Lakehead UNIVERSITY | Paleo-DNA Laboratory
Centre for Analytical Services

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SER066-17 Final Report

File #: SER066-17

Date: 26 January 2018

Report of Expert

Expert's Name: Stephen Fratpietro, M.Sc., B.Ed.
Title: Technical Manager, Paleo-DNA Laboratory

I, the undersigned, as requested by Thierry Jamin, Instituto Inkari-Cusco, submit my professional opinion in reference to the following matter: This examination of exhibits is connected to an ancient DNA analysis.

ITEMS EXAMINED:
The following items (see Table 1) were submitted for genetic analysis by Thierry Jamin, Instituto Inkari-Cusco. These samples were designated the following case and sample number by the Paleo-DNA Laboratory (PDL):

PDL Case Designation	PDL Sample Designation	Sample Type	Comments
SER066-17	1	Tissue and Bone	Palm of right hand, Maria
SER066-17	2	Tissue and Bone	Finger of left foot, Maria
SER066-17	3	Bone	Coccyx 1, Maria
SER066-17	4	Bone	Left arm, Maria
SER066-17	5	Bone	Coccyx 2, Maria
SER066-17	6	Bone	Vertebrae, Maria

Table1. Samples submitted to the Paleo-DNA Laboratory.

EXAMINATION REQUESTED: Ancient DNA Analysis: extraction of DNA, nuclear DNA feasibility test, and autosomal DNA profiling

REQUIREMENTS REQUESTED: Determine if any genetic information could be extracted from the sample. Unless otherwise discussed, the industry standard extraction, purification and amplification protocols were to be used and attempted in this case.

The Paleo-DNA Laboratory agreed to work on the project in accordance with high scientific and professional standards, but as we had not been involved with the collection and storage of the sample, nor have we inspected the sample, nor have we assessed the condition of the sample, the Paleo-DNA Laboratory did not promise success in achieving any desired result. The Paleo-DNA Laboratory undertook this project giving no warranty of fitness for a particular purpose, or any other warranty,

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expressed or implied, on the results of your project or the tests carried out pursuant to your project. This includes no guarantee or warranty that the recommended protocol will achieve your desired results.

EXAMINATION METHODOLOGY:

All aDNA samples are prepared pre-amplification in a room dedicated specifically to limited quantity DNA samples. This environment is monitored quarterly for the presence of DNA. This lab has restricted access and requires protective gear to be worn at all times: tyvek suit covering head and feet, gloves, hairnet, facemask. All persons entering this lab have their DNA profiled and kept for future comparison.

Sample Preparation

All aDNA samples are rinsed in ethanol and allowed to dry. Each specimen is separately milled and dissolved into extraction buffer

DNA Extraction

TNE Extraction [Hansen et al, 1974]: **Sample 1 to 5**

Extraction buffer is made fresh consisting of 2175uL TNE [10mM Tris, 100mM Sodium Chloride(NaCl), 1mM Ethylenediaminetetraacetic acid disodium salt solution(EDTA)], 300uL 20% sodium dodecyl sulphate, 300uL 0.39M Dithiothreitol, 30uL 20mg/mL Proteinase K, and 195uL sterile water. This buffer is incubated with the sample overnight at 56°C for 500rpms agitation. The resulting supernatant is transferred to the *Silica Bead Purification*.

Total Demineralization [Loreille et al, 2007]: **Sample 6**

Approximately 1-2g of sample powder is mixed with 9.0mL 0.5M EDTA, 150uL 20% Lauryl Sarcosinate, and 200uL Proteinase K (20mg/mL) in a sterile 15.0mL tube. This reaction is incubated overnight at 56°C with gentle agitation. The resulting supernatant is transferred to the *Silica Bead Purification*.

Silica Bead Purification [modified Boom et al, 1990]:

The supernatant is mixed with 18mL 4M Guanidinium Thiocyanate and 15uL silica. This is allowed to sit for 4 hours at 4°C [to allow DNA to bind to silica] after which the

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supernatant is removed and the remaining silica washed with Working Wash Buffer (10mM Tris-HCl, 50mM NaCl, 1mM EDTA, anhydrous ethanol) and 100% ethanol, then allowed to dry. The silica is resuspended in 55uL sterile water and incubated for 1 hour at 56°C to allow DNA to unbind from silica and dissolve in the water. The resulting supernatant is transferred to the next step.

Size Exclusion Column Purification [Matheson et al, 2009]:

The purified DNA extract is further filtered using Biorad Micro Bio-Spin P30 Chromatography Columns as per manufacturer's instructions.

It is important to note that an extraction control (negative) is carried through this entire process as a quality control measure.

Quantification

Nuclear DNA is targeted using Life Technologies Quantifiler™ Human DNA Quantification kit as per manufacturer's instructions run on the Cepheid Smart Cycler® II.

Fragment Analysis

Autosomal DNA is amplified in 10uL reactions using the Promega PowerPlex® 21 System using manufacturer's cycling parameters. This PCR reaction batch includes a positive and negative PCR control. Each locus is amplified at least twice for replication.

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RESULTS: The results below relate only to the items tested.

The results for the nuclear DNA feasibility test were as follows:

Sample	Nuclear DNA Feasibility
Palm of right hand, Maria (1)	positive
Finger of left foot, Maria (2)	positive
Coccyx 1, Maria (3)	negative
Left arm, Maria (4)	negative
Coccyx 2, Maria (5)	negative
Vertebrae, Maria (6)	positive

A positive result indicates that there is sufficient nuclear DNA in the extract to proceed to further autosomal DNA testing.

Autosomal Analysis

Autosomal analysis was performed on the following samples from 'Maria': Palm of right hand (1), Finger of left foot (2), vertebrae (6). Each analysis was performed in triplicate. Two pieces of numerical information are produced for each DNA marker analyzed. Since half of the biological mother's and half of the biological father's DNA are passed onto the child, one piece of information from each marker comes from each biological parent.

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DNA Markers Analyzed	Palm of right hand (1)	Finger of left foot (2)	Vertebrae (6)
AMEL	X [Y]	X [Y]	X,Y
D3S1358	15,16,17	15,16 [17]	14,15,16,17
D1S1656	11,14	13,14,15	13,14,15
D6S1043	11	14,21.3 [11,18,19,20.3]	14,17,21.3
D13S317	10,14	9,11,12	10,11,12
Penta E	NR	17	15,17
D16S539	11 [10,12]	10,11	10,11,12
D18S51	13,14,17,18 [20]	14,17,18 [13,16]	17,18,20
D2S1338	17,19	17,19,20	19,20,23 [22]
CSF1P0	NR	10,11,12	12
Penta D	11	NR	10,14 [11]
TH01	7,9.3	7 [9.3]	7 [6,9.3]
vWA	16 [18,19]	16 [14,15,17,19]	14,16
D21S11	29,32.2	32.2	29,30,32.2 [31.2]
D7S820	10	11	11
D5S818	11,12	7,11,12	11
TPOX	11	11	8,11
D8S1179	10,14 [8,16]	10,14 [11,12,13,16]	10,12,14
D12S391	[16,18]19,20,21	16,19 [21]	16,19 [18,21]
D19S433	14[13,15,21]	13,15,17	13,14,15 [15.2]
FGA	24,25,26	21,25,26	21,26

Summary of results from three distinct analyses. In some cases more than two alleles were present at one marker. Results from the three analyses of each sample were combined. Any and all major (high level) alleles are stated as such. Any and all minor (low level) alleles are denoted with '[ ]'. 'NR' means no results were obtained.

The following conclusions were drawn from the data obtained:

- There is evidence of DNA contamination.
- Palm of right hand (1) contains DNA from more than one individual.
- Finger of left foot (2) contains DNA from more than one individual.
- Vertebrae (6) contains DNA from more than one individual.
- The Amelogenin marker [AMEL] (the marker used for sex identification within this genotyping kit) shows that for each of the three samples tested, there is a major component of female DNA and a minor component of male DNA.
- For each of the samples tested, there is a presence of, at least, one female individual and one male individual.
- Finger of left foot (2) and Vertebrae (6) show evidence of sharing a common source of DNA.
- There is not sufficient data to include nor exclude Palm of right hand (1) having a common source of DNA to Finger of left foot (2) and Vertebrae (6) with any confidence.

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NOTES:

Controls were run at every step of the analysis and gave expected results. The above profiles do not match any staff member or laboratory user at the Paleo-DNA Laboratory, past or present. This analysis complies with the requirements requested by the client. Details of the experimental procedures and analysis of this case are found in the case file of the Paleo-DNA laboratory, case number SER066-17. Your feedback is important to us! Please fill out our customer survey at http://lucas.lakeheadu.ca/customer-survey.

Technical Manager: Stephen Fratpietro

Stephen Fratpietro

Date: 06 Feb 2018

Sample Name	Panel	SQO	SQ
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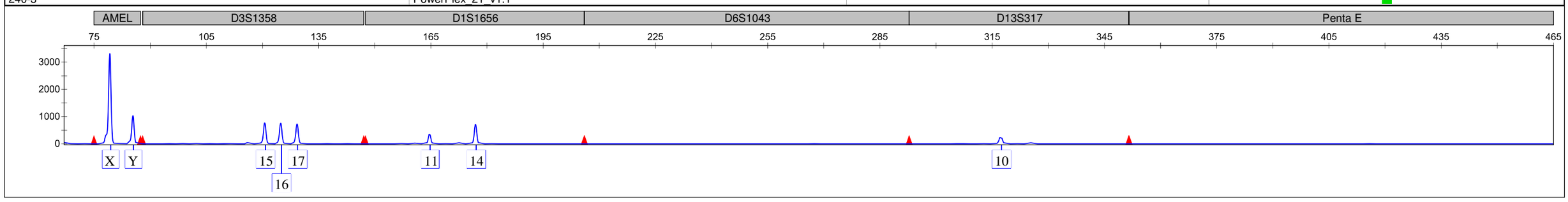

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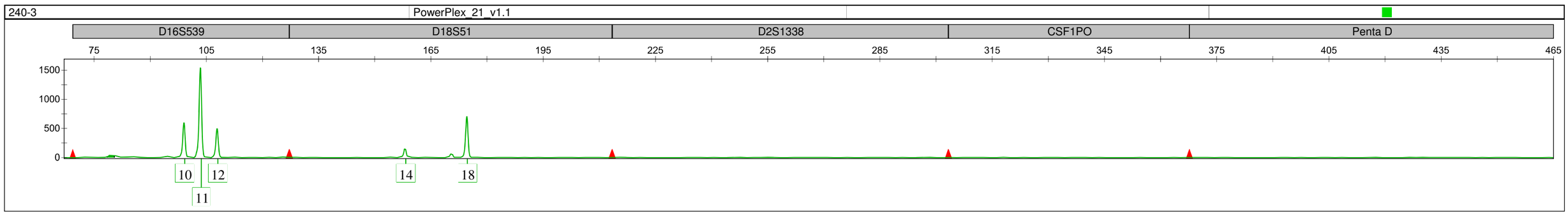

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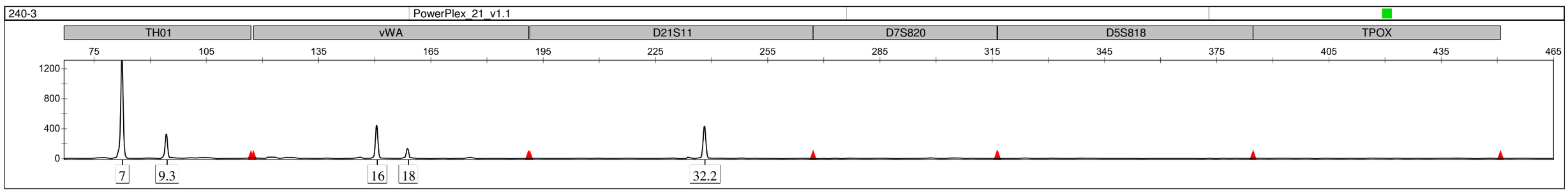

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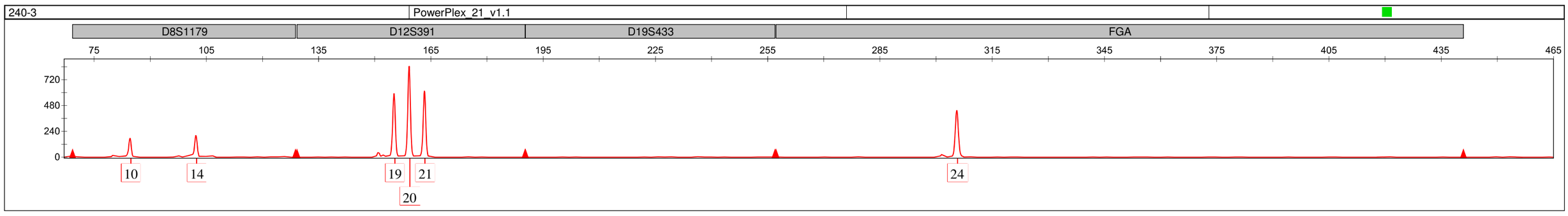

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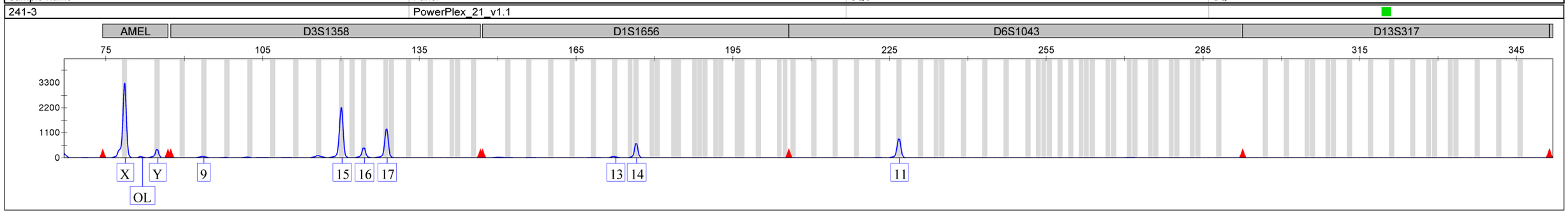

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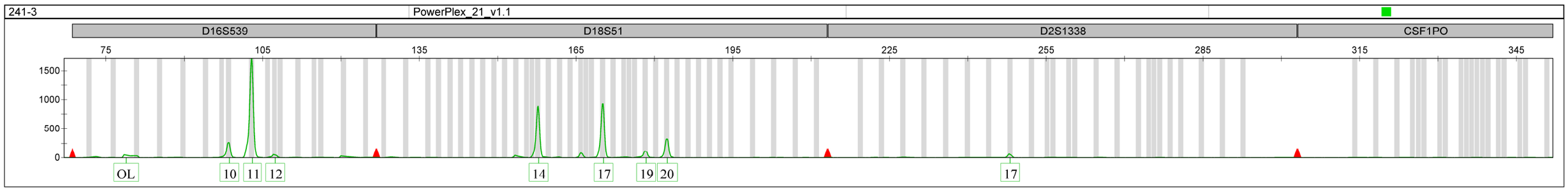

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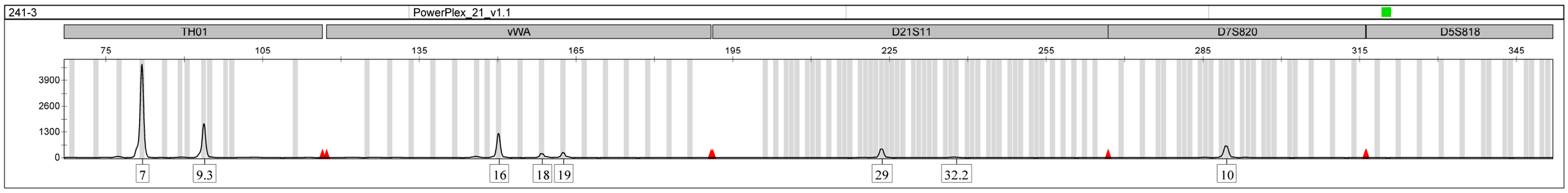

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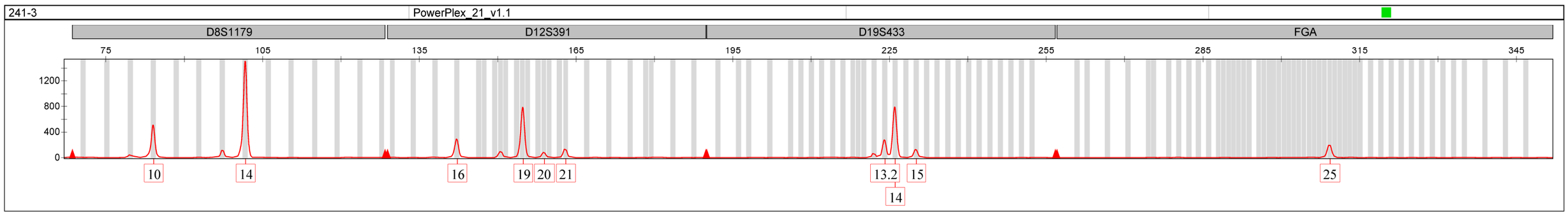

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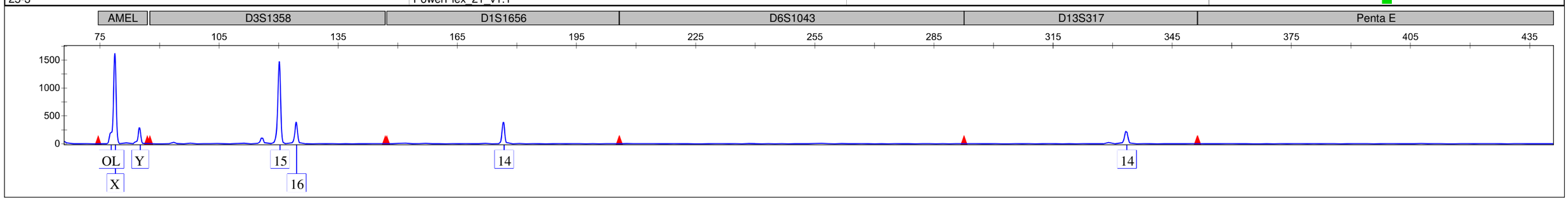

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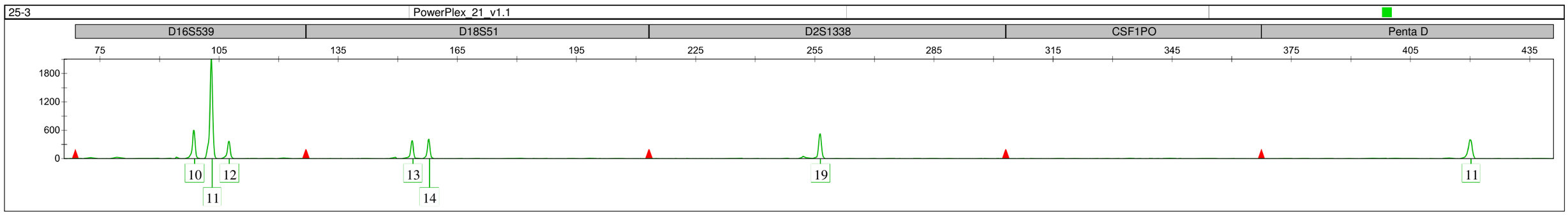

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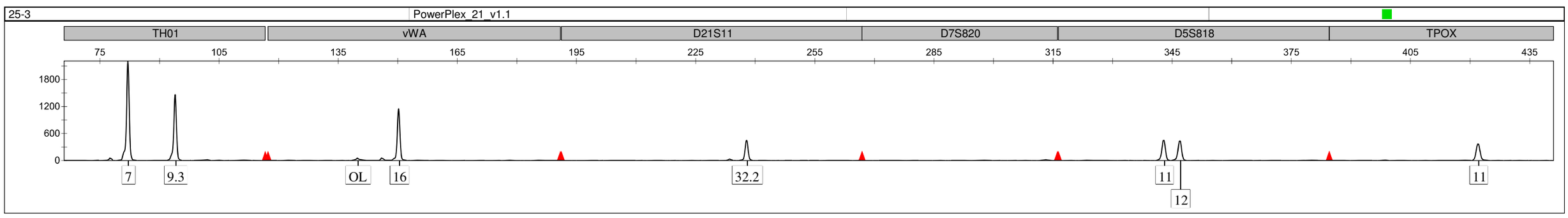

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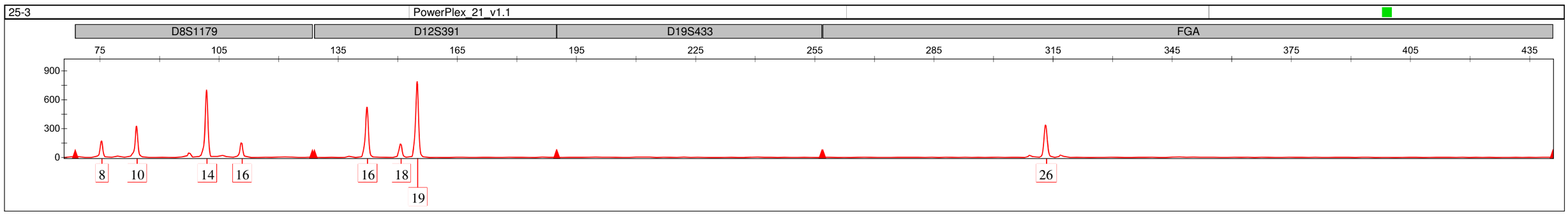

Sample Name	Panel	SQO	SQ
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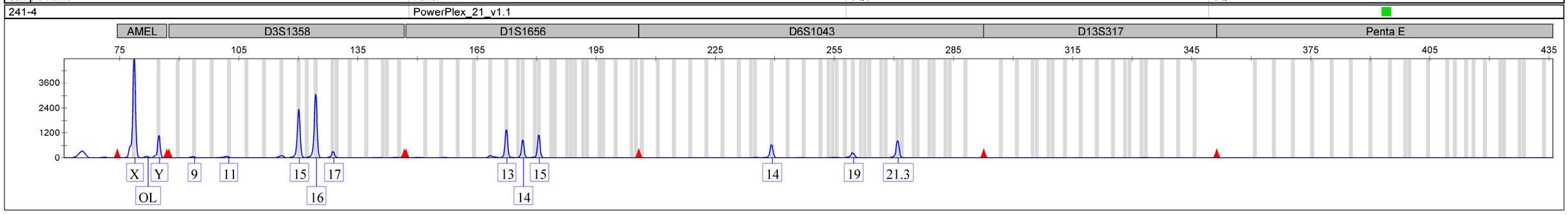

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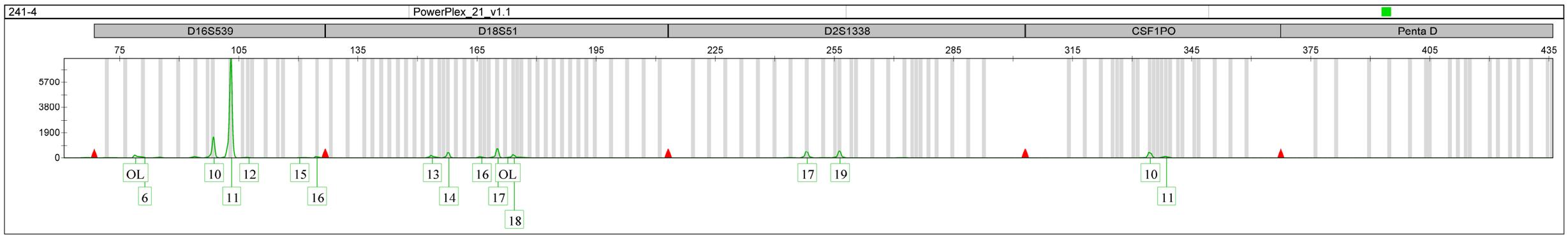

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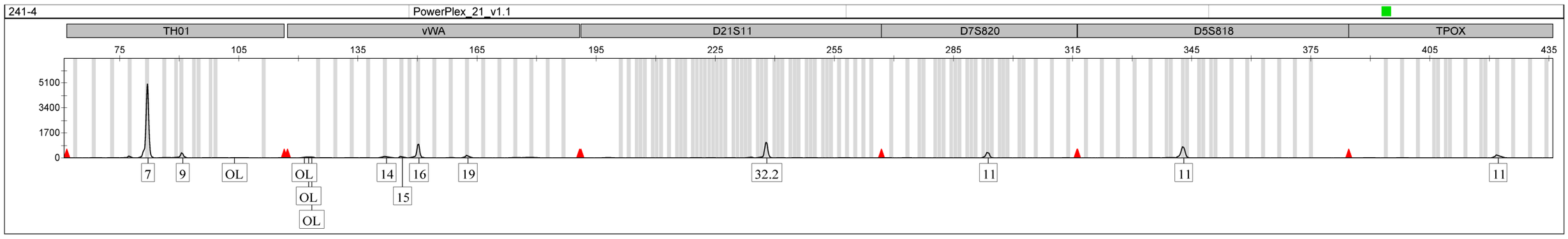

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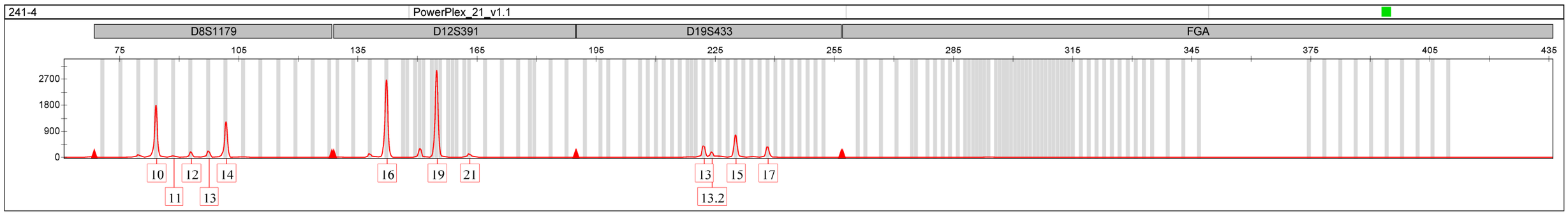

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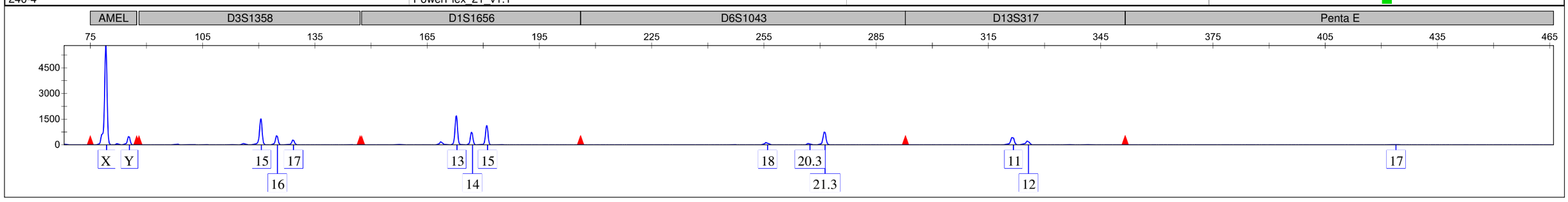

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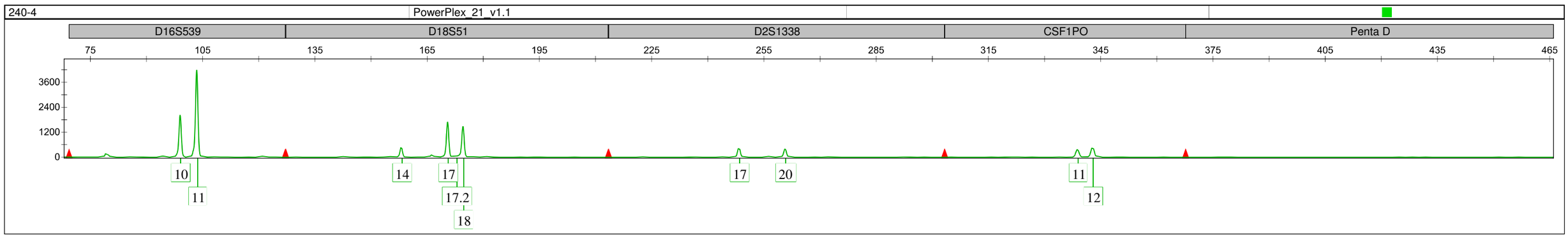

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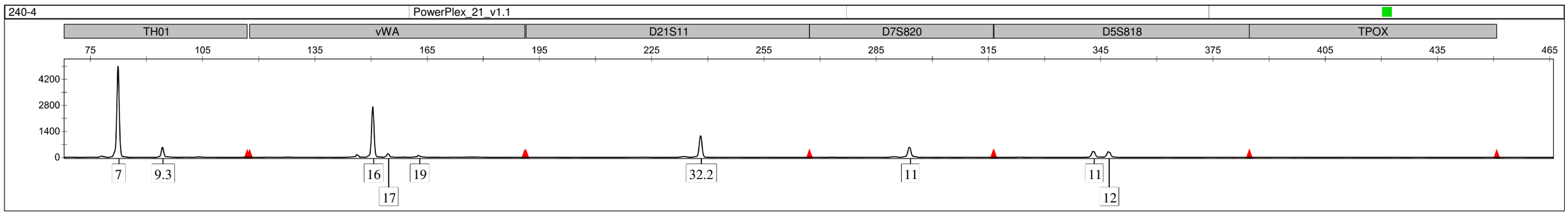

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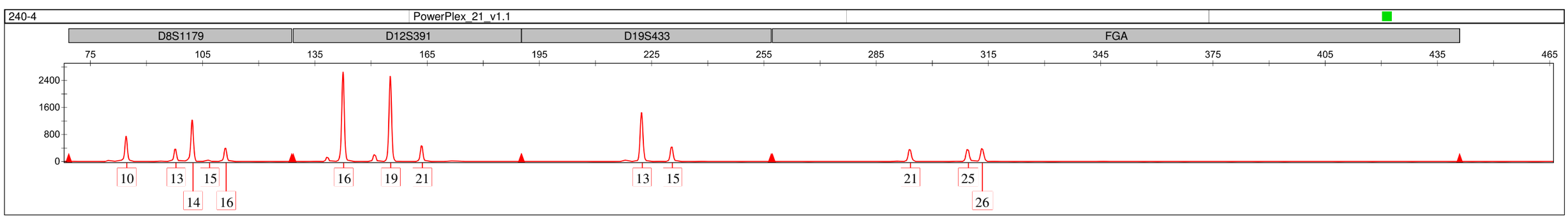

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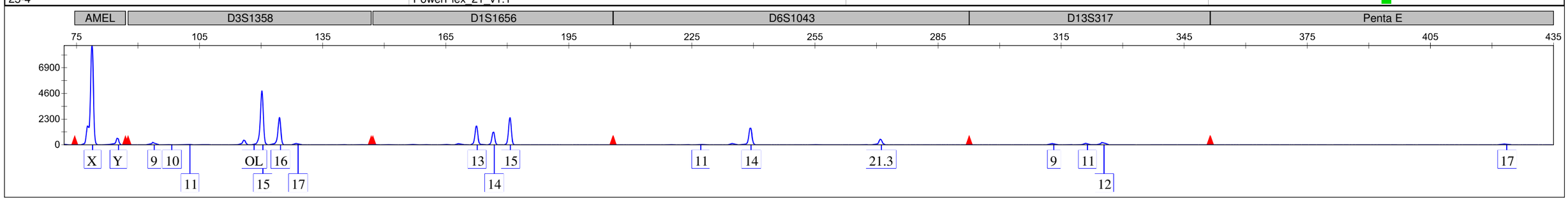

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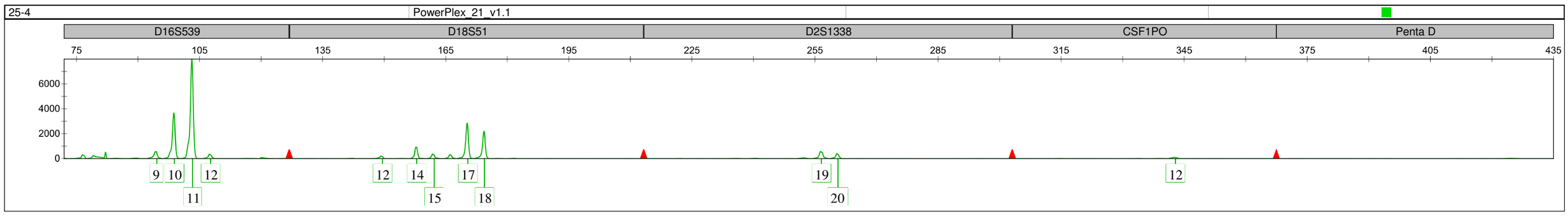

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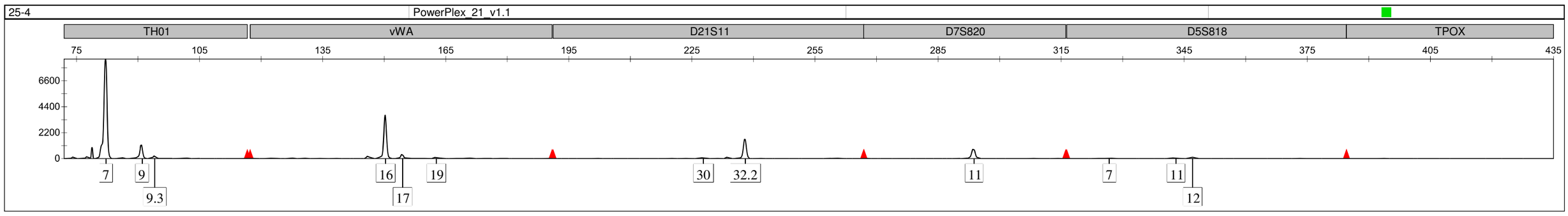

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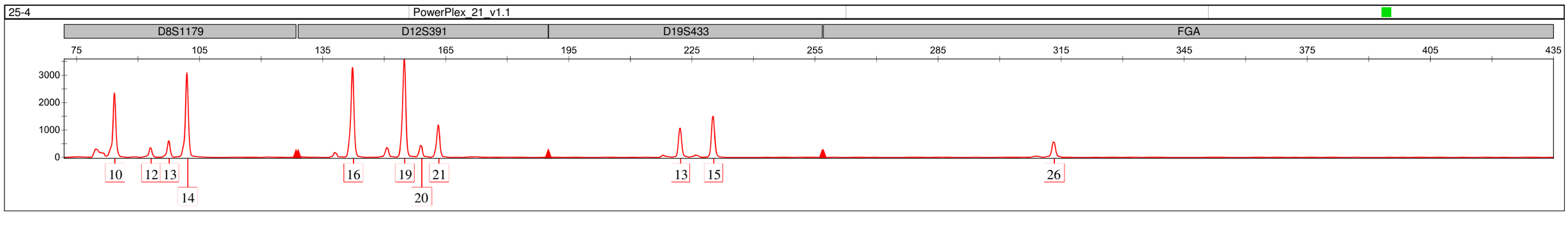

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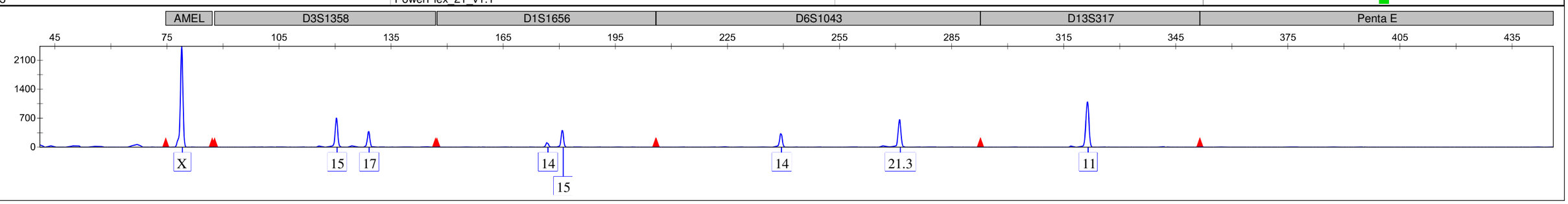

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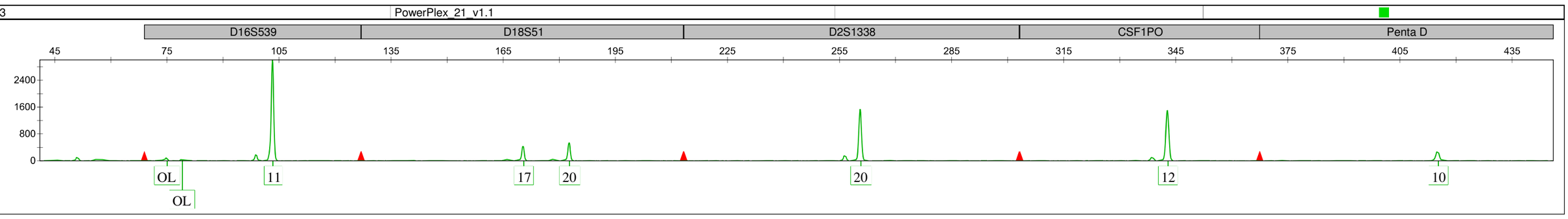

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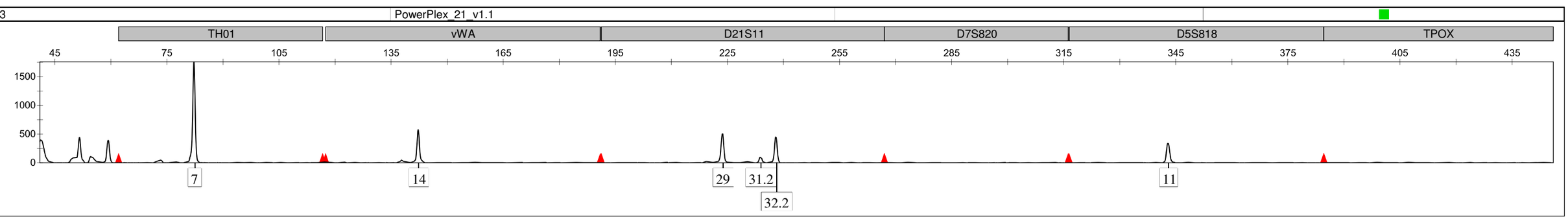

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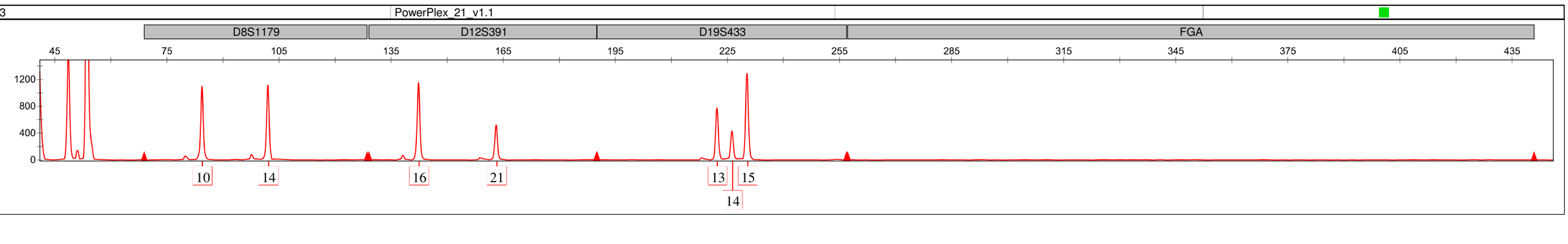

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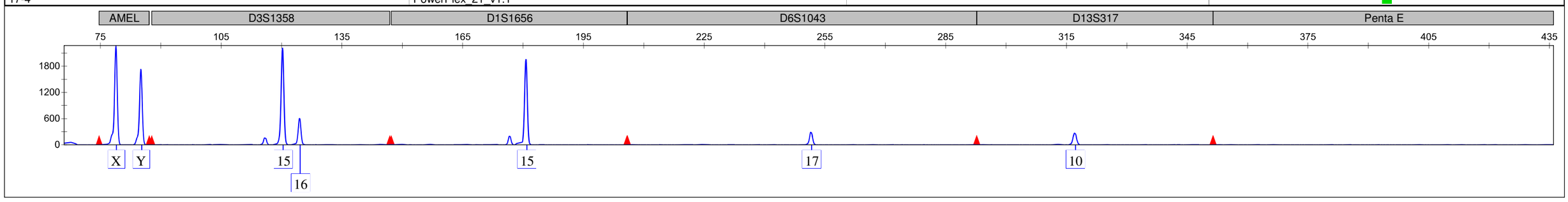

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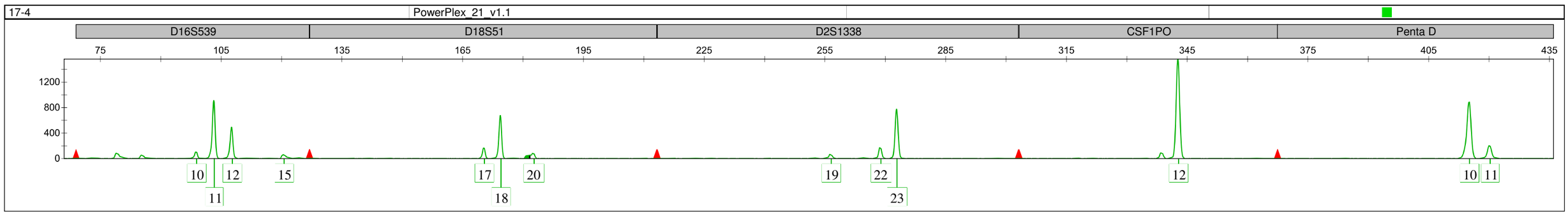

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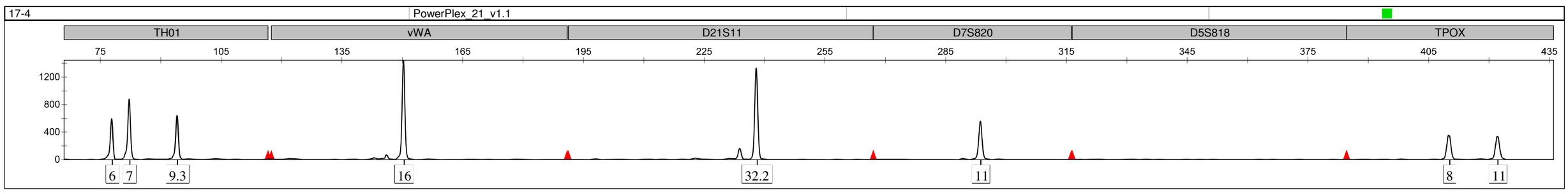

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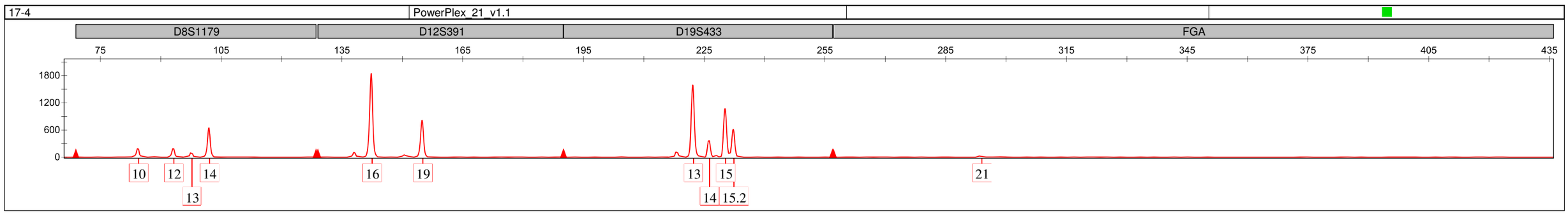

Sample Name	Panel	SQO	SQ
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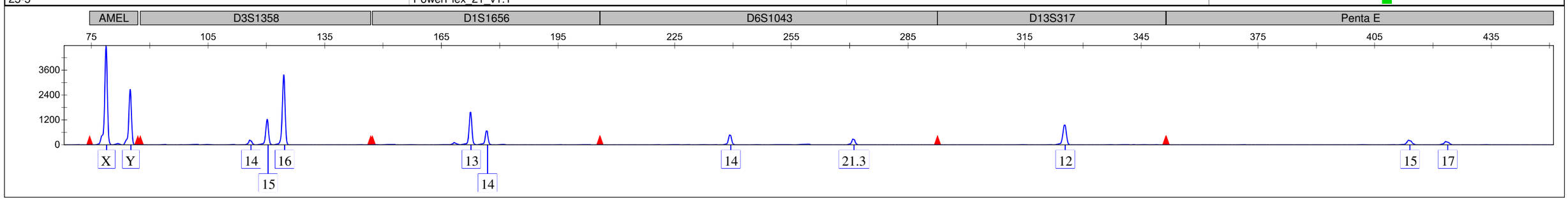

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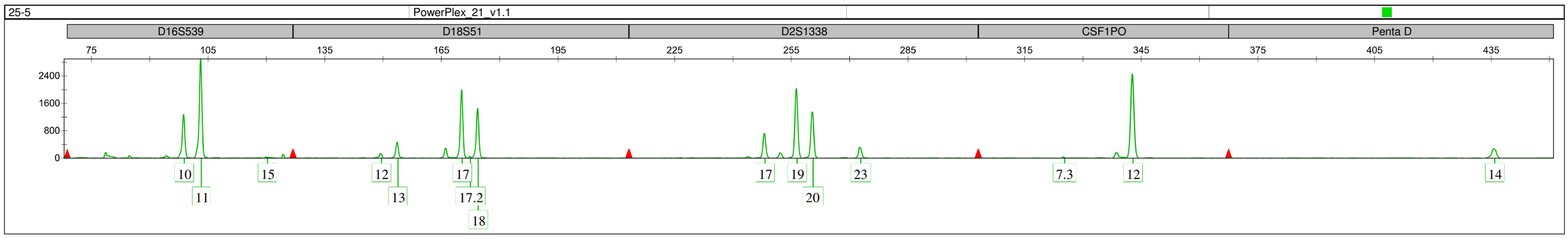

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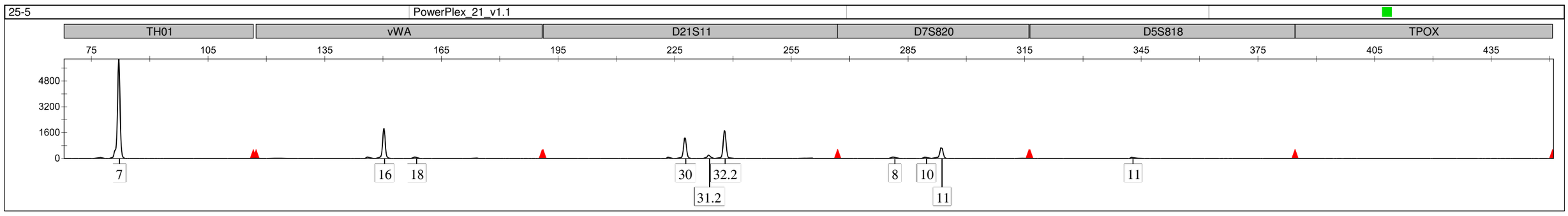

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