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# Multiplex Nested PCR for Vibrio Cholera

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GEMS - Genomic Environ…

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

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## 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The following primers were designed by Dr Alex Shaw and are unpublished: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 Materials, Step 1.1

## 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 |
| | 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** |

| | A | B | C | D |
|---|---|---|---|---|
| | 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 |

| | A | B | C | D |
|---|---|---|---|---|
| | 1 | Final Extension | 72 | 10 minutes |
| | - | Hold | 10 | - |

1.5 Check amplicons using an agarose gel or tapestation.

## Protocol references

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