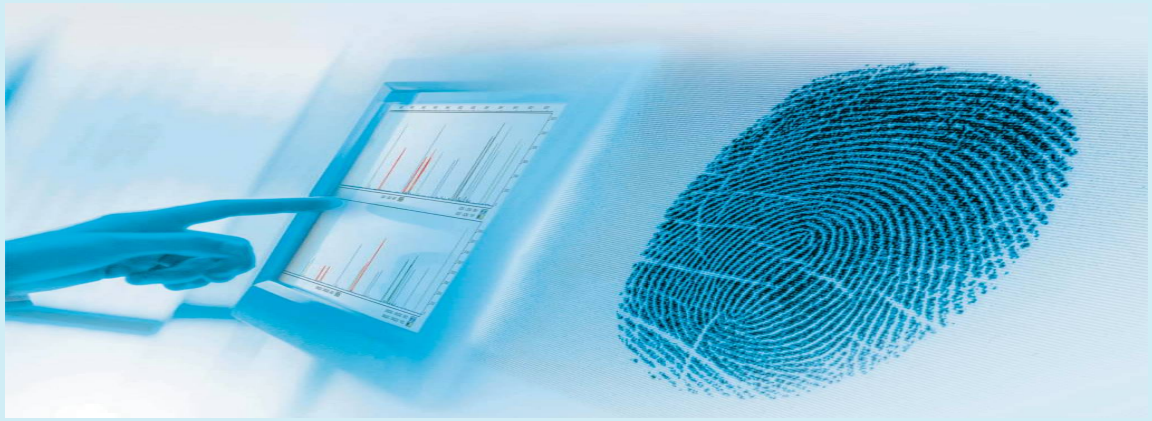

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Introduction to Post-PCR Cleanup

Overview

- **Why post-PCR amplification cleanup?**
- **Enhancing human identity testing**
  - **Introduction to QIAGEN® MinElute® post-PCR cleanup technologies**
  - **MinElute® as a tool to manage throughput and growth**
  - **MinElute® as a tool to enhance DNA forensic capabilities and to aid in special cases**
- **Customer validation data and examples**
  - **Custom fit is key to optimal lab efficiency**
  - **Online support**
- **Automation concepts (tomorrow)**

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Why Post-PCR Amplification Cleanup?

- **Increasing number of touch samples (B & E cases, etc.)**
  - **Increased samples --> managing throughput**
  - **Triaging samples (high/low template DNA)**
  - **Low copy number (LCN) DNA**
    - **< 100 pg DNA**
    - **Few cells, low quantity**
- **Increasing demand/need to process specialized, challenging sample types (bone, hair fragments)**
- **Degraded DNA**

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Why Post-PCR Amplification Cleanup?

- **Various techniques to handle special DNA applications**
  - **LCN PCR (increased cycle number and nested PCR)**
  - **MiniFiler™**
  - **Whole genome amplification (WGA)**
  - **Post-amp cleanup**
  - **Others**
    - **mtDNA**
    - **Y-STRs**

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Why Post-PCR Amplification Cleanup?

- **Possible problems to troubleshoot**
  - **Allele drop-out**
  - **Increased stutter**
  - **Heterozygous peak imbalance**
- **Implications**
  - **(Re)Validation of STR analysis rules**
  - **Specialized personnel or routine task, validation and competency testing**
  - **Ensuring enough reagent blank for the procedure**

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Enhancing Human Identity Testing

Introduction to QIAGEN® Post-PCR Cleanup Technologies

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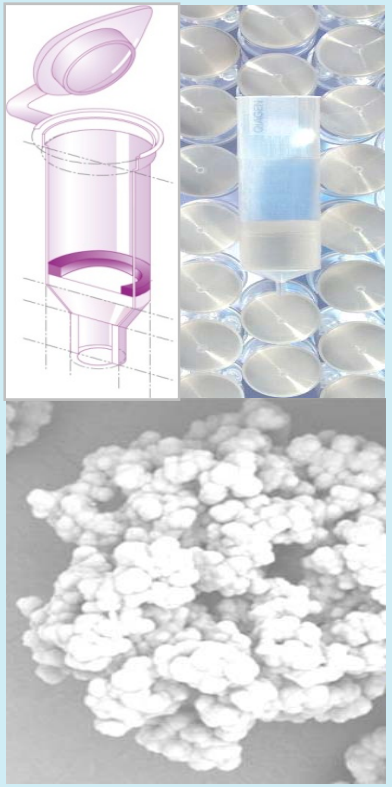
QIAGEN® Kits for Forensic Use

- **Broad range of protocols for forensic casework/ reference samples**
  - **Purification of genomic DNA from…**
    - **Hair**
    - **Cigarette butts**
    - **Body fluids on swabs**
    - **Blood, sperm, and saliva stains on fabric**
    - **Nail clippings and scrapings**
    - **Chewing gum**
    - **Urine, feces**
    - **Tissue biopsy**
    - **Laser-microdissected tissue**
  - **Extraction cleanup of gDNA (P:C)**
  - **Post-PCR amplification cleanup**



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Goals of MinElute® for Forensic Casework

- **Increase sensitivity by increasing alleles detected**
  - **Enhanced low copy number (LCN) interpretations?**
  - **Enhanced mixture interpretations?**
- **Preserve sample by eliminating need for reduced volume PCR (RV-PCR)**
  - **Elimination of increased cycle numbers**
- **Reduce complex and expensive techniques like laser-capture microdissection (LCM)**
- **Automation friendly, automate on the QIAcube®**

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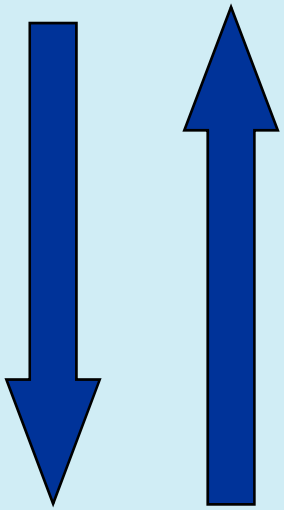
Silica Adsorption Nucleic Acid Purification

Nucleic acid (aqueous)

Adsorption
Chaotropic salt
Alcohol
Lower pH



Elution
No chaotrope
Low salt
Higher pH

Nucleic acid (adsorbed to silica)

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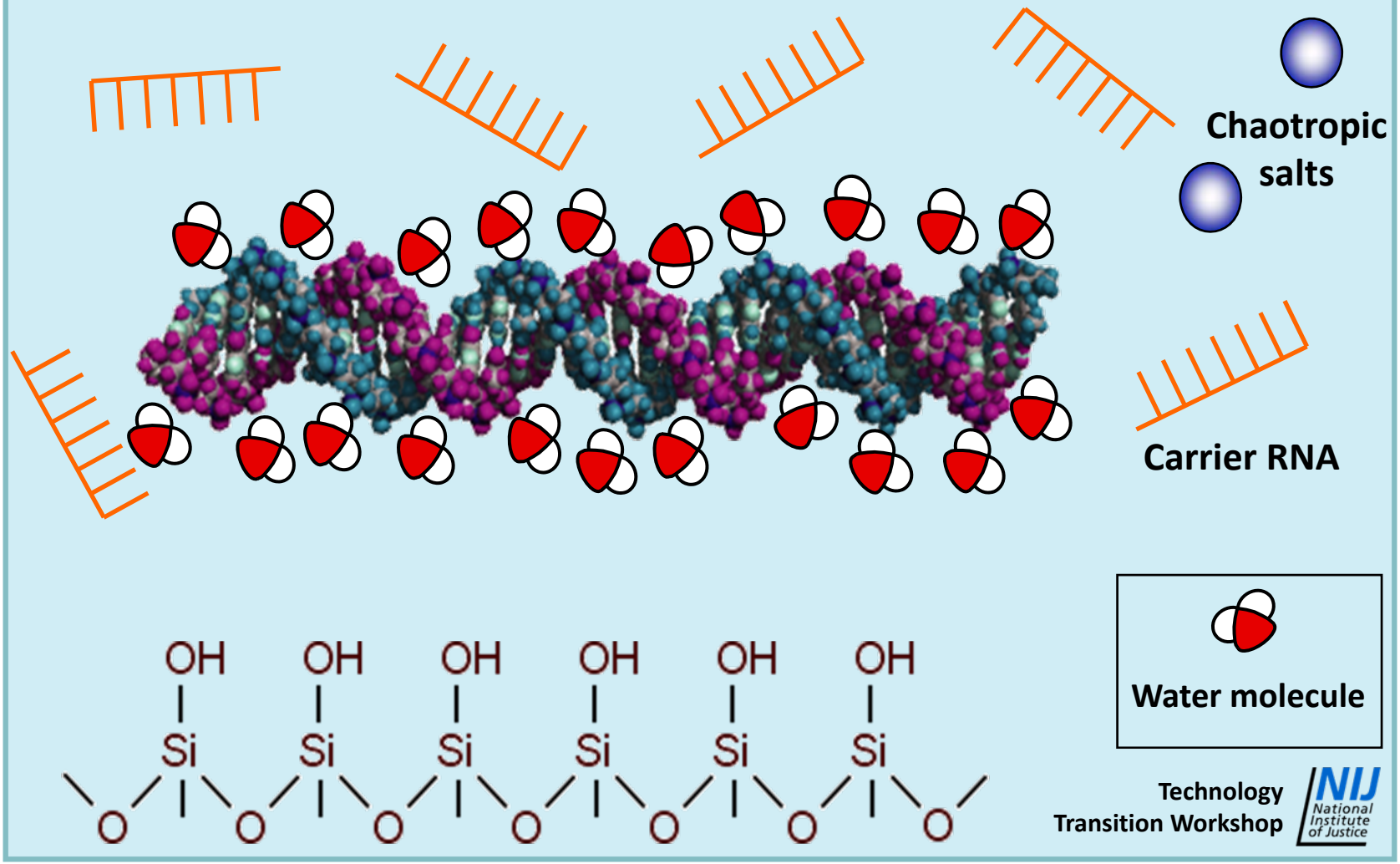
Silica Chemistry – Lysis and Binding



Wash Away Contaminants With Chaotropic Salt Washes

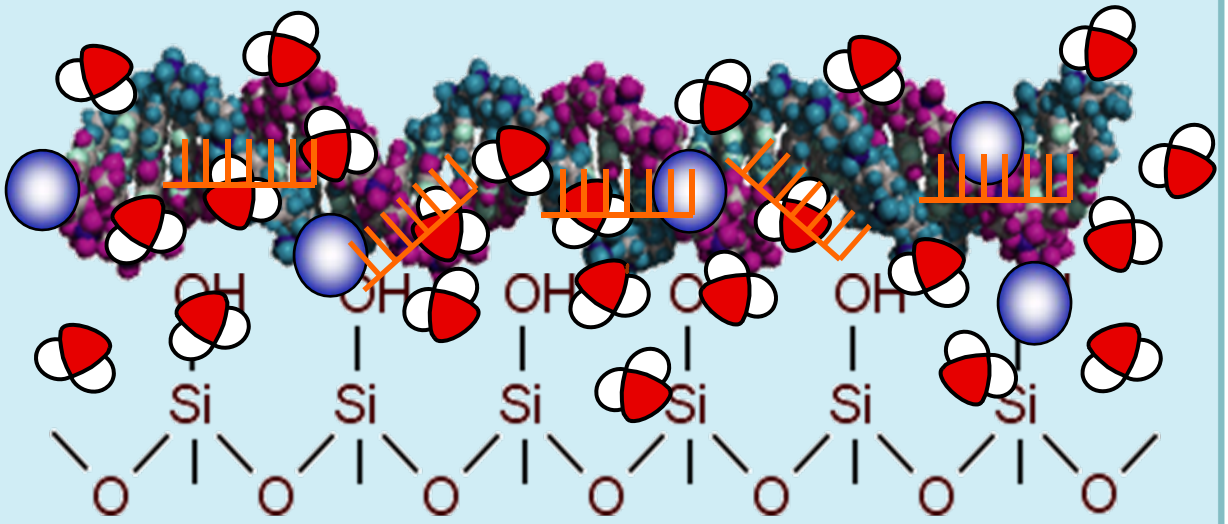
Water molecule

Chaotropic salt

Proteins

Inhibitory contaminants

Carrier RNA



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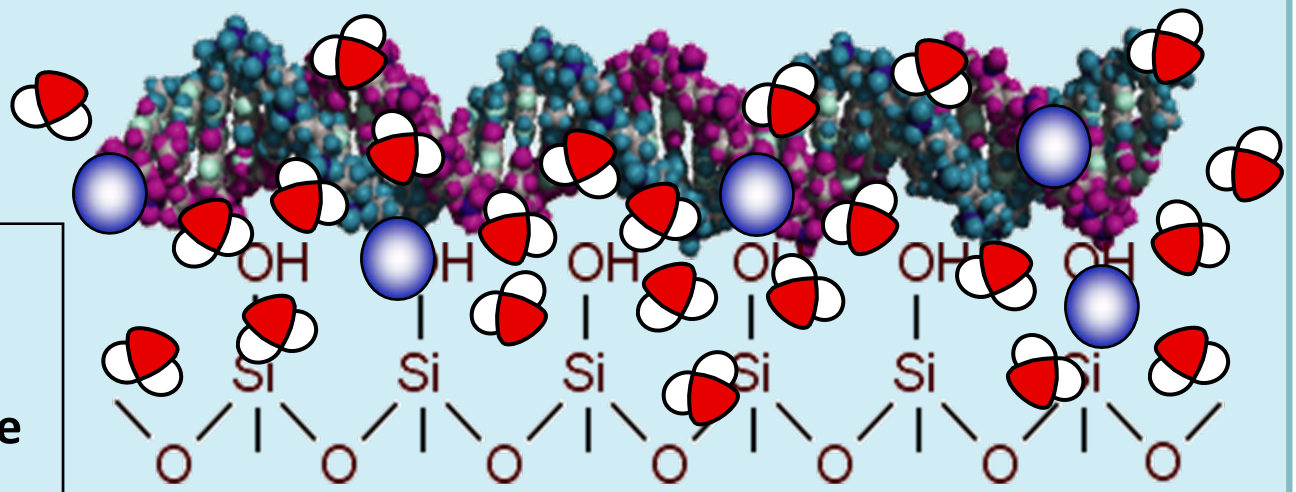
Wash Away Chaotropic Salt With Ethanol



Water molecule

Chaotropic salt

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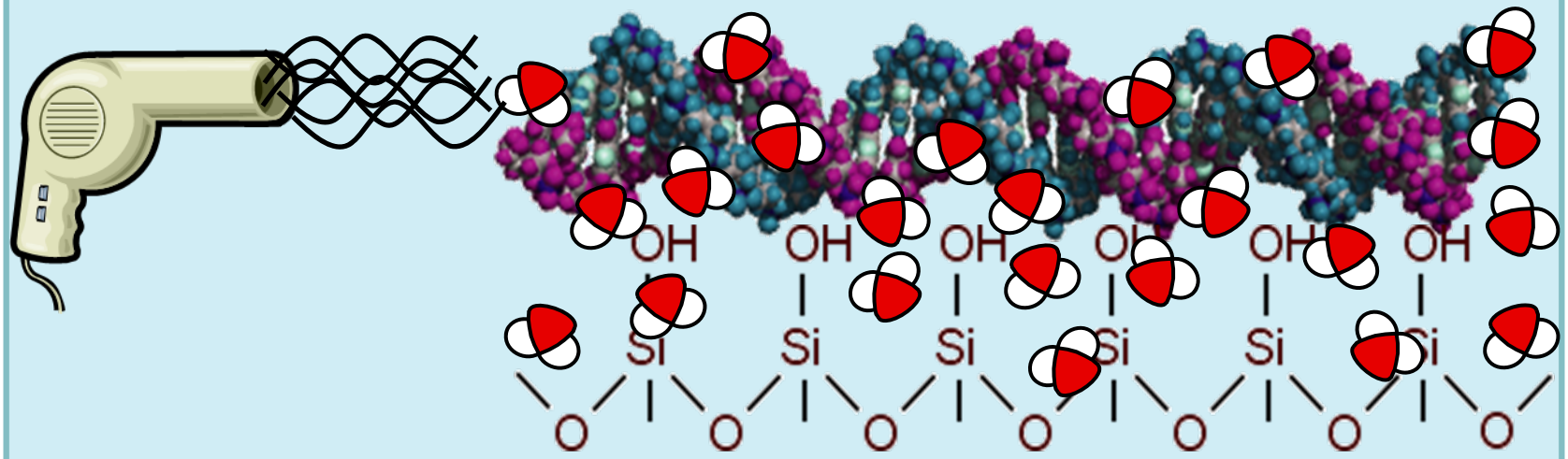
Dry the Silica (Evaporate any Ethanol)



Water molecule

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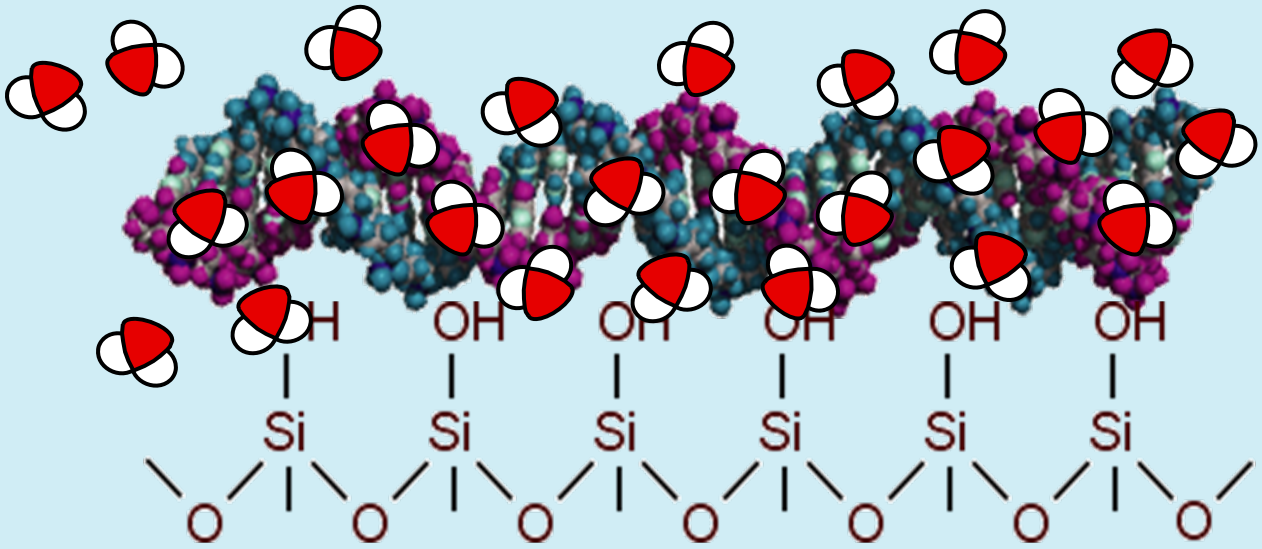
Elute in Water or Low Salt Buffer, pH 7-9



Soluble DNA

Silica (membrane or magnetic beads)

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JOURNAL OF COLLOID AND INTERFACE SCIENCE 181, 635–644 (1996)
ARTICLE NO. 0421

Driving Forces for DNA Adsorption to Silica in Perchlorate Solutions

KATHRYN A. MELZAK, CHRIS S. SHERWOOD, ROBIN F. B. TURNER,* AND CHARLES A. HAYNES†,[1]

*Biotechnology Laboratory, *Department of Electrical Engineering, and †Department of Chemical Engineering, 237 Wesbrook Building, 6174 University Boulevard, The University of British Columbia, Vancouver, British Columbia V6T 1Z3, Canada*

- **Shielded intermolecular electrostatic forces**
- **Dehydration of the DNA and silica surfaces**
- **Intermolecular hydrogen bond formation in the DNA-silica contact layer**

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MinElute® Kit Contents

Kit Contents

MinElute PCR Purification Kits Catalog no.	(50) 28004	(250) 28006
MinElute Spin Columns	50	250
Buffer PB*	30 ml	150 ml
Buffer PE (concentrate)	2 x 6 ml	55 ml
Buffer EB	15 ml	15 ml
pH Indicator	800 µl	800 µl
Collection Tubes (2 ml)	50	250
Loading Dye	110 µl	550 µl
Handbook	1	1

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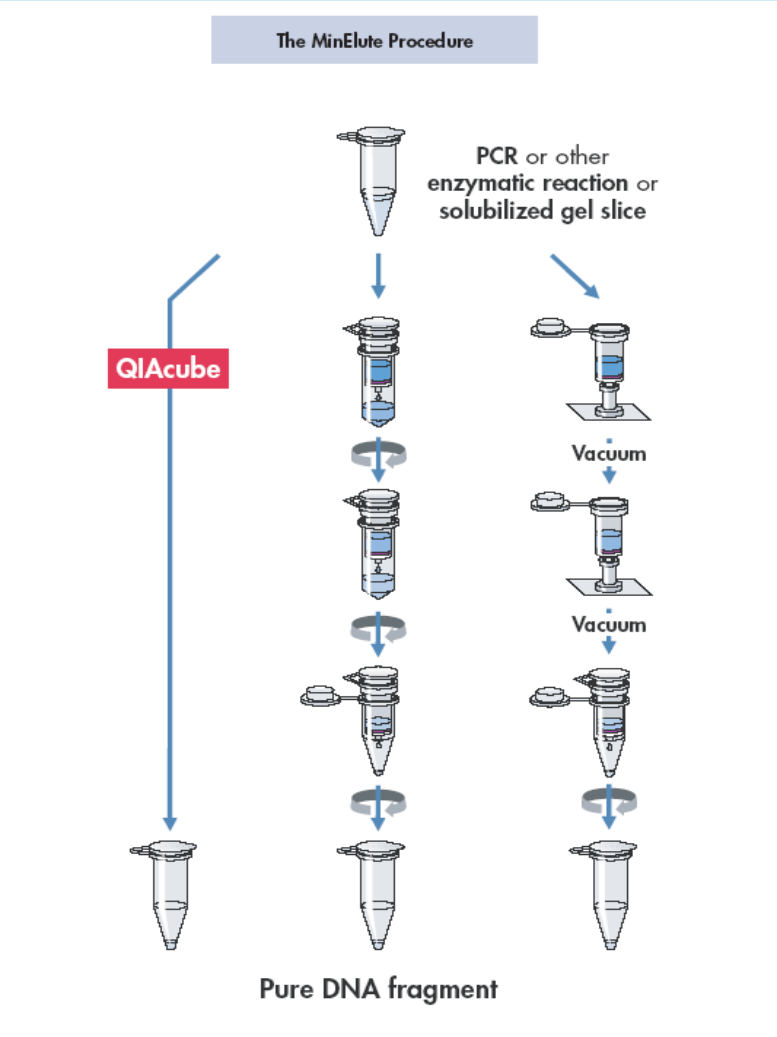
The MinElute® Procedure



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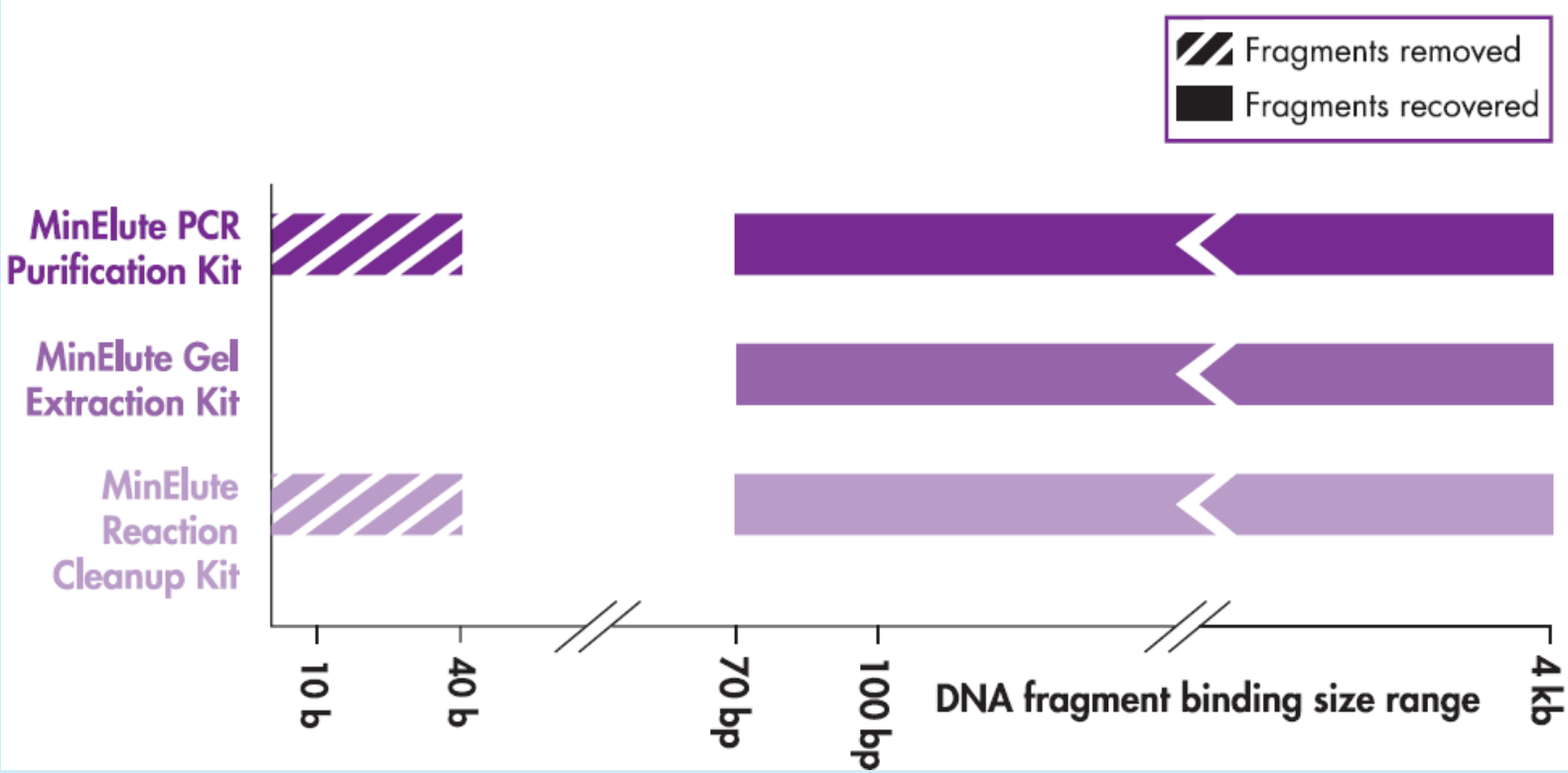
Comparison of QIAGEN® MinElute® Kits



- **Purification of > 70 bp amp products**
- **Primers up to 40 nt long are removed**

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MinElute® PCR Purification Kit Specifications

Product Specifications

	MinElute PCR Purification Kit
Maximum binding capacity:	5 µg
Recovery of DNA:	80% (70 bp – 4kb)
Maximum weight of gel slice:	—
Elution volume:	10 µl
Volume of eluate:	9 µl
Capacity of column reservoir:	800 µl
Recovered: dsDNA	70 bp – 4 kb
Removed: <40mers	**YES**

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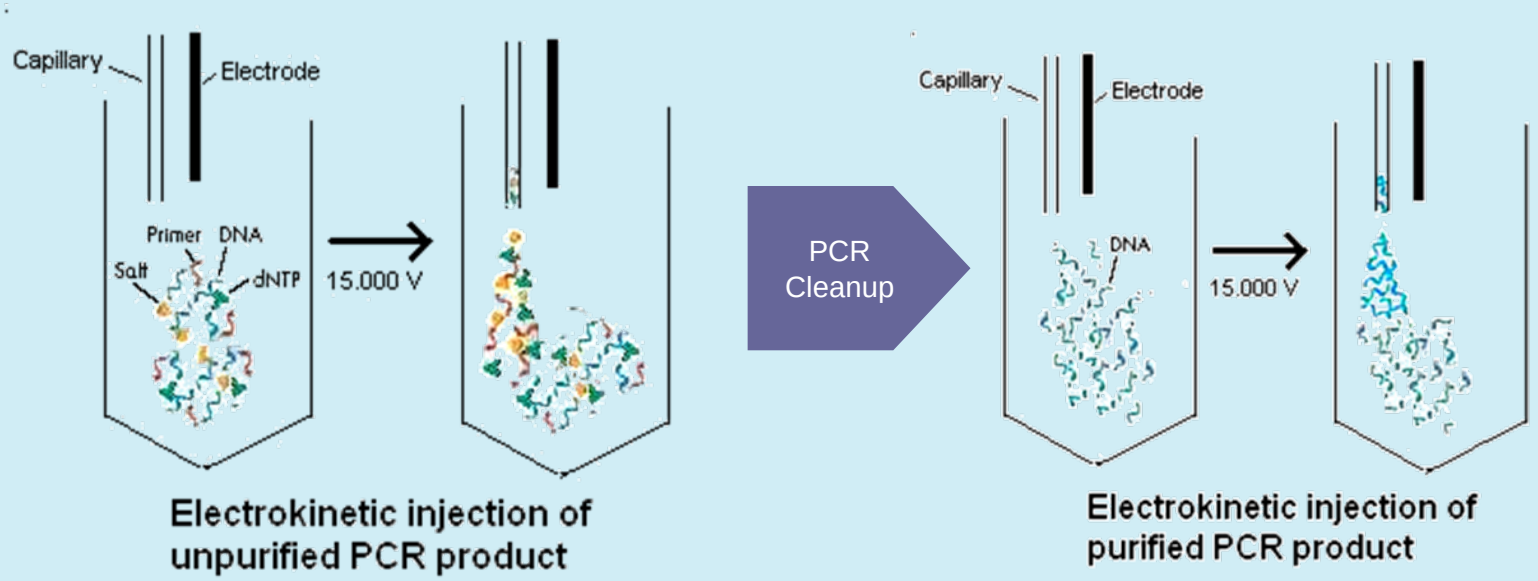
Mechanism for Post-PCR Purification Enhancement of Trace DNA



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Evaluation of Post-PCR Cleanup to Enable LCN Analysis at 28 Amplification Cycles

- **Smith, Pamela J. and Ballantyne, Jack. "Simplified Low-Copy-Number DNA Analysis by Post-PCR Purification." Journal of Forensic Sciences 52 (4) (July 2007): 820-829.**
  - **Smith and Ballantyne demonstrated for the first time that MinElute® post-PCR purification can substantially increase sensitivity, performance**
  - **Simplified LCN DNA analysis where < 100 pg DNA available**
  - **Commonly used techniques to increase sensitivity:**
    - **Increase PCR cycles: 28 to 30-34**
    - **Nested PCR**

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Evaluation of Post-PCR Cleanup to Enable LCN Analysis at 28 Amplification Cycles

- **Smith, Pamela J. and Ballantyne, Jack. "Simplified Low-Copy-Number DNA Analysis by Post-PCR Purification." Journal of Forensic Sciences 52 (4) (July 2007): 820-829.**
  - **Investigate possible issues:**
    - **Allele drop-out, drop-in**
    - **Higher stutter peaks**
    - **Contamination**
  - **Dilution of template DNA from 5 to 600 pg from blood spots on both cotton cloth and FTA cards, analysis in quadruplicates**

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Smith and Ballantyne Study: Comparison of Different Cleanup Procedures

	Signal x Fold	Artifacts (Off-ladder Alleles)	Process Time
Microcon® 50	3 to 6	**Yes, one locus**	**55 minutes** (4 wash steps)
Montage® PCR	6 to 8	**Yes, one locus**	**65 minutes** (4 wash steps)
ExoSAP-IT®	**None**	**Strong**	30 minutes (mostly incubation)
MinElute®	4 to 6	None	6 minutes (2 wash steps)

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Smith and Ballantyne Study: Reliable STR Profiles From 20 pg Template Using MinElute®

Same sample analyzed with and without post-amplification purification

STR profile from 20 pg – <u>no post-PCR cleanup</u>

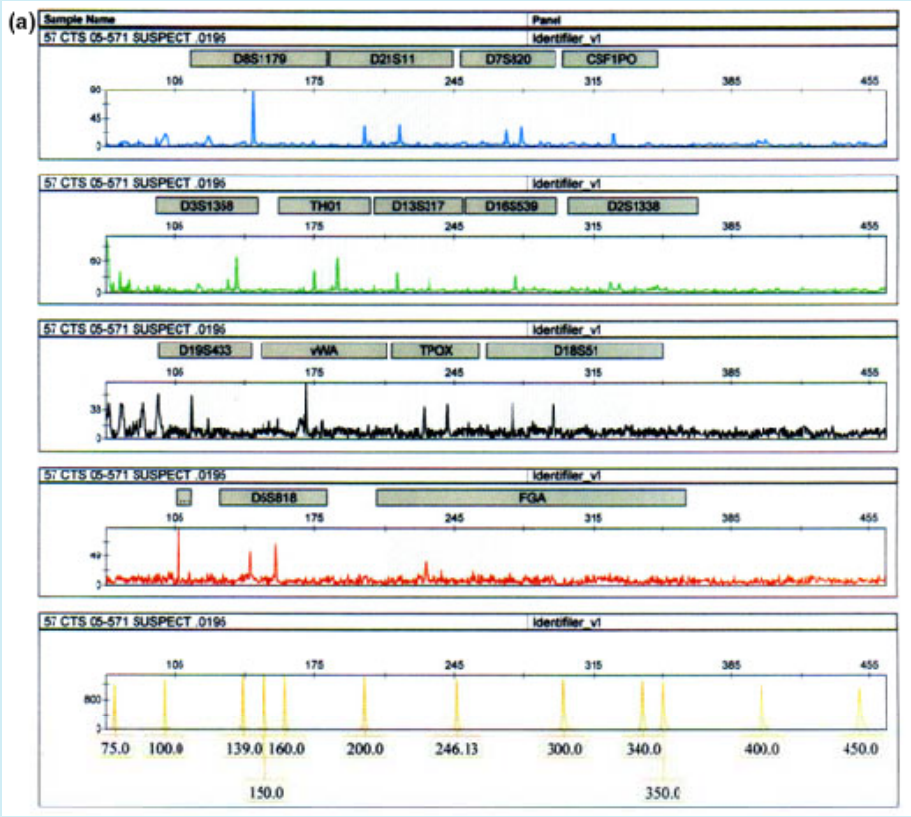

Image courtesy of Smith and Ballantyne

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Smith and Ballantyne Study: Reliable STR Profiles From 20 pg Template Using MinElute®

Same sample analyzed with and without post-amplification purification

Same sample after post-PCR purification and injection of entire concentrated product

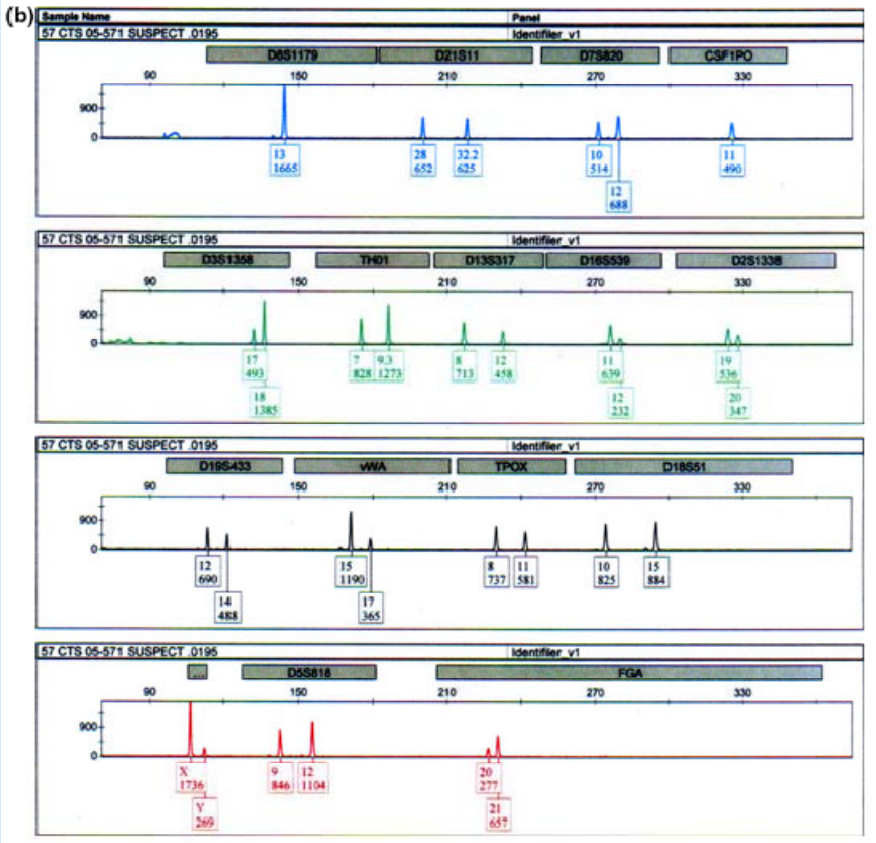

Image courtesy of Smith and Ballantyne

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Smith and Ballantype Study: Evaluation of Post-PCR Purification LCN Analysis

- **Low copy number DNA: 5 to 600 pg evaluated**
- **Approach:**
  - **Evaluated effect of MinElute® Post-PCR Purification Kit**
- **Results:**
  - **With MinElute®-based purification full profiles were consistently obtained from 20 pg with 28 PCR cycles**
  - **Significant data obtained down to 5 pg**
  - **Increase in signal intensity 4 to 6-fold (4-fold on average) at constant DNA amount used for injection**
  - **Up to 19-fold signal increase with increased DNA injection amount**
- **Conclusion:**
  - **LCN DNA analysis can be simplified by using post-PCR purification**

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Supporting Journal Article

- **Gill P., Whitaker J., Flaxman C., Brown N., and Buckleton J. "An Investigation of the Rigor of Interpretation Rules for STRs Derived from Less than 100 pg of DNA." *Forensic Science International* 112 (1) (July 24, 2000): 17-40.**
- **Contact Information for Peter Gill:**
  - **Forensic Science Service, Priory House, Gooch Street North, B56QQ, Birmingham, UK**
  - **dnapgill@compuserve.com**

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Post-amplification Cleanup using the MinElute® Post-PCR Purification Kit:

A Presentation of Two More Papers and Customer Examples

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Summary of Findings

- **Post-PCR cleanup can greatly enhance the sensitivity of STR profiling for low copy number casework applications**
- **Improving sensitivity in trace DNA casework applications by post-PCR purification is increasingly receiving attention**
- **Success rates using standard forensic methods to obtain intelligible profiles from trace DNA samples usually range around only 30-50%**
- **Efforts for method enhancements usually focus on the pre-analytical or pre-PCR process of the analysis**
- **However, there can also be tremendous value in post-PCR method optimization**
- **Two publications in leading peer-reviewed journals, in addition to the Smith and Ballantyne paper previously discussed, demonstrate the value of forensic post-PCR purification**

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Study 1: Forster, et al.

- **Forster, L., et al. "Direct Comparison of Post-28-cycle PCR Purification and Modified Capillary Electrophoresis Methods with the 34-cycle "Low Copy Number" (LCN) Method for Analysis of Trace Forensic DNA Samples." *Forensic Science International - Genetics* 2 (4) (September 2008): 318 – 328.**
- **Forster, et al. challenged increased cycle PCR as a strategy for trace forensic DNA, proposing to combine standard PCR with MinElute® post-PCR purification instead**
- **Direct comparison of post-28-cycle PCR purification and modified capillary electrophoresis methods with the 34-cycle "low copy number" (LCN) method for analysis of trace forensic DNA samples**

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Study 1: Forster, et al.

- **Results:**
  - **The combination of 28-cycle PCR with post-PCR purification using the MinElute® PCR Purification Kit:**
    - **Provided equivalent results in STR analysis**
    - **Reduced sample consumption**
    - **Reduced stutter peak ratios**
    - **Reduced the frequency of non-donor allele peaks**
  - **"This study demonstrates that gains in profile quality at least equivalent to those seen in 34-cycle PCR can be made by concentrating and increasing the loading of product from a 28-cycle PCR."**

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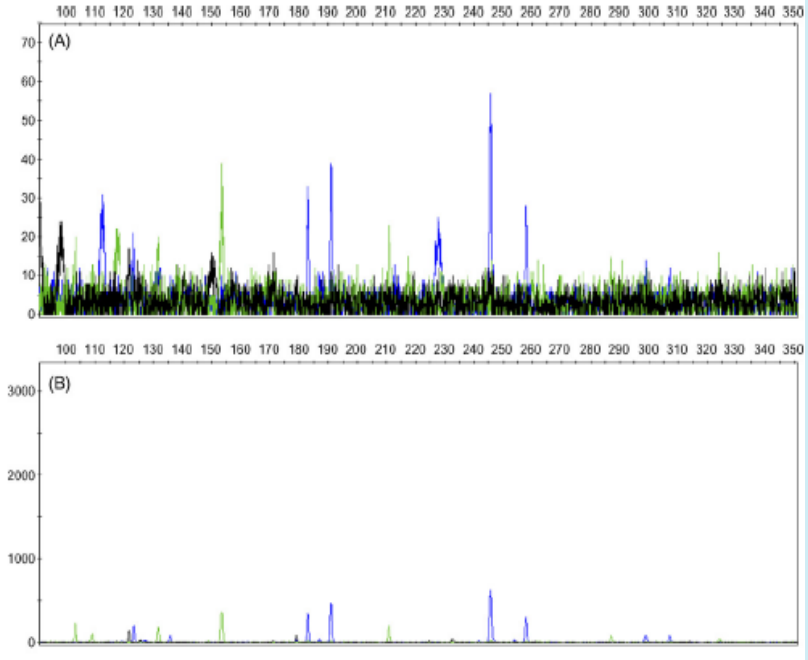
Study 1: Forster, et al.: 50 µl SGM+



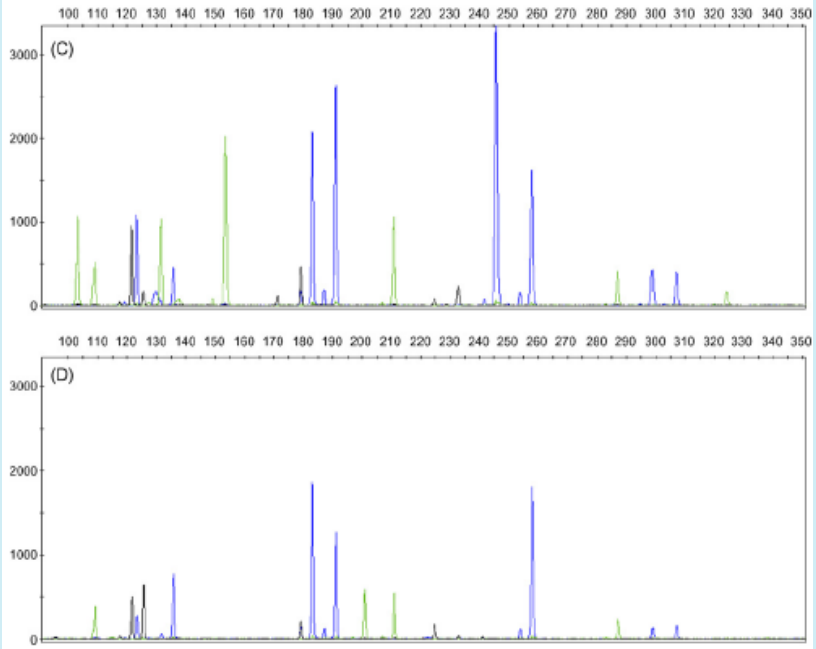


Table 1
Shows a summary of the differences between conditions A, B, C and D

Condition	Number of PCR cycles	Volume of sample loaded onto 3130 (µl)	Post-PCR clean-up	Injection time (s)	Injection voltage (kV)
A	28	1	None	10	3
B	28	2	MinElute	10	3
C	28	2	MinElute	30	4
D	34	1	None	10	3

Images courtesy of Forster, et al.

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Study 1: Forster, et al.

- **Summary:**
  - **Trace DNA analysis utilizing standard 28-cycle PCR combined with MinElute® post-PCR purification achieves equal or better results than increased cycle number procedures with 34-cycle PCR**
  - **Common problems of increased cycle PCR, such as elevated stutter peak ratios and increased non-donor allele peaks frequencies, can be avoided**

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Study 2: Mayntz-Press, et al.

- **Mayntz-Press, K.A., et al. "Y-STR Profiling in Extended Interval (+/- 3 days) Postcoital Cervicovaginal Samples." Journal of Forensic Sciences 53 (2) (March 2008): 342–348.**
- **Mayntz-Press, et al. applied MinElute®-based post-PCR purification after differential extraction in sexual assault analysis**

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Study 2: Mayntz-Press, et al.: Results

- **MinElute® purification not only increased peak heights with partial profiles, but also the appearance of loci or allele call rates**
- **Profiles with results at 10 to 17 loci were obtained from 6-day postcoital samples that, prior to purification, yielded only 6 to 11 locus profiles**
- **The incorporation of a simple post-PCR purification process significantly increased the ability to obtain Y-STR profiles, particularly from 5- to 6-day postcoital samples**
- **Post-PCR purification increases the sensitivity of allele detection resulting in:**
  - **Increased peak heights with partial profiles**
  - **The appearance of loci that otherwise were not apparent in the corresponding non-MinElute® purified samples**

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Study 2: Mayntz-Press, et al.: Study Summary

- **The post-PCR purification procedure significantly increased the ability to obtain Y-STR profiles**
- **Post-PCR purification increased the sensitivity of allele detection in sexual assault sample analysis**

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Study 2: Mayntz-Press, et al.: Continuation at AZ Dept. of Public Safety

- **Milne, S.C. and Mayntz-Press, K.A. "Strategies for Increasing Allele Calls in Forensic Casework Using PCR Enhancements and Commercial Post-amplification Cleanup Systems." (2008) Poster at the 19th International Symposium on Human Identification**

Image courtesy of Milne and Mayntz-Press

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Study 2: Mayntz-Press, et al.: Continuation at AZ Dept. of Public Safety Results Summary

- **MinElute® PCR Purification Kit results:**
  - **Increased the number of loci "called"**
  - **Showed better sensitivity than other methods compared**
  - **Useful tool for difficult casework samples**
  - **Cleaner baseline noted**

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Study 3: North Louisiana Crime Lab

- **Study Parameters:**
  - **Analysis of pulverized bone powder amplified with the PowerPlex® 16 System kit**
  - **Capillary electrophoresis using the Applied Biosystems 3130 Genetic Analyzer**
  - **Analysis at 150 RFU using GeneMapper® ID (v.3.2) software**
  - **The cleanup procedure was performed as described in the QIAGEN® MinElute® Handbook (03/2008) with a 10 µL elution volume**

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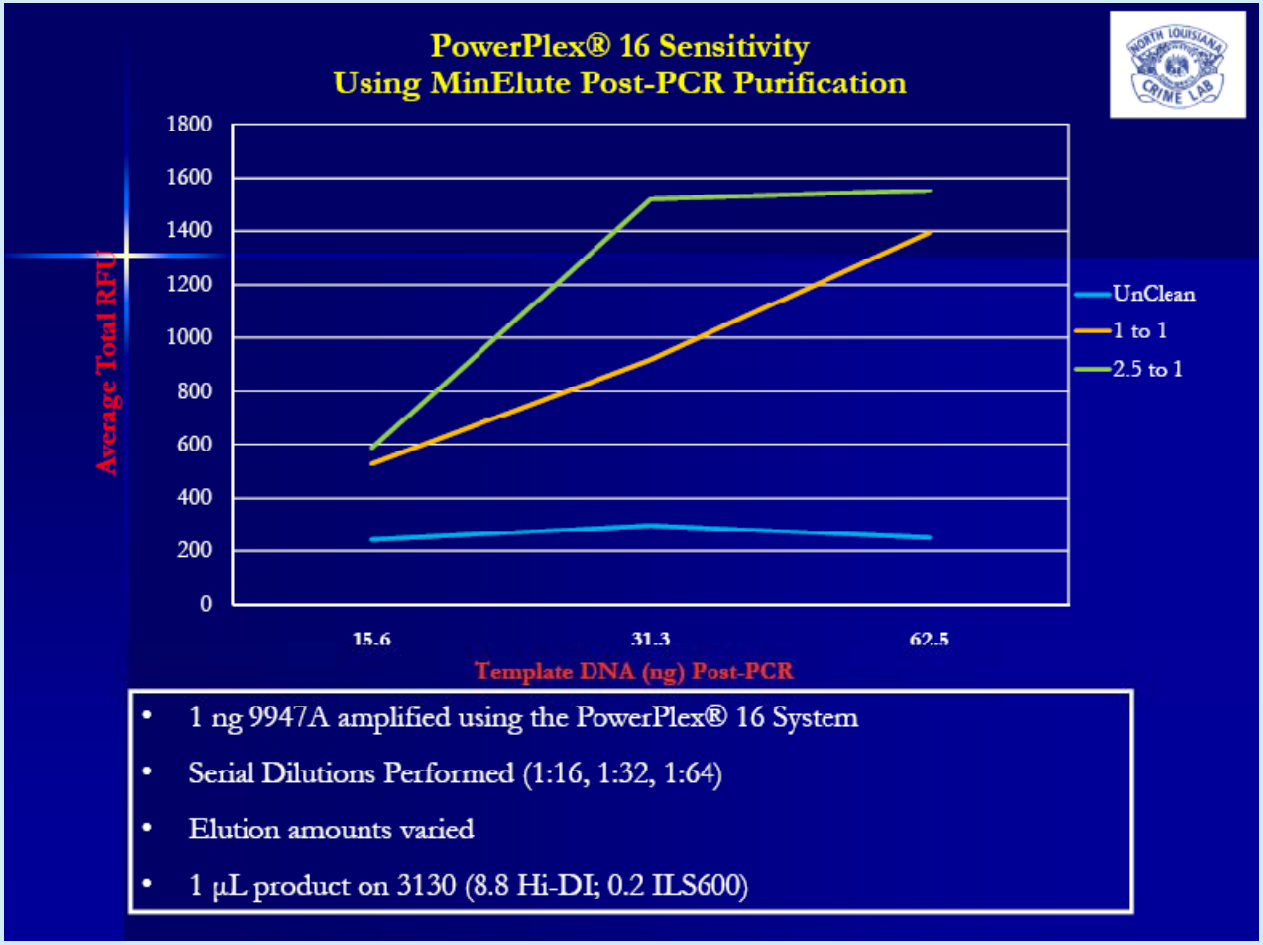
Study 3: North Louisiana Crime Lab



All data generated at the NLCL-Shreveport Headquarters Laboratory

Image courtesy of Jessica Esparza, Ph.D.

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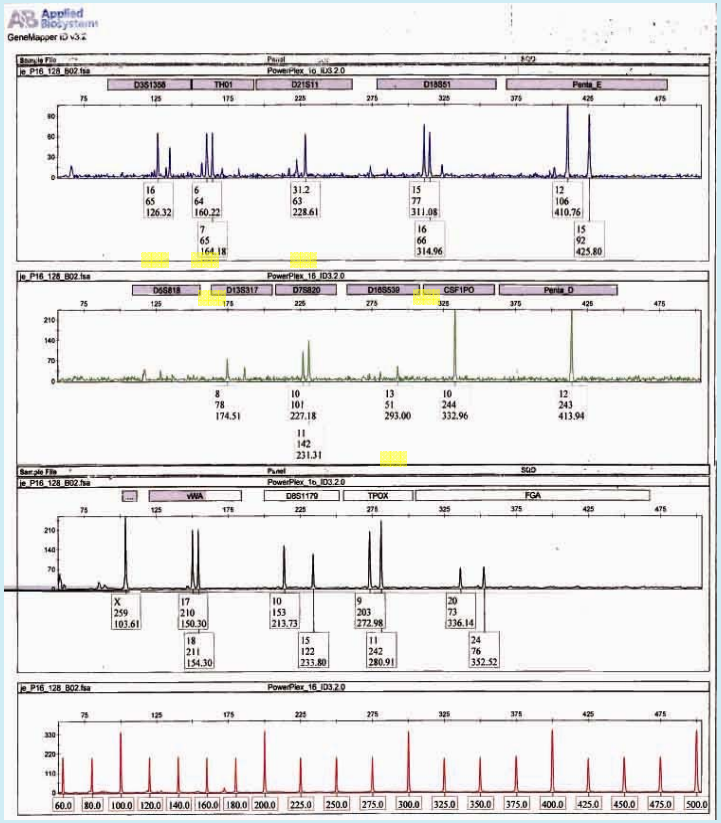
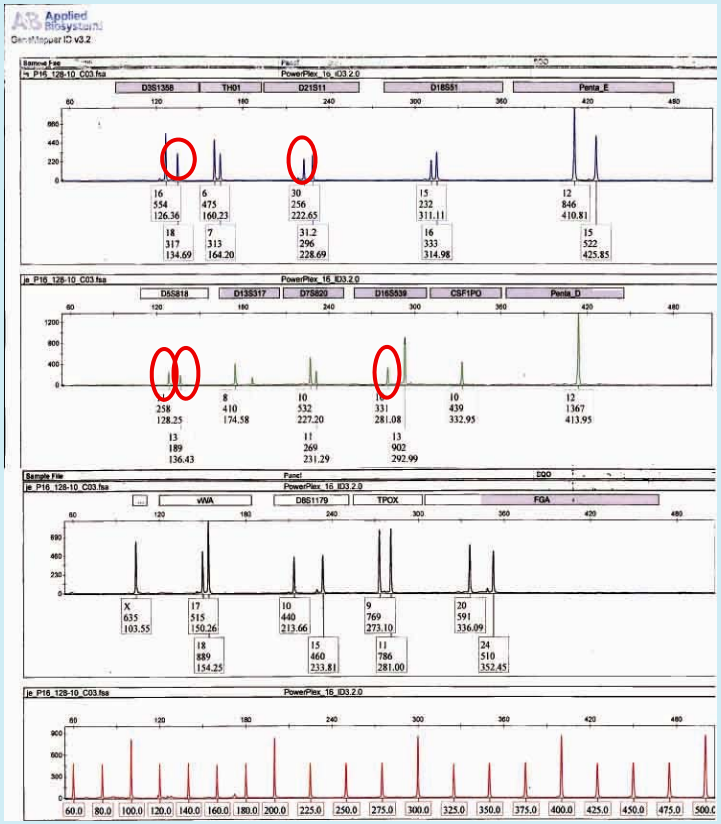
Study 3: NLCL MinElute® Results

7.8 pg Template Using PowerPlex® 16

Without MinElute®



With MinElute®



Images courtesy of Jessica Esparza, Ph.D.

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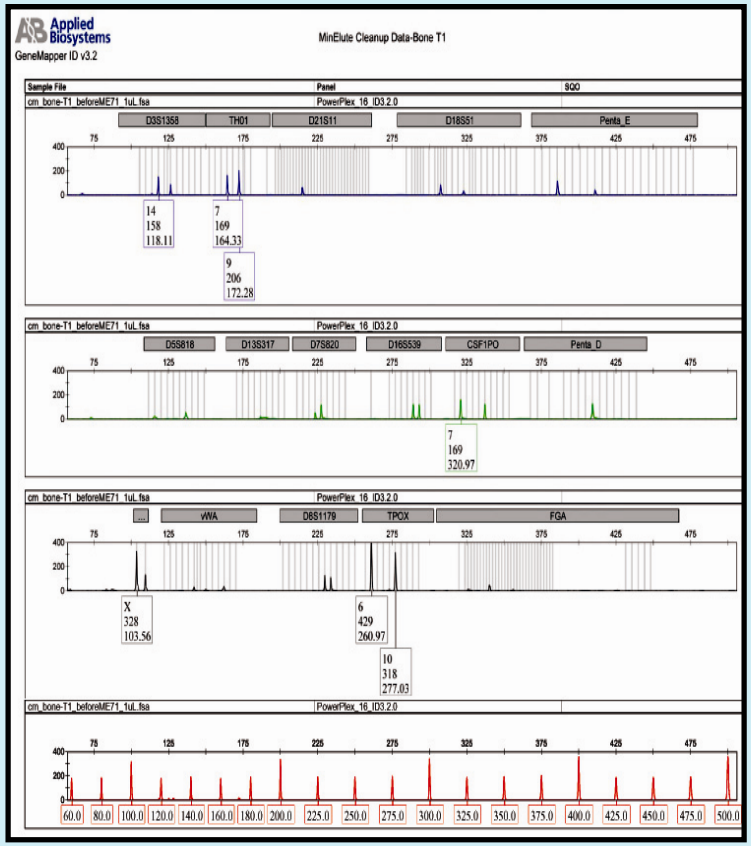
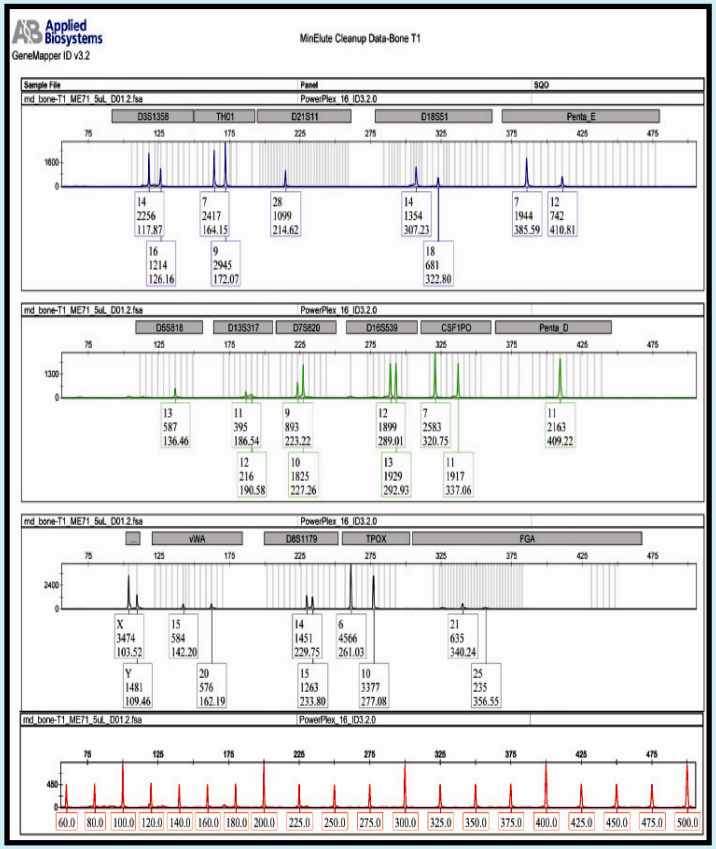
Study 3: NLCL MinElute® Results

Without MinElute®



Images courtesy of Jessica Esparza, Ph.D.

With MinElute®



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Study 3: NLCL MinElute®

- **Summary of Study Results:**
  - **Total RFU value increased approximately 24-fold after samples subjected to MinElute® cleanup**
  - **Average RFU for alleles detected increased from 241 RFU to 1610 RFU, or approximately 7-fold**
  - **100% of alleles were detected following cleanup compared to only 26% of the alleles detected without MinElute® cleanup**

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Study 4: NFSTC MinElute®: Coming Next

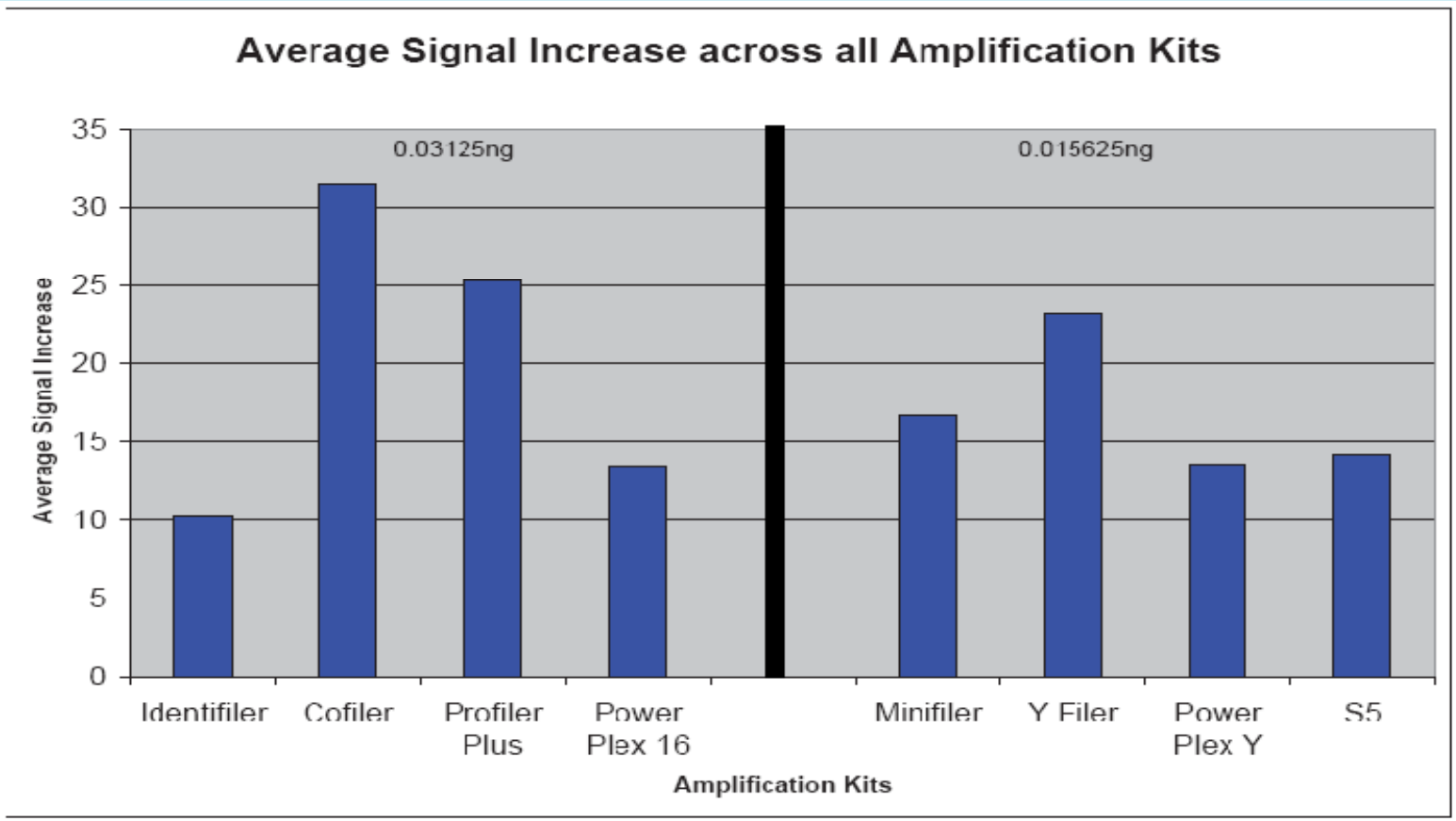

Image courtesy of NFSTC

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Conclusions: Enhancing Forensic Performance with QIAGEN MinElute Post-PCR Cleanup

- **Provides ability to enhance DNA forensic performance, data analysis, conclusive results**
- **Provides ability to handle special applications**
  - **Special sample types: bone, hair fragment, etc**
  - **Low template DNA samples (touch, B & E, etc.)**
  - **Degraded DNA**
- **Ability to gain increased RFU values without increased PCR cycles or nested (LV) PCR reactions**
- **Fast, convenient way to improve results, automatable**
- **Comparable or less complex than other techniques (e.g. LCM)**
- **Has to be validated like any other new method**

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Online Support

- **QIAGEN Webinar: Better STR Profiles from Trace Evidence – Validation of MinElute® for Post-amplification Cleanup of Forensic Samples**
  - **http://www1.qiagen.com/events/WebSeminars/MinElute/default.aspx**
  - **Jay Caponera of the New York State Police Forensic Investigation Center**
    - **Validation of the MinElute® procedure**
  - **Bruce Heidebrecht of Maryland State Police Forensic Sciences Division present on their validation experiences:**
    - **Validation of the MinElute® procedure on the QIAcube® instrument**
- **MinElute® and QIAcube® demo videos can be found at:**
  - **http://www.youtube.com/watch?v=M8TmmL1pcH0**
  - **http://www.youtube.com/watch?v=Li4lSMqSNO8&NR=1**

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Questions?

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Note: All images are courtesy of QIAGEN® unless otherwise noted.

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