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EVALUATION OF A NOVEL 'CARRIER' SPERM METHOD FOR IMPROVING THE YIELD OF HUMAN SPERM CELL DNA DURING DIFFERENTIAL EXTRACTION ANALYSIS

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EVALUATION OF A NOVEL 'CARRIER' SPERM METHOD FOR IMPROVING THE YIELD OF HUMAN SPERM CELL DNA DURING DIFFERENTIAL EXTRACTION ANALYSIS

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Abstract

Conventional differential extraction (DE) has been known to sacrifice percent yield and purity of sperm cell DNA due to the drastic variation in the quality of the separation (i.e., sperm cell retention) due to the multiple lysis, centrifugation, liquid transfer, and washing steps involved in the process. This method has also been shown to result in the loss of 94-98% of male sperm cells (Vuichard et al., 2011). Thus, over thirty years of research has been conducted to improve conventional DE, yet no new methods have been widely adopted despite their shown increase in speed, sensitivity, and accuracy. Like conventional DE, most of these new methods still sacrifice sperm cell DNA yield and purity which are two key criteria which should be considered. This lack of improvement in conventional DE methodology is a significant shortcoming of the forensic science community and is a major explanation why forensic crime laboratories are slow to adopt any new extraction method.

Nevertheless, this research aimed to improve upon human sperm cell DNA retention in both the lysis and wash steps of an organic extraction methodology with the use of two separate 'carrier' sperm. This 'carrier' sperm, in the form of purified salmon sperm DNA or intact horse sperm cells, was meant to act as a "barrier" to human sperm cell loss during the extraction steps of the DNA analysis process and allow for an increased retention of human sperm cell DNA. However, increased retention was not demonstrated due to an overall quantitative decrease in human DNA (ng/µL) of those samples supplemented with 'carrier' sperm versus those samples of only human semen when using qPCR analysis. Thus, our hypotheses were not supported since this novel one-step modification to organic extraction demonstrated a negative advancement towards improving conventional differential extraction for forensic sexual assault casework.

Chapter 1 – Introduction

According to the Bureau of Justice Statistics in 2020, 0.12% of persons twelve years of age or older were victims of completed or attempted rape, or of completed sexual assault with injury or force in the United States (Morgan & Thompson, 2021). In 2020, the population of the United States was estimated to be 331.4 million people (Bureau, U.S.C., 2021). Therefore, approximately 397 thousand people were victims of sexual assault or rape in 2020 alone. Of those assaults, it is estimated that only 22.9% were reported to the police (Morgan & Thompson, 2021). This large quantity of sexual assault casework typically requires the rapid and precise use of DNA analysis in forensic laboratories. Most DNA laboratories use a method known as conventional differential extraction (DE) to analyze sexual assault sample types; however, as will be discussed, this method has been shown to result in the loss of approximately 94-98% male sperm cells (Vuichard et al., 2011). Nevertheless, the use of conventional DE for DNA analysis is crucial for helping our communities by identifying those who are responsible for victimizing the life of another human.

If the incident surrounding a sexual assault requires DNA testing, then a sexual assault evidence kit (SAEK) will be collected from the victim. According to the National Institute of Justice, SAEKs typically contain swabs, test tubes, microscope slides, and collection envelopes for hairs and fibers. The type of evidence collected in these kits depend on what occurred during the assault. Typically, the victim is swabbed for samples of skin cells, saliva, semen, or other bodily fluids. The victim's clothing, bedsheets, or other items from the crime scene may also be collected after a reported assault. Sexual assault evidence may contain a mixture of cells from two or more separate donors. This review focuses on female epithelial cell donors and male

contributor sperm cell donors. The intention of collecting these evidentiary items is that they may contain the male contributor's DNA.

Collected SAEKs are typically sent to forensic crime laboratories for DNA analysis. As mentioned, most forensic laboratories favor a DNA extraction method called conventional differential extraction to separate the two cell types (i.e., epithelial and sperm cells). This method was developed by Gill, Jeffreys, and Werrett in 1985 and is accomplished by first subjecting the evidentiary item (i.e., swab, cutting of clothing, etc.) to a preferential lysis buffer containing 0.01 M Tris-HCl pH 8.0, 0.01 M EDTA, 2% sodium dodecyl sulfate (SDS), 0.1 M NaCl, and 20 μg ml-1 proteinase K (Pro K) to break open all non-sperm cells (i.e., epithelial cells), releasing their DNA while leaving the sperm cells intact (Gill et al., 1985). This non-sperm cell fraction (i.e., epithelial DNA) is removed for later analysis. The undigested sperm cells in the remaining fraction are then purified by a series of wash and centrifugation steps to remove any remaining non-sperm cell DNA. Finally, sperm cell lysis is accomplished with SDS, Pro K and the addition of 0.039 M dithiothreitol (DTT) which facilitates the breakdown of disulfide bonds within the sperm's nuclear membrane, allowing for the release of sperm cell DNA (i.e., male contributor DNA) (Timken et al., 2019). Non-sperm and sperm fractions are then separately quantified, amplified, and analyzed for genetic profiling, thus leading to possible male contributor identifications based on their DNA profiles. However, not always is there enough male sperm cell DNA to generate a DNA profile due to the shortcomings of this methodology. While conventional differential extraction is successful at isolating most sperm cells from epithelial cells, it has failures when considering the overall effectiveness of the procedure. Conventional DE falls short in that it is a time-consuming process, taking up to eight hours to perform per sample. This method also does not provide high sensitivity for low quantity DNA

samples, which are common sample types seen in forensic sexual assault casework. Plus, conventional DE sacrifices percent yield and purity due to the drastic variation in the quality of the separation (i.e., sperm cell retention) due to the multiple lysis, centrifugation, liquid transfer, and washing steps involved in the process (Timken et al., 2019).

For any DNA extraction procedure there are two key criteria which should be considered before the protocol can be considered useful: percent yield of the extracted DNA and purity of the extracted DNA. Percent yield compares the amount of DNA obtained to the total amount of DNA which existed in the whole or portion of the samples used for the extraction (Cotton & Fisher, 2015). Percent yield can also be thought of as the quantity of DNA obtained. Not always is there an abundant amount of DNA present. This is common when targeting male DNA in sexual assault evidence due to the higher ratio of victim epithelial cells in comparison to sperm cells present. Therefore, it is important to retain as many sperm cells as possible throughout the DNA extraction process. Purity is a measure of the extent that non-nucleic acid cellular components and substrate extractable components are removed from the DNA solution (Cotton & Fisher, 2015). Purity can also be thought of as the quality of DNA. DNA can become degraded due to several reasons like excessive exposure to heat, UV, or alkaline pH conditions. For sexual assault casework specifically, sperm cell DNA quality can be compromised due to the conditions of the female vaginal tract before sample collection as leukocytes begin attacking the foreign sperm cells due to the female's immune response (Schjenken & Robertson, 2020). Plus, mixtures of cell types (i.e., epithelial and sperm cells) can cause the purity of the extraction of each cell type to be compromised. In general, poor quality or quantity DNA can cause issues in the analysis of a DNA profile such as allelic dropout due to low DNA concentration which results in less discriminatory results. For any differential extraction procedure, the percent yield

and purity of the sperm cell DNA retained from cell separation is crucial. Yet, high purity and percent yield can be difficult to obtain while performing conventional DE due to carryover of sperm cell DNA into the epithelial fraction or epithelial cell DNA into the sperm fraction.

There are two possible sources of sperm cell DNA presence in the epithelial cell fraction. First, is from the expected lysis of male round cells which will lyse along with the epithelial cells (Cotton & Fisher, 2015). Second, is from premature sperm cell lysis caused by damage to the sperm cell wall or sperm DNA during post collection storage (Clark et al., 2020). While a small amount of carryover of sperm cell DNA into the epithelial cell fraction is common, it does compromise the yield of male DNA for analysis.

Carryover of epithelial cell DNA into the sperm fraction is more likely to occur when a large ratio of epithelial cells to sperm cells is present in the starting material, which is common for forensic sexual assault evidence. Reduction of epithelial cell DNA carryover into the sperm pellet involves repeated washing of the sperm pellet by addition of buffer, re-centrifugation, and removal of the supernatant (Cotton & Fisher, 2015). However, the sperm DNA yield can be diminished with the use of these multiple washing, centrifugation, and transfer steps. Generally, sperm DNA yield can be broken down into loss of sperm in the differential cell separation process and then loss of sperm DNA in the purification steps (Clark et al., 2020).

Conventional DE results in the loss of 94-98% of male sperm cells (Vuichard et al., 2011). Therefore, several other differential extraction methods have been researched to aid in the efficiency of differential extraction. Some of these newly developed methods utilize chemical separation techniques while others use physical separation to separate the two cell types. These methods include, but are not limited to: mild preferential lysis (Wiegand et al., 1992), an improved two-step method (Yoshida et al., 1995), the Differex™ system (Mudariki et al., 2013,

Tereba et al., 2004, and Tsukada et al., 2006), buffer modifications (Norris et al., 2007 and Lounsbury et al., 2014), selective degradation of epithelial cells (Garvin et al., 2009, Klein & Buoncristiani, 2017, and Hudlow & Buoncristiani, 2012), antibody based capture (Schoell et al., 1999, and Li et al., 2014), microfluidic sorting (Horsman et al., 2005 and Inci et al., 2018), and laser capture microdissection (Elliott et al., 2003, Meredith et al., 2012, and Sanders et al., 2007). Each of these methods, mentioned above, will be examined in later sections.

Over thirty years of research has been conducted to improve conventional differential extraction, yet no new methods have been adopted despite their shown increase in speed, sensitivity, and accuracy. Like conventional DE, most of these new methods still sacrifice sperm cell DNA yield and purity. This lack of improvement in conventional DE methodology is a significant shortcoming of the forensic science community and is a major reason why forensic crime laboratories are slow to adopt any new method. Nevertheless, by examining literature that demonstrates how DE methods have evolved and how these novel methods aimed to improve sperm cell DNA yield and purity, an understanding of how the use of these methods to help improve DE efficiency can be made. Lastly, a future research idea with aim to improve human sperm cell DNA yield will be described.

Chemical Separation Methods

Mild Preferential Lysis –

In 1992, Wiegand et al. developed a modified differential extraction method to reduce sperm cell lysis and increase epithelial cell lysis by adjusting the conditions during the preferential lysis stage. Preferential lysis buffer is used first to lyse open epithelial cells. This buffer typically contains SDS and Pro K. SDS is an anionic detergent that facilitates the breakdown of cell membrane proteins to help lyse open epithelial cells (Butler, 2010). Pro K is

similar in that it digests proteins, but it is also an important enzyme that breaks down nucleases which would otherwise degrade the free DNA. Sperm cells are generally resistant to both of these chemicals. In this method, the amount of Pro K added to the lysis buffer was dependent on the number of sperm cells present on the swab sample, but to facilitate better epithelial lysis, the concentration of Pro K used was always greater than what was used by Gill et al. (1985), (Wiegand et al., 1992). Advantages of this method included reducing the number of washing stages and transfer steps during preferential lysis to minimize sperm loss and creating a less stringent lysis condition to help reduce sperm cell lysis compared to conventional DE. Although the results of mild preferential lysis showed a reduction of epithelial DNA concentrations while sperm DNA concentrations remained fairly constant, sperm cells were still lost during the preferential lysis step. This loss of cells caused a decrease in the percent yield of sperm obtained. Another disadvantage to using this method is that it requires knowing the concentrations of epithelial and sperm cells prior to differential extraction. Need for this prior knowledge makes this method incompatible with forensic testing of sexual assault evidence because cell concentrations are not known prior to extraction.

Two-Step Method –

Yoshida et al. (1995) developed a different conventional DE modification called the two-step DE method. This method is similar to that of Wiegand et al. (1992) because this method also used elevated Pro K concentrations with SDS during the initial proteolytic step. It is dissimilar in that this method used a higher digestion temperature, 70°C, compared to 37°C used in both Wiegand et al. (1992) and Gill et al. (1985). Adoption of 70°C as a digestion temperature was used to inhibit DNAase activity; therefore, the sperm DNA remains relatively intact (Yoshida et al., 1995). As will be examined later, DNAase is an enzyme that degrades DNA, so heat

inactivation of this enzyme would help achieve a pure male fraction. However, sperm DNA was detected within epithelial DNA samples on polyacrylamide gels after PCR amplification. This result suggests that there was slight disruption of the sperm heads during the first step of digestion; therefore, sperm DNA percent yield was decreased (Yoshida et al., 1995). Despite this loss in percent yield, an advantage of this modified two-step approach was an increase in sperm fraction purity. Results showed that sperm DNA gave no traces of contamination by epithelial cells when the epithelial cells were exposed to elevated digestion temperatures. Thus, in the modified method of the two-step differential extraction procedure, sperm DNA and epithelial cell DNA could be favorably isolated from a sample which contained a greater amount of vaginal epithelial cells than sperm (Yoshida et al., 1995).

Differex™ System –

In the early 2000s, Promega Corporation developed the Differex™ system to separate sperm and epithelial cells from sexual assault samples in as little as 2 hours, including DNA purification (Tereba et al., 2004). This method involves Pro K selective digestion of epithelial cells followed by differential centrifugation and phase separation. The digested sample (i.e. a cotton swab containing lysed epithelial cell debris, epithelial cell DNA, and sperm cells) and buffer are placed in a spin basket seated in a tube containing a nonaqueous Separation Solution. The Separation Solution is denser than water, but less dense than sperm (Tereba et al., 2004, p. 9). During centrifugation, the sperm are pulled from the sample (i.e., cotton swab) and rapidly move through the Separation Solution to form a tight pellet at the bottom of the tube. The epithelial DNA remains in the aqueous buffer, which forms a layer on top of the denser Separation Solution (Tereba et al., 2004). The supernatant containing epithelial DNA can then be removed for further purification. The remaining sperm pellet can be treated with DTT and

purified using a DNA purification kit. The Differex™ system was designed to be coupled with Promega Corporation's DNA IQ™ kit for further DNA purification, although it can also be coupled with an organic/phenol chloroform method (Tereba et al., 2004).

Mudariki et al. (2013) compared the Differex™ system coupled with DNA IQ™ to Qiagen's QIAamp® DNA mini kit. They found that there was no significant difference between the two kits based on the amounts of DNA extracted and DNA profile classifications; however, the Differex™ system used fewer tubes with less liquid transfers and took approximately half the time taken using the Qiagen kit (Mudariki et al., 2013).

Another study by Tsukada et al. (2006) compared signal peak heights on electropherograms using the Differex™ system coupled with several different extraction methods. They demonstrated that the Differex™ system coupled with the QIAamp® DNA Micro kit showed the highest peaks and the lowest peaks were observed when Differex™ was coupled with organic phenol/chloroform extraction. Tsukada et al. (2006) also evaluated the Differex™ system in comparison to the two-step method created by Yoshida et al. (1995). They showed that the use of the Differex™ system could successfully extract male DNA from mixed stains with similar efficiency as the two-step approach (Clark et al., 2020).

In general, the Differex™ protocol eliminates the multiple wash steps that are required with conventional DE. Yet, incomplete Pro K digestion results in epithelial cells pelleting with the sperm, thus decreasing sperm purity (Tereba et al., 2004). Plus, sperm lysis still occurs during the Pro K digestion of epithelial cells which decreases sperm percent yield (Tereba et al., 2004).

Buffer Modifications –

Thus far modifications of Pro K concentrations in the preferential lysis buffer have been discussed, but other research has been conducted in which other elution or lysis buffers have been tried.

Norris et al. (2007) researched several different lysis detergents to determine which would be the best for cell elution and recovery from cotton swabs compared to conventional DE detergent. They found that the use of Sarkosyl with SDS yielded twofold higher sperm cell recoveries than conventional DE detergent (Pro K and SDS) (Norris et al., 2007). Their results also indicated that the solo use of SDS enhanced the release of sperm and epithelial cells from cotton swabs as compared to conventional DE (Pro K and SDS), thus improving sperm cell yield (Norris et al., 2007). Overall, Norris et al. (2007) determined that the use of an anionic detergent (i.e. SDS, Sarkosyl) for cell elution provided twofold enhancement of sperm cell yield over conventional DE.

Additionally, Lounsbury et al. (2014) aimed to combine elution (from cotton swabs) and lysis steps, creating a one-step method. To do so, they created a buffer that contained 10 mM Tris (hydroxymethyl) aminomethane pH 8.5, 10 mM 2-(N-morpholino) ethane sulfonic acid (MES), 1% SDS, and 10 mg/mL Pro K. They also wanted to increase sperm yield by refining the buffer pH, incubation temperature, incubation time, and detergent used (Lounsbury et al., 2014). The study found that sperm cell yield increased from 80% at pH 8.0 to 89% at pH 8.5. They also determined that the optimal temperature for sperm cell recovery was 42°C and the optimal incubation time was 30 minutes (Lounsbury et al., 2014). The anionic detergent SDS is widely used in cell lysis applications because of the ability to disrupt cell membranes by interfering with the protein–protein interactions required for membrane integrity (Lounsbury et al., 2014, p. 87). It has previously been shown that SDS provides higher sperm cell recoveries than cationic,

zwitterionic and non-ionic detergents (Norris et al., 2007). When compared to sodium lauroyl sarcosinate (Sarkosyl), an anionic detergent with a different head group than SDS (sarcosinate vs. sulfate), SDS was again shown to yield higher recoveries (Norris et al., 2007). To determine if an alternative sulfate-based detergent would provide similar sperm cell recoveries as SDS, sodium octyl sulfate (SOS) was added to the one-step buffer, at pHs 8.25 and 8.5, in place of SDS (Lounsbury et al., 2014). Results showed that sperm cell recoveries were higher when using SDS (81%) at pH 8.25 and 85% pH 8.5 than SOS in the one-step buffer 70% at pH 8.25 and 72% pH 8.5, thus reiterating the results shown by Norris et al. (2007) (Lounsbury et al., 2014). Overall, Lounsbury et al. (2014) concluded that 1) by increasing the pH of the initial incubation buffer, the recovery of intact sperm cells was increased to approximately 90%, a nearly 200% increase as compared to a conventional DE buffer, and 2) use of the optimal incubation conditions along with the optimized one-step buffer provided nearly double the recovery of sperm cells in half the incubation time, as compared to a conventional DE buffer.

Anionic surfactants like SDS play the most significant role in buffer solutions as expressed by Norris et al. (2007) and Lounsbury et al. (2014), but other modifications like pH, temperature, or the use of sodium hydroxide are also factors that can affect the results. Therefore, from the research that was conducted using different elution, lysis, or detergent buffers, it can be noted that these buffer modifications play a role in the increase of both sperm cell recovery and yield.

Selective Degradation of Epithelial Cells –

As stated, the presence of epithelial cells in the sperm cell fraction can greatly reduce sperm cell purity. Thus, research has been conducted to specifically target the epithelial cells

within the sperm cell fraction for DNA degradation. This can be accomplished with the use of nucleases. Nucleases are enzymes that, under specific conditions, can degrade soluble cell DNA.

Garvin et al. (2009) used a specific nuclease called DNase I to selectively degrade the soluble epithelial cell DNA from the sperm fraction. DNase I is highly selective for the degradation of epithelial cell DNA and is not selective for the degradation of sperm DNA present in intact sperm heads. DNase I is active in a modified Triton X-100 and Pro K buffer for the elution of sperm off of cotton swabs. Triton X-100 is used in place of SDS as the usual detergent because SDS interacts with DNase I. This method works by eluting both the epithelial and sperm cells off of the cotton swab with the modified buffer. The epithelial cells will then lyse in this buffer which allows the soluble, free epithelial cell DNA to be present. A single centrifugation step will pull the sperm cells to the bottom of the tube and leave the epithelial cell DNA in solution. An aliquot of the epithelial cell DNA is then taken to serve as the victim's DNA fraction. The remaining solution and sperm cell pellet is then treated with DNase I to degrade all the remaining epithelial cell DNA, thus rendering it useless for DNA analysis and leaving behind a purified sperm pellet. The sperm pellet is then treated with DTT to lyse open the sperm cells and EDTA to inactivate any remaining DNase I; therefore, providing the sperm cell DNA fraction. The authors concluded that the nuclease method provided a superior male fraction compared to both the Differex™ system and conventional DE, because unlike the other methods, their method does not require the washing or separation steps which can cause sperm cell loss. Garvin et al. (2009) summarized their findings by stating that their nuclease method generated male DNA profiles of equal or higher quality than conventional DE.

Similarly, Hudlow & Buoncristiani (2012) and Klein & Buoncristiani (2017) both used DNase I to obtain more purified sperm cell DNA fractions for analysis. Hudlow & Buoncristiani

(2012) paired the use of a sodium hydroxide lysis buffer with DNase I and found that their methods also provided successful male DNA profiles that are comparable to those obtained using conventional DE. Hudlow & Buoncristiani (2012) stated that their method is rapid, easy to use, and relatively inexpensive which makes it an effective method for reducing epithelial cell DNA carryover into the sperm fraction compared to conventional DE. Klein & Buoncristiani (2017) used the Erase Sperm Isolation Kit from Paternity Testing Corporation© which effectively utilizes the same methods as Garvin et al. (2009). Klein & Buoncristiani (2017) performed a comparative study between this kit, conventional DE, and the two-step method developed by Yoshida et al. (1995). Klein & Buoncristiani (2017) demonstrated that, when testing mock sexual assault samples with each method, the Erase Sperm Isolation Kit produced more purified sperm fractions but had a 6-fold lower yield in total male DNA (Clark et al., 2020).

In general, the use of nucleases has become a popular method for supplementing differential extraction. Nucleases can provide a more purified sperm DNA fraction due to the enzyme digestion of the epithelial cells which allow for the sperm cell DNA to be less compromised within the sample. Yet, the sperm cell yield can still be compromised due to loss of male DNA in the initial lysis stage due to liquid transfer steps.

Physical Separation Methods

Antibody Based Capture –

Schoell et al. (1999) introduced the use of flow cytometry to separate male sperm and epithelial cells in sexual assault samples. The flow cytometer used is a device that will sort the two cell types by manipulating the differences in properties of cell size and shape, surface phenotype, cytoplasm, and ploidy (Schoell et al., 1999). This study utilized major histocompatibility class I antigens, CD45, and cytokeratin. All three are found on the surface of

epithelial cells and can be detected with monoclonal antibodies combined with fluoroisothyocyanate, a green fluorescent dye (Schoell et al., 1999). Thus, sperm cells are not stained green, whereas all epithelial cells will show a distinct green fluorescence on the flow cytometer. In this study, cells from vaginal lavages were collected and used for flow cytometric cell sorting of the two different cell types. The study found that at a dilution of 10 epithelial cells to 1 sperm cell, this newer method had a sensitivity of 92% compared with 56% using conventional lysis method for the detection of sperm cells (Schoell et al., 1999). At a dilution of 80 epithelial cells to 1 sperm cell, the sensitivity of this sorting procedure and the conventional lysis method was 40% and 0%, respectively (Schoell et al., 1999). Lastly, at a dilution of 160 epithelial cells to 1 sperm cell, DNA typing for the male fraction was successful in 30% of cases after the cell sorting procedure and in none of the cases after the conventional lysis method (Schoell et al., 1999). Therefore, the use of flow cytometry for separation of cell mixtures containing approximately 1 µl of ejaculate was possible (Schoell et al., 1999). Compared to conventional DE, this method has greater sensitivity towards the detection of sperm cells in low concentrations.

Another method in which antibodies can be used for cell sorting was researched by Li et al. (2014). Sperm cells contain a protein called MOSPD3 in the sperm head, neck, mid-piece, and flagella. This protein is not found in epithelial cells. Li et al. (2014) used biotin labeled MOSPD3 antibodies to bind the protein on the sperm cell surface. Once bound to the sperm cell, MOSPD3 antibodies bind to an avidin-coated magnetic bead. Therefore, mixtures of sperm and epithelial cells were incubated with biotinylated MOSPD3 antibodies along with avidin-coated magnetic beads. The magnetic beads can be retained during washing and purification steps. The sperm cells are then eluted from the beads, which will leave a separated sperm cell fraction from

the epithelial cell fraction (Li et al., 2014). The authors tested 30 mock sexual assault cases with equal parts sperm and epithelial cells, as well as 52 true forensic SAEKs. The results showed that a full single-source male STR profile could be obtained in 80% of the mock mixed samples containing 103 sperm cells per milliliter and 100% of mock samples with ≥ 104 sperm cells per milliliter (Li et al., 2014). As for the true sexual assault kit evidence, male single-source profiles could only be obtained from 16% of the samples tested. Li et al. (2014) noted that the reduction in sperm cell retention was due to sperm cell degradation within the tail, flagellum, and mid-piece regions. Degradation of the sperm cell areas associated with antibody binding sites decreased sperm cell capture. It is common in real-world samples, that the tail, flagellum, and midpiece are frequently not observed from SAEKs; therefore, explaining why only a small percentage of the sexual assault evidence was successful (Clark et al., 2020).

While the method proposed was successful in mock cases for increasing sperm cell DNA yield, it was not successful for real-world applications. Additionally, the mock cases contained magnitudes greater concentrations of sperm cells compared to the sperm cell concentrations that are common in SAEKs. Thus, the likelihood of sperm cell yield increase was due to the presence of more starting material in this study. Overall, antibody-based capture where the antibodies are focused on the capture of epithelial cells are more successful in increasing sperm DNA purity, than methods where the antibodies are used for sperm cell capture. Additionally, flow cytometry as proposed by Schoell et al. (1999) has potential to increase sperm cell retention; therefore, increasing the DNA yield and purity compared to conventional DE.

Microfluidic Sorting -

Inci et al. (2018) produced better results than Li et al. (2014) when they looked at the use of a carbohydrate-ligand, Sialyl-LewisX (SLeX), paired with a microfluidic device for sperm cell

specific binding. SLeX is used by the sperm cells to bind to female egg cells during sexual reproduction; therefore, epithelial cells do not contain this carbohydrate-ligand. A microfluidic device, also referred to as a chip, is an instrument in which the mixed cell sample can be loaded onto. Once on the chip, sperm and epithelial cells are separated into separate chambers due to SLeX sorting. After being sorted into defined channels on the chip, both cell types are lysed open to free their DNA. The DNA from the epithelial and sperm cells are then separately collected from the chip and used for downstream STR profile analysis. The authors tested four simulated forensic sexual assault samples of differing cell concentrations. Using their method, Inci et al. (2018) succeeded to differentially capture sperm cells in channels with a high capture efficiency of 70–92% and a 60-92% reduction in the epithelial fraction. The authors concluded that microfluidic sorting coupled with sperm cell specific binding allowed for a method that that 1) differentially isolated sperm and lysed them on-chip, and extracted sperm DNA for downstream genetic analyses; 2) reduced the differential extraction time from 8 h to 80 min; 3) minimized the need for manual labor; and 4) increased capture efficiency of immuno-based separation of sperm assays from ≈16% (Li et al., 2014) to 70–92%; therefore, increasing both DNA yield and purity.

Similarly, Horsman et al. (2005) used their created microfluidic sorting device to selectively adsorb the epithelial cells to the bottom of an inlet reservoir. Separation of the epithelial cells was driven by the differences in cell size, specific gravity, surface proteins, charge, and proclivity for adsorption compared to sperm cells (Horseman et al., 2005). Once the epithelial cells had become sedimented, buffer was flowed through the inlet reservoir to cause the sperm cells to migrate towards the outlet reservoir while the epithelial cells remained in the inlet reservoir. While their results were successful in separation of the two cell types, sperm cell

recovery was approximately 25% or less and there was carryover of epithelial cells into the sperm cell fraction, thus decreasing sperm cell purity (Vandewoestyne et al., 2010).

Opposite of the results found using antibody-based capture, microfluidic sorting was found to be more successful for obtaining higher sperm DNA yield and purity when the device was created to selectively capture sperm cells and not epithelial cells. The research by Inci et al. (2018) was approximately 3 times more sensitive to sperm cell capture than previous research which used antibodies (Li et al., 2014). Compared to conventional DE, microfluidic devices provide a much faster and efficient way to separate sexual assault evidence mixtures to obtain single-source STR profiles (Horsman et al., 2005).

Laser Capture Microdissection –

Laser capture microdissection (LCM) is one of the most recent techniques for isolating sperm and epithelial cells within mixtures. LCM works by fixing the mixed cells onto a glass microscope slide then using direct microscopic visualization and laser illumination to selectively dissect single sperm cells one-by-one from the slide and place them into a separate reservoir (Sanders et al., 2007). Once a sufficient amount of sperm cells have been captured, the cells are lysed, and downstream DNA amplification and analysis are performed. This method has been shown to outperform conventional lysis techniques by increasing sperm cell yield (Elliott et al., 2003, Meredith et al., 2012, and Sanders et al., 2007).

Elliott et al. (2003) were the first to publish findings on the use of LCM and recovery of sperm cells from microscope slides. The authors tested 16 paired separate low copy number mixed sample slides. Sixteen of those samples were analyzed using LCM while the other 16 were evaluated using conventional lysis. Each slide contained 300 or fewer sperm cells to try and imitate real-world sexual assault scenarios. The results were based on a likelihood ratio, which is

the ratio between if the contributors to the mixture are from the victim and the suspect or if the contributors to the mixture are from the victim and an unknown person. A greater likelihood ratio supports the hypothesis that the mixture contains suspect sperm cells and not a random individual's sperm cells. The analysis showed that 15 out of 16 sample pairs had greater likelihood ratios when LCM was used over conventional lysis (Elliott et al., 2003). The authors concluded that LCM increased the sperm cell yield since the extraction did not require sperm cells to be released from a fibrous swab and that LCM relatively increased sperm DNA purity due to minimal female DNA crossover. Yet, Elliott et al. (2003) did state that in some samples epithelial DNA from lysed cells adhered to the sperm head. LCM is a physical separation process therefore it cannot selectively remove epithelial DNA attached to sperm cells during DNA extraction like preferential lysis can. This decrease in sperm DNA purity is a downfall of LCM.

In 2007, Sanders et al. built upon the previous work and incorporated hematoxylin/eosin slide staining with LCM. Eosin is a dye that will selectively stain sperm cells and hematoxylin is a dye that stains nuclei (Kondracki et al., 2017). Therefore, once the mixed cell sample is fixed onto the slide, staining allows the analyst to visualize sperm that may have epithelial cells attached. This staining method along with LCM achieved pure separation of sperm with no DNA contamination from exogenous epithelial cells (Sanders et al., 2007). Like Elliot et al. (2003), Sanders et al. (2007) tested low copy number mixtures but with greater sensitivity. Their study showed full STR profiling results with as little as 150 and 75 sperm cells after increased PCR cycling from 28 cycles to 34. Therefore, the authors concluded that LCM in conjunction with staining for sperm identification is an effective technique for sperm cell recovery from mixtures for standard forensic STR analysis (Sanders et al., 2007).

Meredith et al. (2012) wanted to define the limit of detection using LCM to identify the number of cells at which 50% or more of the alleles could be obtained from any of the samples tested. Three forensic STR kits, AmpF1STR Identifiler™, Profiler Plus™, and ForenSeq™, were used for the processing of LCM samples and their detection limits were established. Meredith et al. (2012) found that full STR profiles could be obtained with AmpF1STR Identifiler™ after 28 PCR cycles with as little as 15 epithelial cells and 30 sperm cells. Profiler Plus™ kits provided full STR profiles using 34 PCR cycles with only 75 sperm cells. As for the ForenSeq™ kit, full STR profiles could be obtained with 25 epithelial and 25 sperm cells. The authors concluded that there was evidence of reduced sperm DNA yield that may have been due to some of the collected sperm containing degraded DNA, thus they recommend that the optimum number of sperm collected be 150 to allow for this possibility (Meredith et al., 2012).

Overall, laser capture microdissection improves the recovery of sperm DNA from microscope slides. The one-tube extraction methods of LCM minimize the loss of DNA from swab elution, liquid transfers, and contamination compared to conventional lysis. While this method is capable of isolating sperm cells selectively; it is time-consuming, labor-intensive, requires expensive equipment, and is not likely to be amenable to high-throughput applications (Horsman et al., 2005).

Despite three decades of research, conventional differential extraction is the only original DNA extraction method to have remained unchanged over time. Many new methods have been developed to try and improve upon DE. As shown, these new methods are an array of whether or not they are truly effective for increasing sperm cell yield or purity. Some of these novel methods could increase DE efficiency by decreasing the time spent on analysis or being amenable to high throughput processes. Yet, sacrifices are still made to sperm cell DNA yield

and purity. Those methods which do show an increase in sperm cell yield are also coupled with the need for expensive equipment and specialized analyst training which can burden funding for most crime labs. Because of these sacrifices, no novel method has proven to be an advantageous replacement for conventional differential extraction. Therefore, forensic crime laboratories are reluctant to spend time validating any new method that is not guaranteed to be more effective or efficient than conventional DE.

Conventional DE results in the loss of 94-98% of male sperm cells (Vuichard et al., 2011). In order to increase the sperm cell yield and retention, the use of 'carrier' sperm is proposed. By simply adding horse sperm cells or UltraPure™ salmon sperm DNA in specific concentrations to low copy sperm dilutions before or during the extraction process, less human sperm may be lost during the initial lysis and washing steps. Thus, we hypothesize that the use of 'carrier' sperm will increase the human sperm cell yield during differential extraction analysis. Since DNA amplification, the creation of copies of DNA after separation, is human specific, horse or salmon sperm DNA will not be amplified or detected in downstream DNA analysis. This idea was brought to our attention by research from Shaw et al. (2009) who used carrier RNA to increase the total DNA yield and purity in their experiment.

Overall, this research not only aims to improve sperm cell yield, but to provide a cost effective, non-laborious, and quick addition to conventional DE. Furthermore, if the addition of 'carrier' sperm can improve the quality of the male DNA profiles obtained while expediting SAEK analysis, then more perpetrators can be identified and prosecuted. Therefore, the need for continual improvement of differential extraction extends beyond a scientific issue. Continued research like the above proposal is crucial for helping our communities by identifying those who are responsible for victimizing the life of another human.

Section 2 - Methodology

Obtainment of Samples

Two different sources of 'carrier' sperm deoxyribonucleic acid (DNA) were utilized in both the pilot study and experimental study (as discussed later). The first source was from horse semen (intact sperm cells). This semen was purchased from Colorado State University's Equine Sciences Center. The second source was UltraPure™ Salmon Sperm DNA which was purchased from Invitrogen. Single donor human unvasectomized semen was purchased through Innovative Research Inc. The use of these samples was declared exempt from IRB approval by the University of Central Oklahoma Institutional Review Board (IRB # 2021-012) given it was not human subjects research (45 CFR 46.102d).

Sample Creation

In order to obtain a single-source DNA profile for the human semen samples only, the samples were made in two sets of triplicate dilutions of 1:10 to 1: 2,000 using TE^{-4} buffer. Once diluted, 20µl of each dilution was pipetted onto a microscope slide and heat fixed by warming the slide on a hot plate. This allowed for the sperm cells to become immobile and permanently heat-fixed to the microscope slide. The sperm cells were then stained using a differential stain known as Christmas Tree Staining (Nuclear Red Fast/ Picroindigocarmine) which dyes the heads of the sperm red and the tails green, respectively. Bright field microscopy was used to visualize and count the number of human sperm cells present on the entire slide. A single field of view was not utilized. The sperm cells were visualized under 20X magnification and confirmed under 40X magnification. If more than 200 sperm cells were visualized on at least one half of the slide, the dilution was labeled as "too numerous to count" (TNTC) (Table 1). The purpose of the sperm

Dilution	Dilution Factor	Actual Cell Count	Theoretical Cell Count	Theoretical DNA Yield (ng)
1:5	0.2000	TNTC	32437	97.31
1:10	0.1000	TNTC	16190	48.57
1:20	0.0500	TNTC	8067	24.20
1:30	0.0333	TNTC	5354	16.06
1:40	0.0250	TNTC	4005	12.02
1:50	0.0200	TNTC	3193	9.58
1:60	0.0167	TNTC	2657	7.97
1:75	0.0133	TNTC	2104	6.31
1:100	0.0100	TNTC	1568	4.70
1:125	0.0080	TNTC	1243	3.73
1:150	0.0067	TNTC	1032	3.10
1:175	0.0057	TNTC	870	2.61
1:200	0.0050	TNTC	756	2.27
1:250	0.0040	TNTC	593	1.78
1:500	0.0020	284	284	0.85
1:750	0.0013	129	-	0.39
1:1000	0.0010	108	-	0.32
1:1500	0.0007	58	-	0.17
1:1750	0.0006	41	-	0.12
1:2000	0.0005	27	-	0.08

Table 1: Theoretical DNA yields in nanograms based on theoretical cell counts using linear regression. Those dilutions chosen for testing are shaded in blue. TNTC stands for “too numerous to count.”

cell count was to identify those dilutions which contained approximately 100 sperm cells. One hundred sperm cells would correlate to the theoretical DNA concentration of approximately 0.3ng which is considered “low copy number” and would simulate the low amount of male DNA typically found in sexual assault casework. The theoretical DNA yield in nanograms (ng) was calculated for each sample using linear regression from samples 1:500 through 1:2,000 (Figure 1) and applying the slope-intercept equation (y = 162469x - 56.569) for samples 1:5 through

1:250 (Figure 2).

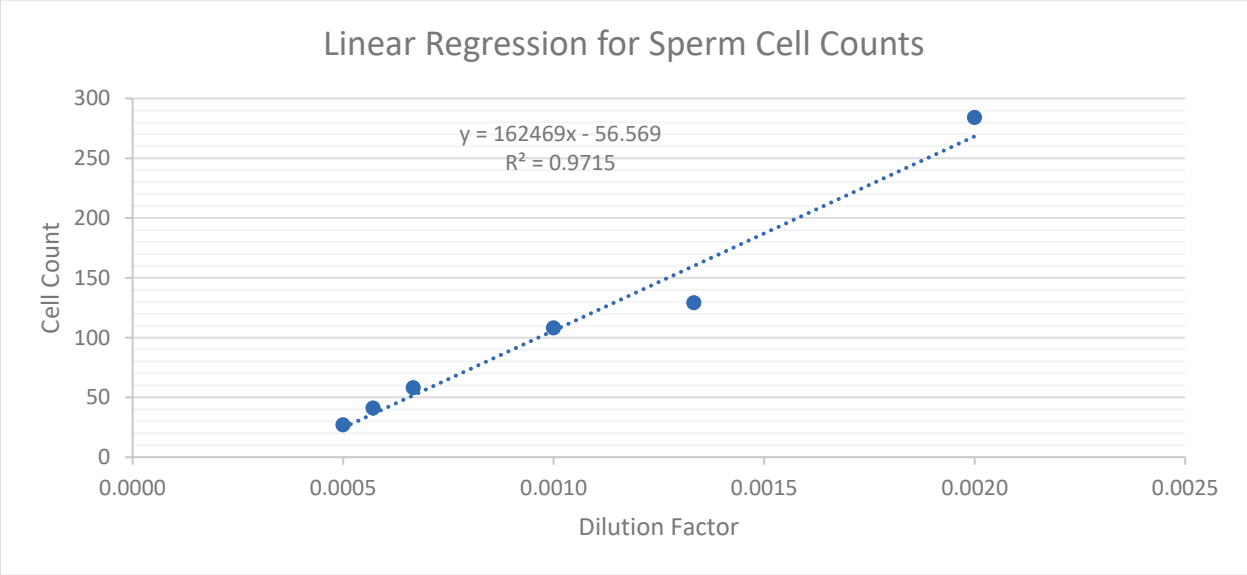

Figure 1: Linear regression was used and calculated from samples 1:500 through 1:2,000. The slope-intercept equation calculated here was used to predict the sperm cell counts and subsequent theoretical DNA yield for samples 1:5 through 1:250.

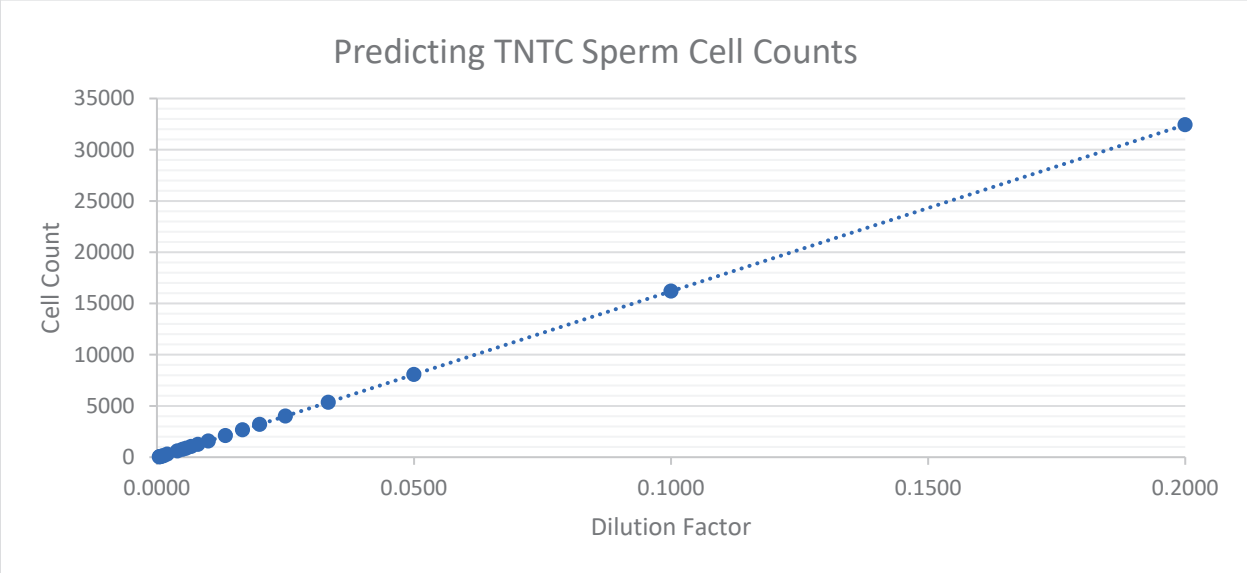

Figure 2: Using the slope-intercept equation from figure 1, the sperm cell counts were able to be predicted for dilutions 1:5 through 1:250. The theoretical sperm cell counts could be multiplied by 0.003 to obtain the theoretical DNA yield (Table 1) since one haploid sperm cell contains ~3pg of DNA (Abbasi et al., 2018).

From this data, the decision was made to continue forward with the following dilutions: 1:10, 1:30, 1:50, 1:75, 1:125, 1:175, 1:250, and 1:500 (Table 1, shaded in blue). These dilutions were chosen because their DNA concentrations were slightly above 0.3ng, which would potentially

avoid downstream allelic dropout during genetic analysis which can occur when too little DNA is within the sample.

Extraction and Purification

Pilot Study – DNA IQ™ Extraction -

The human semen samples were extracted and purified using the DNA IQ™ kit from Promega Corporations. Known semen samples served as positive controls to verify the reliability and consistency of the extraction process. A reagent blank was used as the negative control to monitor contamination throughout the process from extraction to analysis. To each 2mL microcentrifuge tube containing 20µL of human semen sample, 250µL of lysis buffer was added and incubated at 70°C for 30 minutes. After incubation, 7µL of the magnetic resin was added to the tubes. The tubes were then vortexed for 2 seconds and incubated at room temperature for 5 minutes. Following that the tubes were vortexed for 2 seconds and then placed in the magnetic stand. While exercising caution to not disturb the sperm pellet, the lysis buffer was removed and discarded to eliminate any non-sperm cell DNA. To the remaining sperm pellet, 100µL of lysis buffer combined with DTT was added; the tubes were vortexed for 2 seconds, returned to the magnetic stand, and the lysis buffer was removed. Then, 100µL of 1X wash buffer was added; the tubes were vortexed for 2 seconds, returned to the magnetic stand, and the wash buffer was removed. The wash step was repeated two more times for a total of three washes. All wash buffer was removed after the final wash; with the tubes still in the magnetic stand and the lids open, the resin was allowed to air dry for 5 minutes. After air drying, 50µL of elution buffer was added; the tubes were vortexed for 2 seconds and then incubated at 65°C for 5 minutes. After incubation, the tubes were vortexed for 2 seconds and returned to the magnetic stand. The sperm

cell DNA was then eluted off of the magnetic resin. The solutions inside of the tubes were transferred into new 2mL microcentrifuge tubes and constituted as the purified DNA samples.

The 'carrier' horse samples (horse semen plus human semen dilution) were extracted by utilizing a single modification to the previously explained DNA IQ™ extraction protocol. The first step was modified to add both 20µL of human semen and 50µL of horse semen before the addition of the lysis buffer and subsequent incubation. All other steps were followed as previously described.

The 'carrier' salmon samples (purified salmon sperm DNA plus extracted human semen DNA) were extracted by utilizing a single modification to the previously explained DNA IQ™ extraction protocol. After the lysis step, but before the addition of the wash buffer, 50µL salmon sperm DNA was added to the samples. Subsequently, the salmon sperm DNA was added before all 3 wash cycles. All other steps were followed as previously described.

Experimental Study – Organic Extraction -

The human semen samples were extracted and purified using an organic phenol-chloroform extraction method. This method was chosen over a solid phase extraction method (i.e., DNA IQ™) since it has been published that "the resin has a defined DNA-binding capacity in the presence of excess DNA and will only bind a certain amount of DNA" (Promega Corporation, 2016). Thus, due the possibility of an unknown binding capacity for seminal fluid the decision was made to use an extraction method which does not limit the DNA to a binding capacity for the experimental study.

Known semen samples served as positive controls to verify the reliability and consistency of the extraction process. A reagent blank was used to monitor contamination throughout the process from extraction to analysis. The samples consisted of 20µL of the human semen dilution,

225µL of stain extraction buffer (SEB) and 5µL of Proteinase K. The samples were incubated at 55°C for 10 minutes and centrifuged. After incubation, 250µL of phenol/chloroform/isoamyl alcohol was added. The samples were vortexed for 2 seconds and centrifuged at room temperature for 60 seconds at maximum speed. The aqueous layer was carefully transferred into a separate microcentrifuge tube and only the aqueous supernatant was retained. To this, 22µL (1/10 volume) of 3M sodium acetate, pH 5.2 was added and inverted to mix. Next, 284µL (2 times the volume) of ice-cold 100% ethanol was added, vortexed for 2 seconds, then placed on ice for 30 minutes. Following the ice bath, samples were centrifuged for 10 minutes at maximum speed. After centrifugation, the supernatant was discarded. Next, 1mL of 70% ethanol was added. The samples were then inverted and centrifuged for 10 minutes at maximum speed. After centrifugation, the supernatant was discarded. The samples air dried with the tube lids open for 5 minutes. Next, the DNA pellet was dissolved in 50µL of TE^{-4} buffer. Finally, the samples were vortexed for 15 seconds at maximum speed. The solutions inside of the tubes constituted the purified DNA samples.

Two modifications were made to the previously explained organic extraction protocol to extract both 'carrier' horse and salmon sample sets. The first step was modified to add 50µL of horse semen or 50µL of salmon sperm DNA to each 20µL human semen sample. The second modification was to change the SEB volume in the second step to 175µL instead of 225µL.

Quantification

Pilot Study – DNA IQ™ Extraction -

The purified human sperm cell DNA samples were quantified using both a Thermo Scientific NanoDrop™ 2000c Spectrophotometer and Applied Biosystems Quantifiler™ HP

DNA Quantification Kit. The actual yields of each human sperm DNA sample using both instruments were recorded (Table 2).

For quantification using the NanoDrop™ 2000c Spectrophotometer, 1µL of each purified sample was placed onto the pedestal and read using the 260-nanometer nucleic acid setting. This instrument calculates the amount of total DNA in the sample and gives the readings in ng/µL. The standard deviation was also calculated for each dilution set (Table 2). This method of quantification is not human specific. For quantification using Quantifiler™ HP, a master PCR mix was prepared containing 10.5µL of Quantifiler™ Human Primer Mix and 12.5µL of Quantifiler™ PCR Reaction Mix per sample. This master PCR mix (23µL) was dispensed into each well of the sample plate prior to adding 2µL of samples, standards, or controls. Once the wells were filled, the plate was sealed with an Optical Adhesive Cover, centrifuged to remove bubbles, and then placed into a CFX96 Touch Real-Time PCR Detection System. The following thermal cycler conditions were used: 50°C for 2 minutes, 95°C for 10 minutes, then 39 cycles of 95°C for 15 seconds followed by 60°C for 1 minute. Once completed, the quantification data was viewed using CFX Maestro™ Software by Bio-Rad Laboratories and the amount of DNA in ng/µL was recorded. The standard deviation was also calculated for each dilution set (Table 2). This method of quantification is human specific.

NanoDrop™					
	Set 1	**Set 2**	**Set 3**		
Dilution	DNA Concentration	DNA Concentration	DNA Concentration	Average (ng/µL)	Standard Deviation
1:10	4.60	14.60	4.50	7.90	5.80
1:30	12.60	3.70	4.50	6.93	4.92
1:50	1.00	3.40	7.10	3.83	3.07
1:75	2.40	2.30	5.10	3.27	1.59
1:125	1.30	3.00	1.80	2.03	0.87
1:175	2.40	1.20	2.60	2.07	0.76
1:250	2.20	1.50	14.60	6.10	7.37
1:500	3.60	2.90	2.10	2.87	0.75

Quantifiler™ HP					
	Set 1	**Set 2**	**Set 3**		
Dilution	DNA Concentration	DNA Concentration	DNA Concentration	Average (ng/µL)	Standard Deviation
1:10	6.23	2.11	3.91	4.08	2.07
1:30	2.49	0.70	2.21	1.80	0.96
1:50	0.32	0.75	0.78	0.61	0.26
1:75	0.75	0.41	0.03	0.39	0.36
1:125	0.20	0.15	0.17	0.17	0.03
1:175	0.04	0.03	0.07	0.05	0.02
1:250	0.06	0.14	0.08	0.09	0.05
1:500	0.07	0.08	0.10	0.08	0.01

Table 2: Non-human specific (NanoDrop™) quantitation data and human specific (Quantifiler™ HP) quantitation data for the triplicate dilution sets extracted with DNA IQ™. The average DNA concentration and standard deviations were also calculated.

Experimental Study – Organic Extraction -

The purified human sperm cell DNA samples were quantified using Applied Biosystems Quantifiler™ HP DNA Quantification Kit. The actual yields of each human sperm DNA sample were recorded (Table 3). An average DNA concentration (ng/µL) and standard deviation were calculated for each dilution set (Table 3). For quantification using Quantifiler™ HP, a master PCR mix was prepared containing 10.5µL of Quantifiler™ Human Primer Mix and 12.5µL of Quantifiler™ PCR Reaction Mix per sample. This master PCR mix (23µL) was dispensed into each well of the sample plate prior to adding 2µL of samples, standards, or controls. Once the wells were filled, the plate was sealed with an Optical Adhesive Cover, centrifuged to remove bubbles, and then placed into a CFX96 Touch Real-Time PCR Detection System. The following thermal cycler conditions were used: 50°C for 2 minutes, 95°C for 10 minutes, then 39 cycles of 95°C for 15 seconds followed by 60°C for 1 minute. Once completed, the quantification data was viewed using CFX Maestro™ Software by Bio-Rad Laboratories and the amount of DNA in ng/µL was recorded. This method of quantification is human specific.

Quantifiler™ HP					
	Set 1	**Set 2**	**Set 3**		
Dilution	DNA Concentration	DNA Concentration	DNA Concentration	Average (ng/µL)	Standard Deviation
1:10	13.66	2.21	0.89	5.59	7.02
1:30	5.47	0.33	0.26	2.02	2.99
1:50	1.29	0.23	0.14	0.55	0.63
1:75	0.65	0.30	0.15	0.37	0.26
1:125	0.21	0.17	0.10	0.16	0.06
1:175	0.04	0.03	0.04	0.04	0.01
1:250	0.07	0.04	0.04	0.05	0.02
1:500	0.02	0.04	0.06	0.04	0.02

Table 3: Human specific (Quantifiler™ HP) quantitation data for the triplicate dilution sets using an organic extraction. The average DNA concentration and standard deviations were also calculated.

Amplification and Genetic Analysis

The human sperm DNA samples were amplified using Applied Biosystems GlobalFiler™ PCR Amplification Kit. A reaction mix was prepared with 7.5µL of Master Mix and 2.5µL of Primer Set per reaction. Then, 10µL of the reaction mix was placed into 0.5mL PCR tubes prior to adding 1µL of each sample or control. Normalization, the process of concentrating or diluting the DNA concentration before genetic analysis based on quantitation results, was not performed in order to compare dilution sets between all three sample types (i.e., human only versus human plus carriers). The total reaction volume per tube was 25µL: 10µL of the reaction mix, 1µL of DNA extract, and 14µL of TE^{-4} buffer. The tubes were vortexed and centrifuged at 3,000 RPM for 20 seconds. The samples were amplified using the GeneAmp PCR System 9700. The thermal cycling conditions were as follows: a 95°C hold step for 1 minute, 29 cycles of a 94°C denaturation step for 10 seconds followed by a 59°C annealing/extension step for 90 seconds, and a final 60°C extension step for 10 minutes. The samples were held at 4°C until electrophoresis.

The amplified human sperm cell DNA samples were run on an Applied Biosystems™ 3500 Genetic Analyzer. A reaction mix was prepared which contained 0.4µL of GeneScan™ 600 LIZ™ size standard v2.0 per sample and 9.6µL of Hi-Di™ Formamide per sample. Into each well of a MicroAmp™ Optical 96-Well Reaction Plate, 10µL of the reaction mix was dispensed followed by 1µL of PCR product or allelic ladder. The plate was sealed with septa, vortexed briefly, and then centrifuged. The plate was heated in a thermal cycler at 95°C for 3 minutes followed immediately by resting on ice for 3 minutes. The plate was then placed onto the autosampler and run. Afterwards, the data was analyzed using GeneMapper *ID-X* Software to generate the electropherograms for each sample.

Section 3 – Results and Discussion

Pilot Study – DNA IQ™ Extraction -

Whenever horse semen was added to dilutions of human semen and processed through the modified DNA IQ™ extraction process, quantitation revealed an average percent decrease of 99.45% (Figure 3). Since horse semen was added before the lysis step of the extraction procedure, our hypothesis did not support the notion that the addition of horse semen could improve the overall human sperm cell DNA yield by acting as a barrier or 'carrier' to the human sperm during lysis. Whenever salmon sperm DNA was added to dilutions of human semen DNA and processed through the modified DNA IQ™ extraction process, quantitation revealed an average percent decrease of 98.36% (Figure 3). Although this method was not as detrimental as horse semen, our hypothesis was still not supported. The addition of salmon sperm DNA before the washing steps of the extraction procedure decreased the overall human sperm DNA yield; therefore, acting as a barrier or 'carrier' to the human sperm DNA during the wash steps of the extraction procedure. Genetic STR analysis using Globalfiler™ proved to be consistent between all sample sets thus resulting in data that was consistent in allelic dropout and/or failure to determine a DNA profile. This was most likely due to low DNA concentrations within the samples.

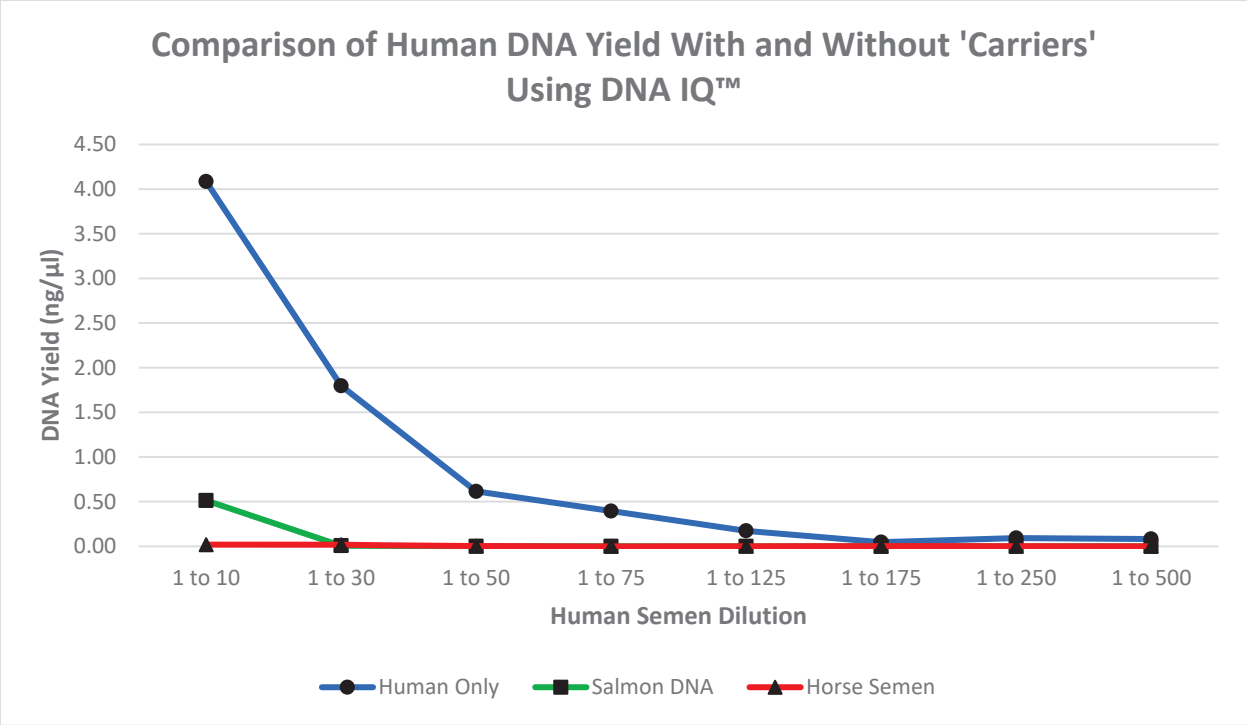

Figure 3: Comparison of human DNA yield with and without 'carriers' using DNA IQ™. The DNA yield for each triplicate dilution set was averaged (y-axis) and compared to the other sample sets at the individual dilutions (x-axis).

While this pilot study was unsuccessful, further experimentation using a different extraction method was conducted in order to determine if these results could be improved. It has been published through Promega Corporation, the company that manufactures DNA IQ™, that "the resin has a defined DNA-binding capacity in the presence of excess DNA and will only bind a certain amount of DNA". Promega also states that the DNA yields "will be consistent within a single sample type but will differ with different sample type" meaning that the DNA binding capacities for blood or seminal fluid will be consistent between themselves but may differ between each other (Promega Corporation, 2016). Unfortunately, Promega has established DNA binding capacities for FTA® blood-cards, liquid blood, and buccal swabs but not for seminal fluid. Thus, due the possibility of an unknown binding capacity for seminal fluid the decision was made to use an extraction method which does not limit the DNA to a binding capacity. This other method is known as an organic (phenol-chloroform) extraction and has been around for

many years. At one time it was the most widely used method for DNA extraction. Additionally, Conventional DE is a variant of an organic extraction although some chemicals differ slightly. This method involves the serial addition of many chemicals. The first chemicals are typically sodium dodecylsulfate (SDS) and proteinase K which will break open the cell walls in order to break down the proteins that protect the DNA molecules while in chromosome configuration. In order to break open sperm cells specifically, an additional chemical, dithiothreitol (DTT), must also be added since DTT facilitates the breakdown of disulfide bonds within the sperm's nuclear membrane allowing for the release of sperm cell DNA. Next, a phenol-chloroform mixture is added to separate the proteins from the DNA. The DNA is more soluble in the aqueous portion of the organic-aqueous mixture. When centrifuged, the unwanted proteins and cellular debris are separated away from the aqueous phase and double-stranded DNA molecules can be cleanly transferred for analysis (McKiernan & Danielson, 2017). Because this free DNA is suspended in the aqueous layer, no binding capacity is present due to an extraneous kit component like the magnetic beads in DNA IQ™. However, utilizing an organic extraction does come with disadvantages. This method has typically been replaced with new extraction methods, like DNA IQ™, because the chemicals used are less toxic than phenol and these newer methods are less time consuming. Despite this, we found it important to use a method that was limitless in DNA binding capacity.

Experimental Study – Organic Extraction -

All three sets of dilutions were quantified and the concentrations of DNA were averaged (Table 4). Using the average DNA concentrations, the percent difference for each dilution set between the human only and 'carrier' samples was calculated and then the average percent increase was determined (Table 4). Whenever horse semen was added to dilutions of human

semen and processed through the phenol-chloroform organic extraction, quantitation revealed an average percent decrease of 88.40% (Figure 4). Whenever salmon sperm DNA was added to dilutions of human semen DNA and processed through the phenol-chloroform organic extraction, quantitation revealed an average percent decrease of 84.25% (Figure 4). Additionally, controls of only horse semen and salmon sperm DNA were tested and resulted in 0.00 ng/µL of DNA using qPCR and Quantifiler™ HP, proving that the 'carrier' DNA could not be quantified using human specific qPCR methods.

Human Semen Only Controls						
	Set 1	**Set 2**	**Set 3**			
Dilution	DNA Concentration	DNA Concentration	DNA Concentration	Average (ng/µL)	% Difference	Average % Decrease
1:10	13.66	2.21	0.89	5.59		
1:30	5.47	0.33	0.26	2.02		
1:50	1.29	0.23	0.14	0.55		
1:75	0.65	0.30	0.15	0.37		
1:125	0.21	0.17	0.10	0.16		
1:175	0.04	0.03	0.04	0.04		
1:250	0.07	0.04	0.04	0.05		
1:500	0.02	0.04	0.06	0.04		
Human Semen + Salmon DNA						
	Set 1	**Set 2**	**Set 3**			
Dilution	DNA Concentration	DNA Concentration	DNA Concentration	Average (ng/µL)	% Difference	Average % Decrease
1:10	0.48	0.17	0.12	0.26	-95.42	-84.25
1:30	0.04	0.10	0.06	0.07	-96.60	
1:50	0.09	0.03	0.03	0.05	-91.31	
1:75	0.13	0.01	0.02	0.05	-85.01	
1:125	0.03	0.01	0.02	0.02	-87.35	
1:175	0.02	0.01	0.02	0.02	-54.48	
1:250	0.01	0.01	0.01	0.01	-81.06	
1:500	0.01	0.01	0.00	0.01	-82.80	
Human Semen + Horse Sperm Cells						
	Set 1	**Set 2**	**Set 3**			

Dilution	DNA Concentration	DNA Concentration	DNA Concentration	Average (ng/µL)	% Difference	Average % Decrease
1:10	0.07	0.06	0.07	0.07	-98.77	-88.40
1:30	0.39	0.03	0.03	0.15	-92.58	
1:50	0.05	0.04	0.04	0.04	-92.28	
1:75	0.01	0.03	0.01	0.02	-95.21	
1:125	0.04	0.01	0.06	0.03	-78.71	
1:175	0.01	0.01	0.01	0.01	-82.95	
1:250	0.02	0.01	0.01	0.01	-72.66	
1:500	0.00	0.00	0.01	0.00	-94.01	

Table 4: Individual and average DNA concentrations using Quantifiler™ HP, the percent difference, and the average percent increase calculated for each triplicate dilution set for all three sample types.

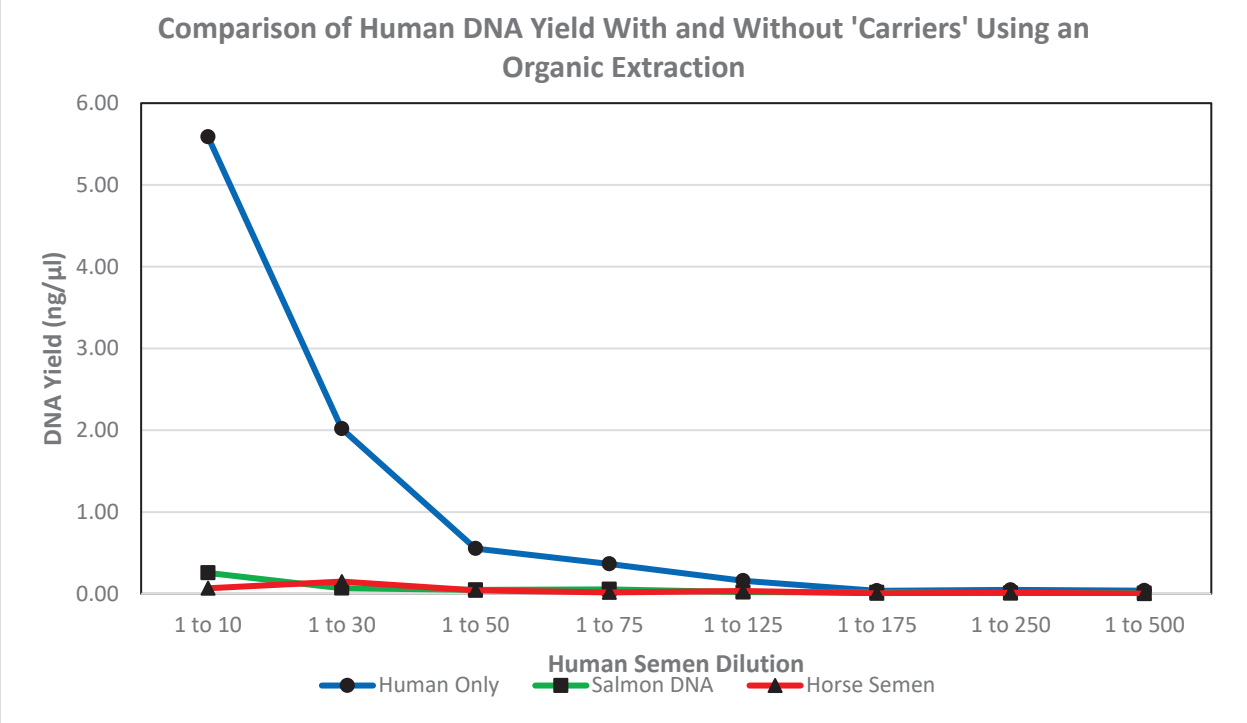

Figure 4: Comparison of human DNA yield with and without 'carriers' using an organic extraction. The DNA yield for each triplicate dilution set was averaged (y-axis) and compared to the other sample sets at the individual dilutions (x-axis).

Since horse semen was added before the lysis step of the extraction procedure, our hypothesis did not support the notion that the addition of horse semen could improve the overall human sperm cell DNA yield by acting as barrier or 'carrier' to the human sperm during lysis. Additionally, the concept that the addition of salmon sperm DNA before the washing steps of the extraction procedure also opposed our hypothesis by demonstrating an overall decrease in human

sperm DNA yield during the wash steps. It is common for sexual assault evidence to have few sperm cells present due to the higher ratio of epithelial cells within samples. Therefore, it is important to retain as many sperm cells as possible throughout the DNA extraction process. However, the research presented here was not able to demonstrate a significant, successful use of 'carrier' DNA within differential extraction.

Despite three decades of research, conventional differential extraction is the only original DNA extraction method to have remained unchanged over time. Many new methods have been developed to try and improve upon DE. However, these new methods vary in effectiveness for increasing sperm cell yield or purity. Some of these novel methods could increase DE efficiency by decreasing the time spent on analysis or being amenable to high throughput processes. Yet, sacrifices are still made to sperm cell DNA yield and purity. Those methods which do show an increase in sperm cell yield are also coupled with the need for expensive equipment and specialized analyst training which can burden funding for most crime labs. Because of these sacrifices, no novel method has proven to be an advantageous replacement for conventional differential extraction. Forensic crime laboratories are reluctant to spend time validating any new method that is not guaranteed to be more effective or efficient than conventional DE. The developmental method proposed here had intent to demonstrate that the addition of a 'carrier' DNA molecule could improve the yield of human sperm cell DNA during differential extraction analysis and potentially increase the efficiency of that method.

Conventional DE results in the loss of 94-98% of human sperm cells (Vuichard et al., 2011). By adding horse sperm cells or UltraPure™ salmon sperm DNA in specific concentrations to low copy sperm dilutions before the extraction process, more human sperm DNA was lost during the initial lysis and washing steps, thus decreasing the efficiency of the

tested extraction methods. This research was able to demonstrate that neither horse or salmon sperm DNA can be amplified or detected in downstream DNA analysis. Therefore, the risk of possible contamination with 'carrier' sperm would not play a factor in forensic genetic analysis if future research were to pursue variations of these methods.

In addition to this research failing to demonstrate the effectiveness of 'carrier' sperm or sperm DNA for improving human sperm cell DNA yield, there are other limitations. Organic extraction has long since been replaced with safer, faster, and more cost-effective extraction techniques, therefore future research into utilizing 'carrier' sperm in different extraction methods would be necessary in order to adapt this method for those extraction methods that are more commonly used today, if pursued. Within our research, genetic STR analysis using Globalfiler™ proved to be consistent between sample sets thus resulting in data that contained allelic dropout and/or complete failure to produce DNA profiles. This failure was most likely caused by the low DNA concentrations within the samples (Appendix I).

Chapter 3 – Conclusions

Research into the use of 'carrier' DNA was inspired by past research using carrier DNA/RNA known as polyadenylic acid or poly(A). Poly(A) is a synthetic homopolymer composed of a long single-stranded sequence of adenine (A) nucleotides (Li, 2021). Poly(A) tails are generated within nuclei through a process called polyadenylation, and are important for transcription termination, mRNA stability, and translation. However, poly(A) has been researched for other uses as carrier DNA/RNA for the quantitative precipitation of DNA and RNA in samples (Li, 2021). It has been shown useful in the purification of DNA and RNA from samples that have low DNA concentrations such as blood and semen dilutions (Kishore et al., 2006). Yet, research that has been conducted using poly(A) was utilizing extraction techniques such as BioRobots® EZ1 or BioRobots® M48 which are robotic extraction techniques that consistently produce lower DNA recovery rates than standard organic extractions (Kishore et al., 2006). Once the poly(A) was added to the low concentration DNA samples after the cell lysis step, the DNA yields increased from four-to-20-fold using the robotic extraction method; however, most forensic laboratories are not utilizing robotic techniques for differential extraction due to the increased cost associated with them (Kishore et al., 2006). Additionally, within the same research it was stated that the DNA yields obtained by robotic extraction in the presence of the carrier RNA were as high, or higher, as those obtained by an organic extraction without carrier RNA (Kishore et al., 2006). Thus, they were able to show they could increase DNA yield using a robotic extraction with carrier RNA but did not attempt to utilize carrier RNA with only an organic extraction. Additional research into poly(A) carrier RNA was also successful on human buccal cells; however; this research was utilizing solid-phase extraction in the form of microfluidic silica monoliths which are not common in forensic laboratory applications (Shaw et

al., 2009). This research was able to demonstrate that the addition of poly(A) to the microfluidic device increased the recovered amount of DNA from 5ng to 25ng within their experiment but no comparison was made to other types of solid-phase or organic extraction methods (Shaw et al., 2009).

Unlike poly(A), our 'carrier' sperm decreased human sperm cell DNA yield during DNA extraction. As mentioned, the most common extraction method utilized by forensic laboratories is conventional differential extraction. This research tested the use of a novel version of 'carrier' DNA in the form of intact horse sperm cells or salmon sperm DNA in both a solid-phase extraction method, DNA IQ™, and an organic phenol-chloroform extraction. These types of extraction methods were chosen to more closely mimic what is commonly used in forensic laboratories and reduced the need for expensive equipment or extensive specialized training. More specifically, this research focused on an organic extraction in order to test our method using a technique that has been utilized for decades but also with the hope that future research could research and adapt the potential use of 'carrier' sperm to the different extraction techniques that are becoming more prevalent. Furthermore, the use of horse sperm cells and salmon sperm DNA were not chosen specifically due to their species of origin. This research wanted to utilize what was available in the form of non-human intact sperm cells and non-human sperm DNA. Future research may adapt and find that other animal species besides horse and salmon could work just as effectively, if not more, and become even easier to access.

Pilot Study – DNA IQ™ Extraction -

DNA IQ™ is a solid-phase extraction technique that was utilized to demonstrate that our methodology could be adapted to a technology that utilizes magnetic resin to capture extracted DNA. The method used for DNA IQ™ was adapted to test both the effectiveness of the lysis and

wash steps for human sperm cell DNA recovery. Intact horse sperm cells were added to human sperm cell dilutions in the very beginning and taken through the entire extraction process to test our hypothesis that the horse sperm cells would act as a 'carrier' to the human sperm cells so less human DNA would be lost during the initial lysis and subsequent liquid handling steps. Our results demonstrated that the addition of intact horse sperm cells resulted in an overall percent decrease in human sperm DNA concentration of 99.45% when compared to the DNA concentration of the human semen only controls. Yet, because literature also debates that the washing steps of differential extraction cause the loss of human sperm cell DNA, we tested a separate hypothesis that adding salmon sperm DNA before each wash step would improve human sperm cell DNA yield. Our research opposed our hypothesis and showed that the addition of salmon sperm DNA resulted in an average percent decrease of 98.36% when compared to the DNA retention of the human semen only controls. However, comparison between the intact horse sperm cells and salmon sperm DNA demonstrates that the addition of already extracted and purified 'carrier' DNA before the wash steps resulted in less loss of human sperm cell DNA yield, yet not significantly.

While this pilot study was unsuccessful considering the qPCR results, genetic STR analysis using Globalfiler™ proved to be consistent between sample sets thus resulting in data that demonstrated consistent allelic dropout and/or failure to produce a DNA profile. Failure was most likely due to low DNA concentrations within the samples. Therefore, additional experimentation using a different extraction method was conducted in order to determine if these results could be improved. This other method was an organic phenol-chloroform extraction.

Experimental Study - Organic Extraction -

An organic extraction results in a limitless amount of DNA. It does not rely on a solid-phase to capture the DNA, but through a series of chemical additions, incubations, and centrifugation steps the DNA is separated by a difference in density. Therefore, unlike DNA IQ™ the 'carriers' did not need to be added at separate stages of the extraction process, hence why both the intact horse sperm cells or the salmon sperm DNA was added to the human sperm cell dilutions at the beginning of the extraction procedure. Yet, despite the stark difference between adding whole cells which needed to be lysed open or adding purified DNA, each 'carrier' resulted in an overall percent decrease in the yield of human sperm cell DNA recovered. The addition of intact horse sperm cells resulted in an 88.40% yield decrease while the salmon sperm DNA resulted in an average percent decrease of 84.25% when compared to the sperm cell DNA yield of the human only controls. Thus, demonstrating that our methodology was ineffective when using one of the most commonly utilized and basic DNA extraction techniques.

Conventional differential extraction was created in 1985 and is considered the most widely used DNA extraction techniques in forensic science for over thirty years. Yet, it is known that conventional DE results in the loss of more than 90% of human sperm cells and that the majority of this loss comes from the initial lysis steps and multiple wash steps. Thus, this research project was created in hopes of increasing sperm cell yield and retention with the use of 'carrier' sperm. By adding intact horse sperm cells or salmon sperm DNA in specific concentrations to low copy sperm dilutions before the extraction process, the intent was for the overall percentage of human sperm cell DNA retention to increase. Our method failed to result in a quick, easy, and simple single-step modification to a commonly used organic extraction method, as was intended. However, since DNA amplification is human specific, we were able to demonstrate that horse or salmon sperm DNA was not amplified or detected in downstream

DNA analysis. Therefore, the risk of possible contamination with ‘carrier’ sperm would not play a factor in forensic genetic analysis if pursued in future research.

Organic extraction has long since been replaced with safer, faster, and more cost-effective extraction techniques; therefore, future research into utilizing ‘carrier’ sperm in different extraction methods would be necessary in order to adapt this method for those extraction methods that are more commonly used today. Within our research, genetic STR analysis using Globalfiler™ proved to be consistent between sample sets; thus, utilizing this technique with simulated sexual assault-like samples in order to more closely mimic sexual assault casework would potentially demonstrate this method’s implications on mixed sample quantitation and subsequent genetic STR analysis.

Although results of this research did not show improvement upon DE the intent was to jumpstart a new era of forensic research into the use of ‘carrier’ sperm. These research results showed an overall percent decrease in human sperm DNA concentration of greater than eighty percent when human semen only controls were supplemented with ‘carrier’ salmon sperm DNA or ‘carrier’ intact horse sperm cells. Thus, our novel one-step modification to organic extraction demonstrated a negative advancement towards improving conventional differential extraction for forensic sexual assault casework. However, future research into utilizing ‘carrier’ sperm into different extraction methodologies and their application in simulated sexual assault-like samples is necessary and would result in a more meaningful understanding of ‘carrier’ sperm and their potential implications.

References

Abbasi, M., Smith, A. D., Swaminathan, H., Sangngern, P., Douglas, A., Horsager, A., Carrell, D. T., & Uren, P. J. (2018). Establishing a stable, repeatable platform for measuring changes in sperm DNA methylation. *Clinical Epigenetics*, *10*(1). https://doi.org/10.1186/s13148-018-0551-7

Bureau, U. S. C. (2021, November 23). *Decennial Census 2020*. Census.gov. Retrieved July 9, 2022, from https://www.census.gov/programs-surveys/decennial-census/about/rdo.html

Butler, J. M. (2010). DNA Extraction. In Fundamentals of Forensic DNA Typing (pp. 105-106). Burlington, MA: Academic Press/Elsevier.

Clark, C., Turiello, R., Cotton, R., & Landers, J. P. (2020). Analytical approaches to differential extraction for sexual assault evidence. *Analytica Chemica Acta*. doi:https://doi.org/10.1016/j.aca.2020.07.059

Cotton, R. W., & Fisher, M. B. (2015). Review: Properties of sperm and seminal fluid, informed by research on reproduction and contraception. *Forensic Science International: Genetics, 18*, 66-77. http://dx.doi.org/10.1016/j.fsigen.2015.03.009

Elliott, K., Hill, D. S., Lambert, C., Burroughes, T. R., & Gill, P. (2003). Use of laser microdissection greatly improves the recovery of DNA from sperm on microscope slides. *Forensic Science International, 137*, 28-36. doi:10.1016/S0379-0738(03)00267-6

Garvin, A. M., Bottanelli, M., Gola, M., Conti, A., & Soldati, G. (2009). DNA Preparation from Sexual Assault Cases by Selective Degradation of Contaminating DNA from the Victim. *Journal of Forensic Sciences, 56*(6). doi:10.1111/j.1556-4029.2009.01180.x

Gill, P., Jeffreys, A. J., & Werrett, D. J. (1985). Forensic Application of DNA 'fingerprints'. *Nature, 318*, 577-579.

Horsman, K. M., Barker, S. L., Ferrance, J. P., Forrest, K. A., Koen, K. A., & Landers, J. P. (2005). Separation of Sperm and Epithelial Cells in a Microfabricated Device: Potential Application to Forensic Analysis of Sexual Assault Evidence. *Analytical Chemistry, 77*(3), 742-749. doi:10.1021/ac0486239

Hudlow, W. R., & Buoncristiani, M. R. (2012). Development of a rapid, 96-well alkaline based differential DNA extraction method for sexual assault evidence. *Forensic Science International: Genetics, 6*, 1-16. doi:10.1016/j.fsigen.2010.12.015

Inci, F., Ozen, M. O., Saylan, Y., Miansari, M., Cimen, D., Dhara, R., . . . Demirci, U. (2018). A Novel On-Chip Method for Differential Extraction of Sperm in Forensic Cases. *Advanced Science, 5*. doi:10.1002/advs.201800121

Kishore, R., Reef Hardy, W., Anderson, V. J., Sanchez, N. A., & Buoncristiani, M. R. (2006). Optimization of DNA extraction from low-yield and degraded samples using the biorobot ez1 and Biorobot M48. *Journal of Forensic Sciences*, *51*(5), 1055–1061. https://doi.org/10.1111/j.1556-4029.2006.00204.x

Klein, S. B., & Buoncristiani, M. R. (2017). Evaluating the efficacy of DNA differential extraction methods for sexual assault evidence. *Forensic Science International: Genetics, 29*, 109-117. http://dx.doi.org/10.1016/j.fsigen.2017.03.021

Kondracki, S., Wysokińska, A., Kania, M., & Górski, K. (2017). Application of two staining methods for sperm morphometric evaluation in domestic pigs. *Journal of Veterinary Research, 61*, 345-349. doi:10.1515/jvetres-2017-0045

Li, Q. (2021, September 22). *The ubiquitous 'poly(a)' and its applications in life science*. J&K Scientific LLC. Retrieved July 10, 2022, from https://www.jk-sci.com/blogs/resource-center/poly-a-and-applications-in-life-science

Li, X., Wang, Q., Feng, Y., Miao, Y., Wang, Y., & Li, H. (2014). Magnetic bead-based separation of sperm from buccal epithelial cells using a monoclonal antibody against MOSPD3. *International Journal of Legal Medicine, 128*, 905-911. doi:10.1007/s00414-014-0983-3

Lounsbury, J. A., Nambiar, S. M., Karlsson, A., Cunniffe, H., Norris, J. V., Ferrance, J. P., & Landers, J. P. (2014). Enhanced recovery of spermatozoa and comprehensive lysis of epithelial cells from sexual assault samples having a low cell counts or aged up to one year. *Forensic Science International: Genetics, 8*(1), 84-89. doi:10.1016/j.fsigen.2013.06.015

McKiernan, H. E., & Danielson, P. B. (2017). Molecular diagnostic applications in forensic science. *Molecular Diagnostics*, 371–394. https://doi.org/10.1016/b978-0-12-802971-8.00021-3

Meredith, M., Bright, J., Cockerton, S., & Vintiner, S. (2012). Development of a one-tube extraction and amplification method for DNA analysis of sperm and epithelial cells recovered from forensic samples by laser microdissection. *Forensic Science International: Genetics, 6*(1), 91-96. doi:10.1016/j.fsigen.2011.02.007

Morgan, R. E., & Thompson, A. (2021). *Criminal Victimization, 2020*. Bureau of Justice Statistics. Retrieved July 9, 2022, from https://bjs.ojp.gov/library/publications/criminal-victimization-2020

Mudariki, T., Pallikarana-Tirumala, H., Ives, L., Hadi, S., & Goodwin, W. (2013). A comparative study of two extraction methods routinely used for DNA recovery from simulated post coital samples. *Forensic Science International: Genetics Supplement Series, 4*(1). doi:10.1016/j.fsigss.2013.10.100

Norris, J. V., Manning, K., Linke, S. J., Ferrance, J. P., & Landers, J. P. (2007). Expedited, Chemically Enhanced Sperm Cell Recovery from Cotton Swabs for Rape Kit Analysis. *Journal of Forensic Sciences, 52*(4), 800-805. doi:10.1111/j.1556-4029.2007.00453.x

Promega Corporation. (2020). *Differex™ System – For Use with the Differex™ Magnet*. Madison, WI: Author.

Promega Corporation. (2016). *DNA IQ™ System—Database Protocol. Promega Corporation*. Madison, WI: Author.

Sanders, C. T., Reisenbigler, E. K., & Peterson, D. A. (2007, February). *Laser Microdissection Separation of Pure Spermatozoa Populations from Mixed Cell Samples for Forensic DNA Analysis* [PDF]. Washington D.C.: US Department of Justice.

Schjenken, J. E., & Robertson, S. A. (2020). The female response to seminal fluid. *Physiological Reviews*, *100*(3), 1077–1117. https://doi.org/10.1152/physrev.00013.2018

Schoell, W. M., Klintschar, M., Mirhashemi, R., & Pertl, B. (1999). Separation of sperm and vaginal cells with flow cytometry for DNA typing after sexual assault. *Instruments and Methods, 94*(4), 623-627. doi:https://doi.org/10.1016/S0029-7844(99)00373-7

Shaw, K. J., Thain, L., Docker, P. T., Dyer, C. E., Greenman, J., Greenway, G. M., & Haswell, S. J. (2009). The use of carrier RNA to enhance DNA extraction from microfluidic-based silica monoliths. *Analytica Chemica Acta, 652*, 231-233. doi:10.1016/j.aca.2009.03.038

Tereba, A., Flanagan, L., Mandrekar, P., & Olson, R. (2004). DIFFEREX™ SYSTEM A New, Rapid Method to Separate Sperm and Epithelial Cells. *Profiles in DNA*. www.promega.com

Timken, M. D., Klein, S. B., Kubala, S., Scharnhorsta, G., Buoncristiani, M. R., & Miller, K. W. (2019). Automation of the standard DNA differential extraction on the Hamilton AutoLys STAR system: A proof-of-concept study. *Forensic Science International: Genetics, 40*, 96-104. doi:https://doi.org/10.1016/j.fsigen.2019.02.011

Tsukada, K., Asamura, H., Ota, M., Kobayashi, K., & Fukushima, H. (2006). Sperm DNA extraction from mixed stains using the Differex System. *International Congress Series, 1288*, 700-703. doi:10.1016/j.ics.2005.12.059

Vandewoestyne, M., & Deforce, D. (2010). Laser capture microdissection in forensic research: A review. *Internation Journal of Legal Medicine, 24*, 513-521. doi:10.1007/s00414-010-0499-4

Vuichard, S., Borer, U., Bottinelli, M., Cossu, C., Malik, N., Meier, V., . . . Castella, V. (2011). Differential DNA extraction of challenging simulated sexual-assault samples: A Swiss collaborative study. *Investigative Genetics, 2*(11). doi:http://www.investigativegenetics.com/content/2/1/11

Wiegand, P., Schiirenkamp, M., & Schiitte, U. (1992). DNA extraction from mixtures of body fluid using mild preferential lysis. *International Journal of Legal Medicine, 104*, 359-360. doi:10.1007/BF01369558

Yoshida, K., Sekiguchi, K., Mizuno, N., Kasai, K., Sakai, I., Sato, H., & Seta, S. (1995). The modified method of two-step differential extraction of sperm and vaginal epithelial cell DNA from vaginal fluid mixed with semen. *Forensic Science International, 72*, 25-33. http://dx.doi.org/10.1016/0379-0738(94)01668-U

APPENDIX I

Reference Allele Calls for Known Human Semen Donor

Loci	Allele 1	Allele 2
D3S1358	15	17
vWA	18	19
D16S539	12	12
CSF1PO	8	10
TPOX	8	8
D8S1179	9	11
D21S11	31	442
D18S51	12	15
DYS391	11	
D2S441	14	14
D19S433	14	14
TH01	8	9
FGA	22	23
D22S1045	16	17
D5S818	12	12
D13S317	11	11
D7S820	11	11
SE33	20	26.2
D10S1248	13	15
D1S1656	16.3	18.3
D12S391	17	19
D2S1338	24	24
Y indel	2	
Amelogenin	X	Y

Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name SQ

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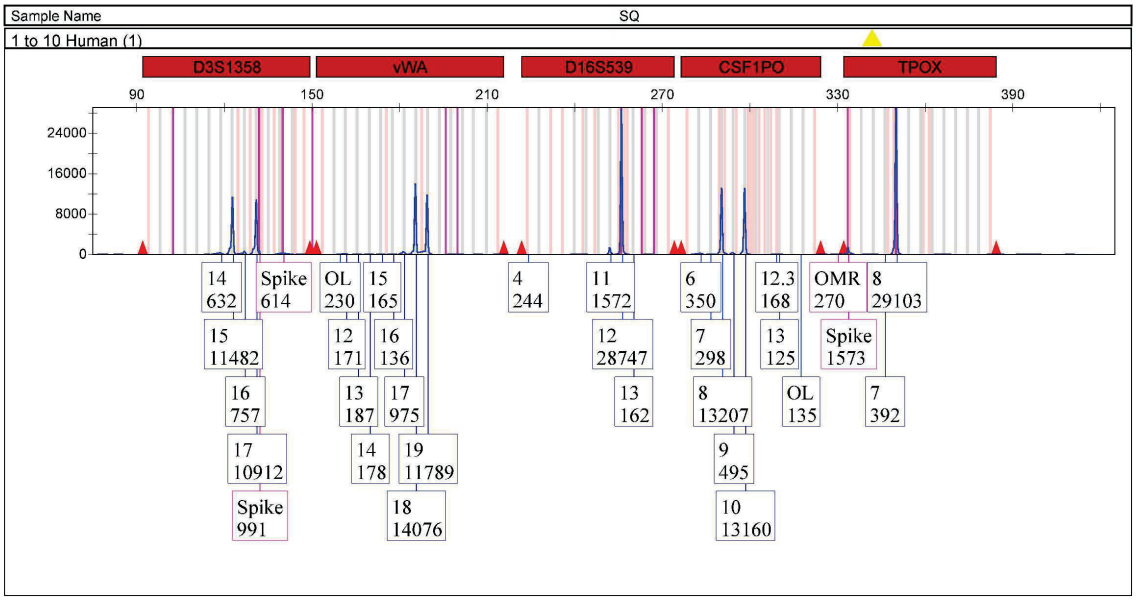

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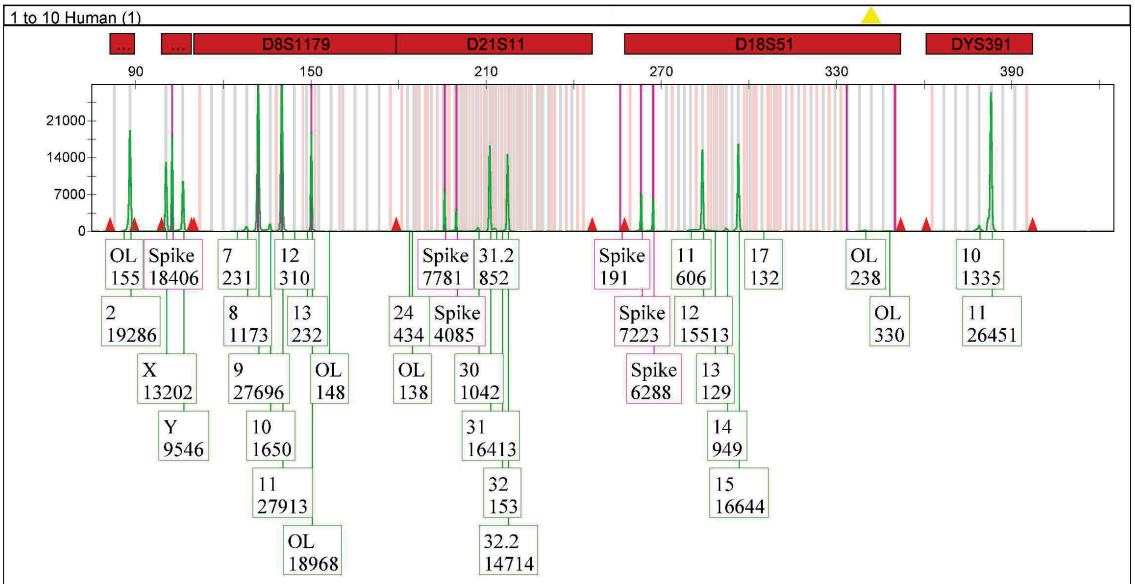

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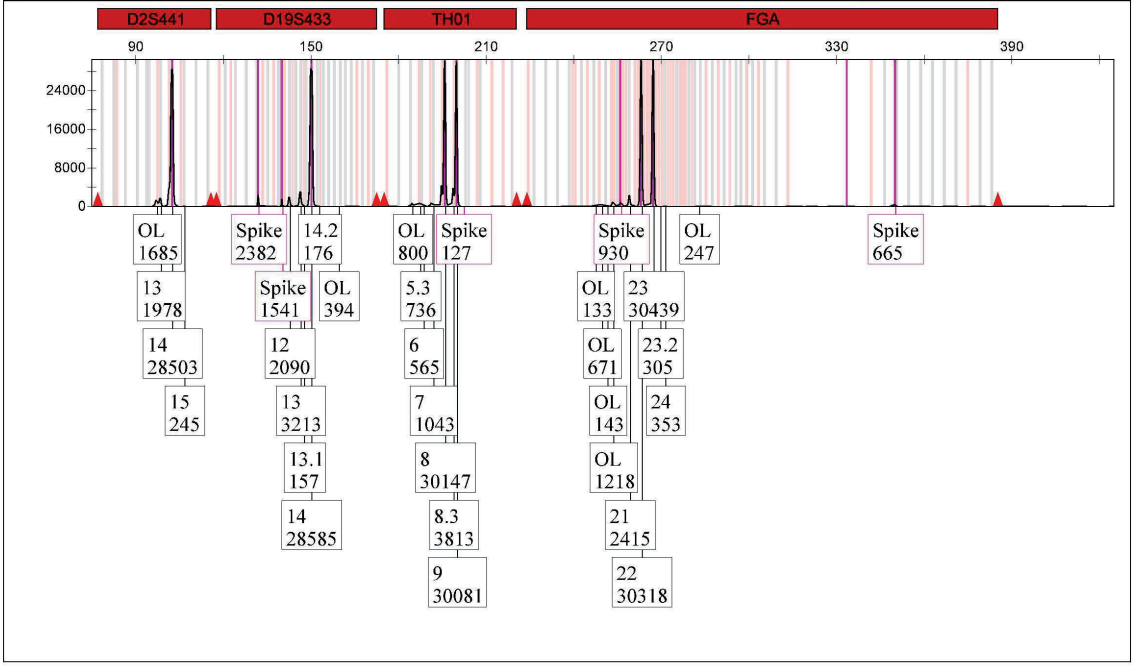

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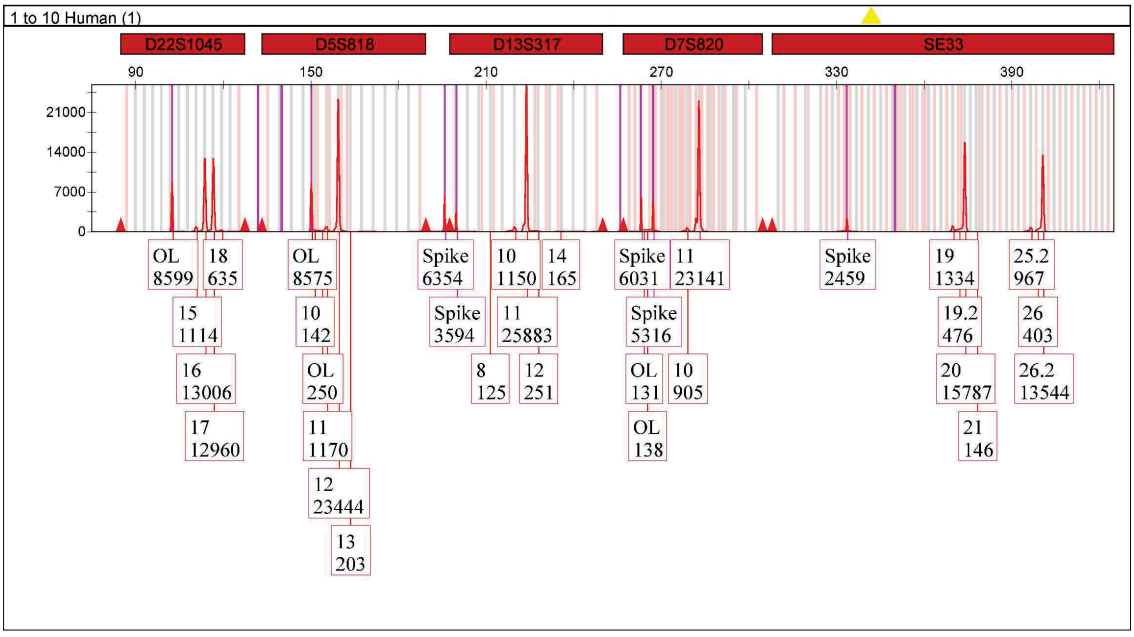

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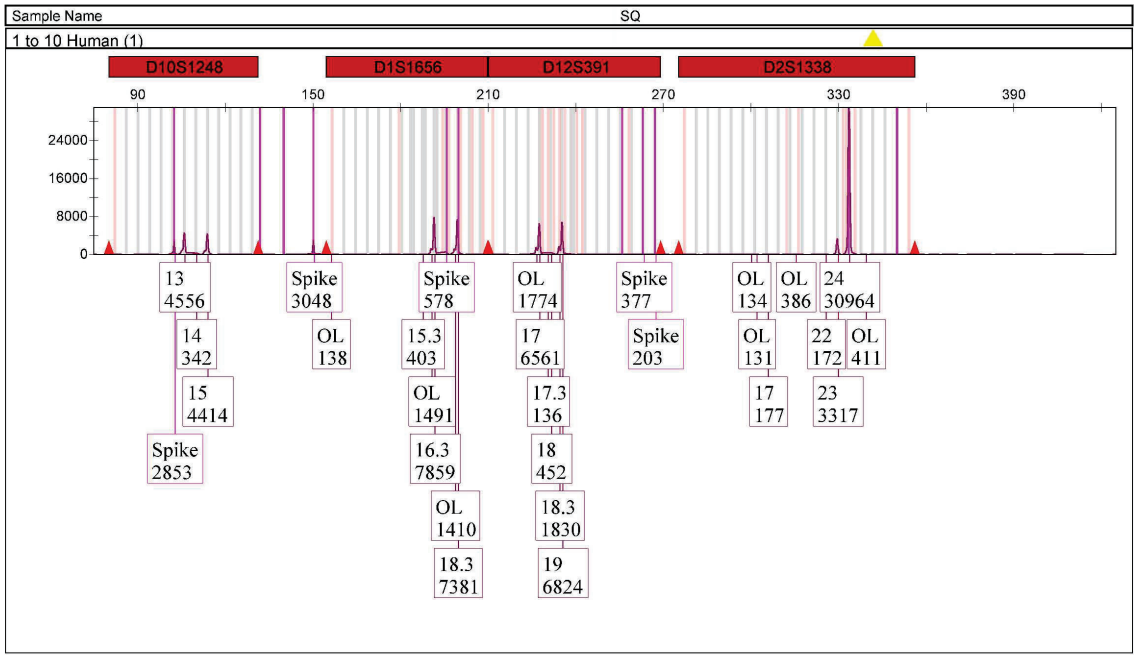

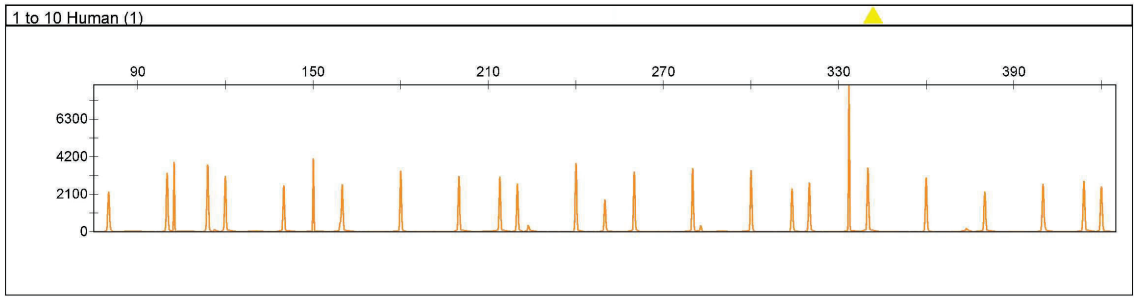

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Project: Organic Extractions CRG 061422

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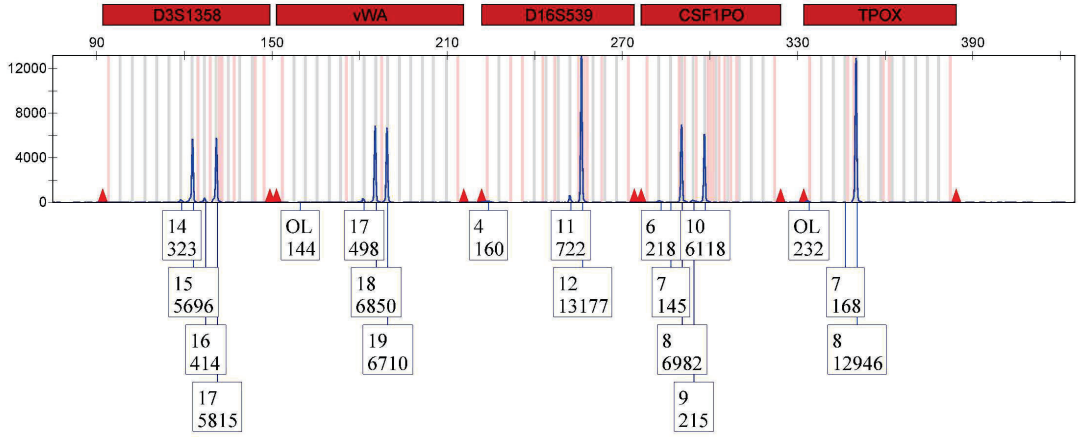

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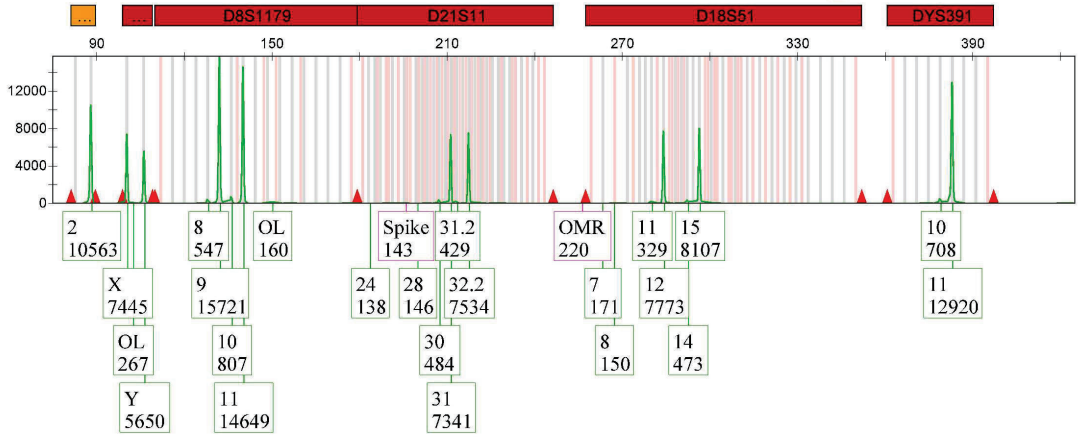

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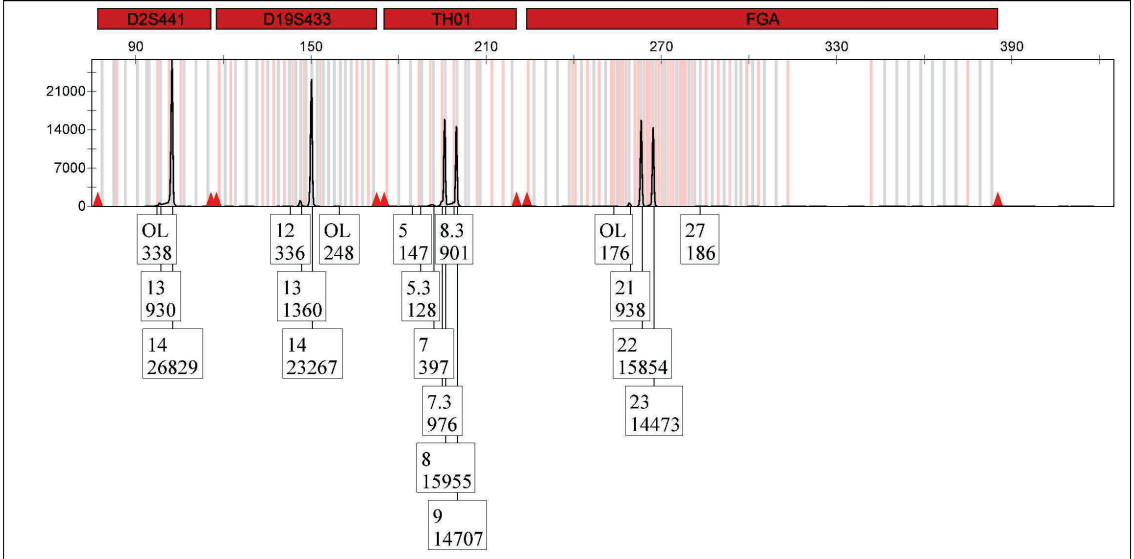

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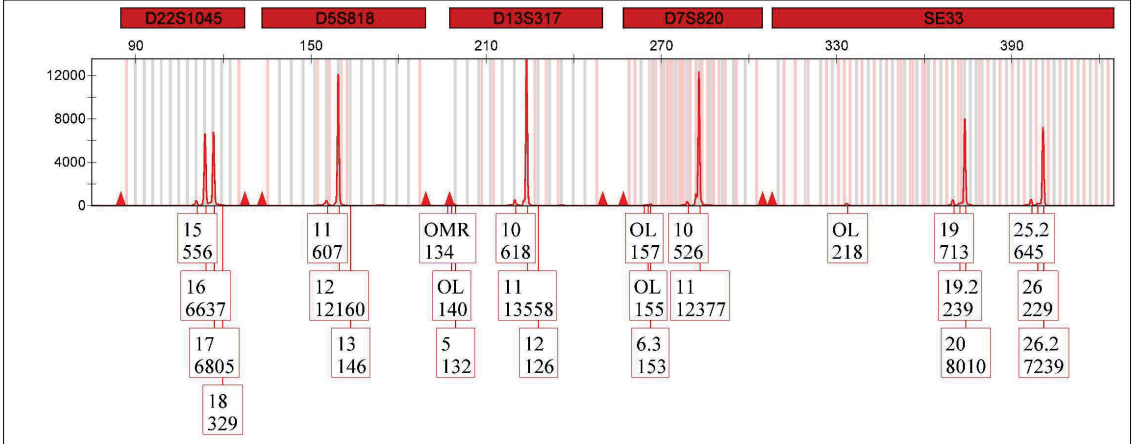

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Project: Organic Extractions CRG 061422

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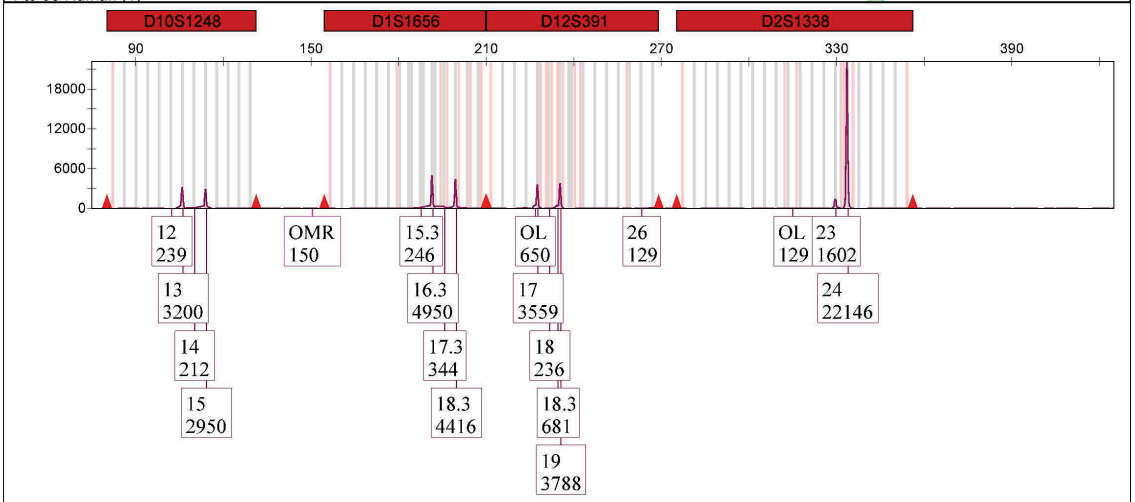

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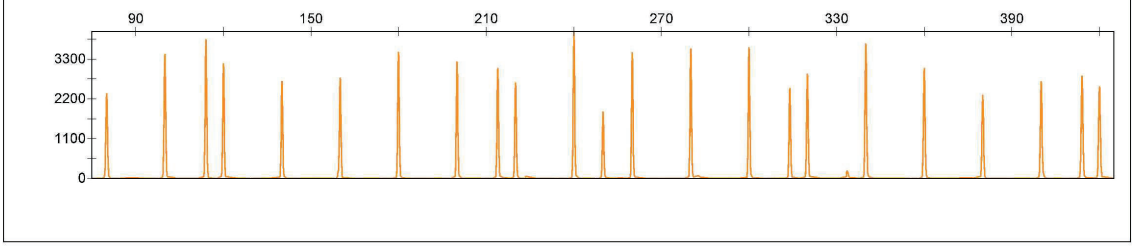

Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ

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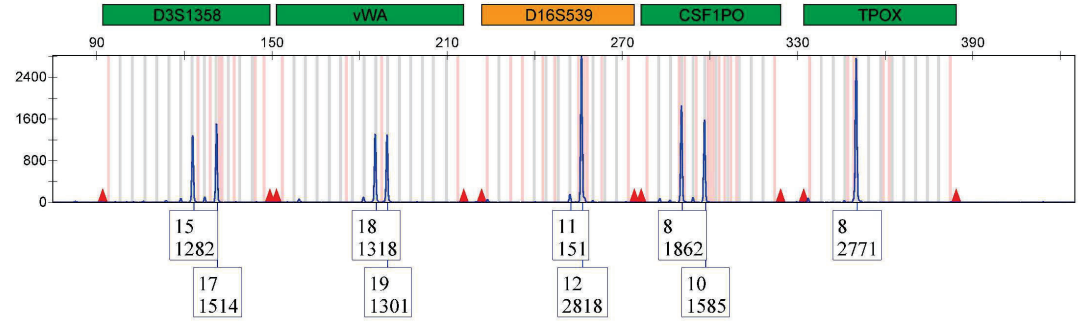

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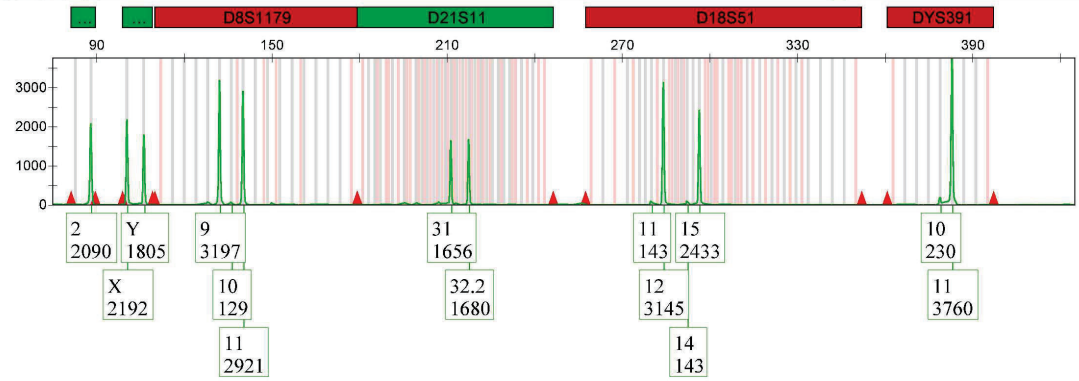

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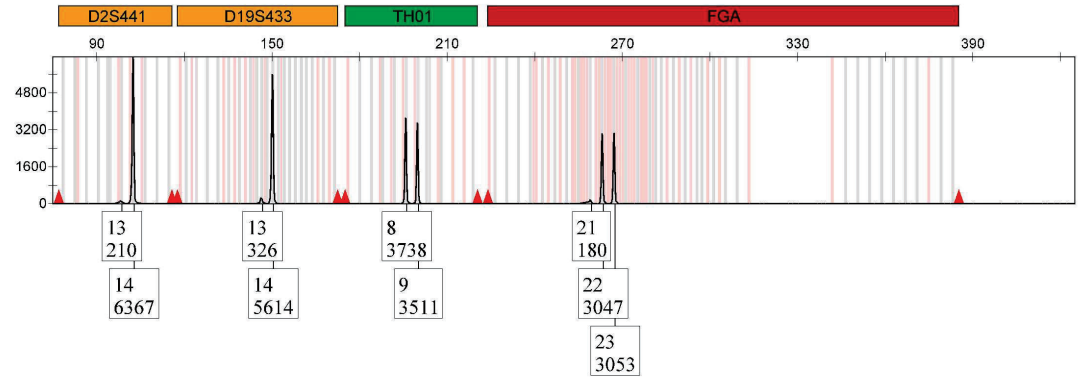

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Project: Organic Extractions CRG 061422

Sample Name	SQ

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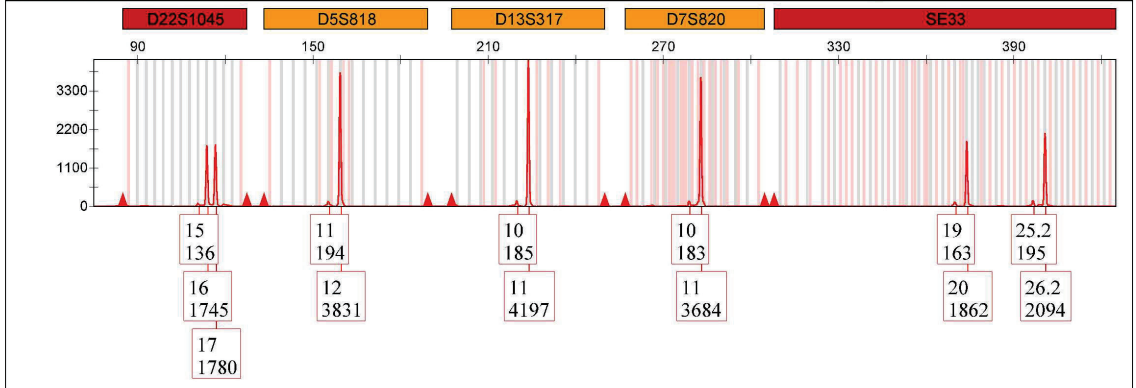
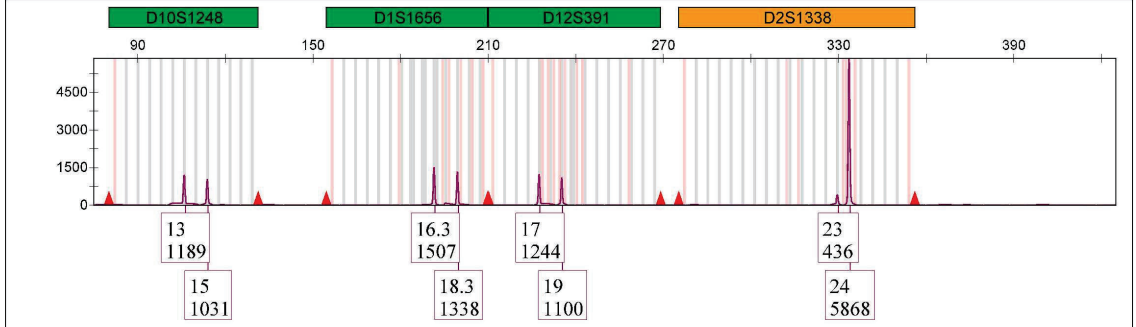
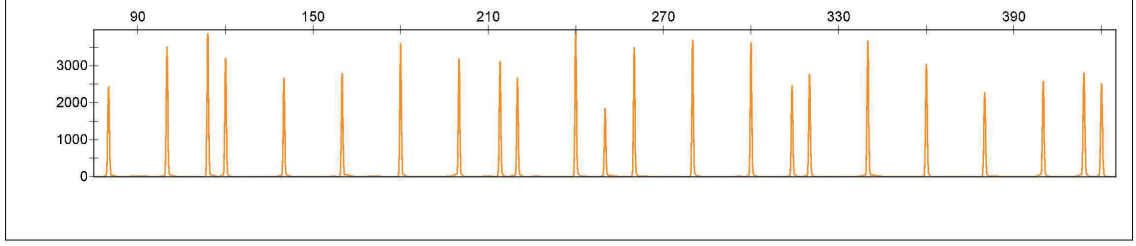


1 to 50 Human (1)



1 to 50 Human (1)

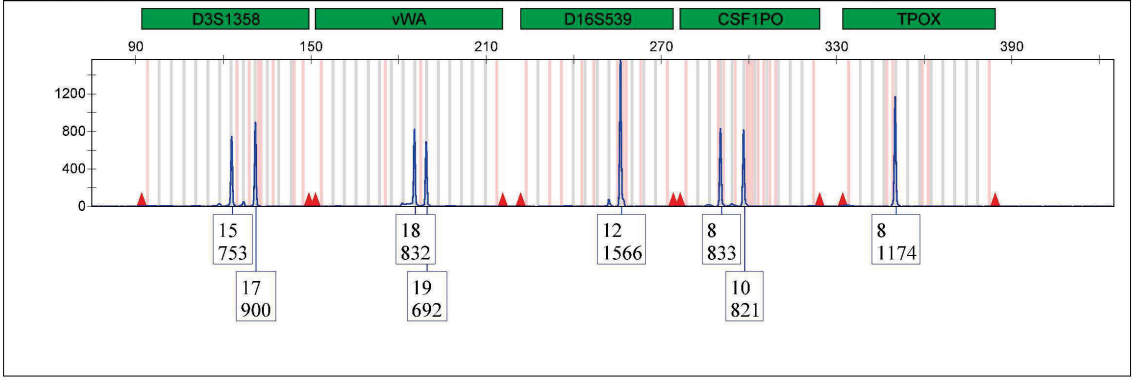


Applied Biosystems
GeneMapper® ID-X 1.4

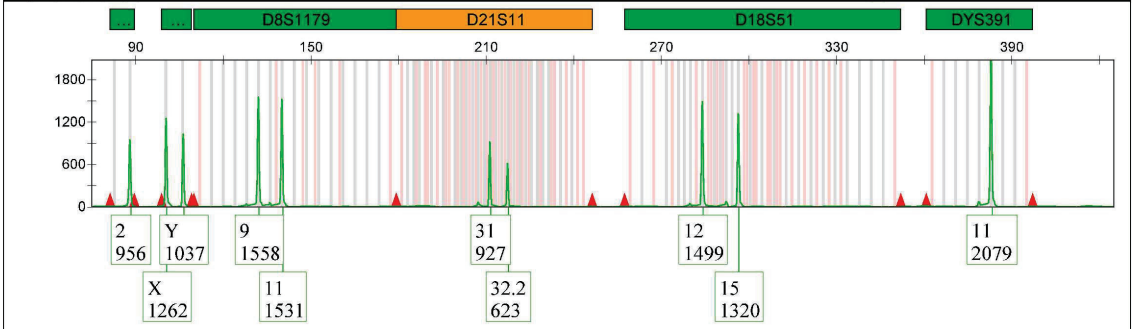
Project: Organic Extractions CRG 061422

Sample Name	SQ

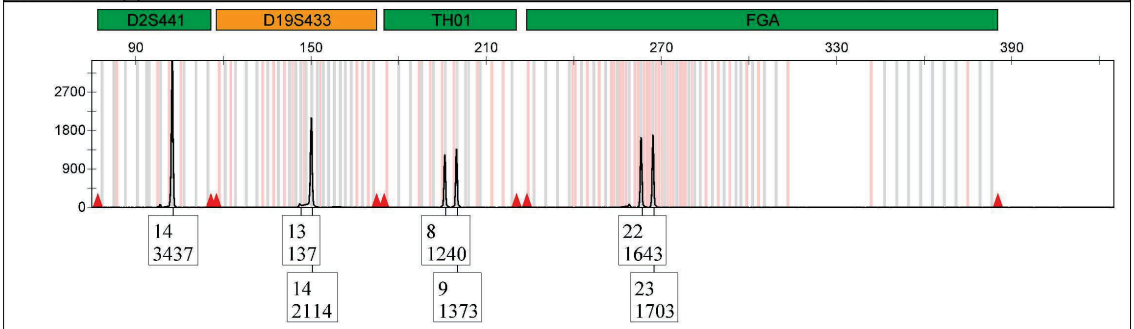
1 to 75 Human (1)



1 to 75 Human (1)



1 to 75 Human (1)

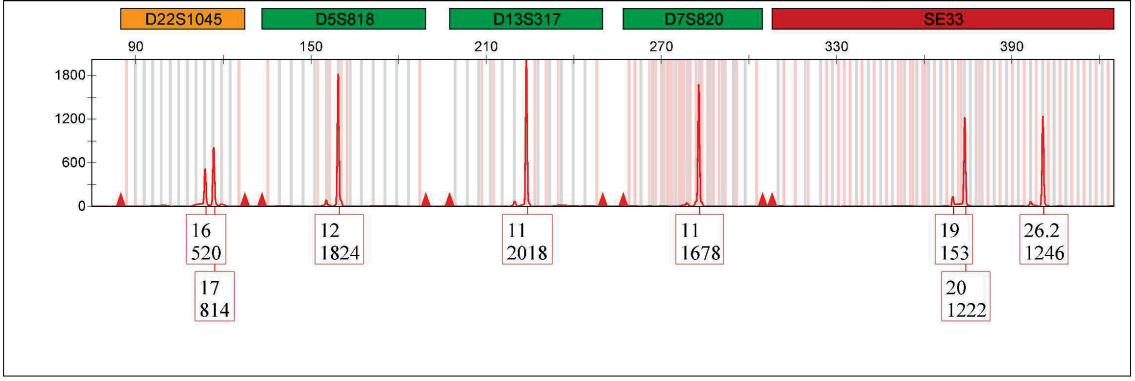


Applied Biosystems
GeneMapper® ID-X 1.4

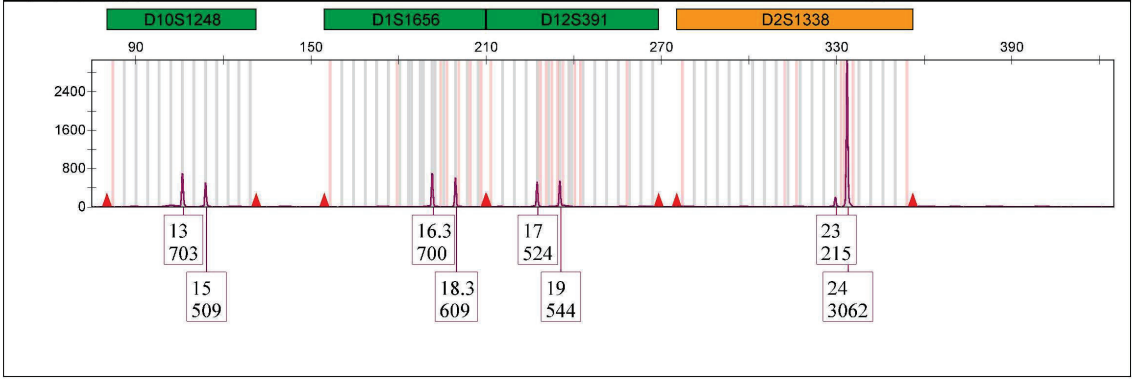
Project: Organic Extractions CRG 061422

Sample Name SQ

1 to 75 Human (1)



1 to 75 Human (1)



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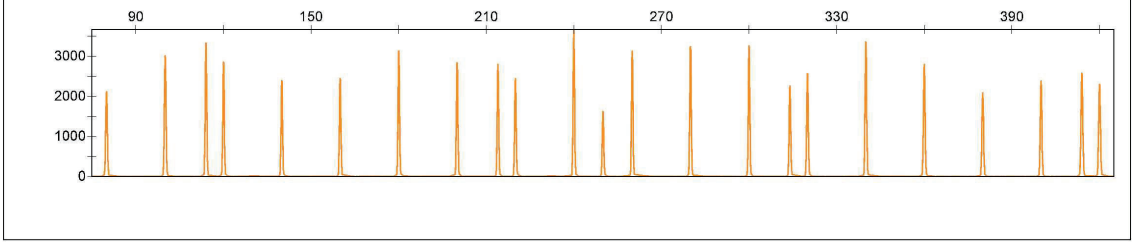
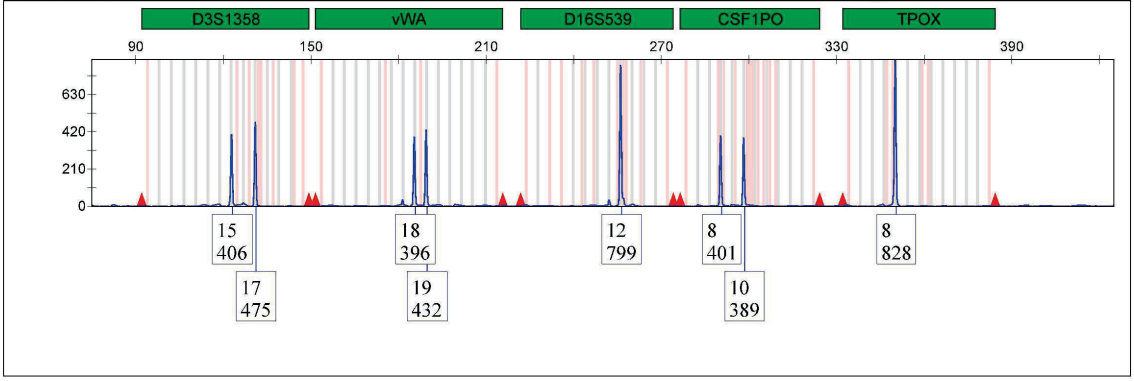


Applied Biosystems
GeneMapper® ID-X 1.4

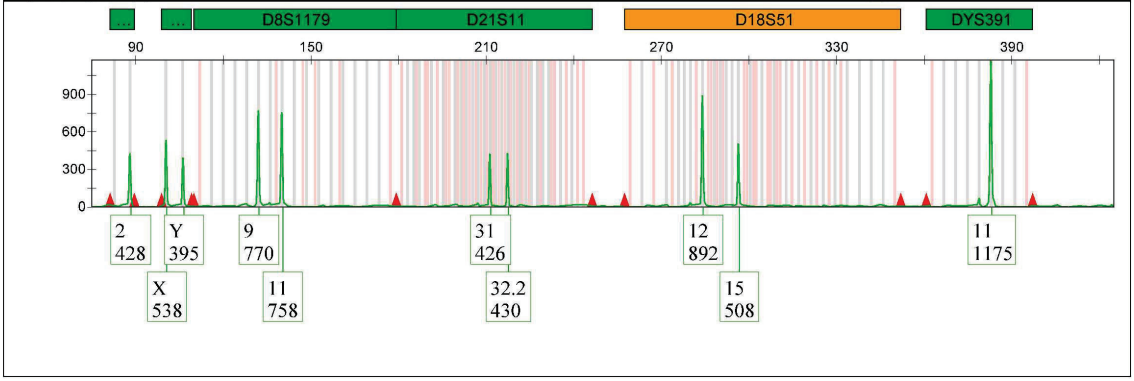
Project: Organic Extractions CRG 061422

Sample Name	SQ

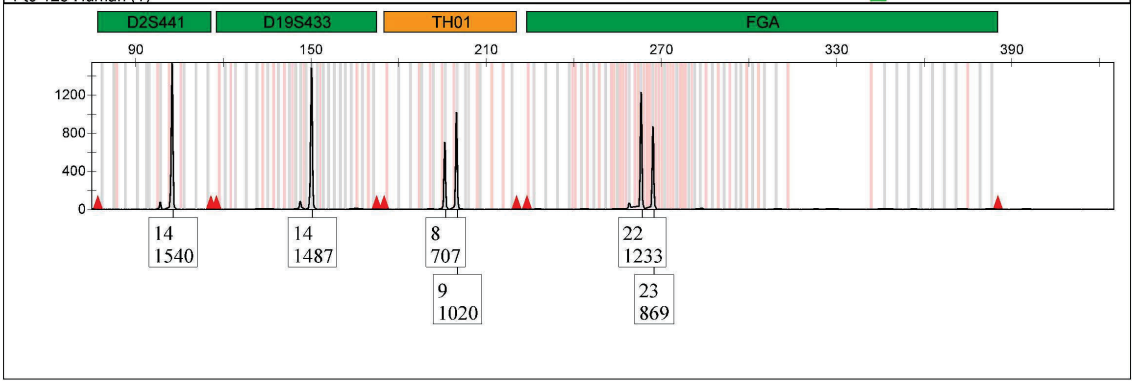
1 to 125 Human (1)



1 to 125 Human (1)



1 to 125 Human (1)

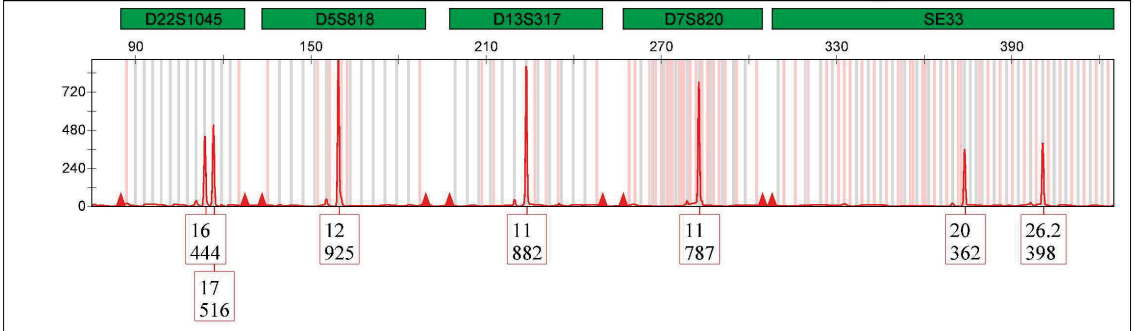


Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 125 Human (1)



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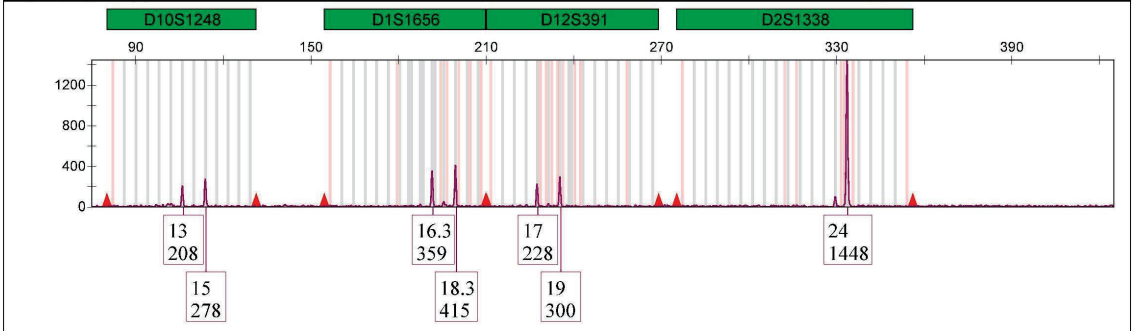

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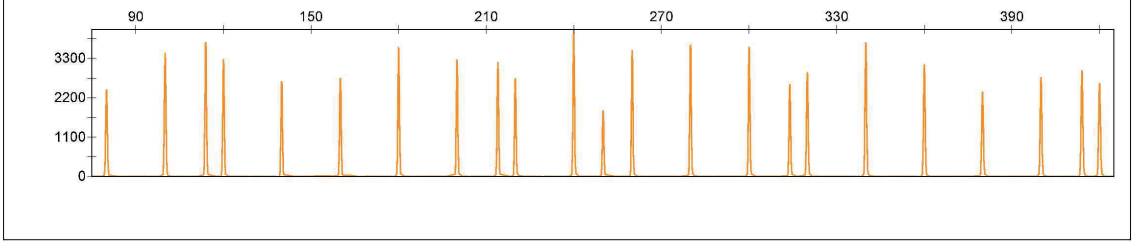
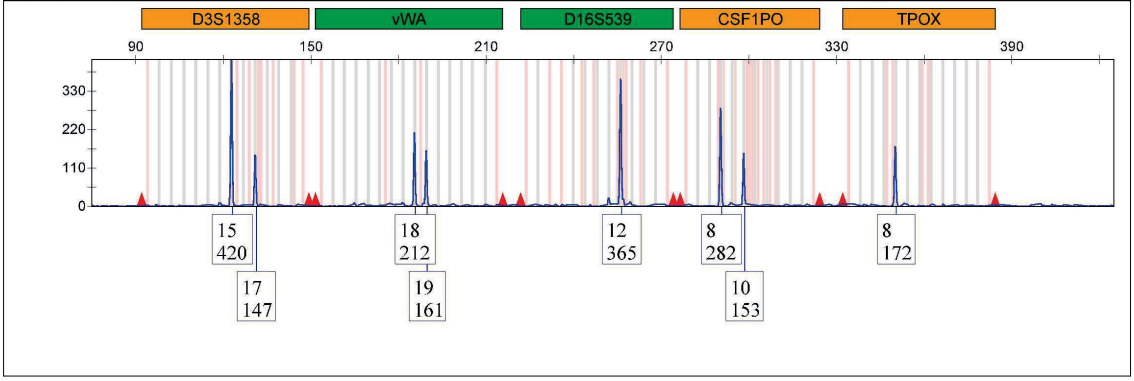


Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 175 Human (1)



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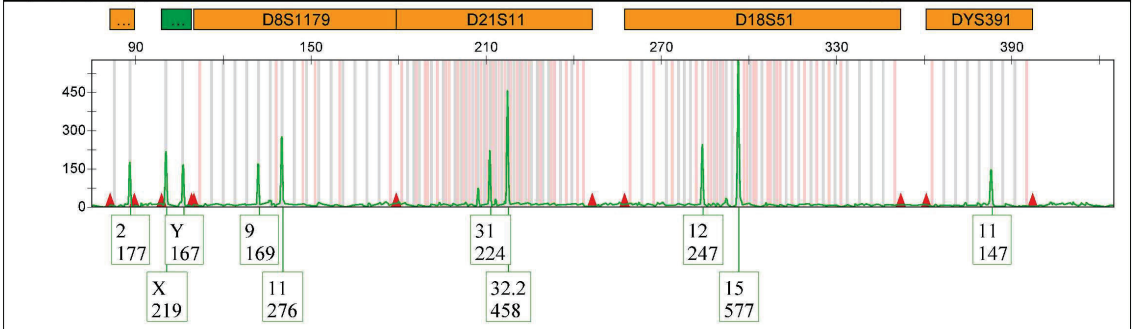
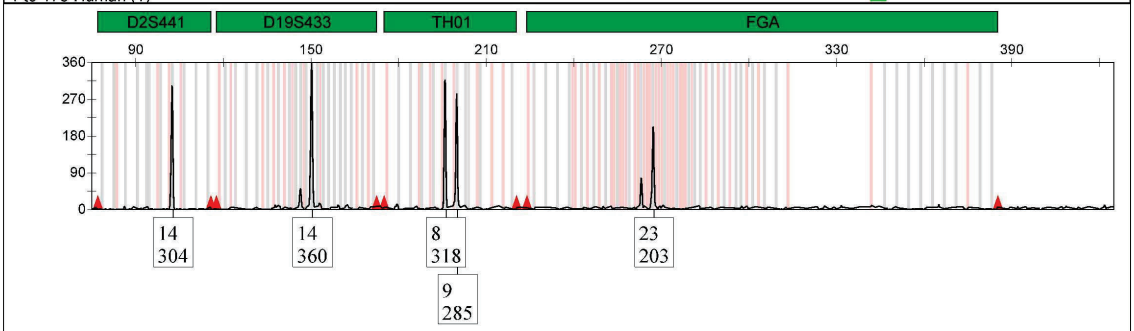


1 to 175 Human (1)

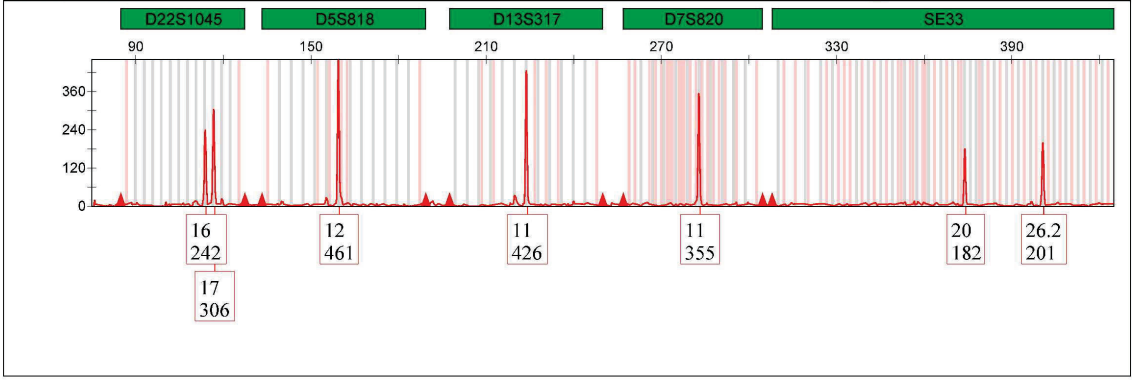


Applied Biosystems
GeneMapper® ID-X 1.4

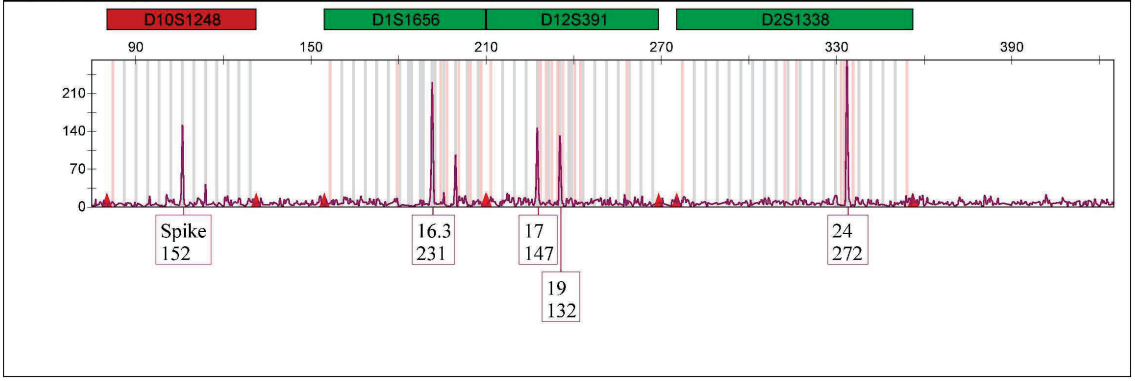
Project: Organic Extractions CRG 061422

Sample Name SQ

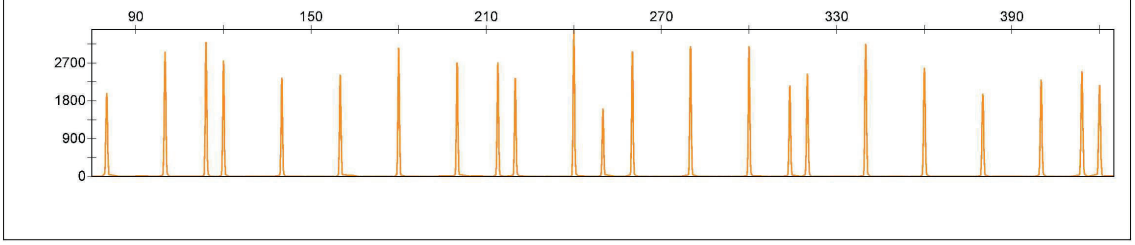
1 to 175 Human (1)



1 to 175 Human (1)



1 to 175 Human (1)

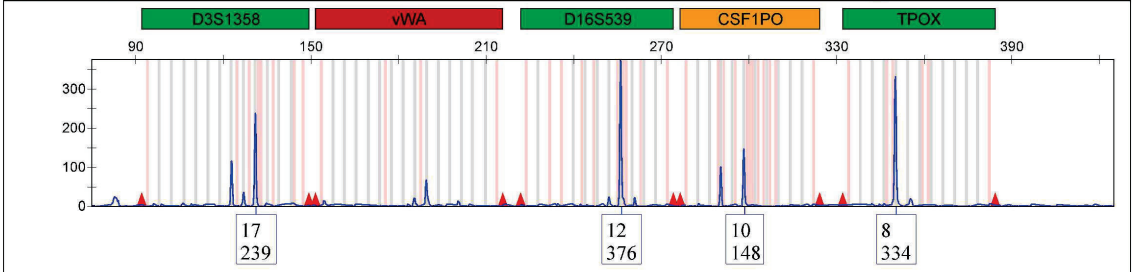


Applied Biosystems
GeneMapper® ID-X 1.4

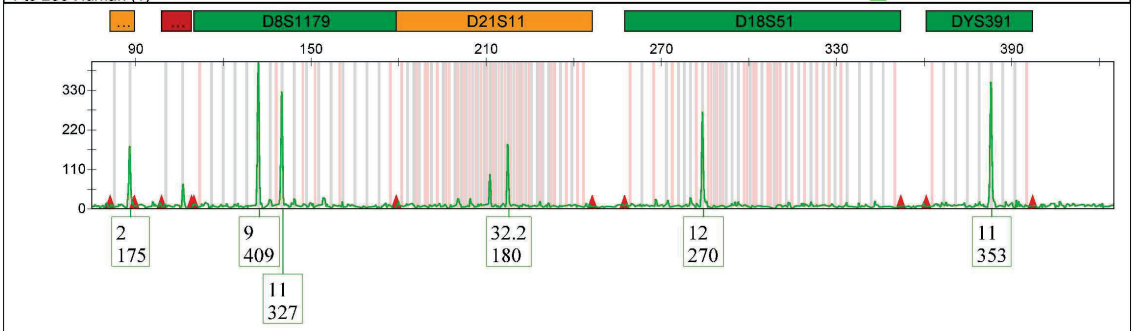
Project: Organic Extractions CRG 061422

Sample Name	SQ

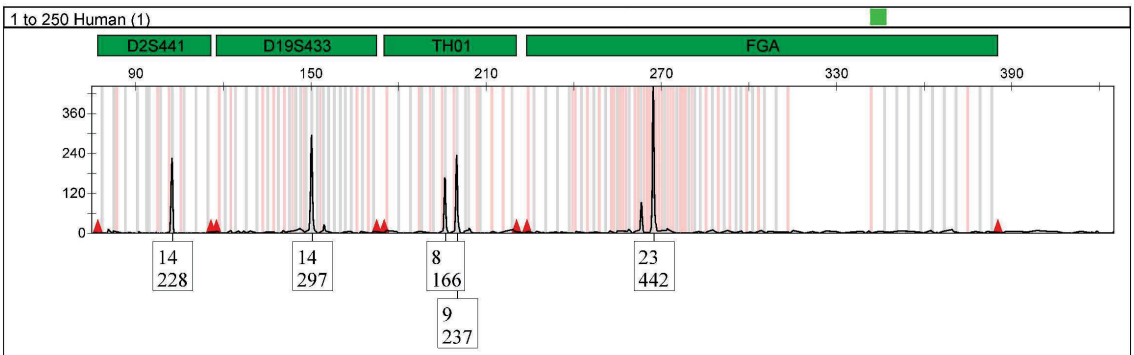
1 to 250 Human (1)



1 to 250 Human (1)



1 to 250 Human (1)

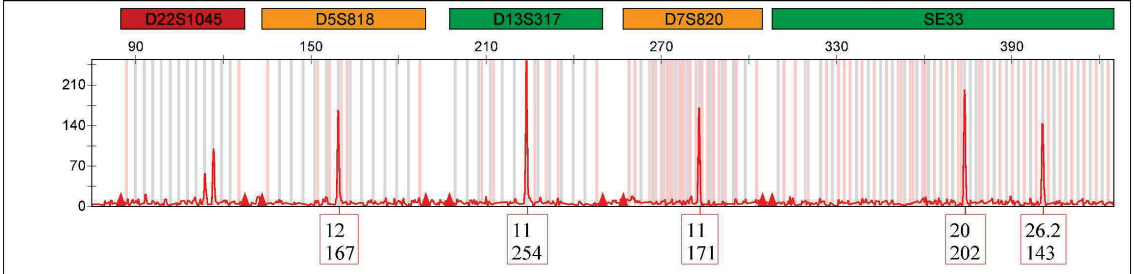


Applied Biosystems
GeneMapper® ID-X 1.4

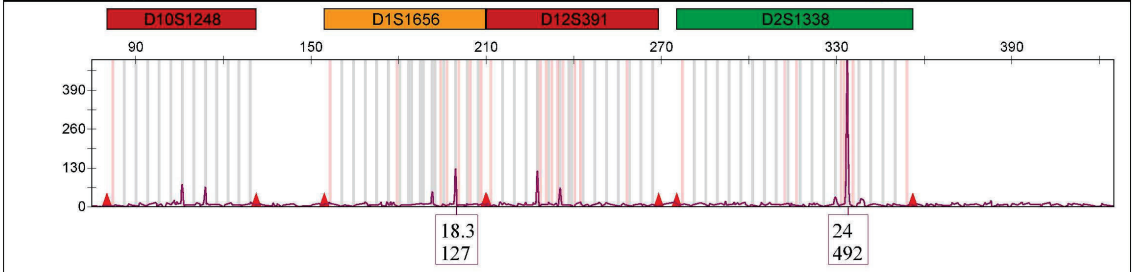
Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 250 Human (1)



1 to 250 Human (1)



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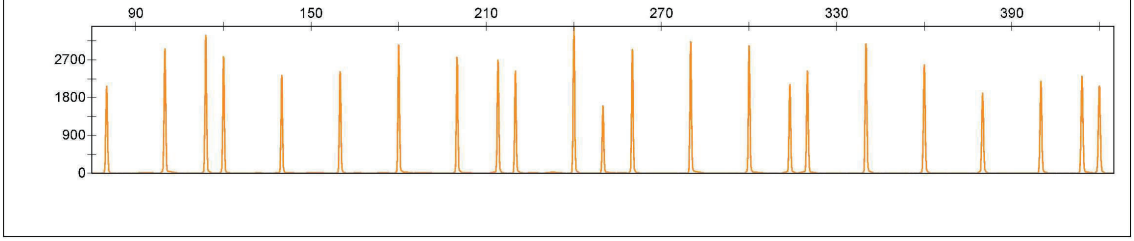
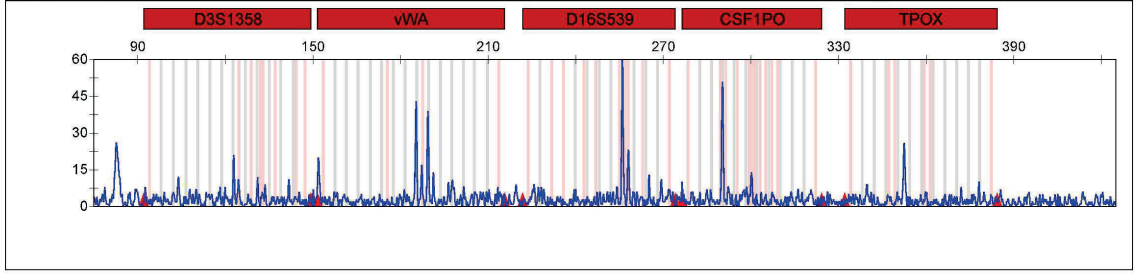


Applied Biosystems
GeneMapper® ID-X 1.4

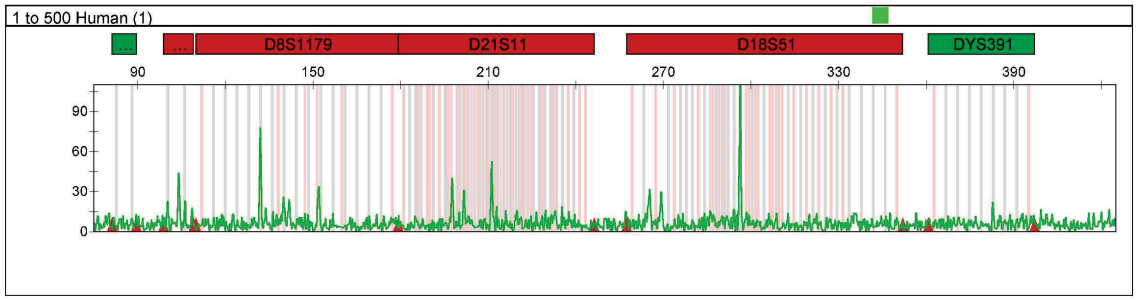
Project: Organic Extractions CRG 061422

Sample Name SQ

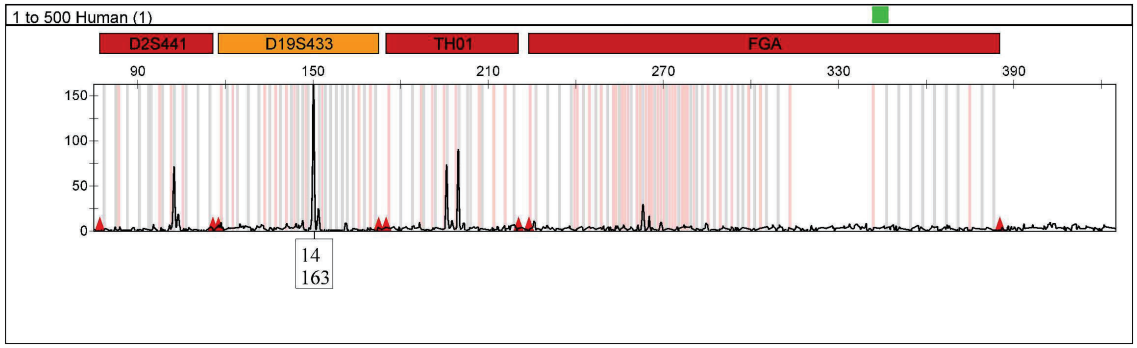
1 to 500 Human (1)



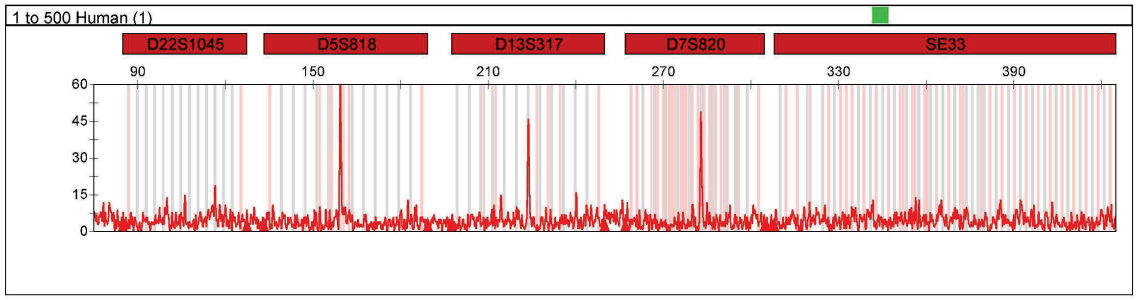
1 to 500 Human (1)



1 to 500 Human (1)



1 to 500 Human (1)

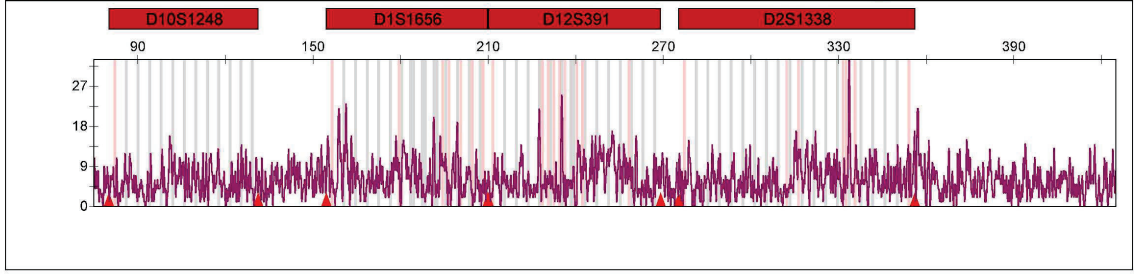


Applied Biosystems
GeneMapper® ID-X 1.4

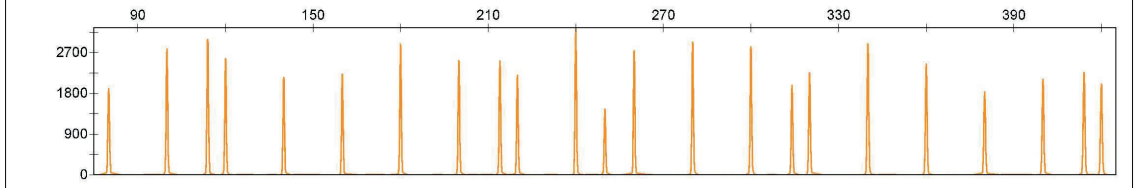
Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 500 Human (1)



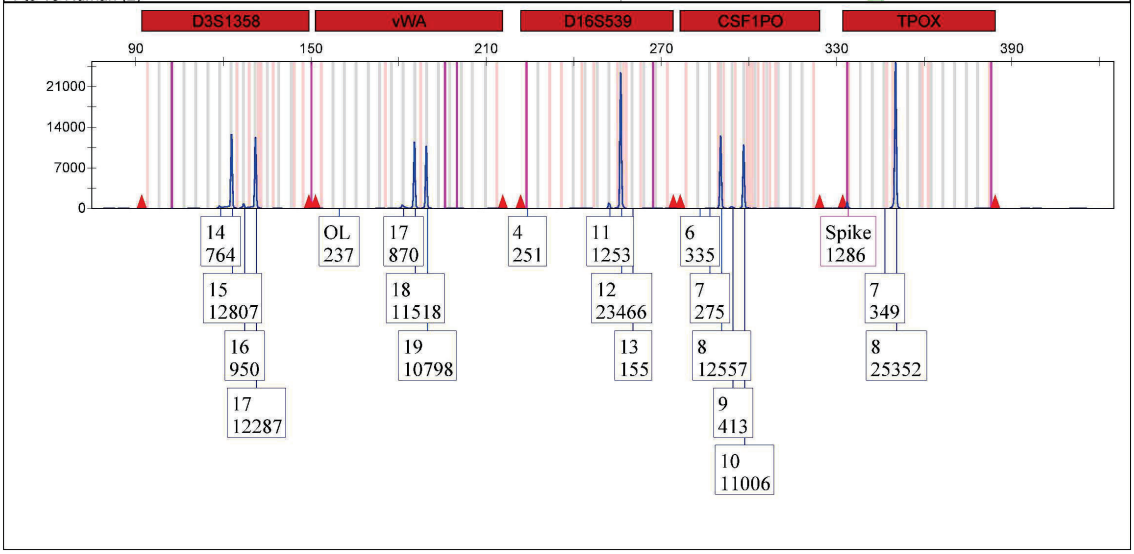
1 to 500 Human (1)



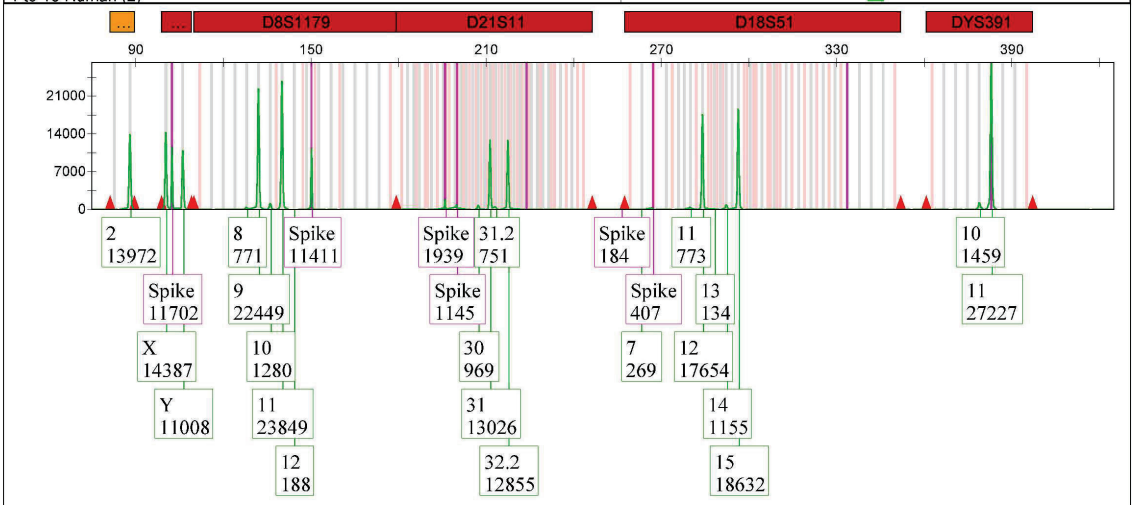
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 10 Human (2)	



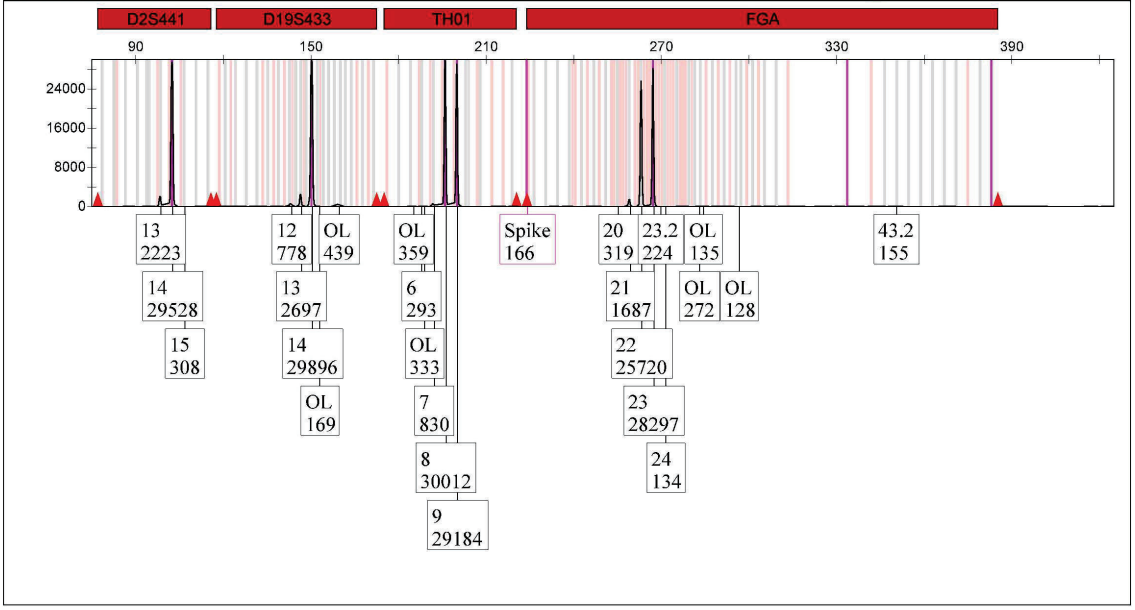
1 to 10 Human (2)	



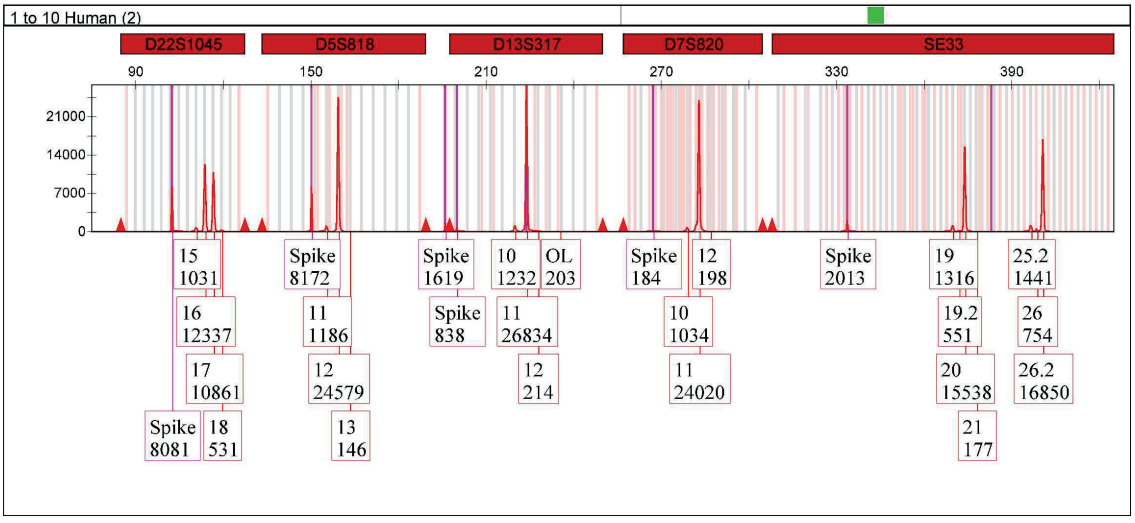
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 10 Human (2)	

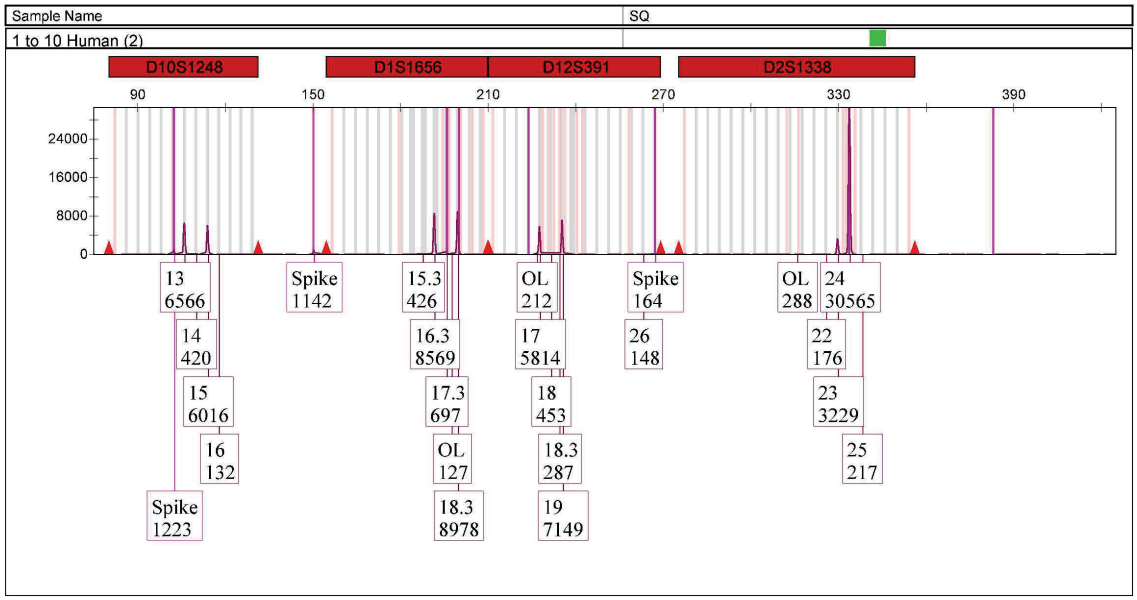


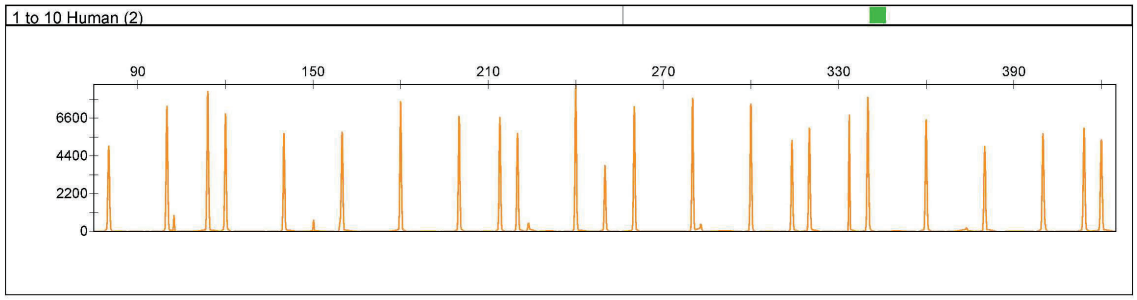
1 to 10 Human (2)	



Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422



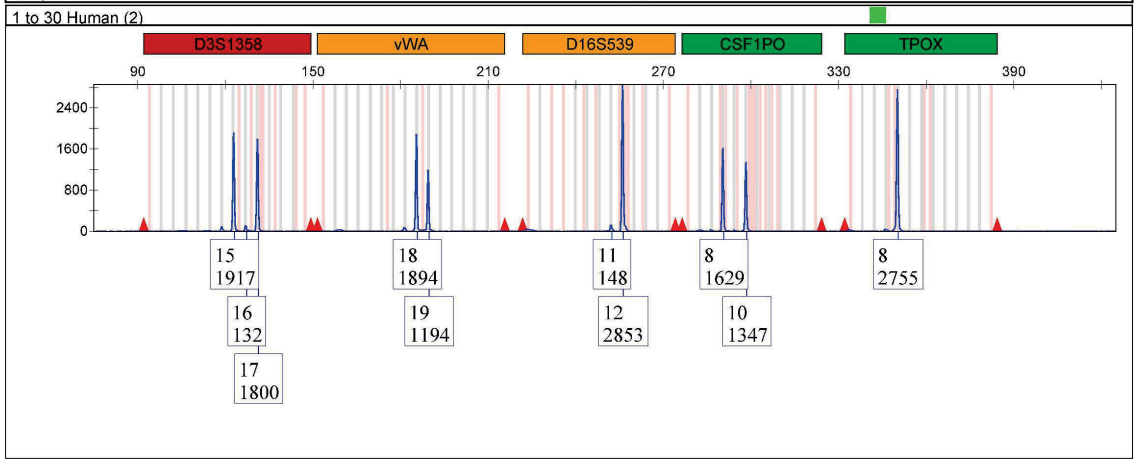


Applied Biosystems
GeneMapper® ID-X 1.4

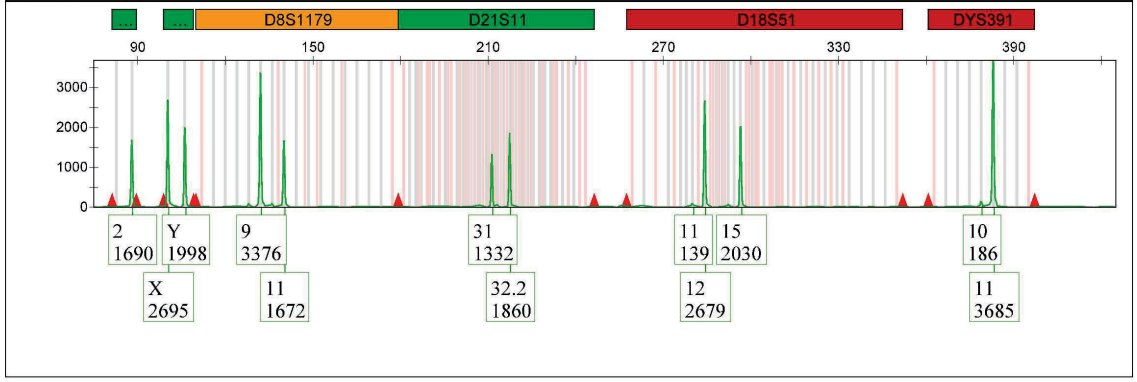
Project: Organic Extractions CRG 061422

Sample Name	SQ

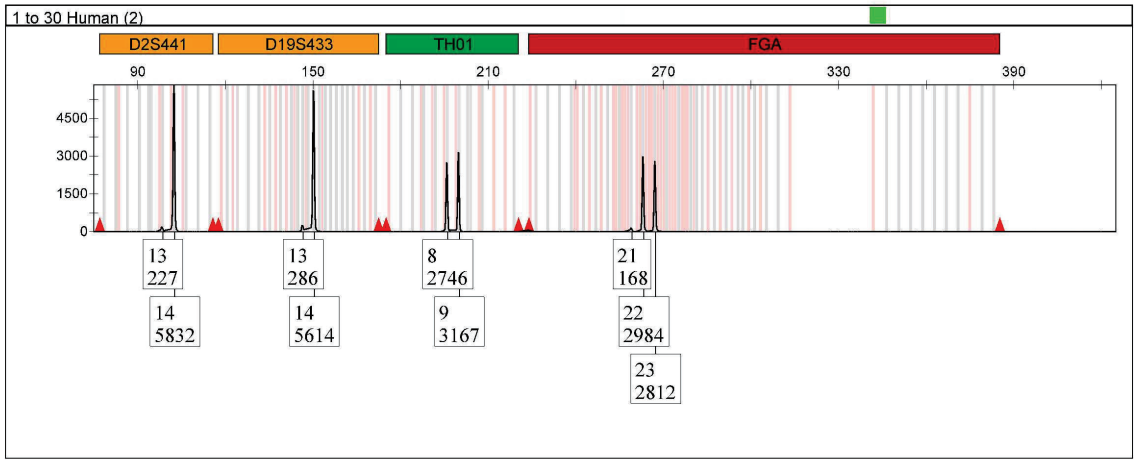
1 to 30 Human (2)



1 to 30 Human (2)



1 to 30 Human (2)

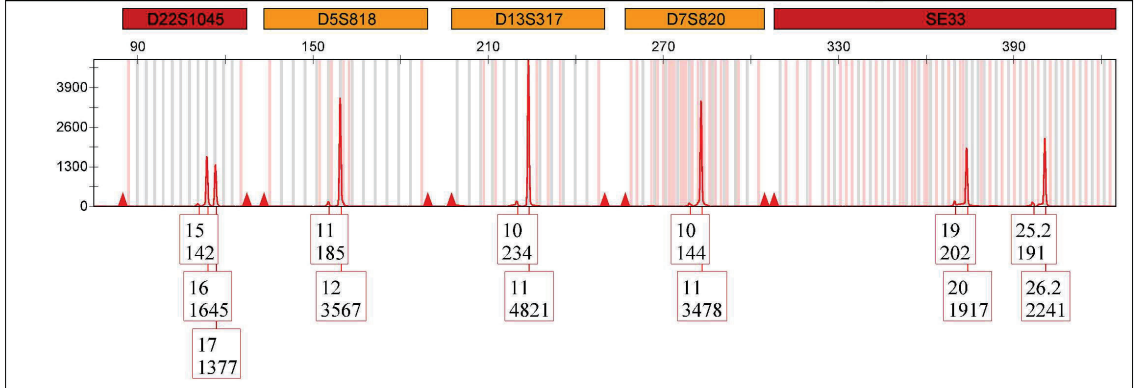


Applied Biosystems
GeneMapper® ID-X 1.4

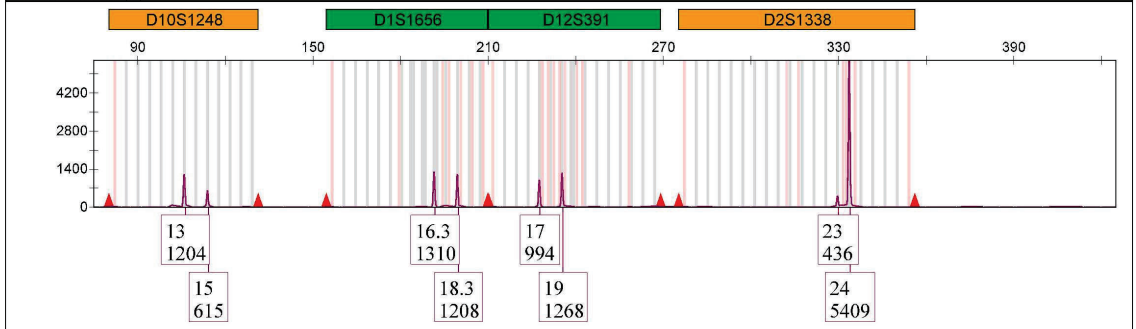
Project: Organic Extractions CRG 061422

Sample Name	SQ

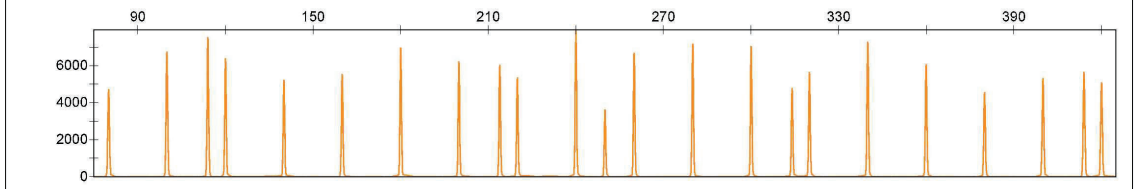
1 to 30 Human (2)



1 to 30 Human (2)



1 to 30 Human (2)

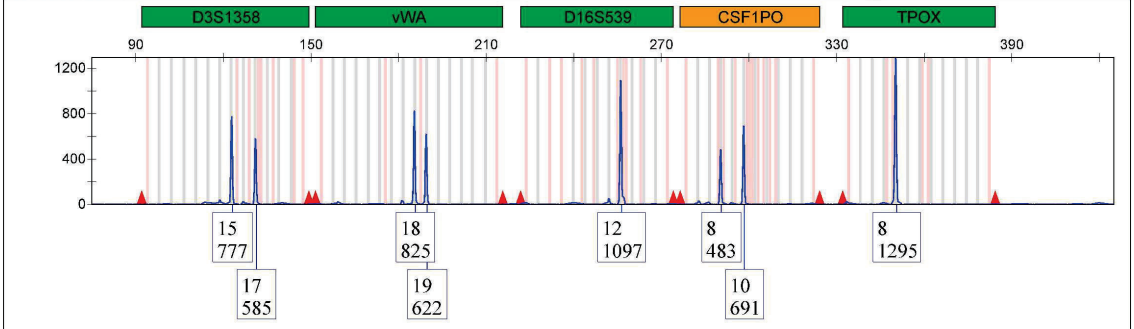


Applied Biosystems
GeneMapper® ID-X 1.4

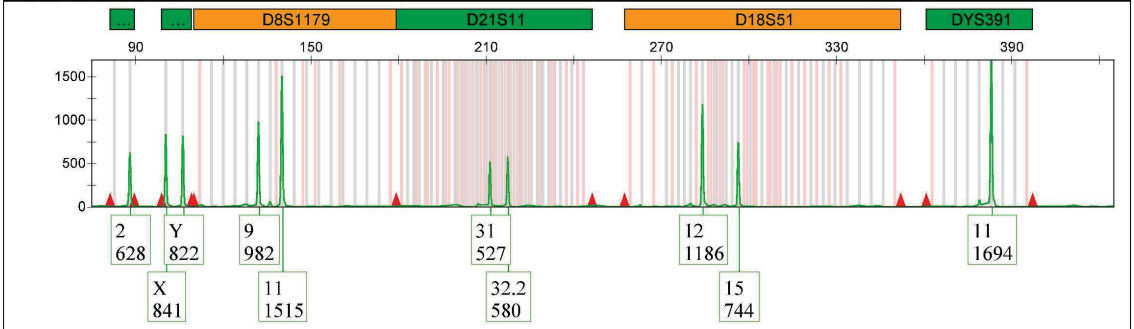
Project: Organic Extractions CRG 061422

Sample Name	SQ

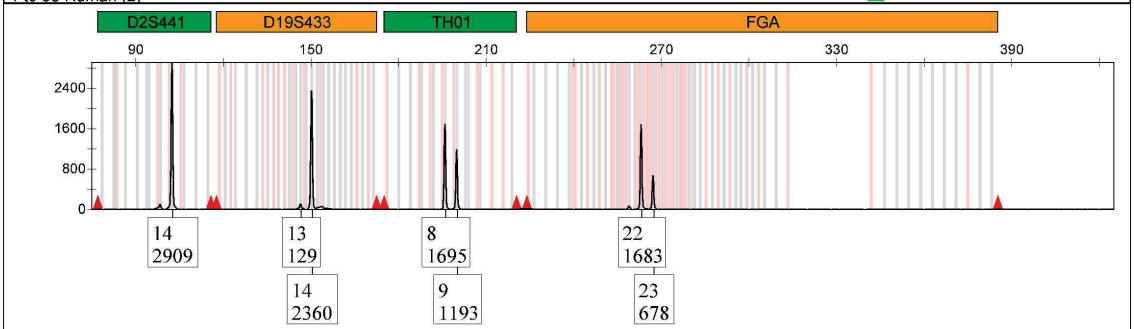
1 to 50 Human (2)



1 to 50 Human (2)



1 to 50 Human (2)

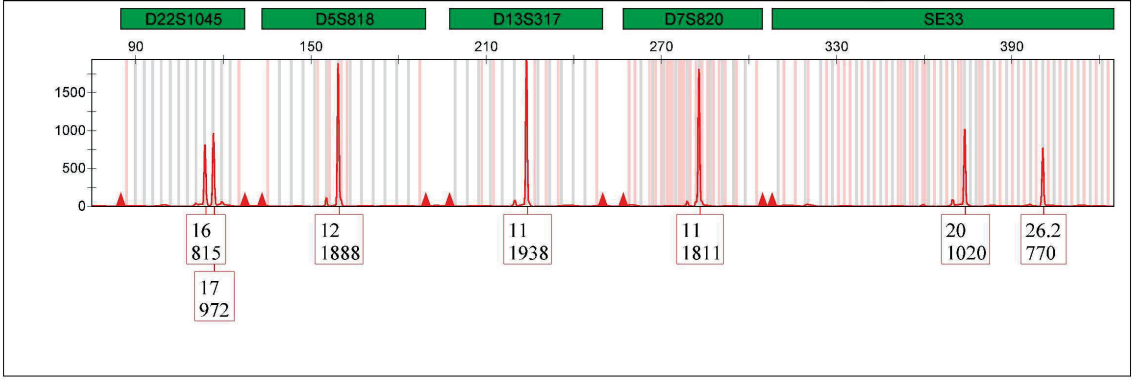


Applied Biosystems
GeneMapper® ID-X 1.4

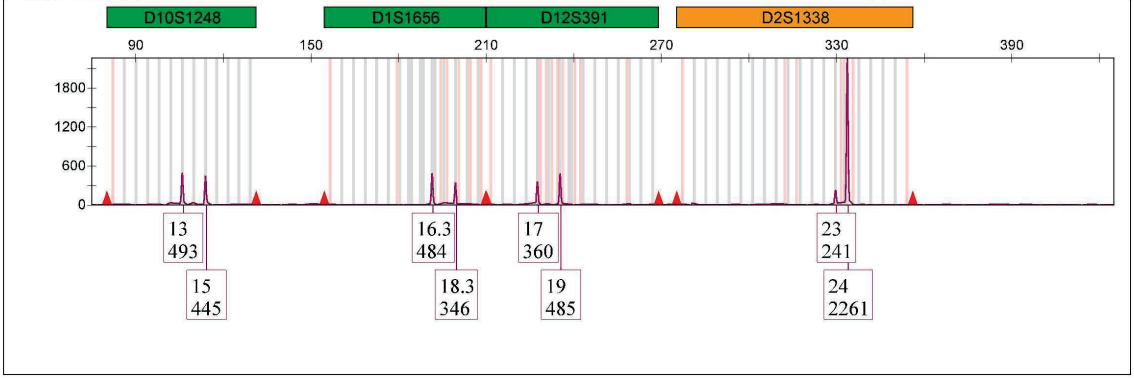
Project: Organic Extractions CRG 061422

Sample Name	SQ

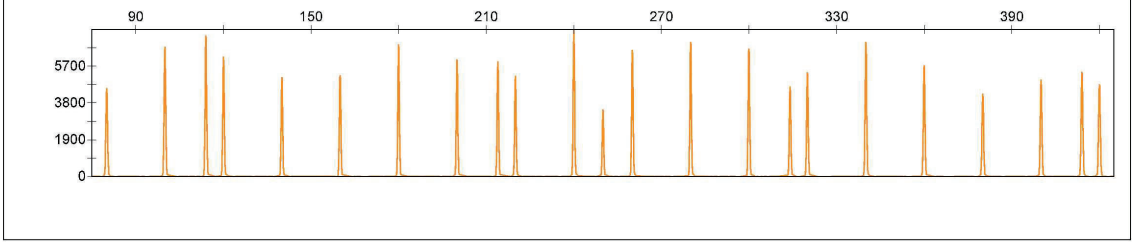
1 to 50 Human (2)



1 to 50 Human (2)



1 to 50 Human (2)

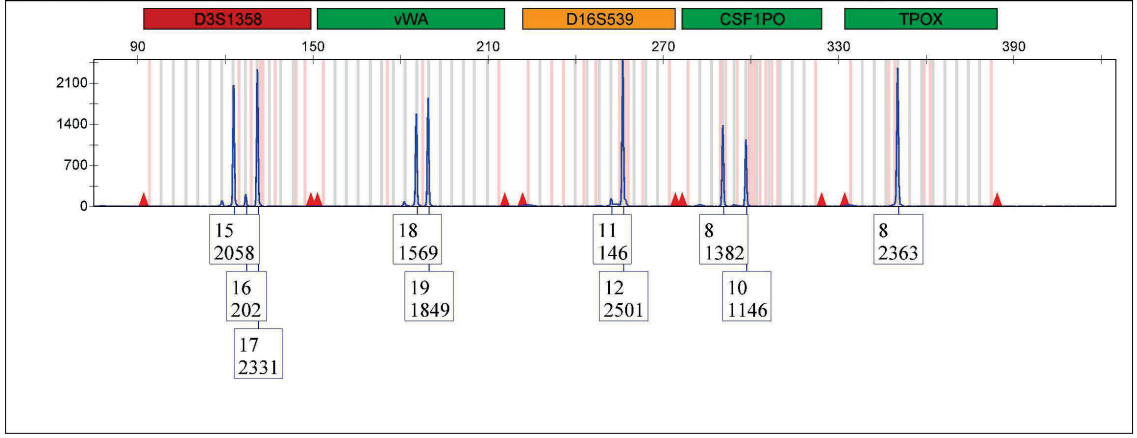


Applied Biosystems
GeneMapper® ID-X 1.4

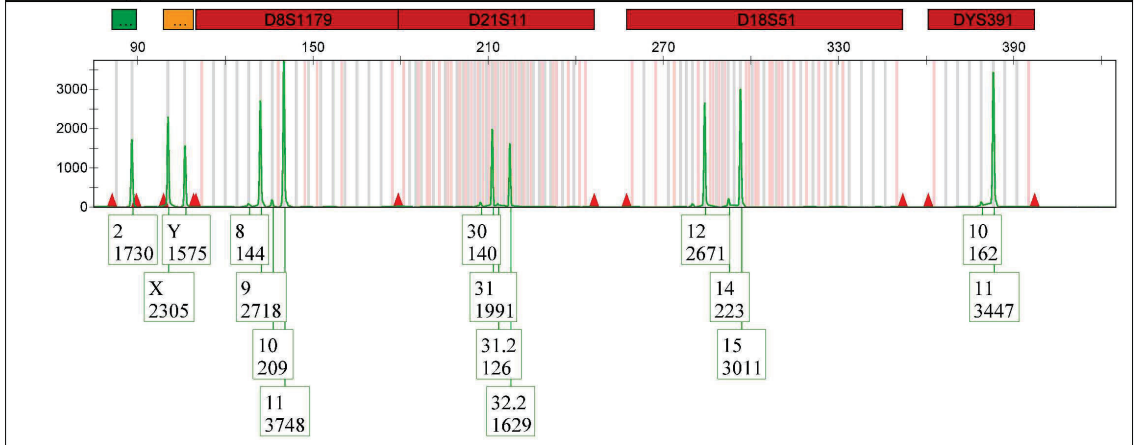
Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 75 Human (2)



1 to 75 Human (2)

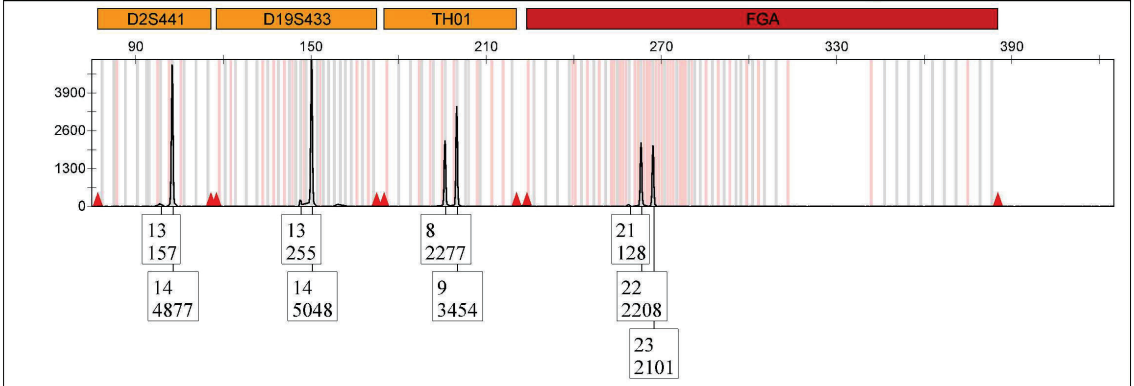


Applied Biosystems
GeneMapper® ID-X 1.4

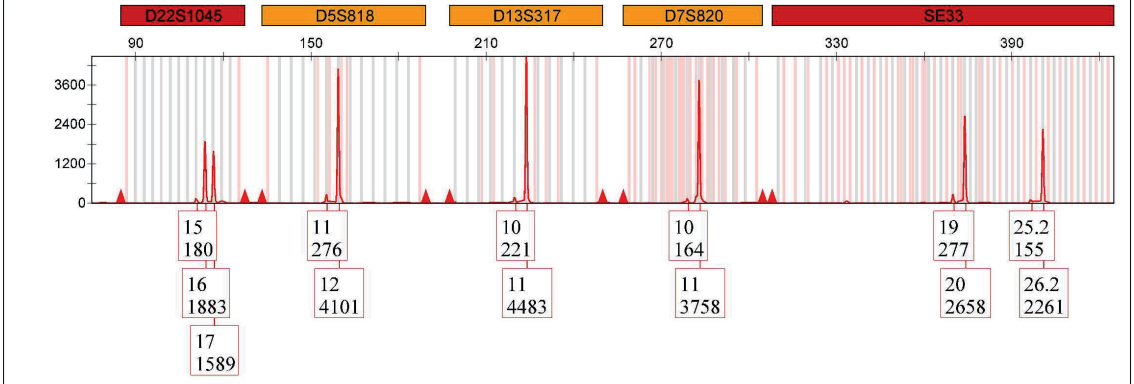
Project: Organic Extractions CRG 061422

Sample Name	SQ

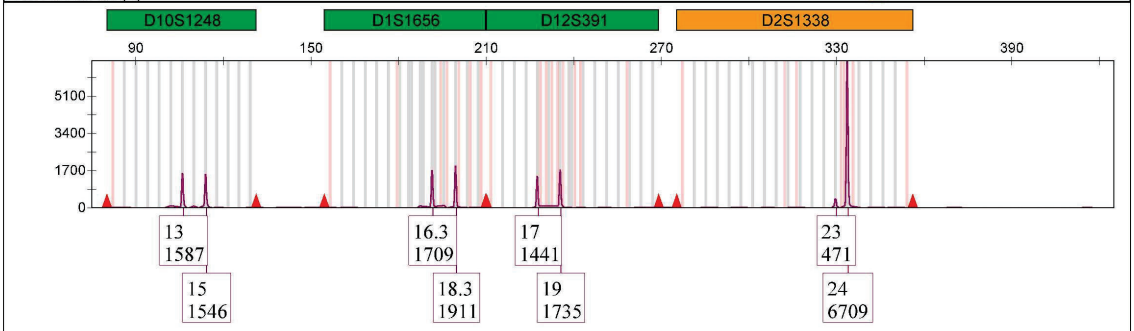
1 to 75 Human (2)



1 to 75 Human (2)



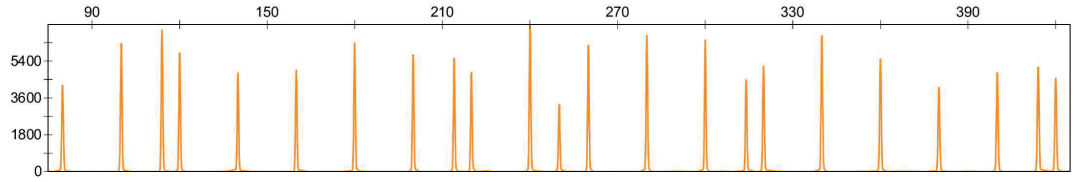
1 to 75 Human (2)



Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 75 Human (2)	

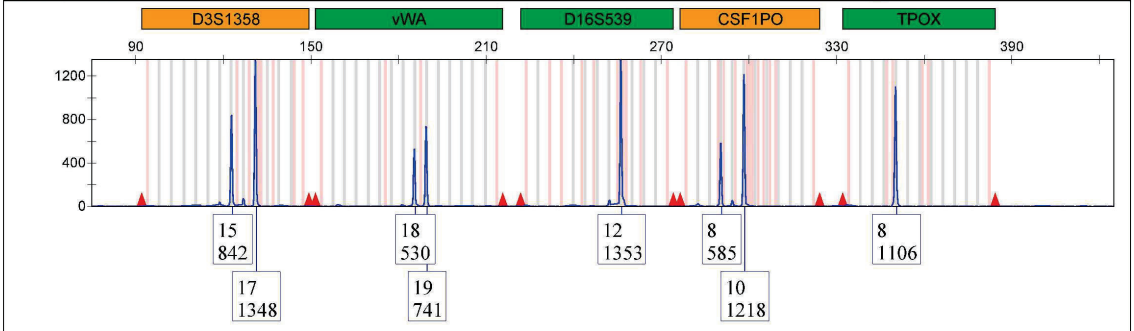


Applied Biosystems
GeneMapper® ID-X 1.4

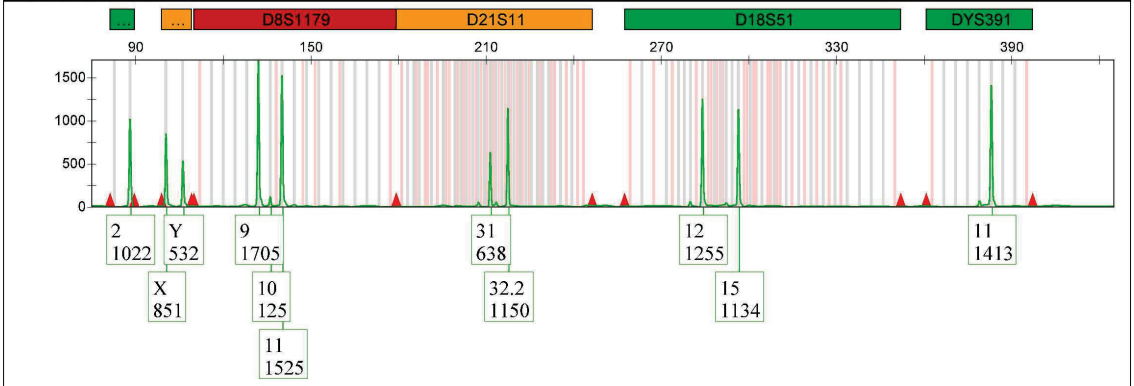
Project: Organic Extractions CRG 061422

Sample Name	SQ

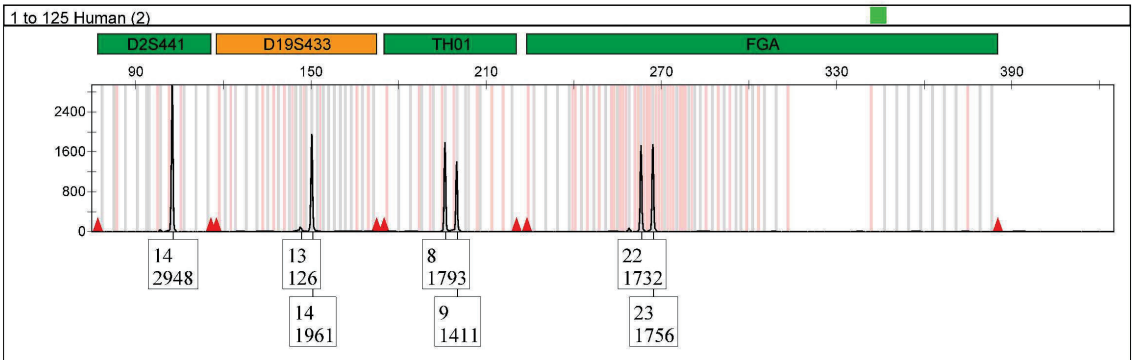
1 to 125 Human (2)



1 to 125 Human (2)



1 to 125 Human (2)

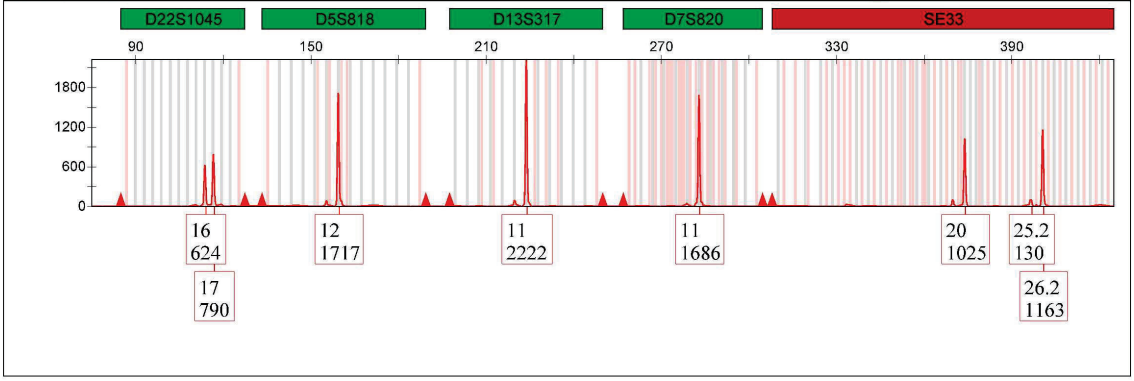


Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 125 Human (2)



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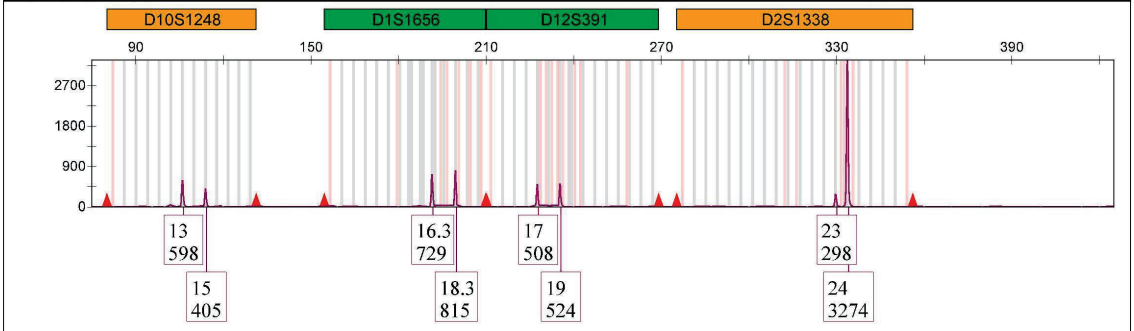
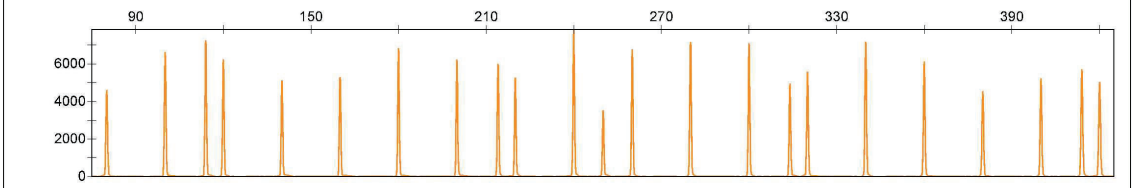


1 to 125 Human (2)

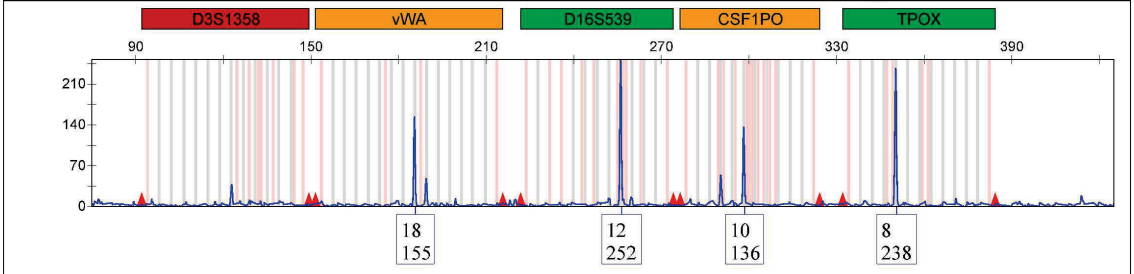


Applied Biosystems
GeneMapper® ID-X 1.4

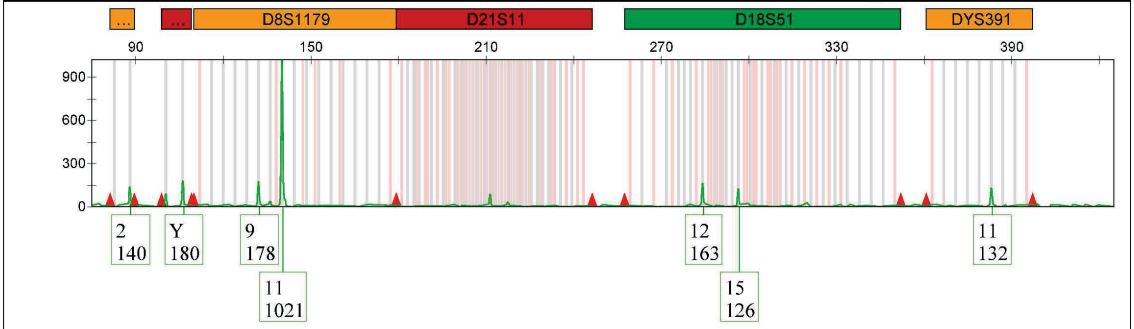
Project: Organic Extractions CRG 061422

Sample Name	SQ

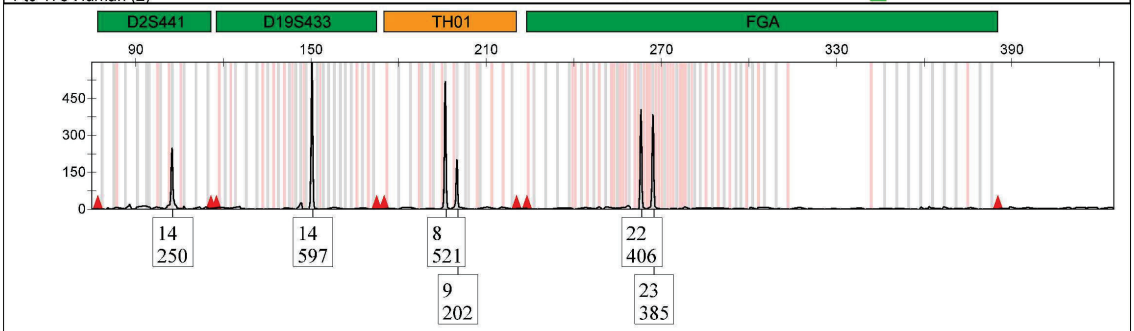
1 to 175 Human (2)



1 to 175 Human (2)



1 to 175 Human (2)

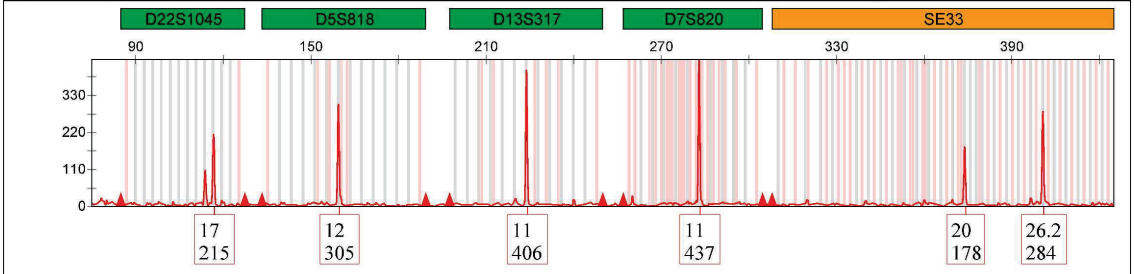


Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 175 Human (2)



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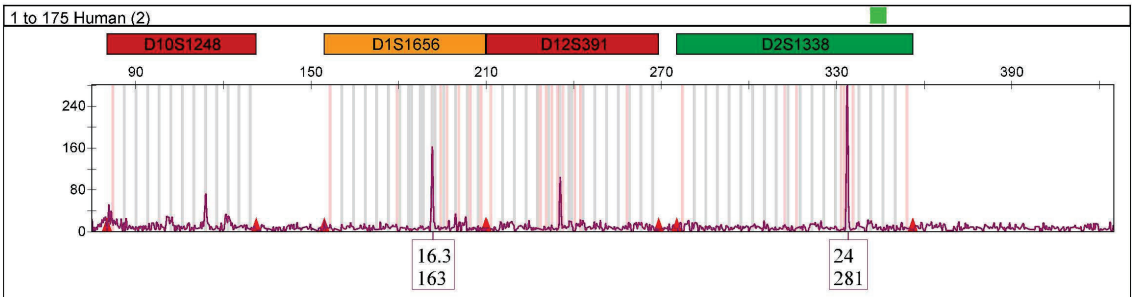
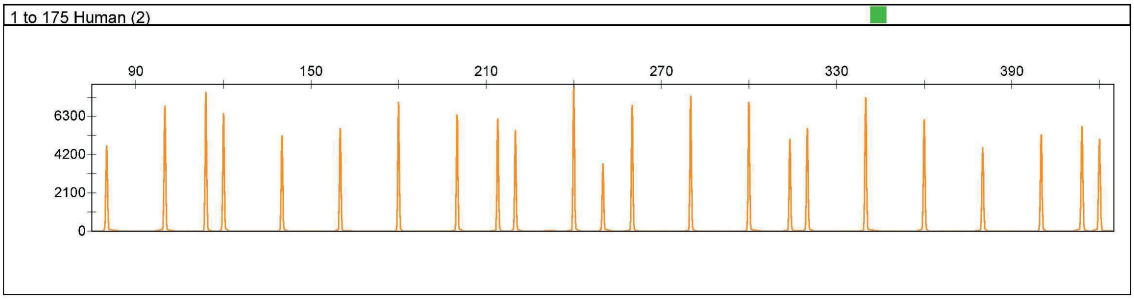


1 to 175 Human (2)

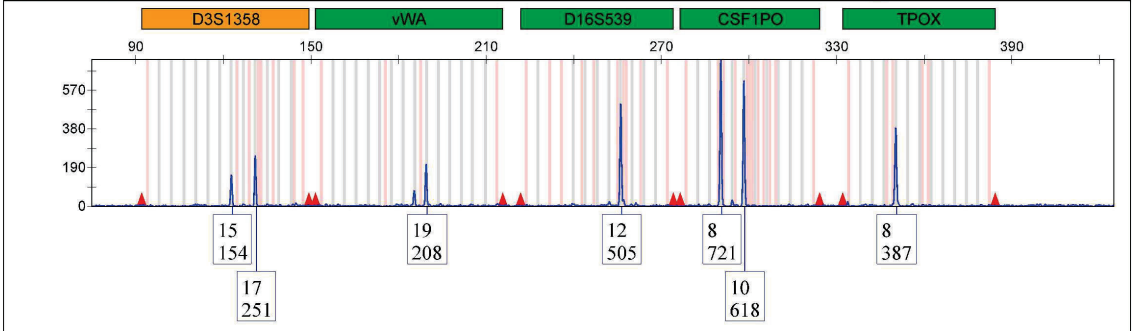


Applied Biosystems
GeneMapper® ID-X 1.4

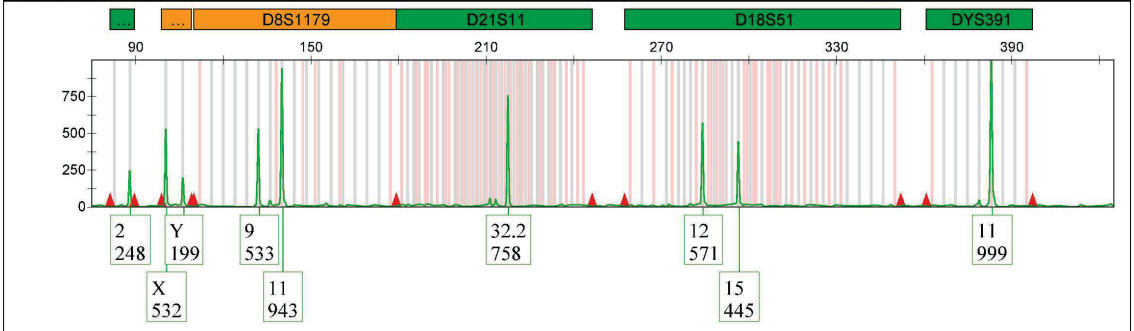
Project: Organic Extractions CRG 061422

Sample Name	SQ

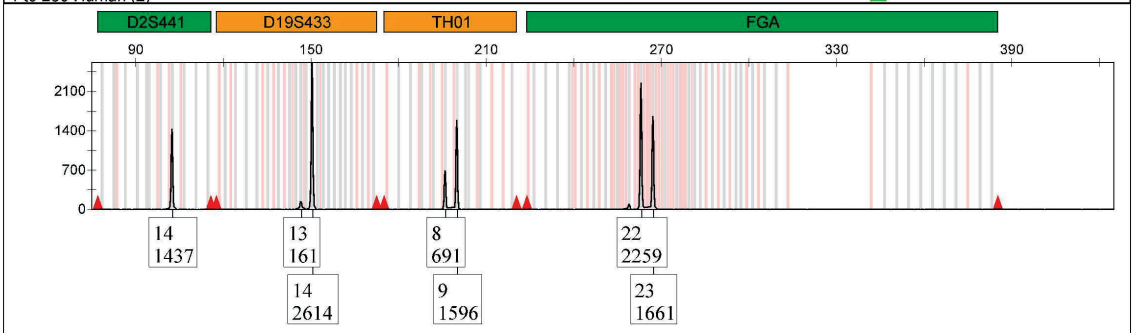
1 to 250 Human (2)



1 to 250 Human (2)



1 to 250 Human (2)

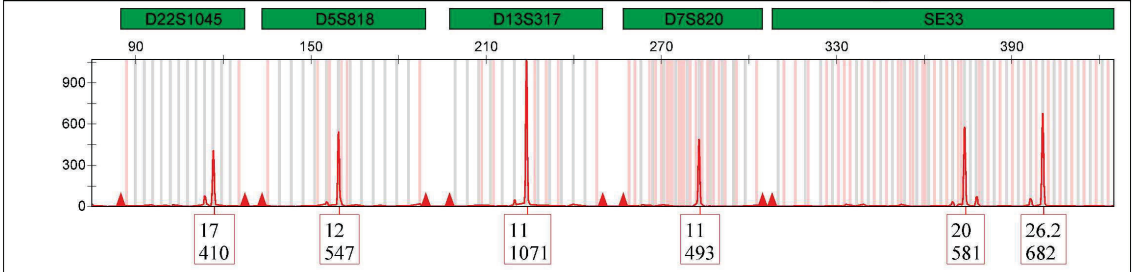


Applied Biosystems
GeneMapper® ID-X 1.4

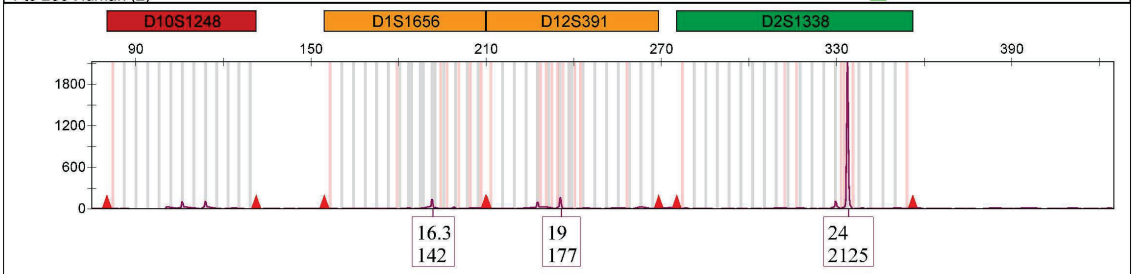
Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 250 Human (2)



1 to 250 Human (2)



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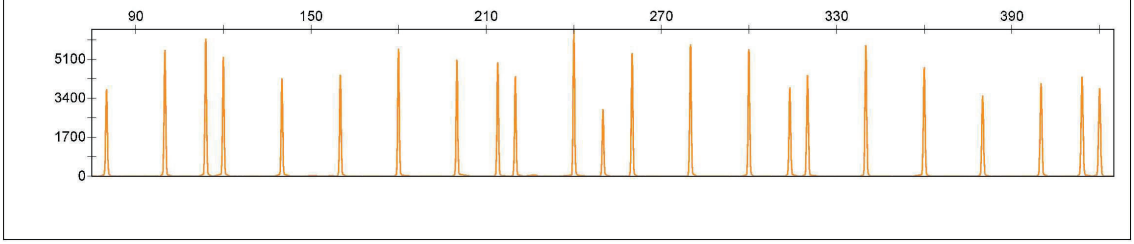
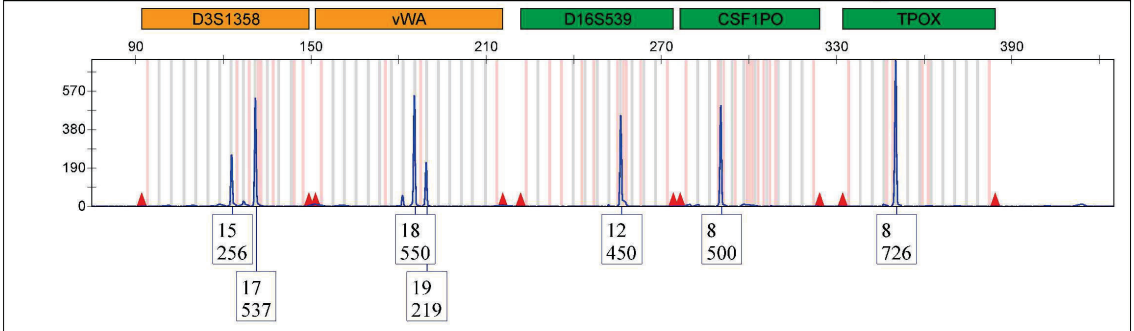


Applied Biosystems
GeneMapper® ID-X 1.4

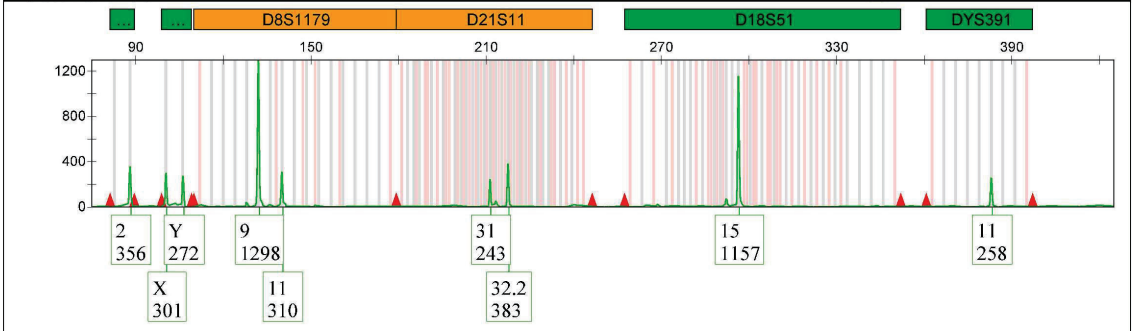
Project: Organic Extractions CRG 061422

Sample Name	SQ

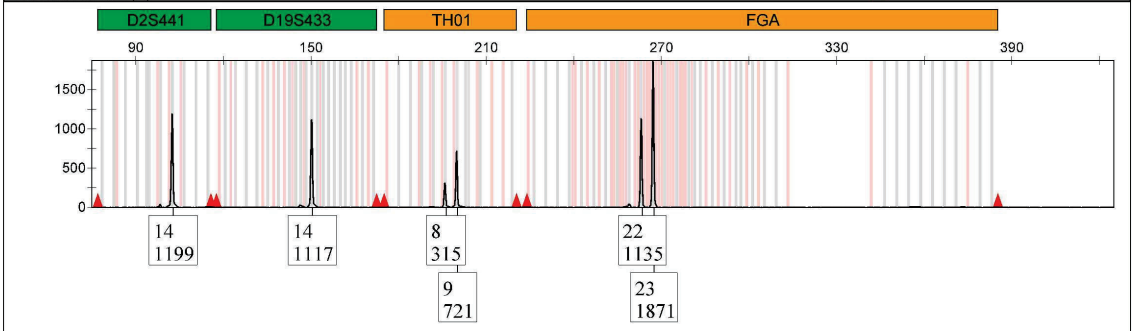
1 to 500 Human (2)



1 to 500 Human (2)



1 to 500 Human (2)

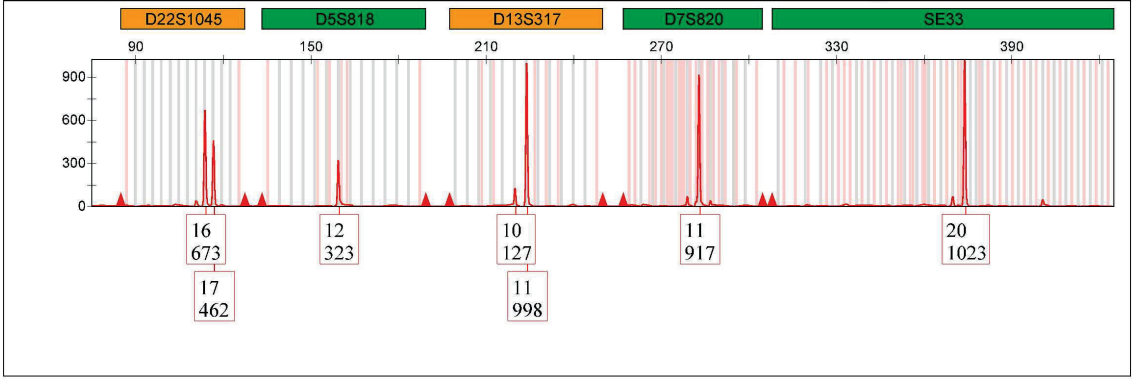


Applied Biosystems
GeneMapper® ID-X 1.4

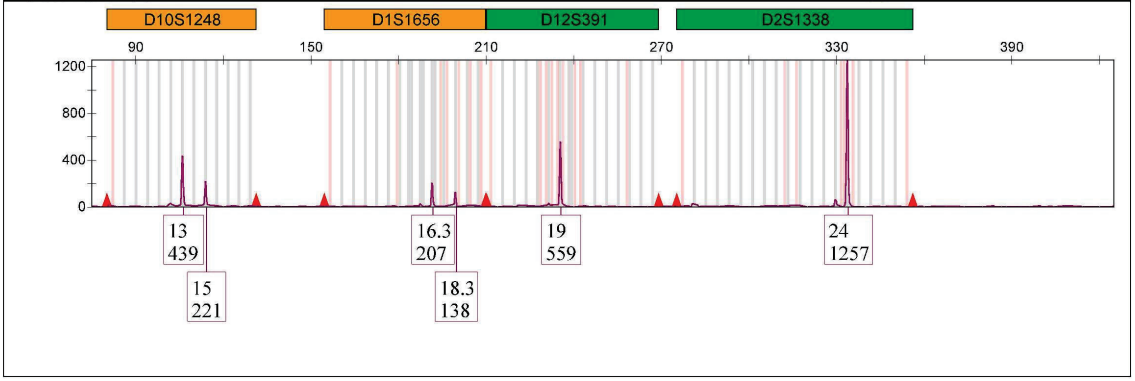
Project: Organic Extractions CRG 061422

Sample Name	SQ

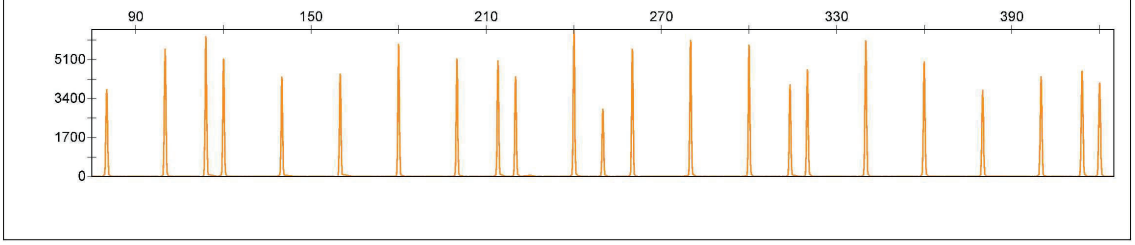
1 to 500 Human (2)



1 to 500 Human (2)



1 to 500 Human (2)

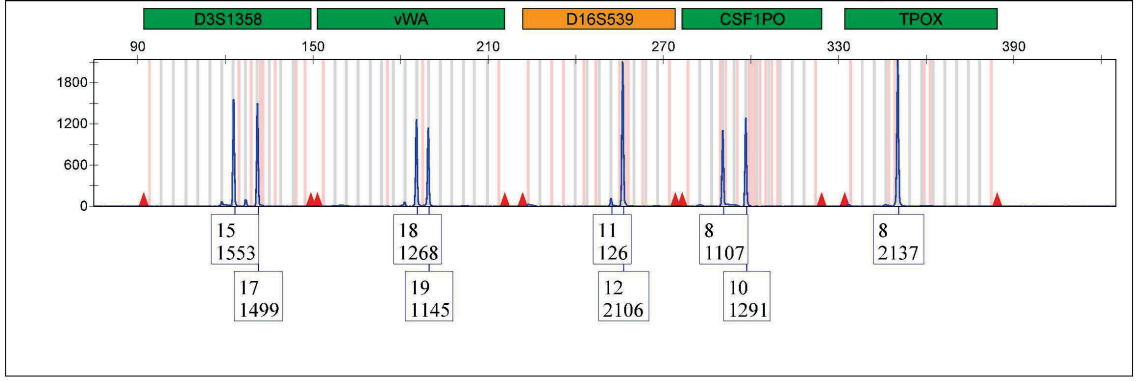


Applied Biosystems
GeneMapper® ID-X 1.4

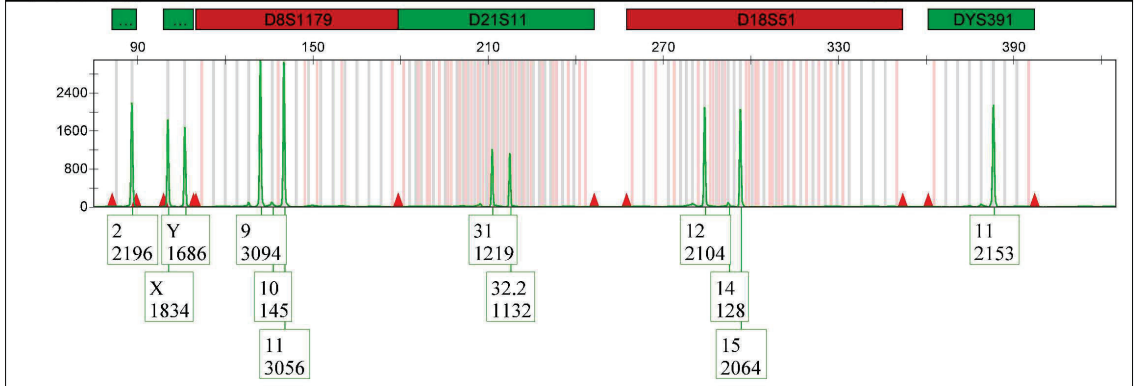
Project: Organic Extractions CRG 061422

Sample Name SQ

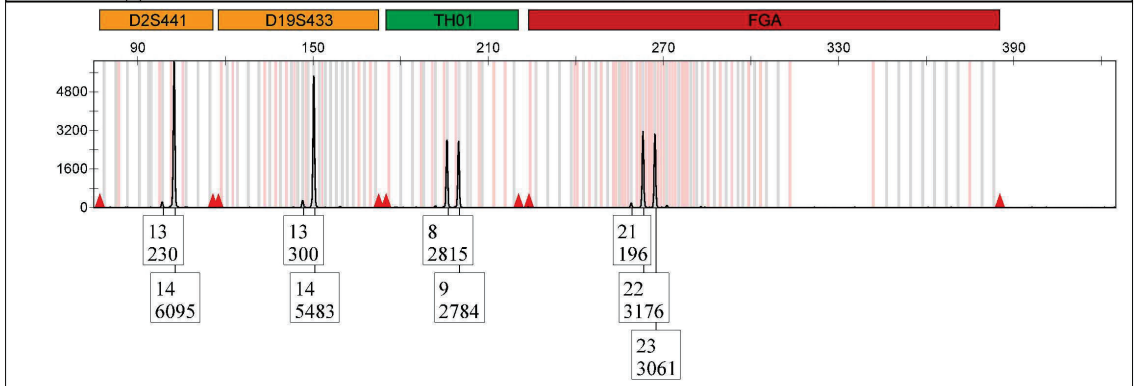
1 to 10 Human (3)



1 to 10 Human (3)



1 to 10 Human (3)

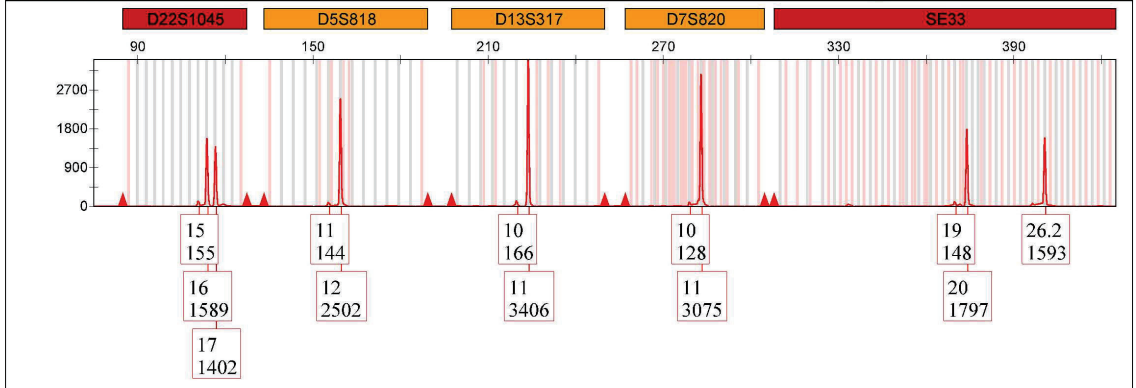


Applied Biosystems
GeneMapper® ID-X 1.4

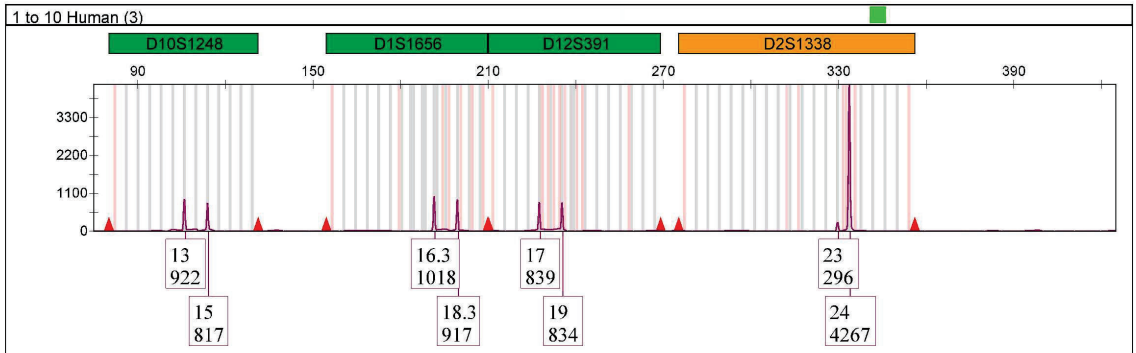
Project: Organic Extractions CRG 061422

Sample Name	SQ

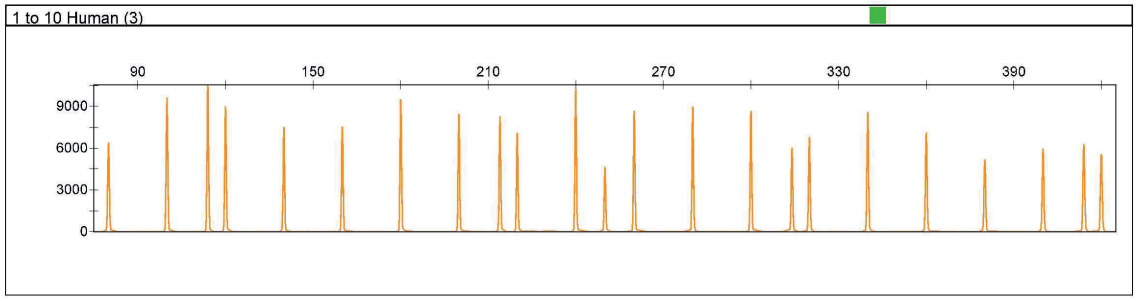
1 to 10 Human (3)



1 to 10 Human (3)



1 to 10 Human (3)

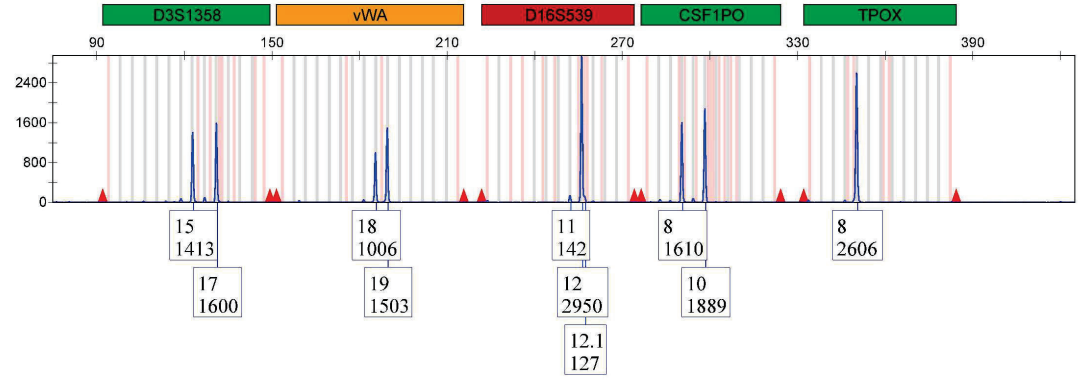


Applied Biosystems
GeneMapper® ID-X 1.4

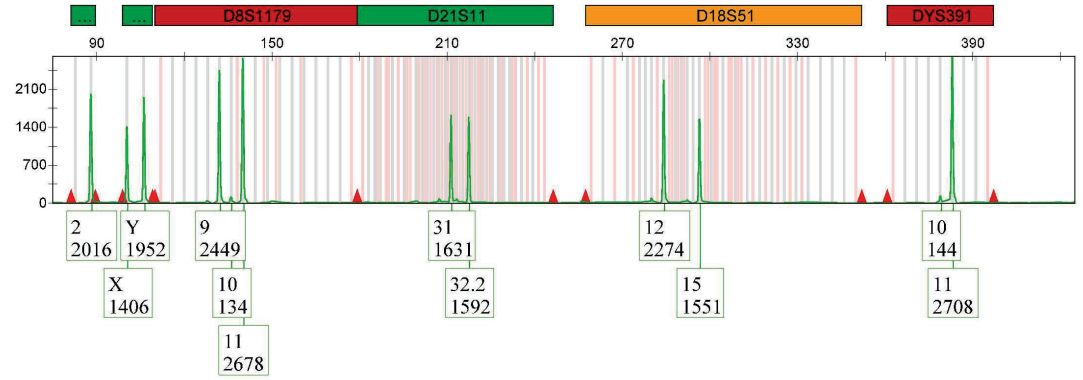
Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 30 Human (3)



1 to 30 Human (3)

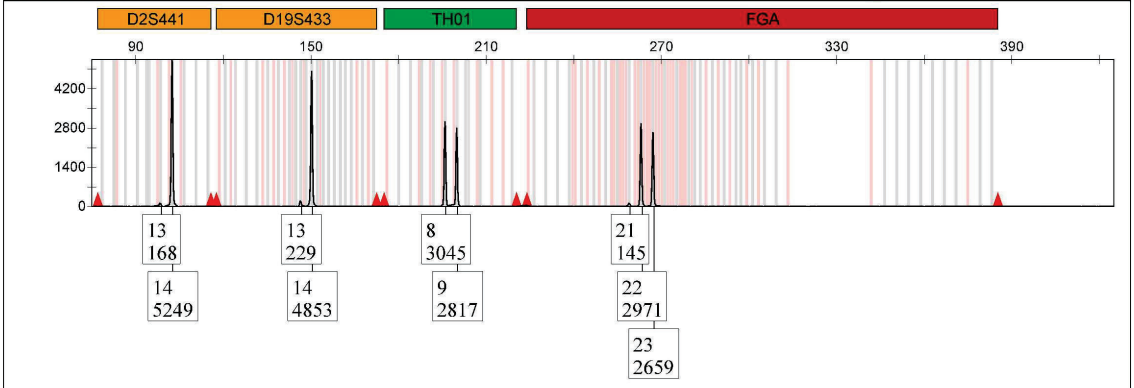


Applied Biosystems
GeneMapper® ID-X 1.4

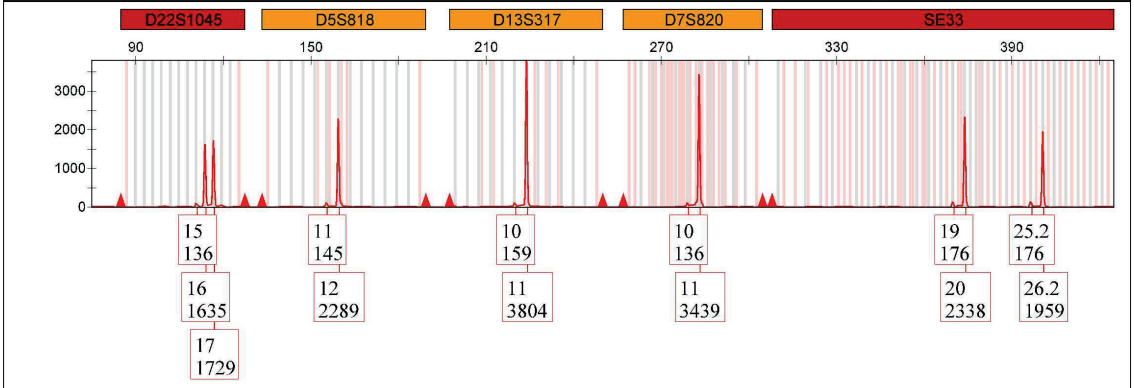
Project: Organic Extractions CRG 061422

Sample Name	SQ

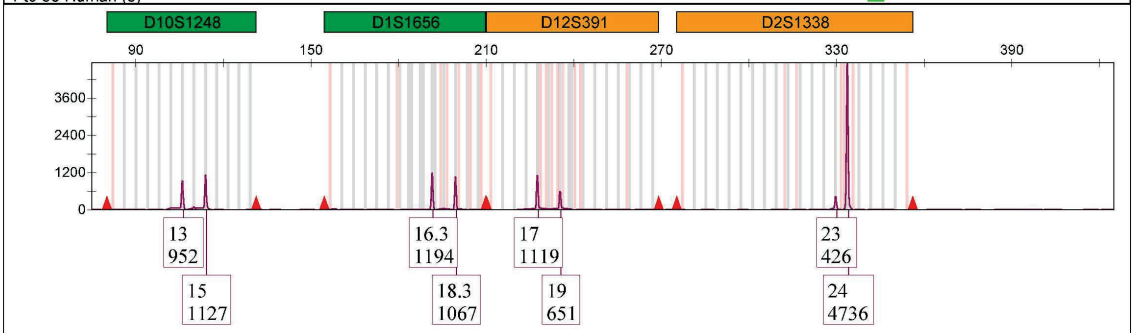
1 to 30 Human (3)



1 to 30 Human (3)



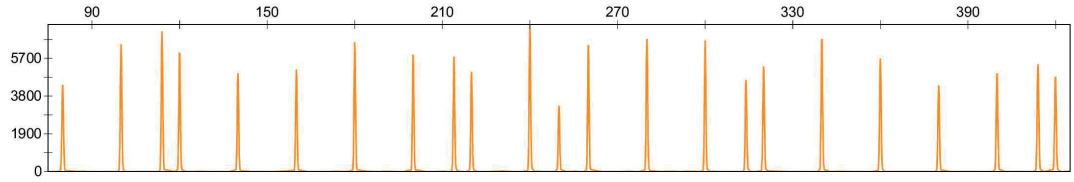
1 to 30 Human (3)



Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 30 Human (3)	

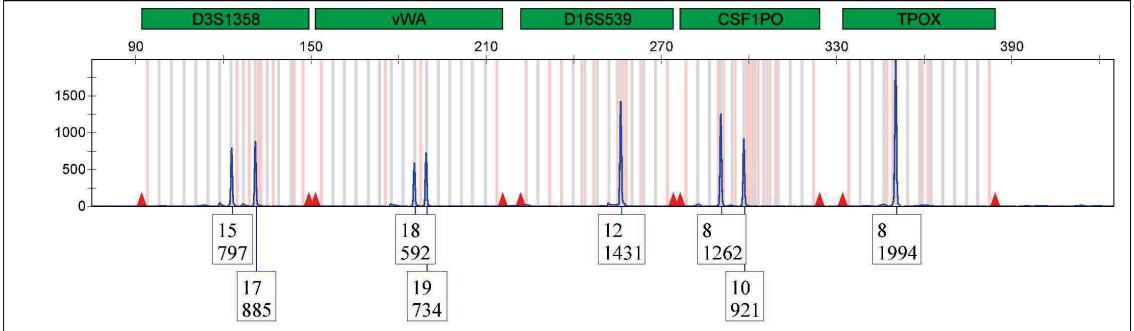


Applied Biosystems
GeneMapper® ID-X 1.4

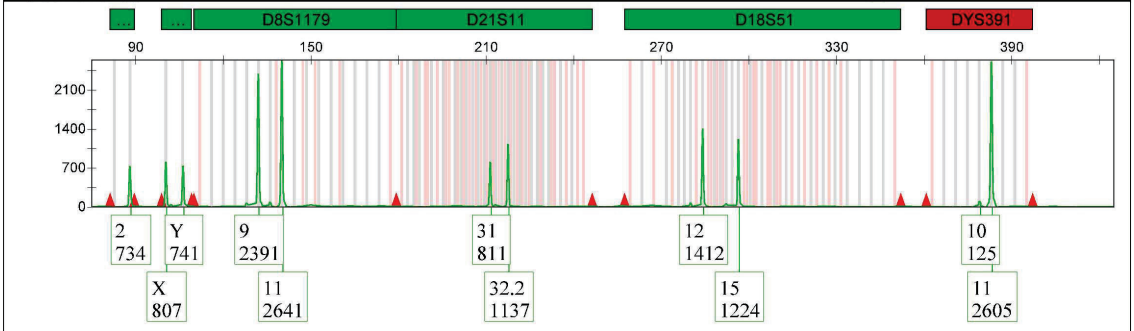
Project: Organic Extractions CRG 061422

Sample Name	SQ

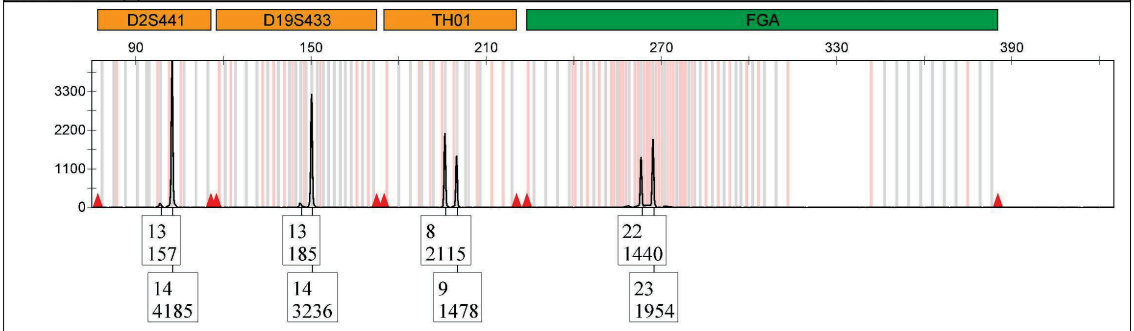
1 to 50 Human (3)



1 to 50 Human (3)



1 to 50 Human (3)

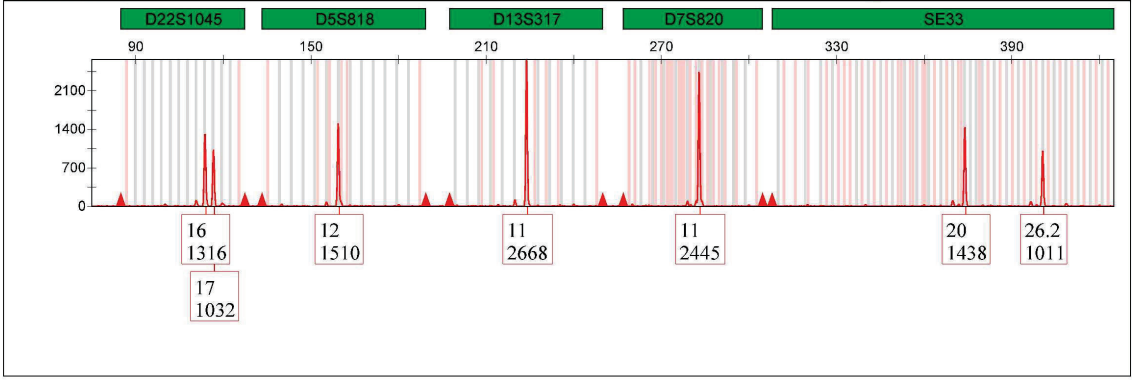


Applied Biosystems
GeneMapper® ID-X 1.4

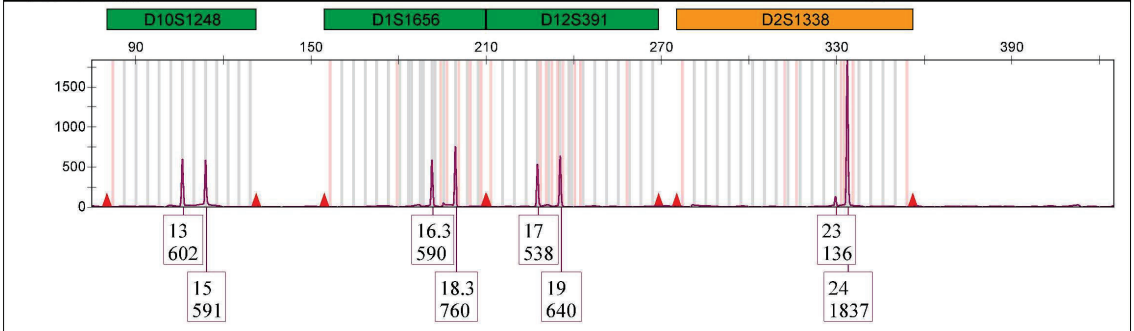
Project: Organic Extractions CRG 061422

Sample Name	SQ

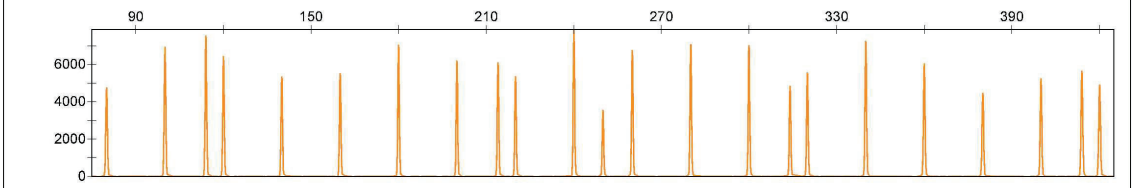
1 to 50 Human (3)



1 to 50 Human (3)



1 to 50 Human (3)

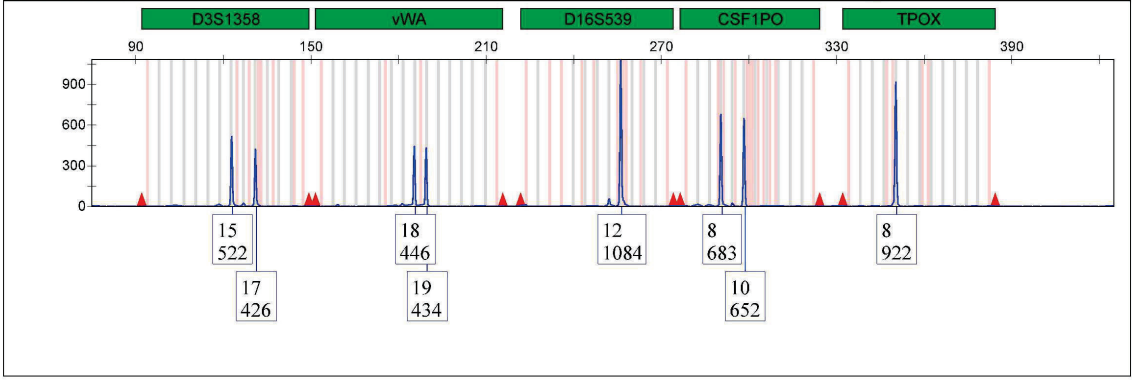


Applied Biosystems
GeneMapper® ID-X 1.4

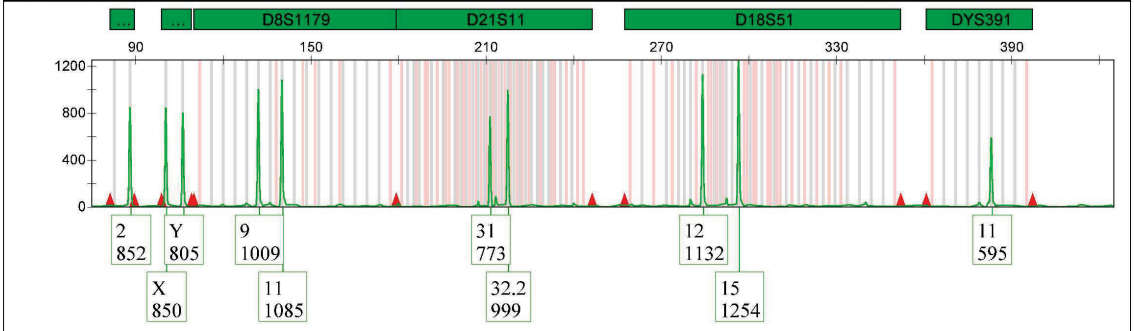
Project: Organic Extractions CRG 061422

Sample Name	SQ

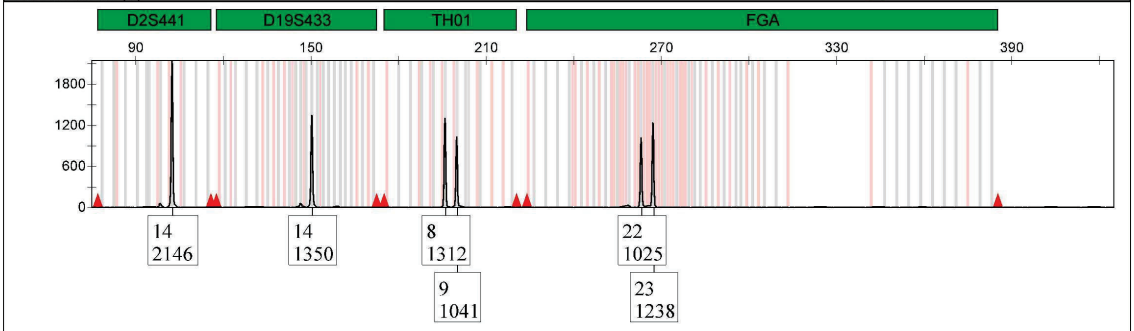
1 to 75 Human (3)



1 to 75 Human (3)



1 to 75 Human (3)

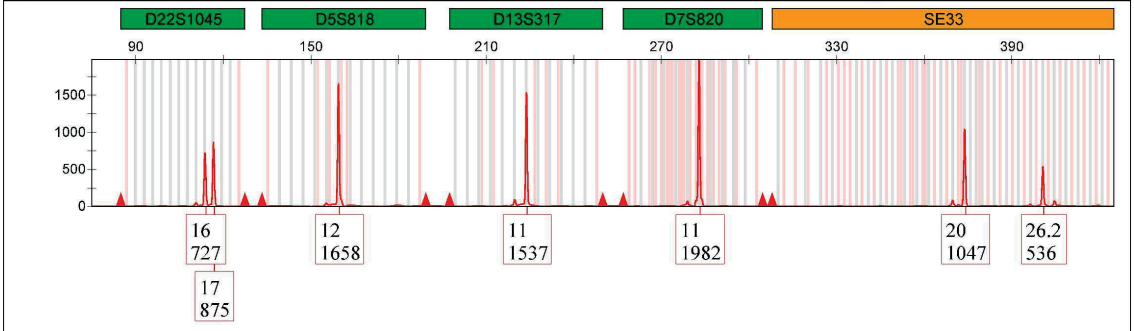


Applied Biosystems
GeneMapper® ID-X 1.4

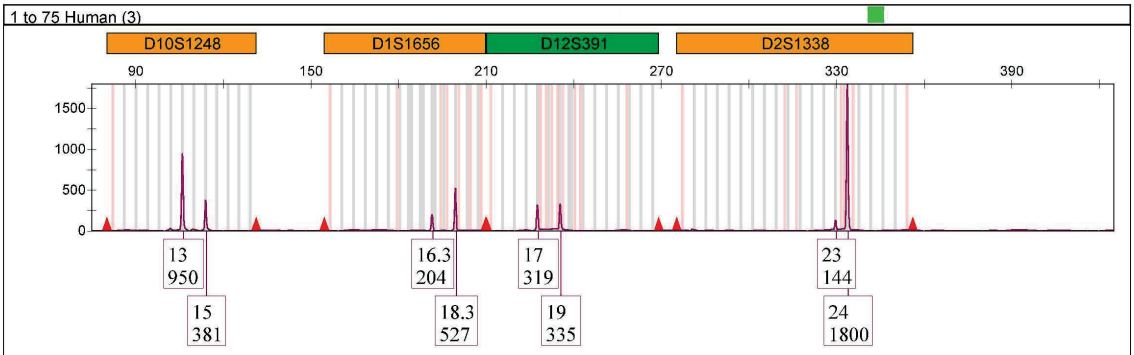
Project: Organic Extractions CRG 061422

Sample Name	SQ

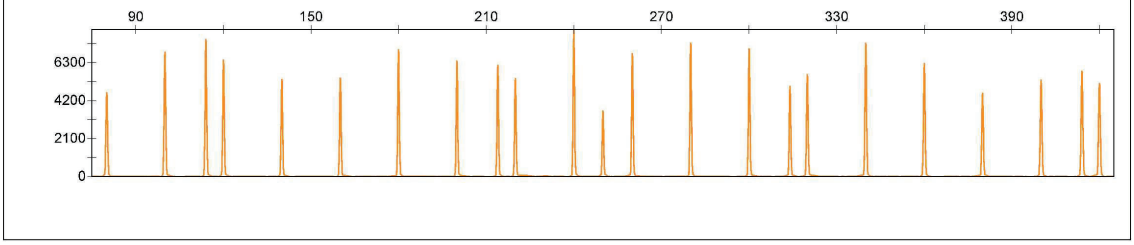
1 to 75 Human (3)



1 to 75 Human (3)



1 to 75 Human (3)

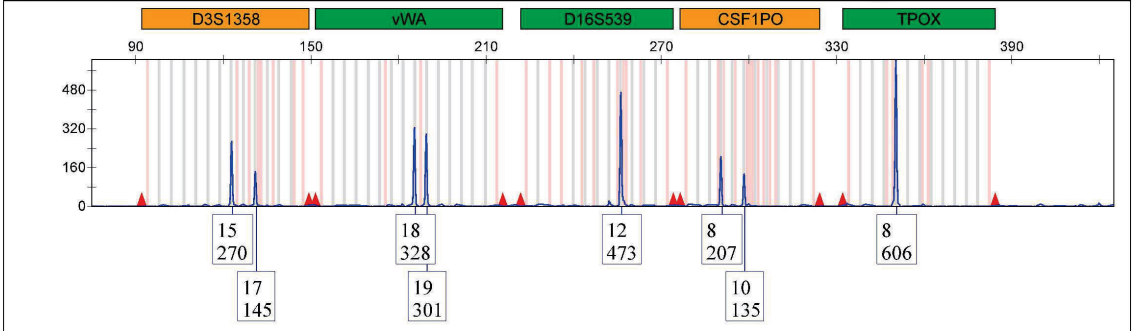


Applied Biosystems
GeneMapper® ID-X 1.4

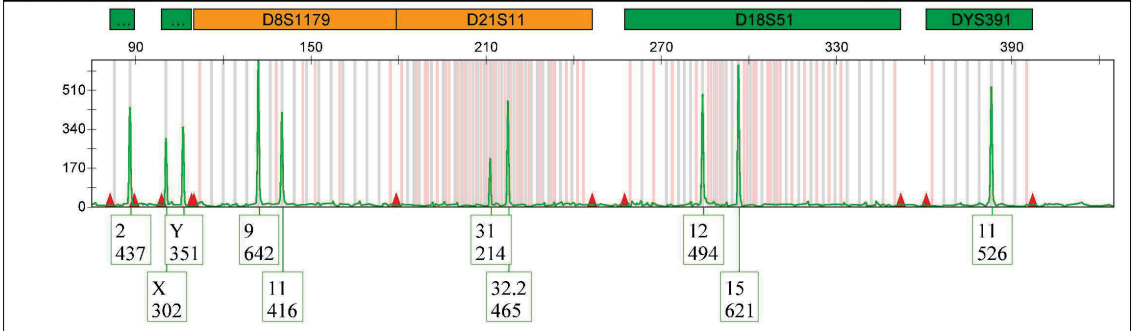
Project: Organic Extractions CRG 061422

Sample Name	SQ

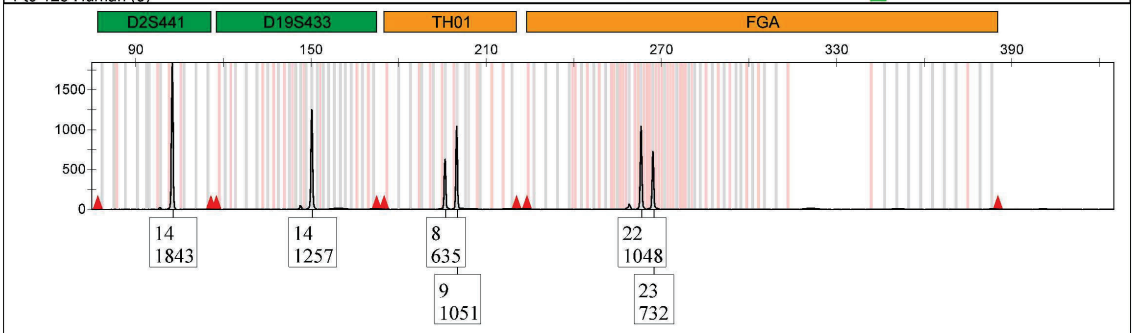
1 to 125 Human (3)



1 to 125 Human (3)



1 to 125 Human (3)

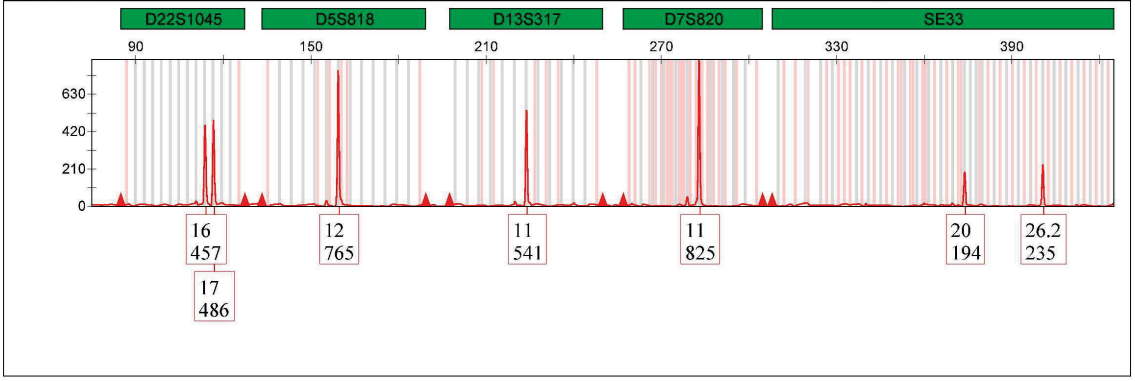


Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name SQ

1 to 125 Human (3)



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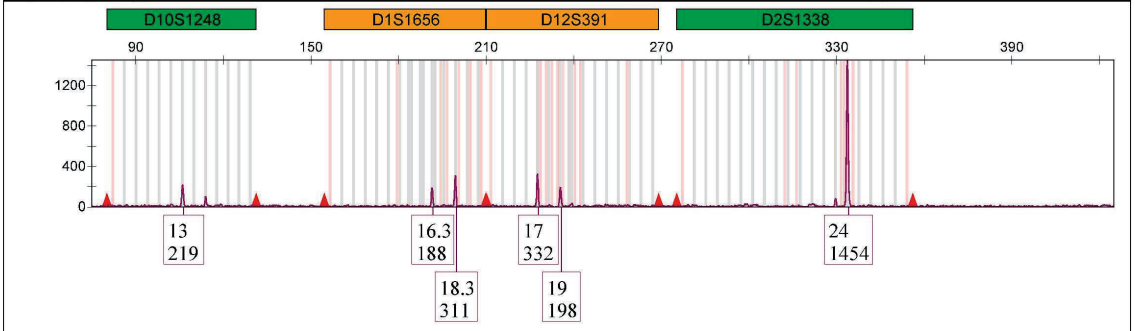
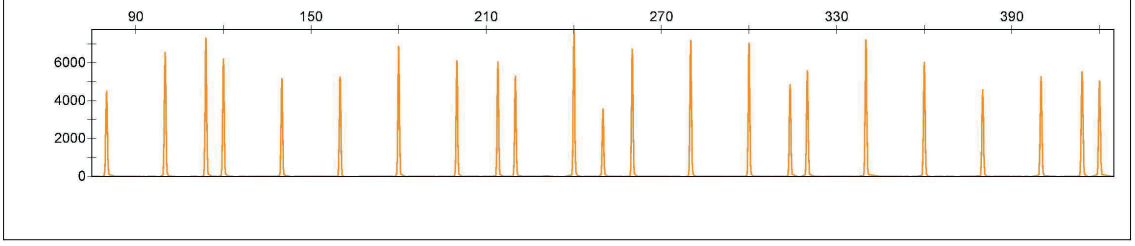


1 to 125 Human (3)

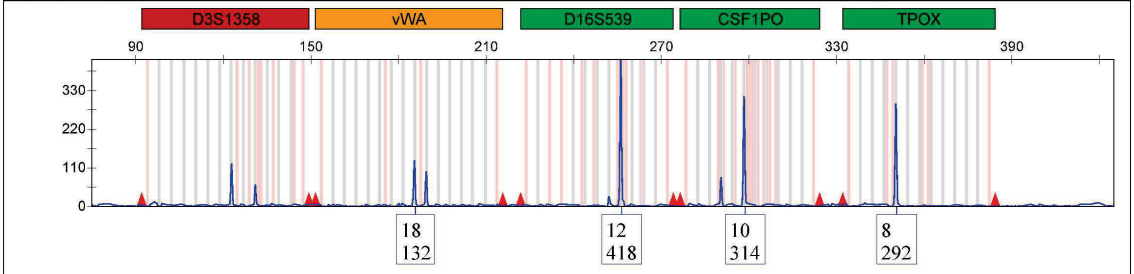


Applied Biosystems
GeneMapper® ID-X 1.4

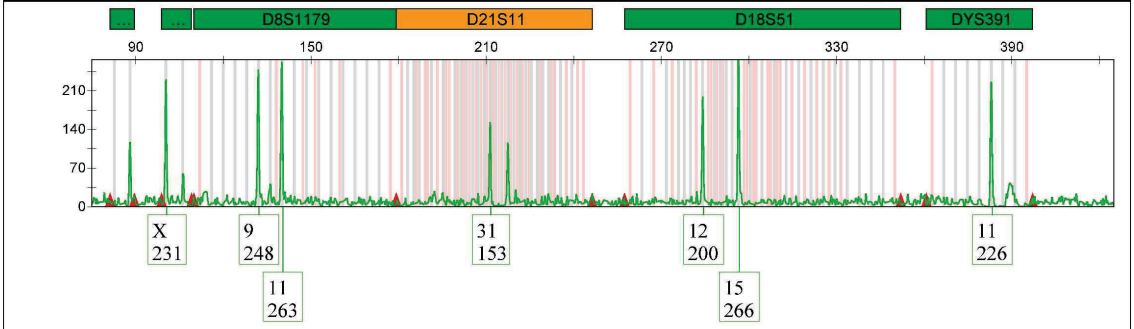
Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 175 Human (3)



1 to 175 Human (3)



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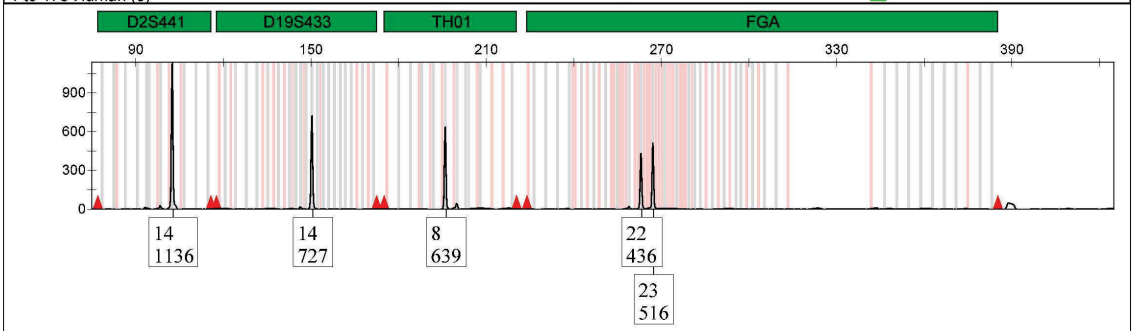
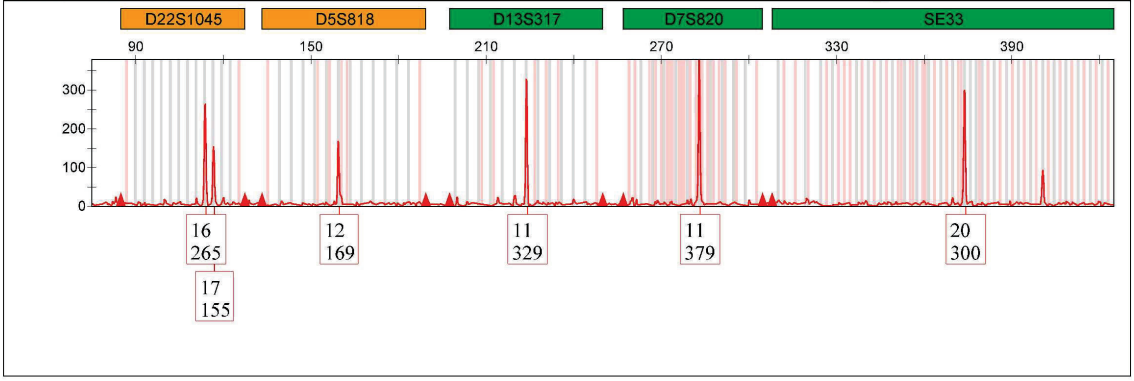


Applied Biosystems
GeneMapper® ID-X 1.4

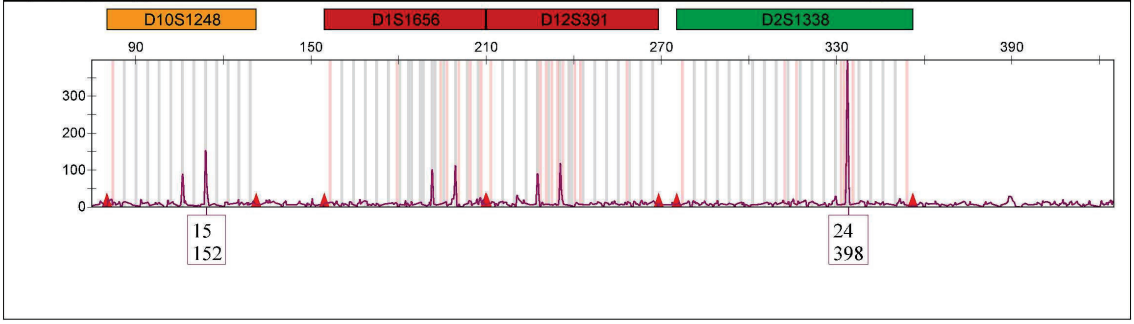
Project: Organic Extractions CRG 061422

Sample Name	SQ

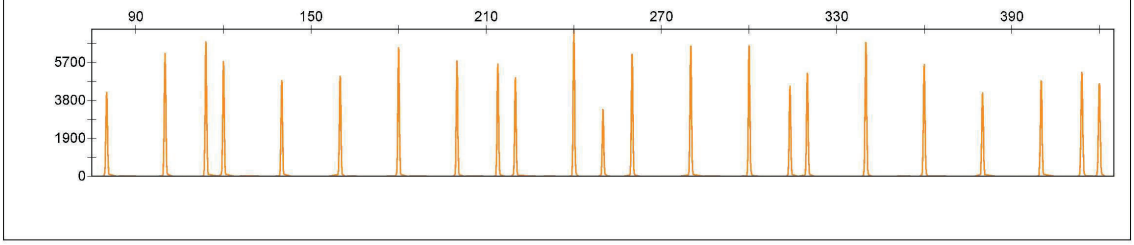
1 to 175 Human (3)



1 to 175 Human (3)



1 to 175 Human (3)

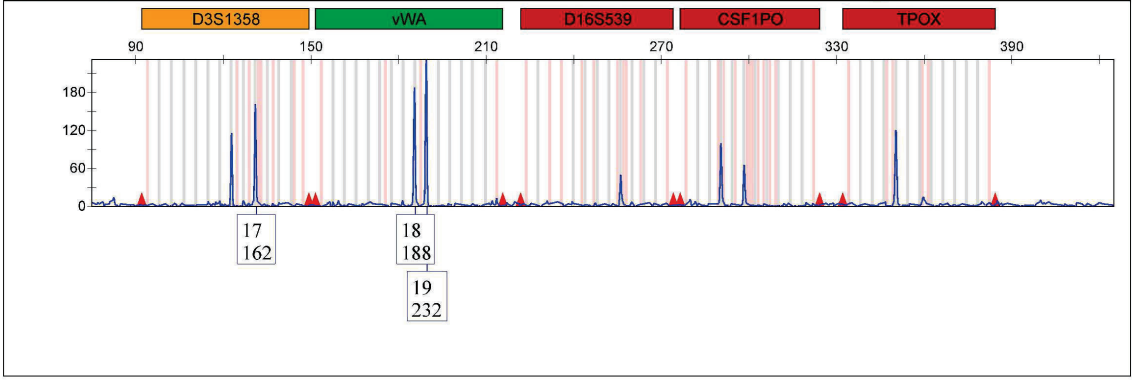


Applied Biosystems
GeneMapper® ID-X 1.4

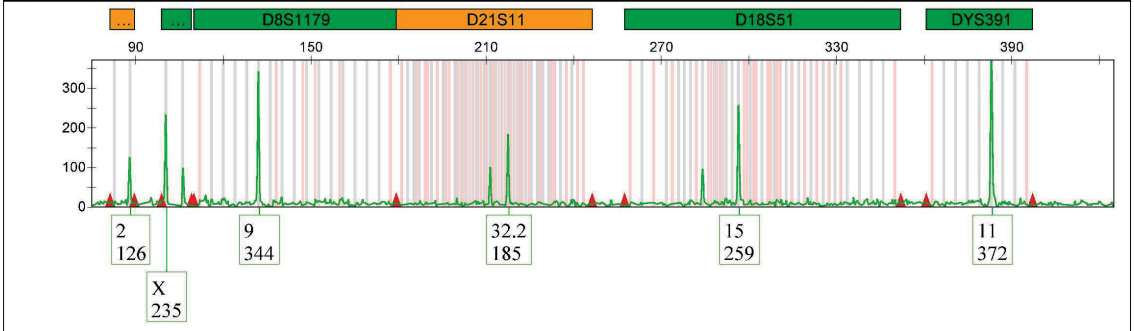
Project: Organic Extractions CRG 061422

Sample Name	SQ

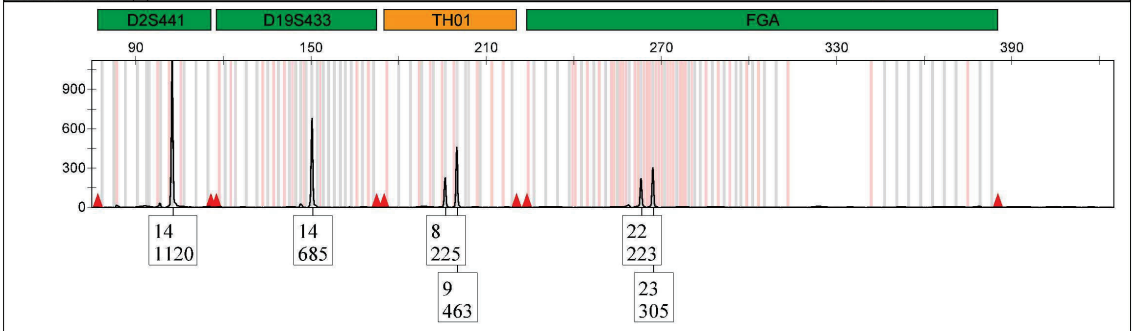
1 to 250 Human (3)



1 to 250 Human (3)



1 to 250 Human (3)

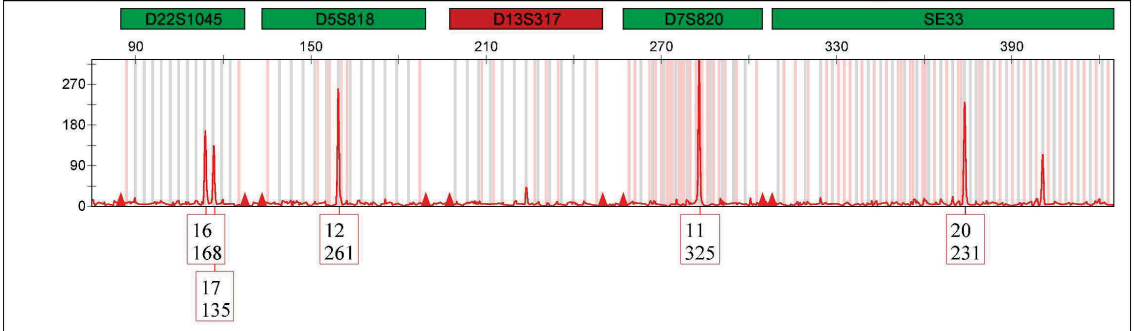


Applied Biosystems
GeneMapper® ID-X 1.4

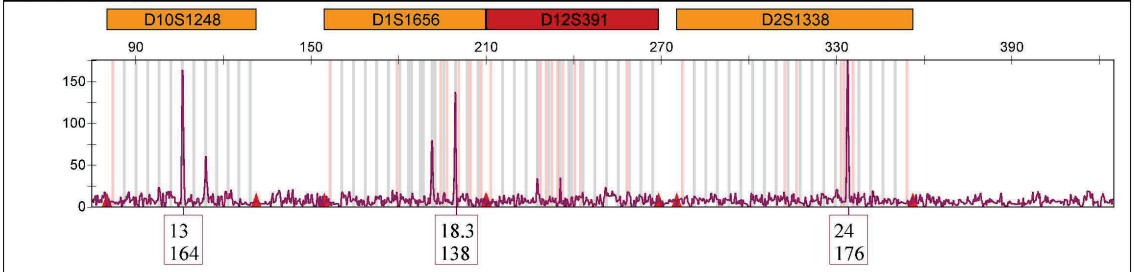
Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 250 Human (3)



1 to 250 Human (3)



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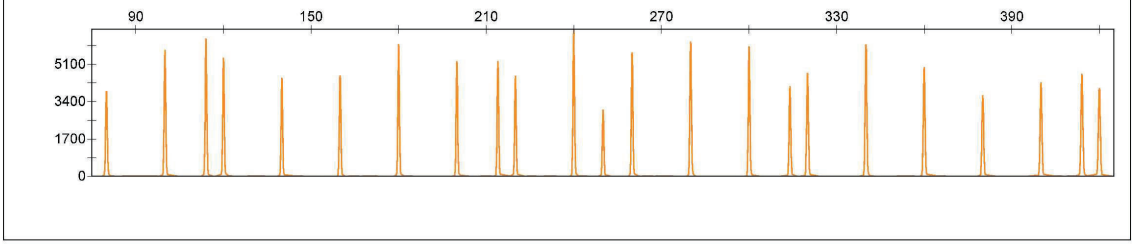
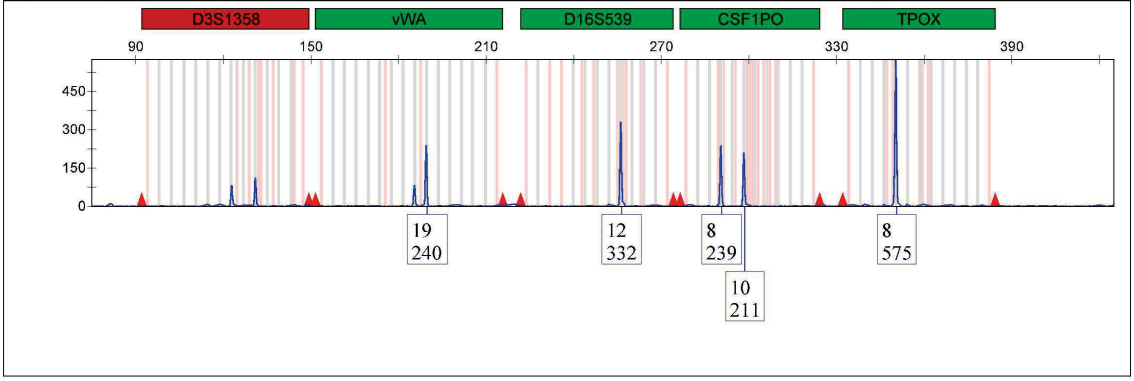


Applied Biosystems
GeneMapper® ID-X 1.4

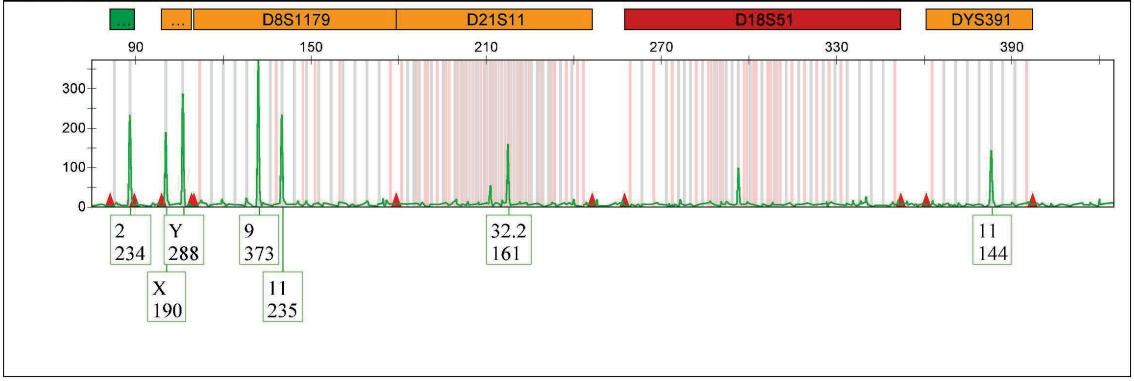
Project: Organic Extractions CRG 061422

Sample Name	SQ

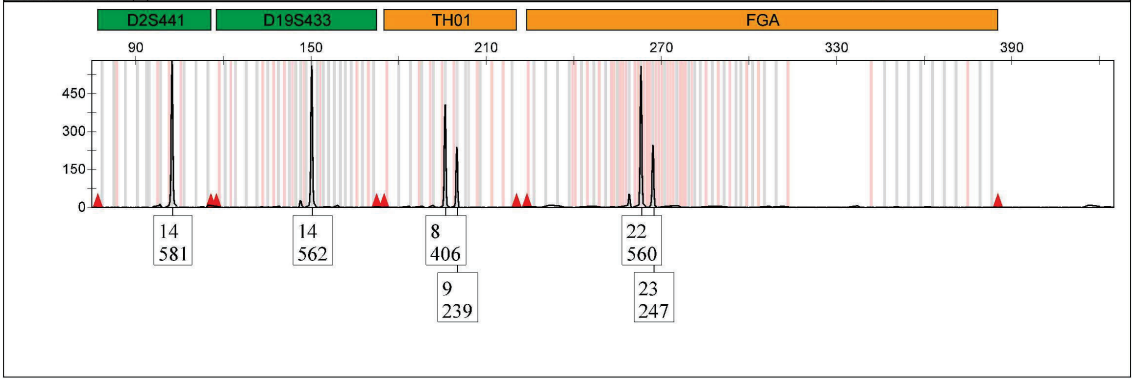
1 to 500 Human (3)



1 to 500 Human (3)



1 to 500 Human (3)

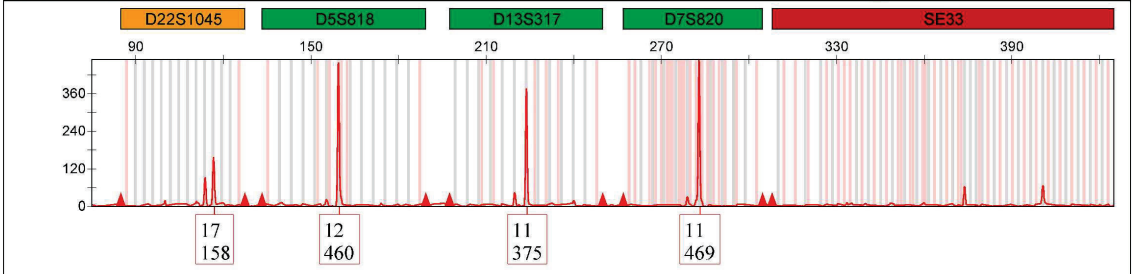


Applied Biosystems
GeneMapper® ID-X 1.4

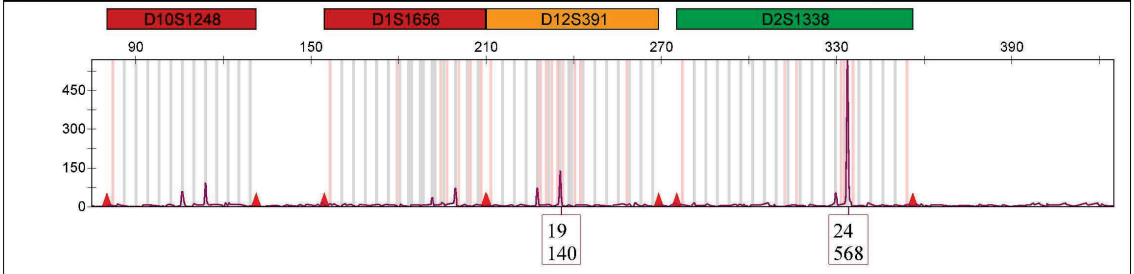
Project: Organic Extractions CRG 061422

Sample Name SQ

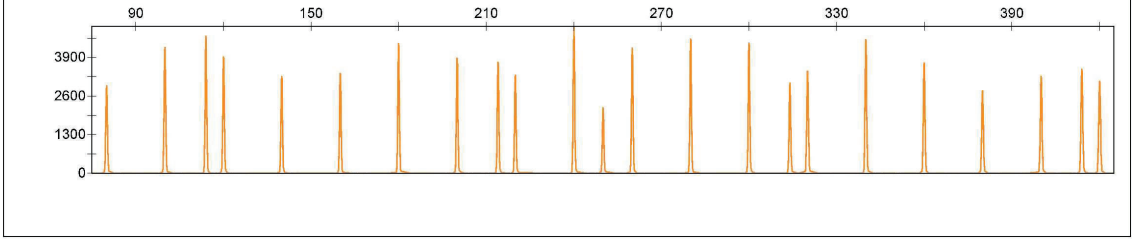
1 to 500 Human (3)



1 to 500 Human (3)



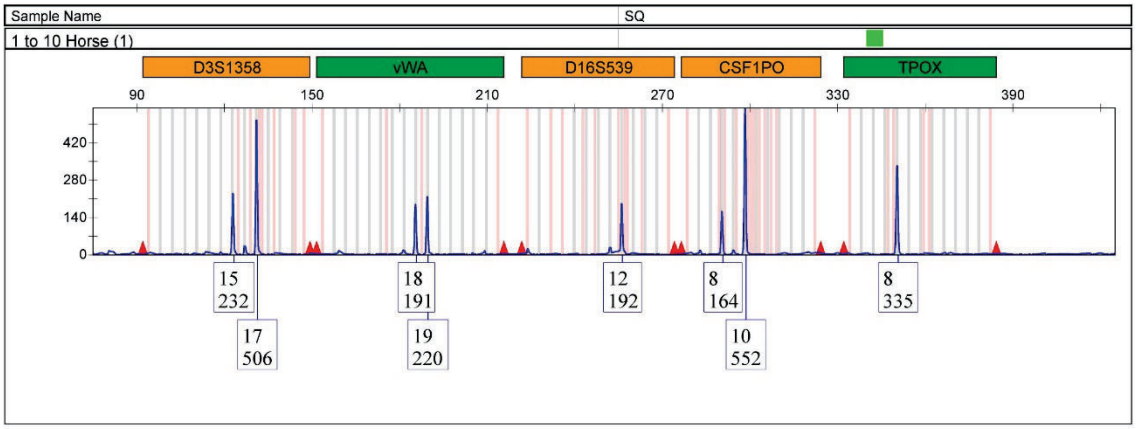
1 to 500 Human (3)

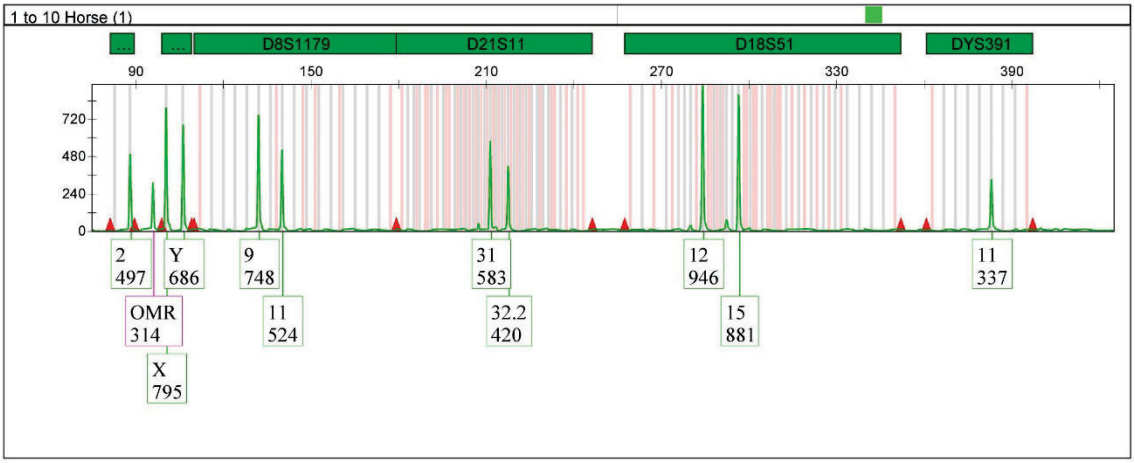


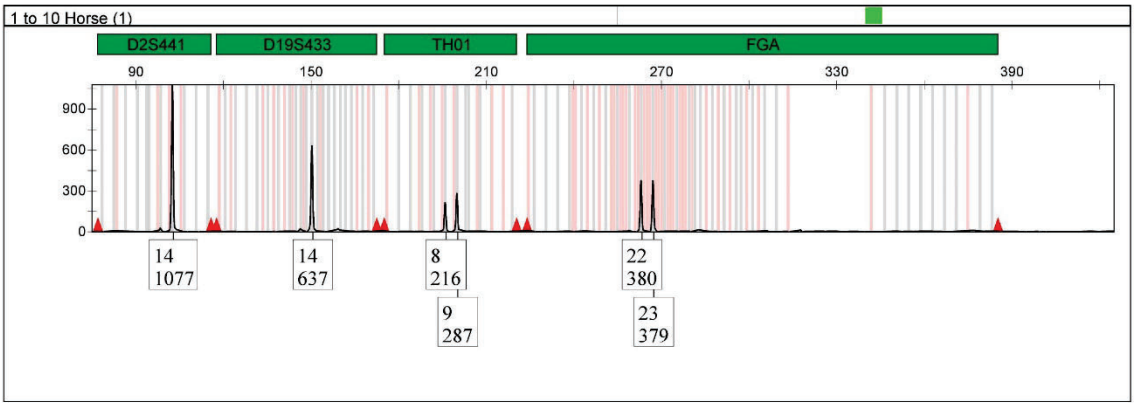
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 10 Horse (1)	





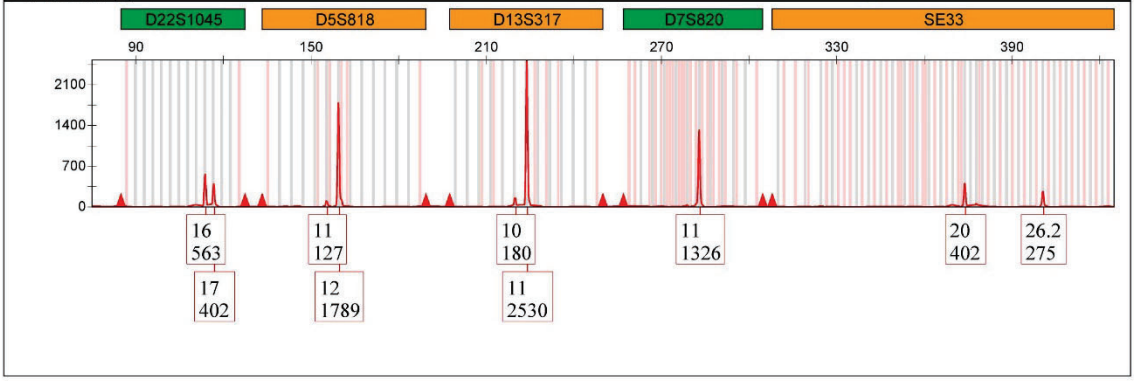


Applied Biosystems
GeneMapper® ID-X 1.4

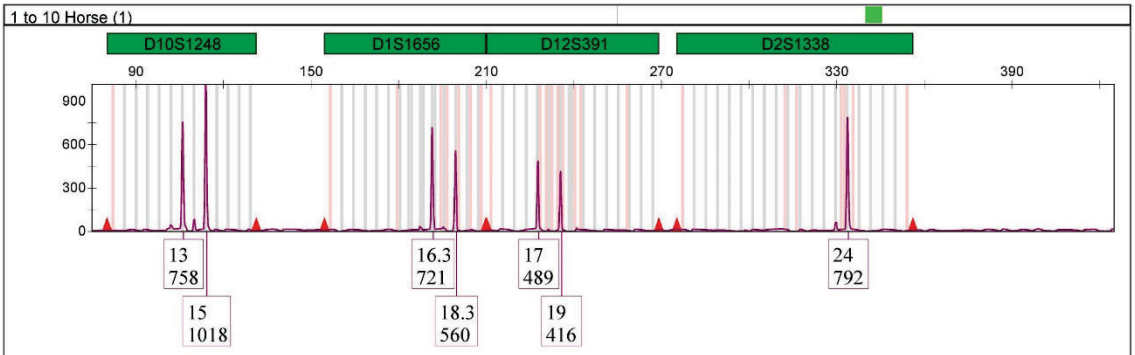
Project: Organic Extractions CRG 061422

Sample Name	SQ

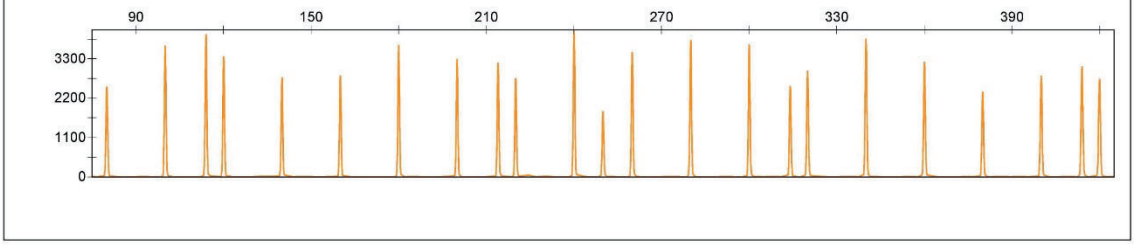
1 to 10 Horse (1)



1 to 10 Horse (1)



1 to 10 Horse (1)

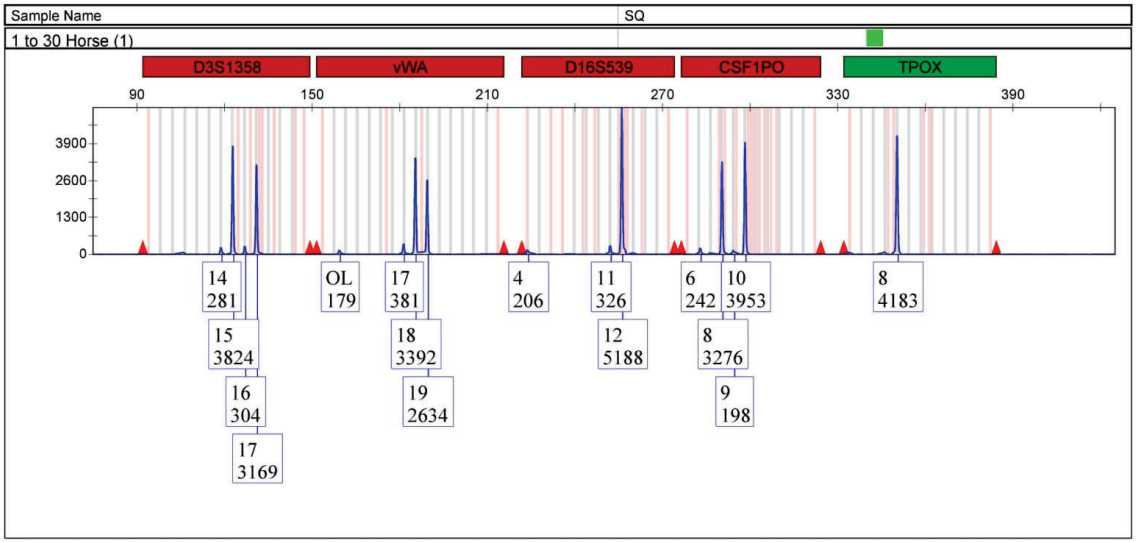


Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 30 Horse (1)

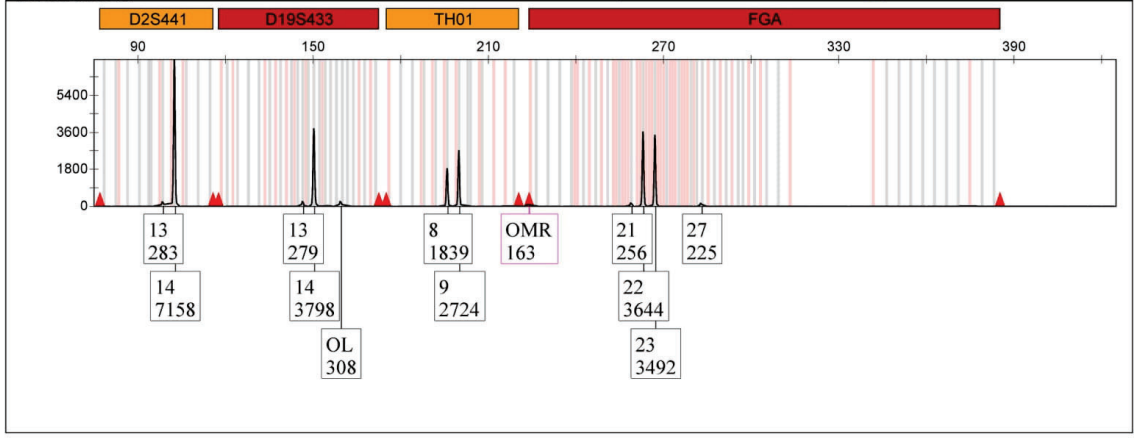


1 to 30 Horse (1)

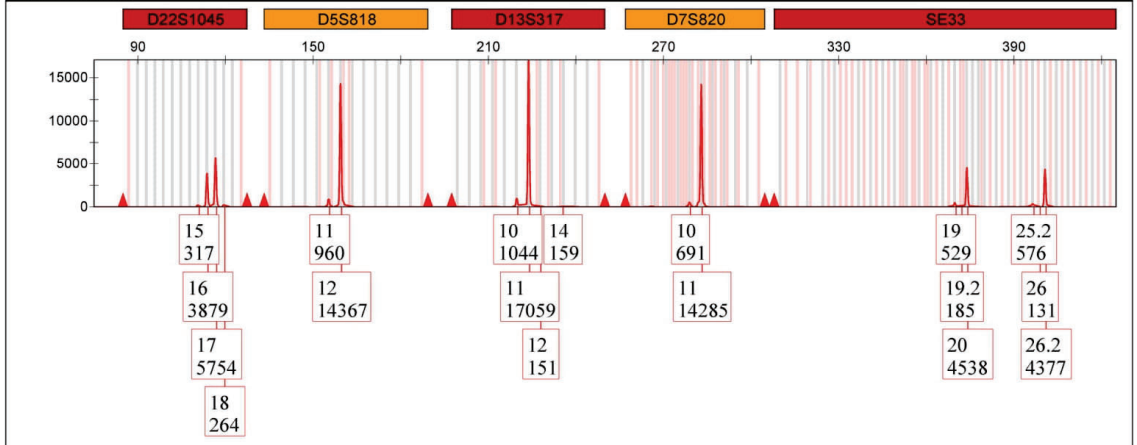
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 30 Horse (1)	



1 to 30 Horse (1)

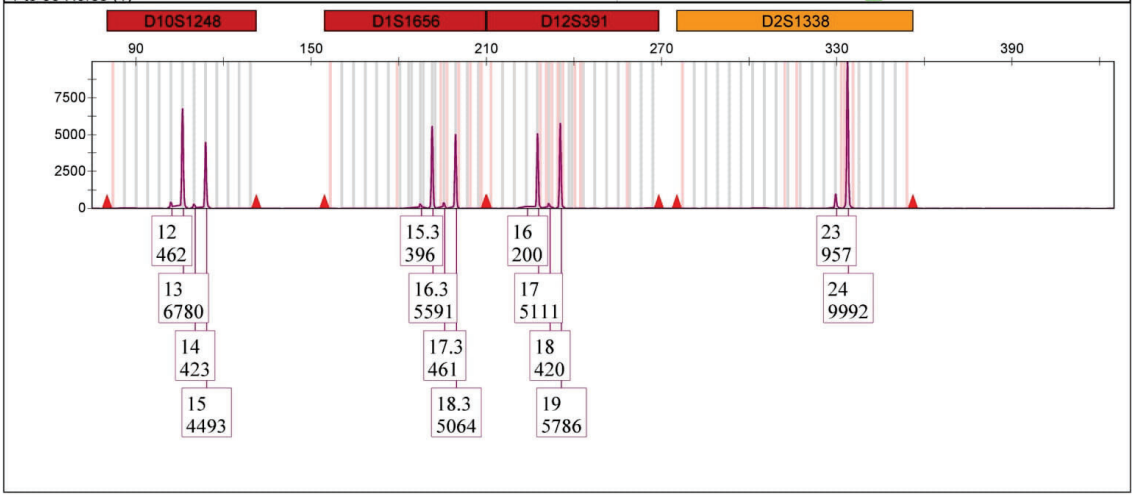


Applied Biosystems
GeneMapper® ID-X 1.4

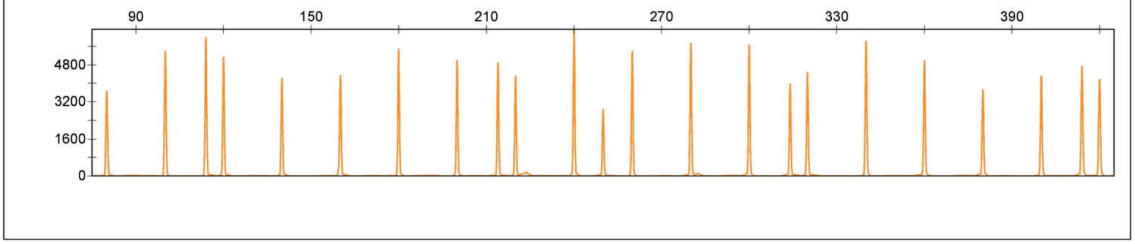
Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 30 Horse (1)



1 to 30 Horse (1)

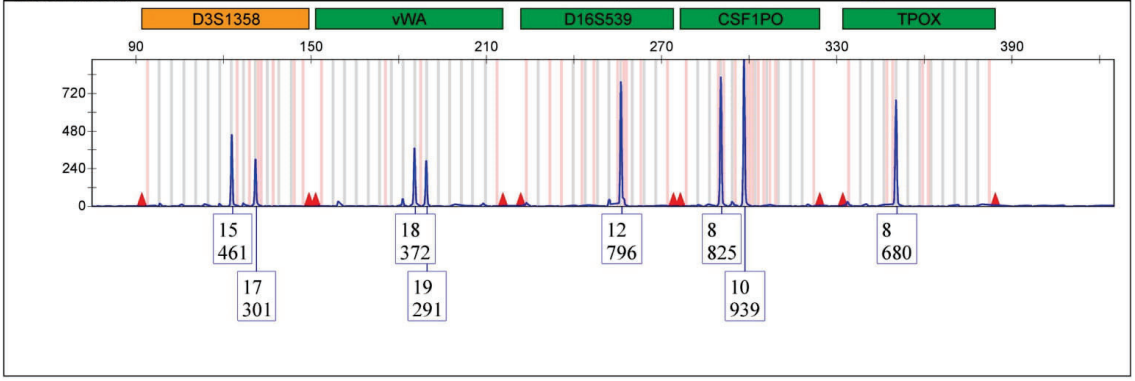


Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

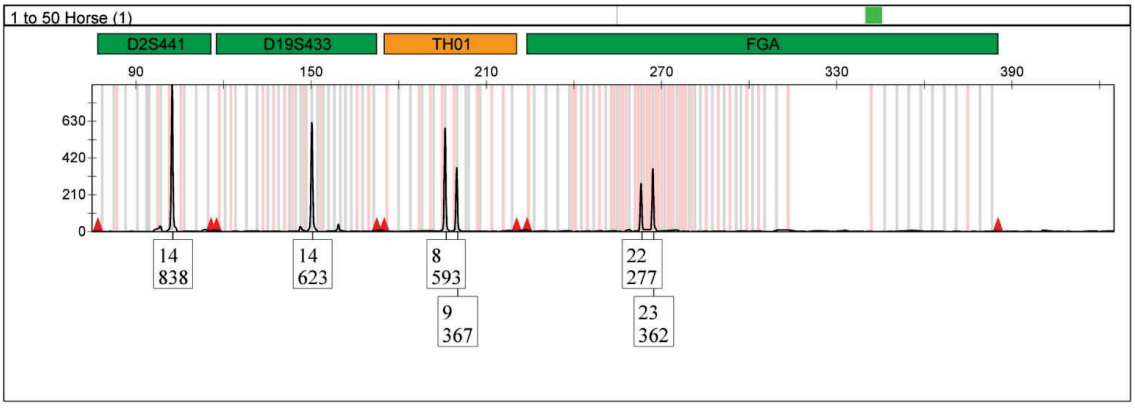
Sample Name	SQ

1 to 50 Horse (1)



1 to 50 Horse (1)

1 to 50 Horse (1)

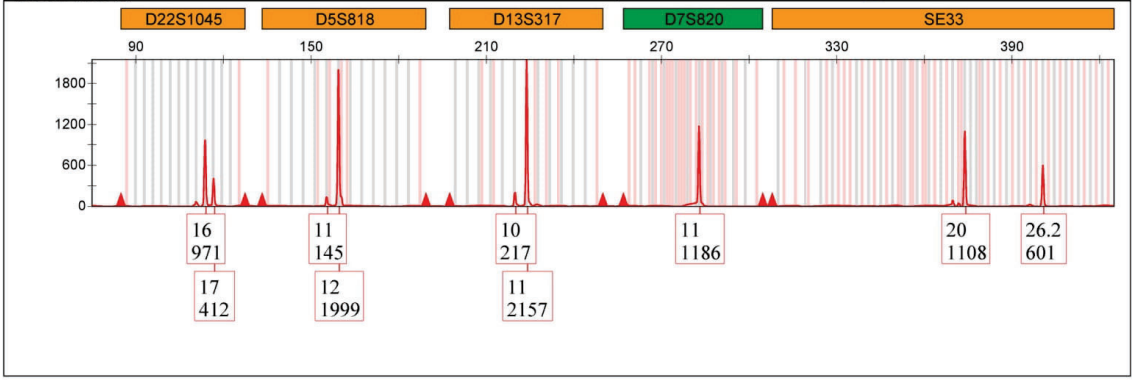


Applied Biosystems
GeneMapper® ID-X 1.4

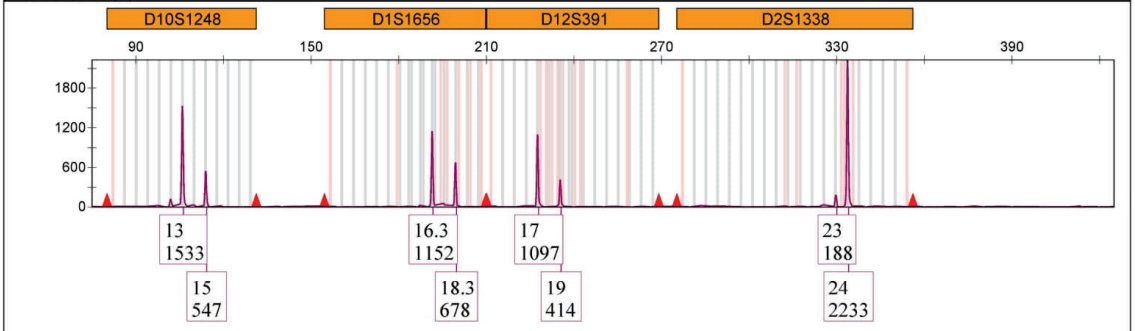
Project: Organic Extractions CRG 061422

Sample Name	SQ

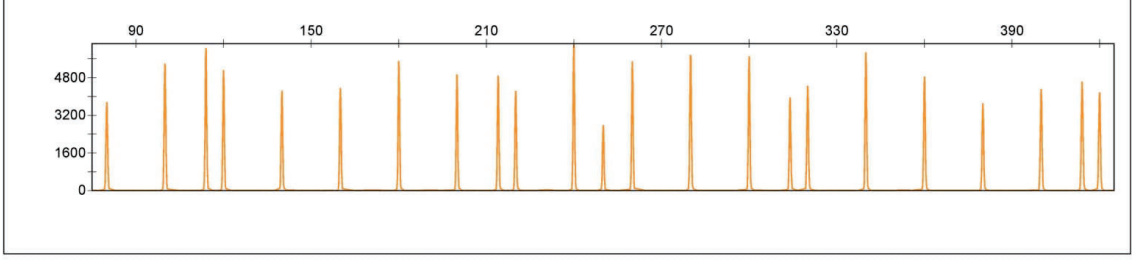
1 to 50 Horse (1)



1 to 50 Horse (1)



1 to 50 Horse (1)

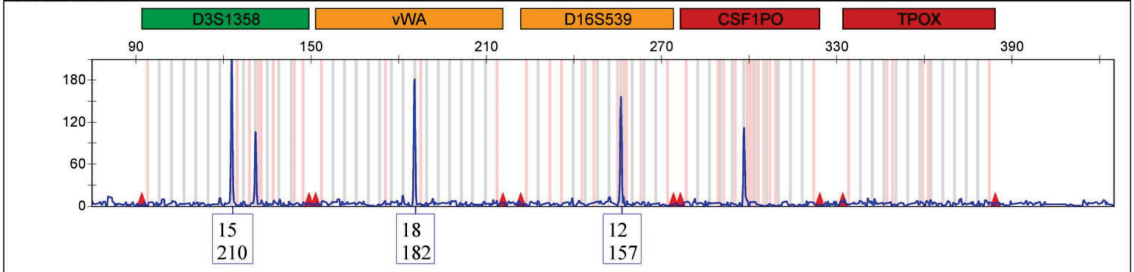


Applied Biosystems
GeneMapper® ID-X 1.4

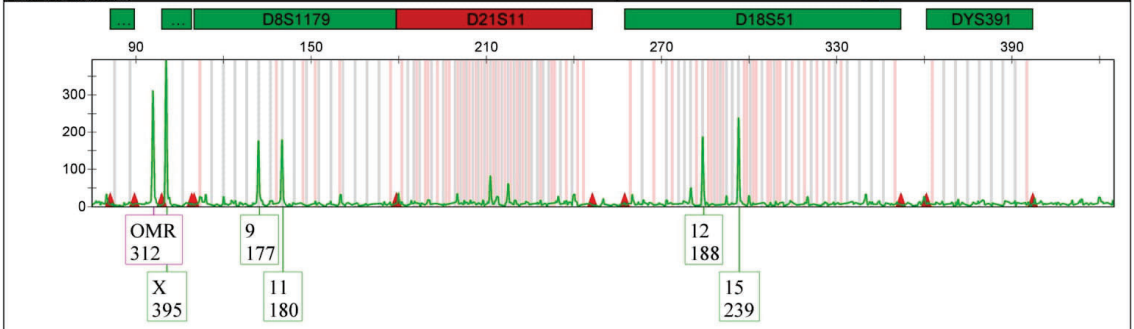
Project: Organic Extractions CRG 061422

Sample Name	SQ

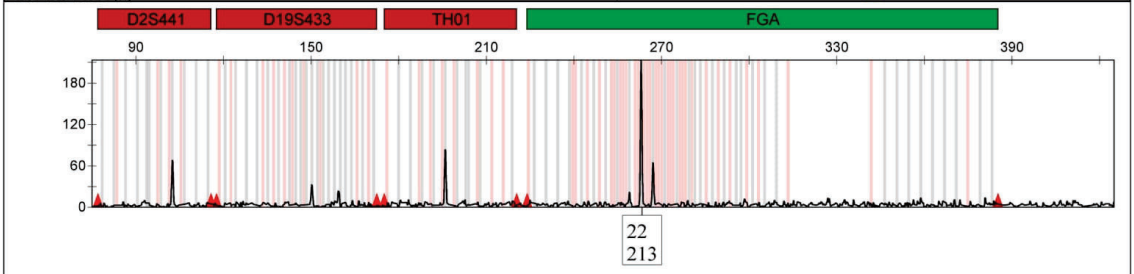
1 to 75 Horse (1)



1 to 75 Horse (1)



1 to 75 Horse (1)

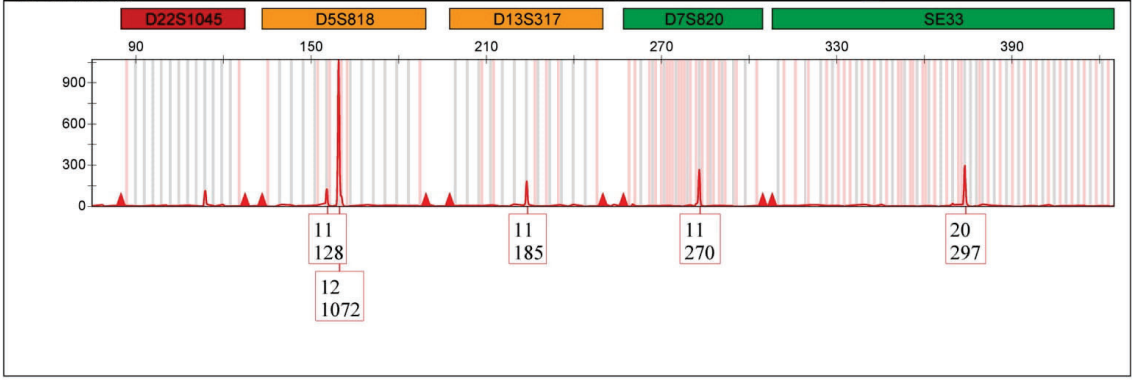


Applied Biosystems
GeneMapper® ID-X 1.4

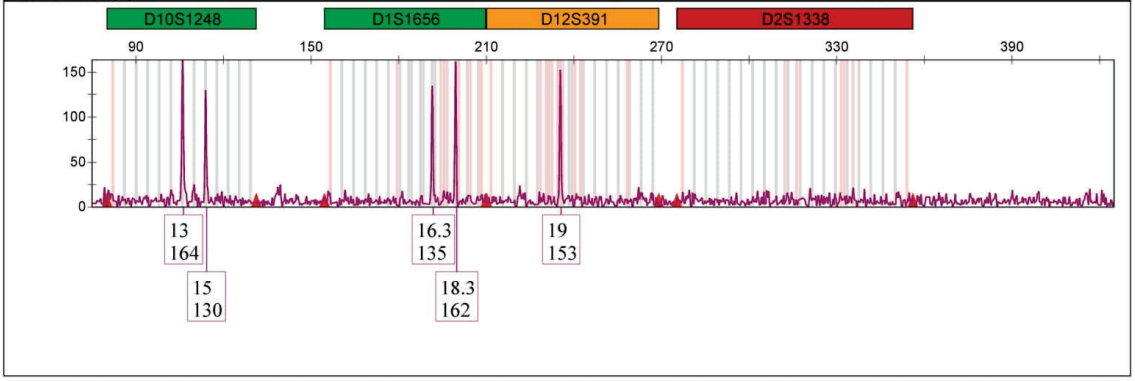
Project: Organic Extractions CRG 061422

Sample Name	SQ

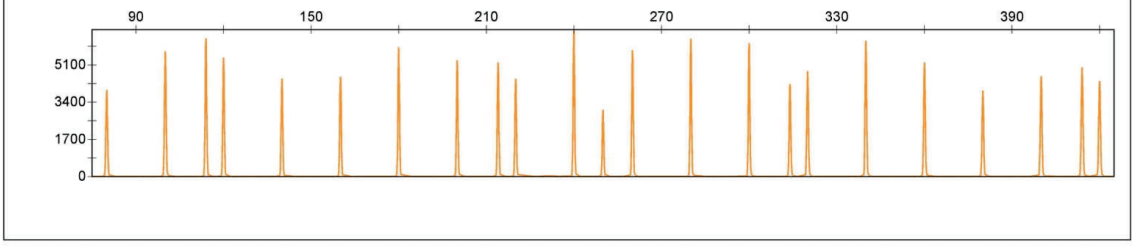
1 to 75 Horse (1)



1 to 75 Horse (1)



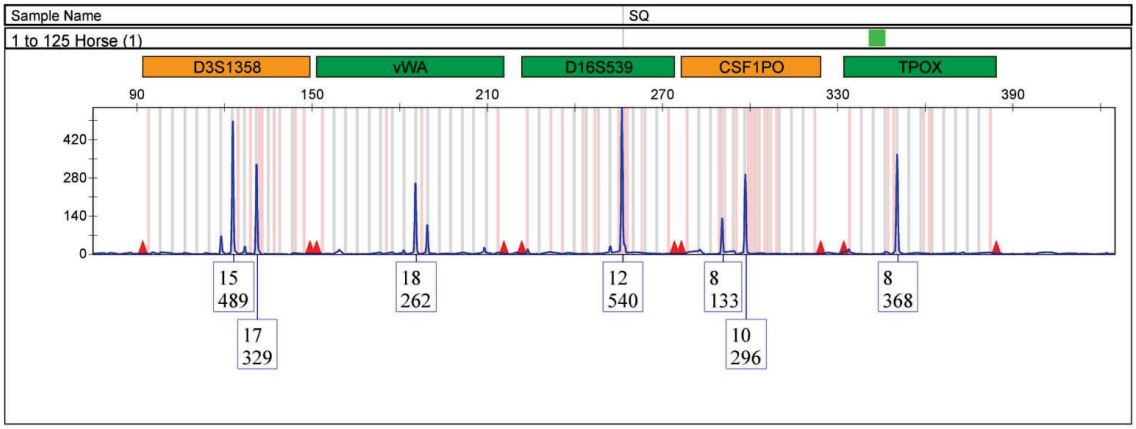
1 to 75 Horse (1)

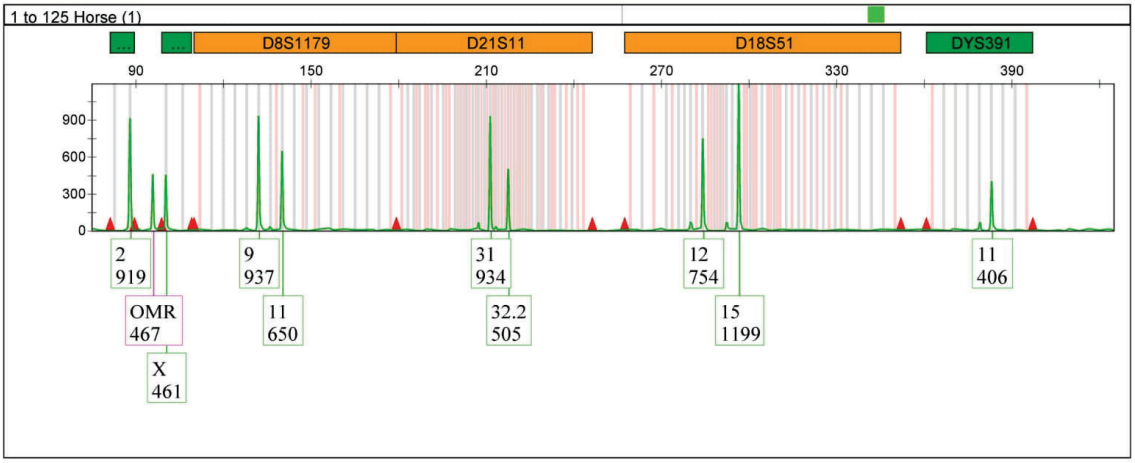


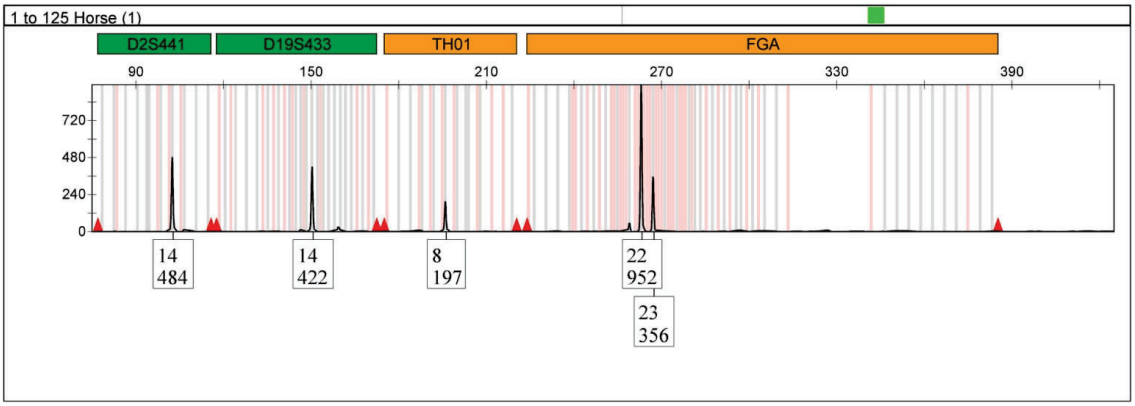
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 125 Horse (1)	



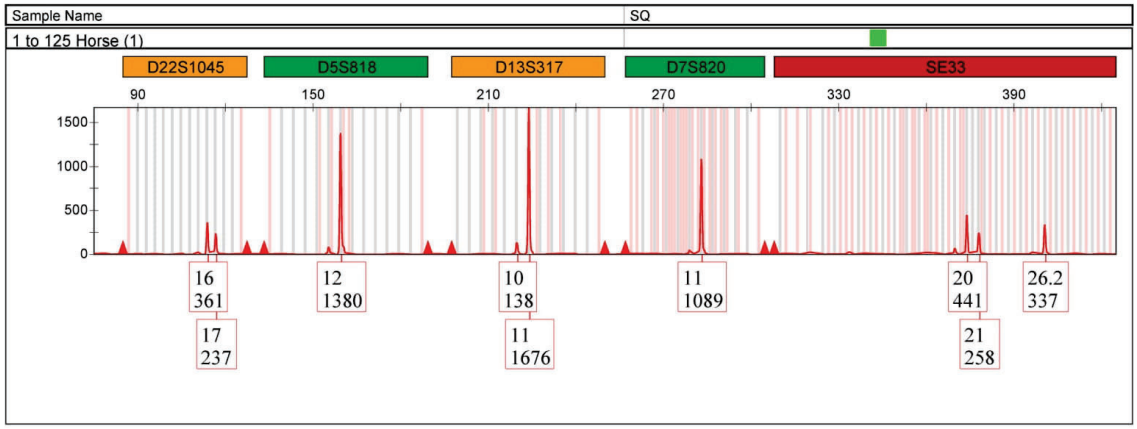




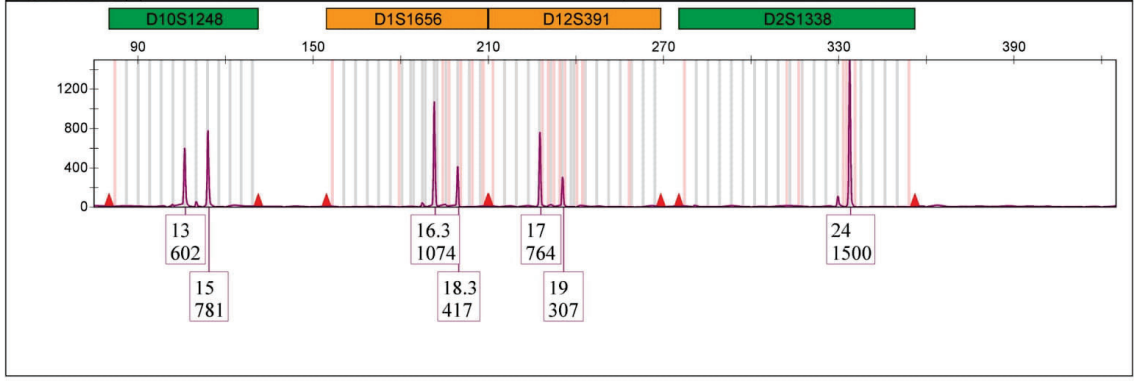
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

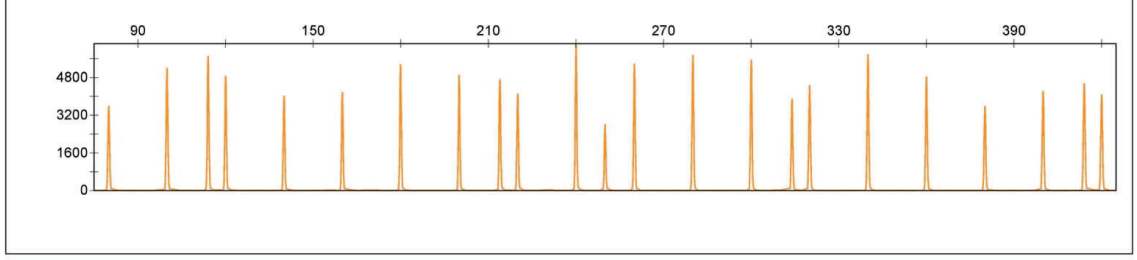
Sample Name	SQ
1 to 125 Horse (1)	



1 to 125 Horse (1)



1 to 125 Horse (1)

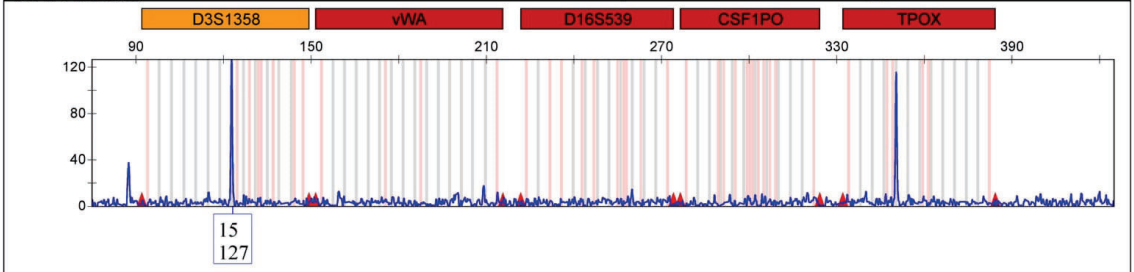


Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 175 Horse (1)



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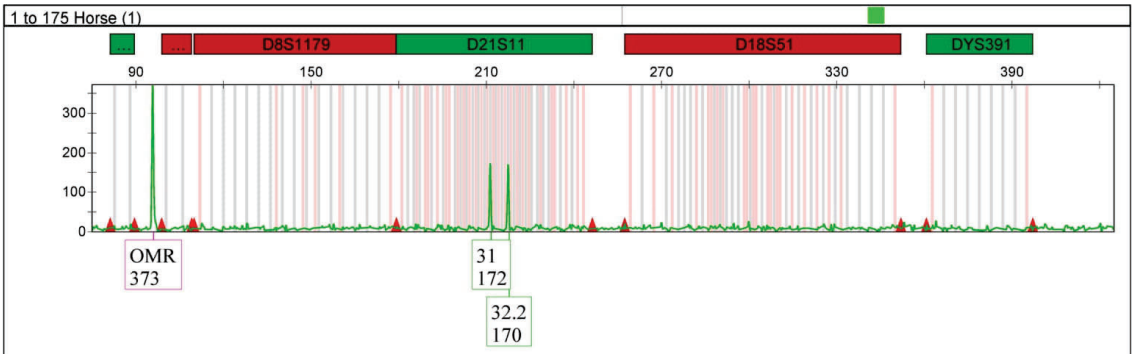
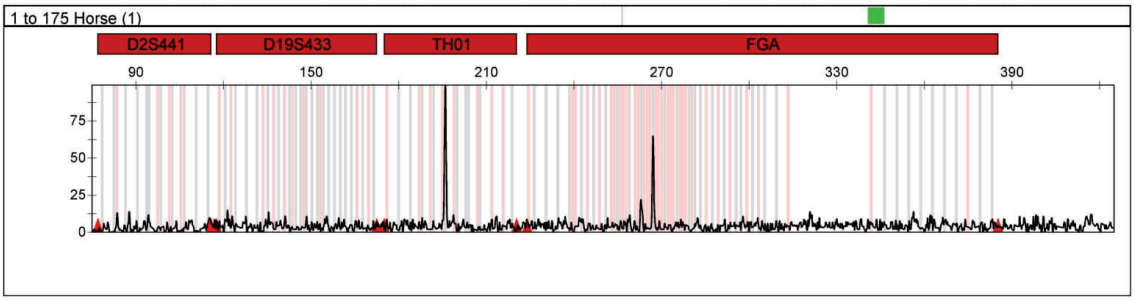


1 to 175 Horse (1)

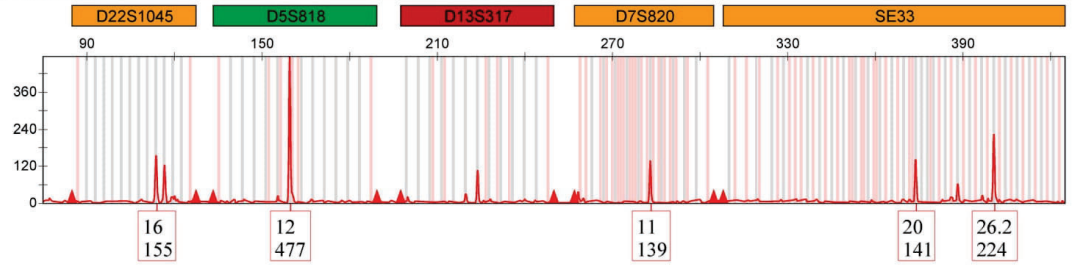


Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 175 Horse (1)



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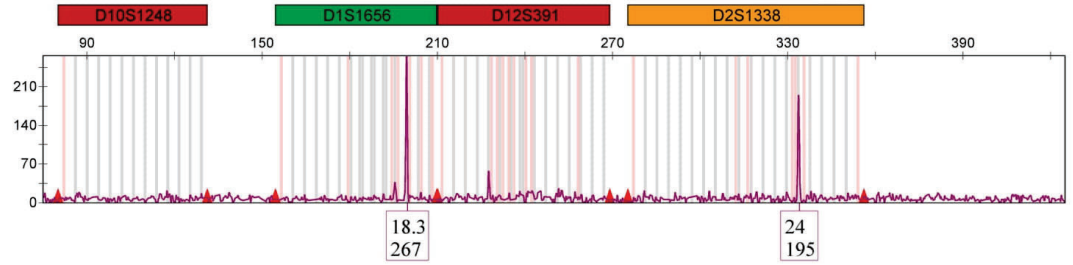
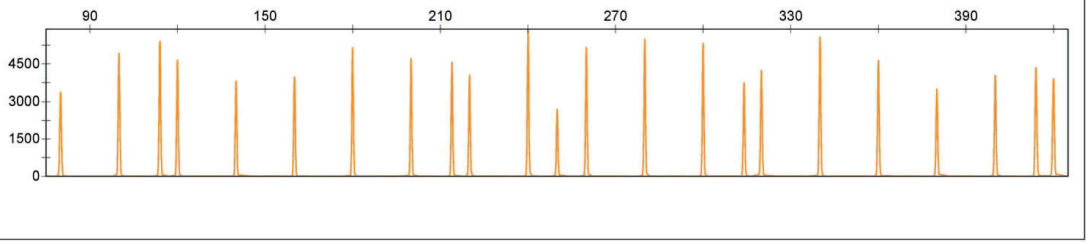


1 to 175 Horse (1)

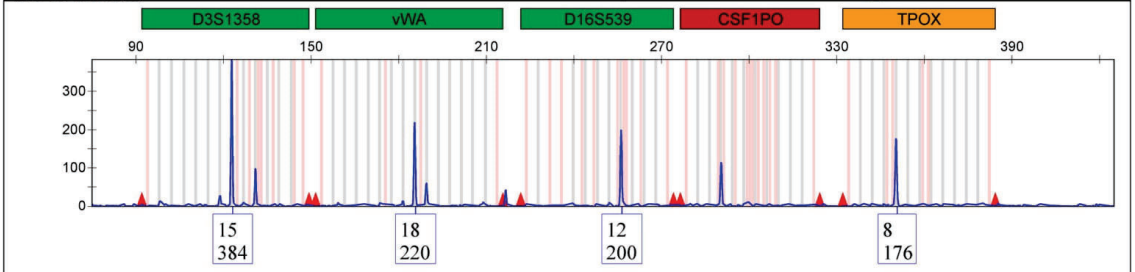


Applied Biosystems
GeneMapper® ID-X 1.4

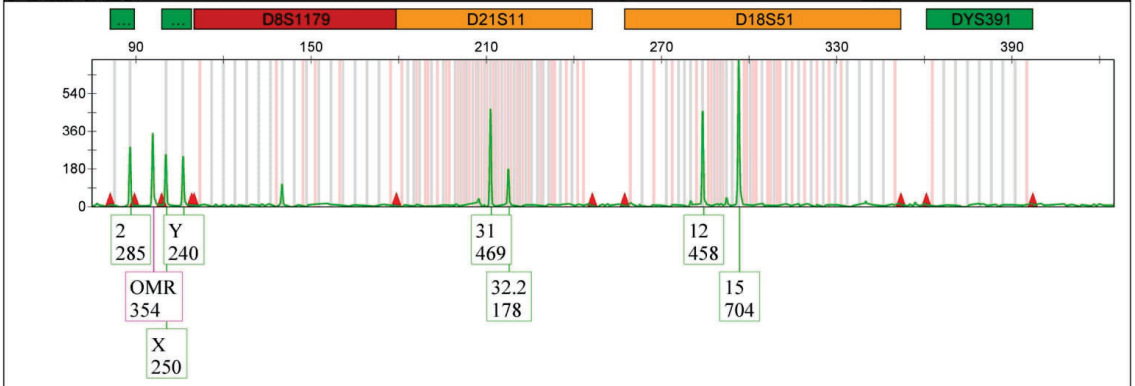
Project: Organic Extractions CRG 061422

Sample Name	SQ

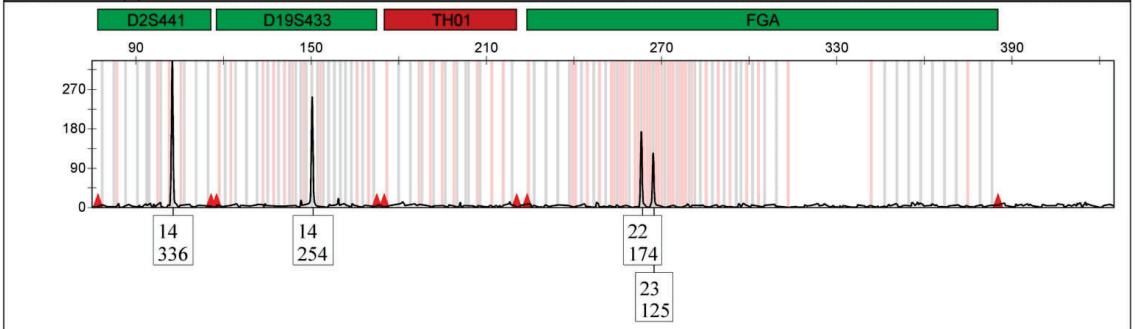
1 to 250 Horse (1)



1 to 250 Horse (1)



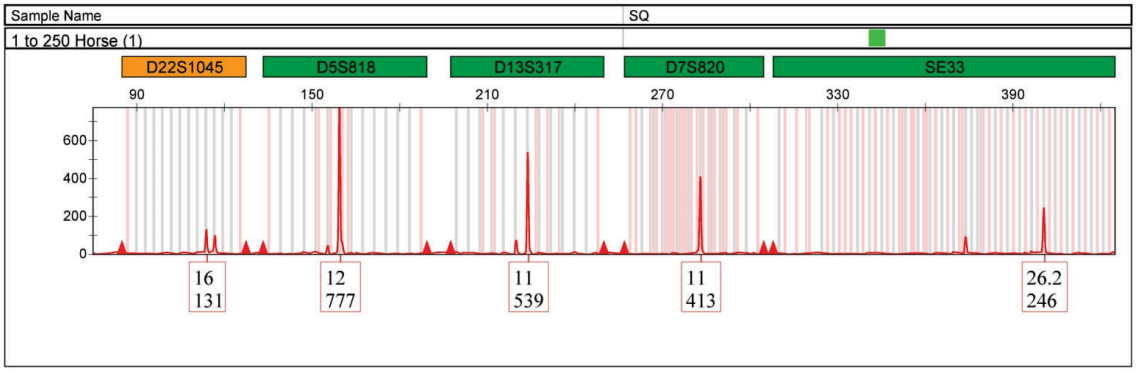
1 to 250 Horse (1)



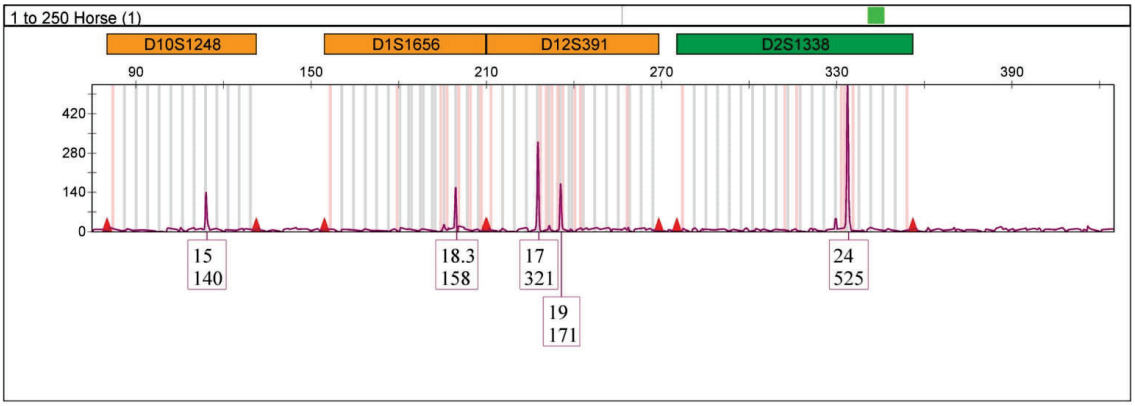
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

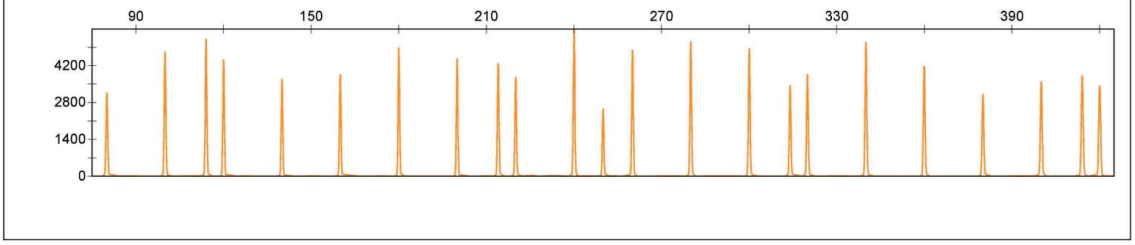
Sample Name	SQ
1 to 250 Horse (1)	



1 to 250 Horse (1)



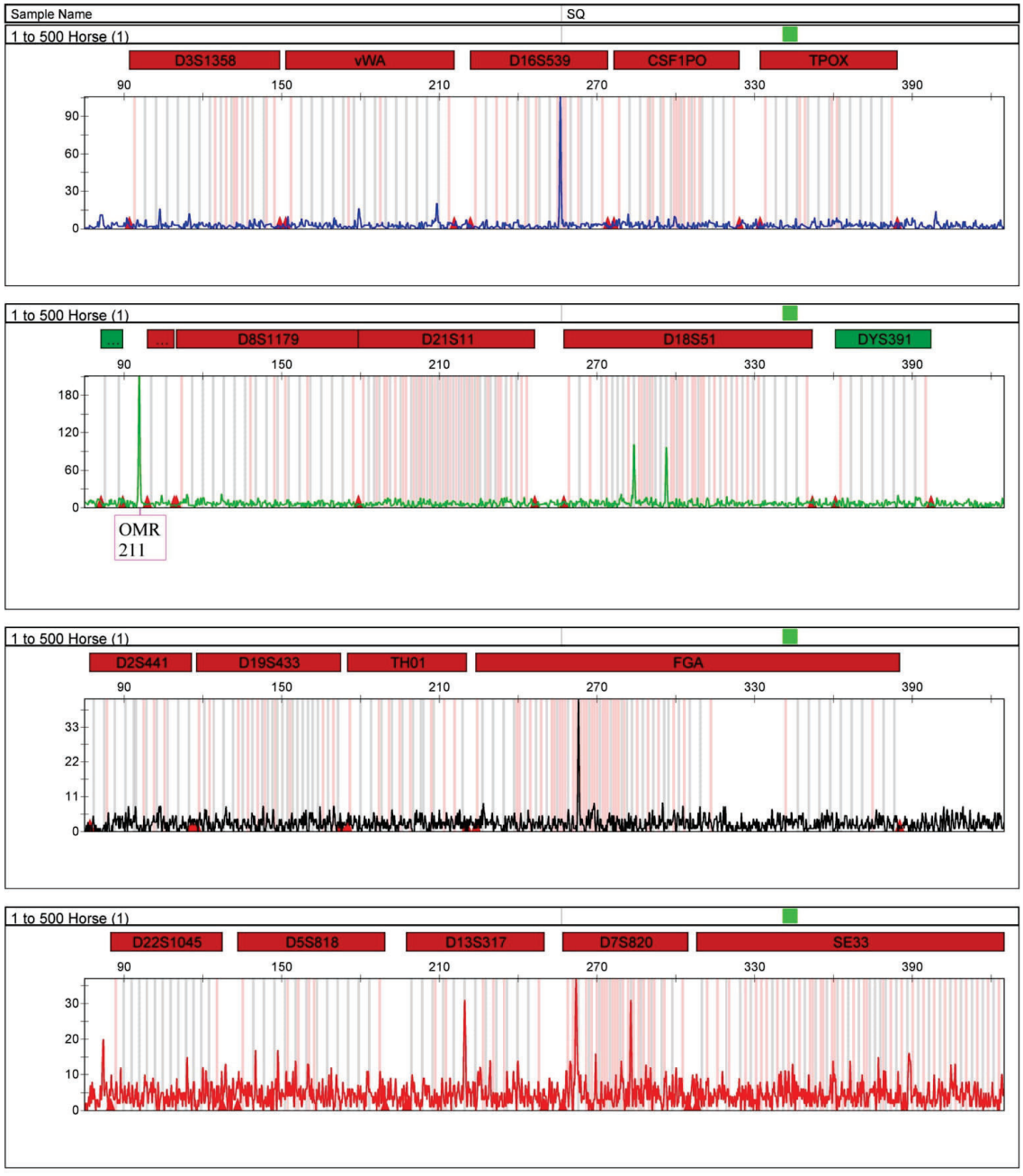
1 to 250 Horse (1)



Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

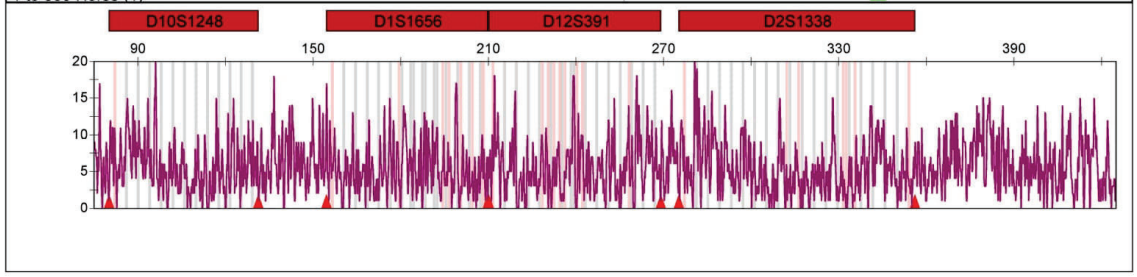
Sample Name	SQ



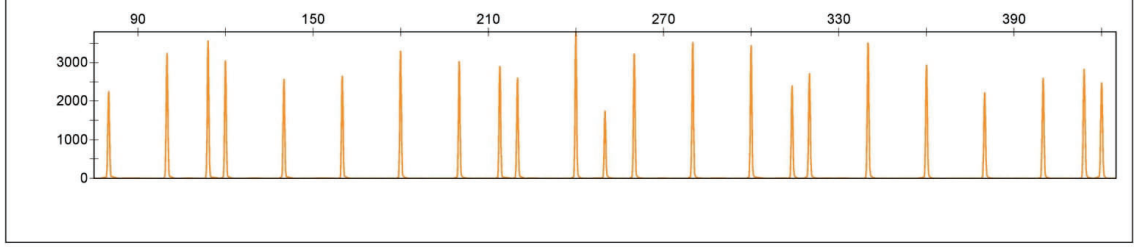
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 500 Horse (1)	



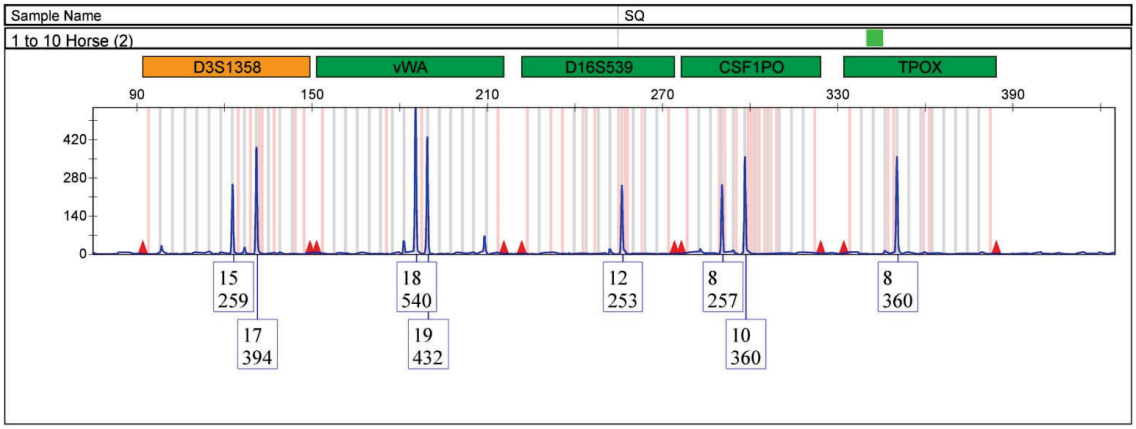
1 to 500 Horse (1)

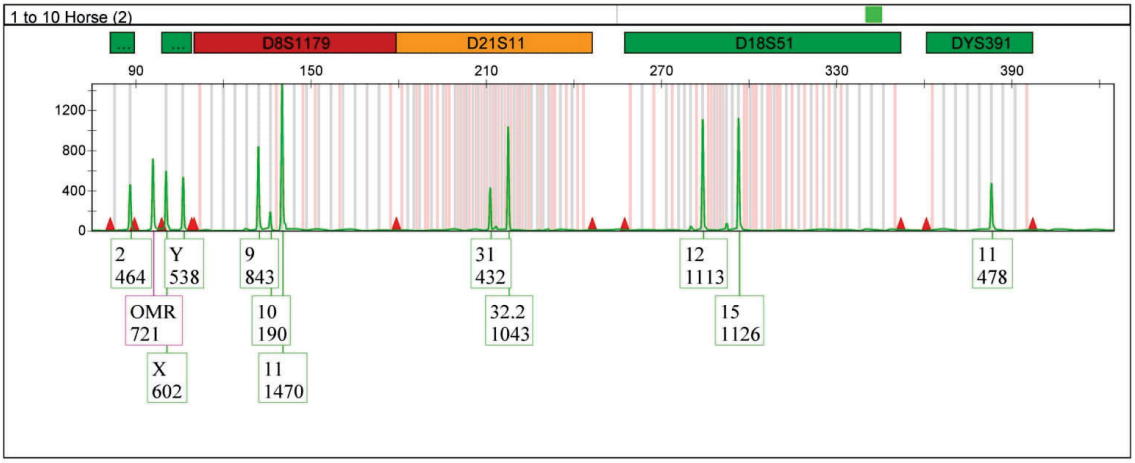


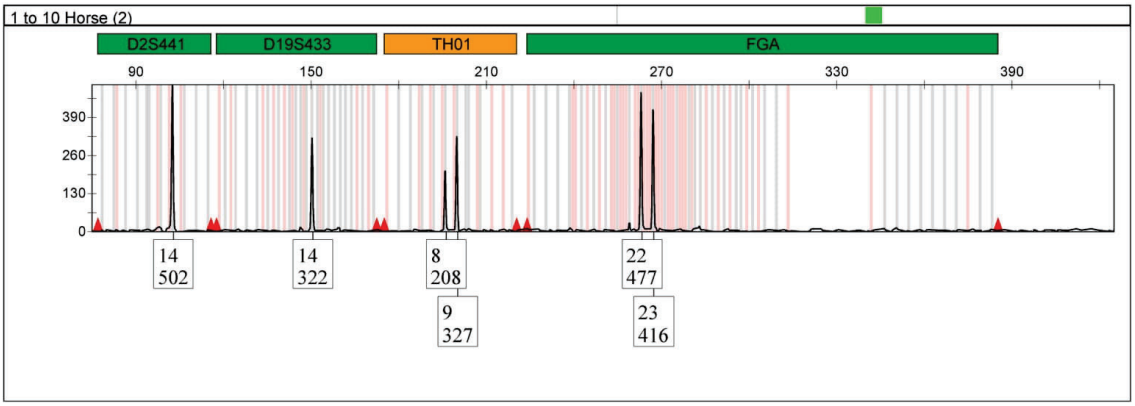
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 10 Horse (2)	





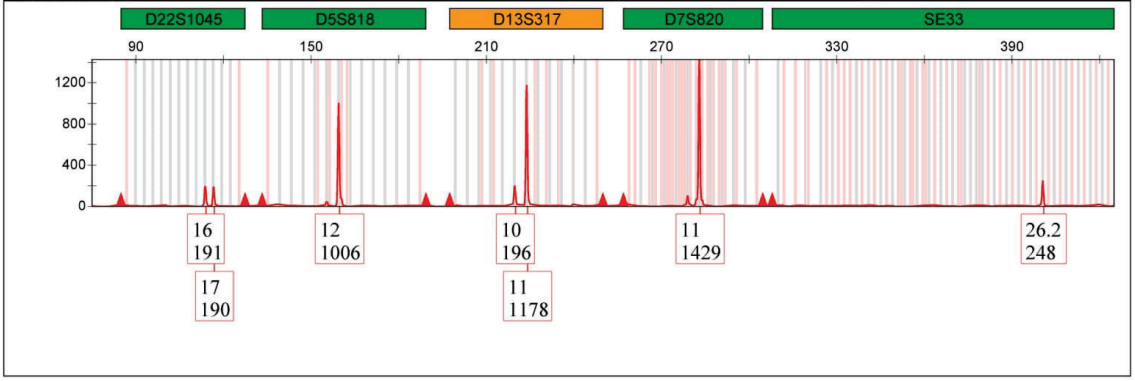


Applied Biosystems
GeneMapper® ID-X 1.4

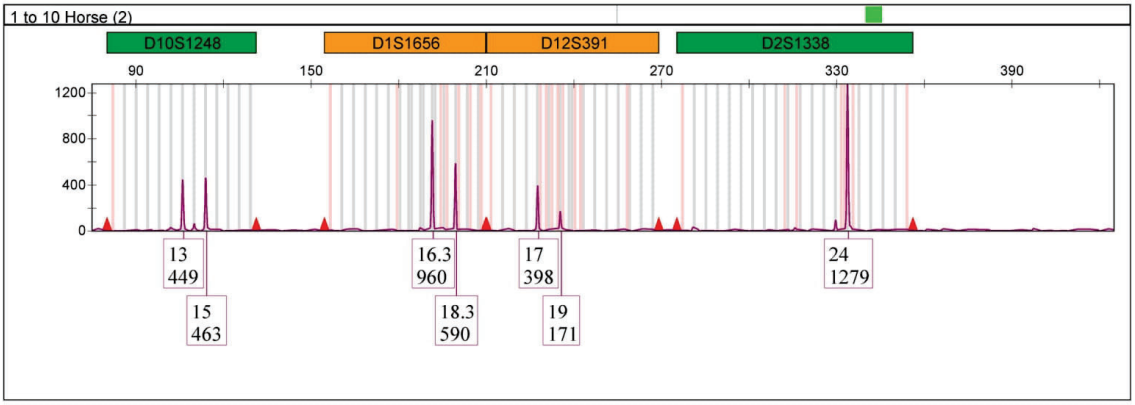
Project: Organic Extractions CRG 061422

Sample Name	SQ

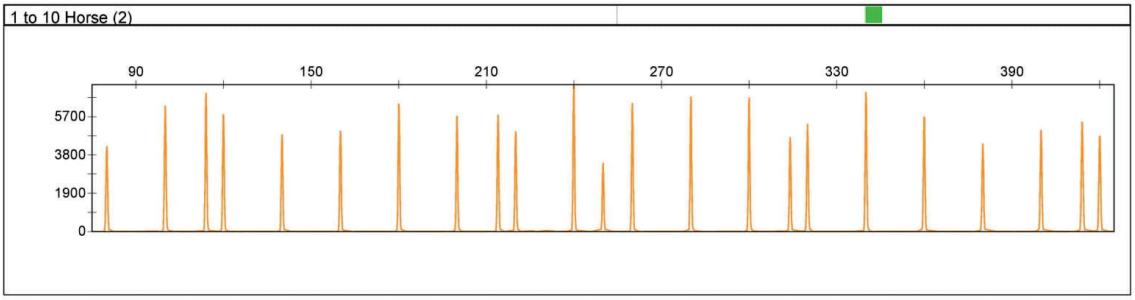
1 to 10 Horse (2)



1 to 10 Horse (2)



1 to 10 Horse (2)

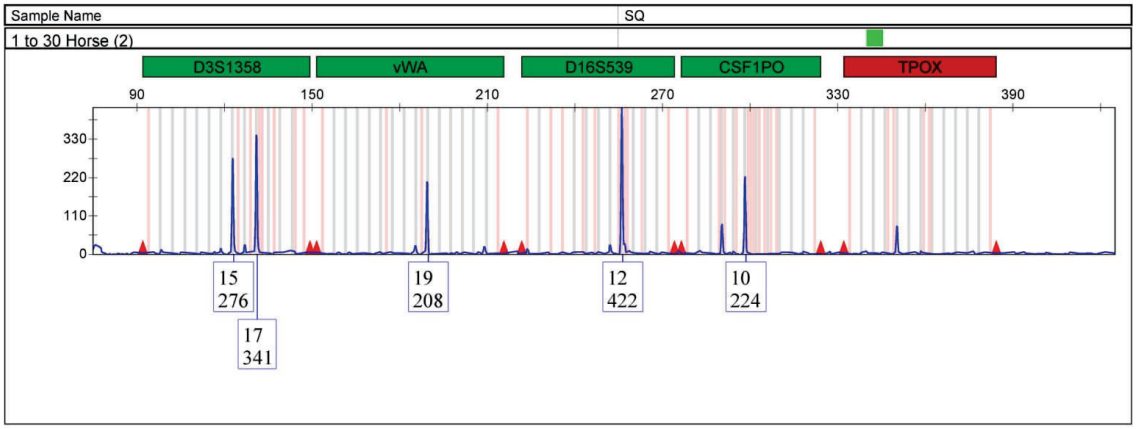


Applied Biosystems
GeneMapper® ID-X 1.4

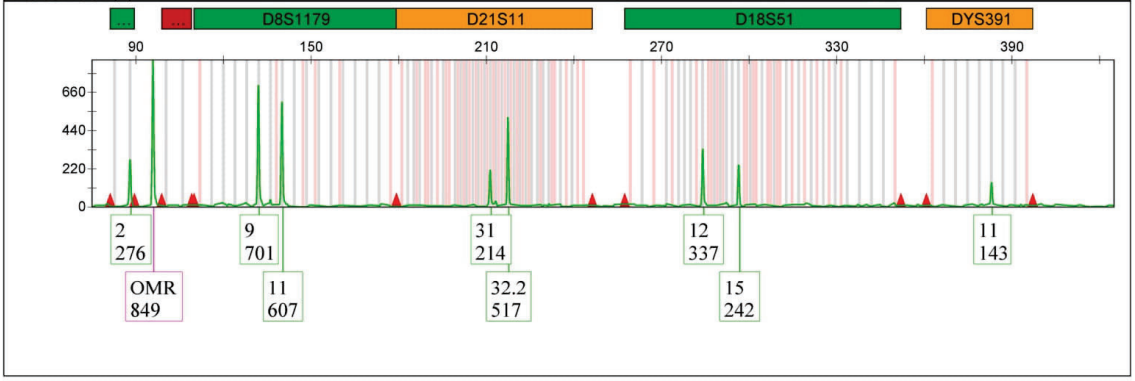
Project: Organic Extractions CRG 061422

Sample Name	SQ

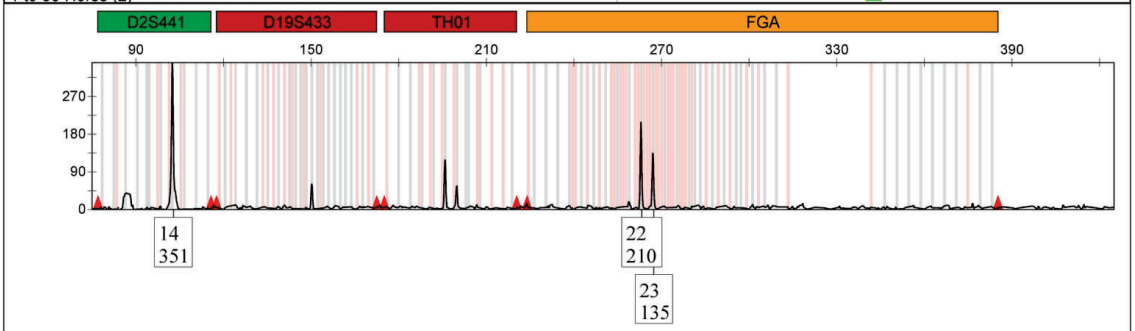
1 to 30 Horse (2)



1 to 30 Horse (2)



1 to 30 Horse (2)

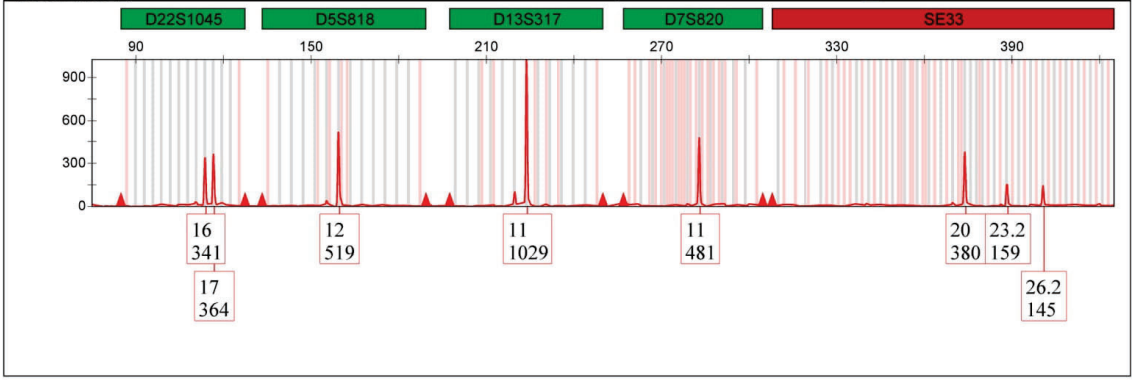


Applied Biosystems
GeneMapper® ID-X 1.4

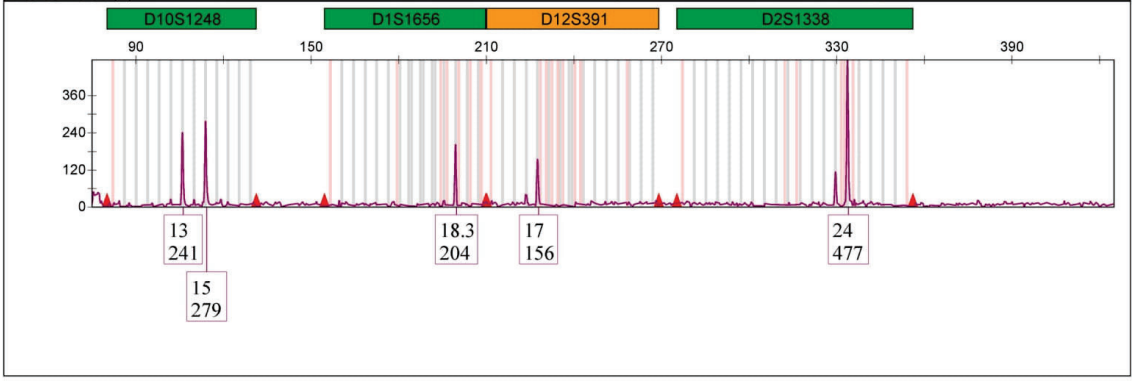
Project: Organic Extractions CRG 061422

Sample Name	SQ

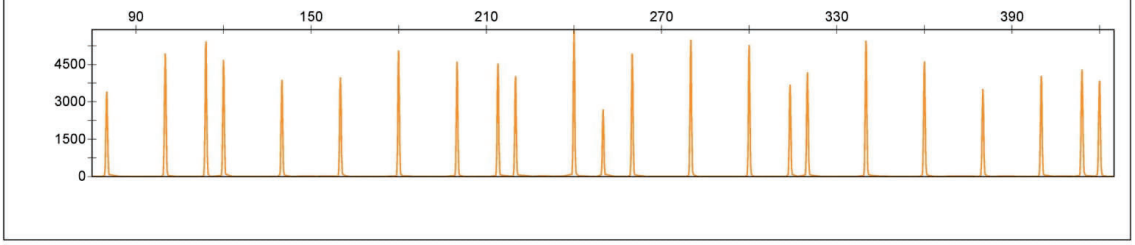
1 to 30 Horse (2)



1 to 30 Horse (2)



1 to 30 Horse (2)

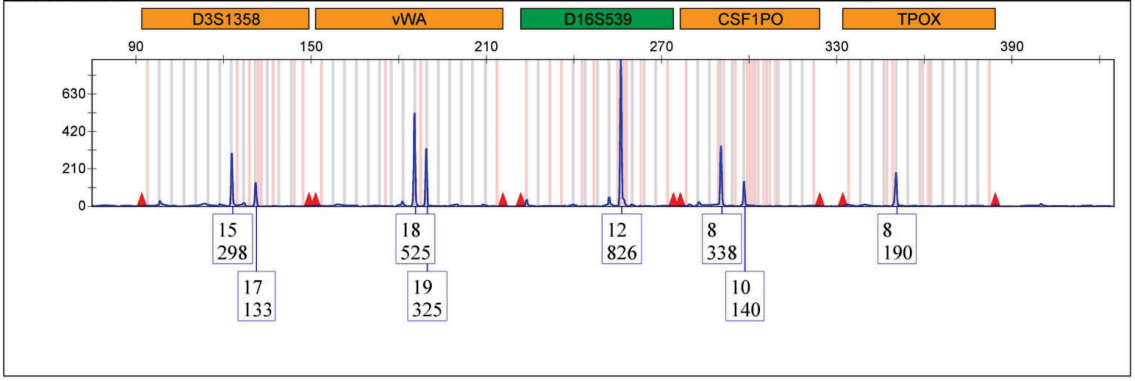


Applied Biosystems
GeneMapper® ID-X 1.4

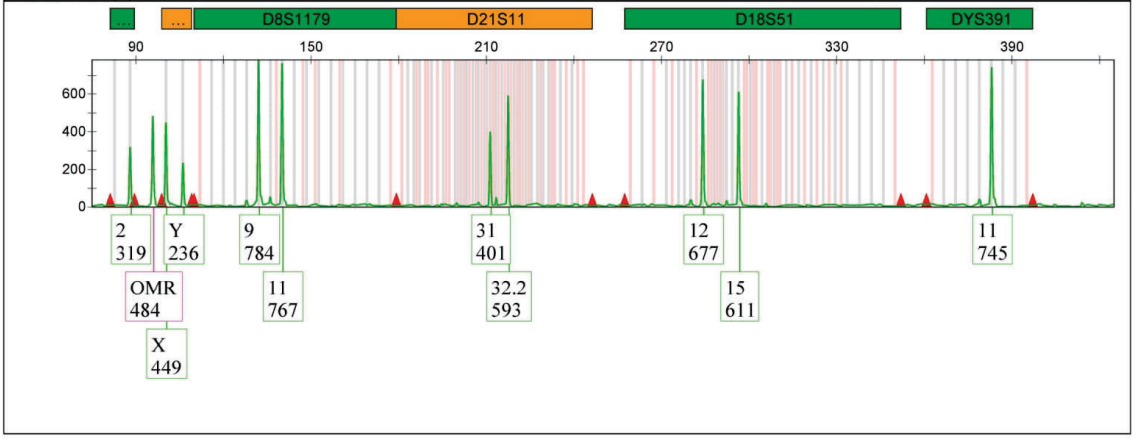
Project: Organic Extractions CRG 061422

Sample Name	SQ

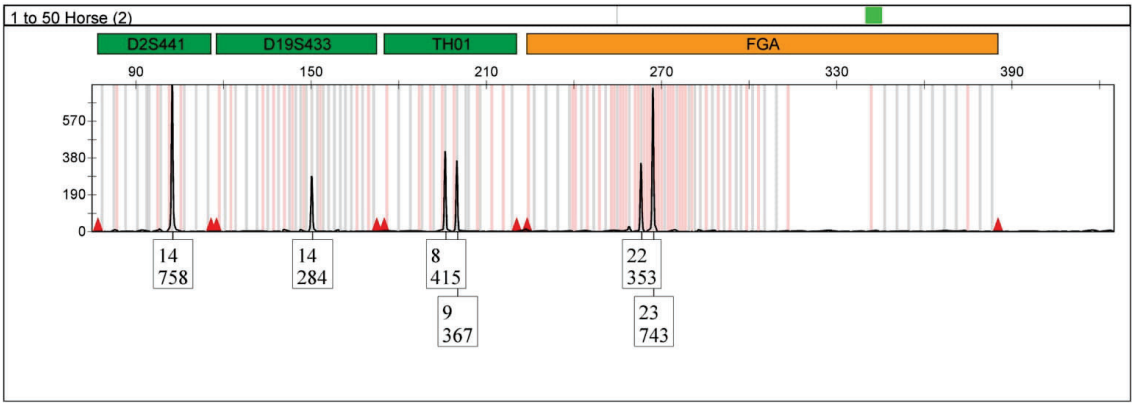
1 to 50 Horse (2)



1 to 50 Horse (2)



1 to 50 Horse (2)

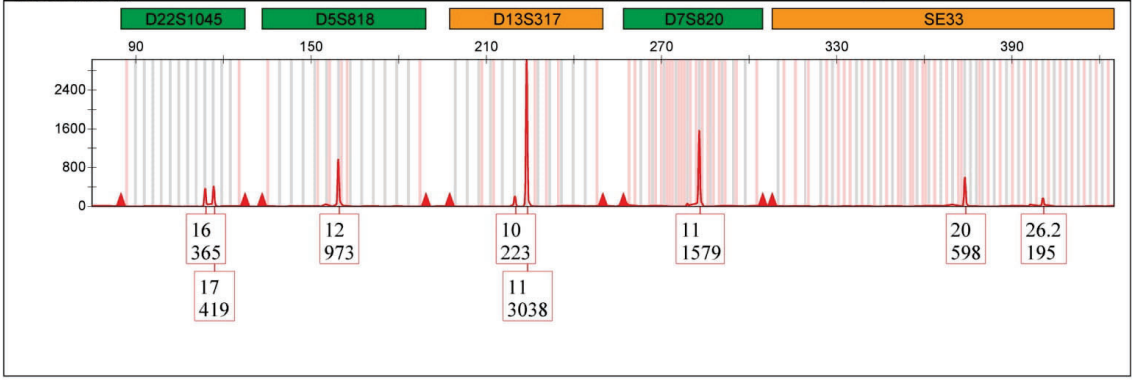


Applied Biosystems
GeneMapper® ID-X 1.4

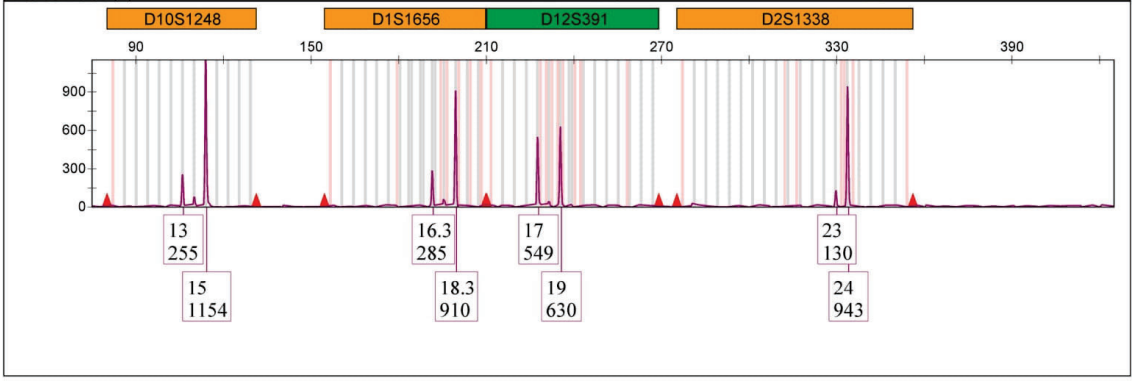
Project: Organic Extractions CRG 061422

Sample Name	SQ

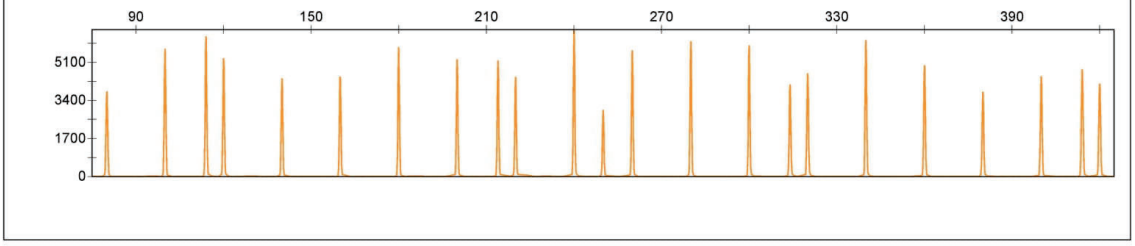
1 to 50 Horse (2)



1 to 50 Horse (2)



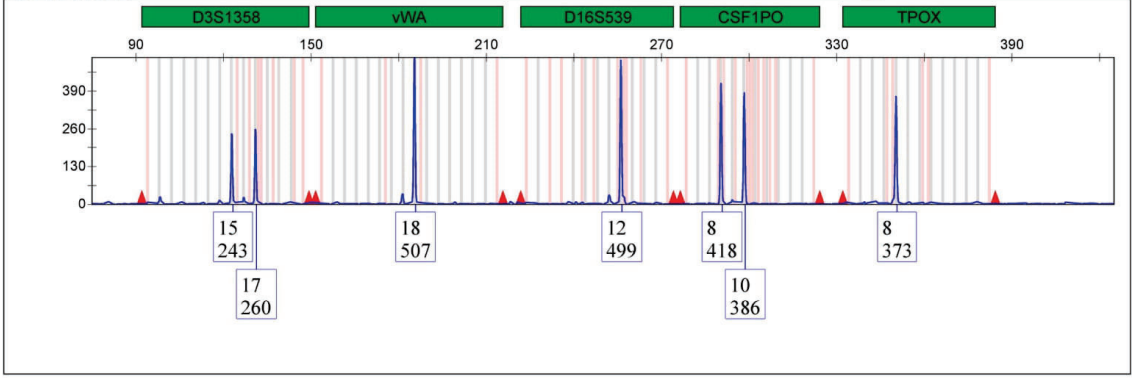
1 to 50 Horse (2)

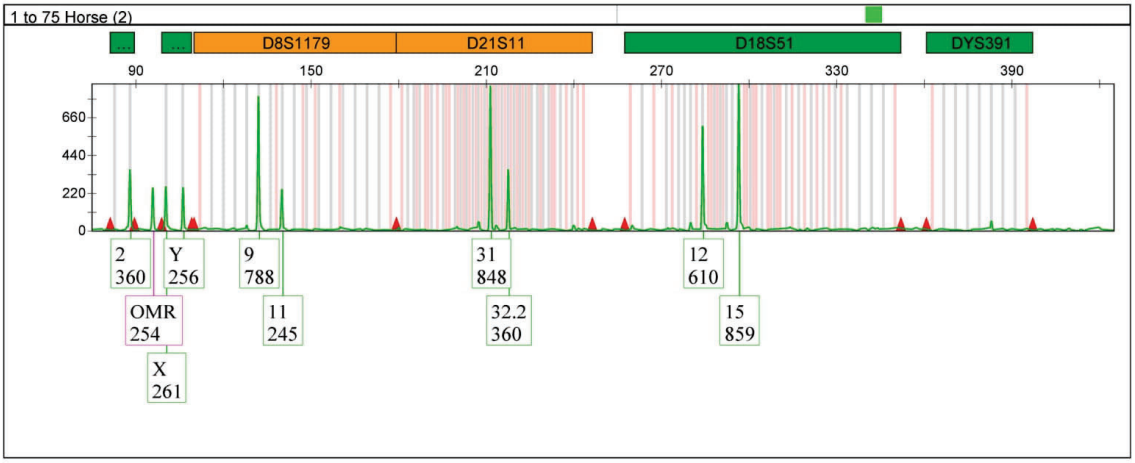


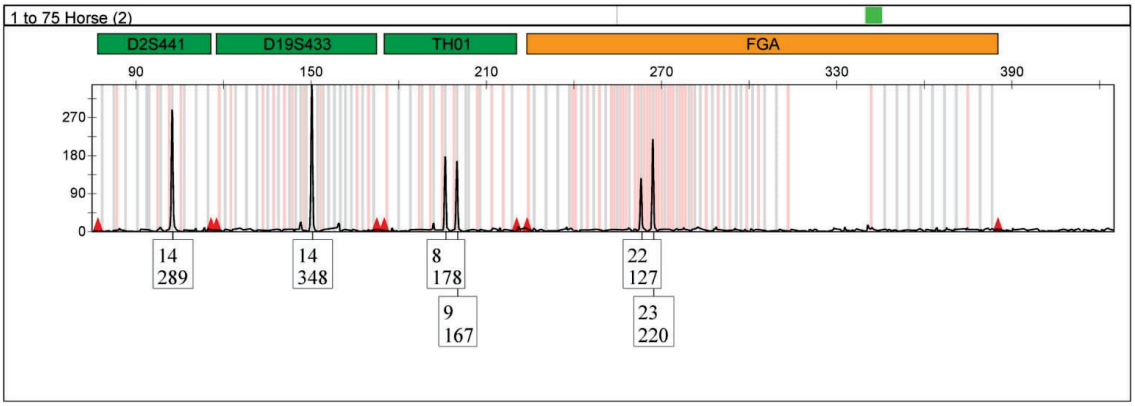
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 75 Horse (2)	





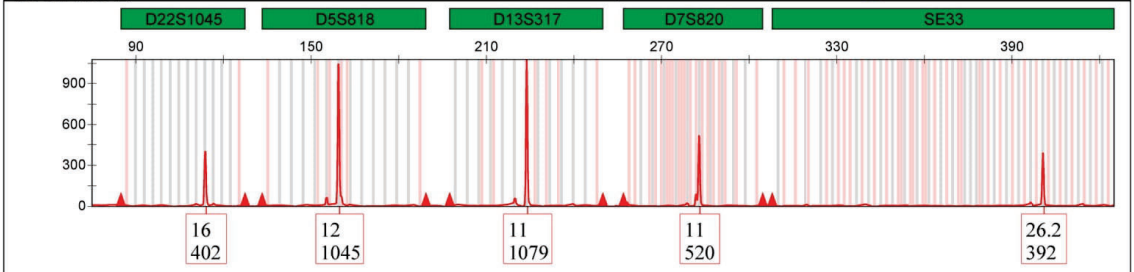


Applied Biosystems
GeneMapper® ID-X 1.4

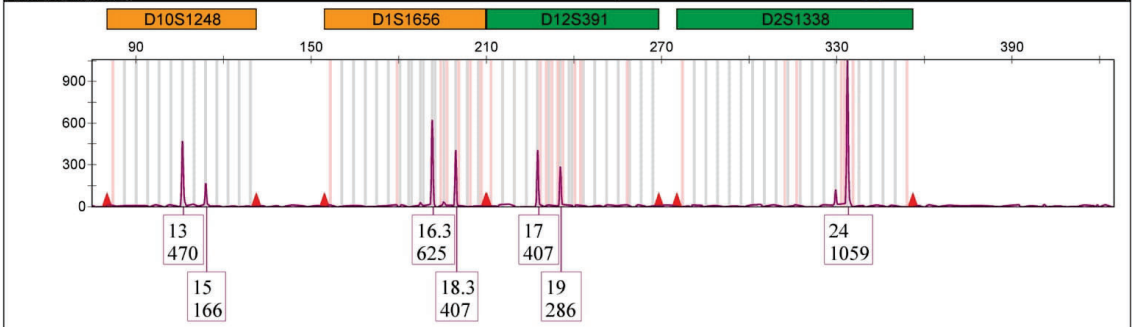
Project: Organic Extractions CRG 061422

Sample Name	SQ

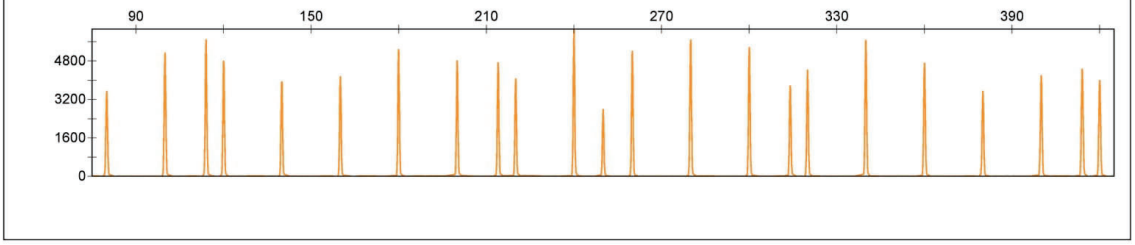
1 to 75 Horse (2)



1 to 75 Horse (2)



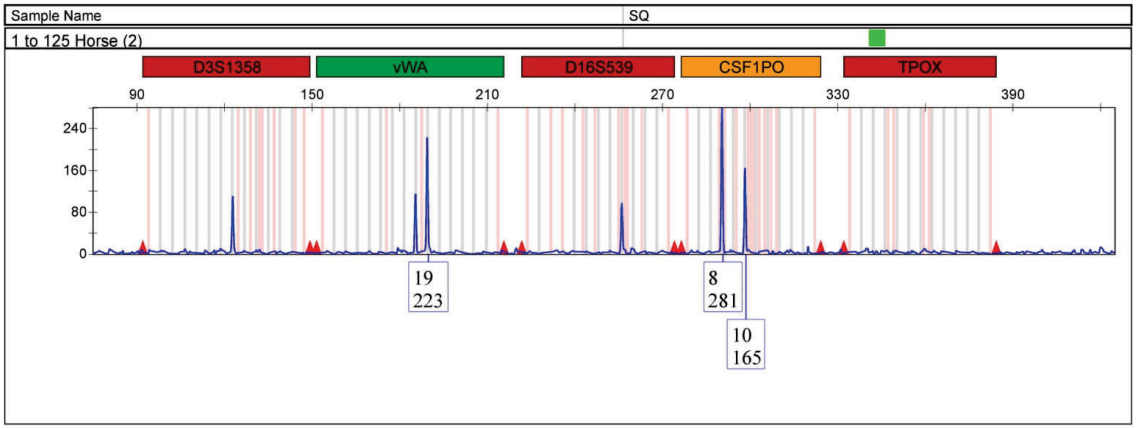
1 to 75 Horse (2)



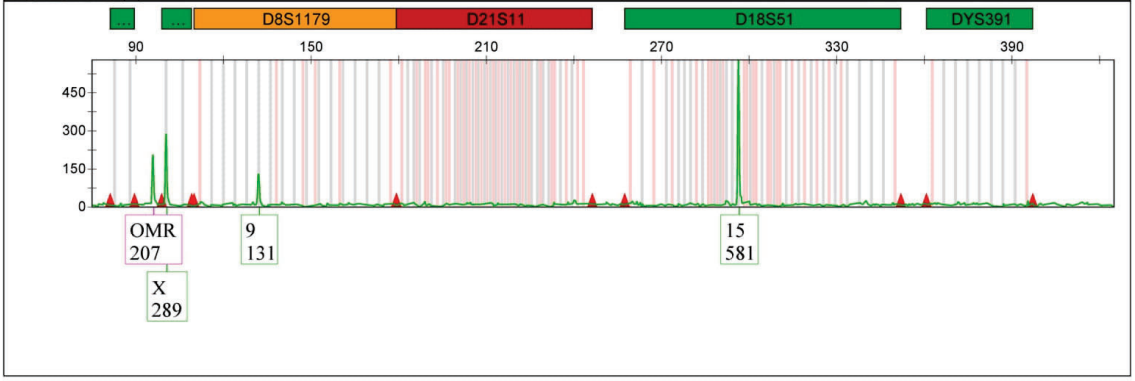
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

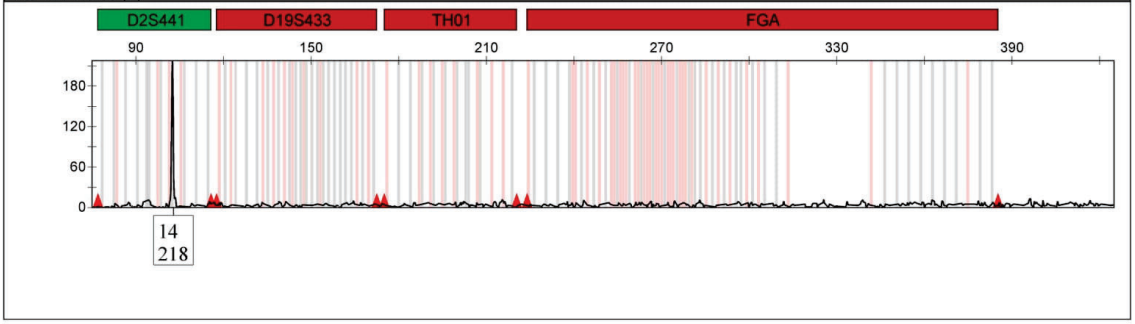
Sample Name	SQ
1 to 125 Horse (2)	



1 to 125 Horse (2)



1 to 125 Horse (2)

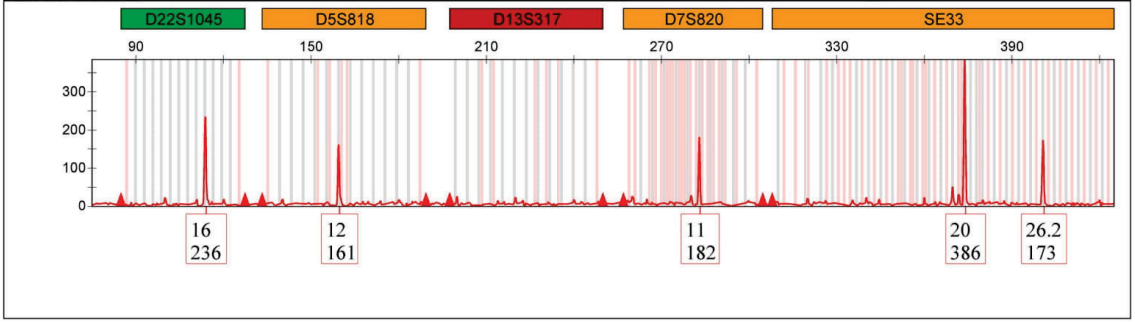


Applied Biosystems
GeneMapper® ID-X 1.4

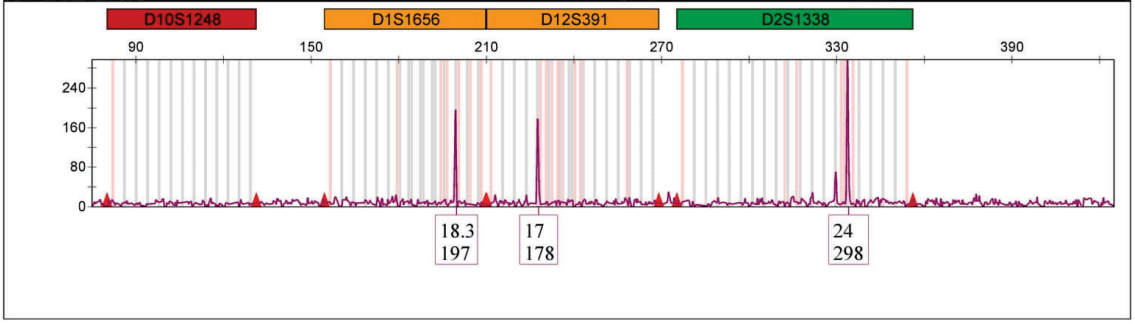
Project: Organic Extractions CRG 061422

Sample Name	SQ

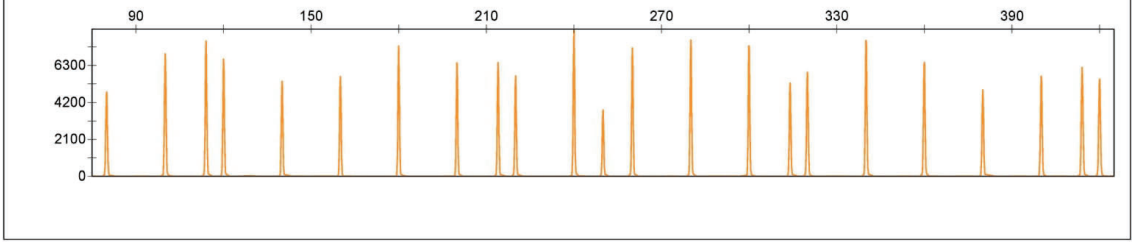
1 to 125 Horse (2)

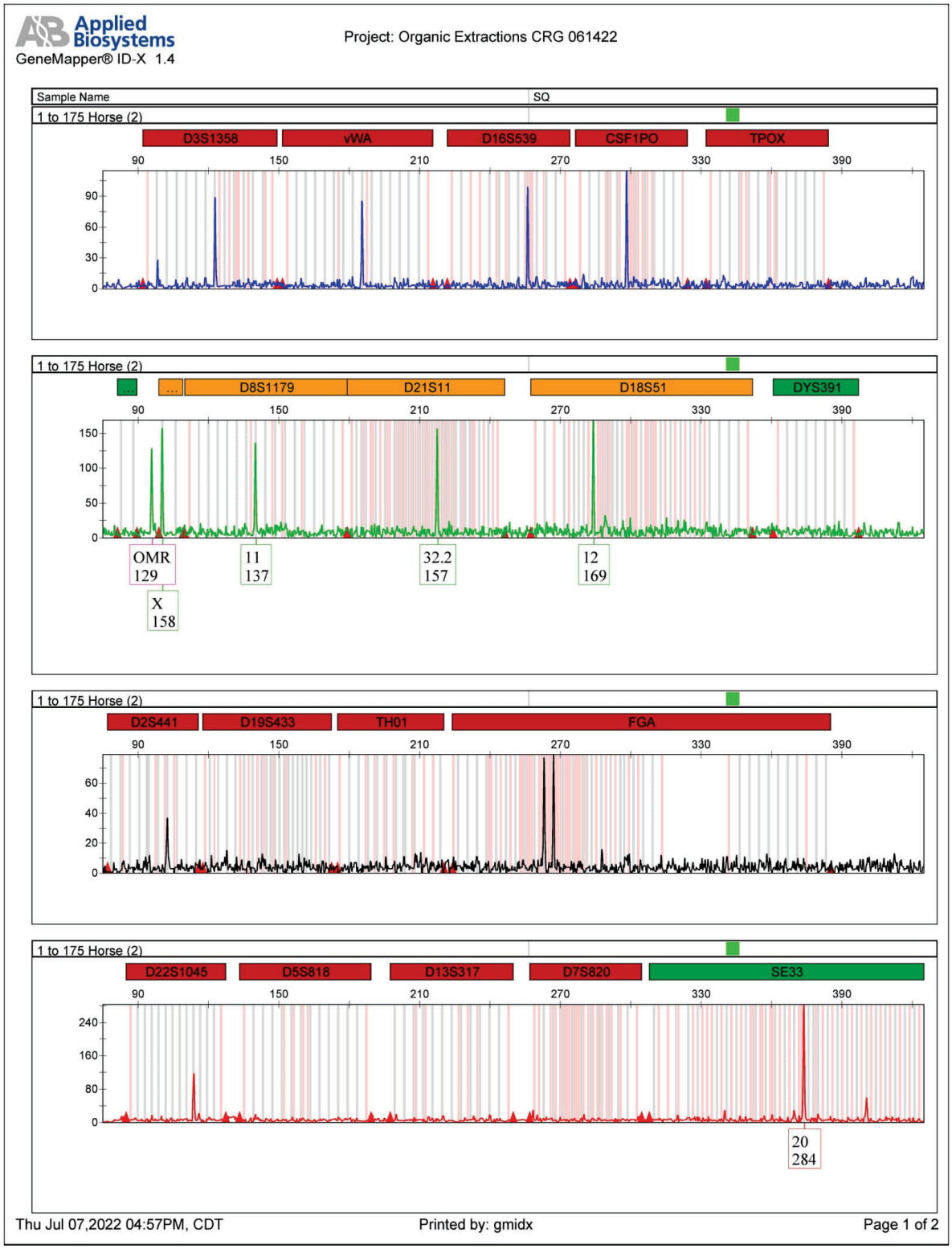


1 to 125 Horse (2)



1 to 125 Horse (2)



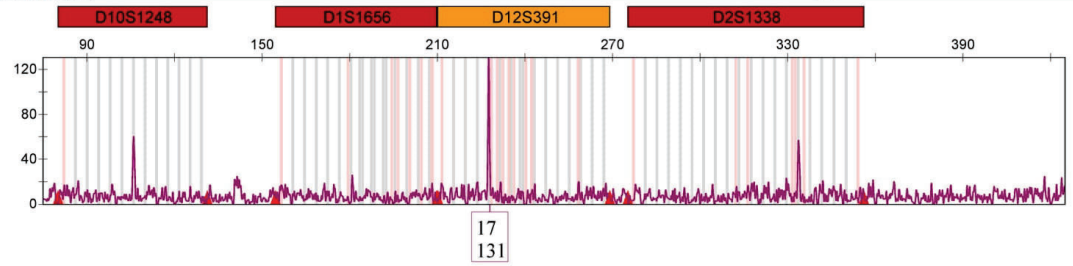

Applied Biosystems
GeneMapper® ID-X 1.4
Project: Organic Extractions CRG 061422
Sample Name
SQ
1 to 175 Horse (2)
D3S1358
vWA
D16S539
CSF1PO
TPOX
90
150
210
270
330
390
90
60
30
0
1 to 175 Horse (2)
D8S1179
D21S11
D18S51
DYS391
150
100
50
0
OMR
129
X
158
11
137
32.2
157
12
169
1 to 175 Horse (2)
D2S441
D19S433
TH01
FGA
60
40
20
0
1 to 175 Horse (2)
D22S1045
D5S818
D13S317
D7S820
SE33
240
160
80
0
20
284
Thu Jul 07,2022 04:57PM, CDT
Printed by: gmidx
Page 1 of 2

Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 175 Horse (2)



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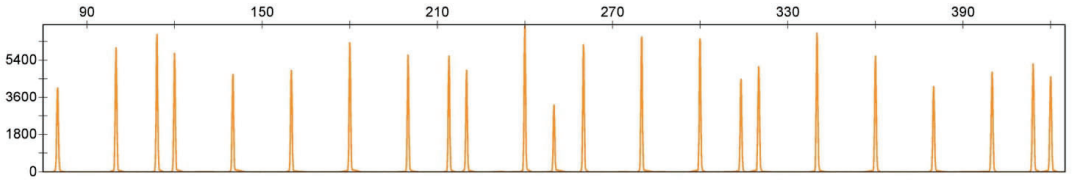
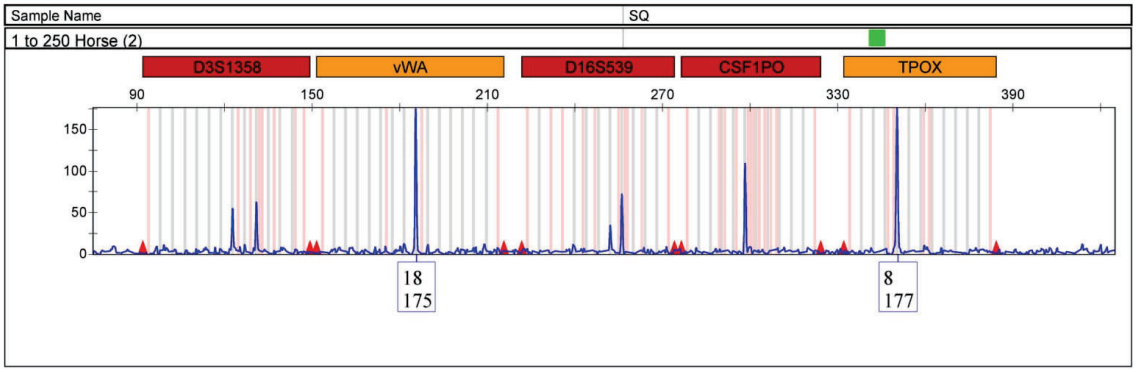


Applied Biosystems
GeneMapper® ID-X 1.4

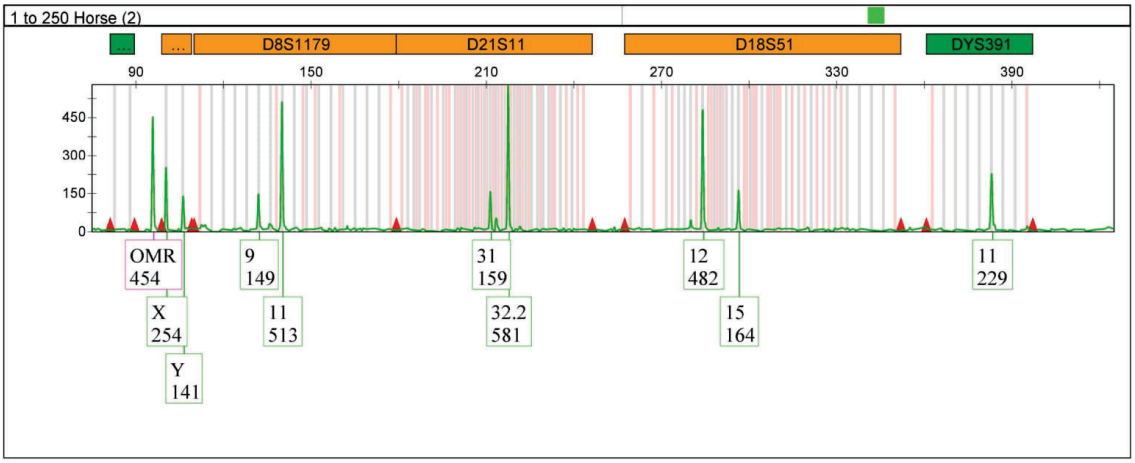
Project: Organic Extractions CRG 061422

Sample Name	SQ

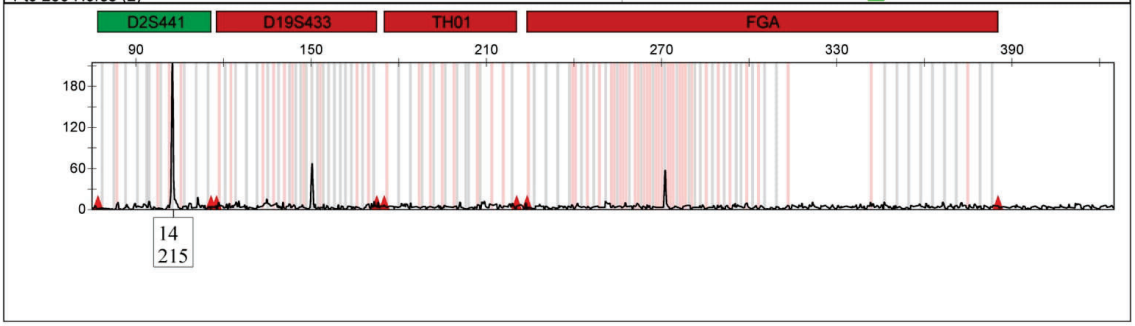
1 to 250 Horse (2)



1 to 250 Horse (2)



1 to 250 Horse (2)

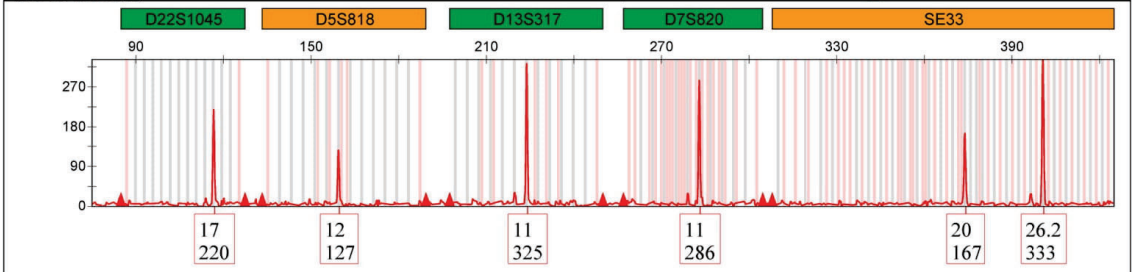


Applied Biosystems
GeneMapper® ID-X 1.4

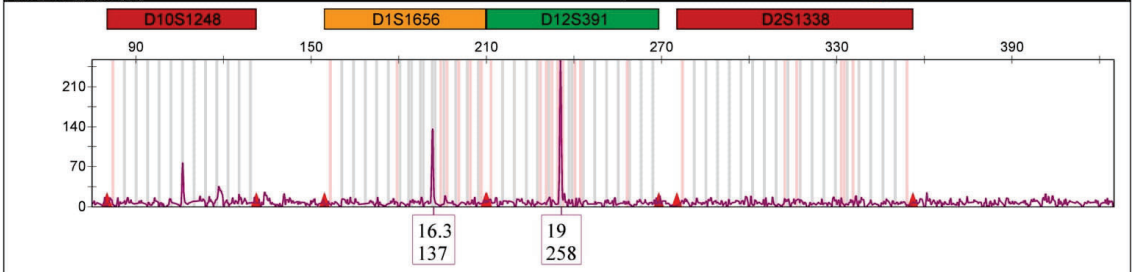
Project: Organic Extractions CRG 061422

Sample Name	SQ

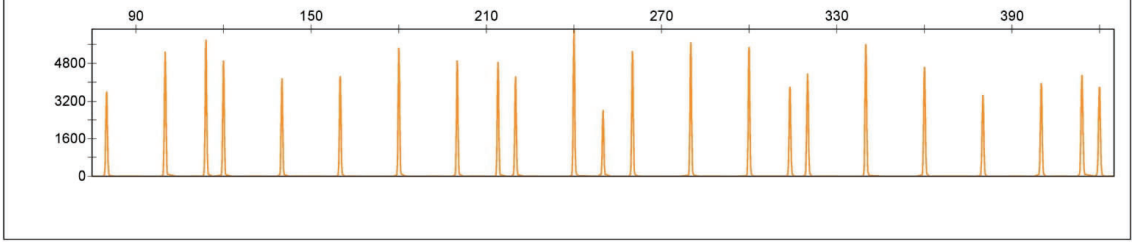
1 to 250 Horse (2)



1 to 250 Horse (2)



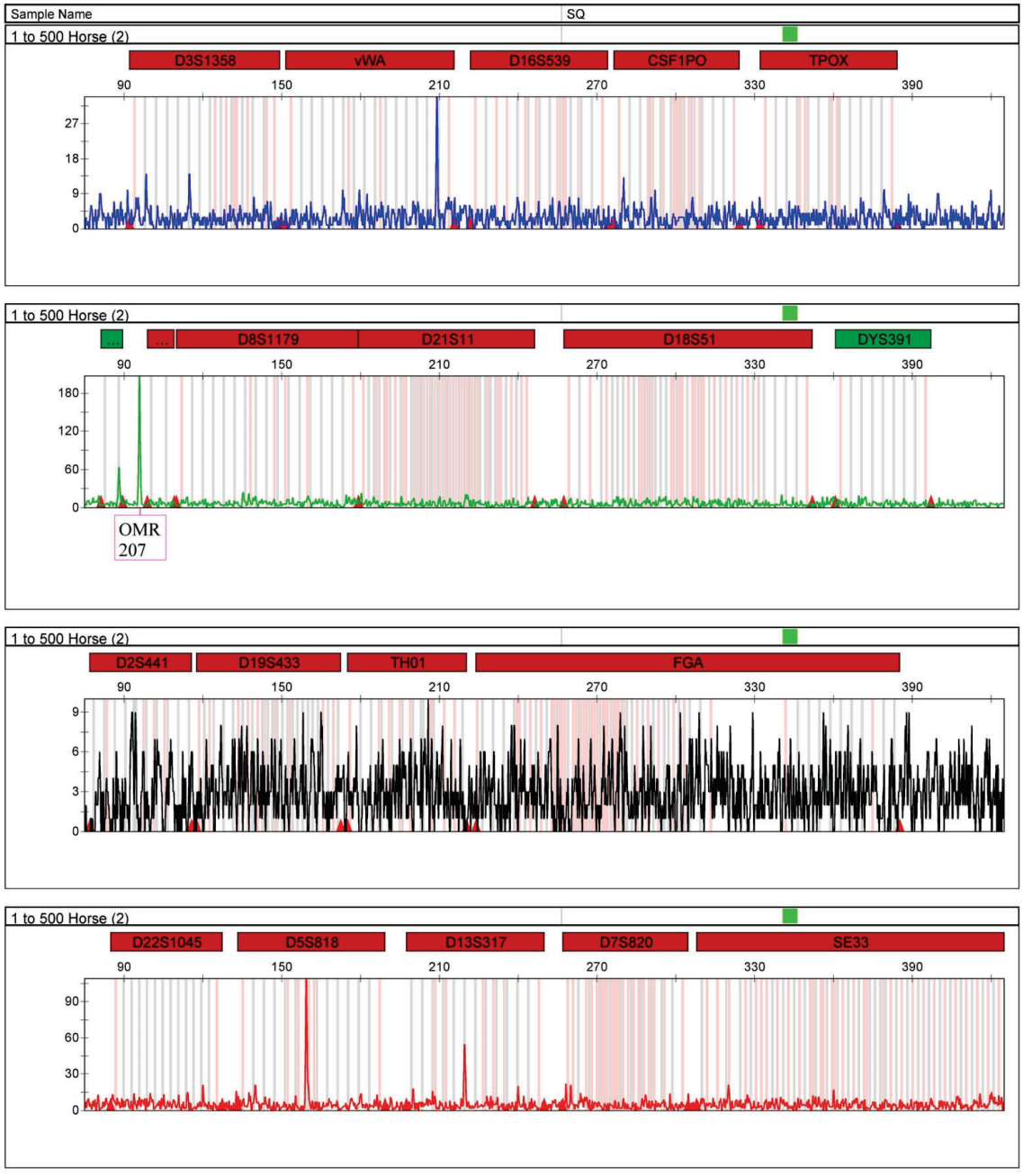
1 to 250 Horse (2)



Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ

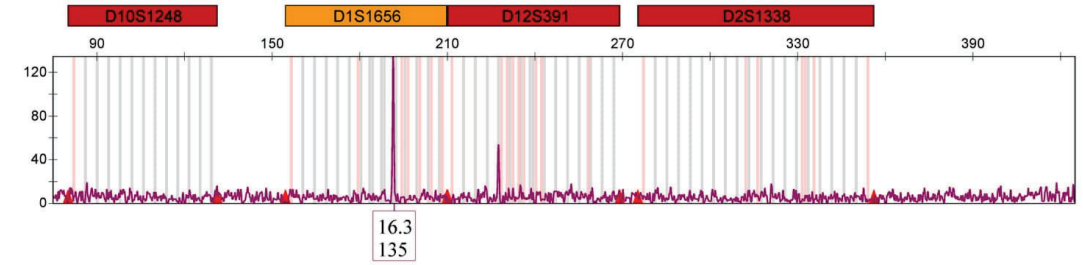


Applied Biosystems
GeneMapper® ID-X 1.4

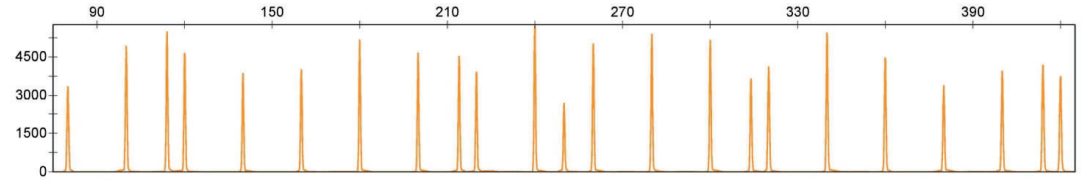
Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 500 Horse (2)



1 to 500 Horse (2)

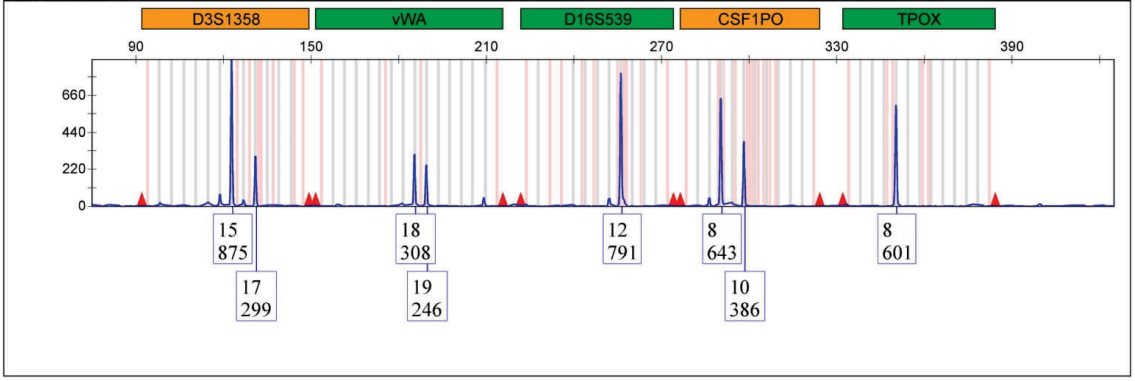


Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 10 Horse (3)



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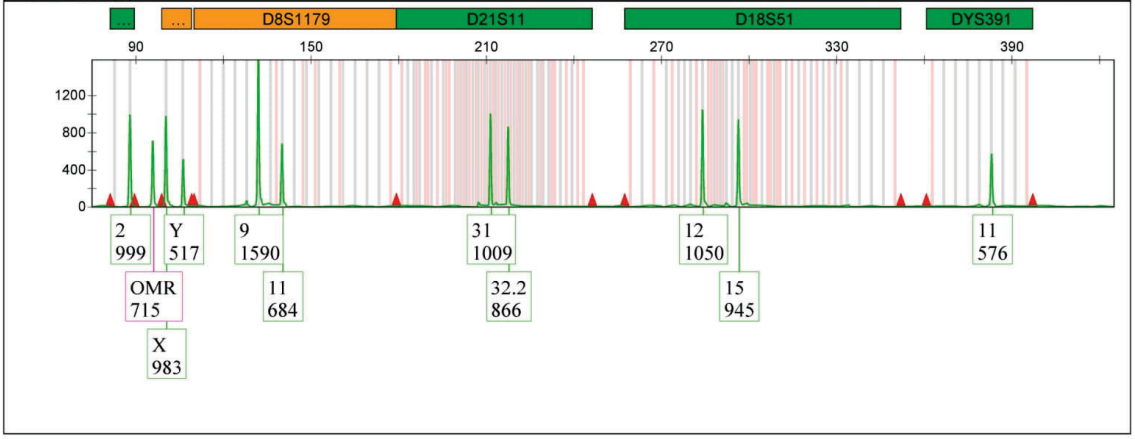

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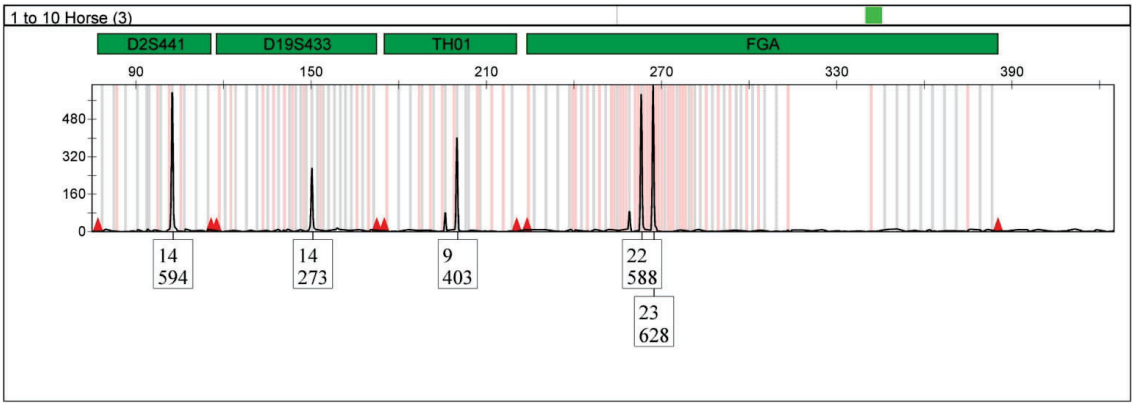
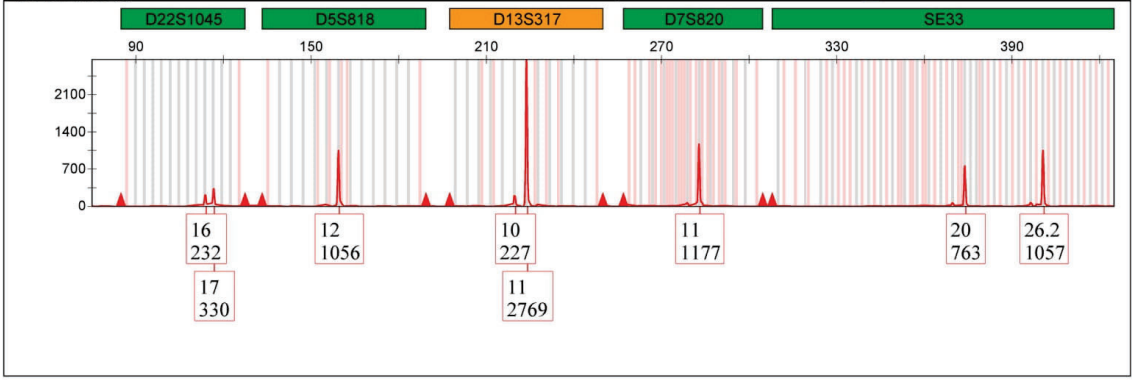


Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 10 Horse (3)



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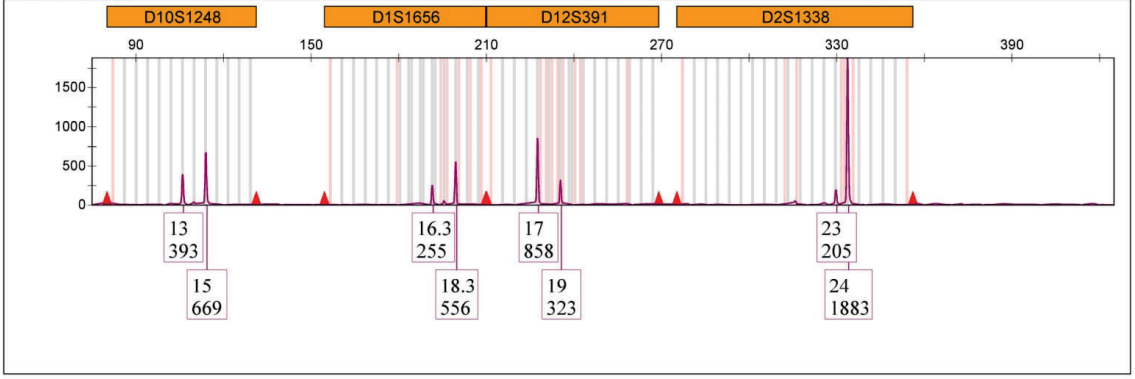

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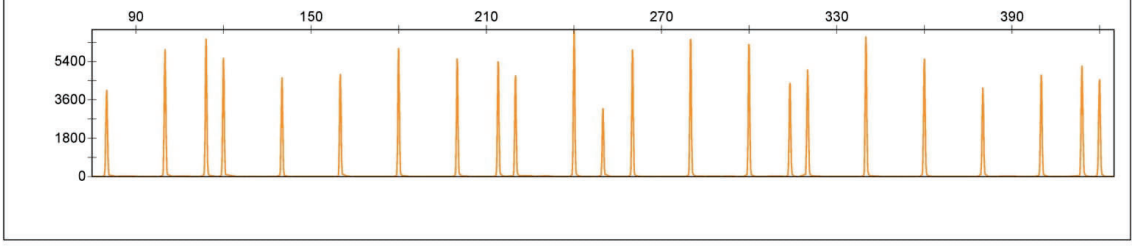
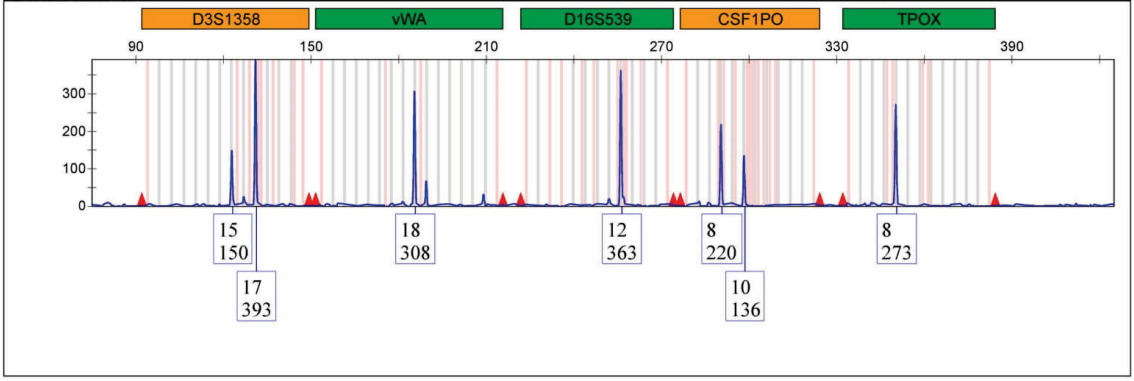


Applied Biosystems
GeneMapper® ID-X 1.4

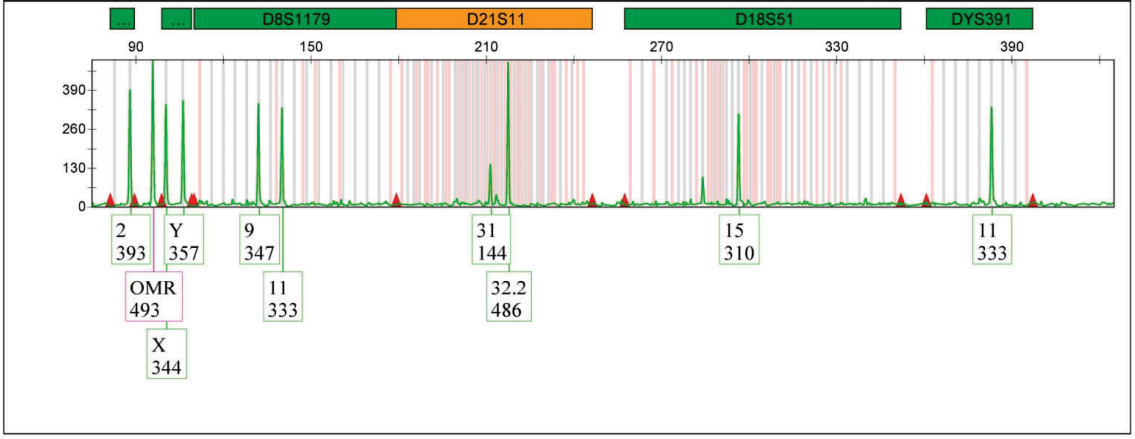
Project: Organic Extractions CRG 061422

Sample Name	SQ

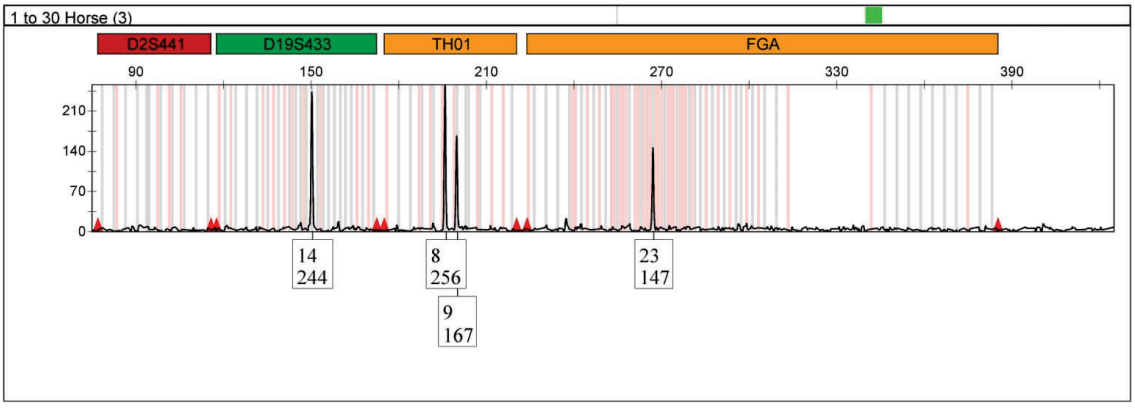
1 to 30 Horse (3)



1 to 30 Horse (3)



1 to 30 Horse (3)

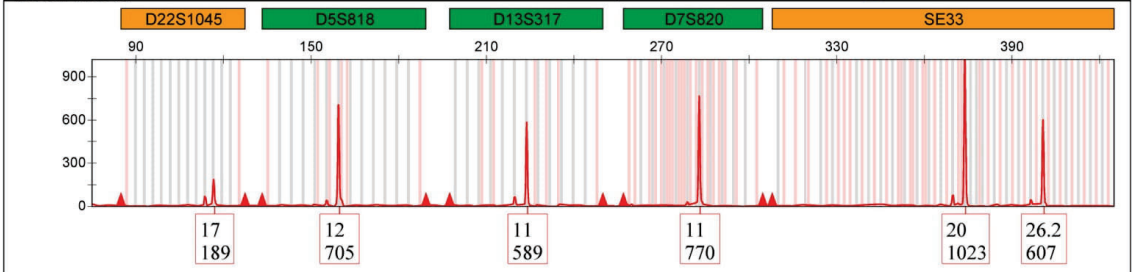


Applied Biosystems
GeneMapper® ID-X 1.4

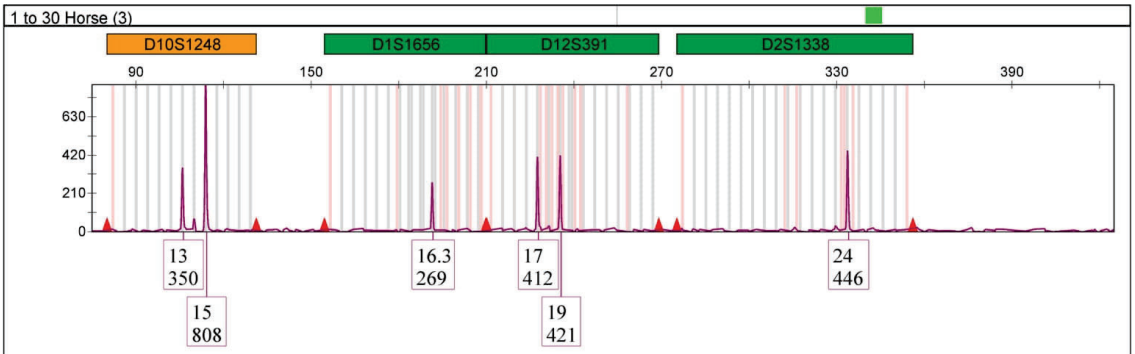
Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 30 Horse (3)



1 to 30 Horse (3)



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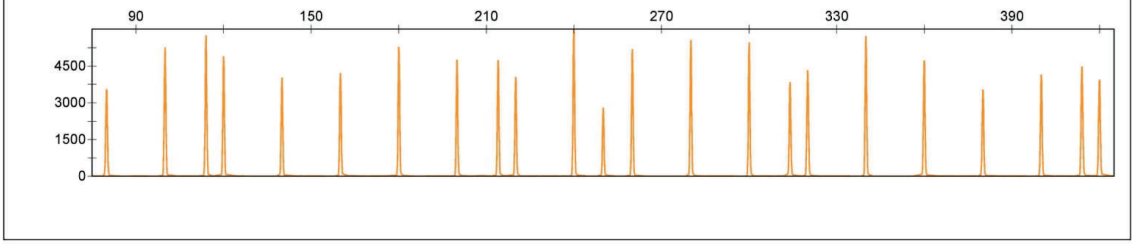
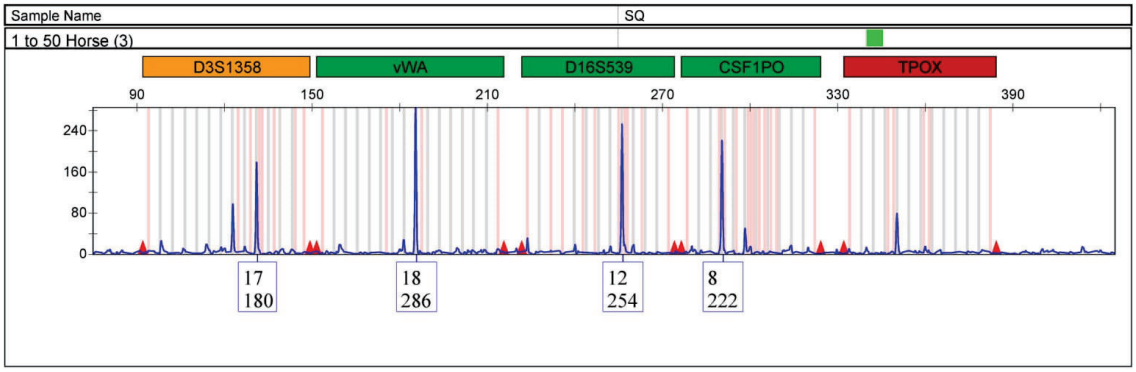


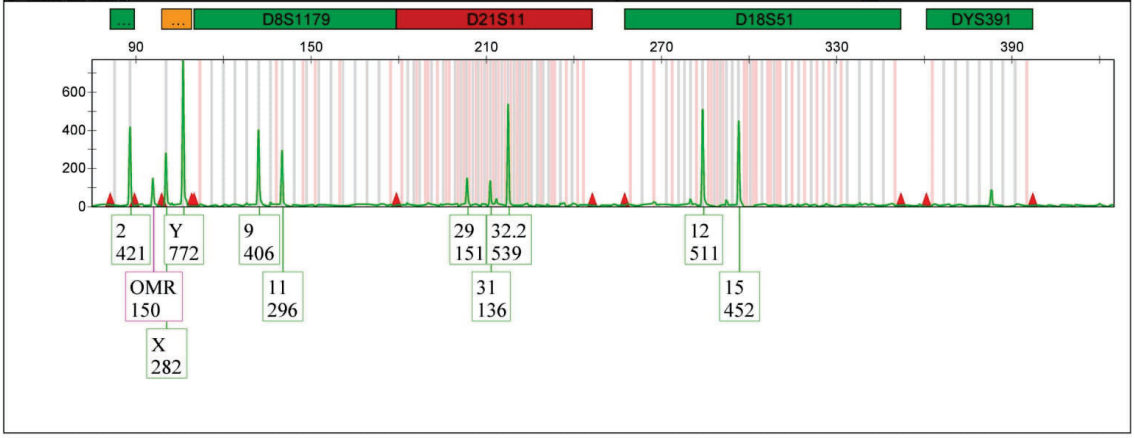
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

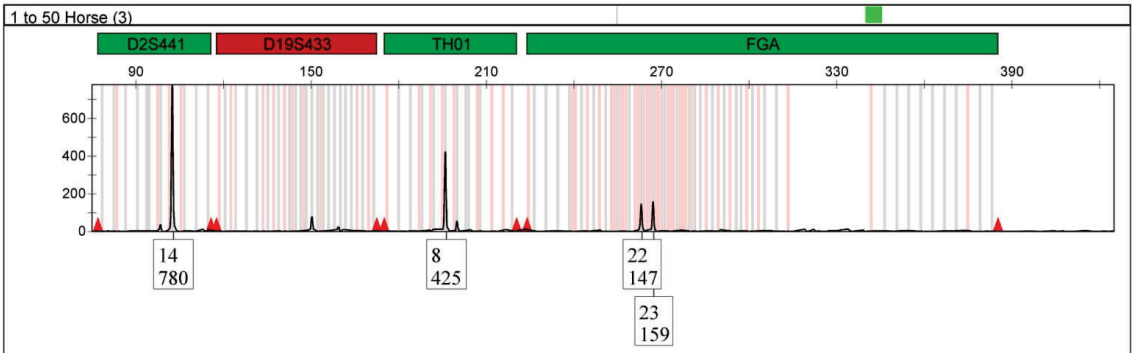
Sample Name	SQ
1 to 50 Horse (3)	



1 to 50 Horse (3)



1 to 50 Horse (3)

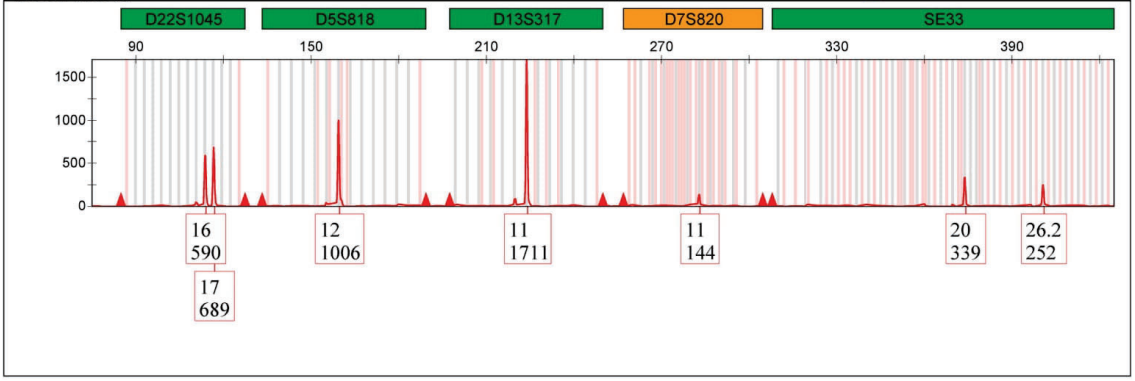


Applied Biosystems
GeneMapper® ID-X 1.4

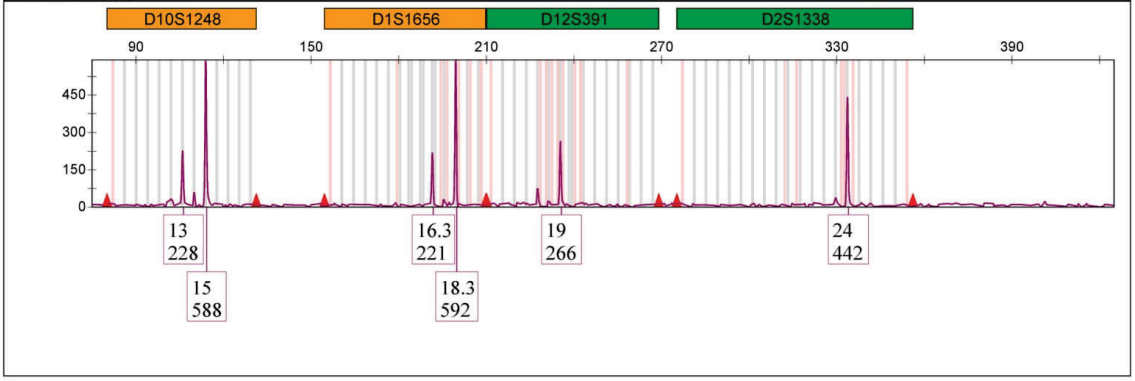
Project: Organic Extractions CRG 061422

Sample Name	SQ

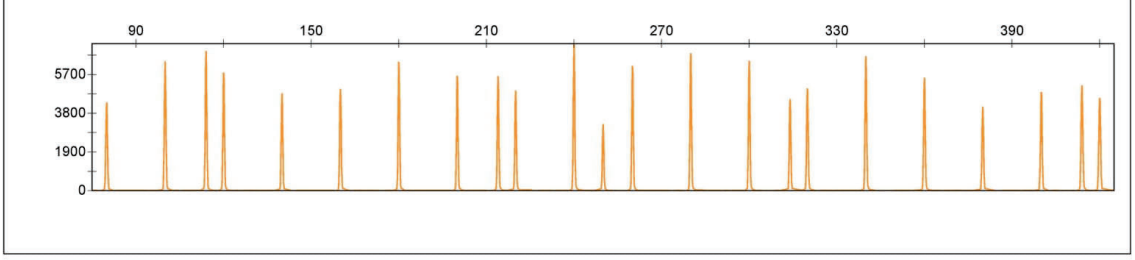
1 to 50 Horse (3)



1 to 50 Horse (3)



1 to 50 Horse (3)

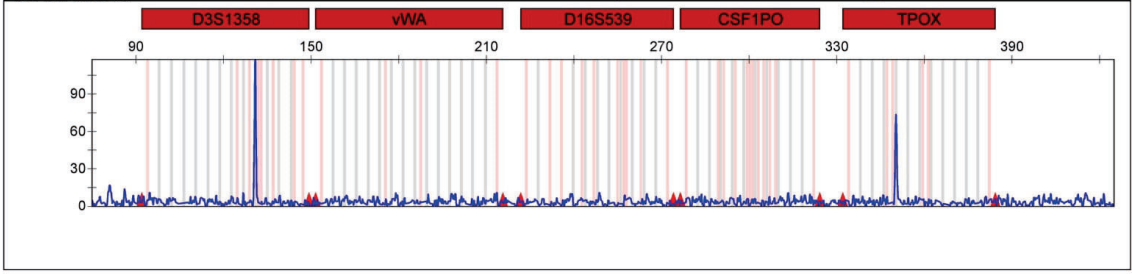


Applied Biosystems
GeneMapper® ID-X 1.4

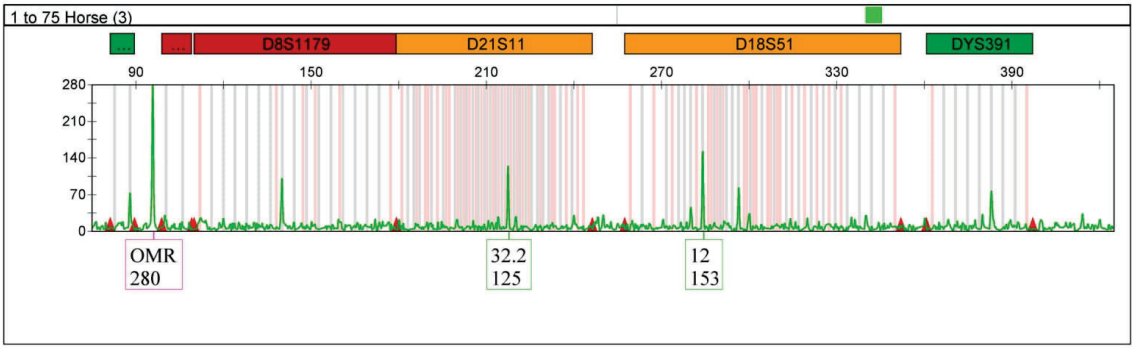
Project: Organic Extractions CRG 061422

Sample Name	SQ

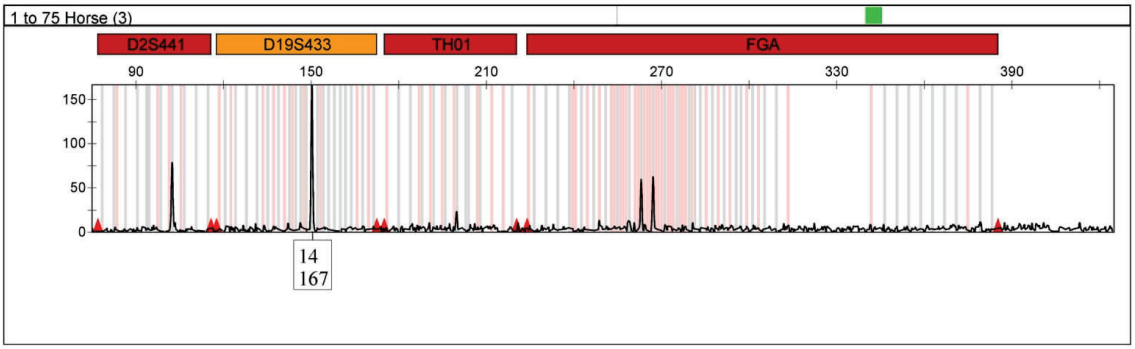
1 to 75 Horse (3)



1 to 75 Horse (3)



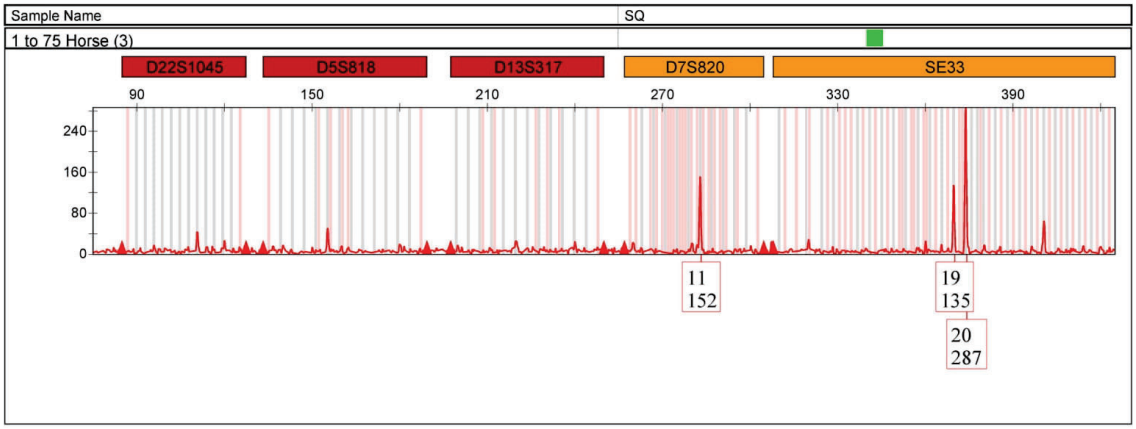
1 to 75 Horse (3)



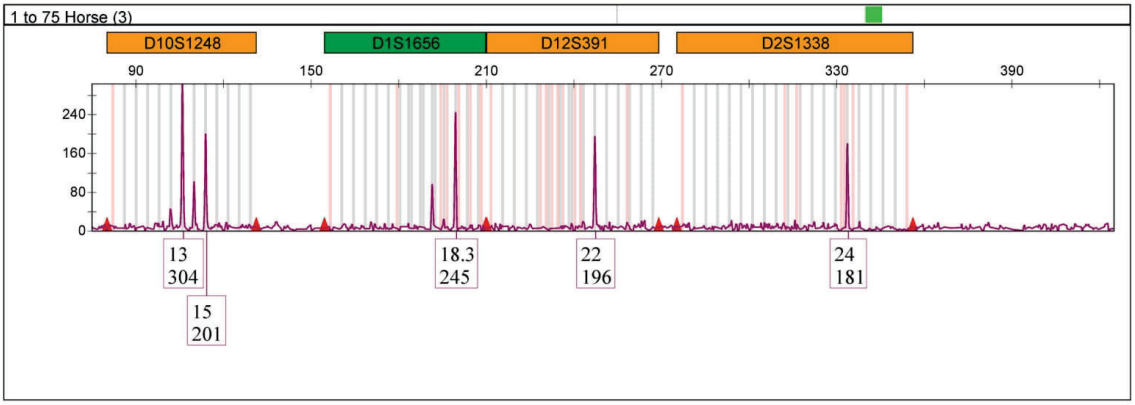
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

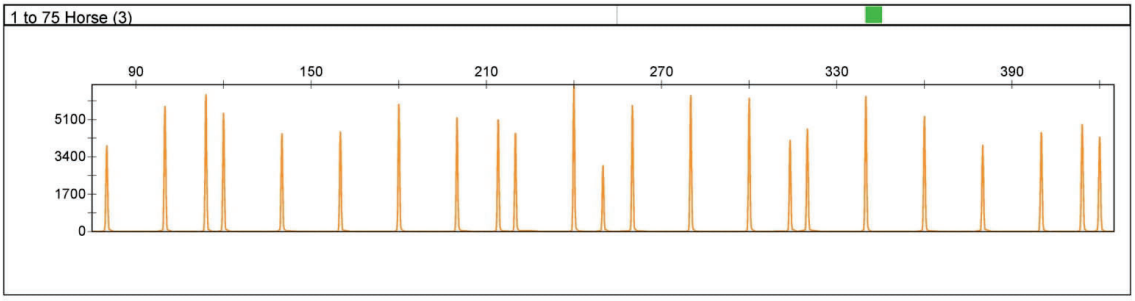
Sample Name	SQ
1 to 75 Horse (3)	



1 to 75 Horse (3)



1 to 75 Horse (3)

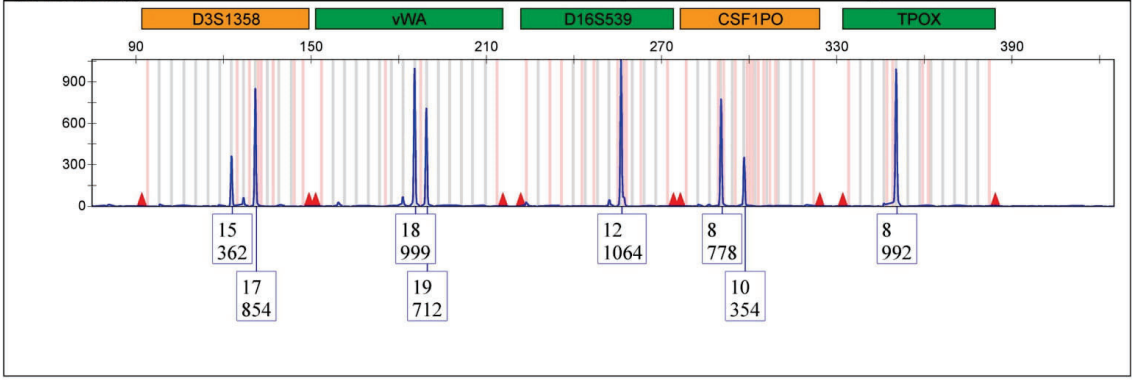


Applied Biosystems
GeneMapper® ID-X 1.4

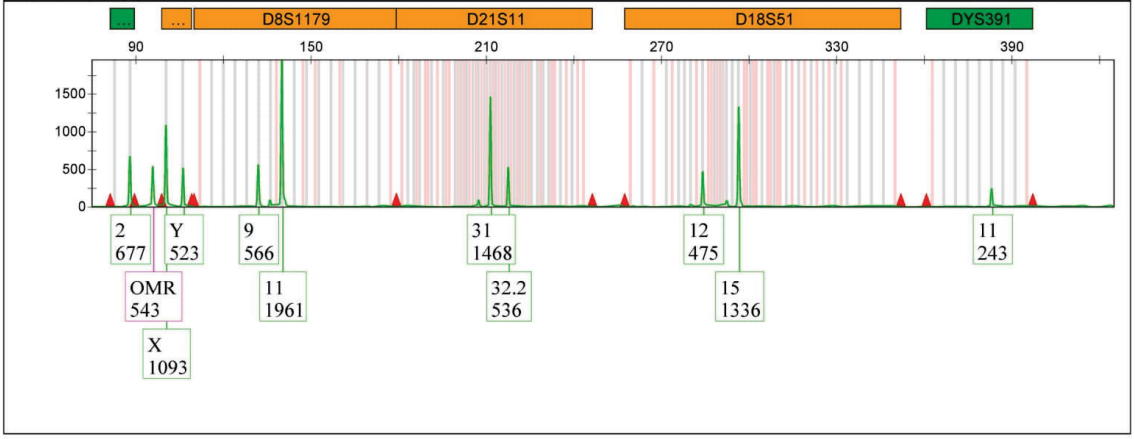
Project: Organic Extractions CRG 061422

Sample Name	SQ

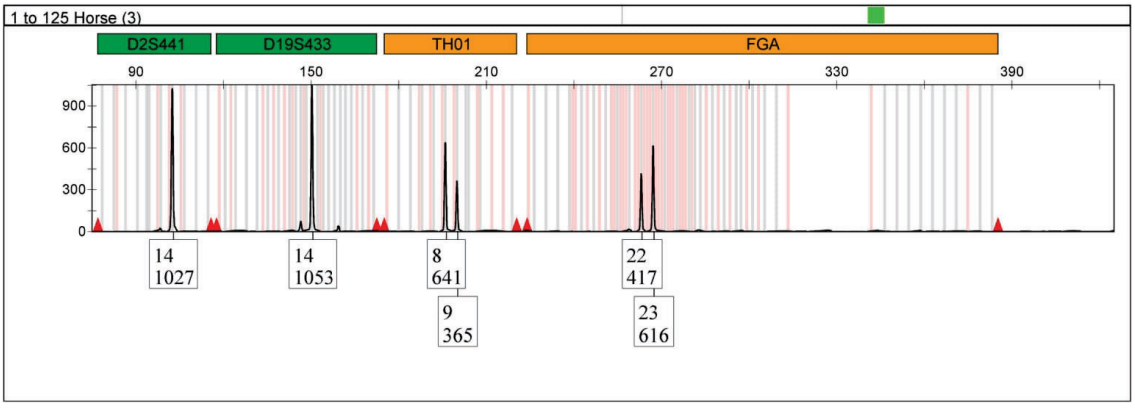
1 to 125 Horse (3)



1 to 125 Horse (3)



1 to 125 Horse (3)

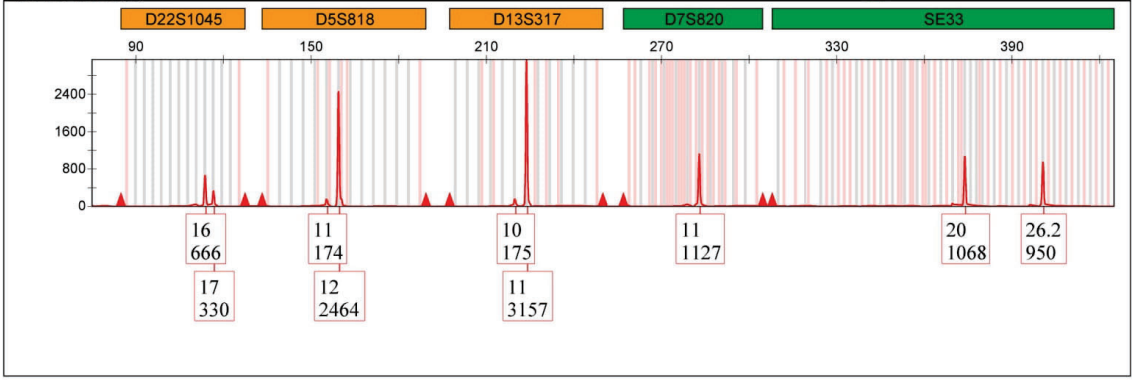


Applied Biosystems
GeneMapper® ID-X 1.4

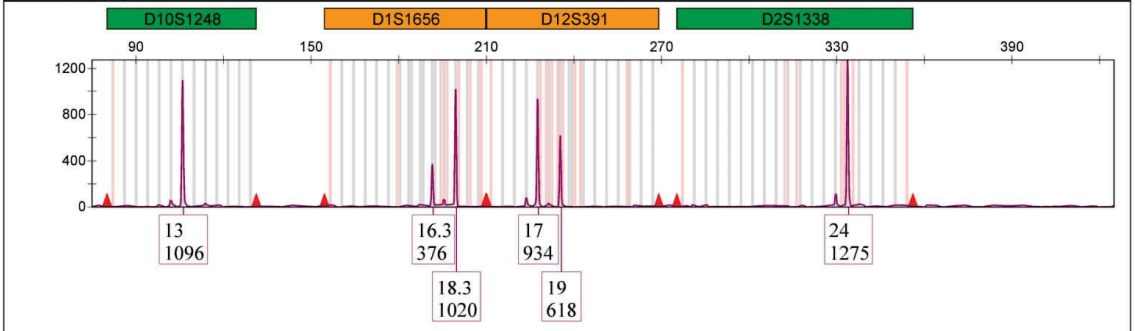
Project: Organic Extractions CRG 061422

Sample Name	SQ

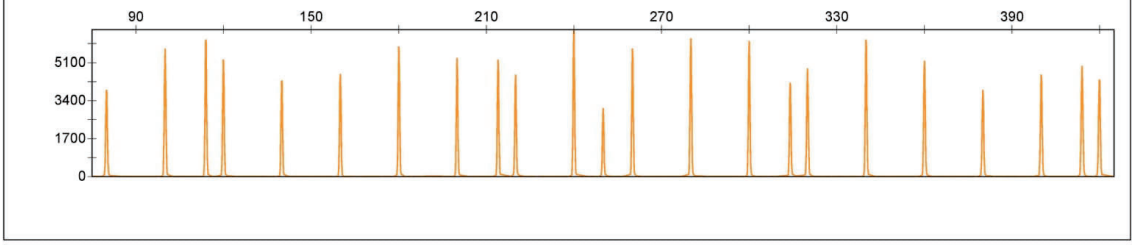
1 to 125 Horse (3)



1 to 125 Horse (3)



1 to 125 Horse (3)

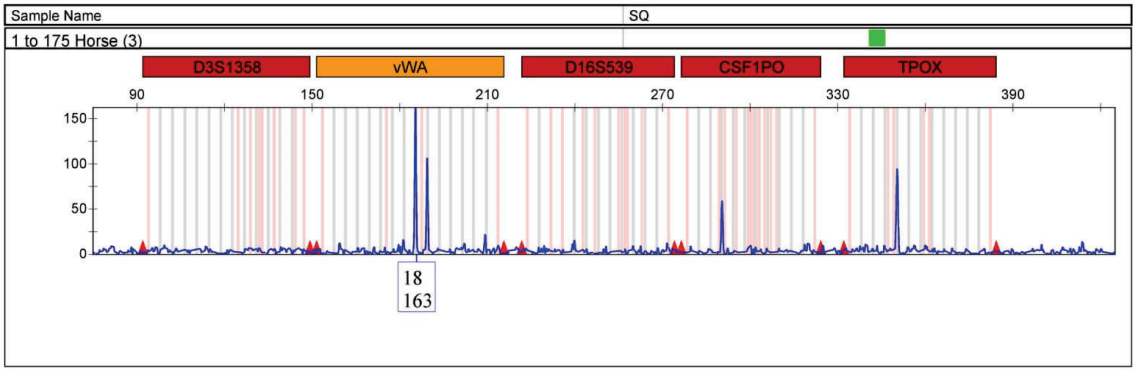


Applied Biosystems
GeneMapper® ID-X 1.4

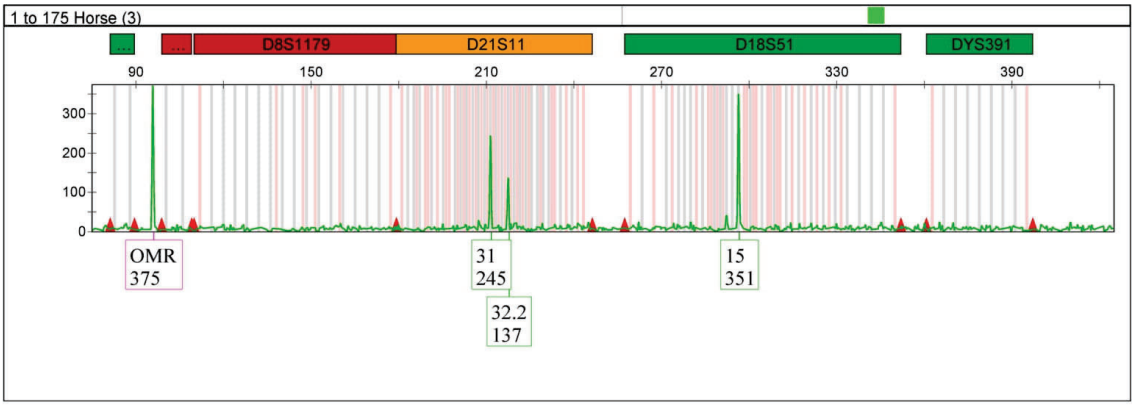
Project: Organic Extractions CRG 061422

Sample Name	SQ

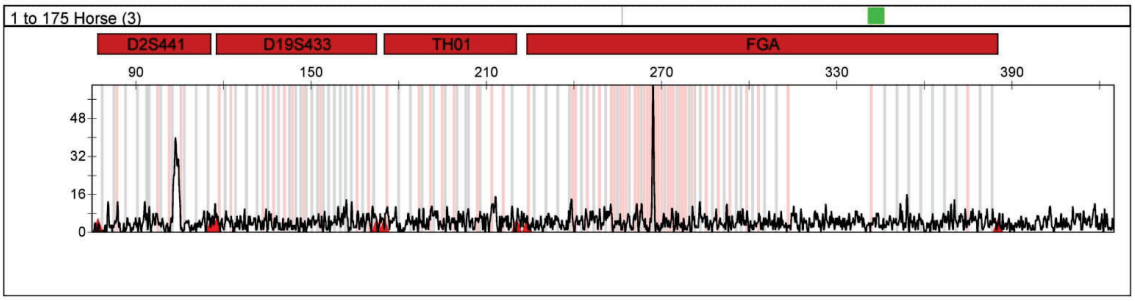
1 to 175 Horse (3)



1 to 175 Horse (3)



1 to 175 Horse (3)

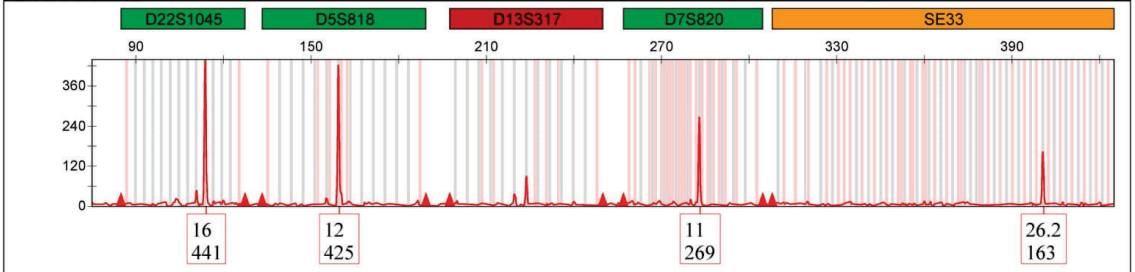


Applied Biosystems
GeneMapper® ID-X 1.4

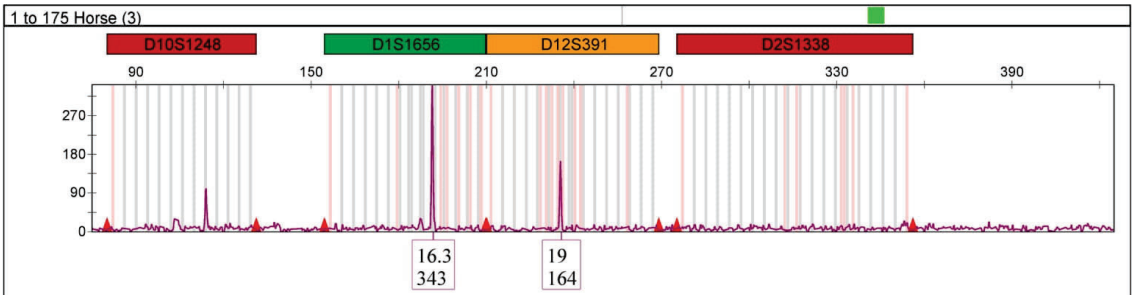
Project: Organic Extractions CRG 061422

Sample Name	SQ

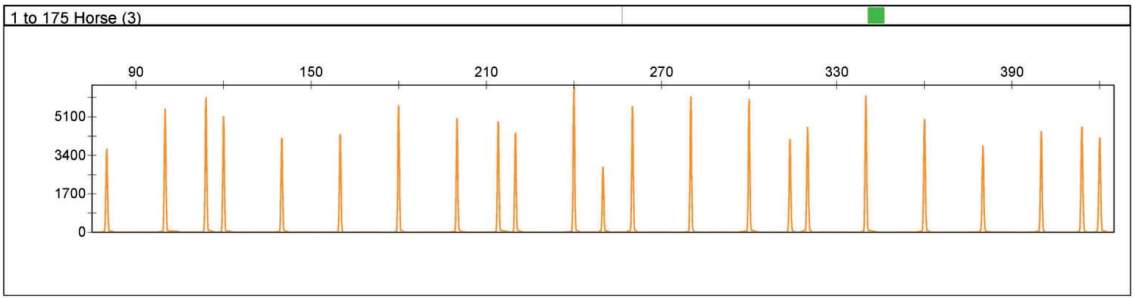
1 to 175 Horse (3)



1 to 175 Horse (3)



1 to 175 Horse (3)

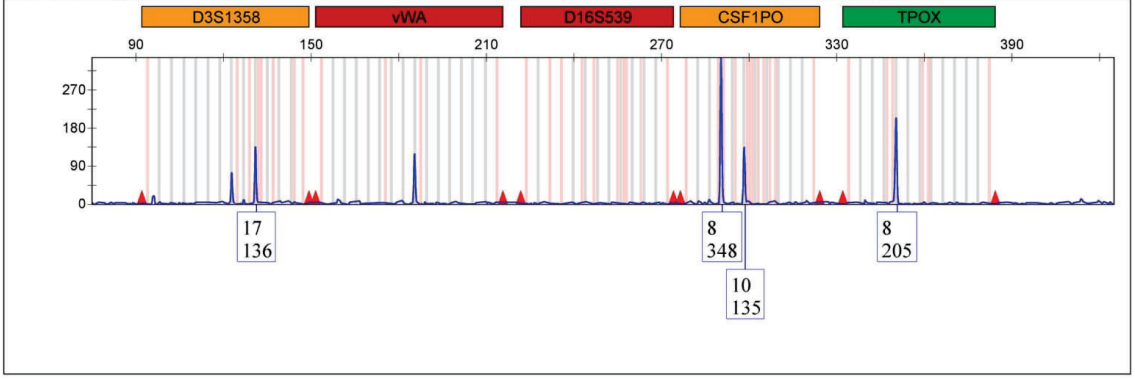


Applied Biosystems
GeneMapper® ID-X 1.4

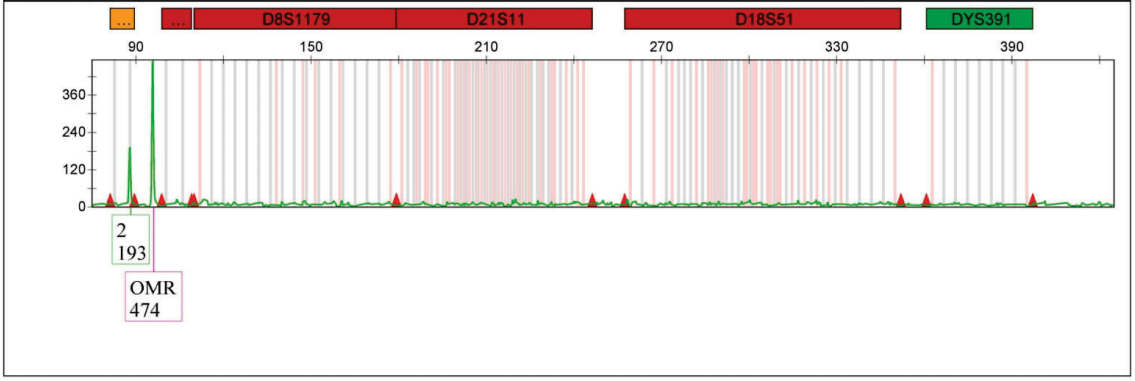
Project: Organic Extractions CRG 061422

Sample Name	SQ

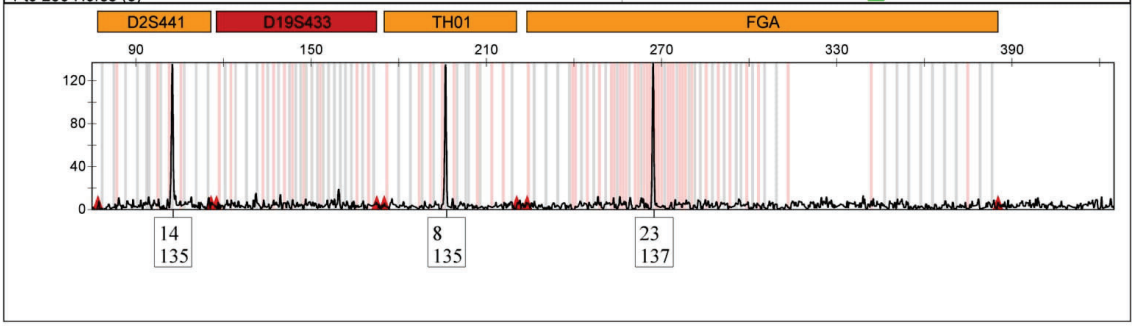
1 to 250 Horse (3)



1 to 250 Horse (3)



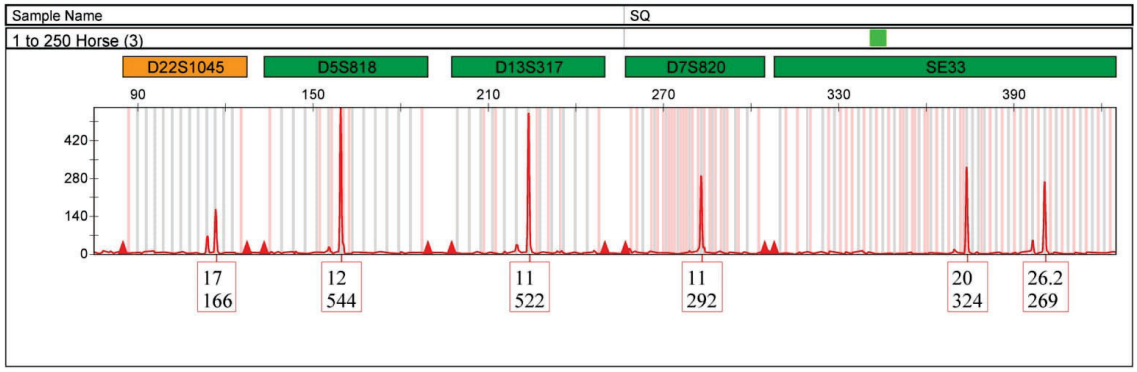
1 to 250 Horse (3)



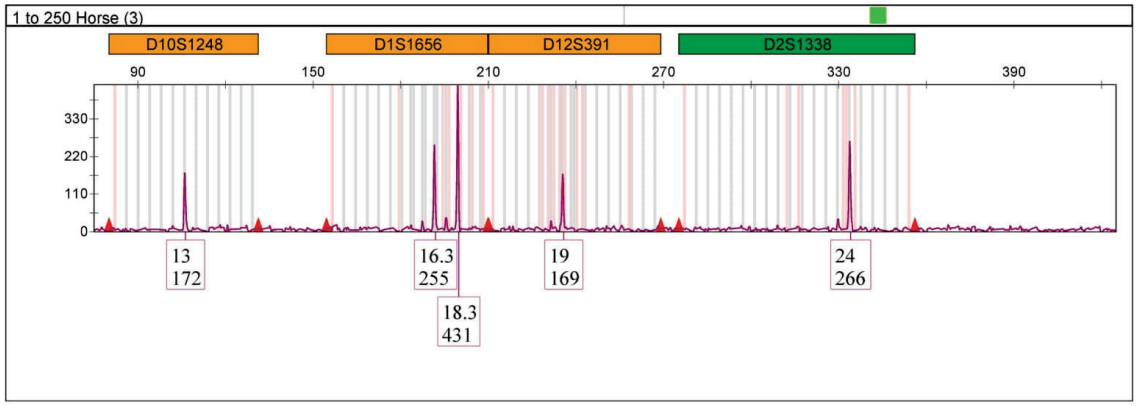
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

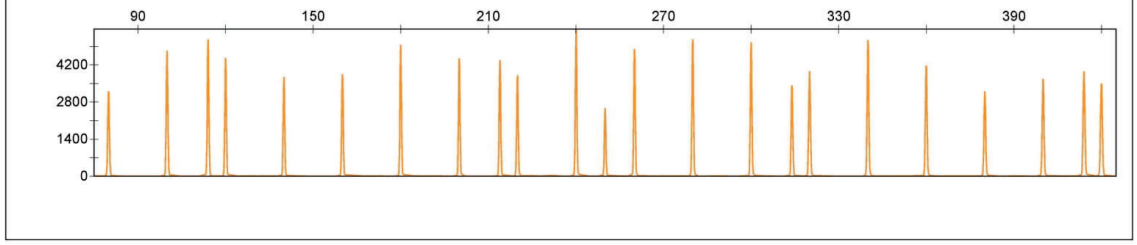
Sample Name	SQ
1 to 250 Horse (3)	



1 to 250 Horse (3)



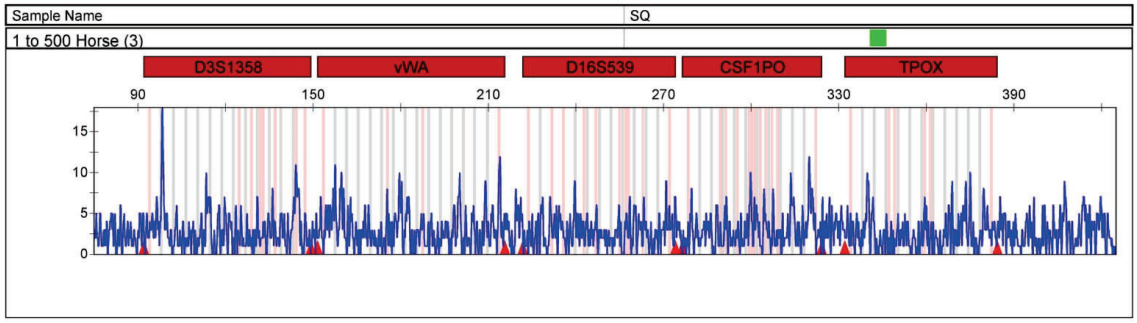
1 to 250 Horse (3)

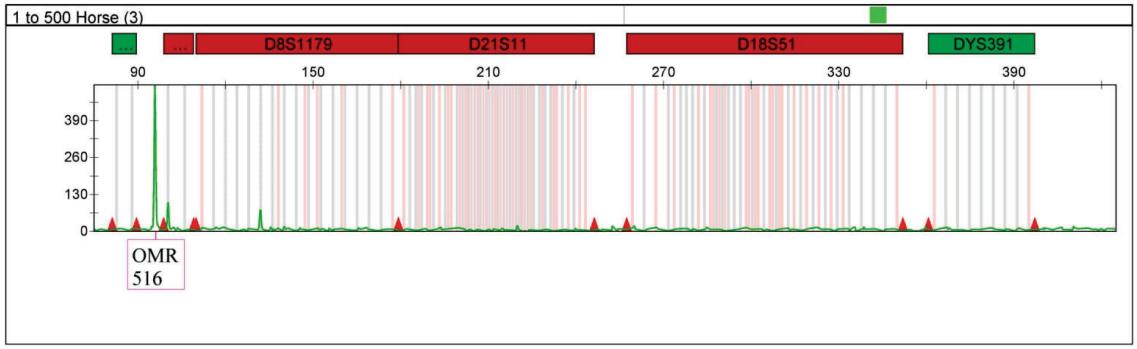


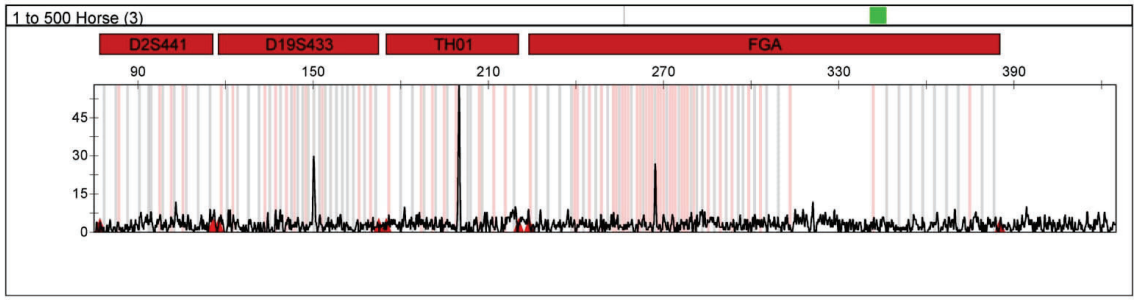
Applied Biosystems
GeneMapper® ID-X 1.4

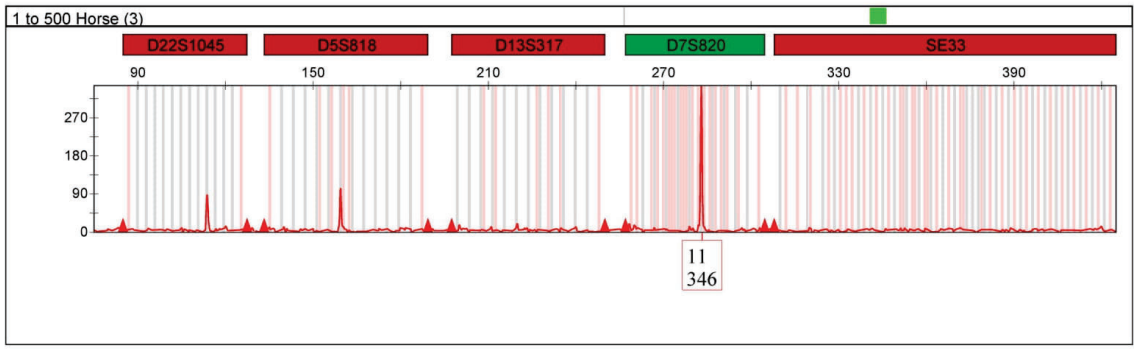
Project: Organic Extractions CRG 061422

Sample Name	SQ





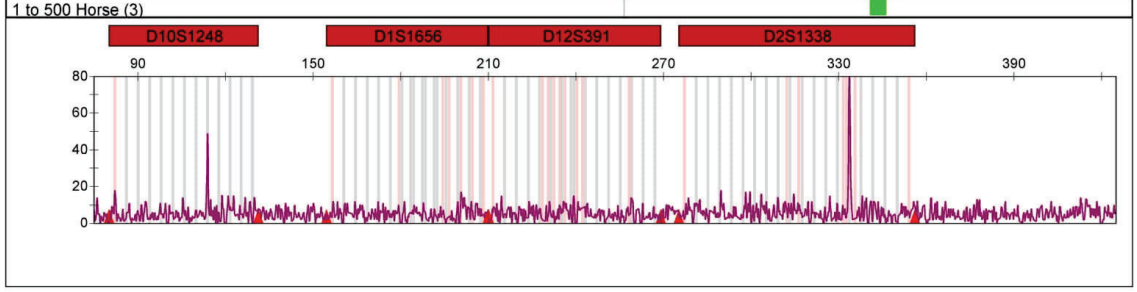




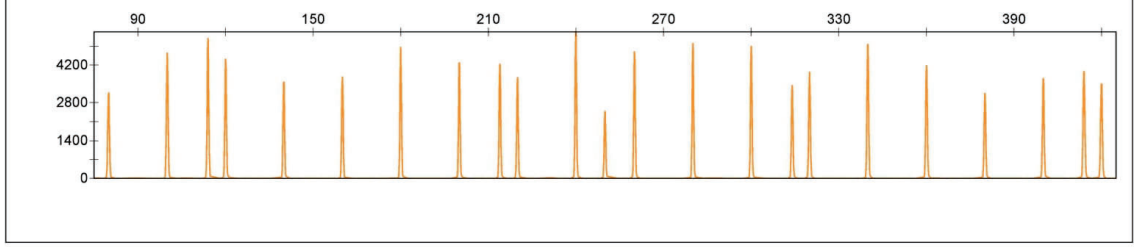
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 500 Horse (3)	



1 to 500 Horse (3)

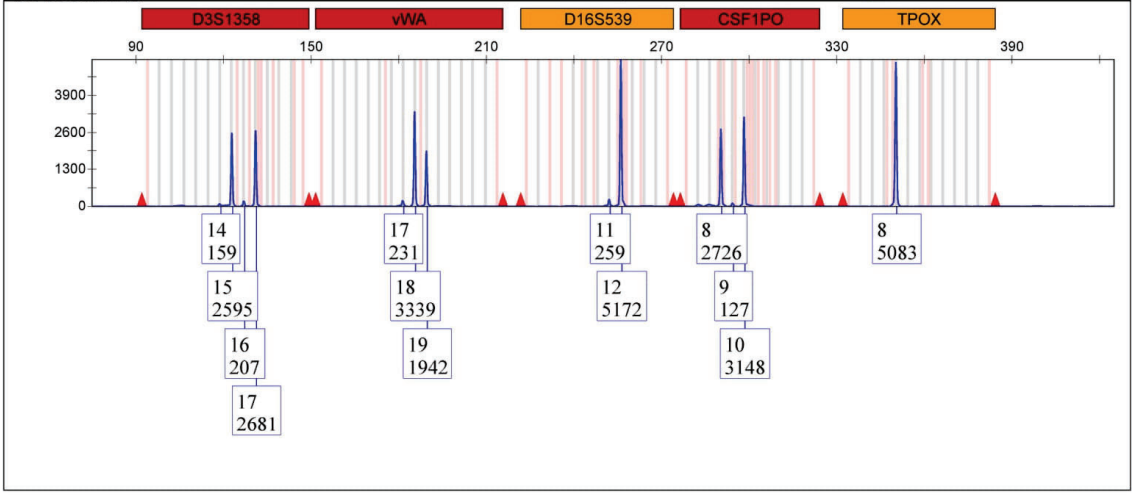


Applied Biosystems
GeneMapper® ID-X 1.4

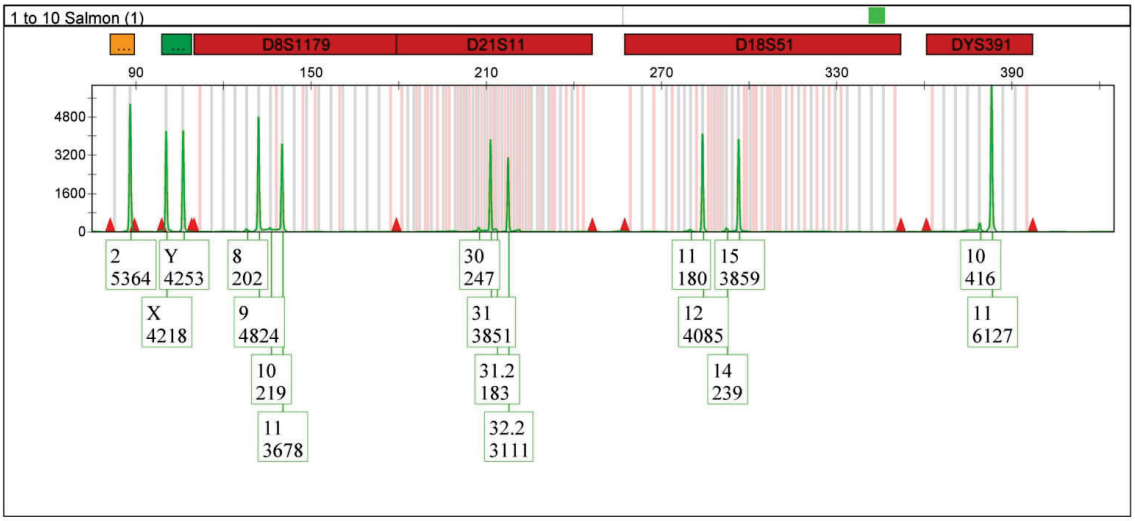
Project: Organic Extractions CRG 061422

Sample Name	SQ

1 to 10 Salmon (1)



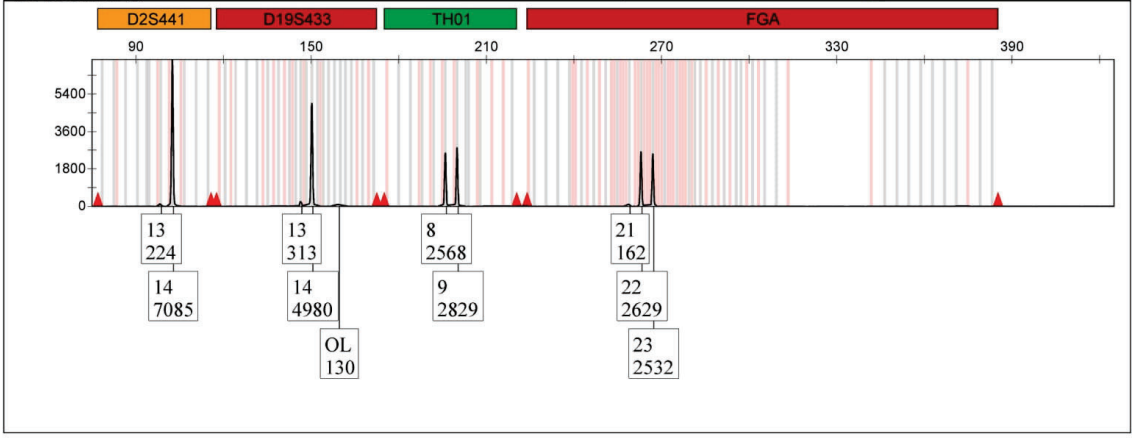
1 to 10 Salmon (1)



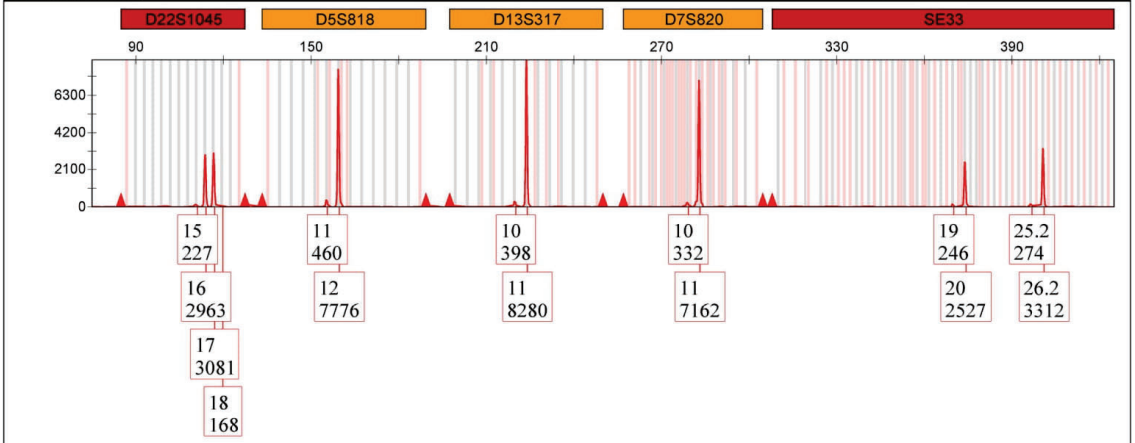
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 10 Salmon (1)	



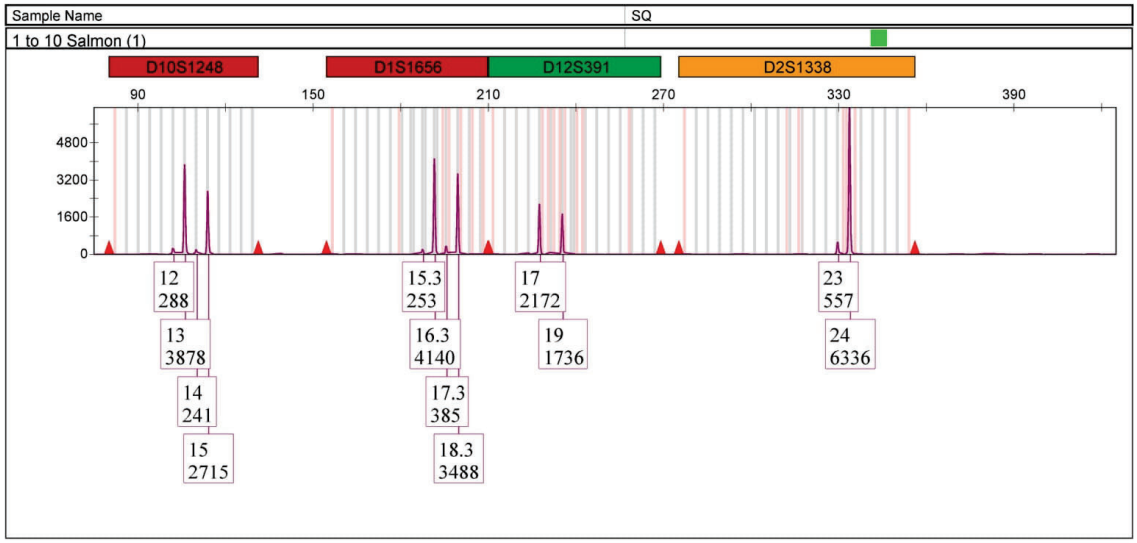
1 to 10 Salmon (1)



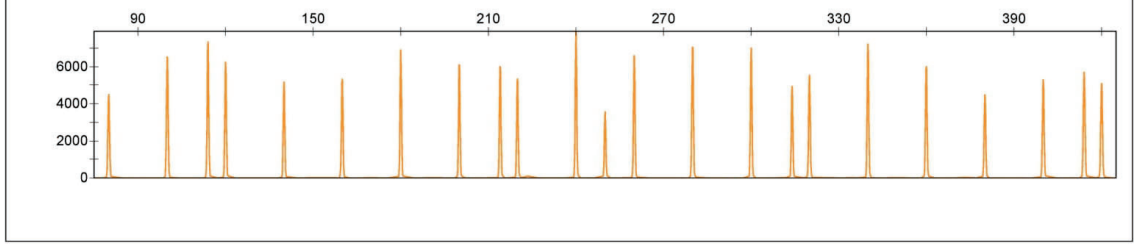
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 10 Salmon (1)	



1 to 10 Salmon (1)

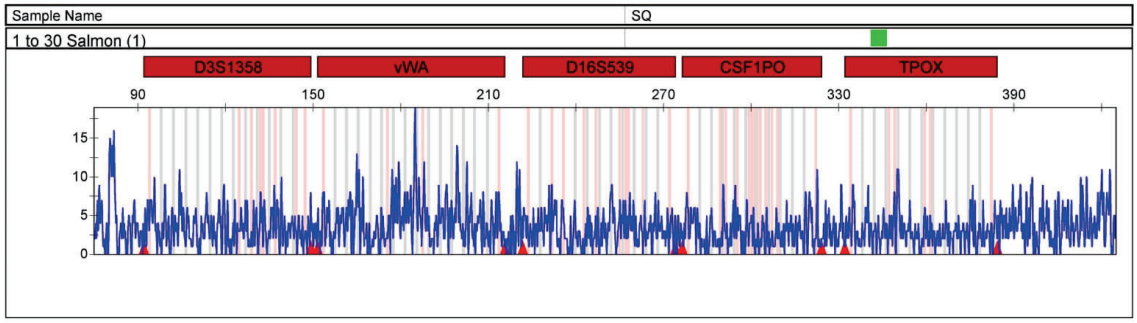


Applied Biosystems
GeneMapper® ID-X 1.4

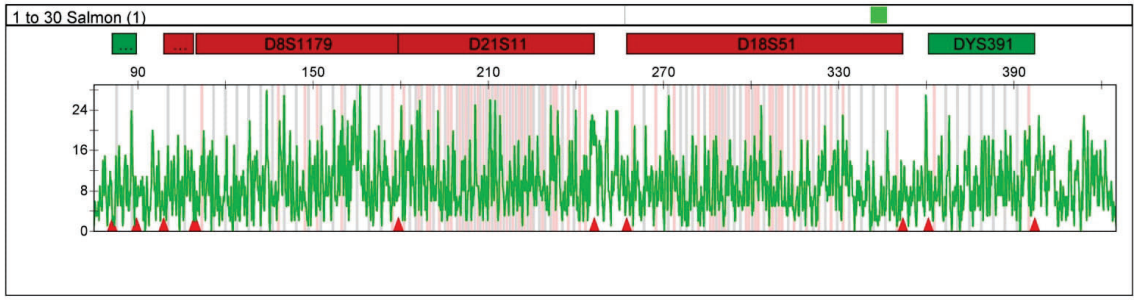
Project: Organic Extractions CRG 061422

Sample Name	SQ

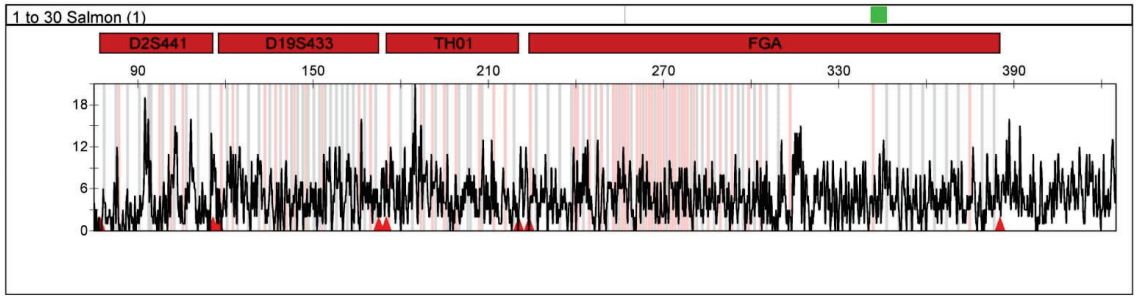
1 to 30 Salmon (1)



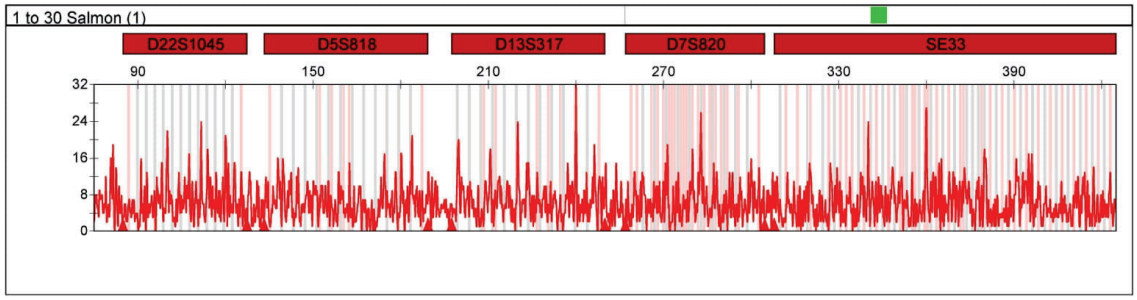
1 to 30 Salmon (1)



1 to 30 Salmon (1)



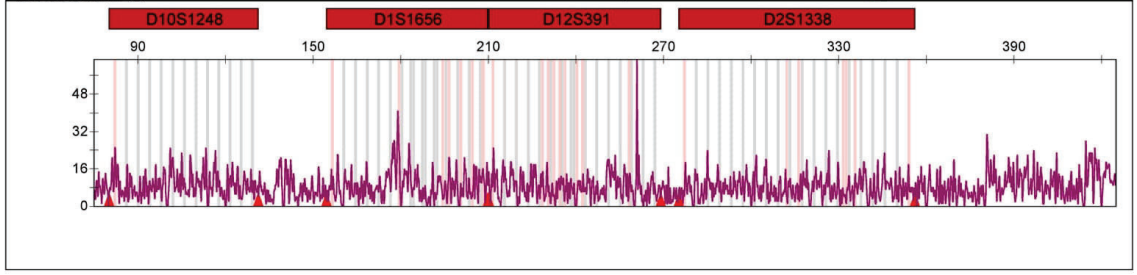
1 to 30 Salmon (1)



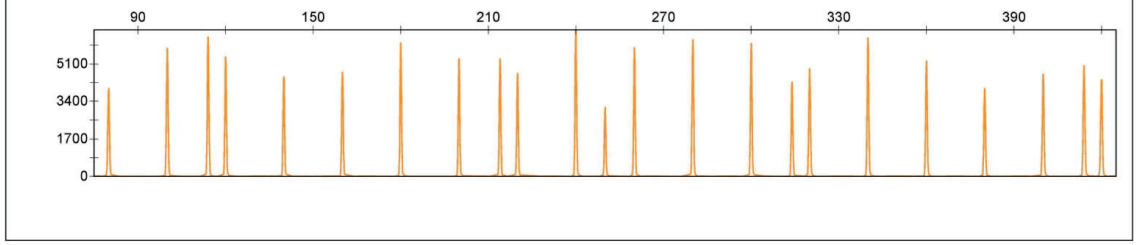
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 30 Salmon (1)	



1 to 30 Salmon (1)

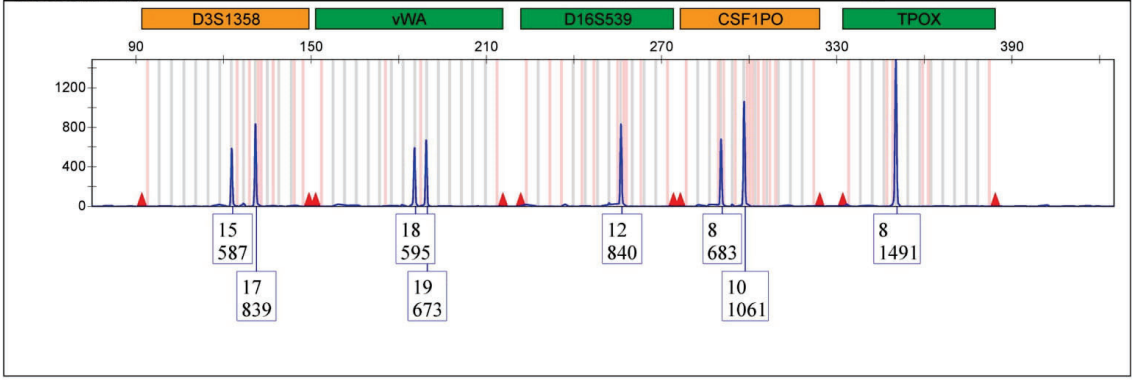


Applied Biosystems
GeneMapper® ID-X 1.4

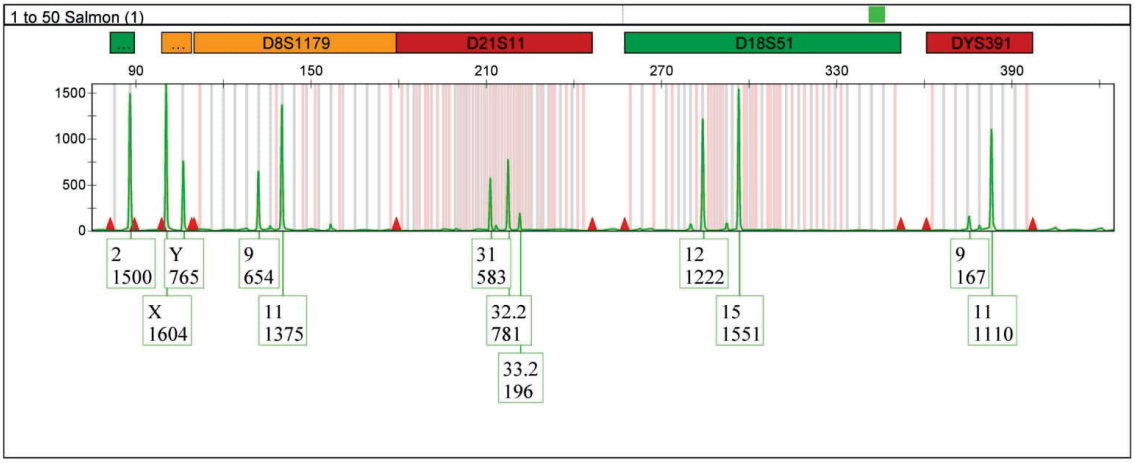
Project: Organic Extractions CRG 061422

Sample Name	SQ

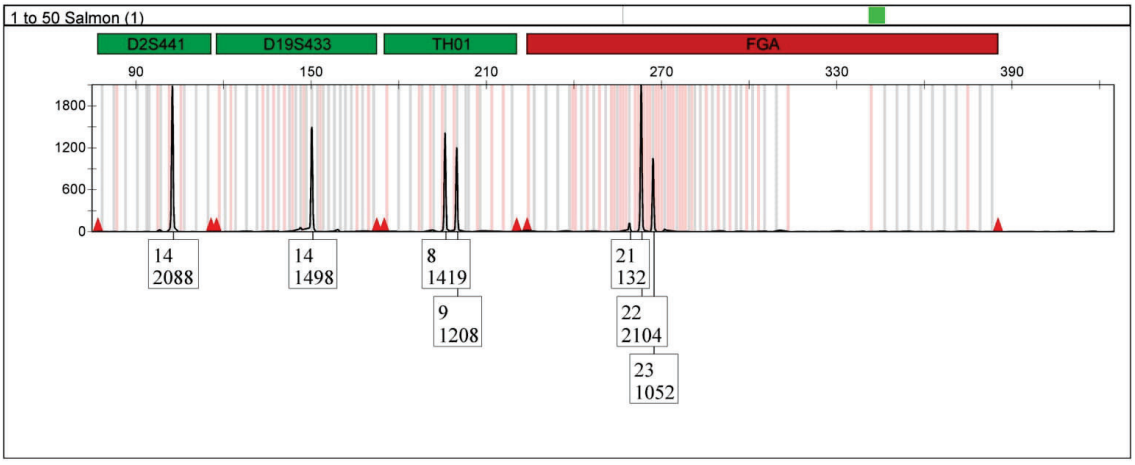
1 to 50 Salmon (1)



1 to 50 Salmon (1)



1 to 50 Salmon (1)

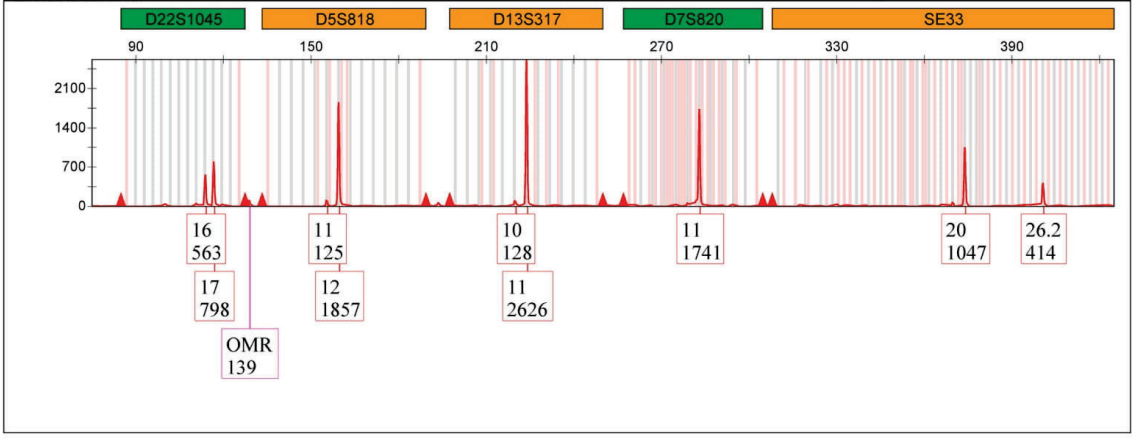


Applied Biosystems
GeneMapper® ID-X 1.4

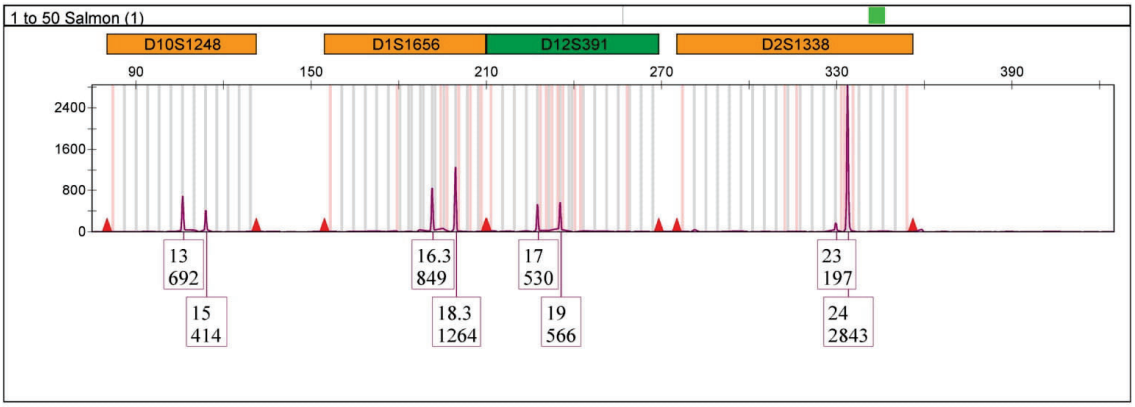
Project: Organic Extractions CRG 061422

Sample Name	SQ

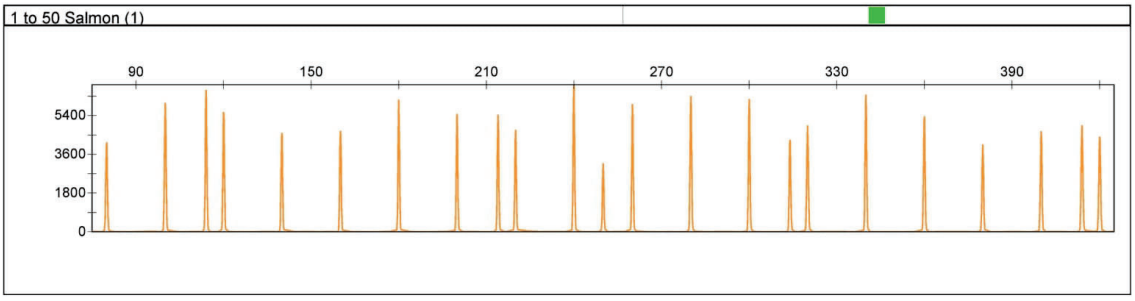
1 to 50 Salmon (1)



1 to 50 Salmon (1)



1 to 50 Salmon (1)

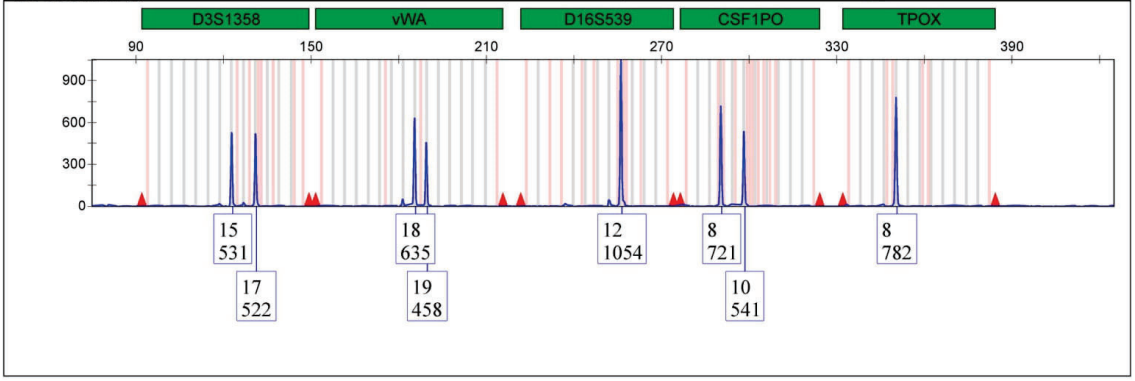


Applied Biosystems
GeneMapper® ID-X 1.4

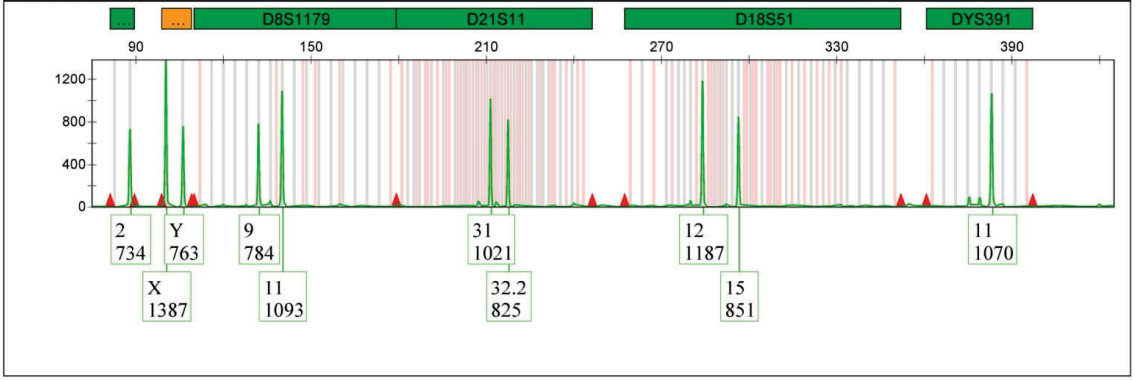
Project: Organic Extractions CRG 061422

Sample Name	SQ

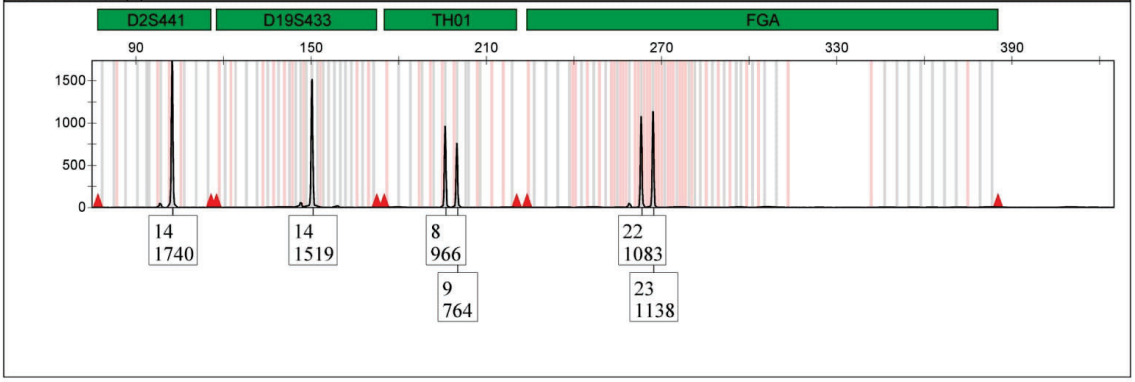
1 to 75 Salmon (1)



1 to 75 Salmon (1)



1 to 75 Salmon (1)

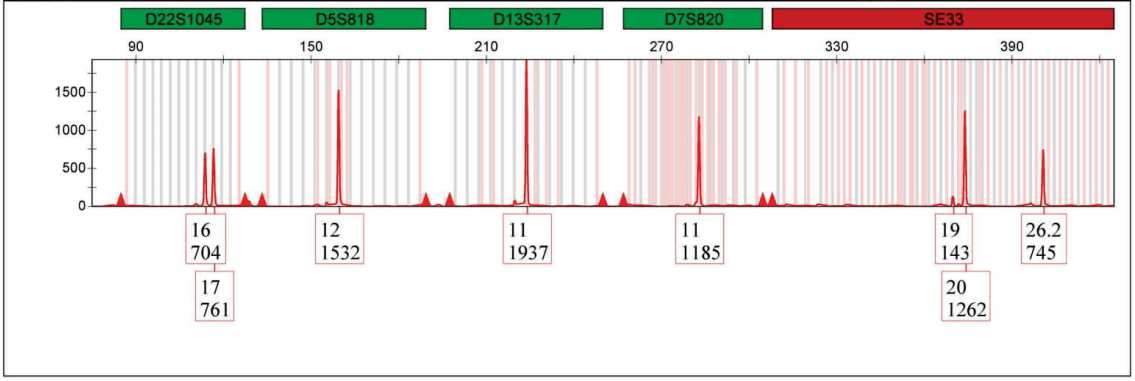


Applied Biosystems
GeneMapper® ID-X 1.4

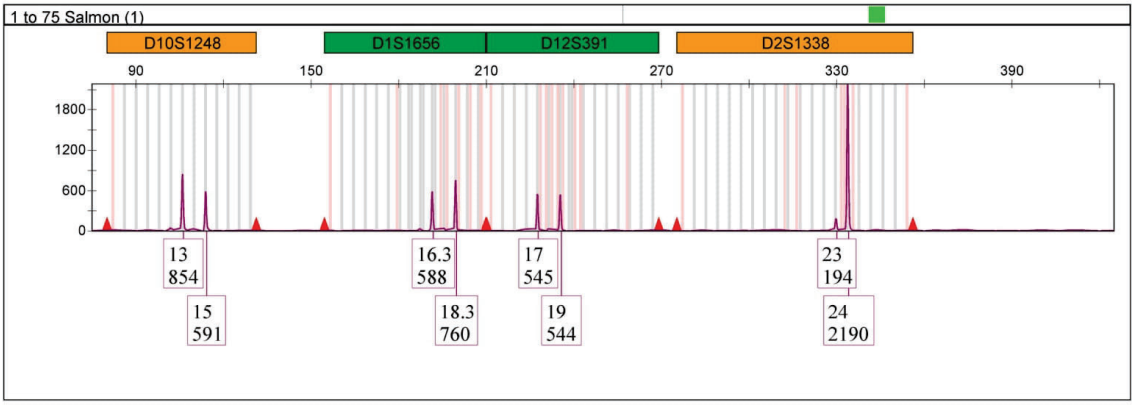
Project: Organic Extractions CRG 061422

Sample Name	SQ

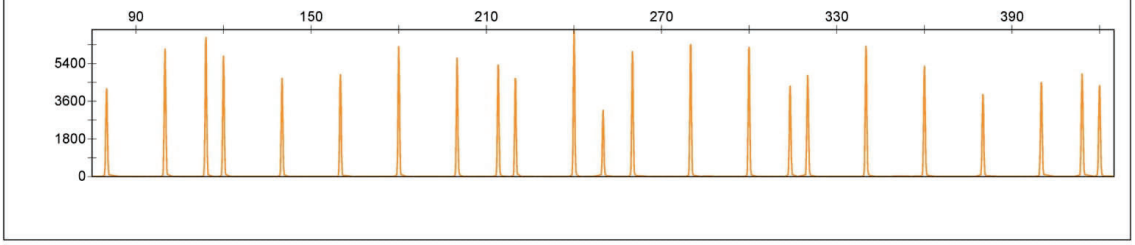
1 to 75 Salmon (1)



1 to 75 Salmon (1)



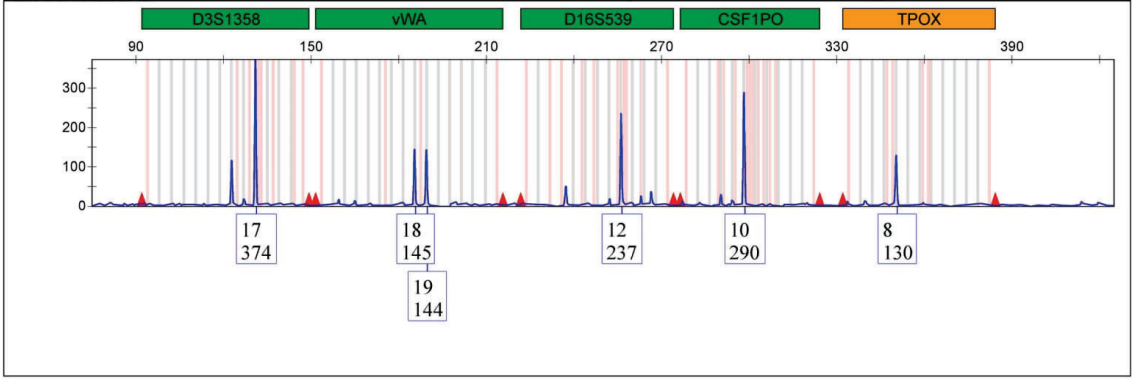
1 to 75 Salmon (1)



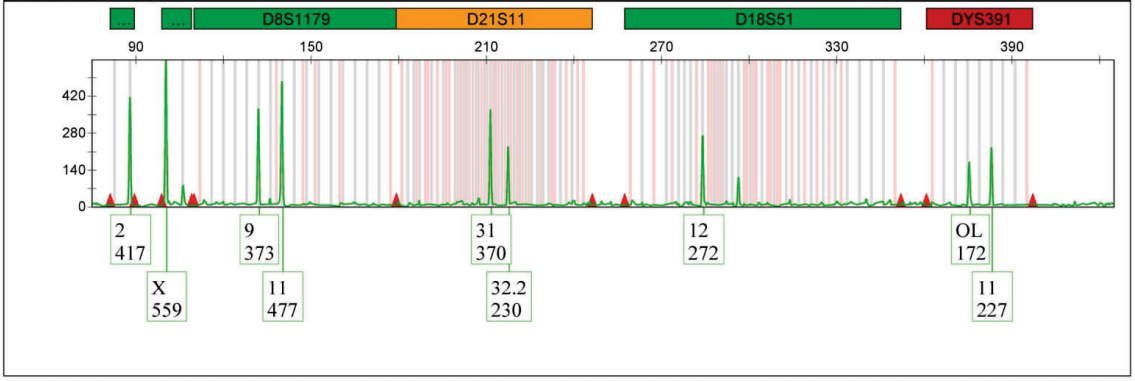
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

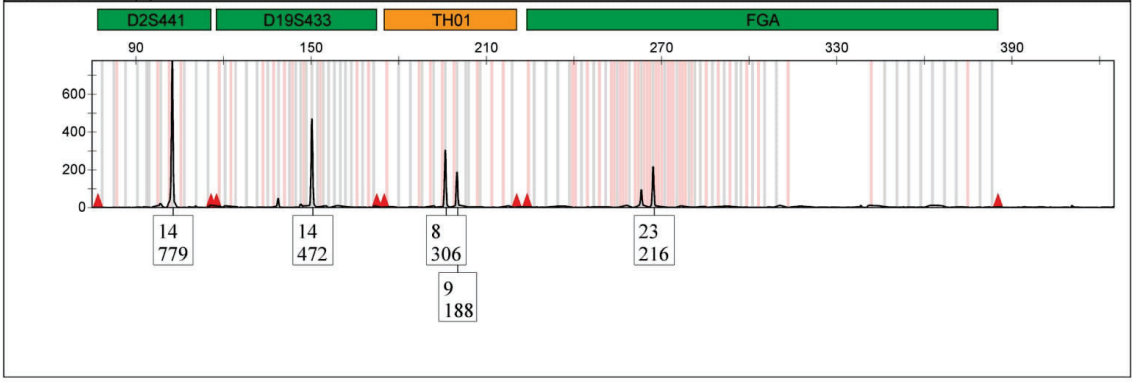
Sample Name	SQ
1 to 125 Salmon (1)	



1 to 125 Salmon (1)



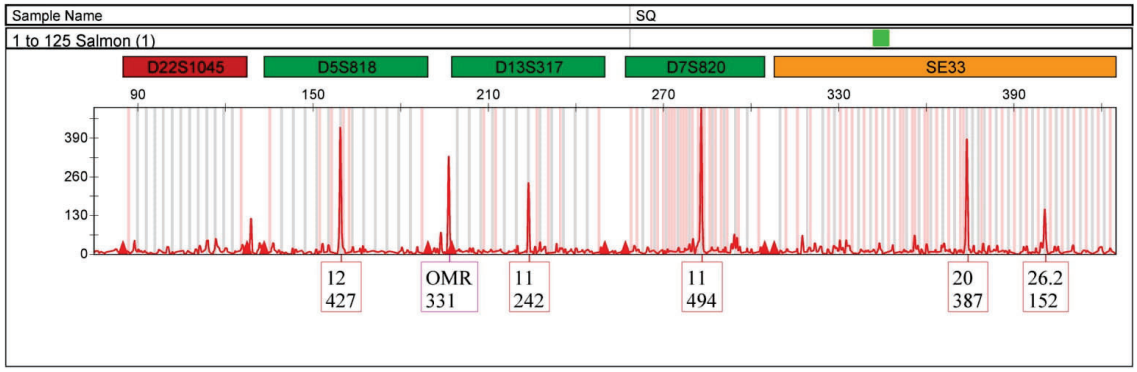
1 to 125 Salmon (1)



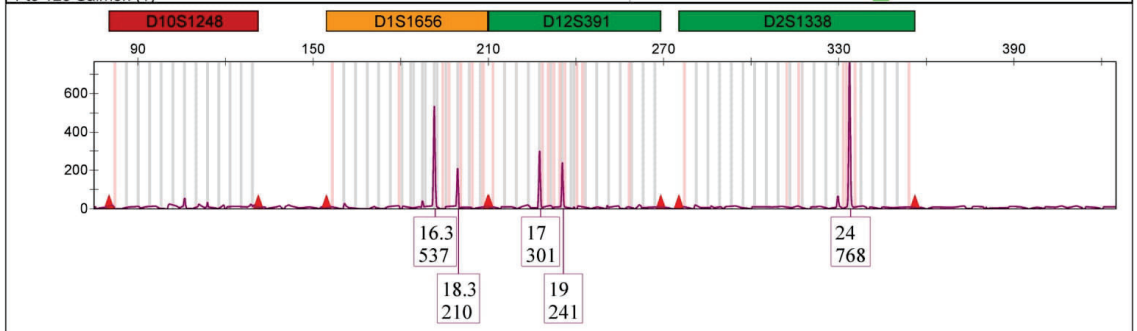
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

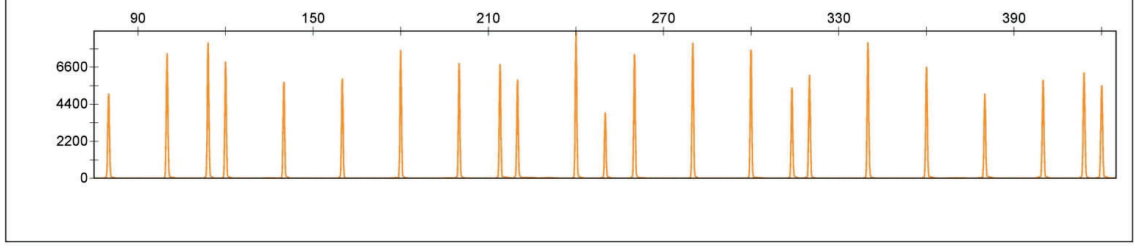
Sample Name	SQ
1 to 125 Salmon (1)	



1 to 125 Salmon (1)



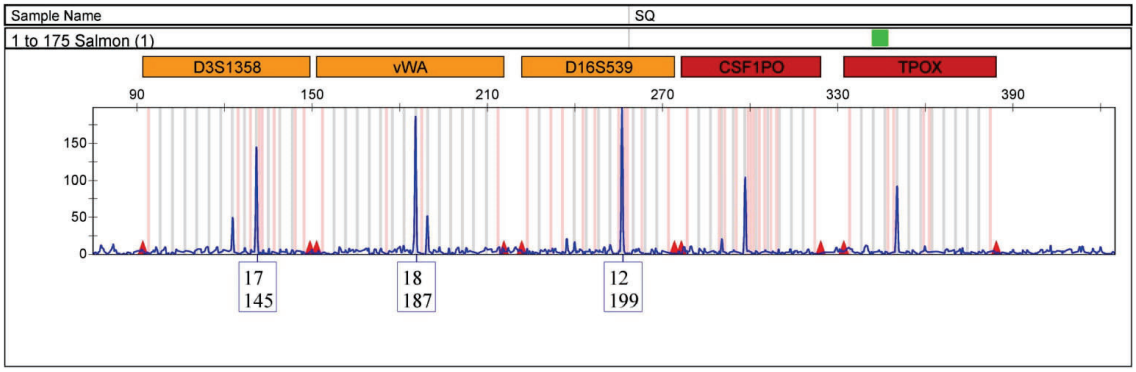
1 to 125 Salmon (1)

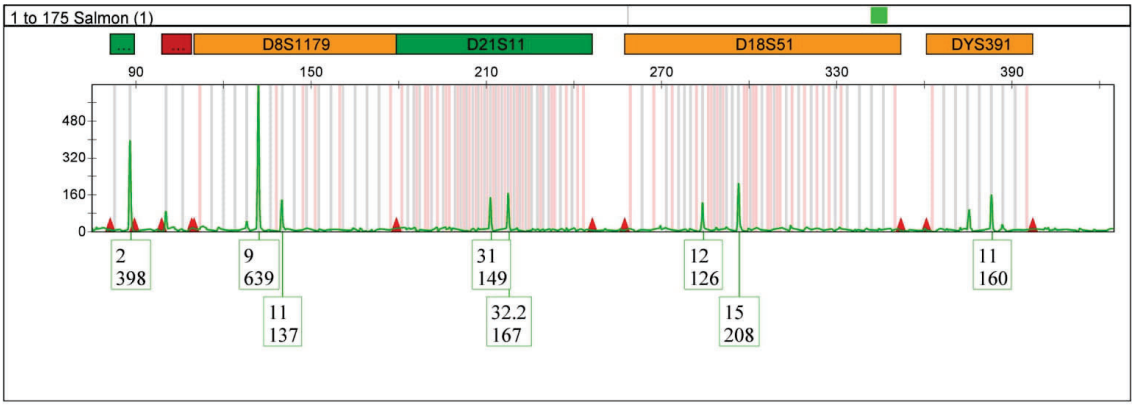


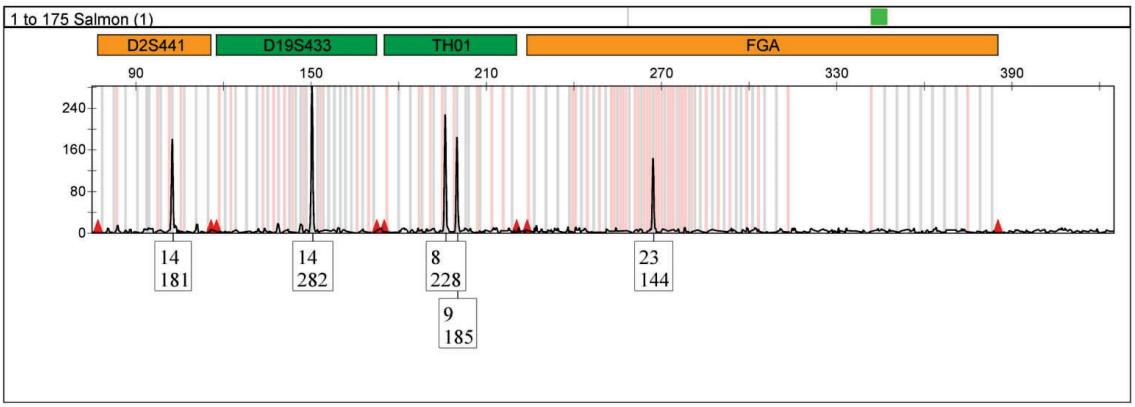
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 175 Salmon (1)	





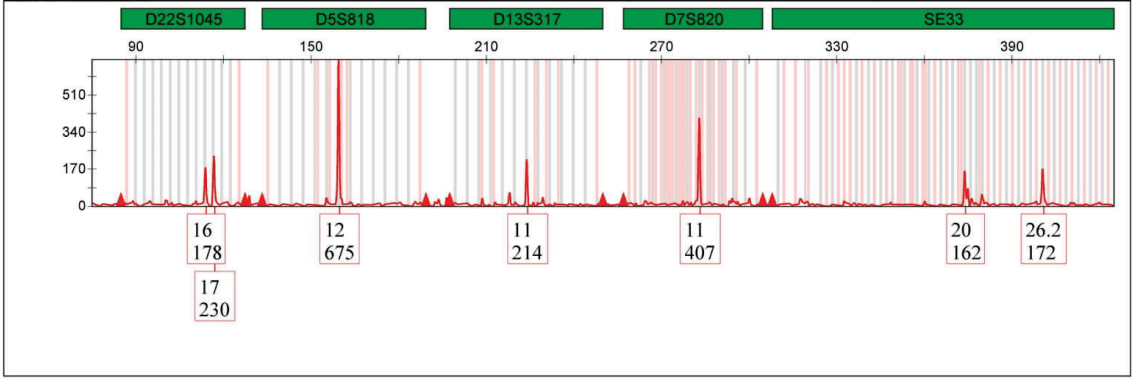


Applied Biosystems
GeneMapper® ID-X 1.4

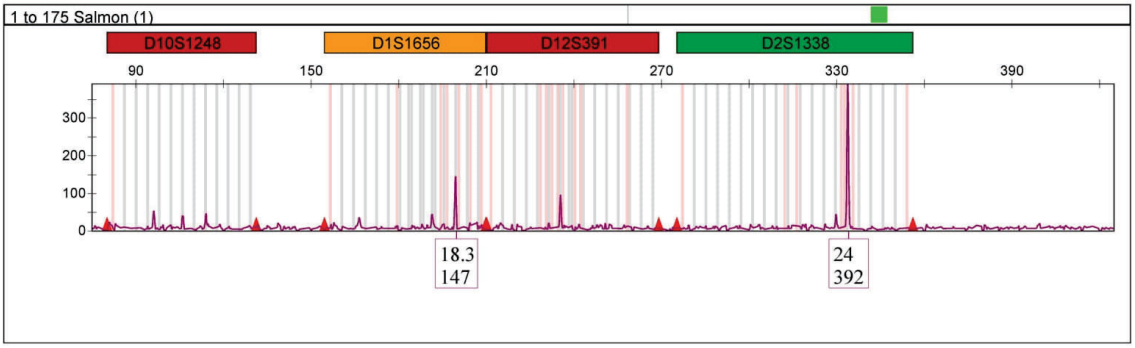
Project: Organic Extractions CRG 061422

Sample Name	SQ

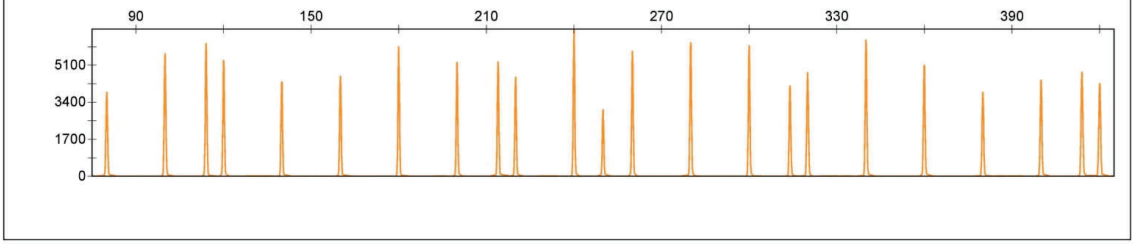
1 to 175 Salmon (1)



1 to 175 Salmon (1)



1 to 175 Salmon (1)

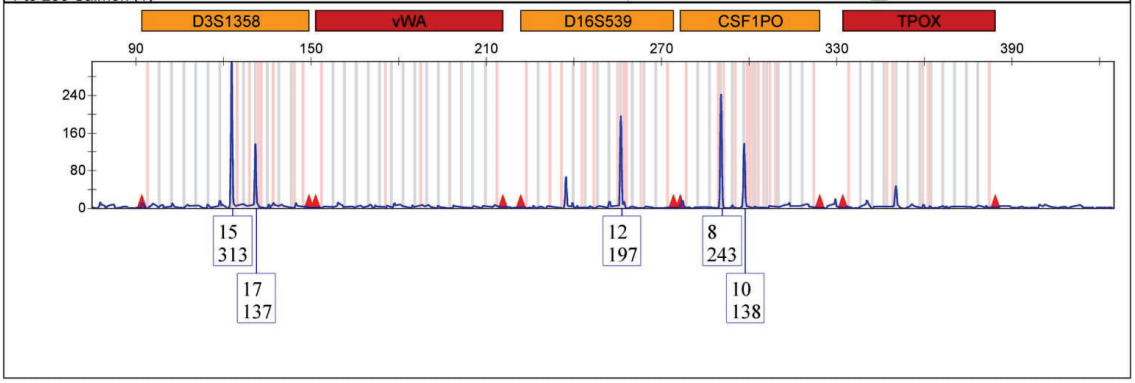


Applied Biosystems
GeneMapper® ID-X 1.4

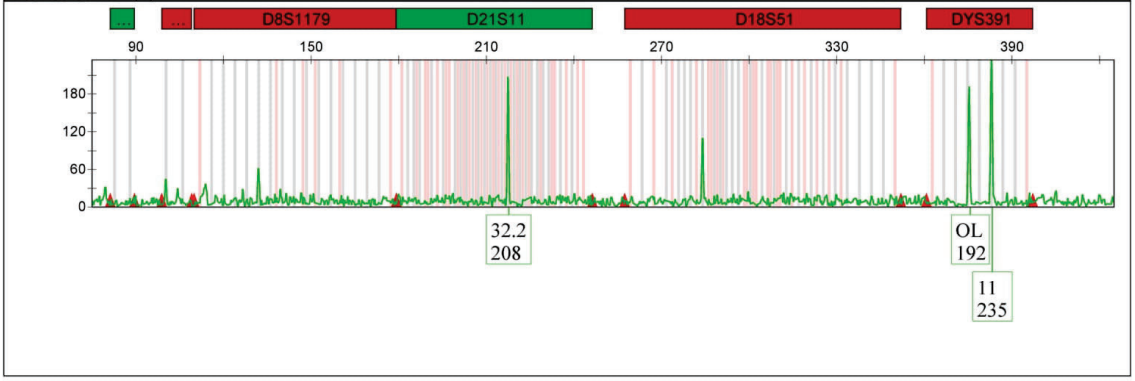
Project: Organic Extractions CRG 061422

Sample Name	SQ

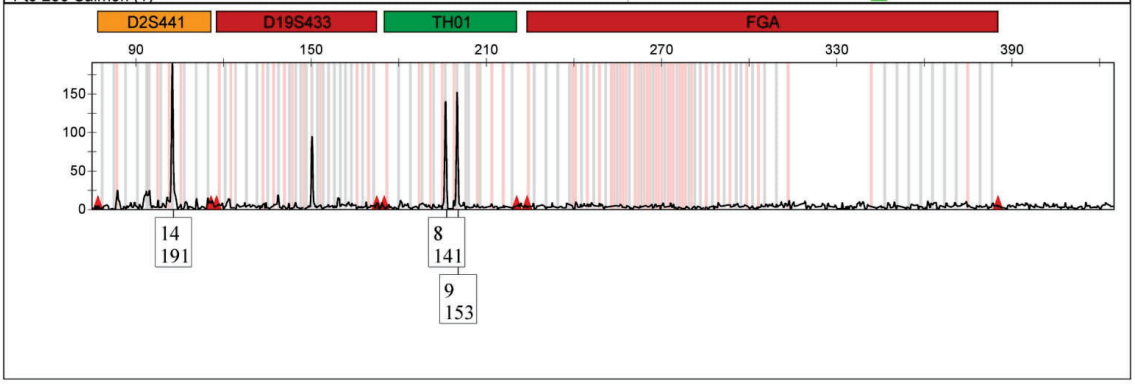
1 to 250 Salmon (1)



1 to 250 Salmon (1)



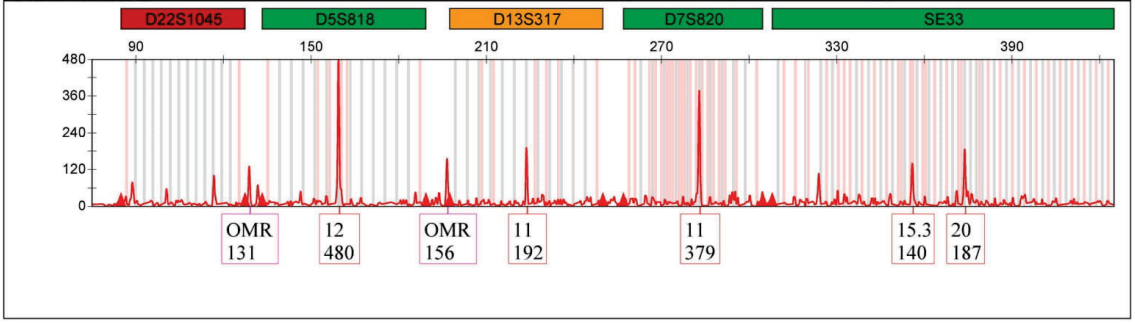
1 to 250 Salmon (1)



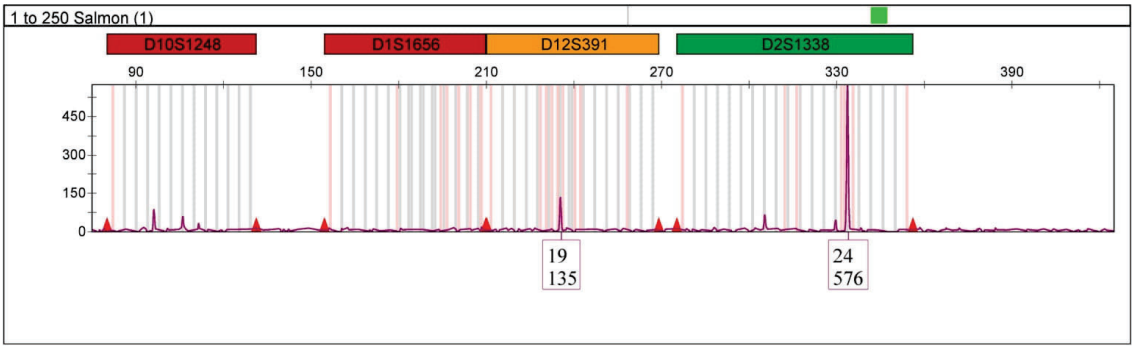
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

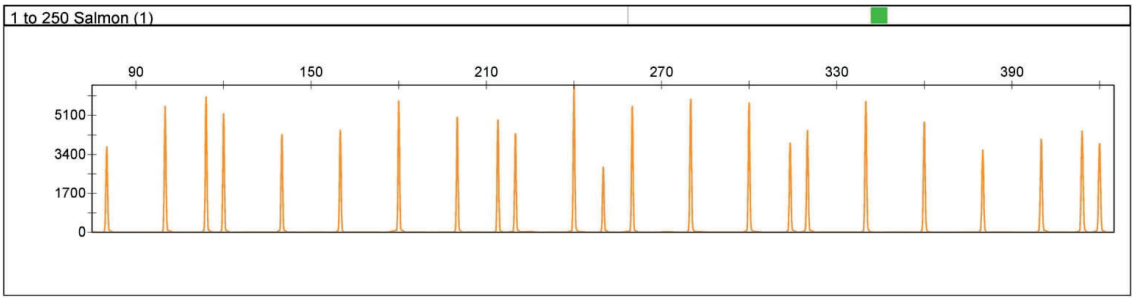
Sample Name	SQ
1 to 250 Salmon (1)	



1 to 250 Salmon (1)



1 to 250 Salmon (1)

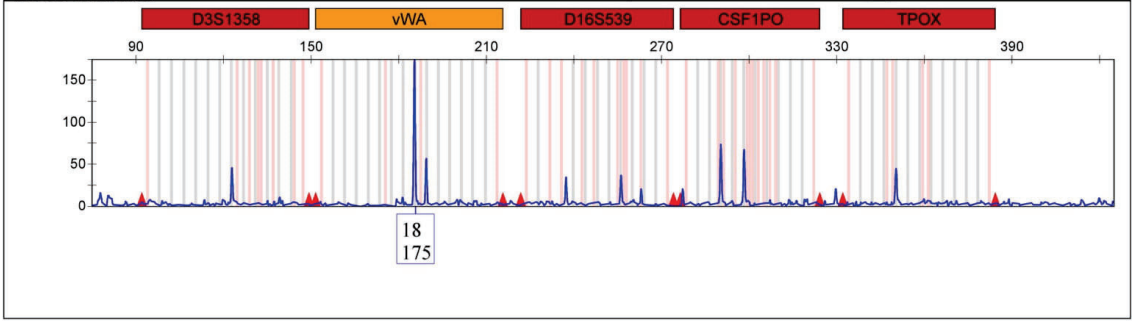


Applied Biosystems
GeneMapper® ID-X 1.4

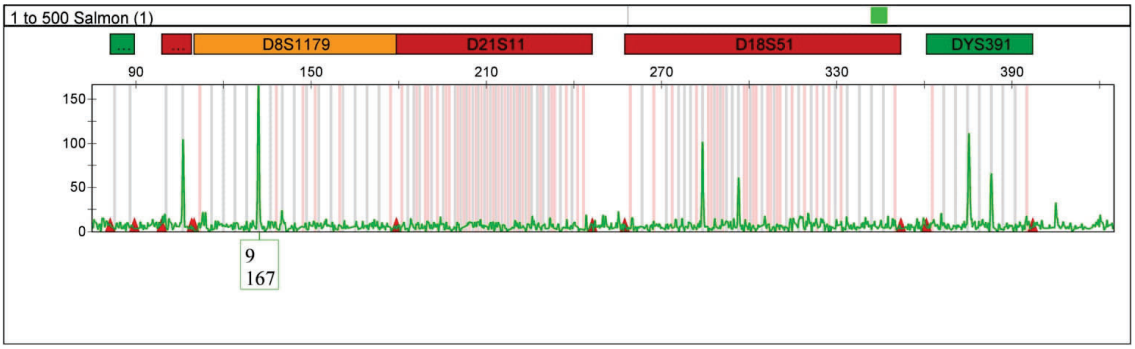
Project: Organic Extractions CRG 061422

Sample Name	SQ

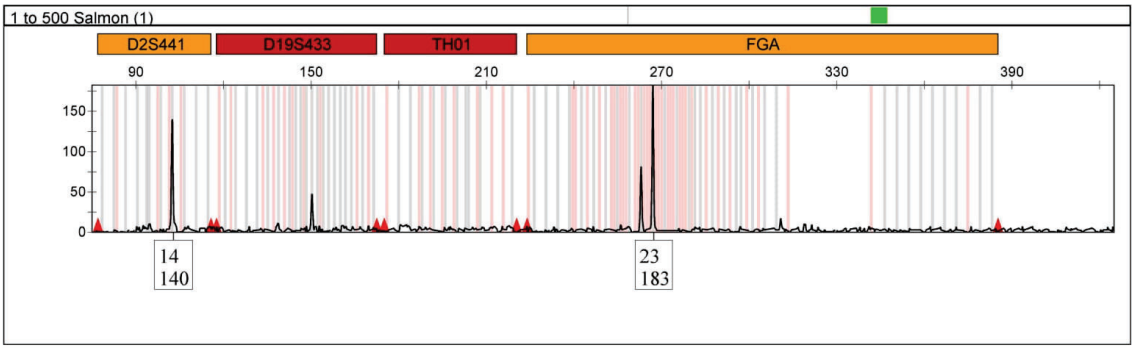
1 to 500 Salmon (1)



1 to 500 Salmon (1)



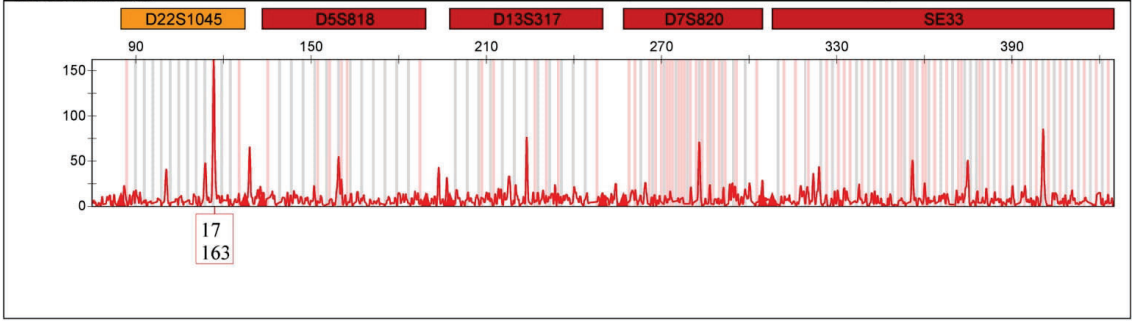
1 to 500 Salmon (1)

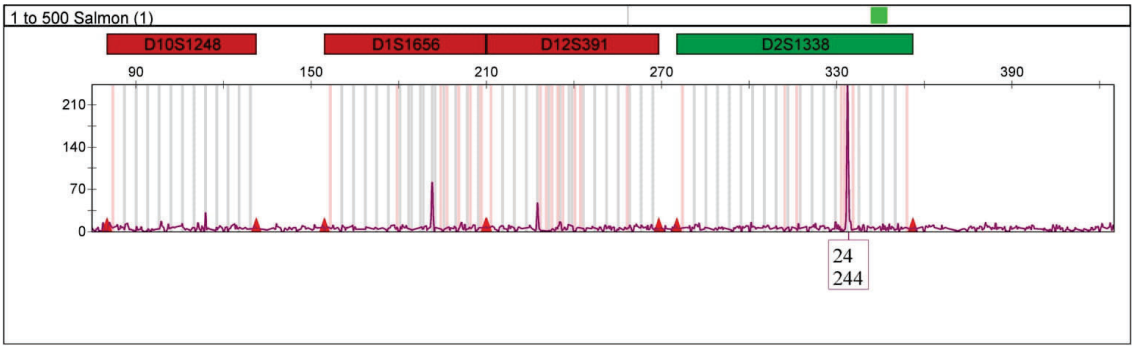


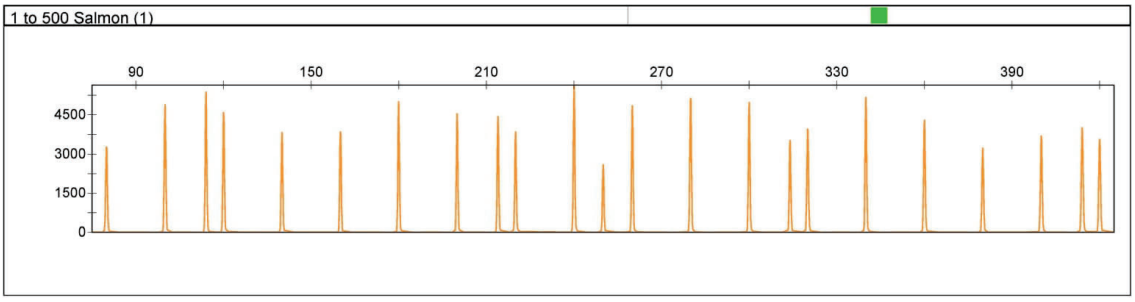
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 500 Salmon (1)	





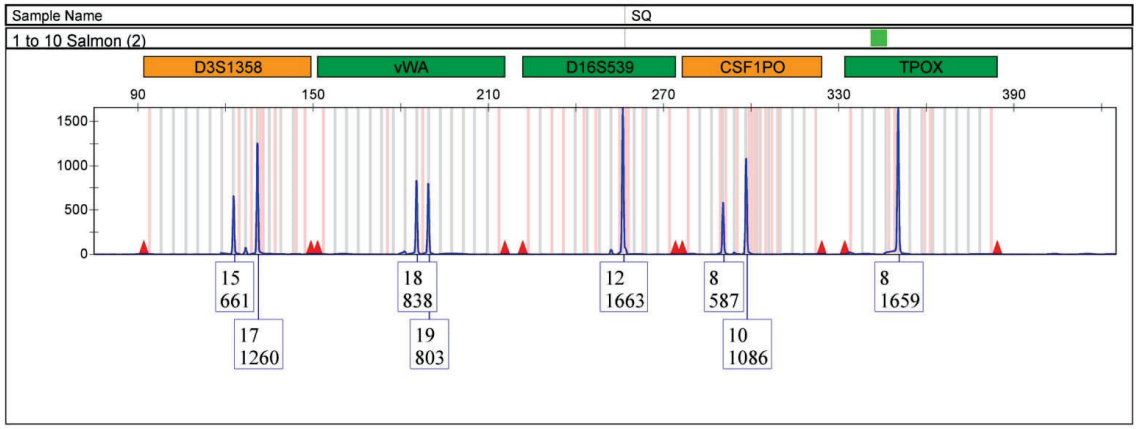


Applied Biosystems
GeneMapper® ID-X 1.4

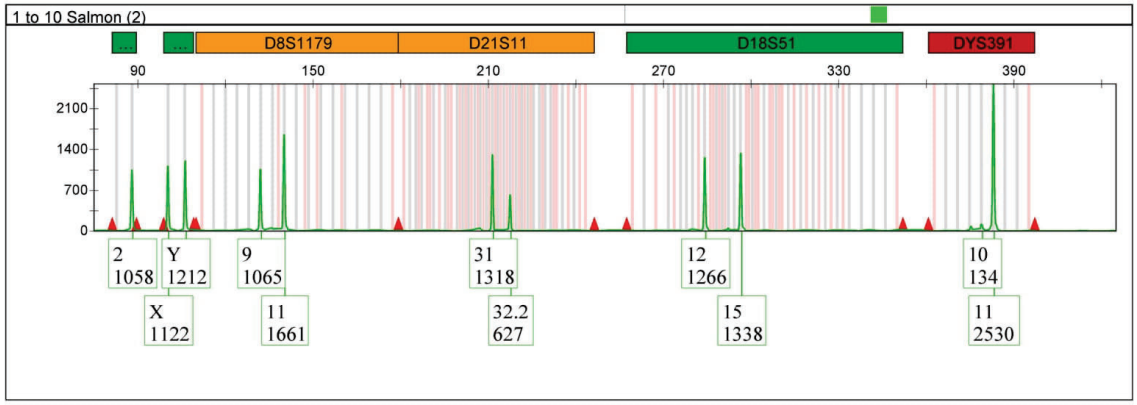
Project: Organic Extractions CRG 061422

Sample Name	SQ

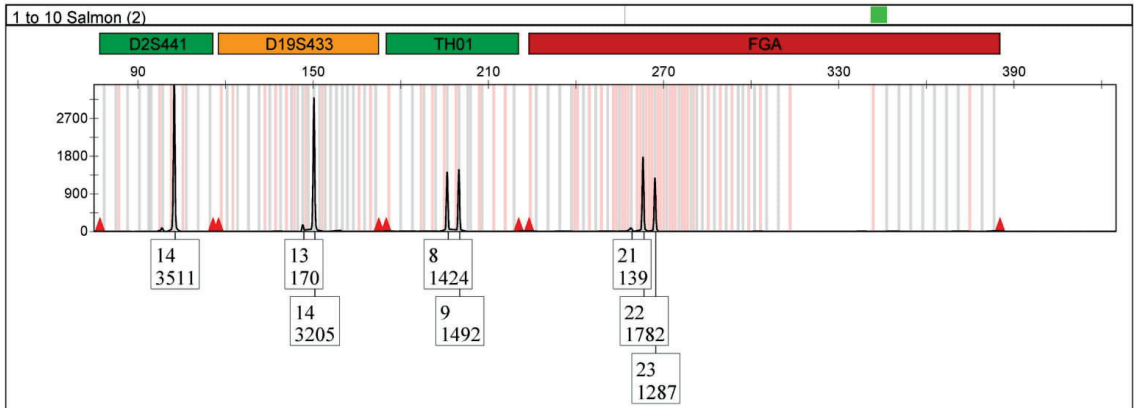
1 to 10 Salmon (2)



1 to 10 Salmon (2)



1 to 10 Salmon (2)

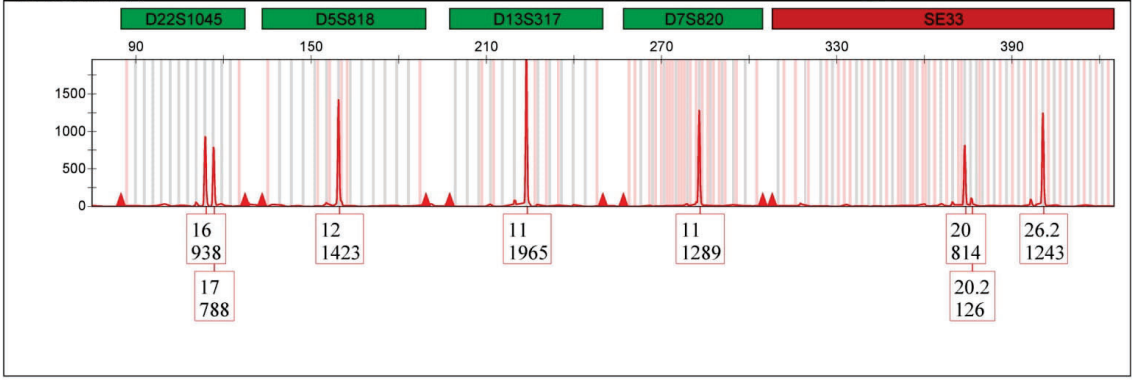


Applied Biosystems
GeneMapper® ID-X 1.4

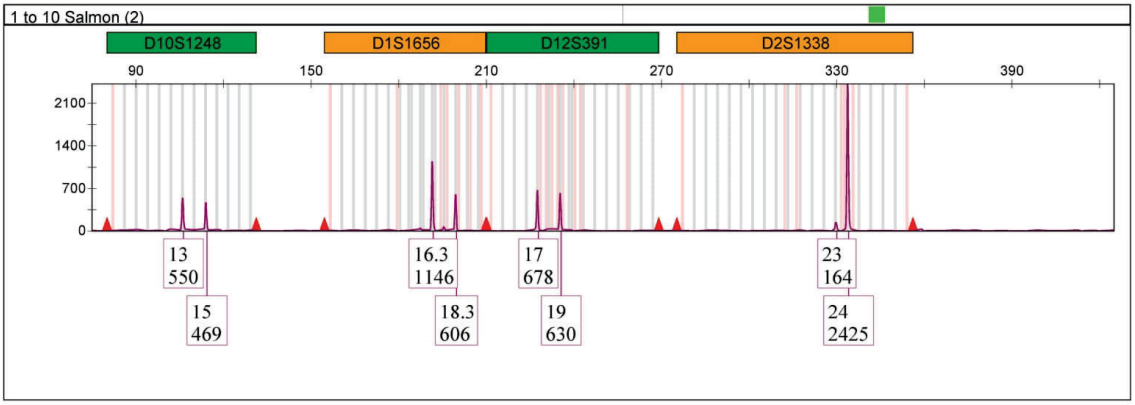
Project: Organic Extractions CRG 061422

Sample Name	SQ

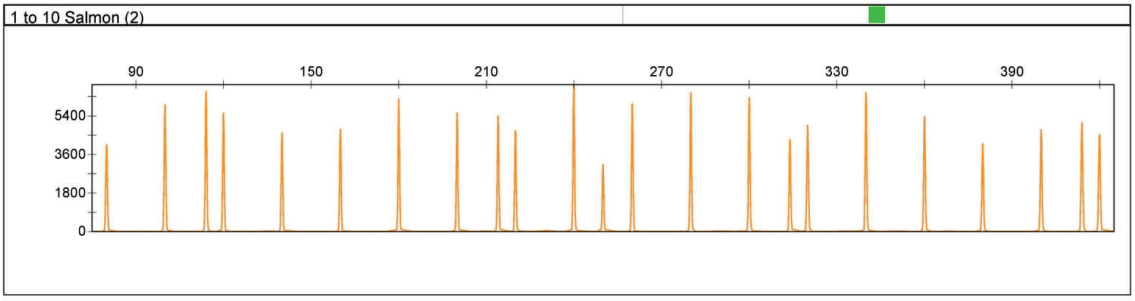
1 to 10 Salmon (2)



1 to 10 Salmon (2)



1 to 10 Salmon (2)

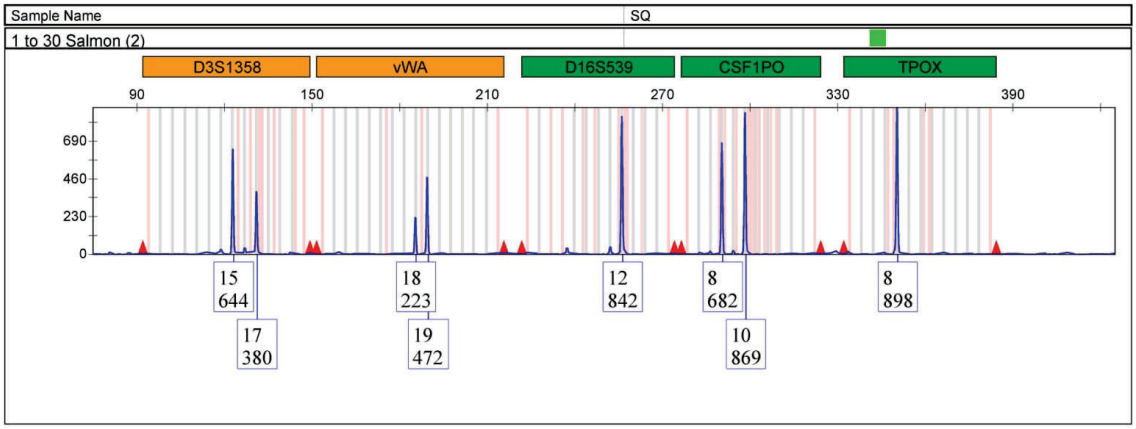


Applied Biosystems
GeneMapper® ID-X 1.4

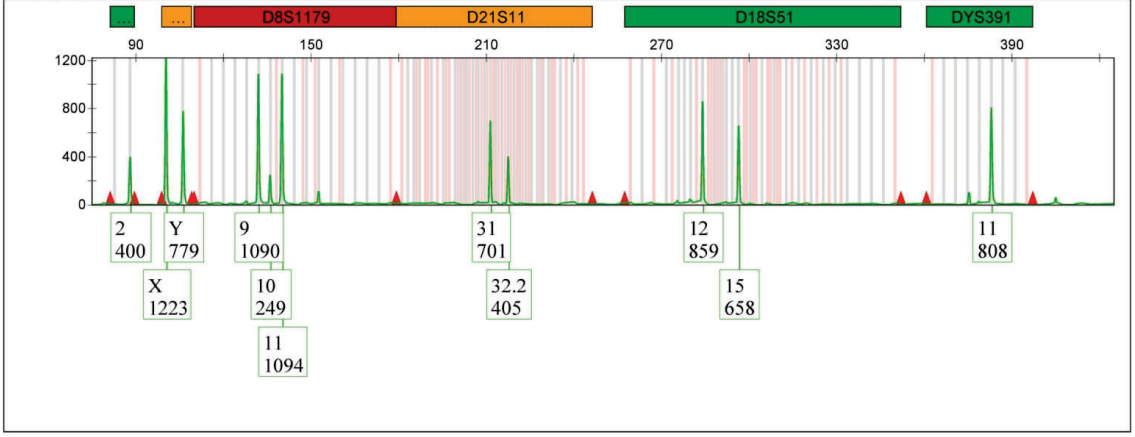
Project: Organic Extractions CRG 061422

Sample Name	SQ

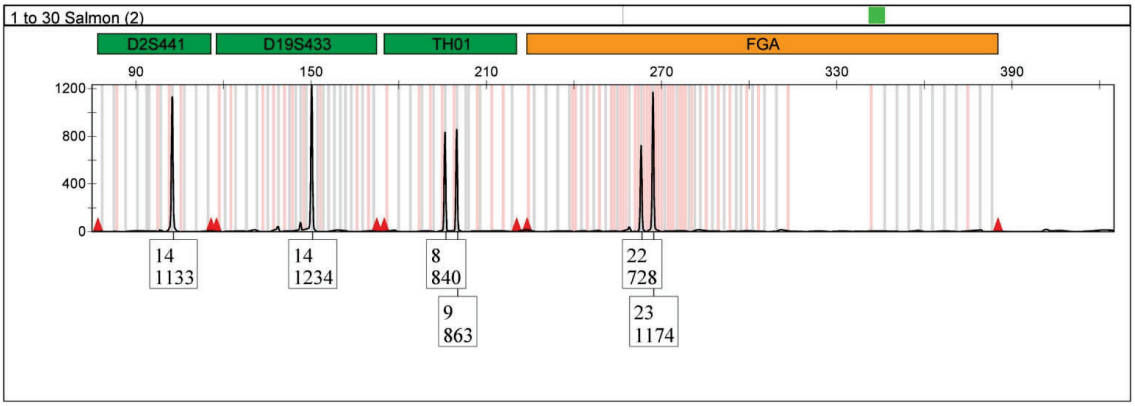
1 to 30 Salmon (2)



1 to 30 Salmon (2)



1 to 30 Salmon (2)

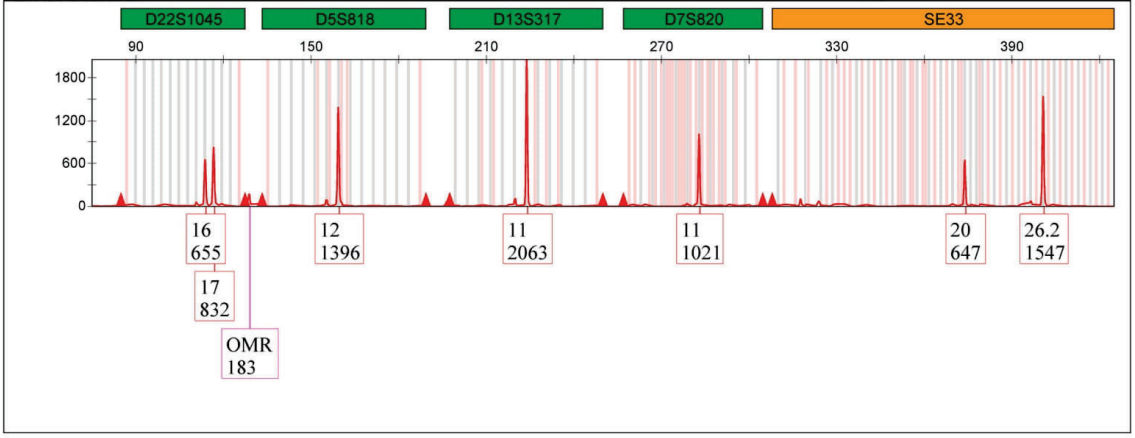


Applied Biosystems
GeneMapper® ID-X 1.4

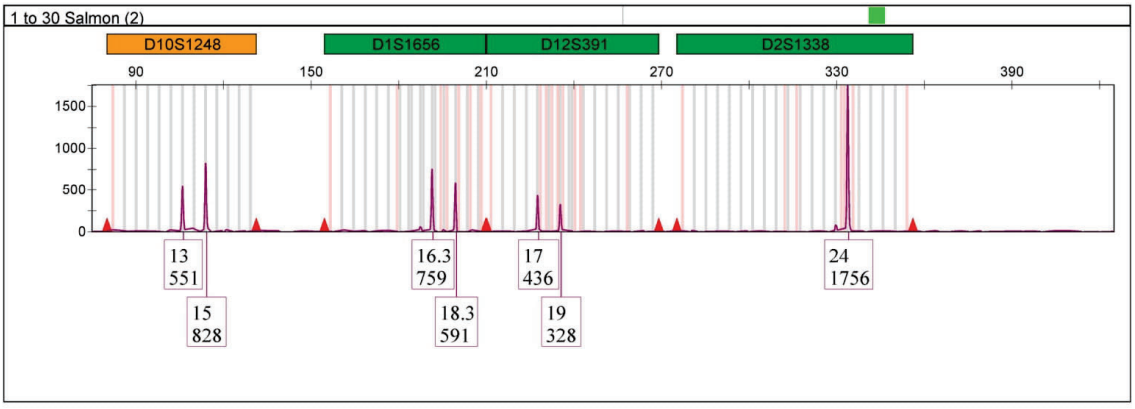
Project: Organic Extractions CRG 061422

Sample Name	SQ

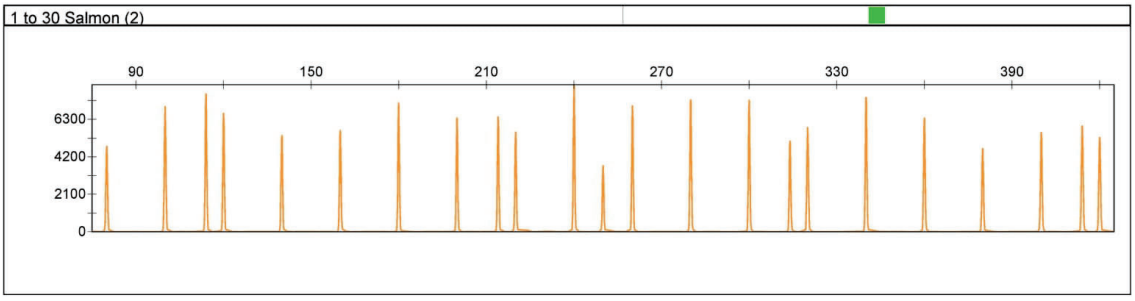
1 to 30 Salmon (2)



1 to 30 Salmon (2)



1 to 30 Salmon (2)

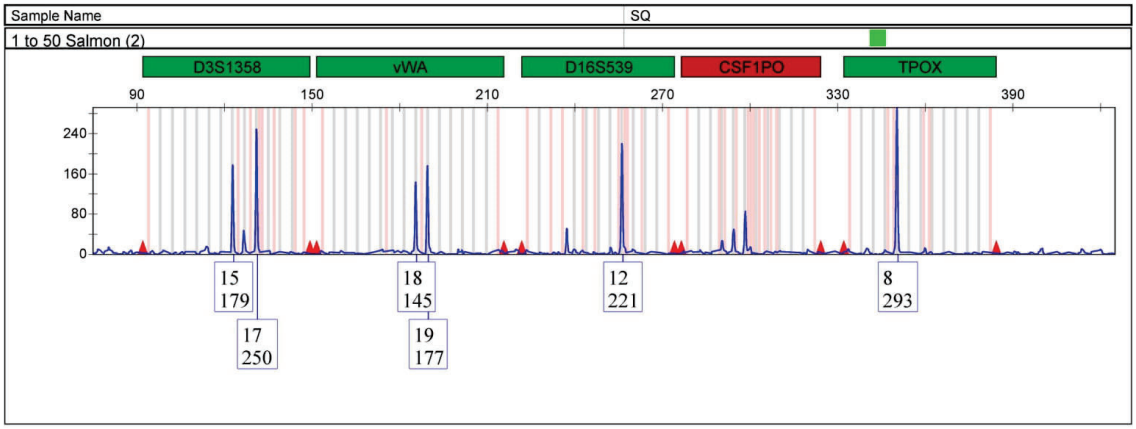


Applied Biosystems
GeneMapper® ID-X 1.4

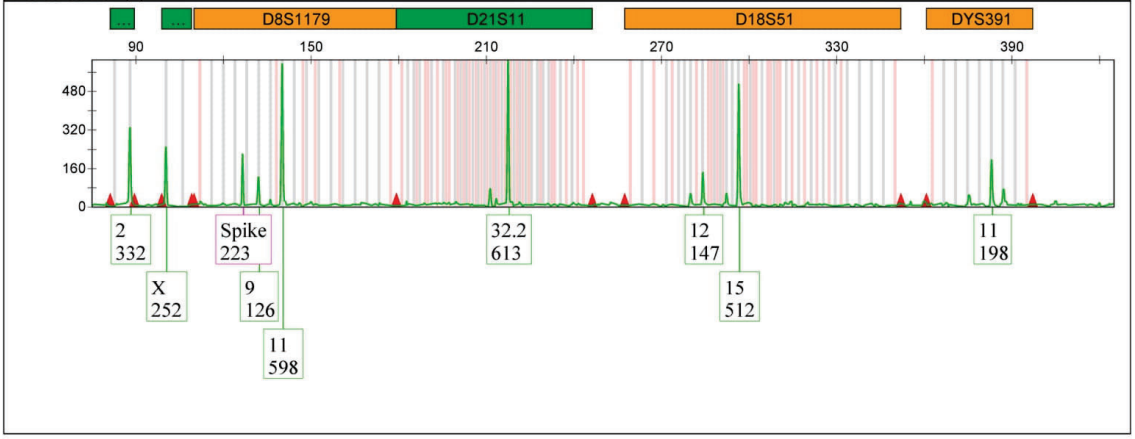
Project: Organic Extractions CRG 061422

Sample Name	SQ

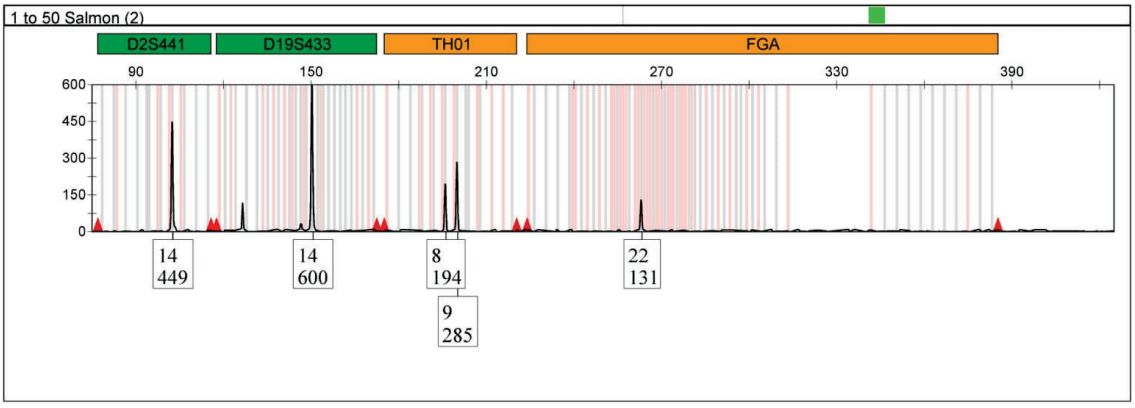
1 to 50 Salmon (2)



1 to 50 Salmon (2)



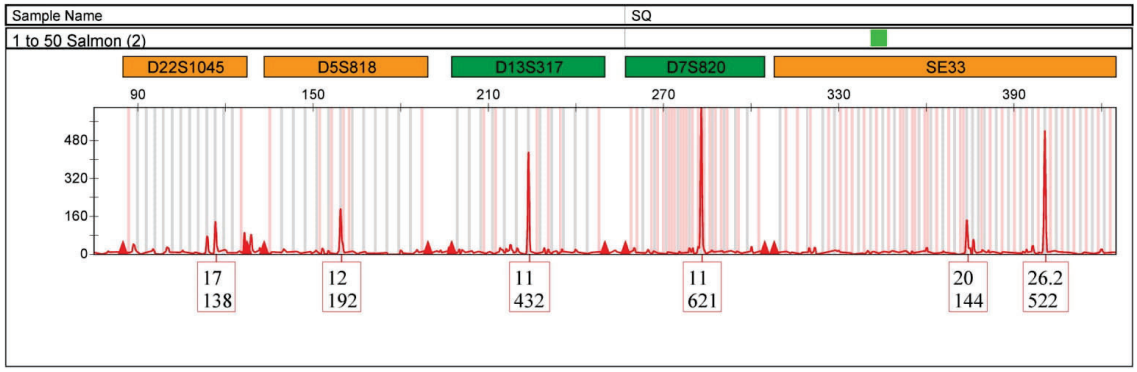
1 to 50 Salmon (2)



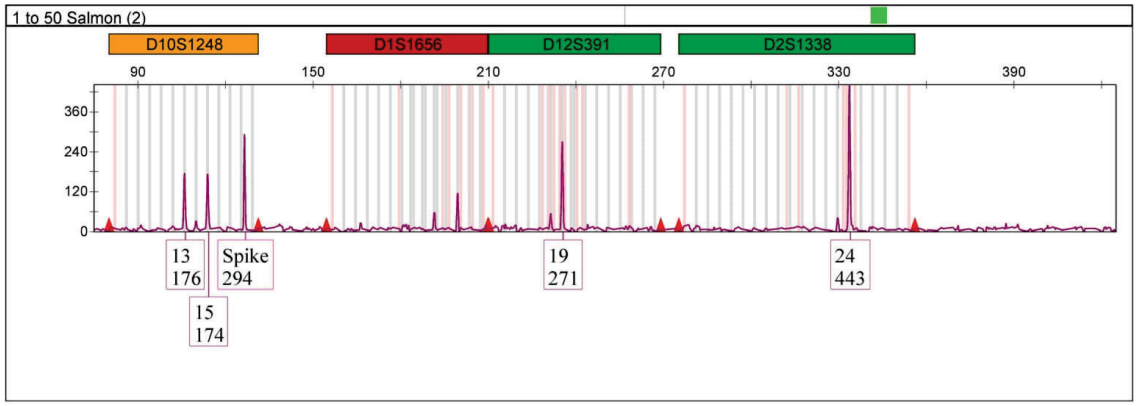
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

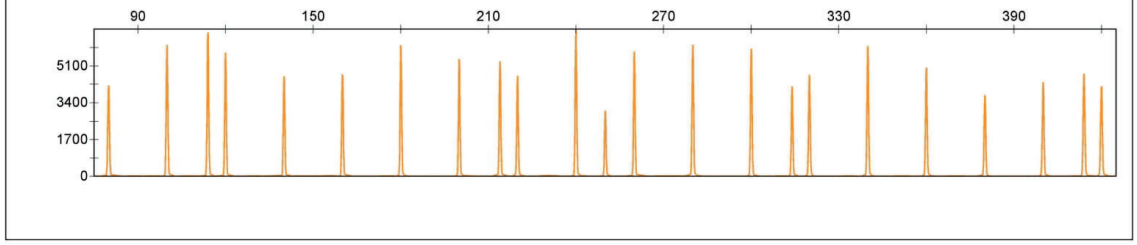
Sample Name	SQ
1 to 50 Salmon (2)	



1 to 50 Salmon (2)



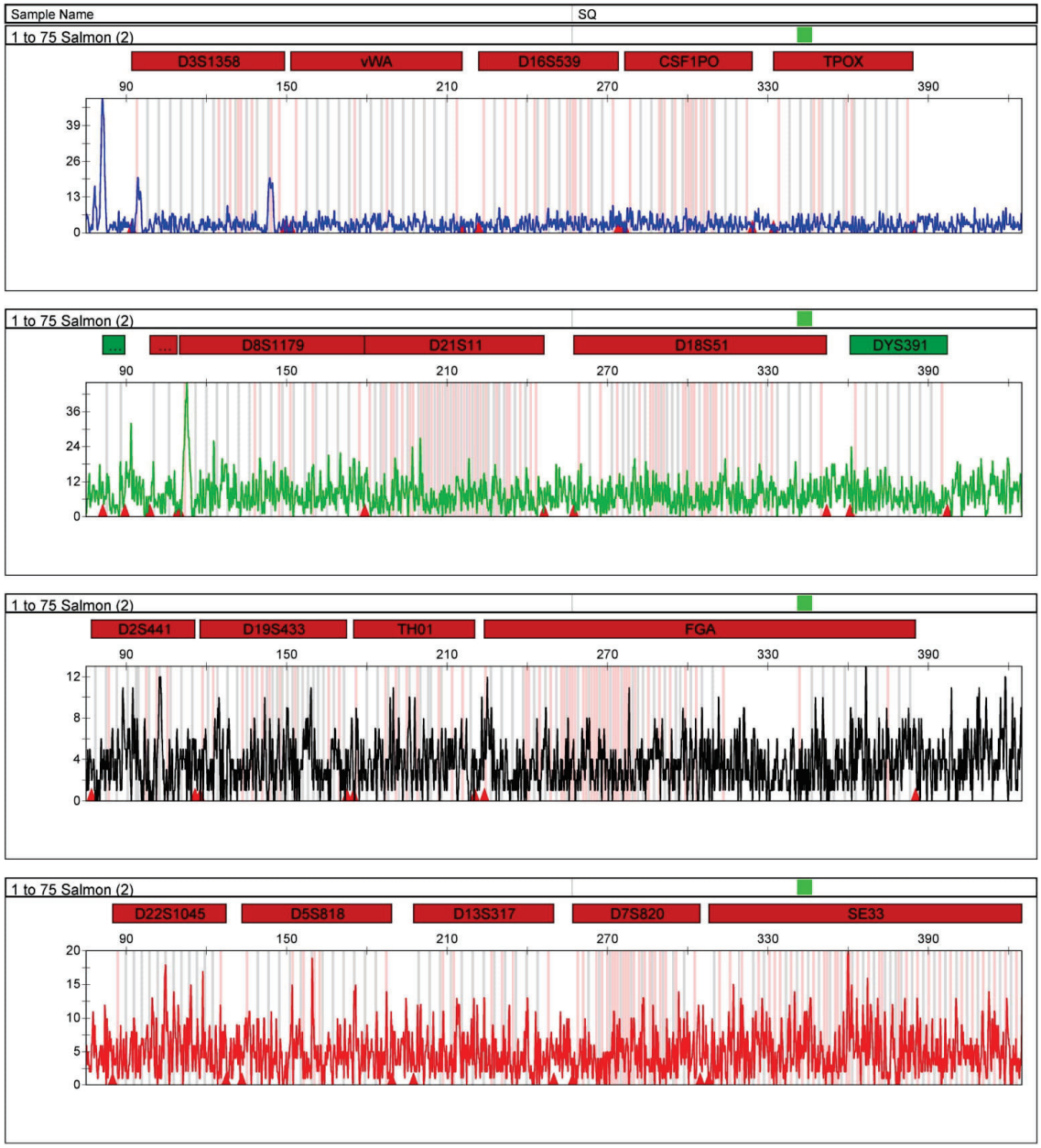
1 to 50 Salmon (2)



Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

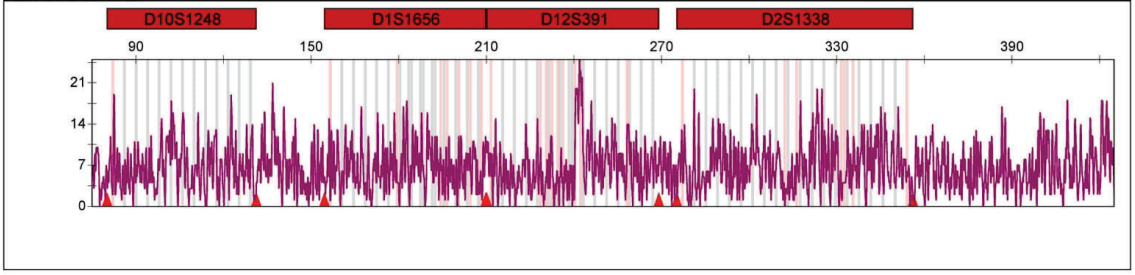
Sample Name	SQ



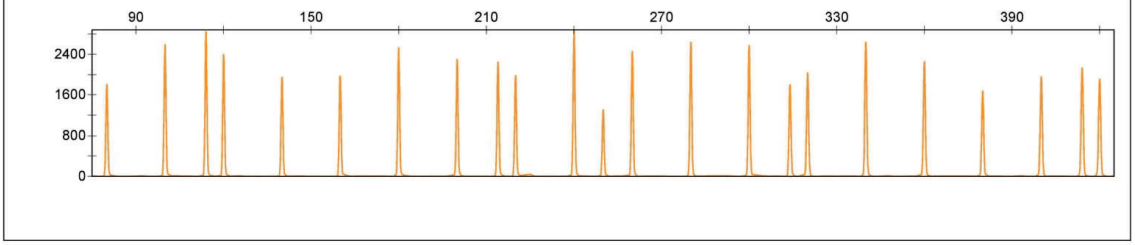
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 75 Salmon (2)	



1 to 75 Salmon (2)



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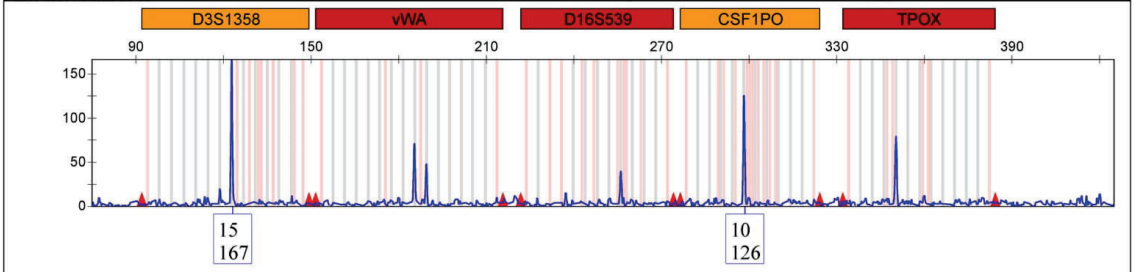
GeneMapper® ID-X 1.4

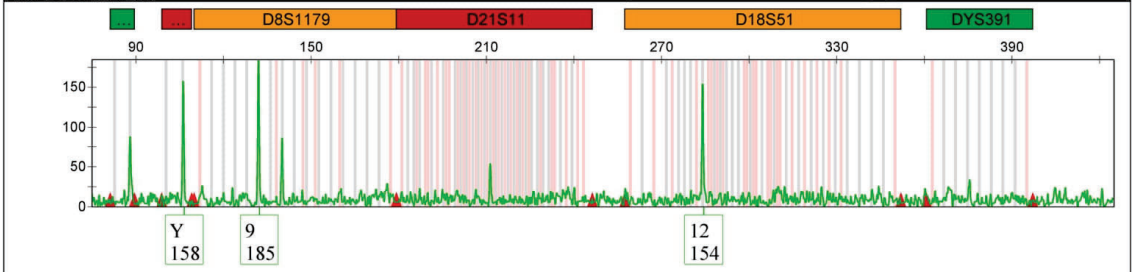
Project: Organic Extractions CRG 061422

Sample Name	SQ

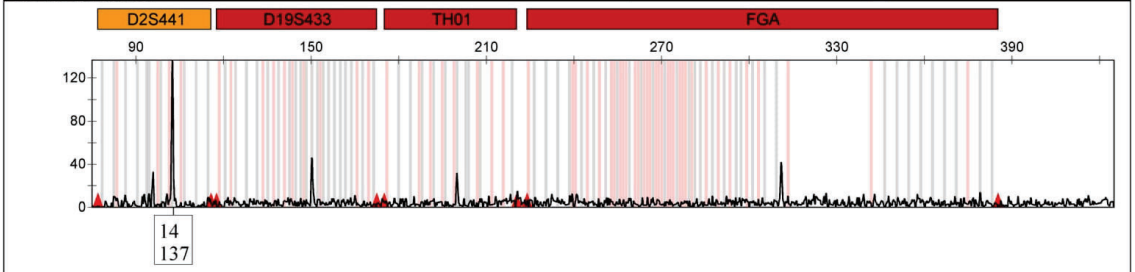
1 to 125 Salmon (2)



1 to 125 Salmon (2)



1 to 125 Salmon (2)

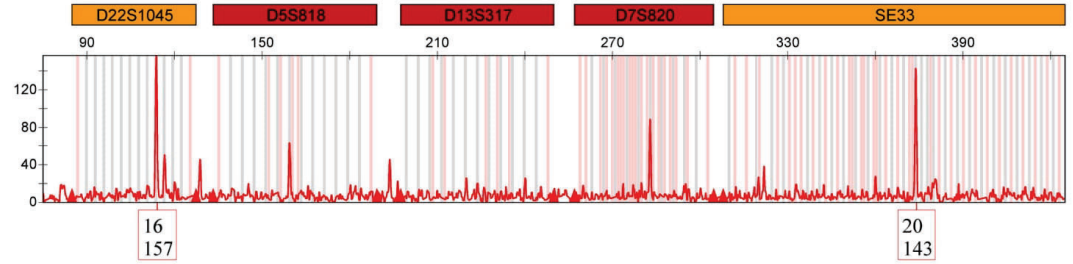


Applied Biosystems
GeneMapper® ID-X 1.4

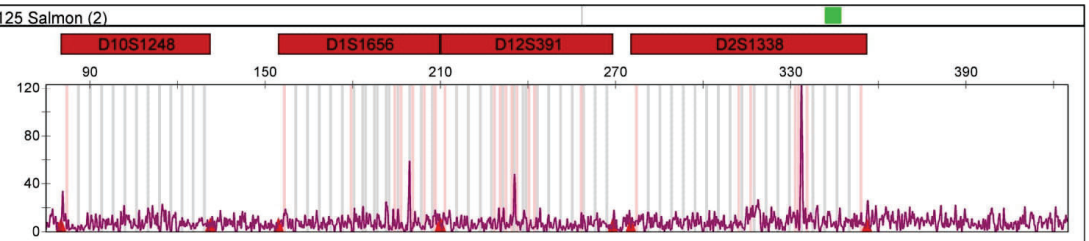
Project: Organic Extractions CRG 061422

Sample Name	SQ

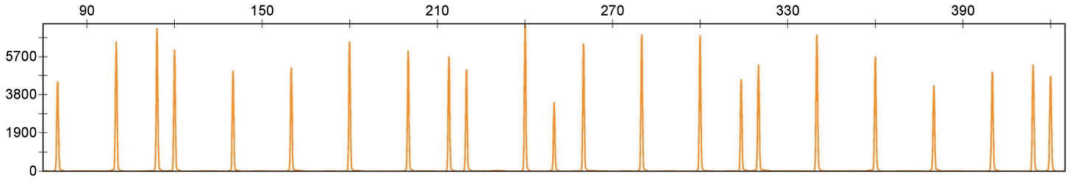
1 to 125 Salmon (2)



1 to 125 Salmon (2)



1 to 125 Salmon (2)



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GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
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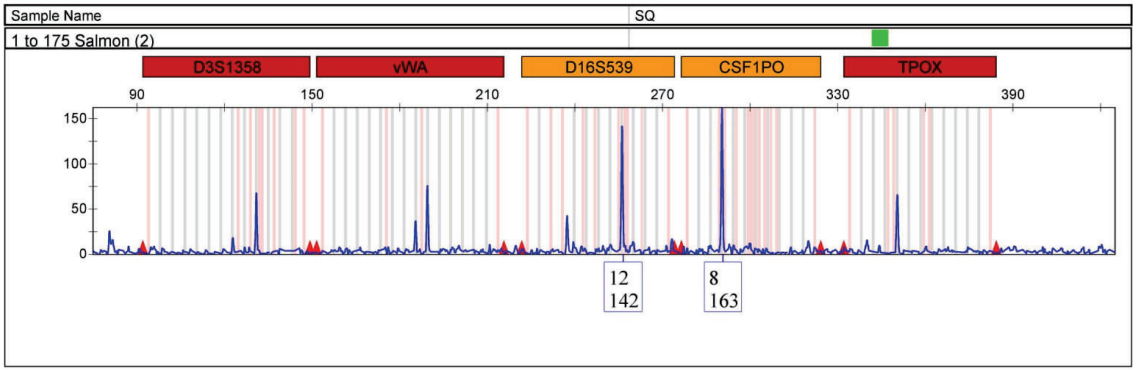
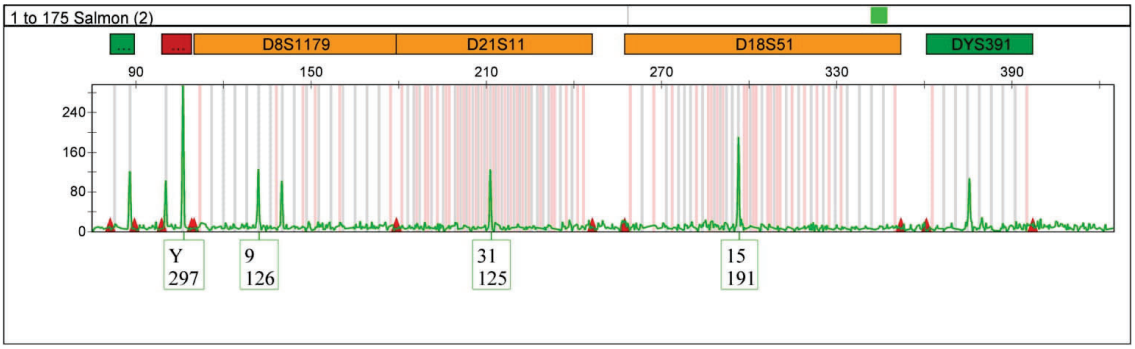
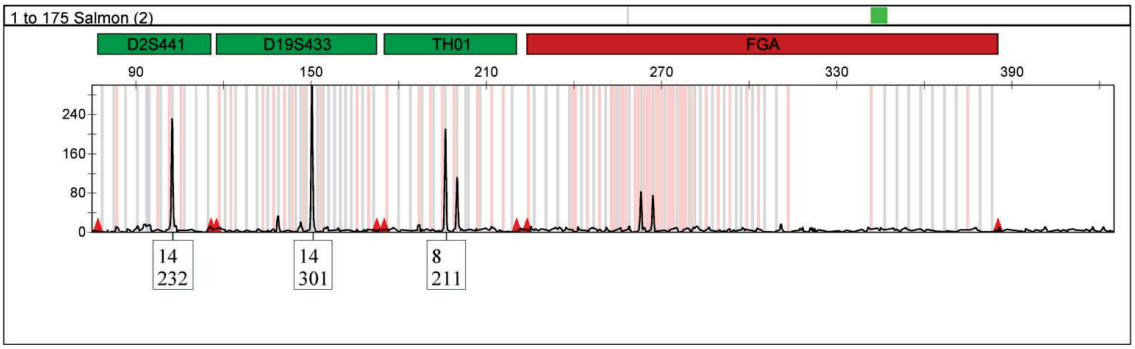


1 to 175 Salmon (2)



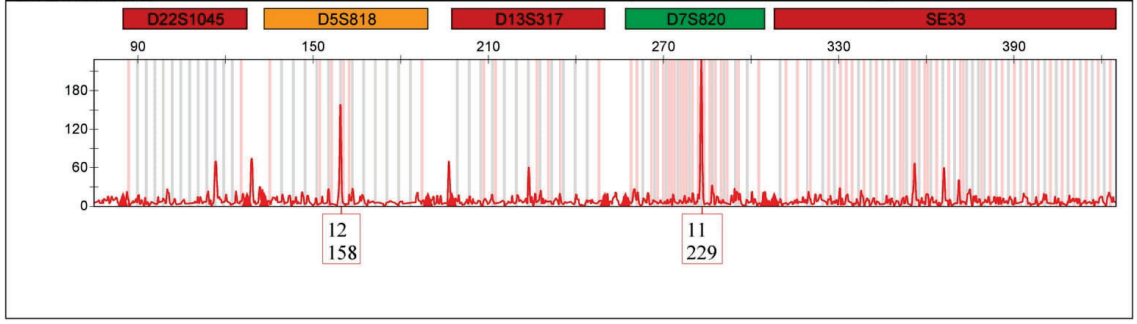
1 to 175 Salmon (2)



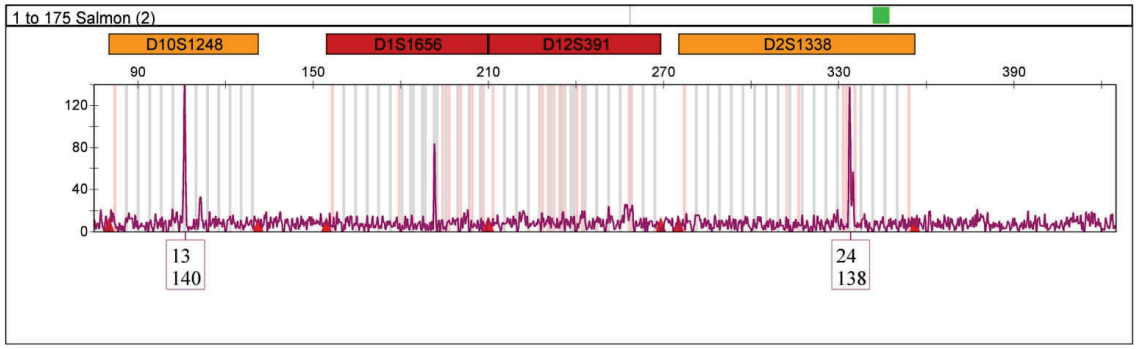
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

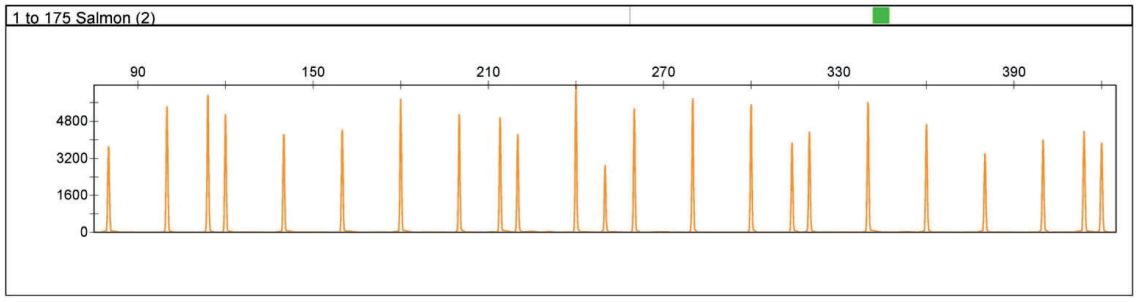
Sample Name	SQ
1 to 175 Salmon (2)	



1 to 175 Salmon (2)



1 to 175 Salmon (2)



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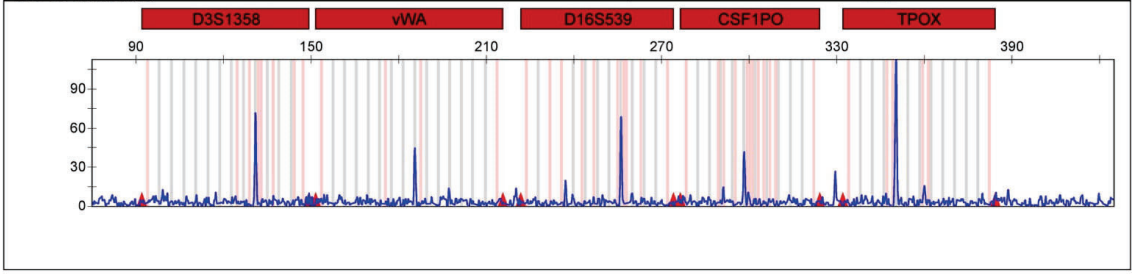
GeneMapper® ID-X 1.4

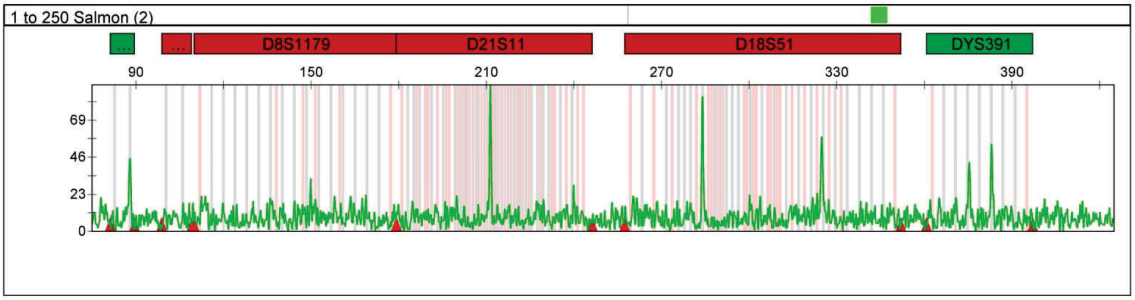
Project: Organic Extractions CRG 061422

Sample Name	SQ

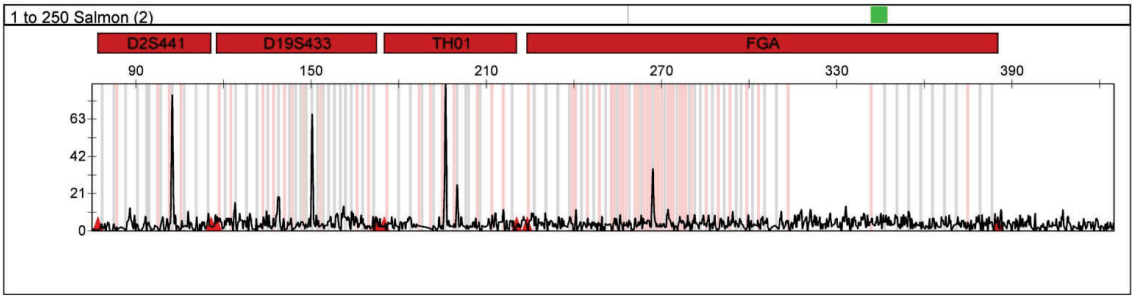
1 to 250 Salmon (2)



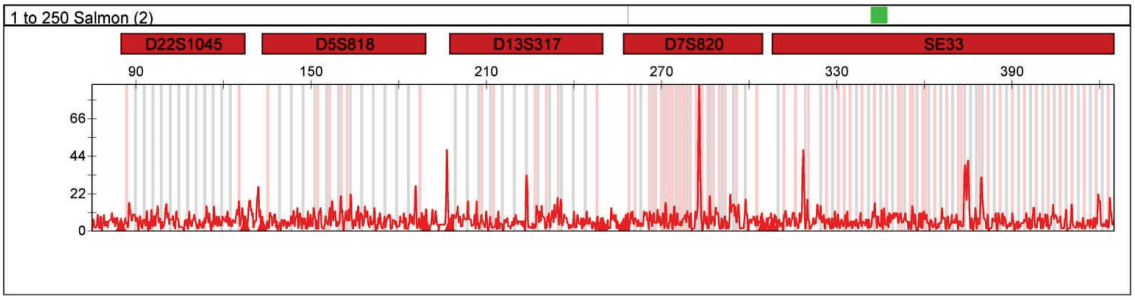
1 to 250 Salmon (2)



1 to 250 Salmon (2)



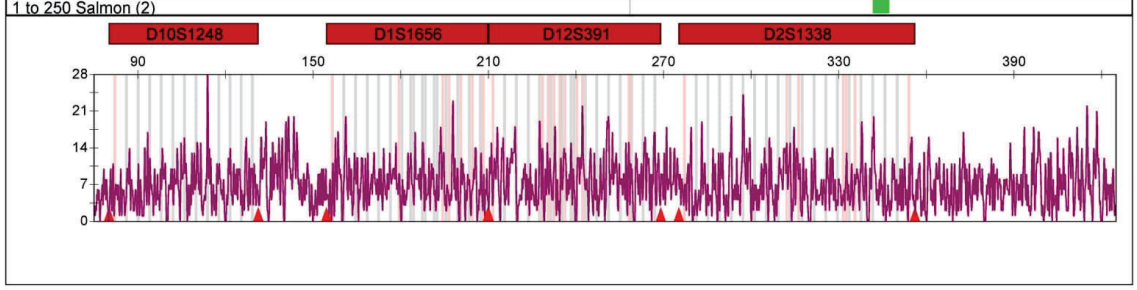
1 to 250 Salmon (2)



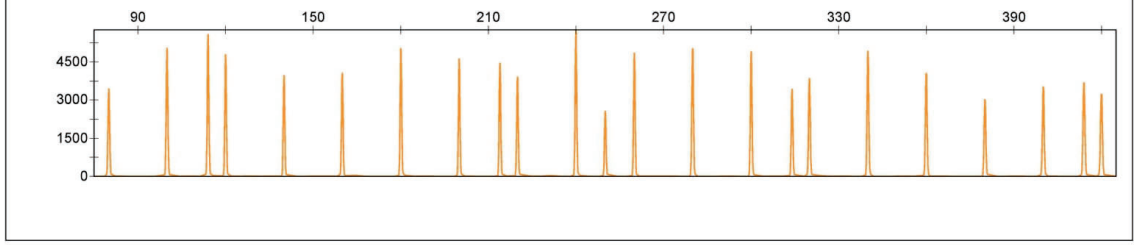
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 250 Salmon (2)	



1 to 250 Salmon (2)



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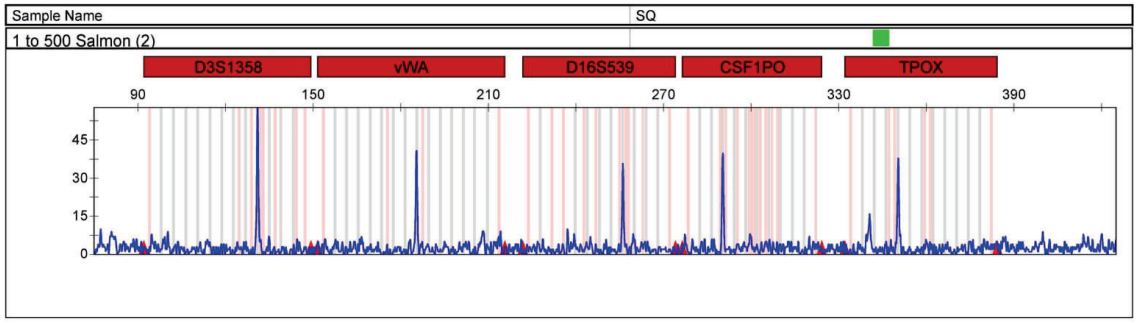
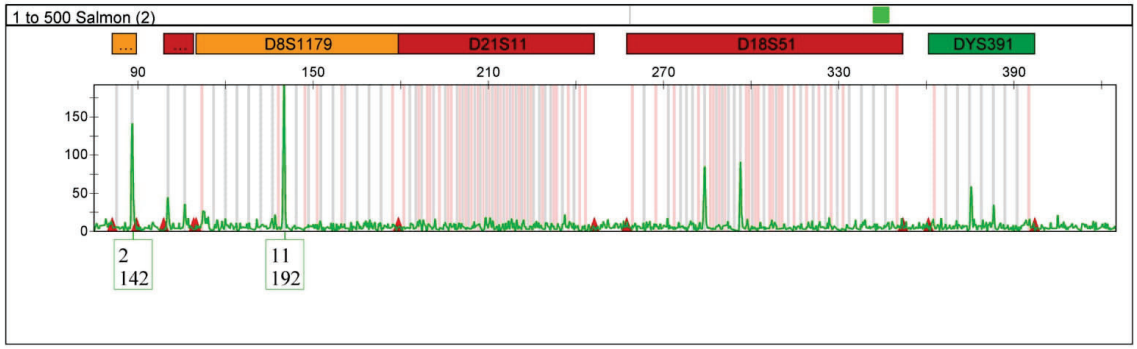
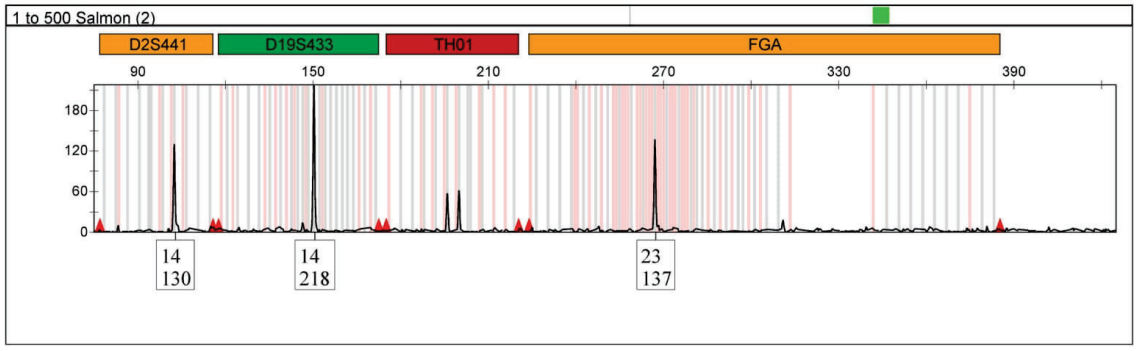
GeneMapper® ID-X 1.4

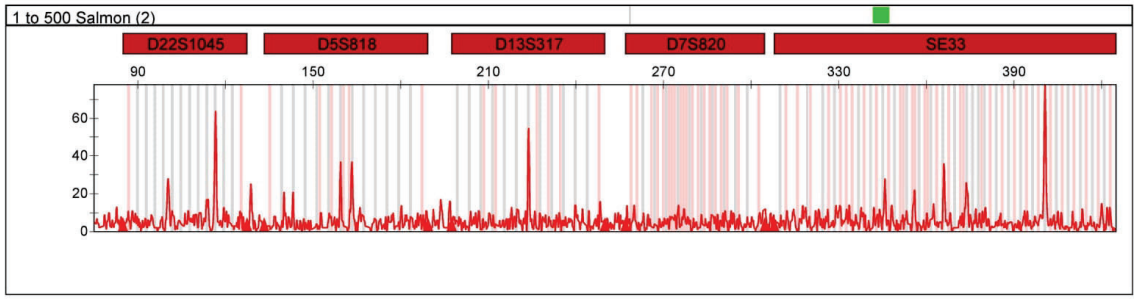
Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 500 Salmon (2)	









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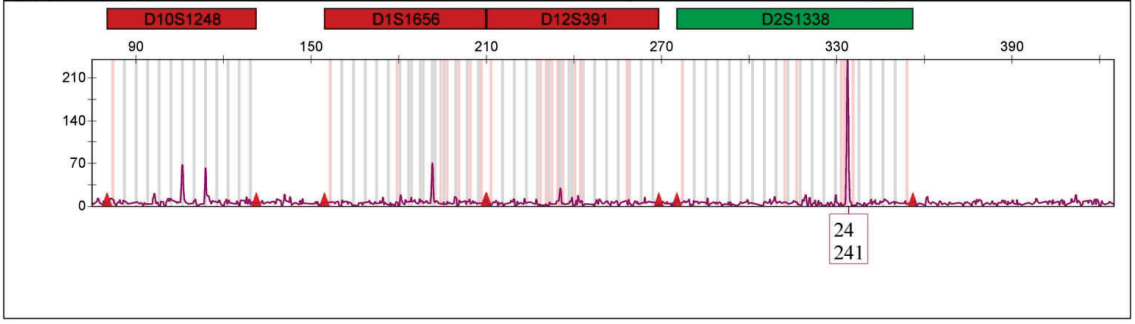
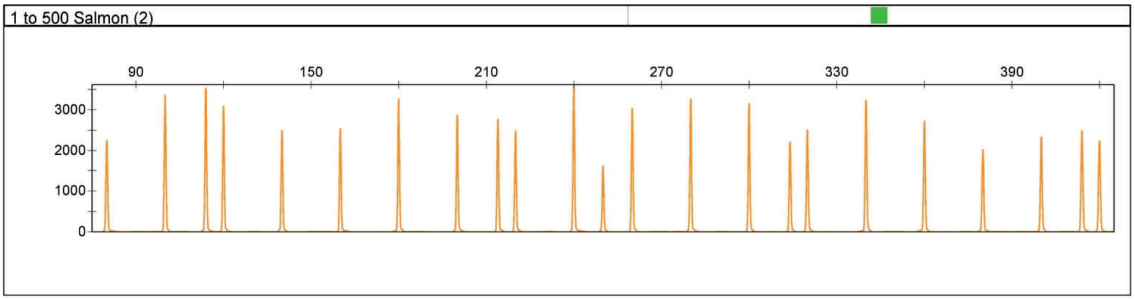
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 500 Salmon (2)	





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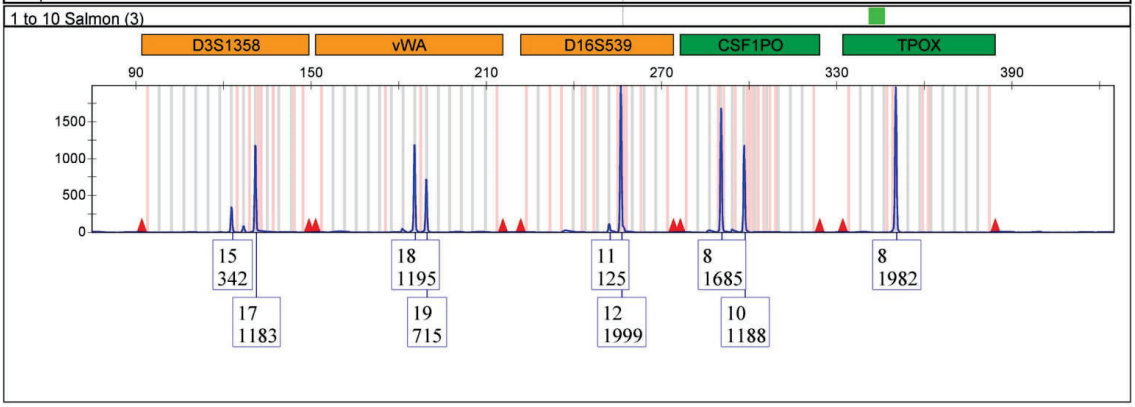
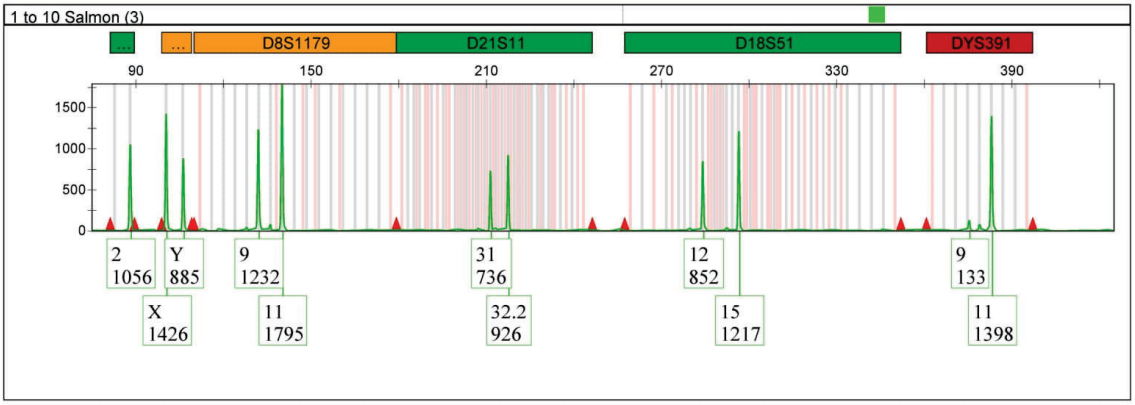
Project: Organic Extractions CRG 061422

Sample Name	SQ

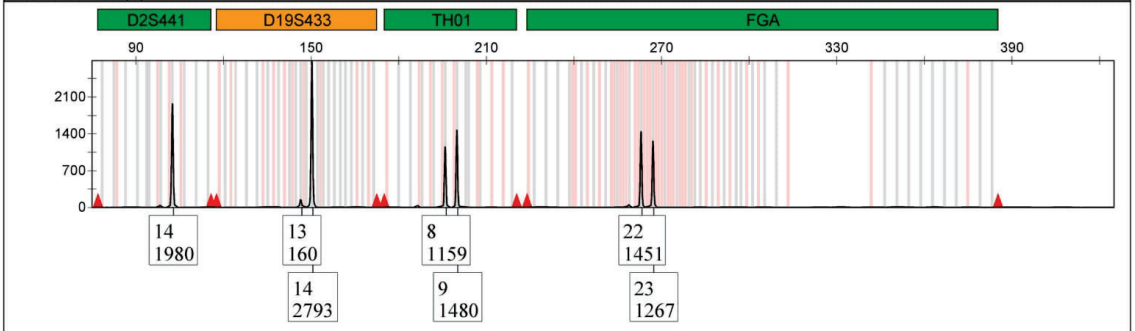
1 to 10 Salmon (3)



1 to 10 Salmon (3)



1 to 10 Salmon (3)

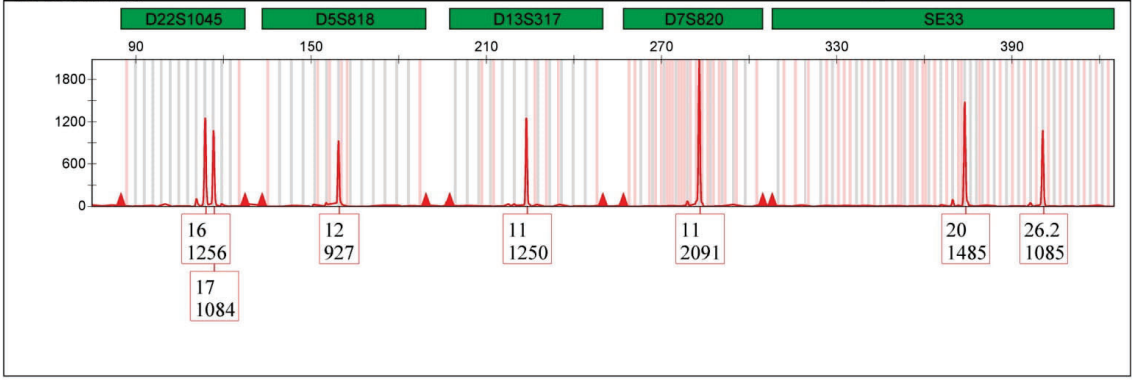


Applied Biosystems
GeneMapper® ID-X 1.4

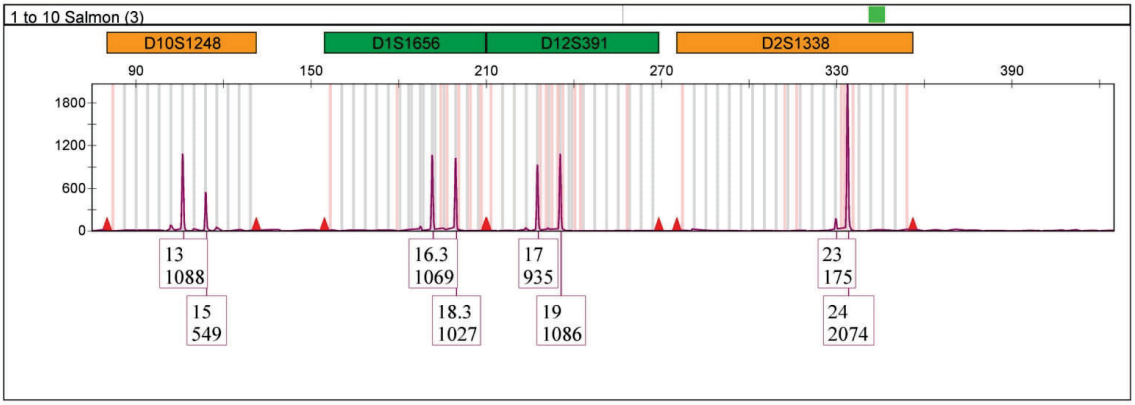
Project: Organic Extractions CRG 061422

Sample Name	SQ

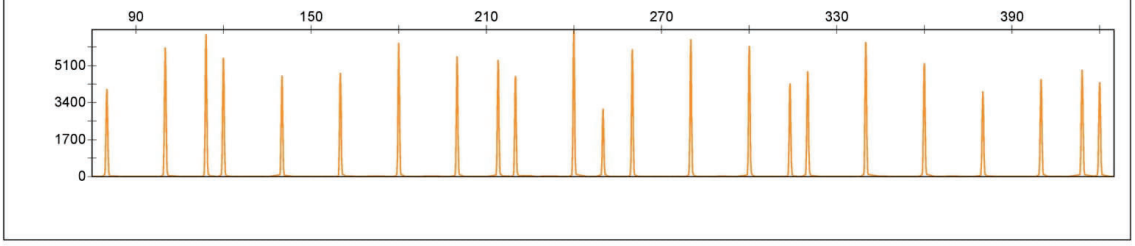
1 to 10 Salmon (3)



1 to 10 Salmon (3)



1 to 10 Salmon (3)



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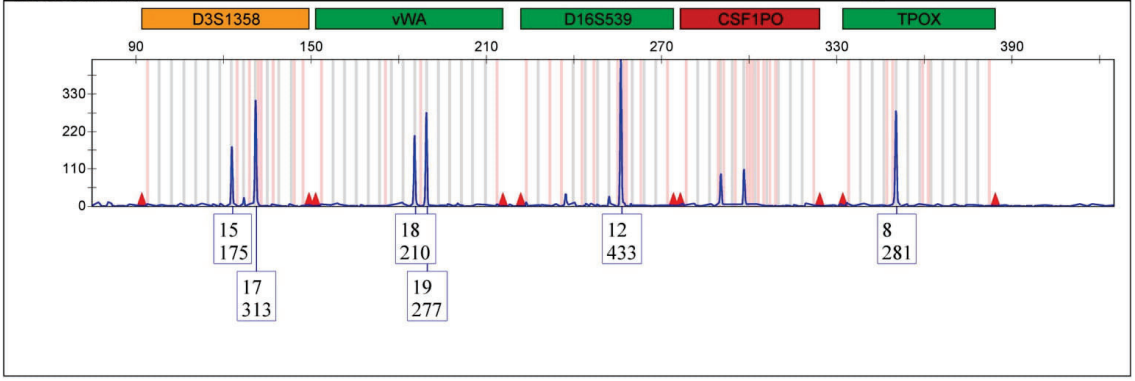
GeneMapper® ID-X 1.4

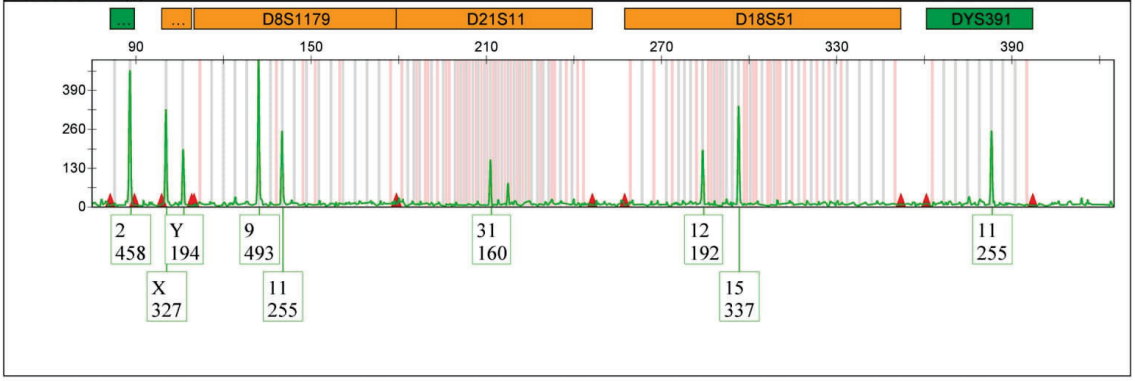
Project: Organic Extractions CRG 061422

Sample Name	SQ

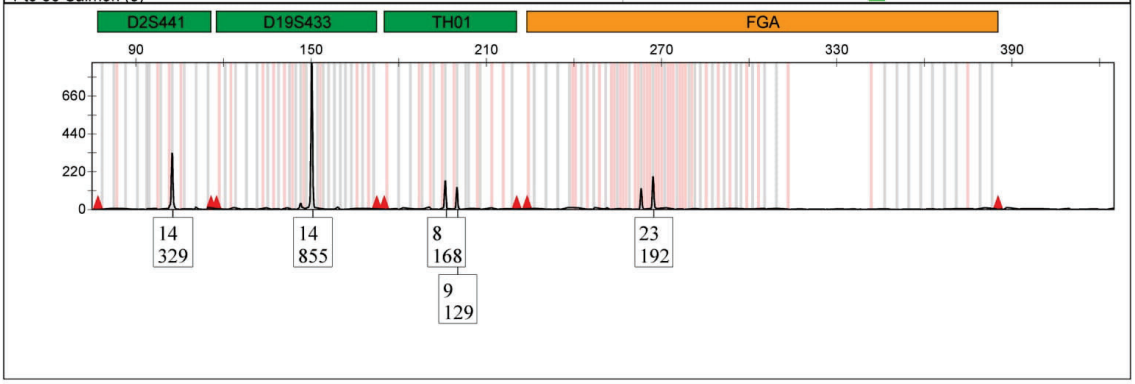
1 to 30 Salmon (3)



1 to 30 Salmon (3)



1 to 30 Salmon (3)

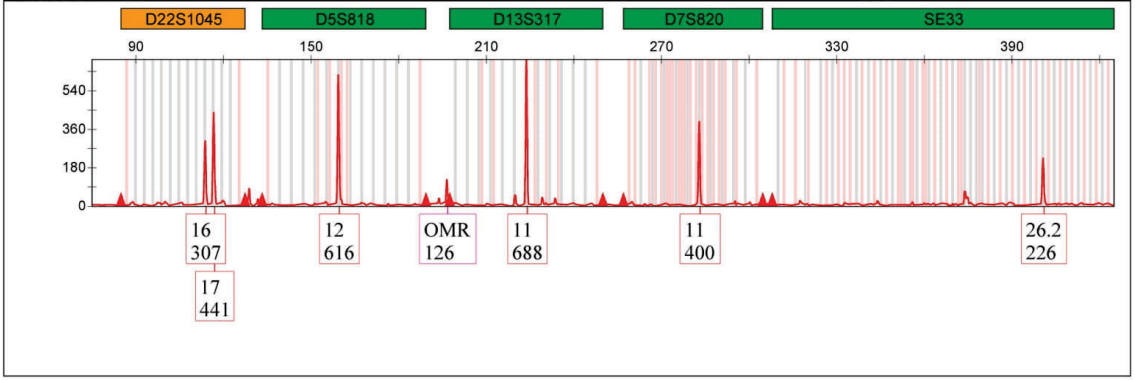


Applied Biosystems
GeneMapper® ID-X 1.4

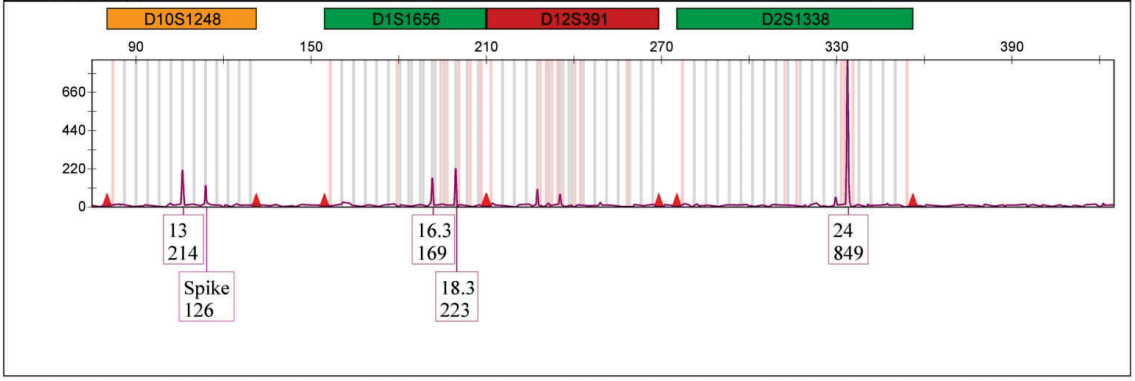
Project: Organic Extractions CRG 061422

Sample Name	SQ

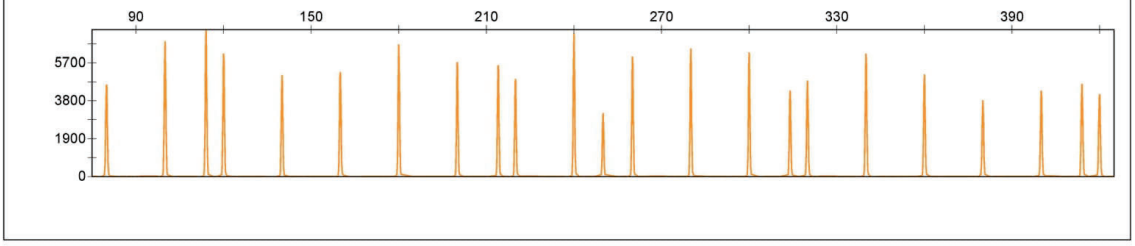
1 to 30 Salmon (3)



1 to 30 Salmon (3)



1 to 30 Salmon (3)

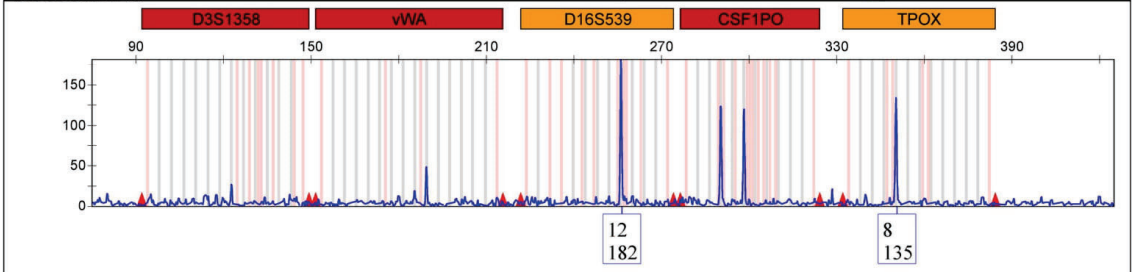


Applied Biosystems
GeneMapper® ID-X 1.4

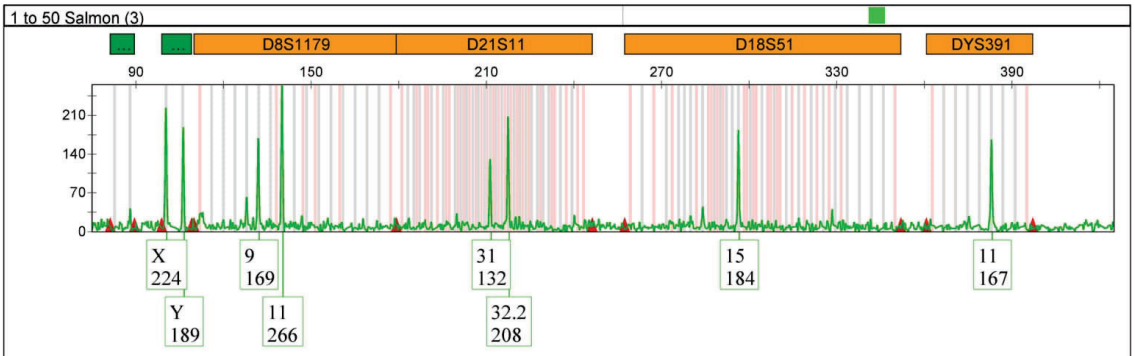
Project: Organic Extractions CRG 061422

Sample Name	SQ

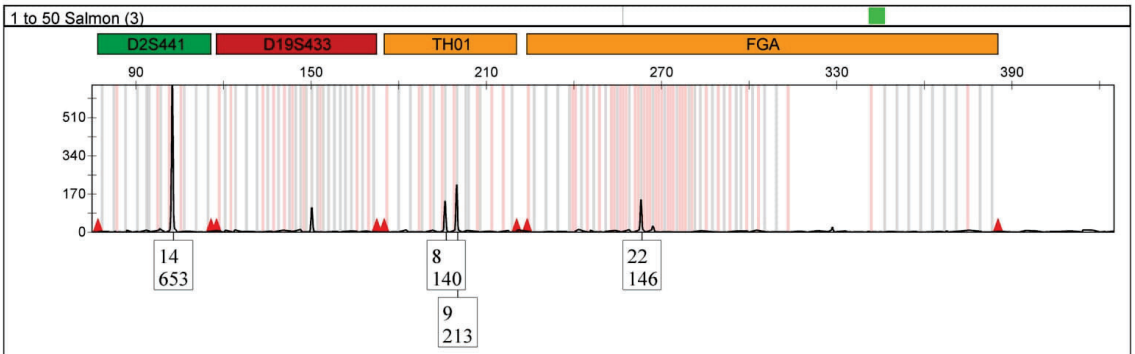
1 to 50 Salmon (3)



1 to 50 Salmon (3)



1 to 50 Salmon (3)

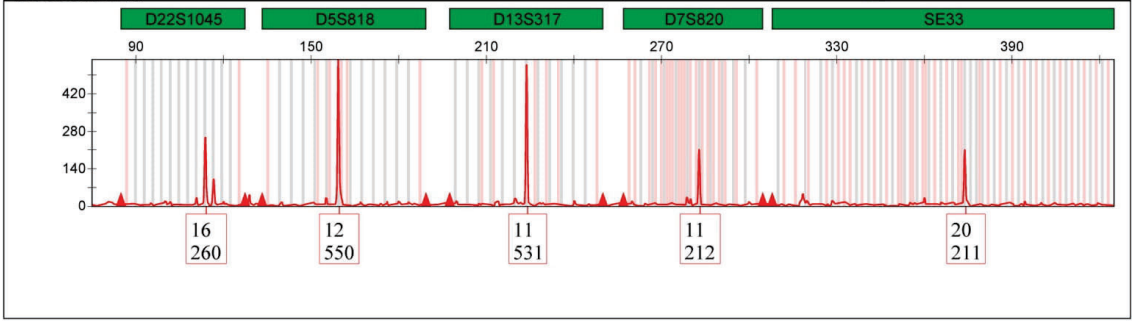


Applied Biosystems
GeneMapper® ID-X 1.4

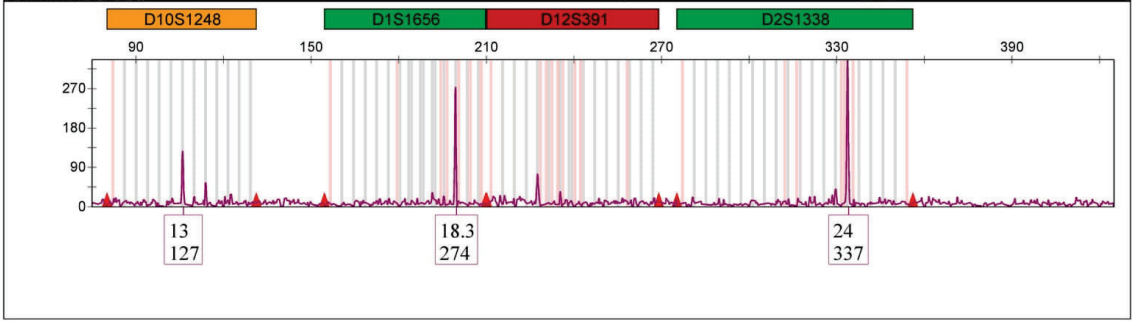
Project: Organic Extractions CRG 061422

Sample Name	SQ

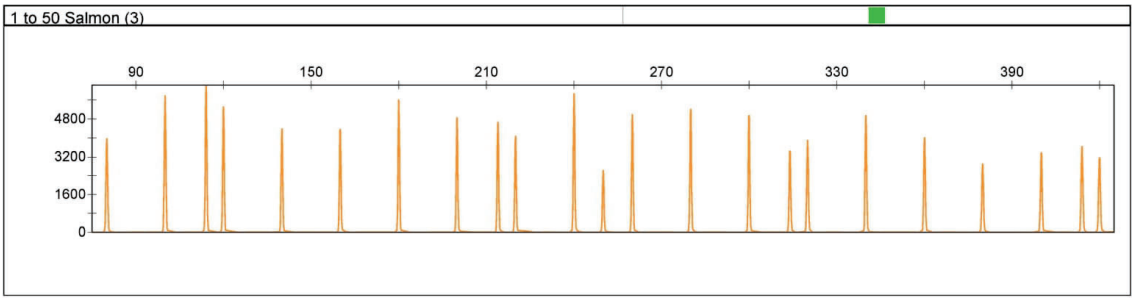
1 to 50 Salmon (3)



1 to 50 Salmon (3)



1 to 50 Salmon (3)

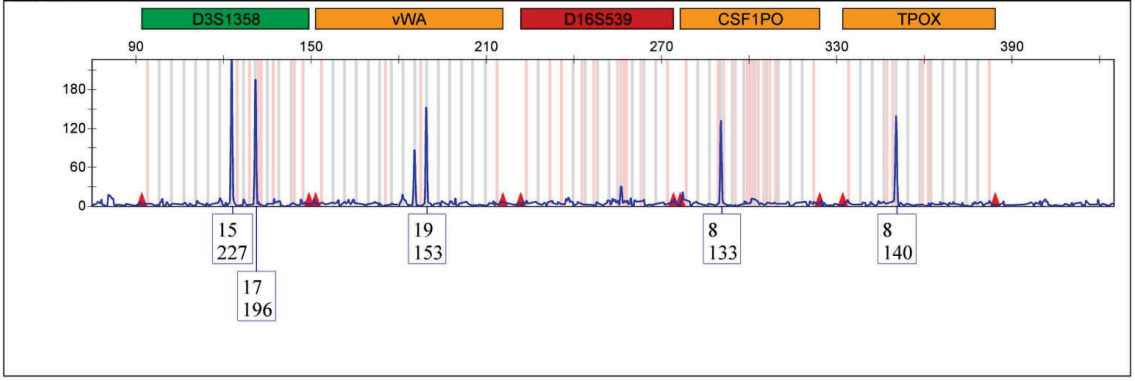


Applied Biosystems
GeneMapper® ID-X 1.4

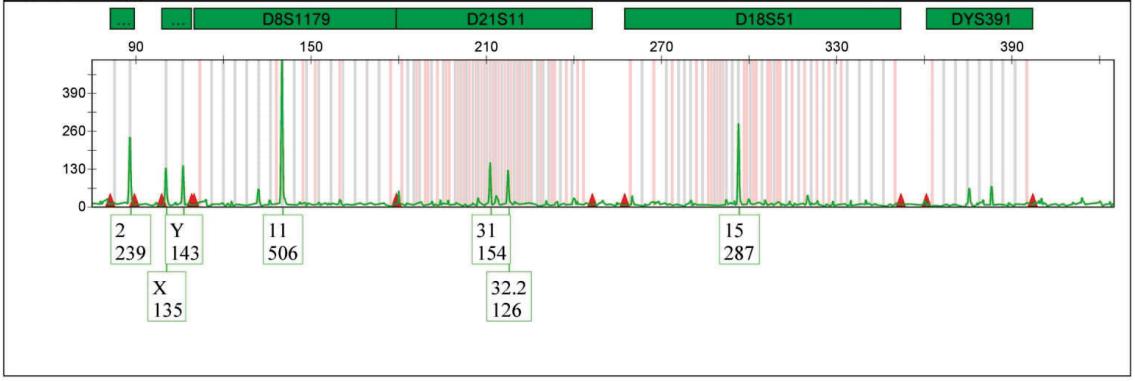
Project: Organic Extractions CRG 061422

Sample Name	SQ

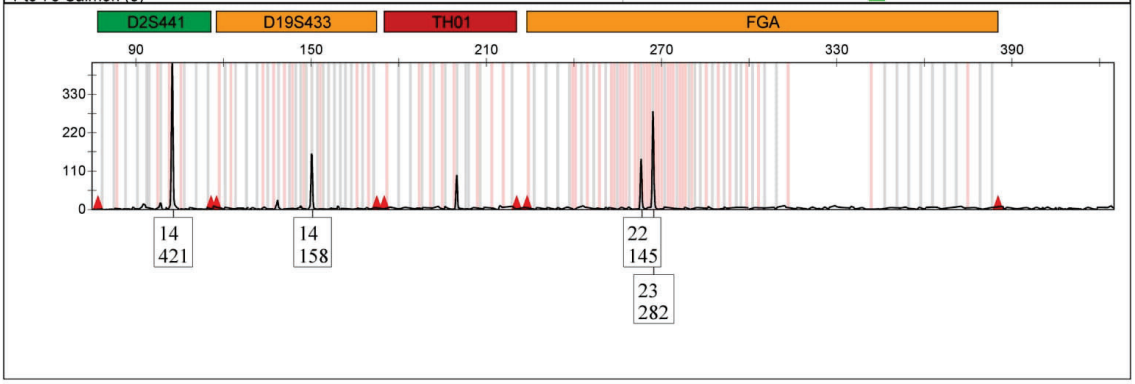
1 to 75 Salmon (3)



1 to 75 Salmon (3)



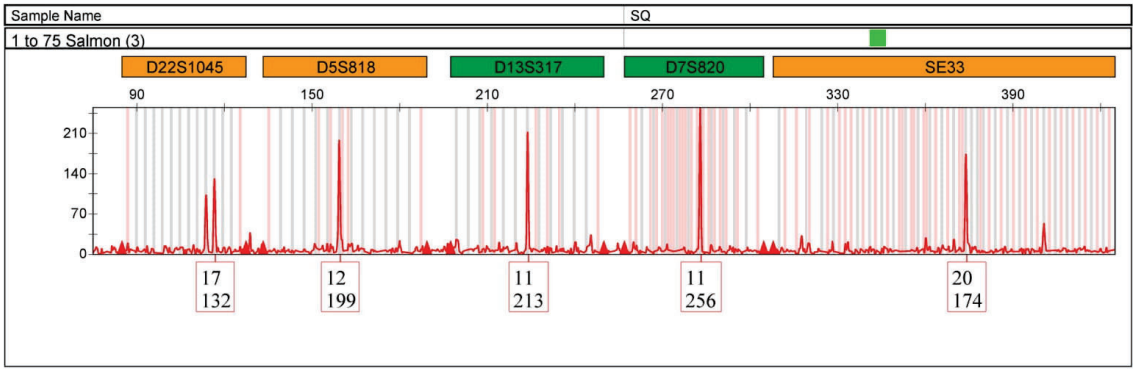
1 to 75 Salmon (3)



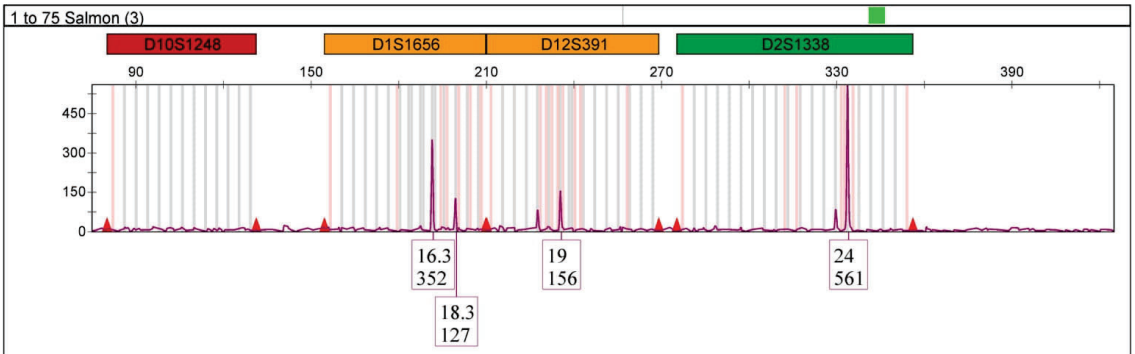
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

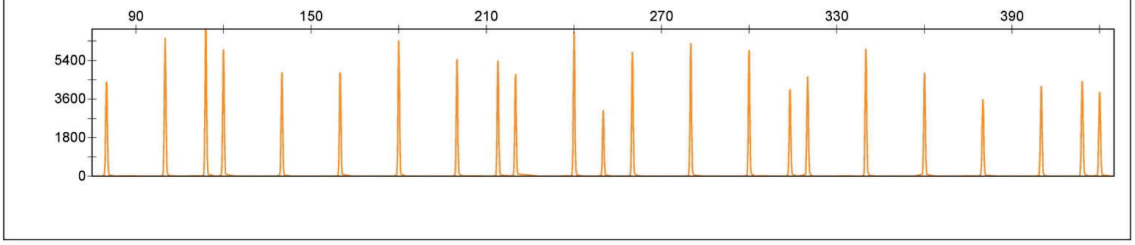
Sample Name	SQ
1 to 75 Salmon (3)	



1 to 75 Salmon (3)



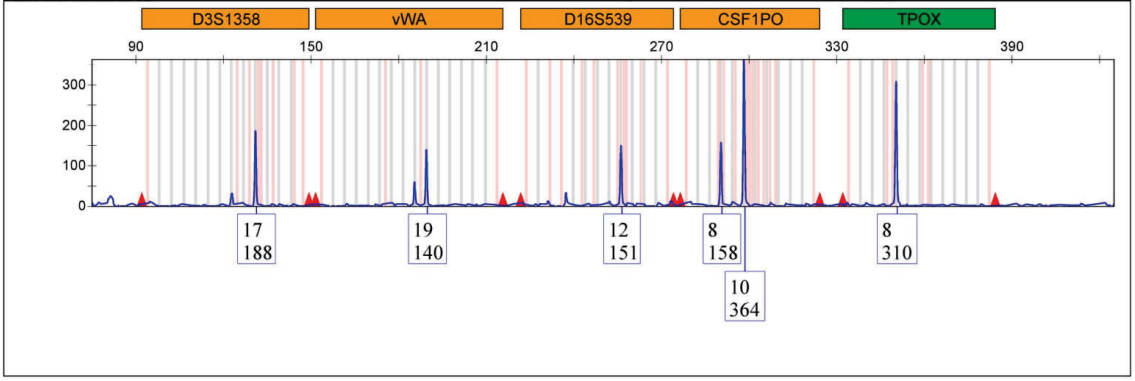
1 to 75 Salmon (3)



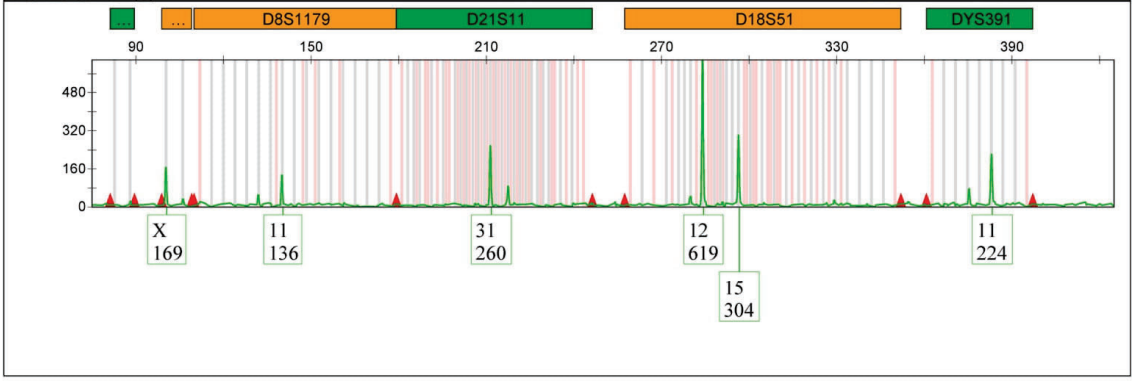
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

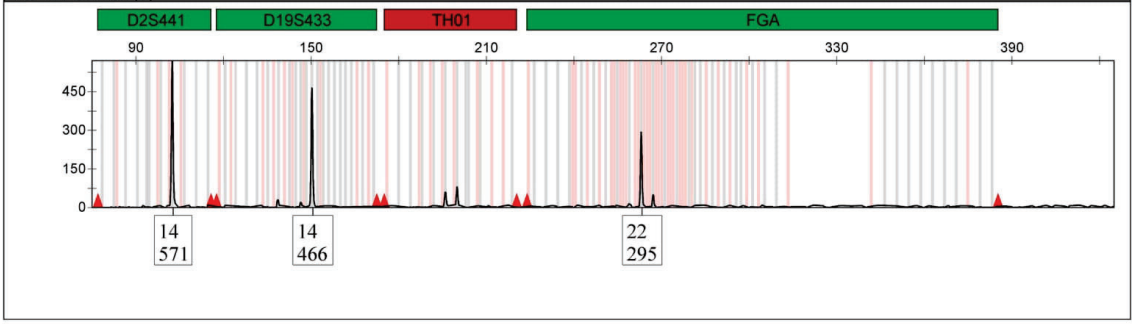
Sample Name	SQ
1 to 125 Salmon (3)	



1 to 125 Salmon (3)



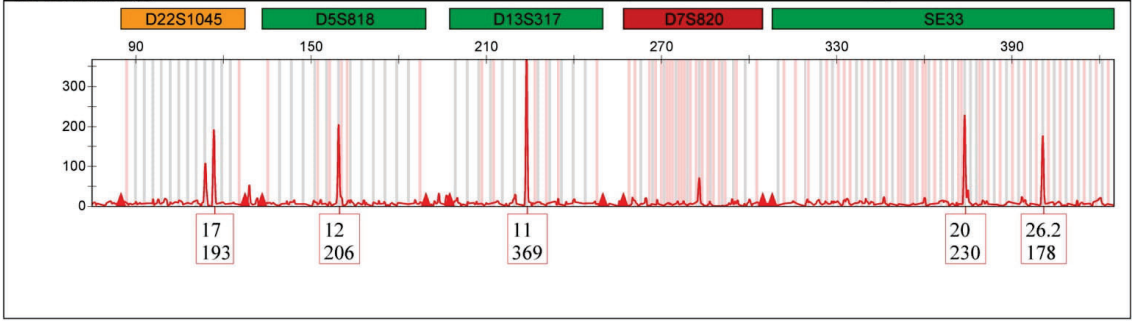
1 to 125 Salmon (3)



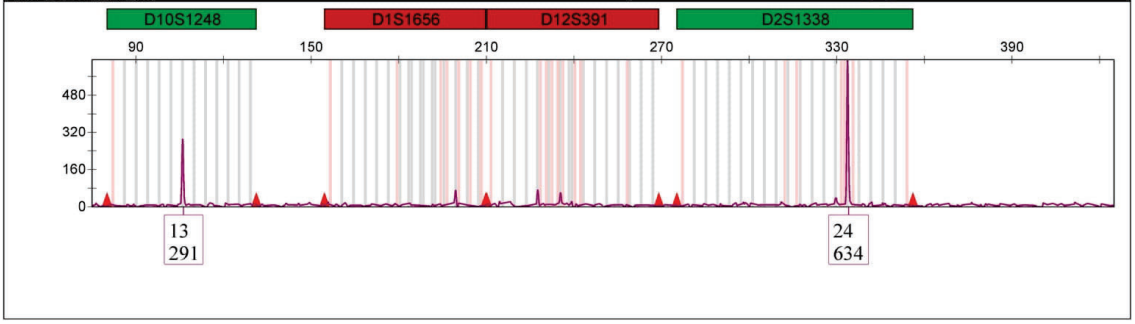
Applied Biosystems
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

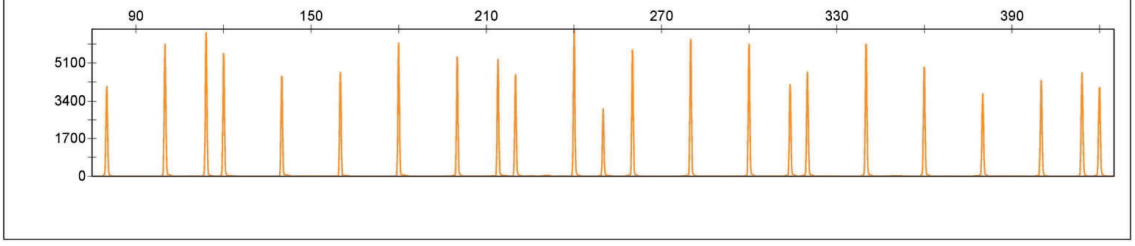
Sample Name	SQ
1 to 125 Salmon (3)	



1 to 125 Salmon (3)



1 to 125 Salmon (3)



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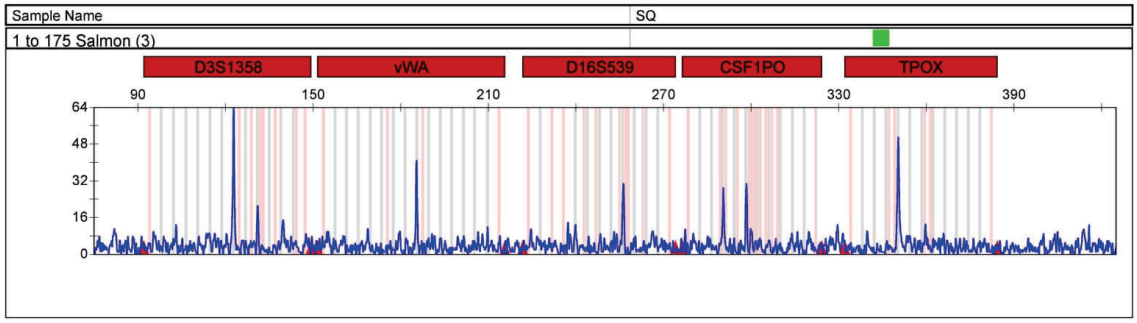
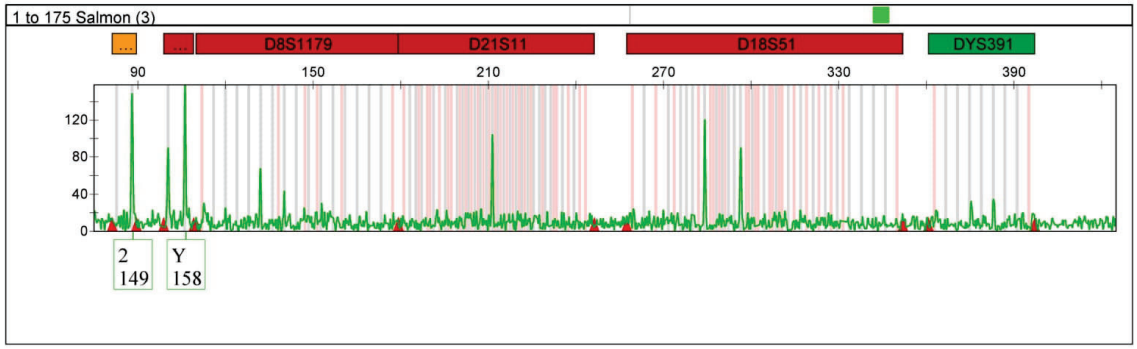
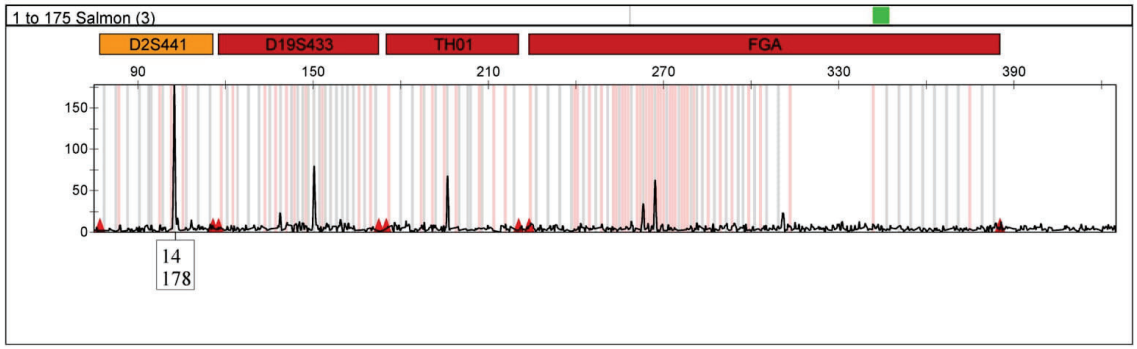
GeneMapper® ID-X 1.4

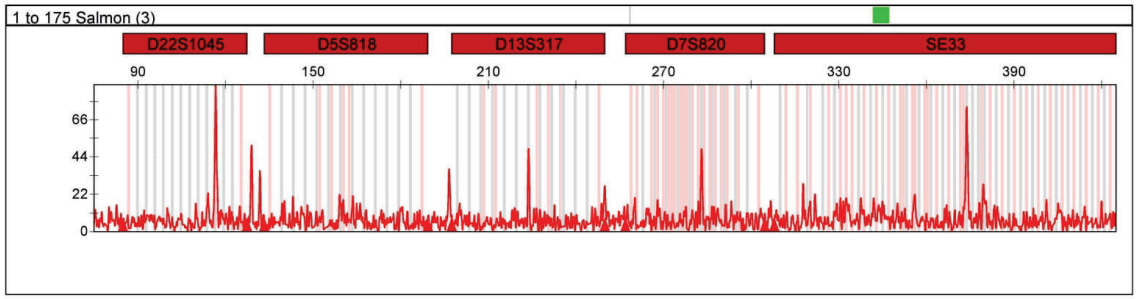
Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 175 Salmon (3)	









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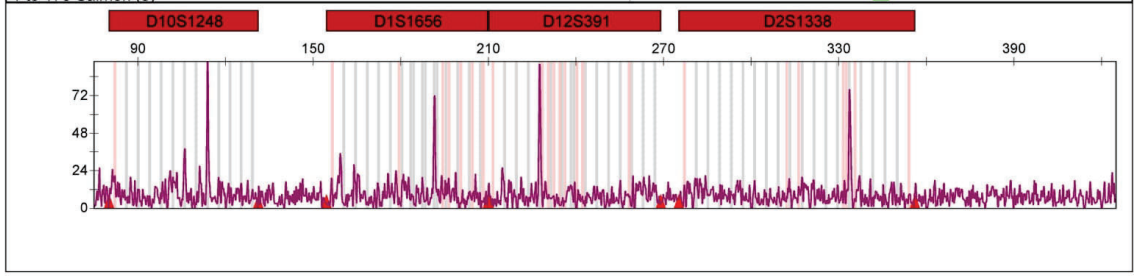
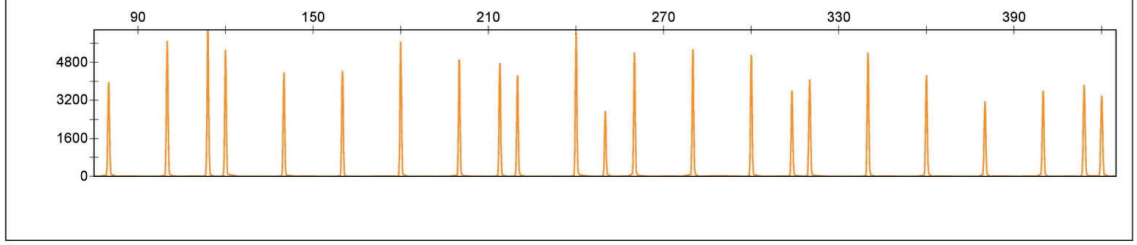
GeneMapper® ID-X 1.4

Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 175 Salmon (3)	



1 to 175 Salmon (3)



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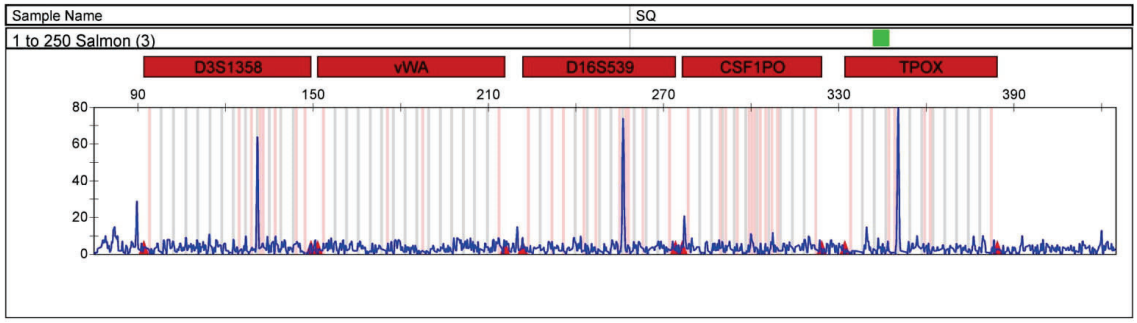
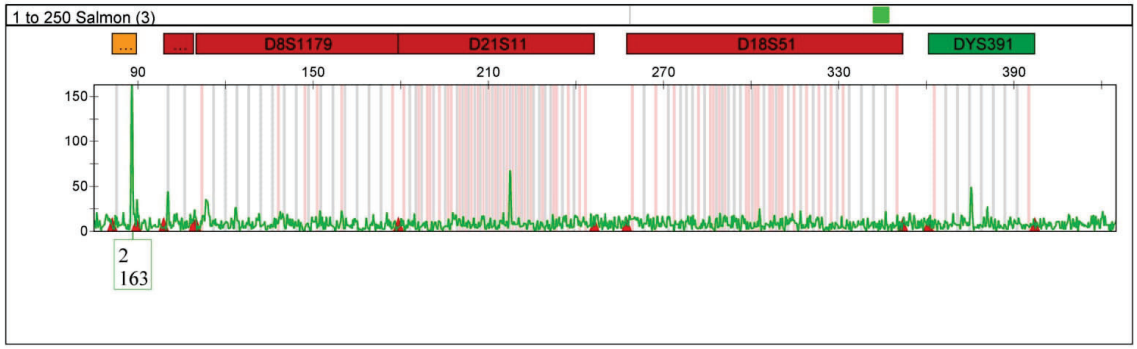
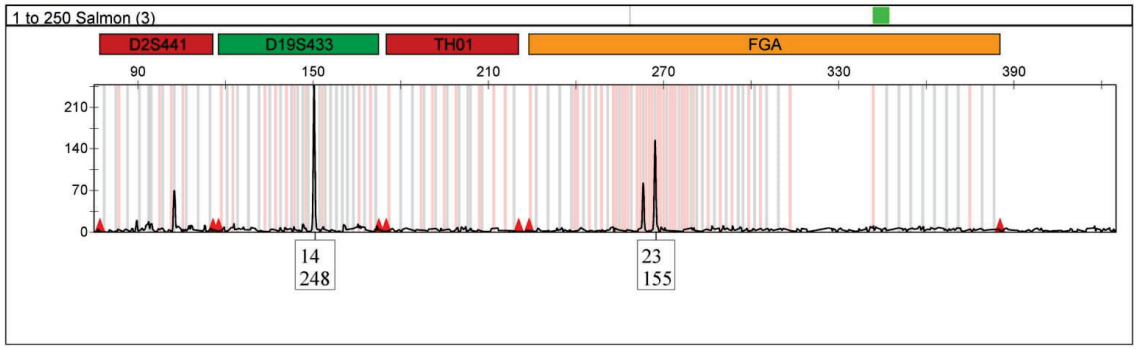
Sample Name	SQ
1 to 250 Salmon (3)	



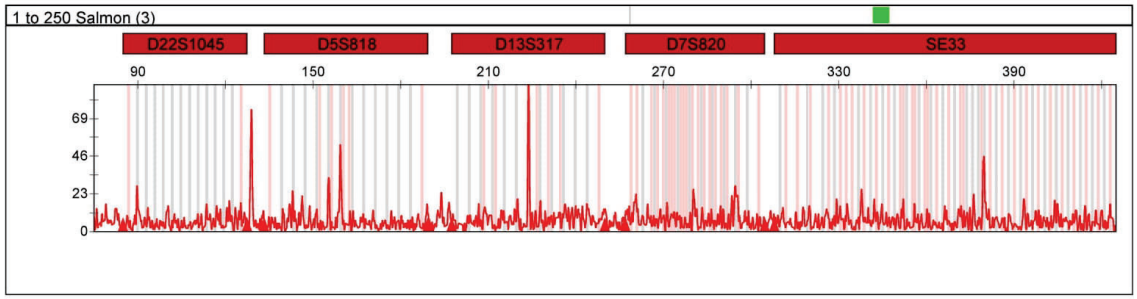
1 to 250 Salmon (3)



1 to 250 Salmon (3)



1 to 250 Salmon (3)



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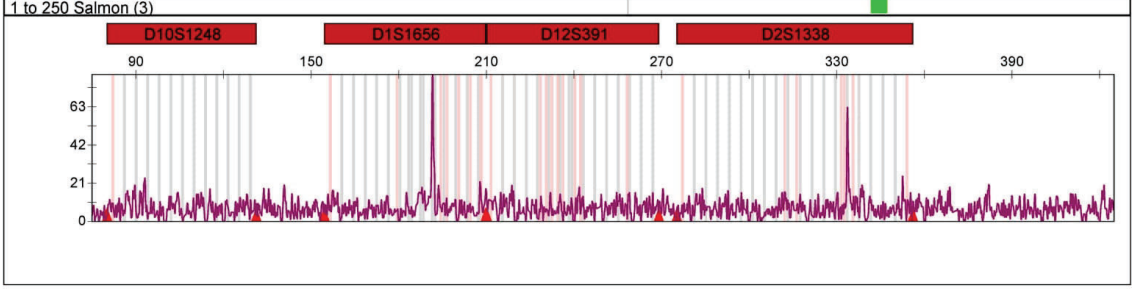
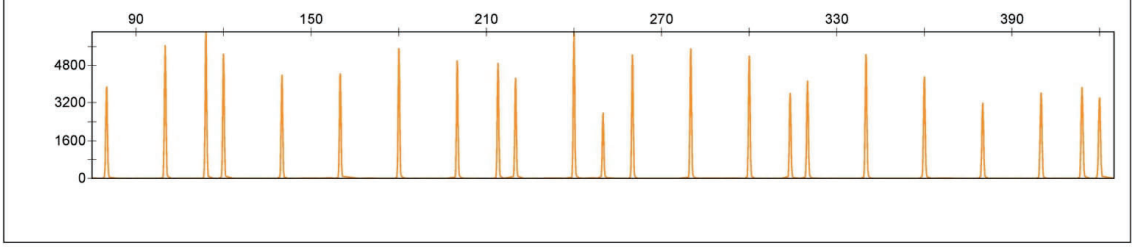
Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 250 Salmon (3)	



1 to 250 Salmon (3)



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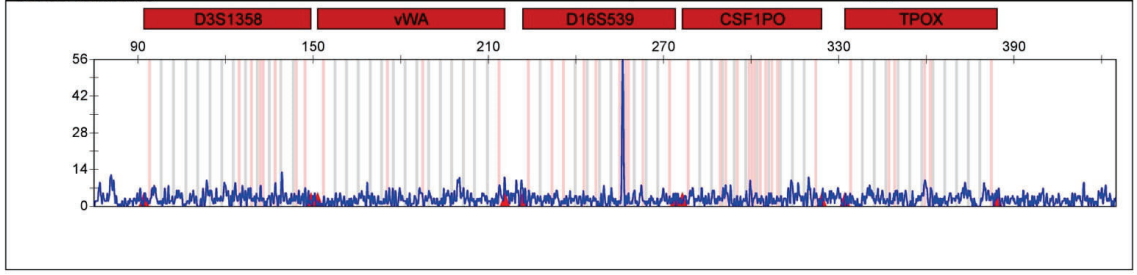
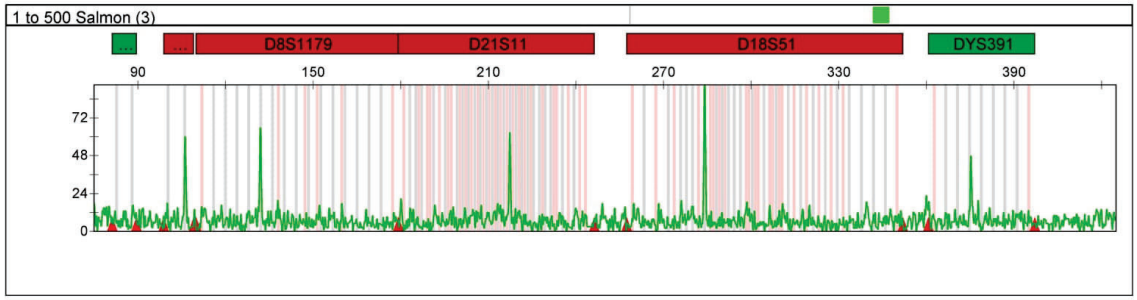
Project: Organic Extractions CRG 061422

Sample Name	SQ

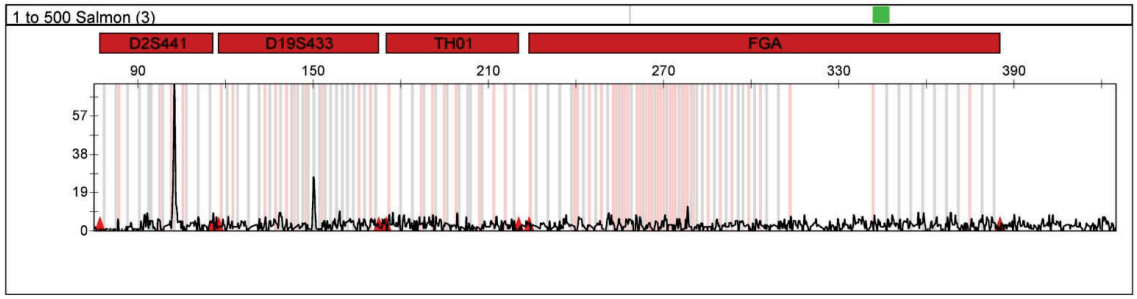
1 to 500 Salmon (3)



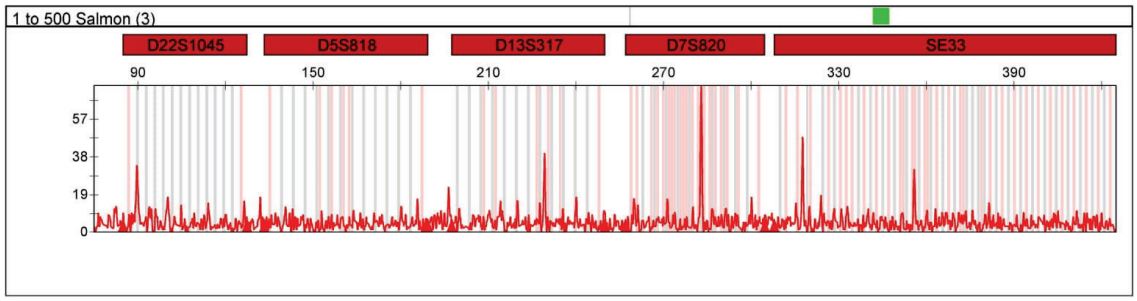
1 to 500 Salmon (3)



1 to 500 Salmon (3)



1 to 500 Salmon (3)



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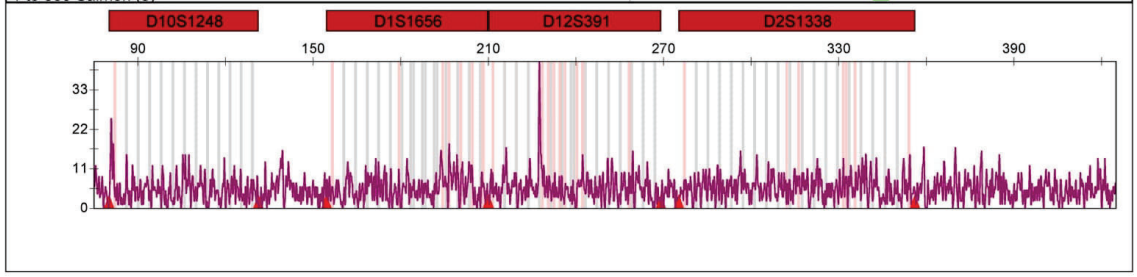
Project: Organic Extractions CRG 061422

Sample Name	SQ
1 to 500 Salmon (3)	



1 to 500 Salmon (3)