B145 Validated and Straightforward Multiplex PCR Method for High-Quality Analysis of the Expanded CODIS STR Loci Set

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After attending this presentation, attendees will know about the benefits of QIAGEN's® Combined DNA Index System (CODIS) expansion Polymerase Chain Reaction (PCR) kit, how to evaluate the amplification efficiency of the PCR in a very convenient way, and how to choose the most appropriate rework strategy.

This presentation will impact the forensic science community by showing new technical features for a Short Tandem Repeat (STR) analysis with the benefits of reducing costs and increasing efficiency, productivity, and quality for laboratory operations by using the unique and novel quality sensor system.

QIAGEN® has developed and validated two multiplex PCR kits for reliable genotyping of the expanded CODIS STR loci set: the Investigator® 24plex QS Kit and the Investigator® GO! Kit. The former kit is designed for purified DNA from casework and reference samples, the latter kit is optimized for direct amplification of reference samples, like blood or buccal cells on FTA® paper or swabs. For buccal swabs, a five-minute lysis protocol is provided to prepare samples for direct amplification.

To verify the quality of the DNA sample and the performance of the PCR, both kits contain a novel quality sensor as an internal performance control. Forensic analysts are often faced with challenges when it comes to interpreting STR results. What is the reason that no peaks are visible in the electropherogram? Did the PCR fail? Was the DNA concentration too low? Was the DNA degraded? The kits of the Investigator® 24plex family contain a unique and novel Quality Sensor that is useful for evaluating the amplification of the PCR reaction. The system consists of two internal PCR controls (Quality Sensor QS1 and Quality Sensor QS2) located at the borders of the purple dye channel at 74bp and 435bp, respectively. To ensure that the quality controls do not cause any unspecific amplification during PCR, the internal control template was designed using a random algorithm and the obtained sequences were checked for absence of significant similarity to any known sequences. The 74bp QS1 shows very stable amplification, even in the presence of extremely high inhibitor concentrations (e.g., 1,000µM hematin). In contrast, the 435bp QS2 is more prone to inhibition and typically drops out before the first STR marker drop out is observed. Relative signal heights of QS1 and QS2 can thus be used to indicate inhibition. In the case where both sensors are unaffected but the sample shows a ski-slope effect with poor amplification of high molecular weight markers, degradation of the DNA sample is most likely. This information can be used to choose the most appropriate rework strategy.

Both assays use a new 6-dye technology to shorten the overall amplicon length and minimize overlap of the 23 markers which might lead to errors in data interpretation. The kits feature a very robust gender typing by offering small amplification fragments both for amelogenin and DYS319, leading to correct gender typing even for difficult samples (degraded or inhibited DNA) of amelogenin null mutant individuals. The developmental validation of the Investigator® 24plex QS kit based on the revised guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDAM) and the recommendations of the European Network of Forensic Science Institutes (ENFSI) has recently been completed. In a sensitivity study, full profiles were consistently obtained with 125pg template DNA. First stochastic allele drop outs were observed at 63pg template. Inhibitor studies revealed full DNA profiles in the presence of up to 200ng/µl humic acid, 750µM hematin, 3mM calcium, 12mM indigo carmine, 4000ng/µl tannic acid, and 200ng/µl collagen. These kits are in the process of being evaluated for approval for use at the National DNA Index System (NDIS).

CODIS, PCR, Quality