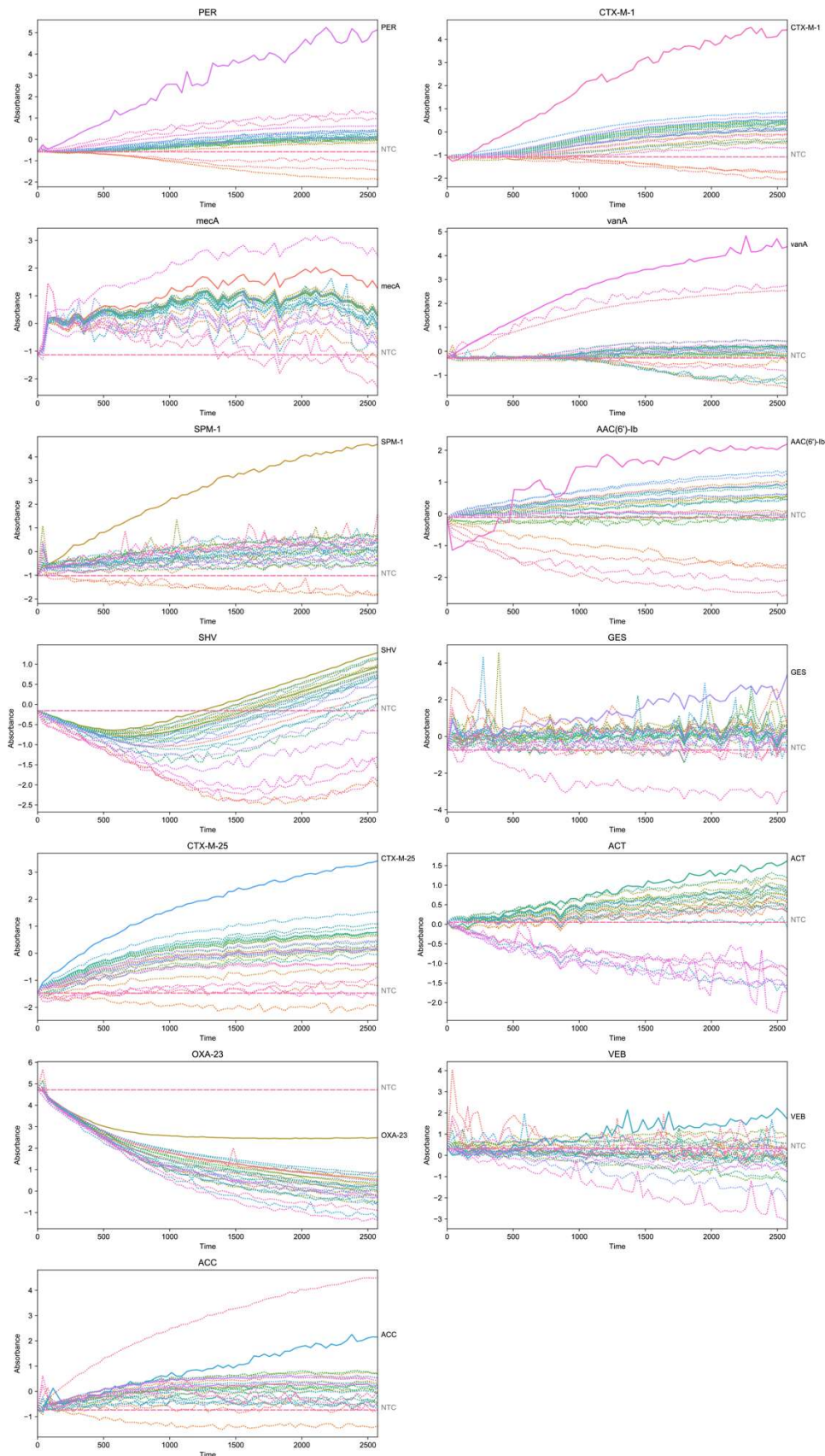


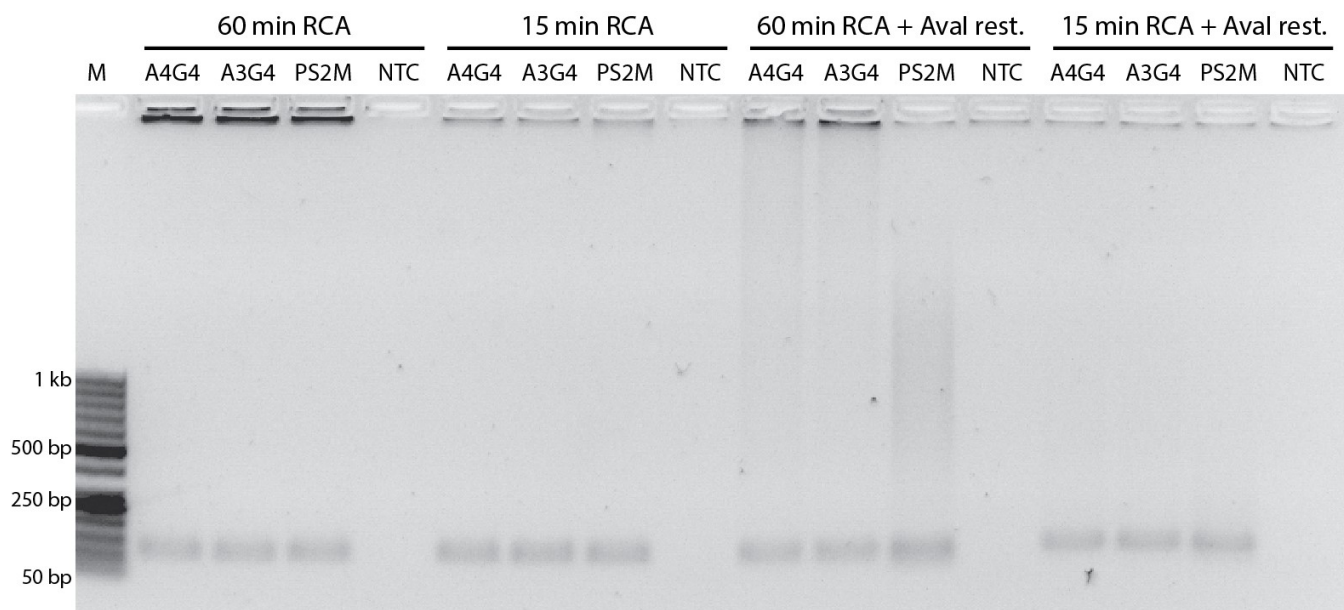
Supplementary information

**Padlock probe-based generation of DNAzymes for the colorimetric detection of
antibiotic resistance genes**

Rick Conzemius, Ariadne Haunold, and Ivan Barišić



Supplementary Figure S1 (cont'd). Specificity evaluation of the padlock probes with synthetic DNA. The padlock probes were ligated, amplified, and finally, the conversion of the ABTS chromophore by the G-quadruplex DNAses was measured. The data was normalized to the first time point and the 'no probe control'. The curve was smoothened using a rolling average (window: 5 data points).



Supplementary Figure S2. Verification of the RCA and restriction digestion by Aval. The agarose gel shows the successful amplification of the CTX-M-1 gene after A) a RCA of 60 min, B) a RCA of 15 min, C) a RCA of 60 min followed by Aval restriction, and D) a RCA of 15 min followed by Aval restriction. The labels above the gel pockets represent different G-quadruplex DNAzyme sequences which were evaluated. The final design used in this publication is A4G4.

Supplementary Table S1. G-quadruplex padlock probes. Each padlock probe consists of the sequence 1, insert 1, filler, insert 2 and sequence 2; all sequences are in the 5'-3' order. The insert 1 sequence corresponds to ACTACTTACCTCGGGC, and the insert 2 sequence corresponds to TTTTCCCCTTTTCCCCTTTTCCCCTTTTCCCC.

Name	Sequence 1	Filler	Sequence 2	Tm 1 [°C]	Tm 2 [°C]	Length [nt]	5' offset
AAC(6')-Ib	CGCTTCCAAGAGCAACG		TCCCACCATCCGTCCC	61	62	81	11
ACC	GGGGATCGTGATGCTGG		CGTTTGTTCCTGCTAAGAAGAT	61	61	87	20
ACT	CGCCAACGCCAGCA		TGGCACAACGCGTCTTTA	61	62	80	13
aph2	GCATAAGGCGCCGGA		GGCAGGAAGGTATTAATAACTATCA	62	62	90	18
CMY	GCGCTTCCCGCCG	A	AGCAAAGTGGCATTGGCA	61	63	80	5
CTX-M-1	TGCGCATACAGCGGC	C	CTGTACGTCCGCGGTT	62	61	80	9
CTX-M-25	CGCCGCTACCGCC		CGTTTCACTTTGCTTAAGCAC	60	61	82	12
CTX-M-9	GCCGCTGGTTCTGGT	TTG	CCGCAGGGTCTGTC	60	61	80	21
DHA	GCGGTTTCTGTGGCGA		TCACAGGTGTGCTGGGT	62	62	81	8
GES	CTACGAGCGGGTTTTCTT		AACGGGAGACGCGACA	61	62	82	17
GIM	GGTAGTTCTTGATAATAATCAAGCC		GGTTAGTTGATTGCAATGGGT	62	61	95	19
IMI	AATCGTTCAATTTGCTTTGTACGA		GCCTTTGAAAGAACTACATAATGGA	62	62	96	11
IMP	ATAGATCGAGAATTAAGCCACTCT		TTCAGATGCATACGTGGGG	62	62	91	5
mecA	AGTTGAACCTGGTGAAGTTGT		CCCAATCATTGCTGTTAATTTTTTG	62	62	96	15
MOX	GGCGAGTTATGCCTATGGC		GCCGAAGCAGGCCAT	63	61	82	19
OXA-1	AGAACCTTACAAAACGGATGGT		GTGCAGGATTCACAGCAAAT	63	61	90	17
OXA-10	TGCGACACCAGGATTTGAC		CCCACCCAAACCCACCA	63	63	83	20
OXA-23	ATATGTGCCAGCCTCTACATTT		AAGCCGCGCAAATACAGA	62	62	88	22
OXA-24	ACTCCACAGGTAGGTTGGT		GGATGGGGAATGGGTGTT	62	61	85	7
PER	TGCAGCAGTTGCTGCA		GTGTGCGAGACCGAGTAT	62	61	82	16
qnrB	AACTCGCCGCCGGA		CGCCAGTCGAAAGTCGAA	62	62	80	20
qnrS	CGGGAAAAGTTGGCACCT		ACTTGATGGGCAAAGTTTGT	62	61	87	12
SHV	GGGTGCGCGCGG	CACTGA	GGAGCTGGCGAGCG	61	61	80	18
SPM-1	CCGCGCCCATCTTCTT		TCGCTCGACCACGTCT	61	62	80	4
TEM	GTGATAACACTGCGGCCA		AGTGCTGCCATAACCATGA	62	61	85	13
vanA	GGTCTAGCCCGTGTGGA		AGCGCTCGGCTGTAGA	62	62	81	20
VEB	CCACGTTTTAGGCAAAAGGT		GGGAATTCCTCTTTAATCGGACT	61	63	91	5

Supplementary Table S2. Primer set targeting antibiotic resistance genes.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Size
AAC(6)-Ib	GCCCCAGTCGTACGTTGC	CTTTGCCCAGTTGTGATGC	112
AAD	GATGAGCGAAATGTAGTG	GGCAGCGACATCCTTCG	84
ACC	CCGATTGTTCCCCGTTA	GATTGAGTAGTTTTTGTAGCCA	141
ACT	GGATGAGGTCACGGATA	CCAAAAAGACCGATGCT	111
aph2	TTTRTGCTTGCTGGATTGTA	TGCTTTTCTTTCTGCTACTTC	106
ccrA	CACAGAAAAGCGTAAAAATA	GCGAGGGATACATAAGT	79
cepA	AGACTTATACATTTATCCATTATC	TATTTCCGGCTTTTTTCCC	117
CMY	TATGTACCAGGGATTAGGCTG	CTCAACGGCGGGAAGC	109
CTX-M-1	AGGAAGTGTGCCGCTGT	AATTATCTGCTGTGTTAATCA	113
CTX-M-2	TGCCGAAATCATGGGTAG	GGTTGGGTAAAGTAGGTCAC	124
CTX-M-25	CAGTAAAGTGATGGCGGTA	AATGGGGTTGTAGTTAATC	112
CTX-M-9	ACTACGGCACCAACCAATG	TGCGGCTGGGTAAAATAG	85
DHA	TAAGCCCTATTATTTCAATTATG	CTCTTTTTTCGCCACAG	139
DIM	GTACATTGGATTTCGTAATAAT	CGTAGAAATTGATTGGTCA	111
ermB	ATCTATTCAACTATCGTCAGA	TCCCAACAATTTTATACCTCT	114
ermF	AATTAGAACCACACAAAAGTTAT	ACCTACCTCATAGACAAG	101
FOX	SAAAGGTTCCGCCTT	GCATCTTGTCATTGCAATC	107
GES	CACCTCGACCCACACCA	CCAACAACCCAATCTTT	93
GIM	AAGTTATAAAAAATTGAAGATGGAGT	AAGGTGTGTCGATAATATAGGCT	129
IMI	GYAAATCATTTTCRTACARAGCA	GTGATGGGTGAATRGAAC	163
IMP	GGAATAGAGTGGCTTAATT	AACTTCAATTTTATTTTAACTAG	144
KPC-2	TCTAGTTCTGCTGTCTTGTC	AGTCCTGTTGAGTTTAG	101
MCR-1	CTCCAAAATGCCCTACAGA	CTTAACAAAAGCCACAAG	117
mecA	AACAAGTTCCAGATTACAAC	GCCAACCTTTACCATCG	121
mecC	GTTCCATACCATTAGTTAATATAT	GCTTTAGAAAATAACGGAAATAT	121
MOX	TGCACCACACCTATGTC	GCTTGTCTCTTTCTGA	75
NDM	CAGCACACTTCCTATCTCG	GGTCCAGGCGGTATCGA	102
OXA-1	GAAAAACACCATAGAGAACA	TATAATAAACCTTCAAACCA	126
OXA-10	TTTTCTGGTGTGGGAAGTGAAG	GATTTTGGTGGGAATGGATTTC	150
OXA-23	TTCAAACAGATAAAAAAATTAATC	CGATCAGGGCATTCAACATT	102
OXA-24	ACTCAAGAAGAAGTTAAAAAATG	CCAGTCAACCAACCTACCT	113
OXA-48	GTATCACAATAAGTTACACGT	CCAGTTTTAGCCCGAATAAT	102
OXA-58	TGCCTTTTAAACCTGAAGT	TTTTCAACAAAACCCACATACCA	142
PER	GAGTTTAGTGTTCAGTGC	GTCTCCTTTATACCCATAGAC	140
qnrA	TATCAGTGTGACTTCAGCC	CCTTGAACTGGCATCGC	127
qnrB	CGTTCAGTGGTTCAGATCT	AATTGGTCAGATCGCAATG	87
qnrS	GTGTGATTTAAAGGKGCC	CCKCTCCATATTGGCATA	121
SHV	TGCAGTGGATGGTGGAC	CCAAGCAGGGCGACAAT	127
SPM-1	TTACAAGAAGATGGGCG	AACAGGATGGGAACCTCAGAATC	145
TEM	CGCCGCATACACTATTCTC	GTCAGAAGTAAGTTGGC	143
tetQ	GTAGAAGATATGGAAGATTTTC	GACGGAGGATTTGAGAG	102
vanA	CGGAAAAAGGCTCWGAA	CGTTATCTTGTAACAAACATATC	150
vanB	GGTAAAAATCCGCAATAGAAAT	CTTCCCATTCCGTACAT	122
VEB	TCAAGACCTTTTGCTAAA	AAGAATTTTGAACAGAATCAGT	168
VIM	GGTCTACCCGTCCAATGG	CCGCTGTGTTTTTCGCA	86

Supplementary Table S3. Single-stranded synthetic targets complementary to the G-quadruplex padlock probes.

Name	Target sequence (5'-3')
AAC(6')-lb_tar	GGGACGGATGGTGGGACGTTGCTCTTGGAAGCG
ACC_tar	ATCTTCTTAGCAGGAACAAACGCCAGCATCACGATCCCC
ACT_tar	TAAAGACGCGTTGTGCCATGCTGGCGTTGGCG
aph2_tar	TGATAGTATTTTAATACCTTCCTGCCTTCCGGCGCCTTATGC
CMY_tar	TGCCAATGCCACTTTGCTCGGCGGGAAGCGC
CTX-M-1_tar	AACGGCGGACGTACAGGCCGCTGTATGCGCA
CTX-M-25_tar	GTGCTTAAGCAAAGTGAAACGGGCGGTAGCGGCG
CTX-M-9_tar	GCACGACCCTGCGGACCAGAACCAGCGGC
DHA_tar	ACCCAGCACACCTGTGATCGCCACAGAAACCGC
GES_tar	TGTCGCGTCTCCCGTTAGGAAAACCCGCTCGTAG
GIM_tar	AACCCATTGCAATCAACTAACCGGCTTGATTATTATCCAGAACTACC
IMI_tar	TCCATTATGTAGTTCTTTCAAAGGCTCGTACAAAGCAAATGAACGATT
IMP_tar	CCCCACGTATGCATCTGAAAGAGTGGCTTAATTCTCGATCTAT
mecA_tar	CAAAAAATATTAACAGCAATGATTGGGACAACTTCACCAGGTTCAACT
MOX_tar	ATGGCCTGCTTCGGCGCCATAGGCATAACTCGCC
OXA-1_tar	ATTTGCTGTGAATCCTGCACACCATCCGTTTTGTAAGGTTCT
OXA-10_tar	TGGTGGGTTGGGTGGGGTCAAATCCTGGTGTGCA
OXA-23_tar	TCTGTATTTGCGCGGCTTAAATGTAGAGGCTGGCACATAT
OXA-24_tar	AACACCCATTCCCATCCACCAACCTACCTGTGGAGT
PER_tar	ATACTCGGTCTCGCACACTGCAGCAACTGCTGCA
qnrB_tar	TTCGACTTTGACTGGCGTCCGGCGGCGAGTT
qnrS_tar	AGCAAACCTTGCCAATCAAGTGGGGCCAACCTTTCCCG
SHV_tar	CCGCTCGCCAGCTCCCCGCGCGCACC
SPM-1_tar	AGACGTGGTCGAGCGAAAGAAGATGGGCGCGG
TEM_tar	TCATGGTTATGGCAGCACTTGCCCGCAGTGTTATCAC
vanA_tar	TCTACAGCCGAGCGCTTCCACACGGGCTAGACC
VEB_tar	AGTCCGATTAAAGAGGAATTCCCACCTTTTGCCATAAACGTGG

Supplementary Table S4. Additional primers.

Name	Sequence (5'-3')
Padlock probe primer	GCCCGAGGTAAGTAGT
Padlock probe primer RC	ACTACTTACCTCGGGC