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Results, Questions, Perspectives of a Study on Human Polyomavirus BK and Molecular Actors in Prostate Cancer Development

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Abstract. Background: Prostate cancer (PC) is a common tumor in Western countries. Several risk factors play significant roles. MYC, BIRC5/survivin, CDC25 and P53 may contribute to PC risk. As demonstrated, human Polyomavirus BK (BKV) could affect cellular homeostasis contributing to PC pathogenesis. Materials and Methods: Biological samples were collected from PC patients. Viral RNA was searched using quantitative polymerase chain reaction (PCR), whereas a qualitative PCR was employed to find particular viral sequences. Proper size amplicons were analyzed. Single nucleotide polymorphisms (SNPs) were detected in p53 coding regions by means of a specific PCR. C-MYC, BIRC5/survivin and CDC25 gene expression was investigated using a Retro Transcriptional Quantitative PCR. Results: Viral DNA copy number was higher in cancer tissues taken from Gleason score 9 patients with Gleason score 7. Different p53 mutated compared to patients exons were found according to tumor advanced stage and a statistical significant correlation was found between Gleason score and p53 mutational rate. C-MYC, BIRC5/survivin and CDC25 expression was de-regulated according to the literature. Conclusion: The presence of BKV and its variants

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in transformed cells does not exclude viral pressure in cell immortalization. Expression of other target genes evidenced a significant change in their regulation, useful for cancer drug discovery and therapies.

Prostate cancer (PC), a multi-factorial disease, is the most common tumor in Western countries (1, 2). Although age is the main risk factor, other factors, such as genes and infections, play significant role (3, 4). A great number of genes, such as *MYC*, *BIRC5*/survivin, *CDC25* and *P53* may contribute to PC risk (5).

MYC mRNA has been found to be elevated in cancer tissue with respect to matched benign prostatic hyperplasia tissues in the majority of cases. Moreover, it has been demonstrated that *MYC* amplification is related to PC progression (6).

BIRC5, encoding for surviving, functions as a negative regulator of apoptosis or programmed cell death. The protein survivin is highly expressed in most human tumors and its up-regulation has also been reported to possibly confer hormone resistance in PC (7-9).

CDC25 is a dual-specificity phosphatase present in mammalian cells in three forms named a, b and c (10). Misregulation of CDC25a levels could lead to genomic instability, a cancer hallmark. Furthermore, CDC25a over-expression often correlates with more aggressive diseases and poor prognosis (10).

P53 is a tumor suppressor gene whose mutations are implicated in the molecular genetics of many malignancies. Single-point mutations have been shown to not only abrogate p53 function but also contribute to the transformed

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phenotype. Certain DNA viruses may also contribute to the progression of invasive cancer in infected tissue acting to p53. Viral oncoproteins interact with p53 inhibiting its action (11). Inactivation of p53 has been proposed as the main mechanism whereby the oncogenic human Polyomavirus BK (BKV) plays a role in cancer progression (12). In the past, our research group demonstrated the presence of BKV in PC tissues suggesting that it could affect cellular homeostasis in the prostate and play the role of a co-factor in PC pathogenesis (13, 14). In particular, the viral large T antigen (TAg) is able to interfere with p53 oncosuppressor blocking apoptosis. Moreover, in laboratory animals, it has been demonstrated that the BKV regulatory region (RR) affects, in a sequence-dependent manner, cellular tropism and oncogenicity. RR rearrangements are associated with higher oncogenicity as a consequence of a more intense induction of cell proliferation (15, 16).

Taking into account this molecular and oncoviral portrait, the aim of the present study was to understand if normal prostate cells are really susceptible to viral infection and if BKV could play a role in a transformation scenario. In addition, p53 mutational analyses were performed and the expression pattern of selected PC genes was studied.

Materials and Methods

Patient population. From March 2012 to October 2013, 71 patients (median age=63) without a history of neoplastic diseases were admitted to the "Umberto I" Hospital of Rome, Italy, and enrolled according to the following criteria: (i) no previous hormonal or radiation therapy; (ii) no previous surgery on the prostate gland; (iii) histologically proven prostate cancer by biopsy and confirmed by radical prostatectomy;

Tumors were selected accounting for histopathological diagnosis, tumor size, grade and stage, the androgen receptor status and family history data.

Urine, blood and fresh tissue samples were collected from patients with clinically proven pT2aN0M0 or pT3N0M0 prostate adenocarcinoma. As controls, 71 non-tumor biopsies of the same patients were analyzed.

BKV DNA was searched in urine, blood and fresh PC samples by means of a quantitative polymerase chain reaction (Q-PCR). BKV RNA corresponding to TAg was searched using a Retro Transcriptional Quantitative PCR (RT-QPCR). Finally, BKV RR was searched by nested PCR and sequenced (17).

The sequencing analysis of p53 specific DNA binding exons (5-9) was carried out to understand if mutations might be correlated with viral infection and/or cancer progression.

Moreover, other target genes (*C-MYC*, *BIRC5*/survivin, *CDC25*) than P53, in PC gene profile, were considered. Genes were chosen using the ONCOMINE database and scientific literature. Their expression was investigated using RT-QPCR.

Processing of clinical specimens. DNA was extracted from 1 ml of urine, 200 μ l of plasma and about 25 mg of fresh PC resections using the DNeasy[®] Tissue Kit (QIAGEN, S.p.A, Milan, Italy) according to the manufacturer's instructions.

RNA was extracted from biopsies using the Total RNA Purification Kit (Norgen Biotek Corporation 3430, Thorold Ontario Canada) according to supplier's protocol. cDNA was obtained by the iScript cDNA Synthesis Kit (BioRad Laboratories S.r.I, Milan, Italy). One microgram of total purified DNA was used for QPCR, whereas two micrograms of total purified cDNA were used for RT-QPCR.

Q-PCR for BKV DNA and viral TAg cDNA. Urine, blood, fresh PC and control specimens were tested using Q-PCR for detection of BKV. Viral genome was quantified from each sample using a Q-PCR Alert Kit (ELITechGroup S.p.A, Trezzano S/N, Milan, Italy) in a 7300 Real-Time PCR System (AB Applied Biosystem, Foster City, CA, USA). Results for urine and plasma specimens were expressed as copies of viral DNA per milliliter (c/ml) and as copies per 10⁵ cells (c/10⁵ cells) for the biopsies. To correct the DNA variable amount in different tissue samples, each sample was subjected to simultaneous TaqMan PCR for the housekeeping gene Glyceraldehyde-3-phosphate-dehydrogenase (*GAPDH*, Accession No. J04038), targeting the region between exons 6-8. Results were considered acceptable only in the presence of *GAPDH*-positivity (18).

Q-PCR for C-MYC, BIRC5/survivin, CDC25. All reagents were spinned for 10-15 sec, then, in a PCR tube, the following reagents were added: 12.5 μ l of RT² SYBR Green Q-PCR Master Mix, 1.5 μ l of high-quality nuclease-free H₂O, 10 μ l of cDNA (template) and 1.0 μ l of gene-specific RT² Q-PCR specific primers. Tubes were quickly centrifuged and placed in a 7300 Real Time PCR System (AB Applied Biosystem, Foster City, CA, USA). Two-step cycling program was used; thermal cycling was initiated with a first denaturation step of 10 min at 95°C followed by 40 cycles of 95°C for 15 s, 60°C for 1 min. SYBR Green fluorescence from every well was detected and recorded during the annealing step of each cycle. Specific gene expression was quantified by using the 2- $\Delta\Delta$ CT method (19). Normalization of gene expression was performed using GAPDH as a reference gene.

PCR for BKV RR and amplicons sequencing. BKV RR was obtained by nested PCR as previously described (17). Correct size amplicons were purified prior to sequencing with QIAquick PCR purification kit (QIAGEN, S.p.A, Milan, Italy). DNA sequencing was performed in service (BioFab research s.r.l., Rome, Italy). Acquired sequences were analyzed by the Basic Local Alignment Search Tool at the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi), whereas alignments were performed with ClustalW2 at the EMBL-EBI website (http://www.ebi.ac.uk/Tools/msa/clustalw2/) using default parameters.

Mutational analysis of P53. P53 mutational analysis was performed according to Das and co-workers (12). Single nucleotide polymorphisms (SNPs) were detected by means of specific PCR in coding regions between exons 5-9. PCR products were separated by 1% agarose gel electrophoresis, purified (QIAquick PCR purification kit; QIAGEN, S.p.A, Milan, Italy) and sequenced (BioFab research s.r.l., Rome, Italy). Acquired sequences were analyzed by the Basic Local Alignment Search Tool at the NCBI website, whereas alignments were performed with ClustalW2 at the EMBL-EBI website using default parameters.

P53 sequence used for alignments corresponds to the genomic sequence NC_000017.10 (NCBI37/hg19, Chr17:7571720..7590917) from GenBank.

Table I. Average	number of vi	al copies cal	lculated in .	BKV-positive	samples.
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	Urine	Blood	Biopsies
Positive BKV samples	6/71 (8%)	4/71 (6%)	31/71 (44%)
Average of viral copies number	1000 c/ml	475 c/ml	Gleason 9: 16,000 c/10 ⁵ cells Gleason 8: 12,500 c/10 ⁵ cells Gleason 7: 8,364 c/10 ⁵ cells Gleason 6: 1,000 c/10 ⁵ cells

Data analysis. Data were summarized as medians and ranges or as mean, as appropriate. If the Z test indicated a non-normal distribution, nonparametric tests such as the Mann–Whitney U-test and Kruskal–Wallis test, were used. Categorical data were analyzed by using the χ^2 test and Student's t-test. Values of p<0.05 were considered statistically significant.

Results

Urine analysis revealed the presence of BKV genome in 6/71 (8%) patients, whereas 4/71 (6%) blood samples were positive for viral DNA. The average number of viral copies calculated in positive urine did not exceed 1,000 c/ml, whereas this value was 475 c/ml in blood samples (Table I).

Examination of biopsies evidenced BKV DNA in 31/71 (44%) (Table I). The average number of viral copies was calculated on biopsies of positive patients belonging to the same Gleason score. Twelve patients with Gleason 9 showed an average of $16,000 \text{ c}/10^5$ cells, whereas in eight patients with Gleason 8 an average of $12,500 \text{ c}/10^5$ cells was found and, finally, nine patients with Gleason 7 showed an average of 8,364 c/105 cells (Table I). Regarding patients with Gleason score 6, two patients were BKV DNA-positive with a low mean copy number of $1,000 \text{ c}/10^5$ cells (Table I). No controls were found positive (data not shown).

Regarding statistical analysis, the comparison between the mean values of BKV DNA copy numbers obtained for each class of Gleason scores over the months of this trial was not statistically significant, although copy numbers decrease from the class with the highest Gleason score (9) to that with lower Gleason score (7) (Table I).

Regarding the P53 mutational analysis, all specimens had at least one mutated exon. It was found that codons 248 (exon 7) and 273 (exon 8) were the most susceptible to mutation (5, 20). Exon 7 was always mutated in patients with Gleason score 9. Mutations in exons 5, 6, 8 and 9 were observed in 12/31 positive specimens. The results are shown in Table II.

It is known that mutations of *P53* are rare in primary PC but more common in PC at a higher tumor stage, higher tumor grade, metastases or androgen-independent tumors. Results confirmed these data and different mutated exons

were found according to tumor advanced stage (5). In particular, statistical analysis proved a significant correlation between Gleason score and the number of p53 mutated exons (14).

In order to understand if PC cells present a particular genotype, target genes *C-MYC*, *BIRC5*/survivin and *CDC25* rather than *P53* were considered. Genes were chosen using the ONCOMINE database and scientific literature. Their expression was investigated using RT-Q-PCR. According to scientific production, results showed the up-regulation of *C-MYC* at the mRNA level (Figure 1). De-regulated myc levels contribute to tumor growth by metabolic reprogramming and by adaptation to the microenvironment (21, 22).

Also survivin expression was enhanced in the analyzed samples (Figure 1). According to molecular profiling studies, high survivin expression in cancer has been correlated to indicate overall survival, resistance to therapy and shorter disease-free survival (23).

Regarding the *CDC25* gene, results showed its overexpression in cancer cells confirming its role as protooncogene (Figure 1). Its increased transcription often correlates with more aggressive diseases and poor prognosis, as demonstrated by Ray and Kiyokawa (24).

To understand if prostate cells are really non-permissive cells for BKV replication, searching of viral messengers was performed. In particular, since TAg is required to support viral DNA replication, its mRNA was investigated. Messenger amount was too low to allow replication (data not shown). The use of fresh samples proved essential for consistent and reproducible amplification of viral DNA, nevertheless to ratify the source of the signal detected by PCR, viral infection of human prostate cancer cell lines DU145 and PC3 is mandatory. These lines are not hormonesensitive, do not express prostate specific antigen (PSA) and differ for their metastatic potential.

Finally, an interesting role in cancer development could be played by RR rearrangements. In this study, two RR variants were found in two patients affected by Gleason 9 prostate cancer (Figure 2). These variants were characterized by enhancement of the oncogenic *c*-myc and the oncosuppressor p53 binding sites. Table II. An example of multiple sequence alignment for P53 gene (exon 5).

	191	
P53 ex.5	TACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGTGCAGCTG	60
Pz.1	TACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGTGCAGCTG	60
Pz.2	TACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGTGCAGCTG	60
Pz.3	TACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGTGCAGCTG	60
Pz.4	TACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGTGCAGCTG	60
Pz.5	TACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGTGCAGCTG	60
Pz.6	TACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGTGCAGCTG	60
Pz.7	TACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGTGCAGCTG	60
Pz.8	TACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGTGCAGCTG	60
Pz.9	TACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGTGCAGCTG	60
Pz.10	TACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGTGCAGCTG	60

P53 ex.5	TGGGTTGATTCCACACCCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAG	120
Pz.1	TGGGTTGATTCCACACCCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAG	120
Pz.2	TGGGTTGATTCCACACCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAG	120
Pz.3	TGGGTTGATTCCACACCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAG	120
Pz.4	TGGGTTGATTCCACACCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAG	120
Pz.5	TGGGTTGATTCCACACCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAG	120
Pz.6	TGGGTTGATTCCACACCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAG	120
Pz.7	TGGGTTGATTCCACACCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAG	120
Pz.8	TGGGTTGATTCCACACCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAG	120
Pz.9	TGGGTTGATTCCACACCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAG	120
Pz.10	TGGGTTGATTCCACACCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAG	120

P53 ex.5	TCACAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCATGAGCGCTGCTCAGATAGC	180
Pz.1	TCACAGCACATGACGGAGGTTGTGAGGC A CTGCCCCCACCATGAGCGCTGCTCAGATAGC	180
Pz.2	TCACAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCATGAGCGCTGCTCAGATAGC	180
Pz.3	TCACAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCATGAGCGCTGCTCAGATAGC	180
Pz.4	TCACAGCACATGACGGAGGTTGTGAGGC A CTGCCCCCACCATGAGCGCTGCTCAGATAGC	180
Pz.5	TCACAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCATGAGCGCTGCTCAGATAGC	180
Pz.6	TCACAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCATGAGCGCTGCTCAGATAGC	180
Pz.7	TCACAGCACATGACGGAGGTTGTGAGGC A CTGCCCCCACCATGAGCGCTGCTCAGATAGC	180
Pz.8	TCACAGCACATGACGGAGGTTGTGAGGC A CTGCCCCCACCATGAGCGCTGCTCAGATAGC	180
Pz.9	TCACAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCATGAGCGCTGCTCAGATAGC	180
Pz.10	TCACAGCACATGACGGAGGTTGTGAGGCGCTGCCCCCACCATGAGCGCTGCTCAGATAGC	180

P53 ex.5	GATG 184	
Pz.1	GATG 184	
Pz.2	GATG 184	
Pz.3	GATG 184	
Pz.4	GATG 184	
PZ.5	GATG 184	
PZ.6	GATG 184	
PZ./	GATG 184	
PZ.8	GATG 184	
PZ.9	GATG 184	
PZ.10	GATG I84	

Table II. An example of multiple sequence alignment for P53 gene (exon 6).

P3 ex.6 GCCGGCCCCCCCAGACTCTATCCGAGGGAACGAAACTTACGCGGGGAGGAACTTGGGGGGGG			141	
PP.1 GECEGGCCCCTCCTCAGCATCTTATCCGAGGGAGGAATTTGCGTGGGAGTATTGG 60 P2.3 GECEGGCCCCCCCCAGCATCTTATCCGAGGGAGGAATTTGCGTGGGAGTATTGG 60 P2.4 GECEGGCCCCCCCCAGCATCTTATCCGAGGGAGGAATTTGCGTGGGAGTATTGG 60 P2.5 GECEGGCCCCCCCCAGCATCTTATCCGAGGGAGGAATTTGCGTGGGAGTATTGG 60 P2.6 GECEGGCCCCCCCCAGCATCTTATCCGAGGGAGGAATTTGCGTGGGAGTATTGG 60 P2.7 GECEGGCCCCCCCCAGCATCTTATCCGAGGGAGGAATTTGCGTGGGAGTATTGG 60 P2.8 GECEGGCCCCCCCCCAGCATCTTATCCGAGGGAGGAATTGCGTGGGAGTATTGG 60 P2.9 GECEGGCCCCCCCCCAGCATCTTATCCGAGGGAGGAATTGCGTGGGAGTATTGG 60 P2.10 GECEGGCCCCCCCCAGCATCTTATCCGAGGGAGGAATTGCGTGGGAGTATTGG 60 P3.0 C.6 ATGCAGGAACACTTTCCGAGGGGGGGCCCTAGGAGGAATTGCGTGGGAGTATTGG 60 P3.1 ATGCAGGAACACTTTCCGACTGGGGGGCCCCTAGGCCCCTGGGGTGGCCTAGGCCCCTGGGGGGCCCTAGGGCCCCTGGGGGGCCCTAGGCCCCCGGGGGGGCCCTGGGGGGCCCTAGGCCGCCGGGGGGGG		P53 ex.6	GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGG	60
P2.2 CTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAGGAATTTGGCTGTGGAGTATTTGG 60 P2.4 GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAGGAATTTGGCTGTGGAGTATTTGG 60 P2.5 GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAGGAATTTGGCTGTGGAGTATTTGG 60 P2.6 GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAGGAAATTTGGCTGTGGAGTATTTGG 60 P2.7 GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAGGAAATTTGGCTGTGGAGTATTTGG 60 P2.8 GTCTGGCCCCTCCTCAGGATCTTATCCGAGTGGAGGAAATTTGGCTGTGGAGTATTTGG 60 P2.10 GTCTGGCCCCTCCTCAGGATCTTATCCGAGTGGAGGAAATTTGGCTGTGGAGTATTTGG 60 P2.11 GTCTGGCCCCTCCTCAGGATCTTATCCGAGTGGAGGAAATTTGGCTGTGGAGTATTTGG 60 P2.10 GTCTGGCCCCTCCAGGATCTTATCCGAGTGGAGGAAATTTGGCTGTGGAGTATTTGG 61 P2.11 TGGCAGGAACACTTTTCGAGTGGAGGCCCTAGGAGCGCCTGGA 113 P2.1 ATGACAGAAACACTTTTCGAGTGGAGTGCCCTATGAGCCGCCCGC		Pz.1	GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGG	60
P2.3 GECEGGECCETCETCAGCATCTTATCCGAGEGAAGGAATTTGCGEGGEGGEGAGEATTTGG 60 P2.5 GECEGGECCETCETCAGCATCTTATCCGAGEGAAGGAATTTGCGEGGEGGEGAGEATTTGG 60 P2.6 GECEGGECCETCETCAGCATCTTATCCGAGEGAAGGAATTTGCGEGGEGGEGAGEATTTGG 60 P2.7 GECEGGECCETCETCAGCATCTTATCCGAGEGAAGGAATTGCGEGGEGGEGAGEATTTGG 60 P2.8 GECEGGECCETCETCAGCATCTTATCCGAGEGAAGGAATTGCGEGGEGGEGAGATATTGG 60 P2.9 GETERGECCETCETCAGCATCTTATCCGAGEGAAGGAAATTGCGETGGAGTATTTGG 60 P2.10 GETERGECCETCETCAGCATCTTATCCGAGEGAAGGAAATTGCGETGGAGTATTTGG 60 P2.11 GETERGECCETCETCAGCATCTTATCCGAGEGAAGGAAATTGCGETGGAGTATTTGG 60 P2.11 ATGCCGAGAACACTTTCCGACTGGEGGEGCCCTATGAGCCGCETGGG 113 P2.2 ATGCCGAAACACTTTCCGACTAGEGTGGGEGCCCTATGAGCCGCCTGAG 113 P2.2 ATGCCGAAACACTTTCCGACTAGEGTGGGEGCCCTATGAGCCGCCTGAG 113 P2.4 ATGCCGAAACACTTTCCGACTAGEGTGGGEGCCCTATGAGCCGCCTGG 113 P2.4 ATGCCGAAACACTTTCCGACTAGEGTGGGEGCCCTATGAGCCGCCTGG 113 P2.4 ATGCCGAAACACTTTCCGACTAGEGTGGGEGCCCTATGAGCCGCCGCGGG 113 P2.4 ATGCCGAAACACTTTCCGACTAGEGTGGGEGCCCTATGAGCCGCCGCGGG 113 P2.4 ATGCCGAAACACTTTCCGACTAGEGTGGGEGCCCTATGAGCCGCCGCGGG 113 P2.4 ATGCCGAAAACACTTTCCGACTAGEGTGGGGEGCCCTATGAGCCGCCGCGGG 113 P2.6 ATGCCGAAACACTTTTCGACTAGEGTGGGGCCCCTATGAGCCGCCGCGGGG 113 P2.6 ATGCCGAAACACTTTTCGACTAGEGTGGGGCCCCTATGAGCCGCCGCGGGG 113 P2.6 ATGCCGAAACACTTTTCGACTAGEGTGGGGCCCCTATGAGCCGCCGCGGG 113 P2.6 ATGCCGAAACACTTTTCGACTAGEGTGGGGCCCCTATGAGCCGCCGCGGG 113 P2.7 ATGCCGAAAACACTTTTCGACTAGCTGGGGGCCCCTATGAGCCGCCGCGGG 113 P2.8 ATGCCGAAACACTTTTCGACTAGCTGGGGGCCCCTATGAGCCGCCGCGGG 113 P2.9 ATGCCCGACGTTTCGACTAGCTGGGGGCCCCTATGGCCGCCGCGGG 113 P2.1 ATGCCGAAACACTTTTCGACTAGCGTGGGGCCCCTATGGCCGCCGCGGG 113 P2.1 ATGCCGGACGTGCCCTATGCCCCCCGCGCGGGG 113 P2.1 ATGCCGGACGCCACTCTCCCCCCCCCCCCGCCGGCGGGG 113 P2.1 ATGCCGGACGCCACTCTCCCCCCCCCCCCGCCGCGGCGGGCG		Pz.2	GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGG	60
P2.4 GTCTGGCCCTCTCTAACCGAGTGAAGAAATTTGCGTGGAGAGAATTTGG 60 P2.6 GTCTGGCCCCTCTCACACATCTTATCCGAGTGAAGAAATTTGCGTGTGAGATATTGG 60 P2.7 GTCTGGCCCCTCTAACACATCTATCCGAGTGAAAGAATTTGCGTGTGAGATATTGG 60 P2.9 GTCTGGCCCCTCCTAACACATCTTATCCGAGTGAAAGAATTTGCGTGTGAGAGTATTTGG 60 P2.9 GTCTGGCCCCTCCTAACACTCTATCCGAGTGAAAGAAATTTGCGTGTGAGAGTATTTGG 60 P2.10 GTCTGGCCCCTCCTAACACTCTATCCGAGTGAAAGAAATTTGCGTGTGAGAGTATTTGG 60 P2.11 GTCTGGCCCCTCCTAACACTCTATCCGAGTGGAAGAAATTTGCGTGTGGAGTATTTGG 60 P2.12 ATGACAGAAACACTTTTCGACATGTGGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.1 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.2 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.3 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.4 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.5 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.5 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.6 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.7 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.7 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.7 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.9 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.9 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.9 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.9 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.9 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.9 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.9 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGACCGCCGCTGAG 113 P2.9 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGACCGCCCTGGG 10 P2.4 GTGGCTGTGACTGTGTGCACCACCACCACCACACACACTACAGTGTGAACGGCCGCGCGGG 60 P2.4 GTGGGCTGGACGTGTGCCCACCACCACCACACACTACAGTGTGAACGGCCGCGCGGG 60 P2.4 GTGGGCTGGACGTGTGCACCACCACCACCACACACTACAGTGTGAACGGTCGGG 60 P2.4 GTGGGCCTGGACGCAGCCCACCCACCACCACCACACACAC		Pz.3	GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGG	60
P2.5 GTTGSCCCTCCTCAGACTCTATCCGAGTGAAGAAATTTGCGTGGAGTATTTGG 60 P2.7 GTCGGCCCCTCCTAGACATCTATCCGAGTGAAGAAATTTGCGTGGAGTATTTGG 60 P2.9 GTCTGGCCCCTCCTAGACATCTATCCGAGTGAAGAAATTTGCGTGGAGTATTTGG 60 P2.9 GTCTGGCCCCTCCTAGACATCTATCCGAGTGAAGAAATTTGCGTGGAGTATTTGG 60 P2.10 GTCTGGCCCCTCCTAGACATCTATCCGAGTGAAGAAATTTGCGTGGAGTATTTGG 60 P2.11 GTCTGGCCCCTCCTACACATCTATCCGAGTGAAGAAATTTGCGTGGAGTATTTGG 60 P2.12 ATGACGAAACACTTTTCCGACTGAAGAAATTTGCGGTGGAGTATTTGG 60 P2.13 ATGACGAAACACTTTTCCGACTGAGAGAAATTTGCGGTGGAGTATTTGG 60 P2.14 ATGACGAAACACTTTTCCGACTGAGAGAAATTTGCGGTGGAGGTGATGG 113 P2.2 ATGACGAAACACTTTTCCGACTATGCTGGTGGCCCTATGAGCCGCCTGAG 113 P2.2 ATGACGAAACACTTTTCCGACTATGCTGGTGGCCCTATGAGCCGCCTGAG 113 P2.4 ATGACGAAACACTTTTCCGACTAGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.4 ATGACGAAACACTTTTCGGACTAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.6 ATGACGAAACACTTTTCGGACTAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.6 ATGACGAAACACTTTTCGGACTAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.7 ATGACGAAACACTTTTCGGACTAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.7 ATGACGAAACACTTTTCGGACTAGTGTGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.9 ATGACGAAACACTTTTCGGACTAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.9 ATGACGAAACACTTTTCGGACTAGTGTGGTGGCCCTATGAGCCGCCTGGG 10 P2.9 GTTGGCTCGGACTGACCACCACCACCACCACACACACACTGGTGAACGGTCCGGGC 60 P2.9 GTTGGCTCGGACTGTGACCACCACCACCACACACACACTGGTGAACGGTCGGGC 60 P2.1 GTGGCTCGGACTGTGACCACCACCACCACCACACACACAC		Pz.4	GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGG	60
P2.6 CTCTGGCCCCCTCCCAGACTCTATCCGAGTGAAAGAATTTCCCGTGGGGATATTTGG P2.7 GTCTGGCCCCCTCCAGACTCTATCCGAGTGGAAGAATTTGCGTGTGGAGTATTTGG P2.8 GTCTGGCCCCTCCTAGACTCTATCCGAGTGGAAGAAATTTGCGTGTGGAGTATTTGG P2.9 GTCTGGCCCCTCCTAGACATCTATCCGAGTGGAAGAAATTTGCGTGTGGAGTATTTGG P2.10 GTCTGGCCCCTCCTAGACACTTATCCGAGTGGAAGAAATTTGCGTGTGGAGTATTTGG P2.11 GTCTGGCCCCTCCTAGACACTTATCCGAGTGGAAGAAATTTGCGTGTGGAGTATTTGG P53 ATGACAGAAACACTTTTCGGACTAGTGTGGTGGCCCTATGAGCCGCCTGAG P53 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG P2.3 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG P2.4 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG P2.5 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG P2.6 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG P2.6 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG P2.6 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGAGCCGCCTGAG P2.6 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGCCCTATGAGCCGCCTGAG P2.7 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGCCCTATGAGCCGCCTGAG P2.6 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGCCCTATGAGCCGCCTGAG P2.7 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGCCCTATGAGCCGCCTGAG P2.6 ATGACAGAAACACTTTTCGACATAG		Pz.5	GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGG	60
P2.7 GTCTGSCCCCTCTCTATCCGARTGAAGAAATTECGTTGSGATATTEG 60 P2.8 GTCTGSCCCCTCTAACCATCTATCCGARTGAAGAAATTEGGTGGGAGATATTEG 60 P2.9 GTCTGSCCCCTCTACCATCTATCCGARTGAAGAAATTEGGTGGAGGATATTEG 60 P2.10 GTCTGSCCCCTCACCACTTATCCGARTGAAGAAATTEGGTGGAGGATATTEG 60 P2.11 GTCTGSCCCCTCACCACTTATCCGARTGAAGAAATTEGGTGGAGGATATTEG 60 P2.11 GTCTGSCCCCTCACACTTTTCGACAGAGAAATTEGGTGGGCGCTATGAGCGGCCTGAG 113 P2.1 ATGACAGAAACACTTTTCGACATAGTGGGGTGGCCCATTGAGCGGCCTGAG 113 P2.2 ATGACAGAAACACTTTTCGACATAGTGGGGGGGCCATAGAGCGGCCTGAG 113 P2.4 ATGACAGAAACACTTTTCGACATAGTGGGGGGCCATAGAGCGGCCTGAG 113 P2.5 ATGACAGAAACACTTTTCGACATAGTGGGGGGGCCATTGAGCGGCCCTAGG P2.6 ATGACAGAAACACTTTTCGACATAGTGGGGGGGCCATAGAGCGGCCTGAG 113 P2.7 ATGACAGAAACACTTTTCGACATAGTGGGGGGGCCATTGAGCCGCCCTGAG 113 P2.6 ATGACAGAAACACTTTTCGACATAGTGGGGGGGGCCATTGAGCCGCCCTGAG 113 P2.7 ATGACAGAAACACTTTTCGACATAGTGGGGGGGGCCATTGAGCCGCCCTGAG 113 P2.8 ATGACAGAAACACTTTTCGACATAGTGGGGGGGGGGCCATTGAGCCGCCCTGAG 113 P2.7 ATGACAGAAACACTTTTCGGACTAGTGGGGGGGGGCCATTGAGCCGCCCTGAG 113 P2.7 ATGACAGAAACACTTTTCGGACTAGGGGGGGGGGGCCATGGGCCCATGGGCCCATGGGCCCATGGGGGGGCCATGGGGCCCATGGGGCCCATGGGGCCCATGGGCCCATGGGCCCATGGGCCCATGGGCCCATGGGCCGGGGGCCCATGGGCCGGGGCCCATGGGCCCATGGGCCCGGGGGCCCCTAGGGCCGGGGCCCATGGGGCCGGCC		Pz.6	GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGG	60
P2.8 GCCCGCCCCCCCCCACACCACCACCACCACCACCACCACC		Pz.7	GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGG	60
P2.9 GCCTGGCCCTCGCAGGATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTGG 60 P2.10 GCCTGGCCCCTCCTCAGGATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTGG 60 P2.11 GCCTGGCCCCTCCTCAGGATCTTATCCGAGTGGAAGGAAATTGCGTGTGGAGTATTGG 60 10 ATGACAGAACACTTTCGACATAGTGGGGGGCCCCAATGAGTGGGGCCCTAG 113 P2.1 ATGACAGAAACACTTTCGACATAGTGGGGGGGCCCCTATGAGCGGCCTGAG 113 P2.2 ATGACAGAAACACTTTCGACATAGTGGTGGTGGCCCCTATGAGCGCGCTGAG 113 P2.1 ATGACAGAAACACTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCGCTGAG 113 P2.2 ATGACAGAAACACTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCGCTGAG 113 P2.4 ATGACAGAAACACTTTCGACATAGTGTGGTGGTGGCCCTATGAGCGCGCTGAG 113 P2.5 ATGACAGAAACACTTTCGACGTAGTGTGGTGGTGCCCTATGAGCGCGCCTGAG 113 P2.6 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTATGAGCGCGCTGAG 113 P2.7 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTAGAGCCCGCGGGGG 113 P2.8 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGCCCTAGAGCCCGGGGGGGG		Pz.8	GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGG	60
P2.10 GTCTGGCCCTCCTCAGGATCTTATCCGGGTGGAAGGAAGTTGGCGTGTGGAAGTATTGG 60 P2.11 GTCTGGCCCTCCTCAGGATATTTGGCGTGGTGGAAGGAAG		Pz.9	GTCTGGCCCCTC G TCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGG	60
Pr.11 GTCTGGCCCTCCAGCATCTTATCCGAGTGGAAGGAAGTTTGCGGTGTGGAGTATTTGG 60 P53 ex.6 ATGACAGAAACACTTTCGACATAGTGTGGTGGTGCCCCTATGAGCGCGCTGAG 113 Pr.1 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCCTATGAGCGCGCTGAG 113 Pr.2 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCCTATGAGCGCGCCTGAG 113 Pr.2 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCCTATGAGCGCGCCTGAG 113 Pr.2 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCCTATGAGCCGCCTGAG 113 Pr.4 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 Pr.5 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 Pr.6 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 Pr.7 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 Pr.7 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 Pr.7 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGTGCCCTATGAGCCGCCTGAG 113 Pr.7 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGTGGTGGTGGTGCCCTATGAGCCGCTGAG 113 Pr.7 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGTGGTGGTGGTGGTGGTGCCCTAGG 113 Pr.10 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGTGGTGGTGGTGGTGGCCTAGAGGAGCCTGAG 113 Pr.11 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGGTG		Pz.10	GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGG	60
P53 ex.6 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCGCCTAG 113 Pz.1 ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCTATGAGCGCCTAG 113 Pz.2 ATGACAGAAACACTTTTCGACATAGTGGTGGTGCCCTATGAGCGCCCTAG 113 Pz.3 ATGACAGAAACACTTTTCGACATAGTGGTGGTGCCCTATGAGCGCCCTAG 113 Pz.4 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCCTAG 113 Pz.5 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCCTAG 113 Pz.6 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCCTAG 113 Pz.7 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCCCTAG 113 Pz.8 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCCCTAG 113 Pz.9 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCCCTAG 113 Pz.10 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCCCTAG 113 Pz.11 ATGACAGAAACACTTTTCGACATACGTGGTGGTGGTGCCCTATGAGCGCCCCTAG 113 Pz.10 ATGACAGAAACACTTTTCGACATACGTGGTGGTGGTGCCCTATGAGCGCCCTAG 113 Pz.11 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCCCTAG 113 Pz.10 GTGGCCTGACGATAGCCCATCCCACCACCACCACCATCCAT		Pz.11	GTCTGGCCCCTCCTCAGCATCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGG	60
P53 ex.6 ATGACAGAACACTTTTCGACATAGTGGTGGTGCCCTATGAGCGCCTGAG 11.3 P2.1 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCCTGAG 11.3 P2.2 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCCCTAG 11.3 P2.3 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCGCTGAG 11.3 P2.4 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCCCTAG 11.3 P2.5 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCGCTGAG 11.3 P2.6 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCGCTGAG 11.3 P2.7 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCGCTGAG 11.3 P2.8 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCGCTGAG 11.3 P2.9 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCGCTGAG 11.3 P2.10 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCGCTGAG 11.3 P2.11 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCCTGAG 11.3 P2.10 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGTGCCCTATGAGCGCCTAGG 11.3 P2.11 GTGGCCTGACGTGACCACACCATCCCATACAACTGTGTGAACAGTCCTGCCATGGC 60 P2.12 GTTGGCCTGACTGACGACACCATCCCATACAACTACATGTGTGAACAGTTCCTGCATGGGC			*****	00
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Pz.2 ATGACAGAAACACTTTTCGACGTAGTGGTGGTGGCCCTATGAGCCGCCTAG 113 Pz.3 ATGACAGAAACACTTTTCGACGTAGTGGTGGTGGCCCTATGAGCCGCCTGAG 113 Pz.4 ATGACAGAAACACTTTTCGACATAGTGGTGGTGGCCCTATGAGCCGCCTGAG 113 Pz.5 ATGACAGAAACACTTTTCGACATAGTGGGTGGTGCCCTATGAGCCGCCTGAG 113 Pz.6 ATGACAGAAACACTTTTCGACATAGTGGGTGGTGCCCTATGAGCCGCCTGAG 113 Pz.7 ATGACAGAAACACTTTTCGACATAGTGGGTGGTGCCCTATGAGCCGCCTGAG 113 Pz.8 ATGACAGAAACACTTTTCGACATAGTGGGTGGTGCCCTATGAGCCGCCTGAG 113 Pz.9 ATGACAGAAACACTTTTCGACATAGTGGGTGGTGGCCCTATGAGCCGCTGAG 113 Pz.10 ATGACAGAAACACTTTTCGACATAGTGGGTGGTGGCCCTATGAGCCGCTGAG 113 Pz.10 ATGACAGAAACACTTTTCGACATAGTGGGTGGTGGCCCTATGAGCGCCGAG 113 Pz.11 ATGACAGAAACACTTTTCGACATAGTGGGTGGTGGCCCTATGAGCGCCGAG 113 Pz.10 ATGACAGAAACACTTTTCGACATAGTGGGTGGTGGCCCTATGAGCGCCGAG 113 Pz.11 ATGACAGAAACACTTTTCGACATAGTGGGTGGTGGCCCTATGAGCGCCGAG 113 Pz.11 ATGACAGAAACACTTTTCGACATAGTGGGTGGTGGCCCTATGAGCGCCTGGGC 113 Pz.11 ATGACAGAAACACTTTTCGACATAGTGGGTGGTGCCCTATGAGCGCCTGGGC 113 Pz.11 ATGACTGGACTGACTGACCACACCACCATCACATACATGTGTAACAGTTCCTGCAGGGG 60		Pz. 1	ATGACAGAAACACTTTTCGACATAGTGTGGTGGTGCCCCTATGAGCCGCCTGAG 113	
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P2.6 ATGACAGAAACACTTTTCGACGTAGTGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.7 ATGACAGAAACACTTTTCGACGTAGTGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.8 ATGACAGAAACACTTTTCGACGTAGTGTGGTGGCCCTATGAGCCGCCCTGAG 113 P2.9 ATGACAGAAACACTTTTCGACGTAGTGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.10 ATGACAGAAACACTTTCGACATAGTGTGGTGGTGCCCTATGAGCCGCCTGAG 113 P2.11 ATGACAGAAACACTTTCGACATAGTGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.11 ATGACAGAAACACTTTTCGACATAGTGTGGTGGCCCTATGAGCCGCCTGAG 113 P2.11 ATGACAGAAACACTTTTCGACATAGTGTGGCCCTATGAGGCCCCTATGAGCGCCGCGGAG 113 P2.11 GTTGGCTCTGACTGTACCACCATCCACTACCACTACATGTGGCCCCTATGAGCGCCCCGCGGG 113 P2.11 GTTGGCTCTGACTGTACCACCATCCACTACCACTACCATGTGTGTAACAGTTCCTGCATGGGC 60 P2.2 GTTGGCTCTGACTGTACCACCACTCCACTACAACTACATGTGTGTAACAGTTCCTGCATGGGC 60 P2.3 GTTGGCTCTGACTGTACCACCACTCCACTACAACTACATGTGTGTAACAGTTCCTGCATGGGC 60 P2.4 GTTGGCTCTGACTGTACCACCACCACCACCACTACAACTACATGTGTGTAACAGTTCCTGCATGGGC 60 P2.6 GTTGGCTCTGACTGTACCACCACCACCACCACTACAACTACATGTGTGTAACAGTTCCTGCATGGGC 60 P2.7 GTGGCTCTGACTGTACCACCACCACCACTACCACTACACTGCATGACAGTCCTGCCACATGGGC 60 P2.8 GTTGGCTCTGACTGTAC		Pz 5		
P2.0 ATGACAGAAACACTITTICGACATAGTIGGTGGGTGCCCTATGAGCCGCCTGAG 113 P2.8 ATGACAGAAACACTITTICGACATAGTIGGTGGTGCCCTATGAGCCGCCCTAGGAGCGCCCTGAGGAGCGCCTGACGAGCGCCCTAGGAGCGCCCTAGGAGCGCCCTGAGGAGCGCCAGCCTGACGAGCGCCTGACGAGCGCCTGACGAGCGCCTGACGAGCGCCTGACGAGCGCCTGACGAGCGCCTGACGAGCGCCTGACGAGCGCCTGACGAGCGCGGGGCCCTGACGAGCGGGGCCCTGACGAGCGGGGCCCTGACGAGCGGGGCGGGGCCGAGCGGGGCGGGGGCGGGGGGG		Pz 6		
P2.7 ATGACAGAAACACTITTICGACGAGTGGTGGGCCCATGAGACCGCCTGAG 113 P2.9 ATGACAGAAACACTITTICGACATAGTGTGGTGGTGCCCATGAGACCGCCCTAG 113 P2.10 ATGACAGAAACACTITTICGACATAGTGTGGTGGTGCCCATGAGCCGCCCTAG 113 P2.11 ATGACAGAAACACTITTICGACATAGTGTGGTGGTGCCCATGAGCCGCCCTAG 113 P2.11 ATGACAGAAACACTITTICGACATAGTGTGGTGGTGCCCATGAGCCGCCCTAG 113 Table II. An example of multiple sequence alignment for P53 gene (cxon 7). P53 ex.7 GTGGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.2 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGGTAACAGTTCCTGCATGGGC 60 P2.3 GTGGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.4 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.5 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.6 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.7 GTGGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.6 GTGGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.7 GTGGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.8 GTGGGCTCTGACCTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.9 GTTGGCTCTGACCTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.10 GTGGCTCTGACCTGTACCACC		FZ.0 Dz 7		
P2.0 ATGACAGAAACACTITTCGACATAGTGGGGGCCCATGGAGCCGCCTGAG 113 P2.10 ATGACAGAAACACTITTCGACATAGTGGGGGGCCCATGGAGCCGCCTGAG 113 P2.11 ATGACAGAAACACTITTCGACATAGTGGGGGGCCCATGGAGCCGCCTGAG 113 P2.11 ATGACAGAAACACTITTCGACATAGTGGGGGGCCCATGGAGCCGCCTGAG 113 P2.11 ATGACAGAAACACTITTCGACATAGTGGGGGGCCCATGGAGCCGCCTGAG 113 Table II. An example of multiple sequence alignment for P53 gene (exon 7). P53 ex.7 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.2 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.3 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.4 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.5 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.6 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.7 GTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.8 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.10 GTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 P2.12 GTGGCTCTGACCGTGACCATCCACCATCCACTACAACTACATGGTAACAGTTCCTGCATGGGC 60 P2.12 GTGGCTCTGACCGTGTACCACCATCCACTACAACTACATGGTAACAGTTCCTGCATGGGC 60 P2.8 <t< td=""><td></td><td>Pz 9</td><td></td><td></td></t<>		Pz 9		
P2.9 ATGACAGAAACACTTTTCGACATAGTGTGGTGCCCCTATGAGCCGCCTGG 113 P2.10 ATGACAGAAACACTTTTCGACATAGTGTGGTGCCCCTATGAGCCGCCTGG 113 P2.11 ATGACAGAAACACTTTTCGACATAGTGTGGTGCCCCTATGAGCCGCCTGG 113 Table II. An example of multiple sequence alignment for P53 gen (exon 7). P53 ex.7 GTTGGCTCTGACTGTACCACCACCACCACACACACACGACGTGCTGACAGTCCGCGAGGGC 60 P2.1 GTTGGCTCTGACTGTACCACCACCACCACCACACACACGACGTGCTGACAGTGCCGCGGC 60 P2.2 GTTGGCTCTGACTGTACCACCACCACCACCACACACACAC		PZ.0		
P2.10 ATGACAGAAACACTITICGACATAGUTGUGUGUGUGUGUGUGUGUGUGUGUGUGUGUGUGU		FZ.9		
P2.11 Alteractor Infinition Calibration Street Contractor Control Calibration Street Control Calibration Street Control Calibration Street Calibration Street Calibration Street Calibratic Calibrate Calibrate Calibratic Calibrate Calibratic Calibratic		PZ.10		
Table II. An example of multiple sequence alignment for P33 gene (exon 7). P53 ex.7 GTTGGCTCTGACTGTACCACCACCACCACTACAACTGCGTGACAGGTTCCTGCATGGGC Pz.1 GTTGGCTCTGACTGTACCACCACCACCACCACACACACGACGACGACGGTCCTGCATGGGC Pz.3 GTTGGCTCTGACTGTACCACCACCACCACACACACACGACGACGGTCCTGCATGGGC Pz.4 GTTGGCTCTGACTGTACCACCACCACCACCACACACACGACGGTCCTGCATGGGC Pz.5 GTTGGCTCTGACTGTACCACCACCACCACCACACACGACGGTCCTGCATGGGC Pz.6 GTTGGCTCTGACTGTACCACCACCACCACCACCACACACGACGGTCCTGCATGGGC Pz.7 GTTGGCTCTGACTGTACCACCACCACCACCACCACCACCACTGCACTGCACGGTGCCGCAGGCC Pz.6 GTTGGCTCTGACTGTACCACCATCCACTACAACTACAGTGTAACAGTTCCTGCATGGGC Pz.7 GTTGGCTCTGACTGTACCACCATCCACTACCACTACCACTGCATGGTAACAGTTCCTGCATGGGC Pz.8 GTTGGCTCTGACTGTACCACCATCCACTACCACTACCACTGCATGCA		PZ.11		
P53 ex.7 GTTGGCTCTGACTGTACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 Pz.1 GTTGGCTCTGACTGTACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 Pz.2 GTTGGCTCTGACTGTACCACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 Pz.3 GTTGGCTCTGACTGTACCACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 Pz.4 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 Pz.5 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 Pz.6 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 Pz.7 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 Pz.8 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 Pz.9 GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60 Pz.10 GTTGGCTCTGACTGTACCACCATCCACCACCACCACCATCAACAGTGTAACAGTTCCTGCATGGGC 60 Pz.11 GTGCATGAACTGGAGGCCCATCCTCACCATCAACACAGTGGAAGACTCCAG 110 Pz.2 GTGGCATGAACTGGAGGCCCATCCTCACCATCAACACAGTGGAAGACTCCAG 110 Pz.1 GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110 Pz.2 GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110 Pz.3 GGCATGA	Tabl	II An axample of multiple	sequence alignment for $P53$ and $(aron 7)$	
P53 ex.7GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.1GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.2GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.3GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.4GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.5GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.6GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.7GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.8GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.9GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.11GTTGGCTCTGACTGTACCACCATCCACTACAACTGTGTAACAGTTCCTGCATGGGC60Pz.12GTTGGCTCTGACTGTACCACCATCCACTACAACTGTGTAACAGTTCCTGCATGGGC60Pz.13GTGGCTCTGACTGTACCACCATCCACTACAACTGTGTAACAGTTCCTGCATGGGC60Pz.14GTTGGCTCTGACTGTACCACCACCACCACCACTCACACTGGAAGACTCCAG110Pz.2GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.3GGCATGAACCGGAGGCCCACCCTCCACCATCATCACACTGGAAGACTCCAG110Pz.4GGCATGAACCGGAGGCCCACCCTCCACCATCATCACACTGGAAGACTCCAG110Pz.5GGCATGAACCGGAGGCCCACCCTCCACCATCATCACACTGGAAGACTCCAG110Pz.6GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGA	Table		sequence augnment for F55 gene (exon 7).	
Pz.1GTTGGCTCTGACTGTACCACCATCACACTACCACTGTGTAACAGTTCCTGCATGGGC 60Pz.2GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.4GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.5GTTGGCTCTGACTGTACCACCACCACCACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.6GTTGGCTCTGACTGTACCACCACCACCACCACACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.7GTTGGCTCTGACTGTACCACCACCACCACCACACACACTGTGTAACAGTTCCTGCATGGGC 60Pz.8GTTGGCTCTGACTGTACCACCACCACCACCACCACCACTGGTAACAGTTCCTGCATGGGC 60Pz.9GTTGGCTCTGACTGTACCACCACCACCACCACCACACAGTGGTAACAGTTCCTGCATGGGC 60Pz.10GTTGGCTCTGACTGTACCACCACCACCACCACACACACTGCATGTGTAACAGTTCCTGCATGGGC 60Pz.11GTTGGCTCTGACTGTACCACCACCACCACCACCACACACA		P53 ex.7	GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC	60
P2.2GTTGGCTCTGACTGTACCACCACCACCACCACACTACATCAACTACATGTGTAACAGTTCCTGCATGGGC60P2.3GTTGGCTCTGACTGTACCACCACCACCACCACACTACATGTGTAACAGTTCCTGCATGGGC60P2.4GTTGGCTCTGACTGTACCACCACCACCACCACACTACATGTGTAACAGTTCCTGCATGGGC60P2.5GTTGGCTCTGACTGTACCACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60P2.6GTTGGCTCTGACTGTACCACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60P2.7GTTGGCTCTGACTGTACCACCACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60P2.8GTTGGCTCTGACTGTACCACCACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60P2.9GTTGGCTCTGACTGTACCACCACCACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60P2.10GTTGGCTCTGACTGTACCACCACCACCACCACCACCACCACCACGTGGTAACAGTTCCTGCATGGGC60P2.12GTTGGCTCTGACTGTACCACCACCACCACCACCACCACTGCAACTACATGTGTAACAGTTCCTGCATGGGC60P2.12GTTGGCTCTGACTGTACCACCACCACCACCACCACCACTGGTAACAGTTCCTGCATGGGC60P2.12GTTGGCTCTGACCGAGGCCCATCCTCACCACCACACTGGAAGACTCCCAG110P2.12GGCATGAACCGGAGGCCCATCCTCACCATCACACTGGAAGACTCCCAG110P2.3GGCATGAACCGGAGGCCCACCCTCACCATCACACTGGAAGACTCCAG110P2.4GGCATGAACCGGAGGCCCACCCTCACCATCACCACCACACTGGAAGACTCCAG110P2.5GGCATGAACCGGAGGCCCACCCTCACCATCACACACTGGAAGACTCCAG110P2.6GGCATGAACCGGAGGCCCATCCTCACCATCACACTGGAAGACTCCAG110P2.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110P2.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110P2.6GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110P2.9<		Pz.1	GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC	60
Pz.3GTTGGCTCTGACTGTACCACCACTCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.4GTTGGCTCTGACTGTACCACCACCACCACCACACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.5GTTGGCTCTGACTGTACCACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.6GTTGGCTCTGACTGTACCACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.7GTTGGCTCTGACTGTACCACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.8GTTGGCTCTGACTGTACCACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.9GTTGGCTCTGACTGTACCACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.10GTTGGCTCTGACTGTACCACCACCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.11GTTGGCTCTGACTGTACCACCATCCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.12GTTGGCTCTGACTGTACCACCATCCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.11GTTGGCTCTGACTGTACCACCATCCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.12GTTGGCTCTGACCGAGGCCCATCCTCACCATCACACTGGAAGACTCCAG110Pz.13GGCATGAACCGGAGGCCCATCCTCACCATCACACTGGAAGACTCCAG110Pz.24GGCATGAACCGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG110Pz.3GGCATGAACCGGAGGCCCATCCTCACCATCACACTGGAAGACTCCAG110Pz.4GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.6GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.8GG		Pz.2	GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC	60
Pz.4GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.5GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.6GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.7GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.8GTTGGCTCTGACTGTACCACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.9GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.10GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.11GTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.12GTTGGCTCTGACTGTACCACCATCCACCATCACACTGCAACTGCAGTGTACCAGTTCCTGCATGGGC 60Pz.11GTGGCTCTGACTGTACCACCATCCACCATCACACTGCAACTGCAGTGTCCTGCATGGGC 60Pz.12GTTGGCTCTGACTGTACCACCATCCACCATCATCACACTGGAAGACTCCGG 110Pz.13GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.24GGCATGAACCGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.5GGCATGAACCGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.7GGCATGAACCGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GCCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GCCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GCCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GCCATGAACCGGAGGCCCATCCTCACCATCATCACA		Pz.3	GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC	60
P2.5GTTGGCTCTGACCTGACCATCACACTACAACTACATGTGTAACAGTTCCTGCATGGGC60P2.6GTTGGCTCTGACTGTACCACCATCACACTACAACTACATGTGTAACAGTTCCTGCATGGGC60P2.7GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60P2.8GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60P2.9GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60P2.10GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60P2.11GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60P2.12GTTGGCTCTGACTGTACCACCATCCACCATCAACTACATGTGTAACAGTTCCTGCATGGGC60P2.12GTTGGCTCTGACTGTACCACCATCCACCATCAACTACATGTGTAACAGTTCCTGCATGGGC60P2.12GGCATGAACCGGAGGCCCATCCTCACCATCAACTACATGTGTAACAGTTCCTGCATGGGC60P2.11GGCATGAACCGGAGGCCCATCCTCACCATCAACTACACTGGAAGACTCCAG110P2.2GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110P2.3GGCATGAACCGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG110P2.4GGCATGAACCGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG110P2.5GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110P2.6GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110P2.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110P2.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110P2.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110P2.10GCCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110P2.12GCCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG <t< td=""><td></td><td>Pz.4</td><td>GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC</td><td>60</td></t<>		Pz.4	GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC	60
Pz.6GTTGGCTCTGACTGTACCACCACTCACACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.7GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.8GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.10GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.11GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC60Pz.12GTTGGCTCTGACTGTACCACCATCCACCATCACACTACATGTGTAACAGTTCCTGCATGGGC60Pz.11GTTGGCTCTGACTGTACCACCATCCACCATCACACTGGTAACAGTTCCTGCATGGGC60Pz.12GTTGGCTCTGACTGTACCACCATCCACCATCACACTGGTGTAACAGTTCCTGCATGGGC60Pz.12GTTGGCTCTGACCGCACCCATCCATCACACACTGGTGTAACAGTTCCTGCATGGGC60Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCCAG110Pz.2GGCATGAACCGGAGGCCCACCCTCCTCACCATCATCACACTGGAAGACTCCCAG110Pz.3GGCATGAACCGGAGGCCCACCCCTCCTCACCATCATCACACTGGAAGACTCCAG110Pz.4GGCATGAACCGGAGGCCCACCCTCCTCACCATCATCACACTGGAAGACTCCAG110Pz.5GGCATGAACCGGAGGCCCACCCTCCTCACCATCATCACACTGGAAGACTCCAG110Pz.6GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.11GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG		Pz.5	GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC	60
Pz.7GTTGGCTCTGACTGTACCACCATCCACTACAACTACAACTGTGTAACAGTTCCTGCATGGGC 60Pz.8GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.9GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.10GTTGGCTCTGACTGTACCACCATCCACCACACACACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.11GTTGGCTCTGACTGTACCACCATCCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.12GTTGGCTCTGACTGTACCACCATCCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.12GTTGGCTCTGACTGTACCACCATCCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.12GTTGGCTCTGACTGTACCACCATCCATCACCACTGGAAGACTCCCAG 110Pz.11GGCATGAACCGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCCAG 110Pz.2GGCATGAACCGGAGGCCCACCCTCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.3GGCATGAACCGGAGGCCCACCCTCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.4GGCATGAACCGGAGGCCCACCCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.5GGCATGAACCGGAGGCCCACCCTCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.6GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.11GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110		Pz.6	GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC	60
Pz.8GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.9GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.10GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.11GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.12GTTGGCTCTGACTGTACCACCATCCACCACCACACACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.12GTTGGCTCTGACTGTACCACCATCCACCACCACACACTGGAACAGTTCCTGCATGGGC 60Pz.12GGCATGAACTGGACGCCCATCCTCCACCACCACCACCATGGAAGACTCCAG 110Pz.2GGCATGAACTGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.3GGCATGAACCGGAGGCCCACCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.4GGCATGAACTGGAGGCCCACCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.5GGCATGAACTGGAGGCCCACCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.6GGCATGAACTGGAGGCCCACCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.7GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.8GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.11GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110		Pz.7	GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC	60
Pz.9GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.10GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.11GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.12GTTGGCTCTGACTGTACCACCATCCACCATCACATGTGTAACAGTTCCTGCATGGGC 60Pz.12GTGGCTCTGACTGTACCACCATCCACCATCACACTACATGTGTAACAGTTCCTGCATGGGC 60P53 ex.7GGCATGAACCGGAGGCCCATCCTCACCATCACACTGGAAGACTCCAG 110Pz.1GGCATGAACCGGAGGCCCATCCTCACCATCACACTGGAAGACTCCAG 110Pz.3GGCATGAACCGGAGGCCCACCCTCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.4GGCATGAACCGGAGGCCCACCCTCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.5GGCATGAACCGGAGGCCCACCCTCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.6GGCATGAACTGGAGGCCCACCCTCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.7GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.11GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110		Pz.8	GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC	60
Pz.10GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.11GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.12GTTGGCTCTGACTGTACCACCATCCACCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.12GTTGGCTCTGACTGTACCACCACCATCCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60P53 ex.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.1GGCATGAACTGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.2GGCATGAACCGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.3GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.4GGCATGAACTGGAGGCCCAGCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.5GGCATGAACTGGAGGCCCAGCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.7GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.8GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110		Pz.9	GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC	60
Pz.11GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60Pz.12GTTGGCTCTGACTGTACCACCATCCACCACTACAACTACATGTGTAACAGTTCCTGCATGGGC 60***********************************		Pz.10	GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC	60
Pz.12GTTGGCTCTGACTGTACCACCATCCACCATCCACACTACATGTGTAACAGTTCCTGCATGGGC 60248251P53 ex.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.1GGCATGAACTGGAGGCCCATCCTCACCATCATCACCACCAGGAAGACTCCAG 110Pz.3GGCATGAACCGGAGGCCCACCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.4GGCATGAACTGGAGGCCCACCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.5GGCATGAACCGGAGGCCCACCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.6GGCATGAACTGGAGGCCCACCCTCCACCATCATCACCACTGGAAGACTCCAG 110Pz.7GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.11GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110		Pz.11	GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC	60
248251P53 ex.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.1GGCATGAACTGGAGGCCCATCCTCACCATCACCACCAGGAAGACTCCAG110Pz.2GGCATGAACCGGAGGCCCACCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.3GGCATGAACCGGAGGCCCACCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.4GGCATGAACTGGAGGCCCACCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.5GGCATGAACCGGAGGCCCACCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.6GGCATGAACTGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.7GGCATGAACTGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.11GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110		Pz.12	GTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTTCCTGCATGGGC	60
248251P53 ex.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.1GGCATGAACTGGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG110Pz.2GGCATGAACCGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG110Pz.3GGCATGAACCGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG110Pz.4GGCATGAACTGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG110Pz.5GGCATGAACCGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG110Pz.6GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.7GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.11GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110			***************************************	
P53 ex.7GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.1GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.2GGCATGAACCGGAGGCCCAGCCTCACCATCATCACACTGGAAGACTCCAG110Pz.3GGCATGAACCGGAGGCCCAGCCTCACCATCATCACACTGGAAGACTCCAG110Pz.4GGCATGAACTGGAGGCCCAGCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.5GGCATGAACCGGAGGCCCAGCCTCACCATCATCACACTGGAAGACTCCAG110Pz.6GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.7GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.11GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110			248 251	
Pz.1GGCATGAAC TGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.2GGCATGAACCGGAGGCCCA CCTCACCATCATCACACTGGAAGACTCCAG110Pz.3GGCATGAACCGGAGGCCCA CCTCACCATCATCACACTGGAAGACTCCAG110Pz.4GGCATGAAC TGGAGGCCCA CCTCACCATCATCACACTGGAAGACTCCAG110Pz.5GGCATGAAC TGGAGGCCCA CCTCACCATCATCACACTGGAAGACTCCAG110Pz.6GGCATGAAC TGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.7GGCATGAAC TGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.11GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110		P53 ex.7	GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110	
Pz.2GGCATGAACCGGAGGCCCAGCCTCACCATCATCACACTGGAAGACTCCAG110Pz.3GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.4GGCATGAACTGGAGGCCCAGCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.5GGCATGAACTGGAGGCCCAGCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.6GGCATGAACTGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.7GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.11GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110		Pz.1	GGCATGAAC $m{r}$ GGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110	
Pz.3GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.4GGCATGAACTGGAGGCCCAGCCTCACCATCATCACACTGGAAGACTCCAG110Pz.5GGCATGAACCGGAGGCCCAGCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.6GGCATGAACTGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.7GGCATGAACTGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.11GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.12GGCATGAACCGGAGGCCCAGCCTCACCATCATCACACTGGAAGACTCCAG110		Pz.2	GGCATGAACCGGAGGCCCAGCCTCACCATCATCACACTGGAAGACTCCAG 110	
Pz.4GGCATGAACTGGAGGCCCAGCCTCACCATCATCACACTGGAAGACTCCAG110Pz.5GGCATGAACCGGAGGCCCAGCCTCACCATCATCACACTGGAAGACTCCAG110Pz.6GGCATGAACTGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.7GGCATGAACTGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.11GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.12GGCATGAACCGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG110		Pz.3	GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110	
Pz.5GGCATGAACCGGAGGCCCAGCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.6GGCATGAACTGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.7GGCATGAACTGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.11GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG110Pz.12GGCATGAACCGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG110		Pz.4	GGCATGAAC T GGAGGCCCA G CCTCACCATCATCACACTGGAAGACTCCAG 110	
Pz.6GGCATGAACTGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.7GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.11GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110		Pz.5	GGCATGAACCGGAGGCCCAGCCTCACCATCATCACACTGGAAGACTCCAG 110	
Pz.7GGCATGAAC GGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.11GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGAGGCCCACCTCCTCACCATCATCACACTGGAAGACTCCAG 110		Pz.6	$GGCATGAAC \mathbf{T}$ GGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110	
Pz.8GGCATGAACCGGAGGCCCATCCTCACCATCATCACCACTGGAAGACTCCAG 110Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.11GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGAGGCCCACCCTCACCATCATCACACTGGAAGACTCCAG 110		Pz.7	GGCATGAAC $\overline{\boldsymbol{r}}$ GGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110	
Pz.9GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.11GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGAGGCCCACCCATCATCACACTGGAAGACTCCAG 110		Pz.8	GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110	
Pz.10GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.11GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGAGGCCCACCCTCATCACCATCACACTGGAAGACTCCAG 110		Pz.9	GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110	
Pz.11GGCATGAACTGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110Pz.12GGCATGAACCGGGAGGCCCAGCCCACCATCATCACACTGGAAGACTCCAG 110		Pz.10	GGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110	
Pz.12 GGCATGAACCGGAGGCCCA G CCTCACCATCATCACACTGGAAGACTCCAG 110		Pz.11	GGCATGAAC T GGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAG 110	
		Pz.12	GGCATGAACCGGAGGCCCA G CCTCACCATCATCACACTGGAAGACTCCAG 110	
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Table II. An example of multiple sequence alignment for P53 gene (exon 8).

	191 273	
DE2		~~
P53 ex.8		60
PZ.1		60
PZ.Z	TGGTAATCTACTGGGACGGAACAGCTTTGAGGTG Z GTGTTTGTGCCTGTCCTGGGAGAGA	60
Pz.3	TGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCATGTTTGTGCCTGTCCTGGGAGAGA	60
Pz.4	TGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTTTGTGCCTGTCCTGGGAGAGA	60
Pz.5	TGGTAATCTACTGGGACGGAACAGCTTTGAGGTGC A TGTTTGTGCCTGTCCTGGGAGAGA	60
Pz.6	TGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTTTGTGCCTGTCCTGGGAGAGA	60
Pz.7	TGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTTTGTGCCTGTCCTGGGAGAGA	60
Pz.8	TGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTTTGTGCCTGTCCTGGGAGAGA	60
Pz.9	TGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTTTGTGCCTGTCCTGGGAGAGA	60
Pz.10	TGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTTTGTGCCTGTCCTGGGAGAGA ******************************	60
P53 ex.8	CCGGCGCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCC	120
Pz.1	CCGGCGCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCC	120
Pz.2	CCGGCGCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCC	120
Pz.3	CCGGCGCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCC	120
Pz.4	CCGGCGCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCC	120
Pz.5	CCGGCGCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCC	120
Pz.6	CCGGCGCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCC	120
Pz.7	CCGGCGCACAGAGGAAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCC	120
Pz.8	CCGGCGCACAGAGGAAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCC	120
Pz.9	CCGGCGCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCC	120
Pz.10	CCGGCGCACAGAGGAAGAATCTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCC	120

P53 ex.8	AGGGAGCACTAAGCGAG 137	
Pz.1	AGGGAGCACTAAGCGAG 137	
Pz.2	AGGGAGCACTAAGCGAG 137	
Pz.3	AGGGAGCACTAAGCGAG 137	
Pz.4	AGGGAGCACTAAGCGAG 137	
Pz.5	AGGGAGCACTAAGCGAG 137	
Pz.6	AGGGAGCACTAAGCGAG 137	
Pz.7	AGGGAGCACTAAGCGAG 137	
Pz.8	AGGGAGCACTAAGCGAG 137	
Pz.9	AGGGAGCACTAAGCGAG 137	
Pz.10	AGGGAGCACTAAGCGAG 137	
	* * * * * * * * * * * * * * *	

Table II. An example of multiple sequence alignment for P53 gene (exon 9).

191	
CACTGCCCAACAACACCAGCTCCTCTCCCCAGCCAAAGAAGAAACCACTGGATGGA	60

ATTTCACCCTTCAG 74	

	191 CACTGCCCAACAACACCAGCTCCTCTCCCCAGCCAAGAAGAAACCACTGGATGGA



Figure 1. Expression of C-MYC, BIRC5/survivin and CDC25 genes in all biopsies analyzed. Data were expressed as copies out of 10^5 cells. According to scientific production, results showed that expression for all genes was up-regulated.



ARCHETYPE BKV REGULATORY REGION (WW)

Figure 2. Schematic representation of BKV RR variants found in samples taken from two patients affected by Gleason 9 prostate cancer. These variants were characterized by enhancement of the oncogenic c-myc and the oncosuppressor p53 binding sites. RR of the proposed archetypal BK strain WW has been arbitrarily divided into five blocks containing the origin of DNA replication (O-block) and four blocks (P, Q, R and S) encompassing binding sequences of cellular transcription factors. These sequences are involved in transcriptional regulation of viral genes. In particular, in no permissive cells, RR undergoes a rearrangement process allowing for selection of viral variants with a transformation potential.

Discussion

PC is the most frequent cancer in men (1-4). Genetic background may contribute to PC risk. In fact, *MYC*, *BIRC5*/survivin, *CDC25* and *P53* genes are the ones most often implicated and investigated for the specific desease (5). Exposure to infectious agents has been reported to have a putative role in tumorigenesis. Among the infectious agents, several lines of evidence converge on the oncogenic human Polyomavirus BK (25, 26).

BKV's role in cancer is controversial (14, 26-28). Tumors of the urinary tract are the most logical target for an etiological association with BKV, since this urotheliotropic virus persists indefinitely as 'latent' in the kidney and urinary system (15).

This study investigated the presence of BKV sequences in urine, blood and fresh PC specimens from radical prostatectomies by means of a quantitative PCR assay.

The presence of detectable viral DNA assesses that persistent infection could contribute to cancer progression.

Although BKV DNA copy numbers obtained for each class of Gleason scores during this investigation was not statistically significant, it is conceivable to speculate that BKV could promote development of tumors cooperating with cellular proteins for a complete "neoplastic phenotype" and progression of metastatic disease. Moreover, it cannot be excluded that low levels of messengers detected could be translated in low copies of protein able to interfere with control of cell replication conferring the infected cells properties characteristics of cancer, such as loss of growth control. In fact, in no permissive cells, BKV activates the cellular DNA replication machinery and other genes involved in S-phase progression; however, virions are not produced. This aberrant stimulation could contribute to an oncogenic transformation. Moreover, TAg, preparing the cellular metabolism to support optimal viral replication, de-regulates the cell's control cycle pathways inducing cell proliferation. Nevertheless, it is clear that, in their natural hosts, tumor induction derives from a combination of circumstances and is not a part of the normal virus life cycle (15).

DNA sequencing analysis of p53 exons (5-9) was carried out to estimate its mutational rate and to understand if mutations might be correlated with viral infection and/or cancer progression. It is known that SNP not only abrogate p53 function but are commonly revealed in PC at a higher tumor stage, higher tumor grade, metastases or androgenindependent tumors (5, 20). In this study, the presence of different mutated exons was found in agreement to tumor advanced stage. In fact, according to the literature, it was found that codons 249 (exon 7) and 273 (exon 8) were more susceptible to mutation for all patients (5, 20). In particular, exon 7 was always mutated in patients with higher Gleason tumor score (Gleason 9). Regarding the possible link between P53 mutations and BKV infection, it is intriguing to hypothesize that, in heterozygote subjects for P53 gene mutations, the poor quantity of wild type protein could be sequestered from viral TAg promoting the development of a neoplastic phenotype. Therefore, BKV may play a role in the progression of PC rather than in its onset.

An interesting oncogenic role could be played by viral RR rearrangements. In this study, two interesting variants were found since they are characterized by an implementation of binding sites for oncosuppressor p53 and oncoprotein c-myc (Figure 2). In no permissive cells, binding sites for oncoproteins or tumor suppressors could be selected to promote cell proliferation; thus, rearrangements could enhance the transformation potential of the virus. For example, c-myc could be bound by virus to prevent its accumulation into the cell that, otherwise, would be eliminated through protective apoptosis. Alternatively, p53 could be sequestered either by TAg either by viral RR to block its role as the "guardian of the genome". In any case, RR rearrangements probably represent adaptive changes

conferring increased "viral fitness" in the host-cell environment.

Finally, C-MYC, BIRC5/survivin and CDC25 expression was investigated.

The finding increased *C-MYC* mRNA could be related to PC progression or metastatization, since in normal cells this messenger has a short half-life (about 30 min). Hence, understanding the role of c-Myc in PC is important to yield insights that might be of therapeutic importance.

Similarly to Myc, survivin can be regarded as an oncogene as its aberrant over-expression in most cancers contributes to a phenotype more resistant to apoptotic stimuli and chemotherapeutic therapies allowing continued proliferation and survival. P53's normal function is to regulate genes that control apoptosis. As survivin is a known inhibitor of apoptosis, it can be implied that P53-induced repression of survivin is one mechanism by which cells can undergo apoptosis upon induction by apoptotic stimuli or signals (29). When loss of wild-type *P53* occurs, survivin is over-expressed in the cells, thus contributing to cancer progression.

The Cdc25 phosphatases function as key regulators of the cell cycle during normal eukaryotic cell division and as mediators of the checkpoint response in cells Cdk/cyclin complexes. Their role in cancer onset is more complicated than that of a simple driver of cell proliferation. It is possible to hypothesize that Cdc25 over-expression in tumors is required to circumvent the checkpoints that would otherwise hinder cell proliferation.

In conclusion, data underline that specific target genes could be a molecular marker for early detection of certain cancers. In particular, understudying how their expression could be edited on changes of their regulators (*i.e.* the signal transducers and activators of transcription) it is of great importance for cancer drug discovery and therapies. About BKV, DNA presence does not exclude viral pressure on cell transformation. In particular the presence of RR variants allows hypothesizing that they could modify cell expression profile aiding immortalization. However, more studies are necessary to ascertain how to use expression of gene patterns for cancer therapy and how BKV could operate on PC susceptibility.

Conflicts of Interest

None declared.

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