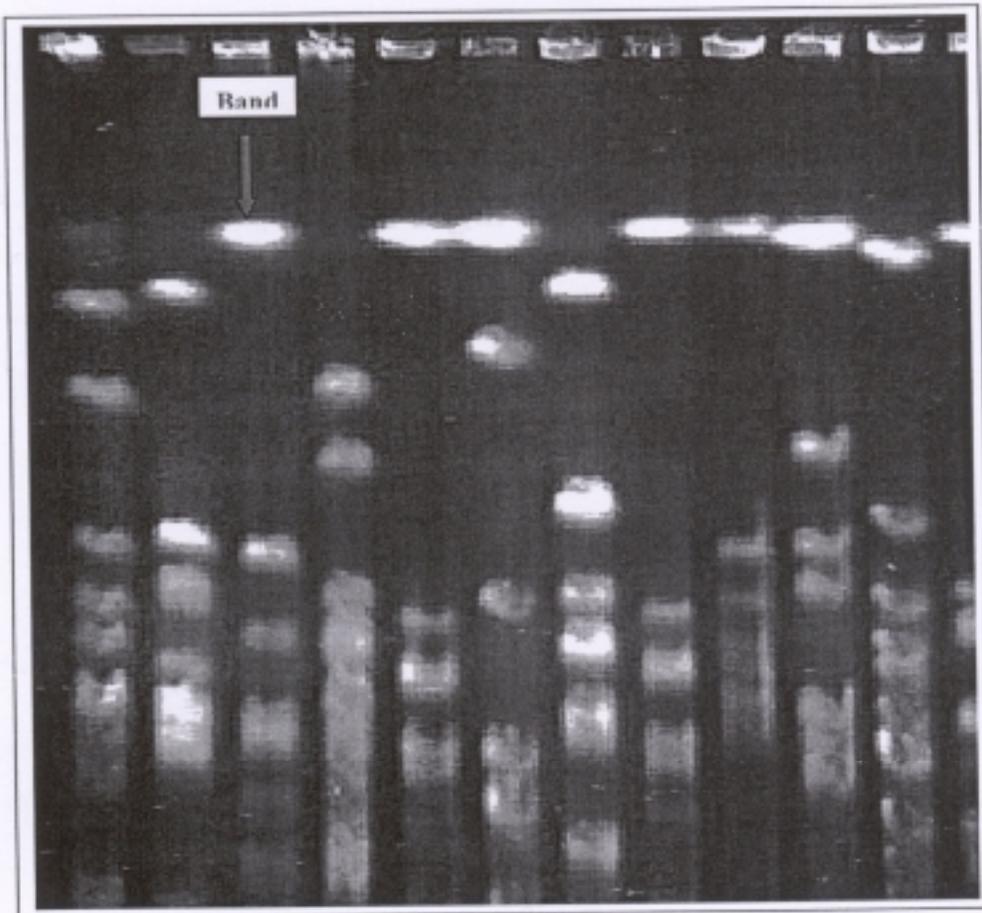


## **APPENDIX D**

### **SAMPLE BST PRINTOUTS AND RESULTS**



Each band of this gel becomes part of a band set. The band type is the actual location of the band as it is modeled within the band set. Many band types make up the band set (database). Once the band set has been created, the database may be run against unknown samples for comparison.

**APPENDIX D**

**BACTERIA SOURCE TRACKING RESULTS USING 6-WAY ANALYSIS**

**(bird vs. cow vs. dog vs. horse vs. bat vs. human)**

**LIBRARY ISOLATES**

Table D-1 Summary Table Of Initial Library (2004) - Percent ARCC And RCC For Six-Way Classifications (Bird Vs. Cow Vs. Dog Vs. Horse Vs. Bat Vs. Human).

%	Human vs. Non-human		Human S vs. Human P vs. Non-human		Human P only vs. Non-human	
	All Antibiotic	Wilks-lambda (stepwise)	All Antibiotic	Wilks-lambda (stepwise)	All Antibiotic	Wilks-lambda (stepwise)
ARCC	38.1	34.2	36.8	31.6	<b>39.9</b>	36.5
Bird	45.6	46.2	45.0	43.3	<b>45.6</b>	41.5
Cow	37.9	38.4	34.2	34.2	<b>36.5</b>	38.8
Dog	37.9	35.2	37.4	34.6	<b>37.4</b>	35.2
Horse	48.5	42.0	47.0	38.5	<b>45.0</b>	36.5
Bat	34.8	20.1	31.9	16.2	<b>35.3</b>	30.4
Human	25.2	25.2				
Human-S			16.0	11.3		
Human-P			39.6	39.6	<b>41.1</b>	37.9
Cross-val. ARCC	34.9	33.4	33.7	30.3	<b>37.3</b>	35.7

Notes:

1. Human-P = human isolates from portable toilets only
2. Human-S=isolates from sewage line only.
3. All antibiotic indicates the analysis was conducted using all the antibiotics.
4. Wilks-lambda (Stepwise) indicates that a stepwise method was used in which the program may remove some antibiotics from the analysis if they do not contribute to the classifications.
5. Abbreviations: ARCC – Average Rate of Correct Classification; RCC – Rate of correct classification

Table D-2 Comparison of Discriminant Analysis Results Of The Revised Library Database Using ARP, CSU And A Combination Of All Data For Six-Way Analysis, Between The Human/Sewage Isolates Collected Only In 2005 And The Human/Sewage Isolates Collected in Both 2004 and 2005.

% Correct classification	ARP only		CSU only		ARP+CSU	
	HS 2004 and 2005	HS 2005 only	HS 2004 and 2005	HS 2005 only	HS 2004 and 2005	HS 2005 only
ARCC	40.2	44.1	62.4	67.2	67.9	72.2
Human	35.2	52.0	71.2	93.0	74.9	94.7
Non human						
Bird	44.3	45.5	59.3	61.7	65.3	65.3
Cow	40.4	41.3	62.4	68.5	70.0	74.6
Dog	40.0	41.2	59.4	62.9	68.2	72.4
Horse	50.8	49.7	56.5	57.0	62.2	63.2
Bat	35.6	35.6	56.5	61.8	59.7	64.4
Cross-val. ARCC	38.0	41.7	52.7	55.7	56.5	59.9

Abbreviations: ARP – antibiotic resistance profiles, CSU – carbon source utilization, HS – Human/Sewage

Note: Using the ARP+CSU combined database with the 2005 Human/Sewage isolates only, 94.7% of the human/sewage isolates were classified correctly. Use of the CSU data alone also provided a high level of correct classification for human; however there was a lower level of correct classification for the animals than the combined database.

Results of cross-validation (also known as resubstitution analysis or leave-one-out method) showed only a slightly reduced ARCC for the ARP while for the CSU the difference was greater. This is partially an effect of the number of variables in the database (95 for CSU, 115 for combined ARP/CSU cf. 20 for ARP).

## **UNKNOWN SAMPLE ISOLATES**

Unknown source sample isolates were analyzed using by six-way classification using the 2005 human/sewage ARA + CSU library as this provided the highest RCCs for the six-way classification.

Six-way classifications found relatively similar proportions of human source isolates as the two-way analysis. (Table D-3; Figures D-1 to D-18) Isolates identified as other animal sources varied by sample. It should be noted that Discriminant Analysis identifies a source for each isolate so isolates from sources other than those included in the library will identify as one of those in the library. For example, the lack of wildlife in the library will result in any wildlife isolates being classified perhaps as bird or bat. There were a limited number of isolates for each water/sediment sample so small differences in percent may only reflect a difference of one or two isolates.

Table D-3 Six-way percent classification of unknown source isolates from each sample as Human (Sewage), Bird, Bat, Cow, Horse or Dog identified using the library including the 2005 sewage only.

Date received	Sample ID	Matrix	%Isolates					
			Human / Sewage	Bird	Cow	Horse	Dog	Bat
8/3/2004 (Dry Weather)	1	Water	17.4	23.9	6.5	10.9	37	4.3
		Sediment	24.1	24.1	3.4	20.7	10.3	17.2
	2	Water	15	35	15	2.5	27.5	5
		Sediment	18.8	21.9	6.3	18.8	3.1	31.3
	3	Water	5.3	42.1	5.3	21.1	15.8	10.5
		Sediment	16.3	20.4	2	8.2	28.6	24.5
7	Water	18.2	13.6	18.2	13.6	18.2	18.2	
	Sediment	20.7	6.9	6.9	20.7	37.9	6.9	
3/16/2005 (Dry Weather)	4	Water	43.3	3.3	18.3	30	5	0
	5	Water	31.9	10.6	10.6	21.3	10.6	14.9
		Sediment	33.3	6.1	3	21.2	15.2	21.2
	9	Water	58.8	3.9	13.7	5.9	7.8	9.8
10	Water	59.1	2	8.2	12.2	10.2	8.2	
4/26/2005 (Wet Weather)	7	Water	42.9	21.4	7.1	8.9	1.8	17.9
	8	Water	41.5	17	3.8	18.9	9.4	9.4
	9	Water	77.6	10.2	0	2	4.1	6.1
	10	Water	52.6	8.8	3.5	8.8	15.8	10.5
5/17/2005 (Dry Weather)	11	Sediment	74.5	2.1	0	17	2.1	4.3
7/13/2005 (Wet Weather)	5	Water	51.9	7.7	15.4	15.4	3.8	5.8
7/14/2005 (Wet Weather)	11	Water	67.3	7.7	3.8	11.5	1.9	5.8

\*as described on chain of custody forms received from UH with the samples