

# AutoPrimer3

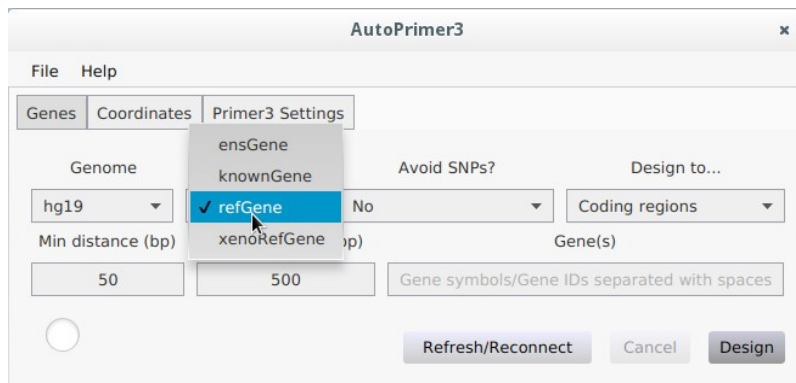
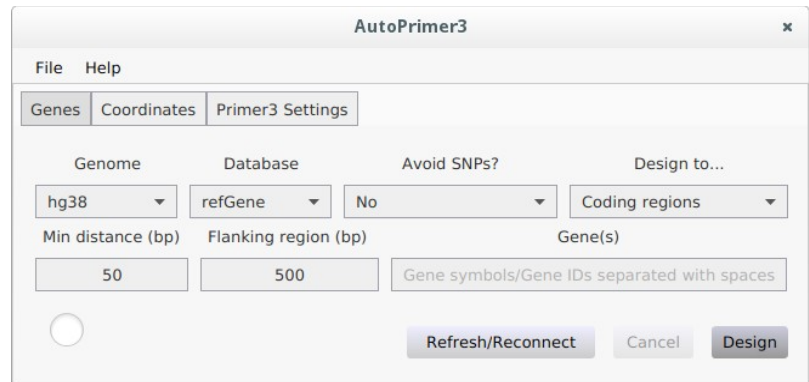
AutoPrimer3 is designed to automatically design primers to genes or genomic coordinate targets. An internet connection is required by the program to retrieve database information from the UCSC genome browser and to retrieve gene information, DNA sequences and SNP annotations from the various hosted genomes.

## Designing

At startup the 'Genes' tab is shown.

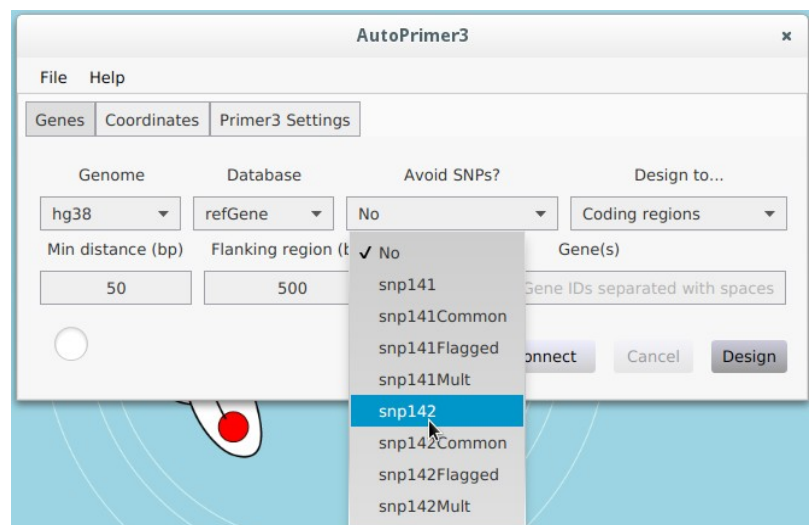
Select the desired genome for the species you want to design primers for using the '**Genome**' menu.

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.

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.

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 '**Primer3 Settings**' tab provides various options to be passed to the primer3 (<http://primer3.sourceforge.net/>) program when designing primers. Three mispriming libraries are available: human, rodent and drosophila.

## 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 first 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.

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 Size	Region	in silico PCR
1	WEE1_ex2_part1	NM_003390_ex1_part1	tgagactggacctgaggaga	cgagaaagagctacccagga	669	chr11:9573682-95...	<a href="#">in-silico PCR</a>
2	WEE1_ex2_part2	NM_003390_ex1_part2	gaagctgatctctcgccct	gccgcttctgtaaccgaac	597	chr11:9574002-95...	<a href="#">in-silico PCR</a>
3	WEE1_ex3	NM_003390_ex2/NM...	aggctcggtgaaggttaggt	ggcttcattgtcttcaccac	448	chr11:9575807-95...	<a href="#">in-silico PCR</a>
4	WEE1_ex4	NM_001143976_ex3/...	tccaataacctaagatggttgggt	tgtgtgaatttaaaacattctacc	289	chr11:9576122-95...	<a href="#">in-silico PCR</a>
5	WEE1_ex5	NM_001143976_ex4/...	ggtagaatggttttaatttac...	gtgtttgaccagctgaaacct	355	chr11:9576384-95...	<a href="#">in-silico PCR</a>
6	WEE1_ex6	NM_001143976_ex5/...	tccttaatggaatctgccttgga	tgtacaagggtgcttcagaaagg	490	chr11:9576957-95...	<a href="#">in-silico PCR</a>
7	WEE1_ex7	NM_001143976_ex6/...	ggttgagatggttggttag	ggccacagaatgagacactg	469	chr11:9581437-95...	<a href="#">in-silico PCR</a>
8	WEE1_ex8	NM_001143976_ex7/...	ggctgaacttgagaatcggc	tgaaaaacttacagtgaagagcaa	294	chr11:9585138-95...	<a href="#">in-silico PCR</a>
9	WEE1_ex9	NM_001143976_ex8/...	cccaaatgctgcctctgaag	tgccctcagatgacggttta	378	chr11:9585286-95...	<a href="#">in-silico PCR</a>
10	WEE1_ex10	NM_001143976_ex9/...	gtcatcttgaggtgtaactgtgt	gctttaccagtgccattgct	392	chr11:9586374-95...	<a href="#">in-silico PCR</a>
11	WEE1_ex11	NM_001143976_ex1...	accacaagtctttccaag	gacacgggaggatagcttga	498	chr11:9586580-95...	<a href="#">in-silico PCR</a>
12	WEE1_ex12	NM_001143976_ex1...	gcaaatatctccaatcgacaat	tgcaactggattctatcagtga	323	chr11:9588359-95...	<a href="#">in-silico PCR</a>
13	AARS_ex2	NM_001605_ex2	tgagaagccagaagtctgtg	ttgcaagaactgaggccc	400	chr16:70282518-7...	<a href="#">in-silico PCR</a>
14	AARS_ex3	NM_001605_ex3	gtggtcgctcttctctgcta	gcaaaattagaaccagttccc	351	chr16:70276883-7...	<a href="#">in-silico PCR</a>
15	AARS_ex4	NM_001605_ex4	gggaactgggtctaattttgc	gtcataaaacccactctggc	497	chr16:70276209-7...	<a href="#">in-silico PCR</a>
16	AARS_ex5	NM_001605_ex5	cactgtgcccgccaatc	tagggccattctgttccag	441	chr16:70271677-7...	<a href="#">in-silico PCR</a>
17	AARS_ex6	NM_001605_ex6	tcacagctgcgaactgttc	agtaggcagatggaaatgggt	377	chr16:70270092-7...	<a href="#">in-silico PCR</a>
18	AARS_ex7	NM_001605_ex7	ggaactctcttgggcaag	accagcaggcgagaggtt	381	chr16:70269546-7...	<a href="#">in-silico PCR</a>
19	AARS_ex8	NM_001605_ex8	gcgaagaagcaggcttagg	agcgtgggtgacagagtg	368	chr16:70268145-7...	<a href="#">in-silico PCR</a>
20	AARS_ex9	NM_001605_ex9	gagctcaggcaatccacct	atgagagccacagtcagtc	391	chr16:70267511-7...	<a href="#">in-silico PCR</a>
21	AARS_ex10	NM_001605_ex10	cagtccagcatccttttgg	aactcatgtcagctctgcag	285	chr16:70265456-7...	<a href="#">in-silico PCR</a>
22	AARS_ex11	NM_001605_ex11	caagaatgactgcccga	gcactctctggaagctaa	305	chr16:70264805-7...	<a href="#">in-silico PCR</a>

[illegible][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.

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