

AutoPrimer3

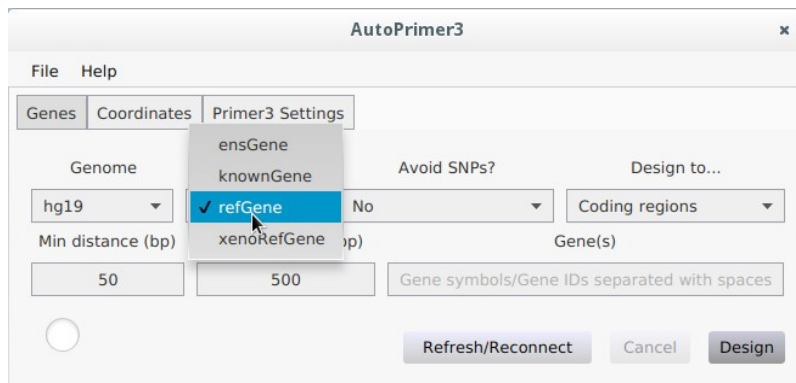
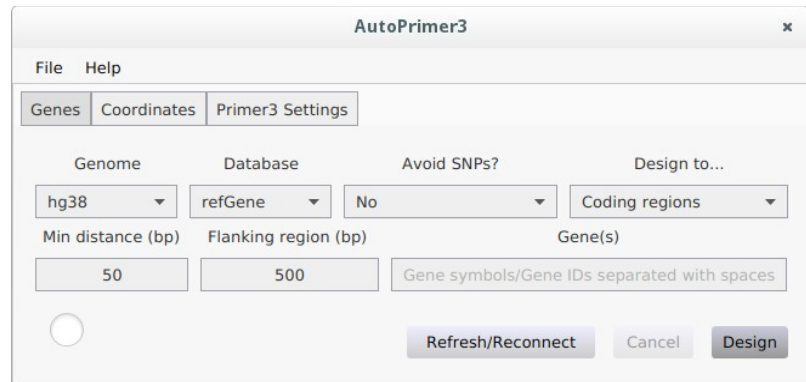
AutoPrimer3 retrieves gene information, DNA sequences and SNP information from the UCSC genome browser and uses primer3 (<http://primer3.sourceforge.net/>) to automatically design primers to genes or genomic coordinate targets. An internet connection is required by the program to retrieve database information from the UCSC genome browser.

Designing

At startup the 'Genes' tab is shown.

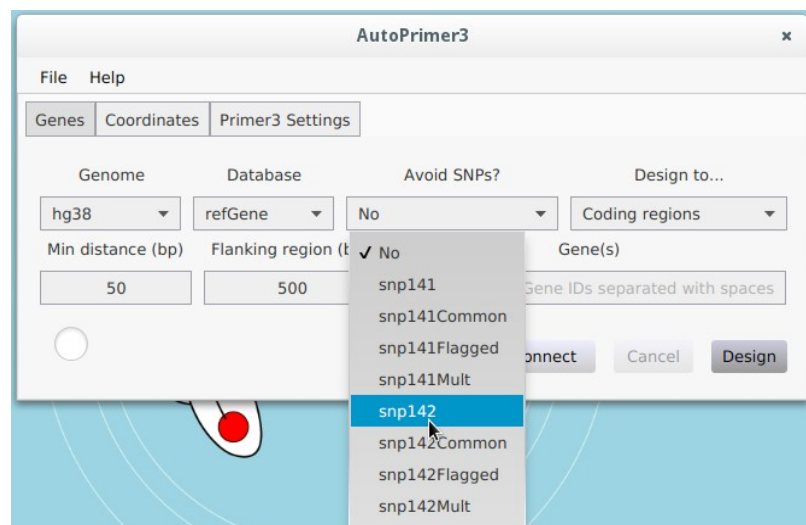
Select the desired genome for the species you want to design primers for using the '**Genome**' menu.

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.

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 input fields: 'Genome' (set to 'hg38'), 'Database' (set to 'refGene'), 'Avoid SNPs?' (set to 'snp142'), and 'Design to...' (set to 'Coding regions'). Below these are 'Min distance (bp)' (set to '50') and 'Flanking region (bp)' (set to '500'). A text box for 'Gene(s)' contains 'AARS WEE1'. At the bottom, there is a radio button labeled '2 genes entered.', a 'Refresh/Reconnect' button, a 'Cancel' button, and a 'Design' button.

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 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 text box for 'Regions/Coordinates' with the following content: 'chr1:10285', 'chr1:565286-565286', and 'chr1:799163-799463'. To the right of this text box are 'Genome' (set to 'hg19') and 'Avoid SNPs?' (set to 'snp142'). Below these are 'Min distance (bp)' (set to '50') and 'Flanking region (bp)' (set to '500'). At the bottom, there is a radio button labeled '3 valid regions', a 'Clear Regions' button, a 'Load File' button, a 'Cancel' button, and a 'Design' button.

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.

The screenshot shows the 'AutoPrimer3' application window with the 'Primer3 Settings' 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 'Primer3 Settings' tab contains several input fields: 'Min Primer Size' (set to '18'), 'Opt Primer Size' (set to '20'), 'Max Primer Size' (set to '27'), 'Max Diff TM' (set to '10'), 'Min TM' (set to '57.0'), 'Opt TM' (set to '59.0'), and 'Max TM' (set to '62.0'). Below these are 'Max Mispriming' (set to '12') and 'Primer Product Size Range' (set to '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 (set to 'human'), a 'Split Targets Larger Than...' input field (set to '300'), a checked 'Autoselect Mispriming Library' checkbox, and a 'Reset' button.

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							
Primers Design Details Gene Reference Sequences							
#	Name	Other IDs	Left Primer	Right Primer	Product Size	Region	in silico PCR
1	WEE1_ex2_part1	NM_003390_ex1_part1	tgagactggacctgaggaga	cgagaaagagctaccagga	669	chr11:9573682-95...	in-silico PCR
2	WEE1_ex2_part2	NM_003390_ex1_part2	gaagctgattctctgccct	gccgcttctgtaaccgaac	597	chr11:9574002-95...	in-silico PCR
3	WEE1_ex3	NM_003390_ex2/NM...	aggctcgttgaaggttaggt	ggcttccatgtcttcaccac	448	chr11:9575807-95...	in-silico PCR
4	WEE1_ex4	NM_001143976_ex3/...	tcacaataacctaagattggttgggt	tgtgtgaataaaaaaccattctacc	289	chr11:9576122-95...	in-silico PCR
5	WEE1_ex5	NM_001143976_ex4/...	ggtagaatggttttaaatccac...	gtgttgaccagctgaaacct	355	chr11:9576384-95...	in-silico PCR
6	WEE1_ex6	NM_001143976_ex5/...	tccttaatggaatctgcctttgga	tgtacaagggtcttcagaaagg	490	chr11:9576957-95...	in-silico PCR
7	WEE1_ex7	NM_001143976_ex6/...	ggttgagattggttggtgag	ggccacagaatgagacactg	469	chr11:9581437-95...	in-silico PCR
8	WEE1_ex8	NM_001143976_ex7/...	ggtagaacttgagaatcggc	tgaaaacttacagtgaagagcaa	294	chr11:9585138-95...	in-silico PCR
9	WEE1_ex9	NM_001143976_ex8/...	cccaaatgctgcctctgaag	tgccctcagatgacggttta	378	chr11:9585286-95...	in-silico PCR
10	WEE1_ex10	NM_001143976_ex9/...	gtcatctttgaggtgtaattctgtt	gctttaccagtgcattgtct	392	chr11:9586374-95...	in-silico PCR
11	WEE1_ex11	NM_001143976_ex1...	accacaagtgtttcccaag	gacacgggaggatagcttga	498	chr11:9586580-95...	in-silico PCR
12	WEE1_ex12	NM_001143976_ex1...	gcaaatatctccaatcgacaat	tgcaaaactgattctatcagtga	323	chr11:9588359-95...	in-silico PCR
13	AARS_ex2	NM_001605_ex2	tgagaagccagaagtcttggtg	ttgcaaaagaaactgaggccc	400	chr16:70282518-7...	in-silico PCR
14	AARS_ex3	NM_001605_ex3	gtggtcgtctcttctgcta	gcaaaattagaaccagttccc	351	chr16:70276883-7...	in-silico PCR
15	AARS_ex4	NM_001605_ex4	gggaactgggttctaattttgc	gtcataaaacccactctggc	497	chr16:70276209-7...	in-silico PCR
16	AARS_ex5	NM_001605_ex5	cactgtgcccgccaatc	taggccactcttgttccag	441	chr16:70271677-7...	in-silico PCR
17	AARS_ex6	NM_001605_ex6	tcacagcctgcaaatgttc	agtaggcagatggaatgggt	377	chr16:70270092-7...	in-silico PCR
18	AARS_ex7	NM_001605_ex7	ggaacctcttgggcaag	accagcaggcagaggtt	381	chr16:70269546-7...	in-silico PCR
19	AARS_ex8	NM_001605_ex8	gcgaaagaagcaggcttagg	agcgtgggtgacagagtg	368	chr16:70268145-7...	in-silico PCR
20	AARS_ex9	NM_001605_ex9	gagctcaggcaatccacct	atgagagcccacagtcaatc	391	chr16:70267511-7...	in-silico PCR
21	AARS_ex10	NM_001605_ex10	cagtcccagcatccttttgg	aactcatgtcagttctcag	285	chr16:70265456-7...	in-silico PCR
22	AARS_ex11	NM_001605_ex11	caagaatgacttgcaccca	gcactctctggaagactaa	305	chr16:70264805-7...	in-silico PCR

31 primer pairs designed. 1 design fai...

Close

Note that gene exons 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 screenshot shows the 'AutoPrimer3 Results' window. The 'Design Details' tab is active, displaying the following information:

- 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	916	20	58.52	55.00	0.00	0.00	0.00	11.00	cgagaaagagtaccaggga

- 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)*:** 150, 389
- EXCLUDED REGIONS (start, len)*:** 15, 1 16, 1 63, 1 99, 1 101, 1 115, 1 121, 1 138, 1 149, 1 160, 1 434, 1 439, 1 472, 1 625, 0 643, 1 655, 1 660, 1 679, 1 691, 1 741, 1 751, 1 763, 1 768,

The sequence alignment shows the primers binding to the target sequence. The left primer binds at position 248-268, and the right primer binds at position 896-916. The sequence is shown in reverse complement for the right primer.

```

      0  cggcgcggcggcgcccccgcgccacggctcgtcatagccccggaaccacacgc
          XX
    60  ccgccccagactggctggcgcgagcgggggtggaagccagcgggttccccgcaaatcg
          X             X   X           X
   120  cgtactggttcctccccgcgggctacgtcgccctctttttttaaacccggag
          X             X       X       X
   240  ctgcactggatttgtggaactggcactcacgtgttaacggtcgcgggaagcgcggag
                                     >>>>>>>>>>>>>>>>
   360  cccgaacctgagactggacctgaggagacctagctcgggtctcggccgcccccctc
                                     >>>>>>>>>>>>>>>>
   480  tgccggaaaagtccgcgcgcgctgcgcacacgtccgcagcccgagcgcgccggagcg
                                     >>>>>>>>>>>>>>>>
   600  caggcgccgcgcgcgagagagcgcgggctgcgactaggcgcgccagcgcacgtggc
                                     >>>>>>>>>>>>>>>>
   720  ggaccgcgcccccagcccgcaagtgtctggaccgccgaggctcgcgtctctgtctcg
          X       X               *****X*****
   840  gcccggtcccagggcgcgATGAGCTTCTTGAGCGACAGCAGCGCCGCCACCCGGCC
                                     >>>>>>>>>>>>>>>>
  1000  GCGCCGGGGCGCCTGCACCTTGGCGGAGAAGCTGATCTTCTGCCCTGCAGCACTGTG
                                     >>>>>>>>>>>>>>>>
  1200  AGGAGGAGGAAGAAGAGGAGGAGGAGGCGCACGCCACAGCACCGGGAGGACTCG
          *****X*****X*****
  
```

At the bottom, it states: "31 primer pairs designed. 1 design failed."

[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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