

## DNA Extraction Efficiency: Is it what you thought?

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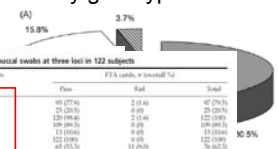
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## Outline

- Define methods used to evaluate extraction efficiencies
- How do we define true extraction efficiency?
- What can we learn from true extraction efficiency?
- How can true extraction efficiency be improved?

## Extraction Efficiency

- Defined using several different methods
  - Full vs. Partial STR Profiles
  - Number of loci successfully genotyped
  - Pass/Fail System



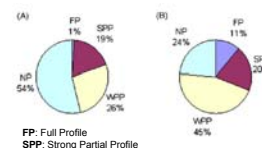
Locus	Buccal swabs			FTA cards		
	Pass	Fail	Total	Pass	Fail	Total
4	48 (39%)	74 (61%)	122 (100%)	42 (34%)	80 (66%)	122 (100%)
3	25 (20%)	97 (80%)	122 (100%)	25 (20%)	97 (80%)	122 (100%)
2	129 (100%)	0 (0%)	129 (100%)	129 (100%)	0 (0%)	129 (100%)
0 or 1	122 (100%)	0 (0%)	122 (100%)	122 (100%)	0 (0%)	122 (100%)
ACE 1/D	48 (39%)	74 (61%)	122 (100%)	42 (34%)	80 (66%)	122 (100%)
STR 18/24	48 (39%)	74 (61%)	122 (100%)	42 (34%)	80 (66%)	122 (100%)

M. Stangegaard et al. "Automated extraction of DNA from reference samples from various biological materials on the Qiagen QIAcube EZ1 Workstation." *Forensic Science International: Genetics Supplement* 2 (2007) 65-70

E. Milne et al. "Buccal DNA Collection: Comparison of Buccal Swabs with FTA Cards." *Cancer Epidemiol Biomarkers Prev* 2006;15(4), April 2006

## Typical Definition of Extraction Efficiency

- The number of observed full STR profiles
- Divided into three categories:
  1. Full Profile
  2. Partial Profile
  3. No Profile



(A) FP 1%, SPP 19%, WPP 26%, NP 54%

(B) FP 11%, SPP 20%, WPP 45%, NP 24%

FP: Full Profile  
SPP: Strong Partial Profile  
WPP: Weak Partial Profile  
NP: No Profile

K.M. Horsman-Hall et al. "Development of STR profiles from firearms and fired cartridge cases." *Forensic Science International: Genetics* 3 (2009) 242-250

## Typical Definition of Extraction Efficiency

- Recovery compared to another method of extraction (often organic)
- The comparison can be of STR loci recovered or by quantitation using real-time PCR methods
- This is a relative efficiency (practical use)

## Limitations of Current Efficiency Metrics

- Measures end point - efficiency of STR genotyping
- Does not reflect the **true efficiency** of the **extraction process**
- Does not account for the initial amount DNA present in the sample
  - However, in case work samples the **true amount of starting material is unknown**

### True 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 true efficiency of the extraction
- The original amount needs to be known

### Testing True Extraction Efficiency

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

- 3 sources of DNA
- 2 extraction methods
- Quantitated with real-time PCR

### Sources of DNA


1. Highly characterized extracted DNA
  - Known quant value: 52.44 ng/μL
2. Primary human cell lines\*
  - MCF 10A: Human epithelial
  - Number of cells can be determined through flow cytometry
3. Whole blood\*
  - Assumed white blood cell count of 4.0 million WBC/mL

\*Assume 6 pg of DNA per cell

### Qiagen EZ1 Advanced


EZ1 Advanced uses magnetic separation and multiple washes to purify DNA

- Swabs & Stains: G2 Buffer and Proteinase K added to sample
- Blood: Total sample volume brought up to 200 μL with G2 Buffer
- Incubated at 56°C for 15 minutes then 95°C for 5 minutes
  - Vortex periodically through incubation (~every 5 minutes)



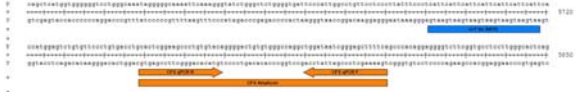
### Modified Salt Out

- 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



### DNA Quantitation Assay

- Targets the STR locus TH01
  - Chromosomal location: 11p15.5; intron 1 of human tyrosine hydroxylase gene
- Modified to run as a SYBR green assay
  - Run on ABI 7500



Richard, M.L., Frappier, R.H. and Newman, J.C. (2003) Developmental validation of a real-time quantitative PCR assay for automated quantification of human DNA. J Forensic Sci, 48 (5): 1041-1046

### Extracted DNA Samples

- Varying amounts added to sterile swab (n=18 per quantity)
  - 1500 ng, 1200 ng, 600 ng, 300 ng, 100 ng
- Swabbing method using a Teflon tube
  - Simulated buccal swab being taken
- Allowed sample to dry in hood overnight

### Extracted DNA Efficiency

- Extraction with EZ1 from swabs
- Highest recovery percentage: 37%
- Lowest recovery percentage: 4%
- n=18 per quantity

True extraction efficiency average: 23%

### Extracted Cell Line Efficiency

Swabbed 100  $\mu$ L of a solution containing DNA in a Teflon tube (n=12 per quantity)

- 50,000 cells (300 ng)
- 100,000 cells (600 ng)
- 200,000 cells (1200 ng)

**EZ1**

Min: 9%

Max: 20%

**Salt Out**

Min: 9%

Max: 41%

True extraction efficiency average: 16% (EZ1) and 20% (SO)

### Blood Extraction Efficiency

- Seven volumes of whole blood tested (n=2 per volume)\*
  - 200  $\mu$ L, 100  $\mu$ L, 50  $\mu$ L, 20  $\mu$ L, 10  $\mu$ L, 5  $\mu$ L, 1  $\mu$ L
  - Ranges from 4800 ng to 24 ng of DNA
- **Liquid blood** extracted without incubation
  - For EZ1 brought to a total volume of 200  $\mu$ L with G2 Buffer
- **For blood stains:**
  - Blood spotted directly onto Whatman 903 paper
  - Cut into small pieces and placed into extraction tube

\*Assuming 4.0 million WBC/mL and 6 pg of DNA per cell

### Liquid Blood Extraction

n=2 per volume

EZ1 Extraction			Salt Out Extraction		
$\mu$ L Blood	ng DNA	% Recovery*	$\mu$ L Blood	ng DNA	% Recovery*
1	0.7	2.8%	1	0.1	0.1%
5	30.9	25.7%	5	1.0	0.8%
10	49.7	20.7%	10	4.4	1.6%
20	108.3	22.6%	20	58.5	12.2%
50	160.5	13.4%	50	78.0	6.5%
100	133.5	5.6%	100	11.6	0.5%
200	55.8	1.2%	200	0.5	0.1%

True extraction efficiency average: 13%

True extraction efficiency average: 3%

\*Assuming 4.0 million WBC/mL

### Blood Stain Extraction

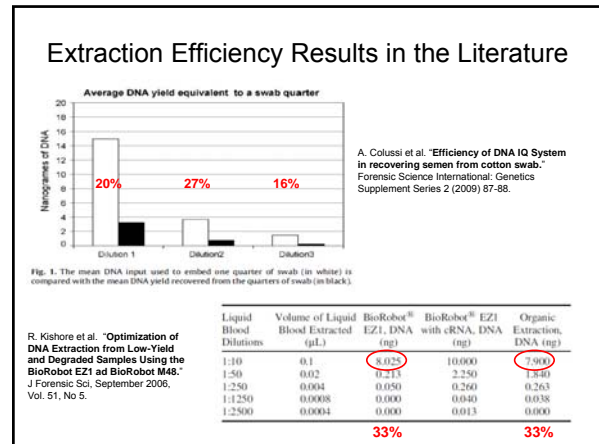
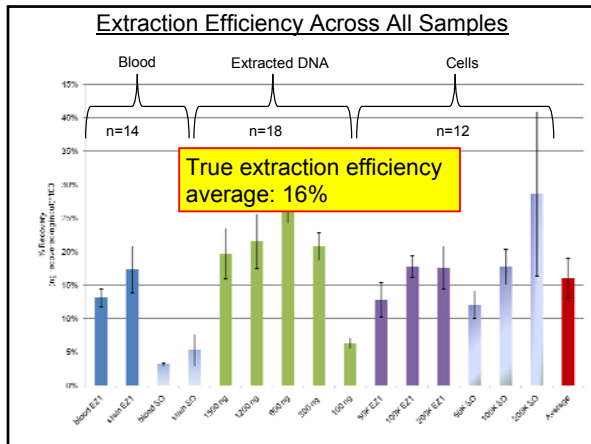
n=2 per volume

EZ1 Extraction			Salt Out Extraction		
$\mu$ L Blood	ng DNA	% Recovery*	$\mu$ L Blood	ng DNA	% Recovery*
1	1.9	8.0%	1	0.2	1.0%
5	20.4	17.0%	5	1.4	1.1%
10	47.0	19.6%	10	3.1	1.3%
20	124.5	26.0%	20	6.3	1.3%
50	292.0	24.3%	50	3.4	0.3%
100	463.0	19.3%	100	486.0	20.3%
200	347.5	7.2%	200	559.0	11.7%

True extraction efficiency average: 17%

True extraction efficiency average: 5%

\*Assuming 4.0 million WBC/mL



### Summary of True Extraction Efficiency

- Our experiments: 16% average true extraction efficiency
- Literature studies: 16-33% true extraction efficiency
- 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)

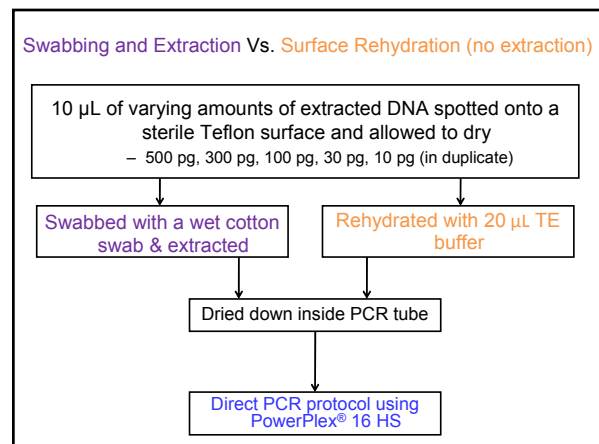
### Why Does This Matter?

- 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 lower sample quantity into the Low Template DNA (L-T-DNA) range

### Is it possible to bypass extraction?


Swabbing and Extraction  
Vs.  
Surface Rehydration (no extraction)

Amplification using Direct PCR



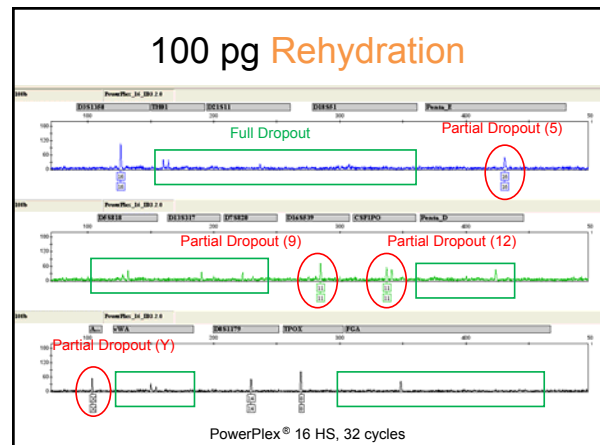
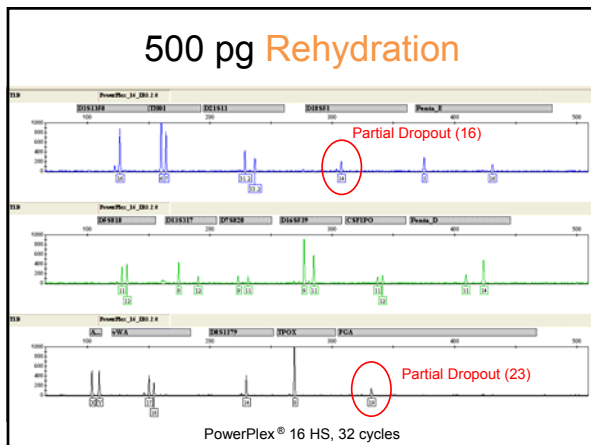
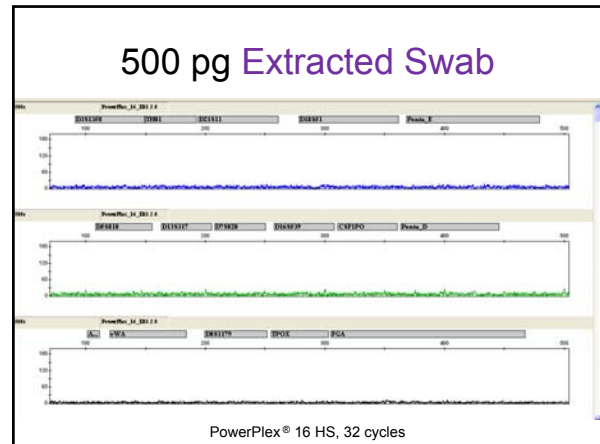
### Direct PCR

- Bypass the extraction and quantitation steps and go directly to PCR
- **Direct PCR** kits are now commercially available
  - Improved polymerase/master mix help limit inhibition in newly released kits
  - Eliminates the need for purification
  - Higher sensitivity



**Direct PCR** Master Mix Recipe  
 1.Reaction Mix  
 2.Primer Mix  
 3.Water

Add to PCR tubes containing DNA extract and amplify



### Surface Recovery Summary

- At 500 pg greater success (STR loci) was observed with the surface recovery method
  - Still not a full profile
  - Not recovering all of the DNA for amplification
- There may be sensitivity issues with surface recovery methods using **direct PCR**
  - Peak height imbalance

### Conclusions

**True Extraction Efficiency**

- **15-30% recovery yield** when evaluating true extraction efficiency
  - Independent of extraction method or DNA source
- Extraction chemistries could be optimized to increase yield

**Surface Recovery**

- For low quantity extracted DNA samples, surface recovery followed by **direct PCR** performed better than traditional swabbing and extraction methods

## Future Work

### Improving Extraction Efficiency

- Alternative extraction methods
  - Liquid based extraction (ZyGem, Isohelix)

### Surface Rehydration and Direct PCR

- Additional rehydration solutions
- Additional sample types
  - Human cell lines
  - Liquid blood and blood stains
- Examine sensitivity of direct PCR and surface rehydration methods

## Acknowledgments

### NIST Team for This Work



Pete Vallone



Margaret Kline

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