Increasing laboratory efficiency with the VeriFiler Plus PCR Amplification Kit

We recently spoke with Luke Ryan, Senior Scientist—Forensic DNA Analysis, from Forensic and Scientific Services, Health Support in Queensland, Australia about their forensic DNA analysis laboratory. The team has validated new STR chemistry and DNA analysis software to improve efficiencies in their workflow.

Luke Ryan, Senior Scientist
Forensic DNA Analysis,
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Thermo Fisher Scientific: Please describe your laboratory.

Luke Ryan: We have 70 people on the staff working in our laboratory. The positions include administration officers, laboratory assistants and technicians, scientists, senior scientists, and team leaders. We provide service to the state of Queensland, Australia, which has a population of 5 million and covers an area 2.5 times as big as Texas. We process approximately 25,000 crime scene samples and 15,000 reference samples annually. All of our processes are automated with large batch processing on robots and liquid handlers. Our goal is to achieve an approximately 10-day turnaround time for all casework samples.

Tell us why you need a primary and secondary-use STR kit.

Right now we are using the Promega™ PowerPlex™ 21 System for both casework and reference analysis. Most of our reference samples are processed using direct amplification using a half-volume PCR reaction. All samples are analyzed using Applied Biosystems™ 3500xL Genetic Analyzers. We are validating the Applied Biosystems™ VeriFiler™ Plus PCR Amplification Kit as a dual-use kit for casework and reference, as we currently do not have a secondary kit.

Our ideal approach is to have a primary and a secondary kit validated and fully supported in our LIMS for two main purposes.

First, having a primary and secondary kit for both types of samples is important for business continuity. If the primary kit is not available or delayed due to manufacturing issues, supply delays, or quality issues, we need to maintain service provision to police, courts, and the Queensland community by switching to the secondary kit. We have experienced these issues before.

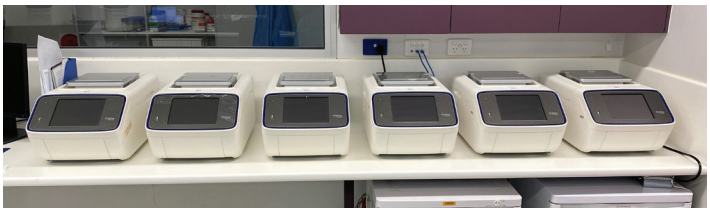
A secondary kit is also important in order to investigate kit- or primer-specific issues: mutations, variants, and locus dropout. If these issues cannot be investigated using a second kit, the entire profile may become unusable, or perhaps one or more loci may need to be removed from the interpretation. The inability to conduct this investigation may have a critical impact on a

case if the issue affects all samples from a suspect or victim in a case. Noting this, we use the primary kit for 100% of routine processing. The secondary kit would be used only for business continuity or for specific sample investigations.

What are the drawbacks to utilizing two different STR kits for processing samples on an ongoing basis?

Physical comparison of the electropherograms (EPG)—different kits have loci in a different order. Although our LIMS matches samples electronically, these comparisons are always confirmed manually by a scientist who compares the EPGs from the casework samples and reference samples. When loci are in a different order, it makes the physical comparison of EPGs difficult, time-consuming, and error-prone.

Kit and consumables—running two kits requires ordering and maintenance of stock levels of two sets of reagents on-site. We also need to manage different expiration dates. Using one kit for both casework and reference samples reduces waste because the same reagents can be used for both amplification types. Different kits require different standard operating procedures and training modules for each kit, which is an additional administrative burden on the lab. Each delivery and lot of an amplification kit undergoes a test amplification for verification before use on real samples. Using two kits means duplication of the kit verification, which is an increased cost per sample.

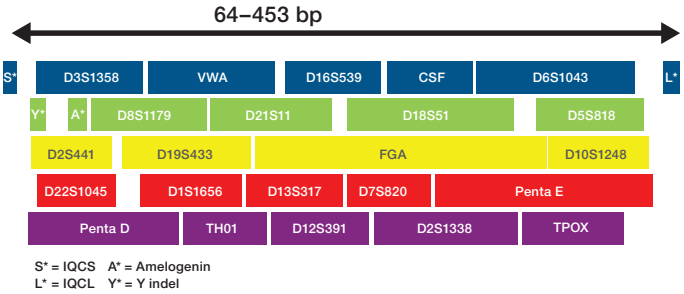


Instruments—different kits will require different protocols, calibrations, and matrices. This introduces additional work for laboratory staff in monitoring and maintaining these. This also introduces the risk of an operator using the incorrect reagents/matrices/calibrations (e.g. use casework protocols/reagents for the reference kit) and the risk is even higher if a single 3500xL Genetic Analyzer is used for both casework and reference workflow.

Validation—running two kits concurrently requires validation of both kits. Validation of amplification kits is the largest and most resource-intensive validation in terms of cost, staff, and time. Therefore, reducing the validation load by using a single kit for both casework and reference is economical. While we aim to maintain a primary and secondary kit, we would never validate two kits concurrently. An ideal model is to have a primary and secondary kit validated and then to assess new kits as they are released to determine what benefits the new kit has over the existing and whether a full validation is warranted. At completion of the validation, a determination is made (based on validation data) whether the new kit will become the primary/secondary or worst case, not implemented.

Training—use of two kits requires staff to be trained and then maintain competency in both amplification kits. This applies to laboratory staff as well as reporting staff performing interpretations.

Reanalysis requirements—scientists may order rework on samples when conducting interpretations: for example, a reamplification, rerunning on the genetic analyzer, or different concentrations of DNA. Having two kits in use introduces the risk of ordering the incorrect test on a sample and therefore consuming and losing valuable sample.



Marker positioning of VeriFiler Plus kit.

Data analysis—validating an amplification kit also requires validation of the statistical package being used to analyze the data. A large component of our validation of the VeriFiler Plus kit is the validation of STRmix™ software, a forensic software, developed by the New Zealand Institute of Environmental Science and Research (ESR) and Forensic Science South Australia (FSSA). This includes generating analysis parameters (stutter files, variance, saturation) and then assessing the ability of the STRmix software to deconvolute the profiles from both single-source and mixture samples. This is extremely time-consuming. Although we are not considering using two different kits for casework samples, it is important to note that moving from one kit to another in a short time frame is undesirable given the validation load. Having a robust

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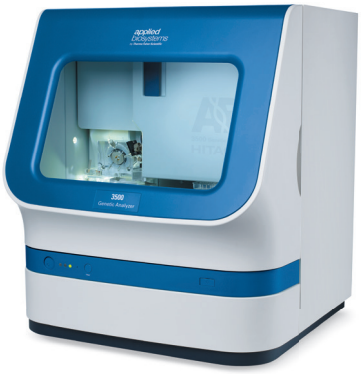
mixture-interpretation software package is increasingly important given the sensitivity of modern amplification kits and the likelihood this sensitivity will result in detection of lower amounts of DNA in samples, thereby increasing the prevalence of higher-order mixed DNA profiles.

What other factors in your workflow help increase efficiency?

Our validation of the VeriFiler Plus kit includes validation of Applied Biosystems™ 3500 Series HID Data Collection Software v4 (DCS) and Applied Biosystems™ GeneMapper™ ID-X Software v1.6. DCS v4 includes new features that are specific to Applied Biosystems™ STR kits and dye sets: pull-up reduction (PUR), and off-scale recovery (OSR) for reference sample analysis.

The decision to validate with PUR and OSR was made following an internal pre-validation assessment of the VeriFiler Plus kit. This was conducted to assess the VeriFiler Plus kit as a new kit and to compare it to our current kit with a view to determining if full validation was warranted.

Twenty-seven constructed mixtures were used to assess PUR (signal optimization was used for all analyses). Each of the 27 samples was run on a 3500xL Genetic Analyzer, and data was collected first with PUR on and then a second time with PUR off. As these are separate CE injections, some variation between runs is expected. Reporting scientists conducted blind interpretation of these 27 samples with PUR on and PUR off for a total of 162 interpretations. This assessment found comparable results between the PUR-on and PUR-off results. PUR did not result in masking of true alleles, nor did there appear to be any loss of data of evidentiary value. A qualitative assessment of PUR on vs PUR off was comparable, with a slightly more favorable assessment for PUR on from the scientists.



OSR provides a more accurate estimation of the height of very large peaks in overloaded samples by increasing the dynamic range, thereby enabling the analysis of these samples and increasing the first pass rate. OSR was assessed by amplifying a set of 10 samples in duplicate, at 0.75 ng DNA input and at 1 ng DNA input. Note the recommended DNA input for the VeriFiler Plus kit is 0.5 ng. These samples were then run on a 3500xL Genetic Analyzer, and data was collected first using OSR on and then a second time with OSR off. As with the previous test, these are different CE runs; therefore, some variation was expected. With OSR off, peak heights are capped at 32,000 RFU. The largest peak height observed with OSR on was 64,072 RFU. Interestingly, all samples amplified at 0.75 ng and 1 ng analyzed with OSR on and OSR off were able to be interpreted. This demonstrates the robustness of the VeriFiler Plus kit to high template input.

In the Forensic DNA Analysis department, we are working toward completing our validation of the VeriFiler Plus kit, including interpretation with STRmix software. We are encouraged by the results we have seen so far, and hope that the new features in 3500 Series HID Data Collection Software v4.0 will provide efficiencies for our workflows.

Find out more about forensic DNA analysis at
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