PulseNet MLVA protocols – General overview

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Multiple-Locus VNTR Analysis (MLVA)

- VNTR = Variable-Number Tandem Repeat

Locus 1
Strain A: VNTR array 4x3
atgggtaatccgtcgACgCACgCACgCgccaatcgatacgat
Strain B: VNTR array 4x5
atgggtaatccgtcgACgCACgCACgCACgCACgCgccaatcgatacgat

Locus 2
Strain A: VNTR array 3x4
ggtaccggtaaagcgcACCACCACCACCttgacactgccggttg
Strain B: VNTR array 3x6
ggtaccggtaaagcgcACCACCACCACCACCACCttgacactgccggttg

Virtually all pro- and eukaryotic genomes contain short repeat sequences that are dispersed throughout the genome. In each repeat sequence locus the repeat copy number can vary between different strains, hence these sequences are often referred to as "variable-number tandem repeats" or VNTRs. When multiple different VNTRs are targeted for analysis, the technology is called "multiple-locus VNTR analysis" or MLVA.

How does MLVA work ?

Primer-F   VNTR array   Offset 2

tgacatgggtaatccgtcgACgCACgCACgCgccaatcgatacgatggcatcg

Offset 1   Primer-R
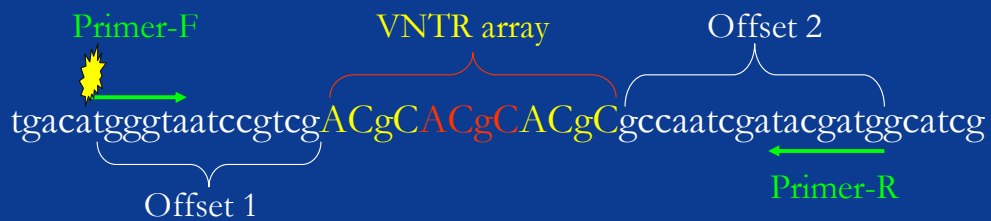
1. Multiplex PCR amplification of the VNTR loci

2. PCR products sized using capillary electrophoresis

3. Data analyzed in BioNumerics

Amplicon size: offset 1+ offset 2 + VNTR array

$$\text{VNTR copy number} = \frac{\text{Amplicon size} - (\text{offset 1} + \text{offset 2})}{\text{repeat size}}$$

In MLVA, the VNTR array is amplified using PCR. Forward primers are fluorescently labeled so that the PCR product can be detected and sized using high resolution capillary electrophoresis (automatic sequencer). Use of multiple different fluorescent tags enables multiplexing targets with overlapping fragment sizes. The fragment size data is exported from the sequencer as a peak file which can be imported into BioNumerics using a dedicated script. A second script is used to assign the copy numbers (allele types) based on the observed fragment sizes.

PulseNet MLVA protocols

Existing protocols
- STEC O157
- *Salmonella* serotype Typhimurium (Group B)
- Supported platforms:
    - Applied Biosystems Genetic Analyzer 3130xl and 3730xl (3500, 3500xl)
    - Beckman Coulter CEQ™8000/8800 (GeXP)

Protocols under validation
- *Salmonella* serotype Enteritidis (Group D)
- *Listeria monocytogenes*

Future
- *Salmonella* serotype Newport
- *Shigella sonnei*
- Non-O157 STEC

Standardized protocols currently available include protocols for STEC O157 and *S. enterica* serotype Typhimurium. Separate protocols for Beckman Coulter and Applied Biosystems platforms have been developed. The newer sequencer versions Genetic Analyzer 3500 and 3500xl and GeXP have not been validated yet so the data generated by those versions may or may not be comparable with the data from the older versions.

Beckman CEQ and Applied BioSystems Genetic Analyzer MLVA protocols – DNA template preparation

- 1-3 colonies of overnight growth in 100 µl of dH_2O
- Vortex to dissolve clumps
- Boil for 10 min
- Spin down 10,000 rpm for 10 min

Beckman CEQ and Applied Biosystems Genetic Analyzer MLVA protocols – PCR procedure

- Two multiplex PCR reactions (R1, R2)
  - dH$_2$O, PCR buffer, MgCl, dNTPs, Taq
  - Primers:
    - CEQ: Forward primers labeled with Quas670, Quas705, and WellRed D2
    - Genetic Analyzer: forward primers labeled with FAM, HEX, and CalRed590
    - Unlabeled reverse primers
  - 1 µl of DNA template in 9 µl of mastermix
  - 35 cycles: 20 s each of denaturation, annealing and extension

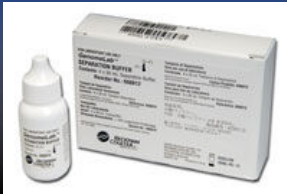## Beckman CEQ MLVA protocols – Fragment analysis reaction set up

- Dilute PCR reactions 1:60 with dH_2O
- Mix GenomeLab SLS and 600 bp DNA size standard → dispense 20 µl per well in a Beckman sample plate
- Add 1 µl of diluted PCR product
- Spin down
- Add 1 drop of mineral oil

Applied Biosystems Genetic Analyzer MLVA protocols – Fragment analysis reaction set up

- Dilute PCR reactions 1:20 with dH_2O
- Mix Hi-Di Formamide and GeneFlo 625 bp size standard → dispense 9 µl per well in a MicroAmp optical reaction plate
- Add 1 µl of diluted PCR product
- Denaturate at 95^oC for 3 min
- Spin down
- Place the plate on a plate base, cover with a rubber septa and insert the plate retainer

Beckman CEQ MLVA protocols – Instrument set up

- Prepare a buffer plate
- Install a gel
- Create a sample plate in the CEQ Sample Plate Set Up module
  - Default "Frag-test" running conditions
- Place the buffer and sample plates in the instrument and start the run

# Applied Biosystems Genetic Analyzer MLVA protocols – Instrument set up

- Install a gel
- Prepare 1x running buffer
- Create a sample plate in the Foundation Data Collection software
  - Default fragment analysis running conditions for the POP7 polymer and 50 cm array
- Place the plate assembly and the buffer in the instrument and start the run

Beckman CEQ MLVA protocols – data export

- View the raw data (chromatograms) for each reaction
- If necessary, filter out background peaks
- Export the peak file in .csv format
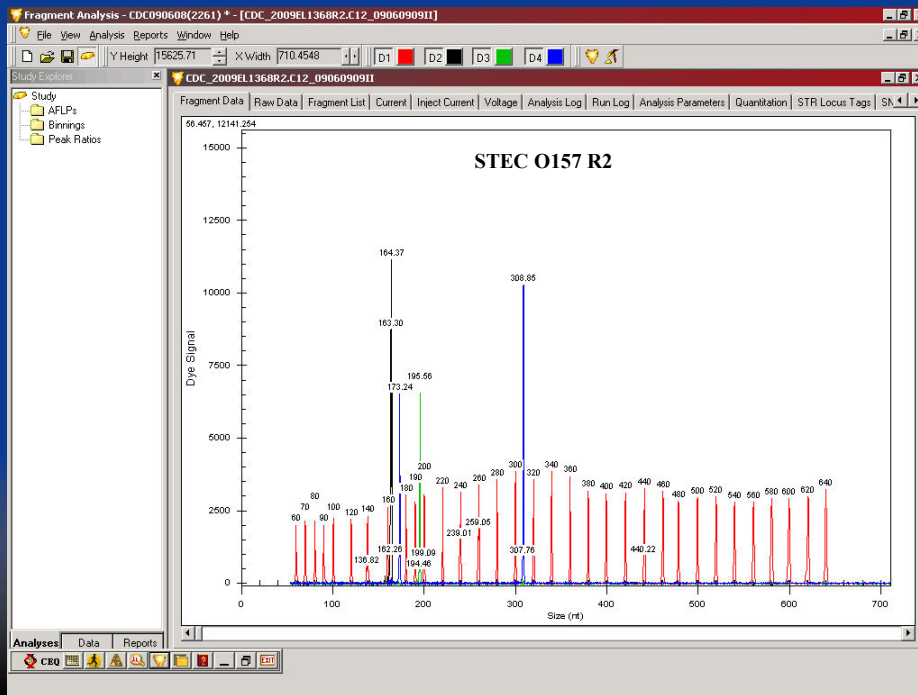
An example of a chromatogram from the Beckman Coulter CEQ platform. The red peaks are the size standard peaks and the blue, green and yellow peaks are the VNTR peaks. The CEQ software labels each peak with the observed size on the chromatogram.

# Applied BioSystems Genetic Analyzer MLVA protocols – data export

- View the raw data (chromatograms) for each reaction
- If necessary, fix sub-optimal DNA size standard profiles (i.e. miscalling of peaks)
- Export the peak file in .txt format

Normal appearance of the GeneFlo 625 size standard
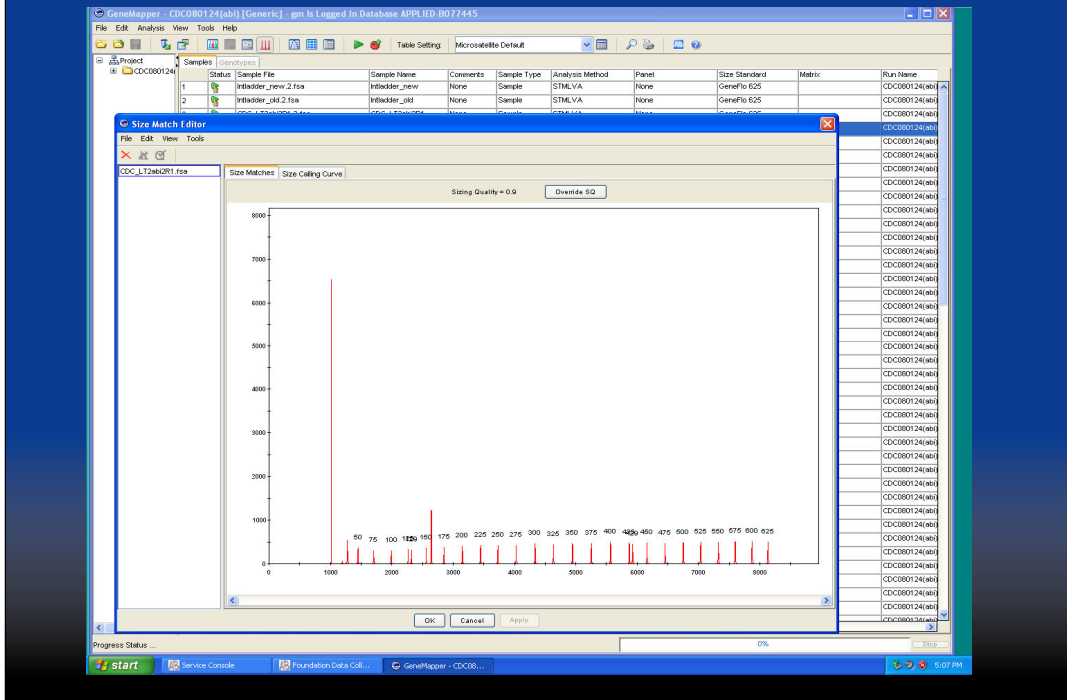
The Size Match Editor view on the Applied Biosystems Genetic Analyzer 3130xl displays the size standard alone. The individual size standard peaks are labeled with size in this view.
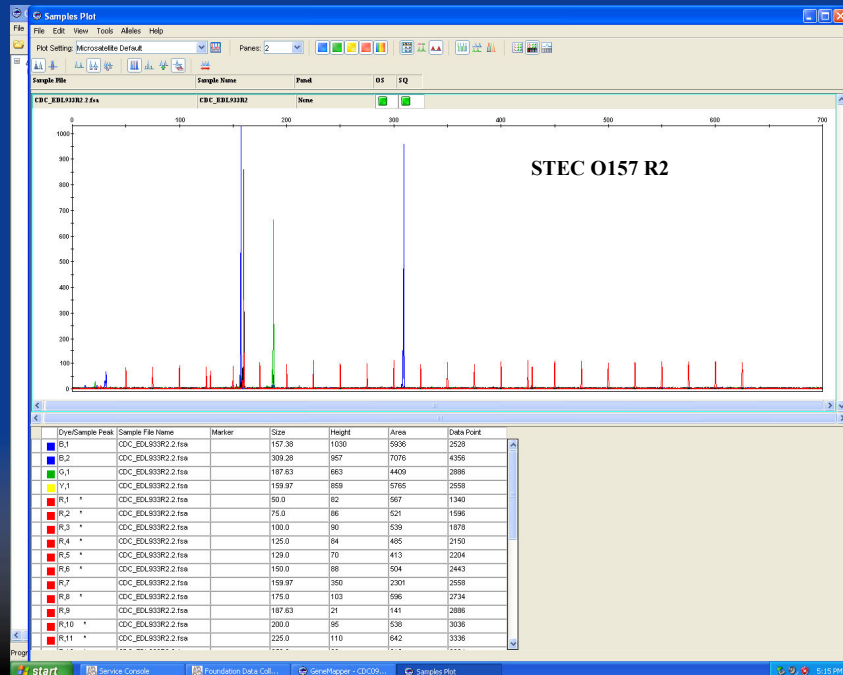
The Display Plots view shows the size standard peaks together with the VNTR peaks without sizes. A separate sizing table can be added to this view.