Applied Biosystems

Methyl Primer Express® Software

Version 1.0

Methyl Primer Express® Software

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Contents

Preface

How to Use This Guide

Purpose of This Guide

The *Methyl Primer Express® Software v1.0 Getting Started Guide* provides step-by-step instructions for using Methyl Primer Express software to find CpG islands and design primers for methylation-focused experiments.

Audience

This guide is intended for novice and experienced researchers designing primers based on DNA methylation patterns.

Assumptions

This guide assumes that:

- *Methyl Primer Express® Software v1.0* has been installed on your computer.
- You have a working knowledge of the Microsoft® Windows XP or Windows 2000 operating system.
- You are familiar with gene sequence databases such as GenBank.

Text Conventions

This guide uses the following conventions:

- **Bold** text indicates user action. For example:

  Type **0**, then press **Enter** for each of the remaining fields.
- *Italic* text indicates new or important words and is also used for emphasis. For example:

  Before analyzing, *always* prepare fresh matrix.
- A right arrow symbol (▸) separates successive commands you select from a drop-down or shortcut menu. For example:

  Select **File ▸ Open ▸ Spot Set**.

  Right-click the sample row, then select **View Filter ▸ View All Runs**.

User Attention Words

Two user attention words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action as described below:

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

How to Obtain More Information

Related Documentation

The following related document is shipped with the software:

Methyl Primer Express® Software v1.0 Quick Reference Card (P/N 4370962) – Provides brief step-by-step procedures for following a typical workflow using the Methyl Primer Express® software.

Note: To open the user documentation included on the software installation CD, use the Adobe® Acrobat® Reader® software available at **www.adobe.com**.

Note: For additional documentation, see "How to Obtain Support" on page viii.

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IMPORTANT! The e-mail address above is only for submitting comments and suggestions relating to documentation. To order documents, download PDF files, or for help with a technical question, go to **http://www.appliedbiosystems.com**, then click the link for **Support**. (See "How to Obtain Support" below).

How to Obtain Support

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Safety Information

This section covers:

Safety Conventions Used in This Document

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

Definitions

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Examples

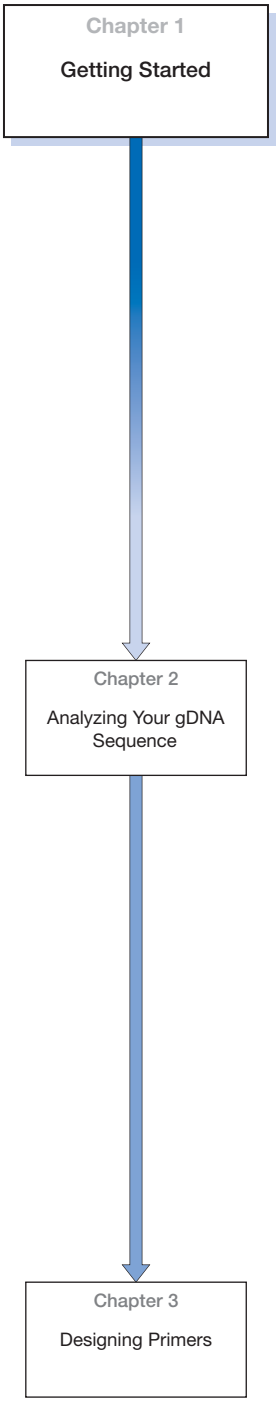
The following examples show the use of safety alert words:

IMPORTANT! The sample name, run folder name, and path name, *combined*, can contain no more than 250 characters.

CAUTION **MUSCULOSKELETAL AND REPETITIVE MOTION HAZARD**. These hazards are caused by potential risk factors that include but are not limited to repetitive motion, awkward posture, forceful exertion, holding static unhealthy positions, contact pressure, and other workstation environmental factors.

WARNING Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

1 Getting Started



This chapter covers:

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About Methyl Primer Express® Software

Methyl Primer Express® Software allows you to design primers for bisulfite sequencing (BSP) and/or for 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.

About DNA Methylation

Epigenetics is the study of inheritable and acquired differences in gene expression patterns that are not attributable to changes in primary DNA sequence. DNA methylation is the most outstanding epigenetic modification.

DNA methylation is one of several post-synthetic modifications that normal DNA goes through after each replication. Only the cytosines adjacent to guanine (CpG sites) are substrates for methylation by methyltransferases in mammalian cells. During DNA replication, the methylation state is conserved by a maintenance methyl transferase. Because not all CG base pairs in DNA are methylated, the maintenance methylase enzyme must recognize the hemimethylated status and ensure that each piece of newly synthesized DNA is methylated appropriately.

Methylation has two distinct functions. It acts as:

a. Protection from endonucleases designed to destroy foreign DNA

b. Regulation in gene expression – the control for turning expression on or off.

Portions of the human genome that are rich in CpG dinucleotides, when compared with their surrounding regions, are referred to as CpG islands. These discrete clusters of unmethylated CpG dinucleotides occur in over half of the known human genome promoters. Hypermethylation at a CpG site can eventually shut down expression of a gene involved in any complex biological processes such as aging. Global hypomethylation at non-CpG sites usually accompanies the hypermethylation of the promoter regions of genes now known to play a crucial role in the development of a variety of cancers.

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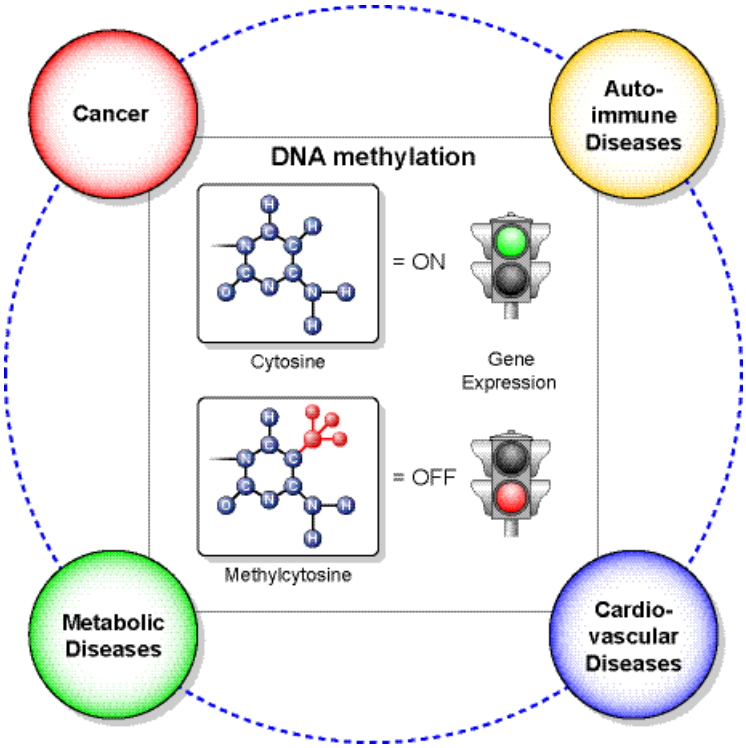

A great amount of interest is paid to the promoter CpG islands because when they become methylated, the gene associated becomes silenced, and this silencing is transmitted through mitosis. CpG island methylation is thought to represent an epigenetic means of inheritance where changes in gene expression are passed on through cell division, without associated DNA sequence alterations. Often studies are designed specifically to determine if a correlation exists between the methylation status of the CpG islands in the promoter regions and the amount of expression in the gene of interest.

One proven method of studying methylation patterns, is to treat gDNA with bisulfite to distinguish methylated cytosine from unmethylated cytosine. Bisulfite treatment converts unmethylated cytosine into uracil (U). As a result, the DNA sequence is changed and any cytosines remaining in a sequence indicate the position of methylated cytosines.

About the Example Workflow

The example gene sequence you use to perform the tasks in this guide is the BRCA1 gene sequence from the GenBank database (File No. U37574).

Using This Guide with Your Own Sequence Files

In addition to using this guide to design primers associated with the example gene (BRCA1), you can use the same or modified workflow with any available gDNA sequence.

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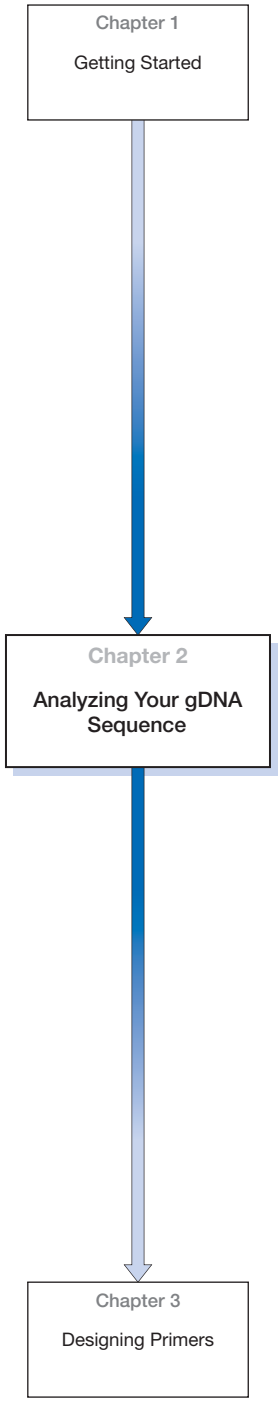
Notes

2 Analyzing Your gDNA Sequence



This chapter covers:

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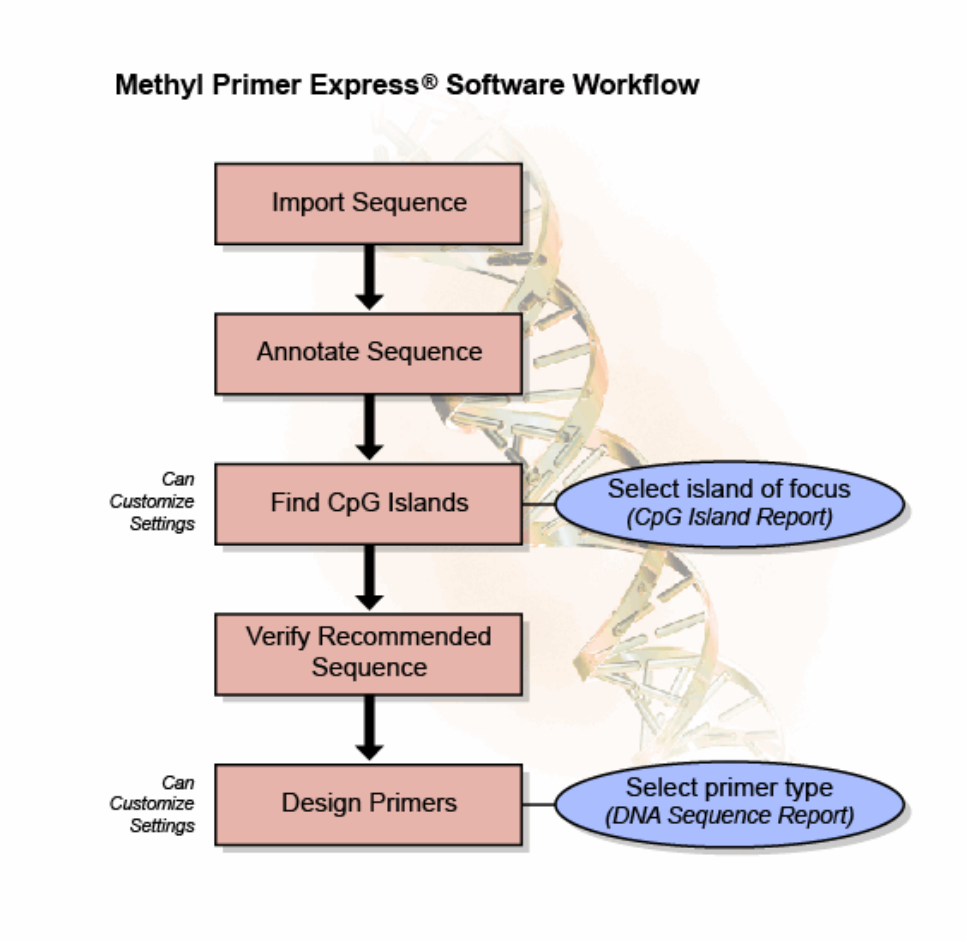
Workflow Overview

In This Chapter

In this chapter 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

The workflow for using Methyl Primer Express® Software to find CpG islands on an imported sequence, and ultimately design PCR primers, is shown below.



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Starting Methyl Primer Express® Software

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

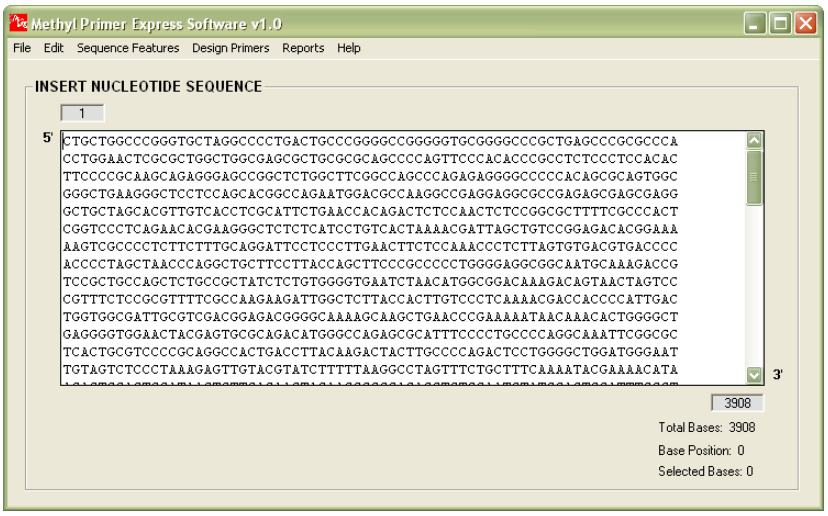
Example Workflow

This guide 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.

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Annotating the Sequence

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

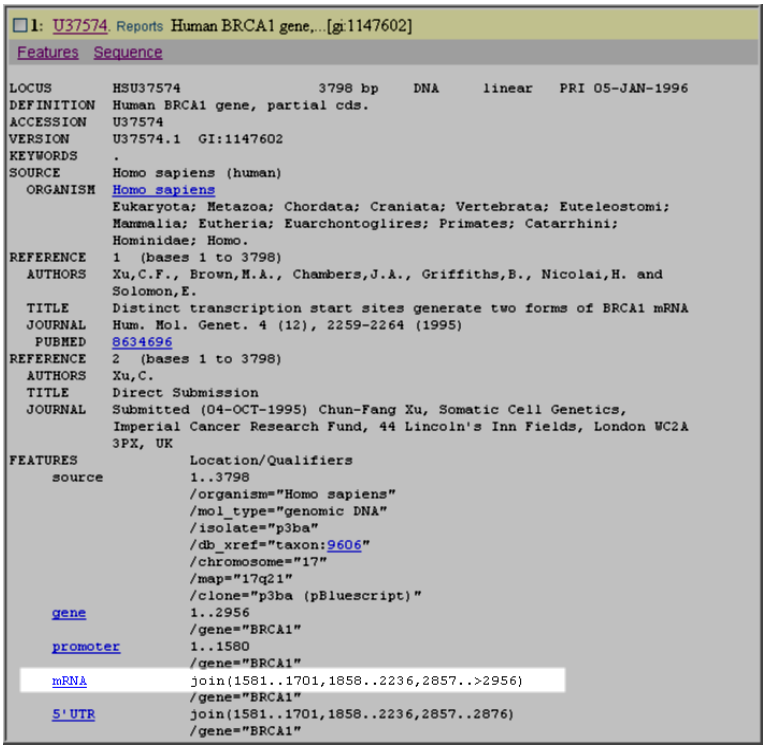
- A user-specified sequence name (page 14)
- The transcription start point of the gene sequence (page 8, page 14)
- The translation start codon of the gene sequence (page 15)

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.

To annotate 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.



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.

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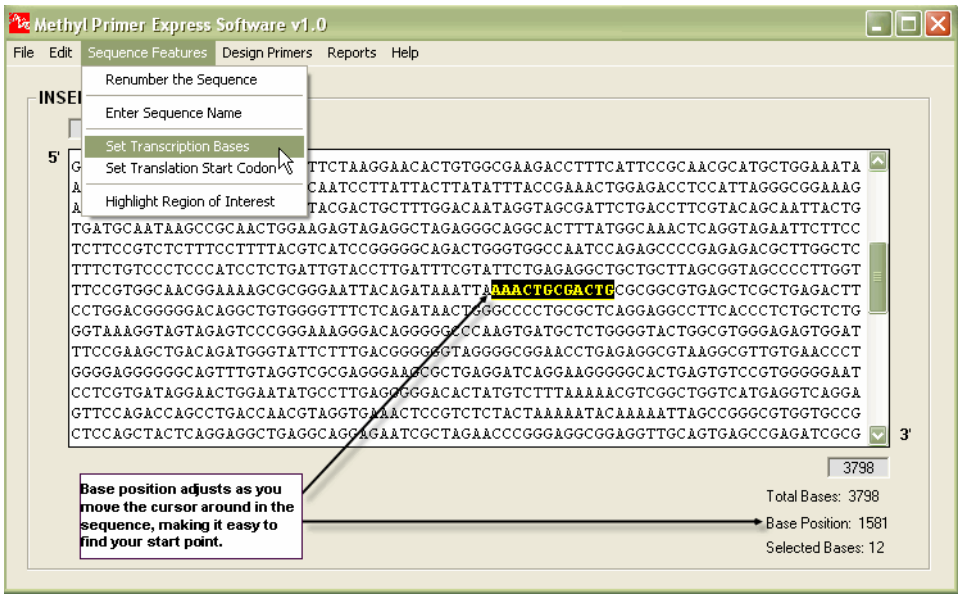
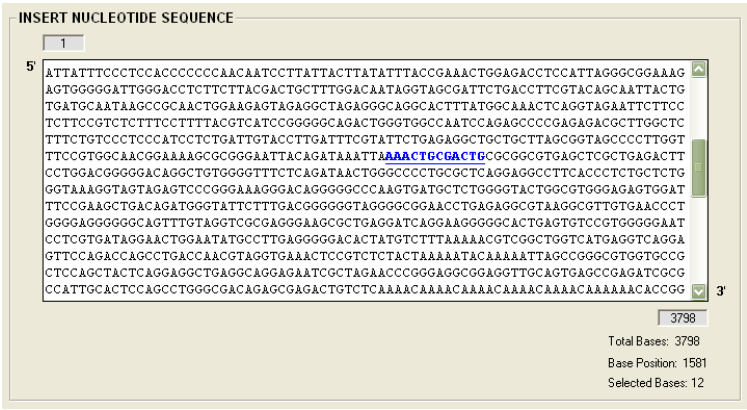


5. Select **Sequence Features ▸ Set Transcription Bases**. The transcription bases are highlighted and underlined in blue. Scroll to see the annotated bases.



Note: As an option, and in addition to annotating the sequence with the transcription start point, you can annotate the sequence with the Translation Start Codon as described on page 15.

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

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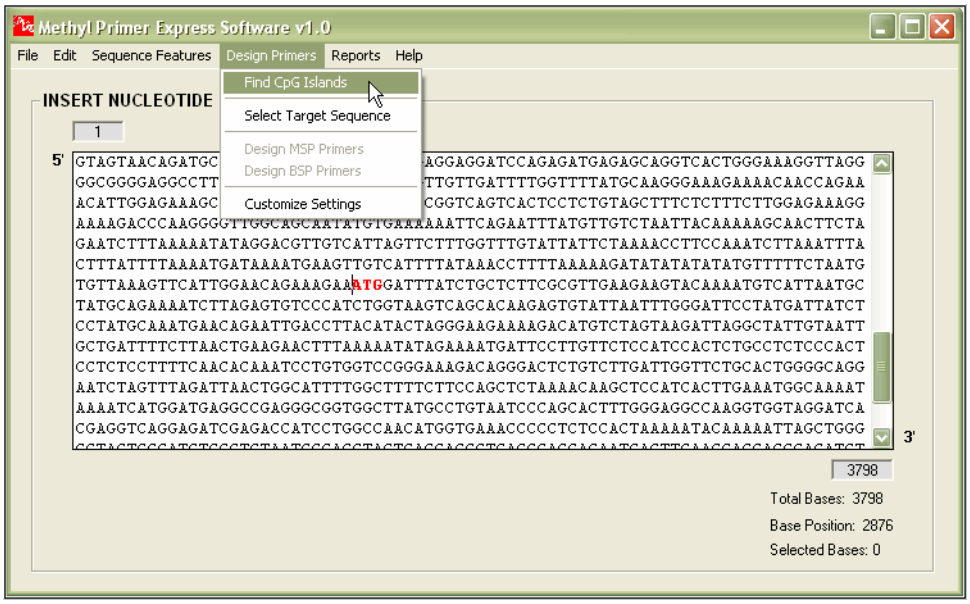
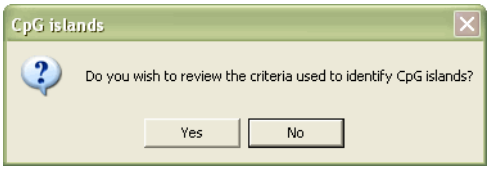
Finding CpG Islands

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.**



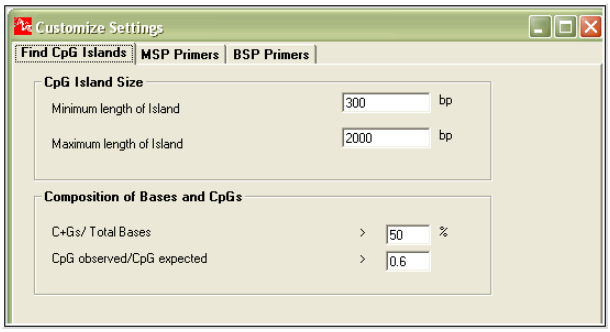
The CpG Islands dialog box prompts you to review the criteria currently set for identifying CpG islands.



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.

Notes



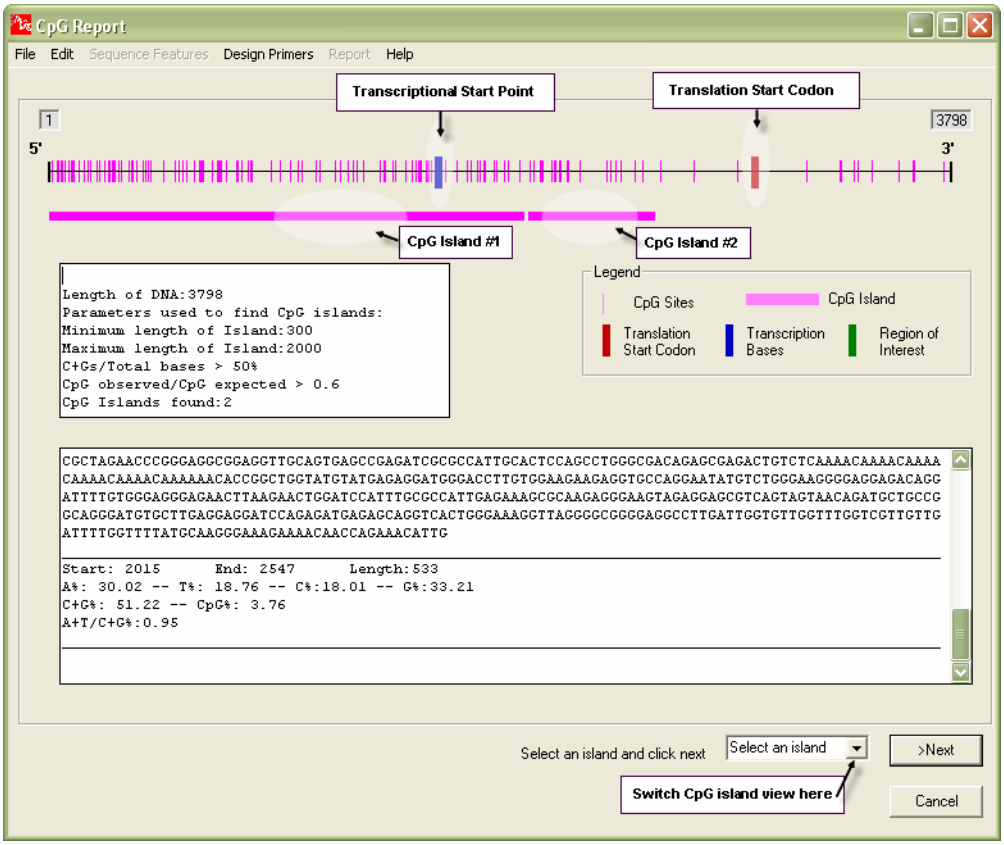
Note: The settings return to the original default settings after you close and reopen the software.

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 page 15 for instructions);
CpG islands are indicated by solid pink bars below the horizontal axis.

Scroll through the report to review the search results.



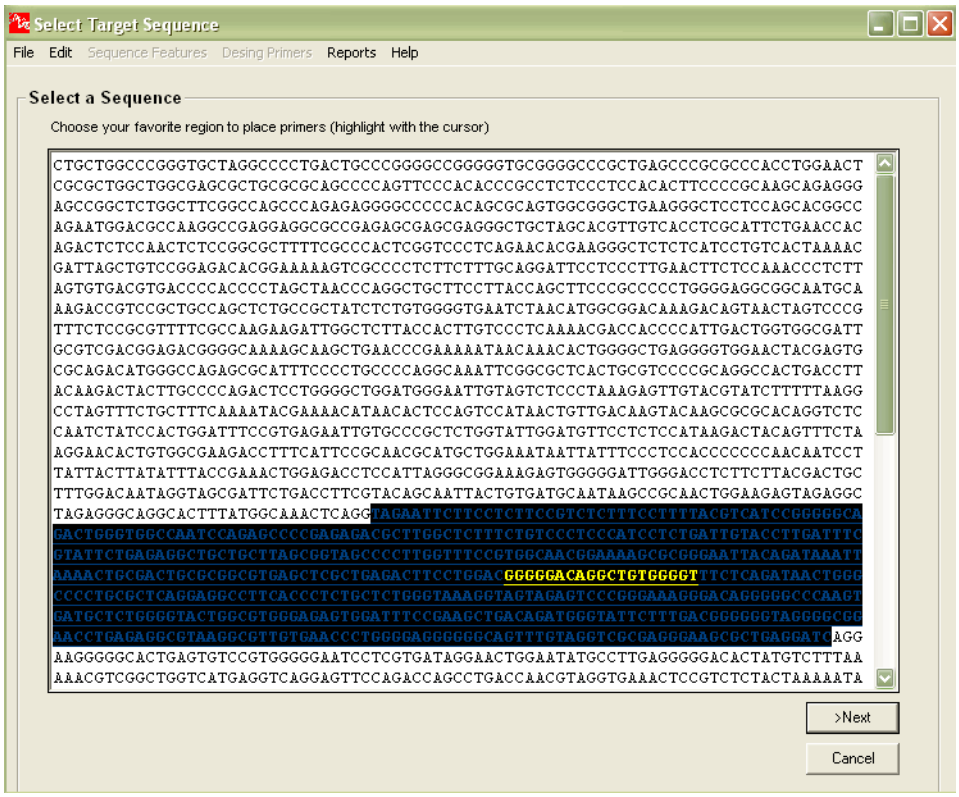
Notes

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**.



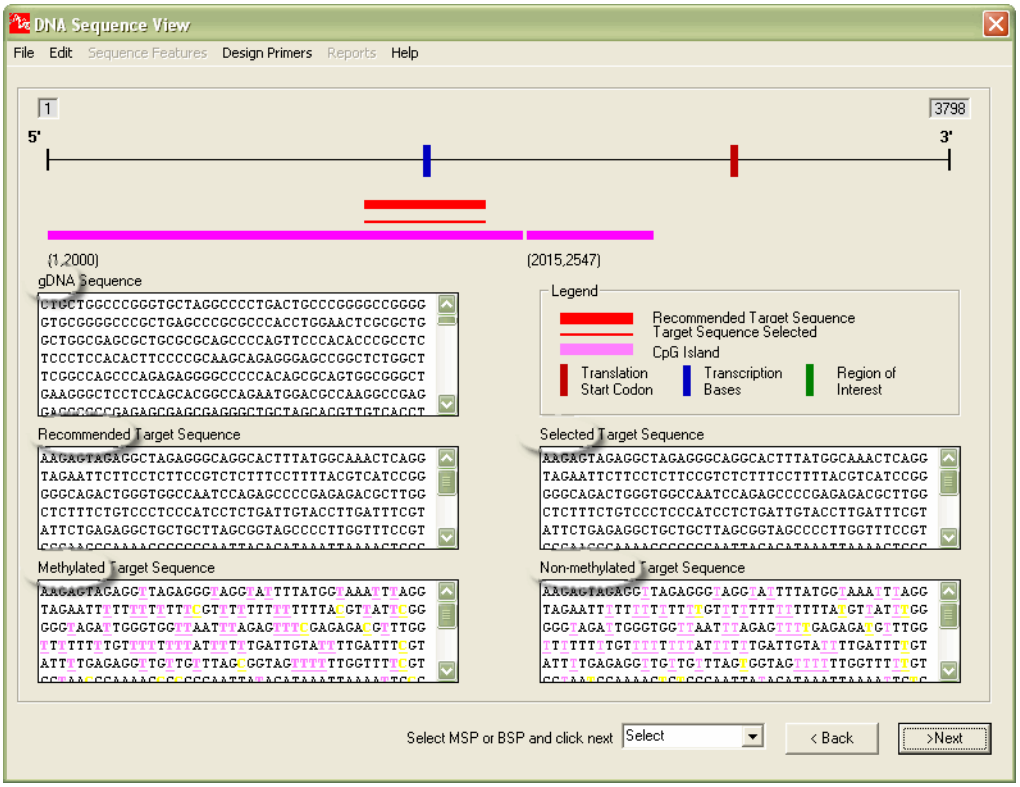
Note: If you annotated the transcription start base, the software automatically selects the target sequence within 500 bases of the transcriptional start point. If you did *not* annotate the transcription base, the CpG island that you see highlighted is automatically set as the target sequence. To overwrite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

Notes

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.



Designing Primers

Design MSP or BSP primers as described in Chapter 3, "Designing Primers."

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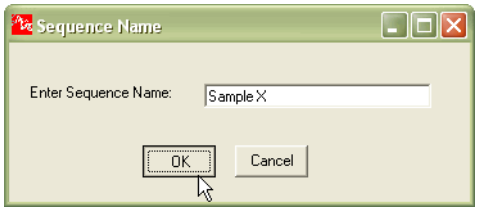
Optional Ways to Annotate a Sequence

The annotation methods in this section are not used in the example workflow described in this guide. However, you can use one or more of these methods for your own sequences according to your experiment requirements.

Naming Your Sequence

To name the imported gDNA sequence:

1. Select **Sequence Features ▸ Enter Sequence Name.**

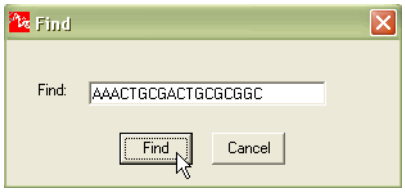


2. Type a name for the sequence, then click **OK**.

Annotating the Sequence with the Transcription Start Point

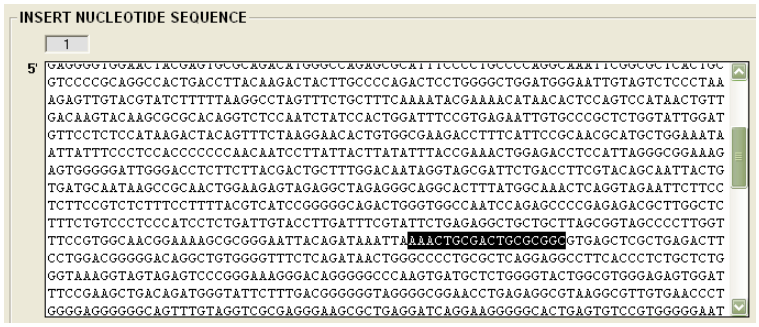
In addition to the method of annotating the sequence with the transcription start base described on page 8, another method is available.

1. Select **Edit ▸ Find.**
2. Paste (or enter) the start codon, plus ten or more supporting bases to make the search sequence unique.



3. Click **Find** to start the search.

   The software locates the transcription start string within the gene sequence and highlights it.



4. Close the Find dialog box.

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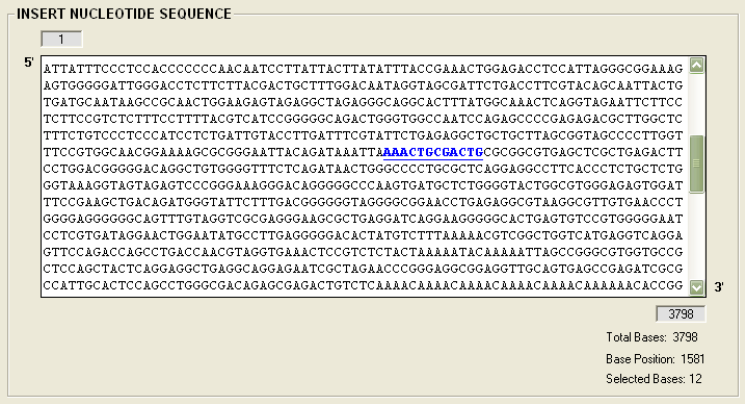
5. Select **Sequence Features ▸ Set Transcription Bases** to annotate the sequence with the transcription start point.

   The software highlights the transcription start point in blue underlined font.

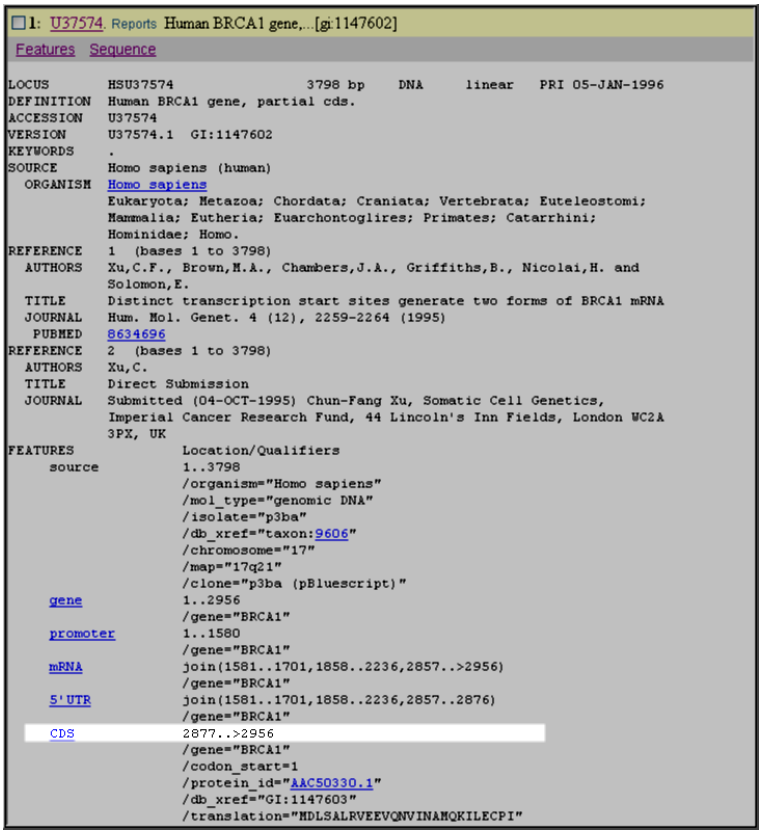


Annotating the Sequence with the Translation Start Codon

You can use one of two methods to annotate the sequence with the translation start codon.

Method 1 for Annotating with the Translation Start Codon

1. Refer to the CDS line in the GenBank record of the imported sequence.

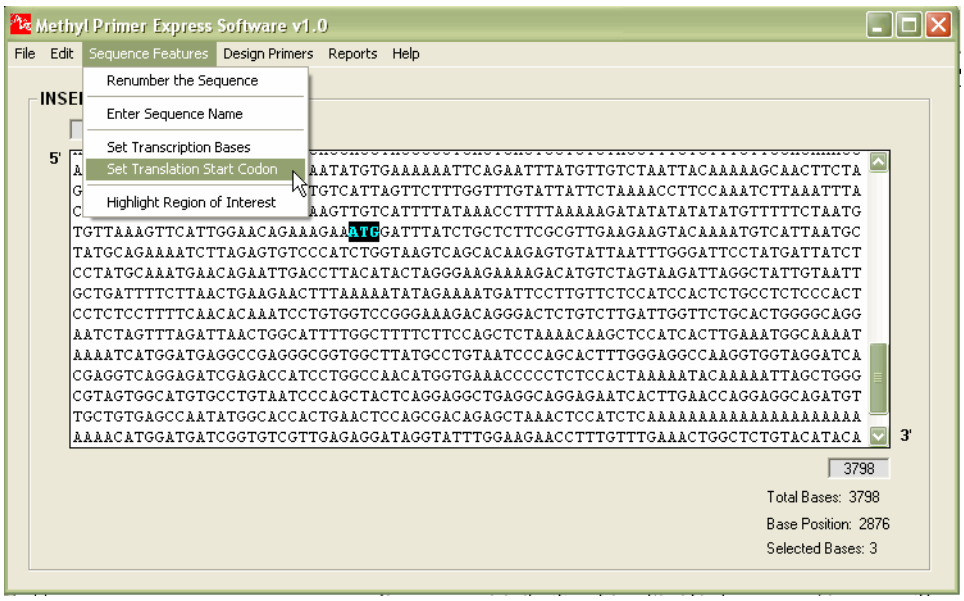


Notes

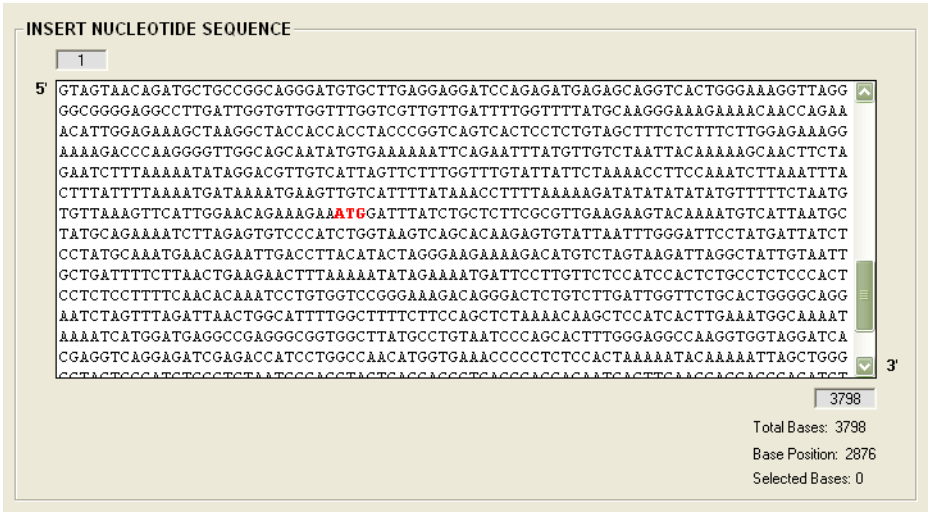
2. Click the pointer anywhere in the imported sequence to view its sequence position in the Base Position at the bottom right corner of the screen.

3. Move the pointer in the sequence until its position matches the CDS number (2877) in the Genbank reference file.

4. Select the matching base position plus the next two bases (ATG).



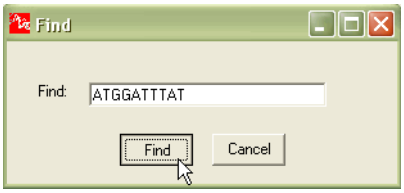
5. Select **Sequence Features ▸ Set Translation Start Codon**. The translation start codon is now highlighted in red. Scroll down in the window to view the codon.



Notes

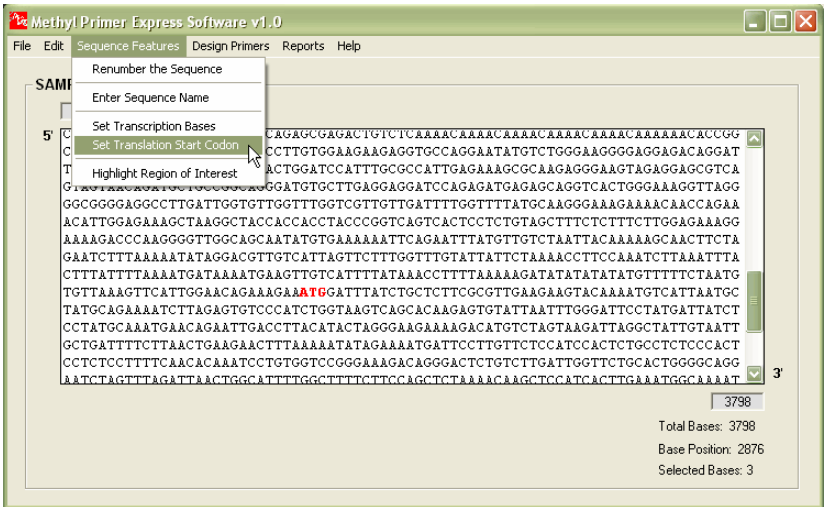
Method 2 for Annotating with the Translation Start Codon

1. In the Find dialog box, select **Edit ▸ Find.**
2. Paste (or enter) the first few bases at the start of the CDS region from your sample.



3. Click **Find** to start the search.

   The software highlights the translation start codon.
4. Close the Find dialog box.
5. Click the pointer at the beginning of the highlighted string of bases, then select *only* the first three nucleotides (ATG).
6. Select **Sequence Features ▸ Set Translation Start Codon**. The codon is highlighted in red.



Notes

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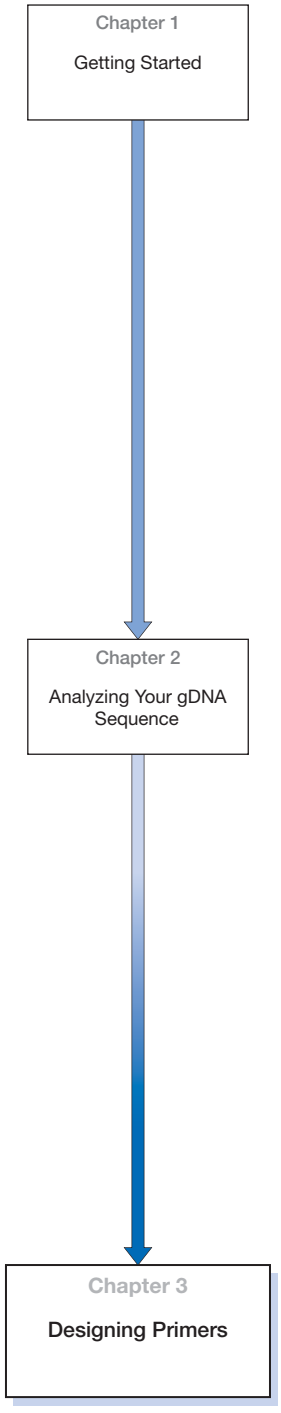
3 Designing Primers



This chapter covers:

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Designing Primers

In This Chapter

In this chapter you will learn to:

- Design a BSP (or MSP) primer
- Create a BSP (or MSP) primer report

Because the two strands of DNA in the sequence are no longer complementary after bisulfite modification, strand-specific primers are used for PCR amplification. Either strand can be selected for methylation studies. Consequently, you can design suitable primers for one strand when the other fails to provide relevant primers.

Due to the conversion of all unmethylated Cs to Ts (U), the bisulfite-treated gDNA has a skewed base composition comprised mostly (or sometimes entirely) of only 3 of the possible 4 bases. The original (bisulfite-converted) strands of DNA are T, G, A-rich, and the reverse complement – once created by PCR – are A, C, T-rich.

Primers designed for amplification of bisulfite-converted gDNA often encounter multiple annealing sites leading to secondary amplicons, and by nature, are more prone to primer-dimer formation. Primer design is a universal challenge for all methods that use PCR to analyze gDNA after the bisulfite conversion.

Guidelines for Selecting a Primer Pair

When designing primers for standard PCR, the most important parameter to consider is the ability of the primers to form a stable duplex strand with the specific site on the target DNA.

When you select primer pairs, be aware that if:

- Multiple islands are found, any of the predicted islands is a target region for amplification.
- A CpG island is smaller than the minimal amplicon size, the primer pair should span the whole island.
- A CpG island is greater than the maximal amplicon size, the primer pair should be within the island.
- A CpG island is between the minimal and maximal amplicon size, the primer pair should cover at least two-thirds of the island.

Methyl Primer Express software considers these rules and automatically applies them to your primer design analysis.

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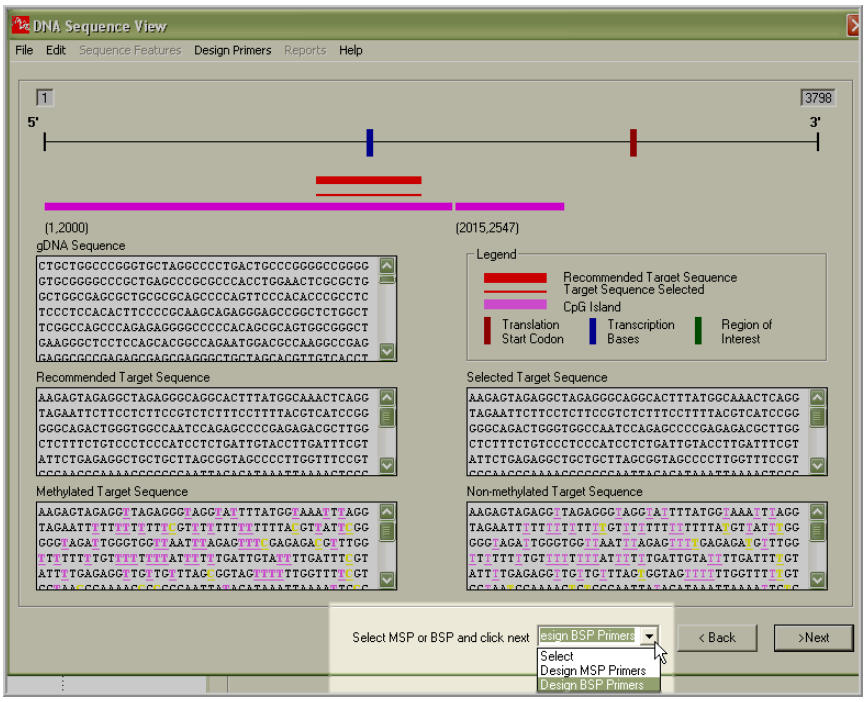
Example Workflow (Continued)

Selecting Primer Type

After you review the DNA Sequence Report as described on page 13, 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.

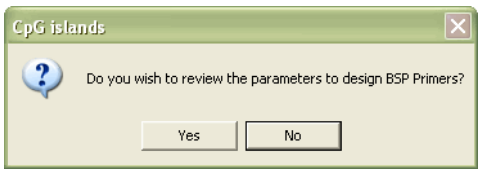


Designing BSP Primers

To design a BSP primer:

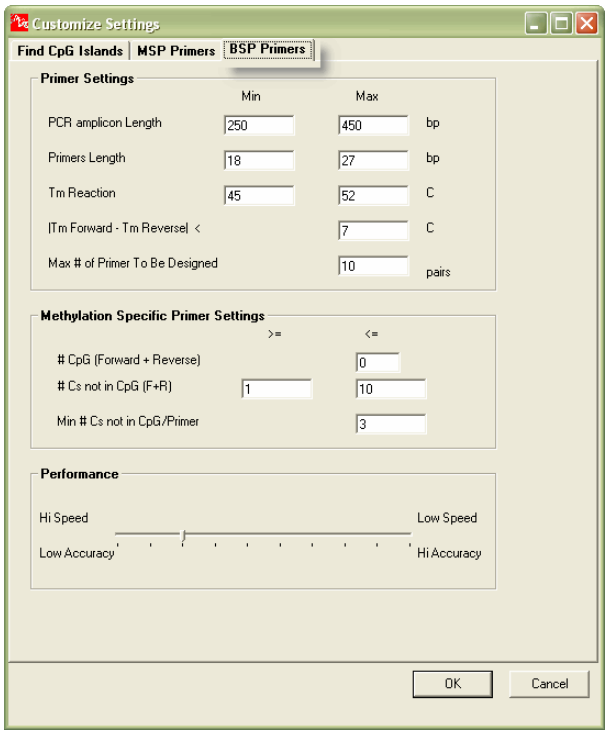
1. Select **Design BSP Primers** from the drop-down list at the bottom of the Sequence Report window, then click **Next.**

   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**.

Notes



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.

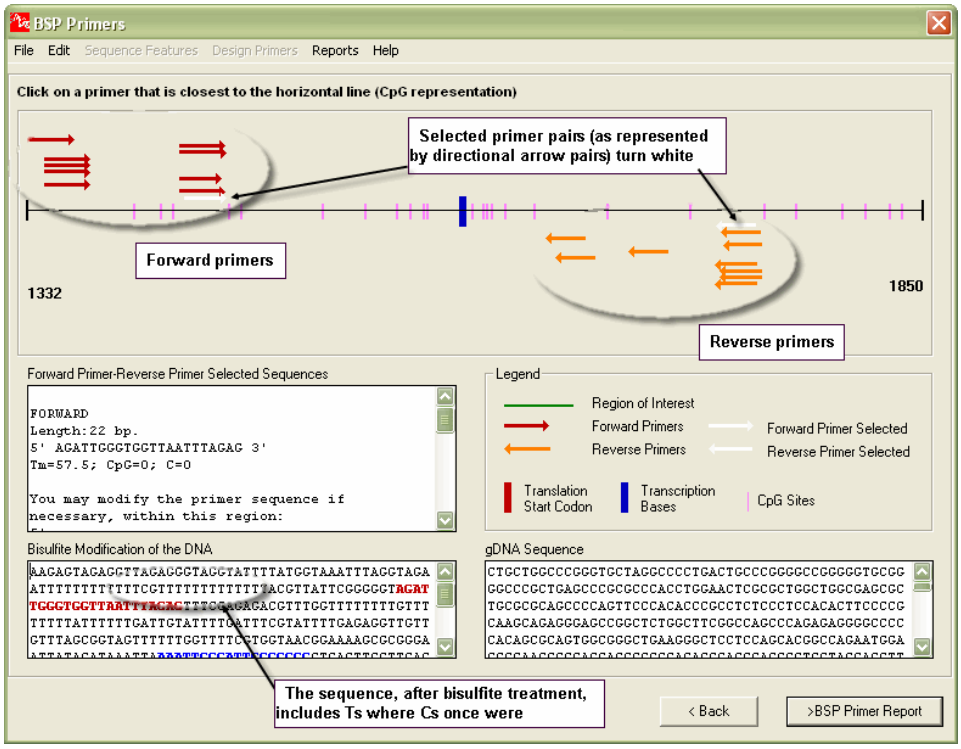
Note: The primer design settings return to the original default settings after you close and reopen the software.

Notes

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.



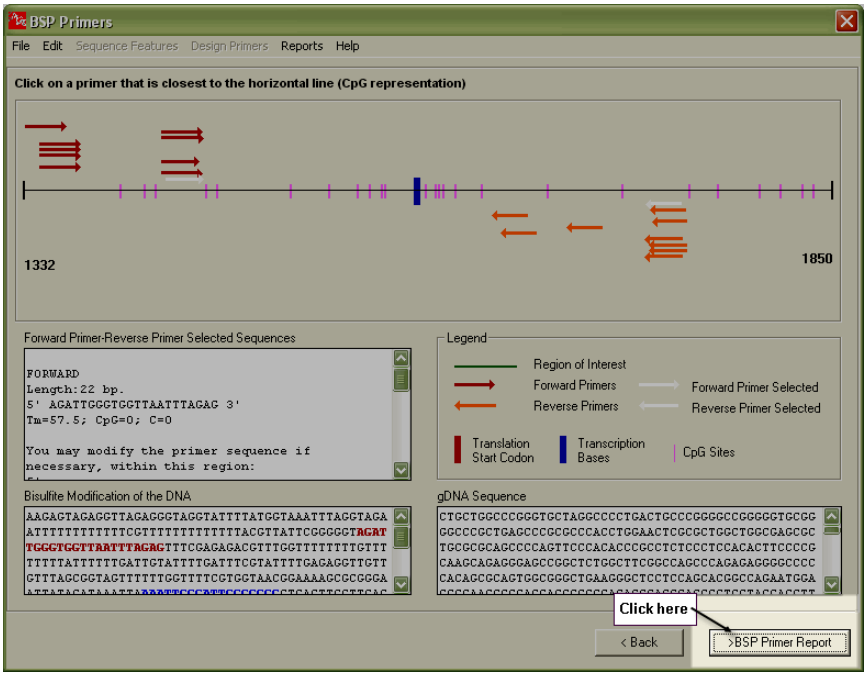
2. Click on an arrow pair to select the best primer. The selected primer pair turns white and the forward primer - reverse primer sequences are displayed in the selected sequences panel on the screen.

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.

Notes

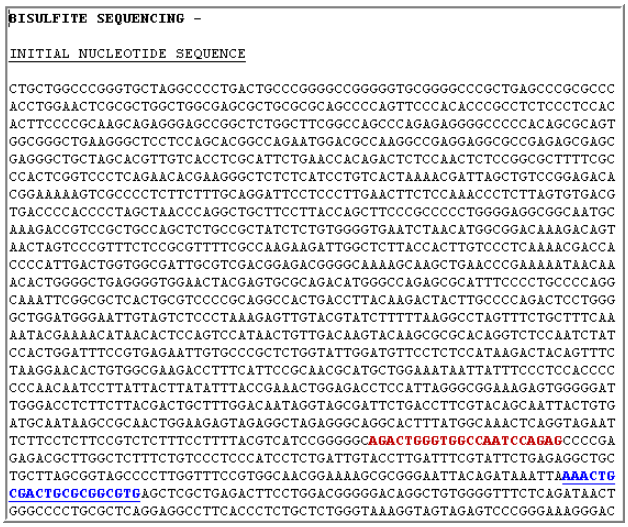
Creating the BSP Primer Report

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



2. Review the BSP primer report by scrolling through each section to see the details of your suggested primer design.

Initial Nucleotide Sequence

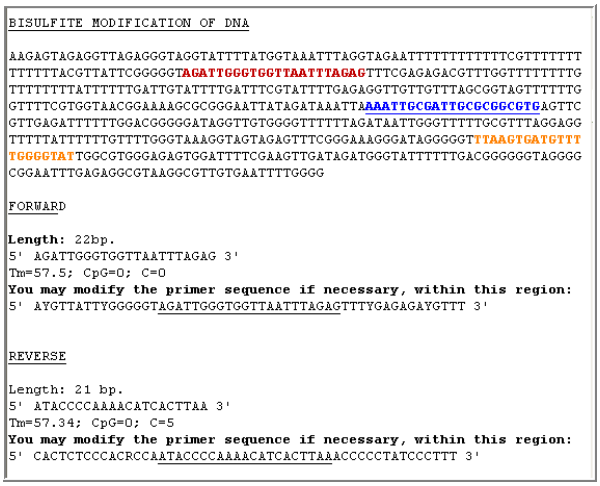


BISULFITE SEQUENCING -

INITIAL NUCLEOTIDE SEQUENCE

CTGCTGGCCCGGGTGCTAGGCCCCTGACTGCCCGGGGCCGGGGGTGCGGGGCCCGCTGAGCCCGCGCCC
ACCTGGAACTCGCGCTGGCTGGCGAGCGCTGCGCGCAGCCCCAGTTCCCACACCCGCCTCTCCCTCCAC
ACTTCCCCGCAAGCAGAGGGAGCCGGCTCTGGCTTCGGCCAGCCCAGAGAGGGGCCCCCACAGCGCAGT
GGCGGGCTGAAGGGCTCCTCCAGCACGGCCAGAATGGACGCCAAGGCCGAGGAGGCGCCGAGAGCGAGC
GAGGGCTGCTAGCACGTTGTCACCTCGCATTCTGAACCACAGACTCTCCAACTCTCCGGCGCTTTTCGC
CCACTCGGTCCCTCAGAACACGAAGGGCTCTCTCATCCTGTCACTAAAACGATTAGCTGTCCGGAGACA
CGGAAAAAGTCGCCCCTCTTCTTTGCAGGATTCCTCCCTTGAACTTCTCCAAACCCTCTTAGTGTGACG
TGACCCCACCCCTAGCTAACCCAGGCTGCTTCCTTACCAGCTTCCCGCCCCCTGGGGAGGCGGCAATGC
AAAGACCGTCCGCTGCCAGCTCTGCCGCTATCTCTGTGGGGTGAATCTAACATGGCGGACAAAGACAGT
AACTAGTCCCGTTTCTCCGCGTTTTCGCCAAGAAGATTGGCTCTTACCACTTGTCCCTCAAAACGACCA
CCCCATTGACTGGTGGCGATTGCGTCGACGGAGACGGGGCAAAAGCAAGCTGAACCCGAAAAATAACAA
ACACTGGGGCTGAGGGGTGGAACTACGAGTGCGCAGACATGGGCCAGAGCGCATTTCCCCTGCCCCAGG
CAAATTCGGCGCTCACTGCGTCCCCGCAGGCCACTGACCTTACAAGACTACTTGCCCCAGACTCCTGGG
GCTGGATGGGAATTGTAGTCTCCCTAAAGAGTTGTACGTATCTTTTTAAGGCCTAGTTTCTGCTTTCAA
AATACGAAAACATAACACTCCAGTCCATAACTGTTGACAAGTACAAGCGCGCACAGGTCTCCAATCTAT
CCACTGGATTTCCGTGAGAATTGTGCCCGCTCTGGTATTGGATGTTCCTCTCCATAAGACTACAGTTTC
TAAGGAACACTGTGGCGAAGACCTTTCATTCCGCAACGCATGCTGGAAATAATTATTTCCCTCCACCCC
CCCAACAATCCTTATTACTTATATTTACCGAAACTGGAGACCTCCATTAGGGCGGAAAGAGTGGGGGAT
TGGGACCTCTTCTTACGACTGCTTTGGACAATAGGTAGCGATTCTGACCTTCGTACAGCAATTACTGTG
ATGCAATAAGCCGCAACTGGAAGAGTAGAGGCTAGAGGGCAGGCACTTTATGGCAAACTCAGGTAGAAT
TCTTCCTCTTCCGTCTCTTTCCTTTTACGTCATCCGGGGGCAGACTGGGTGGCCAATCCAGAGCCCCGA
GAGACGCTTGGCTCTTTCTGTCCCTCCCATCCTCTGATTGTACCTTGATTTCGTATTCTGAGAGGCTGC
TGCTTAGCGGTAGCCCCTTGGTTTCCGTGGCAACGGAAAAGCGCGGGAATTACAGATAAATTAAAACTG
CGACTGCGCGGCGTGAGCTCGCTGAGACTTCCTGGACGGGGGACAGGCTGTGGGGTTTCTCAGATAACT
GGGCCCCTGCGCTCAGGAGGCCTTCACCCTCTGCTCTGGGTAAAGGTAGTAGAGTCCCGGGAAAGGGAC

Bisulfite Modification of DNA with Forward and Reverse Primers



```
BISULFITE MODIFICATION OF DNA

AAGAGTAGAGGTTAGAGGGTAGGTATTTTATGGTAAATTTAGGTAGAATTTTTTTTTTTTCGTTTTTTT
TTTTTTACGTTATTCGGGGGTAGATTGGGTGGTTAATTTAGAGTTTCGAGAGACGTTTGGTTTTTTTTG
TTTTTTTTATTTTTTGATTGTATTTTGATTTCGTATTTTGAGAGGTTGTTGTTTAGCGGTAGTTTTTTG
GTTTTCGTGGTAACGGAAAAGCGCGGGAATTATAGATAAATTAAAATTGCGATTGCGCGGCGTGAGTTC
GTTGAGATTTTTTGGACGGGGGATAGGTTGTGGGGTTTTTTAGATAATTGGGTTTTTGCGTTTAGGAGG
TTTTTATTTTTTGTTTTGGGTAAAGGTAGTAGAGTTTCGGGAAAGGGATAGGGGGTTAAGTGATGTTT
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'
```

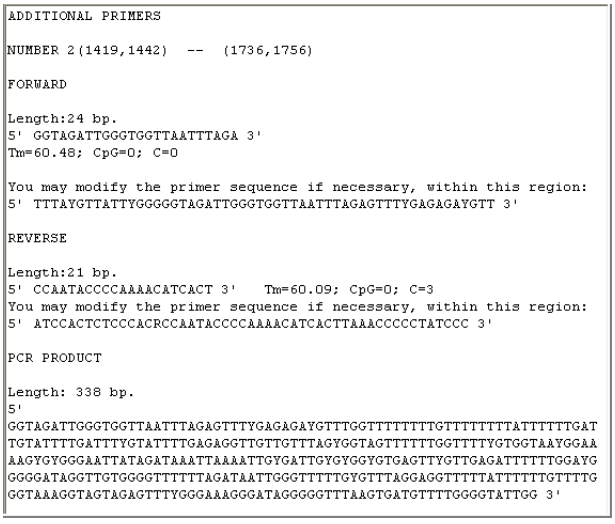
PCR Product

```
PCR PRODUCT

Length: 332 bp.
5'
AGATTGGGTGGTTAATTTAGAGTTTYGAGAGAYGTTTGGTTTTTTTTTGTTTTTTTTATTTTTTGATTGT
ATTTTGATTTYGTATTTTGAGAGGTTGTTGTTTAGYGGTAGTTTTTTGGTTTTYGTGGTAAYGGAAAAG
YGYGGGAATTATAGATAAATTAAAATTGYGATTGYGYGGYGTGAGTTYGTTGAGATTTTTTGGAYGGGG
GATAGGTTGTGGGGTTTTTTAGATAATTGGGTTTTTGYGTTTAGGAGGTTTTTATTTTTTGTTTTGGGT
AAAGGTAGTAGAGTTTYGGGAAAGGGATAGGGGGTTTAAGTGATGTTTTGGGGTAT 3'

%CGs=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

Notes

Recommendations for Designing Primers

Recommendations for Bisulfite Sequencing (BSP)

Sometimes additional sequence content upstream or downstream of the imported sequence (or submission of the reverse compliment sequence of the original gDNA) may help optimize primer recommendations.

Recommendations for Bisulfite Sequencing (BSP)

- Bisulfite sequencing primers are designed to amplify a region regardless of the methylation state, so only a single forward and reverse primer are required.
- Primers designed by Methyl Primer Express software do not contain any CpG sites within their sequences so that both methylated and unmethylated DNA are amplified during PCR. If CpG sites are unavoidable in a primer due to limited sequence input, the software designs a mixed base at the CpG site within the primer sequence. An input "Y" represents "C" and "T" in a forward primer; an "R" represents "G" and "A" in a reverse primer.

  Note: Generally, mixed or degenerate bases are not recommended due to annealing temperature differences that can lead to biased amplification.

- Primers should have a maximum number of non-CpG "Cs" in their sequence to amplify only the bisulfite-modified DNA.
- To map as many CpG sites as possible, a primer pair should span a CpG-rich region.

Recommendations for Methylation-Specific PCR (MSP)

Two primer sets are required for MSP: one assuming a fully methylated sequence and the other a fully unmethylated sequence. The bisulfite-modified and methylated sequence has all Cs except mCs converted to Ts; the corresponding bisulfite-modified and unmethylated sequence represents all Cs including CpGs as Ts.

Recommendations for Methylation-Specific PCR (MSP)

- Each primer should contain at least three CpG sites in its sequence, and one of the CpG sites should be at the 3' end of the sequence to maximally discriminate between methylated DNA and unmethylated DNA.
- Each primer should have a maximum number of non-CpG Cs in its sequence to amplify only the bisulfite-modified DNA.
- The primer pair for the methylated DNA (M pair) and the primer pair for the unmethylated DNA (U pair) should contain the same CpG sites within their sequences. For example, if the forward primer for an M pair has the sequence ATTAGTTTCGTTTAAGGTTCGA, then the forward primer for the U pair must also contain the two CpG sites, although they can differ in length and start position.

Notes

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