Sep 19, 2024

Multiplex Nested PCR for Vibrio Cholera

DOI
https://dx.doi.org/10.17504/protocols.io.36wgqnzbkgk5/v1

Shannon Fitz[1], Alex Shaw[1], Dilip Abraham[2], michael Owusu[3]

[1]Imperial College London; [2]CMC Vellore; [3]Kwame Nkrumah University of Science and Technology

GEMS - Genomic Enviro...

Shannon Fitz
Imperial College London

DOI: https://dx.doi.org/10.17504/protocols.io.36wgqnzbkgk5/v1

Protocol Citation: Shannon Fitz, Alex Shaw, Dilip Abraham, michael Owusu 2024. Multiplex Nested PCR for Vibrio Cholera. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgqnzbkgk5/v1

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

Created: September 19, 2024

Last Modified: September 19, 2024

Protocol Integer ID: 108018

Keywords: PCR, Amplicon, vibrio cholera, multiplex PCR, vibrio cholera primer, multiplex nested pcr assay, nested pcr assay, toxr-for-outer, toxr-for-inner, o139-rfbrev-outer, o1-rfbrev-outer, o1-rfbrev-inner, o139-rfbfor-outer, pcr, genome, o1-rfbfor-outer, tox-rev-outer, ctxa-for, multiplex, following primer, primer

Funders Acknowledgements:
The Gates Foundation
Grant ID: INV-049092 PA4273

Abstract

Primers targeting various regions of the V. Cholera genome were obtained from the literature or designed, and optimized for a multiplex nested PCR assay.

The following primers were extracted from Hoshino et al., 1998: O1_rfbFor_Inner (modified here by Dr Alex Shaw), O1_rfbRev_Inner, O139_rfbFor_Inner, O139_rfbRev_Outer.

The following primers were obtained from Theron et al., 2000: ctxA Inner Rev, ctxA_rev.

The following primers were obtained from Nandi et al., 2000: ctxA_For, ompW_For, ompW_Rev, ompW_Inner_Rev.

The following primers were designed by Dr Alex Shaw and are unpublished:
ToxR_For_Outer, Tox_Rev_Outer, ToxR_For_Inner, O1_rfbFor_Outer, O1_rfbRev_Outer, O139_rfbFor_Outer, O139_rfbRev_Outer.

Materials

DreamTaq PCR Master Mix (2X) **Thermo Fisher Catalog #**K1072

Approximate total cost per sample: £0.9
17 primers required (cost excluded from estimate as primers do not need to be ordered each time)
Dreamtaq cost per sample: ~£0.45 per sample, per PCR run

Extra equipment required:
Vortex, mini centrifuge, thermocycler

Protocol materials

DreamTaq PCR Master Mix (2X) **Thermo Fisher Catalog #**K1072

Multiplex nested PCR for *V.Cholera*

1 Assemble primer pool:

	A	B	C	D
	Primer name	Primer	Pool C	Pool D
	ToxR_For_Outer	AGATGTTCGGATTAGGACAC	C	
	ToxR_Rev_Outer	ATGGCATCGTTAGGGTTAGCA	C	D
	ToxR_For_Inner	GCAGCAACGAAAGCCGAATT		D
	ctxA_For	CTCAGACGGGATTTGTTAGGCACG	C	D
	ctxA_rev	CGATGATCTTGGAGCATTCCCAC	C	
	ctxA_InnerRev	GAGTATGGAATCCCACCTAAAGC		D
	O1_rfbFor_Outer	CCCGACAGCCAGTGAGATAC	C	
	O1_rfbRev_Outer	CGTATTGCGGCGGTAAAAGG	C	
	O1_rfbFor_Inner	GGTTTCACTGAACAGATGGG		D
	O1_rfbRev_Inner	GGTCATCTGTAAGTACAAC		D
	O139 rfbFor_Outer	AACGTAGGCACTTGAGAGGC	C	
	O139 rfbRev_Outer	TCGCCGGTCGACTGTTTAAC	C	
	O139_rfbFor_Inner	AGCCTCTTTATTACGGGTGG		D

	A	B	C	D
	O139_rfbRev_Inner	GTCAAACCCGATCGTAAAGG		D
	ompW_For	CACCAAGAAGGTGACTTTATTGTG	C	D
	ompW_Rev	GAACTTATAACCACCCGCG	C	
	ompW_Inner_Rev	GGTTTGTCGAATTAGCTTCACC		D

Bands for the *V. cholera* reactions:

	A	B	C
		First round amplicon length (bp)	Second round amplicon length (bp)
	ToxR	875	723
	ctxA	444	223
	O1_rfbF	1085	196
	O139_rfb	1018	451
	ompW	586	303

Reconstitute primers to 100 µM using nuclease-free water (or if primer manufacturer recommends otherwise, follow their recommendations for reconstituting primers).

Create working stocks of 10 µM using nuclease-free water. Create 10 µM primer pools C and D by mixing together 10 µL of each primer marked as being part of the pool. Scale up as needed.

1.1 *V. cholera* first round PCR reaction

DreamTaq PCR Master Mix (2X) **Thermo Fisher Catalog #**K1072

Prepare the following Master mix on ice in a 1.5ml Eppendorf Lobind tube per number of samples/controls + 10%:

	A	B	C
		1 Reaction (µL)	Reactions
	DreamTaq 2x master mix	12.5	µL
	Water	6.5	µL
	Primer pool C	1	
	Total volume	20	

Briefly vortex and centrifuge down the master mix and aliquot 20 µL into each PCR tube.

Add 5 µL of extracted DNA from each sample to a tube.

Briefly vortex, and centrifuge down the PCR mixes.

1.2 Amplify using the following cycling conditions:

	A	B	C	D
	CYCLE	**STEP**	**TEMP** (°C)	**TIME**
	1	Initial Denaturation	95	2 minutes
	35	Denaturation	95	30 seconds
		Annealing	56	30 seconds
		Extension	72	3 minutes
	1	Final Extension	72	10 minutes
	-	Hold	10	-

1.3 *V. Cholera* second round PCR reaction

Prepare the following Master mix on ice in a 1.5ml Eppendorf Lobind tube per number of samples/controls + 10%:

	A	B	C
		1 reaction (µL)	Reactions
	DreamTaq 2x master mix	12.5	µL
	Water	6.5	µL
	Primer pool D	1	µL
	Total volume	20	

Briefly vortex and centrifuge down the master mix and aliquot 20 µL into each PCR tube.

Add 5 µL of first round amplicon and vortex briefly to mix. Briefly centrifuge down the PCR mixes.

1.4 Amplify using the following cycling conditions:

	A	B	C	D
	CYCLE	**STEP**	**TEMP** (°C)	**TIME**
	1	Initial Denaturation	95	2 minutes
	35	Denaturation	95	30 seconds
		Annealing	55	30 seconds
		Extension	72	1 minute
	1	Final Extension	72	10 minutes
	-	Hold	10	-

1.5 Check amplicons using an agarose gel or tapestation.

Protocol references

Katsuaki Hoshino, Shinji Yamasaki, Asish K. Mukhopadhyay, Soumen Chakraborty, Arnab Basu, Sujit K. Bhattacharya, G. Balakrish Nair, Toshio Shimada, Yoshifumi Takeda, Development and evaluation of a multiplex PCR assay for rapid detection of toxigenic *Vibrio cholerae* O1 and O139, *FEMS Immunology & Medical Microbiology*, Volume 20, Issue 3, March 1998, Pages 201–207, https://doi.org/10.1111/j.1574-695X.1998.tb01128.x

Nandi B, Nandy RK, Mukhopadhyay S, Nair GB, Shimada T, Ghose AC. Rapid method for species-specific identification of Vibrio cholerae using primers targeted to the gene of outer membrane protein OmpW. J Clin Microbiol. 2000 Nov;38(11):4145-51. doi: 10.1128/JCM.38.11.4145-4151.2000. PMID: 11060082; PMCID: PMC87555.

Theron, J., Cilliers, J., Du Preez, M., Brözel, V.S. and Venter, S.N. (2000), Detection of toxigenic *Vibrio cholerae* from environmental water samples by an enrichment broth cultivation–pit-stop semi-nested PCR procedure. Journal of Applied Microbiology, 89: 539-546. https://doi.org/10.1046/j.1365-2672.2000.01140.x