DEC Primer Mix
Primer Mix for PCR detection of diarrhoeagenic *E. coli* (DEC)

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Application
The DEC Primer Mix is to be used for PCR detection of diarrhoeagenic *E. coli* (DEC).

Description
The primer mix contains 8 primer pairs directed towards the following genes: intimin (*eae*), verocytotoxin 1 (*vtx1*), verocytotoxin 2 (*vtx2*), heat stable enterotoxin, human variant (*estA-human*), heat stable enterotoxin, porcine variant (*estA-porcine*), heat labile enterotoxin (*eltA*), invasive plasmid antigen (*ipaH*) and *16S* rDNA (positive internal control). All primers are synthetic single-stranded oligonucleotides with free 5'- and 3'- hydroxyl ends. The individual concentration of each primer pair is adjusted for optimal performance in the multiplex PCR. The primer mix contains 520 µL enough to perform 100 PCR tests using 4 µL each.

Two control DNA sets are included in the package. Control 1 consists of purified DNA from a non-pathogenic *E. coli* plus DNA from a strain with the *eae*, *vtx1* and *vtx2* genes. Control 2 contains a mixture of purified DNA from a non-pathogenic *E. coli* strain, a strain with the *ipaH* gene and a strain with the *eltA* and *estA* genes. Each control DNA vial contains 1 mL corresponding to at least 100 PCR tests using 8 µL each.

Background
The most important DECs are: verocytotoxin producing *E. coli* (VTEC), enteropathogenic *E. coli* (EPEC), attaching and effacing *E. coli* (A/EEC), enterotoxigenic *E. coli* (ETEC) and enteroinvasive *E. coli* (EIEC)[1]. The genes used for identification of the particular DEC and the amplicon sizes are listed below.

Gene	DEC	Amplicon size (bp)
16S rDNA[a]	-	1062
ipaH[b]	EIEC	647
eltA	ETEC	479
vtx2	VTEC	420
eae	EPEC, A/EEC[c]	377
vtx1[d]	VTEC	260
estA-porcine[e]	ETEC	160
estA-human[e]	ETEC	151

a) Amplifies a fragment from most Gram-negative bacteria, allowing an evaluation of the PCR.
b) Might also be present in *Shigella* spp.
c) EPEC and A/EEC distinction is based on serotype.
d) Might also be present in *Shigella dysenteriae I.*
e) Amplicon sizes cannot be differentiated under standard agarose gelelectrophoresis.

Materials required but not provided
Other reagents may be used, but as the thermocycler conditions are optimized with the listed reagents, changes may require additional optimization. Thermocyclers from MJ Research Tetrad 2, Applied Biosystems 9600 and 9700 have been tested.

TE-buffer (10mM Tris-HCl, 1mM EDTA, pH 8)
10% Chelex-100 in TE-buffer
PCR grade water
10 x PCR buffer (200 mM Tris-HCl (pH 8.4), 500 mM KCl)
50 mM $MgCl_2$
dNTP-mix (each 1.25 mM)
Platinum® *Taq* Polymerase 5 U/µl (*Invitrogen*)
1.5 – 2.0% agarose gel
Loading buffer
DNA marker

Procedure
1. If running only a few PCRs at a time, dispense the primer mix into several aliquots and use only one at a time.
2. Pick up to 10 plate-grown colonies and suspend them in a tube containing 200 µL 10% Chelex-100 dissolved in TE-buffer.
3. Boil the suspension for 5 min. and centrifuge briefly (5 min. at app. 2200 x *g*).
4. Dilute 15 µL of supernatant in 100 µL of 1 x TE-buffer and use 8 µL directly in the PCR (other commercial DNA extraction methods may also be used).
5. Sample preparation and PCR setup should be performed in dedicated areas free of possible contamination. Prepare the total mastermix for the number of samples to be run and dispense 31.6 µL of the mixture in each tube. Each tube has to contain the following amount of reagents:

Mastermix:	
PCR grade water	11.1 µL
10 x PCR buffer	5.0 µL
50 mM Mg Cl_2	3.5 µL
dNTP-mix (each 1.25 mM)	8.0 µL
Primer Mix	4.0 µL
Total	31.6 µL

6. Add 8 µL of template DNA (sample or control) to each tube and mix.
7. At last add 0.40 µL Platinum® *Taq* Polymerase 5 U/µL to each tube and mix.
8. Run the PCR amplification in a thermocycler at the following conditions.

Step	Temp. (°C)	Time
Initial denaturation	95	2 min
35 cycles of:		
Denaturation	94	50 sec
Annealing	62	40 sec
Extension	72	50 sec
Final extension	72	3 min

9. Run 5 – 15 µL of each completed PCR in separate wells on an agarose gel (1.5-2.0%) capable of separating the particular amplicon sizes.

Interpretation of the analysis results
The figure below shows the PCR results containing a variety of different templates compared to a 100 bp DNA marker.

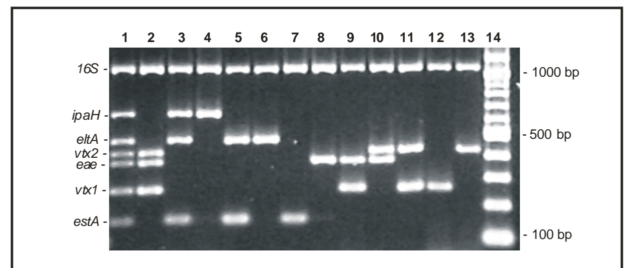

Figure. Analysis of 13 different templates containing: lane 1: *estA, vtx1*, *eae*, *vtx2*, *eltA* and *ipaH*, lane 2: *vtx1*, *vtx2* and *eae*, lane 3: *estA*, *eltA* and *ipaH*, lane 4: *ipaH*, lane 5: *estA* and *eltA*, lane 6: *eltA*, lane 7: *estA*, lane 8: *eae*, lane 9: *vtx1*and *eae*, lane 10: *vtx2* and *eae*, lane 11: *vtx1* and *vtx2*, lane 12: *vtx1*, lane 13: *vtx2* and lane 14: 100 bp DNA marker.

Specificity
A total of 142 reference strains from "The International *Escherichia* and *Klebsiella* Centre (WHO)", Statens Serum Institut, Denmark were tested by the multiplex PCR method. The strains represent a broad variety of different serotypes and virulence gene combinations. Compared to a DNA hybridisation technique targeting the same genes, the PCR method was 100% specific. Also tested were 13 non-*E. coli* species, all of which only produced the *16S* rDNA control band.

Storage and shelf life
The DEC Primer Mix and the DNA controls should be stored at ÷20 ºC. Dispense the primer mix and the DNA controls into several aliquots and store the unused aliquots at ÷20°C while storing the aliquots in use at 4-8° C. The aliquots stored at 4-8° C are stable up to 2 weeks after preparation. The expiry date of the kit is printed on the label.

References
1. Nataro, J. P. and J. B. Kaper. 1998. Diarrhoeagenic Escherichia coli. Clin. Microbiol Rev. 11:142-201.

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