

Methyl Primer Express® Software v1.0

Quick Reference Card

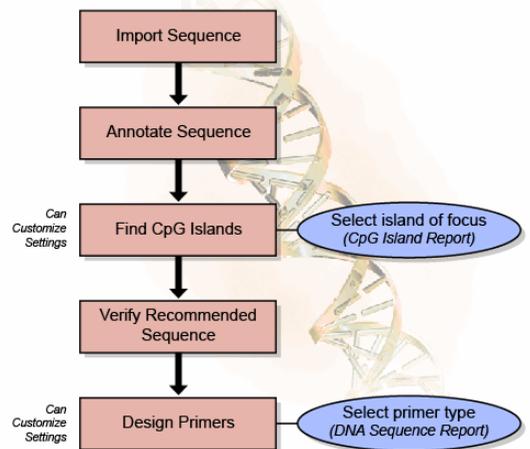
Overview

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 accommodate 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

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.

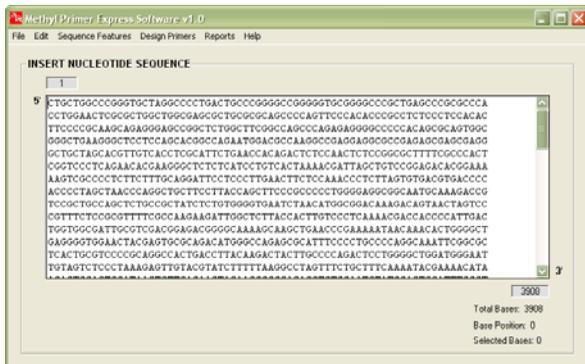
Using an Example Sequence

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.

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, H.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 [8634626](#)

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"
S'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.

MethylPrimer Express Software v1.0

File Edit Sequence Features Design Primers Reports Help

Renumber the Sequence

Enter Sequence Name

Set Transcription Bases

Set Translation Start Codon

Highlight Region of Interest

INSE

5

G TTCTAAGGAACACTGTGGCGAAGACCTTTCATTCGCCAACGCCATGCTGGAAATA

A EAATCCCTTATTACTTATATTTACCAGAACTGGAGACCTCCATTATGGCGGGAAAG

A PACGACTGCTTTGGACAATAGGTAGCGATTCTGACCTTCGTACACGCAATTAATCTG

TGATGCAATAAGCCCACTGGAGAGTAGAGGCTAGAGGGCAGGCACCTTATGGCAAACTCAGGTAGAAATTCCTCC

TCTTCGGCTCTCTTTCCTTTTACGTCATCCGGGGCAGACTGGGTGGCCAATCCAGAGCCCGGAGAGACGCTTGGCTC

TTTCTGCGCCCTCCATCCTCTGATTGTACCTTGATTTTCGTATTTCTGAGAGGCTGCTCTTACGGGTAGCCCTTGGT

TTCCGGTGGCAACGGAAAAGCCGGGAATTACAGATAAATTAAGAGGCTGCGGCTGAGCTCCGCTGAGACTT

CCTGGAGGGGGCAGGCTGTGGGTTTCTCAGATAACTGGCCCTGCGCTCAGAGGCGCTTCAACCTCTGCTCTG

GGTAAAGTAGTAGACTCCGGAAAAGGGACAGGGGGCCAAAGTAGTCTTGGGTAAGGCTGAGGCTGGAGAGTGGAT

TTCCGAAGCTGACAGATGGTATTCTTTCAGCGGGGAGTGGGGCGGAACCTGAGAGGCTAAGGCGTTGTGAACCT

GGGGAGGGGGGCACTTTGTAGTCCGAGGGAAAGCGCTGAGGATCAGGAAGGGGGCACTGAGTGTCCGTGGGGAAAT

CCTCGTATAGGAACCTGGAATATGCCCTTACGGGACACTATGCTTTTAAAAACGTCGGCTGGTCAATGAGGTCAGGA

GTTCCAGACCAGCCCTGACCAACGTAAGGTGAACCTCCGCTCTACTAAAAATACAAAAATTAGCCGGGCGTGGTGGCG

CTCCAGCTACTCAGGAGGCTGAGGAGGAGGAATCGCTAGAACCCGGGAGGCGGAGGTTGCAAGTGAAGCCAGAGATCGCG

3798

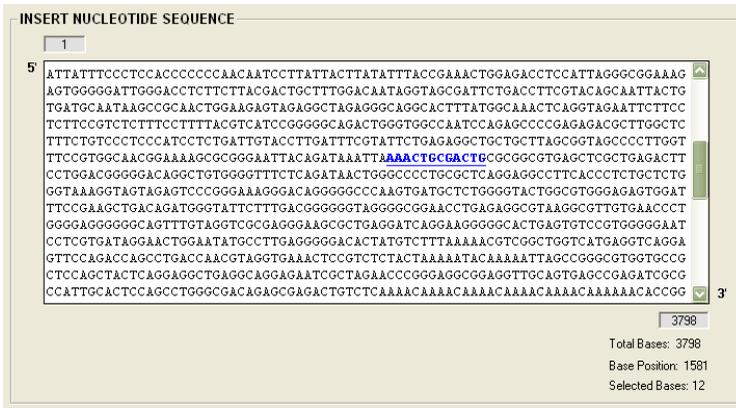
Total Bases: 3798

Base Position: 1581

Selected Bases: 12

Base position adjusts as you move the cursor around in the sequence, making it easy to find your start point.

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

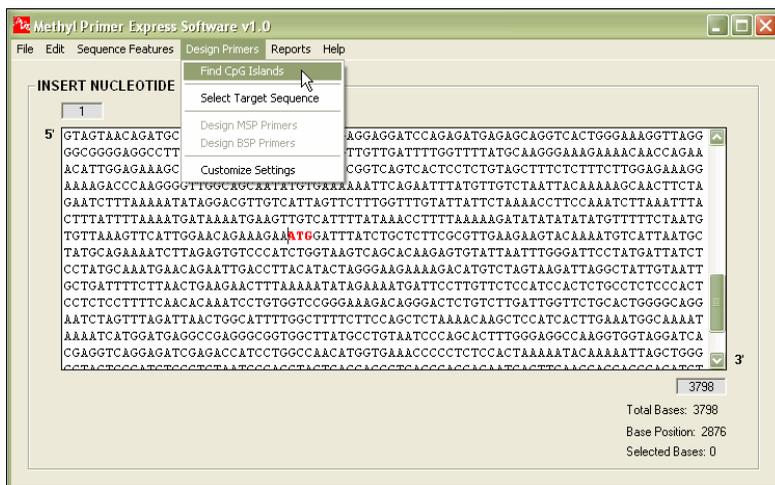


With the sequence imported and annotated, you are ready to have the software find the CpG islands.

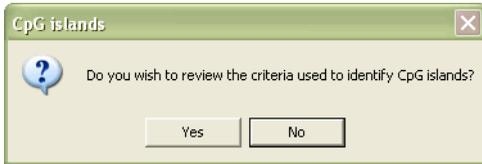
Finding CpG Islands

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:

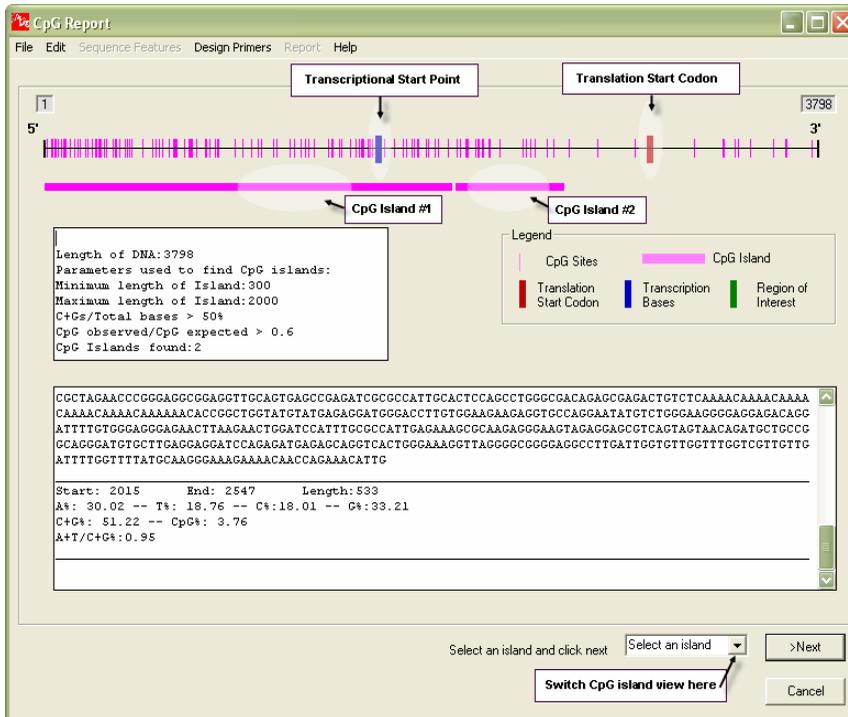
- **No** – The software uses default settings to search for CpG islands and a status window shows the progress.
- **Yes** – In the Customize Settings dialog box that opens, adjust the parameter values set by Methyl Primer Express, then click **OK**. (The Frommer algorithm is used to predict CpG islands).

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.



File Edit Sequence Features Design Primers Report Help

Transcriptional Start Point Translation Start Codon

3798

5' 3'

CpG Island #1 CpG Island #2

Legend

- CpG Sites
- Translation Start Codon
- Transcription Bases
- CpG Island
- Region of Interest

Length of DNA: 3798
Parameters used to find CpG islands:
Minimum length of Island: 300
Maximum length of Island: 2000
C+G/Total bases > 50%
CpG observed/CpG expected > 0.6
CpG Islands found: 2

```
CGCTAGAACCOCGGAGGCGGAGGTTGCAGTGAGCGGAGATCGGCCATTGCCACTCCAGCCTGGGCGACAGAGCGGACTGTCTCAAAAACAAAACAAA  
CAAACAAAACAAAACAAAACCCGGCTGGTATCTATGACAGGATGGCCTTCTGCAAGACAGAGGTGCCAGCAATATCTCTGGGAAGGGGAGGACAGG  
ATTTTGTGGAGCGCAGCAACTTAAACAAGCTGGATCCATTTTCGCCATTGACAAAAGCGCAAGGGAACTAGACGGAGCCTCAGTACTAACAGATGCTGCC  
GCAGCGATGTGTTGACAGGATCCAGAGATGACAGCAGGTCACTGGCAAAGCTTAGGGCCGGGGAGCCCTTGATGTGCTTGGTTGCTGCTGTTGTC  
ATTTTGGTTTTATCCAAAGGCAAAAACAAAACAAACCCAGAAACATTG
```

Start: 2015 End: 2547 Length: 533
A#: 30.02 -- T#: 18.76 -- C#: 18.01 -- G#: 33.21
C+G: 51.22 -- CpG#: 3.76
A+T/C+G: 0.95

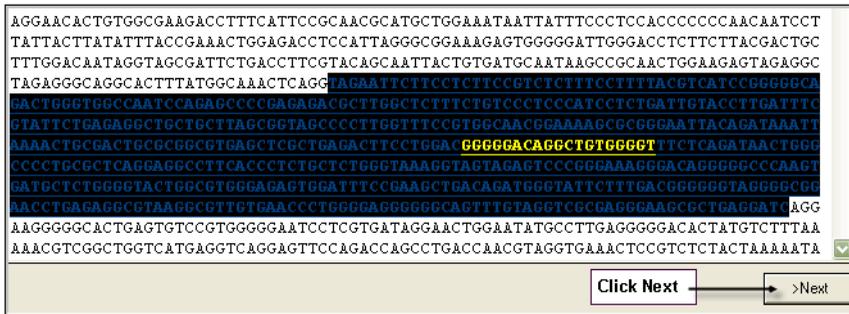
Select an island and click next Select an island >Next

Switch CpG island view here Cancel

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**.



The screenshot displays a DNA sequence within a web-based interface. The sequence is as follows:
AGGAACACTGTGGCGAAGACCTTTCATTCCGCCAACGCATGCTGGAAATAATTATTTCCCTCCACCCCCCAACAATCCT
TATTACTTATATTTACCGAAACTGGAGACCTCCATTAGGGCGGAAAGAGTGGGGGATTGGGACCTCTTCTTACGACTGC
TTTGGACAATAGGTAGCGATTCTGACCTTCGTACAGCAATTACTGTGATGCAATAAGCCGCAACTGGAAGAGTAGAGGC
TAGAGGGCAGGCACCTTTATGGCAAACTCAGGAGBAATTCCTCTCTCCCTCTCTTTCCCTTTACGTCAATCCGGGGGA
GACTGGGTGGCCAAATCCGAGCCCCGAGAGACGCTTGGCTCTTTCTGTCCCTCCCATCTCTGATTGTACCTTGAATTC
GTATTCGAGAGGCTGCTGCTTAGCGGTAGCCCTTGGTTCCGTGGCAACGGAAAAGCGCGGGAAATACAGATAAAAT
AAAACTCCGACTCCGCGGCTGAGCTCGTGAAGCTTCTTGAAGGGGACAGGCTGTGGGTCTCTCAGATAACTGGG
CCCTGCGCTCAGGAGGCTTACCCCTCTGCTCTGGGTAAAGGTAAGTAGAGTCCCGGCAAAAGGGACAGGGGGCCCAAGT
GATGCTCTGGGGTACTGGCGTGGGAGATGGATTCCGAACTGACAGATGGGTATTCCTTTGACGGGGGTAGGGGGGG
AACCTGAGAGGCGTAAGGCGTTGTGAACCTGGGGAGGGGGGCACTTTGTAGGTCGCGAGGGAAAGCGCTGAGGATCAGG
AAGGGGGCACTGAGTGTCCGTGGGGAAATCCTCGTGATAGGAACTGGAAATATGCCTTGAGGGGGACACTATGCTTTAA
AAACGTCGGCTGCTATGAGGTCAGGAGTCCAGACCAGCTGACCAACGTAAGGTGAAACTCCGTCTCTACTAAAAATA

The region from the 13th to the 23rd base of the second line is highlighted in yellow, representing the selected target sequence: **AGBAATTCCTCTCTCCCTCTCTTTCCCTTTACGTCAATCCGGGGGA**. Below the sequence, there is a button labeled "Click Next" with an arrow pointing to a ">Next" button.

Note: If you annotated the transcription start base, the software automatically selects the target sequence within 500 bases of the transcriptional 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.

The screenshot displays the 'DNA Sequence View' application window. At the top, a horizontal line represents the DNA sequence from 5' to 3', with a scale from 1 to 3798. A red vertical bar indicates a CpG Island, and a blue vertical bar indicates a Region of Interest. Below the line, four sequence panels are shown:

- gDNA Sequence (1,2000):** A long DNA sequence with a green bar highlighting a CpG Island.
- Recommended Target Sequence (2015,2547):** A DNA sequence with a red bar highlighting a CpG Island.
- Methylated Target Sequence:** A DNA sequence with yellow bars highlighting methylated CpG sites.
- Non-methylated Target Sequence:** A DNA sequence with green bars highlighting non-methylated CpG sites.

A legend on the right side of the window defines the symbols used:

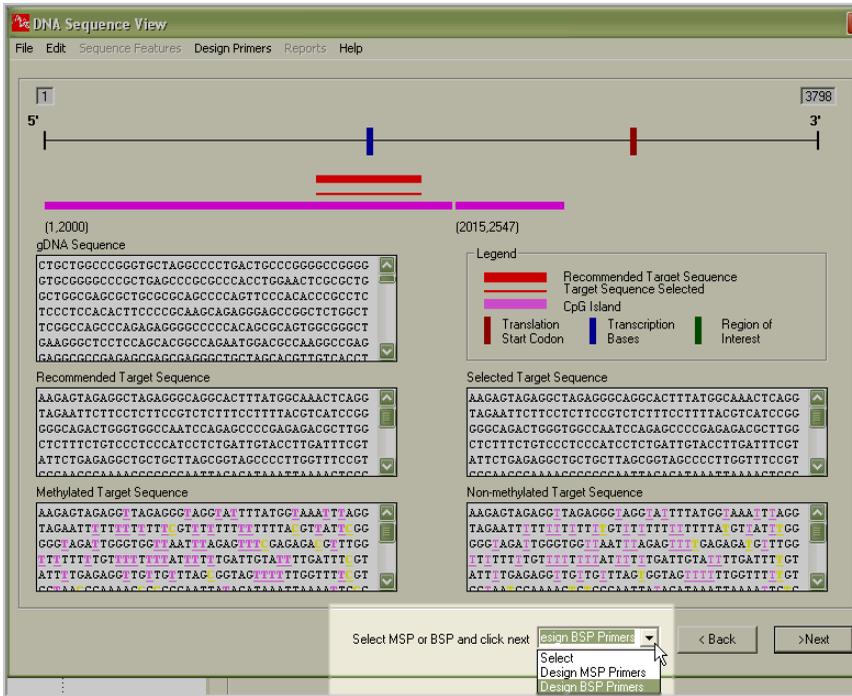
- Red bar: Recommended Target Sequence
- Blue bar: Target Sequence Selected
- Green bar: CpG Island
- Red vertical bar: Translation Start Codon
- Blue vertical bar: Transcription Bases
- Green vertical bar: Region of Interest

At the bottom of the window, there is a dropdown menu labeled 'Select MSP or BSP and click next' with 'Select' chosen, and buttons for '< Back' and '>Next'.

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.

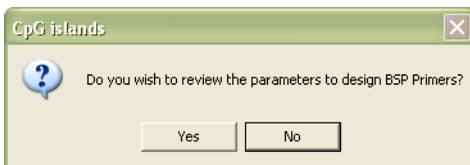


Designing BSP Primers

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.

Click on a primer that is closest to the horizontal line (CpG representation)

Selected primer pairs (as represented by directional arrow pairs) turn white

Forward primers

Reverse primers

Forward Primer-Reverse Primer Selected Sequences

```
FORWARD
Length: 22 bp.
5' AGATTGGGTGCTTAATTTAGAG 3'
Tm=57.5; CpG=0; C=0

You may modify the primer sequence if
necessary, within this region:
```

Bisulfite Modification of the DNA

```
AGACGTAGAGGTTAGACGGTAGGTATTTTATGCTAAATTTAGGTAGA
ATTTTTTTTTTTTCGTTTTTTTTTTTTTTTACGTTATTCGGGGCTAGAT
TGGGTGGTTARTTTCGCTTTCGTAACGGAAAAGCCCGGA
TTTTATTTTTTTCATTGTATTTTCTGTAACGGAAAAGCCCGGA
GTTTACCGGTACTTTTTTGGTTTTTCTGTAACGGAAAAGCCCGGA
ATTATACATAAATAATTCGCAATTCCTCTACTTCTCTC
```

Legend

- Region of Interest
- Forward Primers
- Reverse Primers
- Translation Start Codon
- Transcription Bases
- CpG Sites
- Forward Primer Selected
- Reverse Primer Selected

gDNA Sequence

```
CTGCTGGCCCGGCTAGGCCCTGACTGCCGGGGCCGGGGTTCGG
GGCCCGCTGAGCCCGGCCACCTGGAACTCGGGTGGCGAGCCG
TGGCCGACGCCCACTTCCACACCCGGCTCTCCCTCCACATTCGCC
CAAGCAGAGCCGAGCCGGCTCTGGCTTCGGCCAGCCAGAGCCGCC
CACAGCCAGTGGGGCTCAAGGGCTCCTCCAGCACGCCAGAAATGA
GGCCAAACCCGACCCAGCCGACCCAGCCAGCCCTCTACCACTT
```

The sequence, after bisulfite treatment, includes Ts where Cs once were

< Back

> BSP Primer Report

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.

Initial Nucleotide Sequence

BISULFITE SEQUENCING -
<u>INITIAL NUCLEOTIDE SEQUENCE</u>
<pre>CTGCTGGCCCGGGTGTCTAGGCCCTGACTGCCCGGGGCGGGGGTGGGGGGCCCGTGTAGCCCGCGCC ACCTGGAACTCGCGCTGGCTGGCGAGCGCTGGCGCGAGCCCGAGTTCACACACCCGCTCTCCCTCCAC ACTTCCCGCAAGCAGAGGGGAGCCGGCTCTGGCTTCGGCCAGCCAGAGAGGGGCCCCACACGCGAGT GGCGGGCTGAAGGGCTCCTCAGCAGCGCCAGAAATGGACGCCAAGGCCGAGGAGGGCCGAGAGCGGAGC GAGGGCTGTAGCAGTTGTACCTCGCATTTCTGAAACCCAGCAGCTCTCAACTCTCCGGGCGTTTTGCG CCACTGGTCCCTAGAACAGAAAGGGCTCTCTCATCCGTGCACTAAAACGATTAGCTGTCCGGAGACA CGGAAAAAGTCCGCCCTCTCTTTTTCAGAGATTCCCTCCCTGAACTTCGCCAACCCCTTTAGTGTGAGC TGACCCACCCCTAGCTAACCCAGCGTCTTCTTACAGCCTCCCGCCCGCTGGGAGGGCGGCATGC AAAGACCGTCCGCTGCCAGCTCTCCCGCTATCTCCGGGGTGAATCAACATGGCGGCAAAAGCAGT AACCCTAGCCGTTTTCTCCGGTTTTTCCGCCAAGAAGATTGGCTCTTACCACCTGTCCCTCAAAACGACCA CCCTTTGACTGTGGGATTTGGCTGCAGCGAGCGGGGCAAAAGCAAGTGAACCGAAAAATAACAA ACACTGGGGCTGAGGGTGGAACTAGCAGTGGCGAGCATTGGCCAGAGGGGATTTCCCTCCGCCGAGG CAAAATTCGGCGCTCACTGGCTCCCGCAGGCCACTGACCTTACAAGACTACTTGGCCAGACTCTGGG GCTGGATGGGAATTTAGTCTCCCTAAAGAGTTGTAGCTATCTTTTAAAGGCTAGTTTCTGCTTTCAA AATACGAAAAACATAACACTCAGTCCATAACTGTGACAAGTACAAGCGGCGACAGCTTCCAATCTAT CCACTGGATTTCCGGAGAAATTTGCCCGCTGTGGTATTTGGATGTTCCTCATAAAGACTACAGTTTC TAAGAACACTGTGGCGAAGACTTTTACCTCCGCAACGCATGCTGGAAATAATTTTCCCTCAACCCC CCCAACAATCCTTATTACTTATATTTACGAAACTGGAGACCTCAATTAGGGCGAAAGAGTGGGGAT TGGGACTCTTCTTACGACTGCTTTGGACAAATAGTGTAGCGATTCTGACCTCTGTACAGCAATTAATGTG ATGCAATAAGCGCAACTTGAAGAGTAGAGGCTAGAGGGCAGGCACCTTATGGCAACTCAGGTAGAAT TCTTCTCTCCGCTCTTCTTCTTACGTCAATCCGGGGGCAGACTGGGTGGCCAACTCAGAGCCCCGA GAGAGCTTGGCTCTTCTGCTCCCTCCATCCTCTGATTGTACCTTGAATTCGATTCAGAGAGCGTCG TGTCTAGCGGTAGCCCTTGGTTTTCCGTGGCAACGGAAAGCGGGAAATACAGATAAATAAACTG CGACTGGCGGCTEAGCTCCGTGAGACTTCCGTGACGGGGGACGGCTGGGGTTTTCTCAGATAACT GGGCCCTCGCTCAGGAGGCTTCAACCTCTGCTTGGGTAAAGTAGTAGACTCCGGGAAGGAGC</pre>

Bisulfite Modification of DNA with Forward and Reverse Primers

BISULFITE MODIFICATION OF DNA
<pre>AAAGTAGAGGTTAGAGGGTAGGTATTTTAGTAAATTTAGGTAGAAATTTTTTTTTTTCGTTTTTTT TTTTTTACGTTATTCGGGGTTGATTTGGTGGTAAATTTAGAGTTTCGAGAGACGTTGGTTTTTTTTG TTTTTTTTATTTTTTGATTGTATTTTGATTCGTATTTTGGAGGGTTGTTGTTTAGCGGTAGTTTTTGG TTTTTTCGGTAAACGGAAAAGCGCGGGAATATAGATAAATAAATTTGCGATTCGCGGCGTAGTTC GTTGAGATTTTTTTGGACGGGGATAGGTTGGGGTTTTTGTAGATAATTTGGGTTTTTGGGTTTTAGGAG TTTTTATTTTTTGGTAAAGGTAGTAGAGTTCCGGAAAAGGGATAGGGGGTTTAAGTGAATGTT TGGGGTATTGGCGTGGGAGAGTGGATTTTCAAAGTTGATAGTGGGTATTTTTTTCAGCGGGGGTAGGG CGGAATTTGAGAGCGTAAGCGCTGTGAAATTTGGGG</pre>
<u>FORWARD</u>
Length: 22bp. 5' AGATTGGGTGGTTAATTTAGAG 3' Tm=57.5; CpG=0; C=0 You may modify the primer sequence if necessary, within this region: 5' AYGTATTTGGGGTAGATTGGGTGGTAAATTTAGAGTTTTCGAGAGAYTT 3'
<u>REVERSE</u>
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' CACTCTCCACRCCAATACCCCAAAACATCACTTAAACCCCTATCCCTTT 3'

PCR Product

PCR PRODUCT
Length: 332 bp. 5' AGATTGGGTGGTTAATTTAGAGTT YGAGAGAY GTTCGGTTTTTTTTGTTTTTTTATTTTTTGGATTGT ATTTTGATTT YG TATTTTGGAGAGGTTGTTGTTAG YGGT AGTTTTTGGTTTT YGTGGTAA YGGAAAAG YGYGGG AATTAAGATAAAATTAATAAT YG ATT YGYGG YGTGAGTT YGTGAG ATTTTTGGAYGGG GATAGTTTGGGGTTTTTATAGATAATTTGGTTTTT YGT TTAGAGAGTTTATTTTTTCTGTTTTGGGT AAAGGTAGTAGAGTT YGG AAAAGGATAGGGGGTTAAAGTATGTTTTGGGGTAT 3' %CGs=36.45

Additional Primers

ADDITIONAL PRIMERS
NUMBER 2 (1419,1442) -- (1736,1756)
<u>FORWARD</u>
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' TTTAYGTTATTTGGGGTAGATTGGTGGTTAATTTAGAGTTTTCGAGAGAYTT 3'
<u>REVERSE</u>
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' ATCCACTCTCCCAACCAATACCCCAAAACATCACTTAAACCCCTATCCC 3'
<u>PCR PRODUCT</u>
Length: 338 bp. 5' GGTAGATTGGGTGGTTAATTTAGAGTTTTCGAGAGAYTTGGTTTTTTTTGTTTTTTTATTTTTTGTAT TGTATTTTGGATTYGTATTTTGGAGGTTGTTGTTAGYGGTAGTTTTTGGTTTTYGTGGTAAAYGGA AAGYGGGAATTATAGATAAAATTAATAAT YGYGGT YGYGGYGTGAGTT YGTGAG ATTTTTTGGAY GGGATAGGTTGGGGTTTTTATAGATAATTTGGTTTTT YGYGGT TAGGAGTTTTTATTTTTTGGTTTT GGTAAAGGTAGTAGAGTTT YGG AAAAGGATAGGGGGTTAAAGTATGTTTTGGGGTATTGG 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
- Additional methods to annotate the sequence with the transcription start point
- Methods to annotate the sequence with the translation start codon

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04/2006

