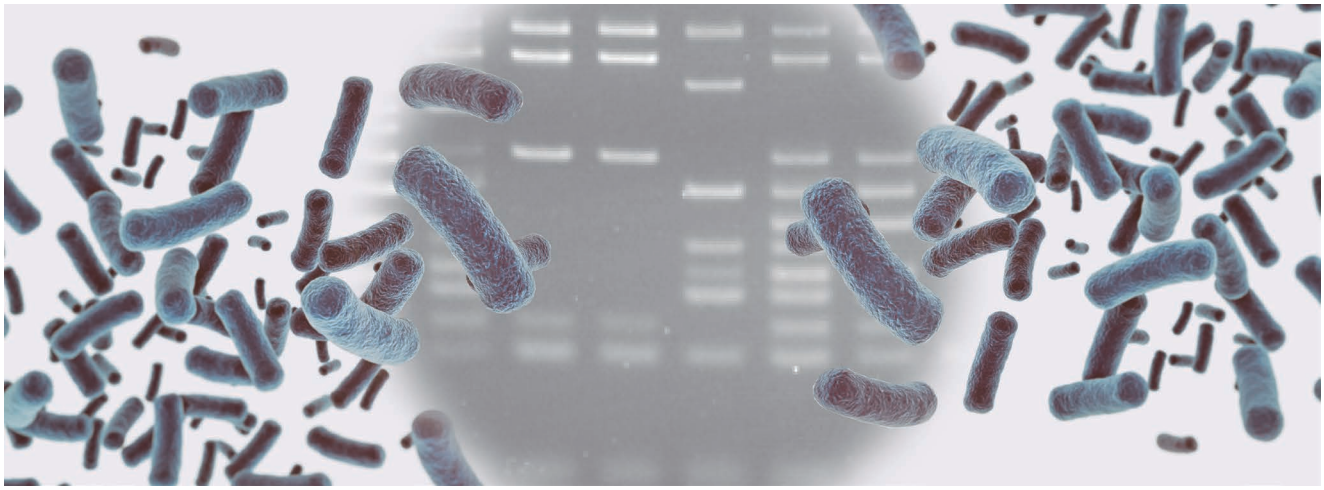
RUO

Cica Geneus™ *E. cloacae* complex POT KIT



The PCR-based ORF typing（POT） is a molecular epidemiological method to amplify plural specific genes by multiplex PCR at the same time, for not only investigations of outbreaks but also for non-outbreak epidemiological surveillance.
Enterobacter cloacae complex (ECC) is a generic name for closely related species of *E. cloacae*, some of which are problematic as Carbapenemase-Producing Enterobacteria (CPE). This kit is intended for ECC and can be used to estimate the species of *E. hormaechei* and *E. asburiae*, identify *E. hormaechei* strains and estimate their ST types, and perform simple ST type estimation of some *E. asburiae*.

*This kit was developed using the results of joint research between Fujita Medical College and Kanto Chemical Life Science Institute.

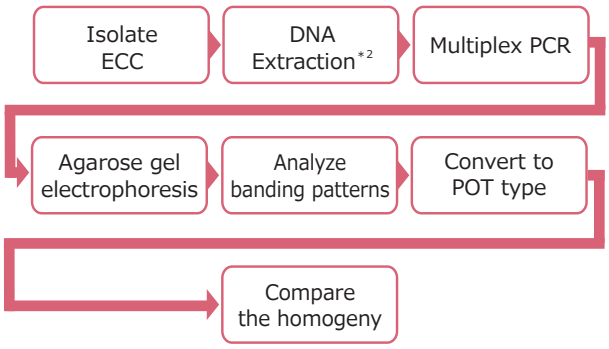
Characteristics

- This POT method can compare the homogeny between strains by digitizing (replace to POT types) an electrocataphoresis result.
- Required for the examination time is approximately 4 hours.
- Nearly the same discriminability as the Multi locus sequencing typing(MLST) in *E. hormaechei*.

Composition (30 rxns)

Reagent		Volume
A	AptaTaq DNA Master (5×Conc.) *1	240μL ×1
B	PCR supplement	240μL ×1
C	Primer mix α	120μL ×1
D	Primer mix β	120μL ×1
E	Positive control	240μL ×1
F	Loading buffer (6xConc.)	240μL ×1

Work Frow



*1 AptaTaq DNA Master（5×Conc.） is the product of Roche Diagnostics K.K.
*2 CicaGeneus® DNA extraction reagent is sold separately.

Kanto Chemical

Analysis example

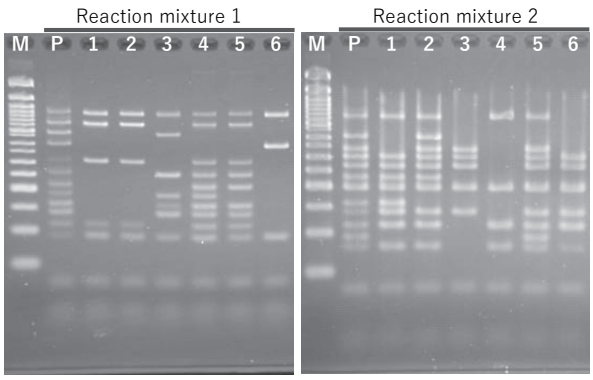


Fig.1 POT electrophoresis pattern
12 primer sets were included in each reaction mixture.
The ORFs detected and size of the amplicon in each reaction mixture are represented in the right column.

M: 50 bp DNA Ladder, P: Positive control (Reagent E),
1-6: Clinical isolate strains of *Enterobacter* sp.

Table.1 Kinds of detection ORF and Amplicon size

	POT No.	Amplicon size (bp)	POT coefficient	Target domain
Reaction mixture 1	PCR PC	668	-	ECC positive control
	E. hormaechei PC	562	1000	*E. hormaechei* positive control
	E. asburiae like 1 PC	477	2000	*E. asburiae* like 1 positive control
	E. asburiae like 2 PC	399	3000	*E. asburiae* like 2 positive control
	POT1-1	313	128	Genomic island-1
	POT1-2	261	64	Genomic island-2, 2a
	POT1-3	216	32	Genomic island-3, 3a
	POT1-4	187	16	Genomic islet-1
	POT1-5	163	8	Genomic island-4
	POT1-6	141	4	Genomic island-5, 5a
	POT1-7	116	2	Genomic island-6
	POT1-8	93	1	bla_{IMP-1} group
Reaction mixture 2	*E. hormaechei* PC	562	-	*E. hormaechei* positive control
	POT2-1	410	1024	Genomic island-7
	POT2-2	344	512	Genomic islet-2
	POT2-3	312	256	Genomic island-8
	POT2-4	274	128	Genomic island-9
	POT2-5	234	64	Genomic island-10
	POT2-6	202	32	Genomic islet-3
	POT2-7	163	16	Genomic island-11
	POT2-8	144	8	Genomic island-12
	POT2-9	118	4	Genomic island-13
	POT2-10	97	2	Genomic island-14
	POT2-11	82	1	Genomic island-15

① Distribution patterns of ORFs are identified by reading electrophoresis banding patterns (Fig.1).
② POT types of two categories are obtained by input electrophoresis banding patterns into the Excel calculation sheet which can be downloaded from Kanto Chemical Co. Inc web site.
i When PCR PC is positive, the sample can confirm that it is *Enterobacter cloacae* complex.
ii The value of POT 1 of *E. hormaechei* become 1000-1999, *E. asburiae* like 1 (Some *E. asburiae* and some *E. roggenkampii*) become 2000-2999, *E. asburiae* like 2 (*Enterobacter* species close to *E. asburiae* and some *E. roggenkampii*) become over 3000, other ECC bocome less than 1000.
iii If the value of POT-1 become off number, the strain has the gene of blaIMP-1 group.
iv By comparing POT types, you can objectively estimate homology between strains..
v As for the strains obtained from outbreaks, all POT types (1,2) become same.
*For *E. asburiae* like 1, only POT1 which is the sum of POT1-2, 1-3, 1-6, and 1-8, is calculated; for *E. asburiae* like 2, only POT1-8 is calculated.

Result

	Sample number in Fig.1					
	1	2	3	4	5	6
POT1	1131	1131	2069	1255	1239	3001
POT2	509	2029	–	37	943	–

Product information

Product No.	Packaging size	Stored at	Product No.
CicaGeneus™ *E. cloacae* complex POT KIT	30 rxns	-25～-20 ℃	08376-97
CicaGeneus™ DNA Extraction Reagent	120 rxns	2 ℃～8 ℃	08178-96

Other POT KIT Seriese

Product No.	Packaging size	Product No.
CicaGeneus™ Staph POT KIT	120 rxns	08180-96
	30 rxns	08180-97
Cica Geneus™ Pseudo POT KIT	50 rxns	08187-96
Cica Geneus™ Acineto POT KIT	30 rxns	08062-96

Product No.	Packaging size	Product No.
Cica Geneus™ E.coli POT KIT	30 rxns	08362-97
Cica Geneus™ C. diff POT KIT	30 rxns	08106-97

★Please contact our sales office or distributors for other necessary equipment and reagents.
★This kit is manufactured under patent license from Fujita Medical College. For information on licenses for other manufacturers' products, please contact the respective manufacturers.

- Please use the products listed in the catalog as reagents (chemicals used for testing or research purpose).
- Product information is subject to change without notice. For the latest information, please have a look at our website "Cica-Web".

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