

# 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).

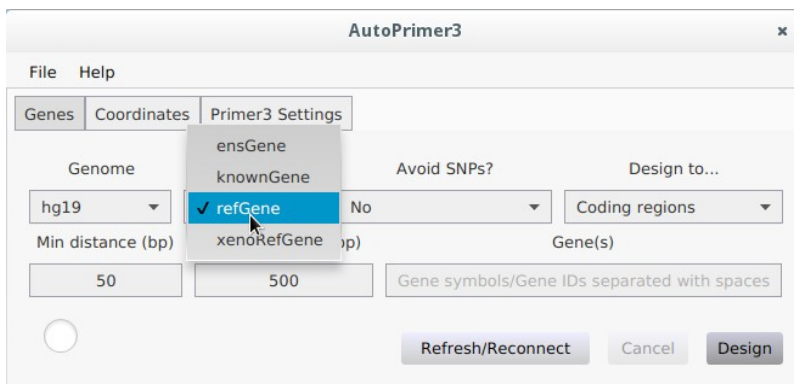
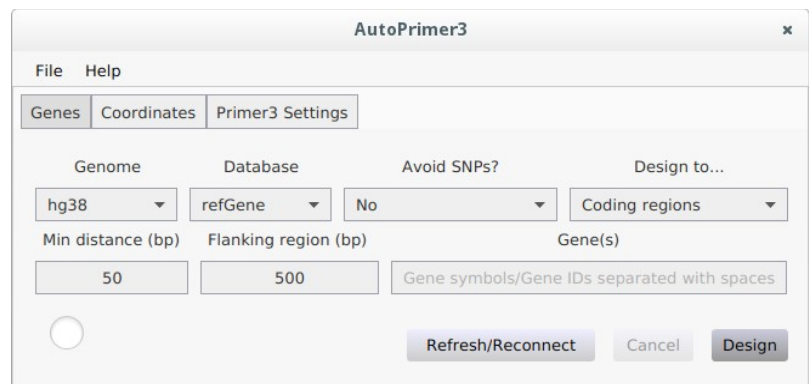
Note, An internet connection is required by the program to retrieve database information from the UCSC genome browser.

## Designing to Genes or Coordinates

At startup the 'Genes' tab is shown.

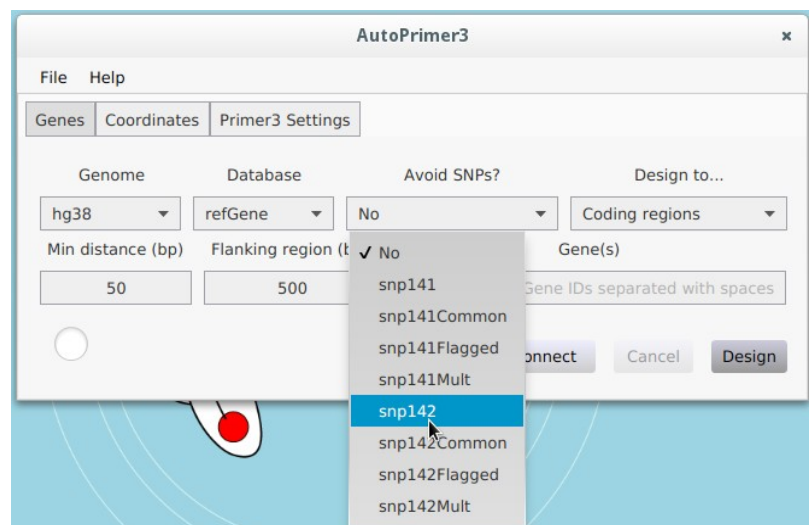
Select the desired genome for the species you want to design primers for using the '**Genome**' menu.

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**.

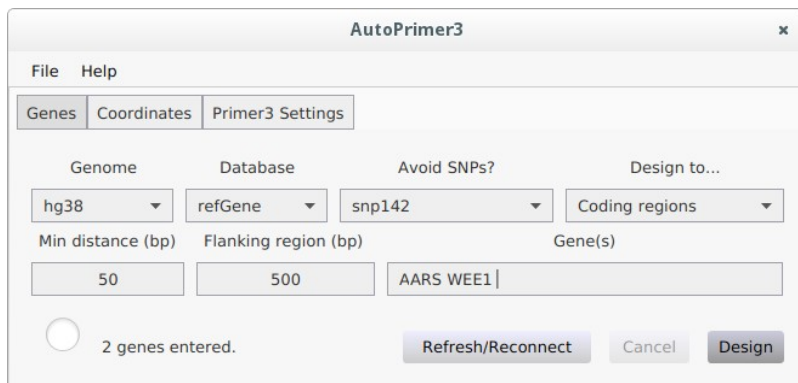
And, if available, you may select a **SNP database** in order to prevent primers from being designed on top of any coordinates that match SNPs in the given database.



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.

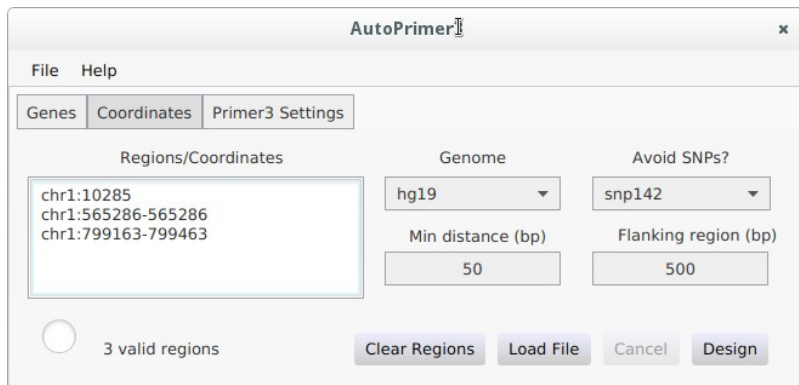
The '**Flanking region**' field tells AutoPrimer3 how many bp of flanking DNA to retrieve either side of target regions to use for its primer designs.



The screenshot shows the 'AutoPrimer3' application window with the 'Genes' tab selected. The interface includes a menu bar with 'File' and 'Help'. Below the menu bar are three tabs: 'Genes', 'Coordinates', and 'Primer3 Settings'. The 'Genes' tab contains several settings: 'Genome' set to 'hg38', 'Database' set to 'refGene', 'Avoid SNPs?' set to 'snp142', and 'Design to...' set to 'Coding regions'. Below these are input fields for 'Min distance (bp)' (50) and 'Flanking region (bp)' (500). A text box labeled 'Gene(s)' contains the text 'AARS WEE1'. At the bottom, there is a status indicator showing a radio button and the text '2 genes entered.', along with three buttons: 'Refresh/Reconnect', 'Cancel', and 'Design'.

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.

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 menu bar are three tabs: 'Genes', 'Coordinates', and 'Primer3 Settings'. The 'Coordinates' tab contains a large text box labeled 'Regions/Coordinates' containing three lines of coordinates: 'chr1:10285', 'chr1:565286-565286', and 'chr1:799163-799463'. To the right of this box are settings for 'Genome' set to 'hg19', 'Avoid SNPs?' set to 'snp142', 'Min distance (bp)' set to '50', and 'Flanking region (bp)' set to '500'. At the bottom, there is a status indicator showing a radio button and the text '3 valid regions', along with four buttons: 'Clear Regions', '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).

## 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.

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.

AutoPrimer3 Results								
File								
<div> <div>Primers</div> <div>Design Details</div> <div>Gene Reference Sequences</div> </div>								
#	Name	Other IDs	Left Primer	Right Primer	Product S...	Region	in silico PCR	
1	WEE1_ex2_part1	NM_003390_ex1_part1	gagacctcagcctcggtg	gccgaagccctctctctc	596	chr11:9573698-95...	<a href="#">in-silico PCR</a>	
2	WEE1_ex2_part2	NM_003390_ex1_part2	gcccttcaagagcccgact	ctgtaaccgaaccaggca	499	chr11:9574093-95...	<a href="#">in-silico PCR</a>	
3	WEE1_ex3	NM_003390_ex2/NM...	aggctcgttgaggttaggt	tctgacagatacatccatgcca	392	chr11:9575807-95...	<a href="#">in-silico PCR</a>	
4	WEE1_ex4	NM_001143976_ex3/...	ccaaataacctaagattggttgg	gtgataggcgctatgtgtgaaattt	300	chr11:9576123-95...	<a href="#">in-silico PCR</a>	
5	WEE1_ex5	NM_001143976_ex4/...	tcacacatagccctatcaccat	gtgttgaccagctgaaacct	335	chr11:9576404-95...	<a href="#">in-silico PCR</a>	
6	WEE1_ex6	NM_001143976_ex5/...	accctctctctctaagcaagt	agtgacagaaatagagcaagattt	356	chr11:9577001-95...	<a href="#">in-silico PCR</a>	
7	WEE1_ex7	NM_001143976_ex6/...	tgggtgagattggttggtag	tggtaagtggatctaaggcc	380	chr11:9581436-95...	<a href="#">in-silico PCR</a>	
8	WEE1_ex8	NM_001143976_ex7/...	ggctagaacttgagaatcggc	tgaaaaacttacagtgaagagcaa	294	chr11:9585138-95...	<a href="#">in-silico PCR</a>	
9	WEE1_ex9	NM_001143976_ex8/...	gctgcctcgaagaaggaga	tgccctcagatgacggttta	371	chr11:9585293-95...	<a href="#">in-silico PCR</a>	
10	WEE1_ex10	NM_001143976_ex9/...	gtcatcttggagggtgaattctgtt	gctttaccagtcgcaattgct	392	chr11:9586374-95...	<a href="#">in-silico PCR</a>	
11	WEE1_ex11	NM_001143976_ex1...	tgaagccctttattgacatggt	ccagcctgatgacagtgaga	375	chr11:9586636-95...	<a href="#">in-silico PCR</a>	
12	WEE1_ex12	NM_001143976_ex1...	tggcatgcaaatatctccca	actgacacaatcgagaaagt	353	chr11:9588353-95...	<a href="#">in-silico PCR</a>	
13	AARS_ex2	NM_001605_ex2	tgtgtactttgactttcaattccct	gggctgtgctttatctggg	295	chr16:70282542-7...	<a href="#">in-silico PCR</a>	
14	AARS_ex3	NM_001605_ex3	gtggtcgctcttctctgcta	agaaccacagttccagtggt	343	chr16:70276883-7...	<a href="#">in-silico PCR</a>	
15	AARS_ex4	NM_001605_ex4	tgtgtctcatgacttggtct	cataaaacccactctggcac	299	chr16:70276405-7...	<a href="#">in-silico PCR</a>	
16	AARS_ex5	NM_001605_ex5	ggagtcgagtaacatgagcc	gctcacacagctccgagttcc	386	chr16:70271658-7...	<a href="#">in-silico PCR</a>	
17	AARS_ex6	NM_001605_ex6	tgaatttttaaccagcagggact	tctttgacagcactgttaagagt	298	chr16:70270118-7...	<a href="#">in-silico PCR</a>	
18	AARS_ex7	NM_001605_ex7	caagaccggcatctttctg	ggcaagatcacgccattgta	397	chr16:70269505-7...	<a href="#">in-silico PCR</a>	
19	AARS_ex8	NM_001605_ex8	agggtgtgcttgggttagga	aacagtatgccctctccac	272	chr16:70268186-7...	<a href="#">in-silico PCR</a>	
20	AARS_ex9	NM_001605_ex9	actgcaccagctaaggag	atgagagcccacagtcagtc	332	chr16:70267570-7...	<a href="#">in-silico PCR</a>	
21	AARS_ex10	NM_001605_ex10	aattcctctgccagtcacca	aactcatgctcagtcgcag	297	chr16:70265444-7...	<a href="#">in-silico PCR</a>	
22	AARS_ex11	NM_001605_ex11	cagagaatgactgagccca	gcactctctgagagactaa	305	chr16:70264805-7...	<a href="#">in-silico PCR</a>	

Note that exons for each gene are counted from the first exon of all relevant transcripts, even if that exon is non-coding and the user has chosen only to design to coding regions. So, the first exon target may not necessarily be numbered '1'.

The **'Design Details'** tab provides output from primer3 for each design attempted. This output should be familiar to users of Primer3 on the web.

```
AutoPrimer3 Results

File

Primers Design Details Gene Expression Sequences

PRIMER PICKING RESULTS FOR WEE1_ex2_part1: NM_003390_ex1_part1

Using mispriming library /home/david/.AutoPrimer3/mispriming_libs/human
Using 0-based sequence positions
OLIGO      start len   tm    gc%  any_th  3' th hairpin rep seq
LEFT PRIMER 248   20   58.92 55.00  5.22  0.00  0.00 10.00 tgagactggacctgaggaga
RIGHT PRIMER 316   20   58.52 55.00  0.00  0.00  0.00 11.00 cgagaagagctaccacaga
SEQUENCE SIZE: 1288
INCLUDED REGION SIZE: 1288

PRODUCT SIZE: 669, PAIR ANY_TH COMPL: 6.48, PAIR 3'_TH COMPL: 0.00
TARGETS (start, len)*: 450,389
EXCLUDED REGIONS (start, len)*: 15,1 16,1 63,1 99,1 101,1 115,1 121,1 138,1 149,1 439,1 472,1 625,0 643,1 655,1 660,1 679,1 691,1 741,1 751,1 763,1 768,1

0 cgcgcggcgccggcggccccgcgcgcccggtctgctcataggccgggaacacacagc
XX
60 cgcggcagacttggtgctgcgcgcgagcgggggtggagcagcgggttcccgcgaatcg
X X X
120 ctgactggtctcttcgcccgggctacgtgcgcctctctttttttcaaacccggag
X X X X
180 ctgcactgggattggtggactgggcactcacgttgttaacggtcgcgggaagccggag

240 ccgaaacctgagactggacctgaggagacctcagctcggtgctcgggccgcgcctc
>>>>>>>>>>>>>>
300 tgcggaaagtccgcgcgcgcgtgcgcacgctccgacgccgagcccccggagcgc

360 caggccgcgcgcgcagagacgcgcggctgcgactaggcgcgccacgccgcagctgc

420 ggacccgccccccaggcccgagtccttggaaccccgaggctccgctctctgtcctg
X X *****X*****
480 gcccgctcccaggccgcgcgATGAGCTTCTTGAGCCGACAGCAGCCGCCGCACCCCGCC
*****
540 GCGCGGGCGGCCGTGCACCTTTGCGCGAGAAGCTGATCTTC TGCCCTGCAGCAGCTGTG
*****
600 AGGAGGAGGAAGAAGAGGAGGAGGAGGAGGCGAGCGGCACAGCACCGGGAGGACTCGG
*****X*****
```

[illegible]



## 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>'.

## License

AutoPrimer3 is free software: you can redistribute it and/or modify it under the terms of the GNU General Public License as published by the Free Software Foundation, either version 3 of the License, or (at your option) any later version.

This program is distributed in the hope that it will be useful, but WITHOUT ANY WARRANTY; without even the implied warranty of MERCHANTABILITY or FITNESS FOR A PARTICULAR PURPOSE. See the GNU General Public License for more details.

You should have received a copy of the GNU General Public License along with this program. If not, see <<http://www.gnu.org/licenses/>>.