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PISMA: COMPUTATIONAL TOOL FOR DNA *MOTIF* DISTRIBUTION

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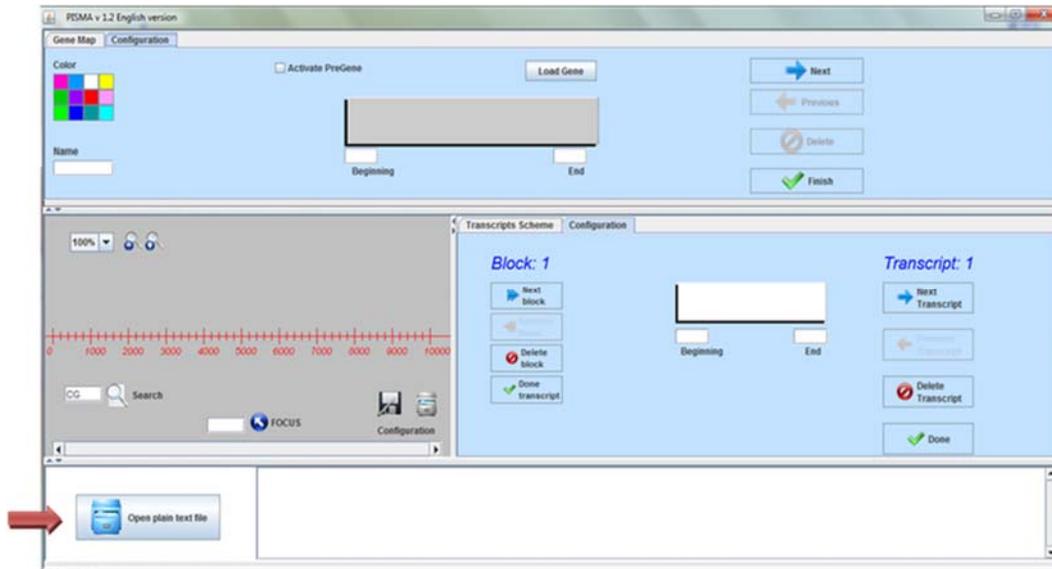
USER MANUAL

CONTENT

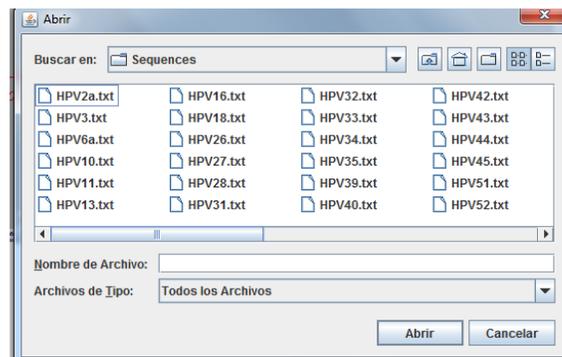
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FILE OPENING

In order to open the input data file, push the file key at the left bottom side.



This opens a window where it is possible to access the file containing the plain text sequence up to 10,000 nucleotides.



Select the file and push the open key.

NOTE. The window language is dependent of the operative system version.

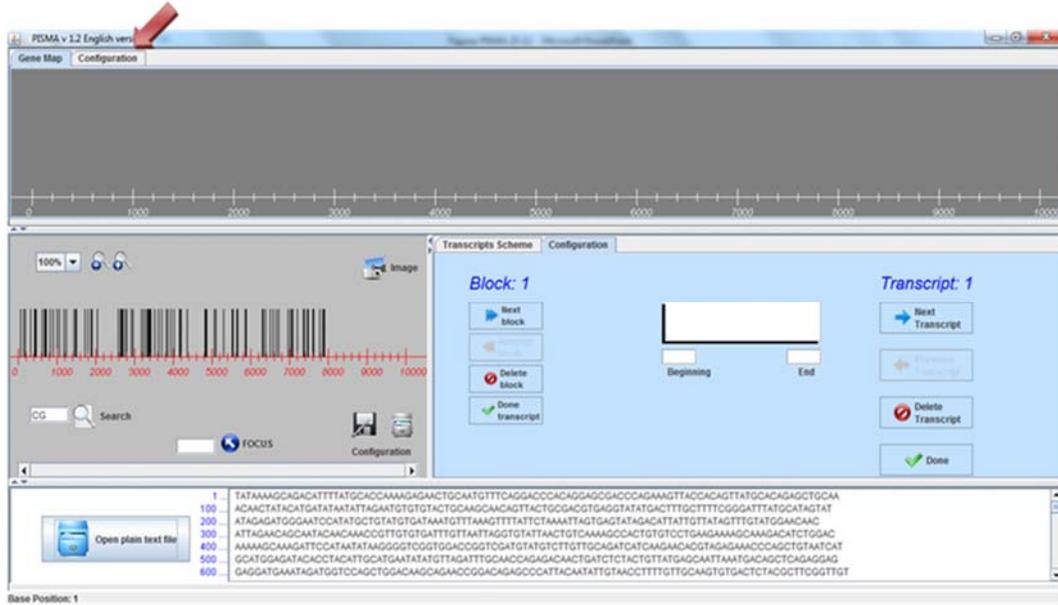
Automatically, In the scale each CpG site appears as a bar and the plain text sequence at the bottom window..

The screenshot displays the PISMA v 1.2 software interface, which is divided into several panels. At the top, the 'Gene Map' panel includes a color selection tool, an 'Activate PreGene' checkbox, a 'Load Gene' button, and navigation buttons for 'Next', 'Previous', 'Delete', and 'Finish'. Below this is a 'Transcripts Scheme' panel with a 'Block: 1' configuration, including 'Next block', 'Previous block', 'Delete block', and 'Done transcript' buttons, along with 'Beginning' and 'End' input fields. The central panel features a scale from 0 to 10000 with a barcode representing CpG sites. A red arrow points to this scale. Below the scale is a search bar for 'CG' and a 'FOCUS' button. The bottom panel shows a 'Transcript: 1' configuration with 'Next Transcript', 'Previous Transcript', 'Delete Transcript', and 'Done' buttons, and a 'Begin' and 'End' input field. At the very bottom, a text window displays a DNA sequence with line numbers 100, 200, 300, 400, 500, and 600. A red arrow points to the first line of the sequence. An 'Open plain text file' button is also visible on the left side of the text window.

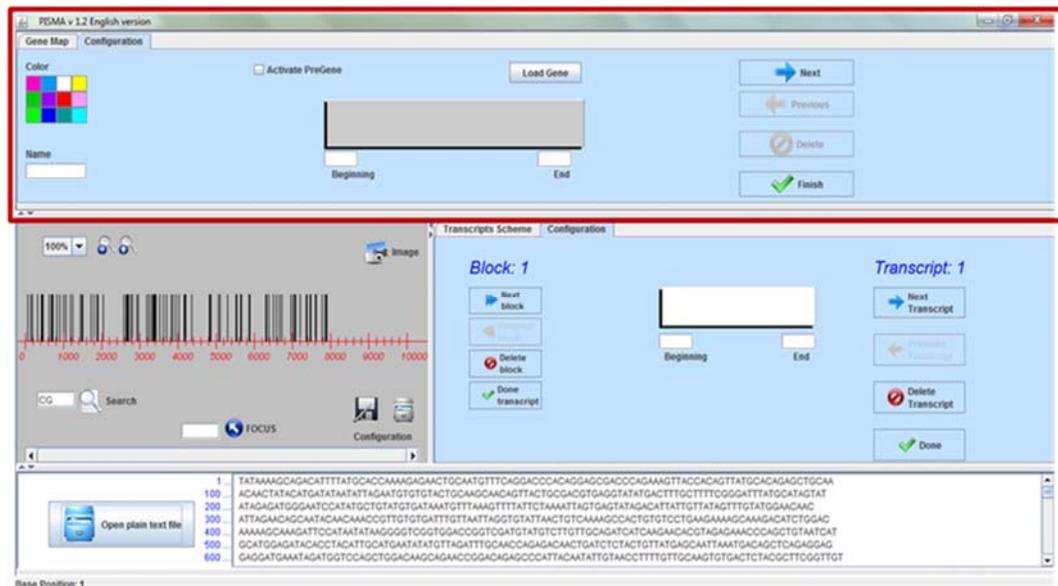
1 TATAAAGCCAGACATTTTATGCACCAAAAGAACTCCATGTTTCAGGACCCACAGGAGCCAGCCAGAAAGTTACACAGTTATGCACAGCTGCAC
 100 AHCACATACATGATATATATAGATGTGTGTACTGCAGGCAACAGTACTGGAGGTGAGTATAGCTTTGCTTTCCGGATTATGCATAGTAT
 200 ATAGAGATGGGAATCCATATGCTGTATGTGATAAATGTTAAGTTTTATTCTAAATAGTGGATAGACATATTGTTATAGTTGTATGGAAACAC
 300 ATTAGAAGCAAGCAATACAAACCCGTTGTGTGATTGTTAATAGGTGATTAACTGTCAAAAGCCACTGTCTCCGAAAGAAAGCAACATCTGGAC
 400 AAAAGCAAGATTCCATATATAGGGGTCGGTGGACCGGTCCGATGTATGCTCTTGTGCAGATCATCAAGAACAGTAGAGAAACCCAGCTGTATCAT
 500 GCATGGAGATACACCTACATTGCATGATATATGTTAGATTTGGAAACAGAGACAGTGTCTCTACTGTTATGAGGAATTAATGACAGCTCAGAGGAG
 600 GAGGATGAATAGATGTTCCAGCTGGCAAGCAGACCCGACAGGCCATACATATTGTAACCTTTTGGAAAGTGTGACTCTACGGTTGGTGTG

GENE MAP CONFIGURATION MENU

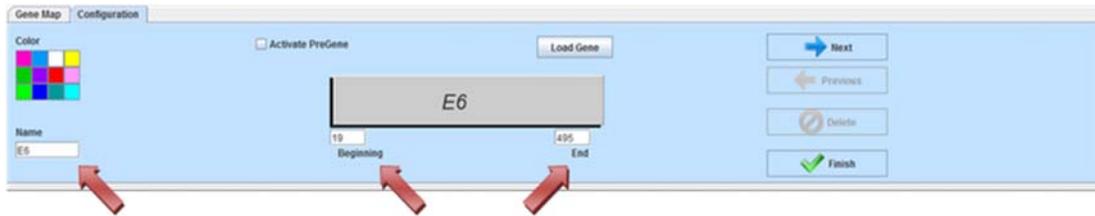
Definition of the gene map-like graph requires pushing on the Configuration key.



This opens the gene window. In here a gene is any user defined region.



Write the gene/region name, the starting and ending nucleotide numbers in the corresponding spaces.



Choose a color for the block representing each gene from the palette.



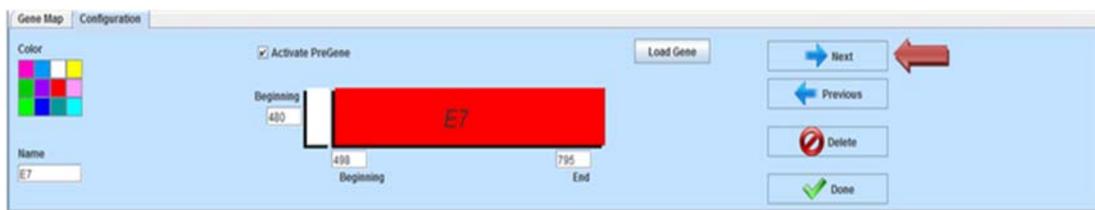
It is possible to activate the Pre-gene button pushing on it. This allows add a coding region previous to the gene. This is particularly useful for human Papillomavirus (HPV) sequences.



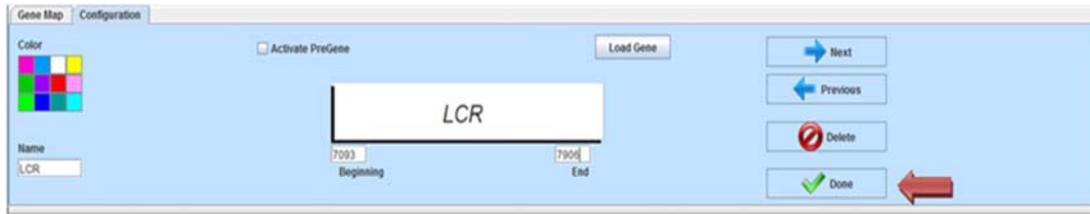
Fill with the starting nucleotide number when the white block rises.



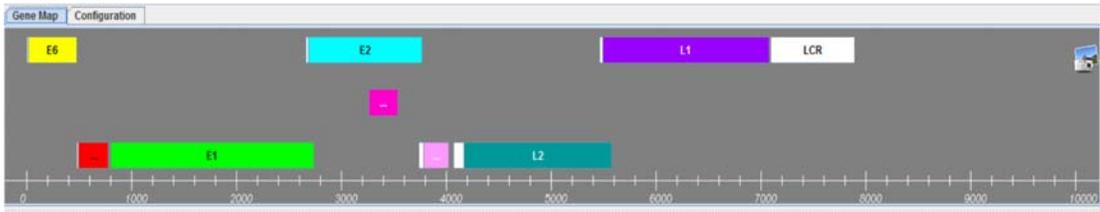
Repeat the same procedure for each desired gene clicking the Next key.



When finish all the genes input, push the Done key.

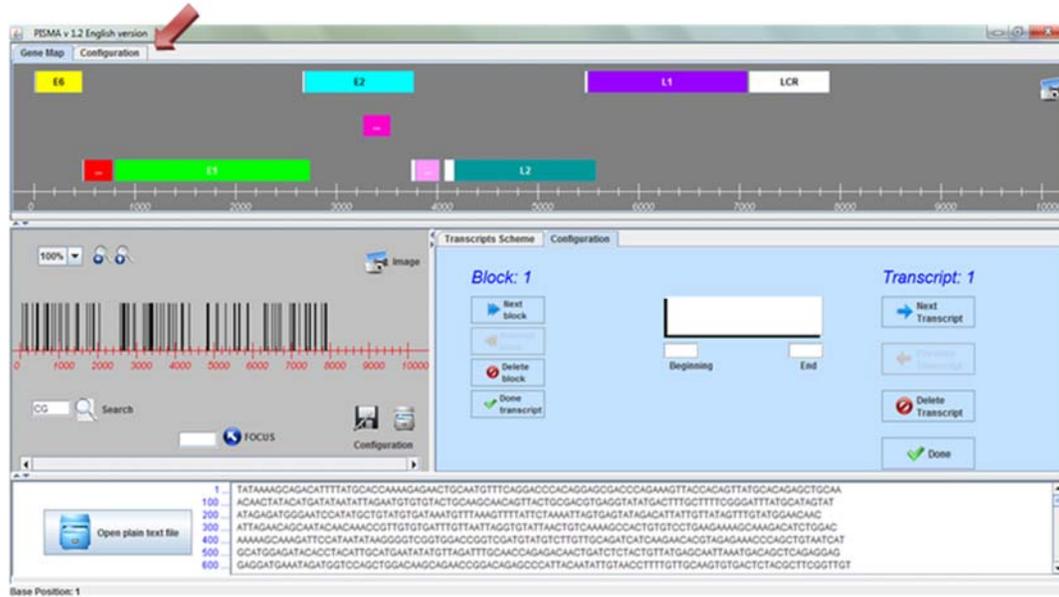


The gene map will be shown:



DELETING GENES

In order to delete a gene, click on the Configuration tab.



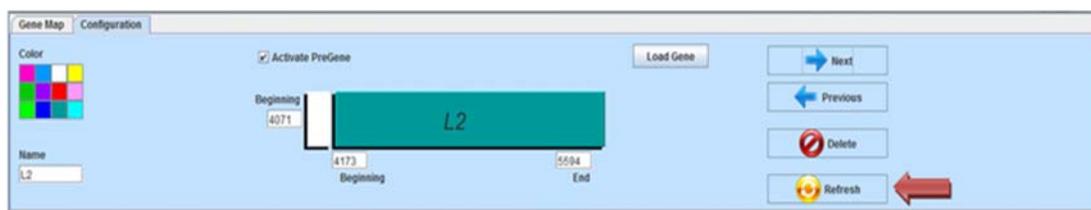
Choose the gene to delete, using the following or previous keys.



When the selected gene is on the screen, push the Delete key.

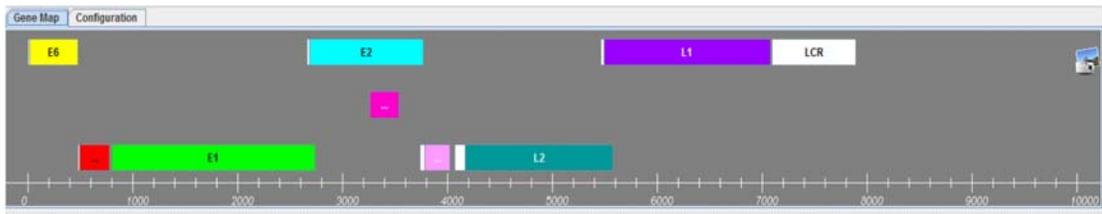


After the modifications are made, push the Refresh key.

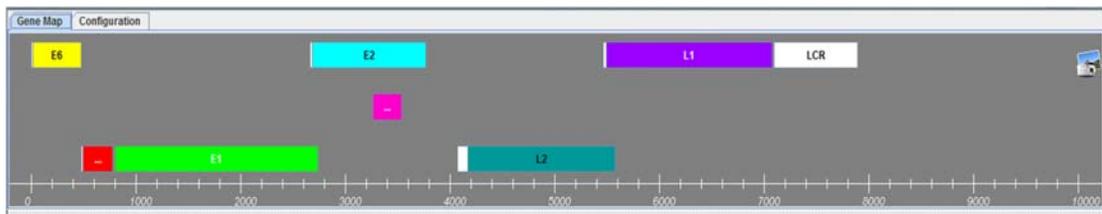


In the next two figures the deletion of E5 gene is shown:

Before deletion of E5:



After deletion of E5:



NOTE: If the Refresh key is no pushed, the changes will not been fixed.

GENE EDITION

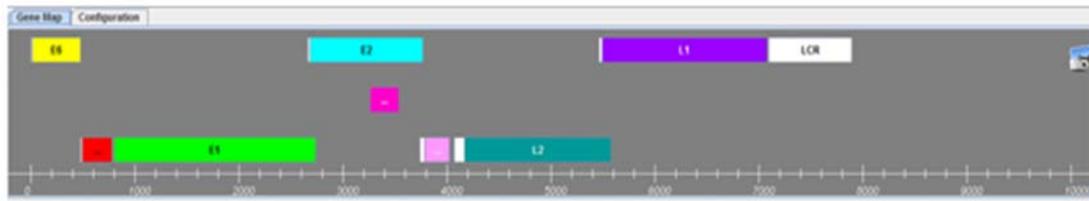
It is possible to make changes in the starting and ending points, in the color and in the gene name every time it is required. After the modifications are made, push the Refresh key.



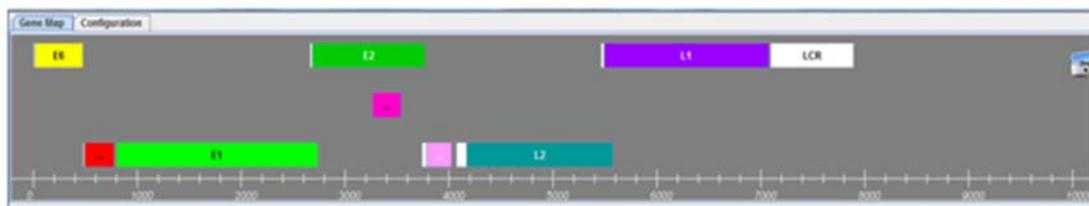
NOTE: If the Refresh key is not pushed, the changes will not be fixed.

In the next two figures a change of the E2 gene color is shown.

Before change:

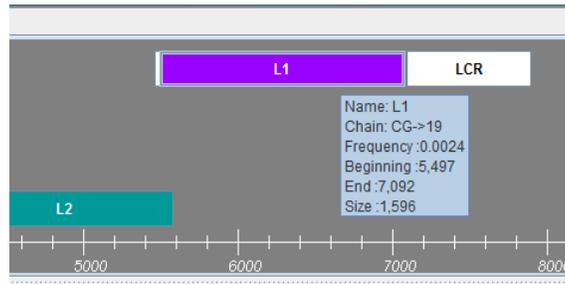


After change:



GENE MAP TOOLTIP

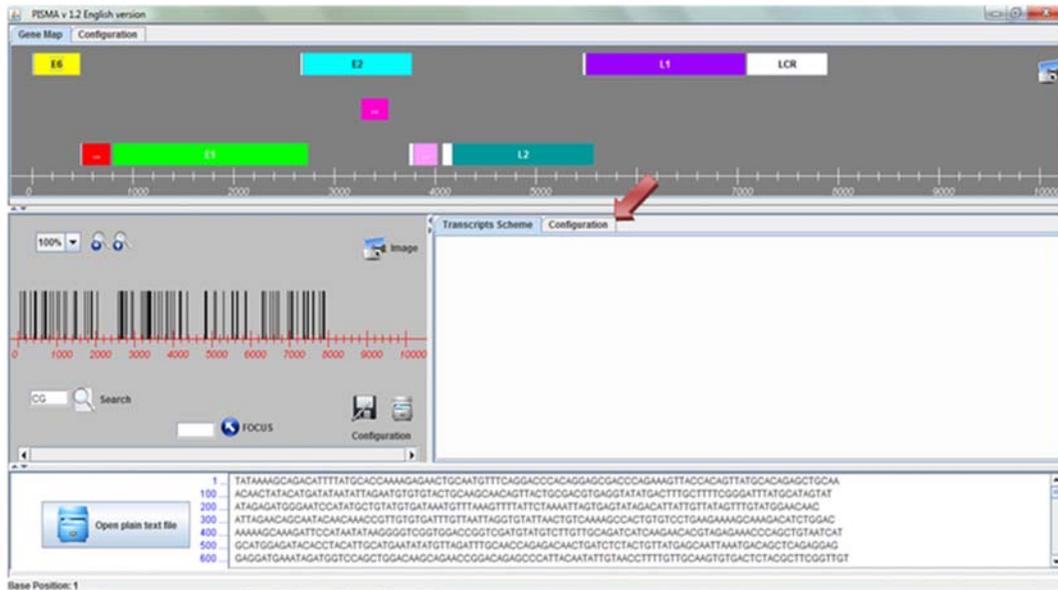
It is possible to display additional information about any selected gene by putting the cursor on the corresponding gene.



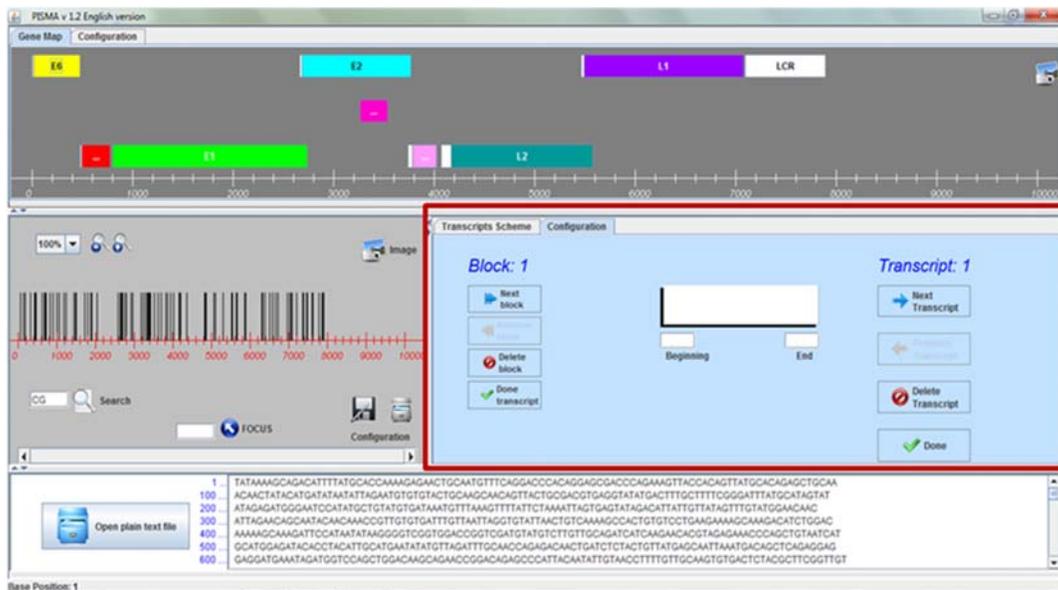
The information on a label includes the gene name, the number of CpG sites, the relative CpG frequency, the starting and ending points, and the gene size, all as nucleotide numbers.

TRANSCRIPT SCHEME CONFIGURATION MENU

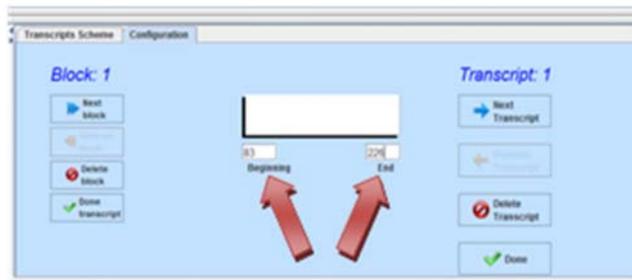
For visualize the transcript scheme it is necessary to push on the Configuration key.



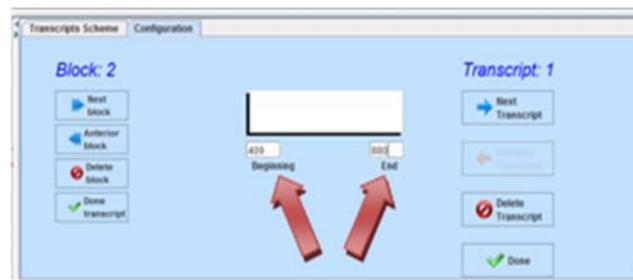
This opens the Blocks-Transcripts window.



Write the starting and ending nucleotide numbers for the first exon/block in the corresponding spaces.



Push the Next block key and write the starting and ending nucleotide numbers for the second exon/block.



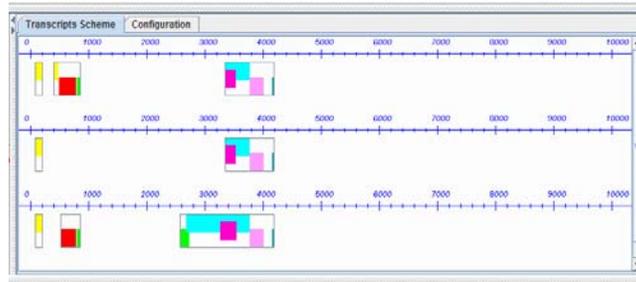
Repeat the same procedure for each exon/block. When finish all exons/blocks from the transcript, click either the Done Transcript (left side) or the Next Transcript (right side) keys.



Repeat the same procedure for each transcript and when finish all transcripts push the Done key.

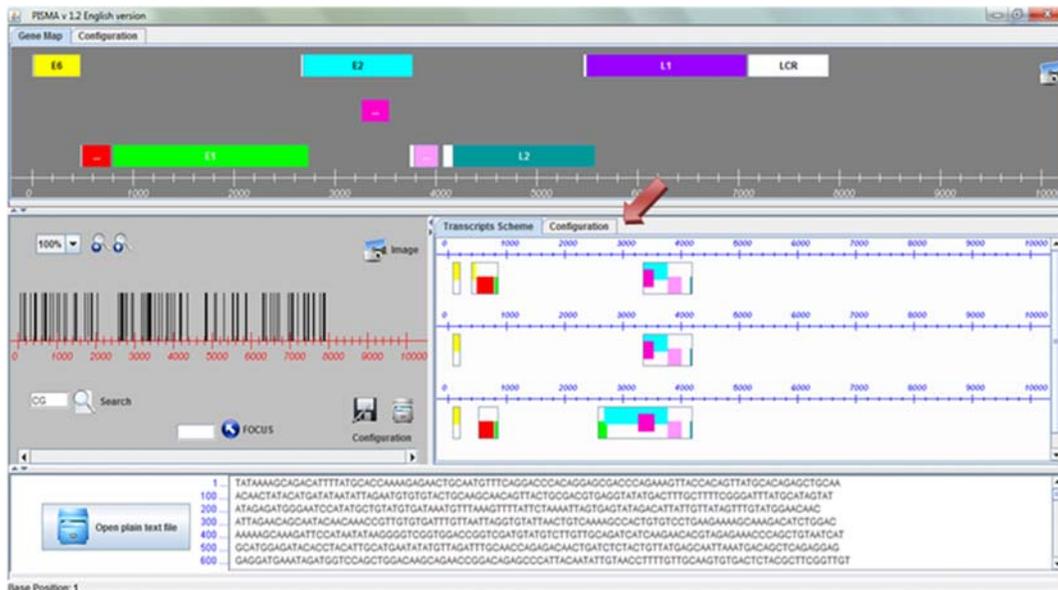


The transcript scheme will be shown:



DELETING EXONS/TRANSCRIPTS

In order to delete either a block (exon) or a transcript, click on the Configuration tab.



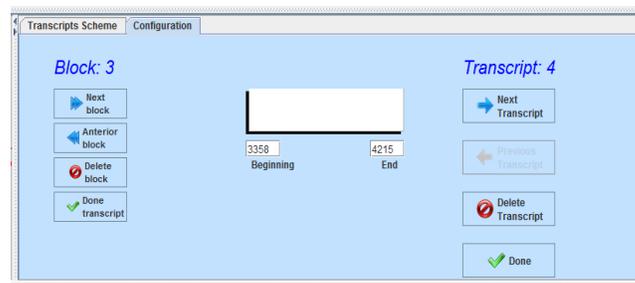
FOR DELETE BLOCKS

Using the Next block or Anterior block keys, choose the block to delete and make sure this belongs to the selected transcript. In the Configuration window the number of block appears up the left side and the number of transcript appears up the right side.

OJO: Done en

Lugar de

Refresh



When the selected block is on the screen, push the Delete block key.

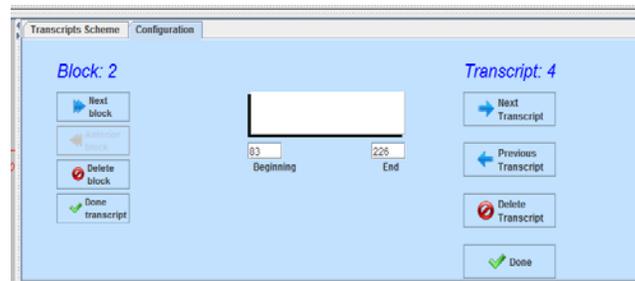


Repeat the same procedure for each block you want to eliminate, and when finish all changes push the Refresh key.

NOTE: If the Refresh key is no pushed, the changes will not be fixed.

FOR DELETE TRANSCRIPTS

Choose the transcript to delete, using the following transcript or previous transcript keys. In the Configuration window the number of transcript appears up the right side.



When the selected transcript is on the screen, push the Delete transcript key.



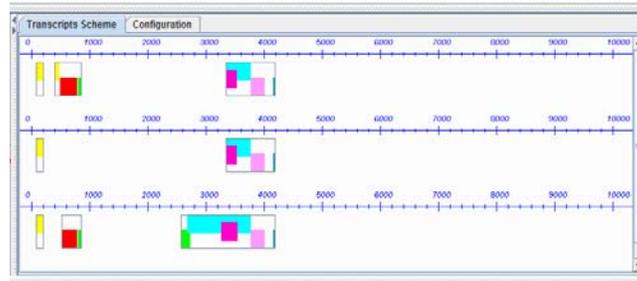
Repeat the same procedure for each transcript and when finish all transcripts changes push the Refresh key.



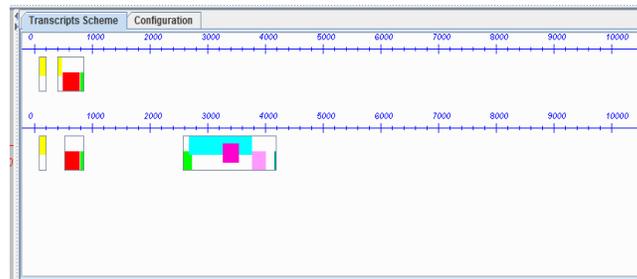
NOTE: If the Refresh key is no pushed, the changes will not be fixed.

In the next two figures the deletion of the block 3 of the transcript 1 and removing of the transcript 2 are shown.

Before change:

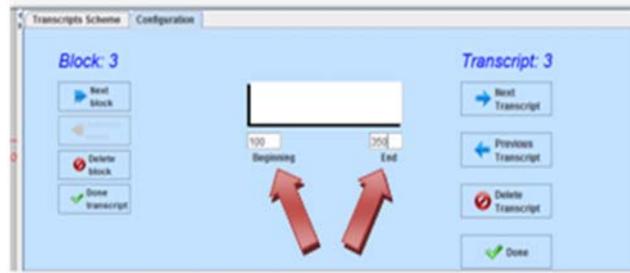


After change



TRANSCRIPT EDITION

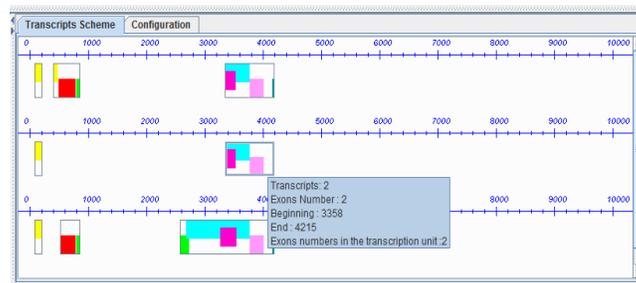
It is possible to change the starting and ending points, of the transcripts every time it is required. After the modifications are made, push the Done key.



NOTE: If the Refresh key is no pushed, the changes will not be fixed.

TRANSCRIPT SCHEME TOOLTIP

It is possible display additional information about any selected block of any transcript putting the cursor on the corresponding block.



The information on a label includes the transcript number, the exon number, the starting and end nucleotides, and the number of exons in the transcript.

LOOKING FOR DIFFERENT *MOTIFS*

It is possible to look for different DNA *motifs* from CpG. In fact this computational tool allows look for any chain from 10 nucleotides or less into a fragment up to 10,000 nucleotides. It is necessary input any desired *motif* in the special block below the scale, in the left middle window, and click the Search key

The screenshot displays the PEDMA v1.2 software interface. At the top, a 'Gene Map' shows exons E1, E2, E3, and E4, and introns I1 and I2. Below this is a 'Transcripts Scheme' window with a search bar containing 'CC' and a 'Search' button. A red arrow points to the search bar. The bottom section shows a DNA sequence alignment with a 'Base Position: 1' label.

Transcripts Scheme Configuration

Search: CC

Base Position: 1

```

1 TATAAAGCAGACATTTATGCACAAAGAGACTGCCAATGTTTCAGGACCCACAGGAGCGACCCAGAAAGTTACCACAGTTATGCACAGAGCTGCA
100 ACAACCTATACATGATATATATAGAAATGTGTACTCCAGACACAGCTTACCGAGCGTAGATATACGTTTCTTTGGGATTTATGCATAT
200 ATAGAGATGGGAATCCATATGCTGTATGTGATAAATGTTAAAGTTTATTCATAAATAGTGAGTATAGACATTTGTTATAGTTTGTATGGAAAC
300 ATTAGAACAGCAATACACAAACCGTTGTGTGATTTGTTAATAGGTGATTAAGTCTCAAAAGCCACTGTCTCAGAAAGCAAGACATCTGGAC
400 AAAAAACAAGATCCATATATAAGGGGTCGGTGGACCGGTGGATGTCTTTGTCAGATCATCAAGAACACCGTAGAGAAACCCAGCTGTAATCAT
500 GCATGGAGATACACCTACATTCATGAATATGTTAGATTTGGACCCAGAGACACTGATCTCTACTGTTATGAGCAATTAATGACAGCTCAGAGGAG
600 GAGGATGAATAGATGTTCCAGCTGCACAGGAGACCGGACAGGACCCATTACATATGTTAACCTTTTGGAAAGTGTGACTCTAGCCTCGGTTGT

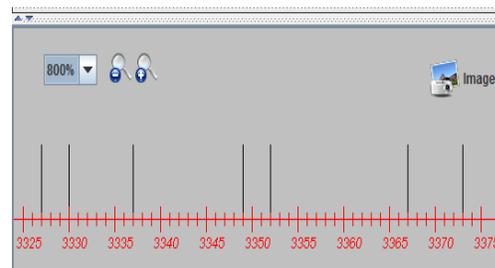
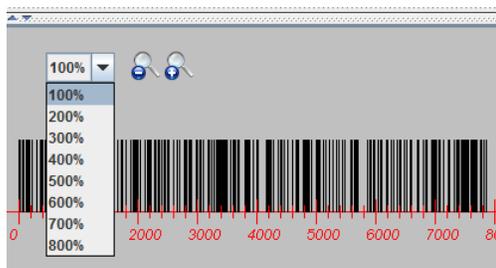
```

ZOOM

A special tool allows amplifying the scale up to 800%. This tool is located at the left upper corner of the scale window.

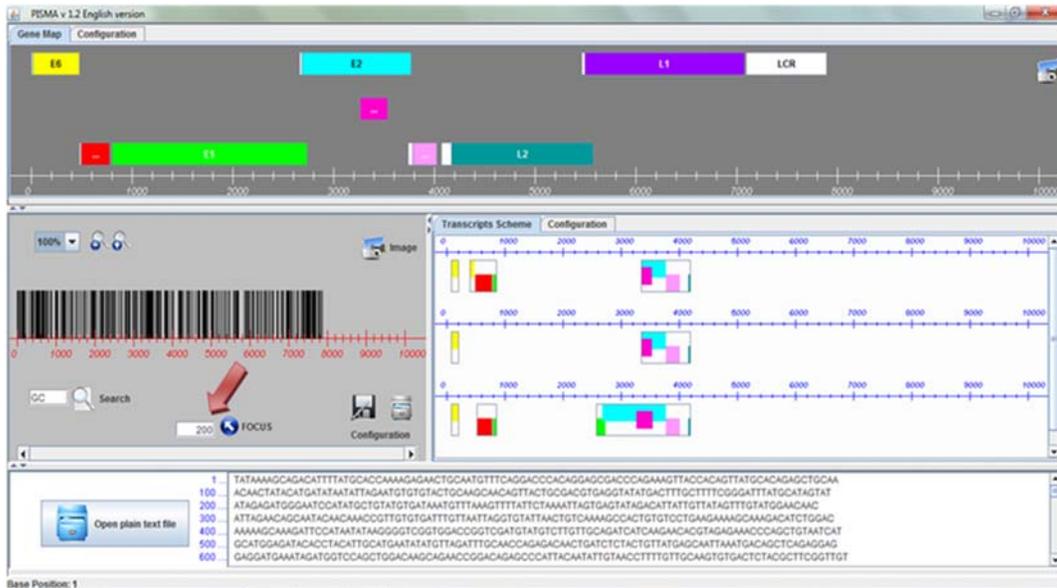


This tool displays the 8 amplification options, from 100% to 800%, shown below.

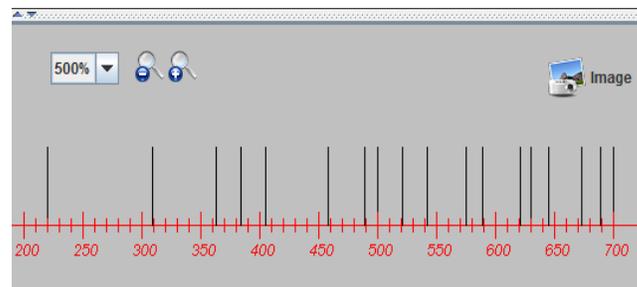


SKIPPING TOOL

There exists the possibility to locate a specific position on the nucleotide sequence using the skipping tool. Just input the number of the specific nucleotide, and push the FOCUS key in the scale window.



In the following figure is shown an example of the skipping tool toward the position 200.



SAVING FILES

In order to save files just click either the image key or the camera icon in the corresponding window.

