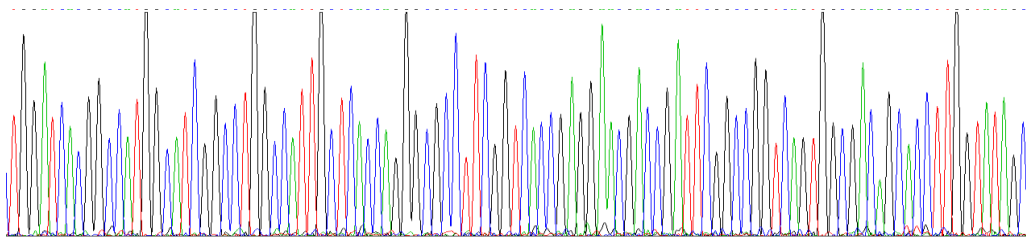


12 个外显子扩增实验进展报告

合同号: PG1-1601005

- 以 P53 基因对应扩增 5、6 号外显子引物对样品进行扩增, 得到的 PCR 产物将疑似目的片段的条带进行切胶回收后送测序, 测序峰图及比对结果如下:

a. 1 号样品

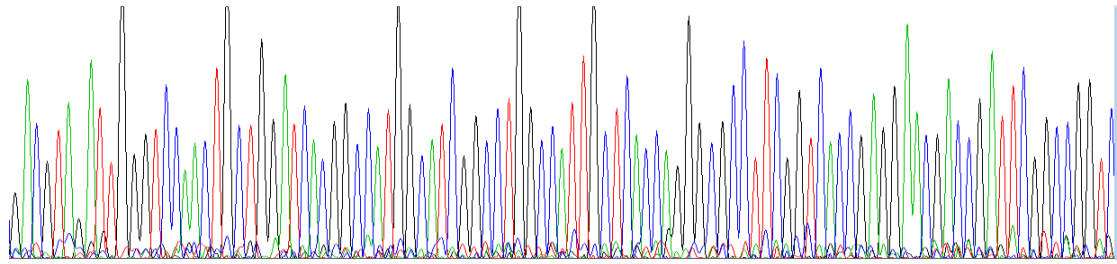


TTGACTGGCGGCGTTGTCGCCGACTTTCTGCAGGCGGCCGCTCTCCAGTTTCGCGACGCAGTTCCA
GCAGTTCCACCGAGGACTGGGTAACGTCGACGTAGATTGGGTCCAAGTCTGGATCACGGCCAT
GGCATCGGCCCTGGCCATTGCTCACCAGGGCGCCTTCGGTCAACGAGGAACGACCGATTTCGGCCG
GTCAGTGGCGACAGCACCTTGGTATAGCGCAGGTTGATCTGGGCGCTTTGCAGCGAGGCTTCCG
ACTGCAGGCGGTTGGCCACGGCAGTGTCTGATTCTTGGCGGCTTACGGCCTGTTCGTTGACCAGT
TGCTTGTAGCGATCAGAAATAGACTTGGTCGATTGCAGATTAGCCTCGGCACTCTTCAGCGTTGC
CTCATAGACCGATGGATCGATCTGATAAAGCTGCTGACCTTCCTTGACGTCTCCACCTTCTTTGA
ACAGACGCTTGAGAATGATCCCGTTTACCTGTGGGCGAACTTCGGCGATGCGGAACGCAGTGGT
CCGGCCCCGGGAGTTCGGAAGTCAAGGTAAAGGCTTGTGGTTGCAGGGTAACGACGCCGACCTG
AGGGGTAAGGGGGGGGATGGTA

Pseudomonas protegens CHA0, complete genome
Sequence ID: [gb|CP003190.1|](#) Length: 6867980 Number of Matches: 1

Range 1: 1527259 to 1527837		GenBank	Graphics	▼ Next Match	▲ Previous Match
Score	Expect	Identities	Gaps	Strand	
1031 bits(558)	0.0	572/579(99%)	0/579(0%)	Plus/Plus	
Query 6		TGGCGGCGTTGTCGCCGACTTTCTGCAGGCGGCCGCTCTCCAGTTTCGCGACGCAGTTCCA		65	
Sbjct 1527259		TGGCGGCGATTGTCGCCGACTTTCTGCAGGCGGCCGCTTCCAGTTTCGCGACGCAGTTCCA		1527318	
Query 66		GCAGTTCCACCGAGGACTGGGTAACGTCGACGTAGATTGGGTCCAAGTCTGGATCACGG		125	
Sbjct 1527319		GCAGTTCCACCGAGGACTGGGTAACGTCGACGTAGATTGGGTCCAAGTCTGGATCACGG		1527378	
Query 126		CCATGGCATCGGCCCTGGCCATTGCTCACCAGGGCGCCTTCGGTCAACGAGGAACGACCGA		185	
Sbjct 1527379		CCATGGCATCGGCCCTGGCCATTGCTCACCAGGGCGCCTTCGGTCAACGAGGAACGACCGA		1527438	
Query 186		TTGCGCCGCTCAGTGGCGACAGCACCTTGGTATAGCGCAGGTTGATCTGGGCGCTTTGCA		245	
Sbjct 1527439		TTGCGCCGCTCAGTGGCGACAGCACCTTGGTATAGCGCAGGTTGATCTGGGCGCTTTGCA		1527498	
Query 246		GCGAGGCTTCCGACTGCAGGCGGTTGGCCACGGCAGTGTCTGATTCTGGCGGCTTACGG		305	
Sbjct 1527499		GCGAGGCTTCCGACTGCAGGCGGTTGGCCACGGCAGTGTCTGATTCTGGCGGCTTACGG		1527558	
Query 306		CCTGTTCTTGACCAAGTTGCTTGTAGCGATCAGAAATAGACTTGGTCGATTGCAGATTAG		365	
Sbjct 1527559		CCTGTTCTTGACCAAGTTGCTTGTAGCGATCAGAAATAGACTTGGTCGATTGCAGATTAG		1527618	
Query 366		CCTCGGCACTCTTACGCTTGCCTCATAGACCGATGGATCGATCTGATAAAGCTGCTGAC		425	
Sbjct 1527619		CCTCGGCACTCTTACGCTTGCCTCATAGACCGATGGATCGATCTGATAAAGCTGCTGAC		1527678	
Query 426		CTTCCTTGACGTCTCCACCTTCTTTGAACAGACGCTTGAGAATGATCCCGTTTACCTGTG		485	
Sbjct 1527679		CTTCCTTGACGTCTCCACCTTCTTTGAACAGACGCTTGAGAATGATCCCGTTTACCTGTG		1527738	

b. 2 号样品



TGGAGTGCTGGCGGCGTTGTGCGCCGACTTCTGCAGGCGGCCGCTCTCCAGTTTCGCGACGCAGTTCCA
GCAGTTCCACCGAGGACTGGGTAACGTCGACGTAGATTGGGTCCAACCTGCTGGATCACGGCCATGGC
ATCGGCCTGGCCATTGCTCACCAGGGCGCCTTCGGTCAACGAGGAACGACCGATTTCGGCCGGTCACT
GGCGACAGCACCTTGGTATAGCGCAGGTTGATCTGGGCGCTTTGCAGCGAGGCTTCCGACTGCAGGC
GGTTGGCCACGGCAGTGTCGTATTCCTGGCGGCTTACGGCCTGTTTCGTTGACCAGTTGCTTGTAGCGA
TCAGAAATAGACTTGGTCGATTGCAGATTAGCCTCGGCACTCTTCAGCGTTGCCTCATAGACCGATG
GATCGATCTGATAAAGCTGCTGACCTTCCTTGACGTCTCCACCTTCTTTGAACAGACGCTTGAGAATG
ATCCCGTTTACCTGTGGGCGAACTTCGGCGATGCGGAACGCAGTGGTCCGGCCCCGGGAGTTCGGAAG
TCAAGGTAAAGGCTTTGTGGTTGCAGGTAACGACGCCGACCTGAGGGGAAGG

Pseudomonas protegens CHA0, complete genome

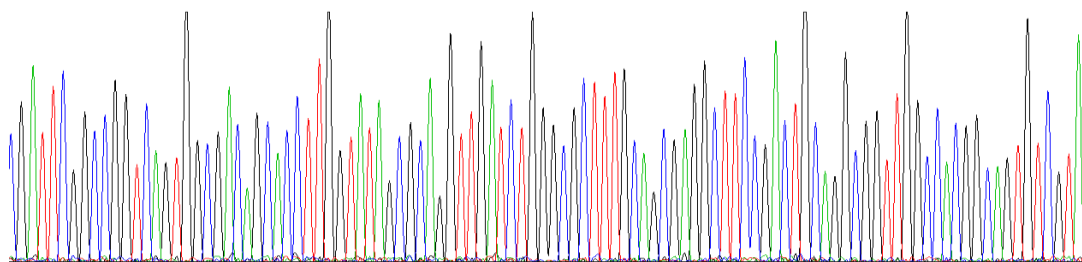
Sequence ID: [gb|CP003190.1](#) Length: 6867980 Number of Matches: 1

Range 1: 1527259 to 1527837 [GenBank](#) [Graphics](#)

▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
1026 bits(555)	0.0	572/580(99%)	1/580(0%)	Plus/Plus
Query 9	TGGCGGCGTTGTGCGCCGACTTCTGCAGGCGGCCGCTCTCCAGTTTCGCGACGCAGTTCCA	68		
Sbjct 1527259	TGGCGGCAATTGTGCGCCGACTTCTGCAGGCGGCCGCTTCCAGTTTCGCGACGCAGTTCCA	1527318		
Query 69	GCAGTTCCACCGAGGACTGGGTAACGTCGACGTAGATTGGGTCCAACCTGCTGGATCACGG	128		
Sbjct 1527319	GCAGTTCCACCGAGGACTGGGTAACGTCGACGTAGATTGGGTCCAACCTGCTGGATCACGG	1527378		
Query 129	CCATGGGATCGGCTTGGCCATTGCTCACCAGGGCGCCTTCGGTCACCGAGGAACGACCGA	188		
Sbjct 1527379	CCATGGGATCGGCTTGGCCATTGCTCACCAGGGCGCCTTCGGTCACCGAGGAACGACCGA	1527438		
Query 189	TTCGGCCGGTCAGTGGCGACAGCACCTTGGTATAGCGCAGGTTGATCTGGGCGCTTTGCA	248		
Sbjct 1527439	TTCGGCCGGTCAGTGGCGACAGCACCTTGGTGTAGCGCAGGTTGATCTGGGCGCTTTGCA	1527498		
Query 249	GCGAGGCTTCCGACTGCAGGCGGTTGGCCACGGCAGTGTCTATTCTTGGCGGCTTACGG	308		
Sbjct 1527499	GCGAGGCTTCCGACTGCAGGCGGTTGGCCACGGCAGTGTCTATTCTTGGCGGCTTACGG	1527558		
Query 309	CCTGTTCGTTGACCAAGTTGCTTGTAGCGATCAGAAATAGACTTGGTCGATTGCAGATTAG	368		
Sbjct 1527559	CCTGTTCGTTGACCAAGTTGCTTGTAGCGATCAGAAATAGACTTGGTCGATTGCAGATTAG	1527618		
Query 369	CCTCGGCACCTTCAGCGTTGCCTCATAGACCGATGGATCGATCTGATAAAGCTGCTGAC	428		
Sbjct 1527619	CCTCGGCACCTTCAGCGTTGCCTCATAGACCGATGGATCGATCTGATAAAGCTGCTGAC	1527678		
Query 429	CTTCCTTGACGTCTCCACCTTCTTTGAACAGACGCTTGAGAATGATCCGTTTACCTGTG	488		
Sbjct 1527679	CTTCCTTGACGTCTCCACCTTCTTTGAACAGACGCTTGAGAATGATCCGTTTACCTGTG	1527738		

c. 3号样品



CGGGGTGGCTGGGCGGCGTTGTGCGCCGACTTCTGCAGGCGGCCGCTCTCCAGTTTCGCGACGCAGTT
CCAGCAGTTCCACCGAGGACTGGGTAACGTCGACGTAGATTGGGTCCAACCTGCTGGATCACGGCCAT
GGCATCGGCCTGGCCATTGCTCACCAGGGCGCCTTCGGTCAACGAGGAACGACCGATTTCGGCCGGTC
AGTGGCGACAGCACCTTGGTATAGCGCAGGTTGATCTGGGCGCTTTGCAGCGAGGCTTCCGACTGCA
GGCGGTTGGCCACGGCAGTGTCGTATTCCTGGCGGCTTACGGCCTGTTTCGTTGACCAGTTGCTTGTAG
CGATCAGAAATAGACTTGGTCGATTGCAGATTAGCCTCGGCACTCTTCAGCGTTGCCTCATAGACCG
ATGGATCGATCTGATAAAGCTGCTGACCTTCCTTGACGTCTCCACCTTCTTTGAACAGACGCTTGAGA

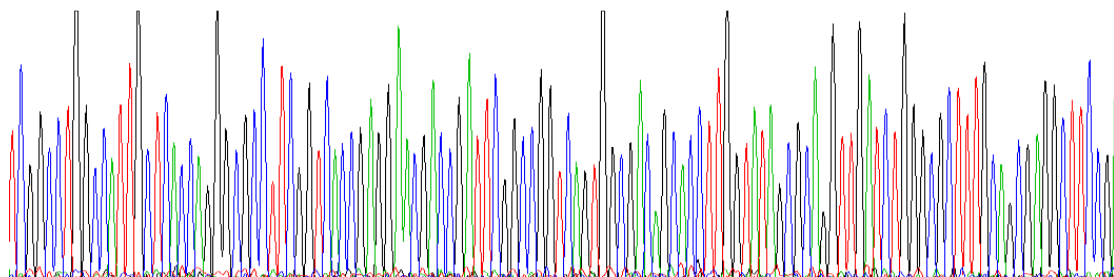


ATGATCCCGTTTACCTGTGGGCGAACTTCGGCGATGCGGAACGCAGTGGTCCGGCCCCGGGAGTTCCG
AAGTCAAGGTAAAGGCTTGTGGTTGCAGGGTAACGACGCCGACCTGAGGGGTTAAGGGGTGGTTGT
A

Pseudomonas protegens CHA0, complete genome
Sequence ID: [gb|CP003190.1](#) Length: 6867980 Number of Matches: 1

Range 1: 1527260 to 1527846					▼ Next Match	▲ Previous Match
Score	Expect	Identities	Gaps	Strand		
1033 bits(559)	0.0	578/587(98%)	1/587(0%)	Plus/Plus		
Query 12	GGCGGCGTTGTCGCCGACTTTCTGCAGGCGGCCGCTCTCCAGTTCGCGACGCAGTTCCAG	71				
Sbjct 1527260	GGCGGCGATTGTCGCCGACTTTCTGCAGGCGGCCGCTTCCAGTTCGCGACGCAGTTCCAG	1527319				
Query 72	CAGTTCCACCGAGGACTGGGTAACGTCGACGTAGATTGGGTCCAACCTGCTGGATCACGGC	131				
Sbjct 1527320	CAGTTCCACCGAGGACTGGGTAACGTCGACGTAGATTGGGTCCAACCTGCTGGATCACGGC	1527379				
Query 132	CATGGCATCGGCGCTGGCCATTGCTCACCAGGGCGCCTTCGGTCACCGAGGAACGACCGAT	191				
Sbjct 1527380	CATGGCATCGGCGCTGGCCATTGCTCACCAGGGCGCCTTCGGTCACCGAGGAACGACCGAT	1527439				
Query 192	TCGGCCGGTCAGTGGCGACAGCACCTTGGTATAGCGCAGGTTGATCTGGGCGCTTTGCAG	251				
Sbjct 1527440	TCGGCCGGTCAGTGGCGACAGCACCTTGGTATAGCGCAGGTTGATCTGGGCGCTTTGCAG	1527499				
Query 252	CGAGGCTTCCGACTGCAGGCGGTTGGCCACGGCAGTGTCGATTCCTGGCGGCTTACGGC	311				
Sbjct 1527500	CGAGGCTTCCGACTGCAGGCGGTTGGCCACGGCAGTGTCGATTCCTGGCGGCTTACGGC	1527559				
Query 312	CTGTTCTGTTGACCAAGTTCCTGAGCGATCAGAAATAGACTTGGTCGATTGCAGATTAGC	371				
Sbjct 1527560	CTGTTCTGTTGACCAAGTTCCTGAGCGATCAGAAATAGACTTGGTCGATTGCAGATTAGC	1527619				
Query 372	CTCGGCACCTCTTCAGCGTTGCCTCATAGACCGATGGATCGATCTGATAAAGCTGCTGACC	431				
Sbjct 1527620	CTCGGCACCTCTTCAGCGTTGCCTCATAGACCGATGGATCGATCTGATAAAGCTGCTGACC	1527679				
Query 432	TTCTTTGACGTCTCCACCTTCTTTGAACAGACGCTTGAGAATGATCCCGTTTACCTGTGG	491				
Sbjct 1527680	TTCTTTGACGTCTCCACCTTCTTTGAACAGACGCTTGAGAATGATCCCGTTTACCTGTGG	1527739				

d. 4 号样品



CTTGAATGGGCGGCGCTCGCCGACTTTCTGCAGGCGGCCGCTCTCCAGTTCGCGACGCAGTTCCAG
CAGTTCCACCGAGGACTGGGTAACGTCGACGTAGATTGGGTCCAACCTGCTGGATCACGGCCATGGCA
TCGGCCTGGCCATTGCTCACCAGGGCGCCTTCGGTCACCGAGGAACGACCGATTCCGGCCGGTCACTG
GCGACAGCACCTTGGTATAGCGCAGGTTGATCTGGGCGCTTTGCAGCGAGGCTTCCGACTGCAGGCG
GTTGGCCACGGCAGTGTCTGATTCCTGGCGGCTTACGGCCTGTTCTGTTGACCAAGTTCGTTGTAGCGAT
CAGAAATAGACTTGGTCGATTGCAGATTAGCCTCGGCACTCTTCAGCGTTGCCTCATAGACCGATGG
ATCGATCTGATAAAGCTGCTGACCTTCCTTGACGTCTCCACCTTCTTTGAACAGACGCTTGAGAATGA
TCCCGTTTACCTGTGGGCGAACTTCGGCGATGCGGAACGCAGTGGTCCGGCCCCGGGAGTTCCGAAAGT
CAAGGTAAAGGCTTGTGGTTGCAGGGTAACGACGCCGACCTGAGGGGCTAGGGGGGGGATGTACCG
CCTCTTCCTTTTACATCCGCTGAGCAGCGATGCCAGGGCGACGGCAGTGACCAGAGCGGTAACAGC
TGGCTTGAGTTGCATGAAAATCCTCGGGTCAGGCACGCAAGATGCGCACAAGAAAAGTGGAAGGGT
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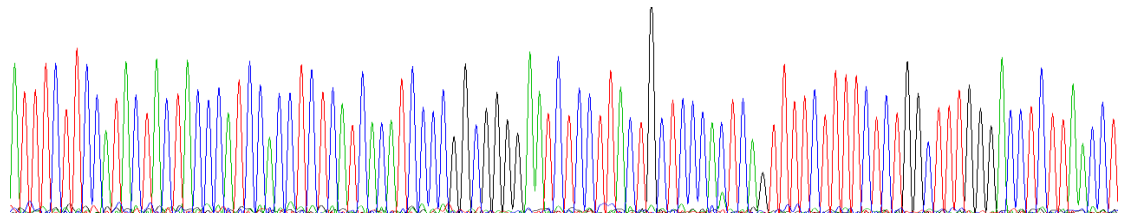
Pseudomonas protegens CHA0, complete genome
Sequence ID: [gb|CP003190.1](#) Length: 6867980 Number of Matches: 1

Range 1: 1527269 to 1528005					▼ Next Match	▲ Previous Match
Score	Expect	Identities	Gaps	Strand		
1295 bits(701)	0.0	726/738(98%)	1/738(0%)	Plus/Plus		
Query 17	GTCGCCGACTTTCTGCAGGCGGCCGCTCTCCAGTTCGCGACGCAGTTCCAGCAGTTCCAC	78				
Sbjct 1527269	GTCGCCGACTTTCTGCAGGCGGCCGCTTCCAGTTCGCGACGCAGTTCCAGCAGTTCCAC	1527328				
Query 77	CGAGGACTGGGTAACGTCGACGTAGATTGGGTCCAACCTGCTGGATCACGGCCATGGCATC	136				
Sbjct 1527329	CGAGGACTGGGTAACGTCGACGTAGATTGGGTCCAACCTGCTGGATCACGGCCATGGCATC	1527388				
Query 137	GGCCTGGCCATTGCTCACCAGGGCGCCTTCGGTCACCGAGGAACGACCGATTCCGGCCGGT	196				
Sbjct 1527389	GGCCTGGCCATTGCTCACCAGGGCGCCTTCGGTCACCGAGGAACGACCGATTCCGGCCGGT	1527448				
Query 197	CAGTGGCGACAGCACCTTGGTATAGCGCAGGTTGATCTGGGCGCTTTGCAGCGAGGCTTC	256				
Sbjct 1527449	CAGTGGCGACAGCACCTTGGTATAGCGCAGGTTGATCTGGGCGCTTTGCAGCGAGGCTTC	1527508				
Query 257	CGACTGCAGCGGTTGGCCACGGCAGTGTCTGATTCCTGGCGGCTTACGGCCTGTTCTGTT	316				
Sbjct 1527509	CGACTGCAGCGGTTGGCCACGGCAGTGTCTGATTCCTGGCGGCTTACGGCCTGTTCTGTT	1527568				
Query 317	GACCAAGTTCCTGAGCGATCAGAAATAGACTTGGTCGATTGCAGATTAGCCTCGGCACT	376				
Sbjct 1527569	GACCAAGTTCCTGAGCGATCAGAAATAGACTTGGTCGATTGCAGATTAGCCTCGGCACT	1527628				
Query 377	CTTCAGCGTTGCCTCATAGACCGATGGATCGATCTGATAAAGCTGCTGACCTTCCTTGAC	436				

剩余 5 号、6 号及 7 号样品结果依次进行比对，发现比对结果都与 *Pseudomonas protegens*（假单胞菌属）的基因序列比对上，而没有与 P53 基因比对上。说明样品 DNA 不纯，含有假单胞菌基因组 DNA 污染。

2. 以 P53 基因对应的扩增 7、8 号外显子的引物进行扩增，将疑似目的片段的条带进行切胶回收送测序，该引物只回收了 4 号样品进行测序，测序结果如下：

a. 7 号样品



CCTTGTTGCTTGATGTACACCATCCACTACAACCTACATGTGTAAACAGTTCCTGCATGGGCGGCAT
GAACCGGAGGCCCCATCCTCACCATCATCACACTGGAAGACTCCAGGTCAGGAGCCACTTGCCAC
CCTGCACACTGGCCTGCTGTGCCCCAGCCTCTGCTTGCCTCTGACCCCTGGGCCCACCTCTTACC
GATTTCTTCCATACTACTACCCATCCACCTCTCATCACATCCCCGGCGGGGAATCTCCTTACTGCT
CCCACTCAGTTTTCTTTCTCTGGCTTTGGGACCTCTTAACCTGTGGCTTCTCTCCACCTACCTG
GAGCTGGAGCTTAGGCTCCAGAAAGGACAAGGGTGGTTGGGAGTAGATGGAGCCTGGTTTTTA
AATGGGACAGGTAGGACCTGATTTCTTACTGCCTCTTGCTTCTCTTTTCTATCTGAGTAGTGG
TAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTGTTGTGCCTGTCCTGGGAGAGACCGGCGC
ACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTCACACGAGCTGCCCCAGGGAGCACT
AAGCGAGGTAAGCAAGCAGGACAAG

Homo sapiens isolate C159 tumor suppressor p53 (TP53) gene, exons 7 through 9 and partial cds
Sequence ID: [gb|JQ751552.1](#) Length: 791 Number of Matches: 1

Range 1: 168 to 775		GenBank	Graphics	▼ Next Match	▲ Previous Match
Score	Expect	Identities		Gaps	Strand
1098 bits(594)	0.0	604/608(99%)		4/608(0%)	Plus/Minus
Query 5		GTT-GCT-TGA-TGTA-CACCATCCACTACAACCTACATGTGTAAACAGTTCCTGCATGGGC		60	
Sbjct 775		GTGGCTCTGACTGTACCACCATCCACTACAACCTACATGTGTAAACAGTTCCTGCATGGGC		716	
Query 61		GGCATGAACCGGAGGCCCCATCCTCACCATCATCACACTGGAAGACTCCAGGTCAGGAGCC		120	
Sbjct 715		GGCATGAACCGGAGGCCCCATCCTCACCATCATCACACTGGAAGACTCCAGGTCAGGAGCC		656	
Query 121		ACTTGCCACCCCTGCACACTGGCCTGCTGTGCCCCAGCCTCTGCTTGCCTCTGACCCCTGG		180	
Sbjct 655		ACTTGCCACCCCTGCACACTGGCCTGCTGTGCCCCAGCCTCTGCTTGCCTCTGACCCCTGG		596	
Query 181		GCCCACCTCTTACCGATTCTTCCATACTACTACCCATCCACCTCTCATCACATCCCCGG		240	
Sbjct 595		GCCCACCTCTTACCGATTCTTCCATACTACTACCCATCCACCTCTCATCACATCCCCGG		536	
Query 241		CGGGGAATCTCCTTACTGCTCCCACTCAGTTTCTTTCTCTGGCTTTGGGACCTCTTAA		300	
Sbjct 535		CGGGGAATCTCCTTACTGCTCCCACTCAGTTTCTTTCTCTGGCTTTGGGACCTCTTAA		476	
Query 301		CCTGTGGCTTCTCCTCCACCTACCTGGAGCTGGAGCTTAGGCTCCAGAAAGGACAAGGGT		360	

以 P53 基因扩增 7、8 外显子的引物对 7 号样品进行扩增，比对结果正确，确认为 P53 基因，将其 7、8 号外显子序列截取之后，片段序列结果如下：

a. 7 号外显子序列

TTGTTGCTTGATGTACACCATCCACTACAACCTACATGTGTAAACAGTTCCTGCA
TGGGCGGCATGAACCGGAGGCCCCATCCTCACCATCATCACACTGGAAGACTC



CAG

b. 8 号外显子序列

TGGTAATCTACTGGGACGGAACAGCTTTGAGGTGCGTGTTTGTGCCTGTCCTG
GGAGAGACCGGCGCACAGAGGAAGAGAATCTCCGCAAGAAAGGGGAGCCTC
ACCACGAGCTGCCCCCAGGGAGCACTAAGCGAG