Methyl Primer Express® Software v1.0

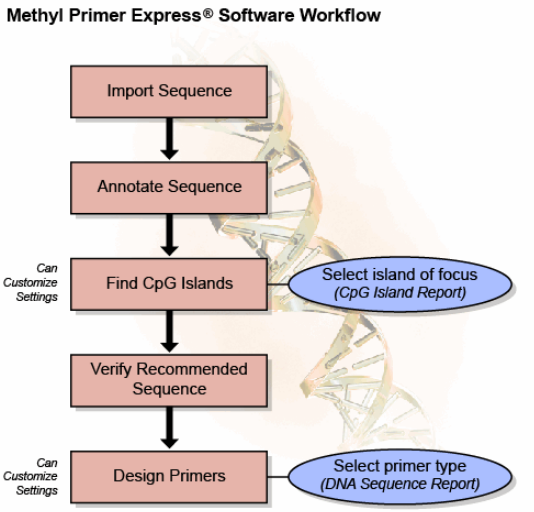
Quick Reference Card

Overview

Methyl Primer Express® Software allows you to design primers for bisulfite sequencing (BSP) and/or methylation-specific PCR (MSP) — the two most commonly used techniques for methylation mapping. The software searches DNA sequences for CpG islands, simulates bisulfite modification on the CpG-containing sequences, then recommends primer pairs for MSP or BSP. Primer design for either method can be adjusted to accomodate variation in experimental design.

The essential steps in designing sequence-specific DNA methylation experiments are substantially automated with Methyl Primer Express. After you insert the selected DNA sequence into the software, the software finds all relevant CpG islands, then provides primer designs based on selected target sequences. You can review all CpG islands found in your sequence, and all suggested primer pairs based on your target sequence, in report views.

Methyl Primer Express® Software Workflow



By using Methyl Primer Express software to design primers you will learn to:

- Import a gDNA sequence
- Annotate the sequence
- Search for CpG islands
- Select a CpG island of interest
- Select a target sequence

Starting the Methyl Primer Express® Software

To start Methyl Primer Express® Software v1.0, click  on the desktop.

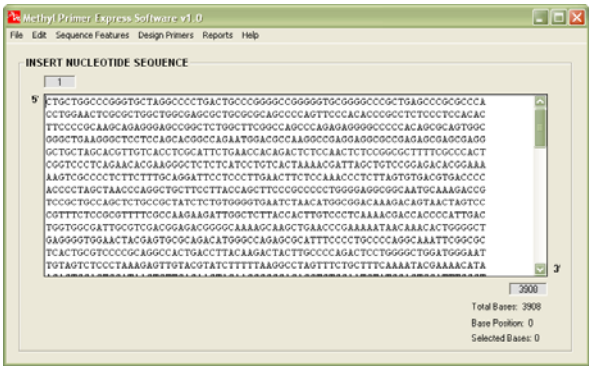
Using an Example Sequence

This Quick Reference Card uses an example human DNA sequence (BRCA1 gene) to show you how to use Methyl Primer Express software.

Importing a Sequence

To enter the sequence into Methyl Primer Express software:

1. Select **File ▸ Import Sequence**.
2. Open the GenBank database, then copy the BRCA1 gene sequence (U37574).
3. Paste the sequence into the Insert Nucleotide Sequence box.



IMPORTANT! If you import a sequence that has one or more N calls, the software does not allow you to proceed to the next step and an error message is displayed. To minimize the chance that a gDNA sequence has N bases to convert, acquire the most recent Genbank sequence you can find.

Note: In the example sequence, N bases were previously converted.

Annotating the Sequence

You can annotate the imported sequence with one or more identifying elements such as:

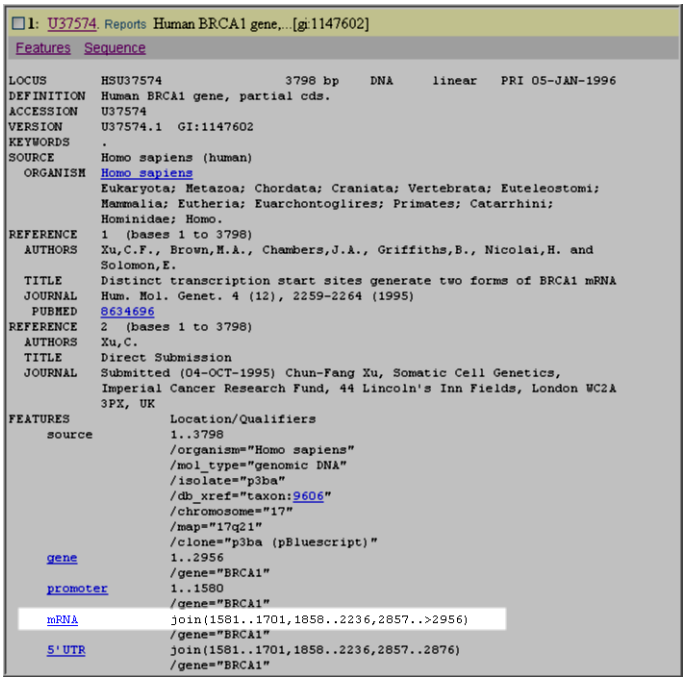
- A user-specified sequence name
- The transcription start point of the gene sequence
- The translation start codon of the gene sequence

In this example workflow, you annotate the sequence only with the transcription start point.

You can determine the transcription start point by referring to the mRNA line in the GenBank record of your imported genomic sequence. The transcription start point begins at the beginning of the gene messenger RNA.

Annotating the Transcription Start Point

1. In the Features section of the GenBank file for the BRCA1 gene (file U37574), find the mRNA start sequence number.

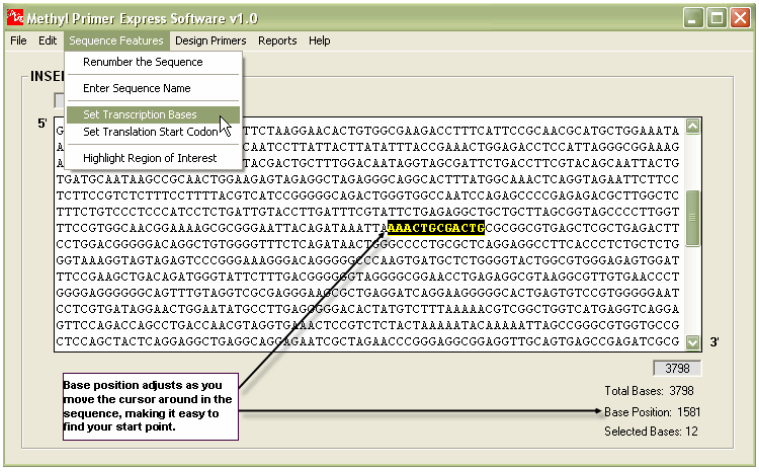


1: U37574 Reports Human BRCA1 gene,...[gi:1147602]

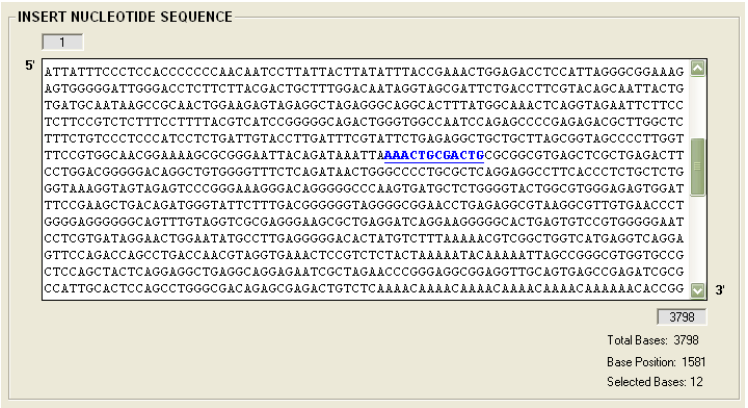
Features Sequence

```
LOCUS       HSU37574                3798 bp    DNA     linear   PRI 05-JAN-1996
DEFINITION  Human BRCA1 gene, partial cds.
ACCESSION   U37574
VERSION     U37574.1  GI:1147602
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Catarrhini;
            Hominidae; Homo.
REFERENCE   1  (bases 1 to 3798)
  AUTHORS   Xu,C.F., Brown,M.A., Chambers,J.A., Griffiths,B., Nicolai,H. and
            Solomon,E.
  TITLE     Distinct transcription start sites generate two forms of BRCA1 mRNA
  JOURNAL   Hum. Mol. Genet. 4 (12), 2259-2264 (1995)
   PUBMED   8634696
REFERENCE   2  (bases 1 to 3798)
  AUTHORS   Xu,C.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-OCT-1995) Chun-Fang Xu, Somatic Cell Genetics,
            Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A
            3PX, UK
FEATURES             Location/Qualifiers
     source          1..3798
                     /organism="Homo sapiens"
                     /mol_type="genomic DNA"
                     /isolate="p3ba"
                     /db_xref="taxon:9606"
                     /chromosome="17"
                     /map="17q21"
                     /clone="p3ba (pBluescript)"
     gene            1..2956
                     /gene="BRCA1"
     promoter        1..1580
                     /gene="BRCA1"
     mRNA            join(1581..1701,1858..2236,2857..>2956)
                     /gene="BRCA1"
     5'UTR           join(1581..1701,1858..2236,2857..2876)
                     /gene="BRCA1"
```

2. Click the pointer anywhere in your imported genomic sequence and view its sequence number position in the Base Position field at the bottom right corner of the screen.
3. Move the pointer as you scroll down the sequence to match the Base Position number to the mRNA start number in the GenBank file. (1581 in the example)
4. Select the start point base plus 12 or more bases after the start point.



5. Select **Sequence Features ▸ Set Transcription Bases**. The transcription bases are highlighted and underlined in blue. Scroll to see the annotated bases.

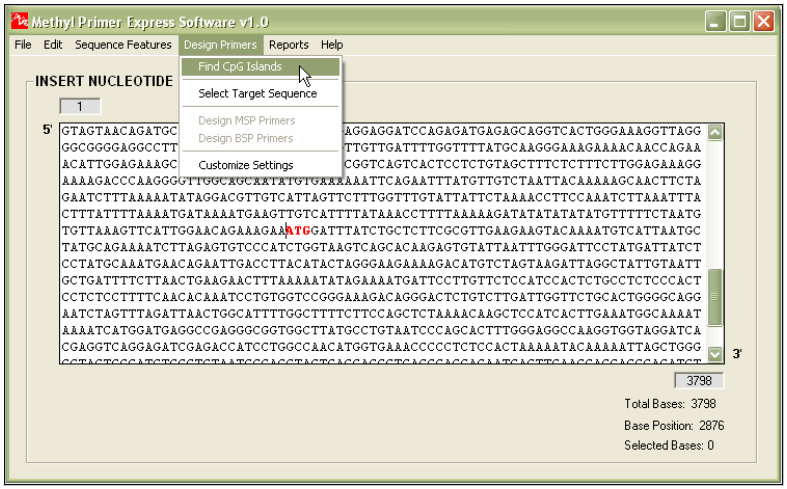


With the sequence imported and annotated, you are ready to have the software find the CpG islands.

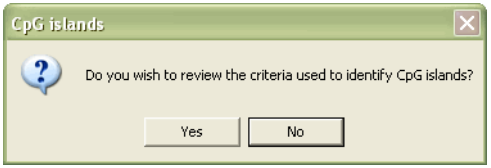
Finding CpG Islands

After annotating the imported sequence, you can search for target CpG islands to begin the process of designing effective primers.

1. Select **Design Primers ▸ Find CpG Islands.**



The CpG Islands dialog box prompts you to review the criteria currently set for identifying CpG islands.

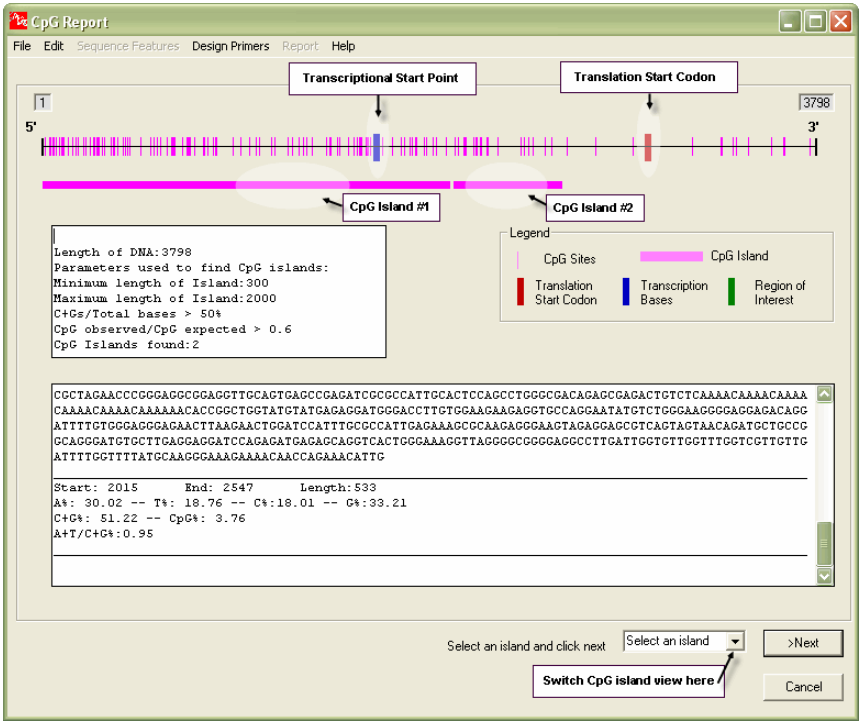


2. If you select:
   - **No** – The software uses default settings to search for CpG islands and a status window shows the progress.
   - **Yes** – In the Customize Settings dialog box that opens, adjust the parameter values set by Methyl Primer Express, then click **OK**. (The Frommer algorithm is used to predict CpG islands).

     IMPORTANT! Although the default settings are the recommended parameter values to run the software, you can modify settings according to the objectives of your experiment. For more details, see the *Methyl Primer Express® Software v1.0 Getting Started Guide.*
3. When the search ends, review the CpG Report as described next.

Reviewing the CpG Report

In the CpG Report, CpG sites are indicated by pink vertical bars along the horizontal axis. The transcription start point is indicated by a blue bar; the translation start codon is indicated by a red bar (if set – see the *Methyl Primer Express® Software v1.0 Getting Started Guide* for all optional step instructions); CpG islands are indicated by solid pink bars below the horizontal axis. Scroll through the report to review the search results.



Selecting a Target Sequence

After reviewing the CpG islands found in your sequence, you can select a portion of your sequence to use as the target sequence for primer design.

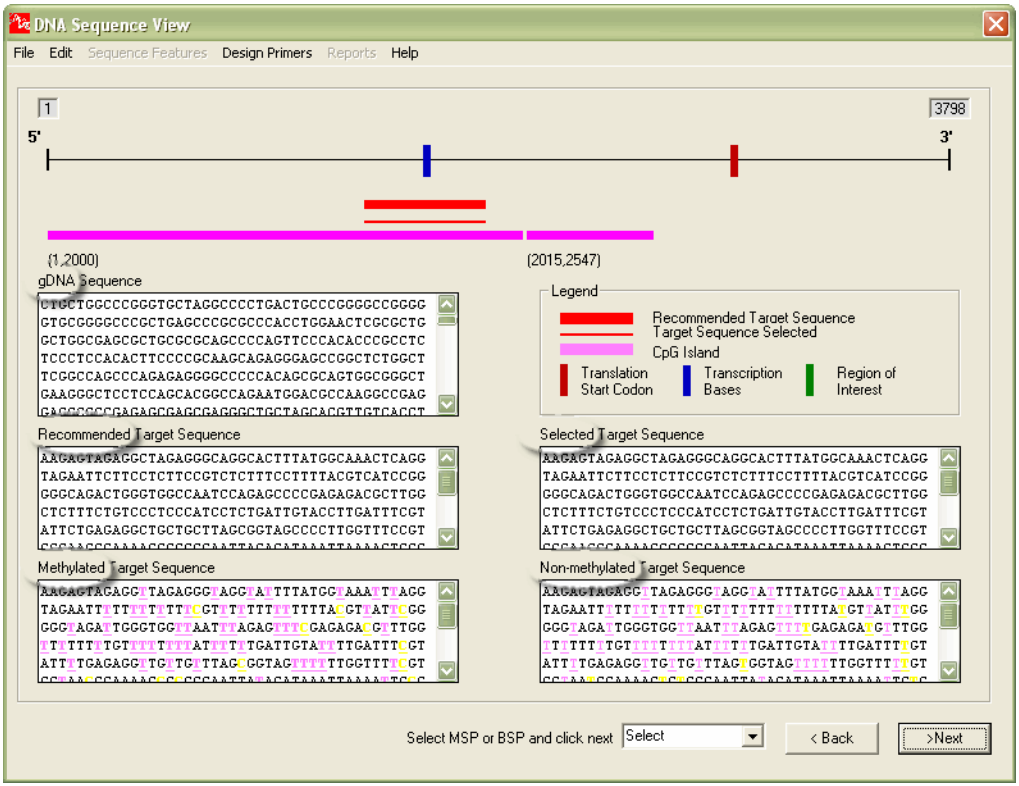
1. In the CpG Report, select the island of interest from the “Select an island” drop-down list at the bottom right of the screen, then click **Next**.
2. Select the region of the CpG island that you want to design primers from, or accept the default area (highlighted), then click **Next**.

Note: If you annotated the transciption start base, the software automatically selects the target sequence within 500 bases of the transciptional start point. If you did *not* annotate the transcription base, the CpG island that you see highlighted is automatically set as the target sequence. To overwrite the selection, select another target sequence, then click **Next**.

Reviewing the DNA Sequence Report

Review the DNA Sequence Report for the recommended target sequence.

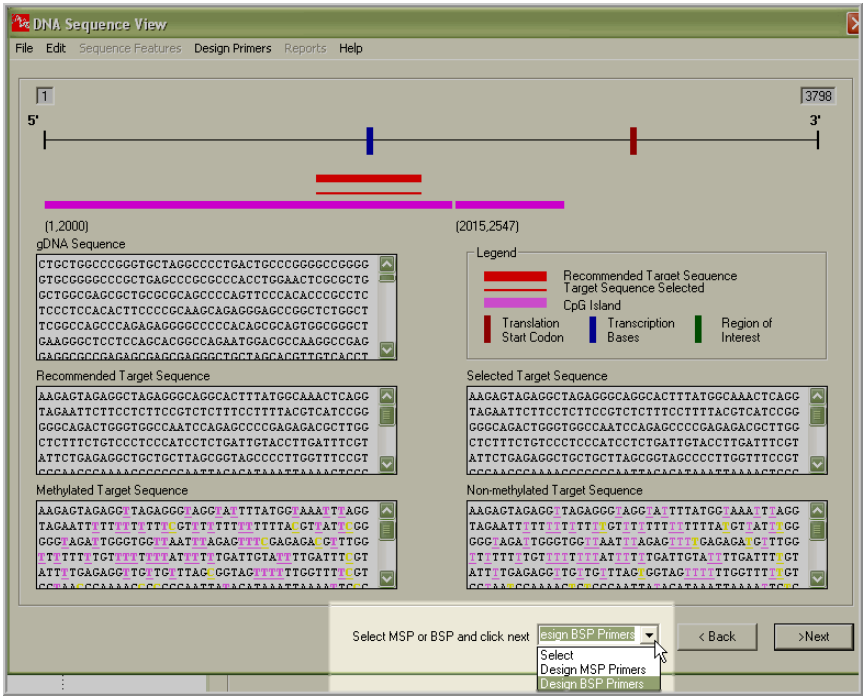
The DNA Sequence Report displays the Recommended, Selected, Methylated and Non-methylated sequences.



Selecting Primer Type

After you review the DNA Sequence Report, you can choose to design primers for either MSP or BSP. The workflow in this guide uses BSP primer design.

Select either MSP or BSP from the drop-down list in the DNA Sequence Report window.

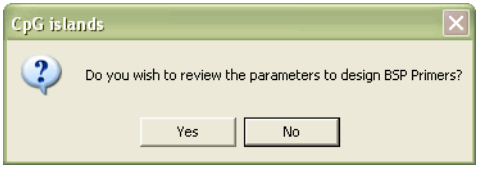


Designing BSP Primers

To design a BSP primer:

1. Select **Design BSP Primers** from the drop-down list at the bottom of the Sequence Report window, then click **Next.**

   A dialog box prompts you to review the criteria to design BSP primers.



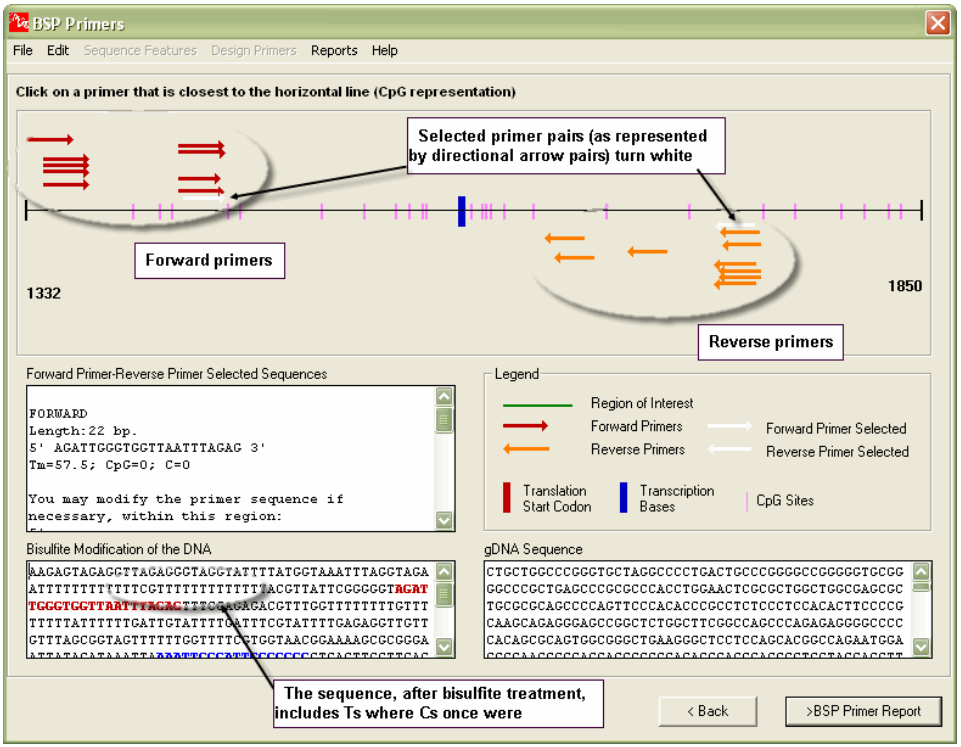
2. If you select:
   - **No** – The software uses default settings to design one or more BSP primers.
   - **Yes** – In the Customize Settings dialog box that opens, adjust the parameter values set by the software, then click **OK**.

     IMPORTANT! Each parameter value in the Customize Settings dialog box is used by the software to design primers. Although the default settings are recommended, you can modify settings according to the objectives of your experiment. For more details, see the *Methyl Primer Express® Software v1.0 Getting Started Guide.*

Selecting a Primer Pair

1. Review the BSP Primer Report to select the primer pair of choice.

   The software indicates the primer pairs by complimentary arrow pairs, as shown below. The best primer pair, as determined by the software, is the pair closest to the CpG island representation along the horizontal axis bar.

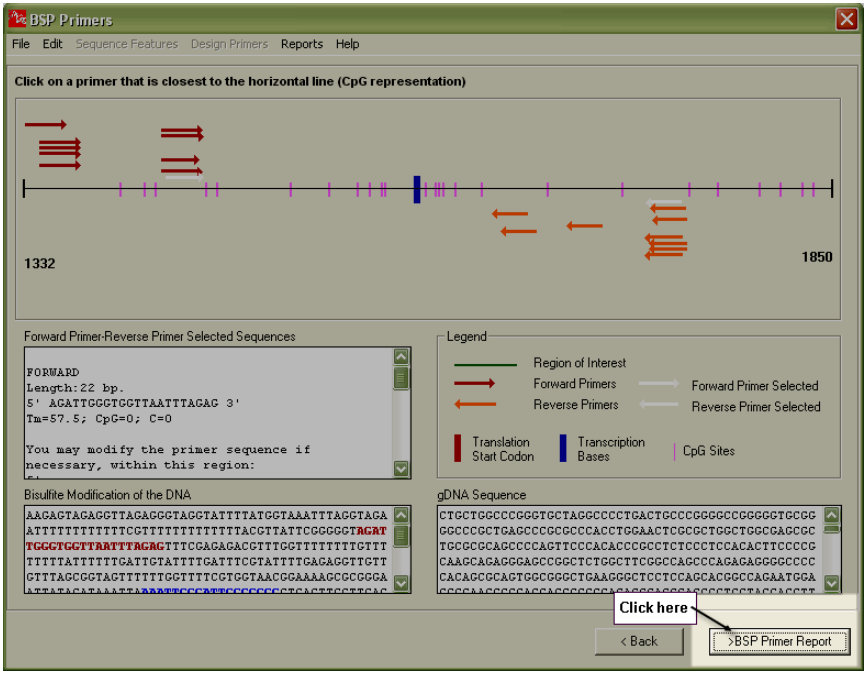


2. Click on an arrow pair to select the best primer. The selected primer pair turns white and the forward primer - reverse primer sequences are displayed in the selected sequences panel on the screen.

   Note: T bases (from simulated bisulfite transformation) are now present in place of C bases. All Cs contained within the CpG sites were methylated and protected against bisulfite transformation.

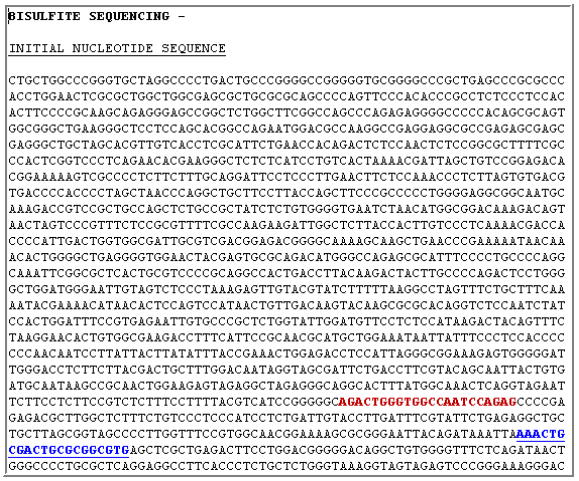
Creating the BSP Primer Report

1. Create a BSP primer report by clicking **BSP Primer Report**.



2. Review the BSP primer report by scrolling to see the details of your suggested primer design. The report is comprised of these four sections:
   - Initial nucleotide sequence
   - Bisulfite modification of DNA with forward and reverse primers
   - PCR product
   - Additional primers

Initial Nucleotide Sequence



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BISULFITE SEQUENCING -

INITIAL NUCLEOTIDE SEQUENCE

CTGCTGGCCCGGGTGCTAGGCCCCTGACTGCCCGGGGCCGGGGGTGCGGGGCCCGCTGAGCCCGCGCCC
ACCTGGAACTCGCGCTGGCTGGCGAGCGCTGCGCGCAGCCCCAGTTCCCACACCCGCCTCTCCCTCCAC
ACTTCCCCGCAAGCAGAGGGAGCCGGCTCTGGCTTCGGCCAGCCCAGAGAGGGGCCCCCACAGCGCAGT
GGCGGGCTGAAGGGCTCCTCCAGCACGGCCAGAATGGACGCCAAGGCCGAGGAGGCGCCGAGAGCGAGC
GAGGGCTGCTAGCACGTTGTCACCTCGCATTCTGAACCACAGACTCTCCAACTCTCCGGCGCTTTTCGC
CCACTCGGTCCCTCAGAACACGAAGGGCTCTCTCATCCTGTCACTAAAACGATTAGCTGTCCGGAGACA
CGGAAAAAGTCGCCCCTCTTCTTTTGCAGGATTCCTCCCTTGAACTTCTCCAAACCCTCTTAGTGTGACG
TGACCCCACCCCTAGCTAACCCAGGCTGCTTCCTTACCAGCTTCCCGCCCCCTGGGGAGGCGGCAATGC
AAAGACCGTCCGCTGCCAGCTCTGCCGCTATCTCTGTGGGGTGAATCTAACATGGCGGACAAAGACAGT
AACTAGTCCCGTTTCTCCGCGTTTTCGCCAAGAAGATTGGCTCTTACCACTTGTCCCTCAAAACGACCA
CCCCATTGACTGGTGGCGATTGCGTCGACGGAGACGGGGCAAAAGCAAGCTGAACCCGAAAAATAACAA
ACACTGGGGCTGAGGGGTGGAACTACGAGTGCGCAGACATGGGCCAGAGCGCATTTCCCCTGCCCCAGG
CAAATTCGGCGCTCACTGCGTCCCCGCAGGCCACTGACCTTACAAGACTACTTGCCCCAGACTCCTGGG
GCTGGATGGGAATTGTAGTCTCCCTAAAGAGTTGTACGTATCTTTTTAAGGCCTAGTTTCTGCTTTCAA
AATACGAAAACATAACACTCCAGTCCATAACTGTTGACAAGTACAAGCGCGCACAGGTCTCCAATCTAT
CCACTGGATTTCCGTGAGAATTGTGCCCGCTCTGGTATTGGATGTTCCTCTCCATAAGACTACAGTTTC
TAAGGAACACTGTGGCGAAGACCTTTCATTCCGCAACGCATGCTGGAAATAATTATTTCCCTCCACCCC
CCCAACAATCCTTATTACTTATATTTACCGAAACTGGAGACCTCCATTAGGGCGGAAAGAGTGGGGGAT
TGGGACCTCTTCTTACGACTGCTTTGGACAATAGGTAGCGATTCTGACCTTCGTACAGCAATTACTGTG
ATGCAATAAGCCGCAACTGGAAGAGTAGAGGCTAGAGGGCAGGCACTTTATGGCAAACTCAGGTAGAAT
TCTTCCTCTTCCGTCTCTTTCCTTTTACGTCATCCGGGGGCAGACTGGGTGGCCAATCCAGAGCCCCGA
GAGACGCTTGGCTCTTTCTGTCCCTCCCATCCTCTGATTGTACCTTGATTTCGTATTCTGAGAGGCTGC
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CGACTGCGCGGCGTGAGCTCGCTGAGACTTCCTGGACGGGGGACAGGCTGTGGGGTTTCTCAGATAACT
GGGCCCCTGCGCTCAGGAGGCCTTCACCCTCTGCTCTGGGTAAAGGTAGTAGAGTCCCGGGAAAGGGAC
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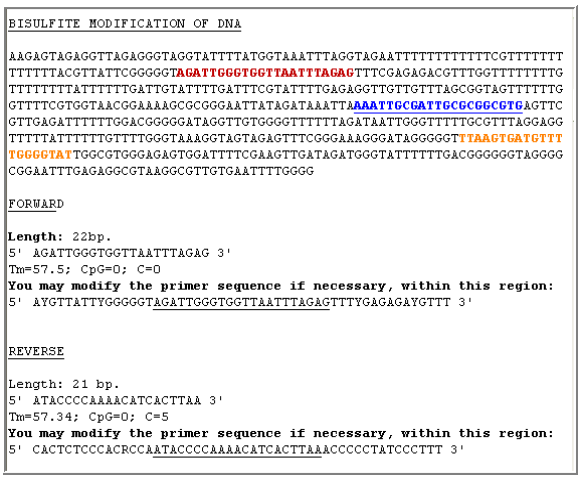
Bisulfite Modification of DNA with Forward and Reverse Primers



```
BISULFITE MODIFICATION OF DNA

AAGAGTAGAGGTTAGAGGGTAGGTATTTTATGGTAAATTTAGGTAGAATTTTTTTTTTTTCGTTTTTTT
TTTTTTACGTTATTCGGGGGTAGATTGGGTGGTTAATTTAGAGTTTCGAGAGACGTTTGGTTTTTTTTG
TTTTTTTTATTTTTTGATTGTATTTTGATTTCGTATTTTGAGAGGTTGTTGTTTAGCGGTAGTTTTTTG
GTTTTCGTGGTAACGGAAAAGCGCGGGAATTATAGATAAATTAAAATTGCGATTGCGCGGCGTGAGTTC
GTTGAGATTTTTTGGACGGGGGATAGGTTGTGGGGTTTTTTAGATAATTGGGTTTTTGCGTTTAGGAGG
TTTTTATTTTTTTGTTTTGGGTAAAGGTAGTAGAGTTTCGGGAAAGGGATAGGGGGTTTAAGTGATGTTT
TGGGGTATTGGCGTGGGAGAGTGGATTTTCGAAGTTGATAGATGGGTATTTTTTGACGGGGGGTAGGGG
CGGAATTTGAGAGGCGTAAGGCGTTGTGAATTTTGGGG

FORWARD

Length: 22bp.
5' AGATTGGGTGGTTAATTTAGAG 3'
Tm=57.5; CpG=0; C=0
You may modify the primer sequence if necessary, within this region:
5' AYGTTATTYGGGGGTAGATTGGGTGGTTAATTTAGAGTTTYGAGAGAYGTTT 3'


REVERSE

Length: 21 bp.
5' ATACCCCAAAACATCACTTAA 3'
Tm=57.34; CpG=0; C=5
You may modify the primer sequence if necessary, within this region:
5' CACTCTCCCACRCCAATACCCCAAAACATCACTTAAACCCCCTATCCCTTT 3'
```

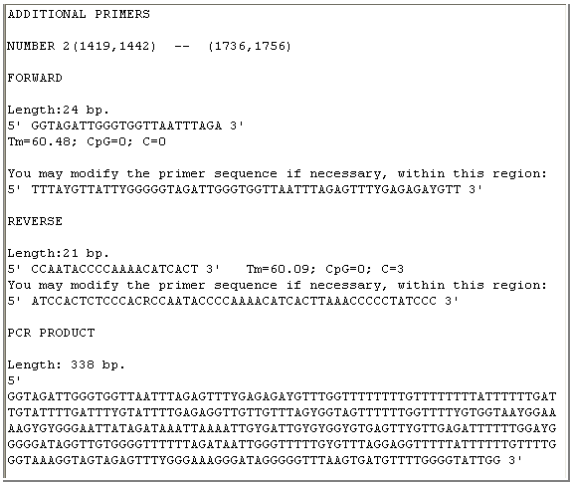
PCR Product

```
PCR PRODUCT

Length: 332 bp.
5'
AGATTGGGTGGTTAATTTAGAGTTTYGAGAGAYGTTTGGTTTTTTTTGTTTTTTTTATTTTTTGATTGT
ATTTTGATTTYGTATTTTGAGAGGTTGTTGTTTAGYGGTAGTTTTTTGGTTTTYGTGGTAAYGGAAAAG
YGYGGGAATTATAGATAAATTAAAATTGYGATTGYGYGGYGTGAGTTYGTTGAGATTTTTTGGAYGGGG
GATAGGTTGTGGGGTTTTTTAGATAATTGGGTTTTTGYGTTTAGGAGGTTTTTATTTTTTGTTTTGGGT
AAAGGTAGTAGAGTTTYGGGAAAGGGATAGGGGGTTTAAGTGATGTTTTGGGGTATT 3'

%CG=36.45
```

Additional Primers



```
ADDITIONAL PRIMERS

NUMBER 2(1419,1442)  --  (1736,1756)

FORWARD

Length:24 bp.
5' GGTAGATTGGGTGGTTAATTTAGA 3'
Tm=60.48; CpG=0; C=0

You may modify the primer sequence if necessary, within this region:
5' TTTAYGTTATTYGGGGGTAGATTGGGTGGTTAATTTAGAGTTTYGAGAGAYGTT 3'

REVERSE

Length:21 bp.
5' CCAATACCCCAAAACATCACT 3'   Tm=60.09; CpG=0; C=3
You may modify the primer sequence if necessary, within this region:
5' ATCCACTCTCCCACRCCAATACCCCAAAACATCACTTAAACCCCCTATCCC 3'

PCR PRODUCT

Length: 338 bp.
5'
GGTAGATTGGGTGGTTAATTTAGAGTTTYGAGAGAYGTTTGGTTTTTTTTGTTTTTTTTATTTTTTGAT
TGTATTTTGATTTYGTATTTTGAGAGGTTGTTGTTTAGYGGTAGTTTTTTGGTTTTYGTGGTAAYGGAA
AAGYGYGGGAATTATAGATAAATTAAAATTGYGATTGYGYGGYGTGAGTTYGTTGAGATTTTTTGGAYG
GGGGATAGGTTGTGGGGTTTTTTAGATAATTGGGTTTTTGYGTTTAGGAGGTTTTTATTTTTTGTTTTG
GGTAAAGGTAGTAGAGTTTYGGGAAAGGGATAGGGGGTTTAAGTGATGTTTTGGGGTATTGG 3'
```

You can now:

- Name the report by selecting **File ▸ Save As**.
- Export the report by saving it as a Microsoft® Word document (.doc)
- Print the report

Optional Ways to Annotate a Sequence

For additional ways to annotate your sequence, see the *Methyl Primer Express® Software v1.0 Getting Started Guide.* In the guide you will find instructions on:

- Naming your sequence
- Addtional methods to annotate the sequence with the transcription start point
- Methods to annotate the sequence with the translation start codon

04/2006