

Tp53 Mutations Screening using High Resolution Melting Analysis

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Introduction

TP53 is a tumour suppressor gene & is known as the *Guardian of The Genome*.

- It is located on chromosome 17 & consists of 11 exons.
- It is mutated in >50% of all human cancers.
- Screening for TP53 mutations is important in some cancers as patients with TP53 mutations are known to have a poor outcome & drug response.
- High resolution melting curve analysis is a fast and post-PCR high-throughput method to screen for sequence variations in a target gene.
- Single-base changes in the target amplicons are detected by their altered melting-properties which is monitored through the release of fluorescent dsDNA binding dye & which give rise to changes in the shape of the melting curve compared to a reference sample.

Results

- 26 colorectal cancer cell lines were screened for TP53 mutations in exons 1,2,3, 5, 6, 7, 8, 9, 10 and 11.
- Mutations detected in exons 1, 2, 3, 5, 8, 9 and 10 were previously reported by other studies.
- LS1034 was reported to have a TP53 in exon 7. However, our HRM results show that LS1034 is a wild type for TP53 and that finding was confirmed by sequencing (Fig 1-B,C,D & F).
- C125-PM was reported to have a TP53 in exon 6. Our HRM data shows that C125-PM is wild type for exon 6 and mutant in exon 7. These findings were confirmed by sequencing C125-PM for exon 6 and 7. (fig 1-B,C,D & E).
- DLD1 showed different melting pattern for exon11. Sequencing shows a hetero. Mutation in intron 10-11.
- The table below shows a summary of our results compared to the published ones.

Aim of the Study

This study was carried out to screen for TP53 mutations in different colorectal cancer cell lines using the ability of High Resolution Melting (HRM) Curve Analysis to distinguish between normal and mutant genomic DNAs on the basis of their melting characteristics.

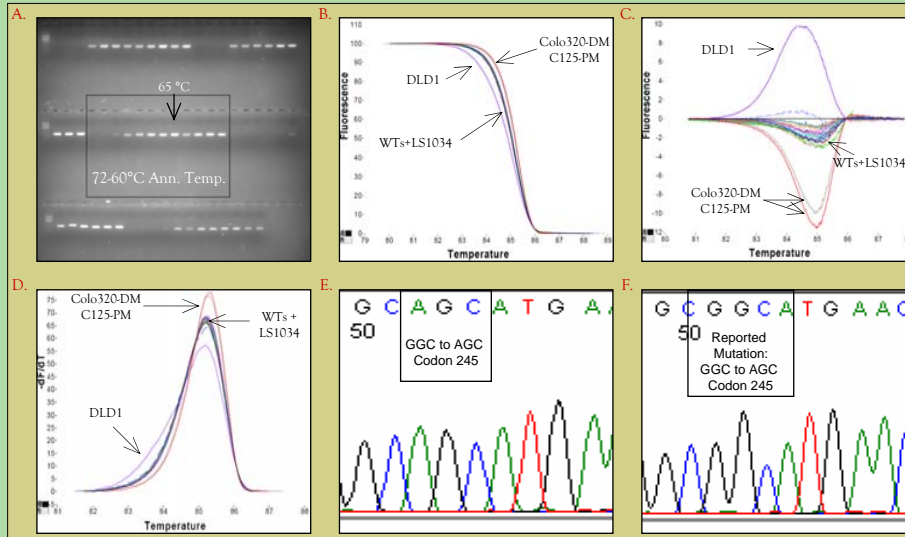


Figure 1: A: Exon7 PCR optimization gel. B, C & D: Exon7 Melting Curves. E: C125-PM exon7 sequencing. F: LS1034 exon7 sequencing.

Discussion

- This study presents an optimized, easy, specific & sensitive method for the screening of the TP53 mutations along the whole coding sequences.
- This assay was able to detect as low as 6% of the mutant sequence in a sample mixed with another wild type sequence.
- This assay is a screening method, i.e. results do not tell the exact mutations and sequencing of samples that give different curves shapes is required.
- Optimization of exon 4 primers conditions is still ongoing.

Methods

- PCR of each primer set was optimized by temperature gradient (fig1-A).
- PCR products of the 26 cell lines were melted using HR-1 instrument. See the table below for primers sequences, optimal annealing temperatures and HR-1 melting profiles.
- Known mutant cell lines' DNAs were mixed with wild type DNAs, 1:1 ratio.
- Sequencing was performed for products with discrepant results from those published.
- Each PCR of 10µl contained: 20ng template DNA, 0.25µM of each primer, 1X LCGreen, 1X HotShot Mastermix (Cadama Medical Ltd.) and appropriate H₂O volume.
- The PCR thermal profile used was: 92°C for 2 min, 45 cycles of 94°C for 30 sec and optimal annealing temp. for 30 sec., 1 cycle of 94°C for 30 sec. and 25 °C for 30 sec.

Exons	Primers sequences	Annealin g Temp.	Product size	Melting Profile
Exon 1	5'CACAGCTCTGGCTTGACAGA3' 5'AGCGATTTCCCGAGCTGA3'	66°C	442 bp	80 - 94°C
Exon 2	5'ACTGCCTCCGGTCACT3' 5'CTTCCAATGGATCCACTCAC3'	65°C	115 bp	80-90°C
Exon 3	5'AGCCCCCTAGCAGAGACT3' 5'AGCCCAACCCCTGTCCCTAC3'	67°C	110 bp	80-90°C
Exon 4	5'TGACTGCTCTTTTACCACATC3' 5'GGTGTAGGAGCTGCTGGT3'	66°C	175 bp	80-91°C
	5'GAATGCCAGAGGCTGCT3' 5'CCCCTCAGGGCAACTGAC3'	Not optimized yet	200 bp	Not Optimized yet
Exon5	5'CTGTCTCCTTCCCTTCCACAG3' 5'GCTGTGACTGCTGTAGATGG3'	65°C	150 bp	80-94°C
	5'GTGCAGCTGTGGTTGATT3' 5'AACCAGCCCTGCTGCTCT3'	66°C	170 bp	80-93°C
Exon 6	5'CCCTGATTCTCACTGATTGC3' 5'CTTAACCCCTCCTCCAGAG3'	63°C	181 bp	80-90°C
Exon 7	5'TTGGCCCTGTGTTATCTCT3' 5'TGGCAAGTGCTCCTGAC3'	65°C	150 bp	70-90°C
Exon 8	5'TTGCTCTCTTTTCTATCTGA3' 5'GCTCTTGTCTGCTGCTTGT3'	63°C	186 bp	80-91°C
Exon 9	5'CCTTCTCTGCTCTTCTCT3' 5'CCACTTGATAAGAGGTCCCAAG3'	63°C	127 bp	70-90°C
Exon 10	5'TCCCCCTCCTGTTGCT3' 5'GAAGGGGCTGAGGCACTC3'	66°C	150 bp	80-92°C
Exon 11	5'TGTCTCTCTCCTCCCTGCT3' 5'CAAGTGGGAAACAAGTGG3'	63°C	142 bp	80-90°C

Many thanks to Dr. Sally Chappell (Clinical Chemistry) & David Harris (Cadama, UK) for helping with HR-1 instrument.

Results were compared to:

• <http://www.p53.iarc.fr/>

• Liu, Y., and Bodmer, W. F., 2005. Analysis of P53 mutations and their expression in 56 colorectal cancer cell

Exon	Mutant Cell lines in this Study	Mutant Cell Lines in Published Data	Sequencing (if done)
Exon1	SW1222	SW1222	None
Exon 2	None	None	None
Exon3	None	None	None
Exon 5	SW1116, VACO10MS	SW1116 ,VACO10MS	None
Exon 6	HT55	HT55, C125-PM	C125-PM: No Mutation Detected.
Exon 7	Colo320-DM, DLD1, C125-PM	Colo320-DM, DLD1, LS1034	C125-PM: GGC to AGC, codon 245. LS1034: No Mutation Detected.
Exon 8	HCA7, HCA46, VACO5, SW480, SW620	HCA7, HCA46, VACO5, SW480, SW620	None
Exon 10	C84	C84	None
Exon 11	DLD1	None	DLD1: heterozygous missense mutation at intron 10-11.