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Nested PCR amplification of Salmonella Typhi from extracted wastewater concentrate

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GEMS - Genomic Environ…

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We use this protocol and it's working

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Abstract

This standard operating procedure describes nested PCR protocols for the generation of amplicons from V. cholerae and S. typhi.

PCR primers have been sourced from the literature or designed using Geneious, with primer pooling tested to minimise primer-primer interactions. Whilst the first round of PCR may be sufficient alone for detection (and will yield larger amplicons) a second round of PCR has been included to increase sensitivity.

Materials

DreamTaq PCR Master Mix (2X) **Thermo Fisher Catalog #**K1071

Cost per sample: £0.90
28 primers required (cost excluded from estimate as primers do not need to be ordered each time)
Dreamtaq cost per sample: ~£0.45 per sample

Extra equipment required:
thermocycler, vortex, mini centrifuge

Primer preparation step

1

	A	B	C	D
	Primer name	Primer	Pool G	Pool B
	3.1.1_4.3.1.1.P1_F	CCGGATATCGATCACCCCAAA	G	B
	3.1.1_4.3.1.1.P1_R	GCTTTCGTGATGTCGCTCAG	G	
	3.1.1_Inner_Rev	ACACGGCCACAGTAAGGG		B
	4.3.1_4.3.1.2_1_F	AGAATTTTCGCATCAGGGGGAT	G	
	4.3.1_4.3.1.2_1_R	CCAGTCATTAACGAGGCGCTT	G	
	4.3.1.2_Inner_For	CGTCGCCTCGGTTTTAACATTA		B
	4.3.1.2_Inner_Rev	CGCTGATGAATCCGCACTC		B
	tviD_842_F	TGCAAGCTGCTTAGTGATCGA	G	B
	tviD_842_R	TGAGTCCGGTAAAACGAGCTC	G	
	tviD_Inner_R	CAGGATGGATATTCCCTGCGT		B
	1_3_4.3.1_F	ACGATGGTACTGAACAACCCT	G	
	1_3_4.3.1_R	TACGCTGTTCAGCCCGATATC	G	
	2.2.2_2_F	AGCACAGTTCATCCGAGTGAT	G	
	2.2.2_2_R	AGCATCAGACTCTGCGACAC	G	
	2.5_4.3.1.2.1_3.3_F	CGGTTCGTTGTCCATTTCGG	G	
	2.5_4.3.1.2.1_3.3_R	GGCGGCTTTCTTCAGTTTTTCA	G	
	3.3.1_3.3_F	GCGAAATCGTTCCCGGAAAAA	G	
	3.3.1_3.3_R	TCCATCGGAAAGCCTTCGTAA	G	
	4.3.1.1_F	TCTGGCCTGATACCTGGATGT	G	
	4.3.1.1_R	CGATCGGATATCCAGCACCA	G	
	gyrA_F	TGACGCCTTCTTCGTACTCAC	G	
	gyrA_R	CTGAAGCTGATCGCCGATAAAC	G	
	parC_F	GCCAGACGACCGAACATATGT	G	
	parC_R	TTCCTCGCAACCTGTCTCAC	G	
	4.3.1.2.1.1_4_F	GTCAGGCCTGGTTTGACAATC	G	
	4.3.1.2.1.1_4_R	CCTGTGAACTAACCCCTGCA	G	
	2.3.2_F	GACGATAAACCGCTTCCGTCA	G	
	2.3.2_R	AGCCGGGTACAGTAGTCCAA	G	

Primer preparation

1. Reconstitute primers to 100 µM using nuclease-free water

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

2 Workstation Preparation

Clean the PCR areas before setting up the reactions; clean room for the master mix and an area for the addition of the template.

- Clean the working area and pipettors with an approved DNAse inhibitor solution.
- Clean the Class II biosafety cabinet and pipettes with 70% ethanol and wipe with a paper towel.

Place a disposal Dispo-safe “sweetie” jar or bio-bin on the bench.

3 Sample Retrieval

Place a rack inside a polystyrene sample box for sample transfer.
Remove tube containing the required samples from the -80 °C freezer.
Close freezer and polystyrene box.
Allow the samples to thaw on ice.

4 Round 1 PCR reaction

Prepare the following Master mix in a 1.5ml Eppendorf Lobind tube:

	1 Reaction (µL)	Reactions
DreamTaq 2x master mix	12.5	µL
Water	6.5	µL
Primer pool G	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 extracted DNA from each sample to a tube, mix well by pipette and label accordingly.
Briefly centrifuge down the PCR mixes and store on ice.

5 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

	A	B	C	D
		Annealing	56	30 seconds
		Extension	72	3 minutes
	1	Final Extension	72	10 minutes
	-	Hold	10	-

6 **Round 2 PCR reaction**

Prepare the following Master mix in a 1.5ml Eppendorf Lobind tube:

		1 Reaction (µL)	Reactions
	DreamTaq 2x master mix	12.5	µL
	Water	6.5	µL
	Primer pool B	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 extracted DNA from each sample to a tube, mix well by pipette and label accordingly.
Briefly centrifuge down the PCR mixes and store on ice.

7 **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	-

8 **Gel electrophoresis bands**

		First round amplicon length (bp)	Second round amplicon length (bp)
	3.1.1.	2991	263

4.3.1.2	2725	398
tviD	843	238

Protocol references

All primers were designed by Dr Anton Spadar, unpublished.