AutoPrimer3

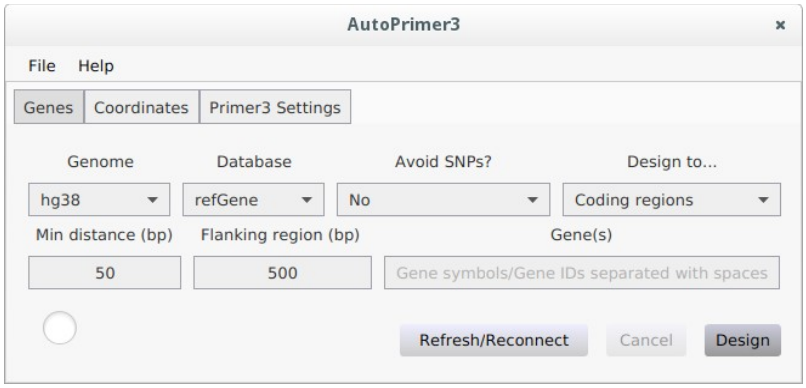
AutoPrimer3 retrieves gene information, DNA sequences and SNP information from the UCSC genome browser and uses primer3 (http://primer3.sourceforge.net/) to automatically design primers to genes or genomic coordinate targets. An internet connection is required by the program to retrieve database information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

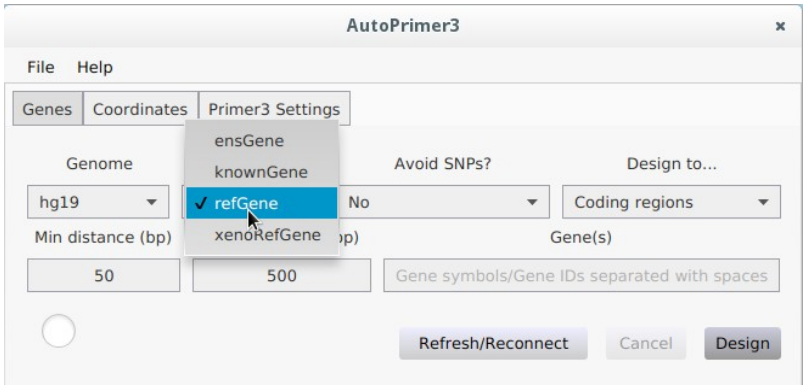
Designing

At startup the 'Genes' tab is shown.

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

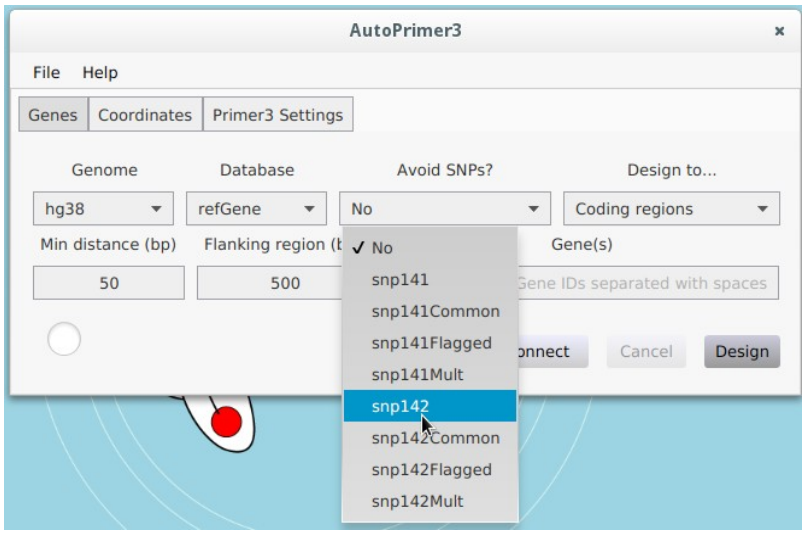
Hover the mouse cursor over the loaded genome for its full description





Once the genome information is loaded you may select the desired **gene database**.

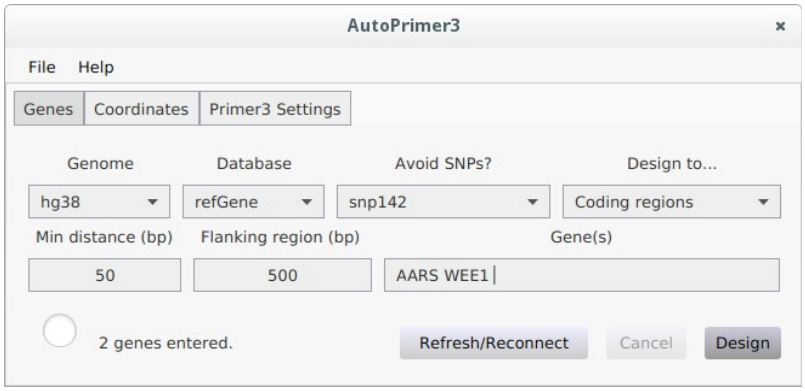
And, if available, you may select a **SNP database** in order to prevent primers from being designed on top of any coordinates that match SNPs in the given database.



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

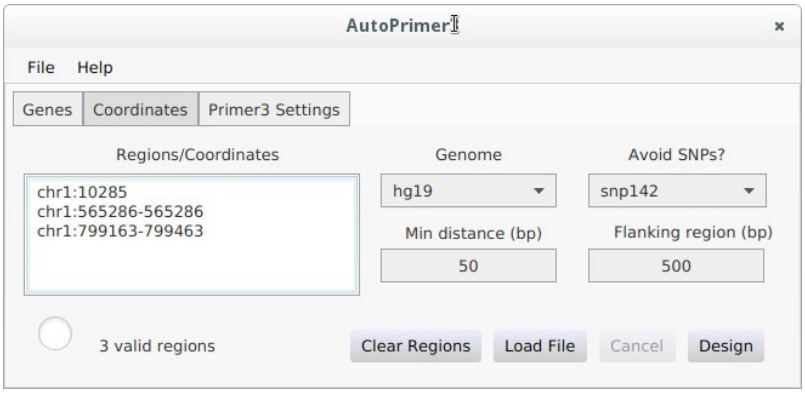
The **'Min distance'** field lets you choose the minimum distance in base pairs (bp) between targets and primers.

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.



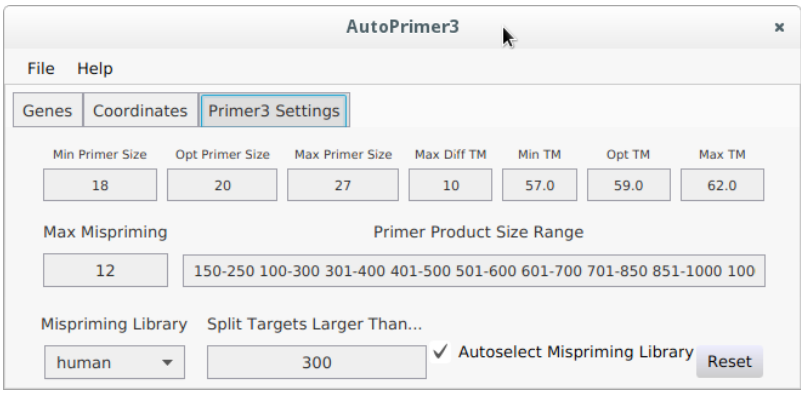
Enter one or more gene symbols or transcript Ids in the **'Gene(s)'** input box and either press Enter or click the **'Design'** button to start designing primers. If a gene symbol is used primers will be designed to all transcripts found for the given gene in the relevant database.

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).

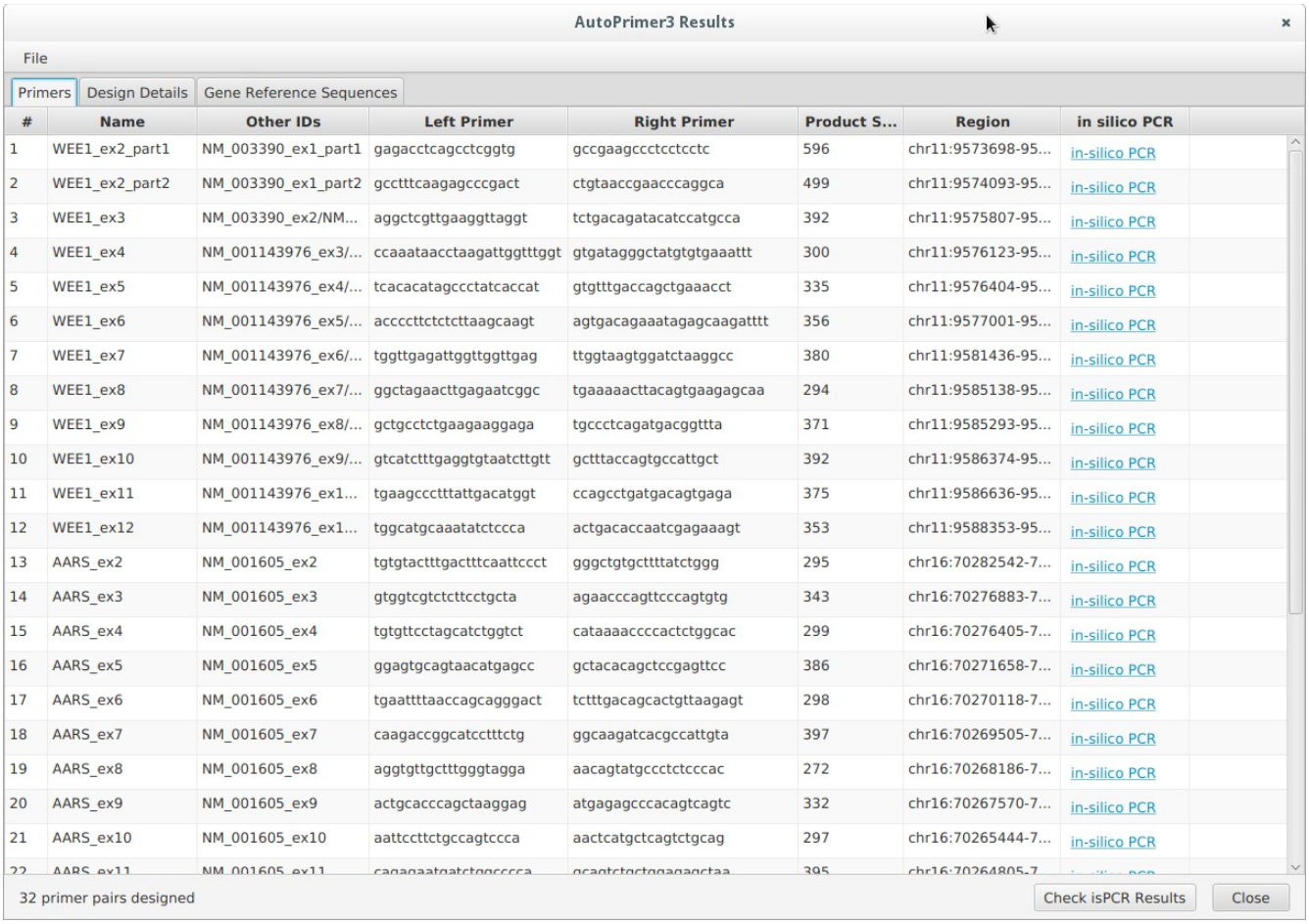
The **'Primer3 Settings'** tab provides various options to be passed to the primer3 (http://primer3.sourceforge.net/) program when designing primers. Three mispriming libraries are available: human, rodent and drosophila.



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.

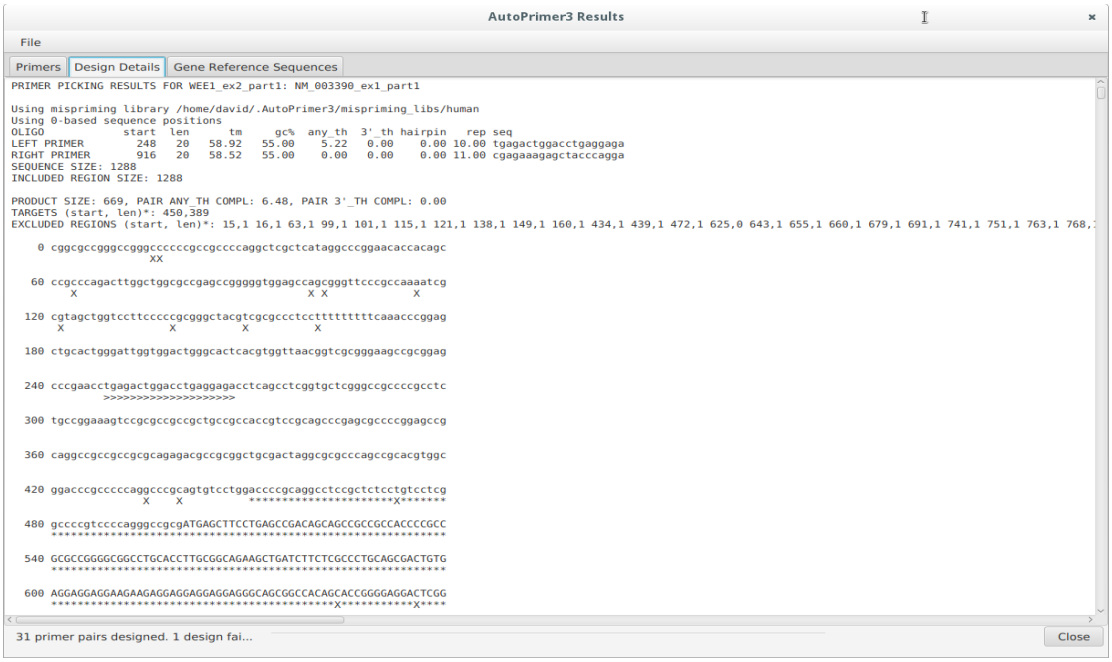


#	Name	Other IDs	Left Primer	Right Primer	Product S...	Region	in silico PCR
1	WEE1_ex2_part1	NM_003390_ex1_part1	gagacctcagcctcggtg	gccgaagccctcctcctc	596	chr11:9573698-95...	in-silico PCR
2	WEE1_ex2_part2	NM_003390_ex1_part2	gcctttcaagagcccgact	ctgtaaccgaacccaggca	499	chr11:9574093-95...	in-silico PCR
3	WEE1_ex3	NM_003390_ex2/NM...	aggctcgttgaaggttaggt	tctgacagatacatccatgcca	392	chr11:9575807-95...	in-silico PCR
4	WEE1_ex4	NM_001143976_ex3/...	ccaaataacctaagattggtttggt	gtgatagggctatgtgtgaaattt	300	chr11:9576123-95...	in-silico PCR
5	WEE1_ex5	NM_001143976_ex4/...	tcacacatagccctatcaccat	gtgtttgaccagctgaaacct	335	chr11:9576404-95...	in-silico PCR
6	WEE1_ex6	NM_001143976_ex5/...	acccccttctctcttaagcaagt	agtgacagaaatagagcaagatttt	356	chr11:9577001-95...	in-silico PCR
7	WEE1_ex7	NM_001143976_ex6/...	tggttgagattggttggttgag	ttggtaagtggatctaaggcc	380	chr11:9581436-95...	in-silico PCR
8	WEE1_ex8	NM_001143976_ex7/...	ggctagaacttgagaatcggc	tgaaaaacttacagtgaagagcaa	294	chr11:9585138-95...	in-silico PCR
9	WEE1_ex9	NM_001143976_ex8/...	gctgcctctgaagaaggaga	tgccctcagatgacggttta	371	chr11:9585293-95...	in-silico PCR
10	WEE1_ex10	NM_001143976_ex9/...	gtcatctttgaggtgtaatcttgtt	gctttaccagtgccattgct	392	chr11:9586374-95...	in-silico PCR
11	WEE1_ex11	NM_001143976_ex1...	tgaagccctttattgacatggt	ccagcctgatgacagtgaga	375	chr11:9586636-95...	in-silico PCR
12	WEE1_ex12	NM_001143976_ex1...	tggcatgcaaatatctccca	actgacaccaatcgagaaagt	353	chr11:9588353-95...	in-silico PCR
13	AARS_ex2	NM_001605_ex2	tgtgtactttgactttcaattccct	gggctgtgcttttatctggg	295	chr16:70282542-7...	in-silico PCR
14	AARS_ex3	NM_001605_ex3	gtggtcgtctcttcctgcta	agaacccagttcccagtgtg	343	chr16:70276883-7...	in-silico PCR
15	AARS_ex4	NM_001605_ex4	tgtgttcctagcatctggtct	cataaaaccccactctggcac	299	chr16:70276405-7...	in-silico PCR
16	AARS_ex5	NM_001605_ex5	ggagtgcagtaacatgagcc	gctacacagctccgagttcc	386	chr16:70271658-7...	in-silico PCR
17	AARS_ex6	NM_001605_ex6	tgaattttaaccagcagggact	tctttgacagcactgttaagagt	298	chr16:70270118-7...	in-silico PCR
18	AARS_ex7	NM_001605_ex7	caagaccggcatcctttctg	ggcaagatcacgccattgta	397	chr16:70269505-7...	in-silico PCR
19	AARS_ex8	NM_001605_ex8	aggtgttgctttgggtagga	aacagtatgccctctcccac	272	chr16:70268186-7...	in-silico PCR
20	AARS_ex9	NM_001605_ex9	actgcacccagctaaggag	atgagagcccacagtcagtc	332	chr16:70267570-7...	in-silico PCR
21	AARS_ex10	NM_001605_ex10	aattccttctgccagtccca	aactcatgctcagtctgcag	297	chr16:70265444-7...	in-silico PCR
22	AARS_ex11	NM_001605_ex11	cagagaatgatctgccccca	gcagtctgctggagagctaa	395	chr16:70264805-7...	

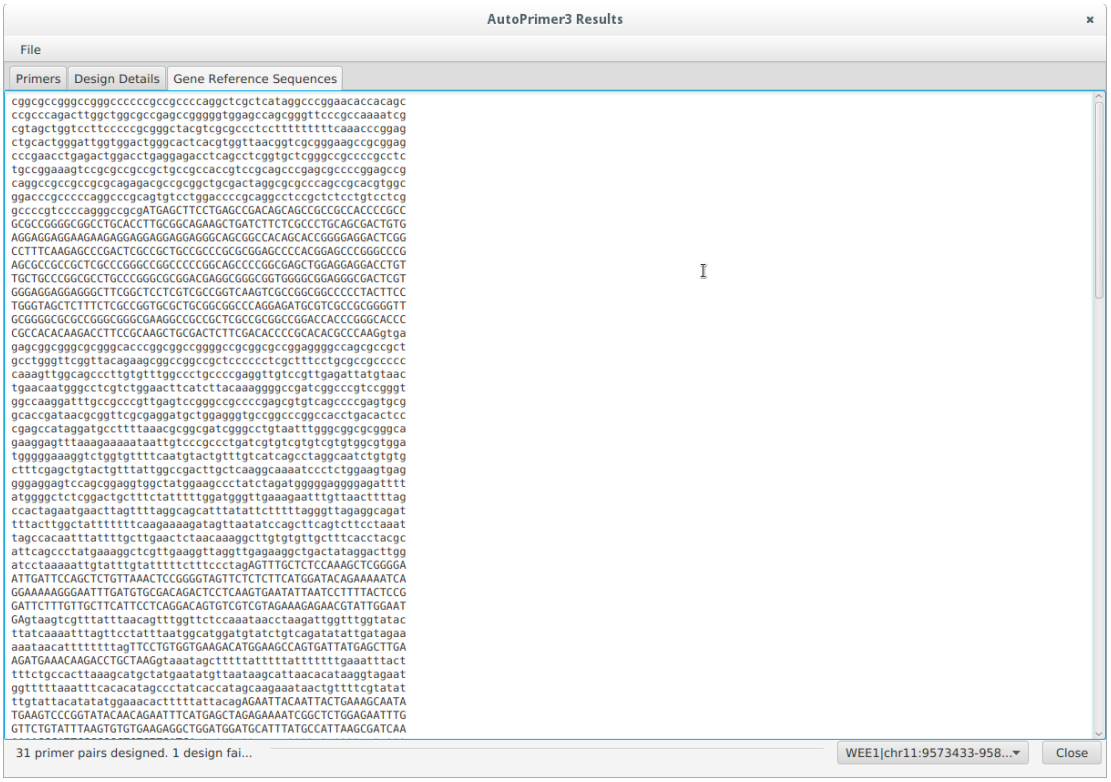
You may also choose to use the **'Check isPCR Results'** button to perform an automatic check of the number of predicted PCR products in your target genome from UCSC's in-silico PCR program. Once complete, a new column will be added with the number of predicted PCR products for each primer pair.

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 **'Design Details'** tab provides output from primer3 for each design attempted.



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/autoprimer3

or alternatively:

https://sourceforge.net/projects/autoprimer3/

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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