

# DRAGEN

(Direct Rapid Analysis Generating Extracted Nucleotides)

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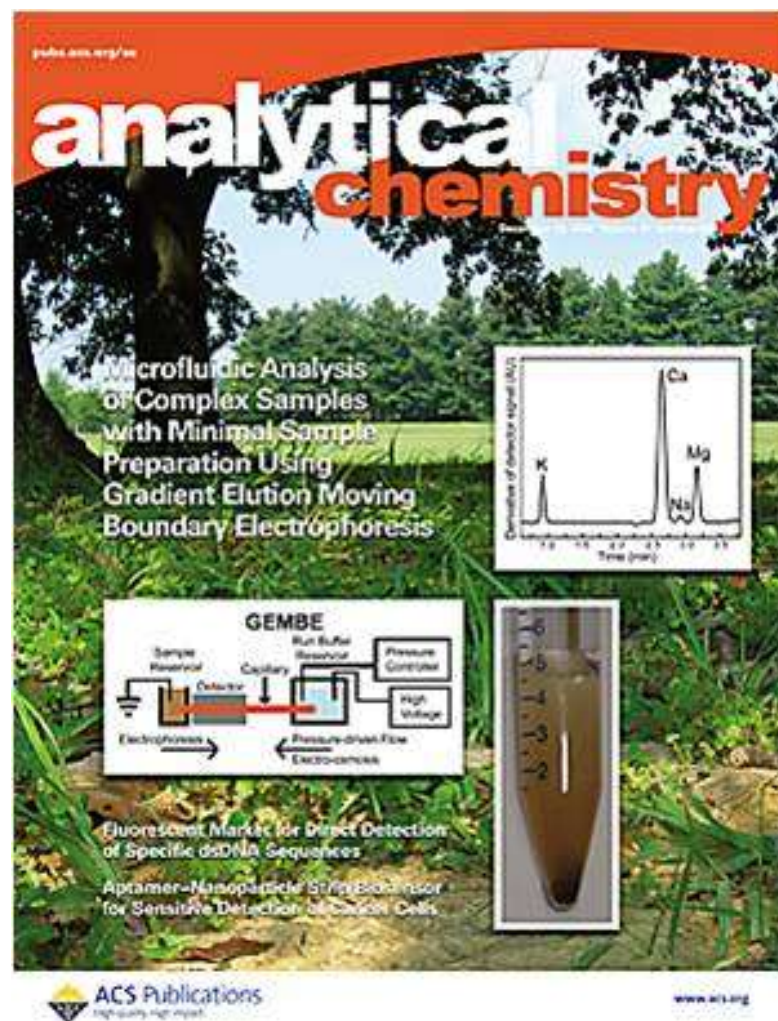
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Research Associates, Inc.)



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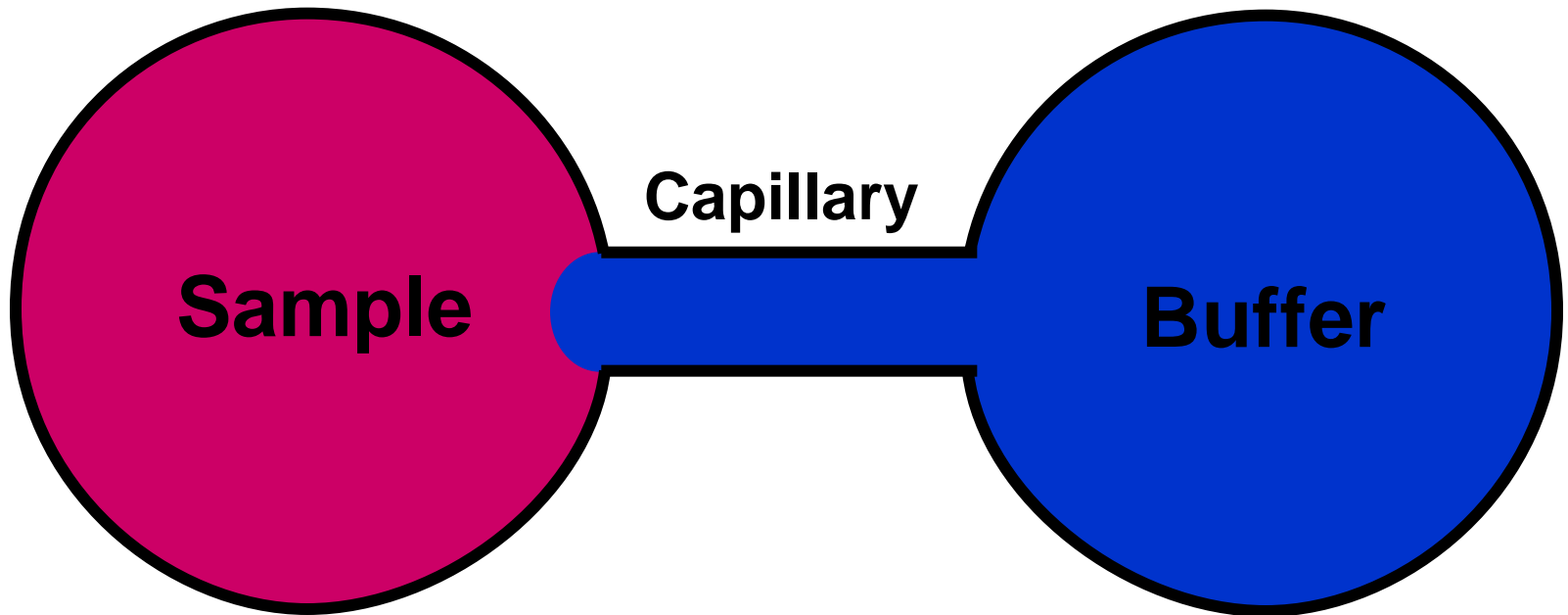
# Gradient Elution Moving Boundary Electrophoresis (GEMBE)

- Simple, Robust System
- High Quality Data
- Minimal Sample Prep Requirements
- Applied to the detection of DoD-relevant species from crude matrices



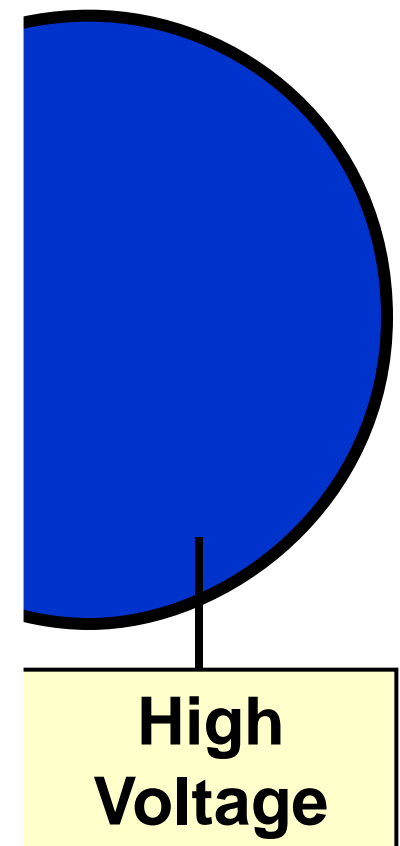
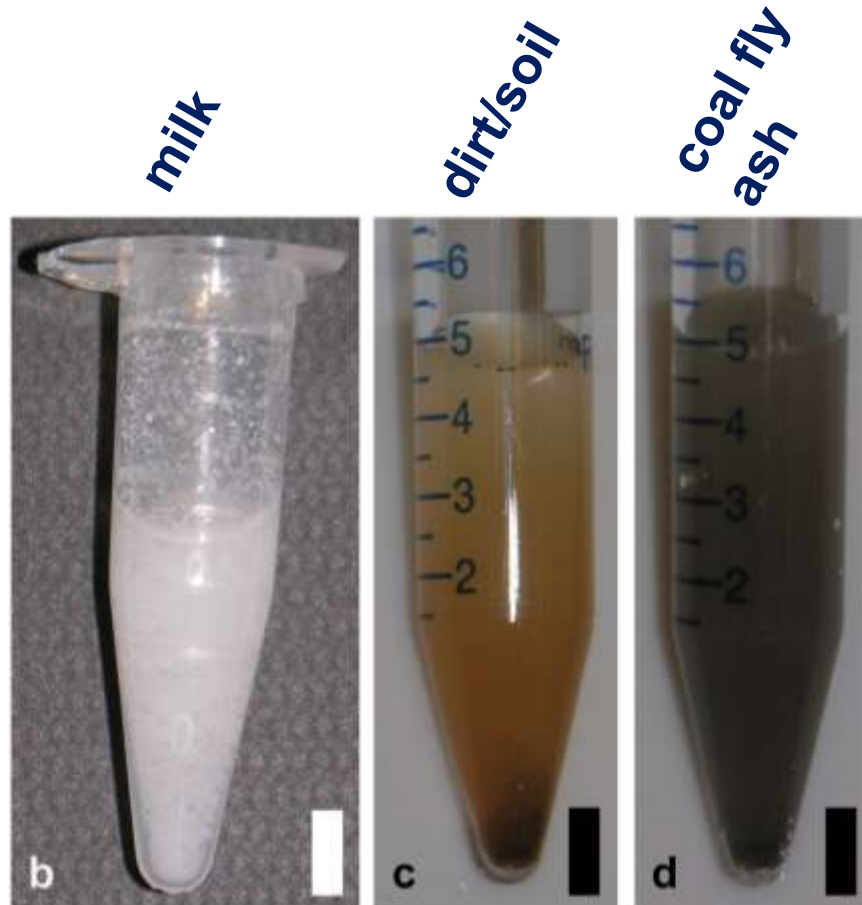
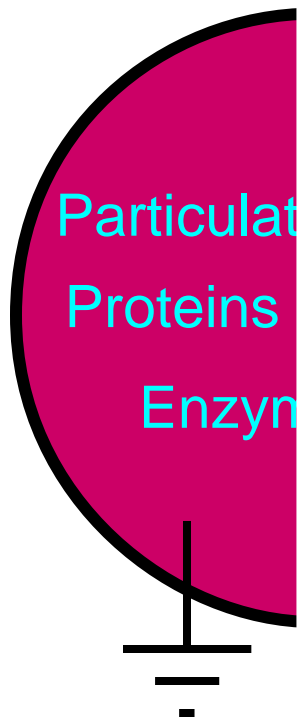
# How GEMBE Works

Simple microfluidic system that provides multi-step chemical analysis functionality

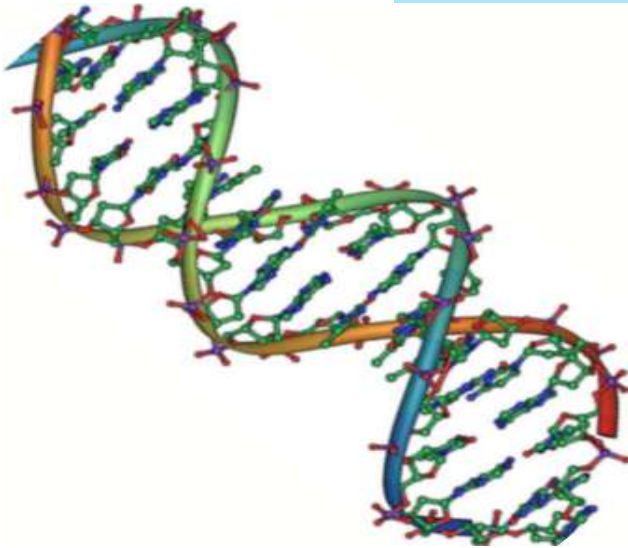


# How GEMBE Works

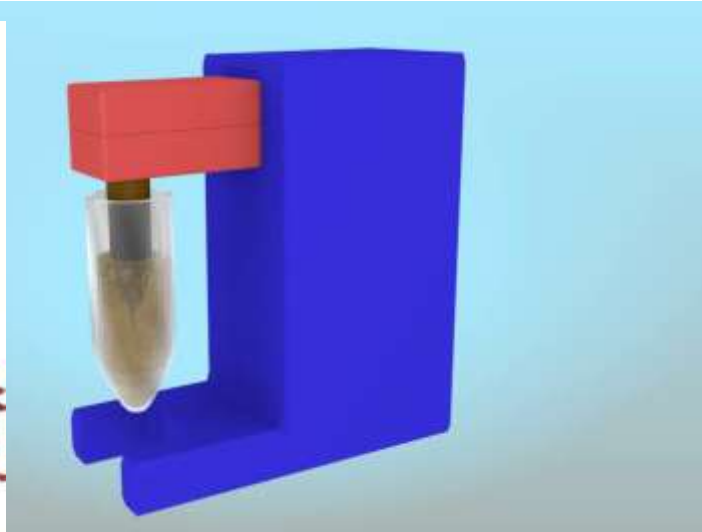
Controlled counterflow prevents proteins, enzymes and particulates from entering and fouling the capillary



# Kit-Based Platform



**DNA Extraction?**



**Bioagent detection**



**Fission product detection**

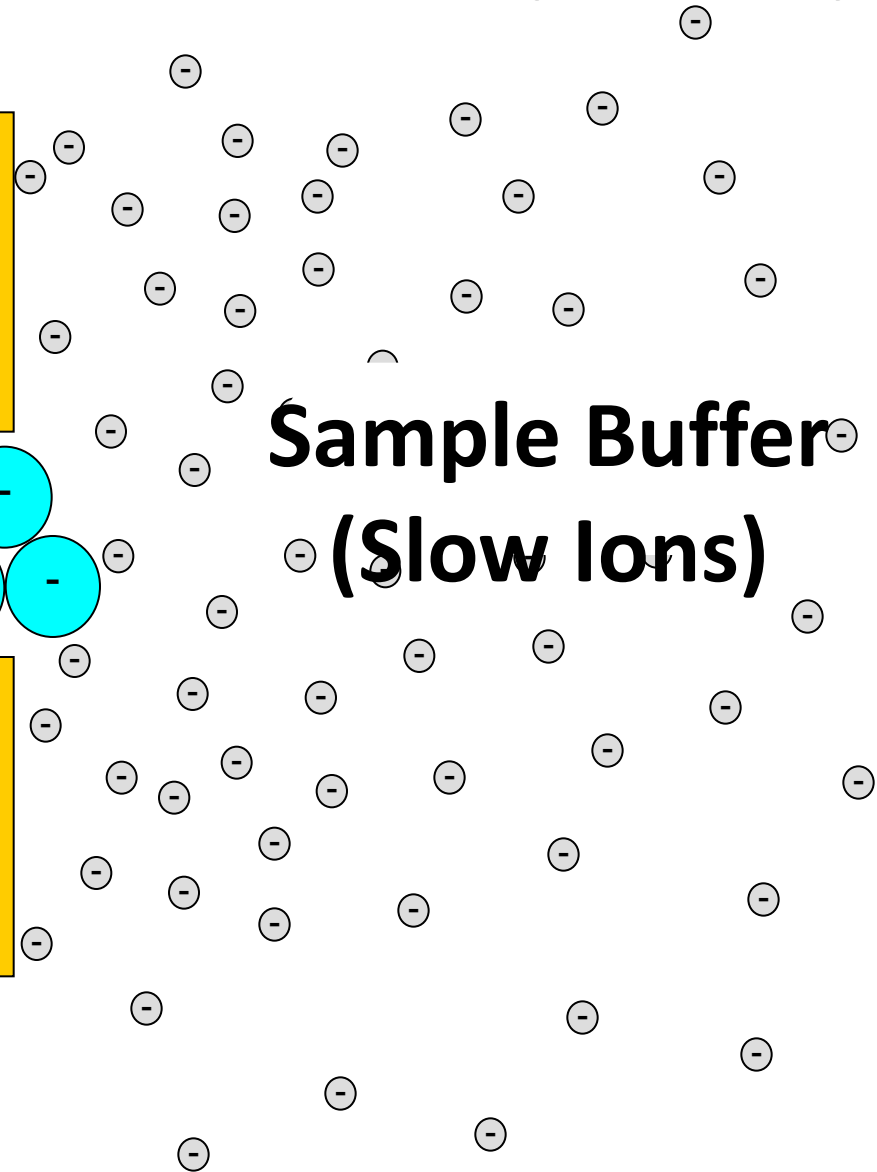
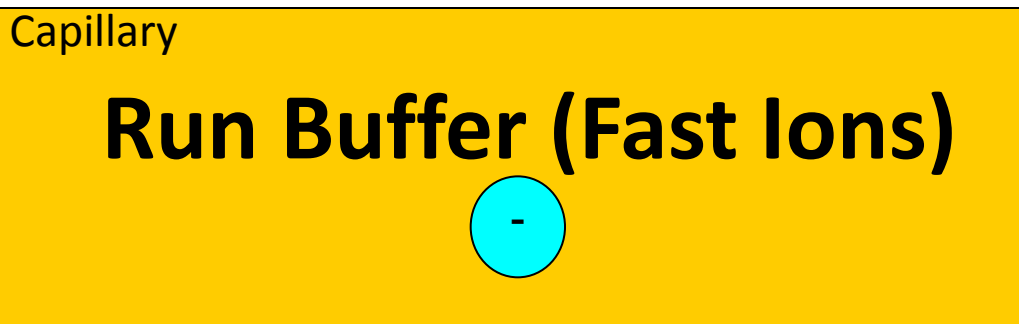
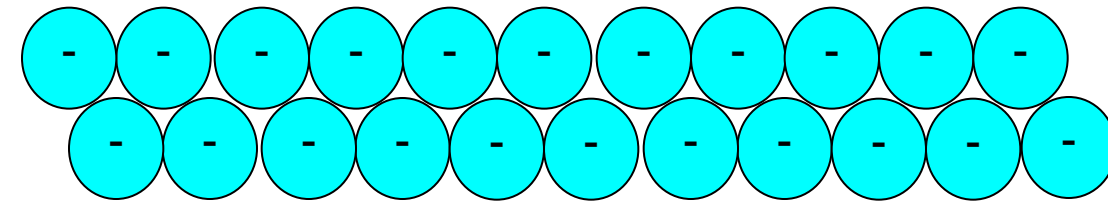


**Nerve agent detection**

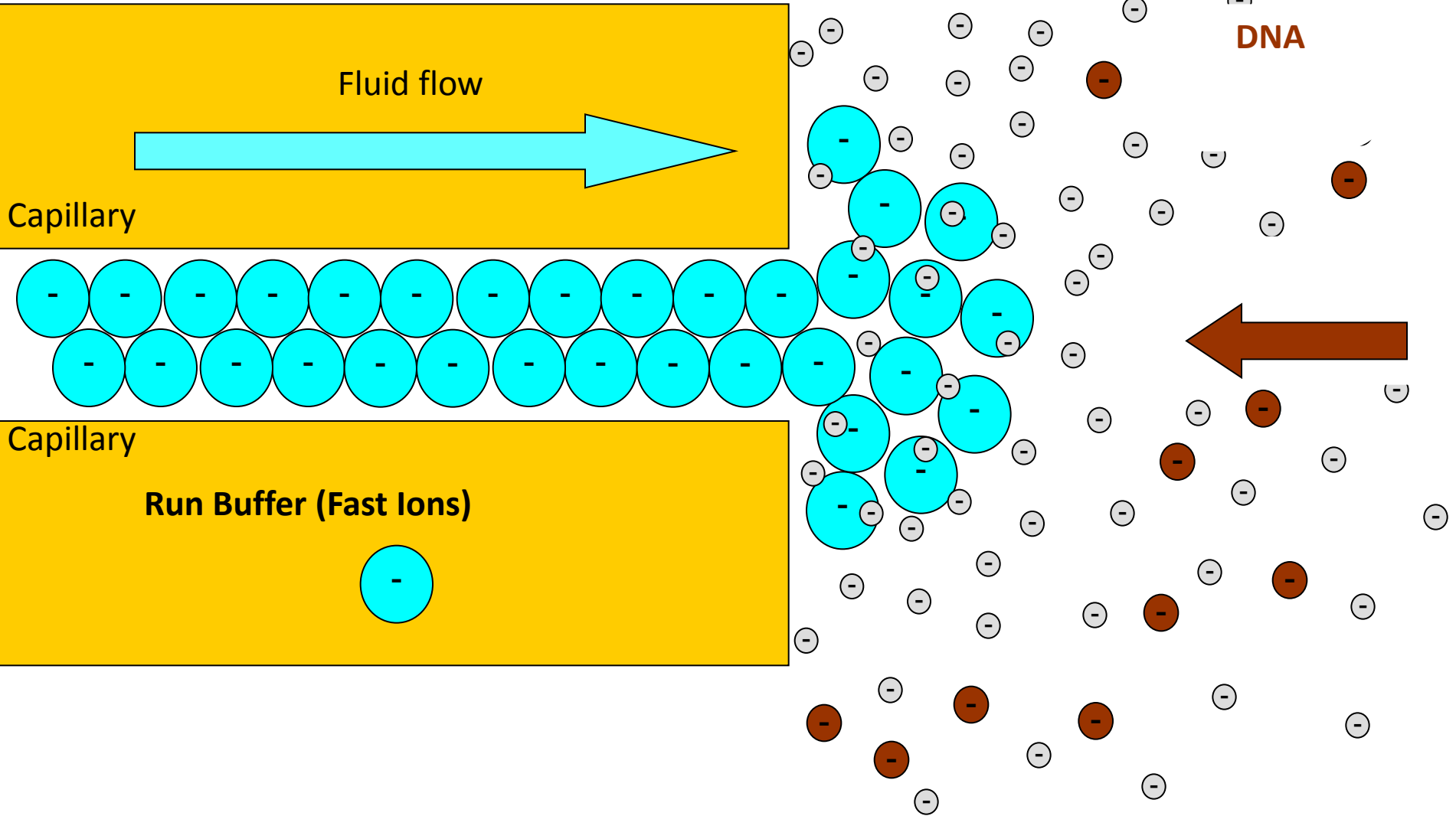


**Explosives detection**

# Gradient Elution Isotachopheresis (GEITP)



# GEITP Theory



# How It Works: GEITP Extraction of Human DNA

movie



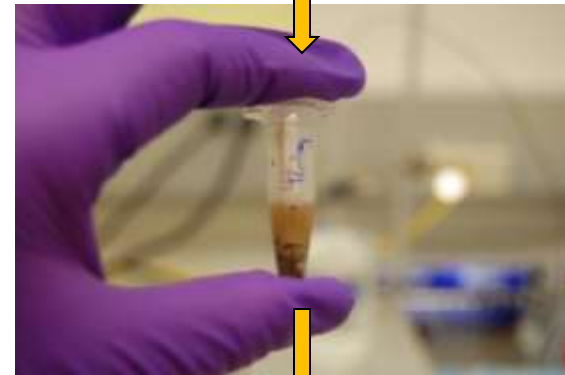


# Phase I Project Goals:

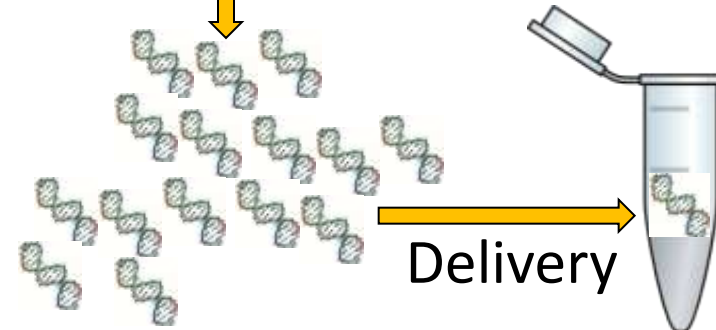
- Extract DNA from Crude Samples
  - High copy number samples, but with PCR inhibitors
  - ‘Dirty Buccal Swab’
- Quantitate DNA
  - On-line fluorescence detection
- Deliver Clean DNA suitable for STR typing
  - Small final volume (10  $\mu$ L)
- Format potentially suitable for field use



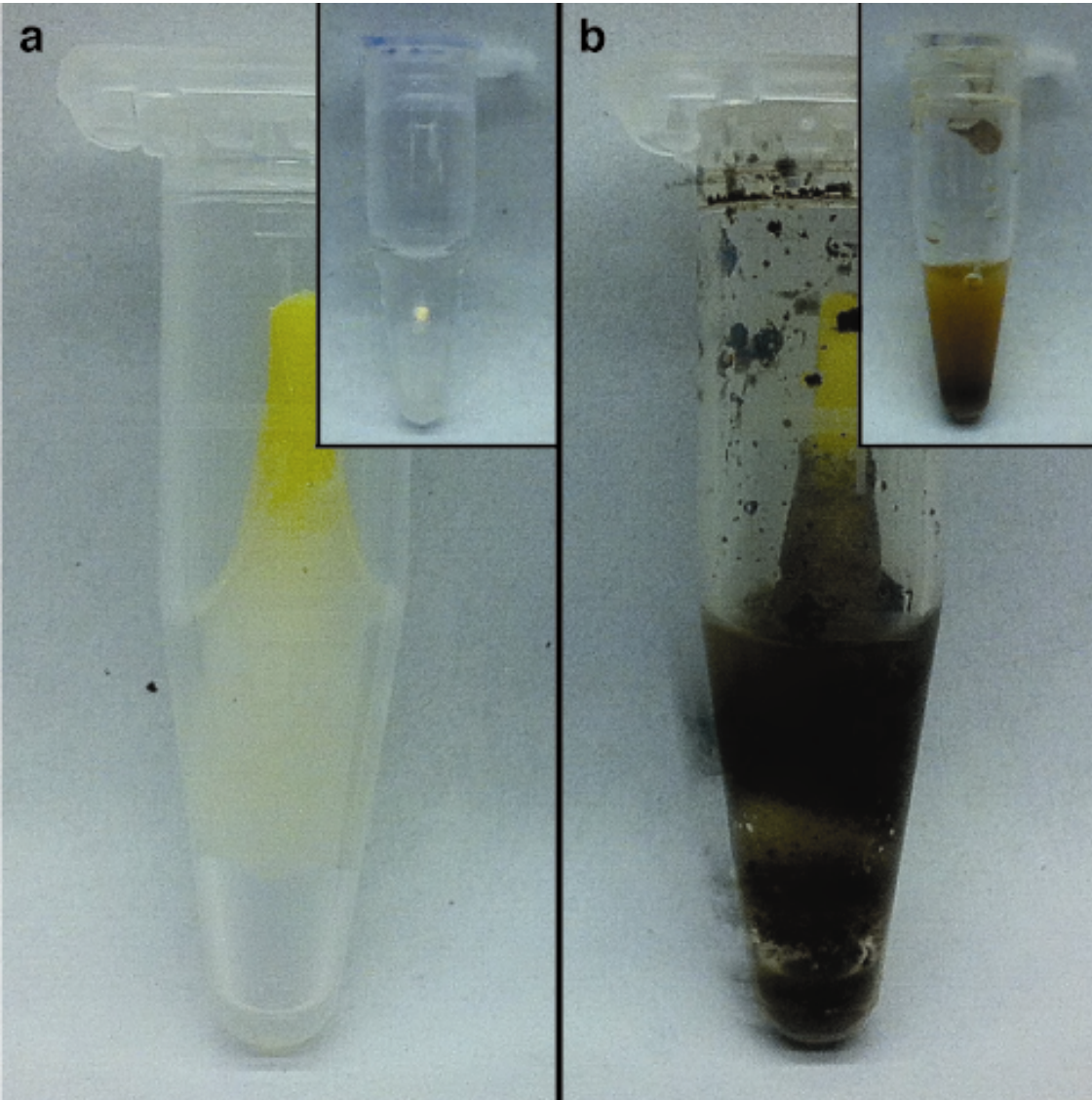
Cell lysis



DNA Extraction

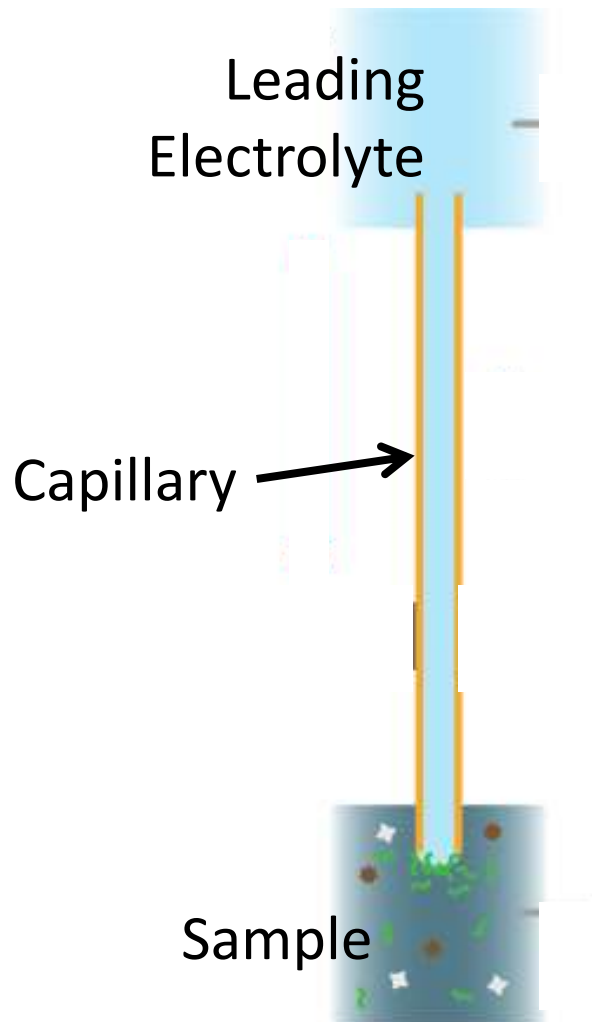


# Dirty Buccal Swab



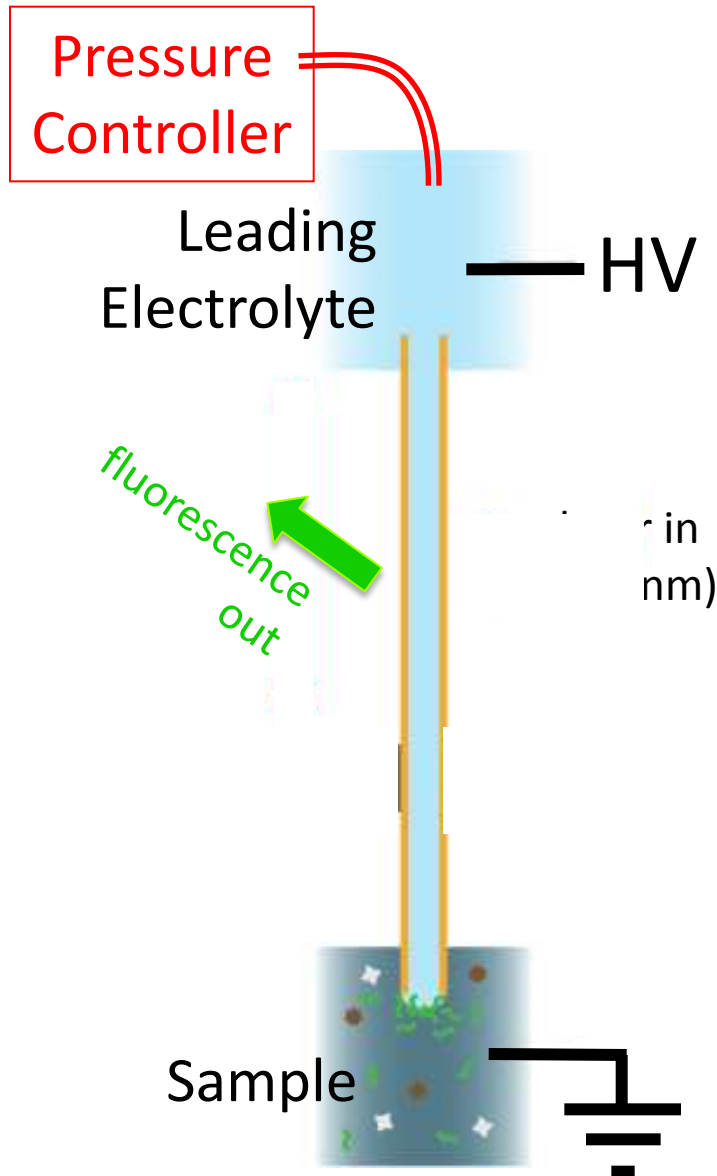
- Buccal Swab plus 50-100 mg soil
  - Soil obtained from under oak tree grove at NIST
- 500 mL lysis buffer solution
  - 485 mL trailing electrolyte (with Triton-X 100)
  - 15 mL Proteinase K
- Incubate at 56 °C for 15 min

# GEITP Hardware



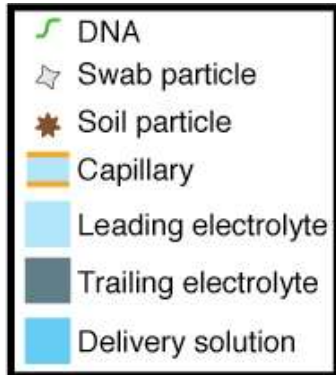
- Leading Electrolyte Reservoir
- Sample
- Capillary

# GEITP Hardware



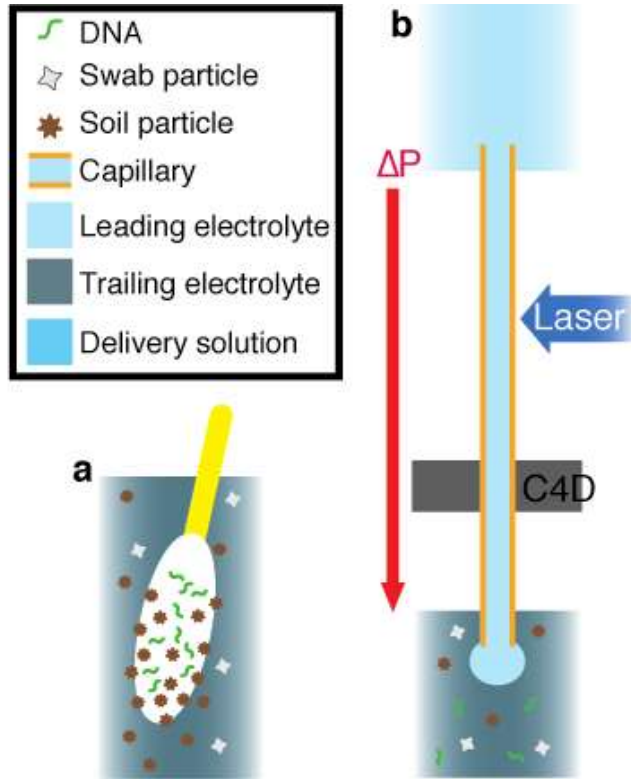
- Leading Electrolyte Reservoir
- Sample
- Capillary
- High Voltage
- Pressure Controller
- Detectors
  - Fluorescence (LIF)
    - SYBR Green Dye
  - Conductivity (C4D)

# GEITP Extraction Method



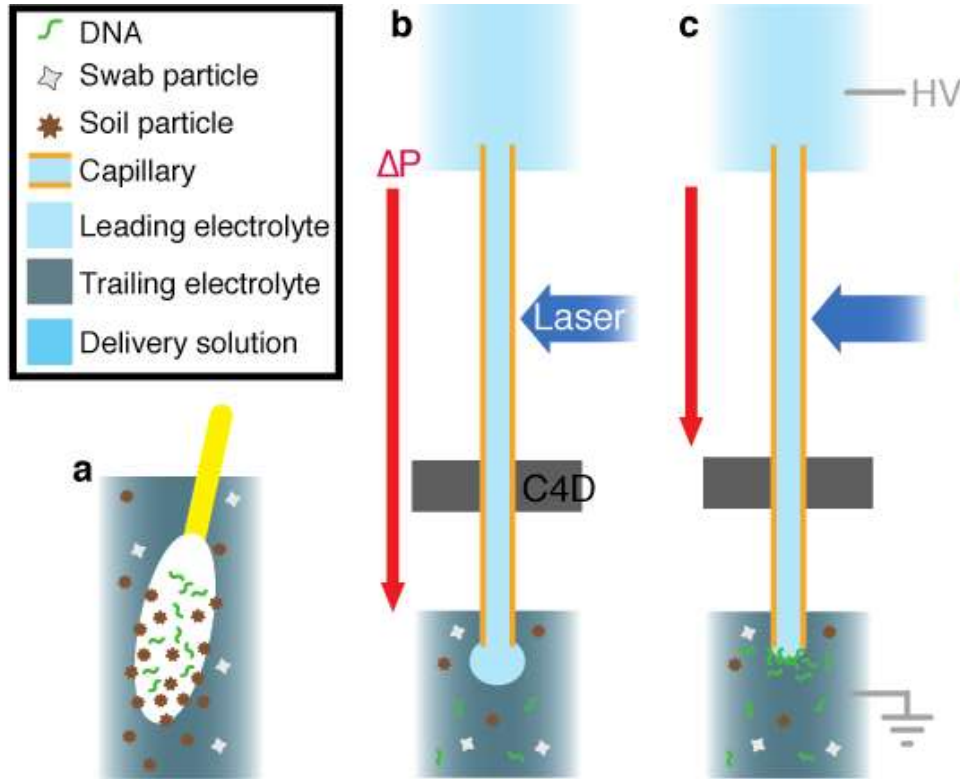
a) Mix buccal swab, dirt, and lysis buffer;  
incubate (15 min, 56 °C)

# GEITP Extraction Method



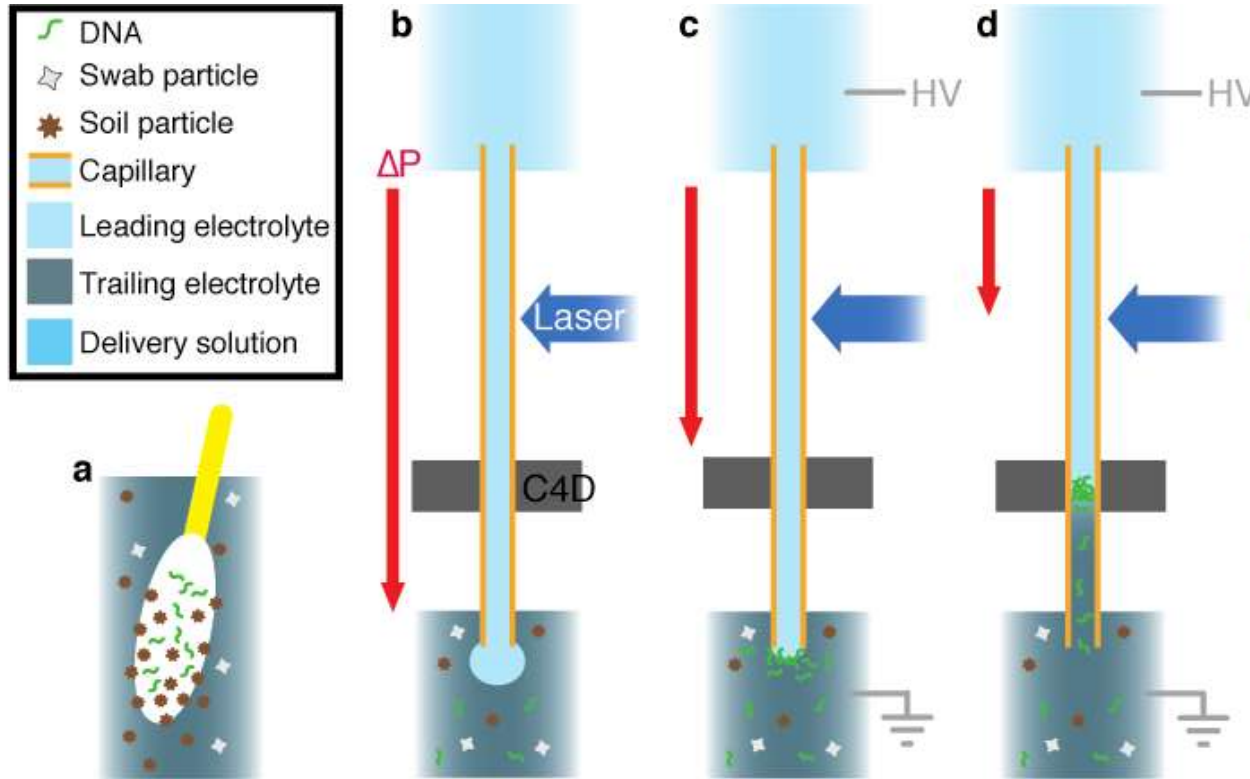
b) Dip capillary into sample; start with positive pressure

# GEITP Extraction Method



c) Turn on High Voltage (2000 V); reduce pressure; focus/extract DNA for 1 minute

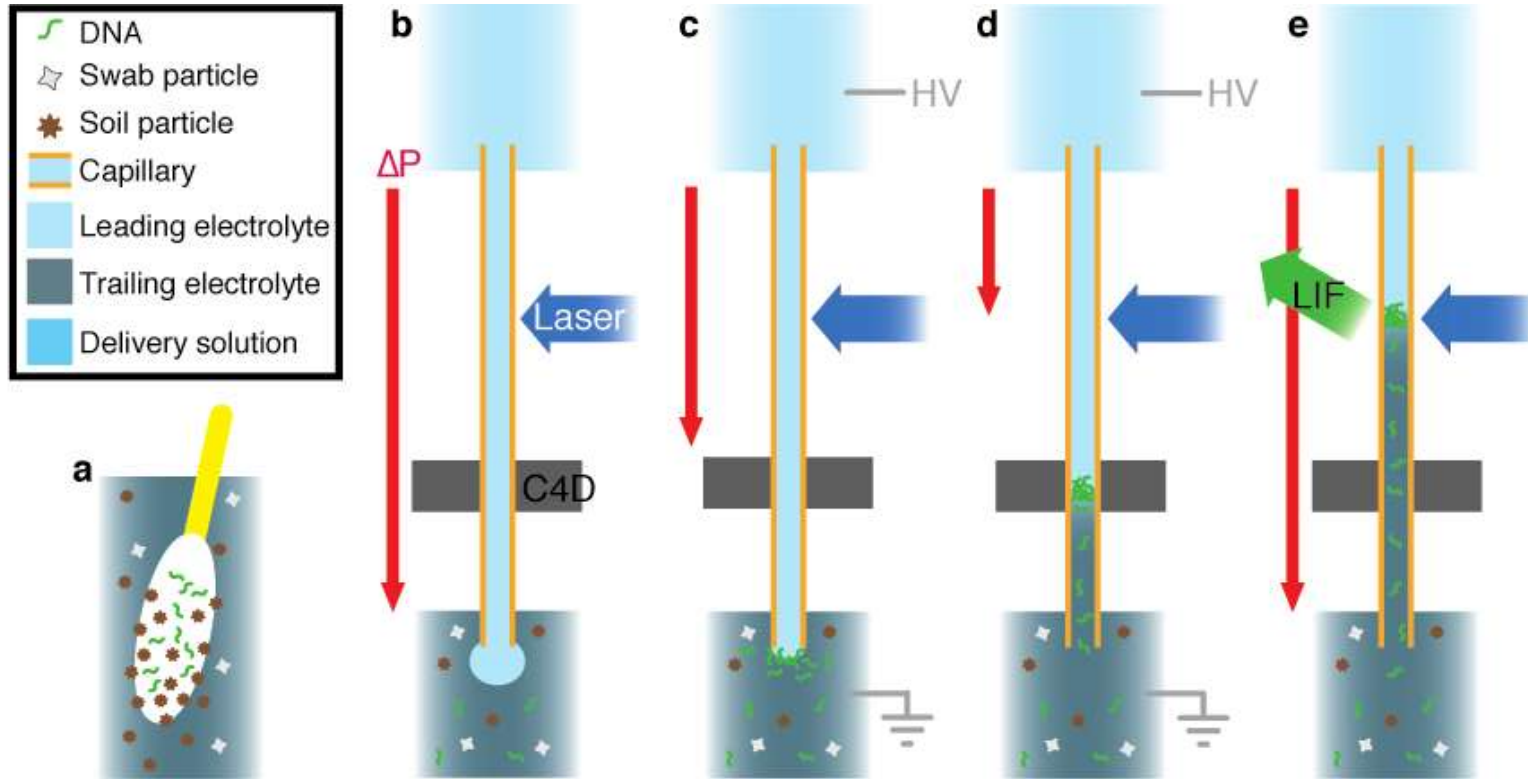
# GEITP Extraction Method



d) Reduce pressure to move DNA through conductivity and LIF detectors

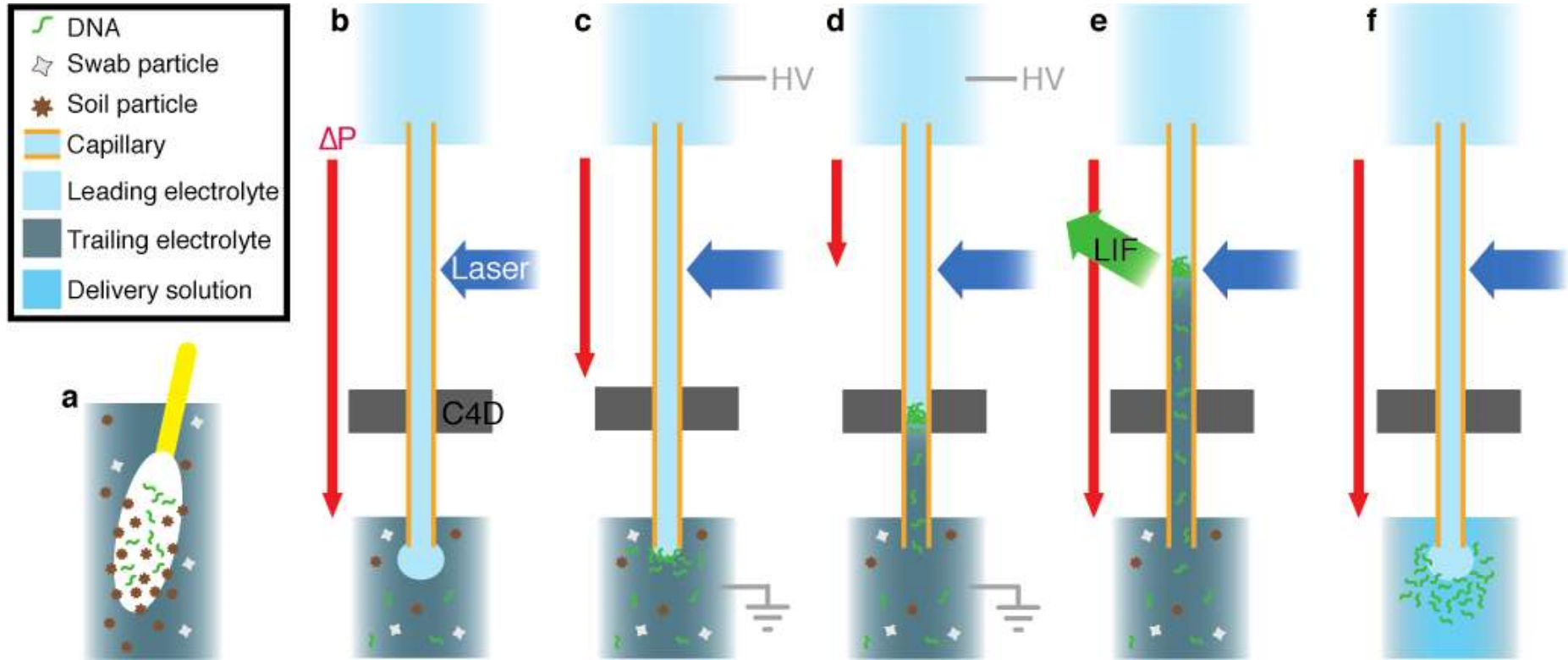


# GEITP Extraction Method



e) Turn off High Voltage; move focused DNA past LIF detector at controlled rate; quantitate DNA

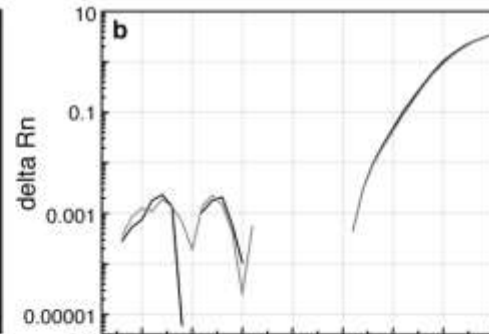
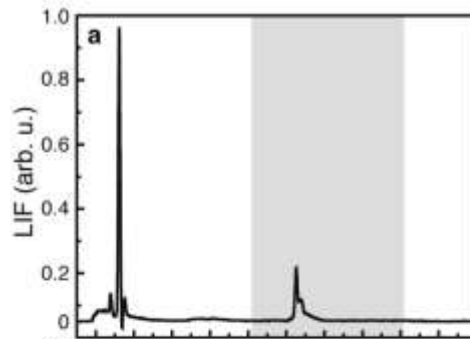
# GEITP Extraction Method



f) Deliver DNA to clean sample tube

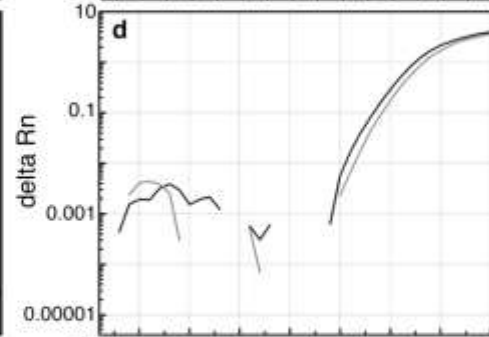
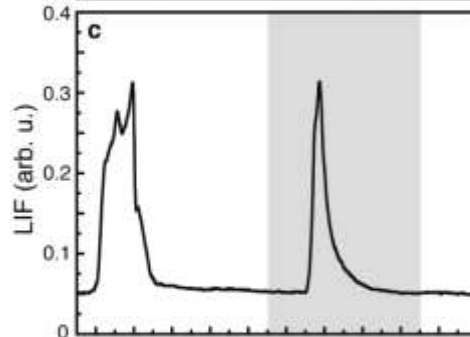
# DNA Quantitation

Standard DNA  
(Human)  
0.5 ng/ $\mu$ L



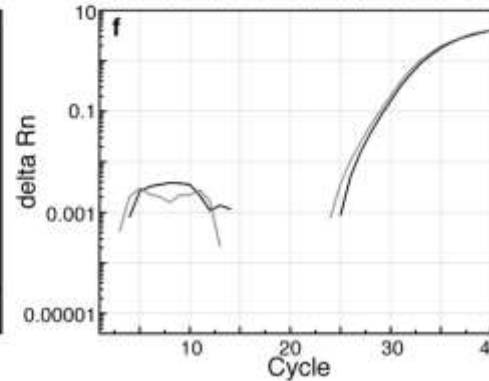
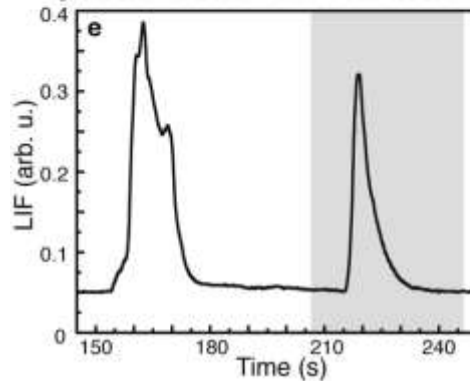
$C_t = 30.7$   
0.024 ng/ $\mu$ L

Clean Buccal  
Swab



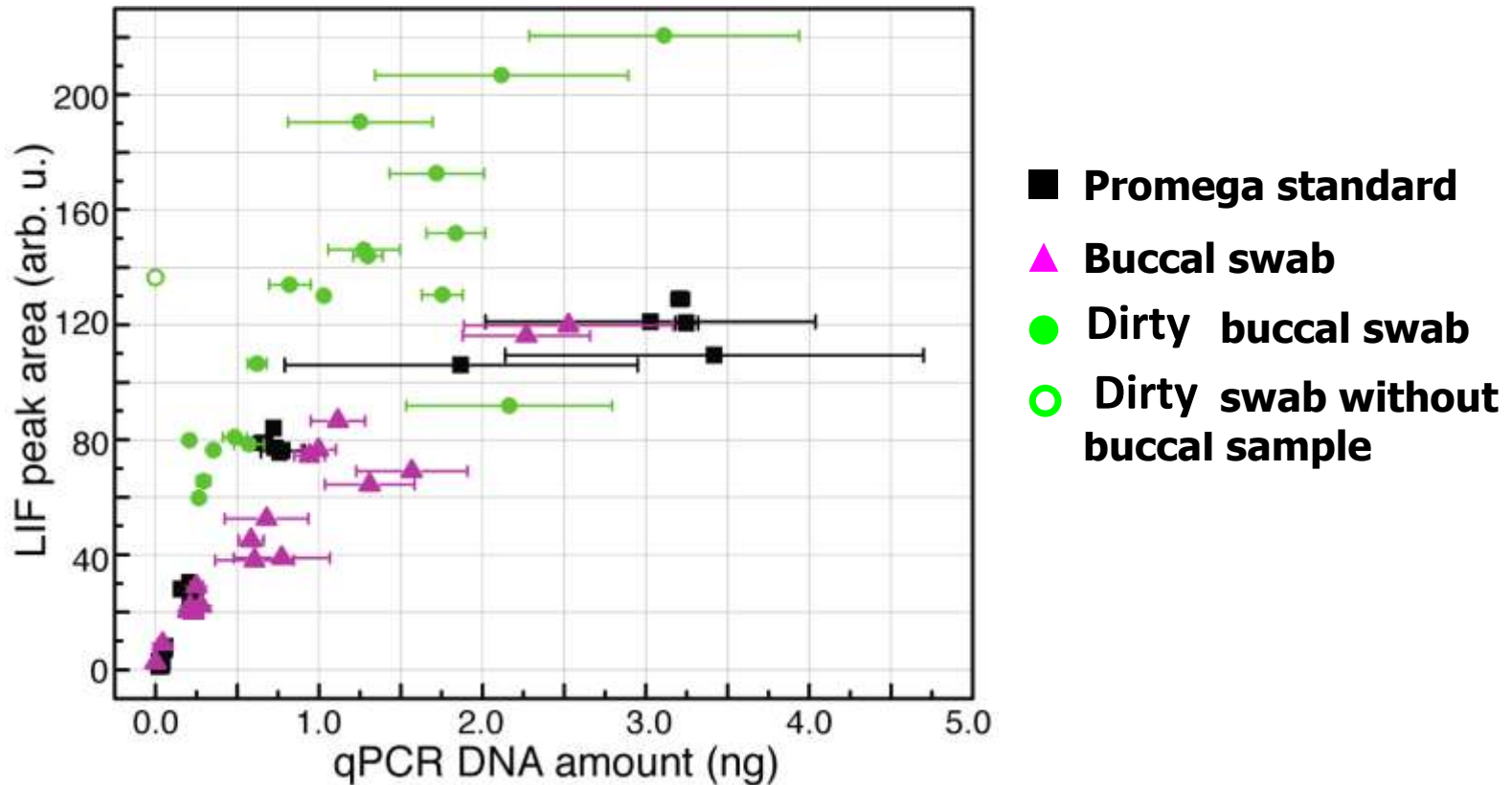
$C_t = 29.8$   
0.216 ng/ $\mu$ L

Dirty Buccal  
Swab



$C_t = 30.2$   
0.157 ng/ $\mu$ L

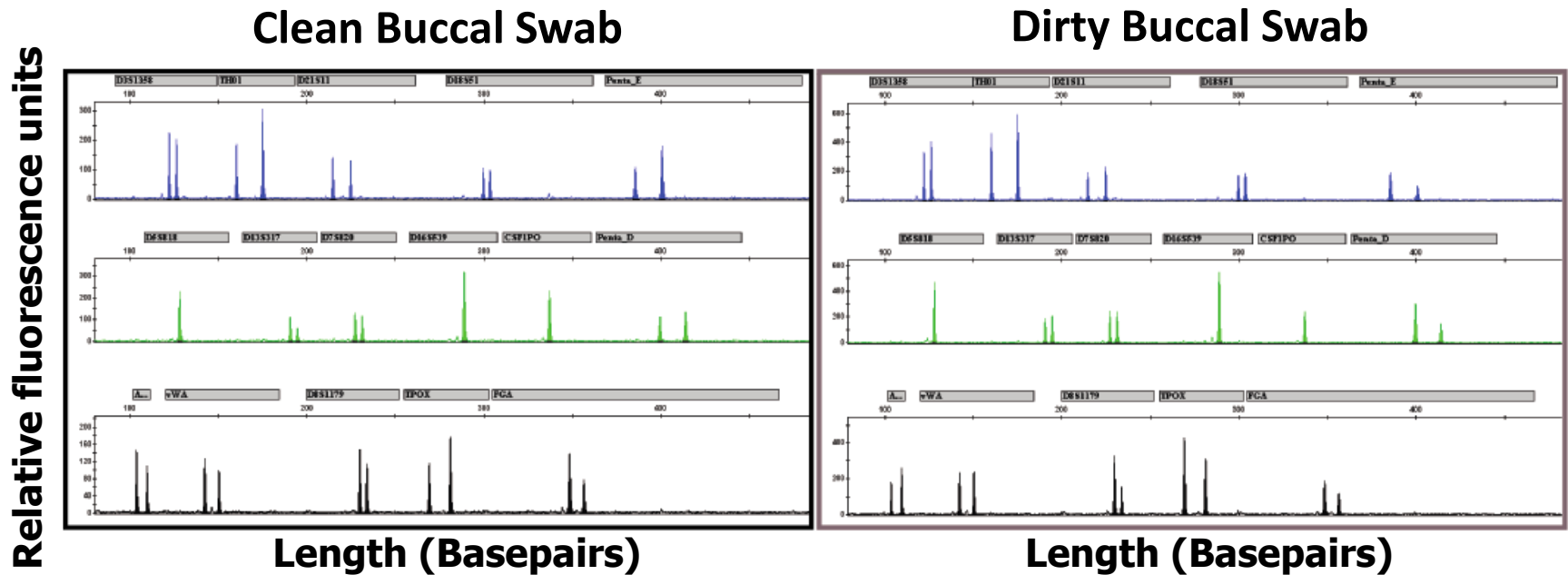
# DNA Quantitation



Data from dirty buccal swabs have larger LIF signals than data from clean samples, for a given qPCR measurement, due to non-human DNA in the soil.

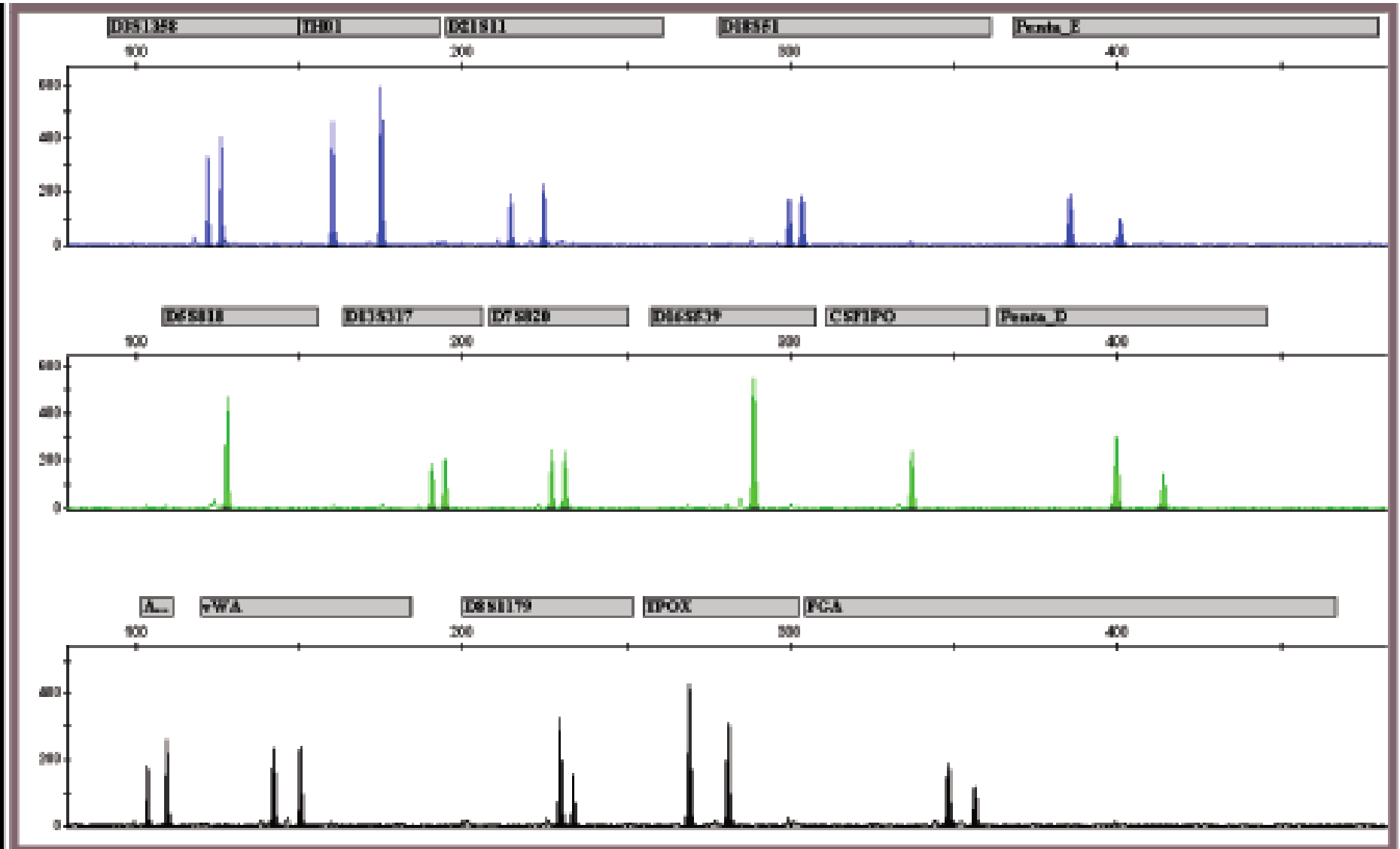
# Human Identification

Delivered DNA was of sufficient amount and purity for qPCR, multiplex PCR, and STR typing for human identification from both clean and dirty buccal swabs.



Comparable STR profiles were obtained from DNA delivered from both clean and dirty buccal swabs from the same person.

# Dirty Swab



# Human Identification

Sample	Concentration in sample	Loci Called	n	Concentration Delivered
DNA Standard	0.5 ng/ $\mu$ L	-mixture-	2	.037 ng/ $\mu$ L
Blank Swab	-	0/16	1	-
Blank Dirty Swab	-	0/16	1	-
Clean Buccal Swabs	1 x	(16 $\pm$ 0)/16	6	0.136 ng/ $\mu$ L
	1/3 x	(14 $\pm$ 2)/16	6	0.057 ng/ $\mu$ L
	1/10 x	(3 + 4)/16	6	0.017 ng/ $\mu$ L
Dirty Buccal Swabs	1 x	(16 $\pm$ 0)/16	6	0.174 ng/ $\mu$ L
	1/3 x	(15 $\pm$ 3)/16	6	0.097 ng/ $\mu$ L
	1/10 x	(10 $\pm$ 4)/16	6	0.031 ng/ $\mu$ L

1x concentrations of both clean and dirty buccal swabs gave STR profiles with 16/16 loci called for every sample.

# Conclusions

- DRAGEN-based DNA extraction and quantitation
  - Time required < 5 minutes
  - DNA concentration and purity good enough for human ID
    - High copy number sample



# What Next?

- Improve Extraction Efficiency
  - Currently ~1%
  - Conventional techniques >20%
- Demonstrate with other sample types
  - Bone
  - Chewing Gum
  - Trace Samples
  - Plant Material

# Acknowledgements

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