

Multiplex PCR Procedure for Genotyping *Clostridium perfringens*

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Materials:

Oligos for the major toxin genes of *Clostridium perfringens*:

cpa GCTAATGTTACTGCCGTTGA
CCTCTGATACATCGTGTAAG

cpb GCGAATATGCTGAATCATCTA
GCAGGAACATTAGTATATCTTC

etx GCGGTGATATCCATCTATTC
CCACTTACTTGTCCTACTAAC

iA ACTACTCTCAGACAAGACAG
CTTTCCTTCTATTACTATACG

cpe GGAGATGGTTGGATATTAGG
GGACCAGCAGTTGTAGATA

cpb2 AGATTTTAAATATGATCCTAACC
CAATACCCTTCACCAAATACTC

Each 50 μ l reaction contains 10x Assay Buffer A [100mM Tris-HCl, pH8.3 (at 25 $^{\circ}$ C); 500mM KCl; 15mM MgCl₂; 0.01%(w/v) gelatin] (Fisher), 0.12mM dNTPs, 0.34 μ M of each *cpe* oligo, 0.36 μ M of each *cpb* oligo, 0.36 μ M of each *cpb2* oligo, 0.44 μ M of each *etx* oligo, 0.5 μ M of each *cpa* oligo, 0.52 μ M of each *iA* oligo, and 5 units of Taq DNA polymerase(Fisher).

	<u>Volume (μl) per 50μl reaction</u>
10x Assay Buffer (Fisher)	5.0
dNTPs (5.0mM)	1.2
MgCl ₂ (25.0mM)	1.0
oligos:	
<i>iA</i>	0.65
<i>cpa</i>	0.63
<i>etx</i>	0.55
<i>cpb</i>	0.45

<i>cpe</i>	0.425
<i>cpb2</i>	0.45
deionized water	28.55
Taq DNA polymerase (5 units/ μ l)	1.0

The volume of each primer set listed refers to a mix of the forward and reverse primers. I resuspended each primer to 40.0 μ M and then mix equal amounts of the forward and reverse primers together. I did this to save time when setting up the reaction.

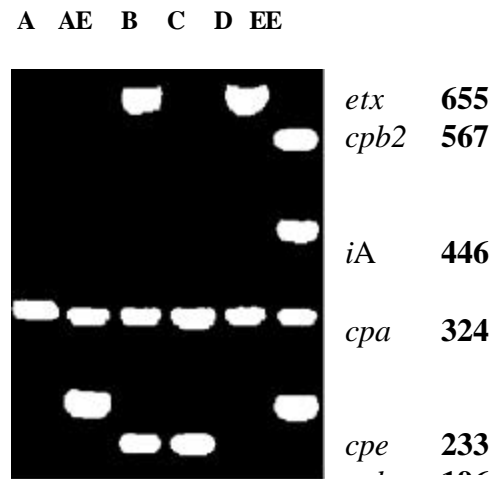
PCR Method:

1. Make up a cocktail containing all PCR reagents, except Taq, for the number of samples being tested. Include positive and negative controls.
2. Incubate on ice for 30-60 minutes. During this time prepare the template (see below).
3. Add Taq to the cocktail, vortex and centrifuge briefly. Aliquot 40 μ l of cocktail to each 0.5ml PCR tube. Place tubes back on ice.
4. Add 10 μ l of supernatant from boiled cells to each 50 μ l PCR reaction. If using genomic DNA as template, use 2 μ l.
5. Overlay each reaction with two drops of mineral oil and process in a Perkin Elmer DNA Thermal Cycler for 35 cycles (1 min @ 94 $^{\circ}$ C, 1 min @ 55 $^{\circ}$ C, 1 min @ 72 $^{\circ}$ C).
6. Use 15 μ l of PCR product from each sample for electrophoresis in a 1.5% TAE-agarose gel stained with EtBr.

Template:

1. Resuspend a single colony from solid medium into 200 μ l of HPLC grade water in a 1.5ml Eppendorf tube. If field samples are being tested, mixed cultures of *C. perfringens* can be used to make the template. I use a type B and a type EE strain as positive controls and water as a negative control.
2. Boil for 20 minutes.
3. Centrifuge for 5 minutes in a microcentrifuge.
4. The supernatant is used as template.

Typical Results:



After electrophoresis, isolates of a given phenotype will appear as shown. Note that the type EE strain is positive for *cpb2*.

References:

Alpha toxin: Titball RW et al. 1989. Molecular cloning and nucleotide sequence of the alpha-toxin (phospholipase C) of *Clostridium perfringens*. *Infection and Immunity*, **57**:367-376.

Beta toxin: Hunter S.E.C. et al., 1993. Molecular genetic analysis of beta-toxin of *Clostridium perfringens* reveals sequence homology with alpha-toxin, gamma-toxin, and leukocidin of *Staphylococcus aureus*. *Infection and Immunity*, **61**:3958-3965.

Epsilon toxin: Hunter S.E.C. et al., 1992. Cloning and nucleotide sequencing of *Clostridium perfringens* epsilon-toxin gene and its expression in *Escherichia coli*. *Infection and Immunity*, **60**:102-110.

Iota toxin: Perelle S. et al., 1993. Characterization of *Clostridium perfringens* iota-toxin genes and expression in *Escherichia coli*. *Infection and Immunity*, **61**:5147-56.

Enterotoxin: Czeczulin J. et al., 1993. Cloning, nucleotide sequencing, and expression of the *Clostridium perfringens* enterotoxin gene in *Escherichia coli*. *Infection and Immunity*, **61**:3429-3439.

Beta2 toxin: Gibert M. et al., 1997. Beta2 toxin, a novel toxin produced by *Clostridium perfringens*. *Gene*, **203**:65-73.