Nov 19, 2025

qPCR Duplex Panel for Detection of Antimicrobial Resistance Genes

DOI
https://dx.doi.org/10.17504/protocols.io.rm7vz9nb4gx1/v1

Leah Lariscy[1], Abbey Wilson[1], Carter Coleman[1], Erin Lipp[1]

[1]University of Georgia

Leah Lariscy
University of Georgia

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.rm7vz9nb4gx1/v1

Protocol Citation: Leah Lariscy, Abbey Wilson, Carter Coleman, Erin Lipp 2025. qPCR Duplex Panel for Detection of Antimicrobial Resistance Genes. **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vz9nb4gx1/v1

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: July 07, 2025

Last Modified: November 19, 2025

Protocol Integer ID: 221902

Keywords: total nucleic acid samples for qpcr detection, antimicrobial resistance genes this protocol, select antimicrobial resistance gene, antimicrobial resistance gene, qpcr detection, qpcr duplex panel for detection, qpcr panel, based qpcr panel, qpcr duplex panel, purified total nucleic acid sample, custom idt primer, custom idt template control

Funders Acknowledgements:
Centers for Disease Control and Prevention Pathogen Genomics Centers of Excellence
Grant ID: NU50CK000626

Abstract

This protocol describes how to prepare purified total nucleic acid samples for qPCR detection of select antimicrobial resistance genes using a duplexed panel. The probe-based qPCR panel utilizes custom IDT primers and probes, as well as custom IDT template controls (gBlocks). This protocol has been validated for use with complex sample matrices such as raw wastewater and stool.

Materials

Reagents

TaqPath qPCR Master Mix, CG (Applied Biosystems, A15297)
Water, Molecular Biology Grade (Fisher, BP2819-1)
Custom primers (IDT)
Custom probes (IDT)
Custom gBlocks (IDT)

Equipment

Micropipettes (p10, p20, p200, p1000)
Microcentrifuge
Microplate centrifuge
Vortex mixer
CFX384 Touch Real-Time PCR Detection System (Bio-Rad, 1855484)

Consumables

1.5 mL tubes, DNA/RNAse free
0.2 mL strip tubes, DNA/RNAse free
Hard-Shell PCR Plates, 384-well, thin-wall (Bio-Rad, HSP3805)
Microseal ‘B’ Seal, optically clear (Bio-Rad, MSB1001)
Micropipette tips, DNA/RNAse free (p10, p20, p200, p1000)

Assay info

1 The three ARG duplexes are described below:

	A	B	C
	Duplex	**Gene Target**	**CARD Accession No.**
	ermB/tetB	ermB (Erm 23S ribosomal RNA methyltransferase)	3000375
		tetB (Tetracycline effux protein)	3000166
	blaKPC/blaSHV	blaKPC (KPC beta-lactamase)	3000059
		blaSHV (SHV beta-lactamase)	3000015
	blaCTX-M-1/QnrS	blaCTX-M-1 (CTX-M beta-lactamase)	3001864
		QnrS (quinolone resistance protein)	3002790

2 Primer and probe sequences for each ARG assay are described below:

	A	B	C
	Gene Target	**Sequence**	**Source**
	ermB	GGATTCTACAAGCGTACCTTGGA (F)	Bockelmann et al., 2009
		GCTGGCAGCTTAAGCAATTGCT (R)	
		FAM-CACTAGGGTTGCTCTTGCACACTCAAGTC-BHQ-1 (Pb)	
	tetB	ACACTCAGTATTCCAAGCCTTTG (F)	Knapp et al., 2010
		GATAGACATCACTCCCTGTAATGC (R)	
		HEX-AAAGCGATCCCACCACCAGCCAAT-BHQ-1 (Pb)	
	blaKPC	GGCCGCCGTGCAATAC (F)	CDC, 2011
		GCCGCCCAACTCCTTCA (R)	
		FAM-TGATAACGCCGCCGCCA ATTTGT-BHQ-1 (Pb)	

	A	B	C
	blaSHV	AACAGCTGGAGCGAAAGATCCA (F)	Bockelmann et al., 2009
		TGTTTTTCGCTGACCGGCGAG (R)	
		HEX-TCCACCAGATCCTGCTGGCGATAG-BHQ-1 (Pb)	
	QnrS	CGACGTGCTAACTTGCGTGA (F)	Calero-Caceres et al., 2014
		GGCATTGTTGGAAACTTGCA (R)	
		FAM-AGTTCATTGAACAGGGTGA-BHQ-1 (Pb)	
	blaCTX-M-1	ATGTGCAGCACCAGTAAAGTGATGGC (F)	Dungan et al., 2018
		ATCACGCGGATCGCCCGGAAT (R)	
		HEX-CCCGACAGCTGGGAGACGAAACGT-BHQ-1 (Pb)	

qPCR reaction preparation

3 Thaw reagents and samples appropriately prior to use.

4 Gently vortex and spin down reagents and samples, **except for the TaqPath Master Mix**.
(Rather than vortex, the TaqPath should be gently mixed via pipette)

5 For each duplex, prepare the reactions as follows:

5.1 For purified nucleic acid samples, combine the following components in an optical reaction plate for each duplex:

	A	B	C
	Component	**Volume (µl)**	**Final Concentration**
	Nuclease-free Water	5.9	N/A
	Forward Primer 1 (20 µM)	0.1	0.2 µM
	Reverse Primer 1 (20 µM)	0.1	0.2 µM

	A	B	C
	Probe 1 (10 µM)	0.1	0.1 µM
	Forward Primer 2 (20 µM)	0.1	0.2 µM
	Reverse Primer 2 (20 µM)	0.1	0.2 µM
	Probe 2 (10 µM)	0.1	0.1 µM
	TaqPath Master Mix (4x)	2.5	1 unit/µL
	Nucleic acid sample	1	N/A

Total reaction volume: 10 µL

5.2 For template controls, combine the following components in an optical reaction plate for each duplex:

	A	B	C
	Component	**Volume (µl)**	**Final Concentration**
	Nuclease-free Water	5.9	N/A
	Forward Primer 1 (20 µM)	0.1	0.2 µM
	Reverse Primer 1 (20 µM)	0.1	0.2 µM
	Probe 1 (10 µM)	0.1	0.1 µM
	Forward Primer 2 (20 µM)	0.1	0.2 µM
	Reverse Primer 2 (20 µM)	0.1	0.2 µM
	Probe 2 (10 µM)	0.1	0.1 µM
	TaqPath Master Mix (4x)	2.5	1 unit/µL
	Template control 1	0.5	N/A
	Template control 2	0.5	N/A

Total reaction volume: 10 µL

5.3 For non-template controls, combine the following components in an optical reaction plate for each duplex:

	A	B	C
	Component	**Volume (µl)**	**Final Concentration**
	Nuclease-free Water	6.9	N/A
	Forward Primer 1 (20 µM)	0.1	0.2 µM
	Reverse Primer 1 (20 µM)	0.1	0.2 µM
	Probe 1 (10 µM)	0.1	0.1 µM
	Forward Primer 2 (20 µM)	0.1	0.2 µM
	Reverse Primer 2 (20 µM)	0.1	0.2 µM
	Probe 2 (10 µM)	0.1	0.1 µM
	TaqPath Master Mix (4x)	2.5	1 unit/µL

Total reaction volume: 10 µL

6 Pipette up and down 10 times to mix, then spin down.

7 Incubate reactions in a real-time PCR machine under the following conditions:

	A	B	C	D
	Step	**Temp**	**Duration**	**Cycles**
	Initial Denaturation	95	10 minutes	1
	Denaturation	95	15 seconds	40

	A	B	C	D
	Annealing/Extension	60	1 minute	40

Imaging step should follow the annealing/extension step

Template control info

8 ARG standard sequences for each target are described below:

	A	B
	Gene Target	**Sequence**
	ermB	TTACCATTTAAGCACACAAATTATTAAAAAAGTGGTTTTTGAAAGCCATGCGTCTGACATCTATCTGATTGTTGAAGAAGGATTCTACAAGCGTACCTTGGATATTCAGACTTGAGTGTGCAAGAGCAACCCTAGTGCAAGTCTCGATTCAGCAATTGCTTAAGCTGCCAGCGGAATGCTTTCATCCTAAACCAAAAGTAAACAGTGTCTTAATAAAACTTACCCGCCATACCACAGATGTTCCAGATAA
	tetB	TTTTCATTAGCGGGTCTTGGTCTTTTACACTCAGTATTCCAAGCCTTTGTGGCAGGAAGAATAGCCACTAAATGGGGCGAAAAAACGGCAGTACTGCTCGGATTTATTGCAGATAGTAGTGCATTTGCCTTTTTAGCGTTTATATCTGAAGGTTGGTTAGTTTTCCCTGTTTTAATTTTATTGGCTGGTGGTGGGATCGCTTTACCTGCATTACAGGGAGTGATGTCTATCCAAACAAAGAGTCATCAGCAAGGTGC
	blaKPC	TCGCCTGGGATGGCGGAGTTCAGCTCCAGCTCCCAGCGGTCCAGACGGAACGTGGTATCGCCGATAGAGCGCATGAAGGCCGTCAGCCCGGCCGGGCCGCCCAACTCCTTCAGCAACAAATTGGCGGCGGCGTTATCACTGTATTGCACGGCGGCCGCGGACAGCTCCGCCACCGTCATGCCTGTTGTCAGATATTTTTCCGAGATGGGTGACCACGGAACCAGCGCATTTTTGCCGTAACGGATGGGTGT
	blaSHV	CCTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGCGGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATCCACTCTATCGCCAGCAGGATCTGGTGGATACTCGCCGGTCAGCGAAAAACATCTTGCCGACGGCATGACGGTCGGCGAACTCTGTGCCGCCGCCATTACCATGAGCGATAACAGCGCCGCCAATCTGCTGCTGGCCACCGT
	QnrS	AACTTTTCACATAAAGACTTAAGTGATCTCACCTTCACCGCTTGCACATTCATTCGCAGCGACTTTCGACGTGCTAACTTGCGTGATACGACATTCGTCAACTGCAAGTTCATTGTCACCCTGTTCAATGAACTCTGCCACTTTGATGTCGCAGATCTTCGTGATGCAAGTTTCCAACAAT

	A	B
		GCCAACTTGCGATGGCAAACTTCAGTAATGCCAATT GCTACGGTATAGAGTTCCGTGCGTGTGATTTAA
	blaCTX-M-1	GATGAGCGCTTTGCGATGTGCAGCACCAGTAAAGTG ATGGCCGTGGCCGCGGTGCTGAAGAAAAGTGAAAG CGAACCGAATCTGTTAAATCAGCGAGTTGAGATCAA AAAATCTGACTTGGTTAACTATAATCCGATTGCGGAA AAGCACGTCGATGGGACACGTTTCGTCTCCCAGCTG TCGGGGGCCGCGCTACAGTACAGCGATAACGTGGC GATGAATAAGCTGATTTCTCACGTTGGCGGCCCGGC TAGCGTCACCGCGTTCGCCCGACAGCTGGGAGACG AAACGTTCCGTCTCGACCGTACCGAGCCGACGTTAA ACACCGCCATTCCGGGCGATCCGCGTGATACCACTT CACCTCGG

See Table 1 for CARD accession numbers.