

# Use of Optical Mapping to Identify Bacteria from Complex Mixture and Clinical Samples

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## ABSTRACT

Rapid identification of bacteria is an important goal in clinical microbiology labs. Current testing procedures most often require pure culture, which significantly lengthens the time required for identification. In contrast, single molecule maps generated by Optical Mapping can theoretically provide more rapid identification, even when multiple organisms are present.

The purpose of this study was to assess the ability of Optical Mapping to identify unknown bacteria in complex mixtures and directly from clinical samples. Four distinct bacterial species were cultured in liquid medium and then adjusted to a target optical density. Eight groups consisting of 2-4 organisms at varying ratios were mixed and DNA extracted. In parallel, 25 clinical unknown urinary tract infections and blood culture bottle samples were obtained, bacteria isolated, and DNA extracted. Identification of bacteria was performed by analysis of DNA samples using Optical Mapping. All 8 of the complex mixtures were correctly identified. Of the 25 samples, 23 were identified to contain a species that was in the identification database and all of these were identified correctly. For the two samples that contained species not represented in the identification database, Optical Mapping correctly called that they were not in the identification database.

The findings confirmed the ability of Optical Mapping to provide identification of clinically relevant bacteria in complex mixtures or directly from clinical samples. In addition, the results provided strong evidence that Optical Mapping could be used to significantly reduce the time necessary to identify bacteria in a clinical laboratory.

## INTRODUCTION

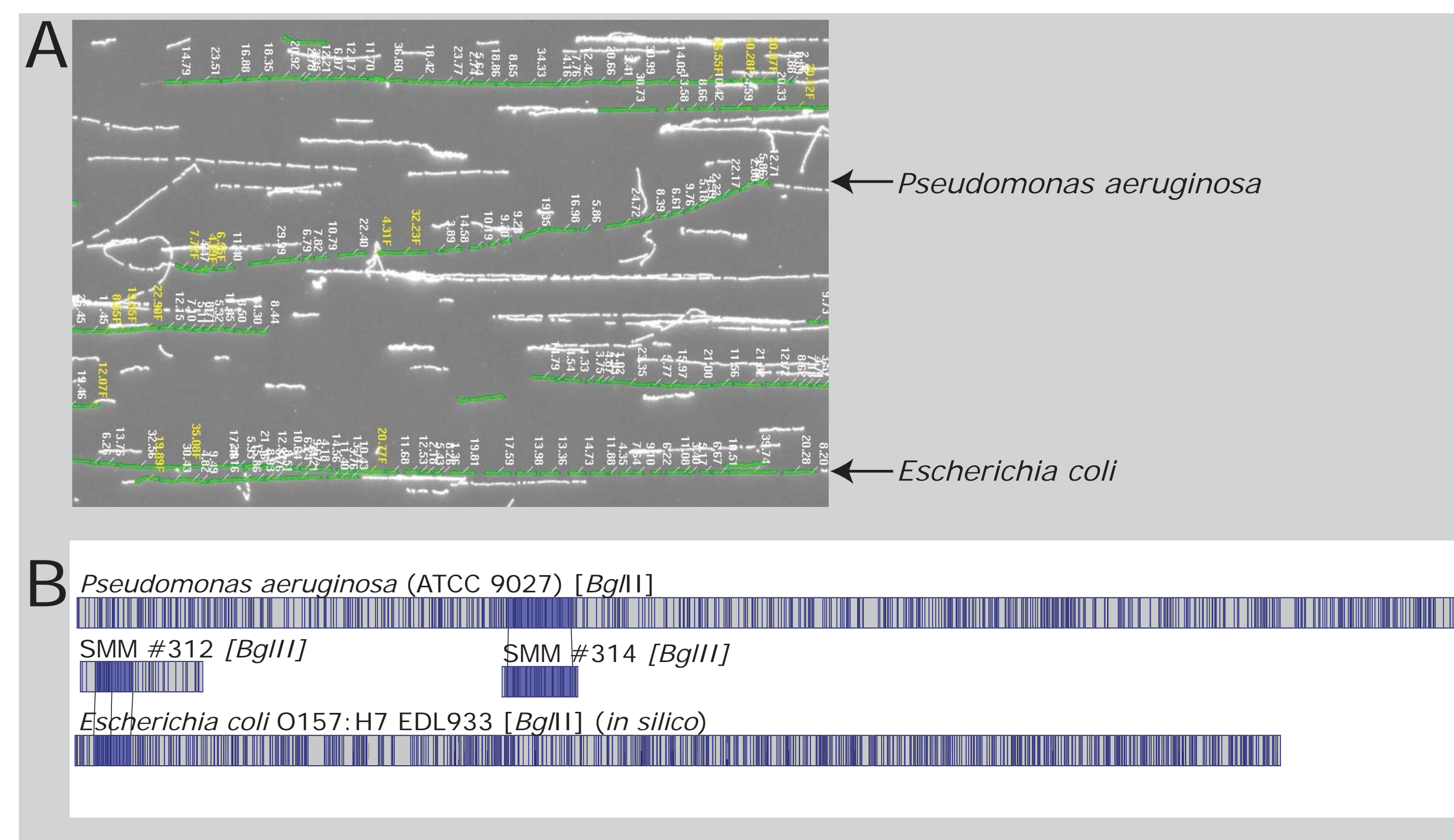
An important goal of clinical microbiology laboratories is the rapid identification of bacteria from clinical samples. However, lengthy culturing steps to obtain enough of a pure culture to allow for identification will slow the time to a result. In contrast, Optical Mapping can potentially provide identifications directly from clinical samples that may contain more than a single organism thereby decreasing the time to a result. Optical Mapping is a technology for rapidly generating whole genome restriction maps of organisms from thousands of single DNA molecules (i.e. single molecule maps). Each single molecule map generated by Optical Mapping contains an ordered set of DNA fragments with distinct sizes. The order and sizes of the fragments within a single molecule map represent a unique signature to a specific genome of a bacterial species. Optical Mapping allows for the ability to collect thousands of single molecule maps in parallel and potentially identify one or more of the bacterial sources of the DNA in a single sample based on the information in the single molecule maps. Optical Mapping also potentially allows for the identification of bacteria directly from clinical samples without the need for growth on primary culture medium. The purpose of this study was to assess the ability of Optical Mapping to identify unknown bacteria in complex mixtures and directly from clinical samples.

Group	Bacterial Species	%
1	<i>Escherichia coli</i> O157:h7 ATCC 35150	50
	<i>Pseudomonas aeruginosa</i> ATCC 9027	50
2	<i>Escherichia coli</i> O157:h7 ATCC 35150	90
	<i>Pseudomonas aeruginosa</i> ATCC 9027	10
3	<i>Staphylococcus aureus</i> ATCC 25923	50
	<i>Escherichia coli</i> O157:h7 ATCC 35150	50
4	<i>Staphylococcus aureus</i> ATCC 25923	90
	<i>Escherichia coli</i> O157:h7 ATCC 35150	10
5	<i>Staphylococcus aureus</i> ATCC 25923	33
	<i>Escherichia coli</i> O157:h7 ATCC 35150	33
6	<i>Pseudomonas aeruginosa</i> ATCC 9027	33
	<i>Staphylococcus aureus</i> ATCC 25923	60
7	<i>Escherichia coli</i> O157:h7 ATCC 35150	30
	<i>Pseudomonas aeruginosa</i> ATCC 9027	30
8	<i>Enterococcus faecalis</i> ATCC 19433	25
	<i>Staphylococcus aureus</i> ATCC 25923	25
8	<i>Escherichia coli</i> O157:h7 ATCC 35150	20
	<i>Pseudomonas aeruginosa</i> ATCC 9027	20

**Table 1: Mixed Culture Constituents & Ratios.** Eight bacterial mixtures (1-8) were prepared with two to four bacterial species to allow for a specific ratio of each bacterium as measured by colony forming units. The percentage of each bacterium within each group is listed.

## METHODS

All bacterial mixes and clinical samples were provided by Gundersen Lutheran Medical Foundation. Bacterial species for the mixtures were normalized to 1x10<sup>9</sup> CFU/ml and mixed in combinations and amounts to yield 8 groups with varying constituents and ratios (Table 1). The five samples for each of five clinical sample types (clinical colony, spiked blood bottles, spiked urine samples, clinical blood bottles, and clinical urine samples) were prepared and the identities blinded. Urine and blood culture bottle samples were processed by OpGen for isolation of bacterial cells. High molecular weight DNA for the samples were prepared directly from isolated bacterial cells using a modified Pulse-Field Gel Electrophoresis method as described in Birren and Lai. Optical Chips for all DNA samples were prepared according to Reslewic et al. Microbial identification was performed by comparing collections of single molecule maps from each DNA sample to the identification database to determine the number of matches by using the OpGen microbial identification algorithm.



**Figure 1: Multiple Single Molecule Maps (SMMs) In a Single Channel.** (A) A representative image from a single Optical Chip channel with DNA from unknown group H. DNA molecules on the Optical Chip processed with *BglII* are represented by the white lines. DNA fragments represented in green were recognized by Pathfinder software and sized in kilobases. The arrows point to DNA molecules that were matched to *P. aeruginosa* and *E. coli*. (B) The DNA molecules that matched *P. aeruginosa* (SMM #314 [*BglII*]) and *E. coli* (SMM #312 [*BglII*]) in panel A were compared to the respective whole genome *BglII* reference maps in the database using OpGen MapViewer software. The whole genome maps and single molecule maps are represented as rectangles with restriction sites indicated as internal vertical lines. Areas of the single molecule maps that match the whole genome reference map are shaded in blue with lines linking the compared regions.

## RESULTS

### Complex Bacterial Mixtures

Collections of single molecule maps for each unknown mixture (Table 1) were analyzed using the OpGen microbial identification algorithm. The OpGen microbial identification algorithm identified matches to the identification database (Table 2). The unblinded group designations indicated that the bacterial constituents of the complex mixtures were identified correctly in 8 of 8 groups. Furthermore, the percentage of contributing bacterial species was identified correctly for 6 of the 8 groups.

Unknown Mix	Enzyme	<i>S. aureus</i> Matches	<i>E. coli</i> Matches	<i>E. faecalis</i> Matches	<i>P. aeruginosa</i> Matches	Max Matches to Untested Species	OpGen 1 <sup>st</sup> Choice	OpGen 2 <sup>nd</sup> Choice
A	<i>NcoI</i>	1330	204	1	0	3	4	3
	<i>BglII</i>	1	78	0	1	2	4	3
B	<i>NcoI</i>	0	594	0	0	2	2	1
	<i>BglII</i>	0	912	0	32	3	2	1
C	<i>NcoI</i>	376	451	0	0	3	6	5
	<i>BglII</i>	29	924	0	127	3	6	5
D	<i>NcoI</i>	425	656	90	0	4	8	7
	<i>BglII</i>	5	198	0	49	3	8	7
E	<i>NcoI</i>	536	1115	170	0	2	7	8
	<i>BglII</i>	0	280	0	80	3	7	8
F	<i>NcoI</i>	301	518	0	0	3	5	6
	<i>BglII</i>	2	245	0	150	3	5	6
G	<i>NcoI</i>	235	923	0	0	2	3	4
	<i>BglII</i>	3	413	0	3	4	3	4
H	<i>NcoI</i>	0	285	0	0	2	1	2
	<i>BglII</i>	0	647	0	777	2	1	2

**Table 2: Microbial Mixture Identification Data.** DNA isolated from eight unknown bacterial mixtures (A, B, C, D, E, F, G, and H) were analyzed by Optical Mapping using the enzyme(s) specified (*NcoI*, *BglII*). The match data was generated using a *p*-value maximum set to 0.01. Data are from representative Optical Chips. The number of matches represents how many single molecule maps matched the database to a specific species. A blue-shaded set indicates a match to a test species at a level of 8-fold or higher above background (i.e. max hit to untested species). The green-shading indicates where a correct group identification was made.

## Clinical Samples

Collections of single molecule maps for each blinded clinical sample were analyzed using the OpGen microbial identification algorithm. Of the 23 clinical samples that contained a representative species in the identification database, 100% identified to the same species as was identified by classical microbiology techniques at the Gundersen Lutheran Medical Foundation laboratory. Furthermore, UTI 1 and CU 4 were correctly identified as not being in the identification database.

Sample Type Group	Unknown Sample	Enzyme (s)	Top Reported Species	Matches to Top Reported Species	Matches to Next Reported Species	Identification by Optical Mapping	Identification by GLMF	Result
Clinical Colony	UTI 1	<i>NcoI/BglII/XbaI</i>	None	-	-	Not in DB	<i>S. marcescens</i>	Not in DB
	UTI 2	<i>NcoI</i>	<i>E. coli</i>	55	0	<i>E. coli</i>	<i>E. coli</i>	Correct
	UTI 3	<i>BglII</i>	<i>E. coli</i>	51	1	<i>E. coli</i>	<i>E. coli</i>	Correct
	UTI 4	<i>NcoI</i>	<i>P. aeruginosa</i>	17	0	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Correct
	UTI 5	<i>BglII</i>	<i>K. pneumoniae</i>	78	1	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	Correct
Spiked Blood Bottle	SB 1	<i>NcoI</i>	<i>S. aureus</i>	64	0	<i>S. aureus</i>	<i>S. aureus</i>	Correct
	SB 2	<i>NcoI</i>	<i>E. faecium</i>	86	1	<i>E. faecium</i>	<i>E. faecium</i>	Correct
	SB 3	<i>NcoI</i>	<i>S. pyogenes</i>	38	1	<i>S. pyogenes</i>	<i>S. pyogenes</i>	Correct
	SB 4	<i>BglII</i>	<i>P. aeruginosa</i>	251	1	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Correct
	SB 5	<i>NcoI</i>	<i>S. agalactiae</i>	122	2	<i>S. agalactiae</i>	<i>S. agalactiae</i>	Correct
Spiked Urine Sample	SU 1	<i>NcoI</i>	<i>E. coli</i>	186	2	<i>E. coli</i>	<i>E. coli</i>	Correct
	SU 2	<i>NcoI</i>	<i>P. mirabilis</i>	53	1	<i>P. mirabilis</i>	<i>P. mirabilis</i>	Correct
	SU 3	<i>NcoI</i>	<i>S. saprophyticus</i>	23	1	<i>S. saprophyticus</i>	<i>S. saprophyticus</i>	Correct
	SU 4	<i>BglII</i>	<i>K. pneumoniae</i>	66	1	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	Correct
	SU 5	<i>BglII</i>	<i>P. aeruginosa</i>	71	1	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	Correct
Clinical Blood Bottle	CB A	<i>NcoI</i>	<i>S. epidermidis</i>	89	1	<i>S. epidermidis</i>	<i>S. epidermidis</i>	Correct
	CB B	<i>NcoI</i>	<i>S. agalactiae</i>	19	0	<i>S. agalactiae</i>	<i>S. agalactiae</i>	Correct
	CB 3	<i>NcoI</i>	<i>E. coli</i>	22	1	<i>E. coli</i>	<i>E. coli</i>	Correct
	CB 4	<i>NcoI</i>	<i>K. pneumoniae</i>	15	2	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	Correct
	CB 6	<i>NcoI</i>	<i>E. coli</i>	100	1	<i>E. coli</i>	<i>E. coli</i>	Correct
Clinical Urine Sample	CU 1	<i>NcoI</i>	<i>S. aureus</i>	200	1	<i>S. aureus</i>	<i>S. aureus</i>	Correct
	CU 2	<i>NcoI</i>	<i>E. faecalis</i>	69	1	<i>E. faecalis</i>	<i>E. faecalis</i>	Correct
	CU 3	<i>NcoI</i>	<i>E. coli</i>	38	1	<i>E. coli</i>	<i>E. coli</i>	Correct
	CU 4	<i>NcoI/BglII/XbaI</i>	None	-	-	Not in DB	<i>C. freundii</i>	Not in DB
	CU 5	<i>BglII</i>	* <i>K. pneumoniae</i>	1	1	<i>K. pneumoniae</i>	<i>K. pneumoniae</i>	Correct

**Table 4: Clinical Identification Data.** DNA isolated from unknown samples from each of five sample type groups (clinical colony, spiked blood bottle, spiked urine sample, clinical blood bottle, and clinical urine sample) were analyzed by Optical Mapping using the restriction enzyme(s) specified. Optical Mapping data were processed through OpGen's microbial identification algorithm, and all match data were generated using a *p*-value maximum set to 0.001. The number of single molecule maps that matched the top reported bacterial species as well as the next reported bacterial species from the ID are listed. The final bacterial species identifications by Optical Mapping for each unknown sample along with the identifications made by Gundersen Lutheran Medical Foundation microbiology laboratory are also represented. Blue-shaded fields indicate where the Optical Mapping made the same identification as Gundersen Lutheran Medical Foundation and the green shaded fields illustrate the samples where Optical Mapping called the correct bacterial species for the unknown sample. An \* symbol represents an unknown sample where the Optical Mapping assembly was used instead of the microbial identification to make an identification. The following abbreviations were used: UTI, urinary tract infection; SB, spiked blood bottle; SU, spiked urine; CB, clinical blood bottle; CU, clinical urine; *E. coli*, *Escherichia coli*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *K. pneumoniae*, *Klebsiella pneumoniae*; *S. aureus*, *Staphylococcus aureus*; *E. faecium*, *Enterococcus faecium*; *S. pyogenes*, *Streptococcus pyogenes*; *S. agalactiae*, *Streptococcus agalactiae*; *P. mirabilis*, *Proteus mirabilis*; *S. saprophyticus*, *Staphylococcus saprophyticus*; *S. epidermidis*, *Staphylococcus epidermidis*; *E. faecalis*, *Enterococcus faecalis*; *S. marcescens*, *Serratia marcescens*; *C. freundii*, *Citrobacter*.

## CONCLUSIONS

- Optical Mapping accurately identified clinically relevant bacteria in complex mixtures or directly from clinical samples.
- Optical Mapping accurately discriminated between samples containing the same organisms but at different ratios.
- Optical Mapping has the potential to rapidly identify organisms from clinical samples.

## REFERENCES

- Birren, B and Lai, E (1993). *Pulsed Field Gel Electrophoresis: A Practical Guide*. San Diego: Academic Press, Inc. p25-74.
- Reslewic S, Zhou S, Place M, Zhang Y, Briska A, Goldstein S, Churas C, Runnheim R, Forrest D, Lim A, Lapidus A, Han CS, Roberts GP, Schwartz DC. (2005). Whole-genome shotgun Optical Mapping of *Rhodospirillum rubrum*. *Appl Environ Microbiol.* 71(9), 5511-22.