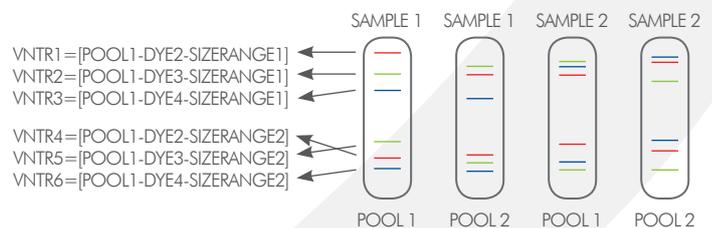# MULTI-LOCUS VNTR ANALYSIS
(MLVA)

What is Multi-Locus VNTR Analysis?

Multi-Locus VNTR Analysis (MLVA) is a method to sub-type microbial isolates based upon the Variable copy Numbers of Tandem Repeats (VNTR). A VNTR typically exhibits a large range of copy numbers, even among highly related bacterial strains. For a selected set of tandem repeats, comparison of the copy numbers between bacterial strains can be used to obtain insight about the relationships at a micro-evolutionary level.

In practice, VNTR loci are selected that are sufficiently and complementary discriminatory for the organisms studied, and conserved primers are designed outside the tandem repeat for each VNTR. Thus, the size in bp of each PCR-amplicon is the sum of the size of the tandem repeat plus the offsets at both ends.
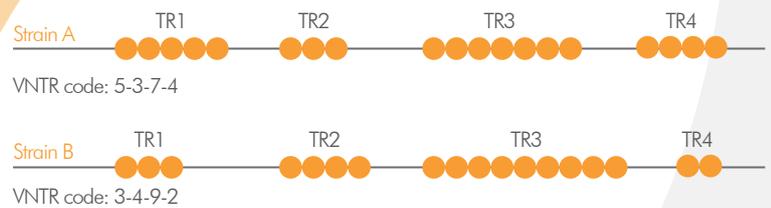
For economical reasons, several VNTRs are sometimes pooled, i.e. they are marked with the same dye and loaded as a mixture in the same column of a capillary sequencer. A condition is that the mixed VNTR PCR products have size ranges that do not overlap. E.g., using 4 dyes and 2 non-overlapping VNTRs, 6 VNTRs can be determined per capillary run (one dye contains a reference marker set for size calculation).


Strain A — VNTR code: 5-3-7-4
Strain B — VNTR code: 3-4-9-2

$$AmpliconSize = OffsetSize + (Copynumber \times RepeatSize)$$

Knowing the repeat size, the copy number can easily be calculated as:

$$\frac{AmpliconSize - OffsetSize}{RepeatSize}$$

VNTR1=[POOL1-DYE2-SIZERANGE1]
VNTR2=[POOL1-DYE3-SIZERANGE1]
VNTR3=[POOL1-DYE4-SIZERANGE1]

VNTR4=[POOL1-DYE2-SIZERANGE2]
VNTR5=[POOL1-DYE3-SIZERANGE2]
VNTR6=[POOL1-DYE4-SIZERANGE2]

SAMPLE 1 — POOL 1
SAMPLE 1 — POOL 2
SAMPLE 2 — POOL 1
SAMPLE 2 — POOL 2

Easy MLVA setup

The MLVA setup is specified in the MLVA management window, which provides direct access to all relevant MLVA settings. Each MLVA schema has its theoretical VNTR bin prediction, based on each VNTR's repeat length, tolerance and offset.

An MLVA schema can have one or more custom mappings to accommodate for deviations in fragment size determination. Distribution plots for individual VNTRs are useful to assess the deviation from theoretical copy numbers and define or refine the VNTR mappings.

When working with more than one capillary sequencer, multiple machine types can be defined. More than one MLVA schema can be used in the same database.
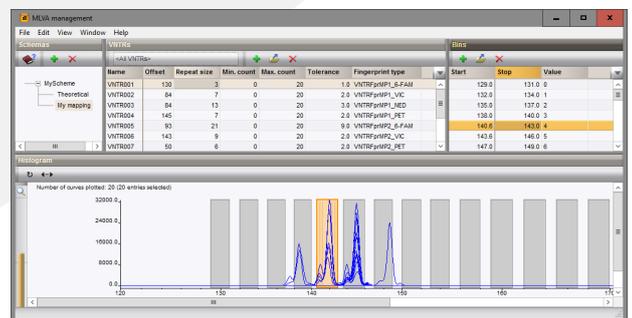
YOUR ADVANTAGES

WHY USE **BioNumerics** FOR YOUR MLVA ANALYSIS?

✓ Easy MLVA setup
✓ Flexible import options
✓ High throughput, detailed reporting
✓ Impressive set of analysis tools

Flexible import options

In case of raw chromatogram files (Applied Biosystems and Beckman), BioNumerics can parse pool, strain and dye information from the file names and file content using an import template defined by the user. In case of GeneMapper or Beckman peak table files, this information is parsed from the tab-delimited peak table. The processing of capillary electrophoresis runs, including peak detection and normalization, can be completed in seconds in the integrated Curve processing window.

High throughput, detailed reporting

Once the settings for VNTRs and parsing have been entered, BioNumerics can automatically process thousands of MLVA runs, thereby creating reports listing unresolved VNTRs, multiple peaks found, and any other problems. Reports can highlight errors, absolute deviations from the expected values and negative and positive deviations.

Double VNTR alleles can be assigned and used in comparison and similarity calculations using a customized similarity matrix.
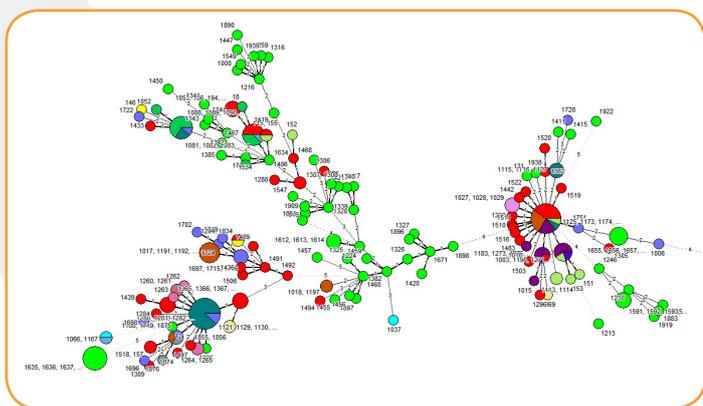
Impressive set of analysis tools

The resulting VNTR information is stored in integer-type character sets where each VNTR represents one character.

Assigning types can greatly facilitate communication e.g. in epidemiologic studies. MLVA types and clonal complexes can be assigned based on the complete set or any subset of VNTRs. To facilitate the use of a uniform and stable nomenclature, MLVA types can be imported and synchronized from external files or URLs.

VNTR data can be analyzed as categorical characters (each different copy number is a different allele) or as quantitative characters. In the latter case, the larger the difference between copy numbers, the less related the organisms are considered.

Population modelling networks can be calculated using the finest and most comprehensive cluster analysis application available today, applying micro-evolutionary criteria as priority rules and displaying branch significance support indication. The Minimum Spanning Tree algorithm applied on multi-locus VNTR data in BioNumerics has proven to be invaluable for epidemiological study and population genetics of bacterial populations.

NOTE: The MLVA plugin is a free add-on. The minimal configuration consists of the "Fingerprint Data", "Character Data" and "Tree and Network Interference" modules. Please contact us for more information.

CONVINCED? INTRIGUED? TRY IT FOR YOURSELF!

www.bionumerics.com
Scan the QR codes to access each step

1. Make sure you have a **BioNumerics** license (also see note above).

2. Watch tutorial movies or download sample data for use in **BioNumerics**.