	4 µl	1X
- dNTP mix (5mM each)	0.8 µl	0.2 mM each
- Primer, forward 10uM	0.8 ul µl	0.4 µM
- Primer, reverse 10 uM	0.8 µl	0.4 µM
- HotStarTaq DNA polymerase (5U/µl)	0.1 µl	0.5 U
- Template DNA	50 ng	
- Water, molecular biology grade	Qsp 20 µl	

PCR programs

A:



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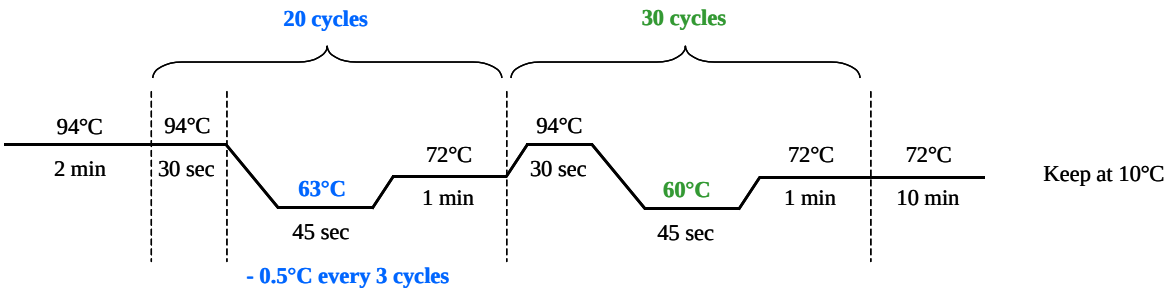

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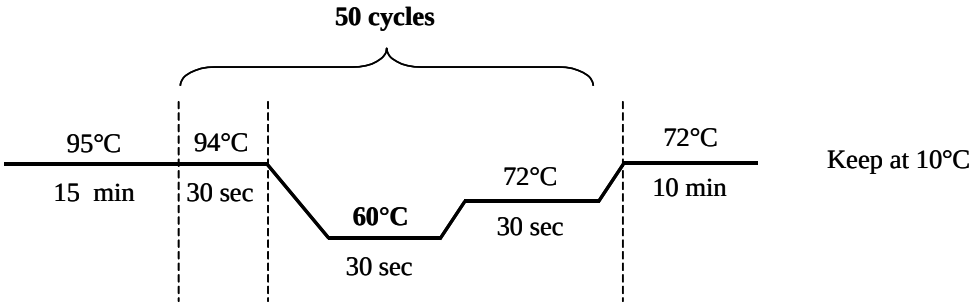

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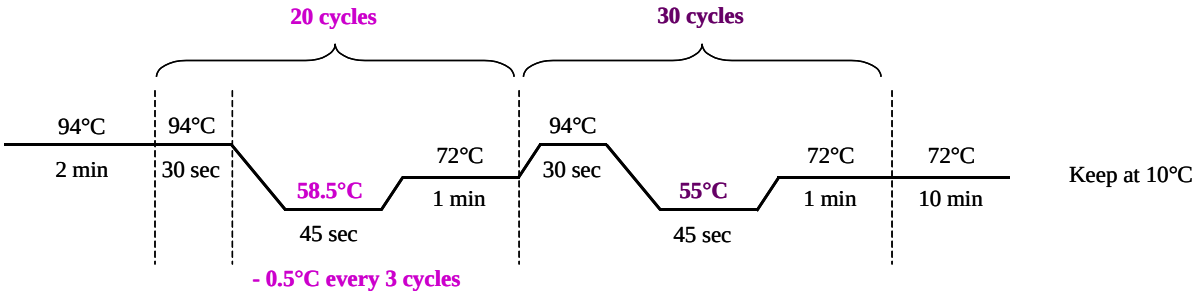

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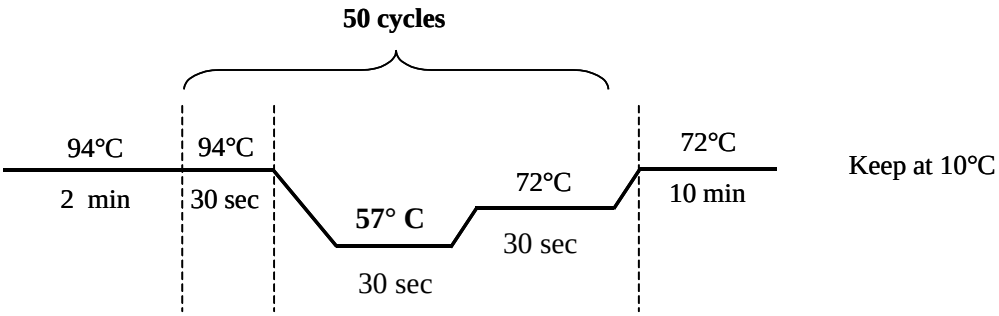

Purification of PCR products

Prior sequence analysis, 5 µl of PCR products are purified with the enzyme ExoSap-IT (USB) for15 min at 37°C and 15 min at 80°C.

You may also use:

- columns (i.e. QIAquick PCR Purification kit, QIAGEN)
- plates (i.e. NucleoFast 96 PCR kit, Clontech)

Sequencing reaction

Sequencing reaction is done with BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) according to the following protocol:

Mix:

- 7 µl of purified PCR product
- 1.25µl Buffer
- 0.5µl primer 10µM*
- 1.5µl Big Dye

Program:

96°C	10 sec	30 cycles
50°C	5 sec	
60°C	4 min	

* Same primers as the ones used for PCR amplification reactions (note that R primer for exon 11 does not work well for sequencing).

Purification of sequencing reaction

Before analysis, purification of the sequencing reaction products is done by the Sequencing Service with 96-well Multiscreen filtration plates (G50-Pharmacia-Millipore).

Sequencing analysis

PCR products are analyzed by the Sanger method on a capillary sequencer.

Result analysis and interpretation

Chromatograms are analyzed semi-automatically by visual inspection of sequences imported in an analysis software using the reference sequence, NC_000017.11, from Genbank (http://www-p53.iarc.fr/TP53sequence_NC_000017-9.html).

Variations can be checked at http://p53.iarc.fr/TP53GeneVariations.aspx that allows checking whether the variation is a known polymorphism or a mutation, and provides frequency data and functional assessment.