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Evaluation of DNA Extraction Efficiency

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Outline

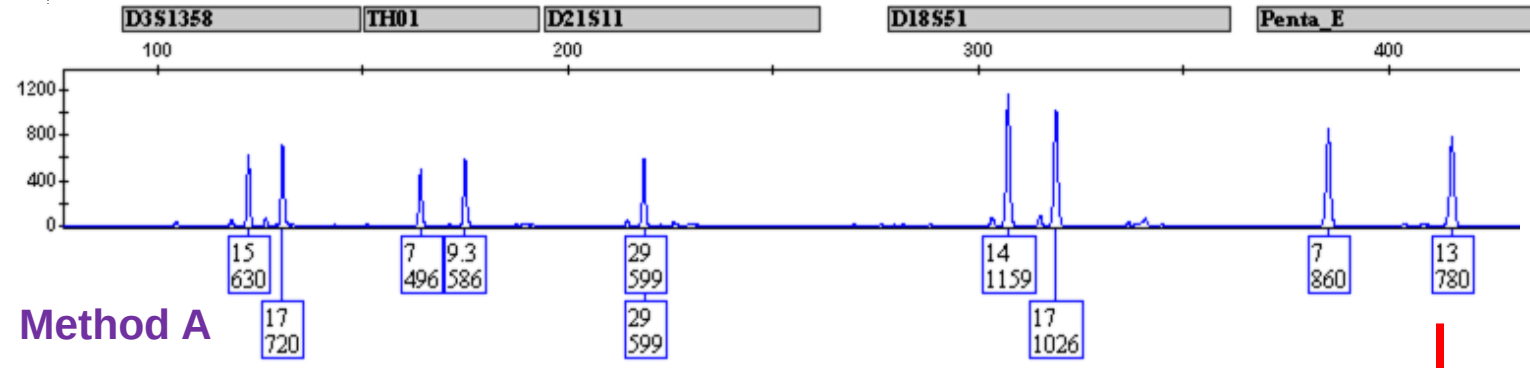
- Methods used to evaluate extraction efficiencies
  - Relative vs. Absolute extraction efficiency
- What can we learn from absolute extraction efficiency?
- Alternate approaches to extraction for multiple substrates

Relative Extraction Efficiency

- Recovery compared to another method of extraction
  - This measurement is relative to another method of extraction (often organic)
- Often defined using several methods within the literature
  - Full vs. Partial STR Profiles
  - Total peak height obtained from samples
  - Number of loci successfully genotyped (percent of profile obtained)

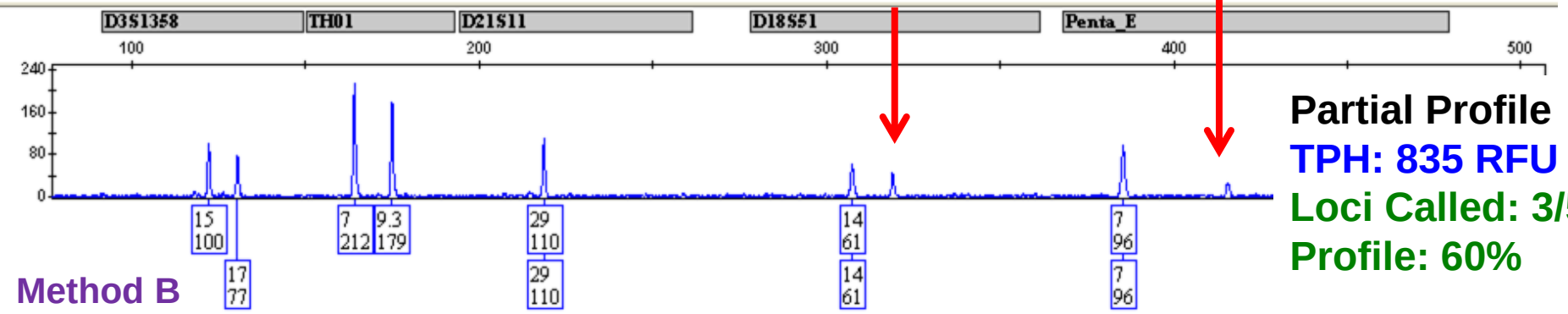
Examples of Relative Extraction Efficiency

- Full vs. Partial STR Profiles
- Total peak height obtained from samples
- Number of loci successfully genotyped (percent of profile obtained)



Method A

Full Profile
TPH: 6856 RFU
Loci Called: 5/5
Profile: 100%



Method B

Partial Profile
TPH: 835 RFU
Loci Called: 3/5
Profile: 60%

Limitations of Relative Efficiency Metrics

- Measures the endpoint of the STR genotyping process
  - Does not reflect the absolute efficiency of 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

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=5 per quantity)

- **Known quant value**: 52.4 ng/µL
- Ranges from 1500 ng to 50 ng

Human cell lines*: 100 µL of a cell suspension in a PCR compatible buffer added to sterile swabs (n=5 per quantity)

- **Number of cells determined through flow cytometry**
- Ranges from 2400 ng to 300 ng of DNA

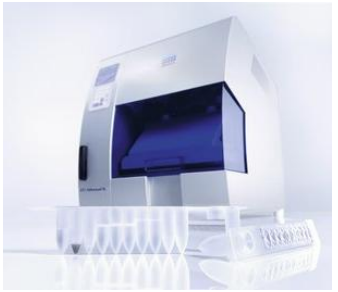
Whole blood*: Four volumes of whole blood tested (n=2 per volume)

- **Assumes 4.0 mil WBC/mL**
- Ranges from 1200 ng to 120 ng of DNA

*Assume 6 pg of DNA per cell

Extraction Methods

Qiagen EZ1 Advanced XL



- Robotic purification process
- ProK digest prior to robotic purification
- Magnetic bead purification process

Modified Salt Out

- Manual extraction process
- ProK digest prior Saturated Ammonium Acetate separation
- DNA precipitation (Ethanol)

Chelex

- Manual extraction process
- Chelating resin used to purify DNA
- Produces single stranded DNA (ssDNA)

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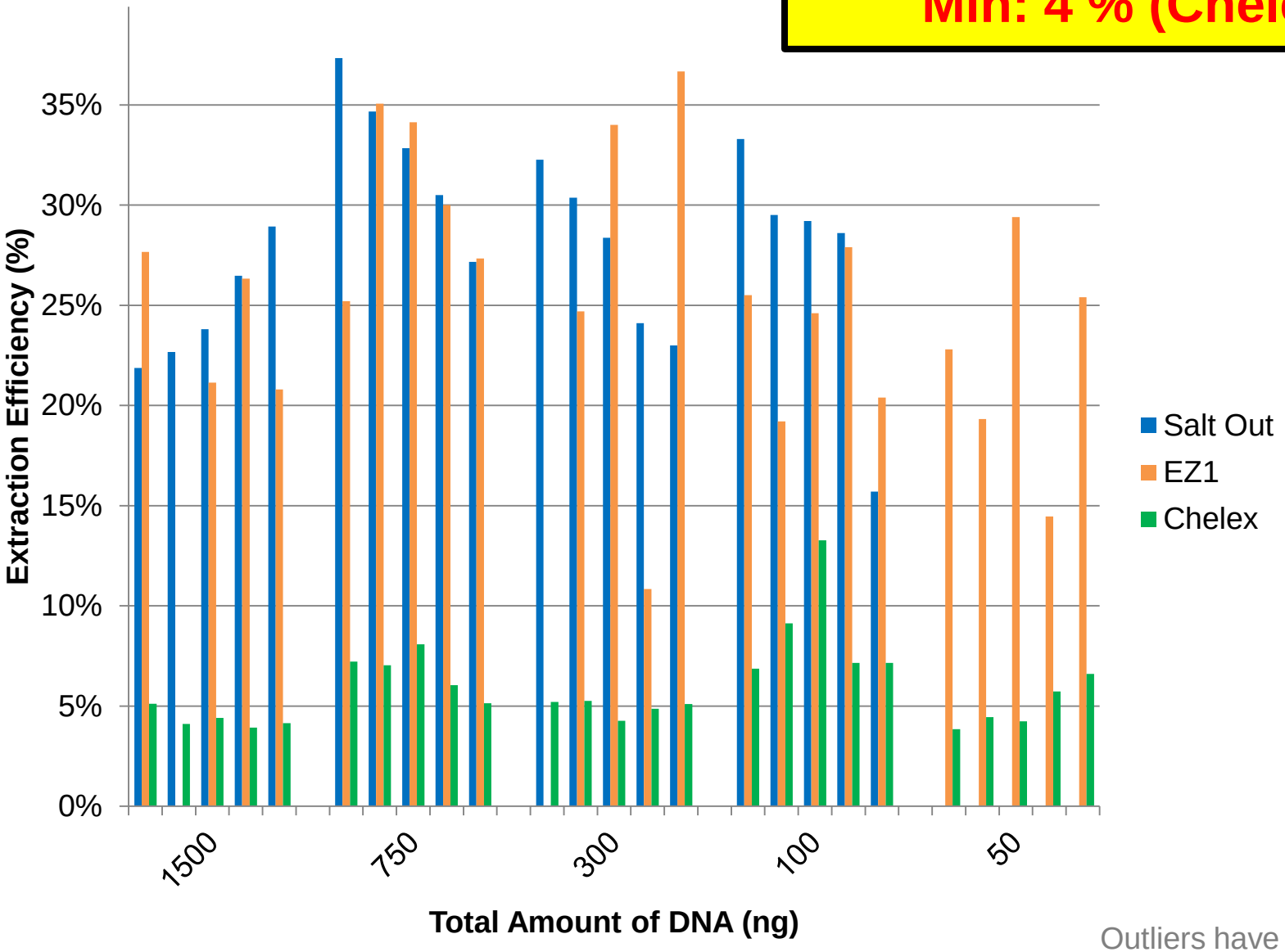
Extracted DNA

Absolute Extraction Efficiency:

Max: 37 % (Salt Out)
Min: 4 % (Chelex)



n=5

Outliers have been omitted

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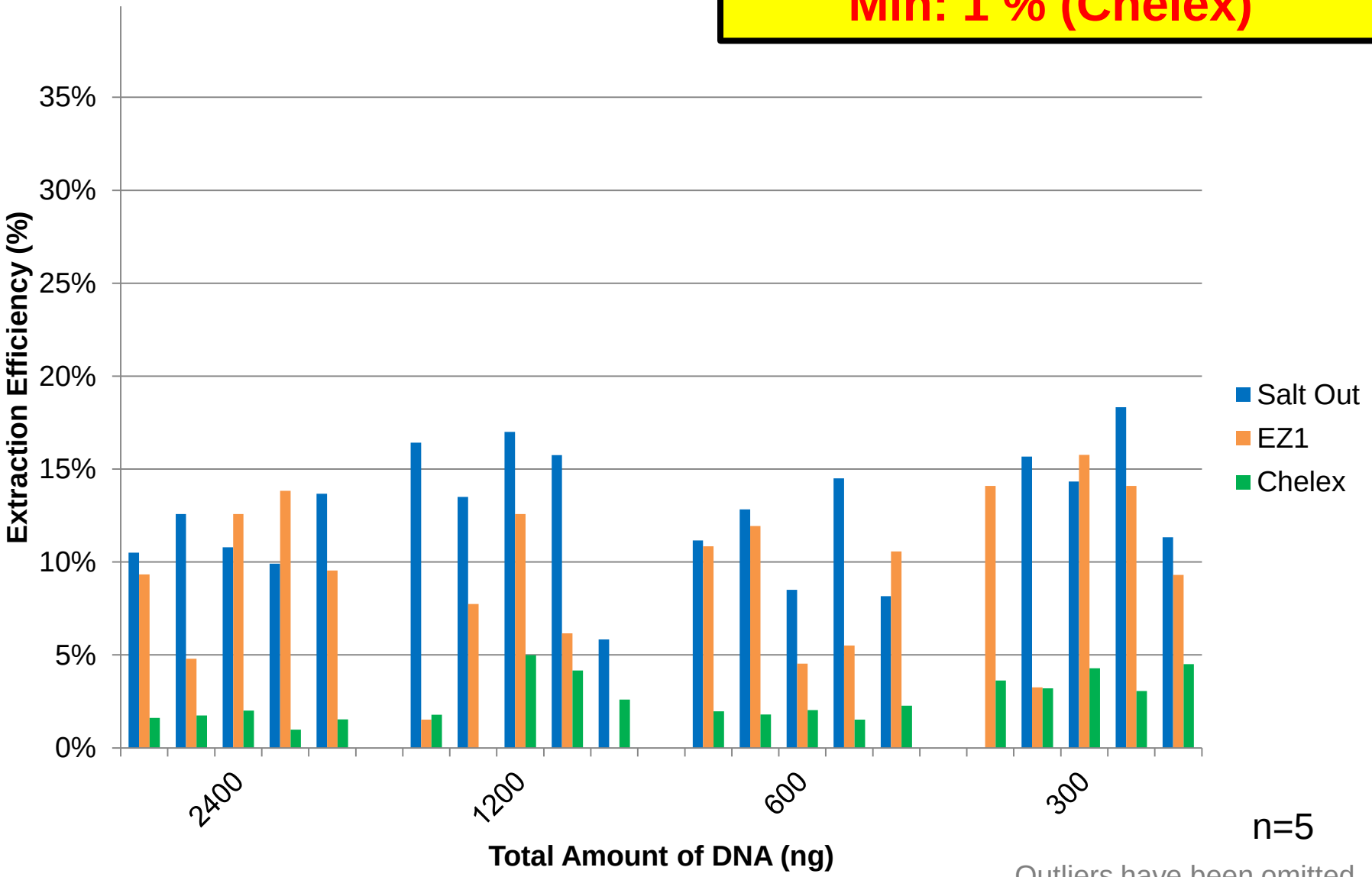
Human Cell Lines

Absolute Extraction Efficiency:

Max: 18 % (Salt Out)
Min: 1 % (Chelex)



n=5

Outliers have been omitted

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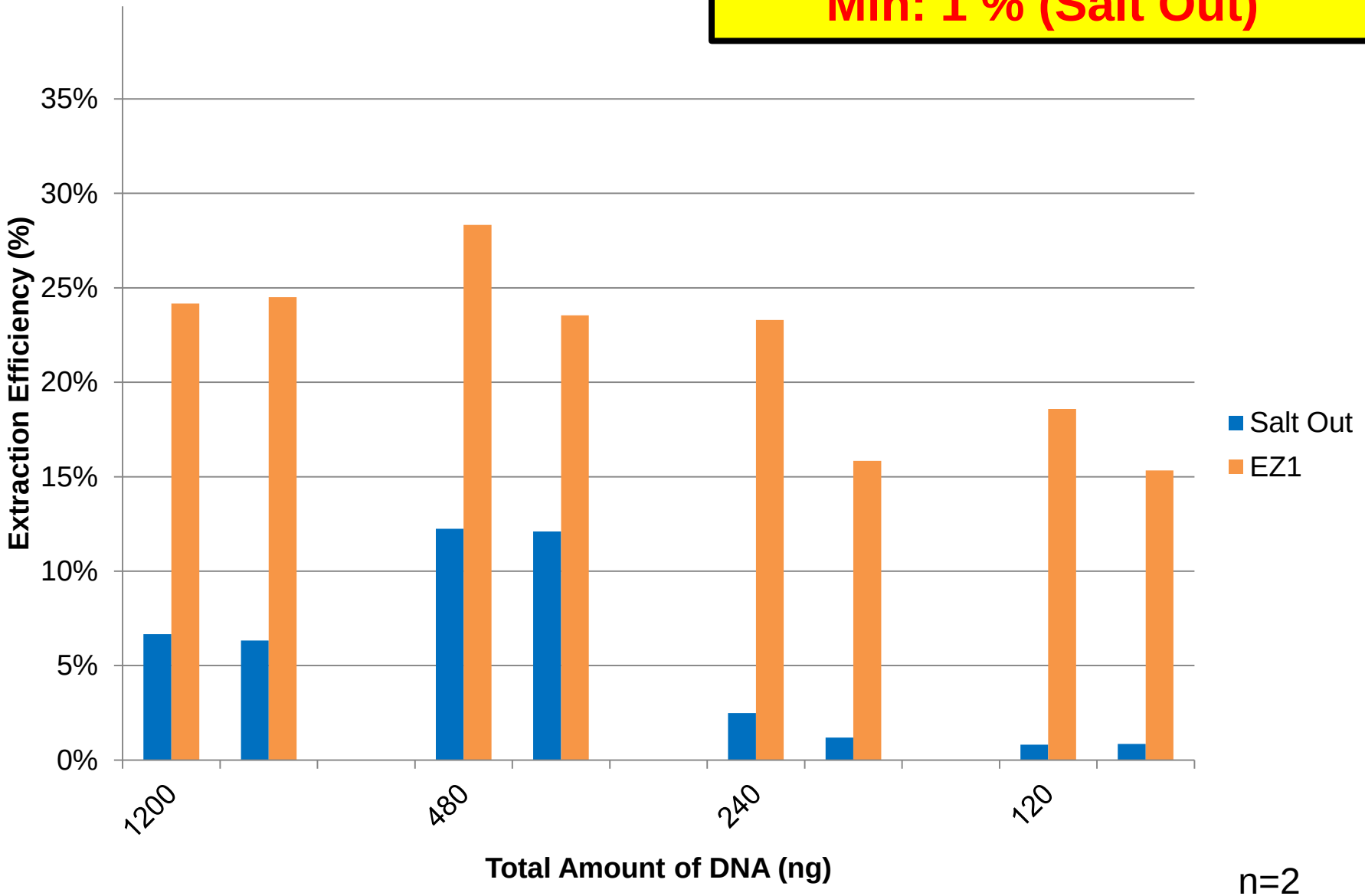
Whole Blood

Absolute Extraction Efficiency:

Max: 26 % (EZ1)
Min: 1 % (Salt Out)



n=2

Summary of Absolute Extraction Efficiency

- **Our experiments**: Range from 1 % to 37 % absolute extraction efficiency
- **Literature studies**: 10-33% absolute extraction efficiency

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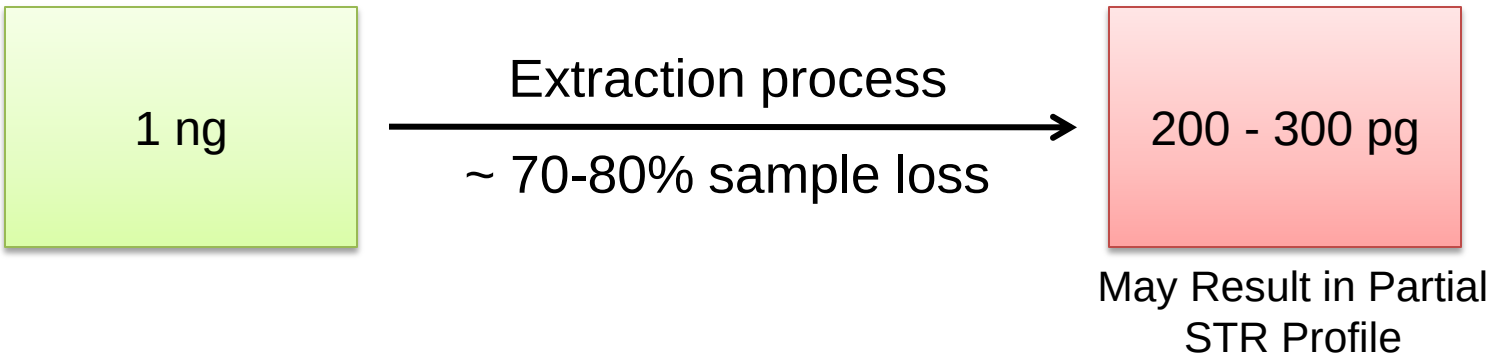
- **Loss of about 70-85%** of initial sample during the extraction process
- Loss is independent of extraction method or source of DNA (i.e. blood, cells, previously extracted)

Why Does This Matter?

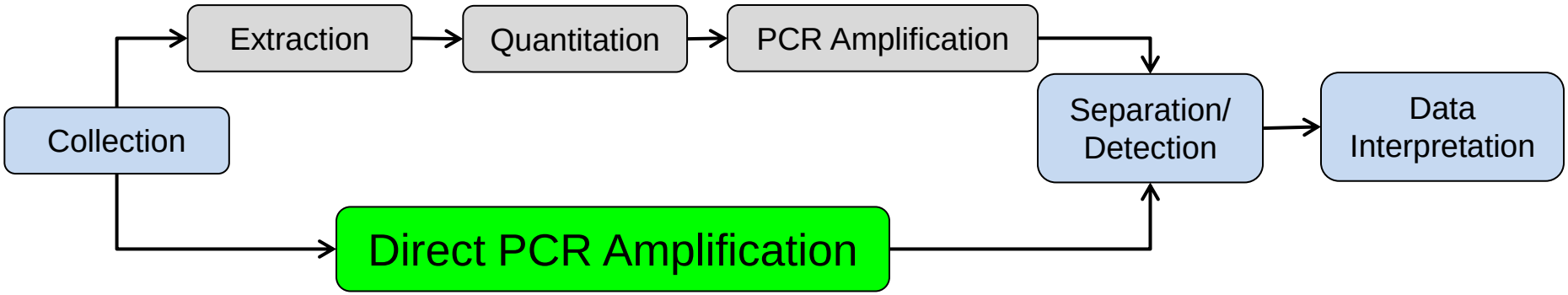
- A majority of sample is lost during extraction
  - Minimal impact on reference samples
  - Enough DNA is recovered for an STR profile
- Low extraction efficiency could result in lower sample quantity which may fail to yield full STR profiles



Is it possible to bypass extraction?

Pretreatment Techniques
&
Direct PCR

Direct PCR



- **Direct PCR** kits commercially available for use with **reference samples**
  - Improved polymerase/master mix help limit inhibition
  - Eliminates the need for sample transfer steps and purification
  - Higher sensitivity
  - Optimized for samples on FTA Cards
    - Pretreatment protocols for other substrates

Pretreatment 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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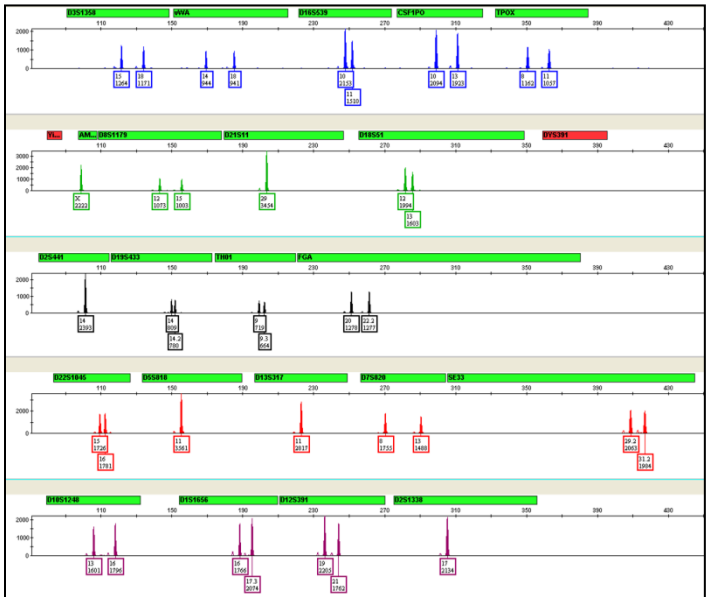
Buccal Swab Pretreatment

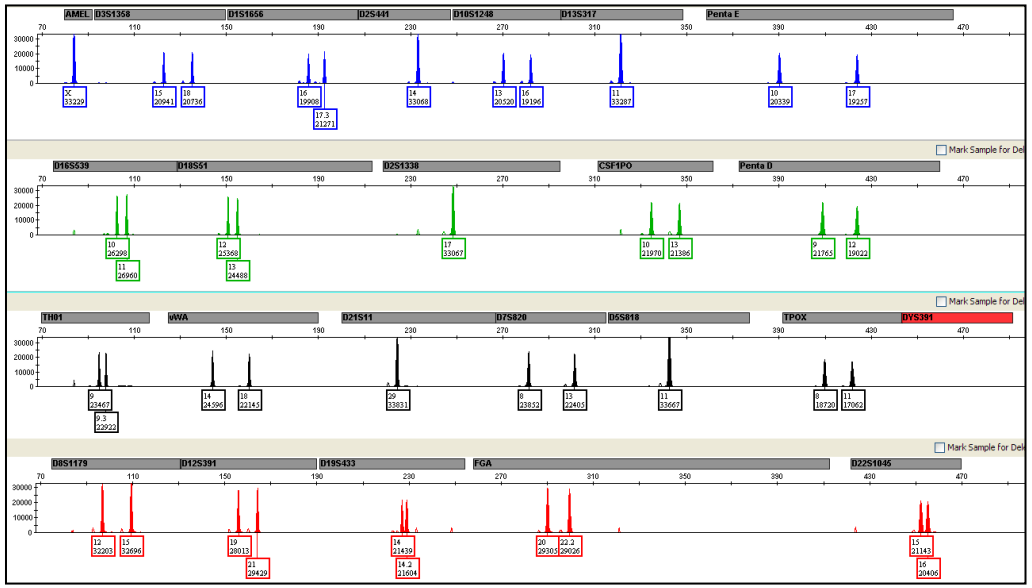
Prep-N-Go Solution

- 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 Express

Promega: PowerPlex Fusion

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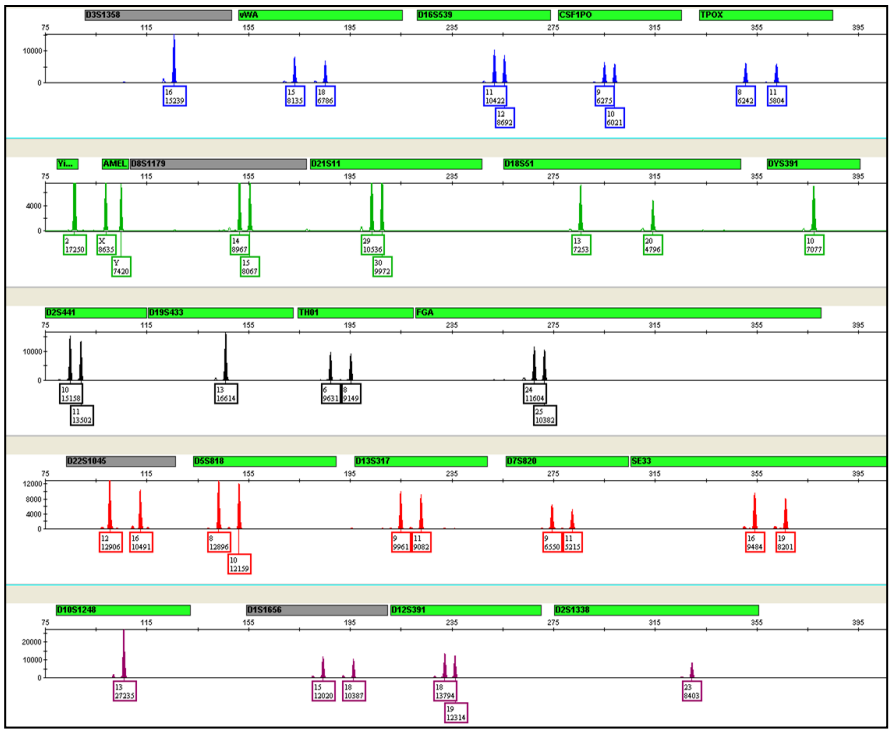
Non-FTA Pretreatment

Prep-N-Go Solution

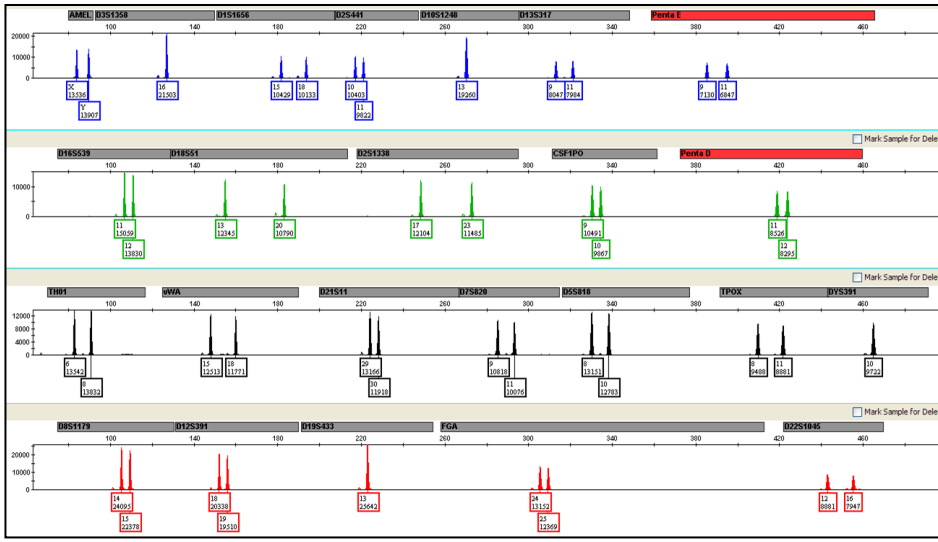
- 3 µL added with PCR setup and one 1.2 mm punch



Life Technologies: GlobalFiler Express

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

- **Range from 1 % to 37 % 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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Thank you for your attention!