



Technology Transition Workshop | *Josh Mann*



## ***QIAcube<sup>®</sup> – Pure Efficiency***

# **QIAcube<sup>®</sup> Overview**



- **What is it all about?**
- **Key features and capabilities**
- **Application data & summary**

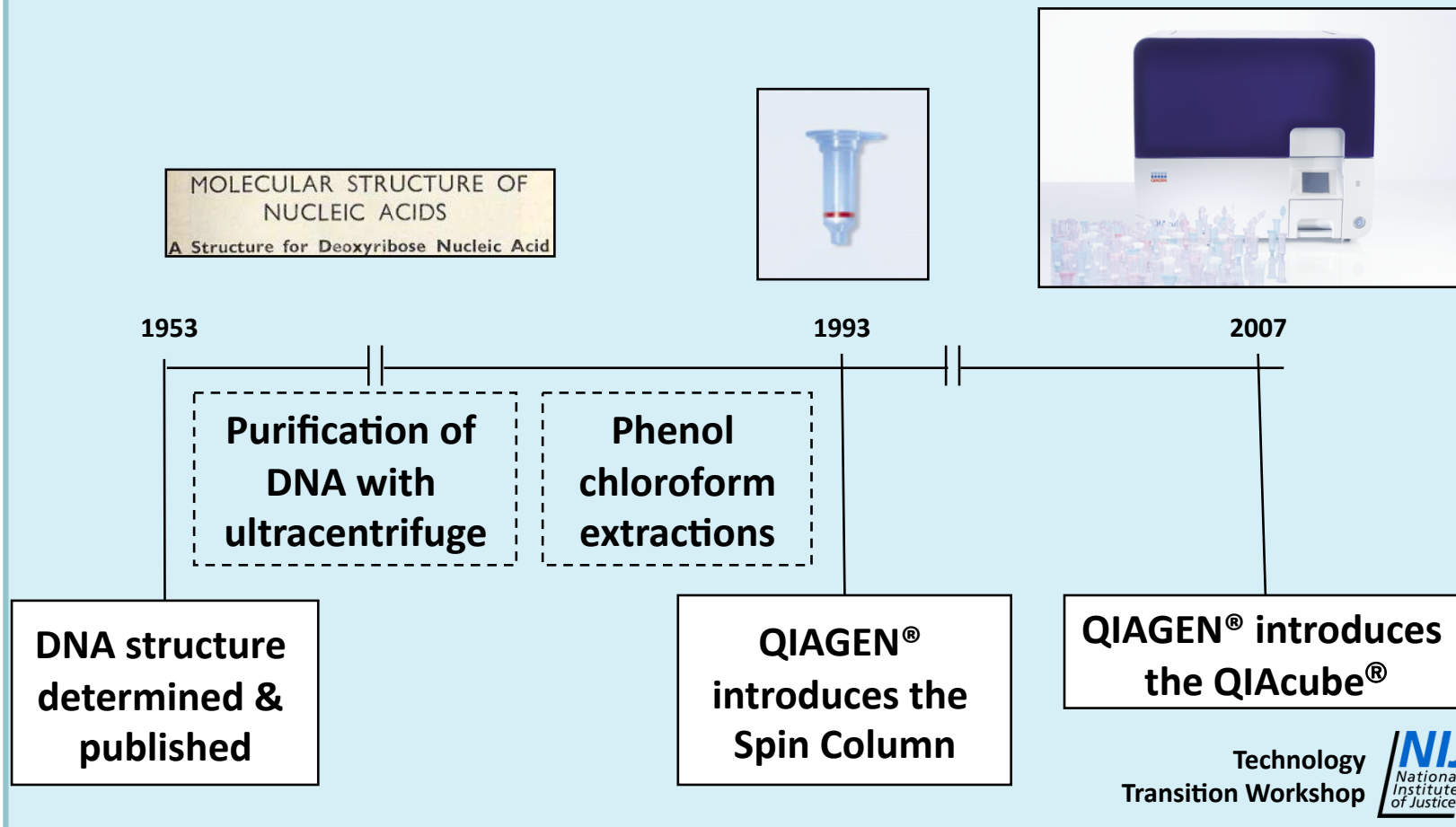


# ***QIAcube®: What Is It All About?***

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# How Everything Began

## The Evolution of Nucleic Acid Purification



# QIAcube<sup>®</sup> – It's Just the Next Logical Step

## The Idea

City Map



Navigation System

Abacus



Calculator

Washing Dishes



Dish Washer

Manual Processing



QIAcube<sup>®</sup>



**The world of sample processing is changing!**

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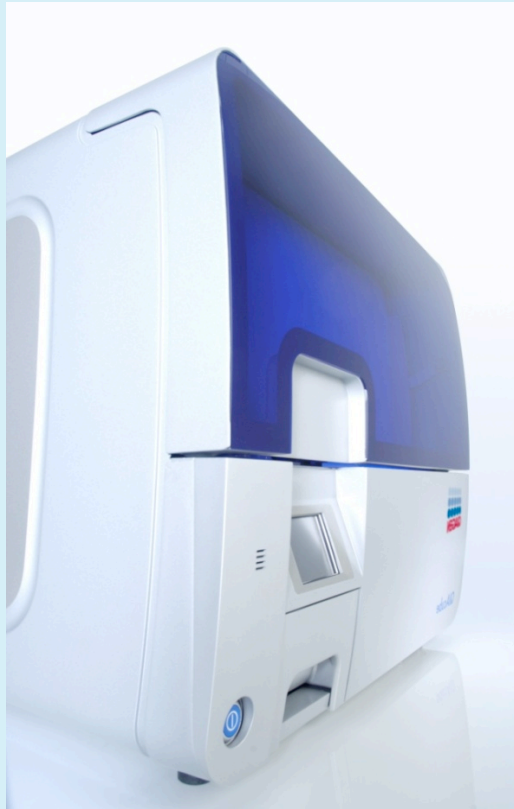
## ***Did You Ever Think About ...?***

- **How much time you spend with spin preps?**
  - Average of 1.5 hours per day
  - 220 days/year
  - → 2 months of continuous spin preps per year!
- **Routine everyday work?**
- **What is your error/failure rate?**
- **Can you use your time more efficiently?**



# ***From Manual to Automation***

*We Make This Step Easy*



- **Eliminating manual sample purification!**
- **Higher efficiency and more time every day for...**
  - Data analysis
  - Writing reports/scientific articles
  - Preparing & designing new experiments
  - Literature search
  - **And much more...**

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# ***The Next Dimension of Sample Purification Technology***

- **Main benefits:**
  - **Safety and convenience**
    - Using the most proven chemistry technology
  - **Application flexibility**
    - More than 100 protocols for sample purification
  - **Time efficiency**
    - Freeing up time from routine work
  - **Standardization**
    - Increased reproducibility through automation





# ***QIAcube<sup>®</sup>: Key Features and Capabilities***

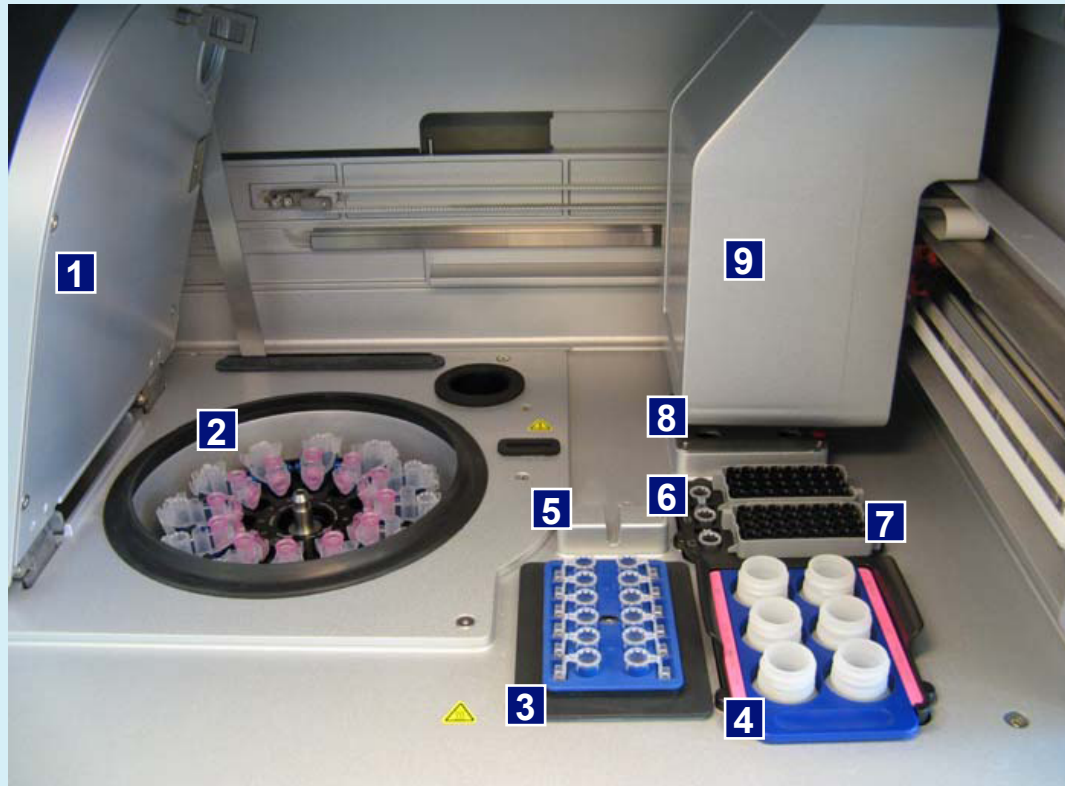
# ***Automation of Routine Processes***

## ***Key Features of the QIAcube®***

- **DNA, RNA and protein purification**
- **1 to 12 samples per run**
- **Sample lysis included**
- **Easy download and transfer of new protocols**
- **Load check before each run**
- **Interaction via touch screen (no computer)**
- **Small bench top system**
- **Individual centrifuge & shaker functionality**



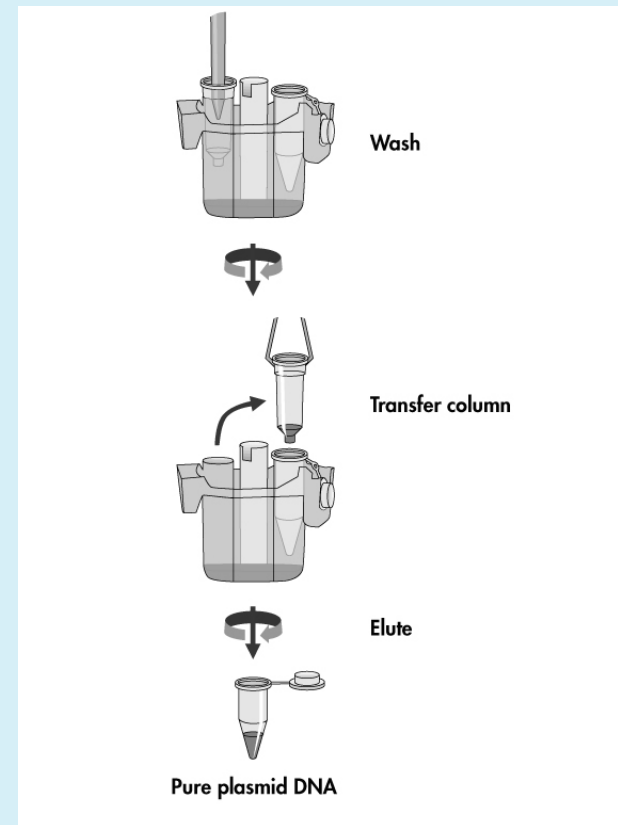
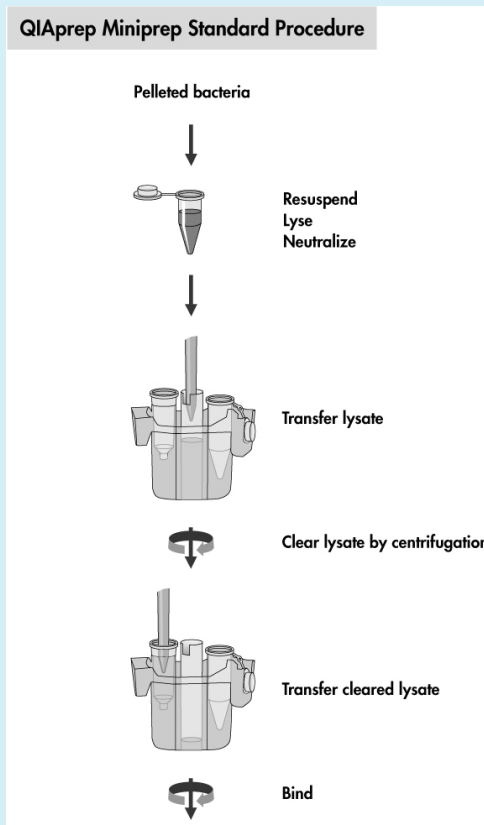
# It's a Little Lab on a Worktable



- |                         |                              |                                            |
|-------------------------|------------------------------|--------------------------------------------|
| <b>1</b> Centrifuge Lid | <b>4</b> Reagent Bottle Rack | <b>7</b> Tip Racks                         |
| <b>2</b> Centrifuge     | <b>5</b> Tip Sensor          | <b>8</b> Disposal Slots for Tips & Columns |
| <b>3</b> Shaker         | <b>6</b> Microtube Slots     | <b>9</b> Robotic Arm                       |

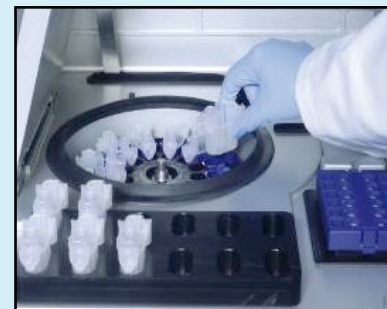
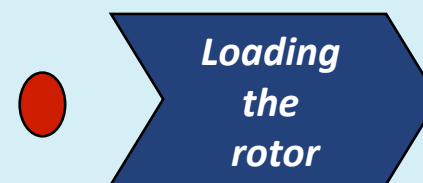
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# Streamlined Sample Processing – Same As Manual – The QIAcube® Workflow



# Three Simple Steps

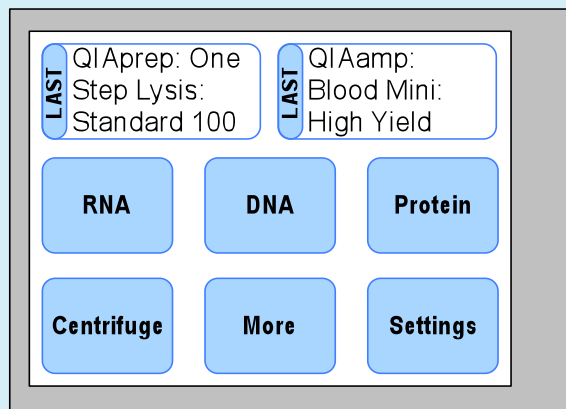
## QIAcube® System Setup Process



# Highest Convenience & Ease of Use

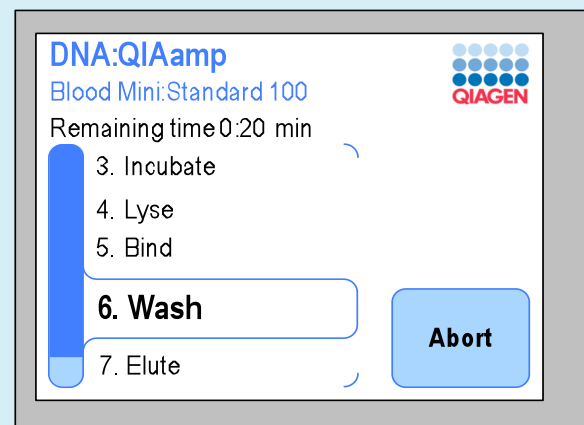
## Protocol & Parameter Selection

- **Easy Selection**
  - Target analyte
  - Protocol
  - Kit
  - (Starting material)



## Monitoring the Process

- **Continuous Information**
  - Active protocol
  - Remaining time
  - Actual processing step



## ***Small, but Smart***

- **Safety features ensuring smooth sample processing**
  - Load check avoiding human errors
  - Verifying the number of samples
  - Checking if buffer volumes are sufficient
  - Proving the right tip size is loaded
  - Verifying the number of available tips
  - Verifying the number of spin columns/rotor adapters
  - **Positive tip check after uptake**



# Over 80 Standard Protocols for Almost 40 Kits – QIAcube<sup>®</sup>, the Multi-Talent



## Genomic DNA from human samples

- QIAamp<sup>®</sup> DNA Blood Mini Kit
- QIAamp DNA Mini Kit
- QIAamp DNA Stool Mini Kit
- QIAamp DNA Micro Kit
- QIAamp MinElute Media Kit\*

## Forensic samples

- QIAamp DNA Investigator Kit

## Viral DNA and RNA purification

- QIAamp MinElute Virus Spin Kit
- QIAamp Viral RNA Mini Kit

## Genomic DNA from animal samples

- DNeasy<sup>®</sup> Blood & Tissue Kit

## Multiple analyte purification

- AllPrep<sup>®</sup> DNA/RNA Kits

## Plasmid DNA purification

- QIAprep<sup>®</sup> Spin Miniprep Kit

## DNA cleanup

- QIAquick<sup>®</sup> PCR Purification Kit
- QIAquick Gel Extraction Kit
- QIAquick Nucleotide Removal Kit
- MinElute PCR Purification Kit
- MinElute Reaction Cleanup Kit
- MinElute Gel Extraction Kit

## RNA purification from a range of samples

- RNeasy<sup>®</sup> Mini Kit
- RNeasy MinElute<sup>®</sup> Cleanup Kit
- RNeasy Plus Mini Kit
- RNeasy Micro Kit
- RNeasy Lipid Tissue Mini Kit
- RNeasy Fibrous Tissue Kit
- RNeasy Protect Bacteria Mini Kit
- miRNeasy Mini Kit
- PAXgene Blood RNA Kit†
- QIAamp RNA Blood Mini Kit

## FFPE samples

- RNeasy FFPE Kit
- QIAamp DNA FFPE Kit

## Plant samples

- DNeasy Plant Mini Kit
- RNeasy Plant Mini Kit

## Protein purification and serum depletion

- Ni-NTA Spin Kit
- Qproteome Albumin/IgG Depletion Kit
- Qproteome Murine Albumin Depletion Kit
- Qproteome Total Glycoprotein Kit
- Qproteome O-Glycan Glycoprotein Kit

## Bisulfite conversion and cleanup

- EpiTect<sup>®</sup> Bisulfite Kit



# Ready to Meet Your Needs

## Easy Extension of Protocol Portfolio

The screenshot shows the QIAGEN Sample & Assay Technologies web portal. The page title is "Search for QIAcube Standard Protocols". Below the title, there are several links: "QIAcube web portal home", "Register/Manage your QIAcube", "QIAcube FAQs", "QIAcube resources", and "QIAcube product information". There is an "Instructions" section with two bullet points: "Click 'More Info' to see the full description of the protocol. You can then choose to download the protocol and/or protocol sheet." and "Find out [how to transfer protocols to your QIAcube](#)". Below this, there are three dropdown menus for "Select an application", "Select a kit", and "Select the starting material", all set to "All". A "Search Again" button is located below the dropdowns. The "Protocols Found" section contains a table with the following data:

Name	Protocol Version	Description
DNeasy Blood&Tissue - Animal blood or cells - Standard	1	This protocol is for purification of total DNA from animal blood or cells using the DNe...
DNeasy Blood&Tissue - Animal tissues and rodent tails - Standard	1	This protocol is for purification of DNA from animal tissues and rodent tails using the...
DNeasy Plant Mini - Plant cells and tissues - Standard	2	This protocol is for purification of total DNA from up to 100 mg fresh or up to 20 mg d...
EpiTect Bisulfite - Cleanup - DNA from FPPE	2	For cleanup of DNA (from FPPE tissues) from bisulfite reactions using the EpiTect Bisul...
EpiTect Bisulfite - Cleanup - Standard	2	For cleanup of DNA from bisulfite reactions using the EpiTect Bisulfite Kit
MinElute PCR - Standard	1	This protocol is for purification of double-stranded DNA fragments (70 bp to 4 kb) from...
MinElute Reaction - Standard	1	This protocol is for purification of double-stranded DNA fragments (70 bp to 4 kb) from...
miRNeasy Mini - Animal tissues and cells - Aqueous phase	2	This protocol is for purification of total RNA, including miRNA and other small RNA mol...
Hi-NTA Spin - Bacterial pellet - Denaturing	1	This protocol is for purification of up to 300 µg of 6xHis-tagged protein from bacteria...

- Web portal including:
  - Standard QIAGEN® protocols
  - Modified protocols
  - Custom protocols



# ***QIAcube®: Post-Amp Cleanup Application Data & Summary***

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# QIAcube<sup>®</sup> for Post-Amp Cleanup



- **The automated spin column**
  - Walk-away automation of established spin column processes, 2-12 samples
- **Post-PCR purification**
  - Protocol for post-PCR purification with MinElute<sup>®</sup> cleanup

# ***Advance Forensic Casework Profiling***

- **Post PCR purification by MinElute® PCR Purification Kit**
- **Post-PCR purification can greatly enhance forensic casework analysis**
- **Conventional methods, such as increasing the PCR cycle number or nested PCR, may complicate analysis due to allele drop-out/drop-in, or increased stutter**

# ***Advance Forensic Casework Profiling***

- **The MinElute® PCR Purification Kit substantially improves performance in casework DNA profiling:**
  - **Higher signal-to-noise ratios, sensitivity, and allele call rates**
  - **Superior results compared to filtration or enzyme-mediated digests**

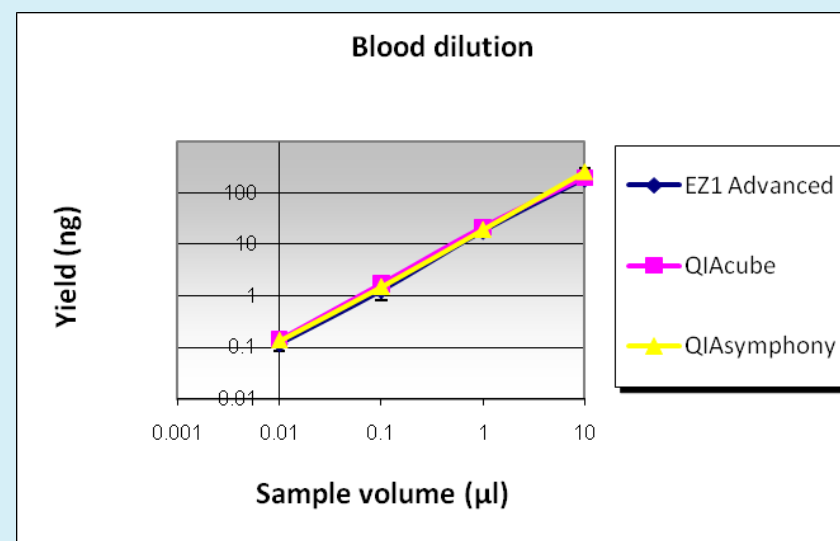
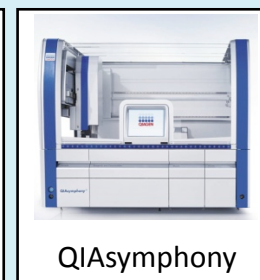
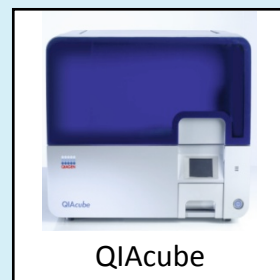
# ***Advance Forensic Casework Profiling***

- **Recent data suggests that standard cycle number PCR followed by post-PCR purification delivers results equivalent to increased cycle number PCR**
- **Forster, et al. concluded:**
  - **The combination of 28-cycle PCR with post-PCR purification using the MinElute<sup>®</sup> PCR Purification Kit**
    - **Provided equivalent results in STR analysis**
    - **Reduced sample consumption**
    - **Reduced stutter peak ratios**
    - **Reduced the frequency of non-donor allele peaks**

# Linearity and Sensitivity

## Trans-platform Concordance

- **Extraction of blood dilutions by:**
  - **QIAcube<sup>®</sup>**
    - Spin column
    - 1 to 12 samples
  - **EZ1<sup>®</sup> Advanced**
    - Bead technology
    - 1 to 6 samples
  - **QIASymphony<sup>®</sup>**
    - Bead technology
    - 1 to 96 samples



# **QIAcube<sup>®</sup> Developmental Validation**

- **The automated spin column**
  - Walk-away automation of established spin column processes, 2 to 12 samples
- **Sample extraction & cleanup**
  - Automation of the established QIAamp<sup>®</sup> Investigator Kit
- **Post-PCR purification**
  - Protocol for post-PCR purification with MinElute<sup>®</sup>

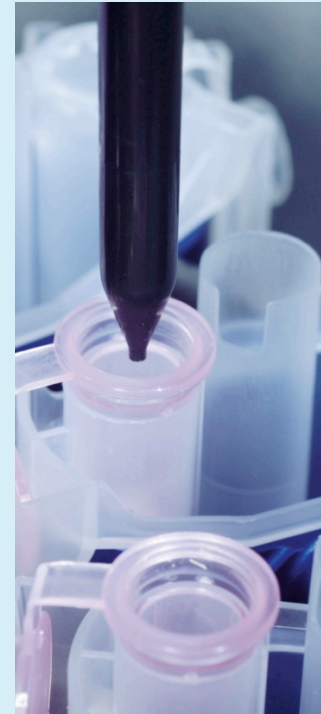




# **QIAcube<sup>®</sup> Developmental Validation**

## *Automated QIAamp<sup>®</sup> DNA Investigator Protocols*

- **Casework samples**
  - Cigarette butts, stains, paper, hair, tape lifts, chewing gum, etc.
    - Optional onboard lysis
- **Surface and buccal swabs**
  - Surface swabs, buccal swabs, blood swabs, etc.
    - Onboard lysis
- **FTA<sup>®</sup> paper and Guthrie cards**
  - Blood card spots
    - Onboard lysis
- **Small volumes of blood or saliva**
  - Up to 100 µl of blood or saliva
    - Integrated lysis



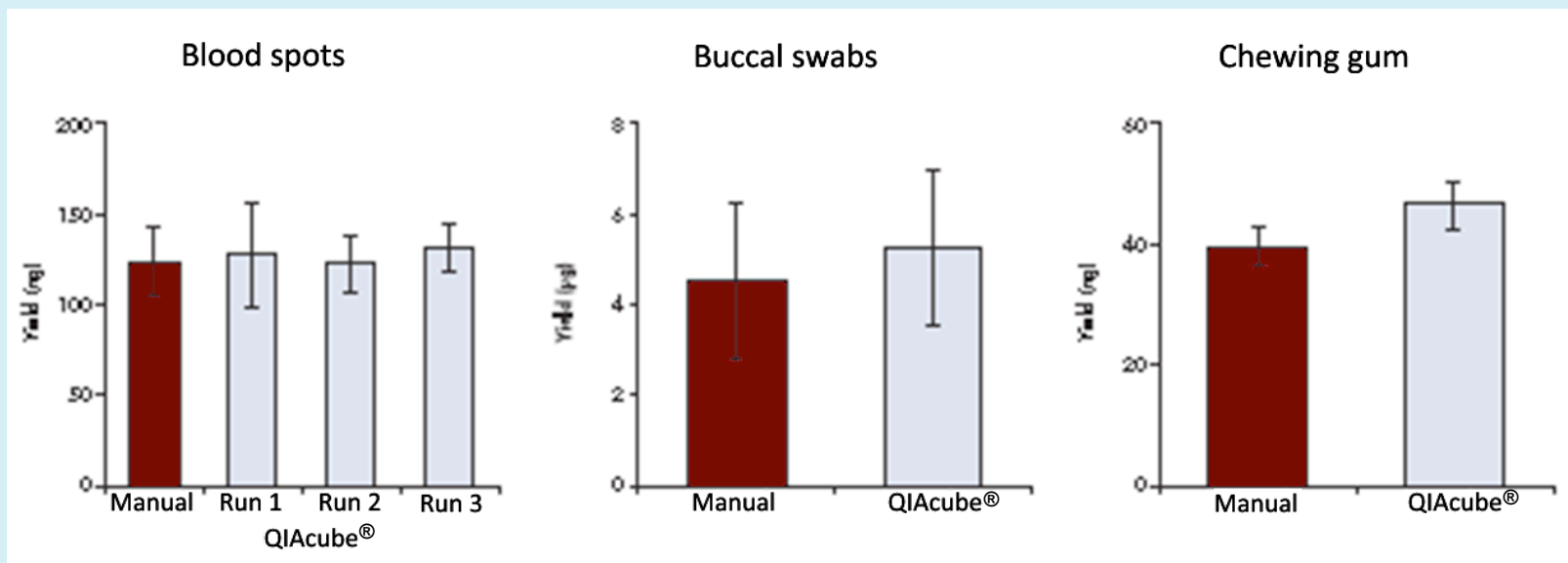
# **QIAcube<sup>®</sup> Developmental Validation**

## *Results - Comparing Manual & Automated Extractions*

- **Samples were processed manually or with the QIAcube<sup>®</sup> using the following automated protocols:**
  - **Blood spots on FTA<sup>®</sup> paper and Guthrie Cards”**
    - 12 manual and 36 automated samples
  - **Surface and buccal swabs**
    - 6 samples each
  - **Chewing gum (30 mg), mock casework sample**
    - 4 samples each
- **DNA was quantified photometrically (swabs) or with real-time PCR (blood and chewing gum)**

# QIAcube<sup>®</sup> Developmental Validation

## Results - Comparing Manual & Automated Extractions



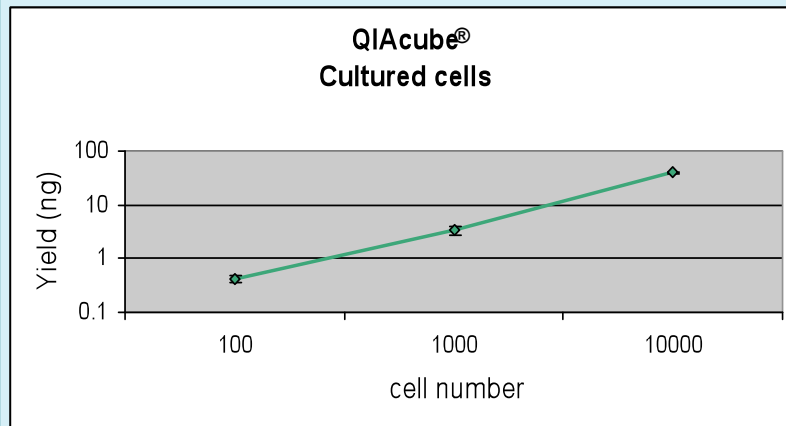
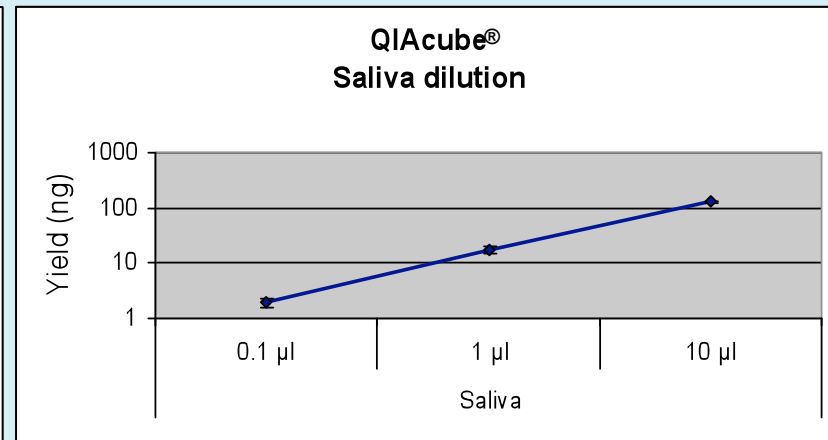
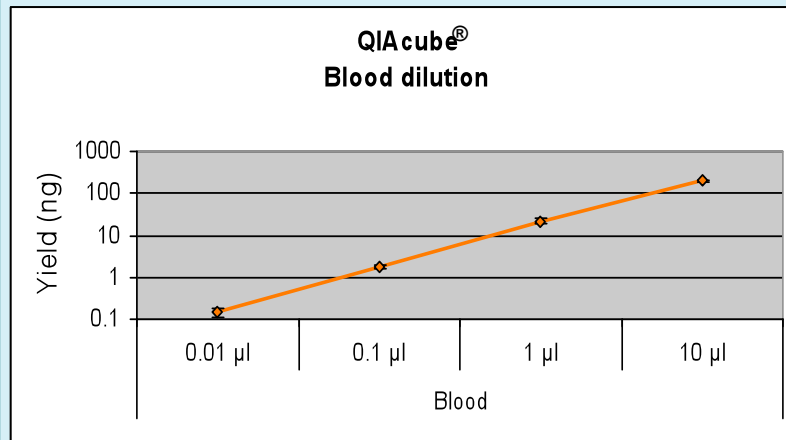
# **QIAcube<sup>®</sup> Developmental Validation**

## *Linearity*

- **Dilution series of blood, saliva and cultured cells**
  - Linear input to yield correlation
- **Experimental setup:**
  - Serial dilution of sample in lysis buffer
  - Pooled lysis
  - Extraction from 100 µl lysate/sample
  - DNA elution volume of 60 µl

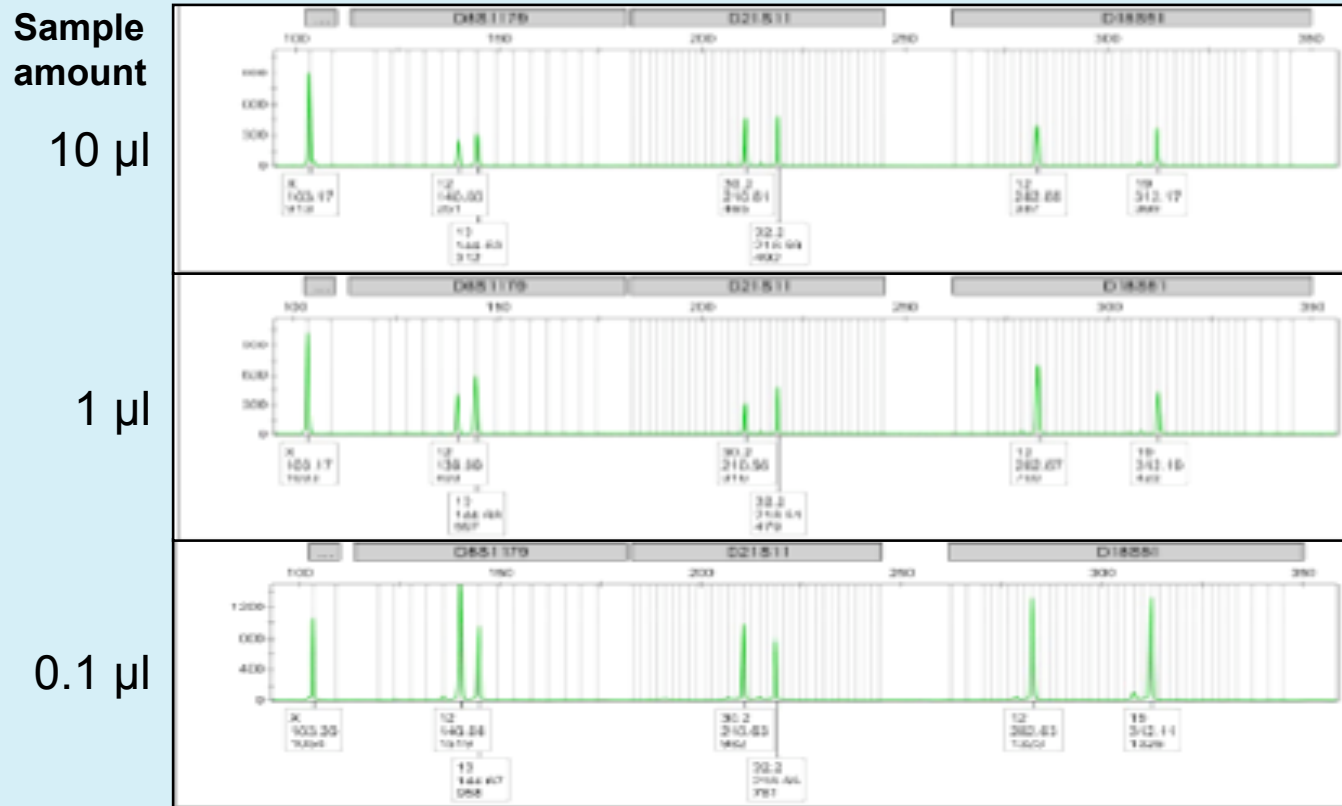
# QIAcube<sup>®</sup> Developmental Validation

## Linearity



# QIAcube<sup>®</sup> Developmental Validation

## Sensitivity, Signal to Noise



- AB SGM Plus<sup>®</sup> 12.5  $\mu$ l reaction
- Full profiles for all dilutions
- 28 or 32 cycles (0.1  $\mu$ l saliva)
- Low noise levels

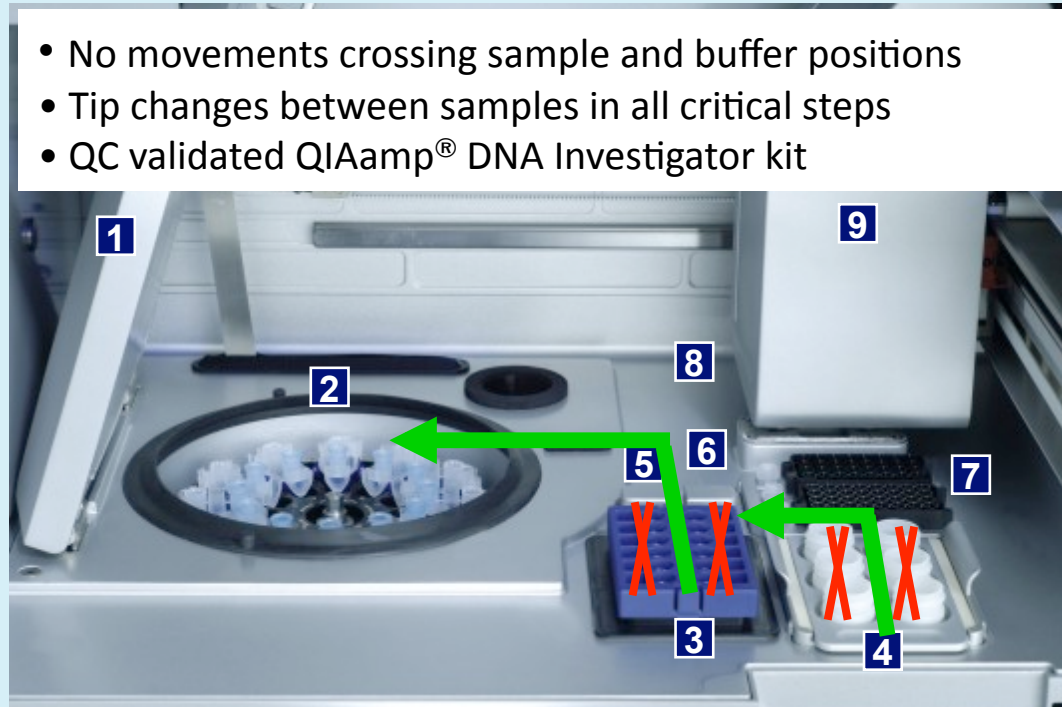
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# QIAcube<sup>®</sup> Developmental Validation

## Cross Contamination (I)

- No movements crossing sample and buffer positions
- Tip changes between samples in all critical steps
- QC validated QIAamp<sup>®</sup> DNA Investigator kit



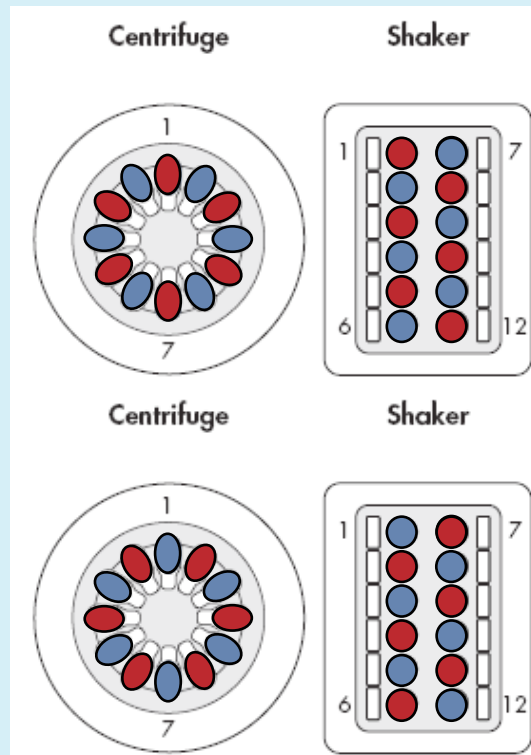
- |                         |                              |                                            |
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| <b>3</b> Shaker         | <b>6</b> Microtube Slots     | <b>9</b> Robotic Arm                       |

# QIAcube<sup>®</sup> Developmental Validation

## Cross Contamination (II)

- 100 µl human whole blood samples
- Mock samples: 100 µl lysis buffer
- Four consecutive runs with an alternating pattern of positive and negative samples (checkerboard)
- 48 samples in total
- Downstream analysis using real-time PCR on repetitive Alu sequences

Runs 1+3



Runs 2+4

● = pos samples

● = neg samples

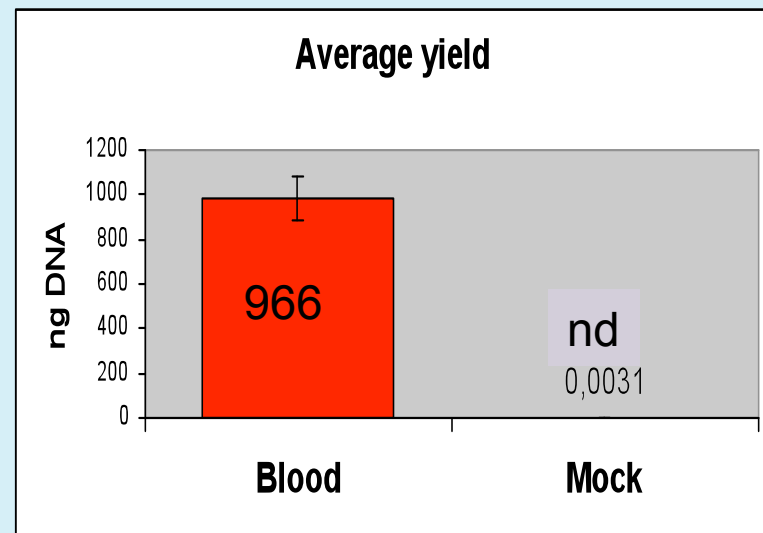
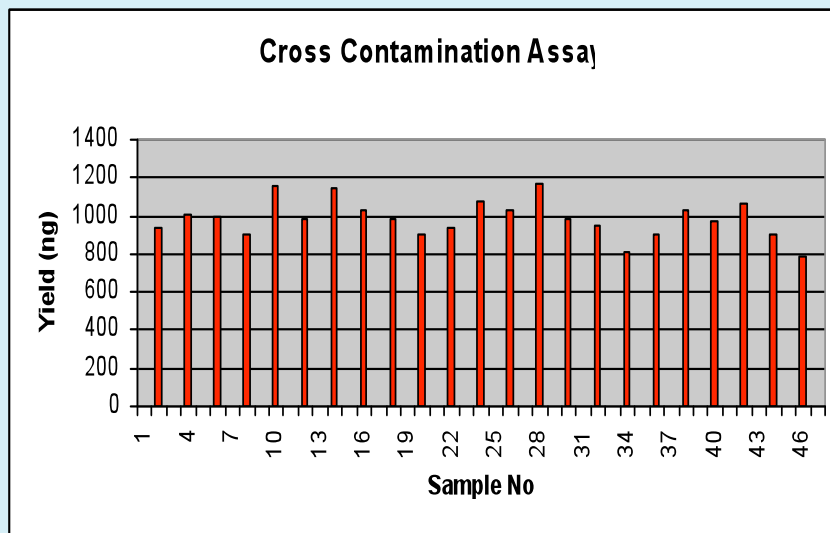
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# QIAcube<sup>®</sup> Developmental Validation

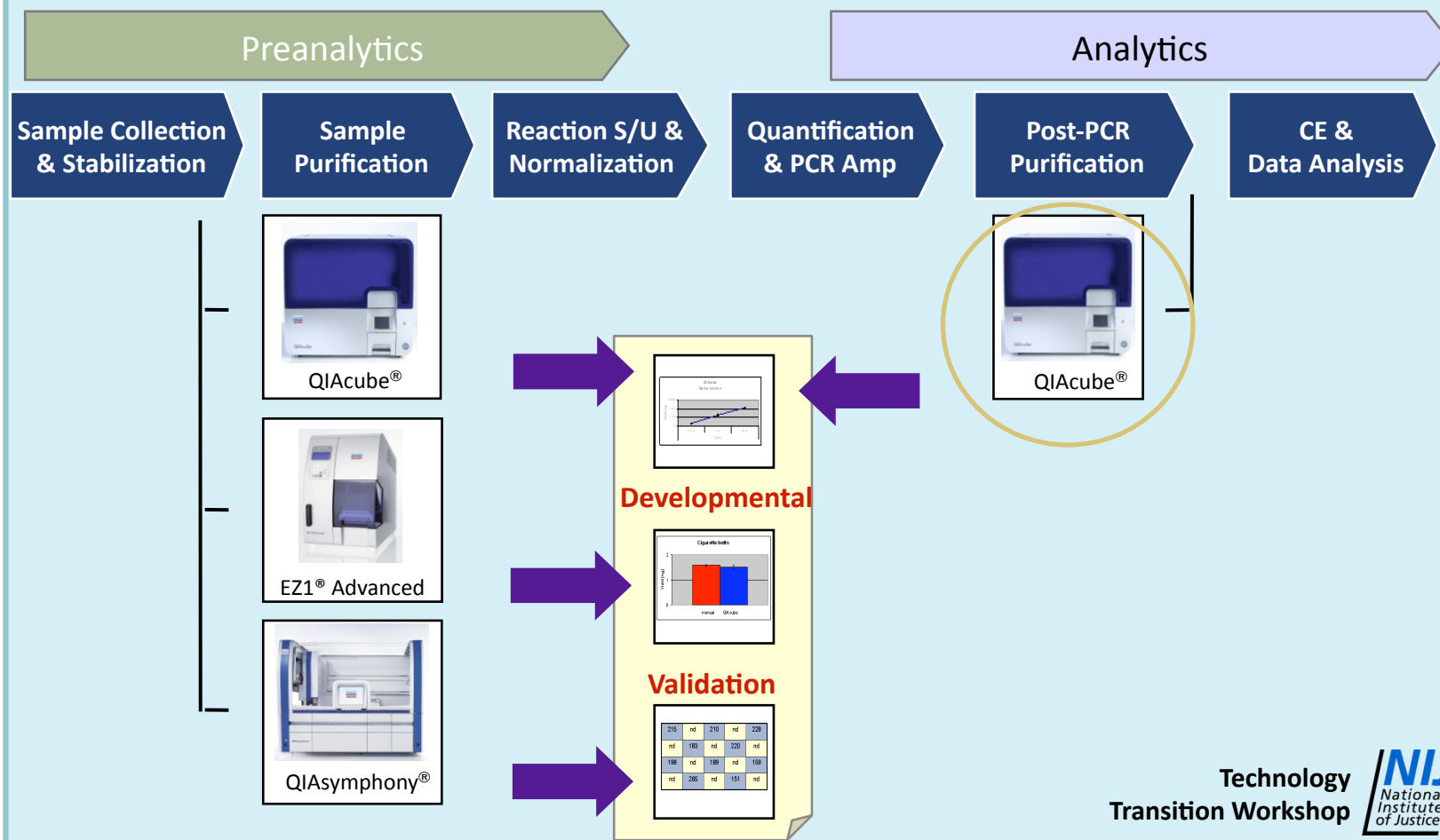
## Cross Contamination (III)

- Uniform yields from 100  $\mu$ l blood samples
- No human DNA detectable in mock sample eluates
- Distribution of yields across runs:



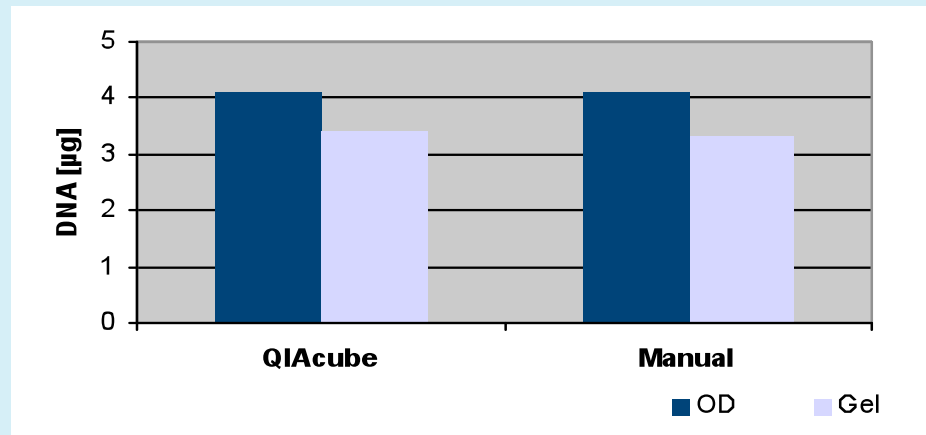
# Preanalytical and Analytical Processes

## Human Identity Testing Workflows

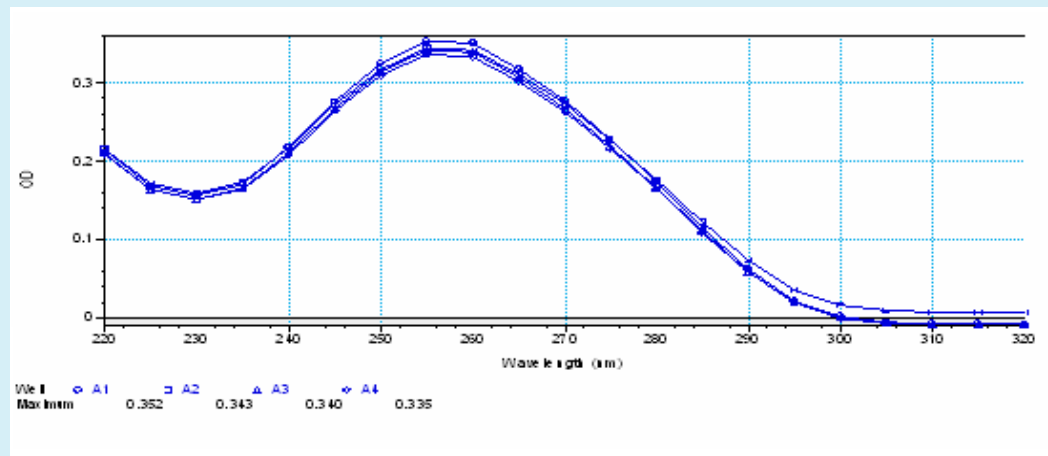


# QIAprep<sup>®</sup> – Standard Protocol

## Good Yields and High DNA Purity



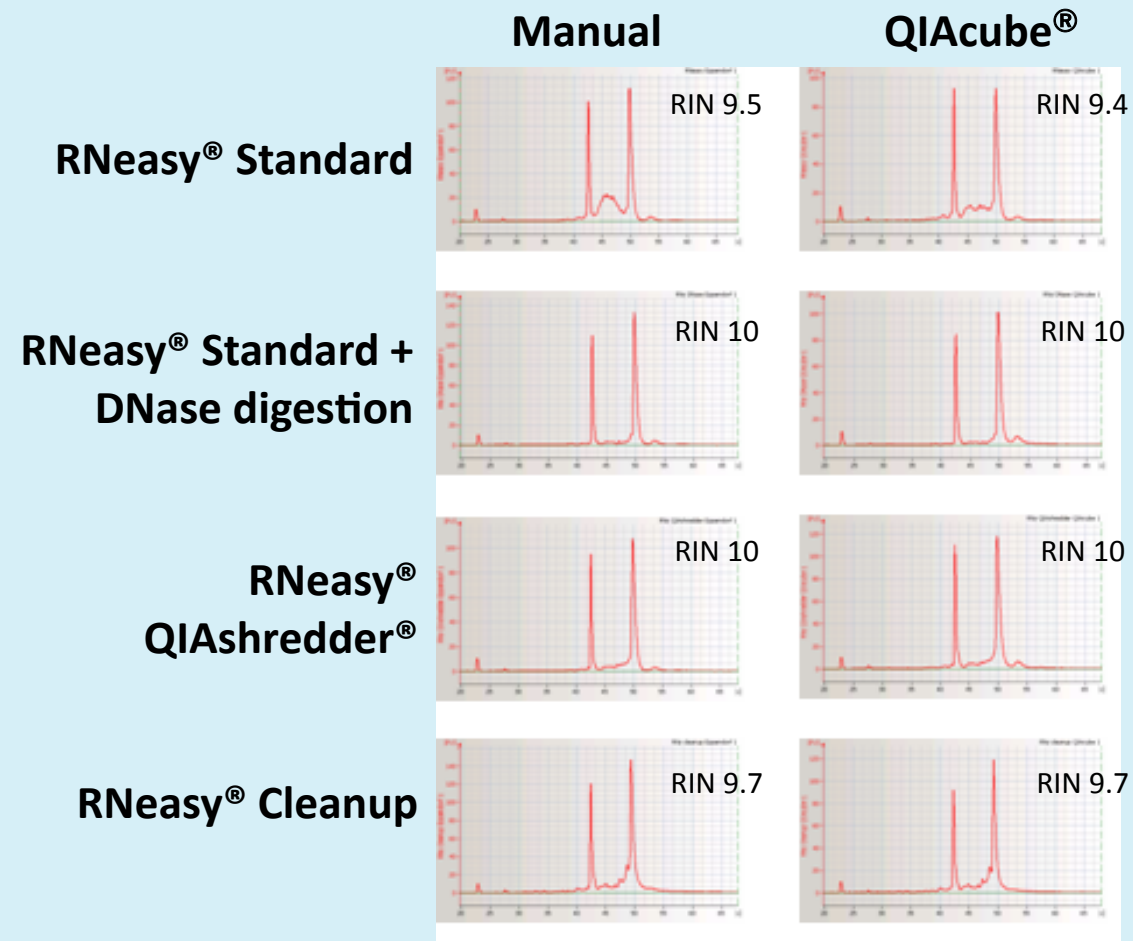
- Yield comparison QIAcube<sup>®</sup> vs. manual:
  - 1.5 ml overnight culture, E. coli DH10B/pUC19 in Lysogeny Broth with ampicillin (LB amp)



- DNA Purity:
  - 5 ml overnight culture, E. coli DH10B/pUC19 in LB amp

# Total RNA Purification

High-quality RNA – High RNA Integrity Numbers (RINs)



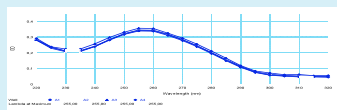
# QIAamp<sup>®</sup> DNA Blood Mini Protocol

## Purification of Highly Pure DNA

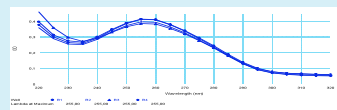
**A**



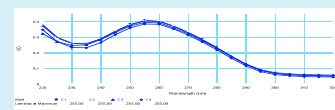
**B**



EDTA

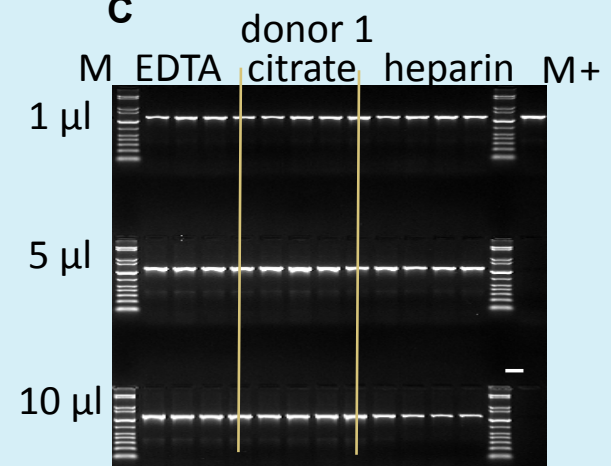


citrate  
donor 1



heparin

**C**



# **QIAcube<sup>®</sup> Regular Maintenance Procedure**

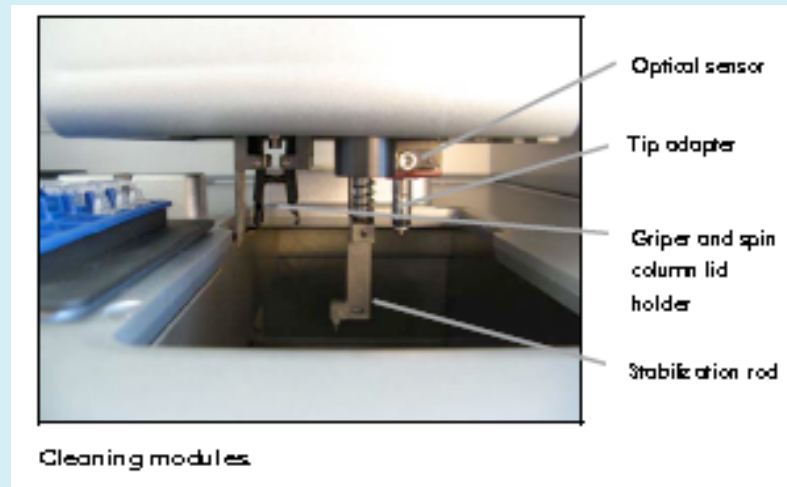
- **After running a protocol, perform the regular maintenance procedure:**
  1. Empty the waste drawer.
  2. Remove used disposable labware and unwanted samples and reagents from the worktable.
    - a. Discard according to local safety regulations.
  3. Replace the lids of the reagent bottles and close tightly.
  4. Store the bottles according to the instructions in the relevant kit handbook.
  5. You can now run another protocol or switch off the QIAcube<sup>®</sup>.

# ***QIAcube<sup>®</sup> Monthly Maintenance Procedure***

- Perform the regular maintenance procedure before you perform the monthly maintenance procedure
- Select the appropriate cleaning agent according to the sample material and downstream assay

# **QIAcube<sup>®</sup> Monthly Maintenance Procedure**

1. Clean the optical sensor, tip adapter, gripper unit (including the gripper), the stabilizing rod, and the spin column lid holder, by carefully wiping these modules with a soft lint-free cloth moistened with water.





# **QIAcube<sup>®</sup> Monthly Maintenance Procedure**

2. Clean the shaker rack, labware tray, heating adapter, and reagent bottle rack with cleaning agent.
  - a. Incubate with cleaning agent, as appropriate.
  - b. Rinse thoroughly with distilled water.
  - c. Wipe dry with paper towels.



# **QIAcube<sup>®</sup> Monthly Maintenance Procedure**

3. Clean the liner of the waste drawer with cleaning agent.
  - a. Incubate with cleaning agent, as appropriate.
  - b. Rinse thoroughly with distilled water.
  - c. Wipe dry with paper towels.
4. Thoroughly wipe the inside and outside of the QIAcube<sup>®</sup> using the cleaning agent.
  - **Important:** Do not use alcohol or alcohol-based disinfectants to decontaminate the QIAcube<sup>®</sup> door.

# ***QIAcube<sup>®</sup> Summary***

- **Increased time efficiency by elimination of routine tasks**
- **Using the most proven sample purification technology**
- **Full flexibility for sample numbers and applications**
- **Reproducibility by automation of the complete process**



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*Questions?*

# ***Contact Information***

**[www.qiagen.com/forensics](http://www.qiagen.com/forensics)**

**Note:** All images courtesy of QIAGEN®

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