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TECHNICAL NOTE

Artifacts Identified Post-Developmental Validation: GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits

The purpose of this document is to assist with data interpretation by providing a repository of artifacts identified and characterized as a result of investigating customer reports following developmental validation of the GlobalFiler kit (Part Numbers 4476135 and 4482815) or after performance verification of the GlobalFiler IQC (Part Number A43565) kit. Many artifacts in this document fall below the peak amplitude threshold (PAT) used during validation or verification, appear outside of the read region, or are attributable to specific sample types not encountered during validation or verification.

The GlobalFiler™ Express (Part Numbers 4476609 and 4474665) PCR amplification kit shares primer sequences with the mutual markers of the GlobalFiler and GlobalFiler IQC kits. Therefore, the artifacts described in this technical note may also be observed in the GlobalFiler Express kit. However, artifact signal intensities and ratios to parent peaks may differ due to differences in primer concentrations.

Background

During developmental validation and performance verification of the GlobalFiler and GlobalFiler IQC PCR Amplification Kits, a 175 RFU PAT was applied to the 3500/xL Genetic Analyzer data. Following internal validations, many laboratories implemented PATs below 175 RFU. As a result of lower analysis thresholds, low-level artifacts not identified in the developmental validation/performance verification have been reported.

Due to the wide variety of sample and substrate types processed in forensic laboratories, it is not feasible to process all sample and substrate type combinations during STR kit development and validation testing. Instead, representative samples and substrates are selected and analyzed during this process. Therefore, it is possible for sample-specific artifacts to be identified through customer reports and subsequently tested and characterized in the Thermo Fisher Scientific HID Laboratory. Such artifacts are included in this document to assist in interpreting data generated from similar sample or substrate types.

Various mechanisms, such as dye byproducts, the formation of secondary structures, nontraditional stutter, non-specific binding, or non-human interaction, can introduce artifacts into PCR STR data. The artifacts' detectable presence and intensity (peak height) can depend upon amplification or electrophoresis conditions, such as elevated PCR cycle numbers or CE system sensitivity differences.

Method

Artifacts included in this technical note were detected and characterized post-product development. Artifacts from standard PCR phenomena, such as traditional stutter or minus A peaks, or observed during the developmental validation species specificity studies are not included. See the GlobalFiler and GlobalFiler IQC User Guide (Publication Number 4477604) for information on these artifacts.

The data herein is specific to GlobalFiler or GlobalFiler IQC kit data run on a 3500 series instrument and, unless otherwise noted, was generated following all manufacturer-recommended PCR and

electrophoresis conditions, as documented in the GlobalFiler and GlobalFiler IQC User Guide. The information provided also applies to GlobalFiler/ GlobalFiler IQC kit data run on other electrophoresis platforms, although peak heights and base pair sizes may differ.

Newly identified and characterized artifacts will be periodically added to this document. Refer to Table 1 for a summary of the artifacts presented here.

Table 1: Summary of Artifacts. Click on the hyperlinks for more information.

Dye Channel	Locus	Artifact ID	Approximate Base Pair (bp) Size	Typical Allele Call
6-FAM™	D3S1358	GF_D3-1	132	OL, 17.2 or 17.3
	D3S1358	GF_D3-2	126-127	Varies
	vWA	GF_vWA-1	204	OL or 22.3
	D16S539	GF_D16-1	228	OL
	TPOX	GF_TPOX-1	N-24	Varies
VIC™	N/A	GF_VIC-1	94	OMR
	N/A	GF_VIC-2	95	OMR
	Y indel	GF_YIndel-1	84	OL
	D8S1179	GF_D8-1	114-121	Varies
	D8S1179	GF_D8-2	124	OL or 7.2
	D8S1179	GF_D8-3	113	OL
	D21S11	GF_D21-1	207	29.3 or 30
	D18S51	GF_D18-1	286	13.1
	D18S51	GF_D18-2	N-2	Varies
	DYS391	GF_DYS391-1	N-5	Varies
NED™	N/A	GF_NED-1	63	N/A
	D2S441	GF_D2NED-1	91-100	Varies
	D2S441	GF_D2NED-2	N-2 to N-2.5	Varies

	D2S441	GF_D2NED-3	76	8
	D19S433	GF_D19-1	N-6	Varies
	D19S433	GF_D19-2	N+2 to N+4	Varies
	TH01	GF_TH01-1	N-10 to N-12	Varies
	FGA	GF_FGA-1	N+2	Varies
	FGA	GF_FGA-2	N-8	Varies
TAZ™	N/A	GF_TAZ-1	250	OMR
	D5S818	GF_D5-1	180	OL
	D5S818	GF_D5-2	142	OL or 7.3
	D13S317	GF_D13-1	215	9
	SE33	GF_SE33-1	N-90 bp	Varies
	SE33	GF_SE33-2	352.5	15.2
	SE33	GF_SE33-3	424	OL
SID™	D10S1248	GF_D10-1	N-2.5	Varies
	D2S1338	GF_D2SID-1	345	OL

Detailed Artifact Descriptions

Dye Channel: 6-FAM

Artifact ID: GF_D3-1

Locus: D3S1358	**Size**: ~132 bp
Probable cause: DNA template-dependent artifact likely caused by a non-STR amplification byproduct.	
Signal Intensity: Relative to DNA input amount (typically <175 RFU with a 1 ng template input).	

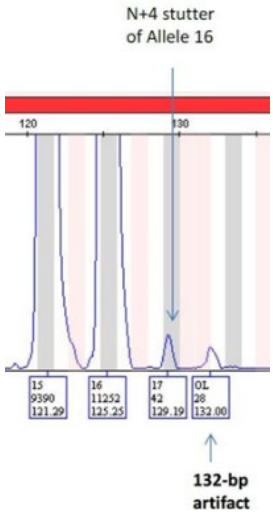


Figure 1: GlobalFiler PCR artifact (GF_D3-1) at 132 bp in D3S1358, obtained from 1 ng 007 control DNA. The y-axis is scaled to 300 RFU, and the artifact is 28 RFU.

Artifact ID: GF_D3-2

Locus: D3S1358	**Size**: ~126-127 bp
Probable cause: DNA template-independent artifact likely caused by a dye derivative, which is a cleavage of the dye from the labeled primer.	
Signal Intensity: Typical signal intensity: <175 RFU.	
Additional information: ▪ May be promoted by post-manufacturing activities, such as repeated freeze/thaw cycles, extended use of the kit beyond recommended storage conditions, or the introduction of chemical factors (for example, cleaning reagents).	

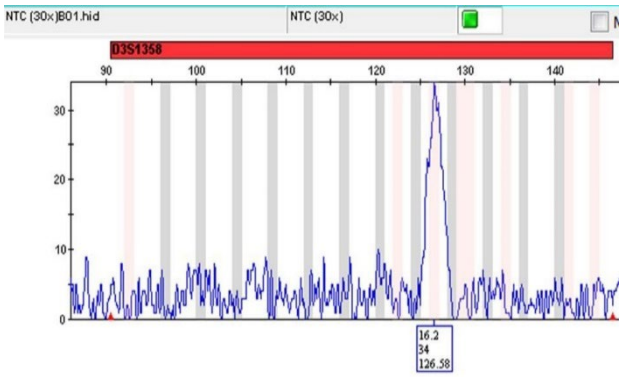


Figure 2: GlobalFiler dye artifact (GF_D3-2) at 126.58 bp in D3S1358, obtained from a non-template control (NTC) amplified with GlobalFiler (30 cycles). The y-axis is scaled to 35 RFU, and the artifact is 34 RFU.

Artifact ID: GF_vWA-1

Locus: vWA	**Size**: ~204 bp
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of non-human DNA in the sample.	
Signal Intensity: Relative to the amount of non-human DNA present.	
Additional information: ▪ Has been observed in a variety of crime scene related sample types (for example, swabs of car parts, clothing, cigarette butts, and swabs of miscellaneous items recovered outdoors). ▪ Sequencing studies have shown that the artifact sequence has partial homology to yeast and fungal species commonly found in the environment with no sequence homology to the human genome.	

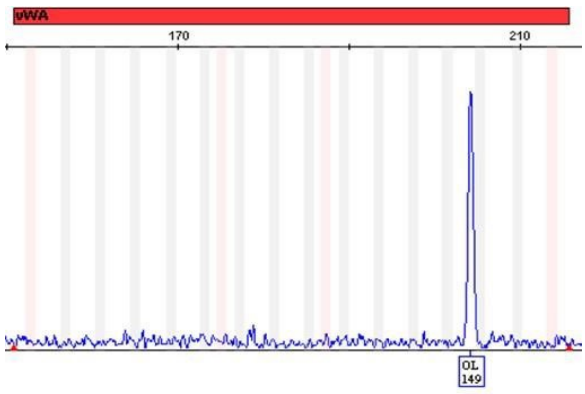


Figure 3: GlobalFiler sample-dependent artifact (GF_vWA-1) at 204.09 bp in vWA, obtained from a swab of a bottle with an undetermined quantity /input amount. The y-axis is scaled to 170 RFU, and the artifact is 149 RFU.

Artifact ID: GF_D16-1

Locus: D16S539	**Size**: ~228 bp
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of non-human DNA in the sample.	
Signal Intensity: Relative to the amount of non-human DNA present.	
Additional information: ▪ Related to the GF_VIC-2 and GF_SE33-3 artifacts also listed in this document. ▪ The combined sequencing and NCBI BLAST search results for the three related artifacts indicate the most likely source of the artifacts is cross reactivity with some species of pig or wild boar DNA. ▪ This artifact has also been observed in the Identifiler Plus kit at ~247-248 bp in the FAM dye channel (IDP_FAM-1).	

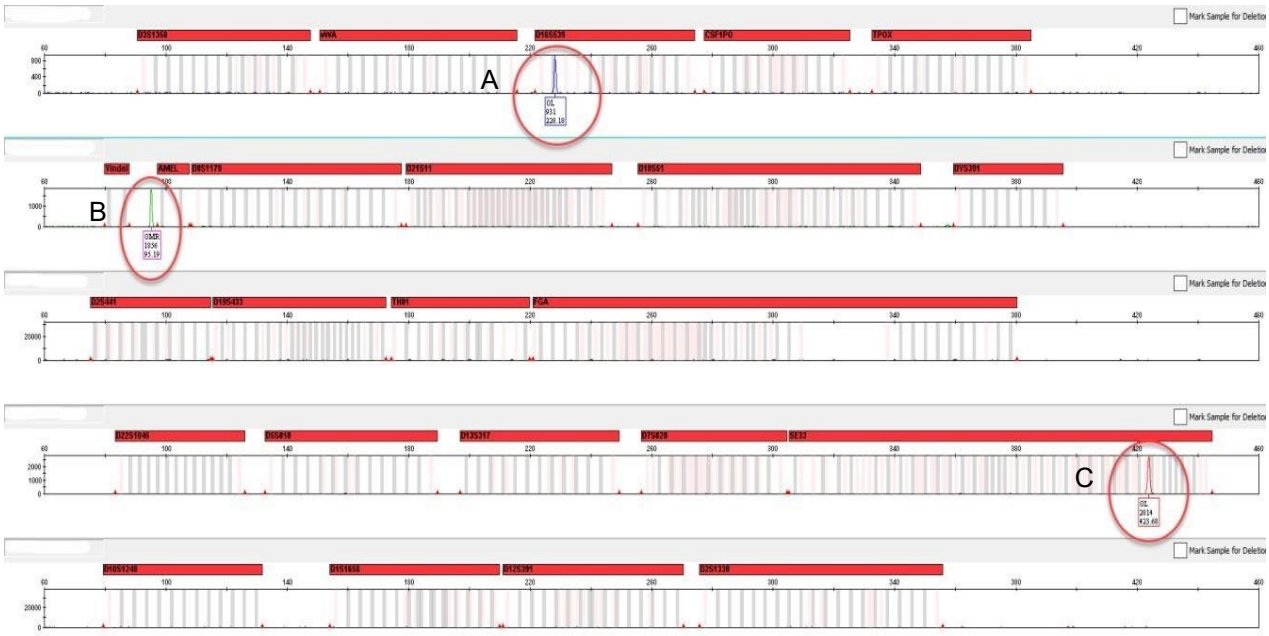


Figure 4: GlobalFiler sample-dependent artifacts obtained from a kidney of an unknown animal species. The artifact peak heights range from 931 RFU to 2814 RFU and are circled in red at (A) 228.18 bp in FAM (GF_D16-1), (B) 95.19 bp in VIC (GF_VIC-2), and (C) 423.69 bp in TAZ (GF_SE33-3).

Artifact ID: GF_TPOX-1

Locus: TPOX	**Size**: N-24 bp (typically observed ~24 nucleotides before the parent TPOX peak(s))
Probable cause: DNA template-dependent artifact likely caused by the formation of a secondary structure in the target sequence.	
Signal Intensity: Relative to DNA input amount (~0.4% to 1% of the parent peak height)*.	
Additional information: ▪ This artifact was also observed in the Identifiler™ Plus kit at ~14 to 15 nucleotides before the parent TPOX peak(s) (IDP_TPOX-1).	

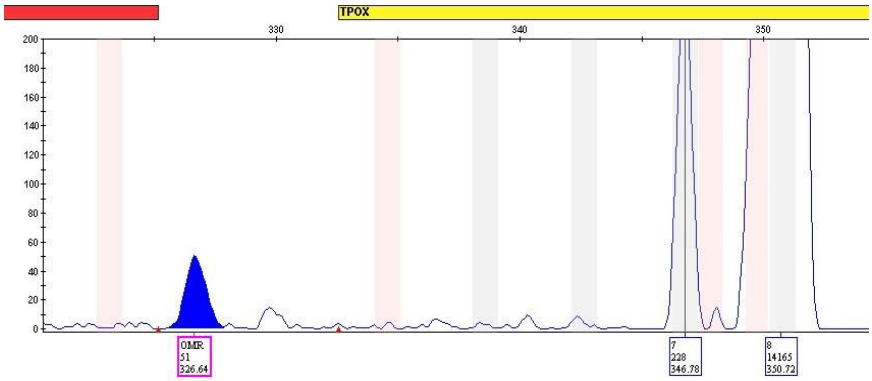


Figure 5: GlobalFiler PCR artifact (GF_TPOX-1) located 24.08 bp before the 8 allele in TPOX, obtained from 1 ng 007 control DNA. The y-axis is scaled to 200 RFU; the artifact is 51 RFU, 0.4% of the parent 8 allele.

Dye Channel: VIC

Artifact ID: GF_VIC-1

Locus: N/A	**Size**: ~94 bp
Probable cause: DNA template-dependent artifact likely caused by a non-STR amplification byproduct or the formation of a secondary structure in the target sequence.	
Signal Intensity: Relative to DNA input amount (~0.3% to 0.7% of the X peak height)*.	

Figure 6: GlobalFiler PCR artifact (GF_VIC-1) at ~94 bp between the Y indel and Amelogenin loci, labeled as an OMR (Outside Marker Range flag). The y-axis is scaled to ~200 RFU; the artifact is 148 RFU, 0.73% of the X allele.

Artifact ID: GF_VIC-2

Locus: N/A	**Size**: ~95 bp
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of non-human DNA in the sample.	
Signal Intensity: Relative to the amount of non-human DNA present.	
Additional information: ▪ Related to the GF_D16-1 and GF_SE33-3 artifacts also listed in this document. ▪ The combined sequencing and NCBI BLAST search results for the three related artifacts indicate the most likely source of the artifacts is cross-reactivity with some species of pig or wild boar DNA. ▪ As per the species specificity section of the GlobalFiler/GlobalFiler IQC user guide, horse DNA produced a similar artifact just before the Amelogenin marker. ▪ Refer to Figure 4, peak B in the VIC channel.	

Artifact ID: GF_YIndel-1

Locus: Y-indel	**Size**: ~84 bp
Probable cause: DNA template-dependent artifact likely caused by a non-STR amplification byproduct.	
Signal Intensity: Can vary in peak height from lot to lot and sample to sample but typically <175 RFU with a 1 ng template.	
Additional information: ▪ May be observed in male and female samples.	

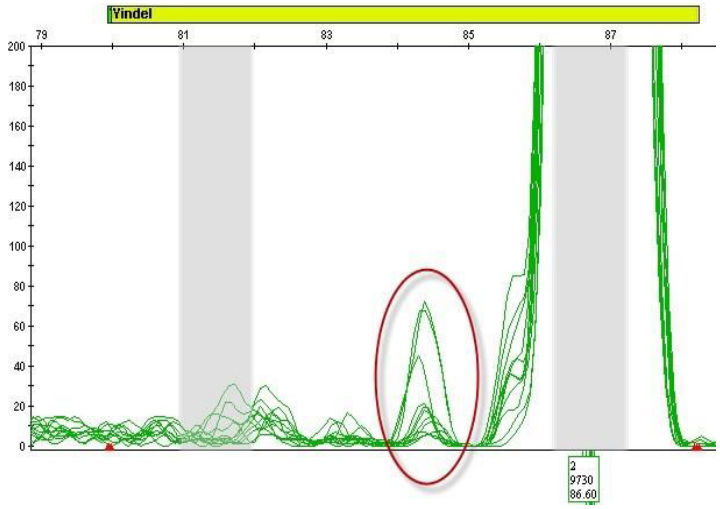


Figure 7: GlobalFiler PCR artifacts (GF_YIndel-1) at ~84.5 bp in the Y indel locus, obtained from multiple replicates of 1 ng 007 control DNA. The y-axis is scaled to 200 RFU, and the artifacts range from <20 RFU to ~70 RFU.

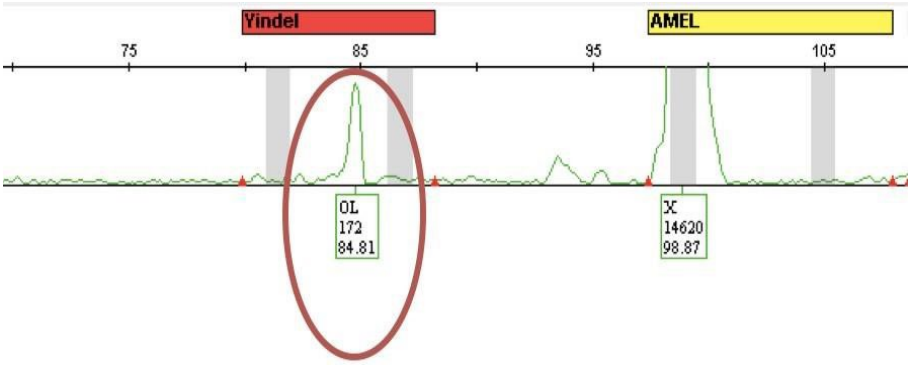


Figure 8: GlobalFiler PCR artifact (GF_YIndel-1) at 84.81 bp in the Y indel locus, obtained from a female sample of unknown input amount. The y-axis is scaled to 200 RFU, and the artifact peak height is 172 RFU.

Artifact ID: GF_D8-1

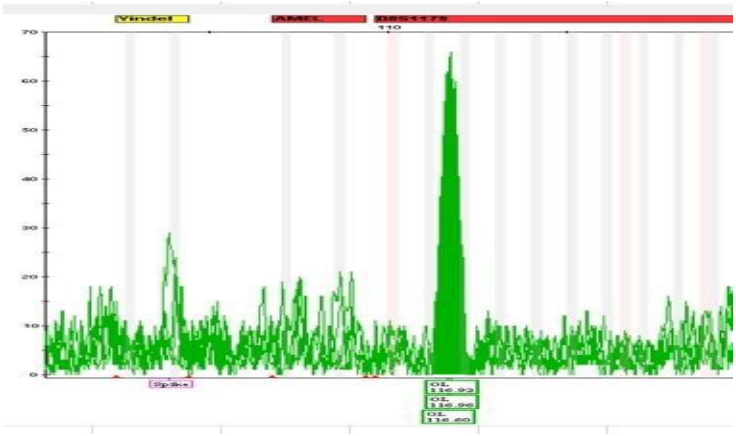
Locus: D8S1179	**Size**: ~114-121 bp
Probable cause: DNA template-independent artifact likely caused by a dye derivative, which is a cleavage of the dye from the labeled primer.	
Signal Intensity: <175 RFU.	
Additional information: ▪ May be promoted by post-manufacturing activities, such as repeated freeze/thaw cycles, extended use of the kit beyond recommended storage conditions, or the introduction of chemical factors (for example, cleaning reagents).	
	

Figure 9: GlobalFiler dye artifacts (GF_D8-1) at ~117 bp in D8S1179, obtained from several amplification non-template controls (NTCs). The y-axis is scaled to 70 RFU, and the artifacts are <70 RFU.

Artifact ID: GF_D8-2

Locus: D8S1179	**Size**: ~124 bp
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of non-human DNA in the sample.	
Signal Intensity: Relative to the amount of non-human DNA present.	
Additional information: ▪ Has been observed in a variety of crime scene related sample types (for example, mop/broom fringe, cigarette butts, skin swabs). ▪ Sequencing studies have shown that the artifact sequence has partial homology to bacterial species commonly found in soil and aquatic environments with no sequence homology to the human genome.	

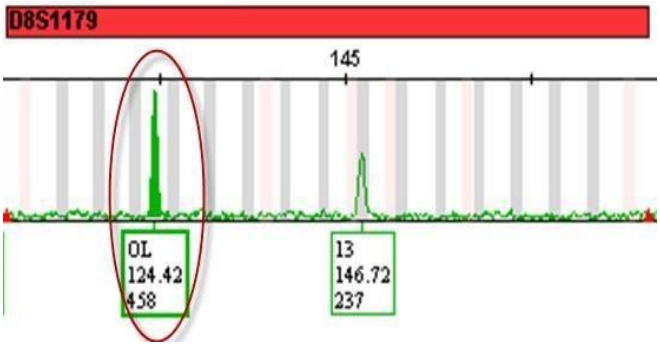


Figure 10: GlobalFiler sample-dependent artifact (GF_D8-2) at 124.42 bp in D8S1179, obtained from a swab of mop/broom fringe. The y-axis is scaled to 500 RFU, and the artifact is 458 RFU.

Artifact ID: GF_D8-3

Locus: D8S1179	**Size**: ~113 bp
Probable cause: DNA template-independent artifact likely caused by a dye derivative, which is a cleavage of the dye from the labeled primer.	
Signal Intensity: <50 RFU, when kit is stored per recommendations.	
Additional information: ▪ Artifact grows slowly over time but is not expected to be higher than 50 RFU unless kit reagents are stored improperly or used beyond the in-use time limits/expiration date.	

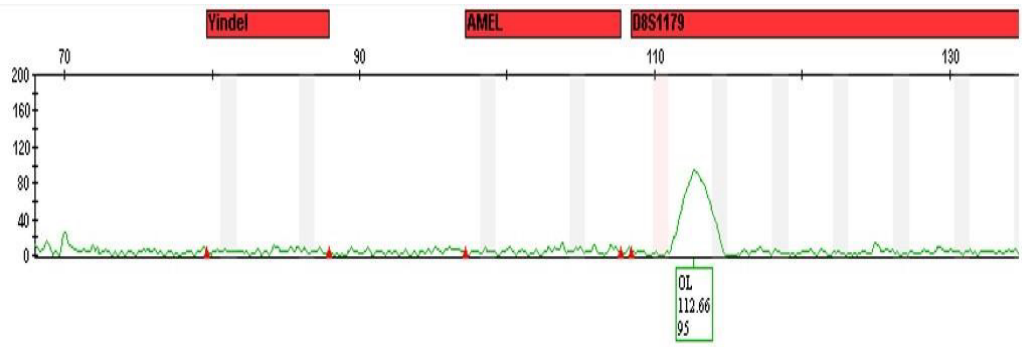


Figure 11: GlobalFiler dye artifact (GF_D8-3) at 112.66 bp in D8S1179, obtained from a non-template control (NTC). The y-axis is scaled to 200 RFU, and the artifact is 95 RFU after completion of an accelerated storage study equal to 10 years at -20º C or 2 years at 4º C.

Artifact ID: GF_D21-1

Locus: D21S11	**Size**: ~207 bp
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of non-human DNA in the sample.	
Signal Intensity: Relative to the amount of non-human DNA present.	
Additional information: ▪ Has been observed in a variety of crime scene related sample types (for example, swabs of car parts and other miscellaneous items). ▪ Sequencing studies have shown that the D21S11 artifact sequence has partial homology to yeast and fungal species commonly found in the environment, such as from plants, soil, and decaying organic matter, with no sequence homology to the human genome. ▪ This artifact was also observed in the Identifiler Plus kit at ~207 bp in the vWA locus (IDP_vWA-4).	

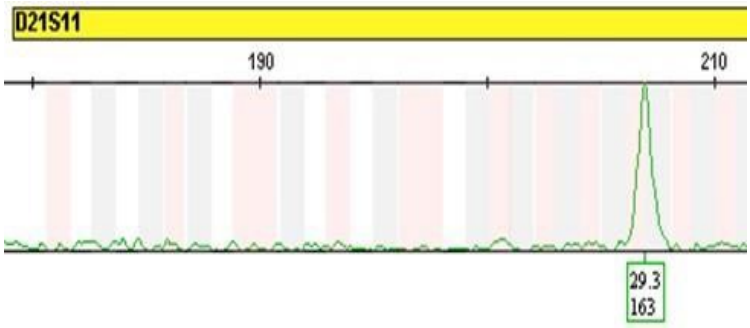


Figure 12: GlobalFiler sample-dependent artifact (GF_D21-1) at ~207 bp in D21S11, obtained from a vehicle swab with an undetermined quantity/ input amount. The y-axis is scaled to 175 RFU, and the artifact is 163 RFU.

Artifact ID: GF_D18-1

Locus: D18S51	**Size**: ~286 bp
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of non-human DNA in the sample.	
Signal Intensity: Relative to the amount of non-human DNA present.	
Additional information: ▪ Observed in a swab of the fringe of a mop/ broom. ▪ Sequencing studies have shown that the artifact sequence has partial homology with the Pseudomonas putida bacterium. At the time of the search, the NCBI BLAST statistics for this match included an E value of 1.0e-138 with 94% query coverage and 99% identity.**	

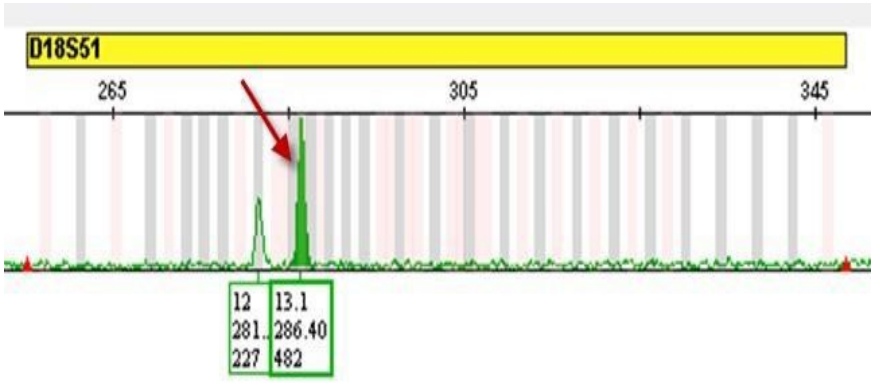


Figure 13: GlobalFiler sample-dependent artifact (GF_D18-1) at 286.40 bp in D18S51, obtained from a swab of mop/ broom fringe. The y-axis is scaled to 500 RFU, and the artifact is 482 RFU.

Artifact ID: GF_D18-2

Locus: D18S51	**Size**: N-2 bp (typically observed ~2 nucleotides before the parent D18S51 peak(s))
Probable cause: DNA template-dependent artifact (N-2 stutter) caused by a dinucleotide repeat stretch in the flanking region.	
Signal Intensity: Relative to DNA input amount (~2-4% of the parent peak height)*.	
Additional information: ▪ This N-2 stutter is observed less frequently than traditional N-4 stutter.	

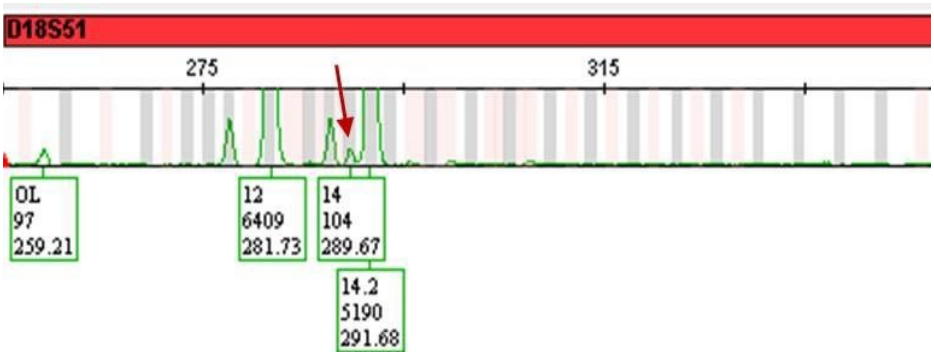


Figure 14: GlobalFilerPCR artifact (GF_D18-2) located 2.01 bp before the 14.2 allele in D18S51, obtained from a reference sample. The y-axis is scaled to 500 RFU; the artifact is at 104 RFU, 2% of the parent peak.

Artifact ID: GF_DYS391-1

Locus: DYS391	**Size**: N-5 bp (typically observed ~5 nucleotides before the parent DYS391 peak(s)).
Probable cause: DNA template-dependent artifact likely caused by polymerase slippage (atypical stutter) due to a repeating nucleotide region within the target sequence range.	
Signal Intensity: N/A – to date, the artifact has only presented as a shoulder of the typical N-4 stutter peak.	
Additional information: ▪ The same repeating region and related artifact is also present at the DYS391 marker in the Yfiler™ Plus PCR Amplification Kit.	

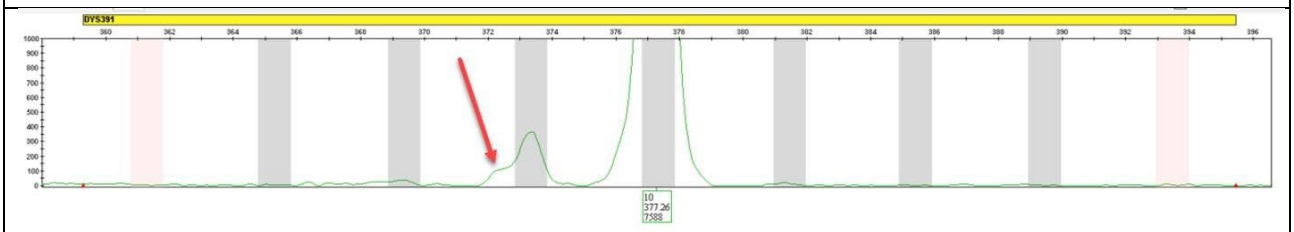


Figure 15: GlobalFiler PCR artifact (GF_DYS391-1) located ~5 bp before the 10 allele, as indicated by the red arrow, obtained from a reference sample. The y-axis is scaled to 1000 RFU. The artifact presented as a shoulder to the typical N-4 stutter peak but was not detected as a unique peak.

Dye Channel: NED

Artifact ID: GF_NED-1

Locus: N/A	**Size**: ~63 bp
Probable cause: DNA template-independent artifact likely caused by a primer dimer (interaction of two or more primers).	
Signal Intensity: Relative to kit storage and usage conditions.	
Additional information: ▪ Not always reproducible upon reamplification. ▪ May be caused or exaggerated by suboptimal kit storage or usage conditions, such as extended bench time at ambient temperature during PCR setup.	

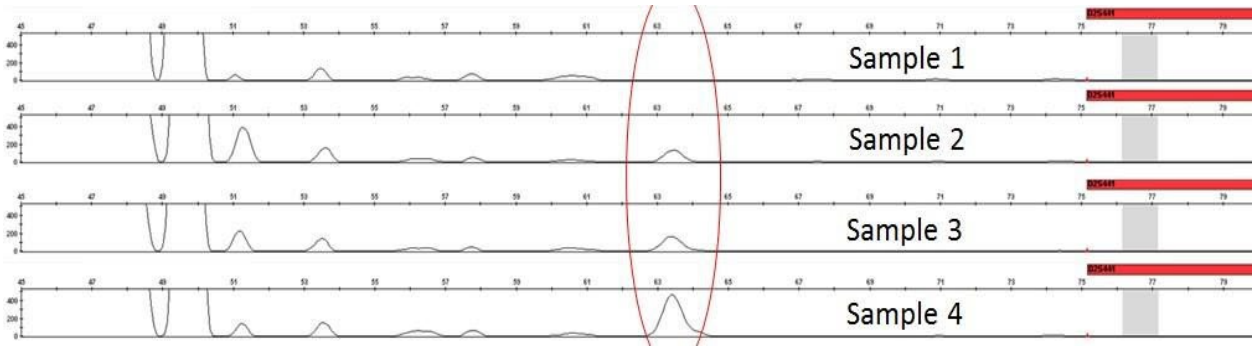


Figure 16: GlobalFiler primer dimer artifacts (GF_NED-1), obtained from four different samples at ~63.5 bp in the NED dye channel, which is before the D2S441 marker range and before the start of the read region. The y-axis is scaled to ~500 RFU, and the artifact heights are variable, ranging from undetected to <500 RFU.

Artifact ID: GF_D2NED-1

Locus: D2S441	**Size**: ~91-100 bp
Probable cause: DNA template-independent artifact likely caused by a dye derivative, which is a cleavage of the dye from the labeled primer.	
Signal Intensity: <175 RFU, when kit is stored per recommendations.	
Additional information: ▪ May be promoted by post-manufacturing activities, such as repeated freeze/thaw cycles, extended use of the kit beyond recommended storage conditions, or the introduction of chemical factors (for example, cleaning reagents).	

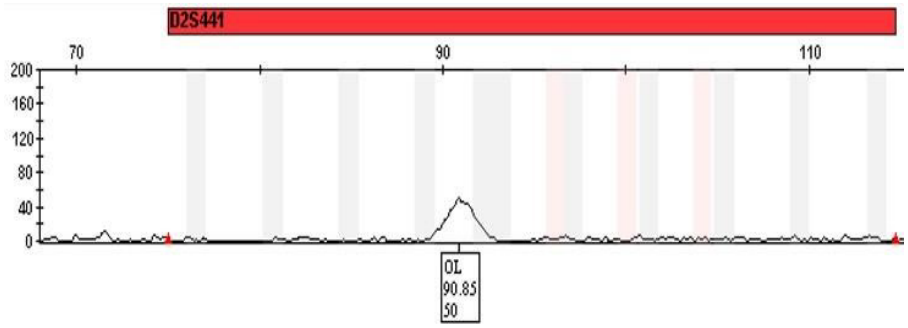


Figure 17: GlobalFiler dye artifact (GF_D2NED-1) at 90.85 bp in D2S441, obtained from a non-template control (NTC). The y-axis is scaled to 200 RFU, and the artifact is 50 RFU after completion of an accelerated storage study equal to 10 years at -20º C or 2 years at 4º C.

Artifact ID: GF_D2NED-2

Locus: D2S441	**Size**: N-2 to N-2.5 bp (typically observed ~2-2.5 nucleotides before the parent D2S441 peak(s))
Probable cause: DNA template-dependent artifact likely caused by N-2 stutter or the formation of a secondary structure in the target sequence.	
Signal Intensity: Relative to DNA input amount (~0.5% of the parent peak height)*.	

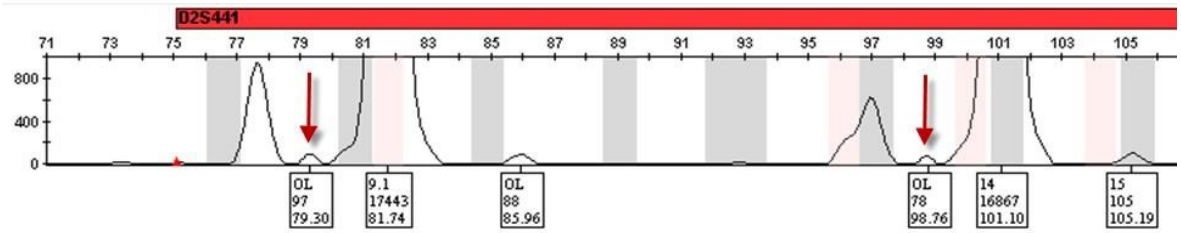


Figure 18: GlobalFiler PCR artifacts (GF_D2NED-2) located 2.44 bp before the 9.1 allele and 2.34 bp before the 14 allele in D2S441, obtained from a reference sample. The y-axis is scaled to 1000 RFU; the artifacts are at 97 RFU and 78 RFU, 0.6% and 0.5% of the respective parent peaks.

Artifact ID: GF_D2NED-3

Locus: D2S441	**Size**: ~76 bp
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of non-human DNA in the sample.	
Signal Intensity: Relative to the amount of non-human DNA present.	
Additional information: ▪ Has been observed in a variety of crime scene related sample types (for example, mop /broom fringe, cigarette butt inside a sink, items near a toilet, skin swabs). ▪ Sequencing studies have shown that the artifact sequence has partial homology with the *Acinetobacter johnsonii XBB1* bacterial strain. At the time of the search, the NCBI BLAST statistics for this match included an E value of $2.0e^{-19}$ with 91% query coverage and 94% identity**.	

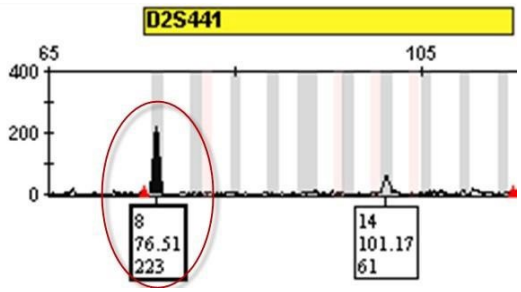


Figure 19: GlobalFiler sample-dependent artifact (GF_D2NED-3) at 76.51 bp in D2S441, obtained from a swab of mop/ broom fringe. The y-axis is scaled to 400 RFU, and the artifact is 223 RFU.

Artifact ID: GF_D19-1

Locus: D19S433	**Size**: N-6 bp (typically observed ~6 nucleotides before the parent D19S433 peak(s)).
Probable cause: DNA template-dependent artifact likely caused by the formation of a secondary structure.	
Signal Intensity: Relative to DNA input amount (<1% of the parent peak height)*.	

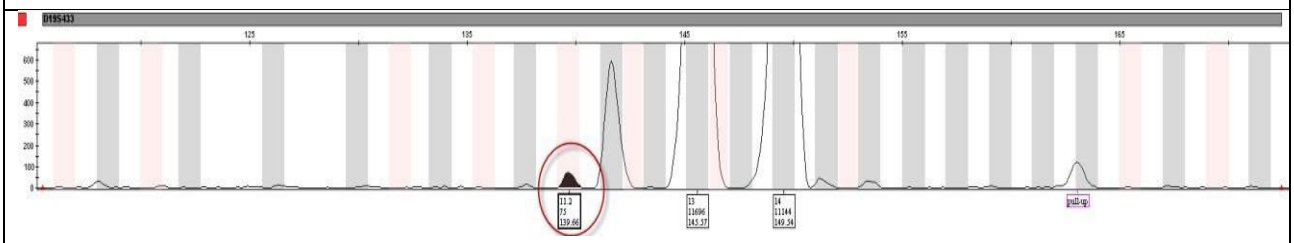


Figure 20: GlobalFiler PCR artifact (GF_D19-1) located 5.91 bp before the 13 allele in D19S433, obtained from 1 ng input reference DNA. The y-axis is scaled to 700 RFU; the artifact is 75 RFU, 0.6% of the parent 13 allele.

Artifact ID: GF_D19-2

Locus: D19S433	**Size**: N+2 to N+4 bp (typically observed ~2 to 4 nucleotides after the parent D19S433 peak(s)).
Probable cause: DNA template-dependent artifact likely caused by a primer derivative, which is a byproduct of the primer manufacturing process.	
Signal Intensity: Relative to both the amount of primer derivative present, which may vary from lot to lot, and DNA input amount.	

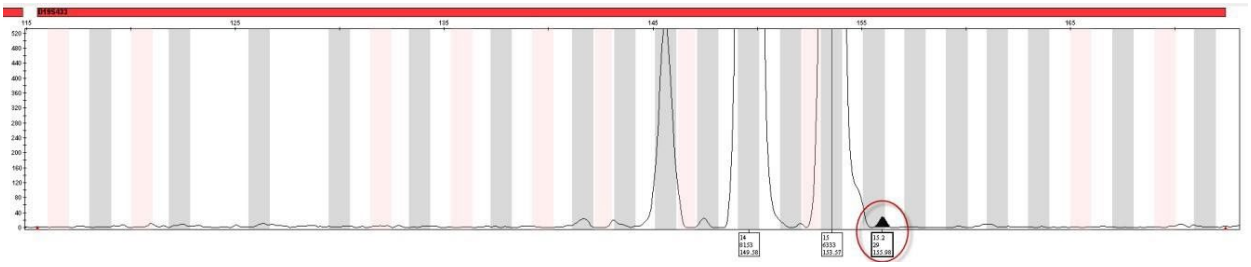


Figure 21: GlobalFiler PCR artifact (GF_D19-2) located 2.41 bp after the 15 allele in D19S433, obtained from 1 ng 007 control DNA. The y-axis is scaled to 540 RFU; the artifact is 29 RFU, 0.5% of the parent 15 allele.

Artifact ID: GF_TH01-1

Locus: TH01	**Size**: N-10 to N-12 bp (typically observed ~10-12 nucleotides before the parent TH01 peak(s)).
Probable cause: DNA template-dependent artifact likely caused by the formation of a secondary structure in the target sequence.	
Signal Intensity: Relative to DNA input amount (~0.4% to 0.9% of the parent peak height)*.	
Additional information: This artifact has also been observed in the Identifiler Plus kit at ~8 to 12 nucleotides before the parent TH01 peak(s) (IDP_TH01-1).	

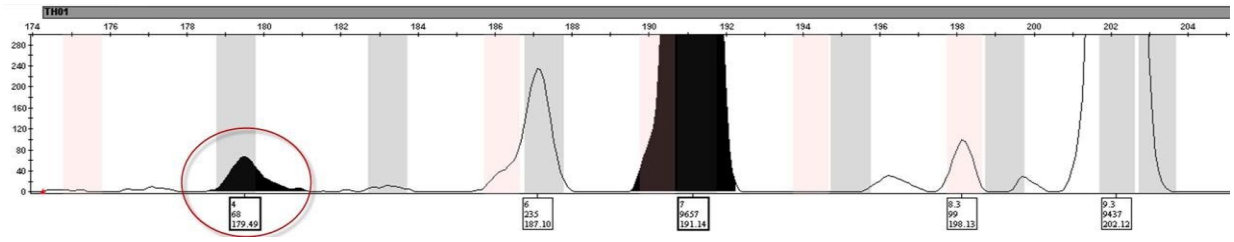


Figure 22: GlobalFiler PCR artifact (GF_TH01-1) located 11.65 bp before the 7 allele in TH01, obtained from 1 ng 007 control DNA. The y-axis is scaled to 300 RFU; the artifact is 68 RFU, 0.7% of the parent 7 allele.

Artifact ID: GF_FGA-1

Locus: FGA	**Size**: N+2 (typically observed ~2 nucleotides after the parent FGA peak(s)).
Probable cause: DNA template-dependent artifact likely caused by a primer derivative, which is a byproduct of the primer manufacturing process.	
Signal Intensity: Varies relative to the amount of primer derivative present, which may vary from lot to lot, and DNA input amount.	

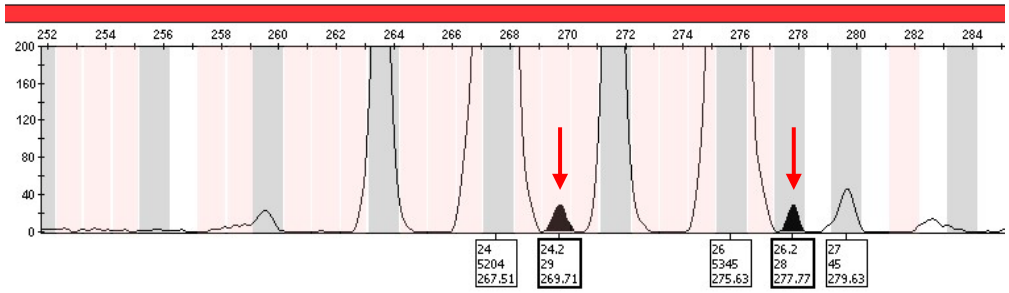


Figure 23: GlobalFiler PCR artifacts (GF_FGA-1) located approximately 2 bp after the 24 and 26 alleles in FGA, obtained from 1 ng 007 control DNA. The y-axis is scaled to 200 RFU; the artifacts are 29 RFU and 28 RFU, ~0.5% of the parent 24 and 26 alleles.

Artifact ID: GF_FGA-2

Locus: FGA	**Size**: N-8 bp (typically observed ~8 nucleotides before the parent FGA peak(s)).
Probable cause: DNA template-dependent artifact (N-8 stutter) caused by a tetranucleotide repeat stretch in the flanking region.	
Signal Intensity: Relative to the DNA input amount present (~0.4% to 0.7% of the parent peak height, reported as high as 2.0%)*.	
Additional information: ▪ This N-8 stutter is observed less frequently than traditional N-4 stutter.	

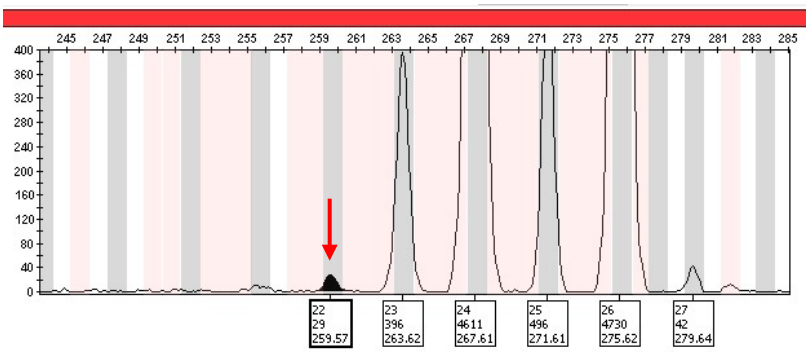


Figure 24: GlobalFiler IQC PCR artifact (GF_FGA-2) located approximately 8 bp before the 24 allele in FGA, obtained from 1 ng 007 control DNA. The y-axis is scaled to 400 RFU; the stutter is at 29 RFU, 0.6% of the parent peak.

Dye Channel: TAZ

Artifact ID: GF_TAZ-1

Locus: N/A	**Size**: ~250 bp
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of non-human DNA in the sample.	
Signal Intensity: Relative to the amount of non-human DNA present.	
Additional information: ▪ Has been observed in a variety of sexual assault samples (for example, labia swabs, condom, etc.). ▪ Sequencing studies have shown that the GlobalFiler_TAZ-1 artifact sequence has partial homology with the Porphyromonas asaccharolytica (gram-negative anaerobic bacteria) genome. At the time of the search, the NCBI BLAST statistics for this match included an E value of 2.0e-56 with 96% query coverage and 82% identity.**. ○ This artifact has also been observed in the Identifiler Plus kit at ~240 bp in the D21S11 locus (IDP_D21-2).	

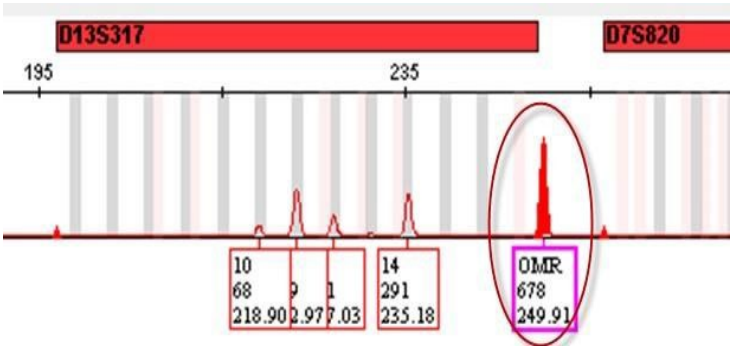


Figure 25: Globalfiler sample-dependent artifact (GF_TAZ-1) at 249.91 bp in the TAZ dye channel between D13S317 and D7S820, obtained from a swab of a condom. The y-axis is scaled to 1000 RFU, and the artifact is 678 RFU.

Artifact ID: GF_D5-1

Locus: D5S818	**Size**: ~180 bp
Probable cause: DNA template-dependent artifact likely caused by a non-STR amplification byproduct.	
Signal Intensity: Relative to DNA input amount (typically <175 RFU with a 1 ng template input).	

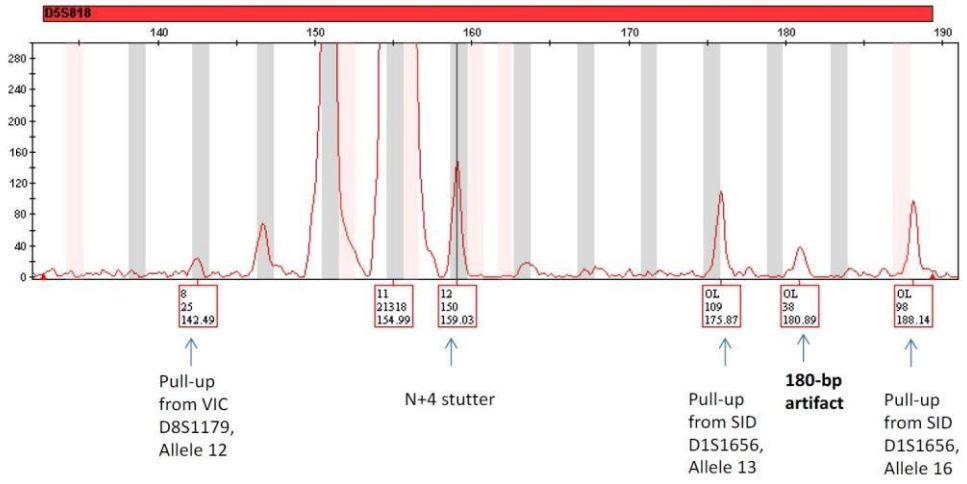


Figure 26: GlobalFiler PCR artifact (GF_D5-1) at 180.89 bp in D5S818, obtained from 007 control DNA. The y-axis is scaled to 300 RFU, and the artifact is 38 RFU.

Artifact ID: GF_D5-2

Locus: D5S818	**Size**: ~142 bp
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of non-human DNA in the sample.	
Signal Intensity: Relative to the amount of non-human DNA present.	
Additional information: ▪ Has been observed in sample types potentially exposed to fecal matter (for example, rectal/anal and peri-anal swabs). ▪ Sequencing studies have shown that the artifact sequence has partial homology with the *Lachnoclostridium* genome. At the time of the search, the NCBI BLAST statistics for this match included an E value of $8.0e^{-37}$ with 95% query coverage and 90% identity**.	

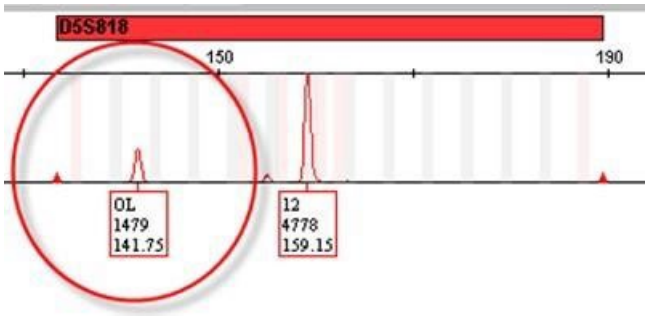


Figure 27: GlobalFiler sample-dependent artifact (GF_D5-2) observed in GlobalFiler, obtained from a peri-anal swab, at 141.75 bp in D5S818. The y-axis is scaled to ~5000 RFU, and the artifact is 1479 RFU.

Artifact ID: GF_D13-1

Locus: D13S317	**Size**: ~215 bp
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of non-human DNA in the sample.	
Signal Intensity: Relative to the amount of non-human DNA present.	
Additional information: ▪ Sequencing studies have shown that the artifact sequences has homology with the *Aspergillus oryzae* genome. At the time of the search, the NCBI BLAST statistics for this match included E values of $8.0e^{-157}$ for the forward sequence and of $2.0e^{-169}$ for the reverse, both with 100% query coverage and 100% identity**.	

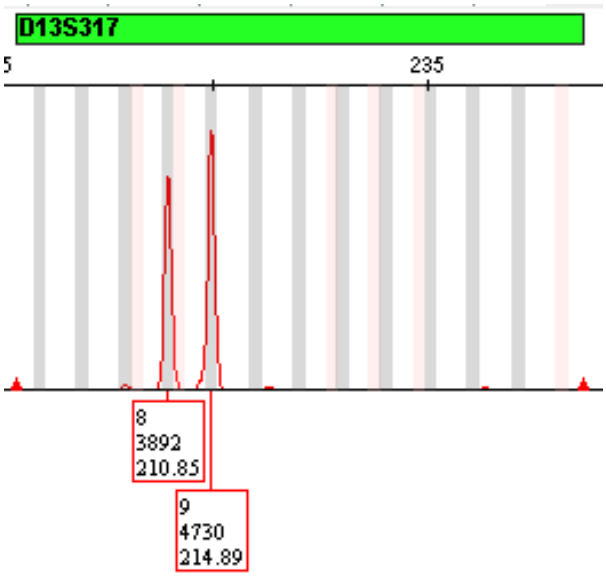


Figure 28: GlobalFiler sample-dependent artifact (GF_D13-1) at 214.89 bp in D13S317, obtained from tissue paper. The y-axis is scaled to ~5500 RFU, and the artifact is 4730 RFU.

Artifact ID: GF_SE33-1

Locus: SE33	**Size**: N-90 bp (typically observed ~90 nucleotides before the parent SE33 peak(s)).
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of human DNA containing a Single Nucleotide Polymorphism (SNP) internal to the SE33 primer binding sites that allows an unlabeled non-SE33 primer to bind.	
Signal Intensity: Relative to the amount of contributor present with the SNP (~15% to 30% of the parent peak height)*	

Additional information:

- Depending on location of the parent SE33 allele containing the SNP, the artifact could appear in D13S317, D7S820 or SE33.
- The SNP exists within a segment of the population of African and European descent (Davis, et al, 2012).
  - Davis, C., Ge, J., King, J., Malik, N., Weirich, V., Eisenbe, A.J., Budowle, B. (2012). Variants observed for STR locus SE33: a concordance study. Forensic Science International. Genetics, 6(4), 494-497. doi:http://dx.doi.org/10.1016/j.fsigen.2011.12.002.

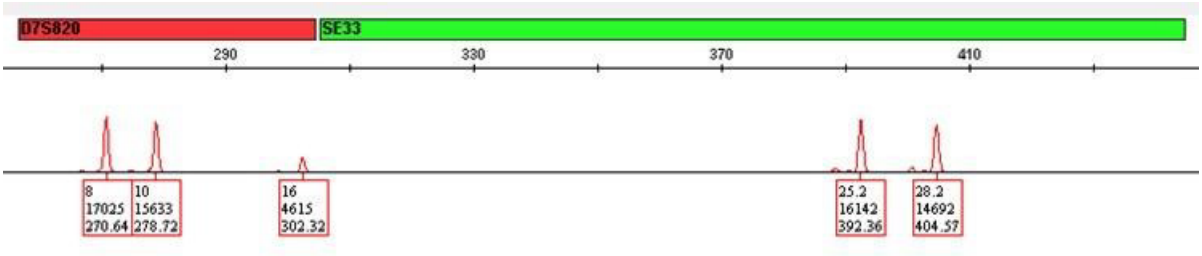


Figure 29: Globalfiler sample-dependent artifact (GF_SE33-1) at 302.32 bp that appears in the D7S820 locus and is 90.04 bp before the SE33 25.2 parent peak, obtained from a reference sample. The y-axis is scaled to ~30,000 RFU; the artifact is 4615 RFU, 29% of the parent 25.2 allele.

Artifact ID: GF_SE33-2

Locus: SE33	**Size**: ~352.5 bp
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of non-human DNA in the sample.	
Signal Intensity: Relative to the amount of non-human DNA present.	

Additional information:

- Observed in a stain from a boot.
- Sequencing studies have shown that the artifact sequence has partial homology with several bacteria, with the Pseudescherichia vulneris, Enterobacter sp. C2, and Kosakonia cowanii genomes giving the 3 closest matches. The NCBI BLAST statistics for these matches were as follows: E value = 2.0e-^{98}, 100% query coverage, 85% identity (P. vulneris), E value = 3.0e-^{76}, 99% query coverage, 80% identity (Enterobacter sp. C3), and E value =- 4.0e-^{55}, query coverage = 95%, 77% identity (Kosakonia cowanii)**.

Figure 30: GlobalFiler sample-dependent artifact (GF_SE33-2) labeled as a 15.2 allele at 352.47 bp in the SE33 locus (red arrow), obtained from a stain on a boot. The y-axis is scaled to 3000 RFU, and the artifact is 2340 RFU. The peak (15 allele at 130 RFU) identified by the green arrow is not stutter and is part of the human DNA profile within the sample.

Artifact ID: GF_SE33-3

Locus: SE33	**Size**: ~ 424 bp
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of non-human DNA in the sample.	
Signal Intensity: Relative to the amount of non-human DNA present.	
Additional information: ▪ Related to the GF_D16-1 and GF_VIC-2 artifacts also listed in this document. ▪ The combined sequencing and NCBI BLAST search results for the three related artifacts indicate the most likely source of the artifacts is cross-reactivity with some species of pig or wild boar DNA. ▪ As per the species specificity section of the GlobalFiler/GlobalFiler IQC user guide, this artifact was observed with pig DNA during developmental validation. Refer to Figure 4, peak C in the TAZ channel.	

Dye Channel: SID

Artifact ID: GF_D10-1

Locus: D10S1248	**Size**: N-2.5 bp (typically observed ~2.5 bp nucleotides prior to the parent peak(s))
Probable cause: DNA template-dependent artifact likely caused by a primer derivative, which is a byproduct of the primer manufacturing process.	
Signal Intensity: Relative to both the amount of primer derivative present, which may vary from lot to lot, and DNA input amount.	

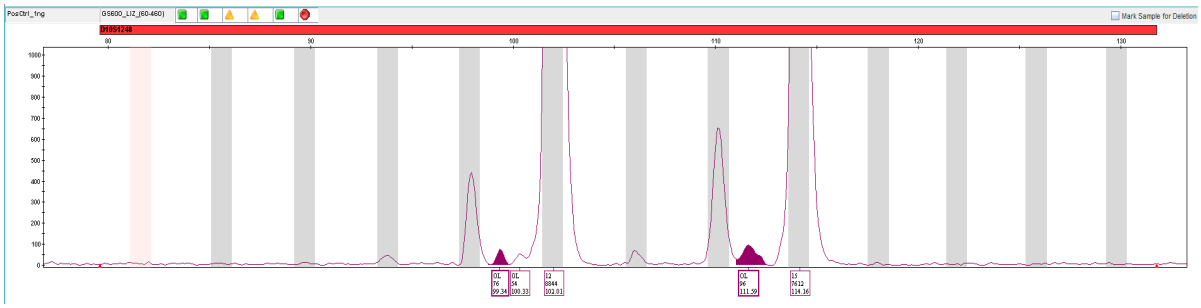


Figure 31: GlobalFiler PCR artifacts (GF_D10-1) ~2.5 bp nucleotides before the parent peak(s) in D10S1248, obtained from 007 control DNA. The y-axis is scaled to 1000 RFU, and the artifacts are 76 and 96 RFU.

Artifact ID: GF_D2SID-1

Locus: D2S1338	**Size**: ~345 bp
Probable cause: Sample-dependent artifact likely caused by the presence and amplification of non-human DNA in the sample.	
Signal Intensity: Relative to the amount of non-human DNA present.	
Additional information: ▪ Has been observed in a variety of crime scene related sample types (for example, swab of moldy hat, fingernail scrapings, boot and gloves found outside, etc.). ▪ Sequencing studies have shown that the artifact sequence has no homology to the human genome, and at the time of the NCBI BLAST search there were no significant matches to genetic sequences in the database**.	

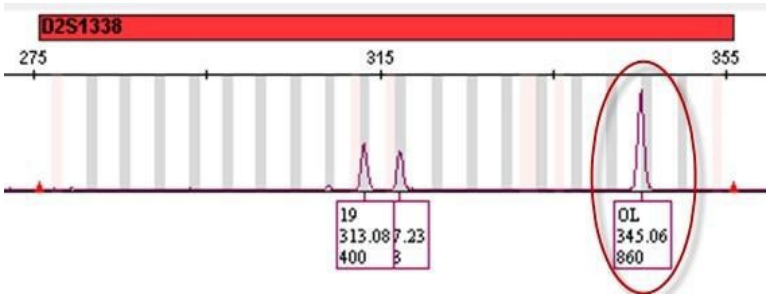


Figure 32: GlobalFiler sample-dependent artifact (GF_D2SID-1) at 345.06 bp in D2S1338, obtained from fingernail scrapings. The y-axis is scaled to 1000 RFU, and the artifact is 860 RFU

Conclusions

Thermo Fisher Scientific's Human Identity STR kits are subjected to rigorous development specifications and criteria to prevent the presence of artifacts. However, the diversity of sample types, variation in storage conditions, and analysis approaches that promote higher sensitivity for the forensic application can lead to artifacts not typically observed during development. This document will be

periodically updated with additional reported and characterized artifacts identified post-product development to assist in the analysis and interpretation of STR results.

Comments

*The observed percentages are provided for guidance only and are based on available data. These percentages have not been confirmed to the same extent as, for example, average marker stutter percentages.

** Refer to https://blast.ncbi.nlm.nih.gov/Blast.cgi for information on BLAST statistics. BLAST statistics and matches observed can change over time as new sequences are added to the database. Match information and the associated statistics are provided for informational purposes only and do not suggest that a particular artifact was conclusively caused by the named organism.

Revision History

Revision	Date	Description
A00	11/29/2017	Initial publication.
A01	4/2/2018	• Updated artifact IDs by adding GF prefix to existing ID • Changed VIC OMR-1 artifact ID to GF_VIC-1 • Added 7 new artifacts (GF_D3-2, GF_D8-2, GF_D18-1, GF_D2NED-3, GF_TAZ-1, GF_SE33-2, GF_D2SID-1) • Added Typical Allele Call column to Summary Table • Changed picture and updated caption for GF_TH01-1 • Added Comments section with updated BLAST info • Various minor wording/grammar edits
A02	2/12/2019	• Added two new artifacts (GF_D18-2 and GF_D19-1)
A03	5/6/2020	• Added references to the GlobalFiler IQC PCR Amplification Kit • Added six new artifacts (GF_D16-1, GF_VIC-2 • , GF_D8-3, GF_DYS391-1, GF_D19-2, GF_SE33-3) • Updated GF_D2NED-1 artifact description and figure • Various minor wording/grammar edits
A04	6/22/2022	• Added two new artifacts (GF_FGA-1 and GF_FGA-2) • Various minor wording, grammar, and formatting edits
A05	5/7/2024	• Added two new artifacts (GF_D13-1 and GF_D10-1) • Updated sequencing studies for GF_SE33-2 • New format and administrative updates applied to the whole document • Updated Revisions format from A to D to A00 to A05