

PULSENET STANDARD OPERATING PROCEDURE FOR ANALYSIS OF MLVA DATA OF SHIGA TOXIN-PRODUCING <i>ESCHERICHIA COLI</i> O157 (STEC O157) AND <i>SALMONELLA ENTERICA</i> SEROTYPES TYPHIMURIUM AND ENTERITIS IN BIONUMERICS-APPLIED BIOSYSTEMS GENETIC ANALYZER 3130/3500 DATA	CODE: PND16		
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1. PURPOSE: To describe the standardized protocol for analysis of MLVA data of Shiga toxin-producing *Escherichia coli* O157 (STEC O157) and *Salmonella enterica* serotypes Typhimurium and Enteritidis in BioNumerics.

2. SCOPE: To provide the PulseNet participants with a single protocol for analyzing MLVA data of STEC O157 and *Salmonella enterica* serotypes Typhimurium and Enteritidis, thus ensuring inter-laboratory comparability of the generated results.

3. DEFINITIONS:

3.1 **MLVA:** Multiple-locus variable-number tandem repeat analysis

3.2 **VNTR:** Variable-number tandem repeat

3.3 **CDC:** Centers for Disease Control and Prevention

3.4 **SOP:** Standard Operating Procedure

4. RESPONSIBILITIES/PROCEDURE

4.1 Software needed for data analysis

4.1.1 BioNumerics version 4.5 or higher

4.1.2 Customized scripts for data import ([VNTRImport_v4.bns](#)), copy number calculation (= allele assignment) ([VNTRCalc_v4.bns](#)), troubleshooting ([VNTRReport.bns](#)) and support functions ([VNTRDetails_v2.bns](#)).

4.1.3 Organism specific assay look-up tables ([ABIEcoli.txt](#), [ABIST.txt](#), [ABISE.txt](#)) for allele size ranges

4.2 General overview

4.2.1 The import process consists of three major steps:

4.2.1.1 Exporting the appropriate data file from the Genetic Analyzer system (see laboratory protocols PNL23 and PNL28 step 4.10.1.15).

4.2.1.2 Importing this file into BioNumerics (using the script [VNTRImport_v4.bns](#)).

4.2.1.3 Determining the copy numbers (assigning alleles) for each VNTR (using the script [VNTRCalc_v4.bns](#) and the organism specific look-up table [ABIEcoli.txt](#), [ABIST.txt](#), or [ABISE.txt](#)).

4.2.1.3.1 [VNTRCalc_v4.bns](#) script facilitates the allele assignment in two different ways:

4.2.1.3.1.1 “ABIEcoli, ABIST, and ABISE” – assign the copy number based on the look-up tables (PND16-1a-c, respectively).

4.2.1.3.1.2 “Predicted” – calculates the copy number based on the mathematical formula: (Observed fragment size – offset)/repeat size (not an option for the ABI data)

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4.3 Required import format

4.3.1 The data should be exported from the GeneMapper software as a txt file (tab-delimited text file) containing the fragment length information. The following columns should appear in the exported table in the following order from left to right: “Dye/Sample peak”, “Sample File Name”, “Marker”, “Allele”, “Size”, “Height”, “Area”, “Data point”. The field “Sample File Name” should contain information designating the BioNumerics key number (isolate identifier) loaded in each lane of the sequencer, as well as which VNTR mastermix (R1 or R2) was loaded. The import script will ignore any text that appears beyond the dot “.” in the “Sample File Name” field.

4.3.1.1 **NOTE:** no spaces or underscores are allowed between the strain ID and the reaction ID (R1 or R2) or between the reaction ID and the dot “.” in the RN field.

4.4 Setting up a new database

- 4.4.1 Click “Create New Database” icon to set up a new database.
- 4.4.2 Type in the name (for example “O157MLVA” for STEC O157, “STMLVA” for *Salmonella* Typhimurium or “SEMLVA” for *Salmonella* Enteritidis) of your new database and click ”Next”.
- 4.4.3 Select the directory (default on CDC training laptops is [HOMEDIR]\O157MLVA for STEC O157; this will save the data on the C-drive) in which you want to set up the new database. Choose the default “Yes (recommended)” when asked “Do you want to automatically create the required directories?” and then click “Next”.
- 4.4.4 Choose “Yes” to enable creation of log files and click “Finish”.
- 4.4.5 In the appearing “Set up new database” pop-up window, choose “Local database (single user only)” and click “Proceed”.
- 4.4.6 Click “Yes” in the next pop-up confirmation window (“Choosing ‘local database’ will restrict some functionality of the software. Are you sure you want to continue?”) and “Proceed” in the following “Plug-in” pop-up window.
- 4.4.7 Close the newly created database.
- 4.4.8 Go to the directory in which you placed your database at step 4.4.3 (on CDC training laptops: C:\Program Files\BioNumerics\Data), open folder for organism specific assay (for example, “O157MLVA” for STEC O157) and create two subfolders named “Scripts” and “VNTRtables”.
- 4.4.9 Save the four MLVA specific scripts ([VNTRImport_v4.bns](#), [VNTRCalc_v4.bns](#), [VNTRReport.bns](#), [VNTRDetails_v2.bns](#)) in the “Scripts”

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folder and the organism specific assay look-up table (e.g. *ABIEcoli.txt*) in the “VNTRtables” folder.

4.4.10 Open the database. The scripts (except the VNTRDetails script) should now appear under the “Scripts” drop-down menu.

4.5 Importing a peak file for the first time

4.5.1 Review the peak file in Excel: make sure that the ROX labelled molecular size standard ran properly without skipping any peaks. Remove any data (failed reactions, controls, internal ladder) that you don’t want to import in the BioNumerics from the .txt file. Re-name and re-save the modified .txt file with correct naming format either on your hard drive or on a flash drive. For example, a modified file of GA140120 would be saved as GA140120-mod.

4.5.2 In BioNumerics, run the script *VNTRImport_v4* from the “Scripts” drop-down menu. The “Import VNTR peak data” dialog box will appear. From the “Peak file format” drop-down menu, select “ABI GeneMapper peak” file. The script pops up a file dialog box, prompting for the name of the file to be imported. Select the appropriate file and click “OK”. A second dialog box pops up, prompting for a variety of other information:

4.5.2.1 *Fingerprint file name.* This is the name of the fingerprint file that will be used in BioNumerics. Leave this unchanged, unless a file has already been imported with the same name. In this case, change the file name into a new, unique and informative name.

4.5.2.2 *Dyes to import.* The dye R (Rox) contains only reference markers and does not need to be imported. Use this list to select what dyes will be imported; they are B (FAM), G (HEX) and Y (Calred 590). Each dye will be stored in a different file, resulting from appending the dye name to the fingerprint file name.

4.5.2.3 *Assign reference positions.* Leave this option checked.

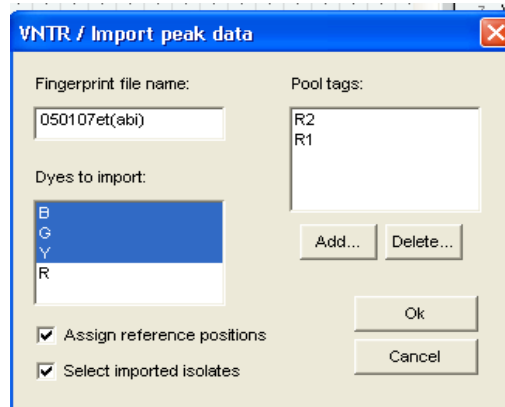
4.5.2.4 *Select imported isolates.* Leave this option checked.

4.5.2.5 *Pool tags.* For this protocol, there are two pool tags representing the two PCR reactions for each isolate (each loaded in different wells) and tagged with names “R1” and “R2“. The two tags should appear in the list. If they do not, they can be added by selecting “add” to update.

4.5.2.6 If everything is filled in appropriately, click “OK” to start the import.

4.5.2.7 **NOTE:** These settings are automatically saved and reloaded the next time this script is run.

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4.5.3 When the script is finished, it has:

- 4.5.3.1 Created a fingerprint type “*VNTRFpr*” with appropriate settings.
- 4.5.3.2 Created fingerprint types with appropriate settings for each dye and each pool used. The names of these fingerprint types are “*VNTRFpr*+”pool name”+”dye” (for example: *VNTRFprR1_Y*).
- 4.5.3.3 Created fingerprint files for each dye in the fingerprint type “*VNTRFpr*”, and imported the band information in these lanes. The individual lanes are assigned to their appropriate pool fingerprint type, according to the known pool tags found in the “Sample File Name” field.
- 4.5.3.4 Created new database entries for all isolates found.
- 4.5.3.5 Linked the fingerprints to their corresponding entries.
- 4.5.3.6 Selected all isolates for which fingerprint data was imported.

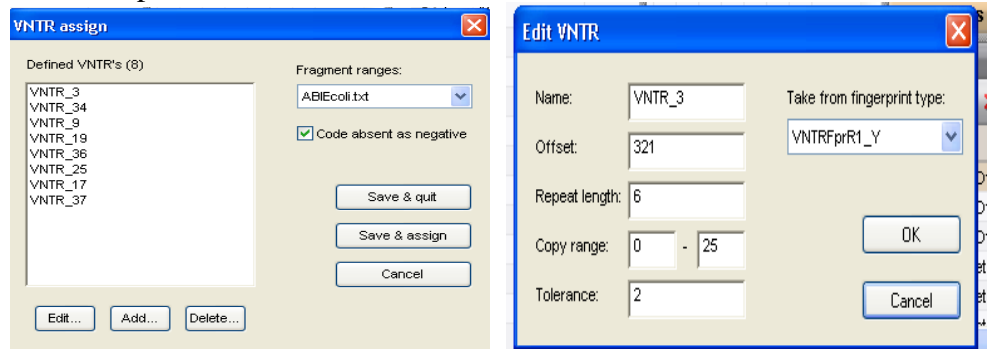
4.6 Determining the copy numbers for the first time (allele assignment)

- 4.6.1 Run the script *VNTRCalc_v4* from the scripts menu. The first time this script is run, it will automatically create a new character set called “*VNTR_vals*”. This character set will hold the copy numbers for each VNTR. The script will also create a second character set called “*VNTR_frgs*” which holds the fragment size with the highest fluorescence for each VNTR. Click “OK” on the two pop up windows notifying that these two character sets will be created.
- 4.6.2 Before the script can determine copy numbers from the fragment length information, it should have sufficient information about what VNTRs can be found in which pool and dye, what are the repeat lengths, etc. To this end, the script pops up a dialog box called “VNTR assign”. This dialog box contains a list of defined VNTRs that is initially empty. To enter the parameters of the first VNTR, click on “Add”. This brings up a second dialog box, prompting for all properties of this first VNTR. Refer to

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Appendices PND16-2a-c for all necessary information to load the specifications for each organism specific VNTR.

Example: STEC O157



4.6.3 The VNTR information includes:

- 4.6.3.1 *Name*. This name will be used for further reference and for the character name in the character type *VNTR_vals*. Each VNTR should have a unique name (for example VNTR_3, VNTR_34, etc).
 - 4.6.3.2 *Offset*. Each fragment consists of a repeat portion and a constant portion, due to the fact that the primers do not locate exactly at the start and the end of the repeat region. This parameter specifies the size (in base pairs) of the constant portion.
 - 4.6.3.3 *Repeat length*. This parameter specifies the size of the repeat block unit (in base pairs).
 - 4.6.3.4 *Copy range*. These parameters specify the minimum and maximum number of copies that the script will consider during the copy number determination.
 - 4.6.3.5 *Tolerance*. This specifies the maximum difference between the expected fragment length (calculated by the software) and the actual length estimated by the sequencer.
 - 4.6.3.6 *Take from fingerprint type*. This drop-down list should be used to indicate on what fingerprint type this VNTR was run. The fingerprint type is determined by the dye and the pool tag.
- 4.6.4 If all parameters are filled in appropriately, click OK to add the VNTR and return to the previous dialog box (“VNTR assign”). Repeat the same actions to enter the information for all VNTRs used for the organism specific database. When all VNTRs are entered, make sure the box for “Code absent as negative” is checked. Select appropriate assay specific look-up table (For example, *ABIEcoli*” for STEC O157) from the “Fragment ranges” drop-down menu and press the “Save&Assign” button to let the script assign copy numbers for the currently selected entries.

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- 4.6.4.1 **NOTE 1:** Pressing “Save&Quit” would store the information without assigning copy numbers for the current selection.
- 4.6.4.2 **NOTE 2:** All information regarding the VNTRs is stored with the database. The next time the script is run, the VNTR definitions will be loaded automatically.
- 4.6.4.3 For every fragment with the highest fluorescence level in the fingerprint type (= the combination of the reaction and the dye), the script will assign an allele type (copy number) based on the fragment size ranges specified in the appropriate look-up table (appendices PND16-1a-c). Note that, within the same fingerprint type, more than one VNTR can be loaded if there is no overlap within the fragment size ranges.
- 4.6.5 When the script has completed, all copy numbers for all VNTRs for the currently selected entries are determined.
 - 4.6.5.1 **NOTE:** Two types of problems may arise during the process (if one or more such errors were encountered during the calculations, an error report is displayed listing all the problems):
 - 4.6.5.1.1 *None of the peaks present in a fingerprint are compatible with the fragment size ranges in the look-up table.* In this case, the corresponding character value will be scored “-2.0”. In this situation, run the “*VNTRReport*” script from the “Scripts” drop-down menu to verify the reason for the problem:
 - 4.6.5.1.1.1 No amplification (a null allele): mutations, insertions or deletions in the primer annealing region, or locus located on a plasmid that was lost. PCR needs to be repeated only if the null allele occurred in a locus in which null alleles have not been previously detected (For STEC O157: VNTR34, VNTR25, VNTR17; and in the case of VNTR19 for which null alleles are extremely rare, and hence should always be confirmed. For *Salmonella* serotype Typhimurium: ST2, ST3, ST6, ST7 and in the case of ST8 for which null alleles are extremely rare, and hence should always be confirmed. For *Salmonella* serotype Enteritidis: VNTR5, VNTR8, VNTR9; and in the case of SE6 for which null alleles are extremely rare and hence always need to be confirmed).
 - 4.6.5.1.1.2 Fragment size outside the acceptable range (either slightly outside of range for a previously detected allele or a possible new allele). Rerun the fragment analysis reaction to confirm the accuracy and reproducibility of the sizing. The isolate should be submitted to CDC for

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confirmation only if a possible new allele is detected. If the sizing is reproducibly slightly outside the range for an existing allele, the CDC database managers will adjust the look-up table based on the evidence the submitting laboratory provides.

- 4.6.5.1.2 *More than one peak in a fingerprint is compatible with an acceptable fragment size range.* In this case, the script will use the solution that corresponds to the peak with the largest “peak height” value. Possible causes for multiple peaks:
 - 4.6.5.1.2.1 Primer stutter: multiple peaks with sizes 1-2 bp from each other. No further action required.
 - 4.6.5.1.2.2 Double alleles: two peaks differing by one or more full repeats from each other. Further actions required for all other loci except *Salmonella* serotype Enteritidis locus SE8 (in a subset of strains double alleles are known to occur and the major (larger) allele always has the highest fluorescence):
 - 4.6.5.1.2.2.1 Repeat the PCR with a freshly made template. If two peaks still observed and the same peak consistently has the highest fluorescence intensity, report the predominant peak only. If the same peak does not consistently have the highest fluorescence intensity, proceed to the next step.
 - 4.6.5.1.2.2.2 Test ten single colony picks from the culture. Report the peak that has the highest fluorescence intensity in the majority of the picks.

4.7 Analyzing a peak file on a routine basis

- 4.7.1 Review the peak file: make sure that the ROX labelled molecular size standard ran properly without skipping any peaks. Remove any data (failed reactions, controls, internal ladder) that you don’t want to import in the BioNumerics from the txt file. Re-name and re-save the txt file either on your hard drive or on the flash drive.
- 4.7.2 In BioNumerics, run the script *VNTRImport_v4* from the “Scripts” drop-down menu. The “Import VNTR peak data” dialog box will appear. From the “Peak file format” drop-down menu, select “ABI GeneMapper peak file”. The script pops up a file dialog box, prompting for the name of the file to be imported. Select the appropriate file and click “OK”. “VNTR/Import peak data” dialog box will appear. De-select “R” from the “Dyes to import” and click “OK”.
- 4.7.3 Run the script *VNTRCalc_v4* from the scripts menu. “VNTR assign” dialog box will appear. Select appropriate look-up table (For example, “*ABIEcoli*”

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for STECO157) from the “Fragment ranges” drop-down menu and click “Save&Assign” to assign allele numbers.

4.8 Verifying the allele assignment

4.8.1 For each isolate in the database that has some VNTR data associated, you can click on the “VNTR frags” and “VNTR vals” entries in the list to open the “Entry edit” windows.

Example: STEC O157

CDC_K7370			CDC_K7370		
Character	Value	Mapping	Character	Value	Mapping
VNTR_3	381.0	<+>	VNTR_3	10.0	<+>
VNTR_34	225.4	<+>	VNTR_34	7.0	<+>
VNTR_9	567.8	<+>	VNTR_9	17.0	<+>
VNTR_25	135.5	<+>	VNTR_25	4.0	<+>
VNTR_17	145.2	<+>	VNTR_17	4.0	<+>
VNTR_19	320.7	<+>	VNTR_19	8.0	<+>
VNTR_36	159.3	<+>	VNTR_36	8.0	<+>
VNTR_37	183.7	<+>	VNTR_37	7.0	<+>

4.8.2 If the *VNTRCalc_v4* script did not detect a fragment for a VNTR, refer to the step 4.6.5.1.1 of this protocol for details on how to proceed. If the fragment size is slightly outside the range specified in the look-up table it is possible to manually assign a temporary allele size and type for that VNTR until an official confirmation has been performed by CDC.

4.8.2.1 **Note:** only the CDC database managers are allowed to modify the look-up table *.txt* files. Once a modification has been made, the modified file will be posted on the PulseNet SharePoint site under the QA/QC manual. An automatic e-mail notification will be sent about the change in the SOP.

4.8.2.2 To manually assign an allele size and type, click on the fragment size in the “VNTRfrags” entry edit window or the allele type in the “VNTR vals” entry edit window. This will open the “Change character value” window in BioNumerics lower than v5.0. In BioNumerics v5.0 the allele size or type can be directly highlighted and changed in the entry edit window (STEC O157 screen shots below).

Character	Value	Mapping
VNTR_3	381.9	<+>
VNTR_34	225.4	<+>
VNTR_9	568.0	<+>
VNTR_25	135.4	<+>
VNTR_17	145.1	<+>
VNTR_19	320.6	<+>
VNTR_36	159.2	<+>
VNTR_37	183.5	<+>

Character	Value	Mapping
VNTR_3	10.0	<+>
VNTR_34	7.0	<+>
VNTR_9	17.0	<+>
VNTR_25	4.0	<+>
VNTR_17	4.0	<+>
VNTR_19	8.0	<+>
VNTR_36	8.0	<+>
VNTR_37	7.0	<+>

4.9 Creating a composite data set to visualize the allele types

4.9.1 In order to display copy numbers next to a dendrogram in a comparison, first create a “*composite data set*” that holds the VNTR data.

4.9.1.1 **NOTE:** The created composite dataset will be automatically saved in the database, and hence only need to be created once before the first analysis in the database.

4.9.1.2 From the “Experiments” drop-down menu, select the option “Create new composite data set...”, enter a name (e.g. *VNTR_cmp*), and click the “OK” button. The “Composite data set ‘VNTR_cmp’” window will appear.

4.9.1.3 Highlight the experiment *VNTR_vals* and from the “Experiment” drop-down menu, select the option “Use in composite data set”. Close the window.

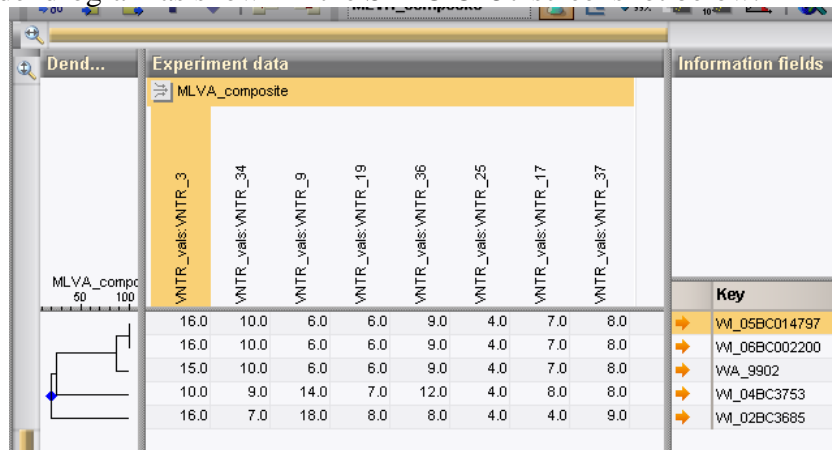
4.9.2 Next time a comparison window is opened (see below step 4.10), there will be a new experiment *VNTR_cmp* listed in the bottom of the window (BioNumerics versions lower than 5.0) or in the top left corner of the window (BioNumerics version 5.0). This experiment will facilitate the display of a spreadsheet-like view of the copy numbers (note that it may be necessary to scroll the experiment list to the right with the arrow button to bring *VNTR_cmp* in display in BioNumerics versions lower than 5.0). This can be shown next to a dendrogram analysis of the data set.

4.10 Performing comparisons based on the VNTR data

4.10.1. The VNTR data contained in the character set *VNTR_vals* can be analysed in BioNumerics with all the tools that are available for character data. That includes cluster analysis with a variety of methods and similarity coefficients. For VNTR data, the coefficients that make most sense are:

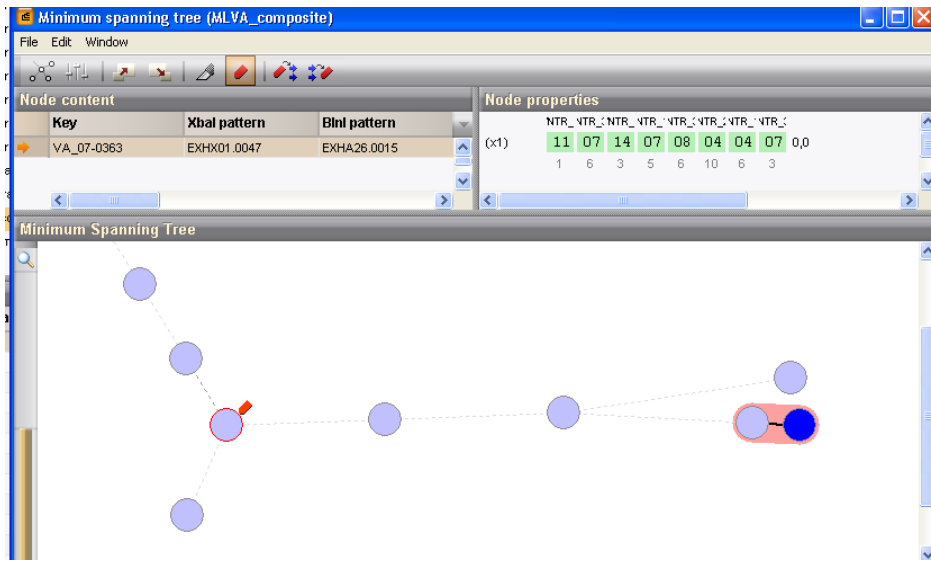
4.10.1.1 *Categorical*: preferred if differences in copy numbers should be treated in a qualitative way. This is the only option for creating dendrograms using MLVA data.

- 4.10.1.2 *Manhattan*: preferred if differences in copy numbers should be treated in a quantitative way (a larger difference means more distantly related organisms). This coefficient can be used to construct minimum spanning trees.
- 4.10.2 In order to create a dendrogram:
 - 4.10.2.1 Select the isolates to be included in the dendrogram.
 - 4.10.2.2 From the “Comparison” drop-down menu, select the option “Create new comparison” and a “Comparison” window will appear.
 - 4.10.2.3 Select “VNTR_cmp” from the bottom of the window in BioNumerics versions lower than 5.0 or from the top left “Experiments” window in BioNumerics 5.0.
 - 4.10.2.4 From the “Clustering” drop-down menu, select the option “Calculate...Cluster analysis (similarity matrix)” and a “Composite data set comparison” dialog box will appear.
 - 4.10.2.5 Select “Categorical” for “Multi-state coefficient” and “UPGMA” for “Dendrogram type”.
 - 4.10.2.6 Click on “OK” button to finish the calculations and the “Comparison” window with the dendrogram will reappear.
 - 4.10.2.7 From the “Layout” drop-down menu, select the option “Show image”.
 - 4.10.2.8 From the “Composite” drop-down menu, select the option ”Show quantification (values)” and the copy numbers will appear next to the dendrogram as shown in the STEC O157 screenshot below.



- 4.10.3 In order to create a minimum spanning tree:
 - 4.10.3.1 Select the isolates to be included in the spanning tree.
 - 4.10.3.2 From the “Comparison” drop-down menu, select the option “Create new comparison” and a “Comparison” window will appear.
 - 4.10.3.3 Select “VNTR_cmp” from the bottom of the window in BioNumerics versions lower than 5.0 or from the top left “Experiments” window in BioNumerics 5.0.

- 4.10.3.4 From the “Clustering” drop-down menu, select the option “Calculate...Minimum spanning tree (population modeling)” and a “Minimum spanning tree” dialog box will appear.
- 4.10.3.5 Make sure the default “Manhattan” is checked for “Coefficient” and click “OK” and the “Minimum spanning tree” window with the tree will appear.
- 4.10.3.6 You can find out the content for each node by clicking on them individually.



5. FLOW CHART:

6. REFERENCES:

6.1 Hyytia-Trees, E., Lafon, P., Vauterin, P., and Ribot, E. (2010) Multi-laboratory validation study of standardized multiple-locus VNTR analysis (MLVA) protocol for Shiga toxin-producing *Escherichia coli* O157 (STEC O157): a novel approach to normalize fragment size data between capillary electrophoresis platforms. *Foodborne Path. Dis.* 7, 129-136.

7. CONTACTS:

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8. AMENDMENTS:

4/9/2013: appendix PND16-2 (BioNumerics specifications for the STEC O157 VNTR loci) was added.

4/9/2013: Additions to step 4.6.5: more clarification for reasons for error messages that occur upon allele assignment and guidance for further follow-up for these errors.

2/26/2014: Merged the SOPs for STEC O157 (PND16), *Salmonella* serotypes Typhimurium (PND17) and Enteritidis (PND18) Analysis for Applied Biosystems Genetic Analyzer 3130/3500 Data into one protocol.

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Appendix PND16-1a

**STEC O157 VNTR allele list and corresponding observed fragment size ranges in
the Applied Biosystems Genetic Analyzer 3130/3500
(*ABIEcoli* look-up table)**

Note: This table is posted on the PulseNet SharePoint under the QA/QC manual as a TXT-file so users may save it locally and use it with the BioNumerics MLVA scripts. Every time the table is updated, the latest version is posted on SharePoint site.

Count	R1				R2			
	VNTR_3	VNTR_34	VNTR_9	VNTR_25	VNTR_17	VNTR_19	VNTR_36	VNTR_37
1			474-475	122-124				
2	339-340			127-128	135-136	283-284		
3	343-346		485-486	132-134	140-142	291-292	123-124	157-158
4	349-352	169-171	491-492	138-140	146-148	296-298	129-131	163-165
5	355-358	187-188	495-498	144-146	153-155	302-304	135-137	169-171
6	361-364	205-206	502-504	150-152	159-161	308-310	141-144	175-176
7	367-370	222-224	508-510	156-158	165-167	314-316	150-151	181-183
8	373-376	242-244	514-516	162-164	172-173	320-322	157-158	187-189
9	379-382	260-262	518-522	168-169	177-179	326-328	163-165	192-194
10	385-388	278-280	525-528	174-175	184-185	332-334	170-172	198-200
11	391-394	295-298	530-534		190-191	338-340	177-179	205-206
12	397-401	315-316	536-540		196-197	344-346	184-185	210-212
13	404-406		543-545	191-192	201-203	350-351	191-192	217-218
14	409-412		548-551			356-357	197-199	223-224
15	415-418		554-557		208-209	362-363	204-206	229-231
16	421-426		559-563				211-213	236-237
17	428-430		565-569				218-219	240-244
18	433-435		572-575		225-228		225-226	249-250
19	440-442		578-581				233-234	255-256
20	447-448		584-586				240-242	262-263
21	453-455		590-591		247-248			
22	459-461		596-597					273-274
23	466-467		601-603					
24	472-473		607-608					
25	478-479		613-614					

Appendix PND16-1b

S. enterica serotype Typhimurium VNTR allele list and corresponding observed fragment size ranges in the Applied Biosystems Genetic Analyzer 3130/3500 (*ABIST* look-up table)

Note: This table is posted on the PulseNet SharePoint under the QA/QC manual as a TXT-file so users may save it locally and use it with the BioNumerics MLVA scripts. Every time the table is updated, the latest version is posted on SharePoint site.

Count	R1				R2		
	ST3	ST5	ST7	ST10	ST2	ST6	ST8
0			121-122				
1			128-130			205-206	
2	177-180	146-147	137-139	322-323			
3		149-153	147-151			216-217	
4	188-192	160-164	157-158	340-341		222-226	
5	200-202	166-168	166-167	345-347		229-231	
6		172-174	175-176	351-354		235-236	
7		178-179	184-185	358-360		241-243	
8		184-185	193-194	364-366	169-170	247-250	
9		190-191	202-203	370-372		253-255	298-299
10		195-197	211-212	376-378		259-261	
11		201-203		382-384	210-211	265-267	331-332
12		207-208	220-221			271-273	
13		213-214	229-230	389-390		277-279	
14		219-220	239-240	394-396	256-257	283-285	
15		225-226	250-251	401-402		289-290	364-365
16		231-233	258-259	407-409	271-272	295-296	377-378
17		237-239		413-415	286-287	300-302	391-392
18		244-245		420-421	298-299	306-308	
19		249-251	286-287	424-427	324-325	312-314	403-405
20		256-257	295-296	431-433	327-334	318-320	424-426
21		261-263	304-305	438-439	351-352	324-326	
22		268-269		444-446	362-366	330-332	435-438
23		273-275	321-322	451-453	370-373	336-337	452-458
24		280-281		458-460	377-380	342-343	464-466
25		286-287		464-466	399-401	348-349	471-474
26		292-293			409-410	354-355	491-492

**PULSENET STANDARD OPERATING PROCEDURE FOR ANALYSIS OF
MLVA DATA OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* O157
(STEC O157) AND *SALMONELLA ENTERICA* SEROTYPES TYPHIMURIUM
AND ENTERITIS IN BIONUMERICS-APPLIED BIOSYSTEMS GENETIC
ANALYZER 3130/3500 DATA**

**CODE:
PND16**

Effective Date:
02 | 26 | 14

27				470-472		360-361	497-499
28				477-478	437-439	366-367	503-504
29		315-316		483-484	448-449	372-373	517-518
30				489-491	469-470		530-532
31				495-496		377-378	537-538
32				501-502		389-390	550-552
33				507-508			556-558
34				514-517			561-565
35				520-521			582-584
36				526-527			589-591
37							609-610
38							615-617
39							622-623
40				545-546			

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Appendix PND16-1c

S. enterica serotype Enteritidis VNTR allele list and corresponding observed fragment size ranges in the Applied Biosystems Genetic Analyzer 3130/3500 (*ABISE* look-up table)

Note: This table is posted on the PulseNet SharePoint under the QA/QC manual as a TXT-file so users may save it locally and use it with the BioNumerics MLVA scripts. Every time the table is updated, the latest version is posted on SharePoint site.

Count	R1				R2		
	SE1	SE2	SE8	SE6	SE9	SE3	SE5
1			346-350		173-175		160-161
2	163-166		433-436		181-184		
3	170-172	300-303			190-192	199-203	172-174
4	177-179	308-309.5			199-201	211-214	177-180
5	184-186	310.4-316.2				224-225	184-185
6	190-193	317.5-324.0					189-191
7	198-199	324.9-331.5					195-197
8	204-206	335-339		408-413		259-260	201-203
9	211-213	342-345		446-447			207-209
10	218-219	348-351		479-482		283-284	213-215
11	225-226	356-358					218-221
12	232-234	363-364		533-534			225-228
13	240-241	370-373		566-567			232-234
14				588-589			238-240
15	255-256	384-385					244-247
16							249-253
17	269-270						258-259
18							264-265
19							268-271
20							274-281
21							
22							287-288
23							
24	317-319						
25							
26							
27							

PULSENET STANDARD OPERATING PROCEDURE FOR ANALYSIS OF MLVA DATA OF SHIGA TOXIN-PRODUCING <i>ESCHERICHIA COLI</i> O157 (STEC O157) AND <i>SALMONELLA ENTERICA</i> SEROTYPES TYPHIMURIUM AND ENTERITIS IN BIONUMERICS-APPLIED BIOSYSTEMS GENETIC ANALYZER 3130/3500 DATA	CODE: PND16		
	Effective Date: <table border="1"> <tr> <td>02</td> <td>26</td> <td>14</td> </tr> </table>	02	26
02	26	14	

28							
29							
30	359-360						

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Appendix PND16-2a

BioNumerics specifications for STEC O157 VNTR loci

Reaction	R1	R2
Name	VNTR_3	VNTR_17
Dye	CalRed590 = Y	CalRed590 = Y
Offset	321	120
Repeat length	6	6
Copy range	0-25	0-30
Tolerance	2	2
Fragment size in EDL933	379-382	159-161
Name	VNTR_34	VNTR_19
Dye	FAM = B	FAM = B
Offset	100	272
Repeat length	18	6
Copy range	0-20	0-25
Tolerance	3	2
Fragment size in EDL933	278-280	308-310
Name	VNTR_9	VNTR_36
Dye	FAM = B	FAM = B
Offset	465	102
Repeat length	6	7
Copy range	0-50	0-20
Tolerance	2	2
Fragment size in EDL933	530-534	157-158
Name	VNTR_25	VNTR_37
Dye	HEX = G	HEX = G
Offset	110	142
Repeat length	6	6
Copy range	0-20	0-25
Tolerance	2	2
Fragment size in EDL933	138-140	187-189

Fragment size ranges are based on independent runs on multiple instruments at CDC and in PulseNet Participating Laboratories.

Appendix PND16-2b

BioNumerics specifications for *S. enterica* serotype Typhimurium VNTR loci

Reaction	R1	R2
Name	ST3	ST2
Dye	HEX = G	FAM = B
Offset	156	62
Repeat length	6*	13**
Copy range	0-20	0-40
Tolerance	2	3
Fragment size in LT2	188-192	370-373
Copy number in LT2	4*	23**
Name	ST5	ST6
Dye	FAM = B	CalRed590 = Y
Offset	139	191
Repeat length	6	6
Copy range	0-60	0-50
Tolerance	2	2
Fragment size in LT2	219-220	271-273
Copy number in LT2	14	12
Name	ST7	ST8
Dye	CalRed590 = Y	HEX = G
Offset	115	196
Repeat length	9	11***
Copy range	0-30	0-50
Tolerance	3	3
Fragment size in LT2	157-158	556-558
Copy number in LT2	4	33***
Name	ST10	
Dye	HEX = G	
Offset	311	
Repeat length	6	
Copy range	0-50	
Tolerance	2	
Fragment size in LT2	376-378	
Copy number in LT2	10	

* A half of a repeat difference considered significant. Actual repeat length 12 bp and actual copy number in LT2 2.5
 ** A third of a repeat difference considered significant. Actual repeat length 39 bp and actual copy number in LT2 8.0
 *** A third of a repeat difference considered significant. Actual repeat length 33 bp and actual copy number in LT2 11.0

Fragment size ranges are based on independent runs on multiple instruments at CDC and in PulseNet Participating Laboratories.

Appendix PND16-2c

BioNumerics specifications for *S. enterica* serotype Enteritidis VNTR loci

Reaction	R1	R2
Name	SE1	SE9
Dye	B (FAM)	B (FAM)
Offset	149	165
Repeat length	7	9
Copy range	0-10	0-5
Tolerance	2	2
Fragment size in K1891	190-193	181-184
Copy number in K1891	6	2
Name	SE2	SE3
Dye	Y (Calred 590)	Y (Calred 590)
Offset	280	165
Repeat length	7	12
Copy range	0-20	0-10
Tolerance	2	3
Fragment size in K1891	335-339	211-215
Copy number in K1891	8	4
Name	SE8	SE5
Dye	G (HEX)	G (HEX)
Offset	261	154
Repeat length	86	6
Copy range	0-3	0-25
Tolerance	4	2
Fragment size in K1891	433-436	201-203
Copy number in K1891	2	8
Locus	SE6	
Dye	B (FAM)	
Offset	149	
Repeat length	33	
Copy range	2-20	
Tolerance	3	
Fragment size in K1891	479-482	
Copy number in K1891	10	

Fragment size ranges are based on independent runs on multiple instruments at CDC and in PulseNet Participating Laboratories.