Cica Geneus™ Staph POT KIT

Kanto Reagents

Methicillin-resistant *Staphylococcus aureus* (MRSA) is pathogens of the hospital-acquired infections, and it is necessary to take appropriate diffusion precaution. The PCR-based ORF Typing(POT)*[1] developed by Dr.Suzuki of Aichi Prefectural Institute of Public Health is a method to amplify plural specific genes by Multiplex PCR at the same time, and then to analyze agarose gel electrophoresis banding patterns.

References

1. Suzuki.M et.al. *J.Appl.Microbiol.* **101(4)**:938-947 (2006)
2. Suzuki.M et.al. *J.Appl.Microbiol.* **107(4)**:1367-1374 (2009)

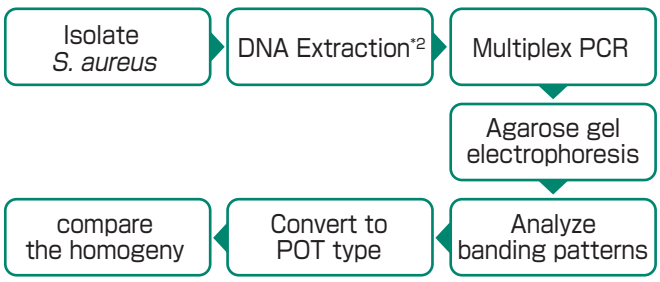
Characteristics

- Required time is approximately 4 hours.
- Nearly the same discriminability as the pulsed field gel electrophoresis (PFGE).
- This POT kit can compare the homogeny between strains by digitizing (POT type) an analysis result.

■ Composition (120 rxns)

Reagent		Volume
A	AptaTaq DNA Master(5×Conc.)*1	1.0 mL×1
B	PCR supplement	1.0 mL×1
C	Primer mix α	0.5 mL×1
D	Primer mix β	0.5 mL×1
E	Positive control	1.0 mL×1
F	Loading buffer(6×Conc.)	1.0 mL×1

■ Work Flow



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

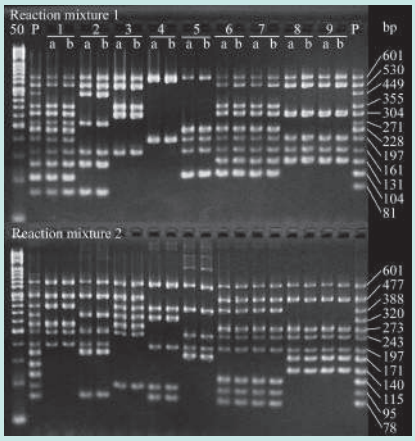


Fig.1 Example of the electrophoretic patterns

50 : 50 bp DNA Ladder, P : Positive control
1 : ATCC™ 700699 (MRSA Mu50)
2 : ATCC™ BAA-1556 (MRSA USA300)
3 : ATCC™ 43300 (MRSA)
4 : ATCC™ 25923 (MSSA)
5 : ATCC™ 29213 (MSSA)
6,7 : Clinical isolate from the outbreaks example1
8,9 : Clinical isolate from the outbreaks example2

Table.1 Kind of detection ORF and Amplicon size

	POT No.	Amplicon size (bp)	Target domain
Reaction mixture 1	*femA*	601	*S. aureus* positive control
	POT 1-1	530	*mecA*
	POT 1-2	449	*mec* gene complex class B
	POT 1-3	355	SCC*mec* type IIa specific
	POT 2-1	304	Tn*554*
	POT 2-2	271	Propharge
	POT 2-3	228	Propharge
	POT 2-4	197	Propharge
	POT 2-5	161	Propharge
	POT 2-6	131	Propharge
	POT 2-7	106	Propharge
	POT 2-8	81	Genomic Island
Reaction mixture 2	*femA*	601	*S. aureus* positive control
	POT 1-4	477	cassette chromosome recombinase A2
	POT 1-5	388	Genetic back ground
	POT 1-6	320	Genetic back ground
	POT 1-7	273	*mec* gene complex class A
	POT 3-1	243	Propharge
	POT 3-2	197	Propharge
	POT 3-3	171	Propharge
	POT 3-4	140	Propharge
	POT 3-5	115	Propharge
	POT 3-6	95	Propharge
	POT 3-7	78	Propharge

① Read banding patterns (Table.1) from an electrophoretic result (Fig.1).
② Using a result of having this electrophoretic band or not, you input it into a calculation sheet for POT type analysis by the binary scale and calculate a POT type of three categories.
i When *femA* is positive, the sample can confirm that it is *staphylococcus aureus,* and In the case of MRSA, the values of POT1 become more than 64.
ii You can suppose the homogeny between strains objectively by comparing the POT type.
iii As for the strains obtained from outbreaks, all POT types (1-3) become same. (6&7、8&9 in Fig.1)

Result

Category	Sample number in Fig.1								
	1	2	3	4	5	6	7	8	9
POT1	93	106	95	6	4	93	93	73	73
POT2	255	77	136	16	58	190	190	156	156
POT3	96	113	66	35	112	103	103	120	120

Product Information

Product No.	Product Name	Package size	Stored at
08180-96	CicaGeneus™ Staph POT KIT	120 rxns	-20 ℃~-25 ℃
08178-96	CicaGeneus™ DNA Extraction Reagent	120 rxns	2 ℃~8 ℃

Multiplex PCR kit series (stored at -20 ℃ ~ -25 ℃)

Product No.	Product Name	Package size
08187-96	CicaGeneus™ Pseudo POT KIT	50 rxns
08062-96	CicaGeneus™ Acineto POT KIT	30 rxns
08087-96	CicaGeneus™ Pathogenesis Gene Detection PCR Kit(for DEC)	100 rxns
08179-96	CicaGeneus™ Staph coaglase detection set	50 rxns
08081-96	CicaGeneus™ Bovine Mycoplasma HI screening kit	100 rxns

Related reagents

Product No.	Product Name	Package size
46510-79	10×TBE buffer	1 L
46509-79	10×TAE buffer	1 L
14575-43	Ethidium bromide solution	10 mL
01089-23	Agarose KANTO HC	100 g
01016-96	AptaTaq DNA Master(5×Conc.)	500 μL

●The example data of the electrophoretic pattern is provided by Dr.Suzuki of Aichi Prefectural Institute of Public Health.
●This product is obtaining the patent licensing from Aichi Prefecture.

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