

AutoPrimer3

AutoPrimer3 retrieves gene information, DNA sequences and SNP information from the UCSC genome browser and uses Primer3 (<http://primer3.sourceforge.net/>) to automatically design primers to genes or genomic coordinate targets.

The default settings should be suitable for the design of primers for use in the amplification and sequencing of small PCR products (< 500 base pairs in length).

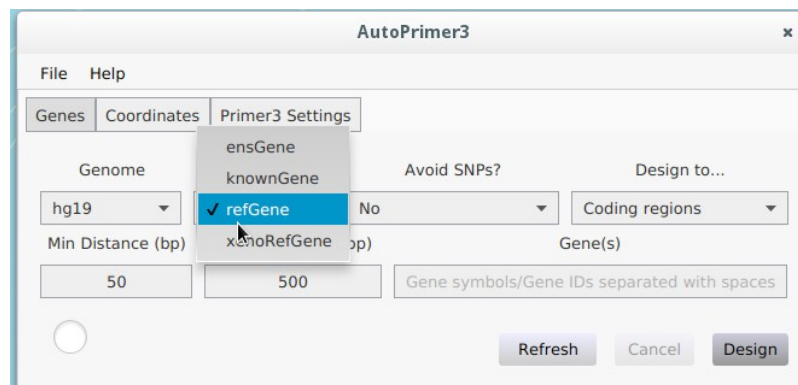
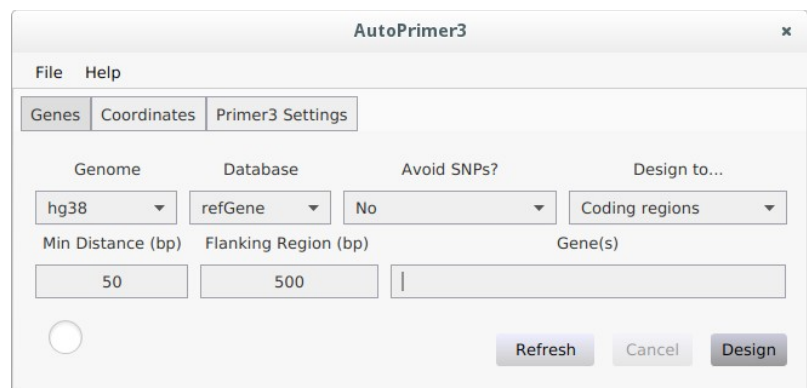
Note: An internet connection is required by the program to retrieve database information from the UCSC genome browser. AutoPrimer3 will not work offline.

Designing to Genes or Coordinates

At startup the 'Genes' tab is shown.

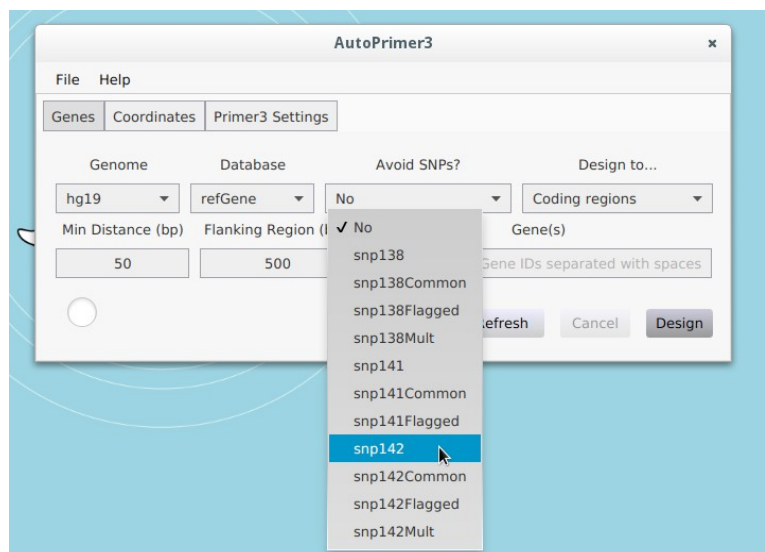
Select the desired genome for the species you want to design primers for using the '**Genome**' menu.

Hover the mouse cursor over the loaded genome for its full description



Once the genome information is loaded you may select the desired **gene database**. If there is an error loading the genome information you may try using the 'Refresh' button to attempt reloading from the UCSC database.

And, if available, you may select a **SNP database** in order to prevent primers from being designed on top of any coordinates that match SNPs in the given database.

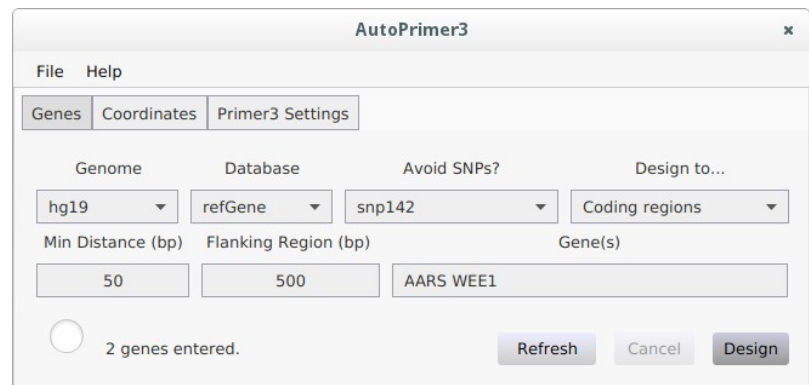


The '**Design to...**' menu allows you to choose between designing only to coding regions or to all exons of gene targets.

The '**Min distance**' field lets you choose the minimum distance in base pairs (bp) between targets and primers.

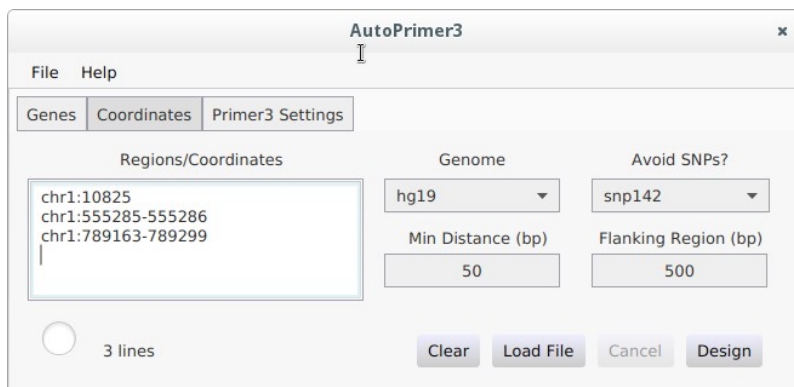
The '**Flanking region**' field tells AutoPrimer3 how many bp of flanking DNA to retrieve either side of target regions to use for its primer designs.

Enter one or more gene symbols or transcript ids in the '**Gene(s)**' input box and either press Enter or click the '**Design**' button to start designing primers. If a gene symbol is used primers will be designed to all transcripts found for the given gene in the relevant database.



The screenshot shows the 'AutoPrimer3' application window with the 'Genes' tab selected. The interface includes a menu bar with 'File' and 'Help'. Below the tabs, there are four dropdown menus: 'Genome' (set to 'hg19'), 'Database' (set to 'refGene'), 'Avoid SNPs?' (set to 'snp142'), and 'Design to...' (set to 'Coding regions'). Below these are two input fields: 'Min Distance (bp)' with the value '50' and 'Flanking Region (bp)' with the value '500'. To the right is a text input field for 'Gene(s)' containing 'AARS WEE1'. At the bottom left, there is a radio button and the text '2 genes entered.'. At the bottom right, there are three buttons: 'Refresh', 'Cancel', and 'Design'.

Alternatively, you may enter coordinates to design primers to as shown below using the input box on the '**Coordinates**' tab. The same options are available for adjusting genome, SNP databases, minimum distance from primer to target and amount of flanking sequence to use as for the 'Genes' tab. Coordinates can be typed into the '**Regions/Coordinates**' input box.



The screenshot shows the 'AutoPrimer3' application window with the 'Coordinates' tab selected. The interface includes a menu bar with 'File' and 'Help'. Below the tabs, there is a text input field for 'Regions/Coordinates' containing three lines of coordinates: 'chr1:10825', 'chr1:555285-555286', and 'chr1:789163-789299'. To the right of this field are two dropdown menus: 'Genome' (set to 'hg19') and 'Avoid SNPs?' (set to 'snp142'). Below these are two input fields: 'Min Distance (bp)' with the value '50' and 'Flanking Region (bp)' with the value '500'. At the bottom left, there is a radio button and the text '3 lines'. At the bottom right, there are four buttons: 'Clear', 'Load File', 'Cancel', and 'Design'.

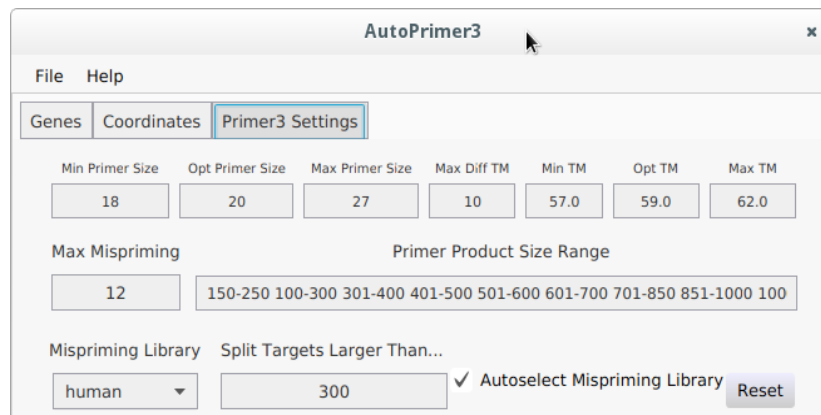
Alternatively, you may use the '**Load File**' button to load up to 100 regions from a BED or VCF file or a text file with regions specified as intervals (i.e. in the format chr1:1000-2000). The 'Regions/Coordinates' input can be cleared using the '**Clear**' button.

Adjusting Primer Design Settings

The '**Primer3 Settings**' tab provides various options to be passed to the Primer3 (<http://primer3.sourceforge.net/>) program when designing primers. The first two rows of options are options specific to the Primer3 tool, and more information can be found in Primer3's own documentation.

AutoPrimer3 makes three mispriming libraries available for use with Primer3: human, rodent and drosophila. By default, when you select a genome if an appropriate mispriming library is available it will be automatically selected. This behaviour can be turned off by unchecking the

'**Autoselect Mispriming Library**' checkbox and you may override the programs choice for a given genome by manually selecting a different mispriming library.



The screenshot shows the AutoPrimer3 application window with the 'Primer3 Settings' tab selected. The window has a menu bar with 'File' and 'Help'. Below the menu bar are three tabs: 'Genes', 'Coordinates', and 'Primer3 Settings'. The 'Primer3 Settings' tab contains several input fields and a checkbox. The fields are: 'Min Primer Size' (18), 'Opt Primer Size' (20), 'Max Primer Size' (27), 'Max Diff TM' (10), 'Min TM' (57.0), 'Opt TM' (59.0), and 'Max TM' (62.0). Below these is a 'Max Mispriming' field (12) and a 'Primer Product Size Range' field (150-250 100-300 301-400 401-500 501-600 601-700 701-850 851-1000 100). At the bottom, there is a 'Mispriming Library' dropdown menu (human), a 'Split Targets Larger Than...' input field (300), a checked 'Autoselect Mispriming Library' checkbox, and a 'Reset' button.

Also of note, is the '**Split Targets Larger Than...**' input field. The default value of 300 is chosen such that with the default '**Min Distance**' value of 50 PCR products should generally be smaller than 500 bp. This is chosen as a sensible default in the expectation that most users will want PCR products of this size due to their relative ease of amplification and suitability for Sanger sequencing of the full length of the target sequence. If the user changes this value they may also want to change the values used in the '**Primer Product Size Range**' field, which has a default maximum of 2000 bp.

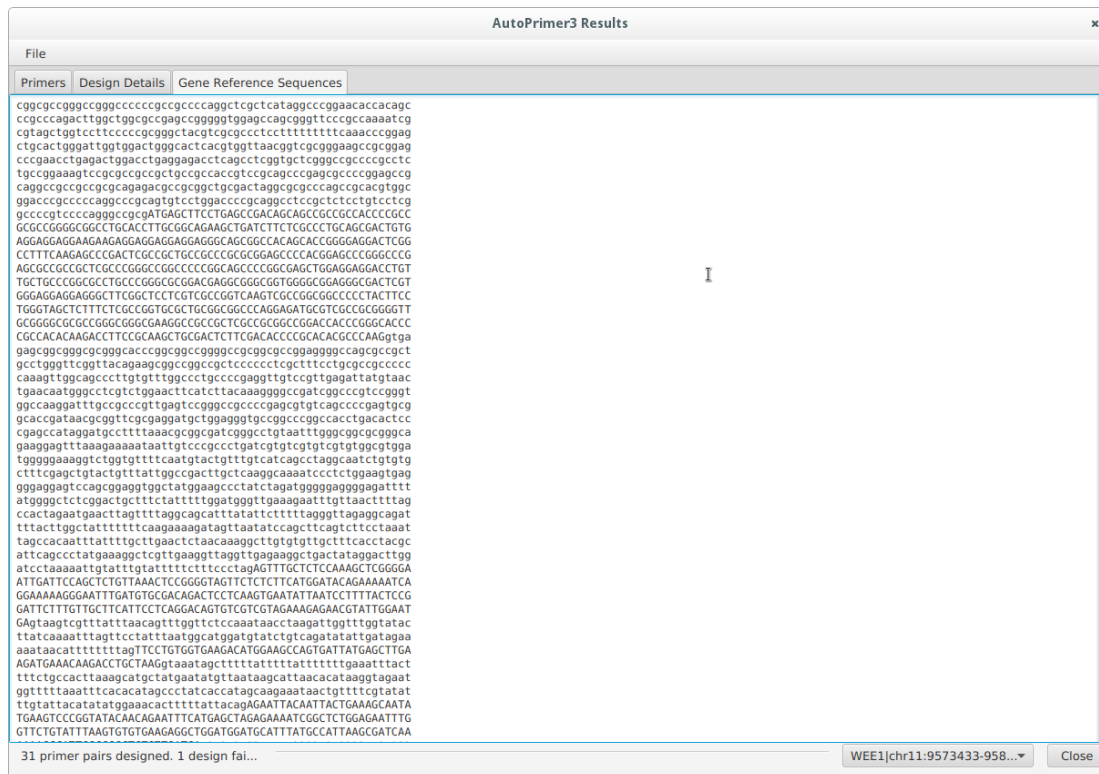
Results

Once designs are completed a new window will appear with the results. You may use the associated '**File**' menu to **save** the output of each tab. Note that if choosing .xlsx output for saving primers the output consists of two worksheets, one giving the primers in a list (one row per primer, which may be useful for copying and pasting into certain primer purchasing websites) and one giving more detailed output like that shown by the AutoPrimer3 Results window.

The '**Primers**' tab of the results window contains a table of the primer targets and designs. Most of the fields are self-explanatory but the final column provides a hyperlink to perform in-silico PCR at the UCSC genome browser in order to check for the specificity of the primer pairs.

You may also choose to use the '**Check isPCR Results**' button to perform an automatic check of the number of predicted PCR products in your target genome from UCSC's in-silico PCR program. Once complete, a new column will be added with the number of predicted PCR products for each primer pair and a warning will be displayed if any primer pair appears to amplify more than one genomic region. Please note that sometimes the in-silico PCR tool appears to fail to find any matches for a pair when one of the primers is short, but this does not necessarily mean that the primers are of no use.

When designing primers to genes, the '**Gene Reference Sequences**' tab provides the DNA sequence for your target exons plus flanking sequence either side (the length of which is determined by the value used in the '**Flanking region**' field when the primers were designed). If there were multiple target regions (either multiple genes/transcripts specified or genes that map to multiple genomic regions) the choice box towards the bottom right of the window can be used to switch between the relevant reference sequences.



Credit

AutoPrimer3 uses primer3 (<http://primer3.sourceforge.net/>). AutoPrimer3 was written by David A. Parry and is available from:

<https://github.com/gantzgraf/autoprimers3>

or alternatively:

<https://sourceforge.net/projects/autoprimers3/>

It was originally available as a perl script and a perl/perl + objective C based GUI application for Windows and Mac OS X. This version is a complete rewrite using java and is available for Windows, Mac OS X and linux.

If you use AutoPrimer3 for primer designs that are used in published work, please cite the URL '<https://github.com/gantzgraf/autoprimers3>'.

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