



BIONUMERICS Tutorial:

Setup of an MLVA scheme

1 Introduction

In this tutorial, we will illustrate the installation of the MLVA plugin and the setup of an MLVA scheme in a new database.

2 Creating a new database

To illustrate the complete setup of the *MLVA plugin* we will start by creating a new, empty database.

1. Double-click on the BIONUMERICS icon () on the desktop.
2. In the *BIONUMERICS Startup* window, press the  button to enter the *New database* wizard.
3. Enter a database name, e.g. "MLVA database".
4. Click <**Next**>.

A new dialog box pops up, asking whether to create a new relational database for data storage or to use an existing one.

5. Leave the default option **Create new** enabled and press <**Next**>.

The next dialog asks which database engine should be used for storing data.

6. Select the default option and press <**Finish**>.

The *Main* window opens with an empty database.

3 Installing the MLVA plugin

1. Select **File** > **Install / remove plugins...** () in the *Main* window to call the *Plugins* dialog box.
2. Select the *MLVA plugin* from the list in the *Applications tab* and press the <**Activate**> button.

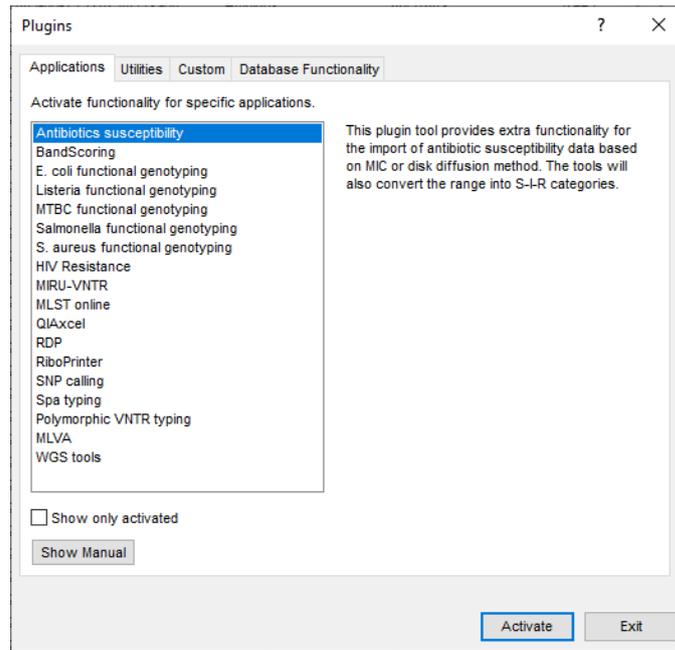


Figure 1: The *Plugins* dialog box.

The program will ask to confirm the installation of the plugin. The installation of the plugin requires administrator privileges on the relational database.

3. Press < **Yes** > to confirm the installation of the *MLVA plugin*.
4. Press < **Yes** > to continue.
5. Press < **OK** >.
6. Close the *Plugins* dialog box by pressing the < **Exit** > button.
7. Close and reopen the database to activate the features of the plugin.

The *MLVA plugin* installs several menu items and buttons in the *Main* window (see Figure 2). In order to visualize the buttons, it might be necessary to select **Window** > **Restore default configuration** from the menu or to restart the software.

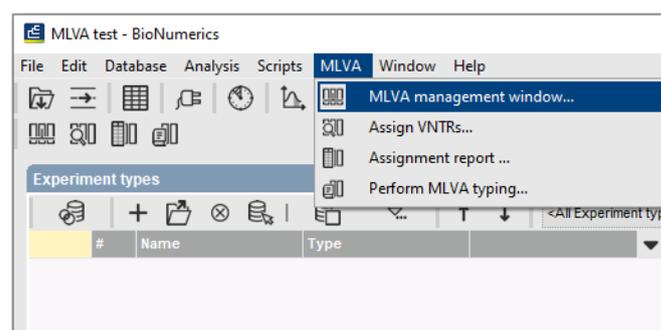


Figure 2: Menu and buttons generated by the *MLVA plugin* in the *Main* window.

4 Setting up an MLVA scheme

1. In the *Main* window, select **MLVA > MLVA management window...** () to call the *Import schemas* dialog box.

There are two options for adding a new MLVA scheme (see Figure 3): either you can create a new MLVA scheme from scratch, or you can import one or more schemes that were defined in another BIONUMERICS database, either by yourself or by somebody else.

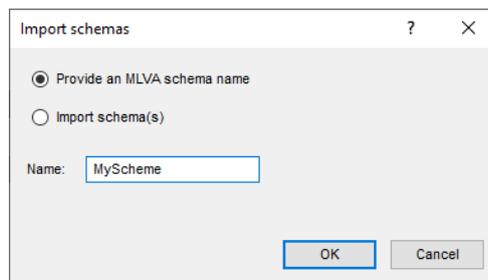


Figure 3: MLVA scheme.

2. In this tutorial we will create a new scheme, so make sure the option **Provide an MLVA schema name** is checked, specify a name (e.g. **MyScheme**) and press **<OK>**.

The character types **[SCHEMA_NAME]_vals** and **[SCHEMA_NAME]_frags** are created automatically in the database. They will be used, respectively, to store the VNTR copy numbers and the molecular sizes (in bp) for each VNTR defined in the schema.

The *MLVA management* window opens. The *Schemas* panel displays a tree-like overview with all MLVA schemas defined in the database. Each schema has at least one mapping ('Theoretical') that corresponds to the theoretical prediction of the VNTR bins. The copy numbers of a VNTR can be theoretically calculated from its metrics values when its offset and repeat size are known.

3. To add a VNTR to a schema, select **Edit > VNTRs > Add VNTR...** (). This action calls the *Add VNTR* dialog box (see Figure 4).

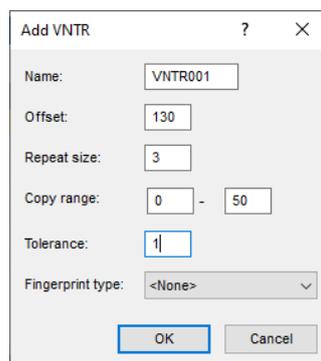
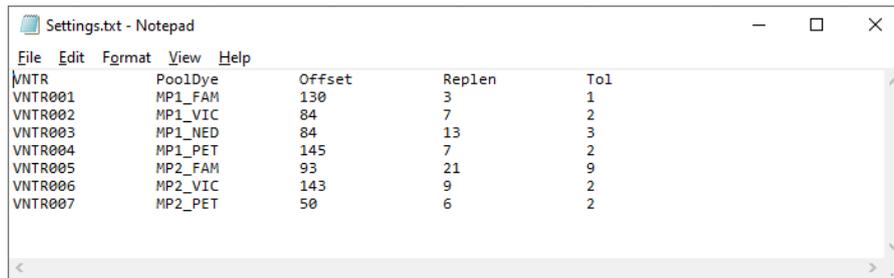


Figure 4: Add a VNTR.

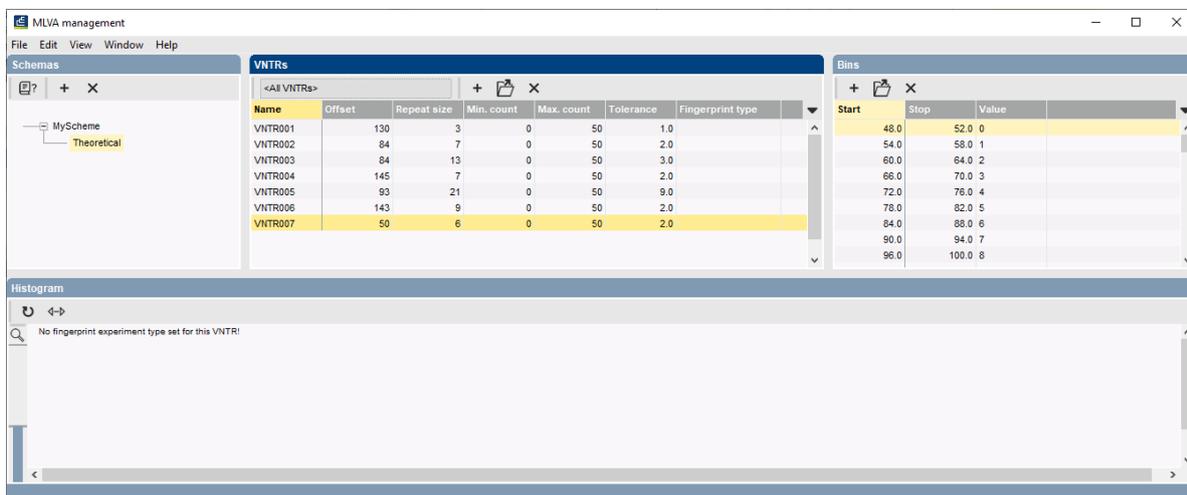
A set of VNTRs and their metric values can be downloaded from the Applied Maths website (<https://www.applied-maths.com/download/sample-data>, click on "VNTR sequencer trace files"). The VNTR settings are stored in the file *Settings.txt* (see Figure 5). Seven VNTR targets per sample were amplified: two pools were generated (**MP1** and **MP2**), containing four and three PCR products respectively, using the color dyes **6-FAM**, **VIC**, **NED**, and **PET**.



VNTR	PoolDye	Offset	Replen	To1
VNTR001	MP1_FAM	130	3	1
VNTR002	MP1_VIC	84	7	2
VNTR003	MP1_NED	84	13	3
VNTR004	MP1_PET	145	7	2
VNTR005	MP2_FAM	93	21	9
VNTR006	MP2_VIC	143	9	2
VNTR007	MP2_PET	50	6	2

Figure 5: VNTR settings.

- Specify the settings for the first VNTR in the *Add VNTR* dialog box (see Figure 4). Specify a copy range from 0 to 50. Since no fingerprint type experiments are yet present in the database, the VNTRs cannot be linked to any experiment. Press <OK> to add the VNTR to the scheme.
- Repeat the previous action to add all seven VNTRs to the scheme (see Figure 6).



The screenshot shows the MLVA management software interface. The **Schemas** panel on the left shows a tree view with 'MyScheme' and 'Theoretical' highlighted. The **VNTRs** panel in the center displays a table of 7 VNTRs. The **Bins** panel on the right shows a table of VNTR bins for the selected VNTR. The **Histogram** panel at the bottom shows a message: 'No fingerprint experiment type set for this VNTR'.

Name	Offset	Repeat size	Min. count	Max. count	Tolerance	Fingerprint type
VNTR001	130	3	0	50	1.0	
VNTR002	84	7	0	50	2.0	
VNTR003	84	13	0	50	3.0	
VNTR004	145	7	0	50	2.0	
VNTR005	93	21	0	50	9.0	
VNTR006	143	9	0	50	2.0	
VNTR007	50	6	0	50	2.0	

Start	Stop	Value
48.0	52.0	0
54.0	58.0	1
60.0	64.0	2
66.0	70.0	3
72.0	76.0	4
78.0	82.0	5
84.0	88.0	6
90.0	94.0	7
96.0	100.0	8

Figure 6: Scheme with 7 VNTRs.

In the *VNTRs* panel, all defined VNTRs for the highlighted schema in the *Schemas* panel are shown in a grid with their Name, Offset, Repeat size, Minimum count, Maximum count, Tolerance and optionally the associated Fingerprint type.

- Select a VNTR from the list in the *VNTRs* panel.

The *Bins* panel shows the VNTR bins for the highlighted VNTR in the *VNTRs* panel in a grid with their Start and Stop metrics and the Value (i.e. the copy number) (see Figure 6).



Each schema has at least one mapping ('Theoretical') that corresponds to the theoretical prediction of the VNTR bins but in practice, often small deviations from the theoretical metrics are observed. To accommodate for this, the user can define one or more custom mappings in an MLVA schema (to create a new mapping for the highlighted schema in the *Schemas* panel, select **Edit** > **Mappings** > **Add mapping...** (+)). This topic will not be covered in this tutorial. More information can be found in the MLVA plugin manual.

- Close the *MLVA management* window.