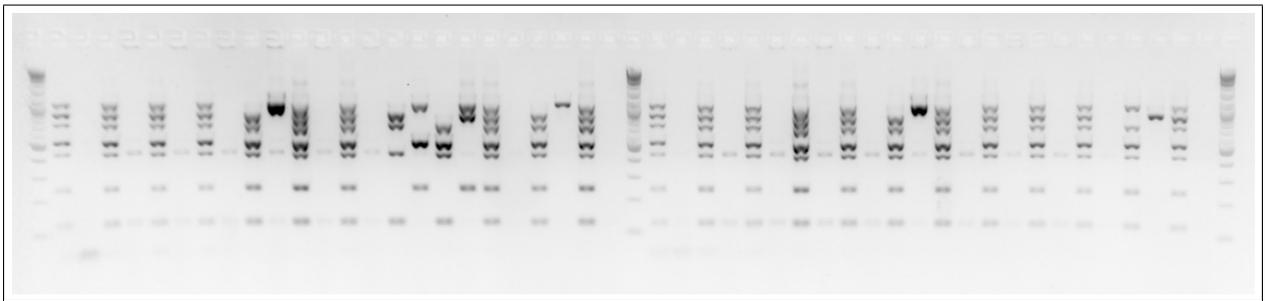


Merlin: Computer-Aided Oligonucleotide Design for Large Scale Genome Engineering with MAGE (Supplementary Info)

A



B

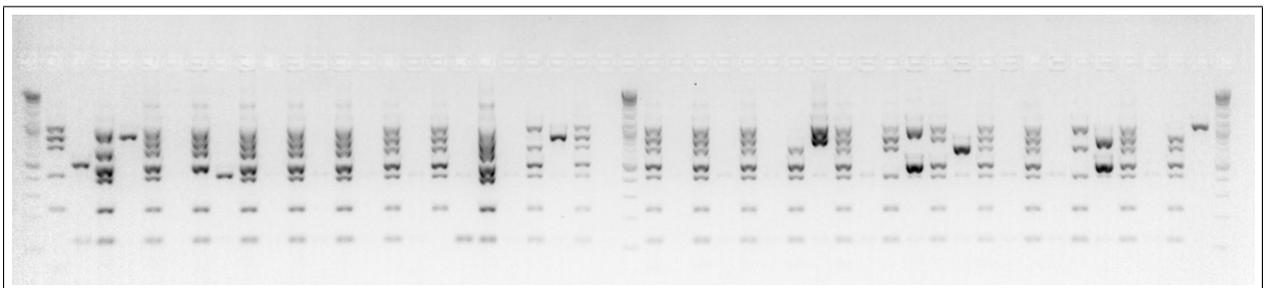


Figure 1: MASC-PCR gel used for primer validation. Images are of mASC-PCR reactions for clones. *A*: wells A1-B12. *B*: wells C1-D12.

Table 1: Sanger Sequencing and PCR validation of Merlin Oligos. 0 = wild type, 1 = mutant.

Well	AvrII01		AvrII02		AvrII03		AvrII04		AvrII05		AvrII06		AvrII07	
	PCR	Seq												
A1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A5	0	0	0	0	0	0	0	0	1	1	0	0	0	0
A6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A8	1	1	0	0	0	0	0	0	1	1	1	1	0	0
A9	1	1	0	0	0	0	1	1	1	1	0	0	0	0
A10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A11	0	0	0	0	0	0	0	0	1	1	0	0	0	0
A12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B6	0	0	0	0	0	0	0	0	1	1	0	0	0	0
B7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B11	0	0	0	0	0	0	1	1	0	0	0	0	0	0
B12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C1	0	0	0	0	0	0	0	0	0	0	1	1	1	1
C2	0	0	0	0	0	0	1	1	0	0	0	0	0	0
C3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C4	0	0	1	1	0	0	0	0	0	0	0	0	0	0
C5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C9	0	0	0	0	0	0	0	0	0	0	0	0	1	1
C10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C11	0	0	0	0	0	0	1	1	0	0	0	0	0	0
C12	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D4	0	0	0	0	0	0	1	1	1	1	0	0	0	0
D5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D6	0	0	0	0	0	0	0	0	1	1	1	1	0	0
D7	0	0	0	0	1	1	0	0	0	0	0	0	0	0
D8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D10	0	0	0	0	0	0	1	1	0	0	1	1	0	0
D11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D12	0	0	0	0	0	0	0	0	1	1	0	0	0	0
Sum	2	2	1	1	1	1	6	6	8	8	4	4	2	2

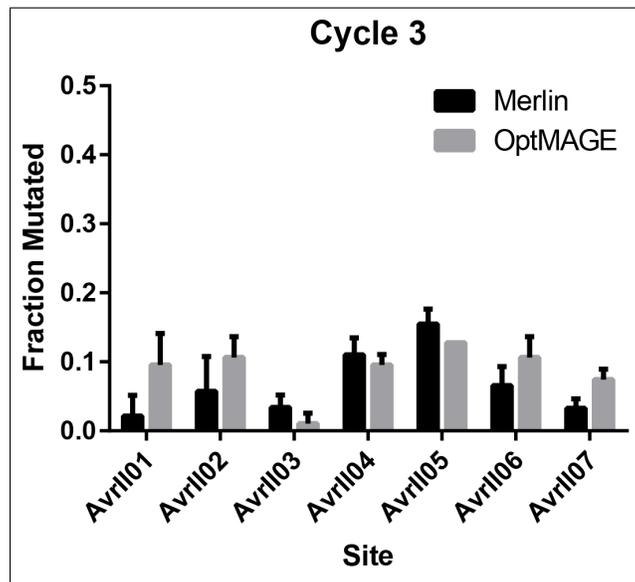


Figure 2: Diversity across all loci after 3 MAGE cycles. Error bars are the range of values for two separate MAGEd populations. $n = 47$.

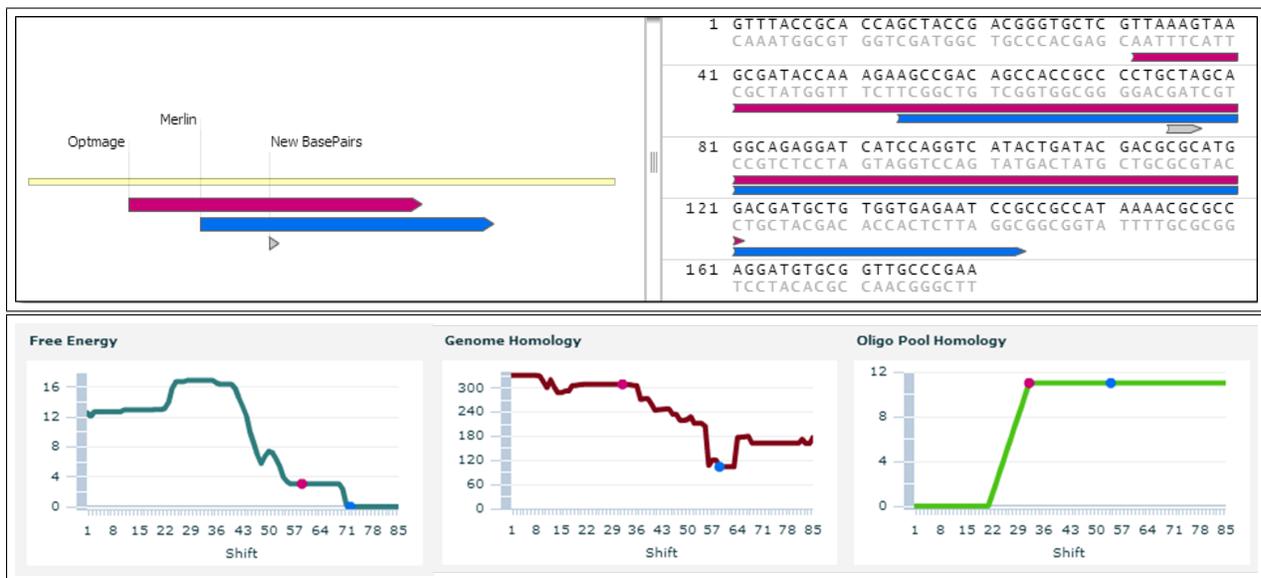


Figure 3: Example graphical output of the oligo optimization process, comparing oligos created by Merlin (blue) and OptMAGE (red). *Top*: Two alternate views of the location of the oligo spans and the targeted position, in this case a TAG insertion. *Bottom*: Plots of the (left to right) ΔG score, genome-wide BLAST score, and inter-oligo BLAST score for each valid oligo start position. The positions used and the resulting scores for the Merlin and OptMAGE oligos are depicted in blue and red, respectively.

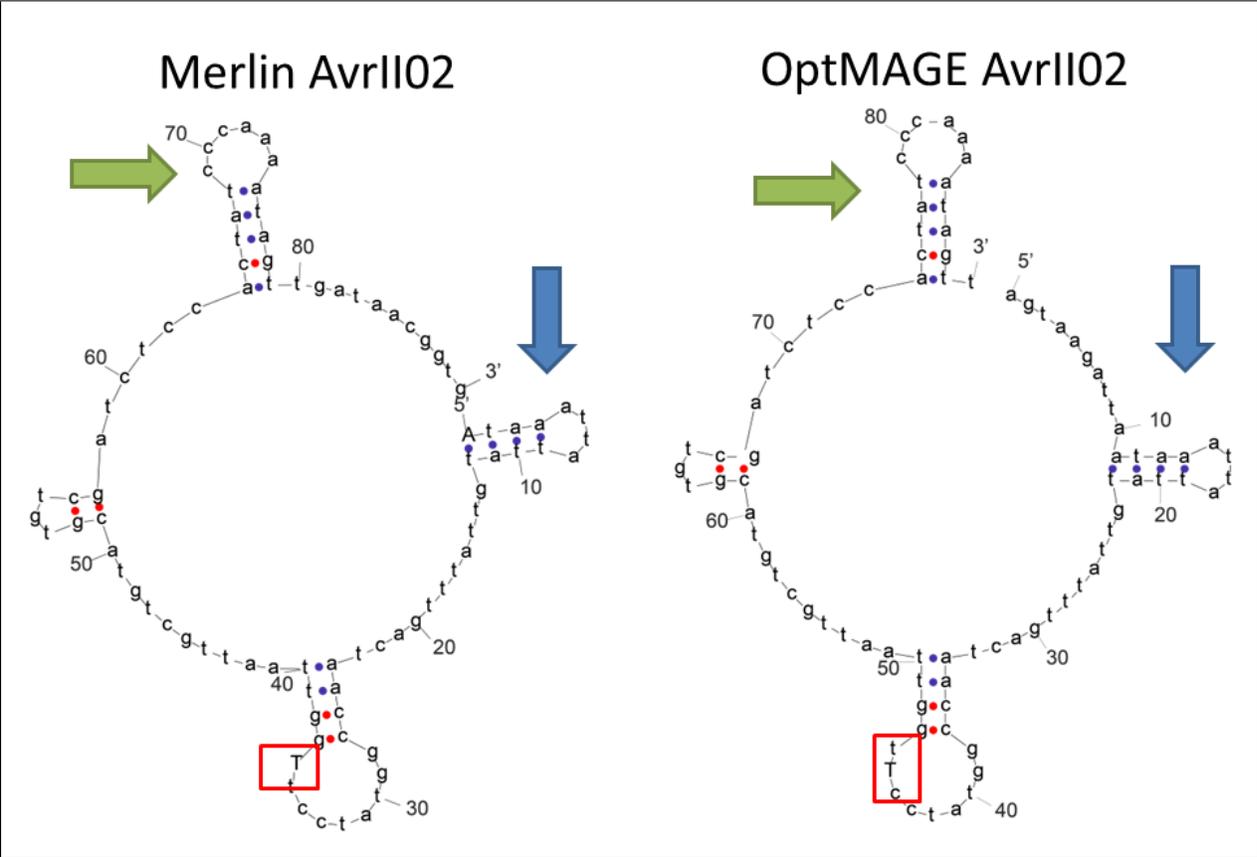


Figure 4: Comparison of predicted structures of oligos targeting AvrII02. The blue arrow indicates the 5' hairpin, the green arrow indicates the 3' hairpin, and the red box indicates the site of mutation. Structure prediction performed with mFold.

Table 2: Sequence and predicted structure in dot-bracket notation of oligos generated by Merlin and OptMAGE. Structure prediction performed with mFold.

Name	dG	Sequence	Structure
avrII01-Mer	-28	tcatcacgtt tateggtcgt cccggcaacg cggttaagag attcttgatc tgcccagtca taacgaccgc ctagGgtgac cagcacttta	...((((((...(((((((...(((...((((((((((((...)))))))).)))))...))) ..)))))))). ..)))).).....
avrII02-Mer	-11.3	ataaattatt atgttatttg actaacgggt atcctTgggtt aattgctgta cgtgctgac tccactatcc caaatagtt gataacgggtg(((((((.....)))))))...((((((...(((... ...((((((... ..)))))))).
avrII03-Mer	-16.3	tgtcactgtt gggagtgtga atgcaatata atacgataat aattccctTg gttaaacagt ctcagtacta aagatgtatg tgaaggggcc	(((((...(((...(((...(((...)))..)))))))).)))...))(((((...(((... ..)))))))).
avrII04-Mer	-10.7	ctgaagtaag aattatTcta ggggatgctg tagtggaagt agcaaacag ggaagaccta tcaataccag aacattgctt gattacattg	..(((((((...(((((((((...(((..((..... ..))..))))..))) ..))).... ..))))))).....
avrII05-Mer	-10.7	ctgaagtaag aattatTcta ggggatgctg tagtggaagt agcaaacag ggaagaccta tcaataccag aacattgctt gattacattg	..(((((((...(((((((((...(((..((..... ..))..))))..))) ..))).... ..))))))).....
avrII06-Mer	-12.7	gaagcattaa atgacttaaa cagttatacc aatgctaata gtgttacttc cctaGgtgca agactggcaa tgtacgaaat tggtttaa (((((((... ..))))))(((((((((((... ..(((... ..))) ...))))). ..)....)))).....
avrII07-Mer	-19.6	caaagggcc agcctttgtc gagcgggtga ccgtgcttta atctgcaatc tttatcaaca caagcccata ttccTtaggt attacgctgg	..(((((((...))))).) .((((...(((((..... ..(((... ..).... ..)).... ..)....))))..))))).
avrII01-Opt	-12.4	gcggttaaga gattcttgat ctgccagtc ataacgaccg cctagCgtga ccagcacttt atcccactgc gectgatcct gaacataaac	((((((((((...)))))).)))...(((.....))((...))..(((...((...).))..)).. ..).....
avrII02-Opt	-8.8	agtaagatta ataaattatt atgttatttg actaacgggt atccTgggtt aattgctgta cgtgctgac tccactatcc caaatagtt	..(((((((...(((...))))))((((((...(((...)))))).. ...)))).. ..((((((... ..)))))).
avrII03-Opt	-15.5	cactgttggg agttgtaatg caatataata cgataataat tcctTgggtt aaacagtctc agtactaaag atgtatgtga aggggccg	..((((((((...(((...)))))).. ..))..)))))))))).... ..).....))..(((((((... ..))))..)) ..).....
avrII04-Opt	-24.7	caccctaaag tggttccctg cgcagcttcc tgaagtaaga attatTctag gggatgctgt agtggaagta gcaaacagg gaagacctat	..((((... ((((((((((((...((((((...(((...))))..))))..)))..))))..)))..) ..)....))).....
avrII05-Opt	-24.7	caccctaaag tggttccctg cgcagcttcc tgaagtaaga attatTctag gggatgctgt agtggaagta gcaaacagg gaagacctat	..((((... ((((((((((((...((((((...(((...))))..))))..)))..))))..)))..) ..)....))).....
avrII06-Opt	-10.8	aatgacttaa acagttatac caatgcta agtgttactt cctaCgtgc aagactggca atgtacgaaa ttggtttaa tatattcata	..(((((((...))))))((((((((((... ..(((... ..)....)))....)))))))).... ..).....
avrII07-Opt	-10.7	accgtgcttt aatctgcaat gtttataaac acaagcccat attccTtagg tattacgctg gaagatatgc gcctgactt taagaaatgc(((... ..))..) .(((...((...((...((((((...((... ..)))..))))).))..).... ..).....