Validation of the MLPA method by EuroGentest

Introduction

Multiplex Ligation-dependent Probe Amplification (MLPA) is a method to establish the **copy number** of up to 40 different DNA sequences in an easy to perform reaction requiring only 20 ng of human DNA (Schouten et al., 2002). This technology is based on the identification of target sequences by hybridisation of pairs of **MLPA probes** that bind to adjacent sequences and are joined by a **ligation** reaction. The sequences are then simultaneously amplified with the use of only one primer pair, resulting in a mixture of amplification products, in which each PCR product of each MLPA probe has a unique length. These products can be electrophoresed on a capillary sequencer or on a gel, resulting in size separated chromatograms. The peak area or peak height of each amplification product reflects the relative copy number of that target sequence. The comparison of this pattern with a control sample enables the **detection of deletions or duplications**.

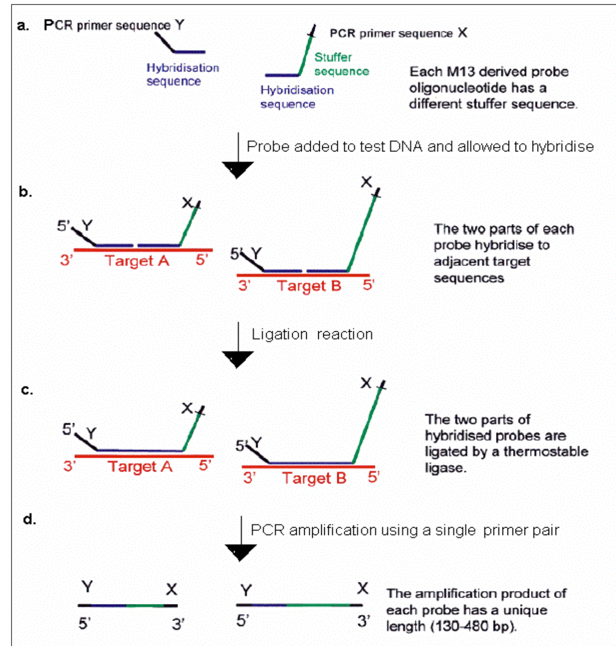

Fig: General overview of the MLPA principle

Aims

So far, no inter-laboratory collaborative programs have been undertaken to fully validate the MLPA method. Within the context of EuroGentest, 10-15 laboratories are willing to cooperate in this study. In addition, MRC-Holland distributes the "BRCA1 MLPA validation kit" to all the participants. DNA samples for positive and negative controls of BRCA1 are coming from NGRL Manchester and UMC Utrecht. They were extracted with the Gentra Autopure LS technology.

The **purpose of this validation** is to:

- ascertain specific performance parameters such as repeatability, reproducibility and uncertainty of measurement.
- evaluate the overall inter-laboratory variability regarding MLPA protocols and data analyses.
- evaluate the performance of the existing Excel sheets and software for the MLPA data analysis.
- help individual users to establish performance data for their own use of the method.

Work flow

In order to achieve these goals, the validation is subdivided in **three different parts**.

1) First of all, the participating laboratories will perform MLPA on **"negative"** DNA samples (without copy number changes) using the "BRCA1 MLPA Validation kit" and their own protocols. These results will be collected to ascertain **precision and performance parameters** and to evaluate the **overall inter-laboratory variability**.
2) Secondly, the laboratories will be asked to use their common Excel sheets and software programs to analyse a given set of MLPA files (DMD). In addition, three companies that develop MLPA analysis software, will perform the same evaluation, i.e. MRC-Holland (Coffalyser), SoftGenetics (GeneMarker) and JSI-Medical-Systems (Sequence Pilot). These results will be collected and compared to evaluate the **IT tools** regarding **performance** and **internal quality control**.
3) In a final step, the participants will perform MLPA on a set of **"positive"** BRCA1 samples with known deletions or duplications, using the same "BRCA1 MLPA Validation kit" and their own protocols. After collection of the data, **precision and performance parameters** will be evaluated.

End result

The final outcome should be:

- a complete validation report, representing the results from this inter-laboratory collaborative study on MLPA.

- a generic SOP, that should enable other interested laboratories to implement MLPA in their system.

References

- Schouten et al. (2002), *Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification*, Nucleic Acids Research, 30(12) e57
- ISO 5725-2 (1994), *Accuracy (trueness and precision) of measurement methods and results – Basic method for the determination of repeatability and reproducibility of a standard measurement method*
- ISO 5725-6 (1994), *Accuracy (trueness and precision) of measurement methods and results – Use in practice of accuracy values*
- ISO 22971 (2005), *Accuracy (trueness and precision) of measurement methods and results – practical guidance for the use of ISO 5725-2:1994 in designing, implementing and statistically analysing interlaboratory repeatability and reproducibility results*