

## Supporting Information

for

### Fluorescent signal amplification of carbocyanine dyes using engineered viral nanoparticles

Carissa M. Soto, Amy Szuchmacher Blum, Gary J. Vora, Nikolai Lebedev, Carolyn E. Meador, Angela P. Won, Anju Chatterji, John E. Johnson, and Banahalli R. Ratna

**Table 1S.** Preparation of NeutrAvidin-dye-virus series.<sup>1</sup>

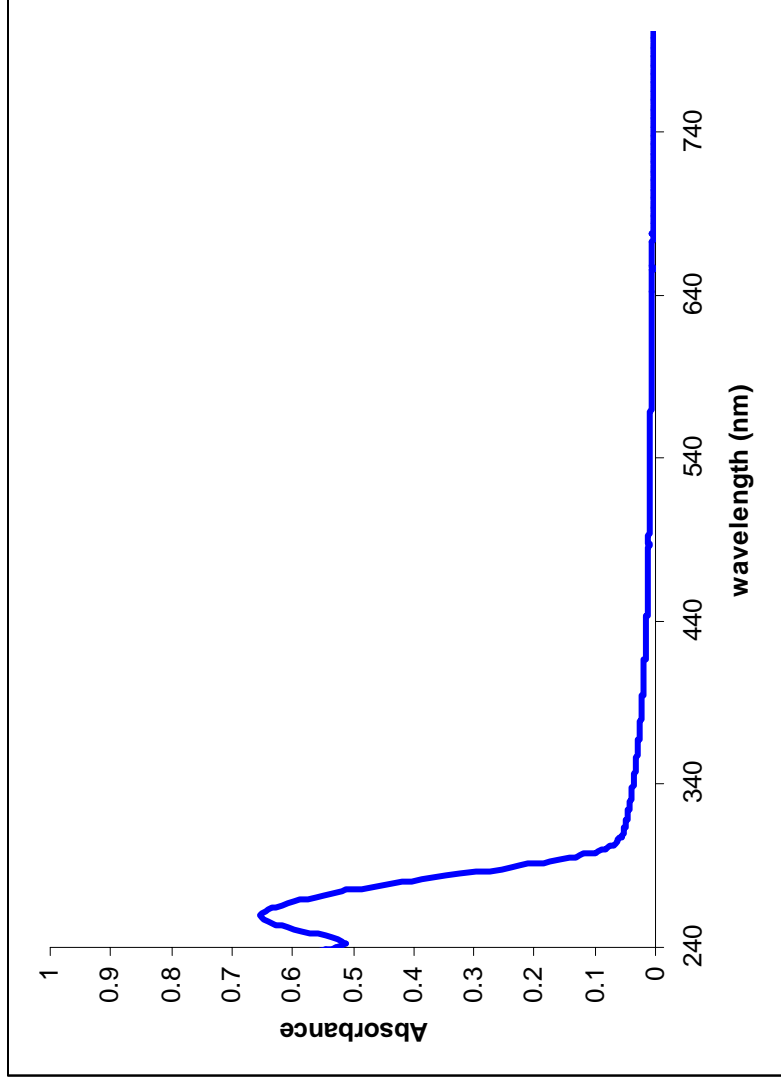
Molar excess of Cy5 <sup>2</sup>	Dye per virus ratio <sup>3</sup>
1	3-5
25	10-15
50	25-30
100*	> 40

<sup>1</sup> NeutrAvidin reacted in a 1:1 molar ratio, NeutrAvidin:thiols

<sup>2</sup> added in reaction mix; excess calculated per total number of thiols on the capsid

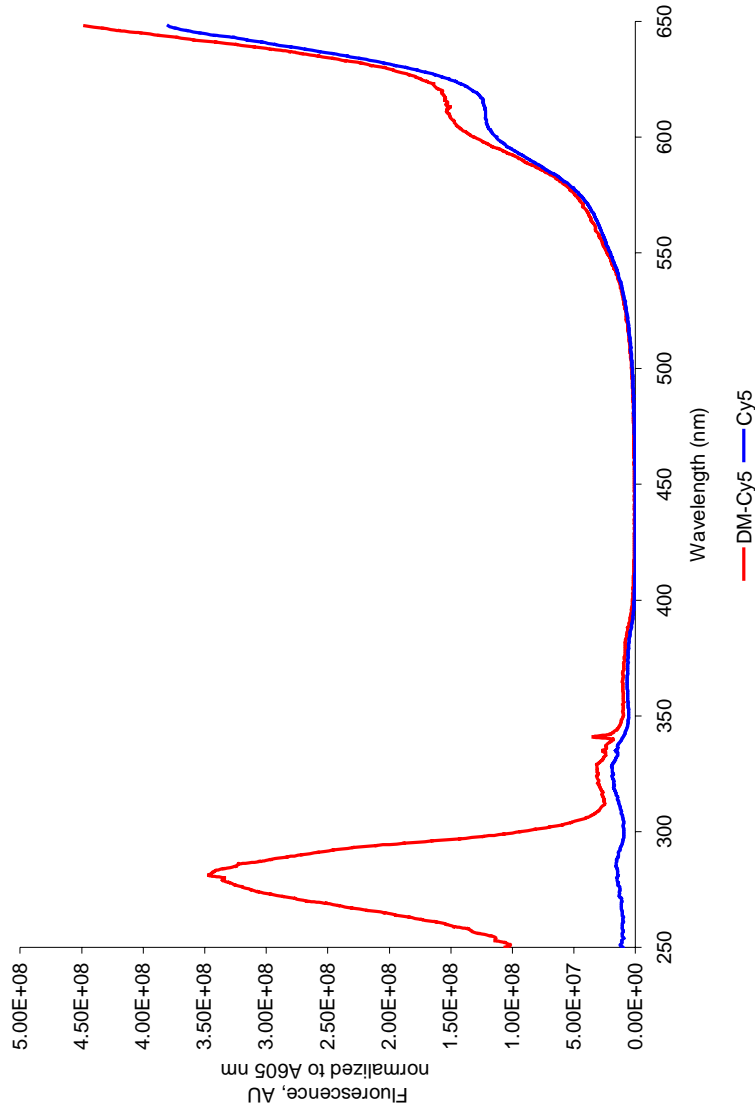
<sup>3</sup> resultant ratio after reaction and purification,

\* extreme conditions: reaction in 30% DMSO, final reaction volume 140  $\mu$ l



**Figure 1S.** UV/Visible absorbance of CPMV virus, showing peak at 260 nm, and no significant absorbance above 400 nm.

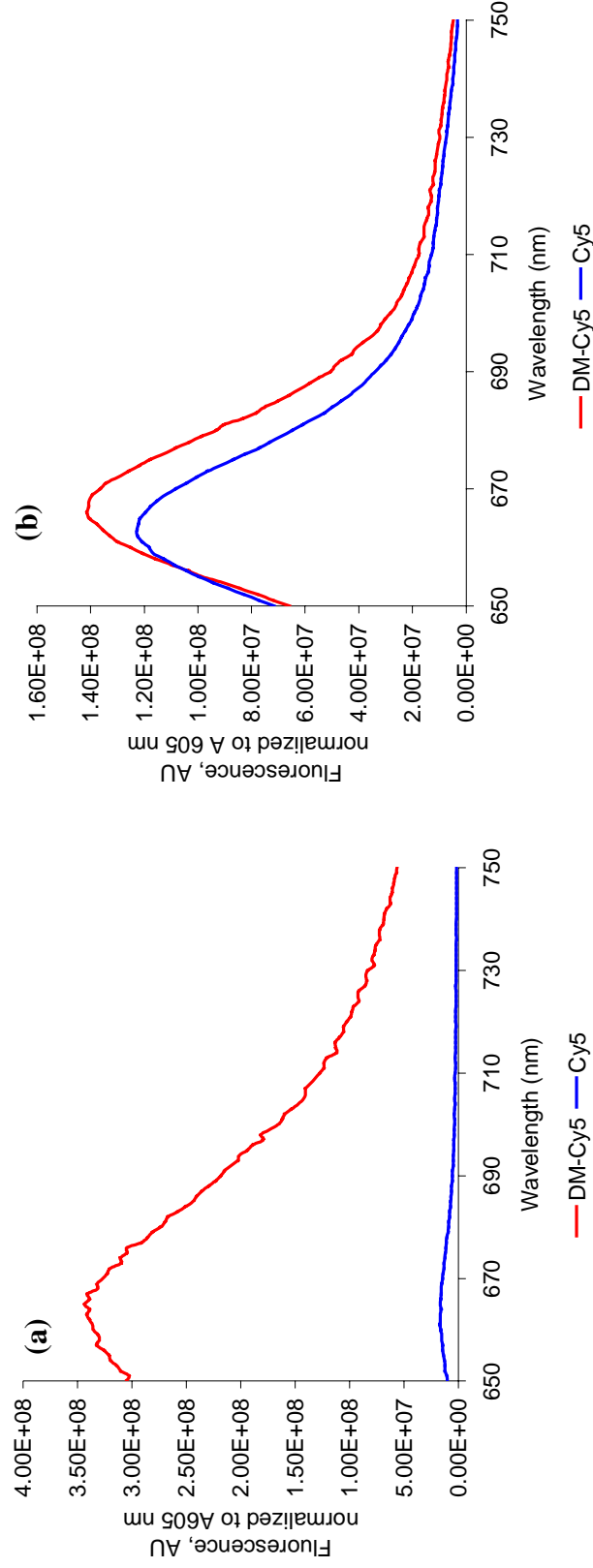
In order to examine energy transfer processes in more detail, a different CPMV mutant (DM), with cysteines inserted at residues 228 and 2102 was also used as a scaffold for binding Cy5. These experiments, which are in a preliminary stage, will help determine environmental effects on Cy5 fluorescence signal enhancement. DM mutant was modified using the same procedure described in the manuscript for EF-CPMV to generate DM-Cy5 complexes. The fluorescence of these complexes was then measured and compared to free Cy5 in solution. Figure 2-S shows an excitation scan for free Cy5 (blue) and DM-Cy5 (red) for emission at 666 nm. The spectra indicate that energy transfer is occurring from the viral scaffold to the Cy5 at 280 nm, confirming coupling of the Cy5 to the virus.



**Figure 2S.** Excitation scan of free Cy5 (blue) and the DM mutant of CPMV with bound Cy5 (red) for emission at 666 nm. Intensity normalized to the Cy5 absorbance at 605 nm. Scan shows energy transfer from CPMV to Cy5 molecules, as in the case for the EF mutant.

Figure 3-Sa shows fluorescence of free Cy5 (blue) and DM-Cy5 (red) under excitation at 280 nm. As expected, the free Cy5 shows very little fluorescence at this excitation wavelength, as Cy5 absorbs only weakly at 280 nm. However, there is a strong fluorescence signal for DM-Cy5 due to energy transfer from aromatic amino acids such as tryptophan to the attached Cy5 molecules on the viral scaffold. Figure 3-Sb shows fluorescence DM-Cy5 (red) and free Cy5 (blue) under excitation at 605 nm. As with EF-Cy5 complexes,

DM-Cy5 complexes show signal enhancement over the same concentration of free Cy5 in solution. Since the excitation wavelength is 605 nm, where the viral scaffold does not absorb, energy transfer alone cannot explain this phenomenon.



**Figure 3S.** (a) Fluorescence of free Cy5 (blue) and the DM mutant of CPMV (cysteines inserted at residues 228 and 2102) with bound Cy5 (red) at excitation 280 nm. Intensity normalized to the Cy5 absorbance at 605 nm. Intensity for DM-Cy5 sample shows energy transfer from DM-CPMV to Cy5. (b) Fluorescence of free Cy5 (blue) and the DM mutant of CPMV (red) at excitation 605 nm. Intensity normalized to the Cy5 absorbance at 605 nm. Spectrum shows signal enhancement for DM-Cy5 sample at an excitation of 605 nm.

**Table 2S.** DNA primers and probes.

Targeted Gene	Primer Name	Primer Sequence (5'-3')	Probe Name	Probe Sequence (5'-3')
groEL	groEL-F	GACGYBGCRRGGTGACGGTACRACMAC	groEL (Vc)P2	ATCGCTTTAGTATCGGCACAAAGGTACAGACAGTCTTCAACTCTTACCCGACGGATAACCGCTTTAT
	groEL-R	CGACGRTCRCCAAAARCCAGGMGCTWT		

16S-23S IGS	16S-23S-F 16S-23S-R	TTAAGS <sup>TTTT</sup> CRCTGAGAA <sup>TG</sup> AGTCAC <sup>TAA</sup> ACCATA <sup>CA</sup> ACC <sup>CG</sup>	16S-23S P2	ATAAA <sup>TG</sup> TTG <sup>TTG</sup> TTTT <sup>TC</sup> AGC <sup>TTT</sup> CCAA <sup>ATTG</sup> TTAAAGAGCAAGA <sup>TTTTCT</sup> GATGAAAA <sup>ACC</sup> ATTTTT <sup>TAAA</sup>
luxO	luxO-F luxO-R	CGCTG <sup>TAT</sup> CGT <sup>TCT</sup> TAC <sup>CT</sup> CA <sup>CACC</sup> GCTCG <sup>CCG</sup> CAGAG <sup>TCA</sup> AT <sup>TGG</sup>	luxO P2	CAGCAG <sup>GAT</sup> TAAG <sup>TC</sup> AGG <sup>CT</sup> CGCAG <sup>GCC</sup> AA <sup>TACT</sup> CTCGAT <sup>GCA</sup> TCGCG <sup>TCC</sup> GGTAC <sup>CC</sup> ACGAT <sup>TTG</sup>
tcpl	tcpl-F tcpl-R	GCCACAGCAACAGCAACTATC GAA <sup>CG</sup> AA <sup>ACT</sup> CA <sup>TCC</sup> GG <sup>TACT</sup> ACAG	tcpl P2	TCGG <sup>TAC</sup> GGAT <sup>GCA</sup> TTAA <sup>TATA</sup> TATAT <sup>ATC</sup> AGAT <sup>CAAA</sup> CTAA <sup>CTTAC</sup> TG <sup>CC</sup> CTAA <sup>TGC</sup> AG <sup>CTAT</sup> CGAGG
ompU	ompU-F ompU-R	TACGCTG <sup>TAT</sup> CGG <sup>TGG</sup> CA <sup>CTTAC</sup> TCCAT <sup>CGG</sup> TAAGAAG <sup>GCG</sup> CTAG	ompU P2	GTACT <sup>CT</sup> GACA <sup>ACGG</sup> TGAAGAT <sup>GTTACT</sup> CTCT <sup>GCT</sup> GCTA <sup>CTAC</sup> ACT <sup>TTC</sup> GG <sup>TG</sup> ACACT <sup>GSC</sup> TTTAA <sup>C</sup>
ompW	ompW-F ompW-R	CACCAAGAAGGTGACTTTATTGTG GAA <sup>CTT</sup> ATA <sup>ACC</sup> CCCG <sup>CG</sup> ATC	ompW P1	TAAC <sup>TGCC</sup> AA <sup>CTC</sup> ACT <sup>TG</sup> AGT <sup>GTTAA</sup> CA <sup>CTTTAT</sup> CG <sup>TACT</sup> GT <sup>CA</sup> T <sup>TAG</sup> GTACT <sup>ACC</sup> GGCA <sup>ATA</sup>
ctxA	ctxA-F ctxA-R	CGGGCAGATTCTAGACCTCCTG CGATG <sup>ATC</sup> TGGAG <sup>CAT</sup> TCC <sup>CA</sup>	ctxA P1	GGCTACAGAGATAGATA <sup>TTAC</sup> AG <sup>TAACT</sup> TAGATA <sup>TTGCT</sup> CCAGCAG <sup>ATGG</sup> TTAT <sup>GGA</sup> TTGGCAGG
ctxB	ctxB-F ctxB-R	ATGCACATGGAACACCTCAAAATATTACTG TCCTCAGGGTATCCTTCATCC <sup>TTTCA</sup> ATC	ctxB P1	AGCTAGAGATTCTGTATACGAAAATATC <sup>TTAT</sup> CA <sup>TTTAG</sup> CGTATATA <sup>TTTTG</sup> TG <sup>TG</sup> TTG <sup>TG</sup> TATTCTGCA
toxR	toxR-F toxR-R	ACCGCAGCCGCCAATGTTG TGGCAATGACTTCTATCGGCTTGAG	toxR P2	GCCGTCAATATGCCGAA <sup>TAA</sup> CCACC <sup>CTG</sup> ATC <sup>TTTCA</sup> AACTG <sup>CTACC</sup> CGTCA <sup>ATCG</sup> AACTG <sup>TCG</sup> CGTTAA <sup>A</sup>
stn/sno	stn/sto-F stn/sto-R	TCGCCCTTAGCCAAACAGTAGAAA GCTGGATTGCAACATATTTCGC	stn/sto P2	GGGCAT <sup>TCTT</sup> CA <sup>TTT</sup> TCAC <sup>T</sup> TAG <sup>TTTTT</sup> TAAT <sup>TTTAC</sup> CTT <sup>GCTTTT</sup> CA <sup>ATTTT</sup> GT <sup>GGTTG</sup> CTG <sup>CTG</sup> CACTG
ace	ace-F ace-R	TGATGGCTTACGTGGCTTGATC GCCTGTGGATAAGCGGATAGTGG	ace P2	AAAAT <sup>TTG</sup> CTCTCA <sup>ATCC</sup> ACATA <sup>ATAC</sup> CGAG <sup>CTTG</sup> ATCA <sup>CAAG</sup> CCACG <sup>TAAAG</sup> CCATCA
epsM	epsM-F epsM-R	GACGGTACTCGCTATCGCTTATTGG ATATCAATCGCATCCACGCTCACC	epsM P1	ATA <sup>TCA</sup> AT <sup>CG</sup> CA <sup>TCC</sup> ACG <sup>CTC</sup> AC <sup>CCCTT</sup> GGC <sup>GCTT</sup> GGCAA <sup>TAC</sup> GC <sup>CAATCC</sup> ATGAGAC <sup>CAAT</sup> TGCGAAA
rxnA	rxnA-F rxnA-R	AATGGTATTGCGGAAGCGGACATC GAATGGATTGACAGAGTGGTGAGTTG	rxnA P1	ACCGTAATGTGAGCTTTAA <sup>TTTTGG</sup> GACACAG <sup>TAA</sup> TGAT <sup>CTG</sup> ATC <sup>TAA</sup> TGAT <sup>GG</sup> TAAG <sup>TG</sup> ATCGATCCCA
zot	zot-F zot-R	ATCTGCC <sup>TAA</sup> CCAG <sup>CC</sup> CTAA <sup>CA</sup> T <sup>TG</sup> ACCGCTT <sup>GCT</sup> CCCCGACAG	zot P2	CAGGGGAAATGATGCAACTATCGAG <sup>TC</sup> AGAGCA <sup>ATCC</sup> GAG <sup>CC</sup> CTCAG <sup>TCAA</sup> AGGC <sup>TACT</sup> GTGGGAATGC
hap	hap-F hap-R	GTCTTACTACCCGCTTAAAGCAGG CACTCGAATGGTGAACATCAATACCCG	hap P1	TCCTCCGGTGGTACGTTATTT <sup>TCG</sup> ATCAG <sup>CCG</sup> TCACG <sup>TGAT</sup> GGCCGCTCGATAGATCAGTCTCACAGT
rfbN	rfbO1-F rfbO1-R	TGGTTTCACTGAACAGATGGG AGGTCATCTGTAAGTACAACAATC	rfbO1 P3	GTAGTGGGCTTACA <sup>AACTCA</sup> AG <sup>TAA</sup> AGC <sup>CTTAC</sup> TTTAC <sup>CTG</sup> CCCCACACACTTCTAGGTT <sup>CG</sup> ATT <sup>CA</sup> TCACG
nanH	nanH-F nanH-R	CTTCTCCAATACGGTCTTGTCTCTTATGC TTCCGGCTACCAATCGGCAACTGTATC	nanH P3	CAGCGTCTATGATGTTGCA <sup>T</sup> CA <sup>GGG</sup> AA <sup>CTGG</sup> CAAG <sup>CG</sup> CTAT <sup>CG</sup> ATG <sup>T</sup> TAC <sup>CG</sup> ATCAGGTGAAAGAA <sup>CG</sup>
wbfR	wbfR-F wbfR-R	AAGCCTCTTTATTACGGGTGG GTCAAACCCGATCGTAAAGGT	rfbO139 P1	TTGATTCGTC <sup>CACT</sup> GTGGTGGG <sup>TATTT</sup> ACA <sup>ATCC</sup> CTCT <sup>ACT</sup> CGTCCGCTCA <sup>AAA</sup> CC <sup>TTT</sup> ACGATC
wbfO	wbfO-F wbfO-R	TGACGGTTCGACTGATAAGACACTAGAG TTTTGCC <sup>TTT</sup> AA <sup>AG</sup> TC <sup>TGG</sup> ATC <sup>T</sup> GG <sup>TTG</sup>	wbfO P2	TCTAG <sup>CCCTT</sup> CA <sup>TTT</sup> AGG <sup>CTTACT</sup> ATTAG <sup>CCCTTT</sup> GT <sup>TTTTCT</sup> TTGAA <sup>ACCA</sup> ATA <sup>CAATAC</sup> CAAC <sup>ATCG</sup> GTCA <sup>CT</sup>
otnA	otnA-F otnA-R	CCATAGCCGAGTGACTACCC CTCAGATTGGTGTACGACCGG	otnA P1	CGTTGAG <sup>TTT</sup> AT <sup>CA</sup> TCAT <sup>GC</sup> CTTGA <sup>ATGG</sup> TC <sup>CA</sup> TG <sup>AT</sup> TAG <sup>CC</sup> GAAG <sup>GG</sup> CTCA <sup>CTCA</sup> CAAC <sup>ATTT</sup> GGC <sup>GTA</sup> AA <sup>TC</sup>
otnB	otnB-F	CCAGTCA <sup>ACC</sup> CCCGCA <sup>A</sup>	otnB P1	TAGCGG <sup>TAA</sup> AGG <sup>TGG</sup> GTAGAT <sup>AAAA</sup> CGAA <sup>ATGG</sup> CGGAT <sup>TGA</sup> AGT <sup>GCT</sup> AAAA <sup>ATCG</sup> CCAA <sup>TTT</sup> ACGACG

otnB-R	GAGCTGATCAGCACTATTGTCC				
hylA-F2	CGGCATTGTGGTTCGCGCACACC	hylA CL P2	GCACGGAGCCGGCTGATCAACTCGGTTATCGTC		
hylA-R3	CCACTCACGGAAATAGGCATCTAACGTC	hylA ET P2	GCACGGAGCCGGCAATCATCTGAATGATCAACTCGGTTATCGTC		
tcpAET-F	AATCATTGTTCTGGGTAATTATGGGT	tcpA ET P1	TTATCAACGTGAAAGAAAGGTGCTTTTCGGCTGCTGTGCTGATCTTTGGTGATTTTCGAAACGAGTGTCCGACGAT		
tcpAET-R	AGCAGCATCTCGCACACTCGTT				
tcpA CL-F	GATCATCGTTCTAGGCCAATTATGGGG	tcpA CL P1	ATATTGCAATCAAAGCTGGTGGCGCAGTAGCACTTGCAGATCTAGGTGATTTTGGAGAAATTTCTGCAGCAGCGG		
tcpA CL-R	CTCAGCCGCTGCTGCAGAAATC				
rsfR CL-F2	CTTACTTCTCATCAGCAAAGCCCTCCATC	rsfR Calc P1	GCACATTAAGACACAGTACGTGGAGAAAAGTTGAGGAAAAAACCCGTAAGCCGTAATCGAGACCAAAAAC		
rsfR ET-F2	GCTAAGCACCATGATTTAAGATGCTC	rsfR CL P1	GCAAAAATCTATGGTGTACCAATAGCTGAACCTTGTACGCATAGCGAAACAAACAATTCAGCGAAAAAGCTG		
rsfR Calc-F2	CTGTAAATCTCTCAATCCTAGGTTG	rsfR ET P1	CCTTGTGAAGATCGCAGATGCTCTGGAGATCTCAATTTGATGAACATA GCGGAAGATGGGCCACACAGATG		
rsfR-R2	GTATCGAGYTGTAATTCRTCAAGAGTG				
aac(6)-lb-F	GAGAAGAA GCACGCCCGCACAC	aac(6')lb P1	CGTAGCACTGGGCATACCCCAATCGGGCTCTCCATTACAGCATTGCAATGTATGGAGTGACGGGACTCTTGCGCG		
aac(6)-lb-R	GGTTACGGTACCTTGCCCTCTC				
aadA1-F	CATCGAGCGCCATCTCGAACCG	aadA1 P1	AGTTTCCAAAAGGTCGTTGATCAAAGCTCGCCCGCGTTGTTTCATCAAGCCTTACGGTCAACCGTAACCAGC		
aadA1-R	GCGGGGAGTCCATAGCGTT				
aadA2-F	CATTGAGCGCCATCTGGAATCA	aadA2 P1	AGCCTCCATAAAGGTCAATTGAGCAATGCTCGCCCGCGTCTTTTCATCAAGCTTTACGGCCACACAGTAACCAAC		
aadA2-R	GCTGCGAGTCCATAGCTTC				
dfrA1-F	CACCTTCGGCTCGATGCTATTG	dfrA1 P2	ACGTTCTCATTGTCAGATGTAAAACTTTGAAACGTTTACGACCCGATACTTTTCGGTTGGGTAATGCTCCCA		
dfrA1-R	TGGCTGTTGGTTGGACGCAAG				
dfrA5-F	GAGTGATTGGTTGGGTCACAC	dfrA5 P1	GAGGCCATGTACGGGCTGGCTGAACCTCACCGATCACGTTATAGTGTCTGGTGGCGGGGAGATTTACAGAG		
dfrA5-R	CATCTCCTCCGGCTCAATATCAATC				
dfrA12-F	CTCGTTGCTGGATGGGAGC	dfrA12 P1	CTACCGCGCCACTGGCTGCGTAGTTGTTTCAACCGCTGTGCGACGCTATCGCTTTGGCATCCGAACTCGGC		
dfrA12-R	GTGCCAGAGTGATATCTCAGCTC				
dfrA15-F	TATGCCGTTGTAACCTCGTTCAAGC	dfrA15 P1	CGATAACGGATCATGTGATTGTGTCTGGTGGTGAAATATACAAAAAGCCTGATCGATAAAAGTTGATAC		
dfrA15-R	CATCACCTTCTGGCTCAATGTCC				
dfr18-F	TCGTATGGGCAGAAAAGACCTTTTG	dfr18 P1	GTGTGAGCACGATGACGTTGCGTCCGGCGAGGCCAATTGGGATGCTCTCAAAAGGCTCTTTCTGCCCATGAC		
dfr18-R	CCGCCACGATGATCTCAGTAAGC				
ereA2-F	GAATCCAAGGGACGACCTAGC	ereA2 P1	GATGTACCTCTCTTGAGGGAGATACTCCGTACGTGACTCGTATATGGCGGGCGTGGTGGATGGAATGG		
ereA2-R	GTAATGATTGTTGTGCGCCAGCAG				
floR-F	GAGCATTGATCGGCGAGTCTTTGG	floR P2	GCGAGCATCGCCAGTATAGCCAAAAGTAATGAATATCGCTGCCATCCCAAGAACTCGCCGATCAATGCTC		
floR-R	AGGTGAGGAATGACGGCGAGC				
qacEΔ1-F	GCCATAGTTGGCGAAGTAATCG	qacEΔ1 P1	GCCTGATCGTGGGGCTTTGTAGGTAATGGGCTCATAAATGCTGCCCTTTTTCGTCGCCCGATCCCATCG		
qacEΔ1-R	GCGTCCGCTCCGACGC				
strA-F	TGATTGCTAACGCCGGAAGAACTG	strA P2	CGCTGTCTCTAATCAATGTAATGGGGATCGAAAGCCCCCGACCGCGAAGCCCTTCCCTTCTATCTGCGGATTTG		
strA-R	GTCCAAATCGCAGATAGAAGGCAAGG				
strB-F	TCGGTCTGGTCCGGTGAAGTGG	strB P1	AAGTCTGCTCTCTGCAGCCGGATCGTAGAACATATTGGCGGCCCAAGCCCACTTACCAGCCAGACCC		
strB-R	GCGTTGCTCCTCTTCTCCATCC				

sull	sull-F sull-R	CGCTCAAGGCAGATGGCATTCC GGCTCGTGTGTGCGGATGAAG	sull P1	CACCGAATGCATAACGACGAGTTTGGCAGATGATTTCCGCCAAATTCCGGATAGAACGCAGCGTCTGGAAA
sull	sull-F sull-R	GCTGCGAGTCGGATCAGACG CCCAACGCCGACTTCAGC	sull P1	GAGGCGCGGGTTTCCGCCCTTCCGACGGAGCGGGTCCGCTGCCGACCCGGCTCATCCTCGATCCCGGGGATGG
vceA	vceA-F vceA-R	TCCACCCGACAAACGGTTATGCTG TCACACGCTGCTCATTTGGCTTC	vceA P1	GCACTCGCAAAACGCCCGTACGTAGCTATCTCGCTAATGACGAAGGTTTAAAGTGCTATGGTGGAAAGCAC
vceB	vceB-F vceB-R	TTGGCGGTGATTGCTGTGATTGG CTGATTCATTTTCGTGCGGTTTGAC	vceB P2	GTCCGGAATACCTTTAAATCCACGACAGGGTTTCCGCTCCGTTAGCTCCCAAAATCAAGAAATGCAATGAAAACC
vcmA	vcmA-F vcmA-R	TGGATCATGTTGCTGCTGCTACTG GCATCGCTAACGCTACCACCAC	vcmA P1	GGTTTAATGACTGGCCTTGGCACTGCTGTGATTAACCGCGTTGCTACCCGTTGTTTTCGTTGAAACAGATC