Solution For Partial Profiles: Amplicon Rx[™] Post-PCR Clean-up Kit

Alex Sinelnikov (alex@ifi-test.com) and Karl A. Reich (karl@ifi-test.com) Independent Forensics, 500 Waters Edge, Suite 210, Lombard, IL 60148, USA

The Problem (1)

Partial Profiles due to:

Low Template Amount Inhibition

Degradation

The Problem (2)

Partial Profiles due to:

Interference with electrokinetic injection of STR amplicons by primers, nucleotides and salts from PCR reaction

Only 4% of PCR reaction is used for electrophoresis

The Solution

Remove primers, nucleotides and salts from post-PCR sample

Use *ALL* of PCR reaction for electrophoresis

Advantages of *Amplicon Rx*™

Quick 3 step procedure (10 min Prep Time)

CE Ready Elution with Formamide/SS mix

95% of PCR reaction is used for CE (ALL what is left after first CE run)

Amplicon Rx™ workflow

PCR Reaction

+

Binding Buffer

| load on column |
| Spin 3 min [Discard run through] |
| +

Formamide + LIZ/ROX [Incubate 5 min, RT°C] |
| Spin 2 min

Validation Method

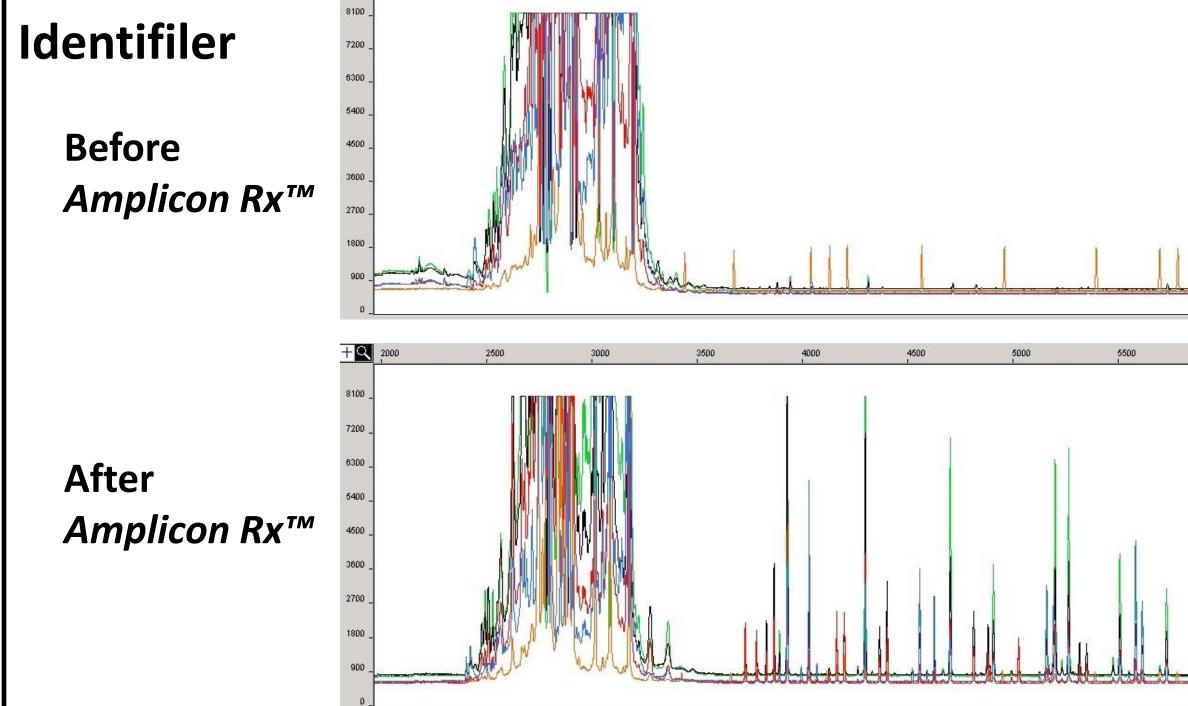
Load Elution on CE

Samples were amplified with either PowerPlex 16, Identifiler, or Y-filer multiplexes. After verification of the DNA profiles, samples were diluted with amplification negative control samples to simulate low copy number (LCN) samples. PCR reaction volumes were varied as shown: quarter (6.25 μ L), half (12.5 μ L), and full (25 μ L) size PCR reaction from which 5 μ L, 11 μ L and 23.5 μ L of LCN Power Plex16, Identifiler, or Y-filer samples were processed with *AmpliconRx*TM.

Results Summary

In all samples analyzed, Amplicon Rx^{TM} boosted peaks which were originally present in the sample. A 50 RFU threshold was used for peak detection. Amplicon Rx^{TM} did not introduce 'new' or extraneous alleles - allelic peak height ratios observed in untreated samples (including mixtures) were preserved using Amplicon Rx^{TM}

Proof of Concept PowerPlex 16 Before Amplicon RxTM After Amplicon RxTM Proof of Concept The proof of



Amplicon Rx™ RFU Boost

	PowerPlex 16	Identifiler
6.25 μL PCR	x 3 - 5	x 3 - 5
12.5 μL PCR	x 7 - 10	x 7 - 10
25.0 μL PCR	x 13 - 20	x 13 - 20

