Figure S1

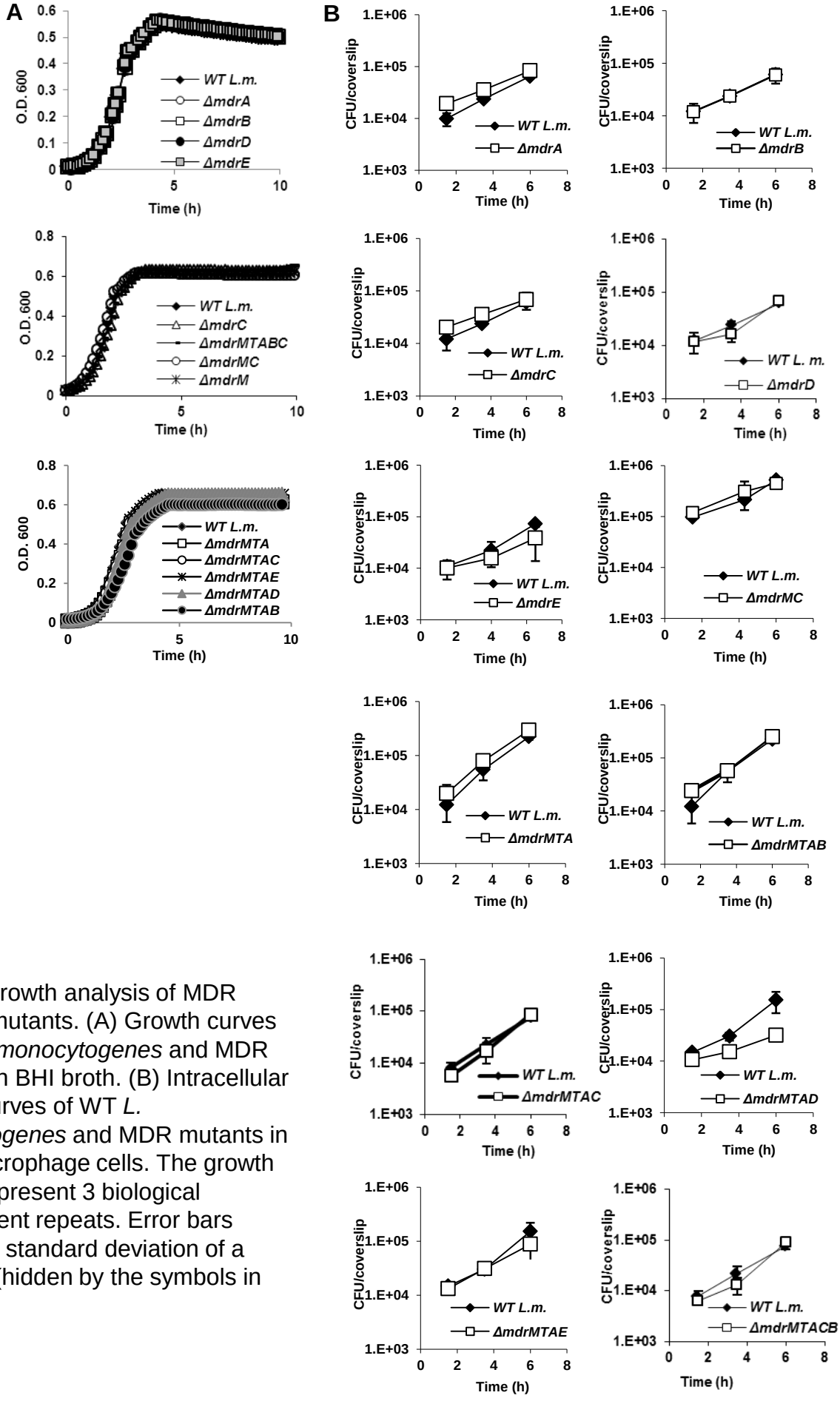

FIG S1 Growth analysis of MDR deletion mutants. (A) Growth curves of WT *L. monocytogenes* and MDR mutants in BHI broth. (B) Intracellular growth curves of WT *L. monocytogenes* and MDR mutants in BMD macrophage cells. The growth curves represent 3 biological independent repeats. Error bars represent standard deviation of a triplicate (hidden by the symbols in panel A).

Figure S2

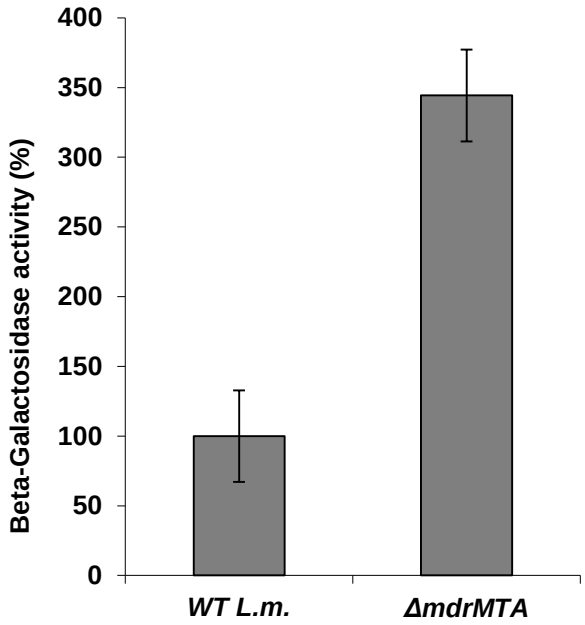

FIG S2 Transcription analysis of *mdrC* gene in *ΔmdrMTA* and WT bacteria. The relative activity of *mdrC* promoter was assayed in WT and *ΔmdrMTA L. monocytogenes* bacteria using the *lacZ* reporter gene. *mdrC* promoter region was cloned up-stream the *lacZ* gene in the integrative plasmid pPL2. Beta-galactosidase activity of WT bacteria was set as 100%.

Figure S3

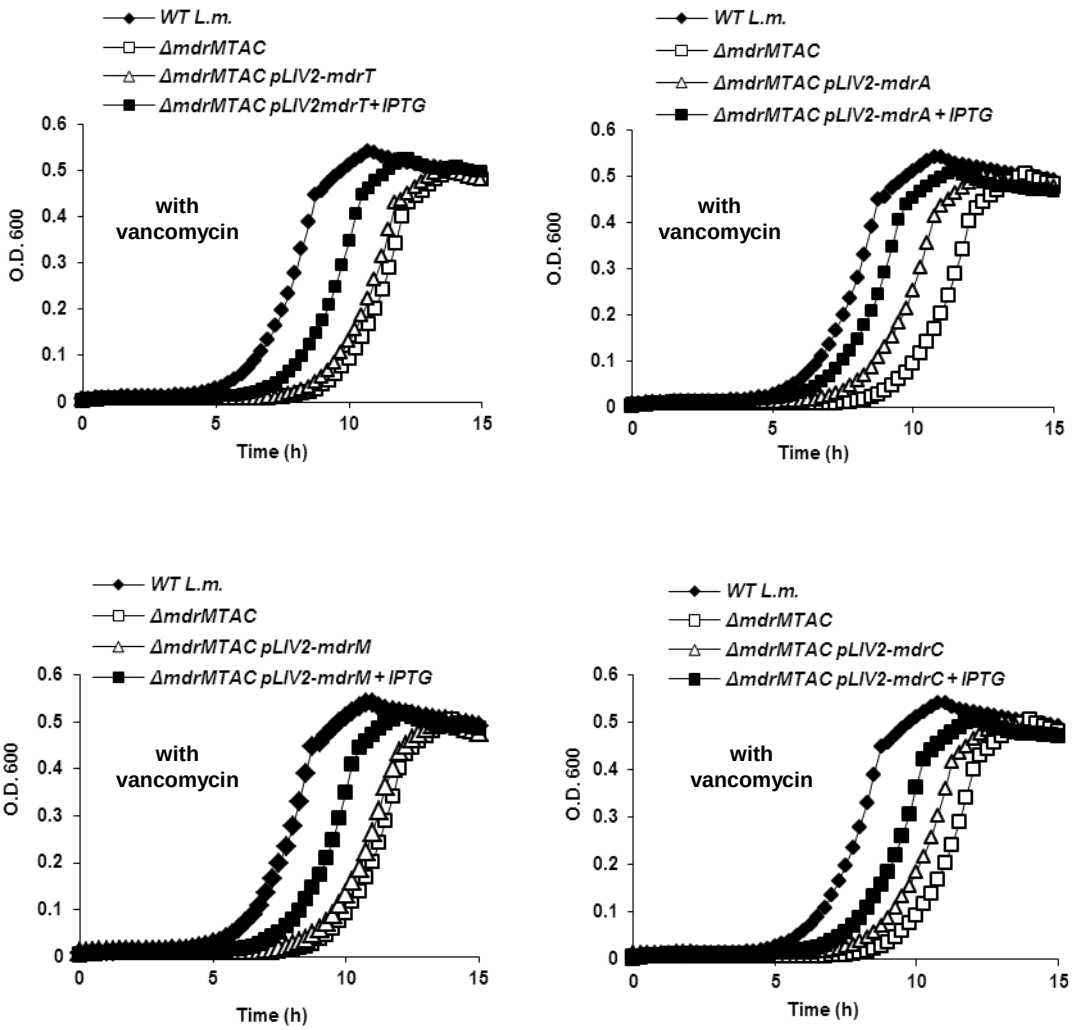

FIG S3 Complementation experiments of *ΔmdrMTAC* mutant. Growth analysis of WT *L. monocytogenes*, *ΔmdrMTAC* mutant and *ΔmdrMTAC* mutant complemented with pLIV2 plasmid expressing each one of the MTAC transporters (with and without IPTG). Experiments were performed in a 96-well format in a Synergy HT Biotek® plate reader. Error bars representing standard deviation of the triplicate are hidden by the symbols. Growth curves from one representative experiment are shown. Experiment was repeated independently 3 times.

Figure S4

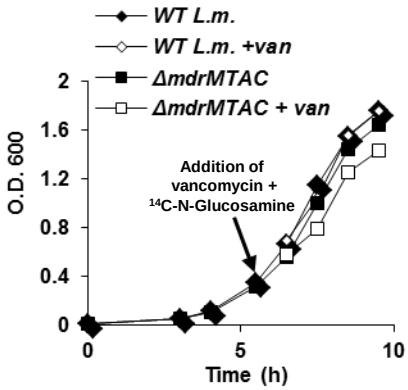

FIG S4 Growth curves of WT *L. monocytogenes* and *ΔmdrMTAC* mutant in BHI media, with and without 0.8 μg ml^{-1} of vancomycin (van). The vancomycin concentration used in this experiment was lower then the one used in figure 5C. Arrow indicates addition of [^{14}C]-N-acetylglucosamine and vancomycin.

Figure S5

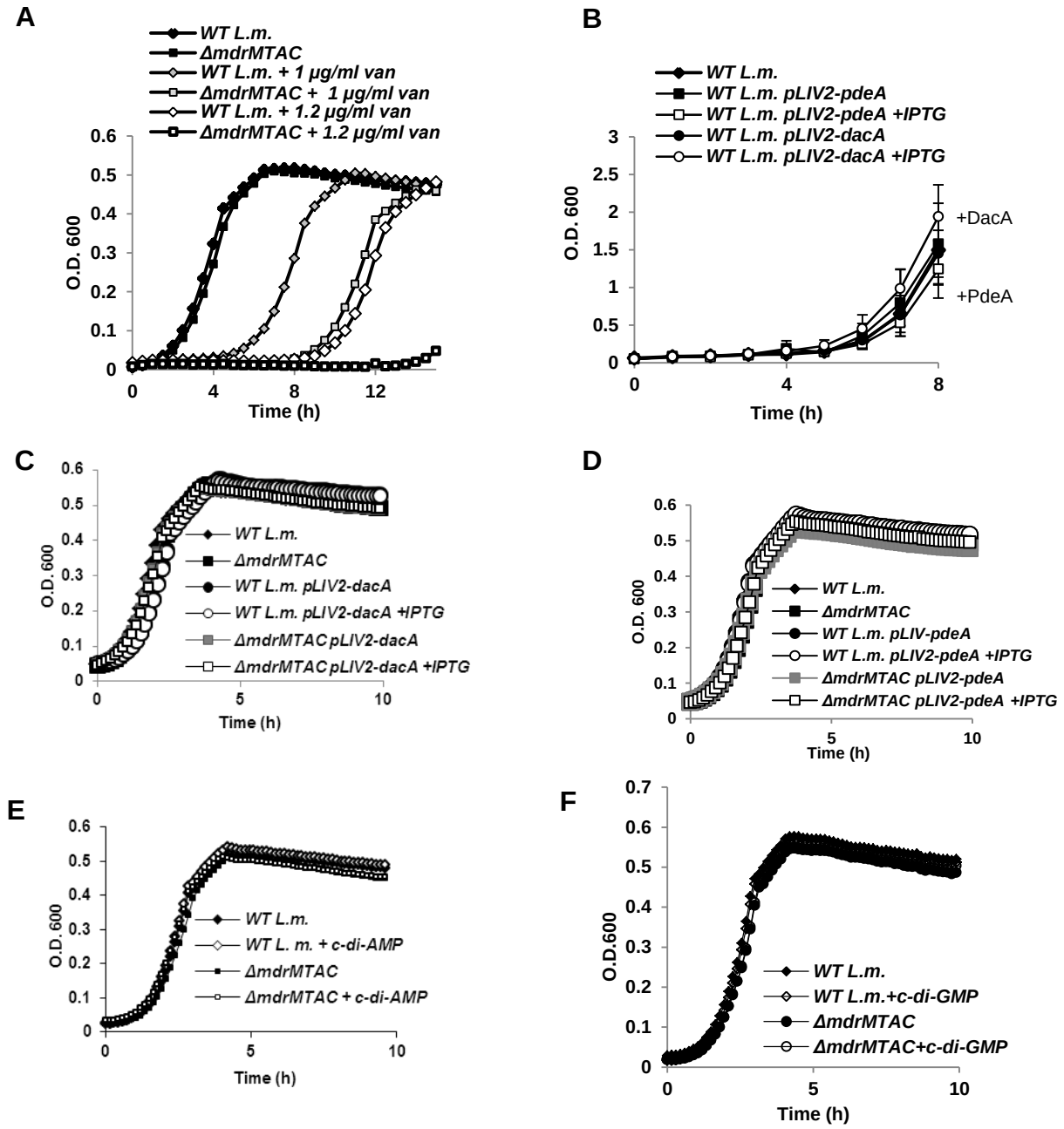

FIG S5 Effect of c-di-AMP on *L. monocytogenes* growth with and without vancomycin stress. (A) Growth curves of WT *L. monocytogenes* and *ΔmdrMTAC* mutant with and without vancomycin. Two vancomycin concentrations were used: 1 and 1.2 µg ml^{-1}. Bacteria were grown in a 96 well microplate reader. (B) Growth curves of WT *L. monocytogenes* strains harboring the pLIV2 plasmid with an IPTG inducible promoter, expressing *dacA* or *pdeA* genes in BHI supplemented with vancomycin (1.2 µg ml^{-1}) with or without IPTG. Experiment was performed in flasks. The data is a mean of 3 independent biological experiments. Error bars represent standard deviation. (C) Growth curves of WT *L. monocytogenes* and *ΔmdrMTAC* mutant harboring pLIV2-*dacA* plasmid in BHI with and without IPTG addition. (D) Growth curves of WT *L. monocytogenes* and *ΔmdrMTAC* mutant harboring pLIV2-*pdeA* plasmid in BHI with and without IPTG addition. (E) Growth curves of WT *L. monocytogenes* or *ΔmdrMTAC* mutant in BHI with and without addition of 3 µg ml^{-1} of purified c-di-AMP or c-di-GMP (F). Experiments were performed in a 96-well format in a Synergy HT Biotek® plate reader. Error bars representing standard deviation of the triplicate are hidden by the symbols. Growth curves from one representative experiment are shown. Experiment was repeated independently 3 times.

Supporting information TableS1. Primers used in this study

A. Bacterial RT-qPCR Primers

Name	Sequence (5'-3')
mdrD-F	TGAATGTGTCTGGTTTGCAACTTTAT
mdrD-R	AAGCCATGCTAACCGTTTCTG
mdrC-F	GGCCGTGCAATCTGACCTT
mdrC-R	CCTGAGAATAGCGCGGTTAAA
mdrB-F	CGCAAATCAACGCCACAAT
mdrB-R	CAGAGCCAAGAATTCCGAAGA
mdrM-F	CAGCAAGTACATCAGTGAAGCGTAA
mdrM-R	GGTAGCGCGACATTCATCAA
mdrT-F	CCGTGCGGTTCTTCGGTAT
mdrT-R	TTTACTGCCGAACCGTGGTT
mdrA-F	GCAACAGGTGGGCAGAAAAT
mdrA-R	GCGCCATGTTAAGAGCAGTTT
hly-F	TAAAAACAATGTATTAGTATACCACGG
hly-R	GATTCACAACTTGAATGTCTGC
rpoB-F	GCGGATGAAGAGGATAATTACG
rpoB-R	TAGTCAATACGTTCTTTTTCTACC
mdrE-F	GTGGAACGCAAATGGAAGCT
mdrE-R	TTCCAACTCCCAGCAATCG
16S rRNA-F	CCTGGTAGTCCACGCCGT
16S rRNA-R	TGCGTTAGCTGCAGCACTAAG
dacA-RT-F	CGTGAACAGCATCATTTAATCGA
dacA-RT-R	GTATCGCGTGCCACTGAAATC
pdeA-F	CCAACTGGGCTAGGGAACATC
pdeA-R	CCTCCGTCAAAAAGGCCATA

B Murine macrophage RT-qPCR primers

Name	Sequence (5'-3')
Ifnβ-F	CCAAGAAAGGACGAACATTCG
Ifnβ-R	CCGCCCTGTAGGTGAGGTT
gapDH-F	TTGTGGAAGGGCTCATGACC
gapDH-R	TCTTCTGGGTGGCAGTGATG
IL1α-F	AGGAGAGCCGGGTGACAGTA
IL1α-R	TCAGAATCTTCCCGTTGCTTG
IL6-F	TTCCATCCAGTTGCCTTCTTG
IL6-R	GAAGGCCGTGGTTGTCACC

C. Primers used for construction of pLIV2 based plasmids

Name	Sequence (5'-3')
mdrM-His-F	CTTGTCGGCTTGATTATTATGG
mdrM-His-R	TATAGTCGACTTAATGATGATGATGATGATGCGTACGTGCTTTTTCCGTTTTAGTAACAATT
M-F58V-ScaI-F	CAGTCAAGGACAATGGTTAAGTACTGGAGTTATGTTAGTTAATGGTGTC
M-F58V-ScaI-R	GACACCATTAACTAACATAACTCCAGTACTTAACCATTGTCCTTGACTG
pLIV2-Oid seq-F	TATACGGTGGATGCATTTCAATTG
dacA-F-EagI-F	GAGGAGCGGCCGATGGATTTTTCCAATATGTCGATATTG
dacA-R-SalI-R	GAGGAGGTCGACATTTAAAATTCGATCCATCATTCGCT
pdeA-pLIV2-EagI	AAAACGGCCGATGTCAGGCTATTTTCAAAAACG
pdeA-pLIV2-SpeI	TTTTACTAGTTTATGTTTCTCCCTTCCAATACG
mdrC-F-BamHI	ATTAGGATCCATGACTTCAACAGCGTATAAA
mdrC-R-PstI-2	TAATCTGCAGCTATTCTTTTGCGGCTTTTAAT
mdrA-F-BamHI	ATTAGGATCCATGCAACAAGAAGCAACAGGTG
mdrA-R-pstI	TAATCTGCAGTTACGAGAAGTTCTCTTCGCT

*Restriction sites are underlined

D. Primers for deletions of bacterial genes

Name	Sequence (5'-3')
mdrB-A-SalI-F	ACTATGTCGACGCAGTAATCACGTTCTTGCGCA
mdrB-B-R	TCGGTAACCGGAATACAAGTAGGTATTACGTTTATTCGTCTGTTCCATGA
mdrB-C-F	TCATGGAACAGACGAATAAACGTAATACCTACTTGTATTCCGGTTACCGA
mdrB-D-PstI-R	ATTACCTGCAGAGCTTGCTGGCAAGTATTTCTT
mdrE-A2-KPNI-F	ATACTGGTACCCTTTGTAATTATCTGGAATCTCCATC
mdrE-B-R	GACAAGACTTTGGACGAAGGACAATAGCTAACATCTCTTGTGAAGTG
mdrE-C-F	CACTTCACAAGAGATGTTAGCTATTGTCCTTCGTCCAAAGTCTTGTC
mdrE-D2-Pst-R	ATAACCTGCAGTAACGAGTCCGCCAGAAGTGG
mdrC-A-SalI-F	ATTATGTCGACTCAGAAATGCCCGTTAGGTACT
mdrC-B-R	AGAATAACTAATGACTTCAACAGCGTAGCGCTCGAATTAAAAGCCGCA
mdrC-C-F	TGCGGCTTTTAATTCGAGCGCTACGCTGTTGAAGTCATTAGTTATTCT
mdrD-A-SalI-F	ATTATGTCGACTCTCATTTATGCGCTAGATTATCC
mdrD-B-R	AAGGCCTATTATTTGAACTATTTATCTTTTCATATCCACATTGTTTCCCCCTA
mdrD-C-F	TAGGGGGAAACAATGTGGATATGAAAAGATAAATAGTTCAAATAATAGGCCTT
mdrD-PstI-R	ATTATCTGCAGTTTCTAGCGCCTTATCGAGCT
mdrA-A-SalI-F	ATTATGTCGACCACGGTCAGTTGTGTTTAGCATTG
mdrA-B-R	TCGCTTTATTATTTAGCTTTACGACCTGTTGCTTCTTGTTGCAT
mdrA-C-F	ATGCAACAAGAAGCAACAGGTCGTAAAGCTAAATAATAAAGCGA
mdrA-D-KpnI-R	ATTATGGTACCGCACAATCGTTTCCGGATCAT

*Restriction sites are underlined