ANKARA ÜNİVERSİTESİ

BİYOTEKNOLOJİ ENSTİTÜSÜ

PCR TEMELLİ GENETİK ANALİZ YAKLAŞIMLARI ÖDEVİ

**PTEN GENİ DİZİ ÇALIŞMASI**

ÖĞRENCİ ADI SOYADI

Öğretim Üyesi

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# 1. GİRİŞ

Bu ödev, kullanımını öğrendiğimiz NCBI, Blast, UCSC, Blat, OMIM ve Ensembl veri tabanlarında, seçtiğimiz bir gen (PTEN geni) üzerinden pratik yapmayı amaçlamaktadır.

# 2. REFERANS DİZİLERİ

## 2.1. NCBI

### 2.1.1. Genomik Dizi (1)

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114481 ctcagaaccc tctatacaat tttattctca ctttatacaa ctctgtacat tagttctgag

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115261 ttttcattct gaatttaatg agactctttt tttctttaac attttacttt gctttagaaa

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115381 aaaagtattg tatatgtccc ttgattttgg cttaagagtt agaatttact tagaatgttt

115441 cctgacccaa cctcattgga atttgaaagt acattccaat agtattgtga gc

### 2.1.2. mRNA Dizisi (2)

Anahtar: Exon

Exon’un başlangıç (ag) –bitiş (gt) bölgeleri

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181 cagagggaga ctccgtctca aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa atttctgcct

241 actctatgaa gcacctggta ggtgtcccaa tattttctgg tatatgggaa ttaccttttt

301 ctattattgg aaggtctaaa aagcaaacaa tgtgctccat atggctagag ttagtattaa

361 ggggaccaag tagaataatt ggtagctagt gttagcctgg agactaacat agtcaaagct

421 gtcttgtcat gttatgtctt ttttatttgt attgctccac ggtccaagac aaaatttctg

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541 caacccttat ttctctgaga atgatgattt catacagcac atctctctac caagatgtga

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721 ttttggagaa tgtcttaaaa ttatctttat tgaaagaaca ataatgtttg ttattaggat

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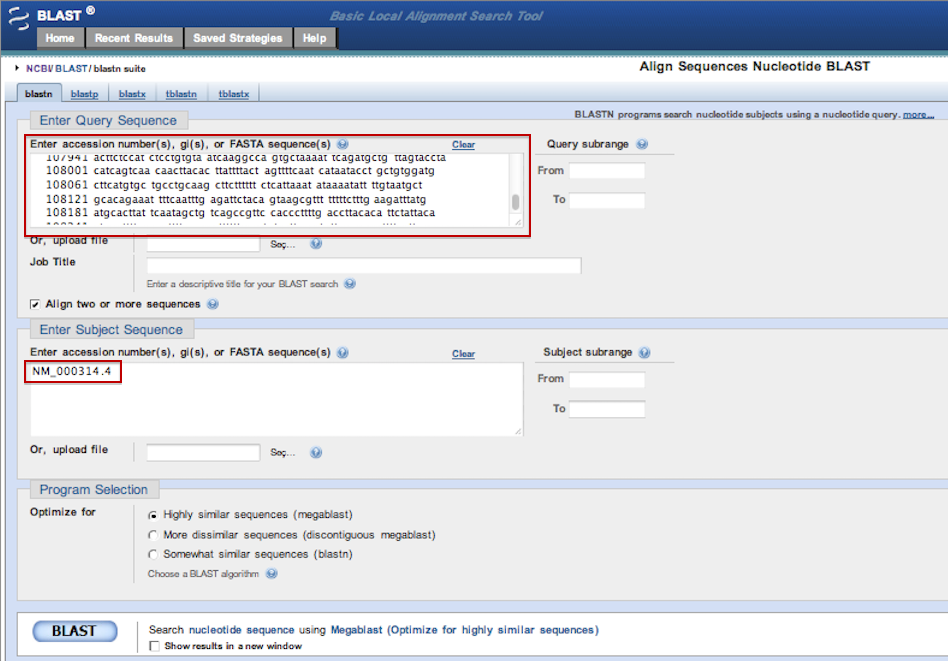
115261 ttttcattct gaatttaatg agactctttt tttctttaac attttacttt gctttagaaa

115321 ggatttaagg caggtgaatg tgtttcccag tgtgagaaaa attgtcatgt aaatttagaa

115381 aaaagtattg tatatgtccc ttgattttgg cttaagagtt agaatttact tagaatgttt

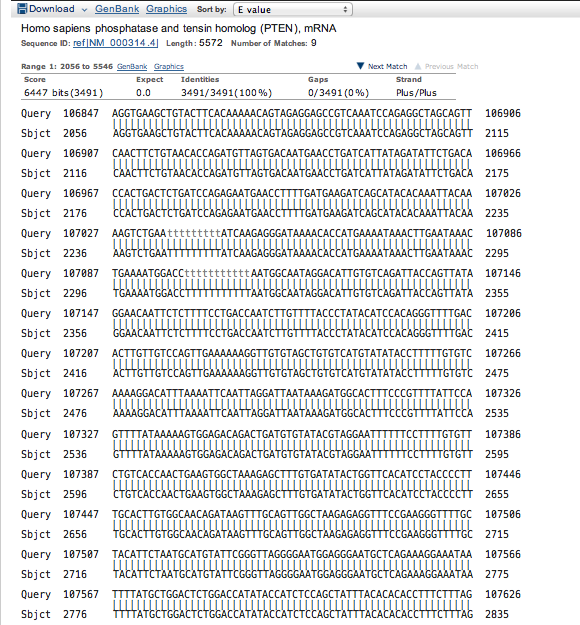
115441 cctgacccaa cctcattgga atttgaaagt acattccaat agtattgtga gc

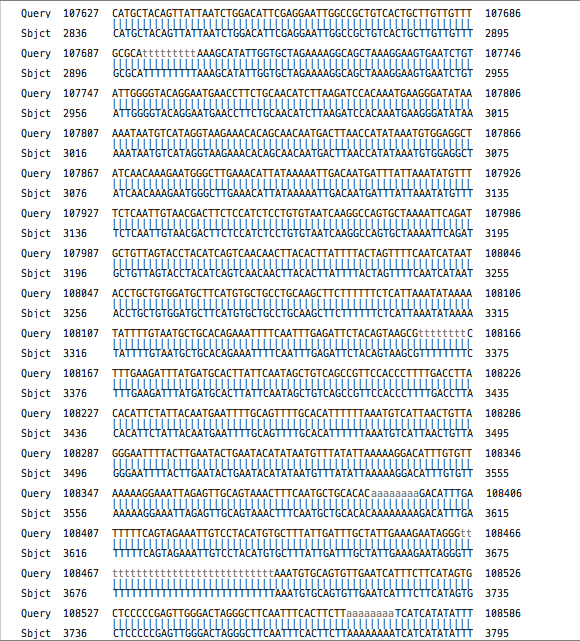
### 2.1.3. Blast Karşılaştırma Analizi (3)

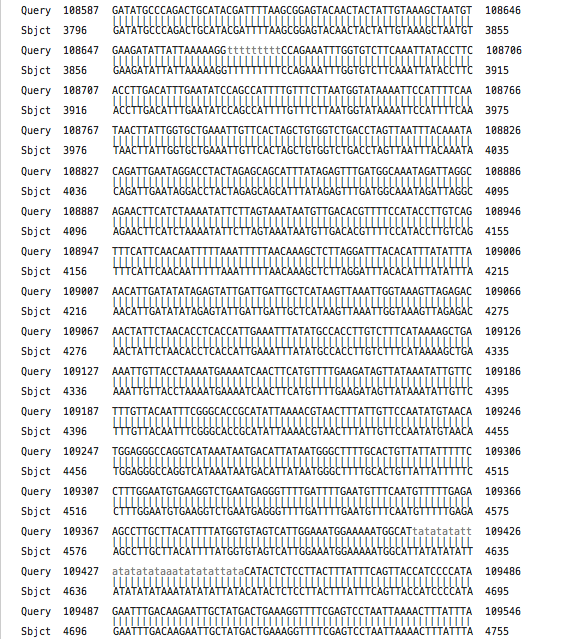


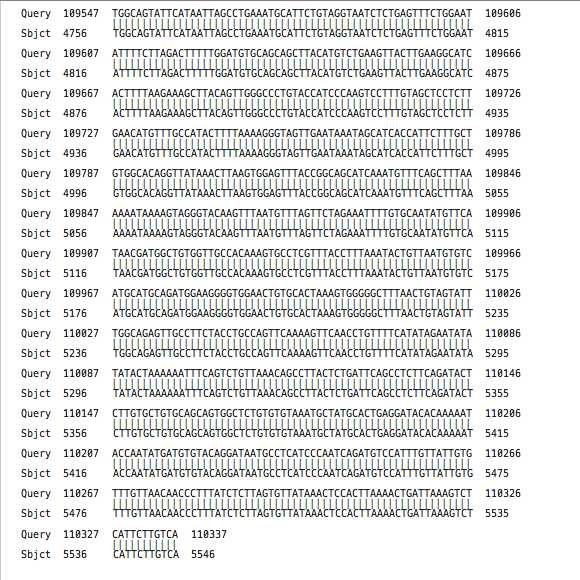
Genomik DNA dizisi ile mRNA dizisinin NCBI kodu BLAST programında karşılaştırıldı.

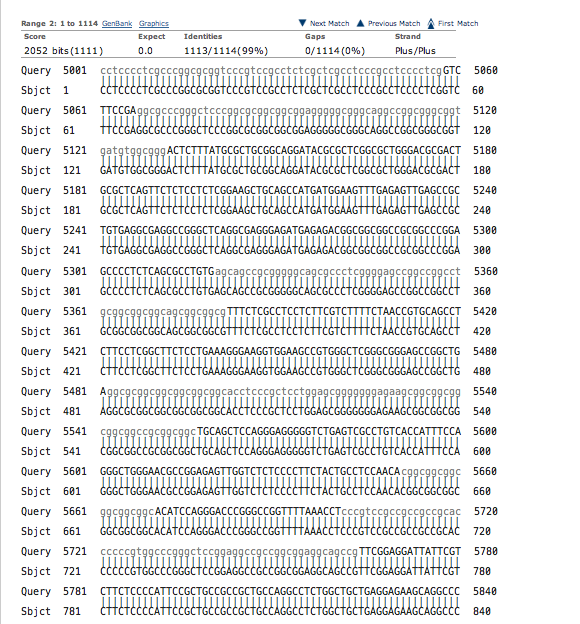
Çakışan bölgeler exon bölgelerini belirtmektedir (4):

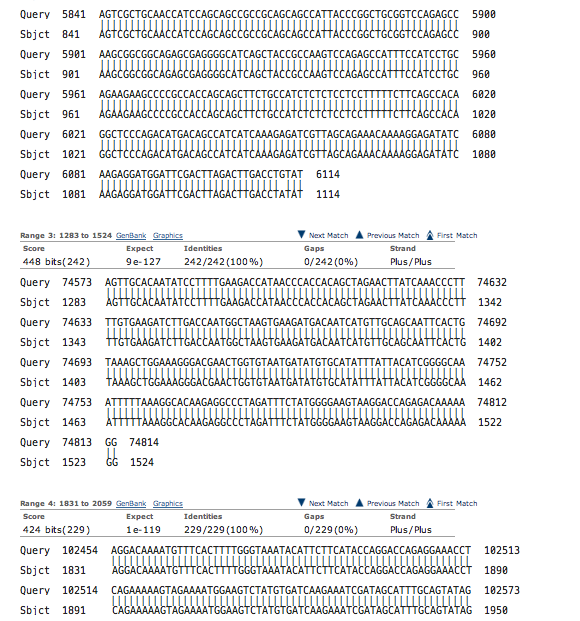


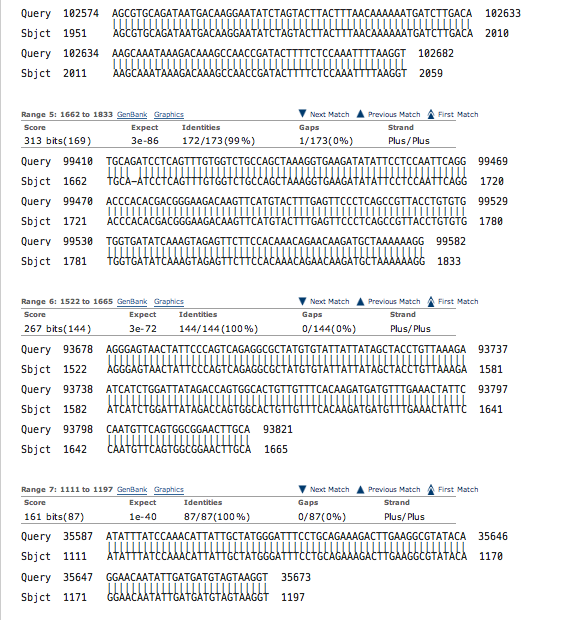


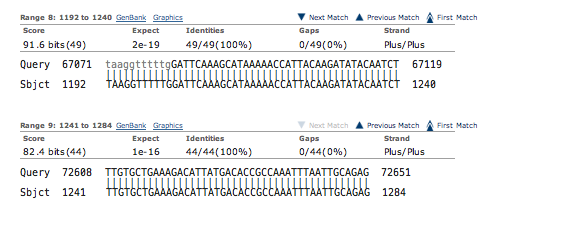






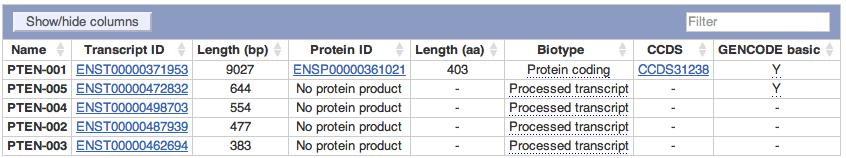


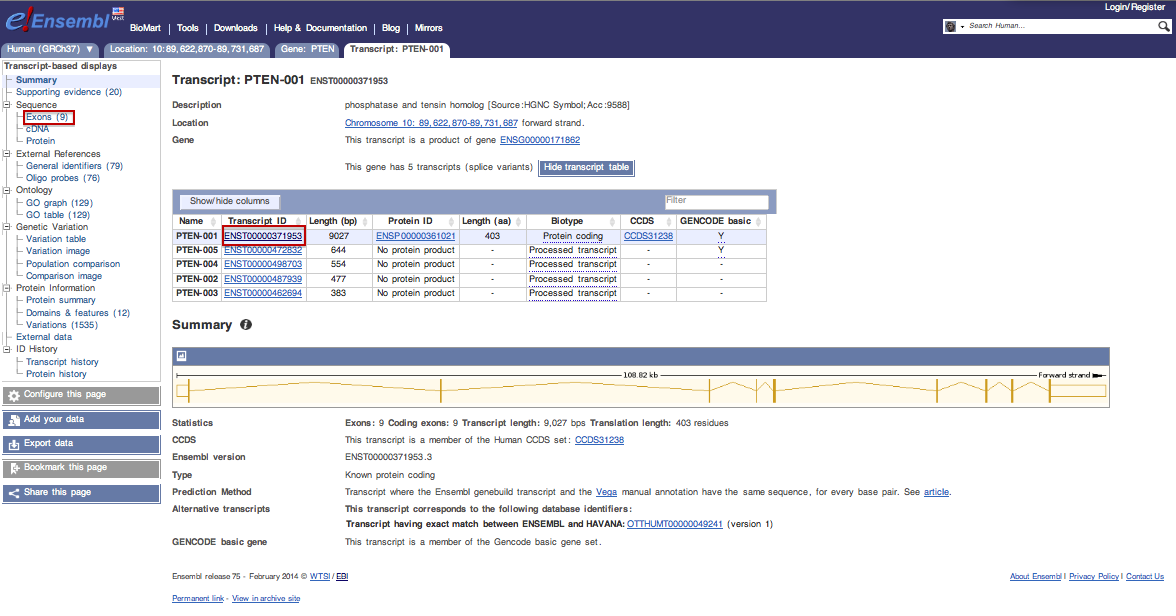




## 2.2. Ensembl (9)

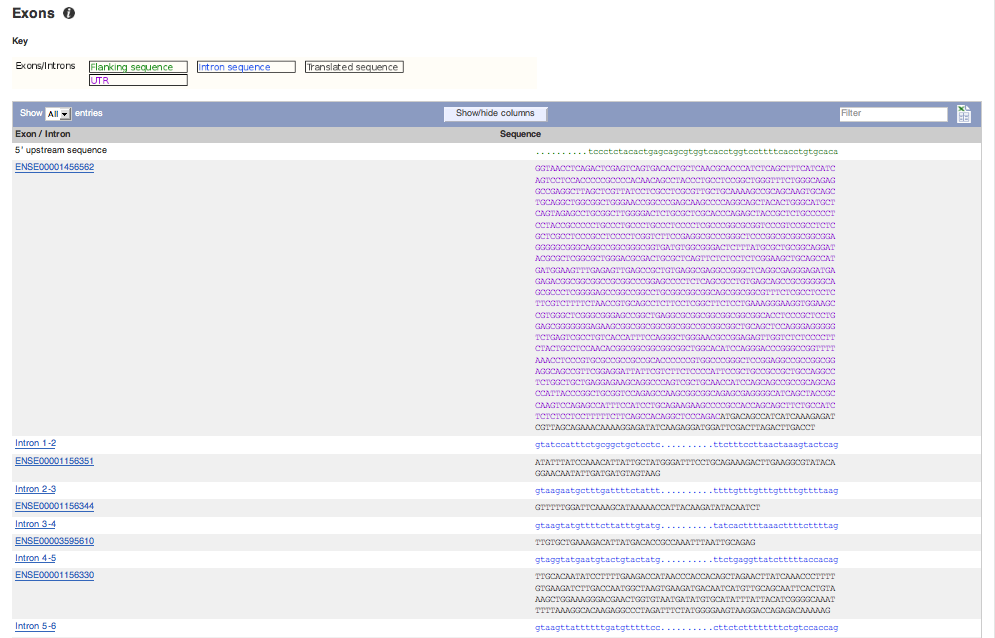
### 2.2.1. Ensembl Ekzon İntron Yapısı (10-11-12)

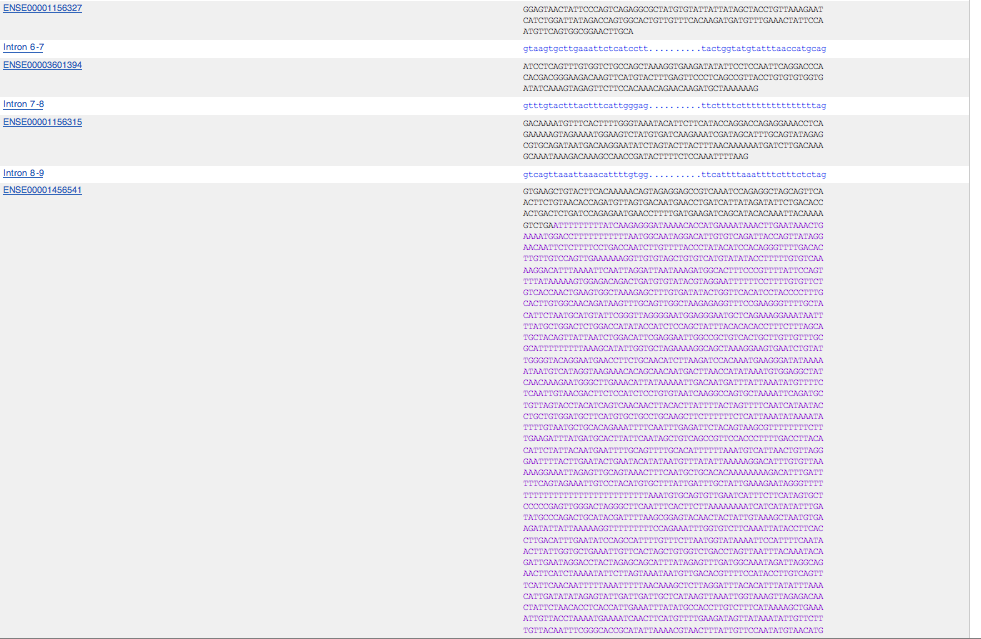


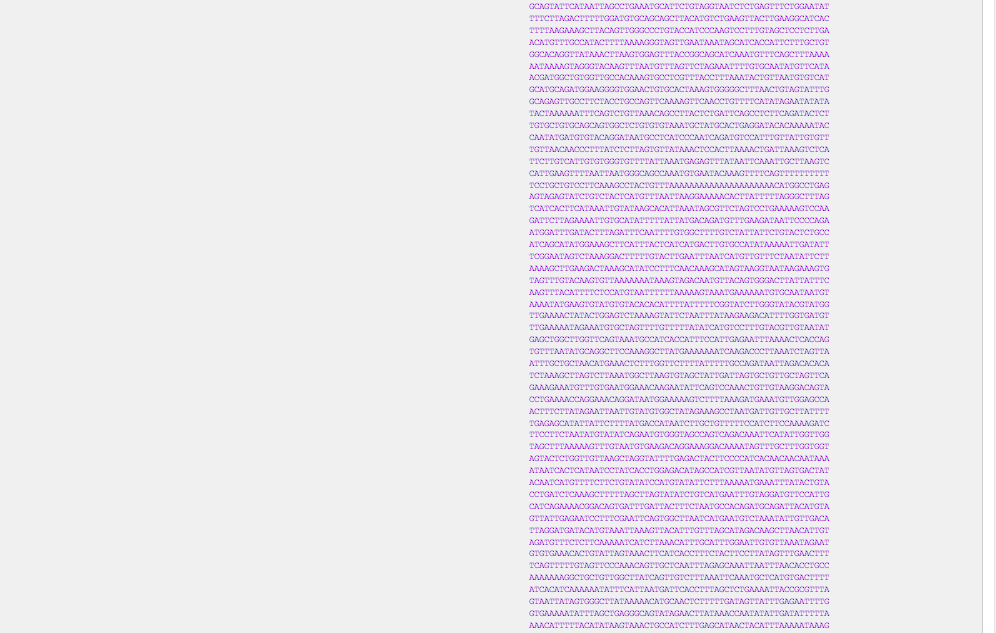


En uzun transkript ID’si örnek olarak alındı ve exonlarına bakldı.

Yukarıdaki şekildeki kırık çizgiler intronları, içleri dolu kutucuklar exonları ve uçlardaki boş kutucuklar 5’ UTR ve 3’ UTR uçlarını göstermektedir.

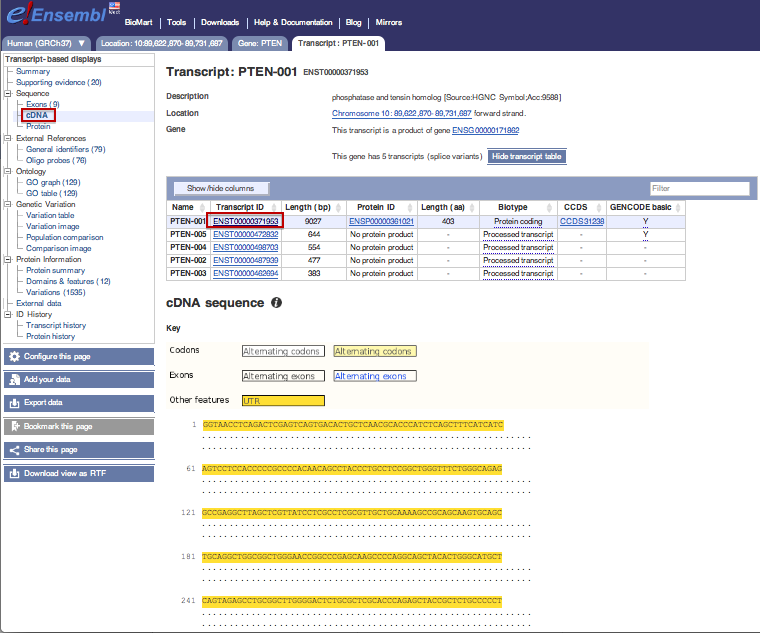


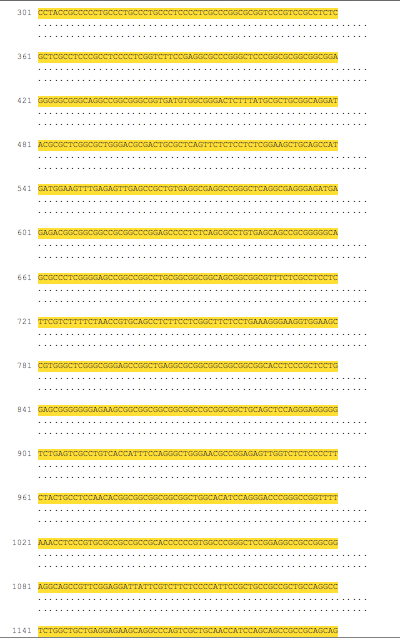




### 2.2.2. Ensembl mRNA, Okuma Çerçevesi, Aminoasit Dizileri (13)

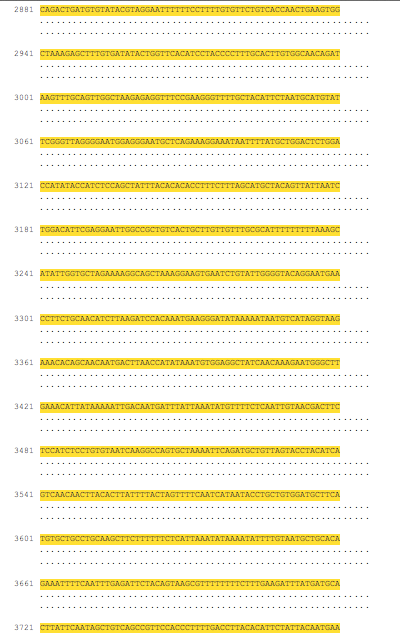
En uzun transkript ID’sinde cDNA’ya bakılarak mRNA, okuma çerçevesi ve amino asit dizileri incelenmiştir. Varyasyonlar gözardı edilmiştir.



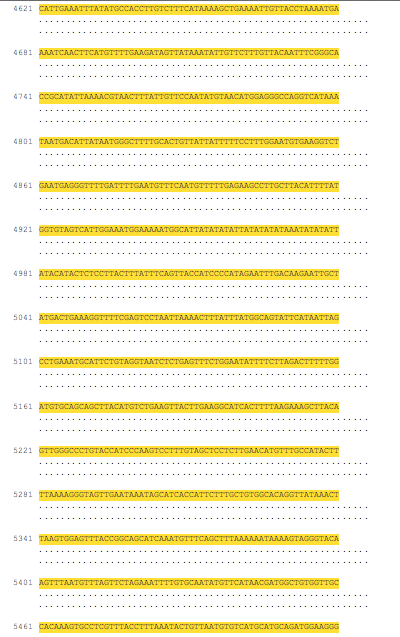


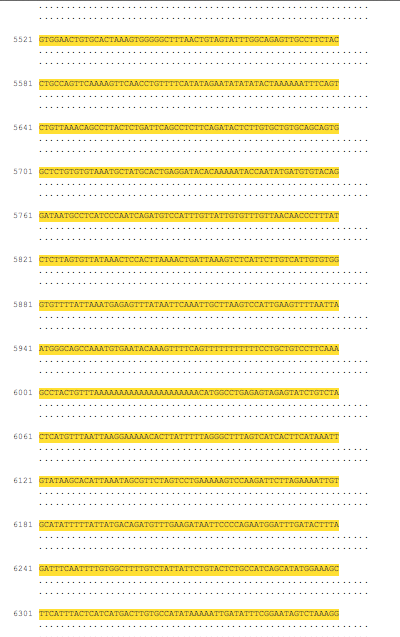


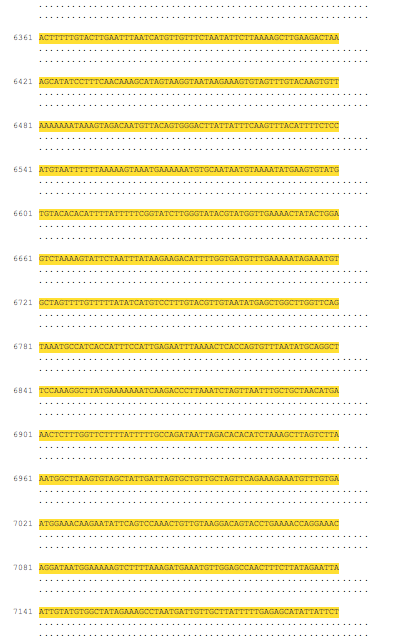


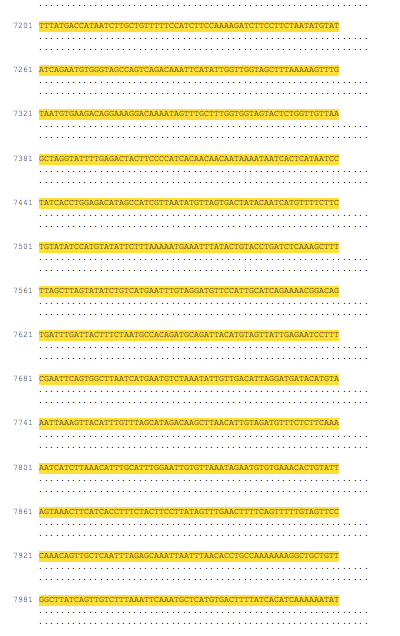


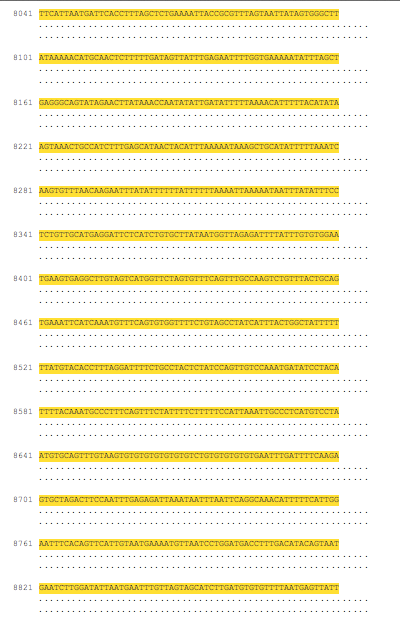


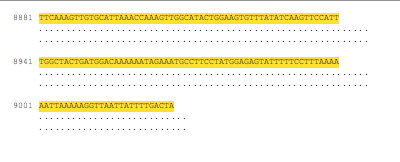












# 3. PRİMER TASARIMI (14)

Primer tasarlanırken çoğaltmak istenilen exon bölgesinin 20-30 baz gerisinden (forward primer için) ve 20-30 baz ilerisinden (reverse primer için) başlanarak tasarlanacak bölge seçilir. Sekans işlemi sırasında ilk 20-30 bazlık bölge düzgün okunamayacağından primer daha uzak bölgede aranır. Klonlama işlemi için primer tasarlanacak olsaydı çoğaltmak istenilen bölgenin hemen yanından seçilmesi gerekirdi.

PTEN geni için, 9 exon bölgesinden 3. ve 8. exon bölgeleri örnek olarak seçilip primerleri, PerlPrimer programında tasarlandı.

3. exon bölgesi için:

66841 ttacttcaga ttgtataaat ctgtgtaatg taataattat ttaagaatga catgattact

66901 actctaaacc catagaaggg gtatttgttg gattatttat tttcacttaa atggtatttg

66961 agattaggaa aaagaaaatc tgtcttttgg tttttcttga tagtattaat gtaatttcaa

67021 atgttagctc atttttgtta atggtggctt tttgtttgtt tgttttgttt taaggttttt

67081 ggattcaaag cataaaaacc attacaagat atacaatctg taagtatgtt ttcttatttg

67141 tatgcttgca aatatcttct aaaacaacta ttaagtgaaa gttatctgct tgttagagtg

67201 aggtagagtt aaagatacat tttaacagaa ttgtattcct aaaccgatta agtcaagaag

67261 tccaagagca ttgttagatc atttagaaag tgtagtgatg aggtaaaaca ttgttggcac

ttacttcaga ttgtataaat ctgtgtaatg taataattat ttaagaatga catgattact

actctaaacc catagaaggg gtatttgttg gattatttat tttcacttaa atggtatttg

agattaggaa aaagaaaatc tgtcttttgg tttttcttga tagtattaat gtaatttcaa

atgttagctc atttttgtta atggtggctt tttgtttgtt tgttttgttt taaggttttt

ggattcaaag cataaaaacc attacaagat atacaatctg taagtatgtt ttcttatttg

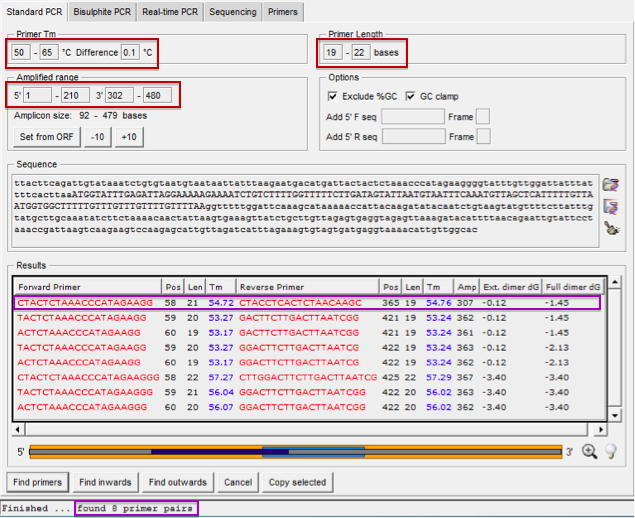
tatgcttgca aatatcttct aaaacaacta ttaagtgaaa gttatctgct tgttagagtg

aggtagagtt aaagatacat tttaacagaa ttgtattcct aaaccgatta agtcaagaag

tccaagagca ttgttagatc atttagaaag tgtagtgatg aggtaaaaca ttgttggcac

1. ve 210. Nükleotidler arasındaki bölgede forward primer,

302. ve 480. Nükleotidler arasındaki bölgede ise reverse aranacaktır.



8. exon bölgesi için:

102241 aaaaatcatg tgaatgaaaa tgcaacagat aactcagatt gccttataat agtctttgtg

102301 tttaccttta ttcagaatat caaatgatag tttattttgt tgactttttg caaatgttta

102361 acataggtga cagattttct tttttaaaaa aataaaacat cattaattaa atatgtcatt

102421 tcatttcttt ttcttttctt tttttttttt tttaggacaa aatgtttcac ttttgggtaa

102481 atacattctt cataccagga ccagaggaaa cctcagaaaa agtagaaaat ggaagtctat

102541 gtgatcaaga aatcgatagc atttgcagta tagagcgtgc agataatgac aaggaatatc

102601 tagtacttac tttaacaaaa aatgatcttg acaaagcaaa taaagacaaa gccaaccgat

102661 acttttctcc aaattttaag gtcagttaaa ttaaacattt tgtgggggtt gttgacttgt

102721 atgtatgtga tgtgtgttta attctaggag tacagctgat gaagaacttg cttgacaagt

102781 ttttaactta tgtattattt cgaagcagtg tttacgtagc agtaacatga aagtttctaa

102841 taaaataccc aatgtacaca gcgtcaaaaa agctgcattt ttccttttcc taattcttcg

aaaaatcatg tgaatgaaaa tgcaacagat aactcagatt gccttataat agtctttgtg

tttaccttta ttcagaatat caaatgatag tttattttgt tgactttttg caaatgttta

acataggtga cagattttct tttttaaaaa aataaaacat cattaattaa atatgtcatt

tcatttcttt ttcttttctt tttttttttt tttaggacaa aatgtttcac ttttgggtaa

atacattctt cataccagga ccagaggaaa cctcagaaaa agtagaaaat ggaagtctat

gtgatcaaga aatcgatagc atttgcagta tagagcgtgc agataatgac aaggaatatc

tagtacttac tttaacaaaa aatgatcttg acaaagcaaa taaagacaaa gccaaccgat

acttttctcc aaattttaag gtcagttaaa ttaaacattt tgtgggggtt gttgacttgt

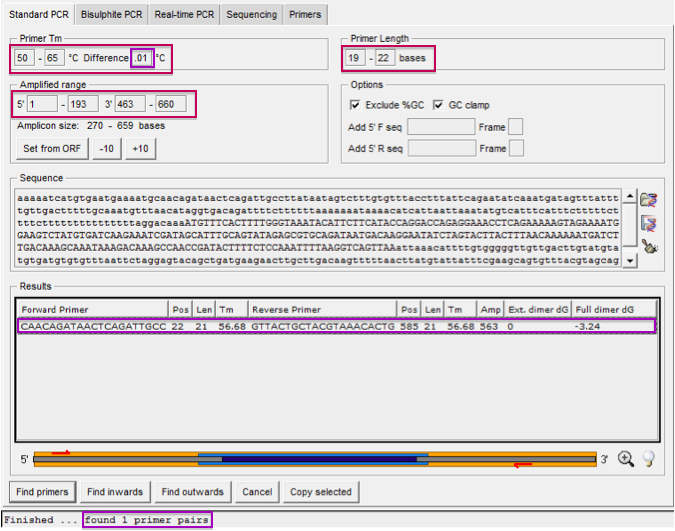
atgtatgtga tgtgtgttta attctaggag tacagctgat gaagaacttg cttgacaagt

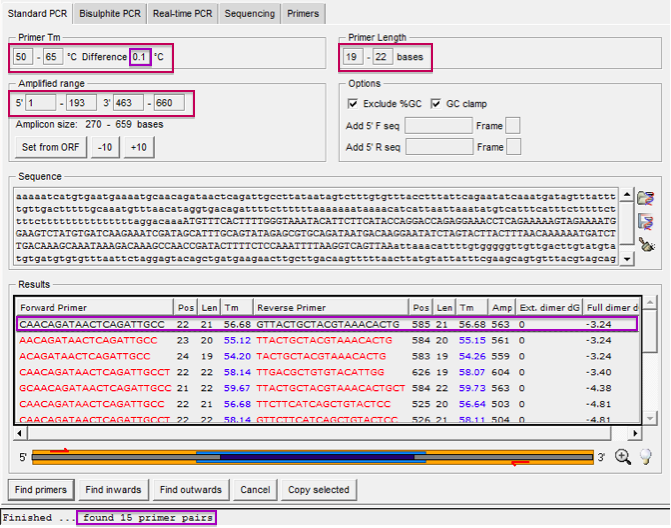
ttttaactta tgtattattt cgaagcagtg tttacgtagc agtaacatga aagtttctaa

taaaataccc aatgtacaca gcgtcaaaaa agctgcattt ttccttttcc taattcttcg

1. ve 193. Nükleotidler arasındaki bölgede forward primer,

463. ve 660. Nükleotidler arasındaki bölgede ise reverse primer aranacaktır.

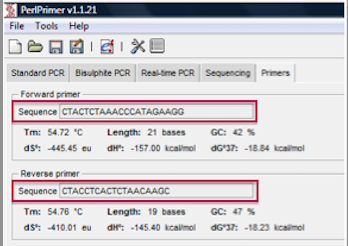




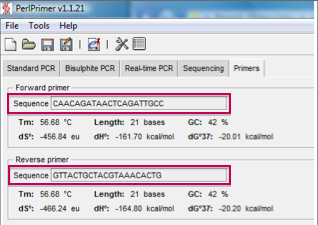
8. exon bölgesi için PerlPrimer’de Tm değeri 0.01**°**C iken 1 tane primer bulunurken, Tm değeri arttırılıp 0.1**°**C seçildiğinde olası primer sayısı 15 olarak bulundu. Primerlerin Tm değerleri arasındaki fark arttıkça olası primer sayısı da artacaktır. Fakat aynı sıcaklıkta çalışabilmeleri için değerler arasındaki farkın mümkün olduğunca az olması gerekir.

## 3.1. Tasarlanan Primer ve Özellikleri (14)

3. exon bölgesi için:



8. exon bölgesi için:



**3.2. Tasarlanan Primerlerin Gen Haritası Üzerindeki Yerleşimleri (14)**

Tasarlanan forward ve reverse primerler kırmızıyla işaretlenmiştir.

3. exon bölgesi için tasarlanan primerler:

ttacttcaga ttgtataaat ctgtgtaatg taataattat ttaagaatga catgattact

actctaaacc catagaaggg gtatttgttg gattatttat tttcacttaa atggtatttg

agattaggaa aaagaaaatc tgtcttttgg tttttcttga tagtattaat gtaatttcaa

atgttagctc atttttgtta atggtggctt tttgtttgtt tgttttgttt taaggttttt

ggattcaaag cataaaaacc attacaagat atacaatctg taagtatgtt ttcttatttg

tatgcttgca aatatcttct aaaacaacta ttaagtgaaa gttatctgct tgttagagtg

aggtagagtt aaagatacat tttaacagaa ttgtattcct aaaccgatta agtcaagaag

tccaagagca ttgttagatc atttagaaag tgtagtgatg aggtaaaaca ttgttggcac

8. exon bölgesi için tasarlanan primerler:

aaaaatcatg tgaatgaaaa tgcaacagat aactcagatt gccttataat agtctttgtg

tttaccttta ttcagaatat caaatgatag tttattttgt tgactttttg caaatgttta

acataggtga cagattttct tttttaaaaa aataaaacat cattaattaa atatgtcatt

tcatttcttt ttcttttctt tttttttttt tttaggacaa aatgtttcac ttttgggtaa

atacattctt cataccagga ccagaggaaa cctcagaaaa agtagaaaat ggaagtctat

gtgatcaaga aatcgatagc atttgcagta tagagcgtgc agataatgac aaggaatatc

tagtacttac tttaacaaaa aatgatcttg acaaagcaaa taaagacaaa gccaaccgat

acttttctcc aaattttaag gtcagttaaa ttaaacattt tgtgggggtt gttgacttgt

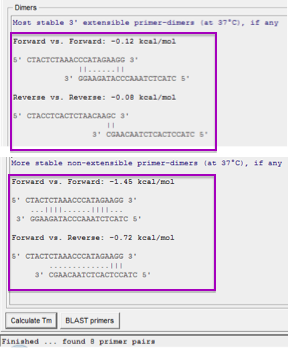
atgtatgtga tgtgtgttta attctaggag tacagctgat gaagaacttg cttgacaagt

ttttaactta tgtattattt cgaagcagtg tttacgtagc agtaacatga aagtttctaa

taaaataccc aatgtacaca gcgtcaaaaa agctgcattt ttccttttcc taattcttcg

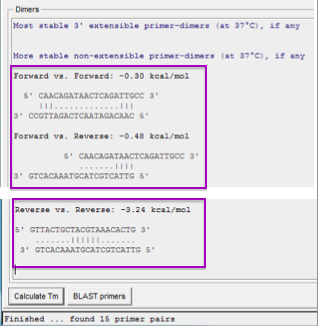
## 3.3. Primer Dimer Analizi (14)

3. exon bölgesi için:



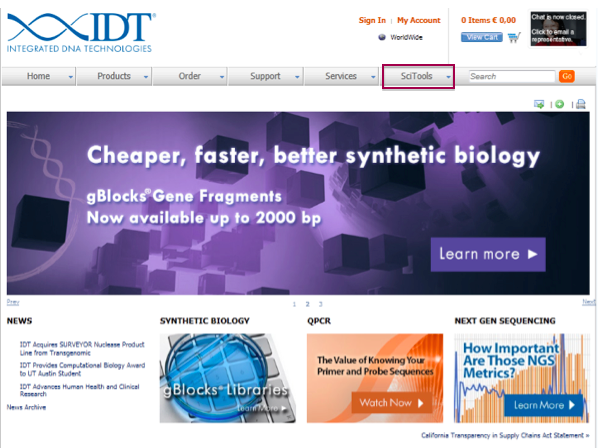
Extensible primer- dimer görülür; fakat sıfıra yakın değerlerdir. Non-extensible primer-dimer’lerin dG’leri diğer olası primer tasarımlarına göre daha uygun sıfıra yakın değerlerdir.

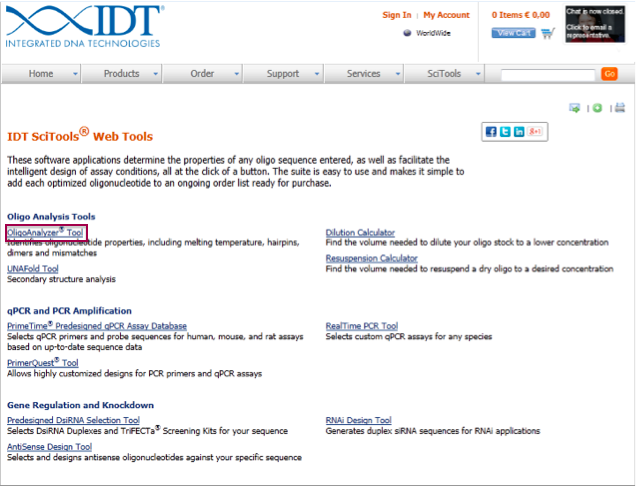
8. exon bölgesi için:



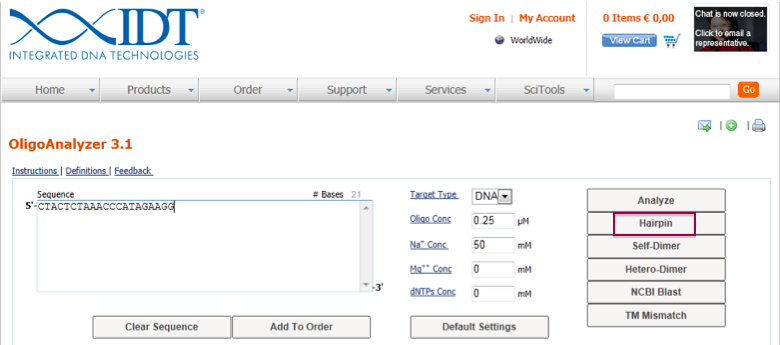
Extensible primer- dimer görülmez. Non-extensible primer-dimer’lerde dG değeri -3.24 olan durum ideal değildir.

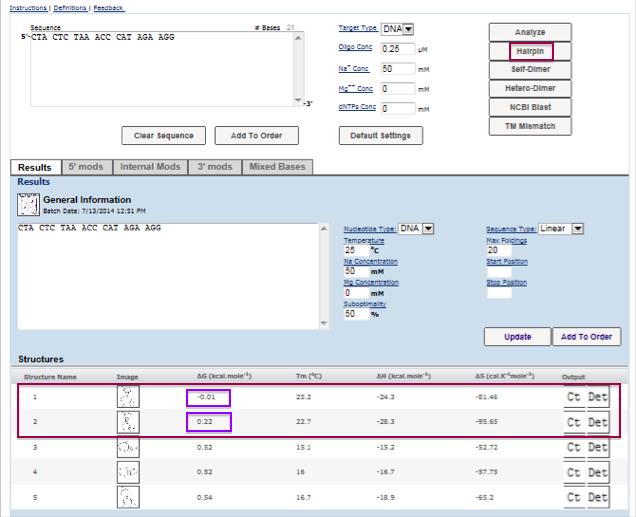
## 3.4. Saç Tokası Yapısı Analizi (15-16)



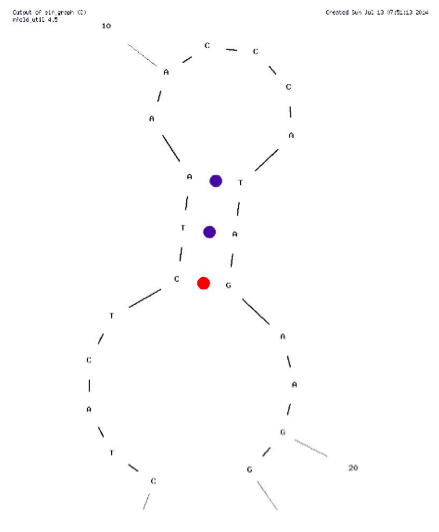


3. exon bölgesi için forward primerin hairpin yapısı (17):

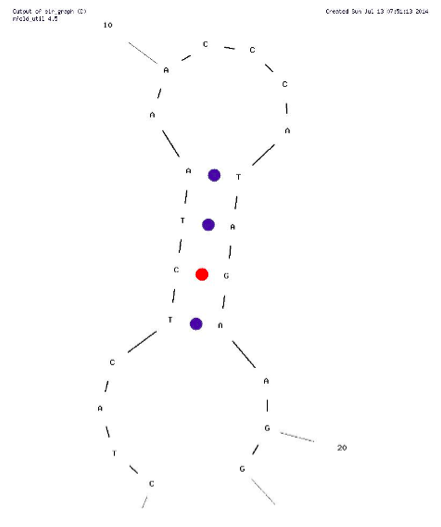




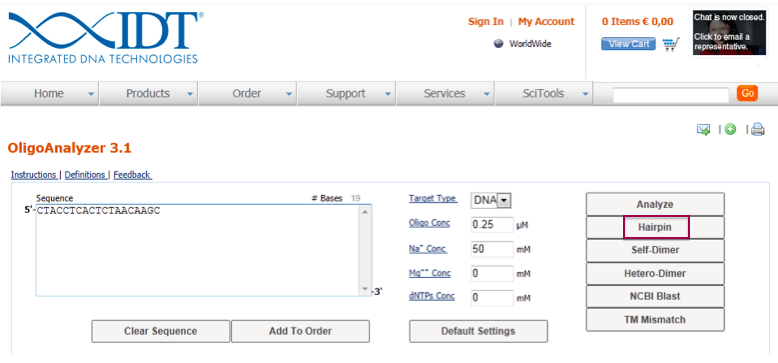
dG: -0.01

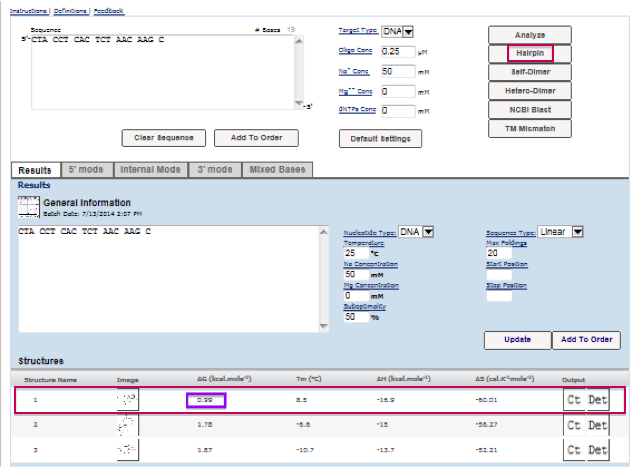


dG:0.22

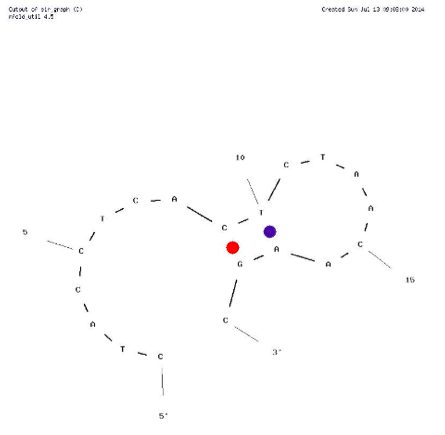


3. exon bölgesi için reverse primerin hairpin yapısı (17):

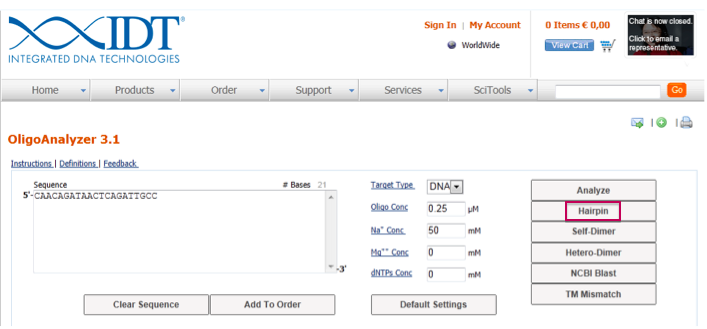


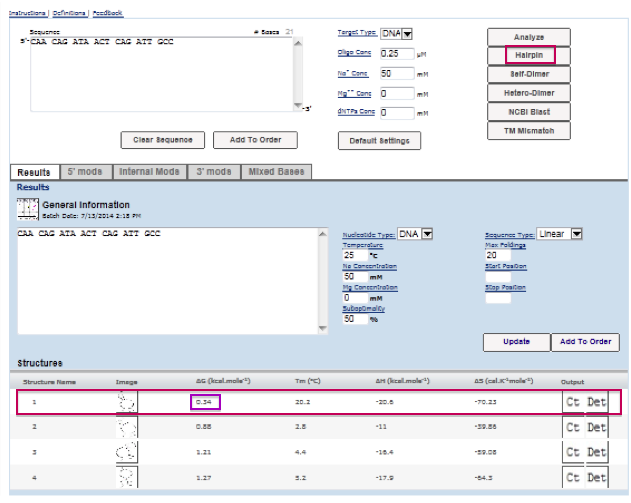


dG: 0.99

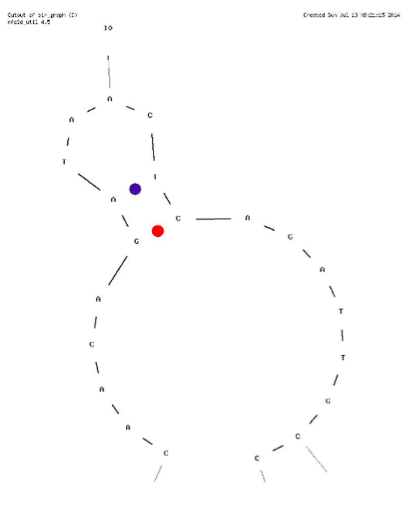


8. exon bölgesi için forward primerin hairpin yapısı (17):

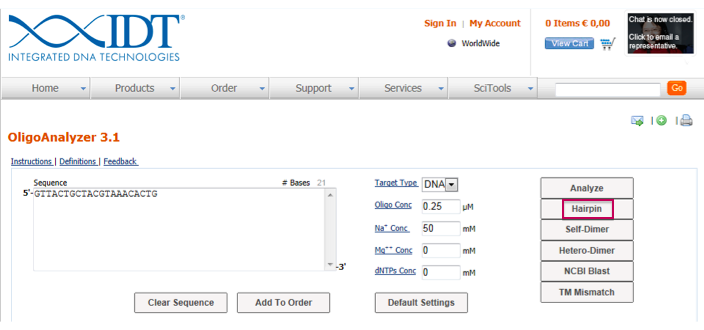


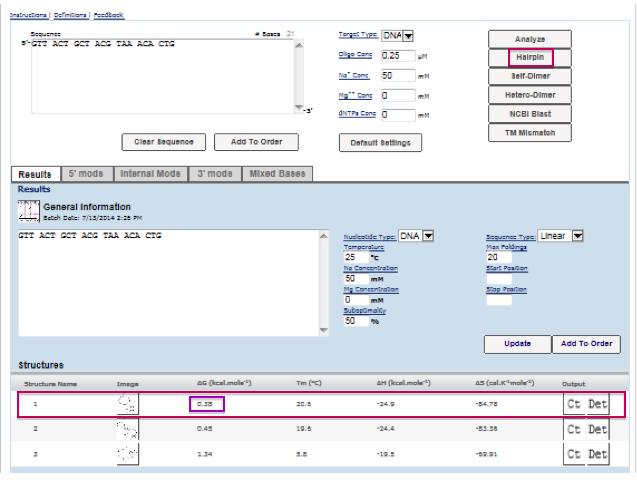


dG: 0.34

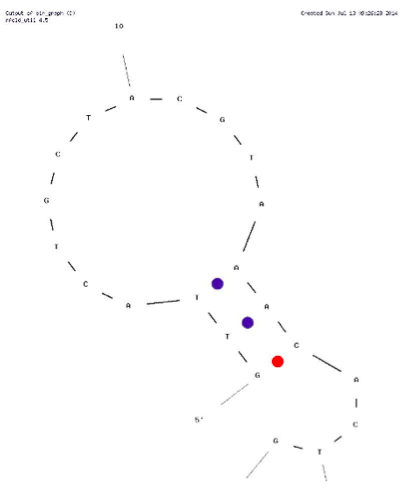


8. exon bölgesi için reverse primerin hairpin yapısı (17):



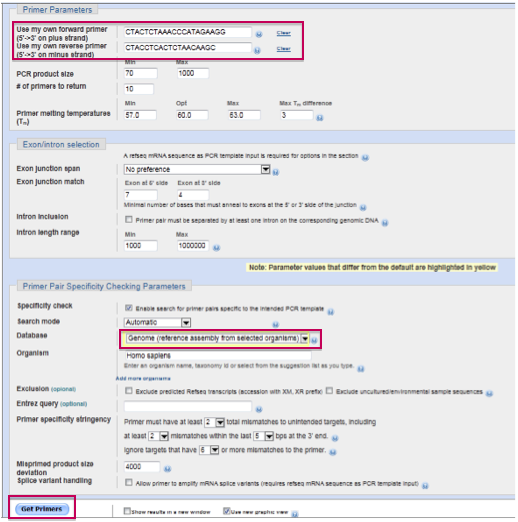


dG: 0.38



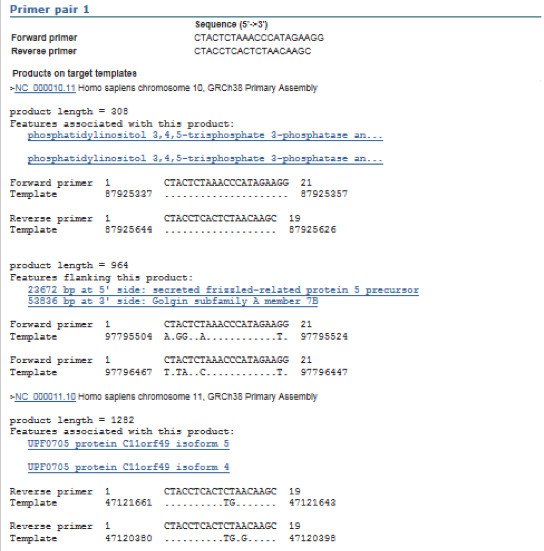
## 3.5. Primerlerin Blast Analizleri (18-19-20)

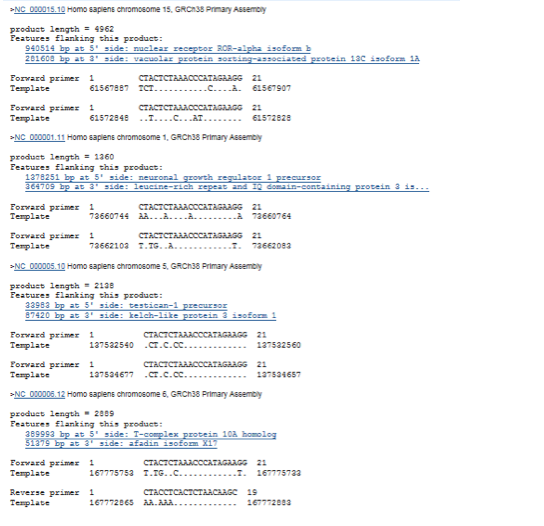
3. exon bölgesi için (18):



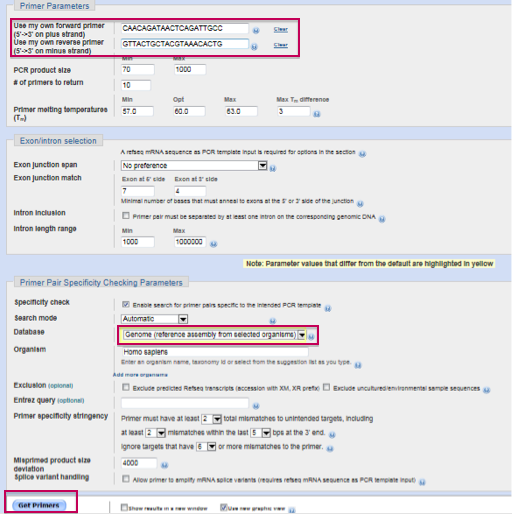
(19)

Macintosh HD:Users:begumkaraoglu:Desktop:Ozdag Odev:ekran resimleri:mac 9. primer 1_blast sonuç 1.png



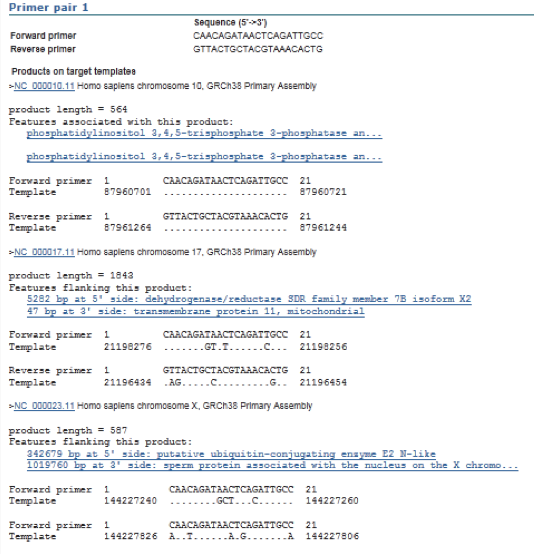


8. exon bölgesi için (18):



(20)





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