Detection of TP53 mutations by direct sequencing

(IARC protocol, 2010 update)

PCR conditions

PCR conditions for amplifying genomic DNA sequences within exons 2-11 of human TP53 gene are summarized in the following table. 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. Nucleotides highlighted in yellow have been described as site of polymorphisms (should not affect PCR according to our experience).

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

PCR mix

1. GoTaq Hot Start Polymerase (Promega)

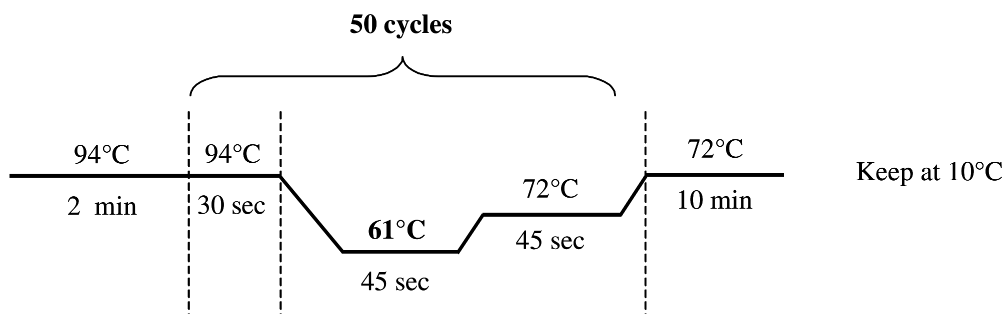
Components	Volume/reaction	Final concentration
- 5X PCR buffer without MgCl2	4 µl	1X
- 25mM MgCl2	1.2 µl	1.5mM
- dNTP mix (5mM each)	0.8 µl	0.2mM each
- Primer, forward 10µM	0.8 µl	0.4µM
- Primer, reverse 10 µM	0.8 µl	0.4µM
- GoTaq DNA polymerase (5U/ul)	0.1 µl	0.5 U
- Template DNA	50 ng	
- Water, molecular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

2. HotStarTaq (Qiagen)

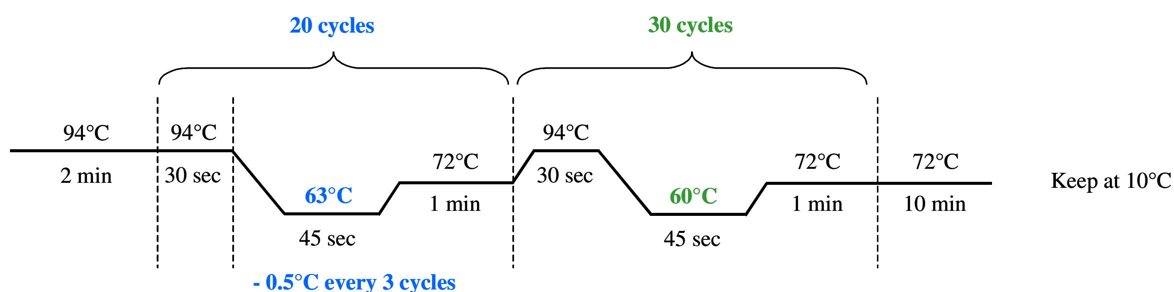
Components	Volume/reaction	Final concentration
- 10X PCR buffer containing 15 mM MgCl2	2 µl	1X
- 5X Q-Solution	4 µl	1X
- dNTP mix (5mM each)	0.8 µl	0.2 mM each
- Primer, forward 10uM	0.8 ul µl	0.4 µM
- Primer, reverse 10 uM	0.8 µl	0.4 µM
- HotStarTaq DNA polymerase (5U/µl)	0.1 µl	0.5 U
- Template DNA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

PCR programs

A:



B:



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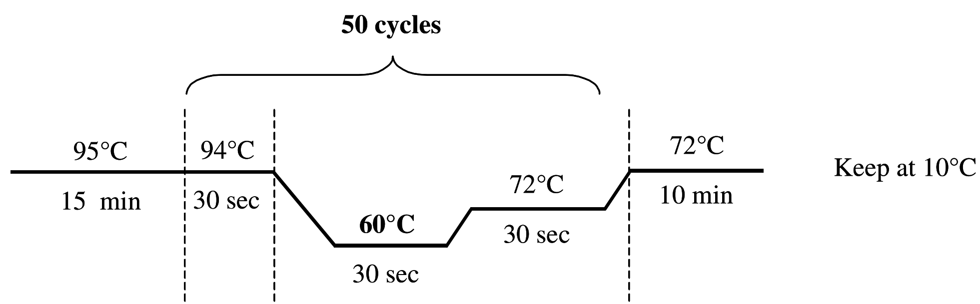

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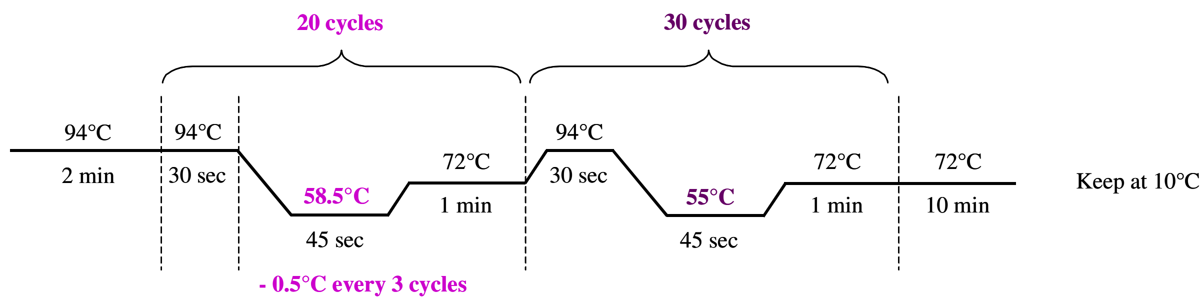
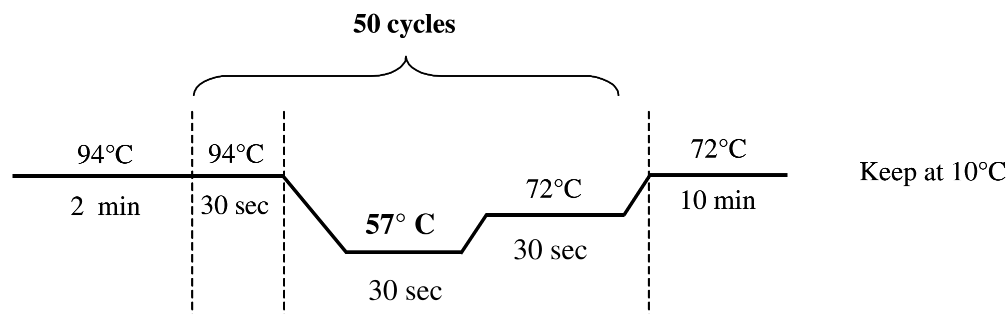


E:



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)

TP53 sequencing

Sequencing is performed by IARC common sequencing service.

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 a 16-capillary automated sequencer (ABI PRISM® 3100 Genetic Analyzer, Applied Biosystems), based on the Sanger method (see principle at: http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/Obenrader/sanger_method_page.htm)

Result analysis and interpretation

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

Variations are checked with the mutation validation tool available at IARC at http://www-p53.iarc.fr/MutationValidationCriteria.asp. This tool allows to check whether the variation is a known polymorphism or a mutation, and provides frequency and functional data as reported in the IARC TP53 database http://www-p53.iarc.fr).