

<b>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 ENTERITIDIS IN BIONUMERICS - BECKMAN COULTER CEQ 8000/8800/GeXP DATA</b>	<b>CODE: PND14</b>		
	Effective Date:		
	02	26	14

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 PulseNet participants with a single protocol for analyzing MLVA data of STEC O157 and *Salmonella* 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 repet

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 Look-up tables (*BeckmanEcoli.txt*, *BeckmanST.txt*, *BeckmanSE.txt*) for allele size ranges

4.2 **General overview**

- 4.2.1 The analysis process consists of three major steps:
  - 4.2.1.1 Exporting the appropriate data file from the Beckman system (refer to SOP PNL19, step 4.10.1.6)
  - 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 *BeckmanEcoli.txt*, *BeckmanST.txt*, *BeckmanSE.txt*)
    - 4.2.1.3.1 *VNTRCalc\_v4.bns* script facilitates the allele assignment in two different ways:
      - 4.2.1.3.1.1 “BeckmanEcoli, BeckmanST and BeckmanSE” – assign the copy number based on the look-up table (PND14-1a-c). This method should be used in all routine analysis.
      - 4.2.1.3.1.2 “Predicted” – calculates the copy number based on the mathematical formula: (Observed fragment size – offset)/repeat size. This method should only be used when a new allele (not specified in the look-up table) is identified

4.3 **Required import format**

- 4.3.1 The data should be exported from the Beckman CEQ/GeXP software as CSV (comma-delimited) text files containing the fragment length information. Multiple columns will be present in the file but only four columns are of interest:

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“RN,” “dye,” “est size (nt)” and “pk height (rfu)”. All other columns will be ignored by the script during import. The field “RN” should contain information designating the BioNumerics key number (isolate identifier) loaded in each well of the sequencer plate run, as well as which VNTR mastermix or reaction was loaded. The import script will ignore any text that appears beyond the dot “.” in the RN field.

4.3.1.1 **NOTE:** no spaces or underscores are allowed between the strain ID and the reaction ID 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”) of your new database and click “Next”
- 4.4.3 Select the directory (default on CDC training laptops is [HOMEDIR]\O157MLVA; this will save the data on the G-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?”. 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 “O157MLVA” 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” folder and the organism specific assay look-up table (e.g. [BeckmanEcoli.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

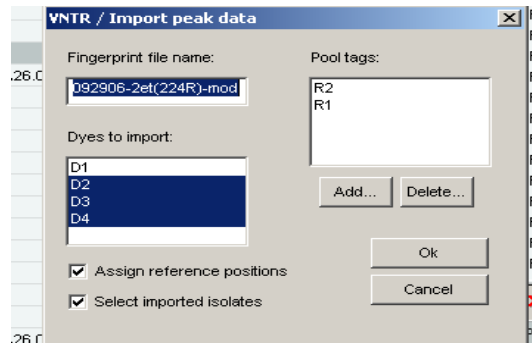
#### 4.5 Importing a peak file for the first time

- 4.5.1 Review the peak file: make sure that the observed size for the D1 labelled molecular size standard peaks is within  $\pm 1$  bp from the expected size. Remove any data (failed reactions, controls, internal ladder) that you don’t want to import in the BioNumerics from the CSV file. Re-name and re-save the CSV file either on your hard drive or on the flash drive.
- 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 “Beckman peak file”. The script pops up a file dialog box, prompting for the name of the file to import. Select the appropriate

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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 first dye (D1) contains only reference markers and does not need to be imported. Use this list to select what dyes will be imported; they are D2, D3 and D4. 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 (all loaded on 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 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.



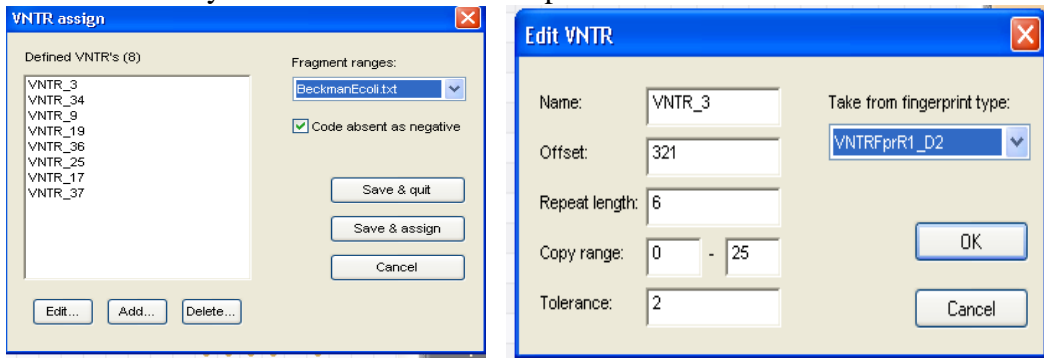
4.5.3 When the script is finished, it has:

- 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\_D2*).
- 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 “RN” 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)**

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- 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\_frags*” 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 types 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 the appendices PND14-2a-c for all necessary information to load the specifications for each VNTR.



- 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 occur 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 a unit repeat block (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.

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- 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 this protocol. When all VNTRs are entered, make sure the box for “Code absent as negative” is checked. Select the appropriate assay specific look-up table (for example “*BeckmanEcoli*” 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.
- 4.6.4.1 **NOTE 1:** pressing “Save&Quit” would store the information without calculating 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 (=a copy number) based on the fragment size ranges specified in the appropriate look-up table (appendices PND14-1a-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 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 observed size for the D1 labelled molecular size standard peaks is within  $\pm 1$  bp from the expected size. Remove any data (failed reactions, controls, internal ladder) that you don’t want to import in the BioNumerics from the CSV file. Re-name and re-save the CSV 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 “Beckman peak file”. The script pops up a file dialog box, prompting for the name of the file to import. Select the appropriate file and click “OK”. “VNTR/Import peak data” dialog box will appear. De-select “D1” 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 the appropriate look-up table (for example “*BeckmanEcoli*” for STEC O157) from the “Fragment ranges” drop-down menu and click “Save&Assign” to assign allele numbers.

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#### 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 of yellow buttons to open the “Entry edit” windows.

Character	Value	Mapping
VNTR_3	381.0 <+>	
VNTR_34	225.4 <+>	
VNTR_9	567.8 <+>	
VNTR_25	135.5 <+>	
VNTR_17	145.2 <+>	
VNTR_19	320.7 <+>	
VNTR_36	159.3 <+>	
VNTR_37	183.7 <+>	

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 <+>	

4.8.2 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 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 5.0. In BioNumerics 5.0 the allele size or type can be directly highlighted and changed in the entry edit window (Screen shots below)

Character	Value	Mapping
VNTR_3	381.0 <+>	
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.

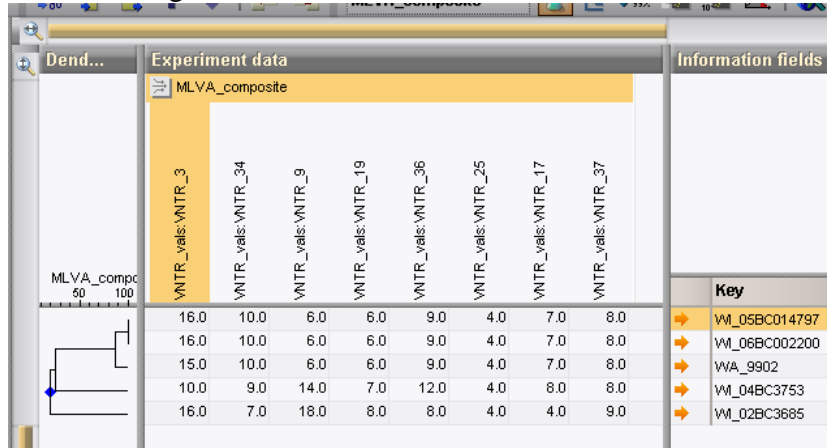
**4.10 Performing comparisons based on the VNTR data**

- 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 (i.e. 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”

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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



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

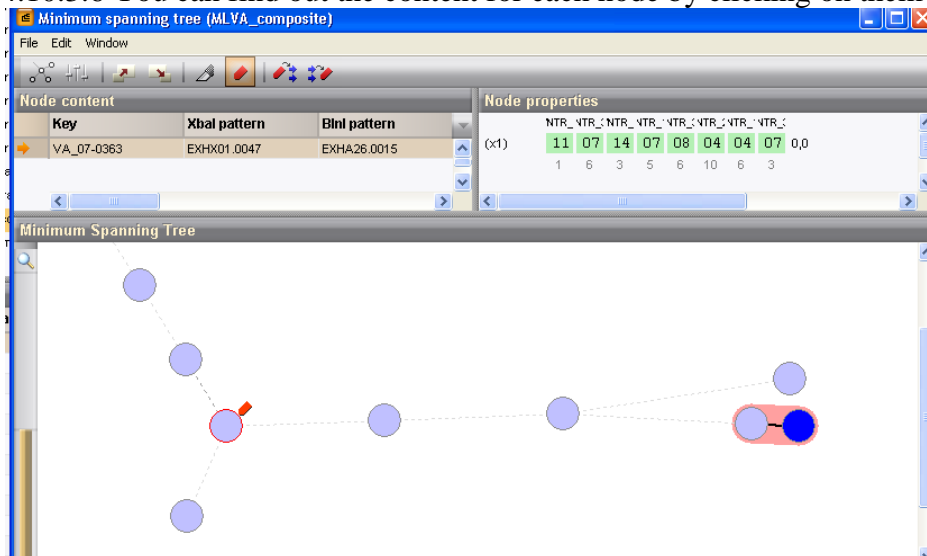
4.10.3.2 From the “Comparison” drop-down menu, select the option “Create new comparison” and a “Comparison” window will appear

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.



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**5. FLOW CHART:**

**6. BIBLIOGRAPHY:**

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**8. AMENDMENTS:**

1/27/2009: the allele (= copy number) assignment will be based on a look-up table listing the actual observed fragment size ranges instead of using the mathematical formula that assigns alleles based on predicted fragment sizes. The look-up table approach in allele assignment will facilitate the comparison of fragment size data from the Beckman Coulter CEQ 8000/8800/GeXP and the Applied Biosystems Genetic Analyzer 3130xl in the same database.

4/8/2013: appendix PND14-2 (BioNumerics specifications for the STEC O157 VNTR loci) was added.

4/8/2013: 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 (PND14), *Salmonella* serotypes Typhimurium (PND15) and Enteritidis (PND19) Analysis for Beckman Coulter CEQ8000/8800/GeXP Data into one protocol.

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**Appendix PND14-1a**  
**STEC O157 VNTR Allele List and Corresponding Observed Fragment Sizes in**  
**the Beckman Coulter CEQ 8000/8800/GeXP (*BeckmanEcoli* look-up table)**

**Note:** This is posted on the PulseNet SharePoint site 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	VNTR_3	VNTR_34	VNTR_9	VNTR_25	VNTR_17	VNTR_19	VNTR_36	VNTR_37
1			471-472	116-119				
2	333-334			122-124	130-133	282-284		
3	339-340	153-154	482-484	128-131	137-140	291-292	123-125	159-161
4	345-346	170-172	488-490	134-136	143-146	296-299	130-132	164-166
5	350-353	188-190	494-497	141-143	149-152	302-304	136-139	170-173
6	356-359	206-209	499-503	146-148	156-159	307-311	143-146	176-179
7	362-365	224-227	506-509	153-154	162-165	314-316	150-153	181-185
8	368-371	241-245	512-515	158-160	169-172	320-322	158-161	187-191
9	373-377	260-262	518-521	165-166	175-178	325-328	165-168	194-197
10	380-383	278-280	524-528	170-171	182-184	332-334	172-175	200-203
11	386-389	295-298	530-534		188-190	337-339	179-182	207-209
12	392-396	313-314	536-540		195-197	343-345	186-189	213-215
13	398-401		543-546	188-190	199-201	349-351	193-196	219-221
14	404-407		549-552			356-357	200-202	225-227
15	410-413		555-558		208-209	361-363	207-210	231-233
16	417-419		561-564				214-216	237-238
17	423-425		566-570				222-223	241-244
18	429-431		572-576		227-228		228-230	249-251
19	435-437		579-582				235-237	255-256
20	442-444		584-587				242-243	261-262
21	448-449		590-594		246-247		250-255	
22	454-456		597-599					273-274
23	460-464		603-605					
24	466-468		608-611					
25	473-474		614-615					
26	476-477							

**Appendix PND14-1b**

***S. enterica* serotype Typhimurium VNTR Allele List and Corresponding Observed Fragment Sizes in the Beckman Coulter CEQ 8000/8800/GeXP (*BeckmanST* look-up table)**

**Note:** This is posted on the PulseNet SharePoint site 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 the SharePoint site.

Count	ST3	ST5	ST7	ST10	ST2	ST6	ST8
0			113-115				
1			124-126			202-203	
2	164-171	146-149	130-136	322-323		207-209	
3		151-153	139-145			213-215	
4	174-182	162-164	151-154	335-336		219-221	
5	187-191	168-170	162-164	341-343		225-227	
6		172-176	172-173	347-349		231-233	
7		179-182	178-183	354-357		234-239	
8		186-188	187-191	360-362	167-168	242-245	
9		191-194	200-201	366-369		247-250	294-296
10		196-199	209-210	373-375		254-257	
11		203-205		379-381	207-209	259-263	326-328
12		208-211	218-219			264-268	
13		214-217	227-229	385-388		272-274	
14		220-223	232-237	392-394	248-252	278-280	
15		226-228	246-247	398-400		284-287	359-360
16		232-234	255-256	404-406	264-266	290-292	373-375
17		238-239	264-265	411-413	279-281	296-298	384-385
18		243-245	273-274	417-419	289-292	302-303	
19		249-251	282-284	424-426	312-316	305-310	399-40
20		255-257	291-293	430-433	319-326	314-315	417-419
21		260-262	301-302	437-439	341-343	320-322	425-426
22		266-268		443-446	351-357	325-327	432-435
23		272-274	319-322	450-452	358-363	331-333	448-452
24		278-279		456-459	365-369	336-339	460-464
25		284-286		463-465	387-392	343-344	471-475
26		290-292			397-407	349-350	485-488
27				470-472		355-357	493-497
28				476-479	425-427	361-362	501-508
29		312-314		483-485	433-438	367-368	512-513
30				489-492		373-374	526-531
31				496-498			532-537
32				503-504		385-386	547-552

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<b>PULSNET 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 ENTERITIDIS IN  BIONUMERICS - BECKMAN COULTER CEQ 8000/8800/GeXP DATA</b>	<b>CODE: PND14</b>		
	Effective Date:		
	02	26	14

33	509-511	553-558
34	516-518	560-565
35	523-526	580-584
36	530-532	587-592
37		599-609
38		610-618
39		622-624
40	551-552	631-636

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**Appendix PND14-1c**

***S. enterica* serotype Enteritidis VNTR Allele List and Corresponding Observed  
 Fragment Sizes in the Beckman Coulter CEQ 8000/8800/GeXP  
 (*BeckmanSE* look-up table)**

**Note:** This is posted on the PulseNet SharePoint site under 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, a latest version of the look-up table is posted on the SharePoint.

Count	SE1	SE2	SE8	SE6	SE9	SE3	SE5
1			344-347		175-176		162-164
2	166-168		433-435		183-186		
3	172-174	295-296			192-195	197-201	171-172
4	179-182	299-303			199-200	209-214	177-179
5	185-189	305-309				224-225	183-185
6	193-196	312-316					189-191
7	200-202	318-322					195-197
8	207-209	325-329		410-413		258-259	201-203
9	214-217	332-335		443-445			207-209
10	222-223	338-342		476-481		280-282	213-215
11	229-230	344-349					219-221
12	236-237	353-354		534-535			225-227
13	244-245	360-365		569-570			231-233
14				593-594			237-239
15	256-257	372-374					243-245
16							249-250
17	270-271						255-257
18							260-263
19							266-268
20							272-274
21							
22							284-285
23							
24	317-318						
24							
25							
26							
27							
28							
29							
30	361-362						

**Appendix PND14-2a  
BioNumerics specifications for STEC O157 VNTR loci**

Reaction	R1	R2
<b>Locus</b>	<b>VNTR_3</b>	<b>VNTR_17</b>
<b>Dye</b>	<b>D2</b>	<b>D2</b>
<b>Offset</b>	321	120
<b>Repeat length</b>	6	6
<b>Copy range</b>	0-25	0-30
<b>Tolerance</b>	2	2
<b>Fragment size in EDL933</b>	373-377	156-159
<b>Copy number in EDL933</b>	9	6
<b>Locus</b>	<b>VNTR_34</b>	<b>VNTR_19</b>
<b>Dye</b>	<b>Quas 670 (D4)</b>	<b>Quas 670 (D4)</b>
<b>Offset</b>	100	272
<b>Repeat length</b>	18	6
<b>Copy range</b>	0-20	0-25
<b>Tolerance</b>	3	2
<b>Fragment size in EDL933</b>	278-280	307-311
<b>Copy number in EDL933</b>	10	6
<b>Locus</b>	<b>VNTR_9</b>	<b>VNTR_36</b>
<b>Dye</b>	<b>Quas 670 (D4)</b>	<b>Quas 670 (D4)</b>
<b>Offset</b>	465	102
<b>Repeat length</b>	6	7
<b>Copy range</b>	0-50	0-20
<b>Tolerance</b>	2	2
<b>Fragment size in EDL933</b>	530-534	158-161
<b>Copy number in EDL933</b>	11	8
<b>Locus</b>	<b>VNTR_25</b>	<b>VNTR_37</b>
<b>Dye</b>	<b>Quas 705 (D3)</b>	<b>Quas 705 (D3)</b>
<b>Offset</b>	110	142
<b>Repeat length</b>	6	6
<b>Copy range</b>	0-20	0-25
<b>Tolerance</b>	2	2
<b>Fragment size in EDL933</b>	134-136	187-191
<b>Copy number in EDL933</b>	4	8

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

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**Appendix PND14-2b**  
**BioNumerics specifications for *S. enterica* serotype Typhimurium VNTR loci**

Reaction	R1	R2
<b>Locus</b>	<b>ST3</b>	<b>ST2</b>
<b>Dye</b>	Quas705 (D3)	Quas670 (D4)
<b>Offset</b>	156	62
<b>Repeat length</b>	6*	13**
<b>Copy range</b>	0-20	0-40
<b>Tolerance</b>	2	3
<b>Fragment size in LT2</b>	174-182	358-363
<b>Copy number in LT2</b>	4*	23**
<b>Locus</b>	<b>ST5</b>	<b>ST6</b>
<b>Dye</b>	Quas670 (D4)	D2
<b>Offset</b>	139	191
<b>Repeat length</b>	6	6
<b>Copy range</b>	0-60	0-50
<b>Tolerance</b>	2	2
<b>Fragment size in LT2</b>	220-223	264-268
<b>Copy number in LT2</b>	14	12
<b>Locus</b>	<b>ST7</b>	<b>ST8</b>
<b>Dye</b>	D2	Quas705 (D3)
<b>Offset</b>	115	196
<b>Repeat length</b>	9	11***
<b>Copy range</b>	0-30	0-50
<b>Tolerance</b>	3	3
<b>Fragment size in LT2</b>	151-154	553-558
<b>Copy number in LT2</b>	4	33***
<b>Locus</b>	<b>ST10</b>	
<b>Dye</b>	Quas705 (D3)	
<b>Offset</b>	311	
<b>Repeat length</b>	6	
<b>Copy range</b>	0-50	
<b>Tolerance</b>	2	
<b>Fragment size in LT2</b>	373-375	
<b>Copy number in LT2</b>	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.

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**Appendix PND14-2c**

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

<b>Reaction</b>	<b>R1</b>	<b>R2</b>
<b>Locus</b>	<b>SE1</b>	<b>SE9</b>
<b>Dye</b>	Quas670 (D4)	Quas670 (D4)
<b>Offset</b>	152	166
<b>Repeat length</b>	7	9
<b>Copy range</b>	0-20	0-5
<b>Tolerance</b>	2	2
<b>Fragment size in K1891</b>	193-196	183-186
<b>Copy number in K1891</b>	6	2
<b>Locus</b>	<b>SE2</b>	<b>SE3</b>
<b>Dye</b>	D2	D2
<b>Offset</b>	271	165
<b>Repeat length</b>	7	12
<b>Copy range</b>	0-20	0-10
<b>Tolerance</b>	2	3
<b>Fragment size in K1891</b>	325-329	209-214
<b>Copy number in K1891</b>	8	4
<b>Locus</b>	<b>SE8</b>	<b>SE5</b>
<b>Dye</b>	Quas705 (D3)	Quas705 (D3)
<b>Offset</b>	260	155
<b>Repeat length</b>	86	6
<b>Copy range</b>	0-3	0-25
<b>Tolerance</b>	4	2
<b>Fragment size in K1891</b>	433-435	201-203
<b>Copy number in K1891</b>	2	8
<b>Locus</b>	<b>SE6</b>	
<b>Dye</b>	Quas670 (D4)	
<b>Offset</b>	148	
<b>Repeat length</b>	33	
<b>Copy range</b>	0-15	
<b>Tolerance</b>	3	
<b>Fragment size in K1891</b>	476-481	
<b>Copy number in K1891</b>	10	

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

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