Obtaining DNA-Short Tandem Repeat (STR) Profiles from Evidentiary Samples with Extremely Limited Amounts of DNA

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

Poster #B9

The Problem

Current Procedures are Not Designed for Limiting Amounts of DNA

Significant losses (>75%) in collection & purification steps

Assaying only ~5% of the PCR reaction products

The Solution

a) collecting the biological material:
mini-swabs and detergent-based buffer*
**secret sauce#1: better swabs and detergent*

b) recovery of collected material:
centrifugation at high speed

c) release of DNA:
proK & detergent at elevated °C

d) purification of DNA:
removal of inhibitors with 80-90% recovery*
**secret sauce#2: NOT bind-wash-elute!*

e) PCR amplification:
modest changes, 29 *v.* 28 cycles, 2x TAQ*
**better amp with no PCR drop-ins*

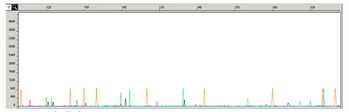
f) assay 100% of the PCR reaction:
post-PCR processing and purification*
**secret sauce#3: use all of the PCR reaction, Amplicon Rx™*

Σ: the most sensitive technique, ever!

Proof of the Pudding is in . . .

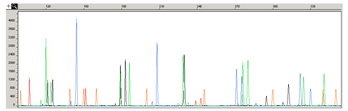
Purification Comparison

bind-wash-elute purification



Dropped alleles 4
Homozygotes <500rfu 4
Total RFU 8,736

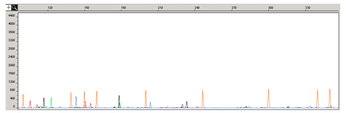
OneTouch™ xS column



Dropped alleles 0
Homozygotes <500rfu 0
Total RFU 48,188

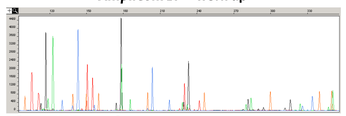
post-PCR Comparison

standard post-PCR work-up



Dropped alleles 5
Total RFU 5,558

AmpliconRx™ work up



Dropped alleles 2
Total RFU 38,854

Tested Samples

Deposited DNA on a slide, 75 pg

Single fingerprint (identified ridge impression)

Bullets, loaded into magazine, unfired

'Touched' Coke can (any soda can actually)

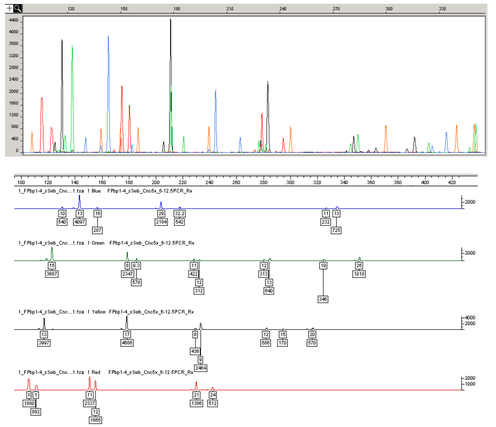
Handled objects, *e.g.*, yearbook, computer mice,

Clothing, *e.g.*, neck collar

Casework Examples

Autosomal STR profile from a single fingerprint

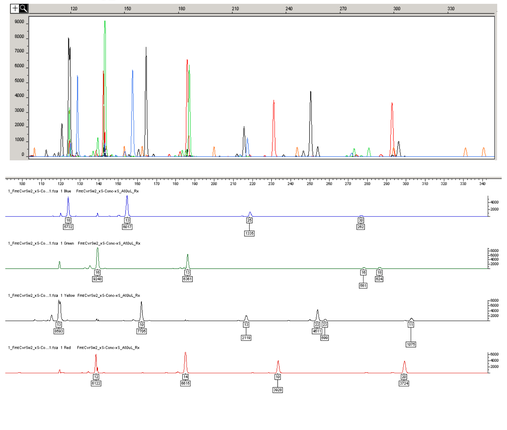
- ✓ Ridge detail identified with DNA-free fingerprint powder
- ✓ Collection with mini-swab and detergent solution
- ✓ proK & detergent digestion
- ✓ xS column purification
- ✓ Concentration in vacuum centrifuge
- ✓ PCR
- ✓ *AmpliconRx*™
- ✓ CE



Dropped alleles 2
Total RFU 38,854

Y STR profile from a 'yearbook' cover

- ✓ Entire cover sampled with full-size swab & detergent solution (wet/dry technique)
- ✓ proK & detergent digestion
- ✓ xS column purification [1st]
- ✓ Concentration in vacuum centrifuge
- ✓ xS column purification [2nd]
- ✓ PCR
- ✓ *AmpliconRx*™
- ✓ CE



Dropped alleles 0
Total RFU 77,532

OneTouch DNA Method

bullets, fingerprints, handled objects. . .

see hand-out for additional examples