

1 **Supplementary Information**

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Melting Temperature Mapping Method: A Novel Method for Rapid Identification of Unknown Pathogenic Microorganisms within Three Hours of Sample Collection

Hideki Niimi^{1*}, Tomohiro Ueno¹, Shirou Hayashi¹, Akihito Abe², Takahiro Tsurue², Masashi Mori³, Homare Tabata⁴, Hiroshi Minami⁴, Michihiko Goto⁵, Makoto Akiyama⁶, Yoshihiro Yamamoto⁷, Shigeru Saito⁸ and Isao Kitajima^{1*}

¹Clinical Laboratory Center, Toyama University Hospital, Toyama 930-0194, Japan, ²Kitami Information Technology Co., Ltd., Hokkaido 090-0813, Japan, ³Research Institute for Bioresources and Biotechnology, Ishikawa Prefectural University, Ishikawa 921-8836, Japan, ⁴Life Science Center, Hokkaido Mitsui Chemicals, Inc., Hokkaido 073-0138, Japan, ⁵Department of Internal Medicine, University of Iowa Carver College of Medicine, IA 52242, USA, and Iowa City Veterans Affairs Medical Center, IA 52246, USA, ⁶Nagaresugi Geriatric Hospital, Toyama 939-8032, Japan, ⁷Department of Clinical Infectious Diseases, Toyama University Hospital, Toyama 930-0194, Japan, ⁸Department of Obstetrics & Gynecology, Toyama University Hospital, Toyama 930-0194, Japan

*Correspondence to:

Hideki Niimi, M.D. Ph.D. E-mail address: hiniimi@med.u-toyama.ac.jp

Isao Kitajima, M.D. Ph.D. E-mail address: kitajima@med.u-toyama.ac.jp

Clinical Laboratory Center, Toyama University Hospital, 2630 Sugitani, Toyama 930-0194, JAPAN

Phone: (+81)-76-434-7759, Fax: (+81)-76-434-7759

1 **Supplemental Table S1**2 **The seven universal primers binding to the bacterial target regions registered in the database**

Bacteria in the database	Primer bindings						
	Reg. 1	Reg. 2	Reg. 3	Reg. 4	Reg. 5	Reg. 6	Reg. 7
<i>Achromobacter xylosoxidans</i>	+	+	+	+	+	+	+
<i>Acinetobacter baumannii</i>	+	+	+	+	+	+	+
<i>Acinetobacter calcoaceticus</i>	+	+	+	+	+	+	+
<i>Aerococcus christensenii</i>	+	+	+	+	+	+	+
<i>Aeromonas hydrophilia</i>	+	+	+	+	+	+	+
<i>Alistipes onderdonkii</i>	+	+	+	+	+	-	+
<i>Anaeroglobus geminatus</i>	+	+	+	+	+	+	+
<i>Arthrobacter cumminsii</i>	+	+	+	+	+	+	+
<i>Atopobium vaginae</i>	+	+	+	+	+	+	+
<i>Bacillus cereus</i>	+	+	+	+	+	+	+
<i>Bacteroides dorei</i>	+	+	+	+	+	-	+
<i>Bacteroides finegoldii</i>	+	+	+	+	+	-	+
<i>Bacteroides fragilis</i>	+	+	+	+	+	-	+
<i>Bacteroides nordii</i>	+	+	+	+	+	-	+
<i>Bacteroides salyersiae</i>	+	+	+	+	+	-	+
<i>Bacteroides thetaiotaomicron</i>	+	+	+	+	+	-	+
<i>Bacteroides uniformis</i>	+	+	+	+	+	-	+
<i>Bacteroides vulgatus</i>	+	+	+	+	+	-	+
<i>Bifidobacterium bifidum</i>	+	+	+	+	+	+	+
<i>Bifidobacterium breve</i>	+	+	+	+	+	+	+
<i>Bilophila wadsworthia</i>	+	+	+	+	+	+	+
<i>Campylobacter curvus</i>	+	+	+	+	+	+	+
<i>Campylobacter jejuni</i>	+	+	+	+	+	+	+
<i>Campylobacter rectus</i>	+	+	+	+	+	+	+
<i>Citrobacter freundii</i>	+	+	+	+	+	+	+
<i>Clostridium difficile</i>	+	+	+	+	+	+	+
<i>Clostridium hylemonae</i>	+	+	+	+	+	+	+
<i>Clostridium perfringens</i>	+	+	+	+	+	+	+
<i>Clostridium tertium</i>	+	+	+	+	+	+	+
<i>Corynebacterium amycolatum</i>	+	+	+	+	+	+	+
<i>Corynebacterium macginleyi</i>	+	+	+	+	+	+	+
<i>Corynebacterium xerosis</i>	+	+	+	+	+	+	+
<i>Eggerthella lenta</i>	+	+	+	+	+	+	+
<i>Eikenella corrodens</i>	+	+	+	+	+	+	+
<i>Enterobacter aerogenes</i>	+	+	+	+	+	+	+
<i>Enterobacter cloacae</i>	+	+	+	+	+	+	+
<i>Enterococcus avium</i>	+	+	+	+	+	+	+
<i>Enterococcus casseliflavus</i>	+	+	+	+	+	+	+
<i>Enterococcus faecalis</i>	+	+	+	+	+	+	+
<i>Enterococcus faecium</i>	+	+	+	+	+	+	+
<i>Escherichia albertii</i>	+	+	+	+	+	+	+
<i>Escherichia coli</i>	+	+	+	+	+	+	+
<i>Eubacterium lentum</i>	+	+	+	-	+	+	+
<i>Eubacterium limosum</i>	+	+	+	+	+	+	+
<i>Finegoldia magna</i>	+	+	+	+	+	+	+
<i>Fusobacterium nucleatum</i>	+	+	+	+	+	+	+
<i>Fusobacterium periodonticum</i>	+	+	+	+	+	+	+
<i>Gardnerella vaginalis</i>	+	+	+	+	+	+	+
<i>Gemella morbillorum</i>	+	+	+	+	+	+	+
<i>Granulicatella adiacens</i>	+	+	+	+	+	+	+
<i>Haemophilus influenzae</i>	+	+	+	+	+	+	+
<i>Halomonas venusta</i>	+	+	+	+	+	+	+
<i>Klebsiella oxytoca</i>	+	+	+	+	+	+	+
<i>Klebsiella pneumoniae</i>	+	+	+	+	+	+	+
<i>Listeria monocytogenes</i>	+	+	+	+	+	+	+
<i>Lactobacillus crispatus</i>	+	+	+	+	+	+	+
<i>Lactobacillus jensenii</i>	+	+	+	+	+	+	+
<i>Lactococcus garvieae</i>	+	+	+	+	+	+	+

Bacteria in the database	Primer bindings						
	Reg. 1	Reg. 2	Reg. 3	Reg. 4	Reg. 5	Reg. 6	Reg. 7
<i>Morganella morganii</i>	+	+	+	+	+	+	+
<i>Mycoplasma genitalium</i>	+	+	+	+	-	+	+
<i>Mycoplasma hominis</i>	+	+	+	+	-	-	+
<i>Odoribacter splanchnicus</i>	+	+	+	+	+	-	+
<i>Pantoea agglomerans</i>	+	+	+	+	+	+	+
<i>Parabacteroides distasonis</i>	+	+	+	+	+	-	+
<i>Parvimonas micra</i>	+	+	+	+	+	+	+
<i>Pasteurella multocida</i>	+	+	+	+	+	+	+
<i>Peptoniphilus gorbachii</i>	+	+	+	-	+	+	+
<i>Peptostreptococcus anaerobius</i>	+	+	+	+	+	+	+
<i>Porphyromonas gingivalis</i>	+	+	+	+	+	-	+
<i>Prevotella bivia</i>	+	+	+	+	+	-	+
<i>Prevotella intermedia</i>	+	+	+	+	+	-	+
<i>Prevotella melaninogenica</i>	+	+	+	+	+	-	+
<i>Prevotella nigrescens</i>	+	+	+	+	+	-	+
<i>Propionibacterium acnes</i>	+	+	+	-	+	+	+
<i>Propionibacterium avidum</i>	+	+	+	+	+	+	+
<i>Proteus mirabilis</i>	+	+	+	+	+	+	+
<i>Proteus vulgaris</i>	+	+	+	+	+	+	+
<i>Providencia rettgeri</i>	+	+	+	+	+	+	+
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+	+	+
<i>Pseudomonas putida</i>	+	+	+	+	+	+	+
<i>Salmonella enterica</i>	+	+	+	+	+	+	+
<i>Serratia marcescens</i>	+	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	+	+	+	+	+	+	+
<i>Staphylococcus capitis/epidermidis</i>	+	+	+	+	+	+	+
<i>Staphylococcus caprae</i>	+	+	+	+	+	+	+
<i>Staphylococcus cohnii</i>	+	+	+	+	+	+	+
<i>Staphylococcus haemolyticus</i>	+	+	+	+	+	+	+
<i>Staphylococcus hominis</i>	+	+	+	+	+	+	+
<i>Staphylococcus lugdunensis</i>	+	+	+	+	+	+	+
<i>Staphylococcus pettenkoferi</i>	+	+	+	+	+	+	+
<i>Staphylococcus schleiferi</i>	+	+	+	+	+	+	+
<i>Staphylococcus warneri</i>	+	+	+	+	+	+	+
<i>Stenotrophomonas maltophilia</i>	+	+	+	+	+	+	+
<i>Streptococcus agalactiae</i>	+	+	+	+	+	+	+
<i>Streptococcus anginosus</i>	+	+	+	+	+	+	+
<i>Streptococcus constellatus</i>	+	+	+	+	+	+	+
<i>Streptococcus dysgalactiae</i>	+	+	+	+	+	+	+
<i>Streptococcus mitis</i>	+	+	+	+	+	+	+
<i>Streptococcus pasteurianus</i>	+	+	+	+	+	+	+
<i>Streptococcus pneumoniae</i>	+	+	+	+	+	+	+
<i>Streptococcus pyogenes</i>	+	+	+	+	+	+	+
<i>Streptococcus salivarius</i>	+	+	+	+	+	+	+
<i>Streptococcus sanguinis</i>	+	+	+	+	+	+	+
<i>Sutterella wadsworthensis</i>	+	+	+	-	+	+	+
<i>Ureaplasma parvum</i>	+	+	+	+	-	+	+
<i>Vagococcus fluvialis</i>	+	+	+	+	+	+	+
<i>Veillonella atypica</i>	+	+	+	+	+	+	+

1

2 +: Primers bind to the bacterial target region

3 -: Primers do not bind or bind very weakly to the bacterial target region

4 Reg. 1 – 7 = Region 1 – 7 primers

1 **Supplemental Table S2**

2 **Low sequence homology between the universal primers and bacterial target regions associated
3 with the unbound primers shown in Table S2**

4

	Forward (5'→3')	Reverse (5'→3')	Difference (bp)	
Region 4 primers	AACAGGATTAGATAACCCTGGTAG	AATTAAACCACATGCTCCACC	Forward	Reverse
<i>Eubacterium lenthum</i>	AACAGGATTAGATAACCCTGGTAG	AATTAA G CCACATGCTCC G CT	0	3
<i>Peptoniphilus gorbachii</i>	AACAGGATTAGATAACCCTGGTAG	AATTAAACCACATGCTCC G CT	0	2
<i>Propionibacterium acnes</i>	AACAGG C TTAGATAACCCTGGTAG	AATTAA T CC G CATGCTCC G CC	1	3
<i>Sutterella wadsworthensis</i>	AACAGGATTAGATAACCCTGGTAG	AATTAA T CCACAT C ATCCACC	0	3
Region 5 primers	TGGTTTAATTCGATGCAACGC	GAGCTGACGACAGCCAT	Forward	Reverse
<i>Mycoplasma genitalium</i>	TTG CTTAATTCGAC GGT ACAC	GAGCTGACGACA ACC AT	6	1
<i>Mycoplasma hominis</i>	TGGTTTAATT TGA AGA TACAC	GAGCTGACGACA ACC AT	5	1
<i>Ureaplasma parvum</i>	TTG CTTAATT TGA CAAT ACAC	GAGCTGACGACA ACC AT	8	1
Region 6 primers	TTGGGTTAACGTCCCAC	CGTCATCCCCACCTTC	Forward	Reverse
<i>Alistipes onderdonkii</i>	T CGGGTTAACGTCCC AT	CGTCATCCCCACCTTC	3	0
<i>Bacteroides dorei</i>	T CGG C TTAAC G CC AT	CGTCATCCCCACCTTC	5	0
<i>Bacteroides finegoldii</i>	T CGG C TTAAC G CC AT	CGTCATCCCCACCTTC	5	0
<i>Bacteroides fragilis</i>	T CGG C TTAAC T GCC AT	CGTCATCCCCACCTTC	6	0
<i>Bacteroides nordii</i>	T CGG C TTAAC T GCC AT	CGTCATCCCCACCTTC	5	0
<i>Bacteroides salyersiae</i>	T CGG C TTAAC T GCC AT	CGTCATCCCCACCTTC	5	0
<i>Bacteroides thetaiotaomicron</i>	T CGG C TTAAC T GCC AT	CGTCATCCCCACCTTC	5	0
<i>Bacteroides uniformis</i>	T CGG C TTAAC T GCC AT	CGTCATCCCCACCTTC	5	0
<i>Bacteroides vulgatus</i>	T CGG C TTAAC T GCC AT	CGTCATCCCCACCTTC	5	0
<i>Mycoplasma hominis</i>	TTTGG T CAAGTCC T GC	CGTCATCCCCACCTTC	3	0
<i>Odoribacter splanchnicus</i>	T CGGGTTAACGTCCC AT	CGTCATCCCC G CCTTC	3	1
<i>Parabacteroides distasonis</i>	T CGG C TTAAC T GCC AT	CGTCATCCCC G CTTC	5	2
<i>Porphyromonas gingivalis</i>	T CGG C TTAAC T GCC AT	CGTCATCC A CACCTTC	5	1
<i>Prevotella bivia</i>	T CGG C TTAAC T GCC AT	CGTCATCCCCACCTTC	5	0
<i>Prevotella intermedia</i>	T CGG C TTAAC T GCC AT	CGTCATCCCCACCTTC	5	0
<i>Prevotella melaninogenica</i>	T CGG C TTAAC T GCC AT	CGTCATCCCCACCTTC	5	0
<i>Prevotella nigrescens</i>	T CGG C TTAAC T GCC AT	CGTCATCCCCACCTTC	5	0

5

1 **Supplemental Table S3 Tm mapping shape similarity (Difference Value: calculated using**
 2 **the registered Tm values) among the 107 species of bacteria in the database.**

3

D: Difference Value

Bacteria in the database	The number of bacteria					Notes; similar bacteria (D ≤ 0.5)
	0 ≤ D ≤ 0.28	0.28 < D ≤ 0.4	0.4 < D ≤ 0.5	0.5 < D ≤ 1.0	1.0 < D	
<i>Achromobacter xylosoxidans</i>	0	0	0	0	106	
<i>Acinetobacter baumannii</i>	0	0	1	0	105	<i>A. calcoaceticus</i> (0.45)
<i>Acinetobacter calcoaceticus</i>	0	0	1	3	102	<i>A. baumannii</i> (0.45)
<i>Aerococcus christensenii</i>	0	0	0	0	106	
<i>Aeromonas hydrophilia</i>	0	0	0	2	104	
<i>Alistipes onderdonkii</i>	0	0	0	0	106	
<i>Anaeroglobus geminatus</i>	0	0	0	0	106	
<i>Arthrobacter cumminsii</i>	0	0	0	4	102	
<i>Atopobium vaginæ</i>	0	0	0	0	106	
<i>Bacillus cereus</i>	0	0	0	3	103	
<i>Bacteroides dorei</i> *	1	0	0	1	104	<i>B. vulgaris</i> (0.26)
<i>Bacteroides finegoldii</i>	0	0	0	2	104	
<i>Bacteroides fragilis</i>	0	0	1	3	102	<i>B. uniformis</i> (0.41)
<i>Bacteroides nordii</i>	0	0	0	3	103	
<i>Bacteroides salyersiae</i>	0	0	0	2	104	
<i>Bacteroides thetaiotaomicron</i>	0	0	0	1	105	
<i>Bacteroides uniformis</i>	0	0	1	2	103	<i>B. fragilis</i> (0.41)
<i>Bacteroides vulgaris</i> *	1	0	0	2	103	<i>B. dorei</i> (0.26)
<i>Bifidobacterium bifidum</i>	0	0	0	0	106	
<i>Bifidobacterium breve</i>	0	0	0	0	106	
<i>Bilophila wadsworthia</i>	0	0	0	0	106	
<i>Campylobacter curvus</i>	0	0	0	0	106	
<i>Campylobacter jejuni</i>	0	0	0	1	105	
<i>Campylobacter rectus</i>	0	0	0	2	104	
<i>Citrobacter freundii</i>	0	0	0	4	102	
<i>Clostridium difficile</i>	0	0	1	0	105	<i>P. anaerobius</i> (0.43)
<i>Clostridium hylemonae</i>	0	0	0	0	106	
<i>Clostridium perfringens</i>	0	0	0	1	105	
<i>Clostridium tertium</i>	0	0	0	1	105	
<i>Corynebacterium amycolatum</i>	0	0	0	0	106	
<i>Corynebacterium macginleyi</i>	0	0	0	0	106	
<i>Corynebacterium xerosis</i>	0	0	0	0	106	
<i>Eggerthella lenta</i>	0	0	0	3	103	
<i>Eikenella corrodens</i>	0	0	0	0	106	
<i>Enterobacter aerogenes</i>	0	0	0	2	104	
<i>Enterobacter cloacae</i>	0	1	0	5	100	<i>P. agglomerans</i> (0.36)
<i>Enterococcus avium</i>	0	0	1	4	101	<i>E. faecium</i> (0.41)
<i>Enterococcus casseliflavus</i>	0	0	0	2	104	
<i>Enterococcus faecalis</i>	0	0	0	2	104	
<i>Enterococcus faecium</i>	0	0	1	3	102	<i>E. avium</i> (0.41)
<i>Escherichia albertii</i>	0	0	0	7	99	
<i>Escherichia coli</i>	0	0	0	9	97	
<i>Eubacterium lentum</i>	0	0	0	0	106	
<i>Eubacterium limosum</i>	0	0	0	0	106	
<i>Finegoldia magna</i>	0	0	0	1	105	
<i>Fusobacterium nucleatum</i>	0	0	1	0	105	<i>F. periodonticum</i> (0.44)
<i>Fusobacterium periodonticum</i>	0	0	1	1	104	<i>F. nucleatum</i> (0.44)
<i>Gardnerella vaginalis</i>	0	0	0	0	106	
<i>Gemella morbillorum</i>	0	0	0	1	105	
<i>Granulicatella adiacens</i>	0	0	0	0	106	
<i>Haemophilus influenzae</i>	0	0	1	8	97	<i>S. caprae</i> (0.43)
<i>Halomonas venusta</i>	0	0	0	0	106	
<i>Klebsiella oxytoca</i>	0	0	0	4	102	
<i>Klebsiella pneumoniae</i>	0	0	0	7	99	
<i>Listeria monocytogenes</i>	0	0	0	0	106	
<i>Lactobacillus crispatus</i>	0	0	0	2	104	
<i>Lactobacillus jensenii</i>	0	0	0	3	103	
<i>Lactococcus garvieae</i>	0	0	0	1	105	
<i>Morganella morganii</i>	0	0	0	8	98	
<i>Mycoplasma genitalium</i>	0	0	0	0	106	
<i>Mycoplasma hominis</i>	0	0	0	0	106	
<i>Pantoea agglomerans</i>	0	1	0	4	101	<i>E. cloacae</i> (0.36)
<i>Odoribacter splanchnicus</i>	0	0	0	0	106	
<i>Parabacteroides distasonis</i>	0	0	0	1	105	
<i>Parvimonas micra</i>	0	0	0	0	106	

4

1

D: Difference Value

Bacteria in the database	The number of bacteria					Notes; similar bacteria ($D \leq 0.5$)
	$0 \leq D \leq 0.28$	$0.28 < D \leq 0.4$	$0.4 < D \leq 0.5$	$0.5 < D \leq 1.0$	$1.0 < D$	
<i>Pasteurella multocida</i>	0	0	0	1	105	
<i>Peptoniphilus gorbachii</i>	0	0	0	0	106	
<i>Peptostreptococcus anaerobius</i>	0	0	1	1	104	<i>C. difficile</i> (0.43)
<i>Porphyromonas gingivalis</i>	0	0	0	0	106	
<i>Prevotella bivia</i>	0	0	0	3	103	
<i>Prevotella intermedia</i>	0	0	0	0	106	
<i>Prevotella melaninogenica</i>	0	0	0	3	103	
<i>Prevotella nigrescens</i>	0	0	0	0	106	
<i>Propionibacterium acnes</i>	0	0	0	0	106	
<i>Propionibacterium avidum</i>	0	0	0	0	106	
<i>Proteus mirabilis</i>	0	0	0	5	101	
<i>Proteus vulgaris</i>	0	0	0	3	103	
<i>Providencia rettgeri</i>	0	0	0	3	103	
<i>Pseudomonas aeruginosa</i>	0	0	0	1	105	
<i>Pseudomonas putida</i>	0	0	0	1	105	
<i>Salmonella enterica</i>	0	0	0	2	104	
<i>Serratia marcescens</i>	0	0	0	11	95	
<i>Staphylococcus aureus</i>	0	1	0	12	93	<i>S. hemolyticus</i> (0.35)
<i>Staphylococcus capitis/epidermidis</i>	0	1	0	7	98	<i>S. pettenkoferi</i> (0.35)
<i>Staphylococcus caprae</i>	0	0	1	10	95	<i>H. influenzae</i> (0.43)
<i>Staphylococcus cohnii</i>	0	0	1	8	97	<i>S. warneri</i> (0.43)
<i>Staphylococcus haemolyticus</i>	0	2	1	12	91	<i>S. lugdunensis</i> (0.32), <i>S. aureus</i> (0.35), <i>S. warneri</i> (0.45)
<i>Staphylococcus hominis</i>	0	0	0	6	100	
<i>Staphylococcus lugdunensis</i>	0	1	1	12	92	<i>S. haemolyticus</i> (0.32), <i>S. warneri</i> (0.43)
<i>Staphylococcus pettenkoferi</i>	0	1	1	8	96	<i>S. capitis/epidermidis</i> (0.35)
<i>Staphylococcus schleiferi</i>	0	0	0	1	105	
<i>Staphylococcus warneri</i>	0	0	3	6	97	<i>S. cohnii</i> (0.43), <i>S. lugdunensis</i> (0.43), <i>S. haemolyticus</i> (0.45)
<i>Stenotrophomonas maltophilia</i>	0	0	0	3	103	
<i>Streptococcus agalactiae</i>	0	0	0	3	103	
<i>Streptococcus anginosus</i>	0	0	0	0	106	
<i>Streptococcus constellatus</i>	0	0	0	4	102	
<i>Streptococcus dysgalactiae</i>	0	0	0	2	104	
<i>Streptococcus mitis</i>	0	0	1	5	100	<i>S. pneumoniae</i> (0.48)
<i>Streptococcus pasteurianus</i>	0	0	0	2	104	
<i>Streptococcus pneumoniae</i>	0	0	1	3	102	<i>S. mitis</i> (0.48)
<i>Streptococcus pyogenes</i>	0	0	0	4	102	
<i>Streptococcus salivarius</i>	0	0	0	1	105	
<i>Streptococcus sanguinis</i>	0	0	0	2	104	
<i>Sutterella wadsworthensis</i>	0	0	0	0	106	
<i>Ureaplasma parvum</i>	0	0	0	0	106	
<i>Vagococcus fluvialis</i>	0	0	0	6	100	
<i>Veillonella atypica</i>	0	0	0	0	106	

2

- 3 This table shows the number of bacterial species registered in the database with a similar shape based
 4 on the Difference Value. In other words, this table shows the specificity of each species of bacteria
 5 based on mutual Tm mapping shape similarity. Each of the Difference Values was calculated using
 6 the Tm values already registered in the database. Bacteria with similar Tm mapping shapes
 7 (Difference Value ≤ 0.28) are shown by asterisks. This similarity does not always interfere with the
 8 Tm mapping identification, as the Difference Value is determined by each of the seven Tm values
 9 (**Fig. S4**). Therefore, *Bacteroides dorei* does not always interfere with the identification of
 10 *Bacteroides vulgatus* and vice versa (**Table S9**).

1 Supplemental Table S4

2 Validation of the measurement errors among three different instruments and trials using the
3 same *E. coli* (ATCC25922) DNA template

4

5 RotorGeneQ (QIAGEN): First instrument

	Triplicate	Reg. 1	Reg. 2	Reg. 3	Reg. 4	Reg. 5	Reg. 6	Reg. 7	Diff.
First Trial	1	89.35	88.60	89.35	89.90	86.25	87.75	87.50	0.21
	2	89.40	88.50	89.40	89.85	86.25	87.75	87.50	0.24
	3	89.35	88.50	89.40	89.90	86.25	87.75	87.50	0.19
Second Trial	1	89.25	88.60	89.40	89.90	86.35	87.75	87.50	0.19
	2	89.40	88.65	89.50	90.00	86.40	87.85	87.60	0.17
	3	89.35	88.65	89.50	90.00	86.35	87.75	87.60	0.13
Third Trial	1	89.35	88.50	89.40	89.85	86.25	87.65	87.40	0.23
	2	89.35	88.50	89.40	89.90	86.25	87.65	87.50	0.19
	3	89.25	88.60	89.50	89.90	86.25	87.75	87.50	0.17

The range of the Difference Values = 0.13 to 0.24

The mean Difference Value = 0.191

8

9 RotorGeneQ (QIAGEN): Second instrument

	Triplicate	Reg. 1	Reg. 2	Reg. 3	Reg. 4	Reg. 5	Reg. 6	Reg. 7	Diff.
First Trial	1	89.35	88.60	89.40	89.90	86.35	87.65	87.50	0.21
	2	89.35	88.60	89.50	90.00	86.40	87.65	87.50	0.13
	3	89.40	88.65	89.50	90.00	86.35	87.75	87.60	0.15
Second Trial	1	89.25	88.50	89.40	89.90	86.35	87.65	87.40	0.16
	2	89.25	88.60	89.50	89.90	86.35	87.75	87.50	0.20
	3	89.35	88.60	89.50	89.90	86.35	87.75	87.60	0.15
Third Trial	1	89.25	88.60	89.50	90.00	86.35	87.65	87.50	0.14
	2	89.35	88.65	89.50	90.00	86.40	87.75	87.50	0.19
	3	89.35	88.65	89.50	89.90	86.35	87.75	87.60	0.15

The range of the Difference Values = 0.13 to 0.21

The mean Difference Value = 0.164

1 LightCycler® Nano (Roche Applied Science)

	Triplicate	Reg. 1	Reg. 2	Reg. 3	Reg. 4	Reg. 5	Reg. 6	Reg. 7	Diff.
First Trial	1	89.46	88.64	89.48	89.93	86.38	87.75	87.63	0.20
	2	89.50	88.62	89.50	89.97	86.38	87.86	87.65	0.21
	3	89.47	88.60	89.47	90.02	86.44	87.88	87.73	0.19
Second Trial	1	89.46	88.64	89.46	89.95	86.33	87.83	87.67	0.17
	2	89.51	88.62	89.49	90.01	86.41	87.80	87.63	0.22
	3	89.47	88.63	89.47	89.99	86.41	87.81	87.72	0.17
Third Trial	1	89.38	88.47	89.32	89.69	86.14	87.57	87.54	0.28
	2	89.45	88.55	89.47	89.93	86.37	87.70	87.58	0.21
	3	89.36	88.57	89.41	89.93	86.34	87.78	87.62	0.14

Range of the Difference Values = 0.14 to 0.28

Mean Difference Value = 0.199

4

5 Reg. 1 – 7 = melting temperatures of the amplicons amplified by the Region 1 – 7 primers

6 Diff. = Difference Value

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1 **Supplemental Table S5**

2 **Range of Difference Values among 15 different trials starting from DNA extraction of the same**

3 ***E. coli* (ATCC25922)**

4

5 **RotorGeneQ (QIAGEN)**

Trials	Reg. 1	Reg. 2	Reg. 3	Reg. 4	Reg. 5	Reg. 6	Reg. 7	Diff.
1 st	89.25	88.50	89.40	89.85	86.35	87.75	87.60	0.16
2 nd	89.50	88.75	89.60	90.00	86.50	87.85	87.75	0.18
3 rd	89.40	88.65	89.50	90.00	86.40	87.75	87.60	0.17
4 th	89.25	88.50	89.25	89.85	86.25	87.65	87.50	0.17
5 th	89.50	88.50	89.50	89.90	86.25	87.75	87.75	0.26
6 th	89.25	88.50	89.40	89.90	86.25	87.75	87.50	0.15
7 th	89.25	88.50	89.40	89.85	86.35	87.65	87.50	0.17
8 th	89.25	88.50	89.35	89.90	86.35	87.75	87.75	0.19
9 th	89.35	88.65	89.50	90.00	86.40	87.75	87.60	0.13
10 th	89.25	88.50	89.40	90.00	86.25	87.75	87.50	0.17
11 th	89.35	88.50	89.35	89.90	86.40	87.75	87.50	0.23
12 th	89.25	88.50	89.35	89.85	86.25	87.65	87.50	0.13
13 th	89.25	88.50	89.40	89.90	86.35	87.75	87.50	0.19
14 th	89.25	88.50	89.50	90.00	86.40	87.75	87.50	0.13
15 th	89.25	88.50	89.50	89.85	86.50	87.65	87.40	0.25

6 Range of the Difference Values = 0.13 to 0.26

7 Mean Difference Value = 0.179

8

9 Reg. 1 – 7 = melting temperatures of the amplicons amplified by the Region 1 – 7 primers

10 Diff. = Difference Value

11

12

1 **Supplemental Table S6**

2 **Validation of limit of identification and limit of detection (LOD) of the Tm mapping method**
 3 **using the same *E. coli* (ATCC25922) DNA template**

4

5 **a) *Escherichia coli* (three independent experiments were performed in triplicate)**

CFU/ PCR Tube	CFU/mL	Tm values							Tm mapping identification results	Difference Value
		Reg. 1	Reg. 2	Reg. 3	Reg. 4	Reg. 5	Reg. 6	Reg. 7		
20.0	1000	89.45	88.70	89.55	90.10	86.20	87.70	87.75	<i>E. coli</i>	0.23
		89.50	88.75	89.65	90.10	86.50	87.75	87.65	<i>E. coli</i>	0.25
		89.85	89.00	89.90	90.25	86.65	88.00	88.10	<i>E. coli</i>	0.26
10.0	500	89.40	88.60	89.50	90.00	86.10	87.65	87.70	<i>E. coli</i>	0.24
		89.50	88.85	89.65	90.15	86.40	87.75	87.75	<i>E. coli</i>	0.22
		89.80	88.90	89.90	90.35	86.65	88.00	88.15	<i>E. coli</i>	0.23
5.00	250	89.40	88.65	89.50	90.00	86.15	87.65	87.60	<i>E. coli</i>	0.23
		89.60	88.80	89.75	90.25	86.50	87.85	87.75	<i>E. coli</i>	0.23
		89.85	89.00	90.00	90.35	86.65	88.00	88.15	<i>E. coli</i>	0.27
2.50	125	89.40	88.70	89.60	90.00	86.25	87.60	87.65	<i>E. coli</i>	0.25
		89.65	88.80	89.75	90.15	86.50	87.80	87.80	<i>E. coli</i>	0.26
		89.80	88.90	89.90	90.25	86.65	88.00	88.15	<i>E. coli</i>	0.25
1.25 ^{*1}	62.5 ^{*1}	89.40	88.65	89.50	89.95	86.10	87.50	87.70	<i>E. coli</i>	0.33
		89.60	88.70	89.65	90.05	86.40	87.65	87.80	<i>E. coli</i>	0.31
		89.85	88.90	89.90	90.25	86.65	87.90	88.15	<i>E. coli</i>	0.34
0.625 ^{*2}	31.3 ^{*2}	N.D.	N.D.	89.70	90.15	N.D.	N.D.	87.70	Not identified	–
		89.70	N.D.	89.65	N.D.	86.50	N.D.	87.75	Not identified	–
		90.00	N.D.	89.90	N.D.	86.75	N.D.	88.15	Not identified	–
0.313	15.6	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Not detected	–	–
		N.D.	N.D.	89.70	N.D.	N.D.	N.D.	87.75	Not identified	–
				90.00	N.D.	N.D.	N.D.	N.D.	Not identified	–

6

*1: limit of identification, *2: limit of detection (LOD)

7

8 Reg. 1 – 7 = melting temperatures of the amplicons amplified by the Region 1 – 7 primers

9 CFU = Colony Forming Unit, N.D. = Not detected

10 The protocol of this validation is described in the methods section under Analytical sensitivity tests.

1 b) *Staphylococcus aureus* (three independent experiments were performed in triplicate)

CFU/ PCR Tube	CFU/mL	Tm values							Tm mapping identification results	Difference Value
		Reg. 1	Reg. 2	Reg. 3	Reg. 4	Reg. 5	Reg. 6	Reg. 7		
20.0	1000	88.30	87.75	88.40	89.00	85.05	86.50	86.00	S. aureus	0.28
		88.25	87.50	88.15	88.75	84.90	86.40	85.75	S. aureus	0.21
		88.50	87.65	88.60	89.00	85.50	86.60	86.15	S. aureus	0.26
10.0	500	88.25	87.65	88.25	88.85	85.10	86.35	85.85	S. aureus	0.23
		88.30	87.65	88.25	88.85	85.10	86.40	85.85	S. aureus	0.21
		88.50	87.75	88.60	89.00	85.40	86.60	86.30	S. aureus	0.26
5.00	250	88.35	87.75	88.35	88.90	85.25	86.40	85.90	S. aureus	0.27
		88.30	87.60	88.25	88.80	85.10	86.35	85.85	S. aureus	0.21
		88.60	87.85	88.70	89.05	85.60	86.70	86.25	S. aureus	0.24
2.50	125	88.40	87.65	88.30	88.90	85.25	86.35	85.90	S. aureus	0.29
		88.30	87.65	88.25	88.80	85.10	86.30	85.85	S. aureus	0.27
		88.50	87.65	88.60	88.90	85.55	86.60	86.15	S. aureus	0.31
1.25 ^{*1}	62.5 ^{*1}	88.40	87.65	88.40	88.90	85.20	86.30	85.90	S. aureus	0.35
		88.30	87.60	88.25	88.80	85.10	86.20	85.85	S. aureus	0.34
		88.60	87.75	88.65	89.00	85.50	86.50	86.25	S. aureus	0.37
0.625 ^{*2}	31.3 ^{*2}	88.40	N.D.	88.45	N.D.	N.D.	N.D.	86.10	Not identified	–
		88.30	N.D.	88.35	N.D.	85.20	N.D.	85.85	Not identified	–
		88.50	N.D.	88.65	N.D.	N.D.	N.D.	86.25	Not identified	–
0.313	15.6	88.40	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Not identified	–
		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Not detected	–
		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Not detected	–

2 *1: limit of identification, *2: limit of detection (LOD)

3 c) *Klebsiella pneumoniae* (three independent experiments were performed in triplicate)

CFU/ PCR Tube	CFU/mL	Tm values							Tm mapping identification results	Difference Value
		Reg. 1	Reg. 2	Reg. 3	Reg. 4	Reg. 5	Reg. 6	Reg. 7		
10.0	500	90.05	89.35	89.95	89.60	86.45	87.45	86.45	K. pneumoniae	0.26
		90.25	89.75	90.15	89.85	86.60	87.50	86.60	K. pneumoniae	0.27
		90.20	89.60	90.20	89.85	86.70	87.70	86.65	K. pneumoniae	0.24
5.00	250	89.95	89.35	89.85	89.60	86.45	87.35	86.35	K. pneumoniae	0.20
		90.20	89.70	90.10	90.05	86.60	87.45	86.55	K. pneumoniae	0.23
		90.25	89.65	90.15	89.95	86.75	87.65	86.65	K. pneumoniae	0.17
2.50	125	89.90	89.50	89.90	89.50	86.40	87.25	86.25	K. pneumoniae	0.30
		90.25	89.80	90.15	89.85	86.50	87.50	86.50	K. pneumoniae	0.32
		90.25	89.75	90.20	89.85	86.70	87.60	86.60	K. pneumoniae	0.23
1.25	62.5	90.05	89.65	90.00	89.55	86.50	87.40	86.40	K. pneumoniae	0.37
		90.15	89.80	90.15	89.85	86.60	87.50	86.60	K. pneumoniae	0.32
		90.25	89.80	90.15	89.80	86.65	87.60	86.50	K. pneumoniae	0.29
0.625 ^{*1}	31.3 ^{*1}	90.00	89.65	89.90	89.55	86.40	87.30	86.30	K. pneumoniae	0.37
		90.25	89.90	90.15	89.85	86.65	87.50	86.60	K. pneumoniae	0.37
		90.35	90.00	90.25	89.90	86.65	87.60	86.65	K. pneumoniae	0.41
0.313 ^{*2}	15.6 ^{*2}	90.25	N.D.	90.25	N.D.	86.65	N.D.	N.D.	Not identified	–
		N.D.	N.D.	90.15	N.D.	86.60	N.D.	86.65	Not identified	–
		90.35	N.D.	90.25	N.D.	N.D.	N.D.	N.D.	Not identified	–
0.157	7.8	N.D.	N.D.	90.15	N.D.	N.D.	N.D.	N.D.	Not identified	–
		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	Not detected	–

4 *1: limit of identification, *2: limit of detection (LOD)

1 **Supplemental Table S7**2 **List of bacterial colonies used in Table 2**

3 140 colonies (51 bacterial species)

Bacterial colonies	No. of trials	Bacterial colonies	No. of trials
<i>Escherichia coli</i>	25	<i>Acinetobacter calcoaceticus</i>	1
<i>Staphylococcus epidermidis</i>	13	<i>Achromobacter xylosoxidans</i>	1
<i>Klebsiella pneumoniae</i>	8	<i>Aerococcus christensenii</i>	1
<i>Staphylococcus aureus</i>	5	<i>Bacteroides salyersiae</i>	1
<i>Staphylococcus haemolyticus</i>	5	<i>Bacteroides thetaiotaomicron</i>	1
<i>Bacillus cereus</i>	4	<i>Corynebacterium macginleyi</i>	1
<i>Citrobacter freundii</i>	4	<i>Enterococcus avium</i>	1
<i>Enterobacter cloacae</i>	4	<i>Enterococcus casseliglauvus</i>	1
<i>Finegoldia magna</i>	4	<i>Eubacterium limosum</i>	1
<i>Staphylococcus capitis</i>	4	<i>Halomonas venusta</i>	1
<i>Streptococcus agalactiae</i>	4	<i>Prevotella bivia</i>	1
<i>Enterococcus faecalis</i>	3	<i>Prevotella intermedia</i>	1
<i>Prevotella melaninogenica</i>	3	<i>Prevotella nigrescens</i>	1
<i>Pseudomonas aeruginosa</i>	3	<i>Prevotella timonensis</i>	1
<i>Staphylococcus hominis</i>	3	<i>Prevotella veroralis</i>	1
<i>Stenotrophomonas maltophilia</i>	3	<i>Proteus mirabilis</i>	1
<i>Anaerococcus vaginalis</i>	2	<i>Serratia marcescens</i>	1
<i>Bacteroides fragilis</i>	2	<i>Staphylococcus cohnii</i>	1
<i>Campylobacter jejuni</i>	2	<i>Staphylococcus lugdunensis</i>	1
<i>Clostridium perfringens</i>	2	<i>Staphylococcus pettenkoferi</i>	1
<i>Enterococcus faecium</i>	2	<i>Streptococcus anginosus</i>	1
<i>Klebsiella oxytoca</i>	2	<i>Streptococcus constellatus</i>	1
<i>Morganella morganii</i>	2	<i>Streptococcus dysgalactiae</i>	1
<i>Pasteurella multocida</i>	2	<i>Streptococcus infantarius</i>	1
<i>Pseudomonas putida</i>	2	<i>Streptococcus mitis</i>	1
<i>Staphylococcus caprae</i>	2		

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1 **Supplemental Table S8**2 **List of Tm mapping results of broad matches with the sequencing results in Table 2**

3

Sequencing results	Identification results using the Tm mapping method					
	Diff. 1	Most similar bacteria	Diff. 2	2 nd most similar bacteria	Diff. 3	3 rd most similar bacteria
<i>Staphylococcus haemolyticus</i>	0.19	<i>Staphylococcus aureus</i>	0.24	<i>Staphylococcus haemolyticus</i>	0.51	<i>Staphylococcus lugdunensis</i>
<i>Staphylococcus aureus</i>	0.28	<i>Staphylococcus aureus</i>	0.28	<i>Staphylococcus haemolyticus</i>	0.48	<i>Staphylococcus lugdunensis</i>
<i>Staphylococcus haemolyticus</i>	0.37	<i>Staphylococcus haemolyticus</i>	0.37	<i>Staphylococcus warneri</i>	0.38	<i>Staphylococcus aureus</i>

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5 Diff. = Difference Value

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1 **Supplemental Table S9**

2 **Blind test results for bacteria with similar Tm mapping shapes (Difference Value ≤ 0.28 shown
3 by asterisks in Table S4) in the database**

4

Bacterial DNA	Triplicate	Identification results using the Tm mapping method				
		Diff. 1	Most similar bacteria	Diff. 2	2 nd most similar bacteria	Diff. 3
<i>Bacteroides dorei</i>	1	0.20	<i>Bacteroides dorei</i>	0.37	<i>Bacteroides vulgatus</i>	0.85
	2	0.23	<i>Bacteroides dorei</i>	0.42	<i>Bacteroides vulgatus</i>	1.10
	3	0.26	<i>Bacteroides dorei</i>	0.47	<i>Bacteroides vulgatus</i>	1.08
<i>Bacteroides vulgatus</i>	1	0.09	<i>Bacteroides vulgatus</i>	0.30	<i>Bacteroides dorei</i>	0.91
	2	0.15	<i>Bacteroides vulgatus</i>	0.36	<i>Bacteroides dorei</i>	0.91
	3	0.20	<i>Bacteroides vulgatus</i>	0.48	<i>Bacteroides dorei</i>	0.96

5

6 Diff. = Difference Value

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1 **Supplemental Table S10**

2 **Comparison of the cell count using flow cytometry method and the colony count using
3 conventional culture method starting from the same bacterial suspensions**

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<i>E. coli</i> suspensions*	Cell count using flow cytometry (cells/ μ L)	Colony count of culture dishes (CFU/ μ L)	Percentage of colony formation (CFU/cells)
<i>E. coli</i> suspension # 1	1081	898	83.1 %
<i>E. coli</i> suspension # 2	971	853	87.8 %
<i>E. coli</i> suspension # 3	1147	1018	88.8 %

5

6 *: Before performing the tests, *Escherichia coli* (ATCC25922) was cultivated in Muller-Hinton Broth
7 at 37°C for 12 hours, then bacterial suspensions were prepared at a concentration of around 1000
8 bacteria/ μ L. Each suspension was prepared separately.

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1 **Supplemental Table S11**2 **Quantification of approximate bacterial concentrations in the blood of patients with sepsis**

3

DNA templates (2 µL/20 µL of PCR reaction mixture)	Ct* ² averages from triplicate experiments	16S ribosomal RNA operon copy number	Blood bacterial concentrations (CFU/mL)	
Control DNA <i>(E. coli)</i>	80 CFU/µL* ¹ 10 CFU/µL* ¹	9.77 13.84	7 7	8,000 1,000
Patient # 1 <i>(E. coli)</i>		15.66	7	395
Patient # 2 <i>(E. coli)</i>		5.44	7	73,200
Patient # 3 <i>(S. aureus)</i>		8.93	5	17,220
Patient # 4 <i>(S. aureus)</i>		5.10	5	121,800
Patient # 5 <i>(S. epidermidis)</i>		10.05	5	9,716
Patient # 6 <i>(K. pneumoniae)</i>		10.84	8	4,051

4 *1: Concentration in a DNA elution buffer. Bacterial DNA was eluted with 100 µL of elution buffer,

5 and 2µL of which was used as a DNA template for one PCR reaction.

6 *2: The Ct (threshold cycle) values amplified by Region 3 primers in the second nested PCR were
7 analyzed using the Rotor-Gene Q software program.

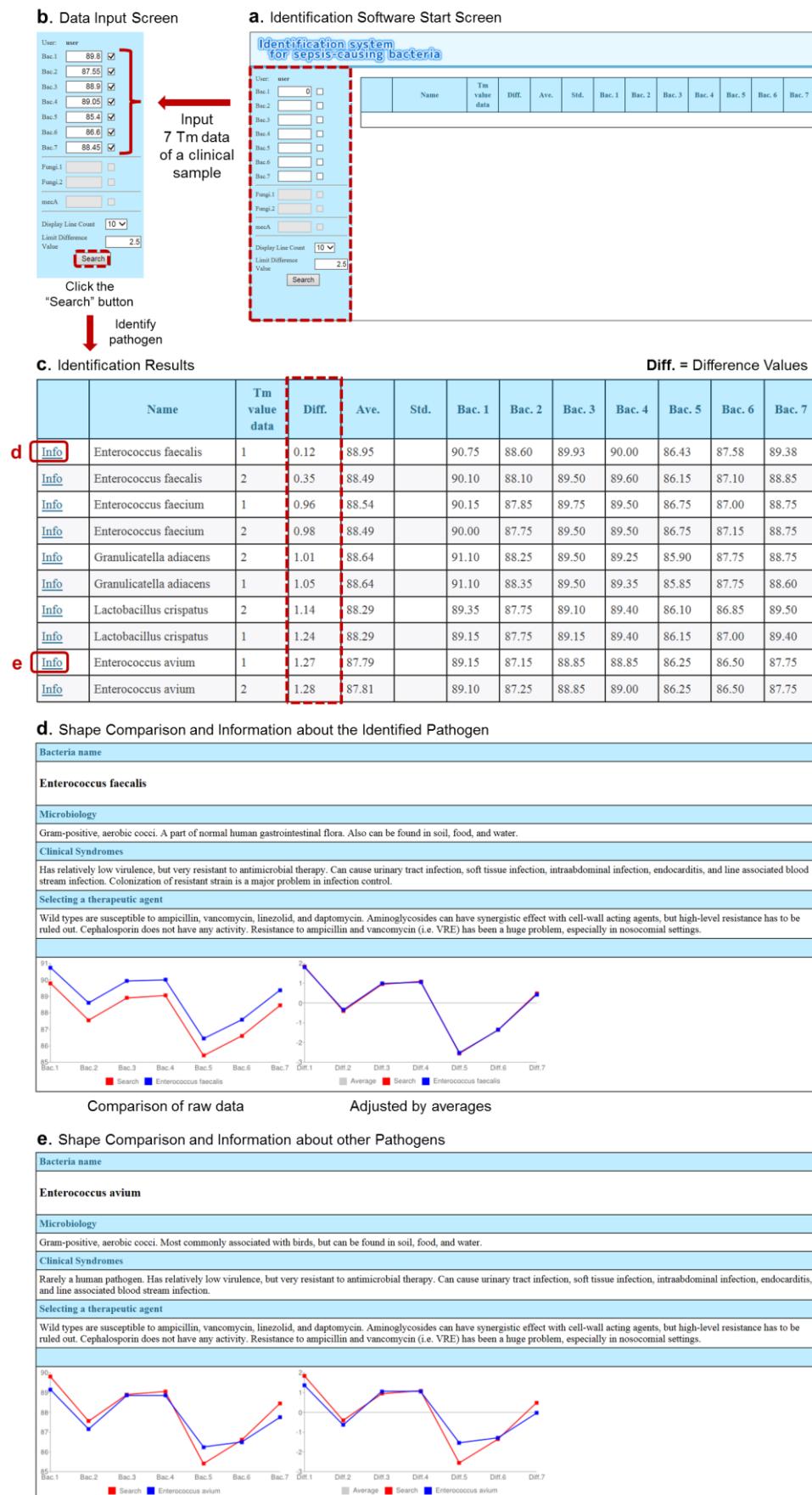
8

9 We did quantitative PCR analysis for relative quantification of bacteria in the blood of patients with
10 sepsis. The pathogenic bacteria had already been identified by both the Tm mapping method and the
11 conventional culture method. In this experiment, the amplification efficiency was 66.7% per PCR
12 cycle, which depends on the enzyme activity of the thermostable DNA polymerase, and the blood
13 bacterial concentrations were finally adjusted by the 16S ribosomal RNA operon copy number.

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1 Supplemental Figure S1



1 **Identification software program on the web**

2 Using the identification software program available on the web, any user can identify bacterial
3 isolates without additional training.

4 (a) Any user can access the identification software program via the internet.

5 (b) First, the user inputs the seven Tm values for a clinical sample and then clicks the “search”
6 button.

7 (c) The identification results are shown promptly. The results are lined up in ascending order
8 according to the Difference Values.

9 (d) Next, the user clicks the “Info” button, where information about the pathogen is provided. It is
10 also possible to compare the raw data to the Tm mapping shape in the database.

11 (e) Information about other pathogens can be seen as well, in addition to the differences between the
12 shapes.

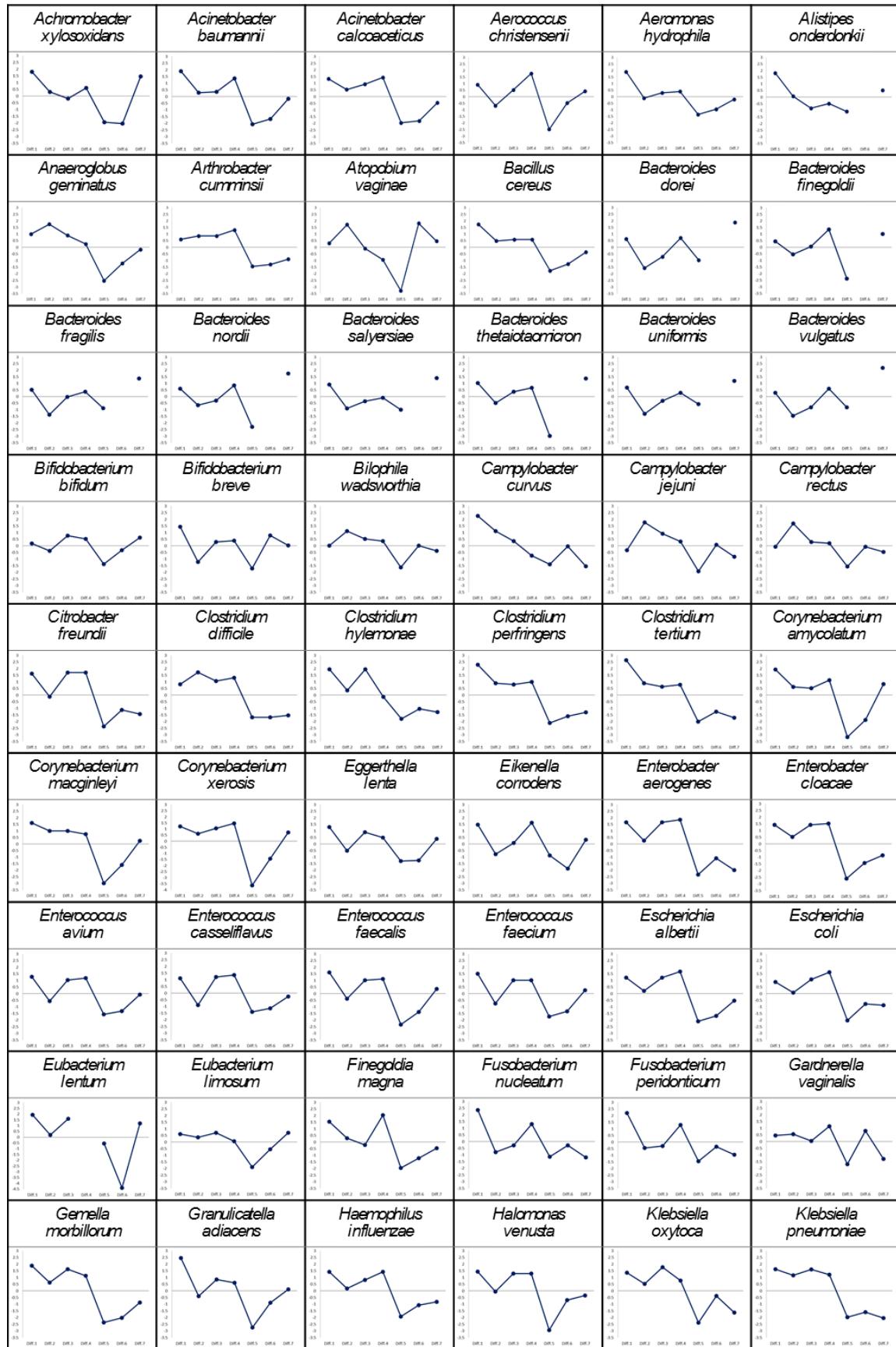
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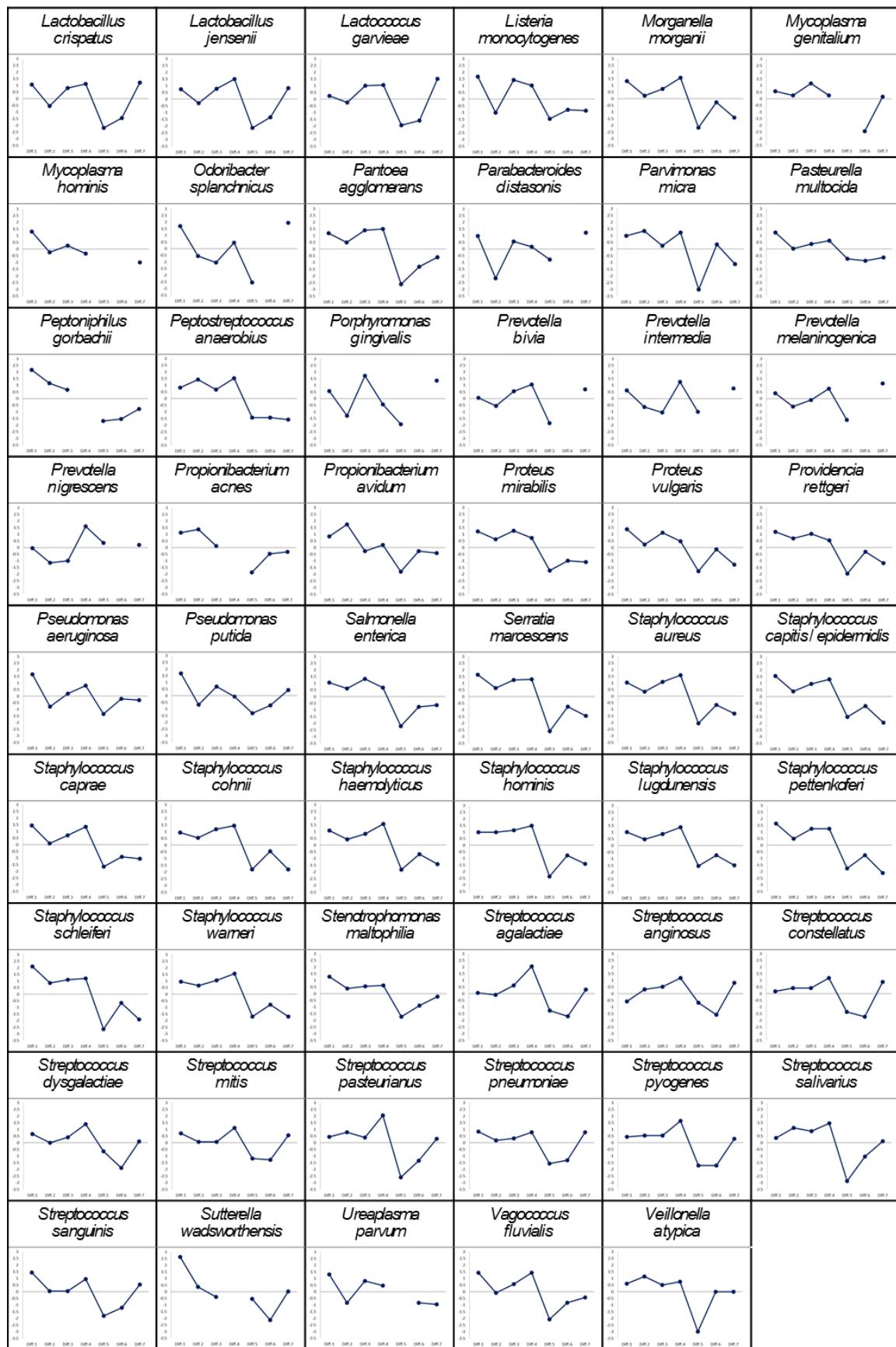
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1 Supplemental Figure S2



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1 **The Tm mapping shapes of the 107 species of bacteria registered in the database**

2 The X-axis shows the average of the seven Tm values.

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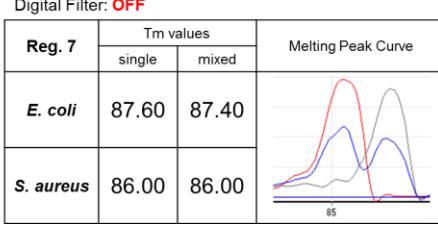
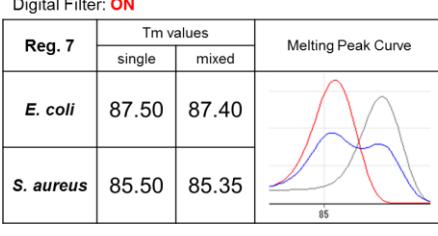
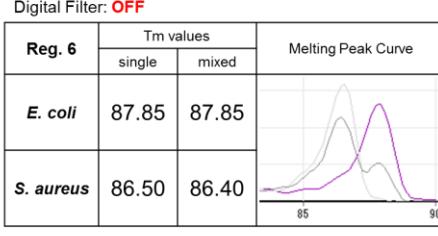
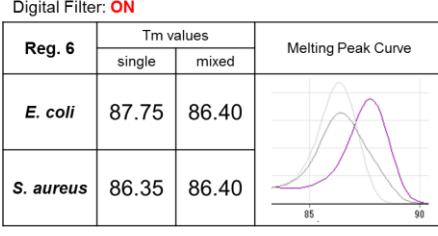
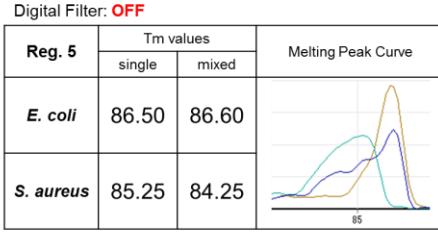
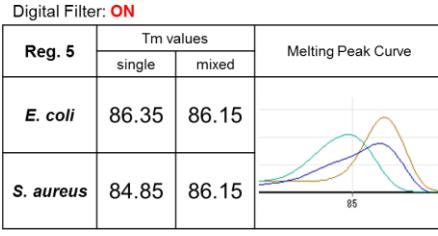
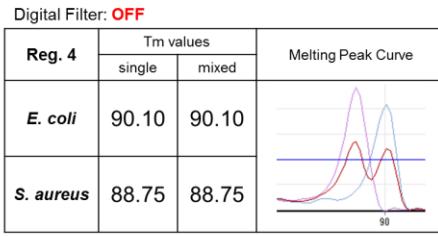
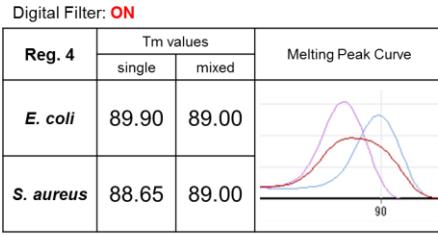
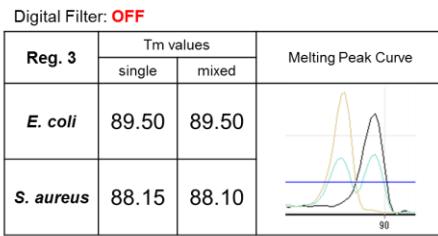
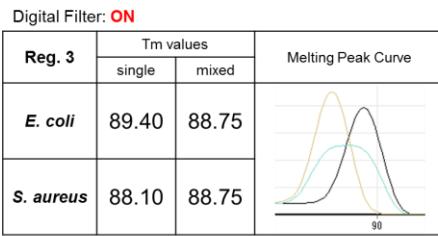
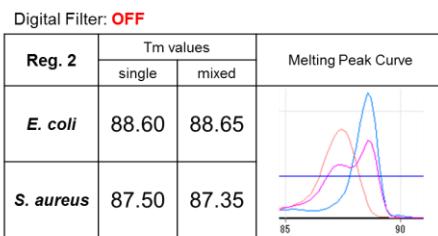
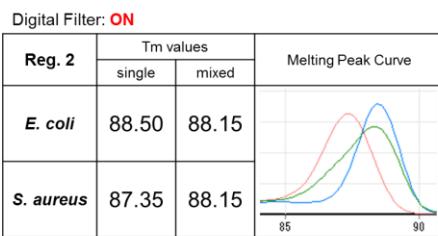
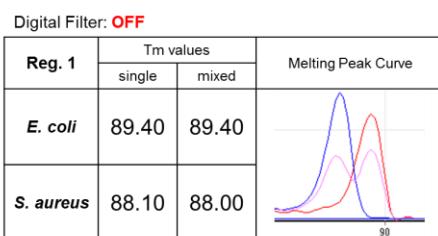
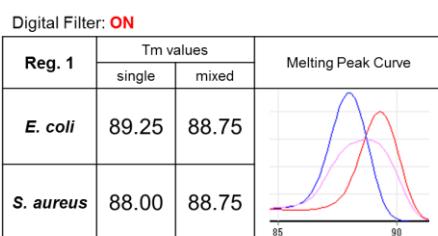
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1 Supplemental Figure S3

a



b

Digital Filter: **ON**

Diff. = Difference Values

Bacterial DNA	sample	Tm mapping identification	Diff.
<i>E. coli</i>	single	<i>Escherichia coli</i>	0.19
	mixed	<i>Escherichia albertii</i>	0.55
<i>S. aureus</i>	single	<i>Staphylococcus aureus</i>	0.21
	mixed	<i>S. capitis / epidermidis</i>	0.67

Digital Filter: **OFF**

Diff. = Difference Values

Bacterial DNA	sample	Tm mapping identification	Diff.
<i>E. coli</i>	single	<i>Escherichia coli</i>	0.15
	mixed	<i>Escherichia coli</i>	0.31
<i>S. aureus</i>	single	<i>Staphylococcus aureus</i>	0.22
	mixed	<i>Escherichia coli</i>	0.73

1

2

3 Tm mapping results for a mixed sample that contains equal amounts of two species of bacteria

4 (*Escherichia coli* and *Staphylococcus aureus*)5 Using a mixed sample containing equal amounts of *E. coli* and *S. aureus*, we performed Tm mapping

6 identification. We used the RotorGeneQ instrument and measured the seven Tm values of the seven

7 amplicons with a Digital Filter ON or OFF, which calculates the melting temperature by

8 approximating the melting peak curve to the normal distribution curve (a). As a result, because the

9 different Tm values overlapped, the equal amounts of the two species of bacteria interfered with

10 each other's Tm mapping identification (b).

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12 Reg. 1 – 7 = amplicons amplified by the Region 1 – 7 primers

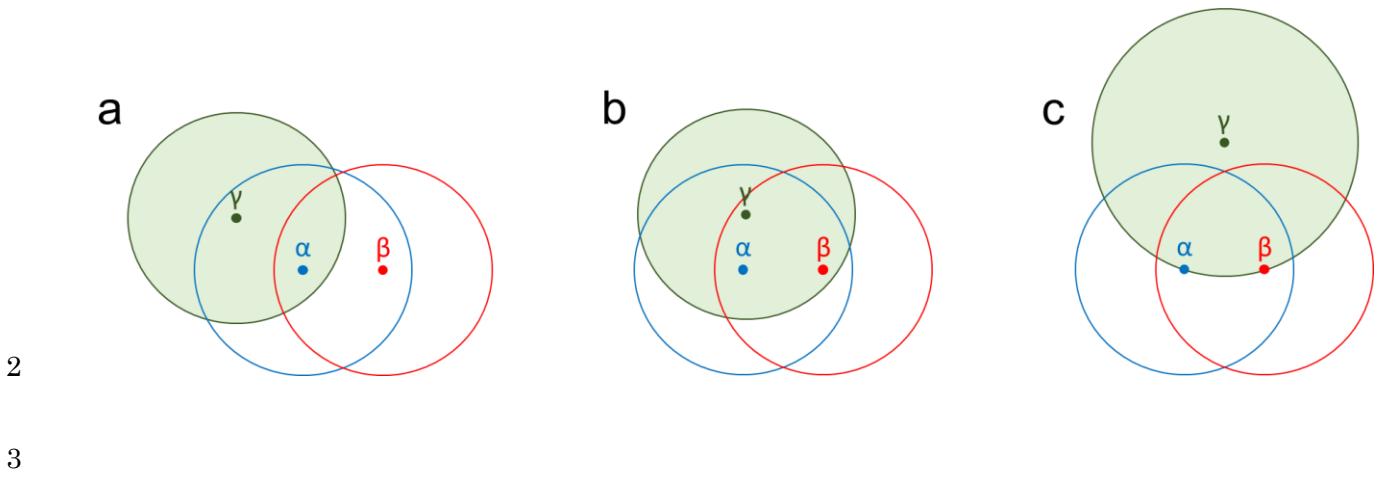
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1 **Supplemental Figure S4**



6 These figures (**a**, **b**, **c**) show seven-dimensional space. From a mathematic point of view, the
 7 Difference Value is the distance between two points in seven dimensions. In this setting, sample γ is
 8 the bacterial isolate from the patient sample that we wish to identify, and point γ is the Tm mapping
 9 result. Bacteria α is the pathogenic bacteria, and bacteria β is the bacteria with a similar Tm mapping
 10 shape (both Difference Values are less than 0.28). Point α is bacteria α 's Tm mapping data in the
 11 database, and point β is bacteria β 's Tm mapping data in the database. The blue and red circles show
 12 the Difference Value boundaries (0.28).

13 (a) The green circle shows the Difference Value boundary (0.28) of sample γ . The Tm mapping result
 14 is bacteria α (Difference Value = 0.26). In this case, bacteria β does not interfere with the Tm
 15 mapping identification. The distance between the two points in seven dimensions provides enough

1 diversity so that bacteria β does not interfere with the Tm mapping identification.

2 (b) The green circle shows the Difference Value boundary (0.28) of sample γ . The Tm mapping
3 result is bacteria α (Difference Value = 0.20) or bacteria β (Difference Value = 0.27). In this case,
4 bacteria β interferes with the Tm mapping identification, such that the result cannot be narrowed
5 down to one bacterial species.

6 (c) In this case, because sample γ is a mutant strain or one dominant strain in a polymicrobial
7 infection, the green circle shows a Difference Value boundary of 0.37. The Tm mapping result is
8 bacteria α (Difference Value = 0.37) or bacteria β (Difference Value = 0.37). In this case ($0.28 <$
9 Difference Value ≤ 0.5), the identification result with the lowest Difference Value is highly likely to
10 be the bacterial isolate (**Table 1**), which means either bacteria α or bacteria β .

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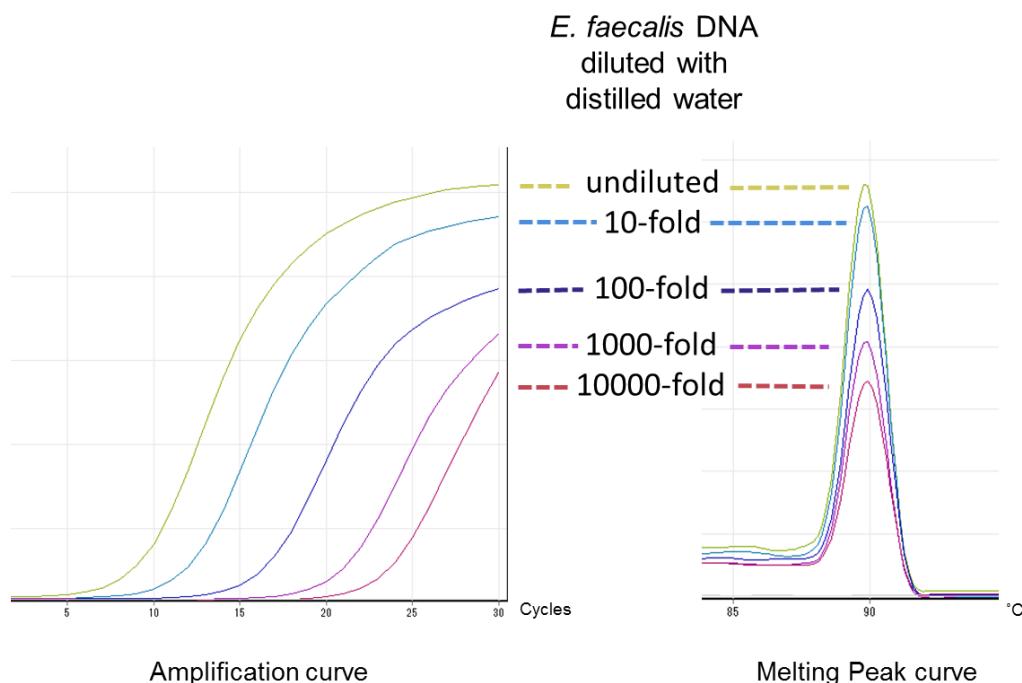
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1 Supplemental Figure S5

2 a

Amplicon	<i>E. faecalis</i> DNA diluted with distilled water	Melting temperature
Amplicon amplified by Region 3 primers	undiluted	89.75
	10-fold	89.85
	100-fold	89.90
	1000-fold	89.85
	10000-fold	89.90



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4

5 b

<i>E. coli</i> DNA diluted with distilled water	Melting Temperature (Duplicate)						
	Reg. 1	Reg. 2	Reg. 3	Reg. 4	Reg. 5	Reg. 6	Reg. 7
undiluted	89.35	88.60	89.50	90.00	86.50	87.75	87.60
	89.40	88.60	89.40	90.00	86.40	87.75	87.60
100-fold	89.35	88.65	89.50	90.00	86.40	87.75	87.60
	89.35	88.60	89.40	90.00	86.40	87.75	87.50
10000-fold	89.35	88.65	89.50	90.00	86.35	87.75	87.60
	89.35	88.60	89.50	89.90	86.40	87.75	87.50

6 Reg. 1 – 7 ≡ Region 1 – 7 primers

1 **Validation of the melting temperatures of the same amplicon using different DNA concentrations**

2 **(a)** The *Enterococcus faecalis* DNA template was diluted from 10 to 10,000-fold with molecular-grade distilled
3 water and amplified using the Region 3 primers. Finally, the melting temperatures of the undiluted and diluted
4 amplicons (five different DNA concentrations) were measured in the same trial.

5 **(b)** The *E. coli* DNA template was diluted from 100 to 10,000-fold with molecular-grade distilled water and
6 then amplified using the Region 1 - 7 primers, respectively. Finally, the melting temperatures of the undiluted and
7 diluted amplicons (three different DNA concentrations) were measured in the same trial.