TAQ MUTANTS ENGINEERED FOR FORENSICS

Final Technical Report to the National Institute of Justice

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TABLE OF CONTENTS

ABSTRACT

Major problems with PCR-based forensic tests are false negative results and low sensitivity caused by residual blood, soil or other PCR inhibitors present in the sample. We proposed to apply our novel genetically-engineered mutants of Taq DNA polymerase, highly resistant to PCR inhibitors, to direct DNA analysis of forensic samples. In many cases, this approach can eliminate the need to purify DNA prior to PCR and decrease the time, lower the cost, and increase the efficiency of forensic DNA testing. In order to achieve this goal, we set the following objectives:

1. Develop and optimize a protocol without the DNA extraction steps for direct PCR-based typing of the human STR loci from crude samples containing blood and soil using our novel OmniTaq and Omni Klentaq enzymes.

2. Develop specific PCR enhancers to improve the detection sensitivity of crude samples.

3. Test the resistance of OmniTaq and Omni Klentaq to PCR inhibitors derived from substances other than blood or soil, such as urine, semen, hair/melanin, tannins, indigo dye, bones, muscle tissue, saliva, and feces/bile salts, and extend the application of the mutant enzymes to testing crude samples of these substances.

4. Formulate and optimize blends of OmniTaq and Omni Klentaq with some members of the Y-family thermophilic polymerases with improved performance on damaged DNA.

In our preliminary tests we found that our cold-sensitive hot-start mutant enzyme, Cesium Taq, performs very well in STR typing, while the Omni Klentaq tends to generate more stutters, therefore we gave a preference to exploring CesiumTaq, along with OmniTaq enzyme.

Before we applied our enzymes and enhancer cocktails to the multiplex DNA typing, we performed a series of systematic testing of various known PCR inhibitors, including blood, treated or untreated with anticoagulants, soil / humic acid, urine /urea, semen, bile salts, tannins,

melanin and indigo dye in monoplex PCR of human gene targets (such as some of the STR alleles, CCR5, beta-actin, DNMT) with OmniTaq and CesiumTaq enzymes, in order to determine the range of tolerance of the mutant enzymes to the particular inhibitor. Along with this line, we tested various formulations of our 3/4 components PCR enhancer cocktails (PECs) , to find the most efficient formulation for each PCR inhibitor tested. Our PECs, which were recently published, typically play a double role, they act as a general PCR enhancer, improving the yield, sensitivity and specificity, especially with tough, GC-rich DNA templates, and they also help overcoming PCR inhibition, thus facilitating DNA amplification from crude samples. The Y-family polymerases, mentioned in Objective 4 and obtained from the NIH through an evaluation license, unfortunately did not meet our performance criteria, showing poor activity and stability with inconsistent results, therefore we dropped this direction from the project.

We successfully developed protocols for STR genotyping of crude samples containing the above mentioned inhibitors, skipping the DNA extraction steps. Some of the specimens were provided from our sub-contractor, Bode Technologies. These protocols predominantly utilize our mutant enzymes Omni Taq and CesiumTaq, optimized PCR buffers, and enhancer cocktails, PEC and PEC-Plus, each of them optimized for a certain group of crude samples. Our protocols are compatible with the primers and cycling conditions of both PowerPlex 16 HS (Promega) and AmpFlSTR Identifiler Plus (Life Technologies) kits, therefore they do not require any extra steps or changes in the established protocols, except that specific components of the master mix provided in these commercial kits are replaced by our components. We present results showing that in comparative tests of direct STR typing with challenging crude samples our protocols outperform significantly the AmpFlSTR Identifiler Plus kit, and to some extent the PowerPlex 16 HS kit.

OBJECTIVES

One. Develop and optimize a protocol without the DNA extraction steps for direct PCR-based typing of the human STR loci from crude samples containing blood and soil using our novel OmniTaq and Omni Klentaq enzymes.

Two. Develop specific PCR enhancers to improve the detection sensitivity of crude samples.

Three. Test the resistance of OmniTaq and Omni Klentaq to PCR inhibitors derived from substances other than blood or soil, such as urine, semen, hair/melanin, tannins, indigo dye, bones, muscle tissue, saliva, and feces/bile salts, and extend the application of the mutant enzymes to testing crude samples of these substances.

Four. Formulate and optimize blends of OmniTaq and Omni Klentaq with some members of the Y-family thermophilic polymerases with improved performance on damaged DNA.

REVIEW OF RELEVANT LITERATURE

Routine forensic DNA typing methods must consider the inherent problems of possible false-negative or reduced sensitivity reactions caused by PCR inhibitors in the specimens. Such inhibitors can compromise most important analyses such as nuclear STR, mitochondrial DNA and Y-chromosome DNA testing (Butler, 2005; Wilson 1997; Radstrom, 2001, La Montagne et al., 2001; Eckhart et al., 2000; Shutler et al., 1999; Lantz et al., 1997, Mahony et al., 1998). Among the most potent PCR inhibitors present in forensic samples are hemoglobin/heme (blood red cells) and humic acid (soil) (Al-Soud et al.,2000, 2001; Tsai et al., 1992; Moreira, 1998). Other known inhibitors found in forensic cases are bile salts and polysaccharides (feces), melanin (tissue and hair), urea (urine), as well as denim textile dyes (clothes) (Lantz et al., 1997; Monteiro, 1997; Eckhart et al., 2000; Mahony et al., 1998; Shutler et al., 1999). Some PCR inhibitors like hemoglobin and humic acid can co-purify with DNA (Akane et al., 1994, De

francis et al., 1998, Radstrom et al., 2004) and cause loss of detection of the larger sized STR loci or even cause complete failure of all loci (Butler 2005). Partial STR profiles obtained in the presence of PCR inhibitors are similar to the profiles from damaged DNA (Applied Biosystems, 1998), which is another major concern with the quality of forensic samples (Schultz et al., 2004; Ballantyne et al., 2007; Swango et al., 2006). The inhibitory effect of blood on PCR is associated primarily with inactivation of the DNA polymerase and/or capturing or degrading the target DNA and primers. (Akane et al., 1994; Al-Soud et al., 2001). AmpliTaq Gold , which is widely used in forensic DNA testing, is unfortunately most sensitive to PCR inhibitors and can be completely inhibited in the presence of less than 0.1% whole blood or traces of heme or humic acid (Al-Soud et al., 2000; Tsai et al., 1992). Therefore, the current DNA typing protocols demand high purity of the evidence DNA. Three primary procedures are currently used to extract DNA from forensic samples, using phenol-chloroform, Chelex resin or FTA paper (Comei et al., 1994; Willard et al., 1998; Kline et al., 2002; Del Valle et al., 2004). In order to neutralize PCR inhibitors, some protocols involve more drastic treatment with sodium hydroxide (Bourke et al., 1999). In general, the DNA extraction procedures are time-consuming or labor-intensive, can cause some DNA losses, and may fail to completely remove critical inhibitors. In addition, multiple sample manipulations involve increased risk of cross-contaminations (Butler, 2005). As a conceptual alternative to DNA extraction procedures, we proposed to utilize our recently developed novel mutants of Taq which are able to overcome PCR inhibitors, along with our PCR enhancer cocktails (PECs), which further increase the tolerance to various PCR inhibitors (Kermekchiev et al., 2009, Zhang et al., 2010). These mutant enzymes also perform with excellent sensitivity and specificity. Our company is a pioneer in genetic modification of Taq with a track record of success. We have already launched the CesiumTaq and Cesium Klentaq cold-sensitive mutants with high specificity Hot-Start performance (Kermekchiev et al., 2003) which are being used in

various applications, including human diagnostic PCR assays.

EXECUTIVE SUMMARY

Our OmniTaq and Omni Klentaq mutant enzymes were originally selected for their capability of overcoming the potent inhibition of blood in PCR. Later we found that they can also tolerate soil and other PCR inhibitors at amounts which are completely inhibitory to the plain wild-type Taq DNA polymerase. **Figures 1 and 2** demonstrate that OmniTaq can efficiently amplify blood genomic DNA, including some STR targets, straight from samples containing whole blood, without DNA extraction. Although we started our tests with monoplex PCR, our main focus was to extend the enzyme application to multiplex systems and make them useful to forensic labs. In our initial tests, our mutant enzymes also remained functional with some crime scene-like probes, when challenged with mixed dry blood and soil samples, where the Taq Gold completely failed (Illustrated with OmniTaq in **Figure 3**). Similar results were obtained with the N-terminal truncated version of the mutant, Omni Klentaq. Furthermore, our enzymes outperformed top commercial Taqs in detection of soil pathogens straight from crude soil extracts (illustrated with Omni Klentaq in **Figure 4**).

We carried a series of experiments comparing the performance of our mutant enzymes with AmpliTaq Gold in amplification of damaged DNA. In these tests we used both partially hydrolyzed (by DNAse I) and UV-crosslinked human DNA. **Figure 5** shows the amplification of a human target of 0.5 kb (about the size of the largest STR targets) from DNAse I-treated DNA. The mutant enzymes, especially OmniTaq, showed progressively better performance with more damaged DNA as compared to AmpliTaq Gold.

We also evaluated four versions of the Y-class (Dpo4) DNA polymerases, which according to earlier reports would perform with high processivity with damaged DNA templates. These enzymes were purchased and obtained from the laboratory of Dr. R. Woodgate at NIH through an evaluation license contract. Unfortunately, in our tests the performance of these enzymes was not satisfactory, due at least to their insufficient thermostability and overall stability during storage. As a result, they actually were not able to perform well in PCR, and (as suggested by Dr. Woodgate's team) they usually require an additional DNA polymerase to exert their effect. Unfortunately, when mixed with our mutant DNA polymerases, these enzymes showed even an inhibitory effect. Therefore, they turned out not to be good candidates for blending our enzymes, and we closed this experimental direction.

In another set of experiments we increased the challenge by combining damaged DNA templates with potent PCR inhibitors found in blood, such as hemoglobin, hemin an IgG (immunoglobulin) fraction. Our Taq mutants, supplemented with PEC, were able to amplify DNAseI-treated DNA in the presence of these PCR inhibitors, while AmpliTaq Gold was only functional with the control, purified and undamaged DNA. These results clearly demonstrated the advantages OmniTaq / Omni Klentaq can have in cases where the samples contain inhibitory substances and DNA is damaged, which often times occurs in forensic practice.

In the beginning of the project, we characterized several compounds which increase the sensitivity of PCR, especially when inhibitors are present in the reaction. These compounds apparently synergize with and further improve the performance of our mutant Taq enzymes. We formulated proprietary PECs for crude samples with GC-rich and regular DNA targets. Our results with various PCR targets indicate that the cocktail acts in four ways: through decreasing the DNA melting temperature, stabilizing the enzyme, helping overcoming PCR inhibitors, and

facilitating cell lysis (in crude reactions containing intact cells or tissue). It contains several components which can be formulated in an optimal proportion for each PCR target, based most of all on the GC-contents of the latter. During the course of the project we identified two more enhancer components, which allowed us to explore various PEC formulations and find the optimal one for direct STR typing of a variety of crude samples

Our enzymes combined with PEC were able to amplify human DNA targets directly in human urine, or samples containing urea, major blood inhibitors (hemin, lactoferrin, IgG), bile salts, milk, humic acid, indigo dye, melanin and tannin, without any DNA extraction steps prior to PCR. On the other hand, some less inhibitory crude samples did not required PEC in conventional PCR, or direct STR genotyping.

Following extensive work on reaction optimization in terms of buffer parameters (pH, salt, magnesium chloride and detergent concentration) and choice and titration of the best mutant enzyme from our collection, as well as testing various PEC enhancer cocktail formulations, we focused our effort on human STR genotyping of a variety of challenging samples.

With samples of damaged human DNA, obtained by partial hydrolysis with DNAse1, the mutant OmniKlentaq generated almost full STR profile, while no profile was obtained with AmpliTaq Gold, which was working quite well with the control, untreated DNA (**Figure 6**).

Next, the two enzymes were challenged with DNA from the same donor, damaged by UV-crosslinking. Both enzymes produced partial profiles, with relatively less alleles amplified with the AmpliTaq Gold (**Figure 7**).

In another series of tests, we challenged our enzymes with direct STR typing in the presence of 1-5% whole blood, using the PowerPlex 16 kit primers. Both OmniTaq and OmniKlentaq yielded complete STR profiles, but we noticed that the full-length Taq mutant, OmniTaq, performed

relatively better, and the truncated OmniKlentaq tended to generate some stutters, usually 4-6 stutters per profile with crude samples This finding could be due to the high speed feature of OmniTaq (able to amplify a 2 kb target in less than 10 seconds), which potentially could reduce the stutters in the STR profile, known to be related to the enzyme speed (Walsh et al., 1996), while the truncated Klentaq (with an N-terminal deletion from the w.t. Taq) is generally 2-3 slower in DNA extension, as compared to the full-length Taq. Also, in addition to the inhibition resistance mutations, OmniTaq and OmniKlentaq possess built-in Cold-Sensitivity phenotype, which adds a hot-start feature to the enzyme performance (like Taq Gold), especially important for STR genotyping.

In parallel tests the AmpliTaq Gold failed to amplify any of the loci from whole blood samples (**Figure 8**).

At the time we submitted our original proposal (early 2008), the 'gold standard" Taq enzyme used in forensic labs for DNA typing was AmpliTaq Gold, included in the two top kits at that time, PowerPlex 16 (Promega) and AmpFlSTR (ABI). Therefore, we originally proposed to focus on parallel tests and comparisons with AmpliTaq Gold. However, in the recent years these two kits evolved, and Promega and ABI (Life Technologies) introduced the improved kit versions PowerPlex16 HS (with a new Hot-Start Taq enzyme replacing AmpliTaq Gold), and AmpFlSTR Identifiler Plus, respectively, which are more robust and can tolerate certain PCR inhibitors, especially blood stains on FTA cards, presumably due to some enhancer additives present in their master mixes. Accordingly, we focused on those newer kits as control standards for the performance of our enzymes. This direction was significantly facilitated by a valuable evaluation agreement that we reached with Promega, whose team kindly provided their PowerPlex 16 HS master mix lacking their Taq HS enzyme, and the enzyme alone. This allowed us to evaluate our enzymes in two directions: by just adding our

enzymes to their master mix, and also we used our optimized buffers and enhancers, combined with their primers from the kit. Some of the results and our reports to Promega were presented in our 2010 progress reports.

We also compared the performance of our protocols with the AmpFlSTR Identifiler Plus kit of Life Technologies by using our master mix (enzymes, buffers, enhancers) supplemented with the kit primers. With both kits, the control reactions were the standard ones with the complete kit, as suggested by the manufacturer.

Besides crude samples with high blood concentrations (5%), we performed comparative tests with the PowerPlex HS kit with very low amounts of blood, 0.001-0.002 ul. This target range should be pretty challenging, considering that the amount of DNA in the blood is only around 30-60 pg. These tests are illustrated in **Figure 9**, where we also included CesiumTaq (our cold-sensitive Taq mutant, which in addition to the hot-start feature shows great sensitivity in general PCR). With these kind of samples we observed relatively more complete profiles with OmniTaq and CesiumTaq as compared to TaqHS. These results were obtained by replacing the Promega Taq HS enzyme by our enzymes in the context of the original Promega master mix, therefore, they reflect the impact of the enzyme only on the performance, and indicate a better sensitivity of the mutant enzymes. We believe these tests could be of practical importance when the evidence blood specimens available for forensic labs are in very low trace amounts, and they demonstrate that one can skip the DNA extraction and potential losses of DNA.

In parallel tests with 1.2 mm punches from standard blood stains on FTA cards, our enzymes matched the performance of the PowerPlex 16HS commercial kit, producing full profiles. The

same results were obtained with EDTA-treated blood stains, while with heparin-treated blood the Promega kit generated only a partial profile (**Figure 10**). This is consistent with our earlier data, showing that in addition to blood, our mutant enzymes can tolerate heparin, a known PCR inhibitor, better than the plain HS Taq.

Under an agreement with the Bode Technology Group, we were collaborating and exchanging samples and protocols, which helped us in gaining more specific experience in DNA typing of forensic samples in achieving our objectives. This collaboration allowed us to challenge our enzymes/enhancer combinations with additional specimens obtained from Bode Tech., such as aged buccal swabs, soda can mouth piece swabs, cigarette butts paper, semen stains on blue jeans, blood and soil stains on T-shirt, for which we found enzyme/enhancer formulations for direct STR genotyping (Figures **11, 12, 13, 15, 23, 24**). On the other hand, some samples provided from Bode Tech., such as laser microdissected cells , fingerprints on glass swabs and DNAse-damaged DNA, did not work, and produced no profiles either with our protocol or with the PowerPlex 16 HS kit, tested both in our lab and Bode's lab. At least for the DNAse-treated DNA we figured that it probably was overdigested.

In exchange we sent to Bode our test samples containing urine, bile salts, humic acids, indigo dye, and aged blood stains on blue jeans.

There were some variations in the results obtained in our lab and Bode, and we realized in our discussions that a likely reason that can explain at least some of those variations was the different storage conditions and progressive degradation of DNA in the crude samples, as well as the timing of the tests.

In the parallel assays our protocols matched the performance of the commercial kits or outperformed them. For example, OmniTaq or Cesium Taq produced complete STR profiles with

heparinized blood stains on FTA cards (1.2 mm punches), aged blood stains on blue jeans (1.2 mm punches), bile salts (at least 60 ug), and indigo dye (at least 200 ug), while the complete PowerPlex 16HS kit generated no, or partial profiles (**Figures 10, 12, 16, 19, 20**).

Generally, the Promega kit was more robust than the Life Tech. In comparisons with our protocol, the Identifiler Plus kit was completely inhibited and produced no profile with semen stains on blue jeans (1.2 mm punches), mixed blood and soil stains on cotton (1.2 mm punches), urine (10% or more) , humic acid (250 ng or more), indigo dye (50 ug or more), tannins (4 ug or more), or showed some allele drop-outs with aged blood stains on blue jeans (1.2 mm punches) and semen+blood stains on blue jeans

(1.2 mm punches), while full STR profiles obtained with our enzymes and the primers from the same kit with all of these samples (**Figures 11, 13, 14, 15, 17, 18, 21, 22**).

Technically, the STR typing was performed in a standard 25 ul reaction, using the Power Plex-16 primers, and displacing the original master mix from the kit with our master mix, containing 2.5 ul 10X PCR buffer, 12.5 ul 2X enhancer cocktail (PEC or PEC-Plus), and 1-3 ul enzyme, OmniTaq or CesiumTaq. The use of enhancer was optional and not needed with relatively less inhibitory crude samples. The type of enhancer used is provided in the figure legends.

The 10X PCR Buffer contains 500 mM tris-HCl, pH 9.2, 160 mM ammonium sulphate, 35 mM MgCl2 and 0.25% Brij-58. The 2X PEC cocktail contains 0.8 M trehalose, 2M L-carnitine and 0.25% Brij-58. The PEC-Plus cocktail contains the same components as PEC, plus 4% polyvinylpyrollidone (PVP). The reactions were performed in a PTC-200 cycler (MJResearch), and the cycling conditions were the standard ones, provided in the Promega PowerPlex16-HS kit manual for Perkin-Elmer cycler.

Taken together, our data on direct STR genotyping of human DNA from crude specimens (summarized in **Table 1**) demonstrate that our genetically engineered enzymes OmniTaq and CesiumTaq, supplemented with the PEC enhancer formulations, outperformed the two top commercial kits available today, generating complete allele profiles with a variety of 17 crude samples relevant to the forensic practice, with one exception in the case of trace amounts (1 nL) of blood, where few allele drop-outs were observed, vs. partial profile with more drop-outs obtained with the PowerPlex 16 HS kit (The ID-kit was not tested). In comparison, out of the 17 crude samples, the Promega kit generated 12 full and 5 partial profiles, and the Life Tech. Identifiler-Plus kit, with 14 of these 17 samples attempted, generated 6 full profiles,2 partial profiles, and no profiles were generated in the other 6 samples (**Table 1**).

In the cases of partial profiles obtained with the commercial kits, we did not observe a strict pattern of the drop-outs, but predominantly missing were usually larger size loci peaks.

We are confident that the potential of our novel enzymes and enhancers does not stop here, and in the near future we can expand the application scope of our reagents to direct STR typing of more crude forensic specimens.

IMPLICATION FOR CRIMINAL JUSTICE POLICY AND PRACTICE

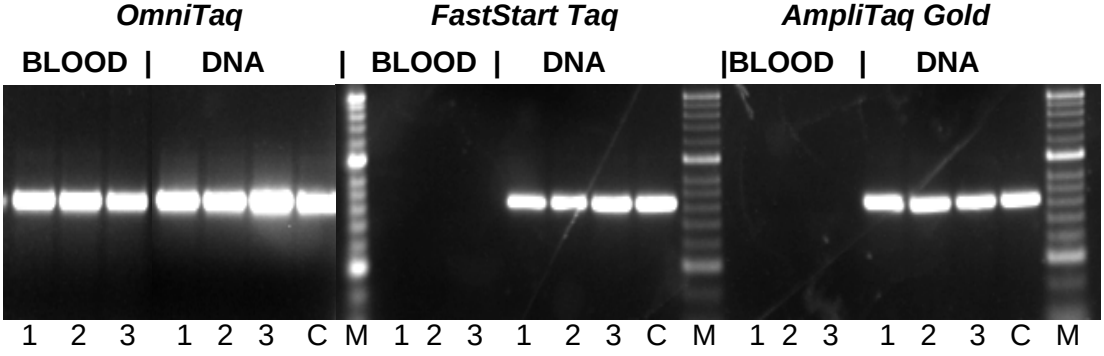
The DNA profiling protocols employing the specific novel features of our mutant Taq enzymes and PECs should save time and cut the cost per sample/case. Our technology should eliminate in most cases the DNA extraction steps currently required for DNA testing. In addition, it will reduce the false-negative results due to the presence of residual PCR inhibitors and/or DNA losses, and will also reduce the risk of cross-contaminations during multiple manipulations of the samples. We have begun to contact the kit suppliers to incorporate our technology into new kit

offerings. Our protocol can be applied with almost no change-over costs or training, as it does not require any additional reagents or equipment, but simplifies the overall procedure without changing the PCR step of the established DNA profiling protocols.

Oftentimes qPCR is used to quantify the evidence DNA prior to profiling. We have already developed unique real-time PCR protocol that skips the extraction step using our enzymes with crude samples. This recently patented protocol overcomes the strong fluorescence quenching effect and high background related to the PCR inhibitors and will help the genomic DNA quantitation of such samples. Therefore, we anticipate that any DNA testing forensic lab will benefit from our technology by eliminating the DNA purification thus far required both for DNA quantitation and genotyping..

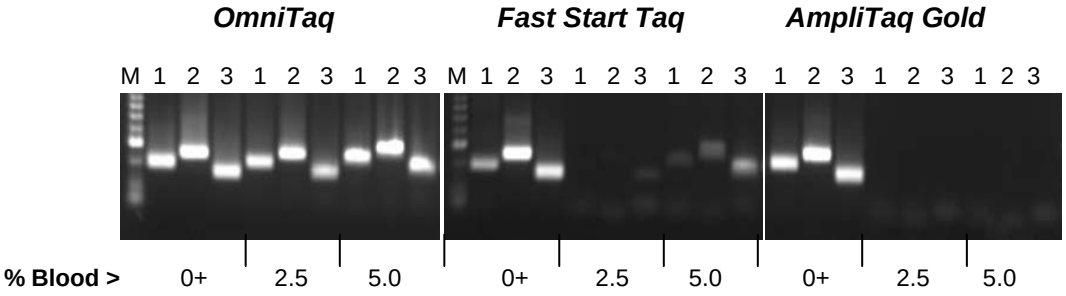
FIGURES

Figure 1. No blood DNA extraction required for PCR with OmniTaq



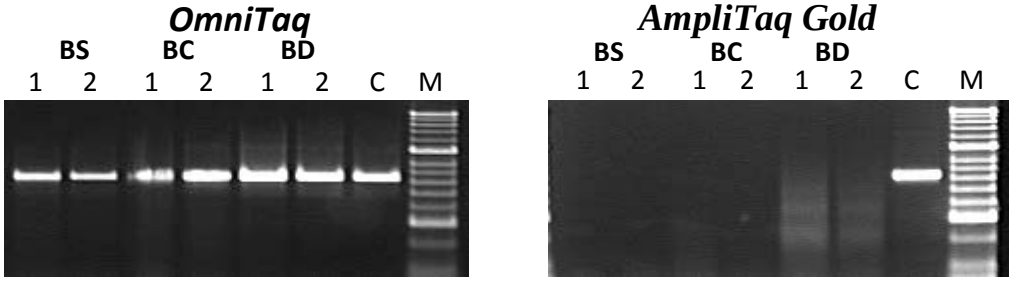
A 630 bp target from the human CCR5 gene was amplified from three 2-fold dilutions of DNA, kit-purified from blood (lanes 1-3), or three equivalent amounts of whole human blood, using OmniTaq, FastStart Taq, or AmpliTaq Gold enzymes. **Results:** With OmniTaq, the test target was detected with similar efficiency both from purified DNA and blood. In contrast, the two commercial enzymes completely failed to work with blood and were dependent on DNA purification prior to PCR.

Figure 2. Human STR PCR of Whole Blood Samples (No DNA Extraction)



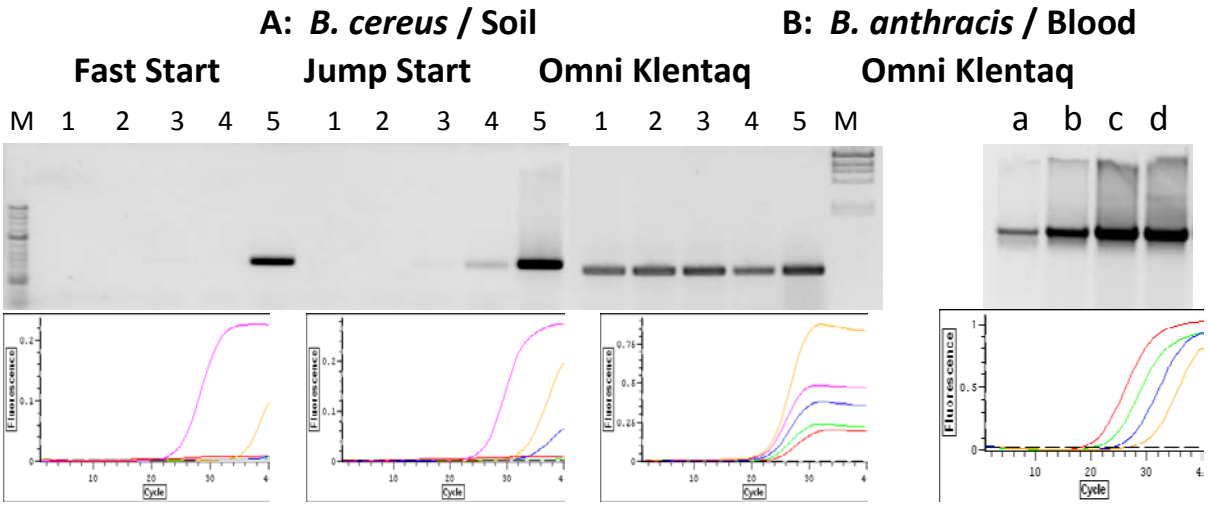
The human STR markers THO1, TPOX and VWA (lanes 1-3) were amplified from 2.5% or 5% whole blood, using Omni Taq (DNAP), Fast Start Taq (Roche) or AmpliTaq Gold (Applied Biosystems). Two units of each enzyme were used according to the manufacturers recommendations, for 35 cycles. Control reactions (0+) contained no blood but 5 ng human DNA purified from blood. The amplified products were resolved in 2% ethidium bromide stained agarose gel. M, DNA standards ladder. **Results:** OmniTaq clearly outperformed the two commercial enzymes, which required purified DNA for efficient amplification of the STR markers from blood cells.

Figure 3. OmniTaq Allows Direct Detection of Blood Genes



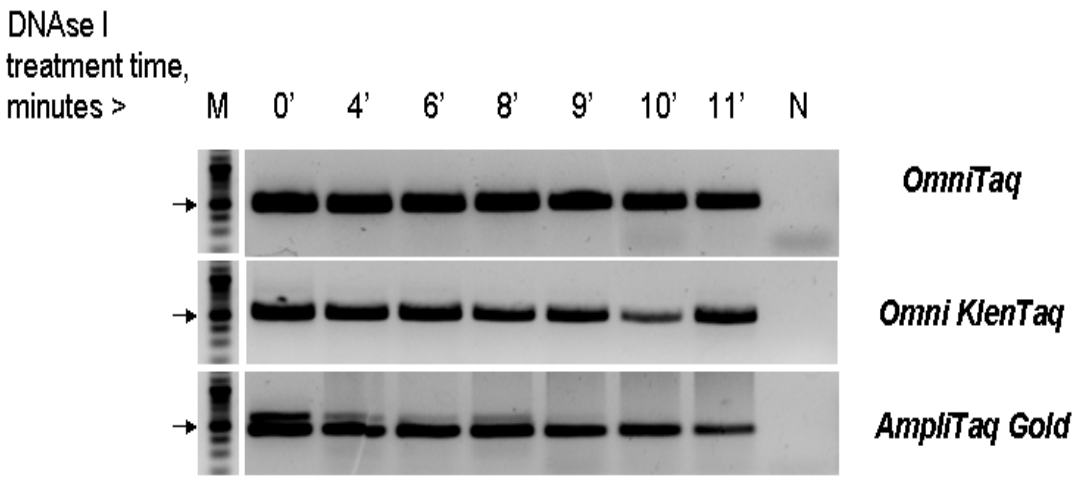
Two days old blood spots on powdered soil (BS), on a cotton tissue (BC), or on a dusty floor surface (BD) were partially eluted in PCR buffer. Then 1.5 or 3 ul (lanes 1 and 2) of each blood sample suspension were used directly in PCR to detect a 630 bp target of the human CCR5 gene, using 2 units of OmniTaq or AmpliTaq Gold enzymes in 33 cycles. Control reactions (C) contained no blood samples but 4 ng purified human DNA. The amplified products were resolved in a 2% ethidium bromide stained agarose gel, along with DNA standards ladder (M). **Results:** Despite the presence of PCR inhibitors, OmniTaq was able to amplify the test gene from all blood samples, even those mixed with soil, while AmpliTaq Gold failed to do so and required DNA extraction.

Figure 4. OmniKlentaq Allows Direct Detection of Pathogen DNA in Crude Soil and Blood Samples



A. Fast Start Taq (Roche), Jump Start Tag (Sigma), and OmnKlentaq enzyme were used to amplify a 600 bp target of *Bacillus Cereus* from four dilutions of a crude soil extract (lanes 1-4). Control reactions (lanes 5) contained 5 ng purified *B. cereus* DNA and no soil extract. PCR was performed in real-time cycler Opticon-2, and the amplified products were analyzed both by SYBR Green dye fluorescence (bottom panels) and gel electrophoresis (top panels). **B.** A 1.3 kb target of *B. anthracis* was amplified from four dilutions (a-c) of the pathogen DNA, mixed with 5% whole human blood and the amplification was analyzed as in A. **Results:** The OmniKlentaq mutant enzyme can tolerate PCR inhibitors much better than plain Taq and amplify pathogen DNA straight from crude soil or blood containing samples.

Figure 5. Comparison of OmniTaq and Omni KlenTaq vs. AmpliTaq Gold in PCR Amplification of Damaged DNA

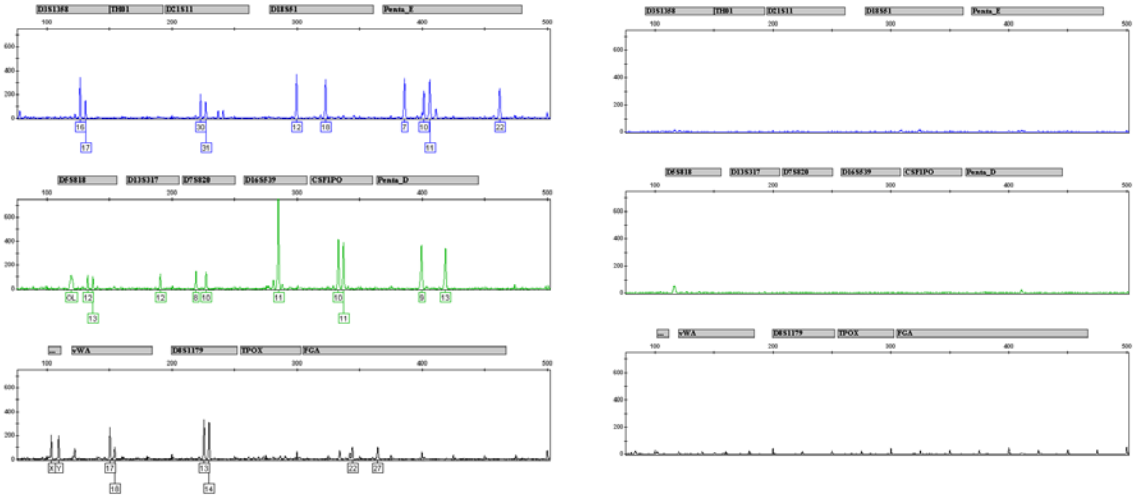


Human genomic DNA (20 ng) was treated with DNase I (0.1 mg/ml DNA) at 25 deg. for 0, 4, 6, 8, 9, 10 and 11 min, respectively. The reactions were stopped with 5 µM EDTA and heating at 75 deg. for 15 min to inactivate the DNase I. A 0.5 kb target of the human DNMT gene was amplified from 1 ng of these DNA samples using OmniTaq, Omni KlenTaq and AmpliTaq Gold. The mutant enzymes were used with an enhancer mix (PEC) optimized for them. Negative reactions (N) contained no DNA. The PCR products were resolved in 1.5% agarose gel and stained with ethidium bromide. Lanes M, DNA ladder.

Results: The mutant enzymes, especially OmniTaq, tend to outperform AmpliTaq Gold when challenged with more damaged DNA. *(Similar results were obtained with UV-crosslinked DNA).

Figure 6. STR GENOTYPING OF DNAse I-TREATED HUMAN DNA (Power Plex 16 kit)

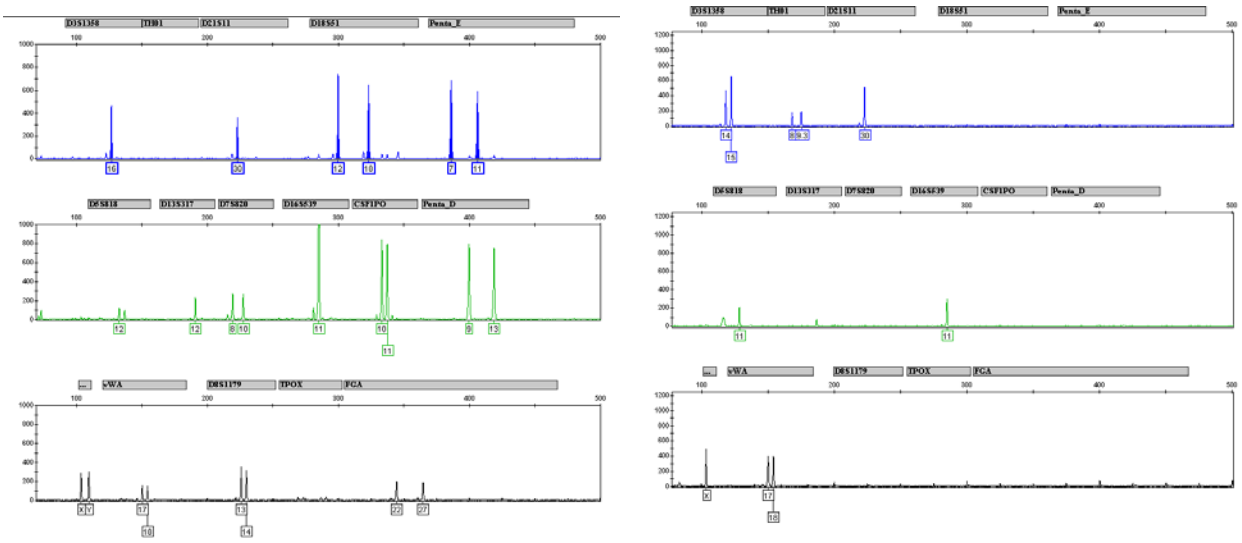
Omni Klentaq (DNAP Tech) AmpliTaq Gold (Life Tech)



OmniKlentaq or AmpliTaq Gold were used in the context of the PowerPlex16 kit (Promega) for STR genotyping of 1 ng DNAse-treated human DNA (0.1 mg/ml DNAse1 For 5 min).
Results: OmniKlentaq produced a partial profile, while no profile was generated with Amplitaq Gold.

Figure 7. STR GENOTYPING OF UV-CROSS LINKED HUMAN DNA (Power Plex 16 kit)

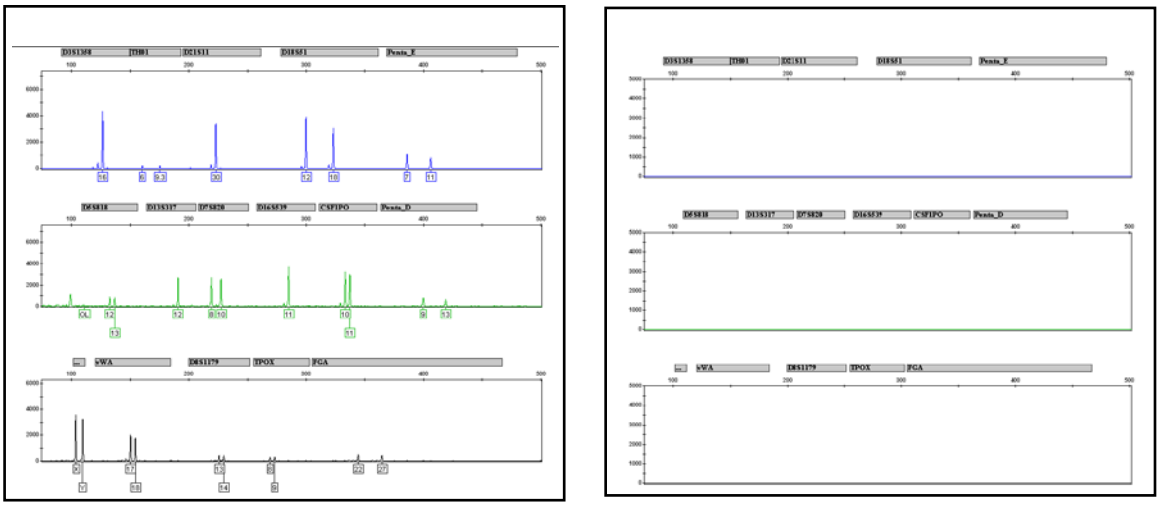
Omni Klentaq (DNAP Tech) | **AmpliTaq Gold (Life Tech)**



OmniKlentaq or AmpliTaq Gold were used in the context of the PowerPlex16 kit (Promega) for STR genotyping of human DNA, UV-crosslinked for 60 sec. **Results:** both enzymes produced partial profiles, with significantly less peaks detected with AmpliTaq Gold.

Figure 8. DIRECT STR GENOTYPING OF 5% BLOOD SAMPLES (Power Plex 16 kit)

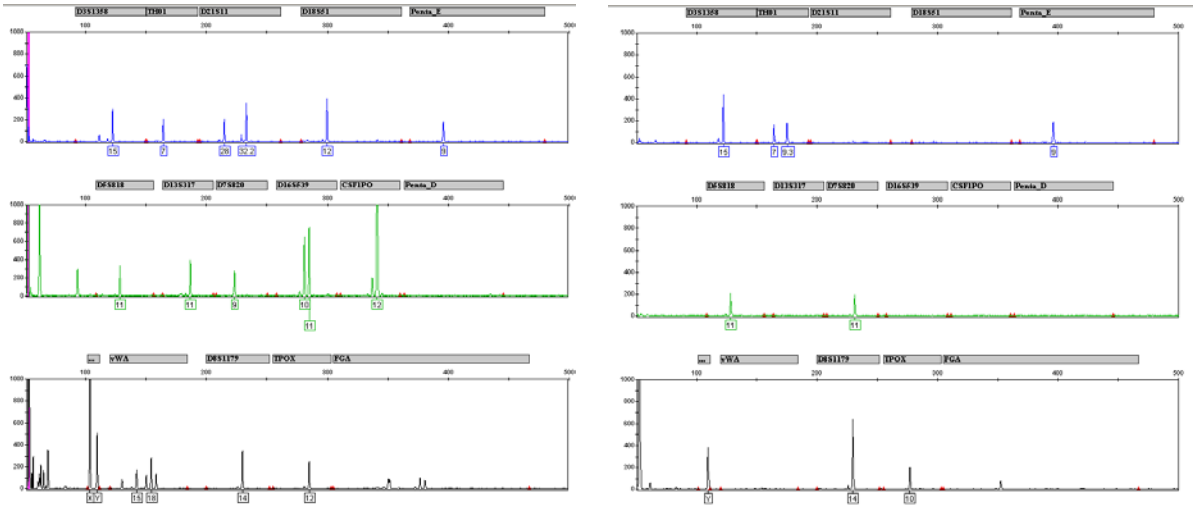
OmniTaq (DNAP Tech) AmpliTaq Gold (Life Tech)



OmniTaq and PEC or AmpliTaq Gold were tested in the context of the PowerPlex 16 kit (Promega) in direct STR genotyping of 5% human blood. **Results:** The OmniTaq mutant enzyme, supplemented with the PEC enhancer, could overcome the blood inhibition and generate full STR profile, while AmpliTaq Golg was completely inhibited, resulting in no profile.

Figure 9. DIRECT STR GENOTYPING OF CRUDE BLOOD SAMPLES AT TRACE AMOUNTS OF BLOOD: DNAP TECH PROTOCOL VS. POWER PLEX 16HS KIT (PROMEGA)

DNAP TECH PROTOCOL | **PROMEGA PP16HS**

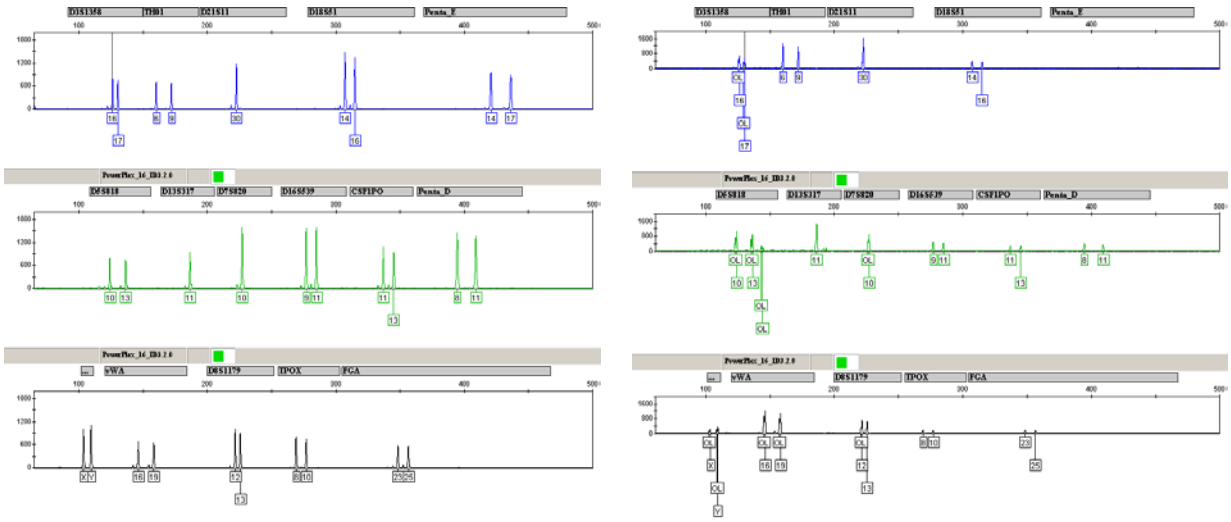


Direct STR typing was performed with 0.001 ul human blood (equivalent to 30-50 pg genomic DNA) without DNA extraction, using Cesium Taq + PEC (left) with the PP 16HS primers, or the PowerPlex 16 HS kit (right). **Results:** Partial profiles were obtained with the two systems, and relatively more allele drop-outs were observed with the PP16HS kit. The results suggest a better sensitivity of the CesiumTaq enzyme in such crude samples, as compared to the Taq HS.

Figure 10. DIRECT STR GENOTYPING OF HEPARINIZED BLOOD ON FTA CARDS: DNAP TECH PROTOCOL VS. POWER PLEX 16HS KIT (PROMEGA)

DNAP TECH PROTOCOL

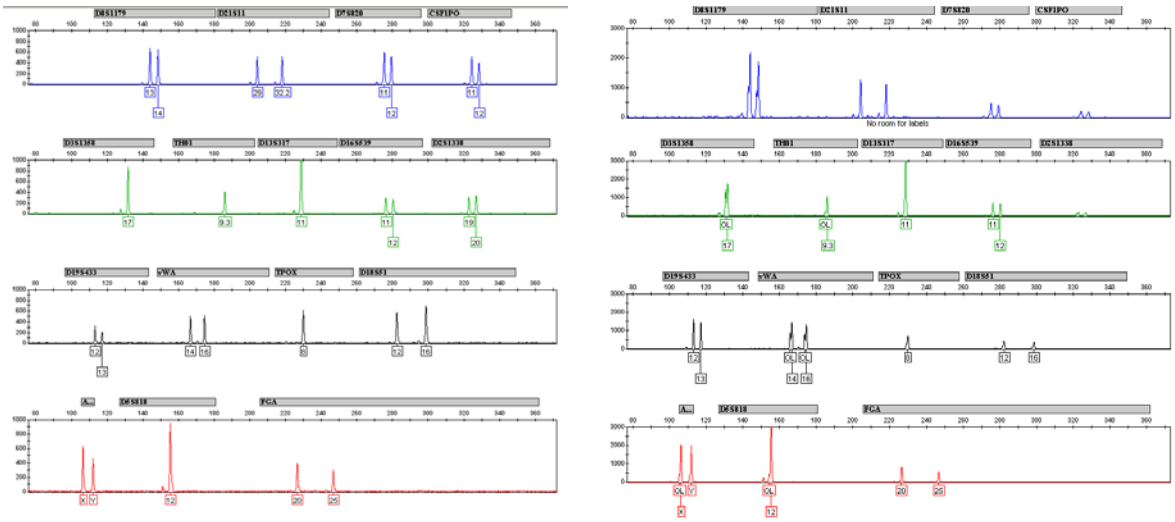
PROMEGA PP16HS



Punches of 1.2 mm from FTA cards with heparinized blood stains were used for direct STR typing without DNA elution, using Cesium Taq + PEC (left) with the PP 16HS primers, or the PowerPlex 16 HS kit (right). **Results:** The mutant enzyme, supplemented with PEC generated full profile, while with the Promega kit a partial profile was produced. Identical results with full profiles were obtained with the OmniTaq mutant enzyme.

Figure 11. DIRECT STR GENOTYPING OF AGED BLOOD STAINS ON BLUE JEANS: DNAP TECH PROTOCOL VS. ID_PLUS KIT (LIFE TECH)

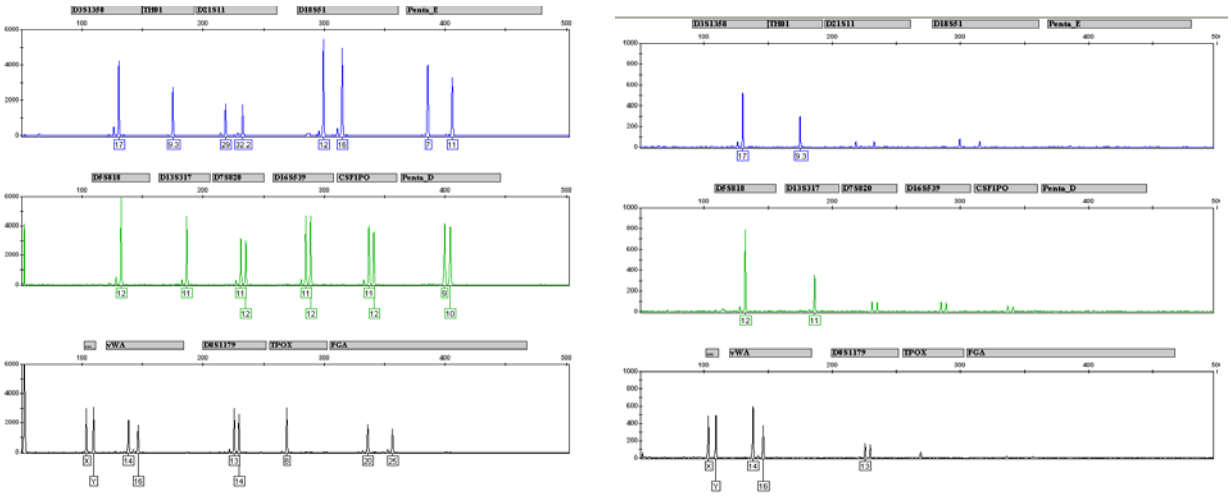
DNAP TECH PROTOCOL **LIFE TECH ID_PLUS KIT**



Punches of 1.2 mm from 2 years aged blood stains on blue jeans were subjected to direct STR typing without DNA extraction, using OmniTaq + PEC (left) with ID_PLUS primers, or the complete ID_PLUS kit (right). **Results:** The ID-PLUS kit generated a relatively less balanced profile, with some allele drop tendency and stutters. The CesiumTaq mutant enzyme worked with such samples as good as OmniTaq (not illustrated).

Figure 12. DIRECT STR GENOTYPING OF AGED BLOOD STAINS ON BLUE JEANS: DNAP TECH PROTOCOL VS. POWER PLEX 16HS KIT (PROMEGA)

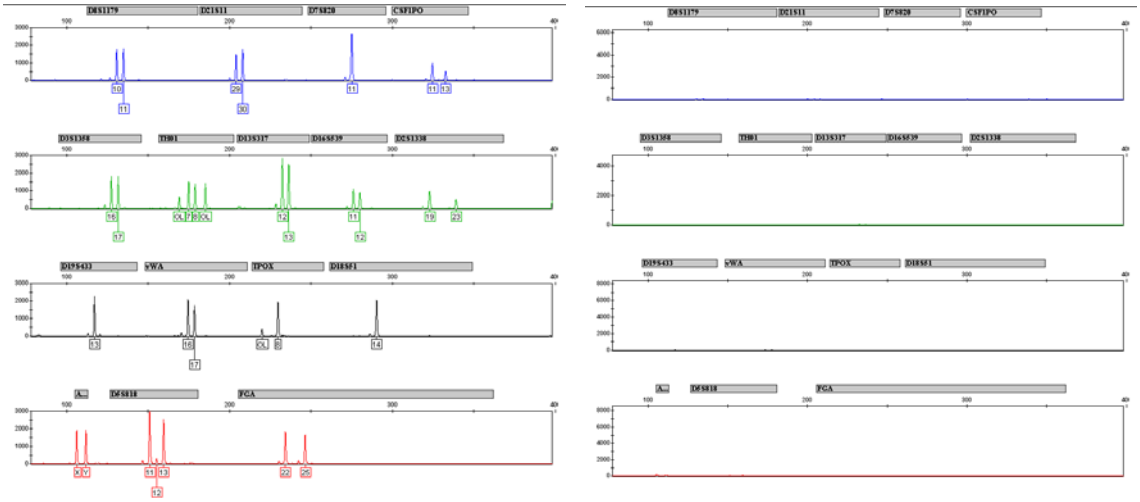
DNAP TECH PROTOCOL | **PROMEGA PP16HS KIT**



Punches of 1.2 mm from 2 years aged blood stains on blue jeans were subjected to direct STR typing without DNA extraction, using OmniTaq + PEC (left) with the PP 16HS primers, or the Power Plex 16 HS kit (right), in 15 ul PCR reactions. **Results:** The mutant enzyme, supplemented with PEC generated full profile, while with the PP16 HS kit a partial profile was obtained in this reduced reaction volume (consistent with its lower tolerance to indigo dye, rather than blood). However, in standard 25 ul reaction the Promega kit yielded full profile as well. Identical to the OmniTaq results with full profiles were obtained with the CesiumTaq enzyme as well (not shown).

Figure 13. DIRECT STR GENOTYPING OF SEMEN STAINS ON BLUE JEANS: DNAP TECH PROTOCOL VS. ID_PLUS KIT (LIFE TECH)

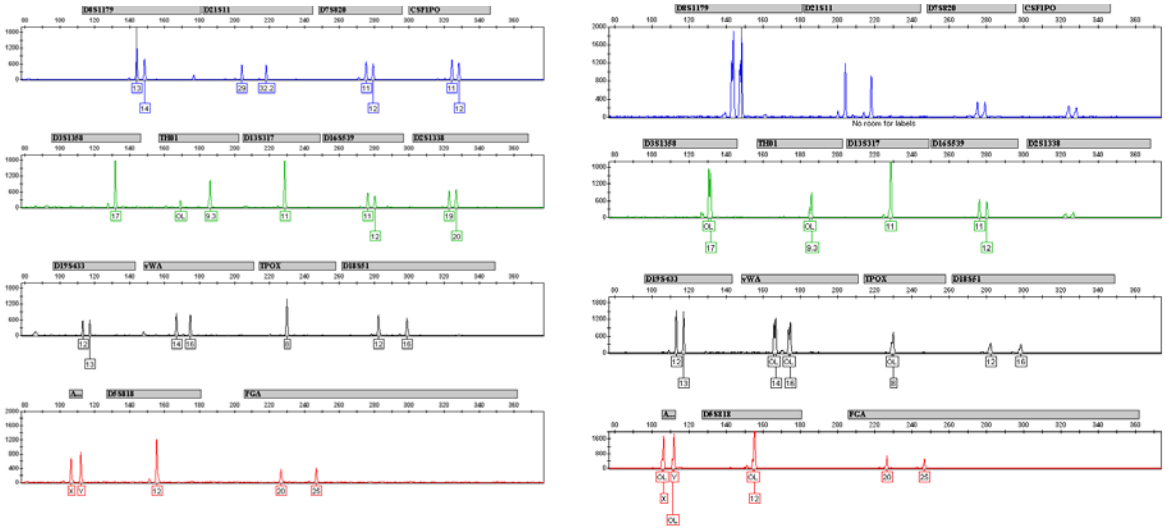
DNAP TECH PROTOCOL | **LIFE TECH ID_PLUS KIT**



Punches of 1.2 mm from sperm stains on blue jeans (samples kindly provided by BODE technology) were subjected to direct STR typing without DNA extraction, using Cesium Taq and PEC enhancer with the ID_PLUS kit primers (left), or the complete ID_PLUS kit (right). **Results:** The mutant enzyme generated a full STR profile, while no profile was obtained with the ID_PLUS kit (consistent with its lower tolerance to at least one of the challenging components here, the indigo dye). The OmniTaq mutant enzyme worked with such samples as good as CesiumTaq (not illustrated).

Figure 14. DIRECT STR GENOTYPING OF MIXED BLOOD AND SEMEN STAINS ON BLUE JEANS: DNAP TECH PROTOCOL VS. ID_PLUS KIT (LIFE TECH)

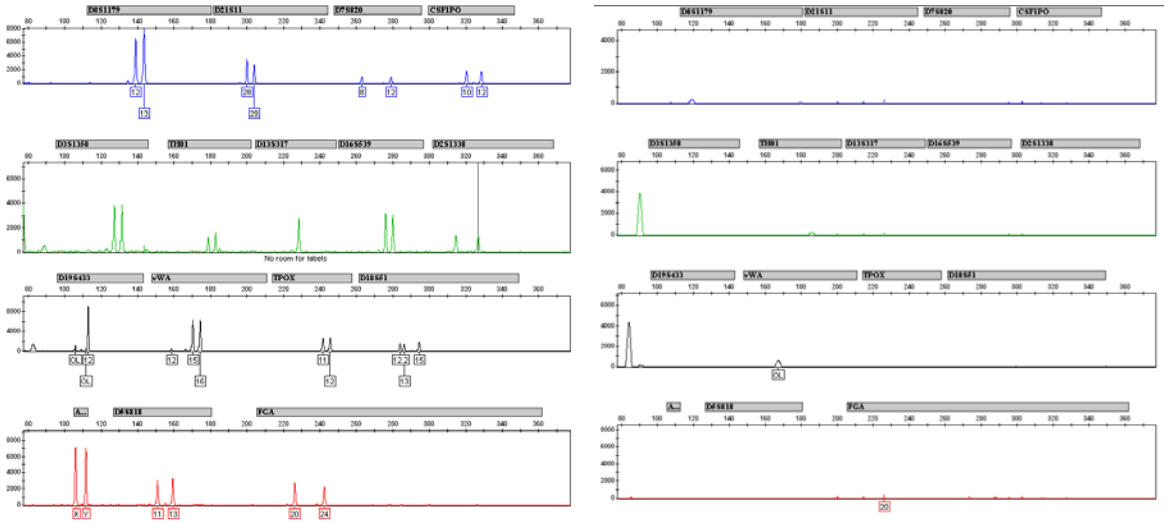
DNAP TECH PROTOCOL | **LIFE TECH ID_PLUS KIT**



Punches of 1.2 mm from mixed blood and sperm stains on blue jeans were subjected to direct STR typing without DNA extraction, using OmniTaq + PEC with ID_PLUS primers (left), or the complete ID_PLUS kit (right). **Results:** When using the ID-PLUS kit, a relatively less balanced profile, with some allele drop-out tendency and stutters was observed

Figure 15. DIRECT STR GENOTYPING OF BLOOD AND SOIL STAINS ON COTTON: DNAP TECH PROTOCOL VS. ID_PLUS KIT (LIFE TECH)

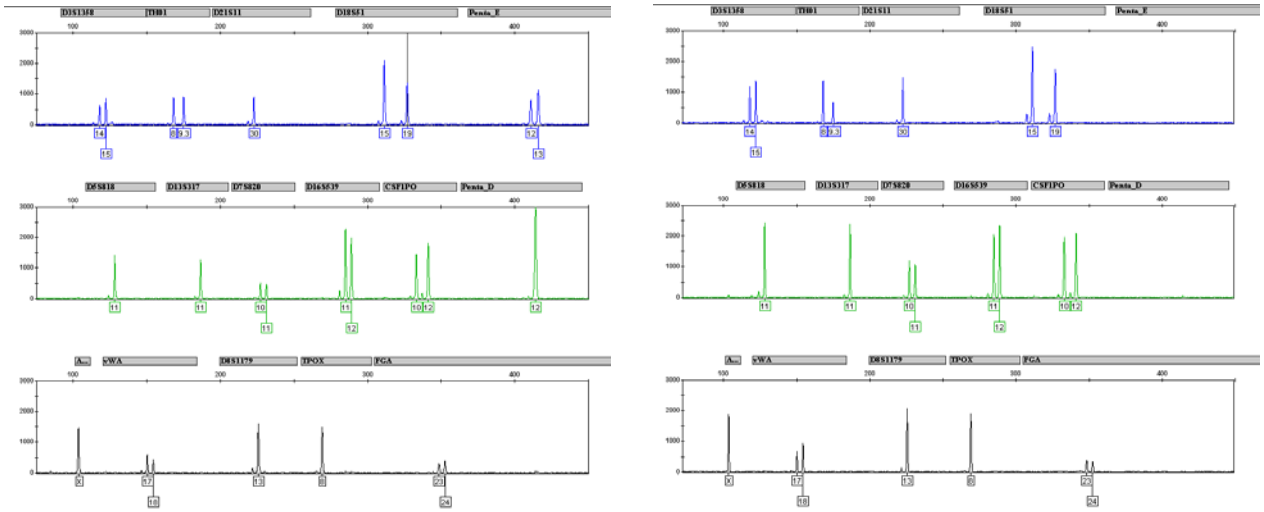
DNAP TECH PROTOCOL | **LIFE TECH ID_PLUS KIT**



Punches of 1.2 mm from mixed blood and soil stains on a cotton T-shirt (samples kindly provided by BODE Technology) were subjected to direct STR typing without DNA extraction, using OmniTaq + PEC (left) with ID_PLUS primers, or the complete ID_PLUS kit (right). **Results:** The inhibition-resistant mutant enzyme, supplemented with PEC enhancer cocktail, generated near to full profile, while no profile was obtained with the ID-PLUS kit (consistent with its low tolerance to humic acid, rather than blood)

Figure 16. DIRECT STR GENOTYPING OF CRUDE URINE SAMPLES: DNAP TECH PROTOCOL VS. POWER PLEX 16HS KIT (PROMEGA)

DNAP TECH PROTOCOL | **PROMEGA PP16HS KIT**

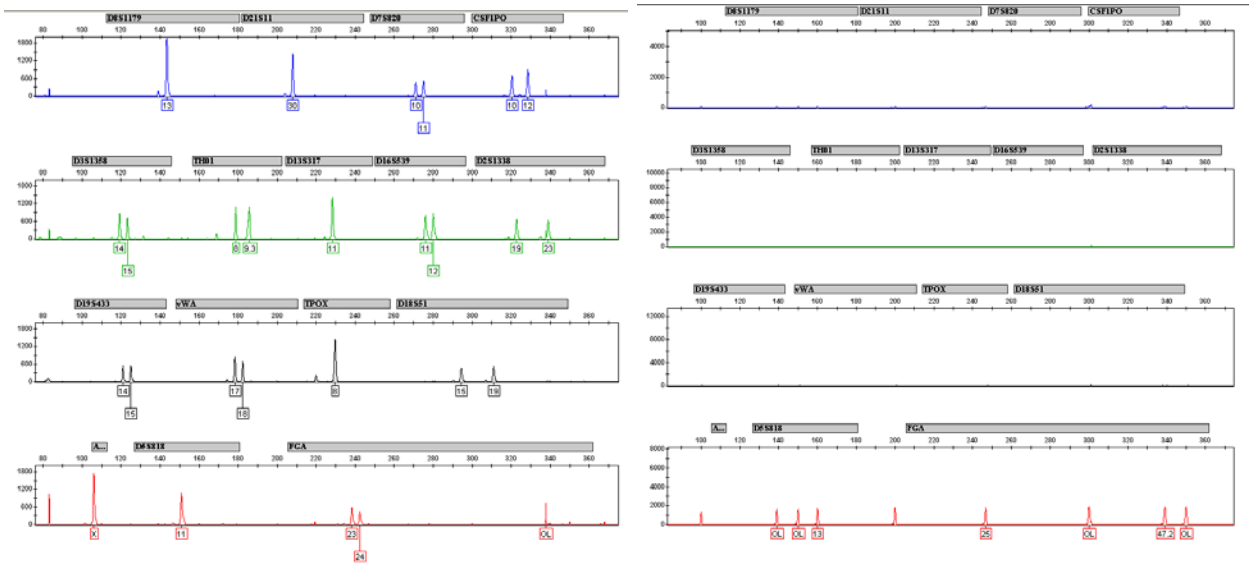


OmniTaq and PEC were used for STR genotyping with the Promega Power Plex 16 kit primers, using 1 ng human DNA in the presence of 5 ul urine and no DNA extraction.

Results: the combination of our novel mutant enzyme and enhancer cocktail can generate a full genotyping profile in the presence of the inhibitory urine skipping DNA extraction. In the profiles generated by the Promega kit some allele drop-outs were observed.

Figure 17. DIRECT STR GENOTYPING OF CRUDE URINE SAMPLES: DNAP TECH PROTOCOL VS. ID_PLUS KIT (LIFE TECH)

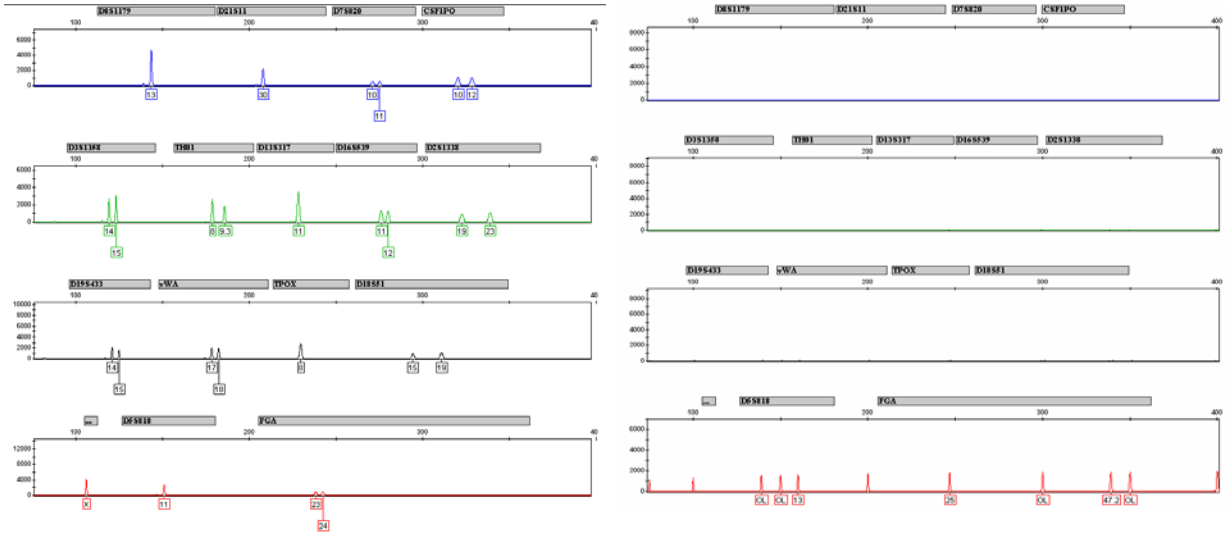
DNAP TECH PROTOCOL | **LIFE TECH ID-PLUS**



OmniTaq and PEC enhancer cocktail were used for STR genotyping with the ID-PLUS kit primers, using 1 ng human DNA in the presence of 2.5 ul urine, and no DNA extraction. In control reactions the complete ID-PLUS kit was used (right panel). **Results:** the novel mutant enzyme, supplemented with the PEC enhancer cocktail can tolerate the urine inhibition and generate a full genotyping profile, while no profile was obtained with the ID_PLUS kit.

Figure 18. DIRECT STR GENOTYPING OF CRUDE SAMPLES CONTAINING HUMIC ACID: DNAP TECH PROTOCOL VS. ID_PLUS KIT (LIFE TECH)

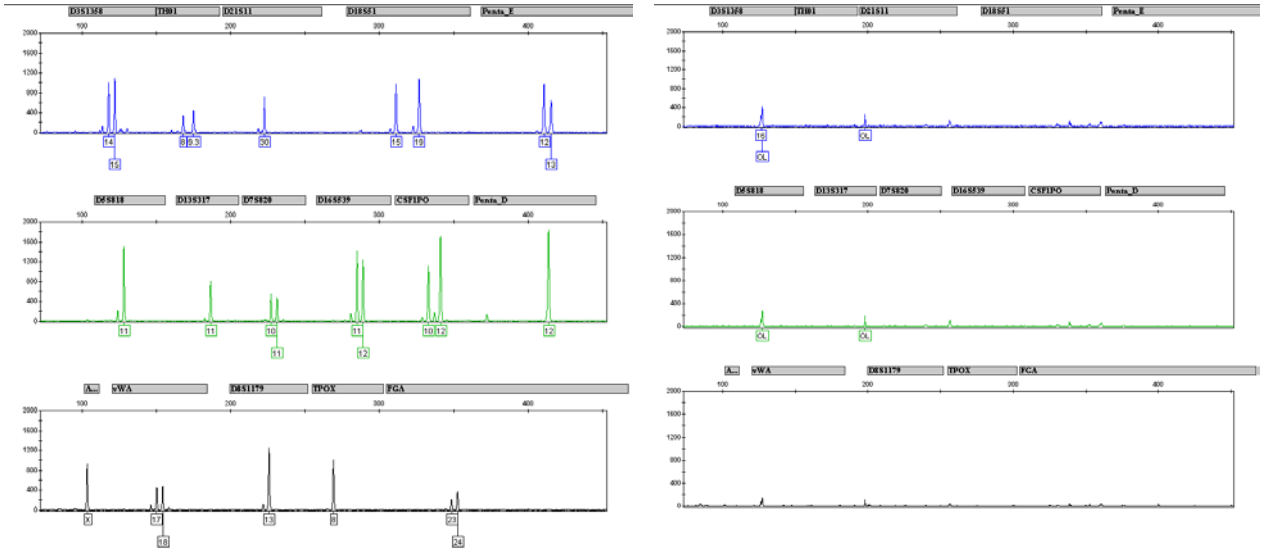
DNAP TECH PROTOCOL | **LIFE TECH ID-PLUS**



OmniTaq and PEC enhancer cocktail were used for STR genotyping with the ID-PLUS kit primers, using 1 ng human DNA in the presence of 250 ng humic acid. In control reactions the complete ID-PLUS kit was used (right panel). **Results:** the combination of the novel mutant enzyme and enhancer cocktail can tolerate the inhibitor and generate a full genotyping profile, while a very partial profile with spurious peaks was produced with the ID_PLUS kit.

Figure 19. DIRECT STR GENOTYPING OF CRUDE SAMPLES CONTAINING BILE SALTS: DNAP TECH PROTOCOL VS. POWER PLEX 16HS KIT (PROMEGA)

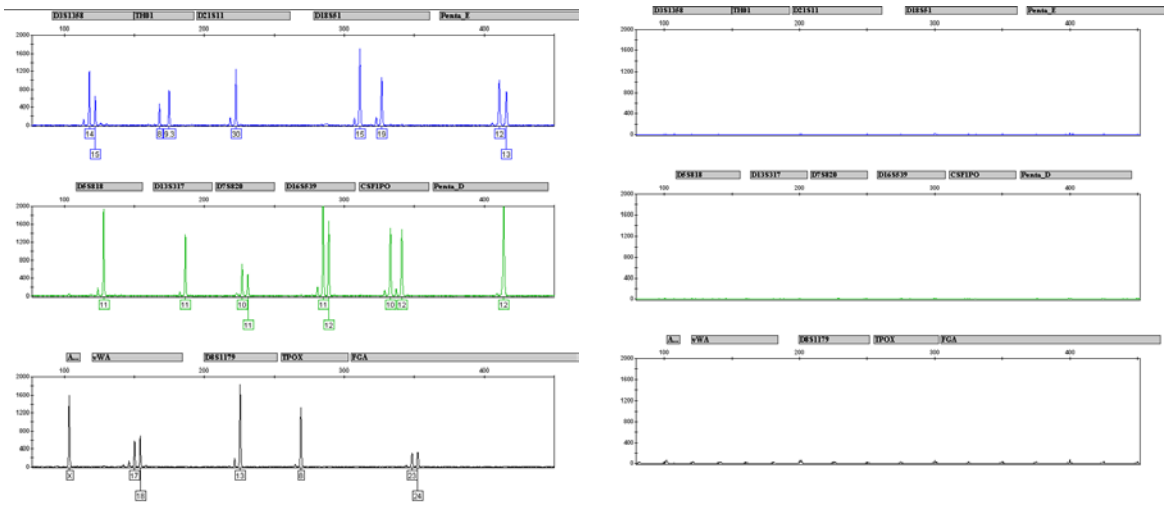
DNAP TECH PROTOCOL | **PROMEGA PP16HS**



OmniTaq and PEC were used for STR genotyping with the Promega Power Plex 16 kit primers, using 1 ng human DNA in the presence of 5 ul bile salts extract (12 ug/ul) and no DNA extraction. In control reactions the complete PP16HS kit was used (right panel). **Results:** Our novel mutant enzyme, supplemented with the PEC enhancer cocktail, can generate a full genotyping profile in the presence of the inhibitory bile salts, while no profile was generated with the PP16HS kit

Figure 20. DIRECT STR GENOTYPING OF CRUDE SAMPLES CONTAINING INDIGO DYE: DNAP TECH PROTOCOL VS. POWER PLEX 16HS KIT (PROMEGA)

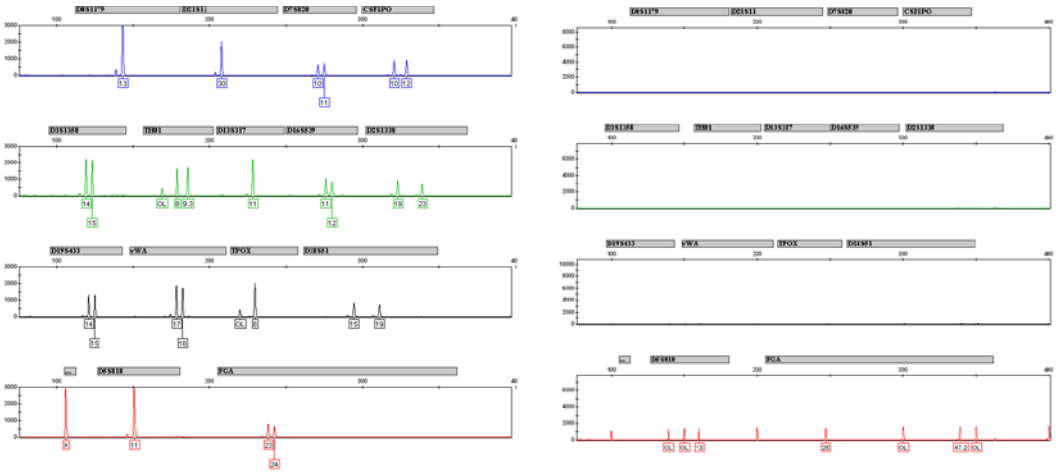
DNAP TECH PROTOCOL | **PROMEGA PP16HS**



OmniTaq and PEC-plus enhancer cocktail were used for STR genotyping with the Promega Power Plex 16 kit primers, using 1 ng human DNA in the presence of 200 ug indigo dye. In control reactions the complete PP16HS kit was used (right panel). **Results:** The novel mutant enzyme, supplemented with the PEC enhancer, can tolerate the inhibitory dye and generate a full genotyping profile, while no profile was produced with the PP16 HS kit.

Figure 21. DIRECT STR GENOTYPING OF CRUDE SAMPLES CONTAINING INDIGO DYE: DNAP TECH PROTOCOL VS. ID_PLUS KIT (LIFE TECH)

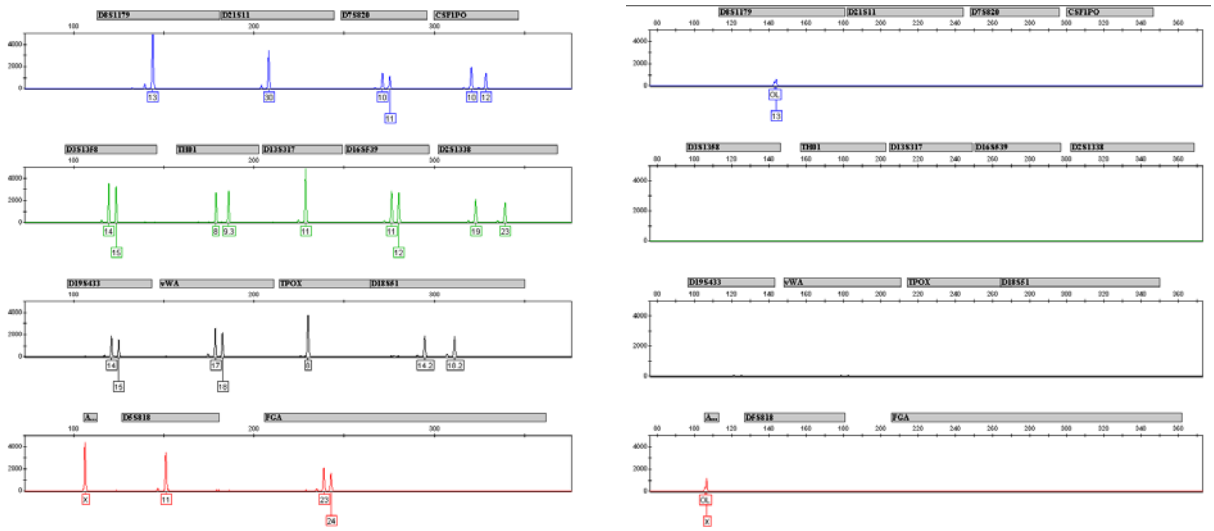
DNAP TECH PROTOCOL | **LIFE TECH ID-PLUS**



OmniTaq and PEC-plus enhancer cocktail were used for STR genotyping with the ID-PLUS kit primers, using 1 ng human DNA in the presence of 50 ug indigo dye. In control reactions the complete ID-PLUS kit was used (right panel). **Results:** the combination of the novel mutant enzyme and enhancer cocktail can tolerate the inhibitory dye and generate a full genotyping profile, while a very partial profile with spurious peaks was produced with the ID_PLUS kit.

Figure 22. DIRECT STR GENOTYPING OF CRUDE SAMPLES CONTAINING TANNINS: DNAP TECH PROTOCOL VS. ID_PLUS KIT (LIFE TECH)

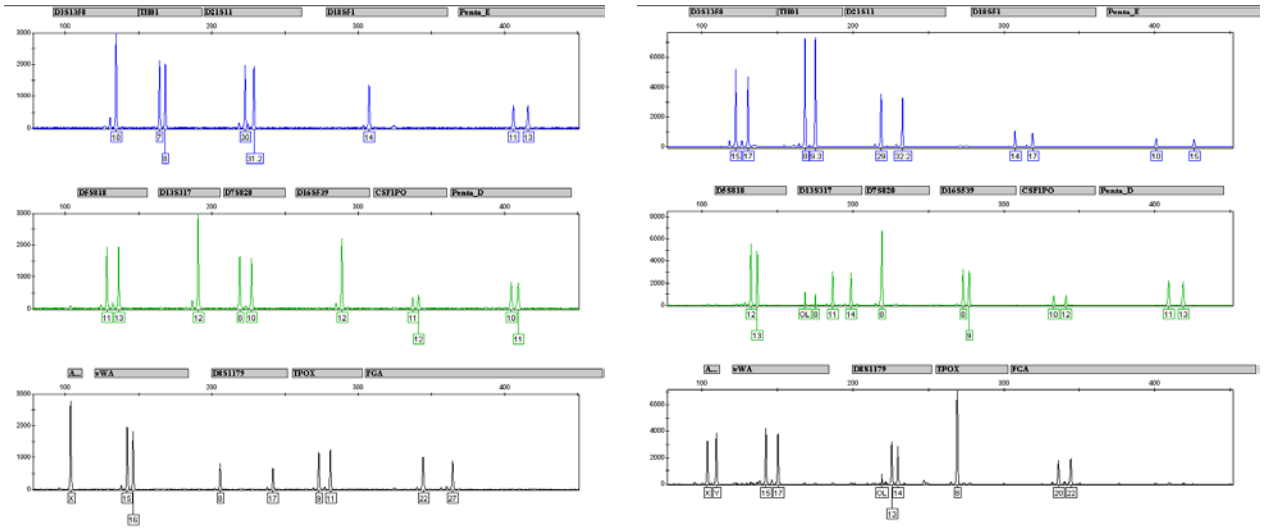
DNAP TECH PROTOCOL | **LIFE TECH ID-PLUS**



Cesium Taq and PEC-plus enhancer cocktail were used for STR genotyping with the ID-PLUS kit primers, using 1 ng human DNA in the presence of 4 ug tannins. In control reactions the complete ID-PLUS kit was used (right panel). **Results:** the combination of the novel mutant enzyme and enhancer cocktail can tolerate the inhibitory and generate a full genotyping profile, while a very partial profile consisting of two peaks was produced with the ID_PLUS kit.

Figure 23. DIRECT STR GENOTYPING OF CIGARETE BUTTS AND SODA CAN SWABS WITH CESIUM TAQ MUTANT ENZYME

Cigarette Butts **Soda Can Swabs**

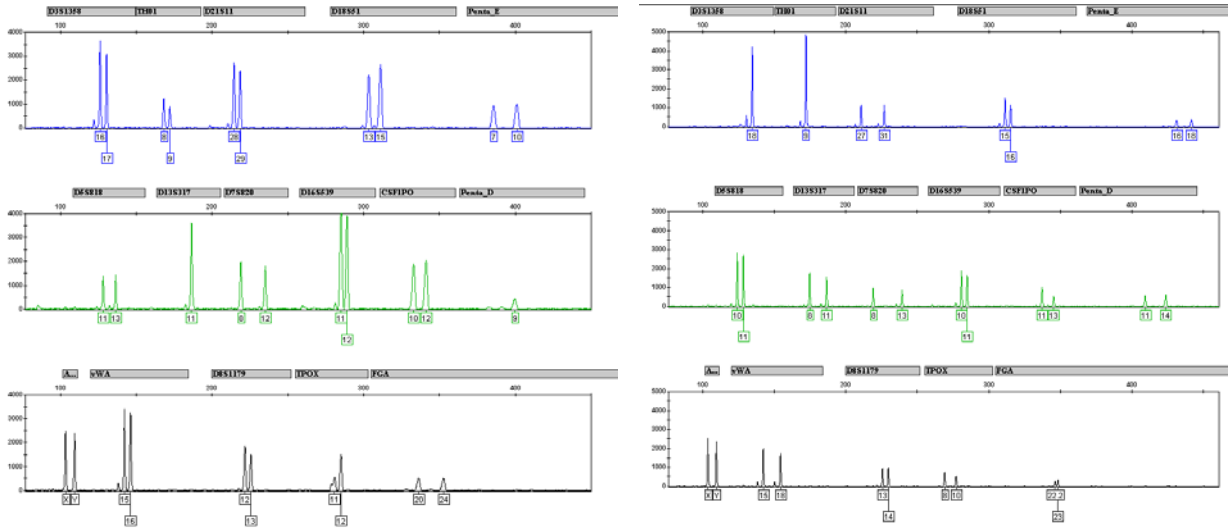


Punches of 1.2 mm from cigarettes butts and mouthpiece soda can swabs (samples kindly provided by BODE Technology) were subjected to direct STR typing without DNA extraction, using Cesium Taq enzyme with its optimal buffer without enhancer, and the Promega PowerPlex 16HS kit primers.

Results: The mutant enzyme can generate complete profiles with such samples without DNA extraction. Similar results were obtained with the OmniTaq mutant enzyme.

Figure 24. DIRECT STR GENOTYPING OF BLOOD STAINS ON BLACK COTTON AND BUCCAL SWABS WITH CESIUM TAQ MUTANT ENZYME

BLOOD STAINS **BUCCAL SWABS**



Punches of 1.2 mm from blood stains on black cotton fabric and buccal swabs (samples kindly provided by BODE Technology) were subjected to direct STR typing without DNA extraction, using Cesium Taq enzyme with its optimal buffer, and the Promega PowerPlex 16HS kit primers. For the blood samples the enzyme was supplemented with our PEC enhancer.

Results: The mutant enzyme can generate complete profiles with such samples without DNA extraction. Similar results were obtained with the OmniTaq mutant.

Table 1. PERFORMANCE OF THE NOVEL PCR MASTERMIXES OF DNA POLIMERASE TECHNOLOGY VS. THE IDENTIFILER PLUS (LIFE TECHNOLOGY) AND POWER PLEX 16 HS (PROMEGA) KITS IN DIRECT STR GENOTYPING OF CRUDE SAMPLES WITHOUT DNA EXTRACTION

CRUDE SAMPLE:	**STR PROFILES GENERATED WITH:**		
	LIFE TECH ***ID-PLUS***	**PROMEGA** ***PP-16 HS***	**DNAP TECH** ***ENZ / PEC***
Whole Blood / 0.1-5%	not tested	FULL	*FULL*
Whole Blood / less than 2 nL	not tested	partial	*few drop-outs*
Blood Stains on Blue Jeans	few drop-outs	FULL	FULL
Blood Stains on Black Cotton	FULL	FULL	FULL
Semen Stains on Blue Jeans	NO	FULL	FULL
Blood+Soil Stains on Cotton	NO	FULL	FULL
Semen+Blood Stains on Jeans	few drop-outs	FULL	FULL
Blood Stains on FTA Cards	FULL	FULL	FULL
Heparin-Blood on FTA Cards	not tested	partial	FULL
Urine / at least 20%	NO	few drop-outs	FULL
Aged Buccal Swabs	FULL	FULL	FULL
Soda Can Swabs	FULL	FULL	FULL
Cigarette Butts	FULL	FULL	FULL
Humic Acid / at least 250 ng	NO	FULL	FULL
Bile Salts / at least 60 ug	FULL	NO	FULL
Indigo Dye / at least 200 ug	NO	NO	FULL
Tannins / at least 4 ug	NO	FULL	FULL

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