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Mutations in Exon 4 of ESR1 Gene in Iraqi Women with Breast Cancer

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Abstract
This study was aimed to determine the mutations and single nucleotide polymorphisms (SNPs) in exon 4 in women with breast cancer from Iraq. Different samples (blood, fresh tissue with blood from same patient, and formalin fixed paraffin embedded, FFPE). Molecular analysis of exon 4 has been studied by using PCR. It was found that exon 4 appeared as a single band with size 370. Single nucleotide polymorphisms (SNPs) were determined in exon 4 *ESR1* using DNA sequence. Then, nucleotide sequences of this exon were aligned with control group (healthy women) and with NCBI. It was detected five polymorphisms (AAA, TTT, AAA, CCG, AAA, and AAC) in exon 4 of *ESR1;* all of them were novel SNPs, all types of polymorphism in exon 4 of *ESR1*were substitution.
Keywords: SNPs in *ESR1*, *ESR1* gene mutations, Breast cancer mutations

1. Introduction

Breast cancer is the most common cause of cancer death and the most common form of cancer in women with a 9% incidence of being diagnosed during a lifetime (1). There was increasing in the incidence rates of breast cancer within the last two decades, which became one of the major threats to Iraqi female health. The estrogen receptor (ER) plays an important role in the pathogenesis and maintenance of breast cancer, it is a ligand-inducible transcription factor which regulates the expression of a variety of genes including some growth factors. The cellular signaling of estrogens mediated through two estrogen receptors, estrogen receptor -alpha (*ESR1*) and estrogen receptor- beta (*ESR*2), both belonging to the nuclear receptor (NR) family of transcription factors (2). The *ESR1* gene is located on chromosome 6q25-27, consists of eight exons and spans more than 140 kb (3). The expression of *ESR1* was studied as a predictive marker of treatment response, it status in breast tumors provided prognostic information and the primary target for endocrine therapy (4). Investigation of the molecular mechanisms of carcinogenesis and development of human breast cancer, the regulation of *ESR1* gene expression was an important issue in breast cancer, and the over expression of ESR1 was an initial significant event in its genesis (5).

2. Materials and Methods

Different samples (blood, frozen tissue and blood from the same patients, and formalin fixed paraffin embedded) were collected from 50 women with breast cancer, with mean age 55.00 ± 10 years, 24 samples recorded with estrogen receptor positive used in this study for detection of mutations in ESR2.Besides, 10 samples of blood from healthy women with median age 45 years as control. The DNA was extracted from blood samples using the Reliaprep blood genomic DNA MiniPrep system from Promega, USA, fresh tissue using Maxwell® 16 Tissue DNA Purification Kit from Promega, USA, and formalin fixed paraffin embedded (FFPE) samples using ReliaPrep™ FFPE genomic DNA Miniprep from promega, USA. The extracted DNA from each sample used as a template for 20µl PCR reactions, and using 10µl Go Taq® Green PCR Master Mix fron Promega, USA, 1µl of 10µm from forward primer: ACCTGTGTTTTCAGGGATACGA and reverse primer: GCTGCGCTTCGCATTCTTAC for exon 4 of ESR alpha (6), and 3µl of DNA template. The mixture volume was completed to 20µl by adding free-nuclease water. PCR process was conducted through 30 cycles with the following steps: denaturation for 30 sec at 95°C, annealing for 30 sec at 57°C and elongation for 40 seconds at 72°C.In order to analyze the nucleotides sequences for all samples, DNA sequencing was performed at the national instrumentation center or environmental management (NICEM), using the ABI prism 3100 xl genetic analyzer from Applied from Biosystems, USA.

3. Result and discussion

- **Amplification of exons in estrogen receptor alpha (*ESR1*) and beta (*ESR2*) genes**

The exon 4 in estrogen receptor alpha (*ESR1*) gene was detected by using PCR and appeared as a band size with 370 bp (Fig. 1).

- **Polymorphisms of exon 4 in *ESR1***

In exon 4 of *ESR1* gene, five polymorphisms (AAA, TTT, AAA, AAA, and AAC) were detected. The type of polymorphisms, position and their effects on gene expression (Table 1). All mutations in exon 4 of *ESR1* were

substitution polymorphisms that converted one base to another and then caused either no changing in the produced protein and this called silent polymorphism (sense mutation), or caused an exchange in the produced protein and this called missense mutation.

- **Sequences profile and alignment of each polymorphism in exon 4 of *ESR1***

1- AAA

The sequencing result displayed the presence of SNP G→ A (Table 1). The identified SNP was missense mutation, it was substitution polymorphism. The common codon AGA was converted to AAA. This point mutation caused alteration in gene expression because of alteration in amino acid; the Arginine was converted to Lysine. This polymorphism found in one (4.1%) sample of FFBE. Then an alignment of nucleotides sequencing of exon 4 in *ESR1* for women with breast cancer compared with control in NCBI center using automated sequencer and analyzed by BLAST data (Fig. 2).

2- TTT

The sequencing result illustrated the presence of SNP C → T (Table 1). The identified SNP was missense mutation, it was substitution polymorphism. The common codon TCT was converted to TTT. This point mutation caused alteration in gene expression because of revision in amino acid; the Aspartic acid was converted to Asparagine. This polymorphism found in 3 (13%) samples of blood. An alignment of nucleotides sequencing of exon 4 in *ESR1* for women with breast cancer was done and compared with control in NCBI center using automated sequencer and analyzed by BLAST data (Fig. 3).

3- AAA

The sequencing result exposed the presence of SNP G → A (Table 1). The identified SNP was a silent polymorphism (sense mutation), it was substitution polymorphism. The common codon AAG was converted to AAA. This point mutation had no effect on gene expression in which the changing codon still encoded the same amino acid, Lysine. This polymorphism found in 1 (4.1%) sample from frozen tissue and it appeared in the blood of the same patient. Then nucleotides sequencing of exon 4 in *ESR1* for women with breast cancer were aligned and compared with control in NCBI center using automated sequencer and analyzed by BLAST data (Fig. 4).

4- AAA

The sequencing result revealed the presence of SNP G →A (Table 1). The identified SNP was a missense mutation, it was substitution polymorphism. The common codon AGA was converted to AAA. This point mutation altered the gene expression because of changing in amino acid has happened; the Arginine was converted to Lysine. This polymorphism found in 6 (25%) samples; 5 samples from blood and only one samples from frozen tissue and also appeared in the blood sample of the same patient. The alignment of nucleotides sequencing of exon4 in *ESR1* for women with breast cancer was done and compared with control in NCBI center using automated sequencer and analyzed by BLAST data (Fig. 5).

5- AAC

The sequencing result revealed the presence of SNP G → A (Table 1). The identified SNP was missense mutation, it was substitution polymorphism. The common codon GAC was converted to AAG. This point mutation caused alteration in gene expression because of changing in amino acid; the Aspartic acid was converted Asparagine. This polymorphism found in 6 (25%) samples, 5 samples from blood and one sample from frozen tissue as well it appeared in the blood sample from the same patient. Then an alignment of nucleotides sequencing of exon 4 in *ESR1* for women with breast cancer compared with control in NCBI center using automated sequencer and analyzed by BLAST data (Fig. 6).

The mutation and polymorphism of cancer-associated *ESR1* gene found to predict tumor formation and prognosis (7). *ERS1* was representing a surrogate marker for predicting breast cancer developing later in life (8). Several functionally important intronic and exonic loci of *ESR1* gene polymorphisms that are associated with breast cancer have been examined (9). As known, estrogen receptor (ER) activation participated in development and progression of breast cancer because of alteration in pathways of *ESR1* occurred during development of breast cancer and that associated with breast cancer risk and investigation (10). The function of ER was as a hormone dependent transcriptional regulator that plays significant role in breast cancer development (11, 12). Identification of a novel acquired mutation of *ESR1* gene in women with metastatic breast cancer may lead to develop resistance to endocrine treatment. The mutations cause a conformational change, which mimics the conformation of activated ligand-bound receptor that lead to change the ligand-independent activity then result in resistance to endocrine treatment (13).The relationship between *ESR1* mutations and resistance to endocrine therapy remains to be investigated, however, there was a significant upregulation of estrogen receptor responsive genes in *ESR1* mutations tumors, suggesting that estrogen receptor signaling was active and may play a role in conferring endocrine therapy resistance (14). The mutations in *ESR1* may prompt a clinician to change the treatment regimen from an aromatase inhibitor to an anti-estrogen, so women who developed resistance to aromatase inhibitors often responded to anti-estrogen therapy (15). Moreover, the SNPs that determined in this study may effect copy number of *ESR1* gene and may cause resistant to treatment because amplification was an abnormal status and normal ER protein expression (ER +ve) was requisite for response to treatment (16, 17).

The somatic mutation may increase sensitivity to estrogen and this may lead to increasing of proliferation at subphysiological level of estrogen and stimulated binding to transcription factor2 at low level of hormone (18). While other studies (19. 20) were showed no association between *ESR1* polymorphism and breast cancer. This may due to the small size of samples or chose only little SNPs.

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Notes

Table (1): Polymorphisms in exon 4 of *ESR1* gene in women with breast cancer

No.	Mutation	Type	Position	Wild type codon	Mutated codon	Chang of amino acid	Effect on translation	Kind of mutation	No. of patie-nts
1	G → A	Substitution	258777	AGA	AAA	R → K	Missense mutation	Point mutation	1
2	C → T	Substitution	258762	TCT	TTT	D → N	Missense mutation	Point mutation	3
3	G → A	Substitution	258826	AAG	AAA	K → K	Sense mutation	Point mutation	1
4	G → A	Substitution	258920	AGA	AAA	R → K	Missense mutation	Point mutation	6
5	G → A	Substitution	258971	GAC	AAC	D → N	Missense mutation	Point mutation	6

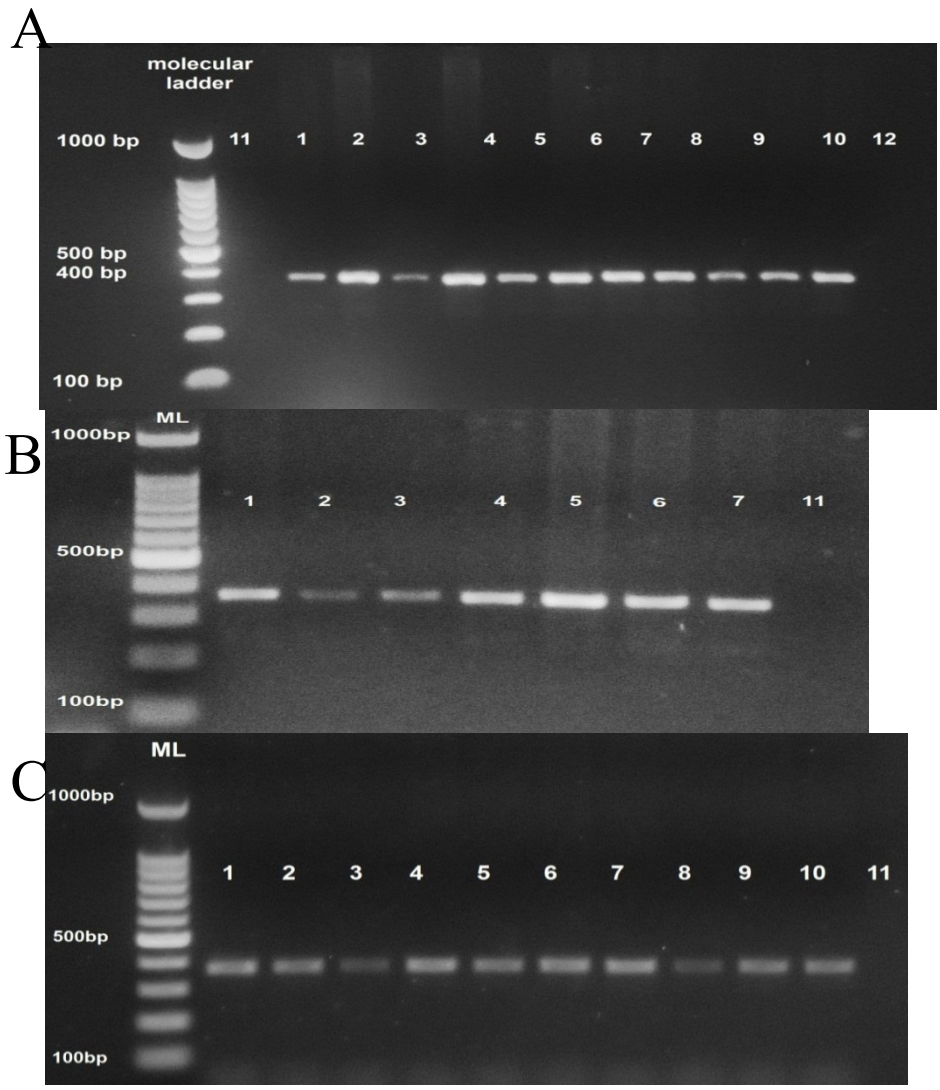

Figure (1): Amplification of exon 4 in estrogen receptor alpha set 1 primer with 370 bp. A: blood samples; Lanes 1-10 represent DNA from women with breast cancer, lane 11 represents DNA of negative control, lane 12 represents DNA from healthy subjects. B: frozen tissue samples; Lanes 1-7 represent samples from women with breast cancer, lane 11 represents DNA in negative control. C. FFPE samples; Lanes 1-10 represent DNA from women with breast cancer, lane 11 represents control negative. Agarose 1.5%, 5V/cm for 45 min, ML: molecular ladder

ref|NG_008493.2| Homo sapiens estrogen receptor 1 (*ESR1*), RefSeqGene on chromosome6
Length:419779

Score	Expect	Identities	Gaps	Strand
582 bits(315)	1e-162	323/325(99%)	2/325(0%)	Plus/Plus

```
Query  15      ATGTTGAAACACAAGCGCCAGAGAGATGATGGGAAGGGCAGGGGTGAAGTGGGGTCTGCT  73
               ||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||
Sbjct  258707  ATGTTGAAACACAAGCGCCAGAGAGATGATGGGGAGGGCAGGGGTGAAGTGGGGTCTGCT  258766

Query  74      GGAGACATGAAAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACGCTCTAAGAAG  133
               |||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258767  GGAGACATGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACGCTCTAAGAAG  258826

Query  134     AACAGCCTGGCCTTGTCCCTGACGGCCGACCAGATGGTCAGTGCCTTGTTGGATGCTGAG  193
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258827  AACAGCCTGGCCTTGTCCCTGACGGCCGACCAGATGGTCAGTGCCTTGTTGGATGCTGAG  258886

Query  194     CCCCCCATACTCTATTCCGAGTATGATCCTACCAGACCCTTCAGTGAAGCTTCGATGATG  253
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258887  CCCCCCATACTCTATTCCGAGTATGATCCTACCAGACCCTTCAGTGAAGCTTCGATGATG  258946

Query  254     GGCTTACTGACCAACCTGGCAGACAGGGAGCTGGTTCACATGATCAACTGGGCGAAGAGG  313
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258947  GGCTTACTGACCAACCTGGCAGACAGGGAGCTGGTTCACATGATCAACTGGGCGAAGAGG  259006

Query  314     GTGCCAGGTAAGAATGCGAAGCGCA  338
               |||||||||||||||||||||||||
Sbjct  259007  GTGCCAGGTAAGAATGCGAAGCGCA  259031
```

Figure (2): Alignment of exon 4 in *ESR1* gene sequence of women with breast cancer using automated sequencer was analyzed by BLAST data, query number represents the current results while the subject represents the reference sequence. Blue color represents exon region, black color represented intron region while the single nucleotide polymorphism represents red color

Homo sapiens estrogen receptor 1 (*ESR1*), RefSeqGene on chromosome 6
Sequence ID: ref|008493.2|Length: 419779Number of Matches: 1
Related Information
Map Viewer-aligned genomic context
Range 1: 258665 to 258986GenBankGraphics Next Match Previous Match First Match
Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
582bits(315)	1e-162	321/322(99%)	1/322(0%)	Plus/Plus

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Query  4       CCTGTGTTTTCAGGGATACGAAAAGACCGAAGAGGAGGGAGAATGTTGAAACACAAGCGC  63
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258665  CCTGTGTTTTCAGGGATACGAAAAGACCGAAGAGGAGGGAGAATGTTGAAACACAAGCGC  258724

Query  64      CAGAGAGATGATGGGGAGGGCAGGGGTGAAGTGGGGTTTGCTGGAGACATGAGAGCTGCC  123
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258725  CAGAGAGATGATGGGGAGGGCAGGGGTGAAGTGGGGTCTGCTGGAGACATGAGAGCTGCC  258784

Query  124     AACCTTTGGCCAAGCCCGCTCATGATCAAACGCTCTAAGAAGAACAGCCTGGCCTTGTCC  183
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258785  AACCTTTGGCCAAGCCCGCTCATGATCAAACGCTCTAAGAAGAACAGCCTGGCCTTGTCC  258844

Query  184     CTGACGGCCGACCAGATGGTCAGTGCCTTGTTGGATGCTGAGCCCCCCATACTCTATTCC  243
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258845  CTGACGGCCGACCAGATGGTCAGTGCCTTGTTGGATGCTGAGCCCCCCATACTCTATTCC  258904

Query  244     GAGTATGATCCTACCAGACCCTTCAGTGAAGCTTCGATGATGGGCTTACTGACCAACCTG  303
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258905  GAGTATGATCCTACCAGACCCTTCAGTGAAGCTTCGATGATGGGCTTACTGACCAACCTG  258964

Query  304     GCAGACAGGGAGCTGGTTCACA  324
               |||||||||||||||||||||||
Sbjct  258965  GCAGACAGGGAGCTGGTTCACA  258986
```

Figure (3): Alignment of exon 4 in *ESR1* gene sequence of women with breast cancer using automated sequencer was analyzed by BLAST data, query number represents the current results while the subject represents the reference sequence. Blue color represents exon region, black color represented intron region while the single nucleotide polymorphism represents red color

A

Homo sapiens estrogen receptor 1 (*ESR1*), RefSeqGene on chromosome 6
Sequence ID: ref|NG_008493.2|Length: 419779Number of Matches: 1
Related Information
Map Viewer-aligned genomic context
Range 1: 258714 to 259026GenBankGraphics Next Match Previous Match First Match
Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
544 bits(294)	8e-151	310/313(99%)	3/313(0%)	Plus/Plus

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Query  22      AACACAAGCGCCAGAGAGATGATGGGGAGGGCAGGGGTGAAGTGGGGTCTGCTGGAGACA  81
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258714  AACACAAGCGCCAGAGAGATGATGGGGAGGGCAGGGGTGAAGTGGGGTCTGCTGGAGACA  258773

Query  82      TGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACGCTCTAAGAAAAACAGCC  141
               ||||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||
Sbjct  258774  TGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACGCTCTAAGAAGAACAGCC  258833

Query  142     TGGCCTTGTCCCTGACGGCCGACCAGATGGTCAGTGCCTTGTTGGATGCTGAGCCCCCCA  201
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258834  TGGCCTTGTCCCTGACGGCCGACCAGATGGTCAGTGCCTTGTTGGATGCTGAGCCCCCCA  258893

Query  202     TACTCTATTCCGAGTATGATCCTACCAAACCCTTCAGTGAAGCTTCGATGATGGGCTTAC  261
               |||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  258894  TACTCTATTCCGAGTATGATCCTACCAGACCCTTCAGTGAAGCTTCGATGATGGGCTTAC  258953

Query  262     TGACCAACCTGGCAGACAGGGAGCTGGTTCACATGATCAACTGGGCGAAAAGGGTGCCAG  321
               |||||||||||||||||||||||||||||||||||||||||||||||||| ||||||||||
Sbjct  258954  TGACCAACCTGGCAGACAGGGAGCTGGTTCACATGATCAACTGGGCGAAGAGGGTGCCAG  259013

Query  322     GTAAGAATGGGAA  332
               |||||||||||||
Sbjct  259014  GTAAGAATGCGAA  259026
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B

Homo sapiens estrogen receptor 1 (*ESR1*), RefSeqGene on chromosome 6
Sequence ID: ref|NG_008493.2||Length: 419779Number of Matches: 1
Related Information
Map Viewer-aligned genomic context
Range 1: 258700 to 259033GenBankGraphics Next Match Previous Match First Match
Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
562 bits(304)	2e-156	332/337(98%)	5/337(1%)	Plus/Plus

```
Query  6       AGGGAGNANATCGTTGAAACACAAGCGCCAGAGAGATGATGGGGAGGGCAGGGGTGAAGT  64
               |||||| | ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258700  AGGGAG-A-ATCGTTGAAACACAAGCGCCAGAGAGATGATGGGGAGGGCAGGGGTGAAGT  258756

Query  65      GGGGTCTGCTGGAGACATGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACG  124
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258757  GGGGTCTGCTGGAGACATGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACG  258816

Query  125     CTCTAAGAAAAACAGCCTGGCCTTGTCCCTGACGGCCGACCAGATGGTCAGTGCCTTGTT  184
               |||||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258817  CTCTAAGAAGAACAGCCTGGCCTTGTCCCTGACGGCCGACCAGATGGTCAGTGCCTTGTT  258876

Query  185     GGATGCTGAGCCCCCCATACTCTATTCCGAGTATGATCCTACCAAACCCTTCAGTGAAGC  244
               |||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
Sbjct  258877  GGATGCTGAGCCCCCCATACTCTATTCCGAGTATGATCCTACCAGACCCTTCAGTGAAGC  258936

Query  245     TTCGATGATGGGCTTACTGACCAACCTGGCAAACAGGGAGCTGGTTCACATGATCAACTG  304
               |||||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  258937  TTCGATGATGGGCTTACTGACCAACCTGGCAGACAGGGAGCTGGTTCACATGATCAACTG  258996

Query  305     GGCGAAGAGGGTGCCAGGTAAGAATGCGAAGCGCAGC  339
               |||||||||||||||||||||||||||||||||||||
Sbjct  258997  GGCGAAGAGGGTGCCAGGTAAGAATGCGAAGCGCAGC  259033
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Figure (4): Alignment of exon 4 in *ESR1* gene sequence of women with breast cancer using automated sequencer was analyzed by BLAST data, query number represents the current results while the subject represents the reference sequence. Blue color represents exon region, black color represented intron region while the single nucleotide polymorphism represents red color; A: blood sample, B: Frozen tissue sample

Homo sapiens estrogen receptor 1 (*ESR1*), RefSeqGene on chromosome 6
Sequence ID: ref|NG_008493.2|Length: 419779Number of Matches: 1
Related Information
Map Viewer-aligned genomic context
Range 1: 258700 to 259031GenBankGraphics Next Match Previous Match
Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand

574 bits(636) 3e-160 330/332 (99%) 2/332(0%) Plus/Plus

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Query  5       AGGGAGAATGTTGAAACACAAGCGCCAGAGAGATGATGGGGAGGGCAGGGGTGAAGTGGG  62
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258700  AGGGAGAATGTTGAAACACAAGCGCCAGAGAGATGATGGGGAGGGCAGGGGTGAAGTGGG  258759

Query  63      GTCTGCTGGAGACATGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACGCTC  122
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258760  GTCTGCTGGAGACATGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACGCTC  258819

Query  123     TAAGAAGAACAGCCTGGCCTTGTCCCTGACGGCCGACCAGATGGTCAGTGCCTTGTTGGA  182
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258820  TAAGAAGAACAGCCTGGCCTTGTCCCTGACGGCCGACCAGATGGTCAGTGCCTTGTTGGA  258879

Query  183     TGCTGAGCCCCCCATACTCTATTCCGAGTATGATCCTACCAAACCCTTCAGTGAAGCTTC  242
               |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  258880  TGCTGAGCCCCCCATACTCTATTCCGAGTATGATCCTACCAGACCCTTCAGTGAAGCTTC  258939

Query  243     GATGATGGGCTTACTGACCAACCTGGCAACAGGGAGCTGGTTCACATGATCAACTGGGC  302
               |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  258940  GATGATGGGCTTACTGACCAACCTGGCAGACAGGGAGCTGGTTCACATGATCAACTGGGC  258999

Query  303     GAAGAGGGTGCCAGGTAAGAATGCGAAGCGCA  334
               ||||||||||||||||||||||||||||||||
Sbjct  259000  GAAGAGGGTGCCAGGTAAGAATGCGAAGCGCA  259031
```

Figure (5): Alignment of exon 4 in *ESR1* gene sequence of women with breast cancer using automated sequencer was analyzed by BLAST data, query number represents the current results while the subject represents the reference sequence. Blue color represents exon region, black color represented intron region while the single nucleotide polymorphism represents red color

Homo sapiens estrogen receptor 1 (*ESR1*), RefSeqGene on chromosome 6
Sequence ID: ref|NG_008493.2|Length: 419779Number of Matches: 1
Related Information
Map Viewer-aligned genomic context
Range 1: 258700 to 259031GenBankGraphics Next Match Previous Match
Alignment statistics for match #1

Score	Expect	Identities	Gaps	Strand
574 bits(636)	3e-160	330/332 (99%)	2/332(0%)	Plus/Plus

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Query  5       AGGGAGAATGTTGAAACACAAGCGCCAGAGAGATGATGGGGAGGGCAGGGGTGAAGTGGG  62
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258700  AGGGAGAATGTTGAAACACAAGCGCCAGAGAGATGATGGGGAGGGCAGGGGTGAAGTGGG  258759

Query  63      GTCTGCTGGAGACATGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACGCTC  122
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258760  GTCTGCTGGAGACATGAGAGCTGCCAACCTTTGGCCAAGCCCGCTCATGATCAAACGCTC  258819

Query  123     TAAGAAGAACAGCCTGGCCTTGTCCCTGACGGCCGACCAGATGGTCAGTGCCTTGTTGGA  182
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  258820  TAAGAAGAACAGCCTGGCCTTGTCCCTGACGGCCGACCAGATGGTCAGTGCCTTGTTGGA  258879

Query  183     TGCTGAGCCCCCCATACTCTATTCCGAGTATGATCCTACCAAACCCTTCAGTGAAGCTTC  242
               |||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
Sbjct  258880  TGCTGAGCCCCCCATACTCTATTCCGAGTATGATCCTACCAGACCCTTCAGTGAAGCTTC  258939

Query  243     GATGATGGGCTTACTGACCAACCTGGCAACAGGGAGCTGGTTCACATGATCAACTGGGC  302
               |||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||
Sbjct  258940  GATGATGGGCTTACTGACCAACCTGGCAGACAGGGAGCTGGTTCACATGATCAACTGGGC  258999

Query  303     GAAGAGGGTGCCAGGTAAGAATGCGAAGCGCA  334
               ||||||||||||||||||||||||||||||||
Sbjct  259000  GAAGAGGGTGCCAGGTAAGAATGCGAAGCGCA  259031
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Figure (6): Alignment of exon 4 in *ESR1* gene sequence of women with breast cancer using automated sequencer was analyzed by BLAST data, query number represents the current results while the subject represents the reference sequence. Blue color represents exon region, black color represented intron region while the single nucleotide polymorphism represents red color