

## PCR multiplex for detection of *bla*CTX-M-U, *bla*PER-2, *bla*CMY genes

Primer name	Gene	Sequence 5'--3'
CTX-MU1	CTX-M-U	ATG TGC AGY ACC AGT AAR GT
CTX-MU2		TGG GTR AAR TAR GTS ACC AGA
PER-2-PLUS	PER-2	GTAGTATCAGCCCAATCCCC
PER-2-MINUS		CCAATAAAGGCCGTCCATCA
CITMF	CMY-2	TGGCCAGAACTGACAGGCAAA
CITMR		TTTCTCCTGAACGTGGCTGGC

Amplicon size	Cycling program
PER-2 = 739 pb CTX-M-U: 593 pb CMY = 462 pb	Initial denaturation = 94°C for 5min; Cycling= 30-35 cycles: 94°C 30sec -- 52° 30sec -- 72°C 60sec; Final extension = 72°C for 10min

PCR Reagents--> Final volume= 25ul	
Reagent	Volume
ADN	2,5 ul
Buffer 10X	2,5 ul
MgCl <sub>2</sub> (50 mM)	0,75 ul
dNTP's (10 mM)	0,5 ul
Taq pol (5U/ul)	0,2 ul
Primer CTX-M (10 µM)	1,5 ul each
Primer PER-2 (10 µM)	0,5 ul each
Primer CIT (10 µM)	0,5 ul each
H <sub>2</sub> O	13,55 ul
<b>Vol. Final</b>	<b>25 ul</b>

References
Pagani, et al, JCM 41(9):4264-4269, 2003. Pérez-Pérez and Hanson, JCM40:2153-2162, 2002)
<b>CTX-M-U:</b> Universal primers. <b>CMY:</b> <b>CMY-2</b> is the most common variant. CMY primers also amplify the following genes and variants: LAT-1/4;CMY-2/7,BIL-1