BIONUMERICS Tutorial:

Calculating and assigning VNTR copy numbers

1 Preparing the database

1. Create a new database, install the *MLVA plugin* and create an MLVA scheme as described in the tutorial: "Setup of an MLVA scheme".

2. Import and pre-process the VNTR sequencer sample trace files as described in the tutorial "Importing and processing VNTR capillary electrophoresis data".

2 The Management window

1. Click somewhere in the *Database entries* panel to make it the active panel and select **Edit** > **Select all** (**Ctrl+A**) to select the 20 entries in the database.

2. In the *Main* window, select **MLVA** > **MLVA management window...** (▥) to call the *MLVA management* window.

In the *VNTRs* panel, the 7 VNTRs are shown in a grid with their Name, Offset, Repeat size, Minimum count, Maximum count and Tolerance.

3. Double-click on the first VNTR in the grid to call the *Edit VNTR* dialog box for this VNTR.

The associated fingerprint type experiment for each VNTR is provided in the "PoolDye" column in the file `Settings.txt` which can be downloaded from the Applied Maths website (https://www.applied-maths.com/download/sample-data, click on "VNTR sequencer trace files") (see Figure 1).
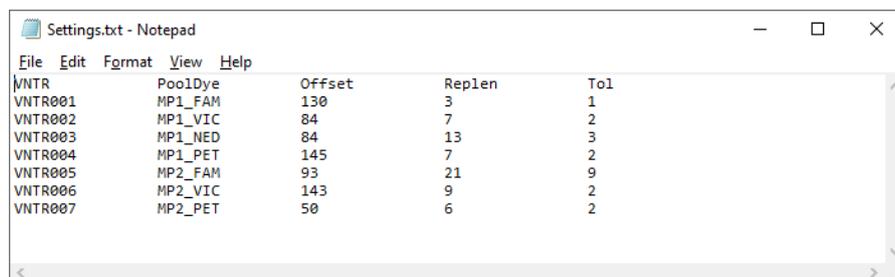
Figure 1: VNTR settings.

4. Select the correct fingerprint type experiment from the list and press <**OK**> to save the changes. Repeat this action for all 7 VNTRs.

The *MLVA management* window should now look like Figure 2.
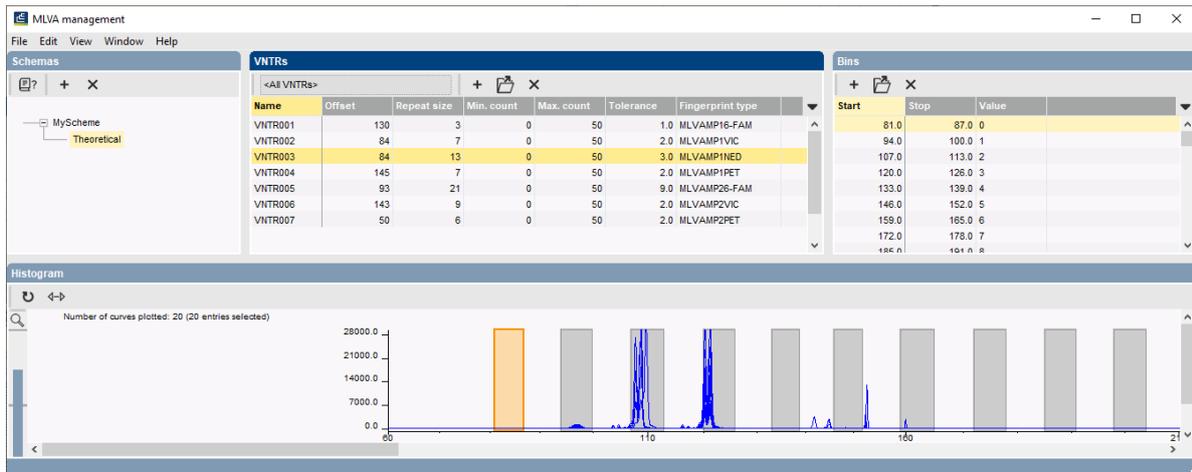
Figure 2: The *MLVA management* window.

In the *Histogram* panel electropherograms for the selected database entries and the corresponding VNTR are plotted, against a backdrop of the VNTR bins for the highlighted VNTR.

     5. Close the *MLVA management* window.

3   VNTR copy number assignment

3.1   Automatic assignment

     1. Make sure all 20 entries are selected in the *Database entries* panel.

     2. Select **MLVA** > **Assign VNTRs...** (⊞).

The *Assign VNTRs* dialog box pops up, listing all schemes and associated mappings defined in the database (see Figure 3).
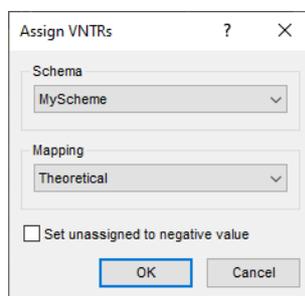
Figure 3: The *Assign VNTRs* dialog box.

Since only one scheme and one mapping is defined in our database, the correct options are automatically selected. Enabling the option **Set unassigned to negative value** will score the absence of a VNTR as negative, rather than absent.

     3. Press <**OK**> to look for VNTRs in the fingerprints of the selected entries.
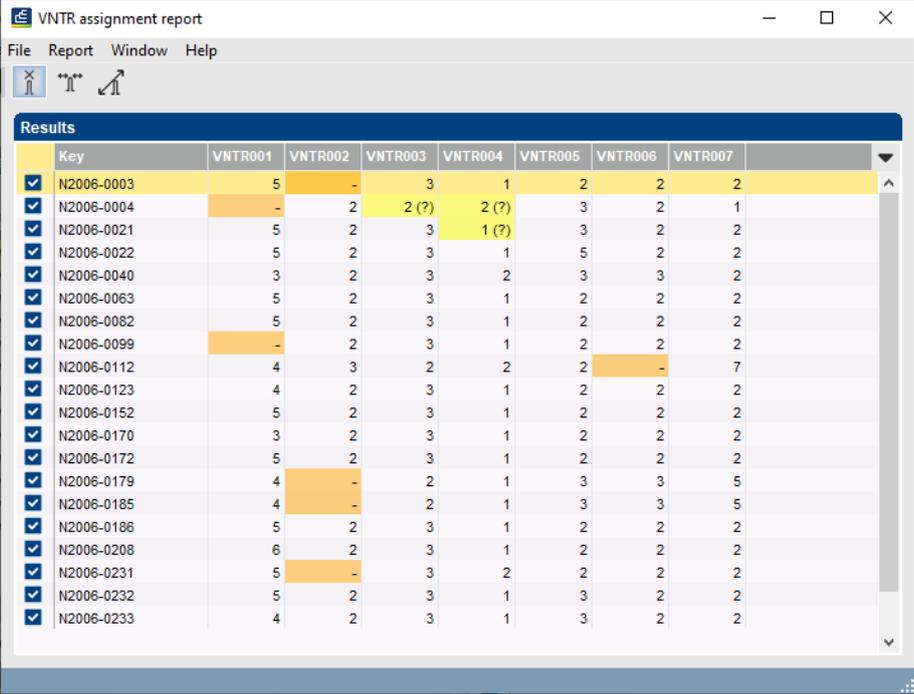
Figure 4: Assignment report.

When the assignment is complete, the *VNTR reporting* window will pop up (see Figure 4).

The *VNTR reporting* window contains a table, listing all selected entries (keys) as rows and all VNTRs from the MLVA scheme as columns. This report window can be called at any time from the *Main* window with **MLVA** > **Assignment report ...** (▯).

Each cell, corresponding to an entry/VNTR combination, provides information about the copy number assignment.

- Any number displayed in a cell corresponds to the copy number determined for the corresponding entry/VNTR combination. When *only* a number is shown, this means that only a single band falls within the expected size ranges (bins) for the selected VNTR mapping and hence that the assignment is unambiguous.

- A number followed by a question mark between brackets (e.g. "2 (?)") means that more than one band in the fingerprint falls within the expected size ranges for the VNTR. The VNTR assignment algorithm selects by default the copy number that corresponds to the band with the largest peak height.

- In case a copy number was manually assigned (see 3.2), the copy number will be followed by "(manual)" to indicate this fact. Please note that double assignments can be only be made manually (e.g. "3/5 (manual)").

- A hyphen ("-") means that no feasible band was found, i.e. none of the bands present in the fingerprint fall within the expected size ranges for the VNTR.

- The text "No data" will be displayed when no fingerprint experiment exists for this entry/VNTR combination.

- The text "N/A" will be displayed when a VNTR assignment has not been performed yet for this entry/VNTR combination.

4. Select ***Report*** > ***View peak heights*** to display the peak heights instead of the copy numbers.

5. Select ***Report*** > ***View peak heights*** again to go back to the default copy number view.

Additional information is visualized via color scales. The default view (***Report*** > ***Show errors*** (🏃)) highlights possible errors: orange when no feasible band is detected and yellow when more than one feasible band is found.

6. Select ***Report*** > ***Show absolute deviation*** (🔝).

The cell colors now provide an indication of the *absolute deviation* of the observed fragment size from the expected fragment size. The cell color ranges between green-yellow-orange-red according to increasing absolute deviation. This makes it easy to detect the magnitude of differences between the observed and expected sizes at a glance.

7. Minimize the *VNTR reporting* window and click on the green dot in the **MyScheme_vals** column of the *Experiment presence* panel to open the character *Experiment card* window for an entry (see Figure 5).

Character	Value	Mapping	▼
VNTR001	5	5	
VNTR002	2	2	
VNTR003	3	3	
VNTR004	1	1	
VNTR005	2	2	
VNTR006	2	2	
VNTR007	2	2	

Figure 5: Character experiment card.

The copy numbers are listed next to the VNTR names.

8. Close the *Experiment card* window by clicking in the small triangle-shaped button in the left upper corner.

3.2 Checking and correcting assignments

9. Double-click on a key/VNTR cell in the *VNTR reporting* window.

This opens the fingerprint profile in the *Fingerprint curve processing* window (see Figure 6 for an example). When the *MLVA plugin* is installed in the database, the *Fingerprint curve processing* window will contain an additional *VNTR assignments panel*. This panel contains a grid with VNTR copy number assignments, identical to the *VNTR reporting* window and with the same display options. In addition, VNTR bins and labels with the assigned copy numbers are drawn on the *Fingerprints* panel, together with the electropherograms.

10. If the VNTR columns are not displayed in the *VNTR assignments panel*, click on the "arrow pointing down" in the upper right corner of the panel and choose ***Restore default settings*** from the list.

11. Select ***Normalization*** > ***Show normalized view*** (⬆, **Shift+N**) to switch to normalized view.
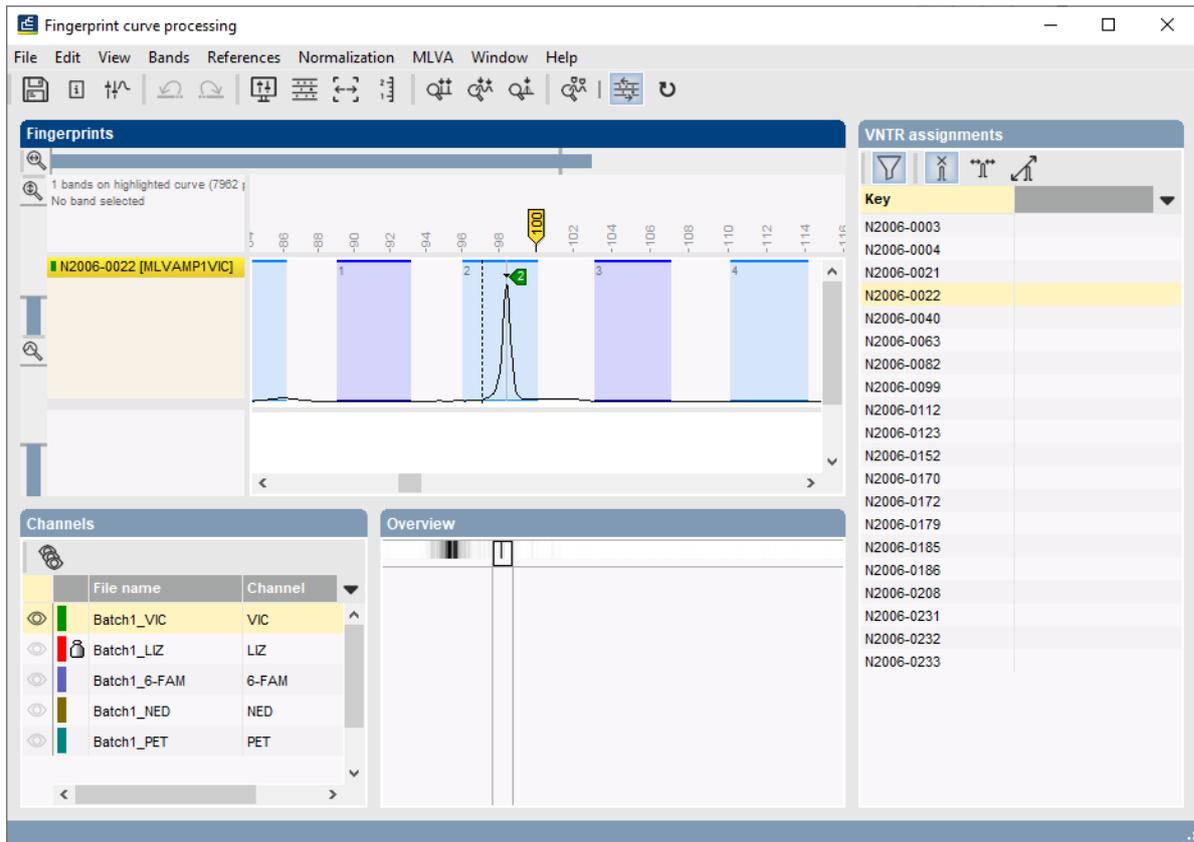
Figure 6: The *Fingerprint curve processing* window for a single electropherogram with VNTR copy number assignment.

The automatic VNTR copy number assignment can be ran again with **MLVA** > **Automatic assignment...**, e.g. to undo any manual assignments to use another mapping.

12. To assign a VNTR copy number for a single band, first select the band using **Ctrl+click** and use **MLVA** > **Manual assignment selected peak (single)** (**Ctrl+M**).

A copy number is assigned that corresponds to the nearest VNTR bin. The copy number will be followed by "(manual)" to indicate that the assignment was made manually (e.g. "2 (manual)").

In some profiles, more than one VNTR peak might occur. The *MLVA plugin* allows you to make double assignments:

13. Select the two bands with **Ctrl+click** and select **MLVA** > **Manual assignment selected peak (double)**.

Copy numbers are assigned that correspond to the nearest VNTR bins. The copy numbers are followed by "(manual)" (e.g. "3/5 (manual)").

14. A VNTR copy number assignment for a selected band can be undone by **MLVA** > **Delete assignment selected peak** (**Ctrl+Shift+Del**).

15. Check the assignments and close the *Fingerprint curve processing* window and *VNTR reporting* window.