Untergasser et al., 2012, Supplementary Data

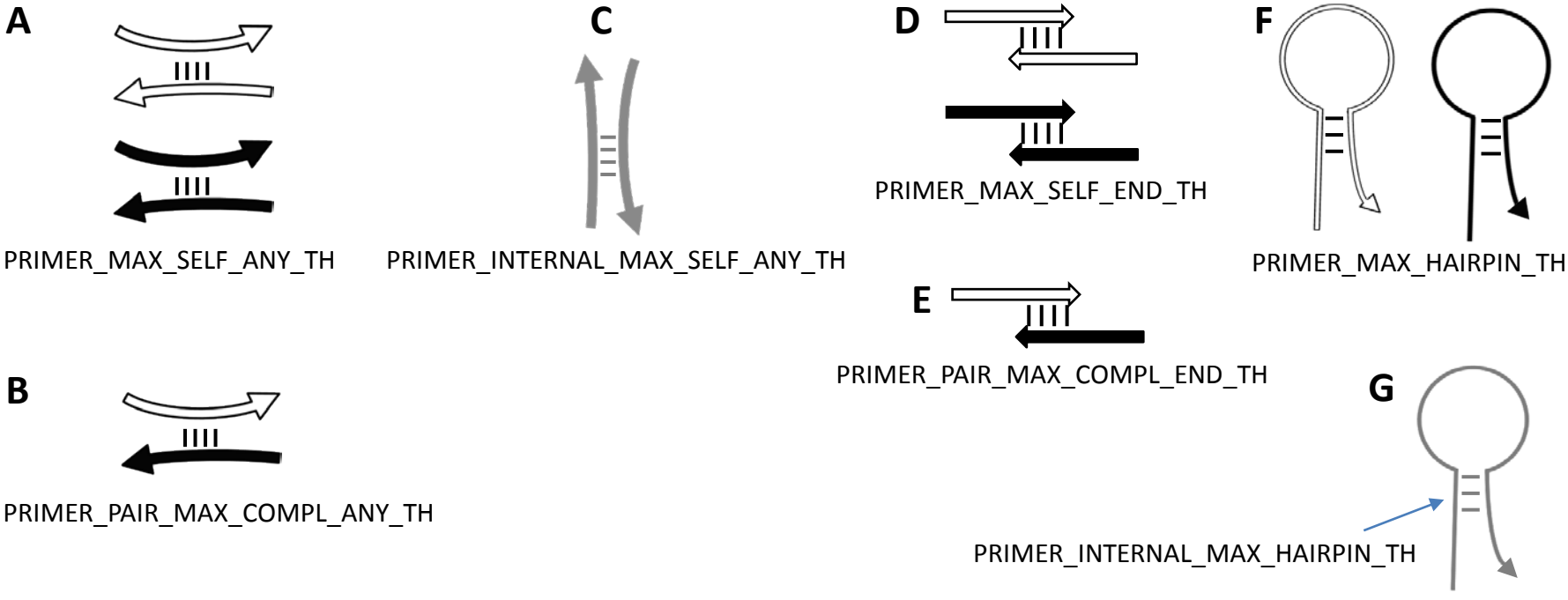
Supplementary table 1. Software and Web Services Incorporating Primer3 (in Addition to Primer3Plus and Primer3web)

Name	W,S (a)	URL	Task and notes	Refer-ences
AcePrimer	W	http://elegans.bcgsc.bc.ca/aceprimer/aceprimer.shtml	Numerous sophisticated design tasks for *C. elegans*	(27)
Autoprime	W	http://www.autoprime.de/AutoPrimeWeb	RT PCR, fetches transcripts automatically	(28)
BatchPrimer3	W	http://probes.pw.usda.gov/cgi-bin/batchprimer3/batchprimer3.cgi	SSR, tetra-primer ARMS PCR, SBE primers, allele-specific primers, multiple primer design tasks from multiple templates in single file	(13)
BioPrimer	W	http://bioinfo.ut.ee/bioprimer/	Designs primers around human SNPs, given SNP rs number or template DNA sequence. Integrated with the GENOMETESTER software, which calculates the number and locations of predicted primer binding sites and number, length and locations of products in the genome.	(22)
ConservedPrimers	W	http://probes.pw.usda.gov/ConservedPrimers/	Pipeline for designing “intron-flanking” primers for large-scale SNP discovery and marker development in un-sequenced species	(29)
EasyExonPrimer	W	http://129.43.22.27/~primer/EasyExonPrimer.html	Easy to use interface to design primers for exons in a gene; fetches genomic sequence template, avoids SNPs	(30)
mPrime	S	http://www.mcardle.wisc.edu/mprime/	Can retrieve human, mouse or rat template sequences from Ensembl; checks primer specificity with Bowtie (31). (Note: there is another Mprime from EC Rouchka, A Khalyfa, NGF Cooper, University of Louisville Bioinformatics Research Group)	(none)
MPprimer	W,S	http://biocompute.bmi.ac.cn/MPprimer , http://code.google.com/p/mpprimer/	Multiplex PCR (multiple PCR reactions in the same tube); not the same mPrimer as used in GENOMEMASKER	(32)
MethPrimer	W	http://www.urogene.org/methprimer/	Design primers for methylation-specific PCR or bisulfite-restriction PCR.	(33)
MsatCommander	S	http://code.google.com/p/msatcommander/	Scans for microsatellites (simple sequence repeats) and optionally designs PCR primers around them	(34)
MultiMPrimer3	W	http://bioinfo.ut.ee/multimprimer3/	Finds species-specific repeats for microbial identification by PCR; primers are checked for specificity using FastaGrep	(35)

Multiple Primer Design with Primer3	W	http://flypush.imgen.bcm.tmc.edu/primer/	Overlapping, tiling primer-pair sets.	(none)
Optimus Primer	W	http://op.pgx.ca/	Primers to amplify exons for next-generation sequencing; uses up to 4 different Primer3 parameter sets and UCSC in-silico PCR utility to check specificity; can take list of gene names as input, thereby relieving user of the burden of pulling out the exon sequences	(36)
PCR Suite	W	http://pcrsuite.cse.ucsc.edu/	Several functions: 1. Multiple overlapping PCR products across one sequence. 2. Primers around exons in genomic sequence. 3. Primers around every SNP in a GenBank file. 4. Primers around open reading frames.	(11)
Primaclade	W	http://www.umsl.edu/services/kellogg/primaclade.html	find conserved PCR primers (possibly degenerate) across multiple species	(37)
PRIMEGENS	W,S	http://primegens.org/	Many different functions; please refer to the web site and references	(8-10,38)
Primer-BLAST	W	http://www.ncbi.nlm.nih.gov/tools/primer-blast/	Genome- or transcriptome-wide specificity using BLAST and design of RT PCR Primers spanning exon junctions	(12)
PrimerIdent	W	http://primerident.up.pt	"[A] tool for the specific amplification of individual members of multigenic families across related species"	(39)
PrimerZ	W	http://genepipe.ngc.sinica.edu.tw/primerz/beginDesign.do	Automatically fetches annotations for templates containing exons, SNPs	(40)
QDD	S	http://www.univ-provence.fr/gsite/Local/egee/dir/meglecz/QDD.html	Pipeline for designing PCR primer pairs for microsatellites (simple sequence repeats)	(41)
QPrimer	W	http://www.bioinformatics.ucla.edu/QPRIMER/	"PCR and RT-PCR primers from multigenome alignments targeting specific exons or introns"	(42)
QuantPrime	W,S	http://quantprime.mpimp-golm.mpg.de/	Quantitative RT-PCR	(43)
RExPrimer	W	http://www4a.biotec.or.th/rexprimer/	Utilizes genome annotations to improve primer design for PCR, SNP genotyping; fetches template sequence and SNP annotations automatically	(44)
SNP Cutter	W	http://bioinfo.bsd.uchicago.edu/SNP_cutter.htm	PCR RFLP	(45)
SNPBox	W	http://www.snpbox.org/	Objective is "high-quality PCR and sequencing primers … generated, preferably in a fast, automated process, while carefully taking repeat sequences into account" with uniform amplification conditions	(46,47)

SNPMASKER	W	http://bioinfo.ut.ee/snpmasker/	SNPmasker does not directly use Primer3 code. However it prepares soft-masked template sequence utilizing the ability of Primer3 to avoid soft-masked positions in template sequence. At the moment human, chimp or mouse SNPs and repeats can be masked with SNPMASKER.	(48)
TOPSI	S	http://www.bhsai.org/downloads/topsi.tar.gz	Pipeline for design of pathogen identification assays	(49)
WebSat	W	http://wsmartins.net/websat/	Microsatellite (STR) marker design	(50)

(a) W = web based, S = standalone



Supplementary figure 1. Primer3's thermodynamic models for the stability of secondary structures and the arguments (boulder-IO tags) that specify maximum stability for each. (**A, B, C, D, E**) Interactions between oligos. (**F, G**) Hairpin structures. (**H**) Undesirable binding of primers to template sequence. Short white and black arrows represent forward and reverse primers. Gray arrows represent hybridization oligos. Long white and black arrows represent forward and reverse template. Curved primers (A, B) and oligos (C) indicate ANY interactions. Straight primers (D, E, H) indicate END (i.e. 3'-anchored) interactions.