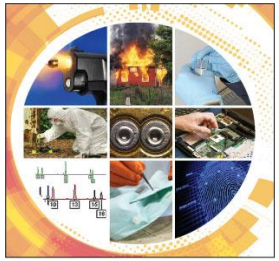

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Exploring DNA Extraction Efficiency

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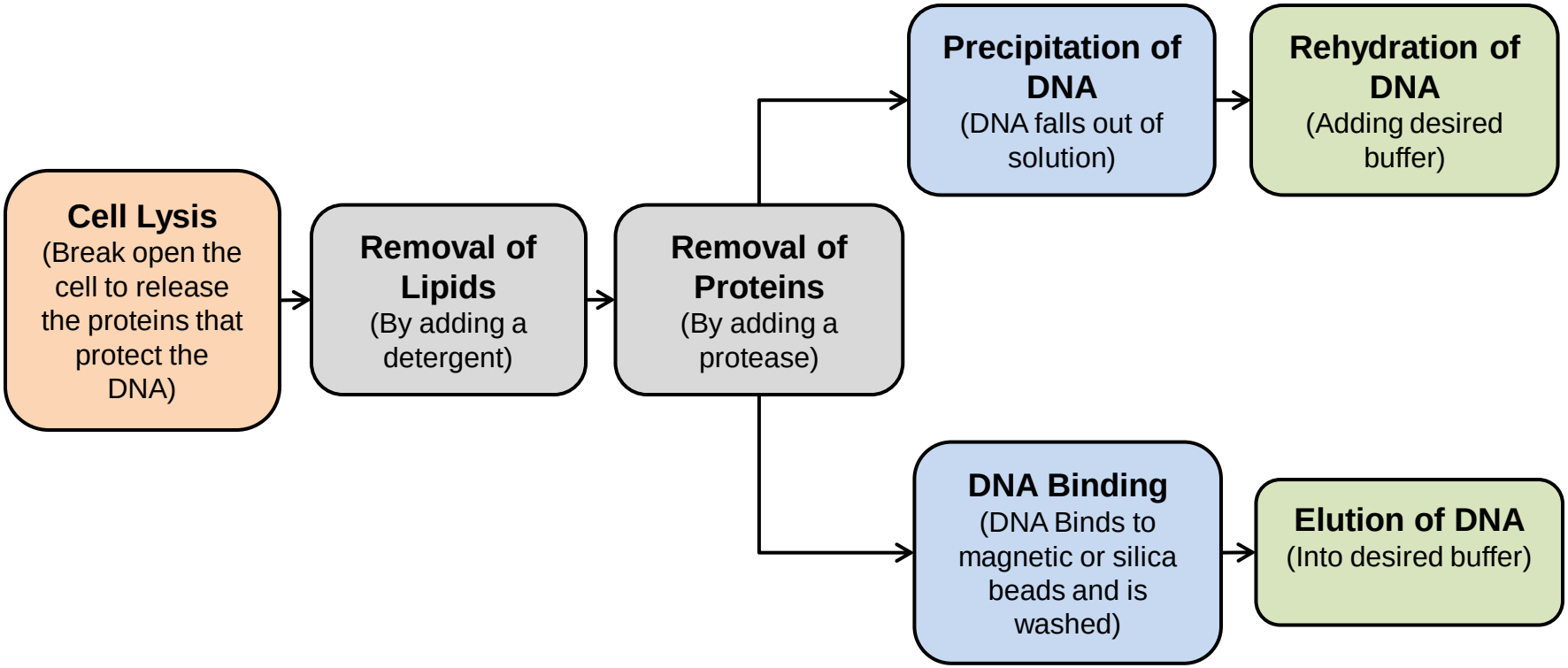
Outline

- Define DNA extraction
- Downstream concerns for DNA extraction
- Methods used to evaluate extraction efficiencies
- Define absolute extraction efficiency
- What can we learn from absolute extraction efficiency?
- Alternate approaches to extraction

DNA Extraction

DNA extraction is the first step after collection in the DNA typing process



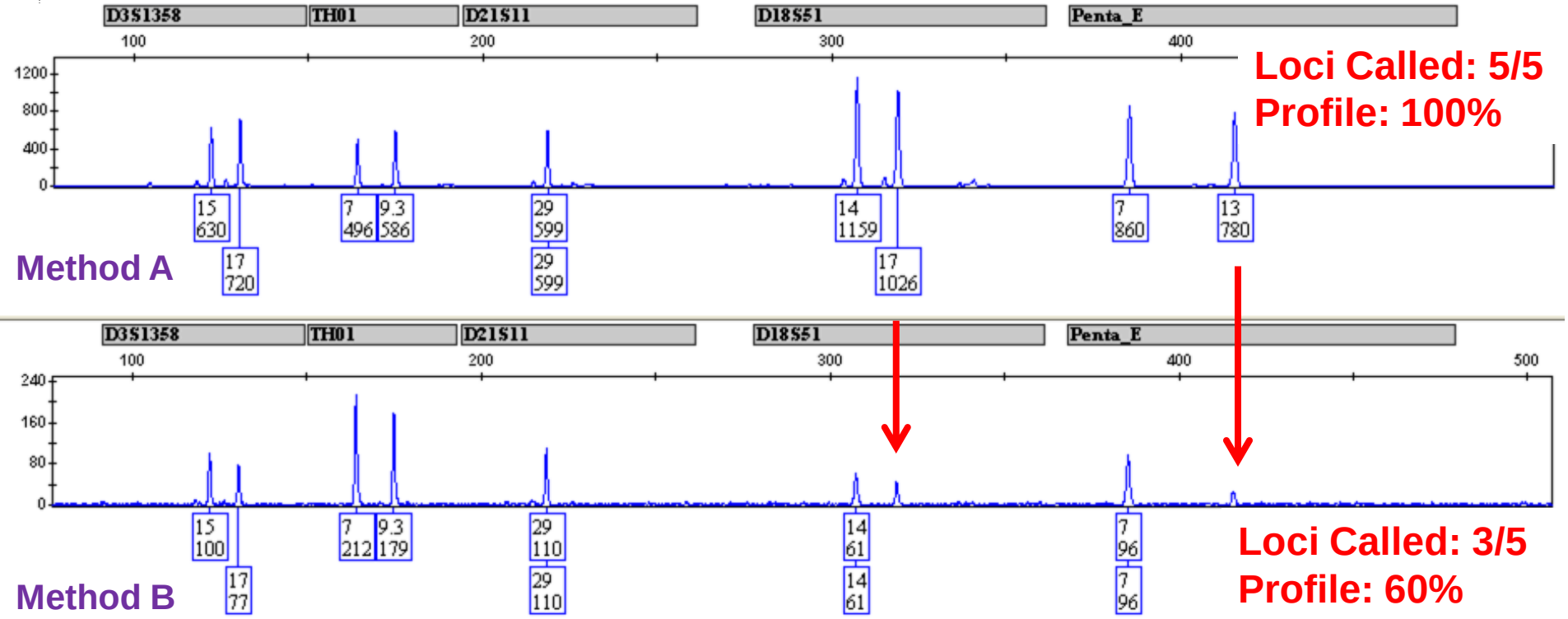
Purification methods are often used to try to eliminate the presence of additional proteins, lipids, and inhibitors

Challenges of DNA Extraction

- Organic extraction processes (manual) involves **hazardous chemicals** such as phenol and chloroform
- Liquid handling steps may increase risk for **contamination** and **loss of sample**
  - Employing robotics can decrease this challenge
- **Inhibitors** found in the original sample may be carried through the extraction process
  - Inhibitors may **reduce the PCR efficiency** of assays being used
- Two common PCR inhibitors found in forensic samples
  - Hemoglobin (Blood)
  - Indigo dye (Denim)

Definition of Relative Extraction Efficiency

- Recovery compared to another method of extraction (often organic)
- Defined using several different metrics
  - Full vs. Partial STR Profiles
  - Number of loci successfully genotyped (percent of profile obtained)



Limitations of Relative Efficiency Metrics

- Measures end point of genotyping process
  - Efficiency of STR genotyping
- Does not reflect the absolute efficiency of only the extraction process
- Does not account for the **initial amount** DNA present in the sample
  - In forensic samples the true amount of starting material is unknown due to the source of the sample

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Absolute Extraction Efficiency

- The ratio of the amount of DNA recovered (quantitated) to the original amount of DNA (known) after extraction
- This offers the ability to evaluate the absolute efficiency of the extraction
- **The original amount needs to be known**

Testing Absolute Extraction Efficiency

Placing a **known amount** of DNA into the extraction process and determine the amount recovered

DNA Sources:

Highly characterized extracted DNA: Varying amounts added to sterile swabs (n=18 per quantity)

- **Known quant value**: 52.44 ng/µL
- Ranges from 1500 ng to 100 ng

Human epithelial cell lines*: 100 µL of a cell suspension swabbed from Teflon tube (n=12 per quantity)

- **Number of cells determined through flow cytometry**
- Ranges from 1200 ng to 300 ng of DNA

Whole blood*: Seven volumes of whole blood tested (n=2 per volume)

- Ranges from 4800 ng to 24 ng of DNA

*Assume 6 pg of DNA per cell

Extraction Methods

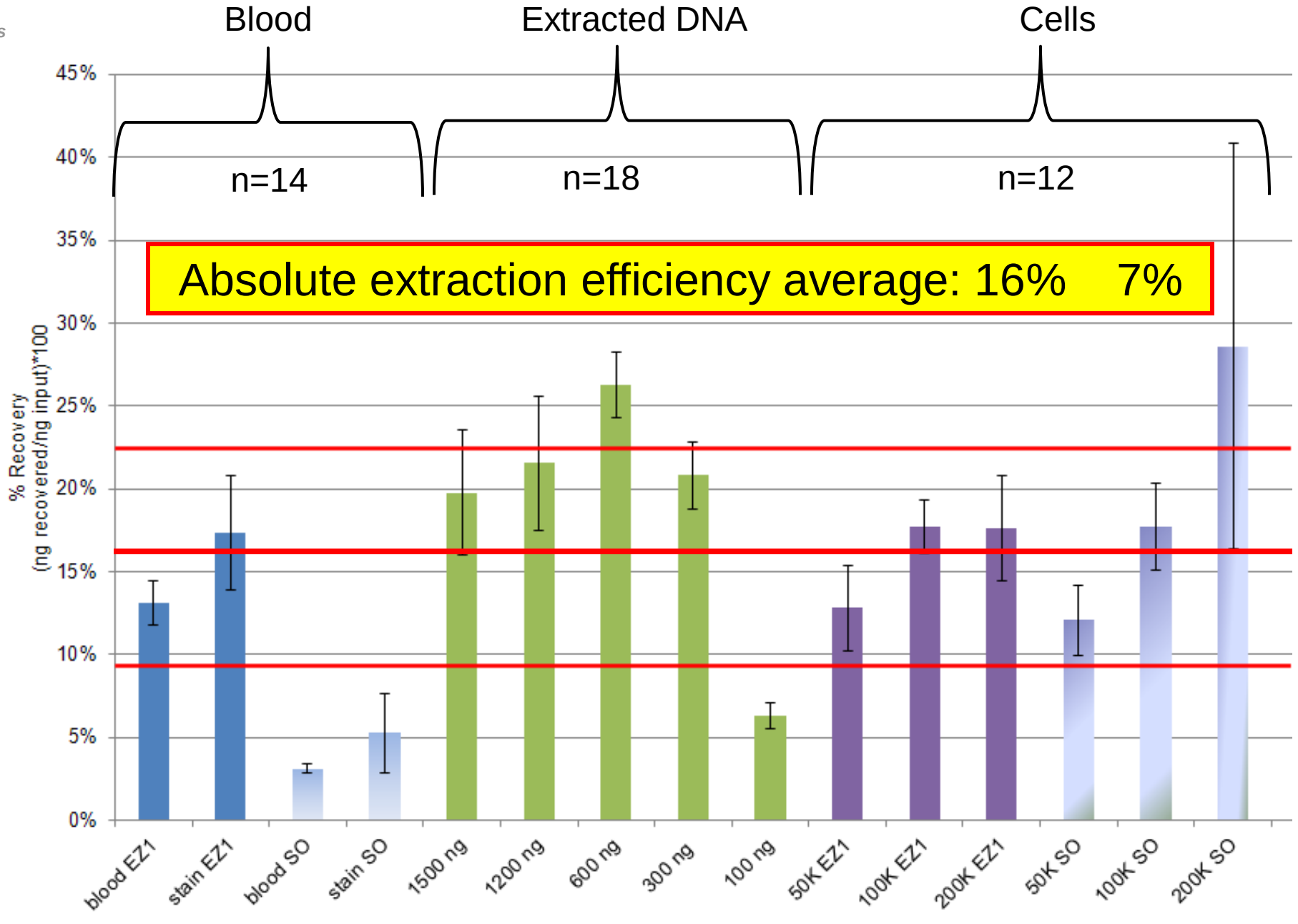
Qiagen EZ1 Advanced XL

- **Swabs & Blood Stains**
  - Pretreated with a Pro K digest and G2 Buffer at 56 C for 15 minutes
  - Pro K inactivation at 95 C for 5 minutes
- **Whole Blood**
  - Total sample volume brought up to 200 µL with G2 Buffer
- Eluted with 100 µL TE

Modified Salt Out

- Manual extraction process
- Involves a Proteinase K digest
- Saturated Ammonium Acetate solution to separate DNA
- Absolute Ethanol wash to precipitate DNA
- Rehydrated with 100 µL TE

Extraction Efficiency Across All Samples

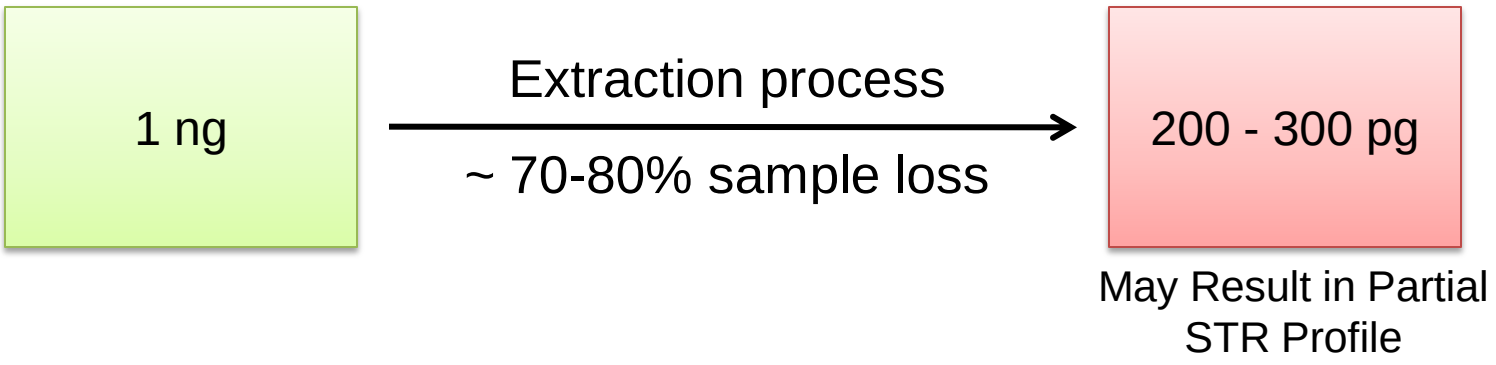


Summary of Absolute Extraction Efficiency

- **Our experiments**: 16% 7% average absolute extraction efficiency (Range from 4% to 41%)
- **Literature studies**: 16-33% absolute extraction efficiency
  - A. Colussi et al. "**Efficiency of DNA IQ System in recovering semen from cotton swab.**" Forensic Science International: Genetics Supplement Series 2 (2009) 87-88.
  - R. Kishore et al. "**Optimization of DNA Extraction from Low-Yield and Degraded Samples Using the BioRobot EZ1 ad BioRobot M48.**" J Forensic Sci, September 2006, Vol. 51, No 5.
  - Y.C. Swaran, L. Welch, "**A comparison between direct PCR and extraction to generate DNA profiles from samples retreived from various substrates."** Forensic Sci. Int. Genetics. May 2012, Vol. 6, No. 3.
- **Loss of about 70-85%** of initial sample during the extraction process
- Loss is independent of extraction method or source of DNA (i.e. blood, cells, previously extracted)

Why Does This Matter?

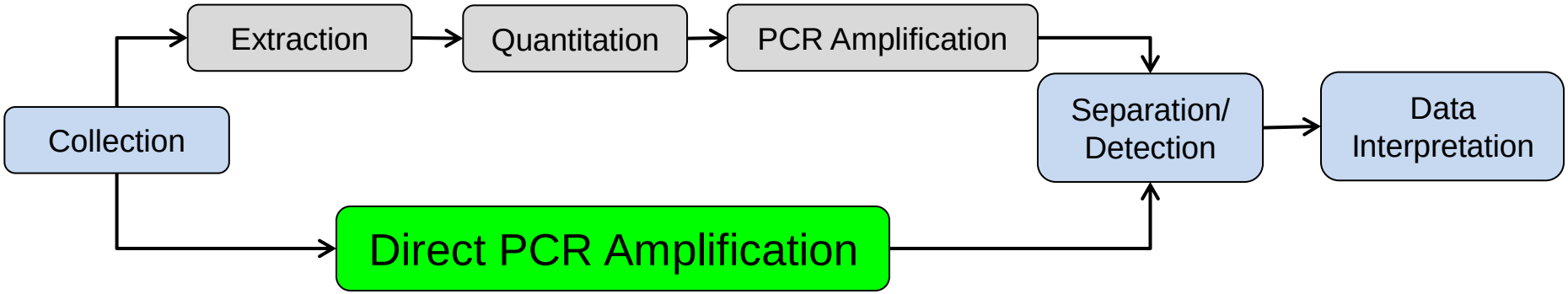
- A majority of sample is lost during extraction
  - Minimal impact on reference samples
  - Enough DNA is recovered for an STR profile
- Low extraction efficiency could result in lower sample quantity which may fail to yield full STR profiles



Is it possible to bypass extraction?

Alternate Extraction Techniques
&
Direct PCR

Direct PCR



- Direct PCR kits commercially available
  - Improved polymerase/master mix help limit inhibition
  - Eliminates the need for purification
  - Higher sensitivity
  - Optimized for samples on FTA Cards
    - Pretreatment protocols for other substrates

Alternate Techniques for Direct PCR

Pretreatment steps aid in breaking open the cell to lyse the DNA without purification

- Buccal Swab Pretreatment for Direct PCR
  - Prep-N-Go Solution (Life Technologies)
  - SwabSolution Reagent (Promega)

- Blood Stains on non-FTA paper Pretreatment for Direct PCR
  - Prep-N-Go Solution (Life Technologies)
  - PunchSolution Reagent (Promega)

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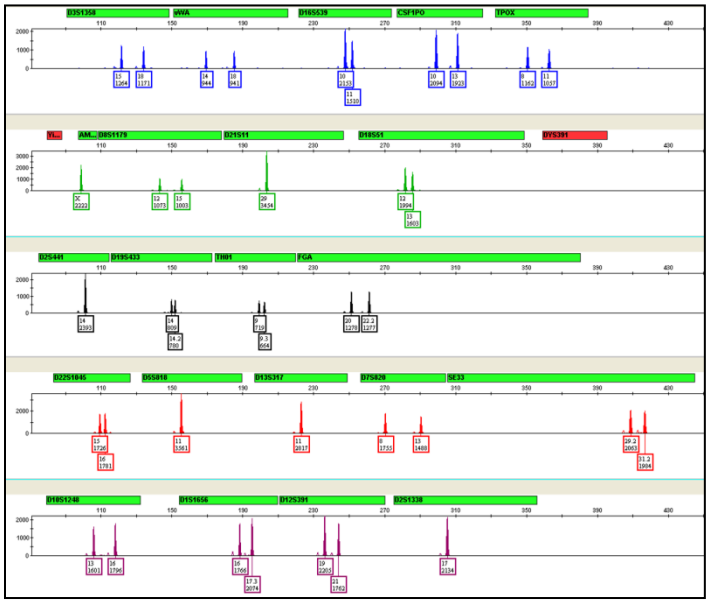
Buccal Swab Pretreatment

Prep-N-Go Solution

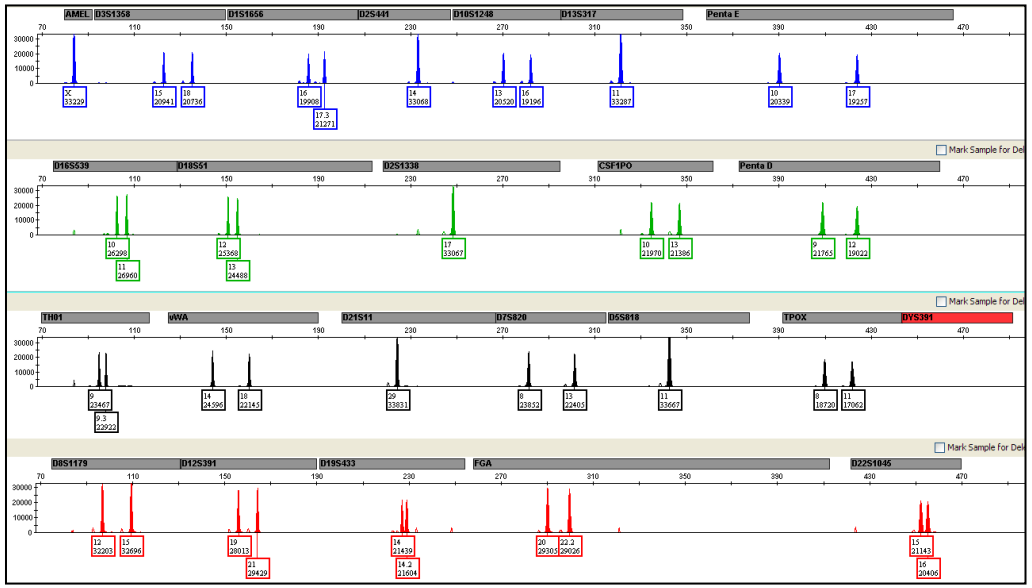
- Incubate swab at room temperature in 400 µL Prep-n-Go Buffer
- 3 µL extract solution added directly to PCR

SwabSolution Reagent

- Incubate swab at 70 °C for 30 minutes in 1 mL SwabSolution Reagent
- 2 µL extract solution added directly to PCR



Life Technologies: GlobalFiler

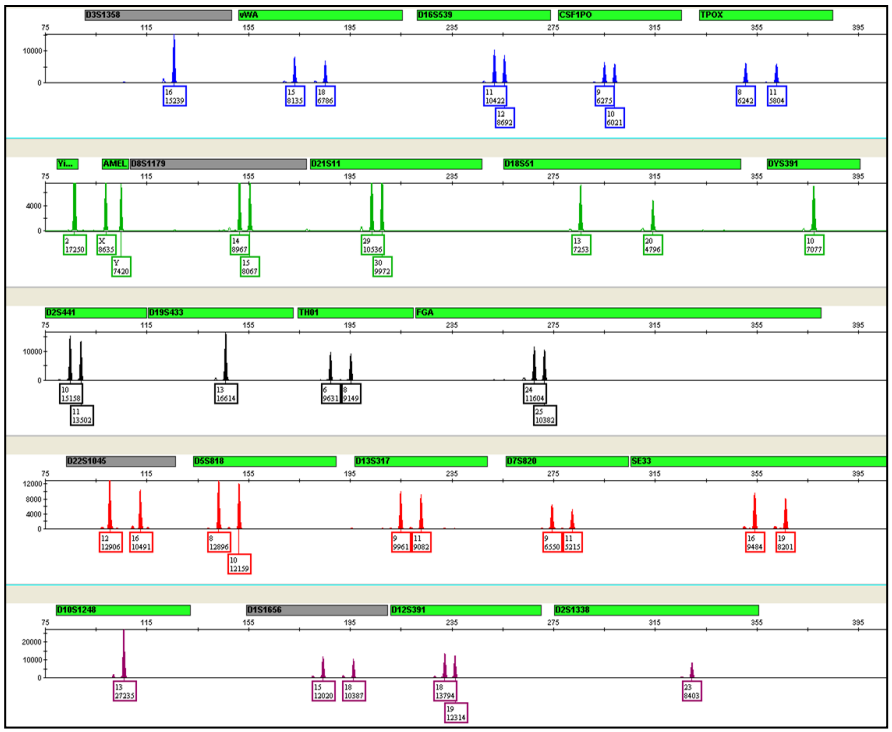


Promega: PowerPlex Fusion

Blood Stains (non-FTA) Pretreatment

Prep-N-Go Solution

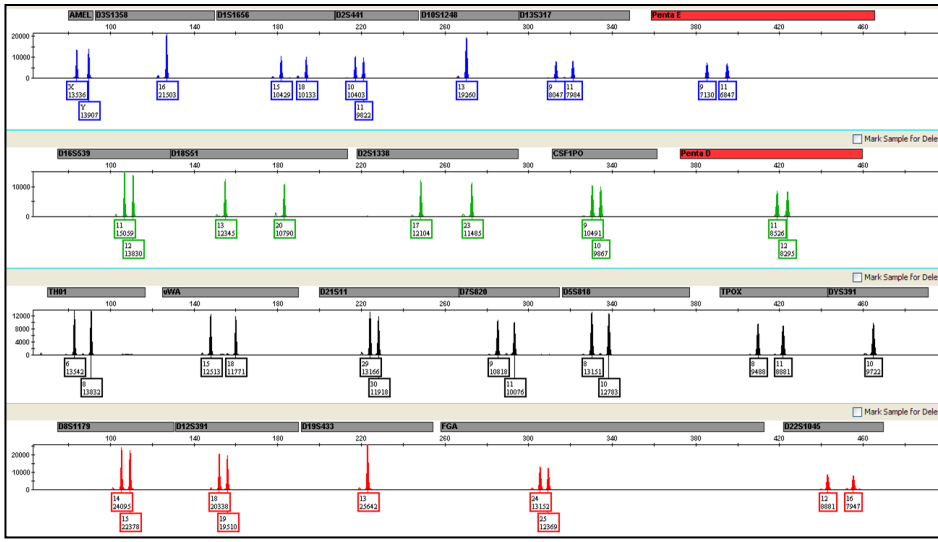
- 3 µL added with PCR setup and one 1.2 mm punch



Life Technologies: GlobalFiler

PunchSolution Reagent

- 10 µL PunchSolution Reagent incubated at 70 °C for 30 minutes until punches are dry



Promega: PowerPlex Fusion

Overall Conclusions

Absolute Extraction Efficiency

- **16% 7% recovery yield** when evaluating absolute extraction efficiency
  - Independent of extraction method or DNA source
- Extraction chemistries could be optimized to increase yield

Direct PCR

- Direct PCR with pretreatment applications are an effective way to **bypass low extraction efficiencies** for reference samples.
  - The need for a quantitation step prevents casework from applying direct PCR techniques
  - Complete STR profiles can be generated from non-FTA substrates

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Acknowledgments

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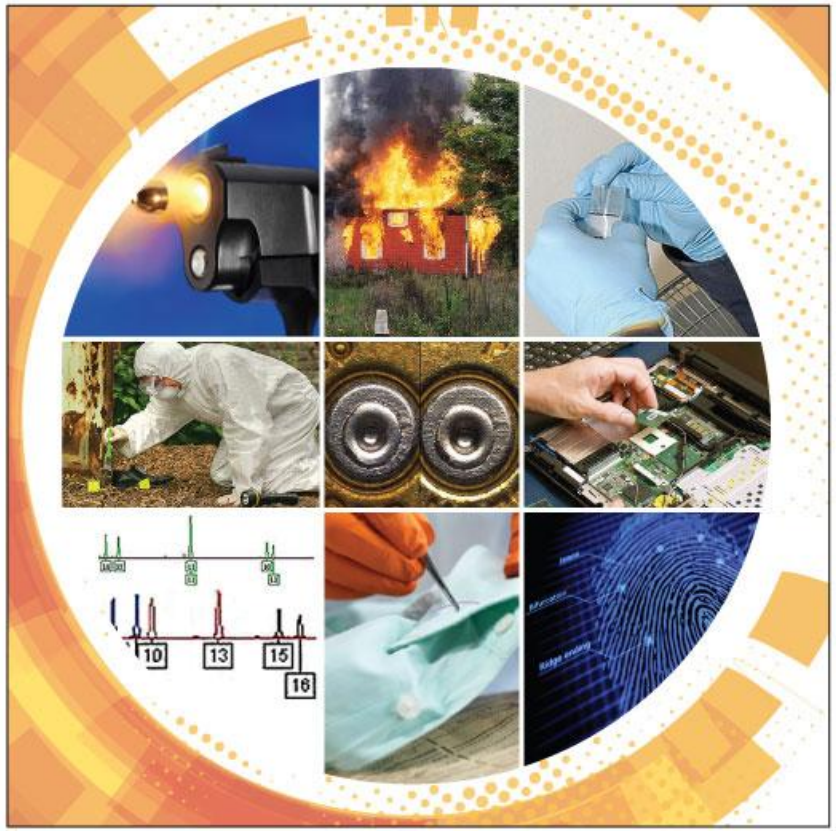
Thank you for your attention!



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