

Instrumentation and Reagents

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Slides prepared by Kelly M. Elkins, Ph.D., Towson University, 2025

Background

In October 2023, Towson University was awarded a cooperative agreement from NIST to develop a standardized DNA training curriculum for the United States that addresses the components in ANSI/ASB Standard 115, *Standards for Training in Forensic Short Tandem Repeat Typing Methods Using Amplification, DNA Separation, and Allele Detection*. 2020. 1st Ed.

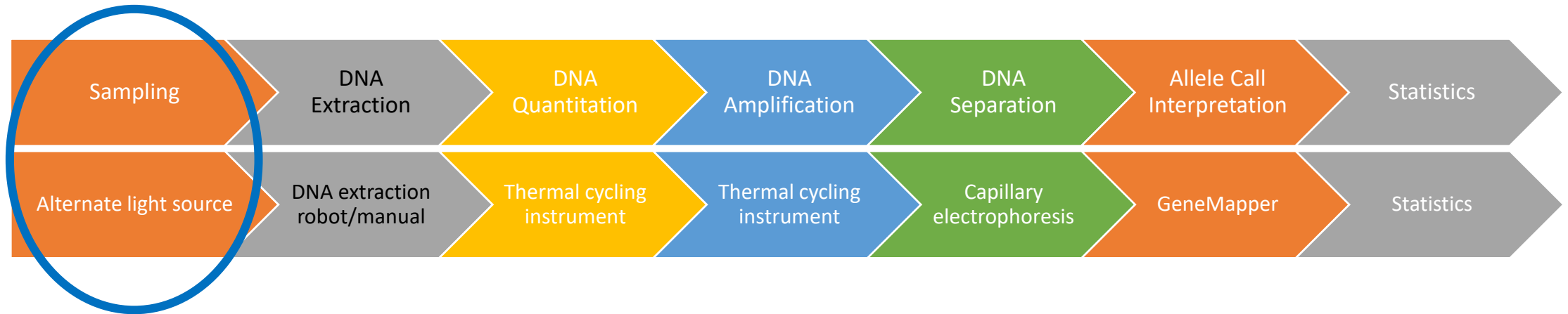
This presentation addresses the knowledge-based portion of the training program and covers the topic outlined in 4.2.3e in ANSI/ASB Standard 115.

Learning Objectives

1. thermal cycling instruments and parameters;
2. DNA separation and detection instruments and parameters;
3. software parameters associated with instruments;
4. maintenance and calibration;
5. storage of STR typing kit and DNA separation reagents.

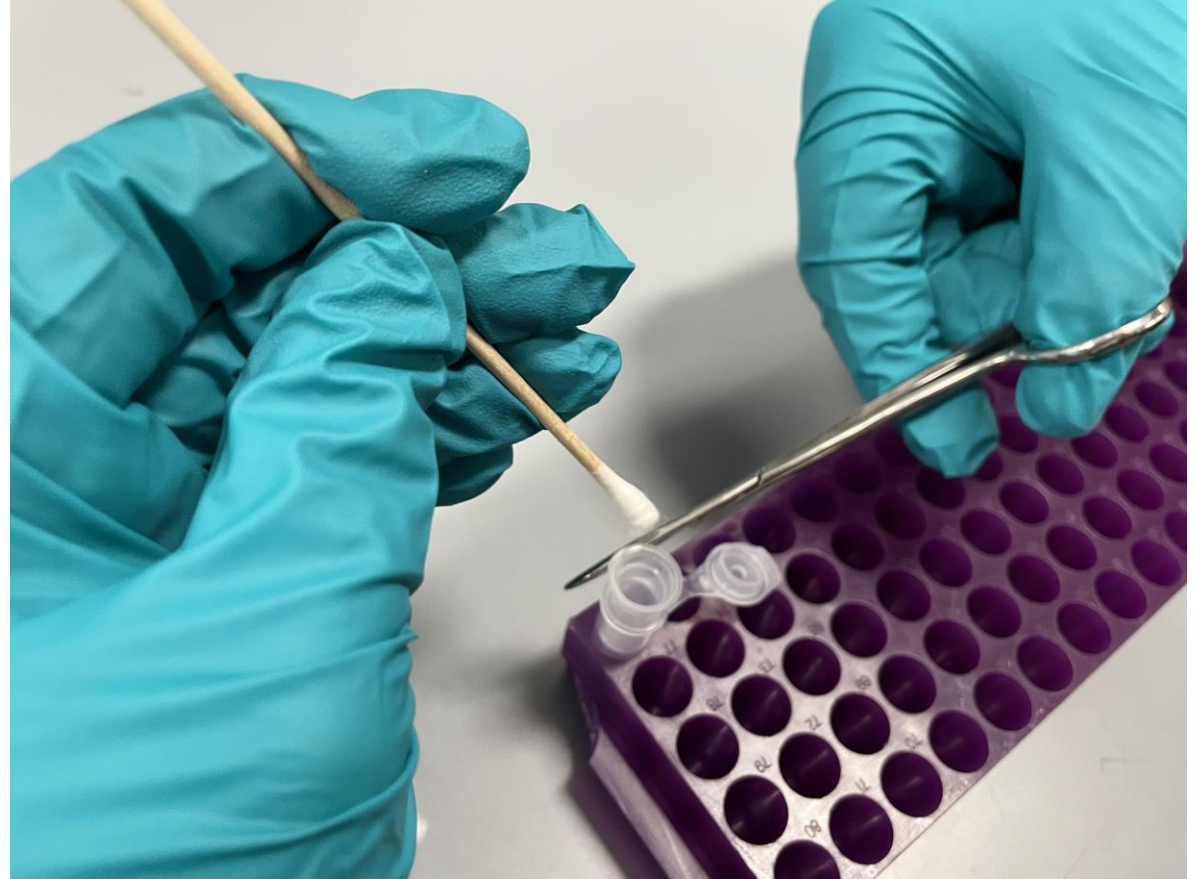


DNA Typing Process & Instrumentation

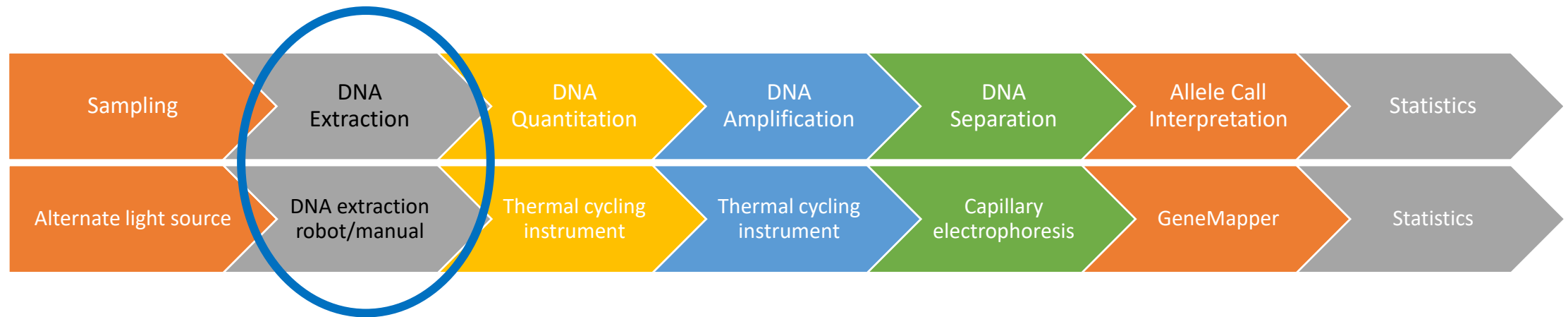


Prior to DNA Extraction: Sampling

- Sampling
 - Swab
 - Scissors
 - Tweezers
 - Scraping tool such as a razor
 - Vacuum filter

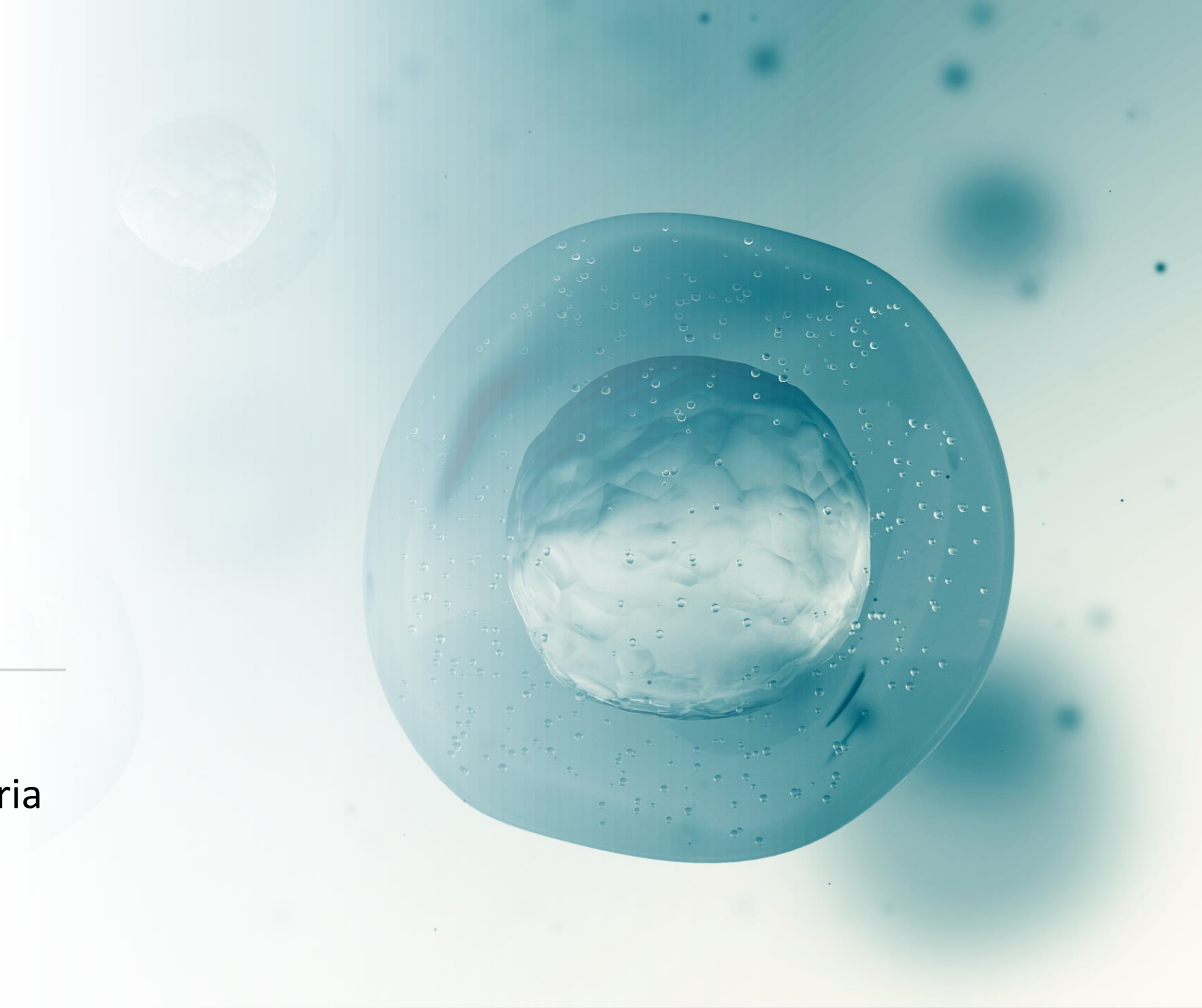


DNA Typing Process & Instrumentation



DNA Extraction

To isolate DNA from the
cellular nucleus, mitochondria
and/or chloroplast



DNA Extraction Methods

- Manual (e.g., Organic / PCIA, DNA IQ, DNA Investigator, PrepFiler, microGEM, Zygem)
 - Pros
 - Cheaper
 - Added flexibility for user-defined protocols
 - Uses standard laboratory equipment
 - Cons
 - Often more time-consuming
 - More opportunity for tube switching, protocol error, and contamination events

DNA Extraction Methods

- Robotic instruments (e.g., EZ1, QIAcube, Maxwell)

- Pros

- Minimize sample handling and risk of tube switching or cross contamination
 - Minimizes hands-on DNA extraction time
 - Higher throughput

- Cons

- Higher upfront and consumable costs
 - Additional training needed to use the instruments

DNA Extraction Instruments

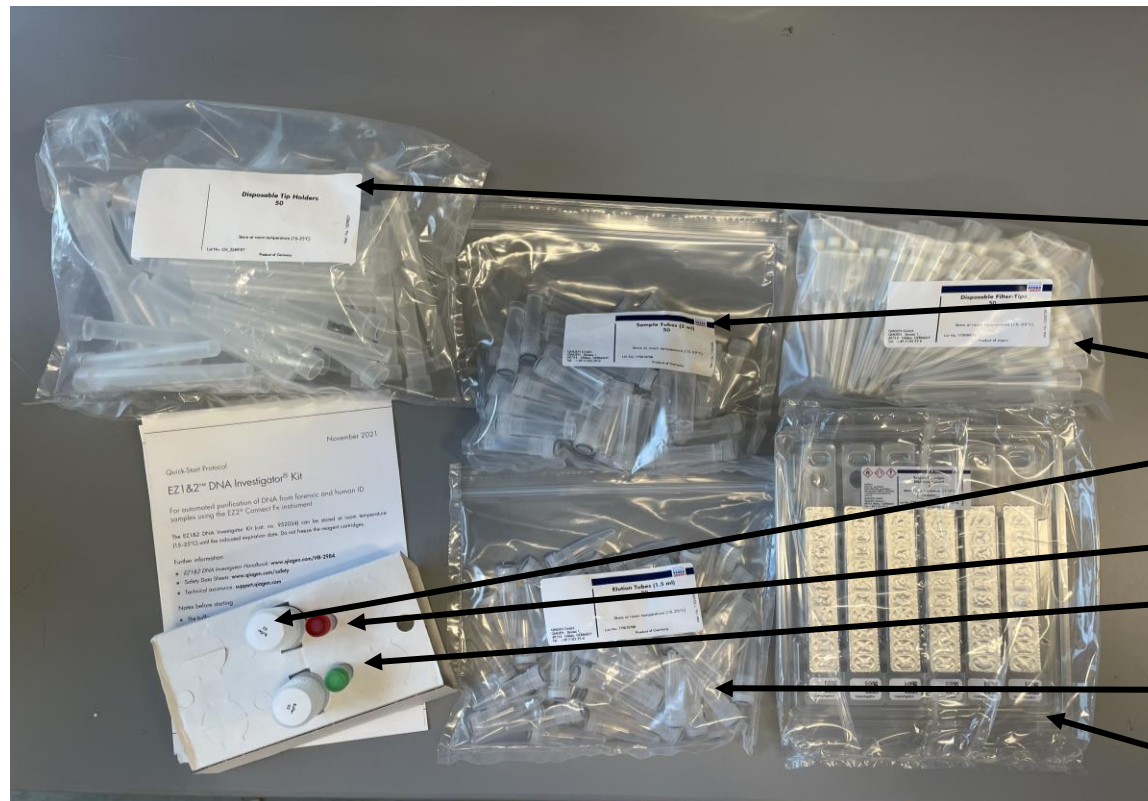
- EZ1
- QIAcube



DNA Extraction Instrument: EZ1

quality seal intact upon receipt

obtain and unpack kit wearing gloves



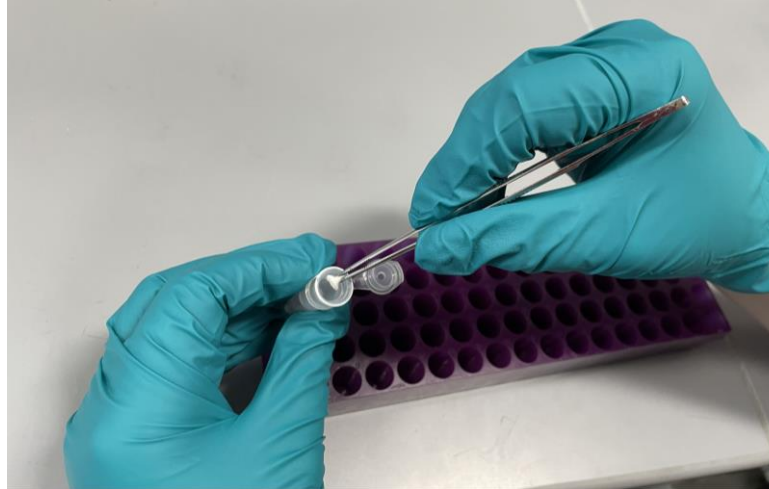
- Tip holders
- Sample tubes
- Pipette tips
- G2 buffer
- Carrier RNA
- Proteinase K
- Elution tubes
- Reagent cartridges

DNA Extraction: Pre-Extraction Steps

- Incubation
 - Thermomixer



- Substrate removal
 - Spin basket and tweezers

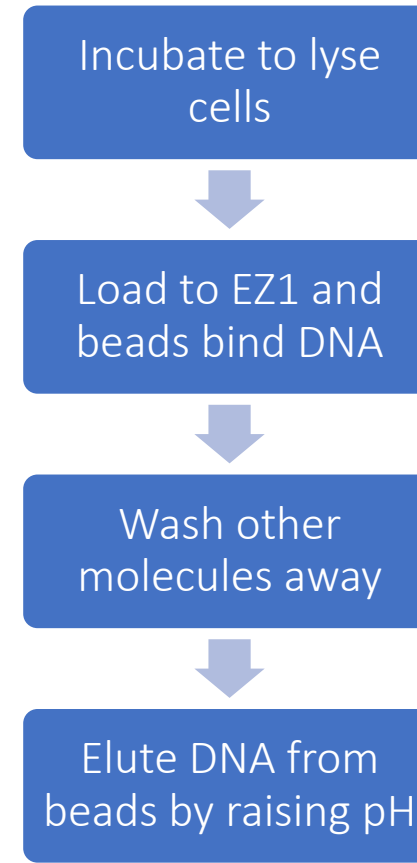


- Mixing
 - Vortex



DNA Extraction: EZ1 Instrument

- Lyse cells- incubation with G2 buffer and proteinase K
 - Ruptures epithelial cells to release DNA but not sperm cells
 - Proteinase K breaks down proteins
- DNA binding and washing
 - Load consumables and sample onto EZ1 instrument
 - DNA is bound, purified and washed using paramagnetic resin beads
 - DNA strands binds to the rough beads (like hair on a tennis ball) in presence of chaotropic salts contained in the reagent cartridge
 - Carrier RNA binds the DNA and increases DNA binding to the beads
 - At low pH, ethyl alcohol washes are used to remove the clinging cellular material and salts
- Elution- DNA binding is decreased from the beads and dissolved in TE buffer or water
 - pH is increased and DNA releases from the beads



DNA Extraction Instrument Run Parameters: EZ1

- Turn power on at the back of the instrument



DNA Extraction Instrument Run Parameters: EZ1

- Select protocols (DNA Extraction), tools or quality assurance tests



DNA Extraction Instrument Run Parameters: EZ1

- Apply validated or manufacturer's protocol
 - Trace- for low quantity samples
 - Trace Tip Dance (TD)- protocol for when sample cutting remains in tube
 - Normalization – eliminates the need to quantify post-extraction
 - Large Volume- protocol for larger input buffer and reagent volumes



DNA Extraction Instrument Run Parameters: EZ1

- Elution solvent
 - Water - no EDTA to chelate Mg^{2+} needed as a cofactor for DNA polymerase activity in PCR and stabilizing DNA duplexes
 - TE - Tris buffer controls the pH and EDTA chelates divalent cations like Mg^{2+} preventing DNase activity



DNA Extraction Instrument Run Parameters: EZ1

- Eluting in a higher volume will yield more but more dilute DNA
- Select a lower volume (e.g., 50 μ L) for more concentrated DNA
- Can use to concentrate DNA from a larger volume to a smaller volume



DNA Extraction Instrument Run Parameters: EZ1

- Pre-run loading steps
 - Load the cartridges to the cartridge area



DNA Extraction Instrument Run Parameters: EZ1

- Pre-run loading steps
 - Load 1.5 mL elution tubes to front row
 - Remove sample and elution tube caps when loading



DNA Extraction Instrument Run Parameters: EZ1

- Pre-run loading steps
 - Load tips and tip holders



DNA Extraction Instrument Run Parameters: EZ1

- Pre-run loading steps
 - Load uncapped sample tube to back row

Sample tubes
Tips and tip holders
Elution tubes



DNA Extraction Instrument Run Parameters: EZ1

- Press START to begin run
- Cartridge reagent foil is pierced by air when instrument starts
- Instrument performs all bind-wash-elution steps
- Run takes ~20 minutes
- Product is high quality dsDNA

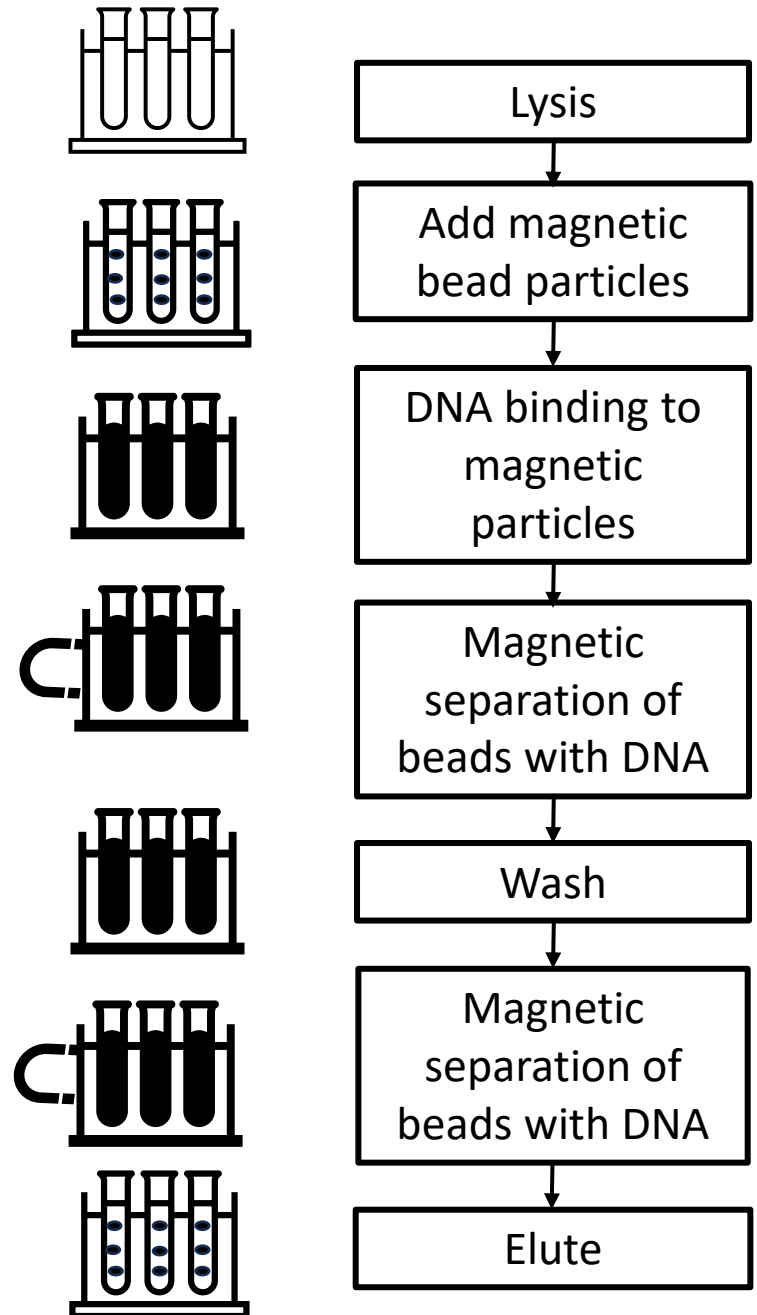


DNA Extraction Instrument EZ1: Binding

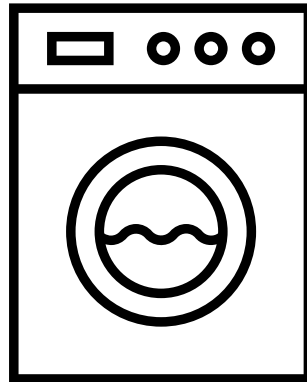


DNA Extraction

- **Lysis** of cell and nuclear membranes
 - Disrupt amphipathic phospholipid bilayers
- **Bind** DNA to magnetic silica resin particles or membrane
 - Isolate DNA from proteins (including DNases), lipids, carbohydrates, and organelles
 - Low pH and high chaotropic salt solution
- **Wash**
 - Remove protein, lipid, and carbohydrate macromolecules and small molecules
- **Elute**
 - Alkaline or basic conditions and low salt solution
 - High quantity and quality for downstream analysis

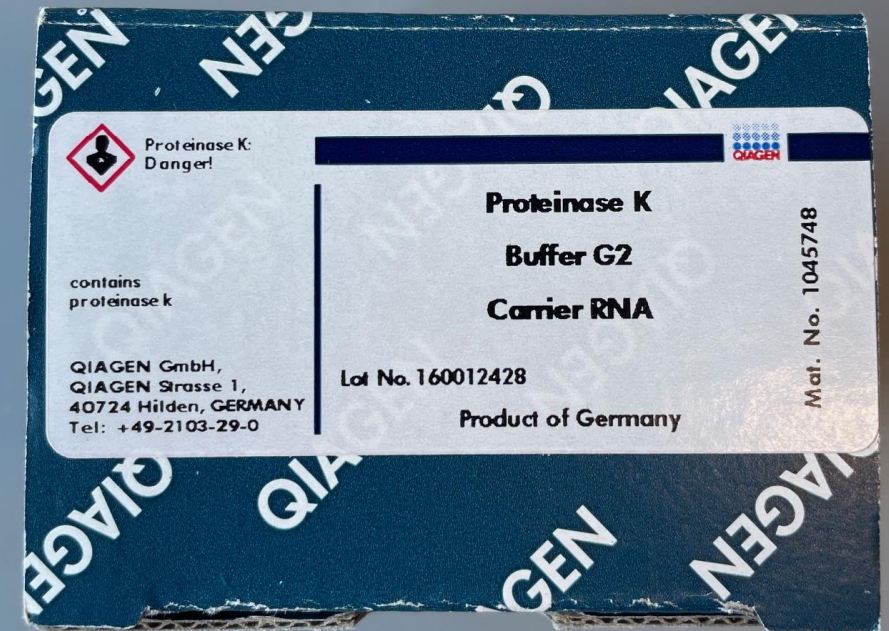


What are the *functions* of the DNA extraction reagents?



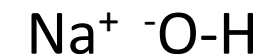
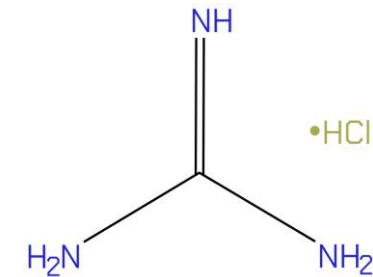
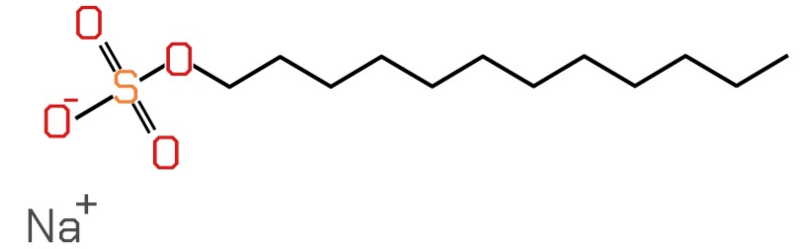
DNA Extraction: Lysis Agents

- Buffer G2
 - 800 mM guanidine hydrochloride; 30 mM Tris•Cl, pH 8.0; 30 mM EDTA, pH 8.0; 5% Tween 20; 0.5% Triton X-100
- Enzymatic - Proteinase K
 - Non-specific serine protease active from pH 4-12.5, at high temperatures and in chemical conditions
 - Digests proteins including histones and enzymes including DNases
 - Activity is enhanced by Tween 20, a non-ionic surfactant that solubilizes proteins
- Binding enhancement - Carrier RNA
 - Improves DNA yield especially for short fragments by increasing surface area binding



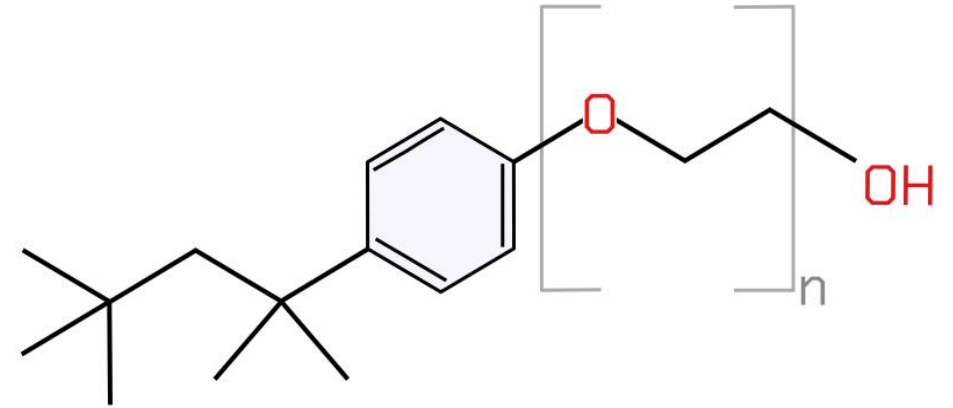
DNA Extraction: Lysis Agents

- Chemical agents
 - Sodium dodecyl sulfate (SDS) - anionic amphipathic detergent that disrupts H-bonding and solubilizes the phospholipids and proteins
 - Guanidinium hydrochloride (GdHCl) - chaotropic agent that denatures proteins and disrupts H-bonding networks among water molecules and in proteins
 - Guanidinium thiocyanate (GdSCN) – chaotropic agent that denatures proteins and disrupts H-bonding networks among water molecules and in proteins
 - NaOH - denatures DNA and proteins by disrupting H-bonding networks



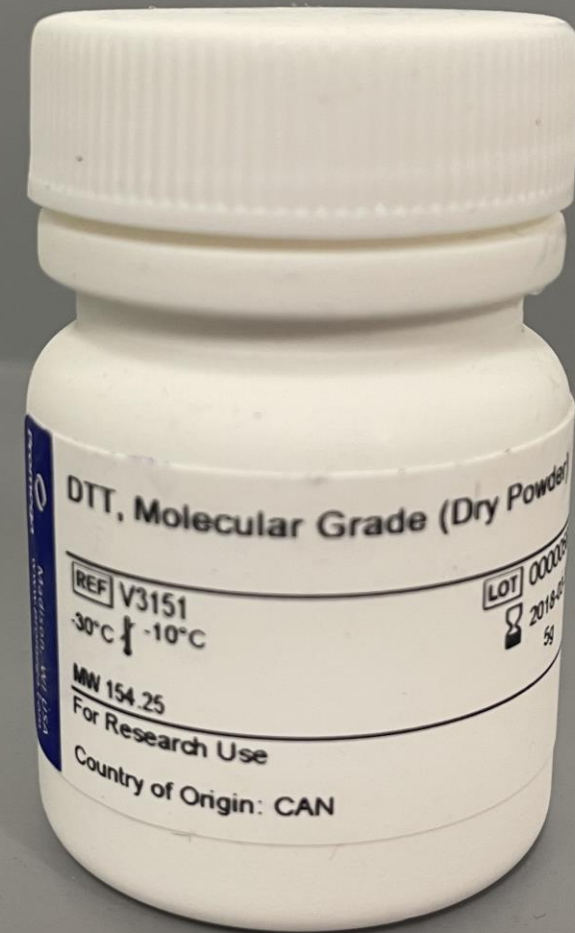
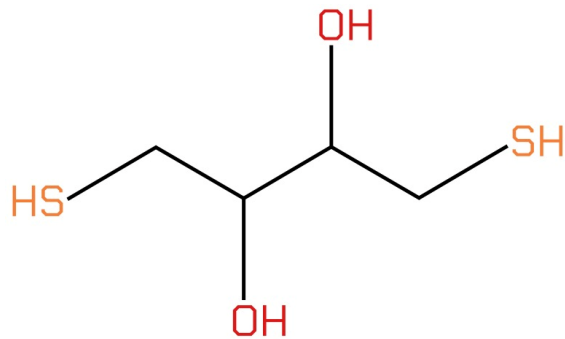
DNA Extraction: Lysis Agents

- Chemical agents
 - Triton X-100 – mild non-denaturing non-ionic surfactant and emulsifier detergent



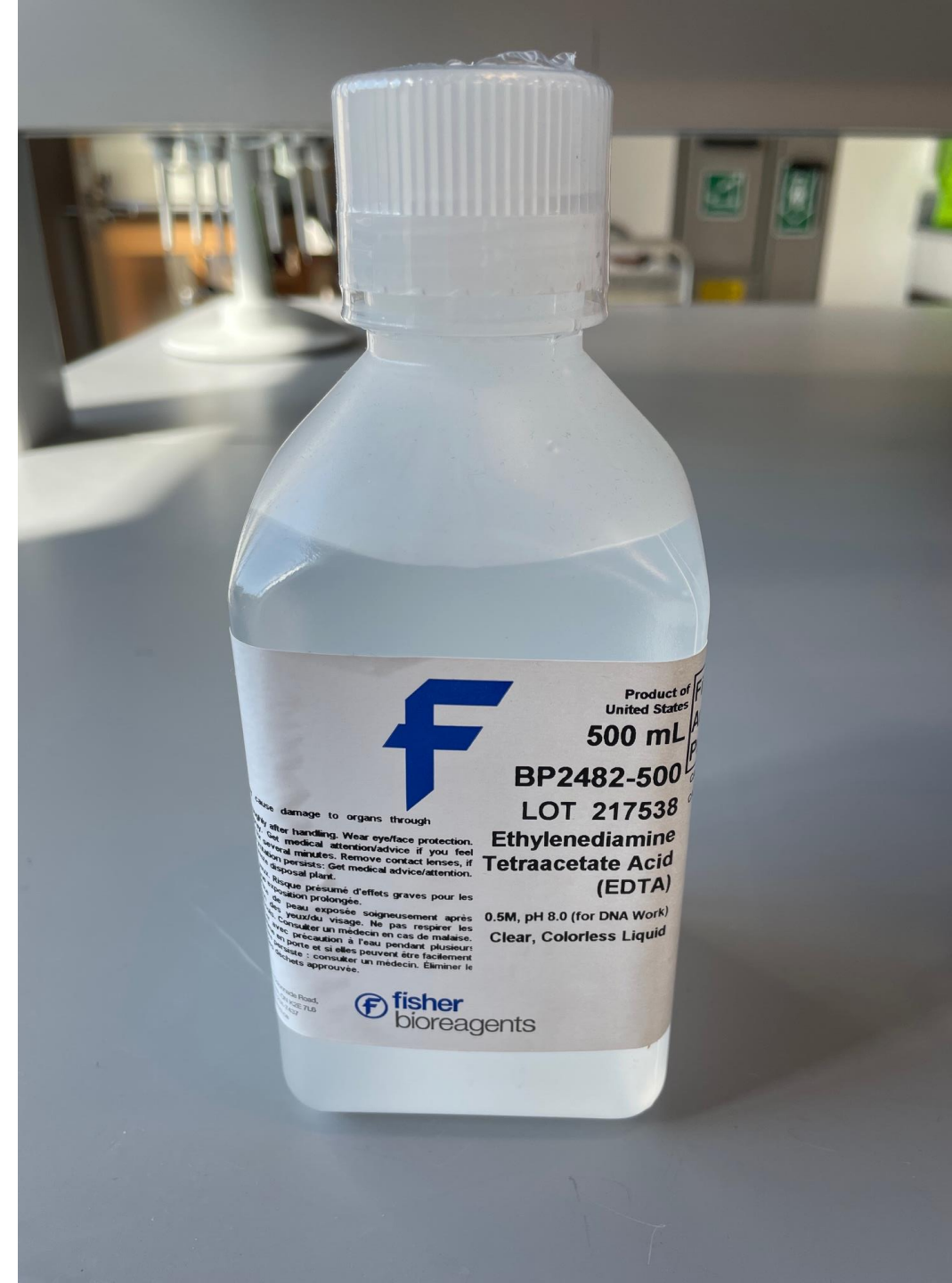
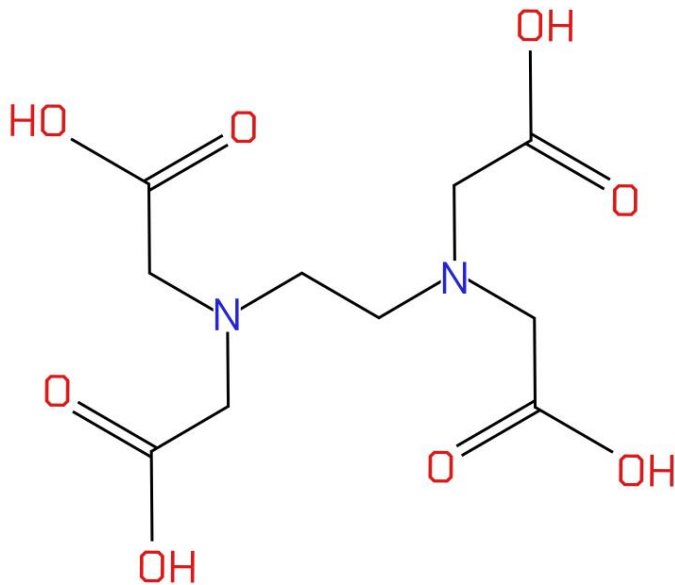
DNA Extraction: Lysis Agents

- Dithiothreitol (DTT)
 - Reduces disulfide bonds in proteins including sperm nuclear membrane proteins



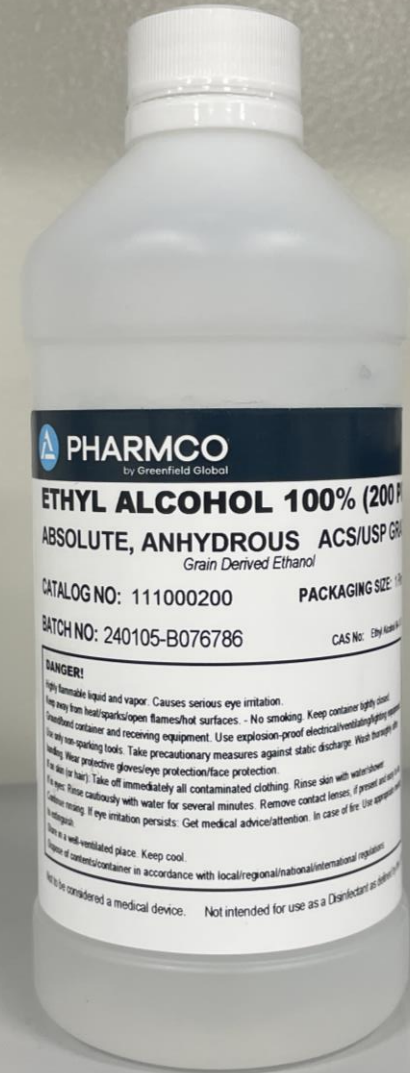
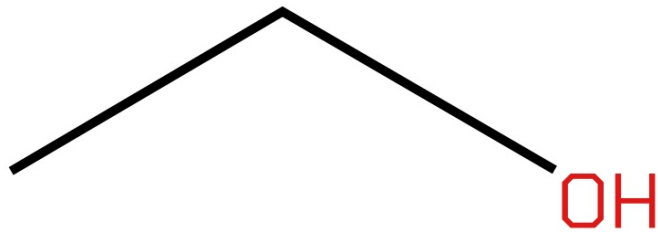
DNA Extraction: Lysis Agents

- Ethylenediaminetetraacetic acid (EDTA)
 - Demineralizes bone and teeth
 - Binds divalent metal ions including calcium and magnesium at its carboxylates



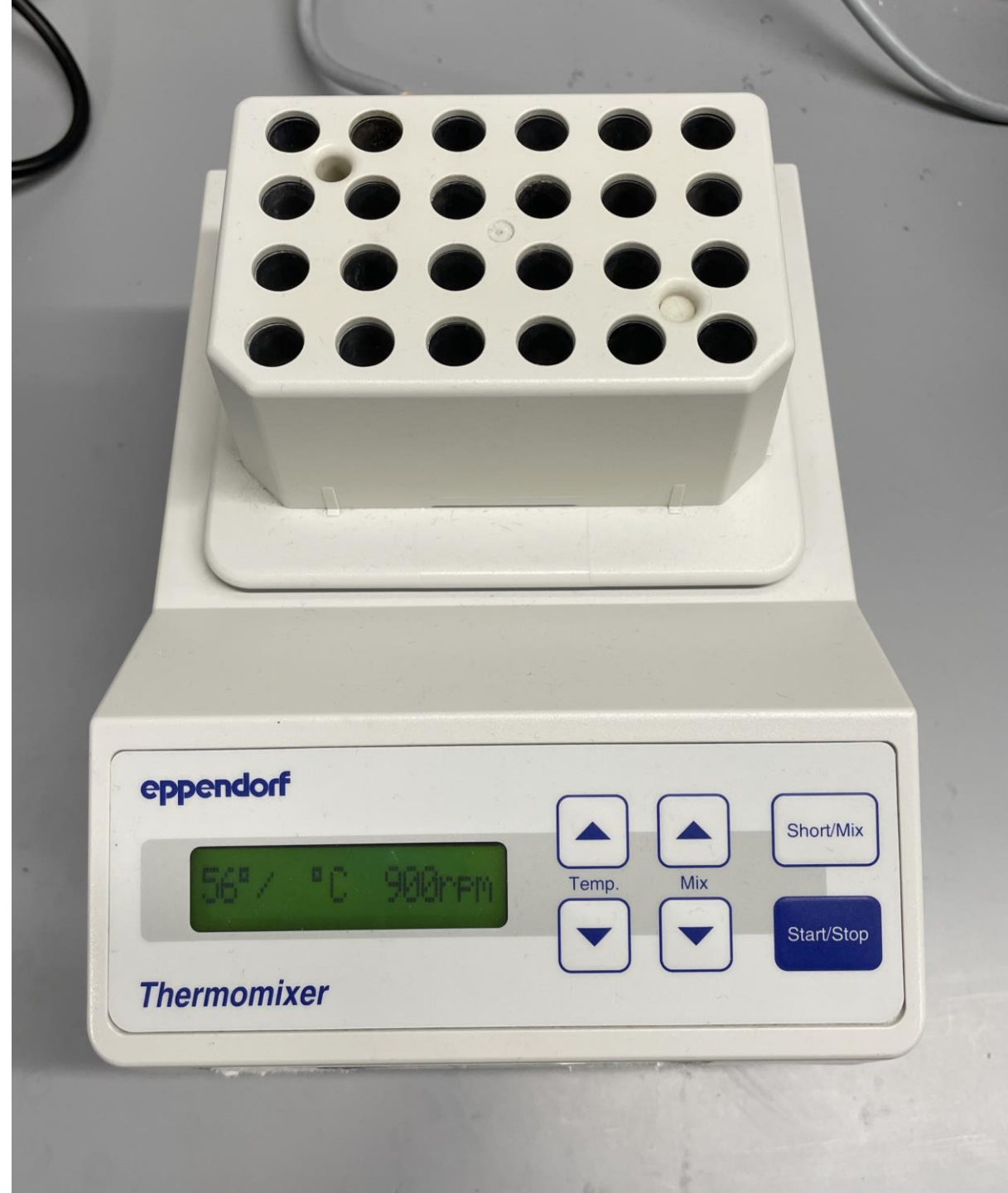
DNA Extraction: Lysis Agents

- Ethanol ($\text{CH}_3\text{-OH}$)
 - Precipitates DNA



DNA Extraction: Lysis Agents

- Heat denaturing agent
 - Membrane fluidifies at 56 °C
 - Proteins are denatured with heat rendering them inactive
- Physical agitation
 - Upsets membrane



DNA Extraction

EZ1 Reagents

Lysis

- General lysis buffer (G2) and proteinase K for forensic samples including semen, blood, saliva, trace cellular material
 - DTT for sperm
- G2 and 0.5 M EDTA followed by MTL for bone and teeth
- Animal tissue lysis buffer (ATL) and proteinase K for epithelial cells

Bind

- Positively charged magnetic silica resin particles
- Magnet
- Chaotropic salt

Wash

Elute

- TE, pH 8.0
- Water

DNA Extraction Incubation Volumes: EZ1

Blood/saliva - Trace
Up to 50 μ L
140-190 μ L G2, 10 μ L ProK

FTA – Trace/Tip Dance
4 x 3 mm punches
290 μ L G2, 10 μ L ProK

Surface swab – Trace/Tip Dance
1 swab
290 μ L G2, 10 μ L ProK

Soil -- Trace/Tip Dance
Up to 0.5 g
100 μ L G2

Blood/saliva stain - Trace
0.5 cm²
290 μ L G2, 10 μ L ProK

Hair - Trace
0.5 - 1 cm
160 μ L G2, 20 μ L ProK, 20 μ L DTT

Nail clipping - Trace
1 clipping
160 μ L G2, 20 μ L ProK, 20 μ L DTT

Semen - Large Volume
>0.5 cm²
455 μ L G2, 25 μ L ProK, 20 μ L DTT

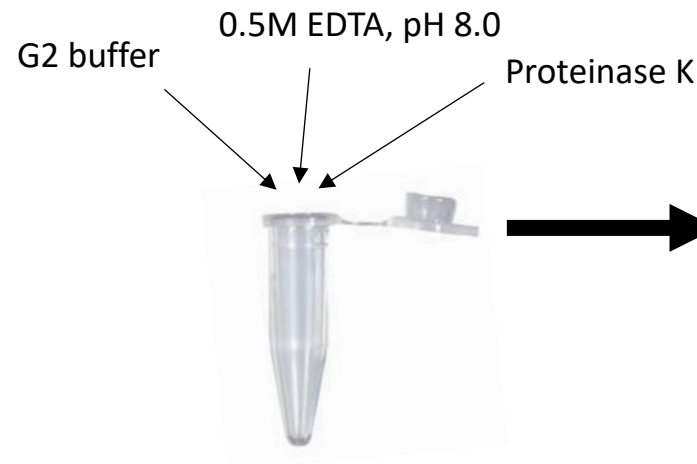
Tissues - Trace
Up to 10 mg
190 μ L G2, 10 μ L ProK

Chewing gum – Trace/Tip Dance
up to 40 mg
190 μ L G2, 10 μ L ProK

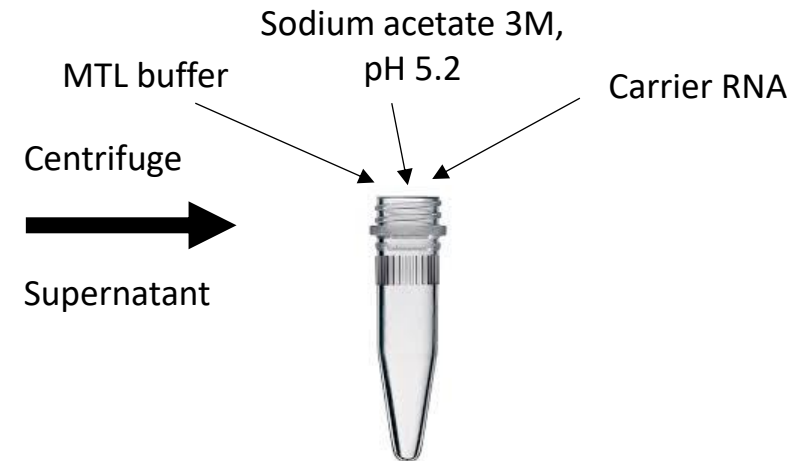
Bone - Large Volume
Up to 150 mg
225 μ L G2, 25 μ L ProK, 250 μ L of 0.5 M EDTA pH 8.0, after incubation 400 μ L MTL buffer, 50 μ L 3 M sodium acetate pH of 5.2, 1 μ L carrier RNA

Cigarette butts – Trace/Tip Dance
1 cm²
190 μ L G2, 10 μ L ProK

Qiagen Supplementary Large Volume Protocol: Extraction of DNA from Bone or Teeth



24 hours @ 56°C
and 750 rpm



< 200 mg bone powder

225 μ L G2, 25 μ L ProK, 250 μ L of 0.5 M EDTA pH 8.0
400 μ L MTL buffer, 50 μ L 3 M sodium acetate pH of 5.2, 1 μ L carrier RNA

DNA Extraction: DNA IQ Manual Kit Steps

To sample, add
386 μL Casework Extraction Buffer, 10 μL ProK (18 mg/ml), 4 μL 1-Thioglycerol

Vortex 5 s and incubate at 56 °C for 30 min

Remove substrate with tweezers and separate with a spin basket

Centrifuge 2 min, add 40 μL of lysis buffer to eluent, vortex 5-10 s

Vortex DNA IQ™ Resin 10 s, add 7 μL to sample

Vortex sample/buffer/resin mixture 3 s
Incubate RT for 5 min
Vortex 3 s once every min and 2 s at end

Place tubes on magnetic stand

Remove liquid carefully (not beads)

Add 100 μL lysis buffer, remove from stand, vortex 2 s

Place on magnetic stand and remove/discard lysis buffer

Add 100 μL 1X wash buffer, remove from stand, vortex 2 s

Place on magnetic stand and remove/discard wash buffer, repeat 2x more for 3 washes

Remove all wash buffer, open the tubes and air-dry resin for 5 min

Add 25-100 μL of elution buffer, vortex 2 s, incubate at 65 °C 5 min, vortex 2 s

Place on magnetic stand and obtain the DNA-containing solution to new tube, store -20 °C

DNA Extraction: Differential Extraction

- Cut out 2 cm² of stain or cut swab tip to a spin basket in a sterile tube.
- Add 15 µL of 20 mg/mL Proteinase K and 285 µL Gill Extraction buffer to each sample.
- Incubate at 60 °C for 1 hour.
- Centrifuge for 5 min and remove substrate and spin basket from tube. Do not disturb pellet.

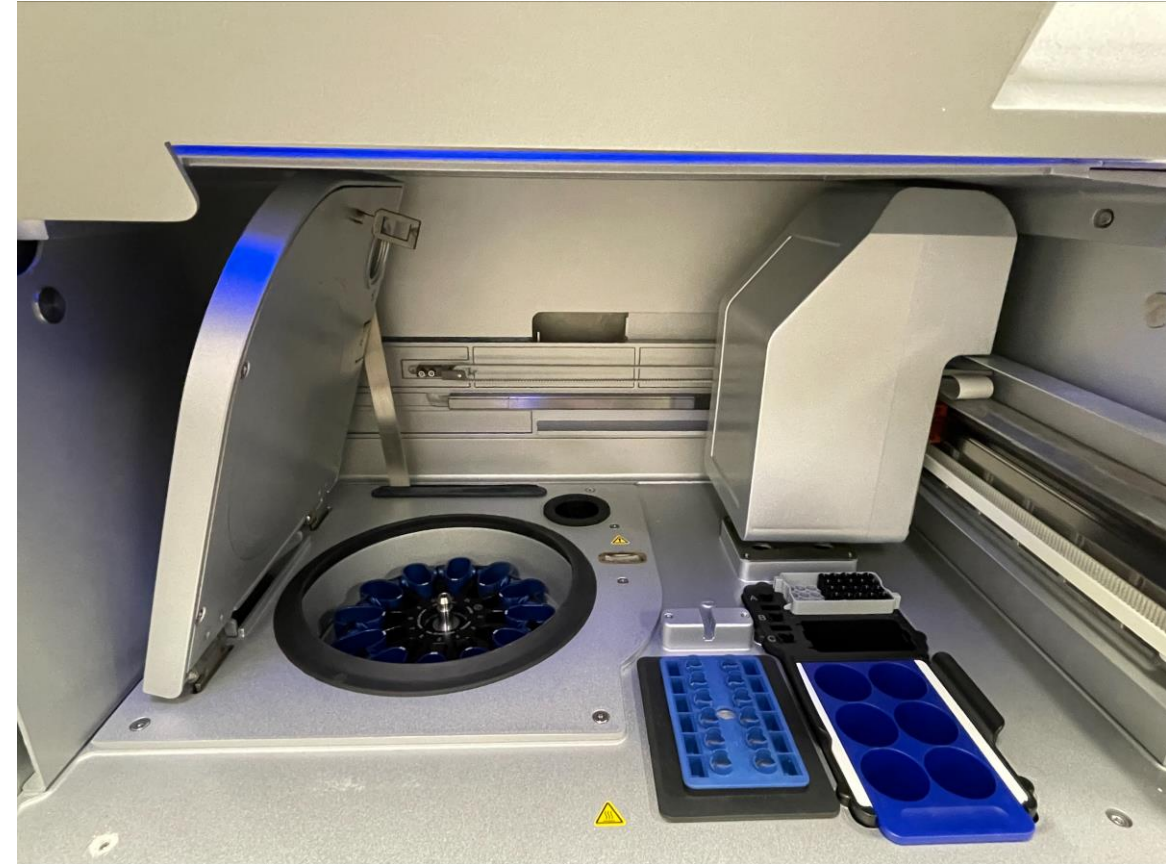
- Remove the **supernatant** to a new **non-sperm fraction** tube.

- Retain and label the tube containing the **pellet** as **sperm fraction**.
- Wash the sperm pellet with 300 µL of Gill Extraction buffer. Centrifuge 3 min. Remove supernatant and discard; if no pellet is visible, retain 15-20 µL of solution.
- Add 10 µL of 20 mg/mL ProK, 250 µL Gill buffer, and 20 µL 0.8 M DTT to each sperm pellet tube.
- Vortex for 20 seconds and incubate at 60 °C for 2 hours.

- In a fume hood, add 300 µL buffer saturated phenol/chloroform/isoamyl alcohol (PCIA) (25:24:1) pH 8.0 to each of the sperm and non-sperm fractions.
- Centrifuge 5 min at top speed and remove the top aqueous layer to a new tube. Reextract sample with additional PCIA reagent and combine aqueous layer in new tube.
- Add 100 µL of TE buffer to a Microcon 100/Microcon concentrator/filter assembly. Centrifuge for 5 min.
- Transfer up to 400 µL of the aqueous phase to the Microcon filter and centrifuge. Discard waste effluent.
- Add 100 µL of TE buffer to Microcon to wash and centrifuge.
- Invert the Microcon filter upside down inside a new Microcon tube. Elute DNA with 50 µL TE buffer or water. Store solution at –20 °C.

DNA Extraction Instrument Parameters: QIAcube

- Apply validated or manufacturer's protocol
- Can perform differential and non-differential extractions
- Fraction separation
- Washes and lyses sperm pellet
- Pre-run reminder
 - Make sure tubes click in or they will dislodge



DNA Extraction Instrument Parameters: Maxwell

- Apply validated or manufacturer's protocol
- Pre-run reminder
 - Maxwell – open reagent kit and tubes



DNA Extraction: Zygem One-Step Sperm Lysis

- Enzymatic, single-tube purification
 - Acrosolv mesophilic and forensicGEM thermophilic proteases
 - 52 - 75 - 95 °C incubation steps
- For use in sexual assault (SA) cases involving a biological male assailant and a biological female victim
- Do not use on SA cases that are:
 - Biological male (s) and biological male (v) cases
 - Biological female (s) and biological female (v) cases
 - Biological female (s) and biological male (v) cases

DNA Extraction: Sonication

- Digestion buffer
- Proteinase K
- Sonicator

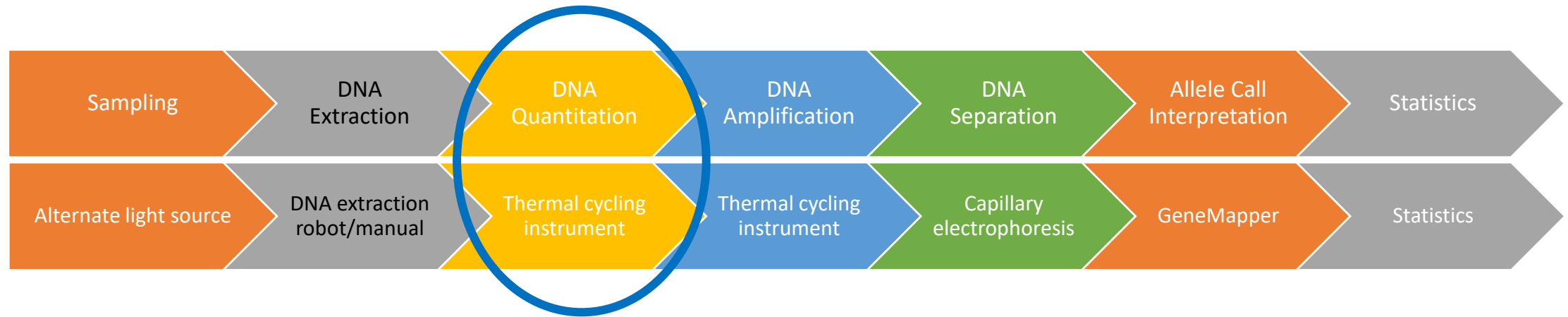


Post-DNA Extraction- Concentration

- Ethanol precipitation
- Microcon
- EZ1 reextraction



DNA Typing Process & Instrumentation



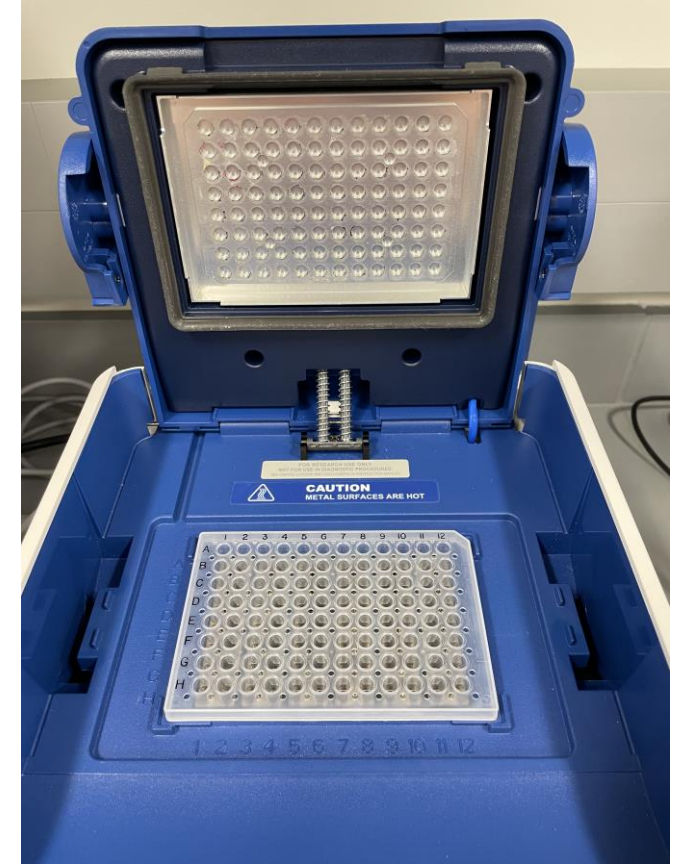
Thermal Cycling Instruments

- Veriti (Applied Biosystems)
- Rotor-gene Q (Qiagen)
- 7500 (Applied Biosystems)
- MultiGene (Labnet)



Thermal Cycling Running Parameters

- Use thin-walled tubes and tubes/plates that fit snugly into the well for best heat exchange
- Apply locking ring to Rotor-Gene prior to starting so that the tube caps do not detach, and the plastic does not get into the instrument, melt and get lodged in
- Apply mineral oil to the surface of the PCR reaction mix to reduce evaporation if the thermal cycler does not have a heated lid
- Input the validated/published PCR temperature and other parameters for DNA quantitation of STR typing

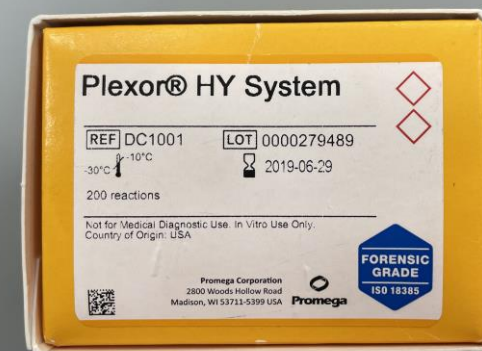


Thermal Cycling Parameters

- Vary by kit polymerases, primer sequences, reagent concentrations, and multiplex optimization
- Variables
 - Temperature
 - Time
 - Number of step repeats
 - Ramp speed
 - Dye detection (for real-time PCR)

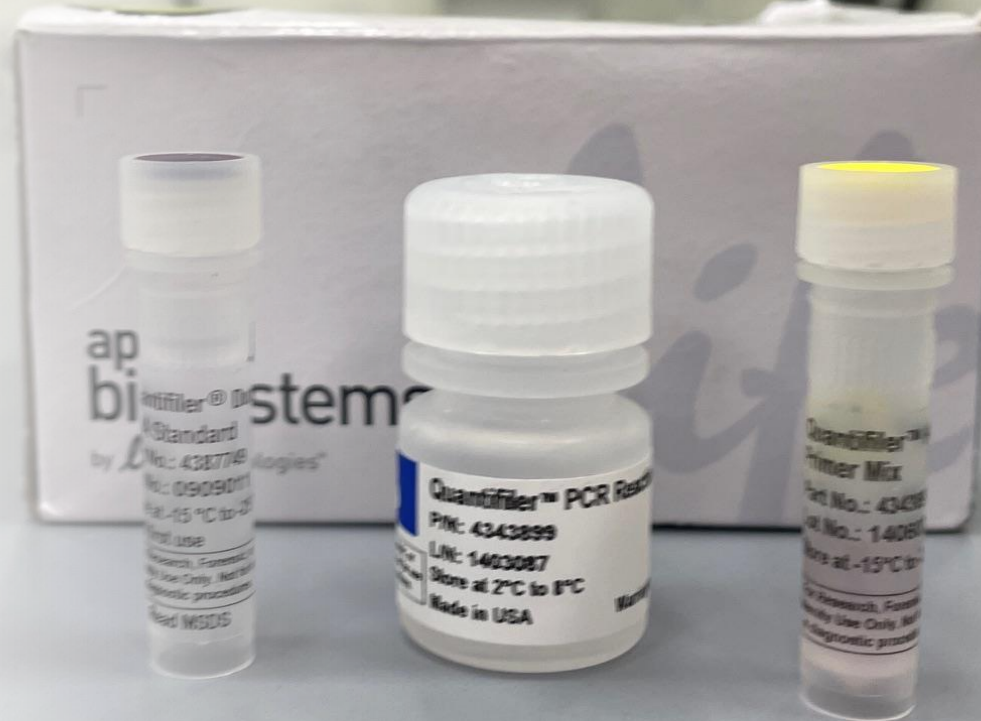
PCR DNA Quantitation Kits for Forensic Applications

- Quantifiler
- Quantifiler Y
- Quantifiler DUO
- Quantifiler TRIO
- Plexor HY
- Investigator Quantiplex Pro RGQ



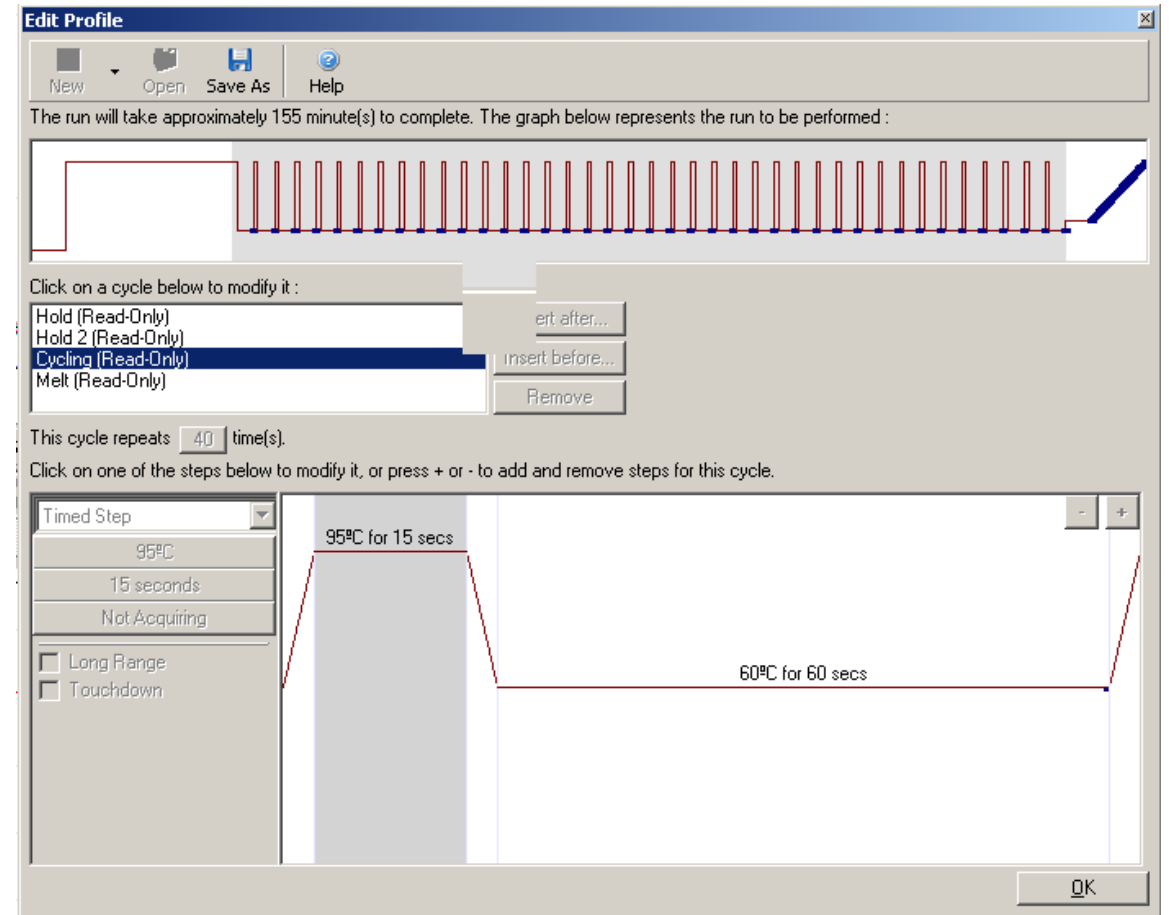
Quantitation Kits

- Master mix
- Primer set
- Standard control DNA
- Nuclease-free water



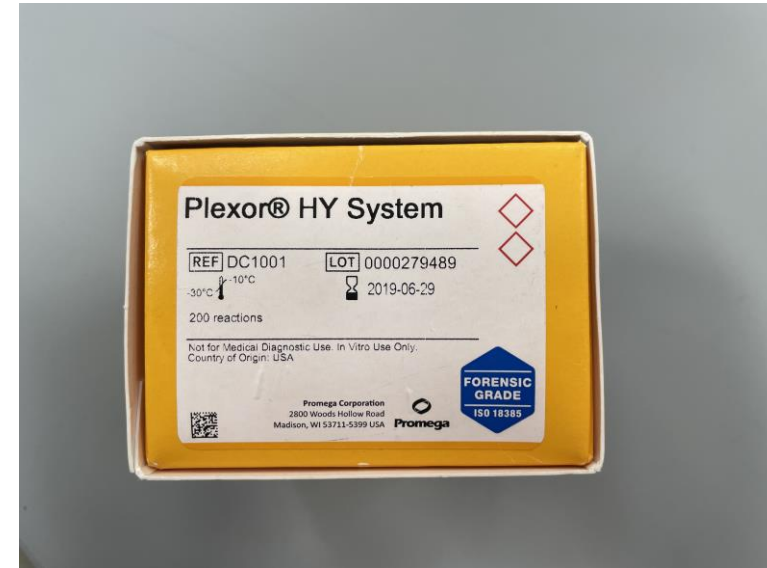
Thermal Cycler Run Parameters: Quantifiler™

- Standards: 0.023-50 ng/mL
- 10 µL primer mix, 12.5 µL PCR reaction mix, 2 µL of sample, standard, or control
- Hold
 - 50 °C for 2 min
- Denature
 - 95 °C for 10 min
- Two-step denature-anneal/extend cycle (40x):
 - 95 °C for 15 s
 - 60 °C for 60 s



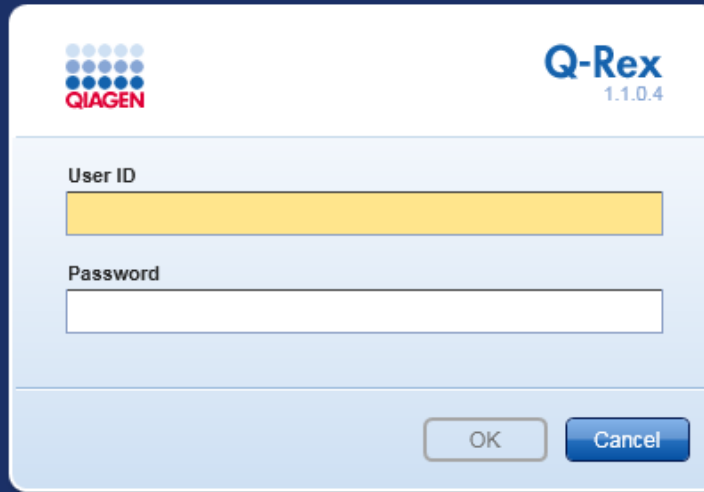
Thermal Cycler Run Parameters: Plexor® HY System

- Standards: 0.0032-50 ng/mL
- 10 µL PCR master mix, 1 µL primer/IPC mix, 7 µL amplification grade water, 2 µL of sample, standard, or control
- Denature
 - 95 °C for 2 min
- Two-step denature-anneal/extend cycle (38x):
 - 95 °C for 5 s
 - 60 °C for 35 s



Thermal Cycler Run Parameters: Quantiplex Pro RGQ in Q-Rex System

- Standards: 0.0025-50 ng/mL
- 9 μ L PCR master mix, 9 μ L primer mix, 2 μ L of sample, standard, or control
- Denature
 - 95 °C for 3 min
- Two-step denature-anneal/extend cycle (40x):
 - 95 °C for 5 s
 - 60 °C for 10 s (detect fluorescence data using the green, yellow, orange, red and crimson channels with auto-gain optimization)



The image shows a login dialog box for the Q-Rex system, version 1.1.0.4. The dialog box has a white header with the QIAGEN logo on the left and the text 'Q-Rex 1.1.0.4' on the right. Below the header, there are two input fields: 'User ID' with a yellow background and 'Password' with a white background. At the bottom right, there are two buttons: 'OK' and 'Cancel'.

Q-Rex

File Help

My Q-RexExperimentsServiceConfiguration

Rotor

Create Experiment

Create experiment by wizard

New Experiment

☆ Favorite Templates

Create experiment from template

Plate layout for 0.005 - 50

Template RGQ 0.0025-50

Create from other template...

🕒 Recent Experiments

Open or create from experiment

20200217_FRSC695_461

20190629_213447_Akt...

20240215_201138_FRS...

20240508_155118_New...

112119_AK_Bone samp...

20240219_173503_Ran...

20240411_165616_Vali...

Open other experiment...

July 12, 2024Administrator Administrator

General

Run Profile

Sample Layout

Run

Analysis

Absolute Quantificat...

Report/Export

Experiment information

Comment

Experiment operator

Administrator Administrator

Creation date

07/12/2024 11:05:17

Assay setup file or worklist

Template

Template RGQ 0.0025-50.qret

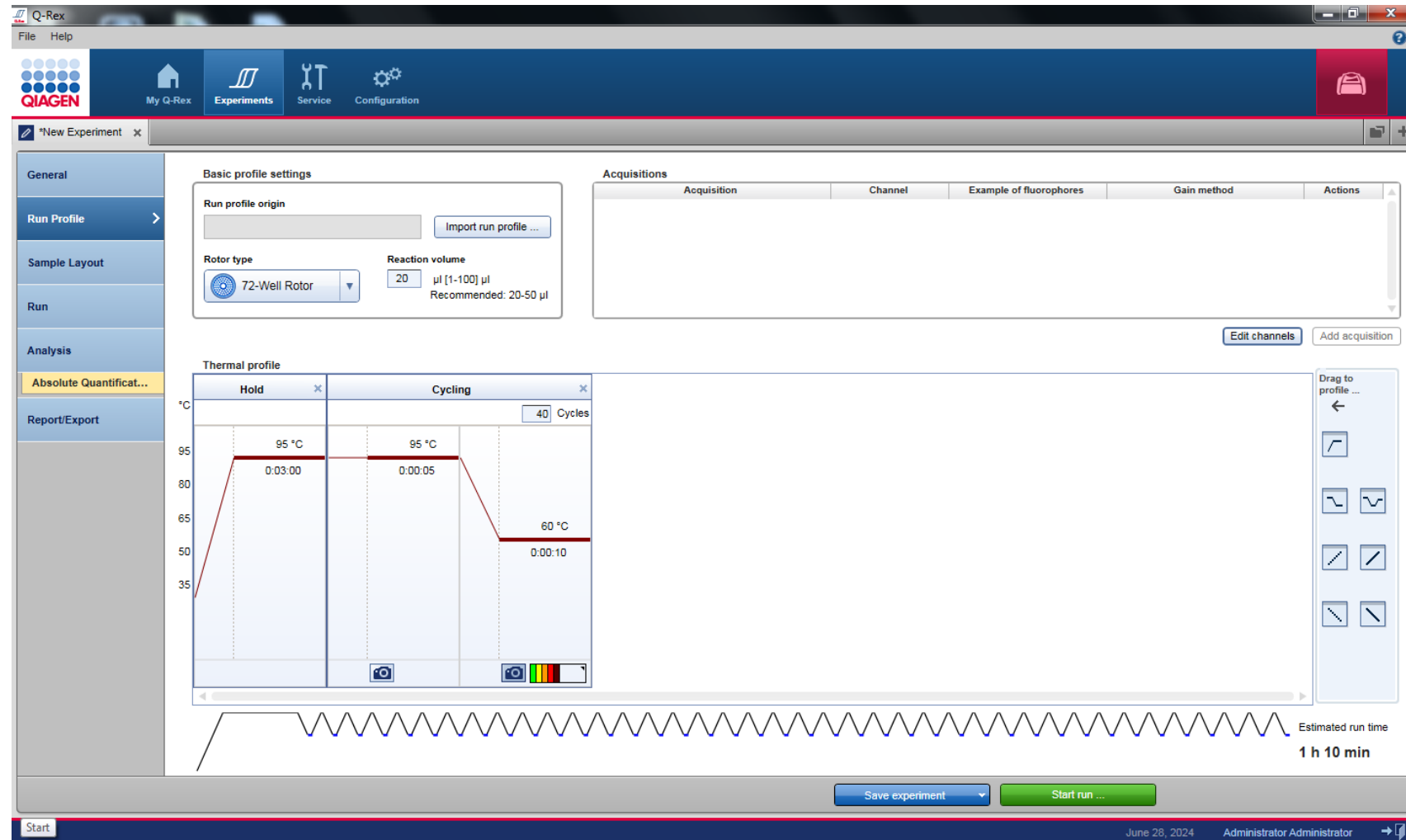
Kit information

Kit name	Catalog / Material number	Lot number	Kit expiry date	
Investigator Quantiplex Pro RGQ Kit	-		9/11/2023	X

Add QIAGEN kit ...

Thermal Cycle Parameters: Quantiplex Pro RGQ in Q-Rex

- Denature
 - 95 °C for 3 min
- Two-step denature-anneal/extend cycle (40x):
 - 95 °C for 5 s
 - 60 °C for 10 s



Thermal Cycle Parameters: Quantiplex Pro RGQ in Q-Rex Standard Input

Q-Rex

File Help

QIAGEN

My Q-Rex Experiments Service Configuration

Rotor

20240712_11... x

General

Run Profile

Sample Layout

Run

Analysis

Absolute Quantificat...

Report/Export

Tube	Color	Style	Name	Type	Green			Yellow			Orange			Red		
					Target	Conc.	Unit	Target	Conc.	Unit	Target	Conc.	Unit	Target	Conc.	Unit
1	Red	—	50	Standard	Green	50	ng/ul	Yellow	50	ng/ul	Orange	50	ng/ul	Red	50	ng/ul
2	Red	—	50	Standard	Green	50	ng/ul	Yellow	50	ng/ul	Orange	50	ng/ul	Red	50	ng/ul
3	Green	—	1.8519	Standard	Green	1.8519	ng/ul	Yellow	1.8519	ng/ul	Orange	1.8519	ng/ul	Red	1.8519	ng/ul
4	Green	—	1.8519	Standard	Green	1.8519	ng/ul	Yellow	1.8519	ng/ul	Orange	1.8519	ng/ul	Red	1.8519	ng/ul
5	Magenta	—	0.0686	Standard	Green	0.0686	ng/ul	Yellow	0.0686	ng/ul	Orange	0.0686	ng/ul	Red	0.0686	ng/ul
6	Magenta	—	0.0686	Standard	Green	0.0686	ng/ul	Yellow	0.0686	ng/ul	Orange	0.0686	ng/ul	Red	0.0686	ng/ul
7	Blue	—	0.0025	Standard	Green	0.0025	ng/ul	Yellow	0.0025	ng/ul	Orange	0.0025	ng/ul	Red	0.0025	ng/ul
8	Blue	—	0.0025	Standard	Green	0.0025	ng/ul	Yellow	0.0025	ng/ul	Orange	0.0025	ng/ul	Red	0.0025	ng/ul
9	Orange	—	NTC	NTC	Green	0	ng/ul	Yellow	0	ng/ul	Orange	0	ng/ul	Red	0	ng/ul
10	Orange	—	NTC	NTC	Green	0	ng/ul	Yellow	0	ng/ul	Orange	0	ng/ul	Red	0	ng/ul
11	Dark Blue	—		Not in use	Green	0	ng/ul	Yellow	0	ng/ul	Orange	0	ng/ul	Red	0	ng/ul
12	Dark Blue	—		Not in use	Green	0	ng/ul	Yellow	0	ng/ul	Orange	0	ng/ul	Red	0	ng/ul
13	Light Green	—		Not in use	Green	0	ng/ul	Yellow	0	ng/ul	Orange	0	ng/ul	Red	0	ng/ul
14	Light Green	—		Not in use	Green	0	ng/ul	Yellow	0	ng/ul	Orange	0	ng/ul	Red	0	ng/ul
15	Dark Grey	—		Not in use	Green	0	ng/ul	Yellow	0	ng/ul	Orange	0	ng/ul	Red	0	ng/ul
16	Dark Grey	—		Not in use	Green	0	ng/ul	Yellow	0	ng/ul	Orange	0	ng/ul	Red	0	ng/ul
17	Blue	—		Not in use	Green	0	ng/ul	Yellow	0	ng/ul	Orange	0	ng/ul	Red	0	ng/ul
18	Light Green	—		Not in use	Green	0	ng/ul	Yellow	0	ng/ul	Orange	0	ng/ul	Red	0	ng/ul
19	Dark Purple	—		Not in use	Green	0	ng/ul	Yellow	0	ng/ul	Orange	0	ng/ul	Red	0	ng/ul
20	Brown	—		Not in use	Green	0	ng/ul	Yellow	0	ng/ul	Orange	0	ng/ul	Red	0	ng/ul
21	Light Green	—		Not in use	Green	0	ng/ul	Yellow	0	ng/ul	Orange	0	ng/ul	Red	0	ng/ul

Define targets

Target	Acquisition	Type
Green	Green	Test
Yellow	Yellow	Test
Orange	Orange	Test
Red	Red	Test

Add target

Define sample groups

Name

Add group

Configure view

Concentration number format

Decimal format

Scientific notation

6

Dec. places

0.123457

Assign well names to tubes

row by row

column by column

Manually define auto-gain tubes

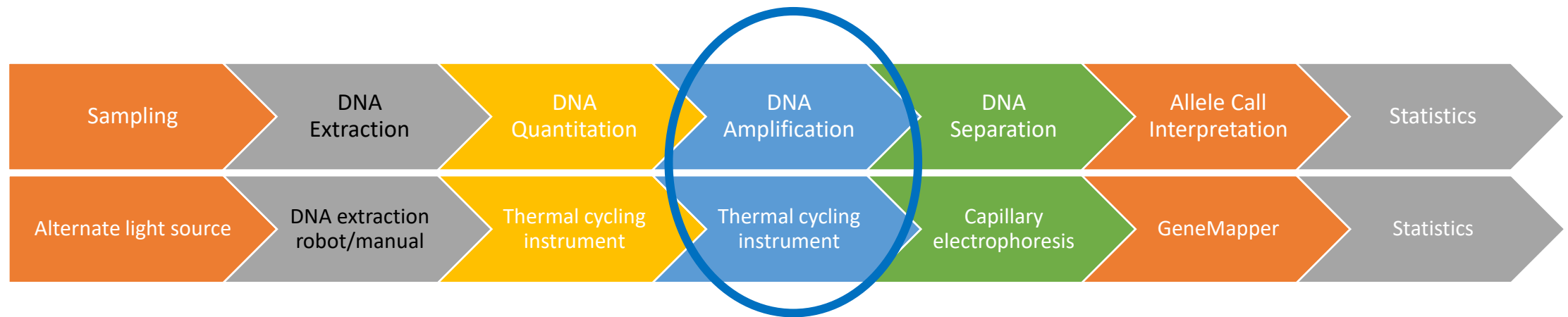
Import sample information

Save experiment

Start run ...

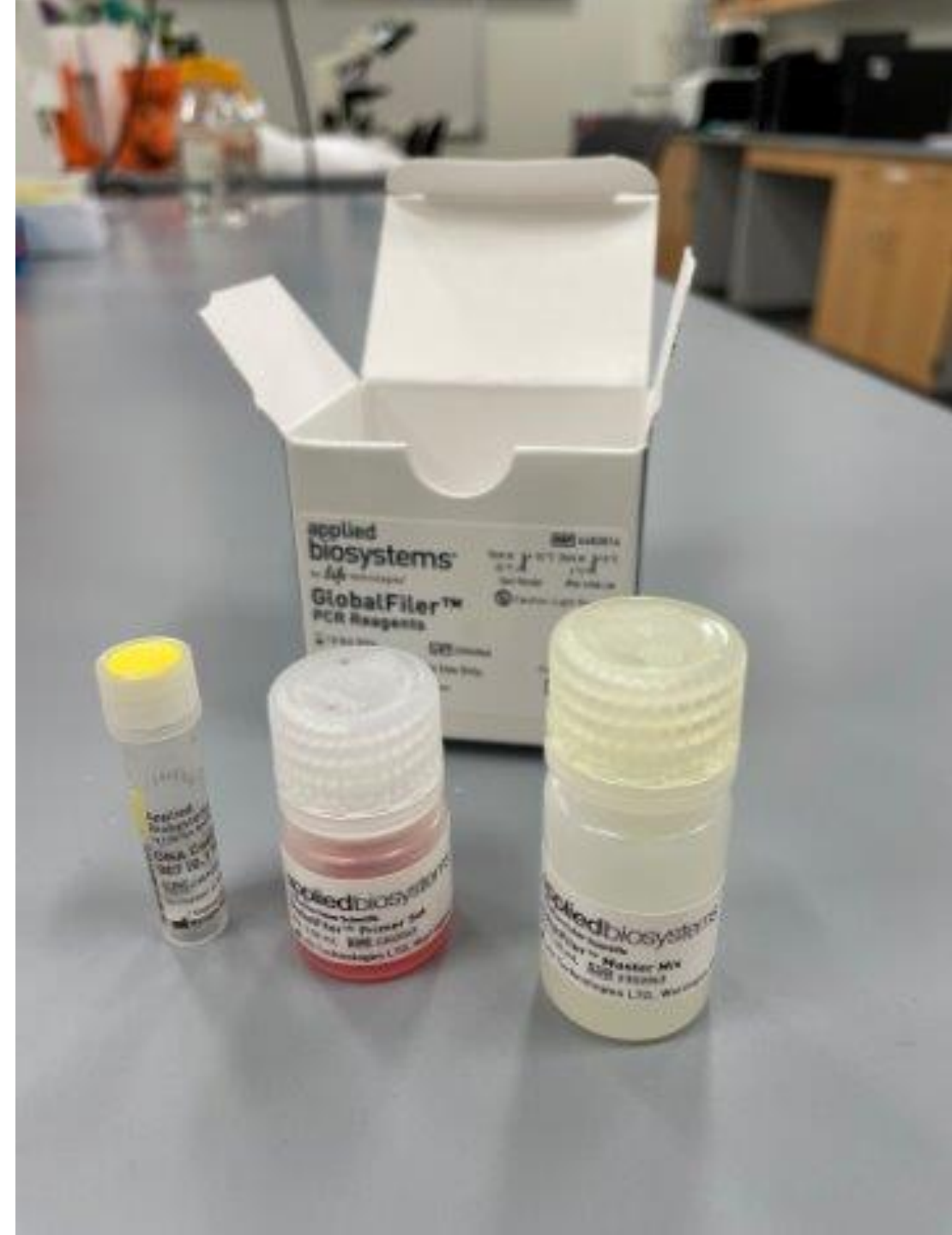
July 12, 2024 Administrator Administrator

DNA Typing Process & Instrumentation



STR Amplification Kits

- Master mix
- Primer set
- Control standard DNA
- Nuclease-free water



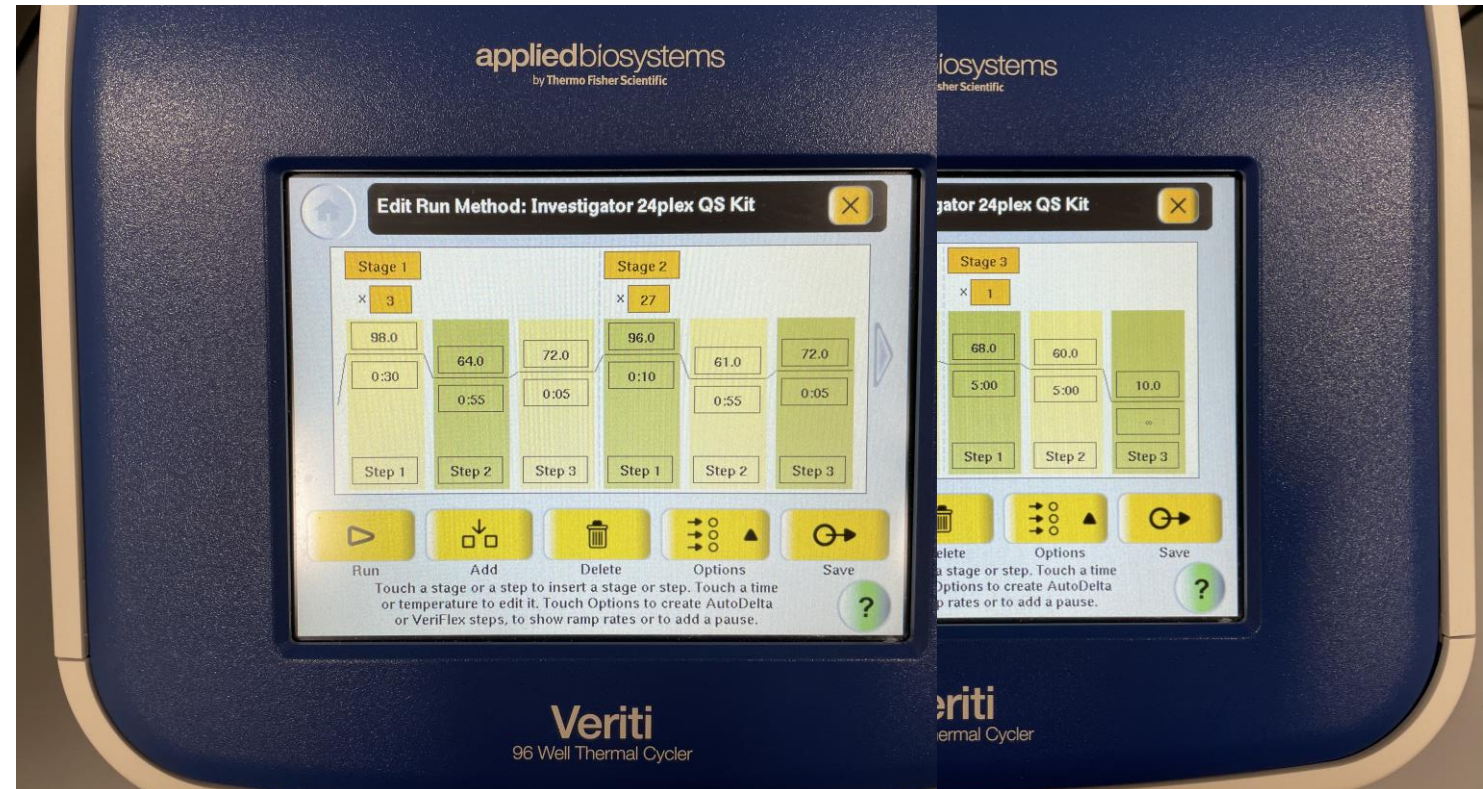
Thermal Cycle Parameters: GlobalFiler™

- Denature
 - 95 °C for 1 min
- Two-step denature-anneal/extend cycle (30x):
 - 94 °C for 10 s
 - 59 °C for 90 s
- Final extension
 - 60 °C for 10 min
- Keep cold / refrigerate
 - 4 °C



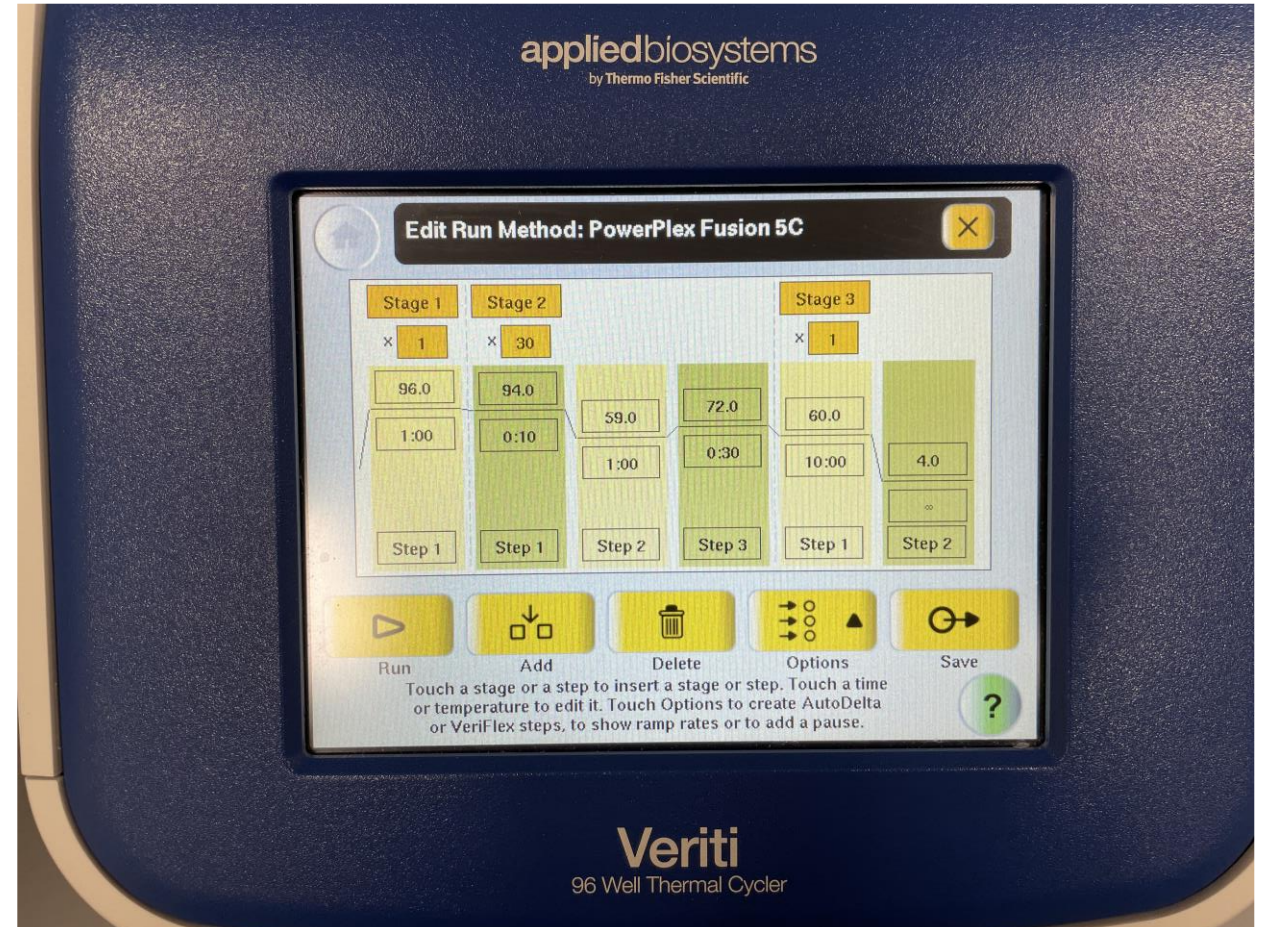
Thermal Cycle Parameters: Investigator 24plex QS

- Denature
 - 98 °C for 30 s
- Anneal
 - 64 °C for 55 s
- Extend
 - 72 °C for 5 s
- Three-step denature-anneal-extend cycle (27x) (lowered annealing-touchdown):
 - 96 °C for 10 s
 - 61 °C for 55 s
 - 72 °C for 30 s
- Final extensions
 - 68 °C for 5 min
 - 60 °C for 5 min
- Keep cold / refrigerate
 - 10 °C



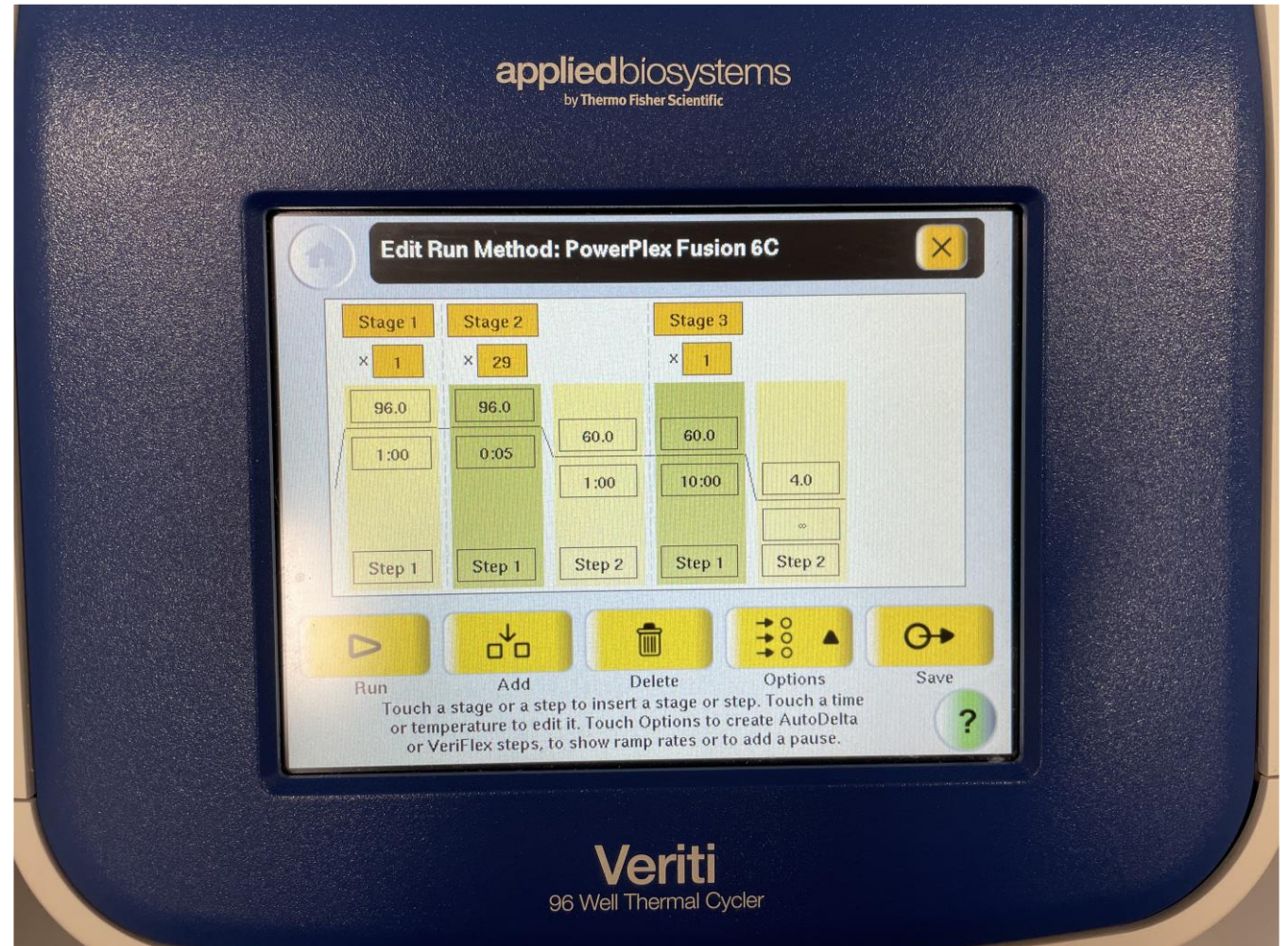
Thermal Cycle Parameters: Fusion 5C

- Denature
 - 96 °C for 1 min
- Three-step denature-anneal-extend cycle (30x):
 - 94 °C for 10 s
 - 59 °C for 60 s
 - 72 °C for 30 s
- Final extension
 - 60 °C for 10 min
- Keep cold / refrigerate
 - 4 °C



Thermal Cycle Parameters: Fusion 6C

- Denature
 - 96 °C for 1 min
- Three-step denature-anneal-extend cycle (30x):
 - 96 °C for 5 s
 - 60 °C for 60 s
 - 72 °C for 30 s
- Final extension
 - 60 °C for 10 min
- Keep cold / refrigerate
 - 4 °C



Thermal Cycle Parameters: PowerPlex 35GY

- Reaction volume: 25 µL
- Denature
 - 96 °C for 1 min
- Three-step denature-anneal-extend cycle (25x, 6 °C / s ramp speed):
 - 98 °C for 5 s
 - 60 °C for 60 s
 - 72 °C for 15 s
- Final extension
 - 60 °C for 10 min
- Keep cold / refrigerate
 - 4 °C

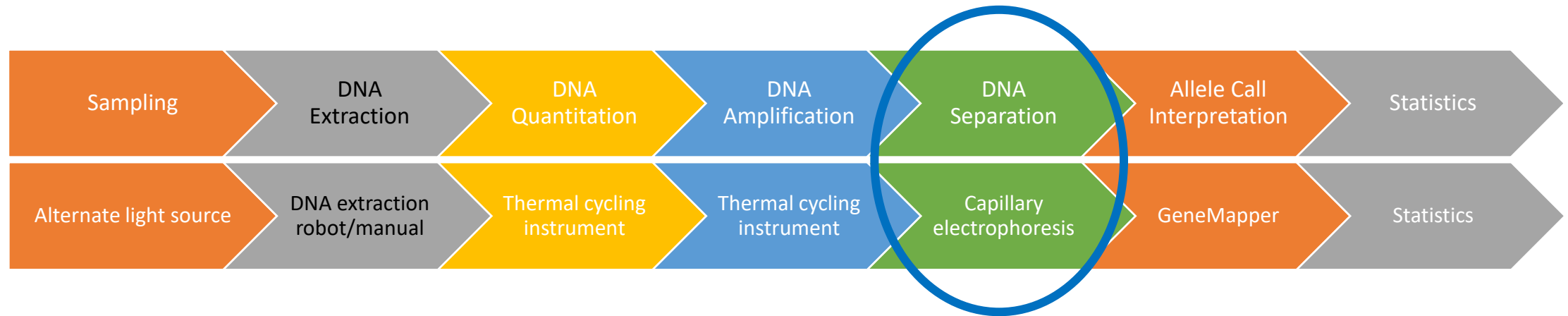


Thermal Cycler Maintenance and Calibration

- Routine, according to requirements for accreditation and lab SOP
- Examples include temperature verification and run cycle performance

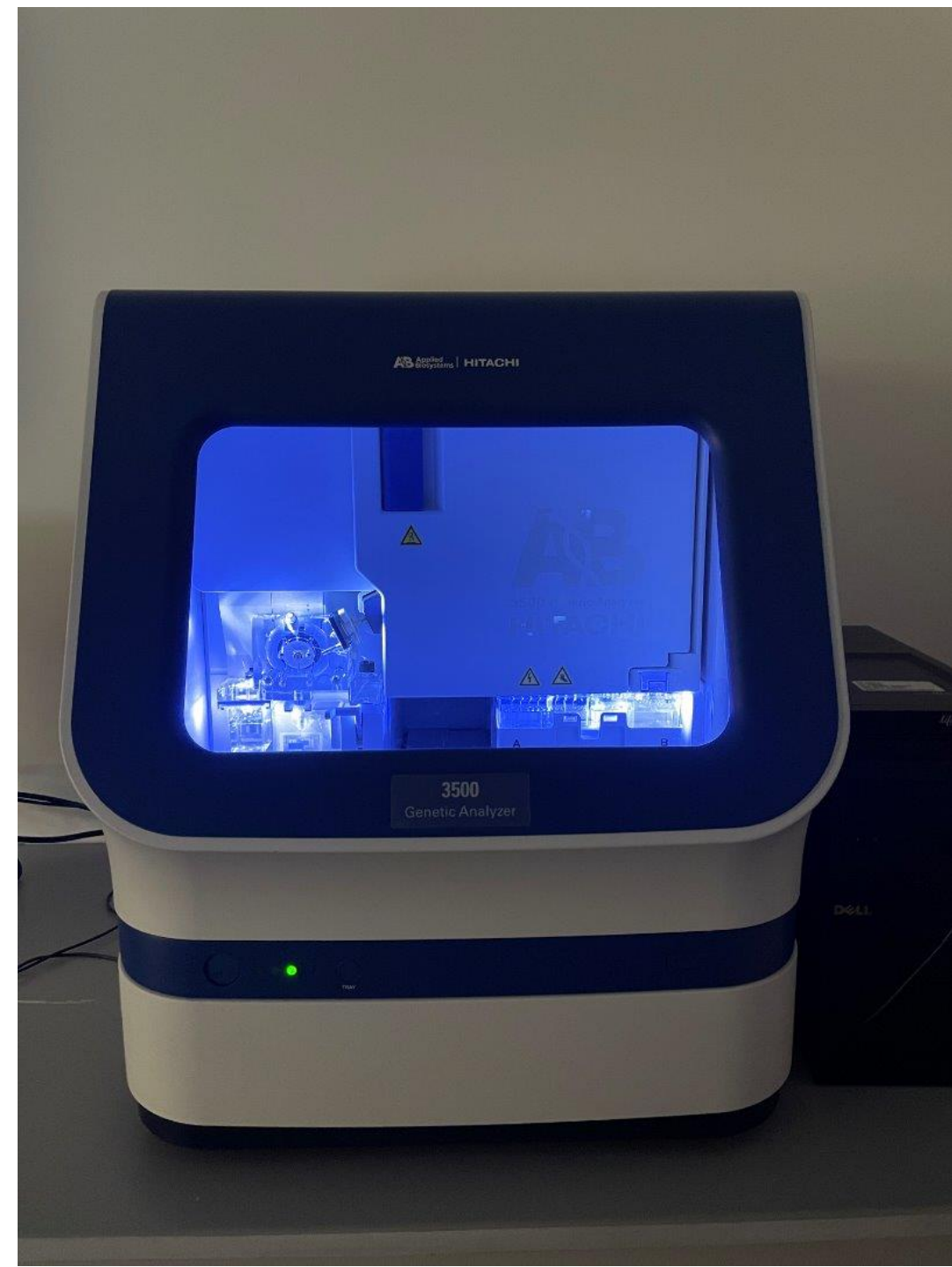


DNA Typing Process & Instrumentation

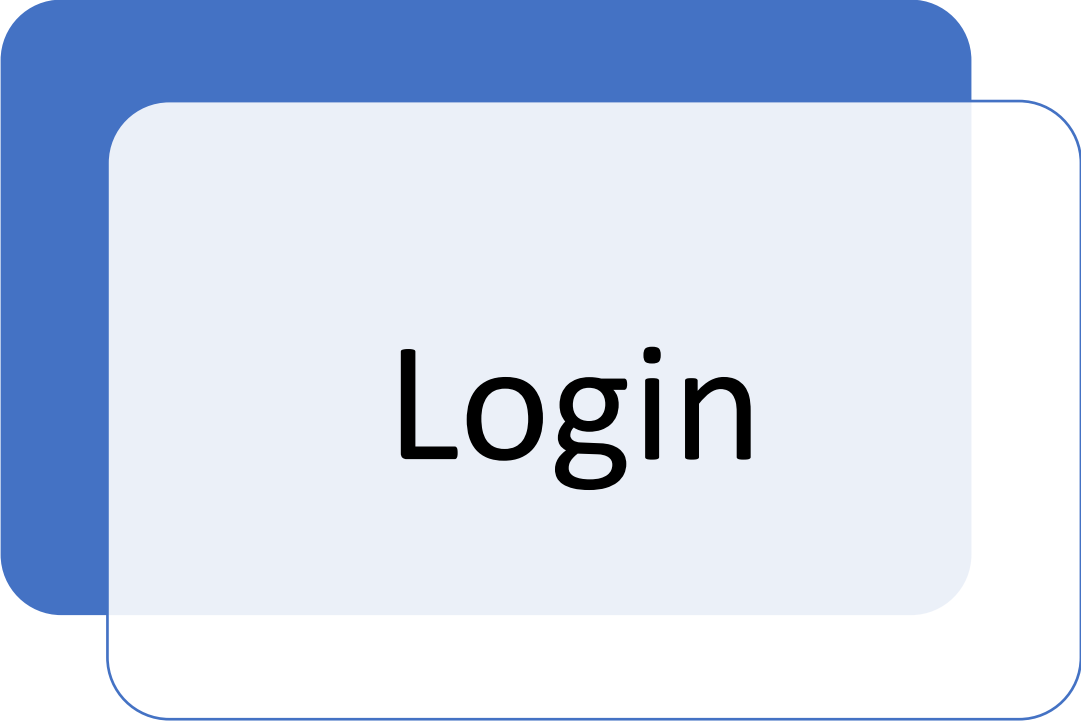


DNA Separation and Detection Instruments

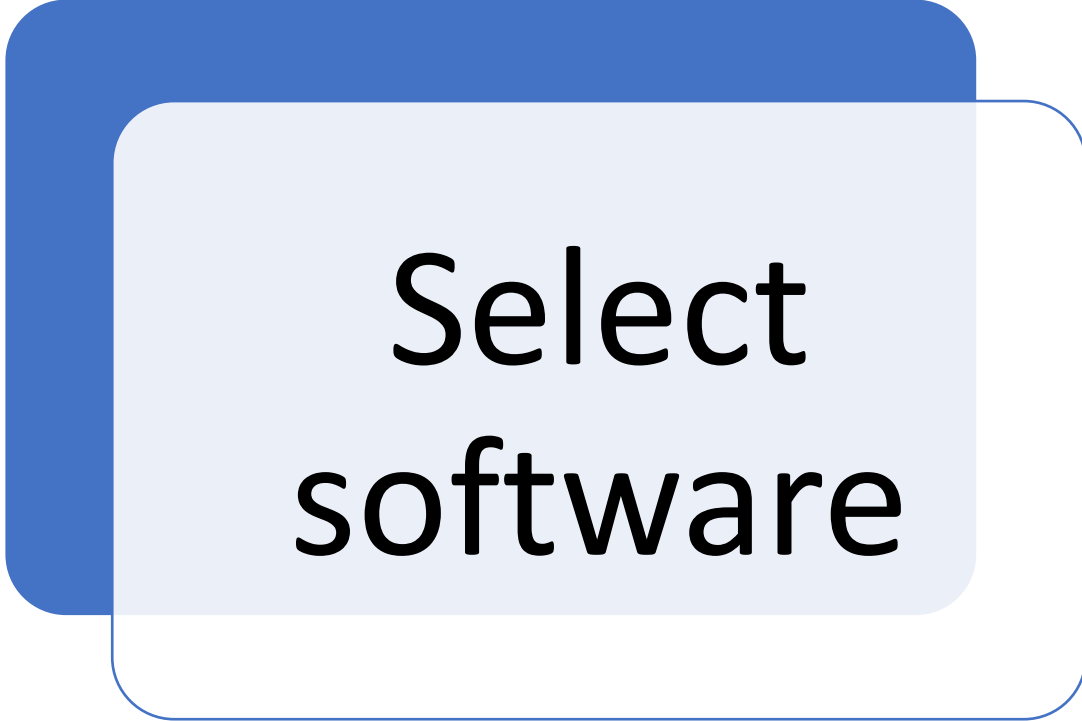
- Applied Biosystems 3130 CE
 - 5 dye
 - 4-16 capillaries
- Applied Biosystems 3500 CE
 - 6 dye
 - 8 capillaries
- Promega Spectrum CE
 - 8 dye
 - 8 capillaries
- Promega Spectrum Compact CE
 - 8 dye
 - 4 capillaries



DNA Separation and Detection Instruments: Preparing to Run



Login



Select
software

applied biosystems®

by *life* technologies™



3500



3500 Results



3500 Series Data Collection Software 3

Version 3.1

The programs included herein are subject to a restricted use license and can only be used in conjunction with this application.

FOR RESEARCH USE ONLY.
Not for use in diagnostic procedures.

Launching 3500 Series Data Collection Software 3...

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AB 3500 Dashboard: Instrument Not Connected

3500 Series Data Collection Software 3 (Research Use Only mode)

Dashboard Workflow Maintenance Library Edit SAE Tools Manage Preferences Help Log Out

AB applied biosystems™
Data Collection Software

Common Operations

Quick Start Run Create New Plate Create Plate from Template Edit Existing Plate View Run Results Wizards

Quick View

Gauges

Unknown Polymer
Unknown Samples Remaining
(Unknown Injections Remaining)

Unknown Buffer - (Anode)
Unknown Days Remaining

Unknown Buffer - (Cathode)
Unknown Days Remaining

Unknown Capillary Array
Unknown Samples Remaining

Instrument: (unknown)
Laser: Laser State
EP: On

State: Unknown
Oven: Off
Oven Door: Open
Instrument Door: Open

View Instrument Sensor Details

Pre-Heat the Oven
Set Temperature to:
60 (°C) Start Pre-Heat

Oven Temperature (°C): XX.X
Detection Cell Temperature (°C): XX.X

Consumables Information

Consumable	Name	Status	Days on Instrument	Expiration Date	Lot Number	Part Number
Pouch	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Anode Buffer	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Cathode Buffer	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Capillary Array	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown

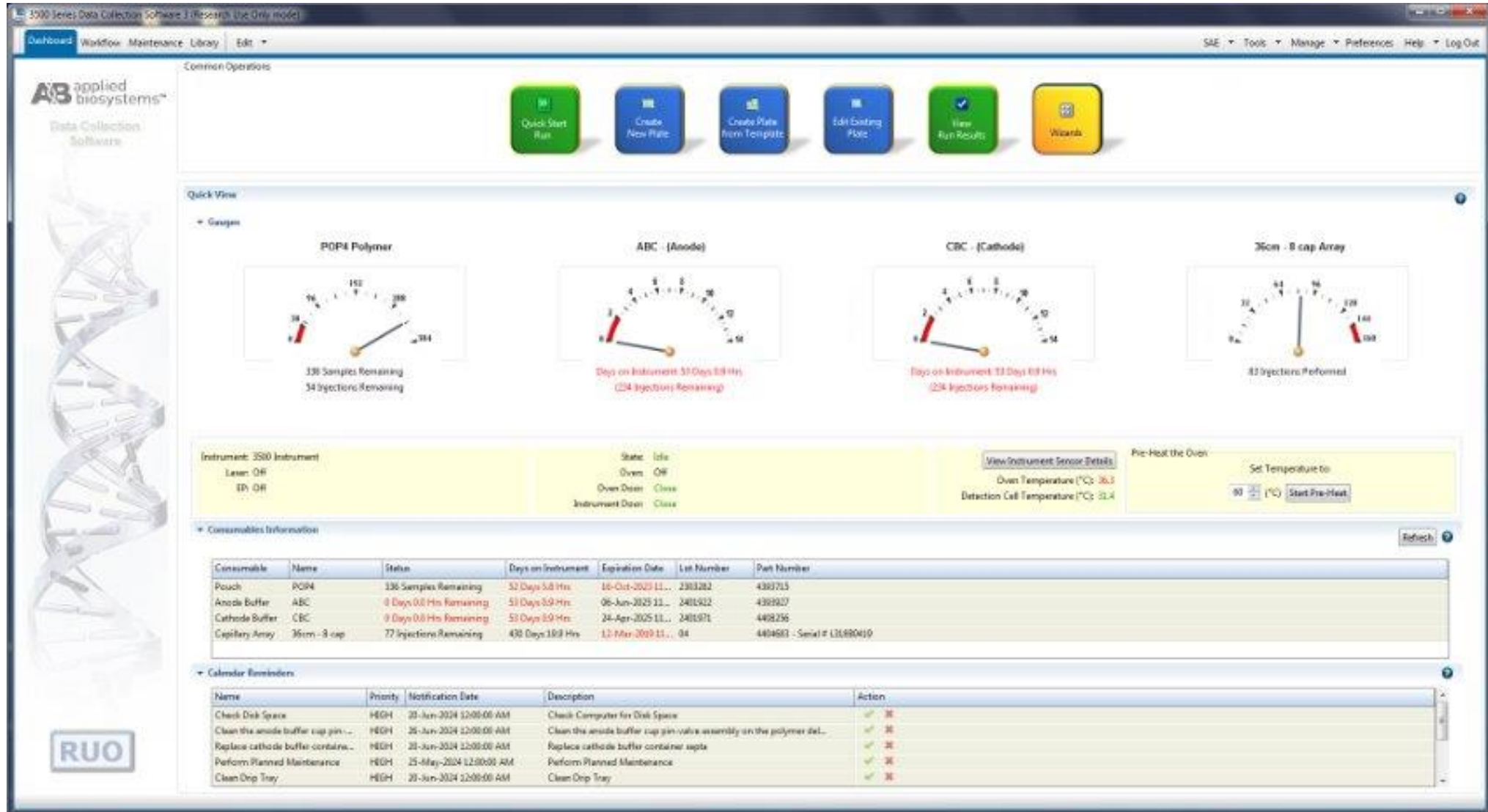
Calendar Reminders

Name	Priority	Notification Date	Description	Action
Check Disk Space	HIGH	20-Jun-2024 12:00:00 AM	Check Computer for Disk Space	✓ ✗
Clean the anode buffer cup pin...	HIGH	26-Jun-2024 12:00:00 AM	Clean the anode buffer cup pin-valve assembly on the polymer del...	✓ ✗
Replace cathode buffer containe...	HIGH	20-Jun-2024 12:00:00 AM	Replace cathode buffer container septa	✓ ✗
Perform Planned Maintenance	HIGH	25-May-2024 12:00:00 AM	Perform Planned Maintenance	✓ ✗
Clean Drip Tray	HIGH	20-Jun-2024 12:00:00 AM	Clean Drip Tray	✓ ✗

RUO

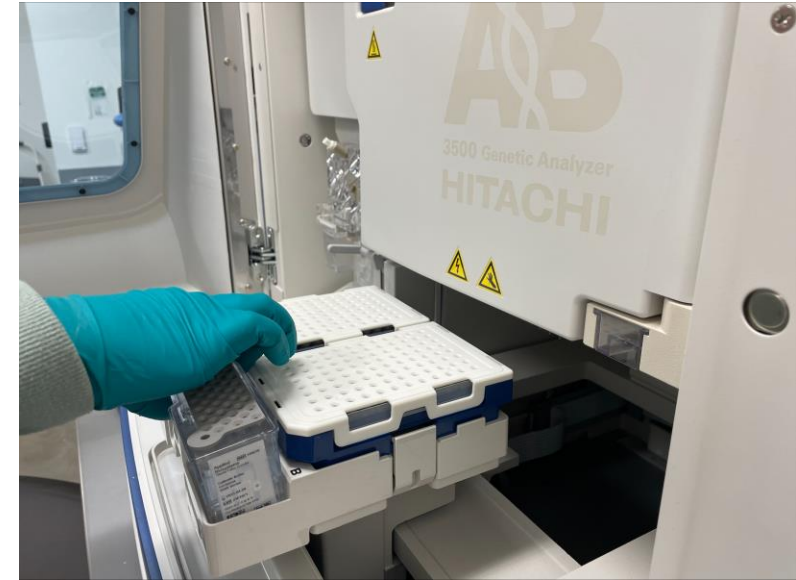
2:02 PM 6/28/2024

AB 3500 Dashboard: Instrument Connected

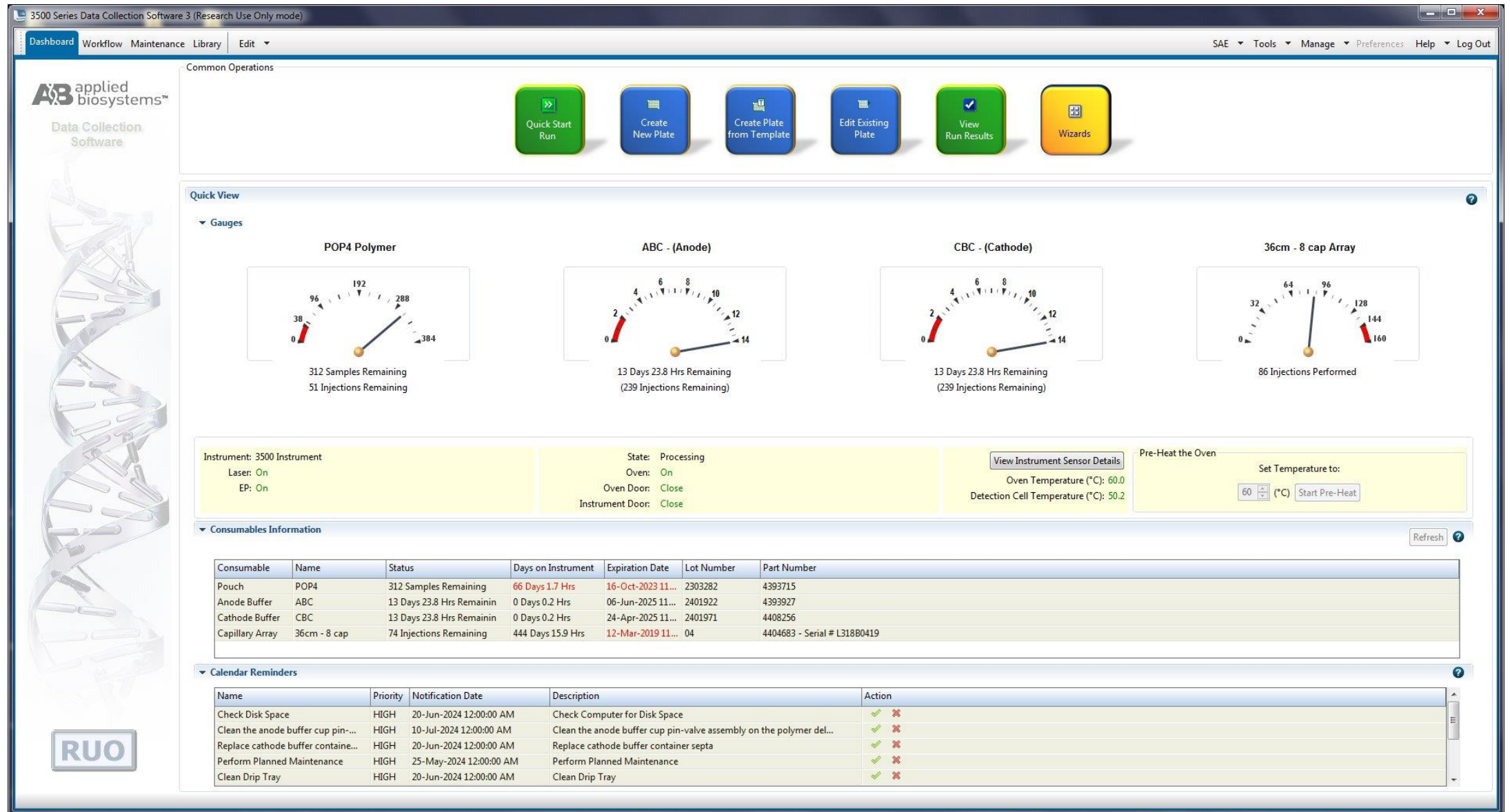


AB 3500 Dashboard: Replacing Anode and Cathode Buffer

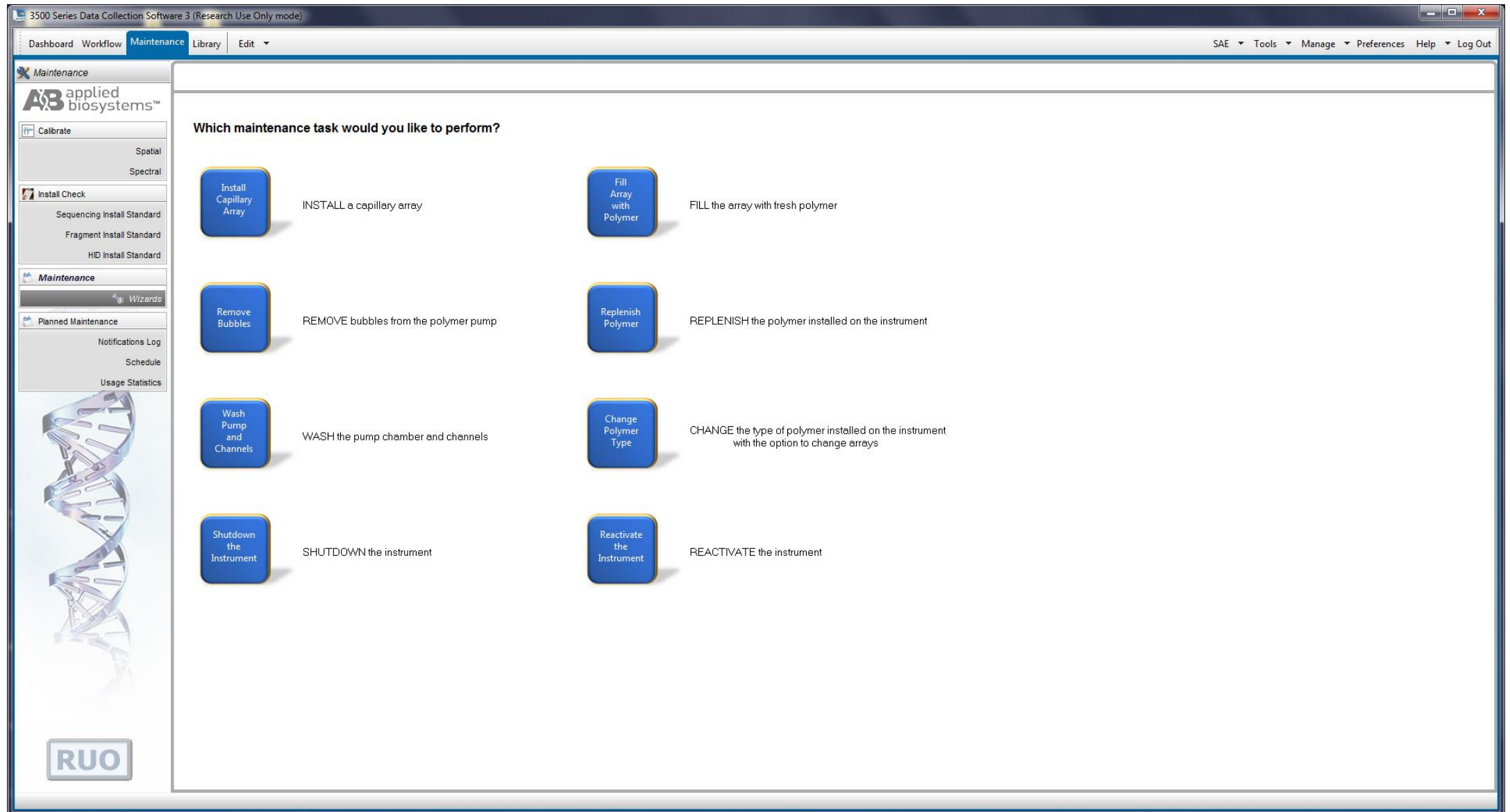
- Replace anode and cathode buffer
 - Remove plastic seals
 - Face barcode to reader in back for anode buffer
 - Add rubber septa to cathode buffer



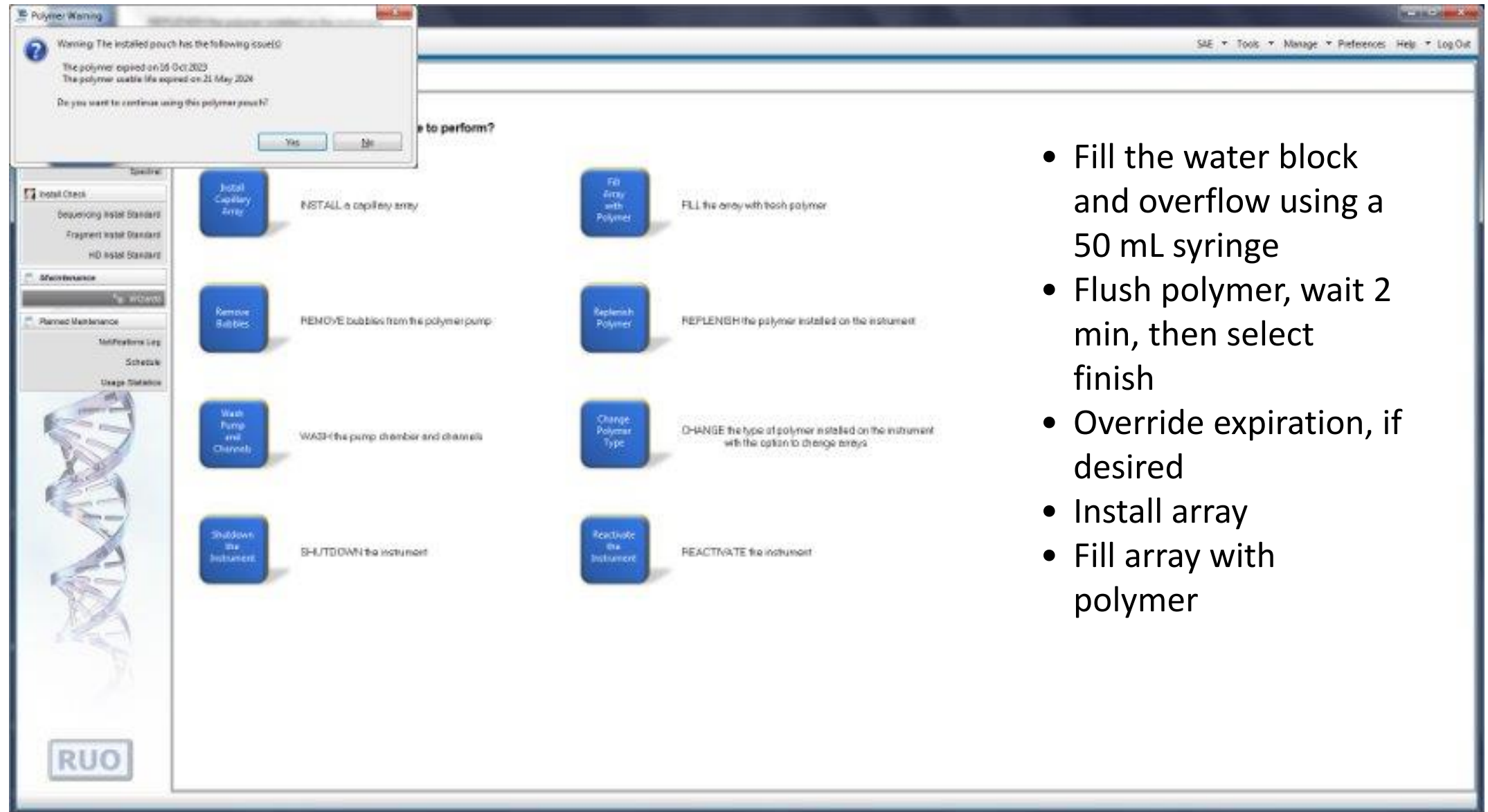
AB 3500 Dashboard: Buffers Replaced



AB 3500 Maintenance Wizard



AB 3500 Expiration Warning



- Fill the water block and overflow using a 50 mL syringe
- Flush polymer, wait 2 min, then select finish
- Override expiration, if desired
- Install array
- Fill array with polymer

AB 3500 Install Array

Install Capillary Array Wizard

Install Capillary Array - Introduction



Welcome to the Install Capillary Array maintenance wizard.

Click 'Next' to continue.

< Back

Next >

Finish

Cancel

SAE Tools Manage Preferences Help Log Out

FILL the array with fresh polymer

REPLENISH the polymer installed on the instrument

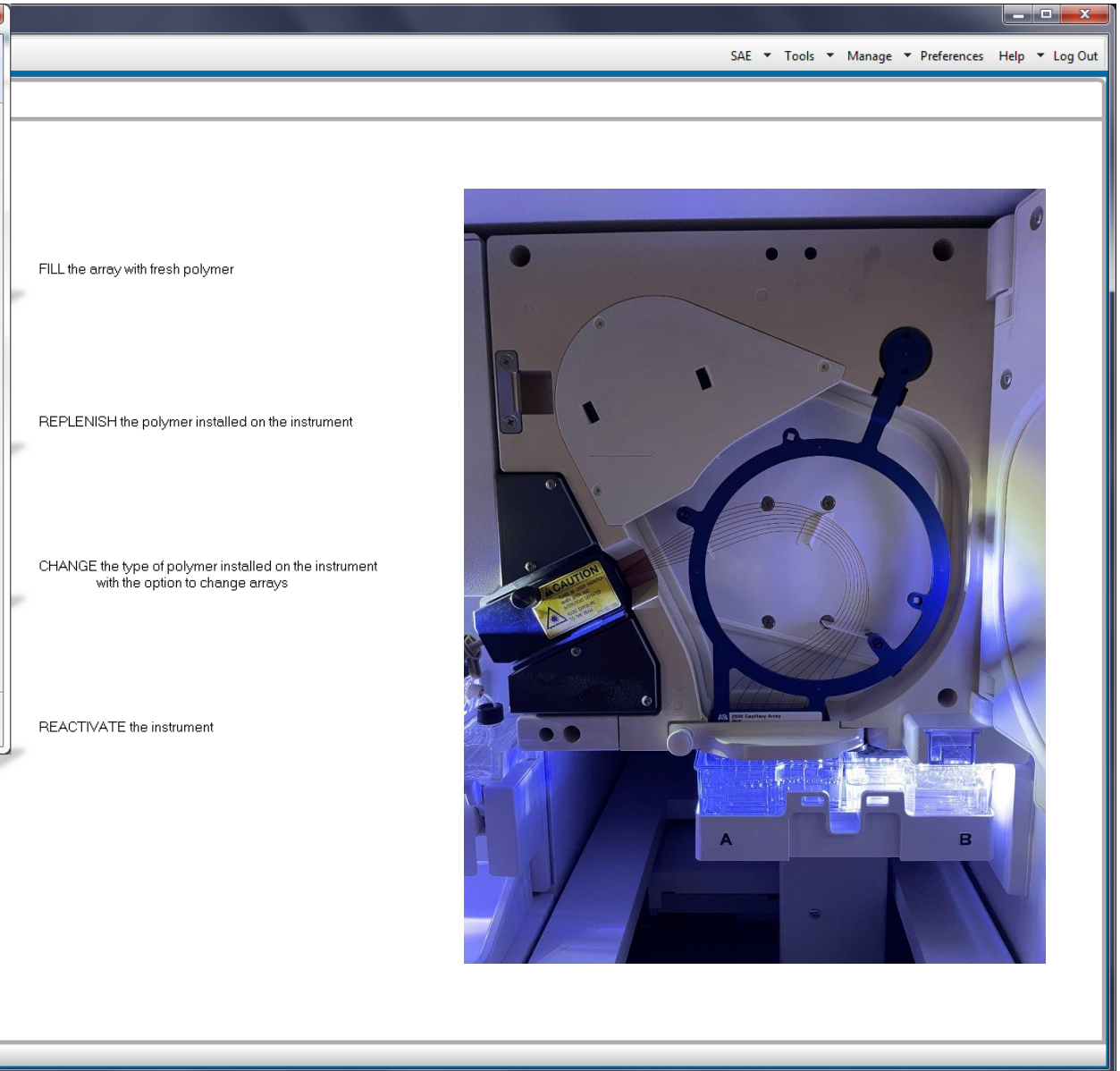
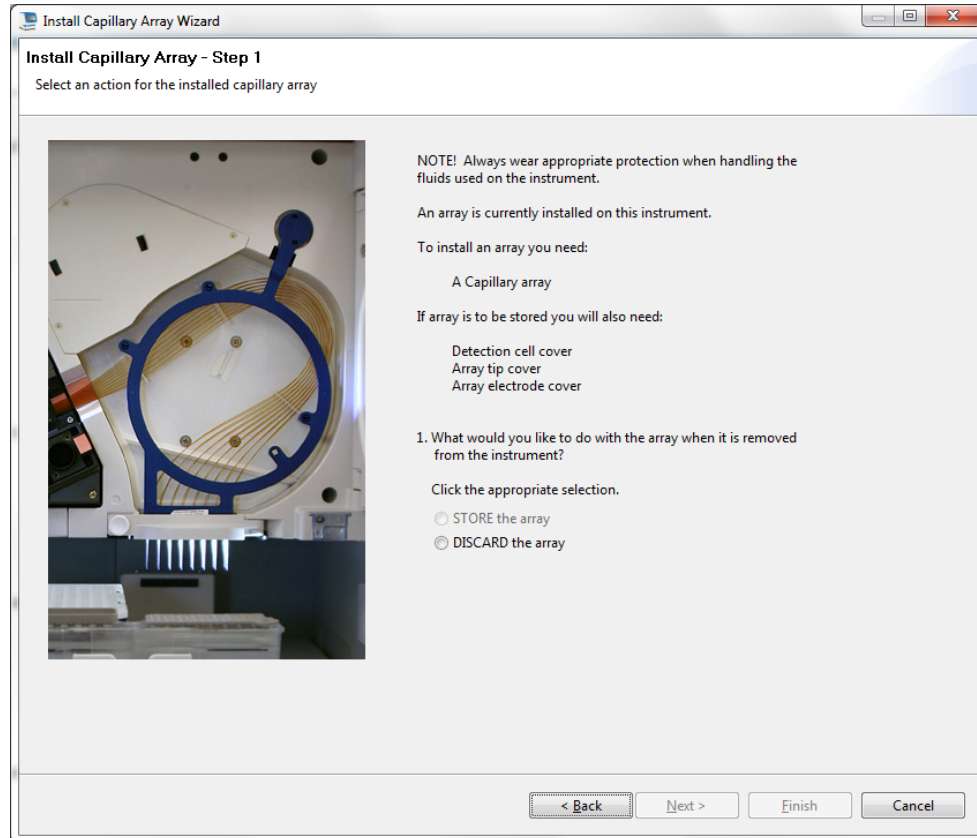
CHANGE the type of polymer installed on the instrument
with the option to change arrays

REACTIVATE the instrument

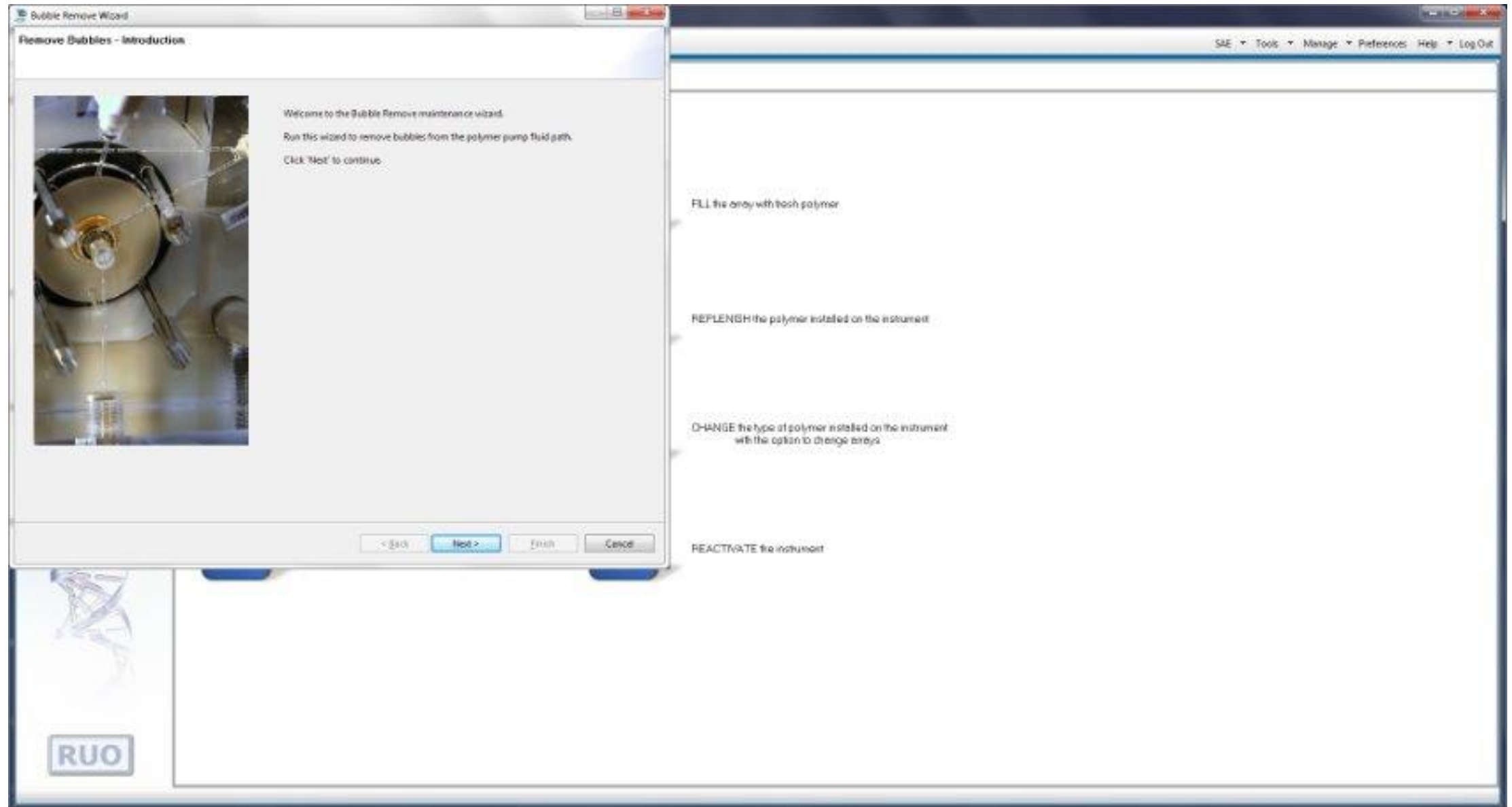


RUO

AB 3500 Install Array



AB 3500 Fill Array & Remove Bubbles



AB 3500 Fill Array & Remove Bubbles

Bubble Remove Wizard

Remove Bubbles - Step 1

Locate and remove bubbles



NOTE: Always wear appropriate protection when handling the fluids used on the instrument.

Trace the fluid pathway from the polymer supply pouch through the pump chamber, array port and ending at the Anode Buffer Container. Locate any bubbles in the channels, pump chamber and array port (bubbles smaller than 0.2 mm diameter may be disregarded).

1. Where are the bubbles?

Click the appropriate selection.

- ☐ At least some bubbles are present BEFORE the array port (total bubble remove).
- ☐ Bubbles are present ONLY AFTER the array port (minor bubble remove).
- ☐ All bubbles are gone.

< Back Next > Finish Cancel

SAE Tools Manage Preferences Help Log Out

FILL the array with fresh polymer

REFLESH the polymer installed on the instrument

CHANGE the type of polymer installed on the instrument with the option to change arrays

REACTIVATE the instrument



AB 3500 Fill Array & Remove Bubbles

Bubble Remove Wizard

Remove Bubbles - Step 2

Fill the array with polymer (optional)



To fill the array with polymer:

1. Close the instrument door and wait for the green status light to illuminate.
2. Click 'Fill Array'.

Wait approximately 2 minutes for the Fill Array procedure to complete.

3. Click 'Finish'.

To skip this step, click 'Finish'.

SAE Tools Manage Preferences Help Log Out

FILL the array with fresh polymer

REPLENISH the polymer installed on the instrument

CHANGE the type of polymer installed on the instrument with the option to change arrays

REACTIVATE the instrument




AB 3500 Spatial Calibration

3500 Series Data Collection Software 3 (Research Use Only mode)

Dashboard Workflow Maintenance Library Edit

SAE Tools Manage Preferences Help Log Out

Maintenance

AB applied biosystems™

Calibrate

Spatial

Spectral

Install Check

Sequencing Install Standard

Fragment Install Standard

HD Install Standard

Maintenance

Wizards

Planned Maintenance

Notifications Log

Schedule

Usage Statistics

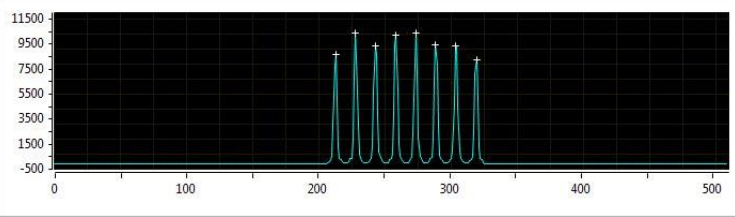
RUO

Show Desktop

View Spatial Calibration Report Print E-Signature

Spatial Calibration

Status: Regular Run in progress



Capillary	Position (pixels)	Spacing	Intensity
1	214	15	8807
2	229	15	10519
3	244	16	9505
4	260	15	10399
5	275	15	10517
6	290	15	9564
7	305	16	9514
8	321	0	8427

Options

☐ Fill ☒ No-Fill

☒ Perform QC Checks

Start Calibration Stop Calibration

Accept Results Reject Results

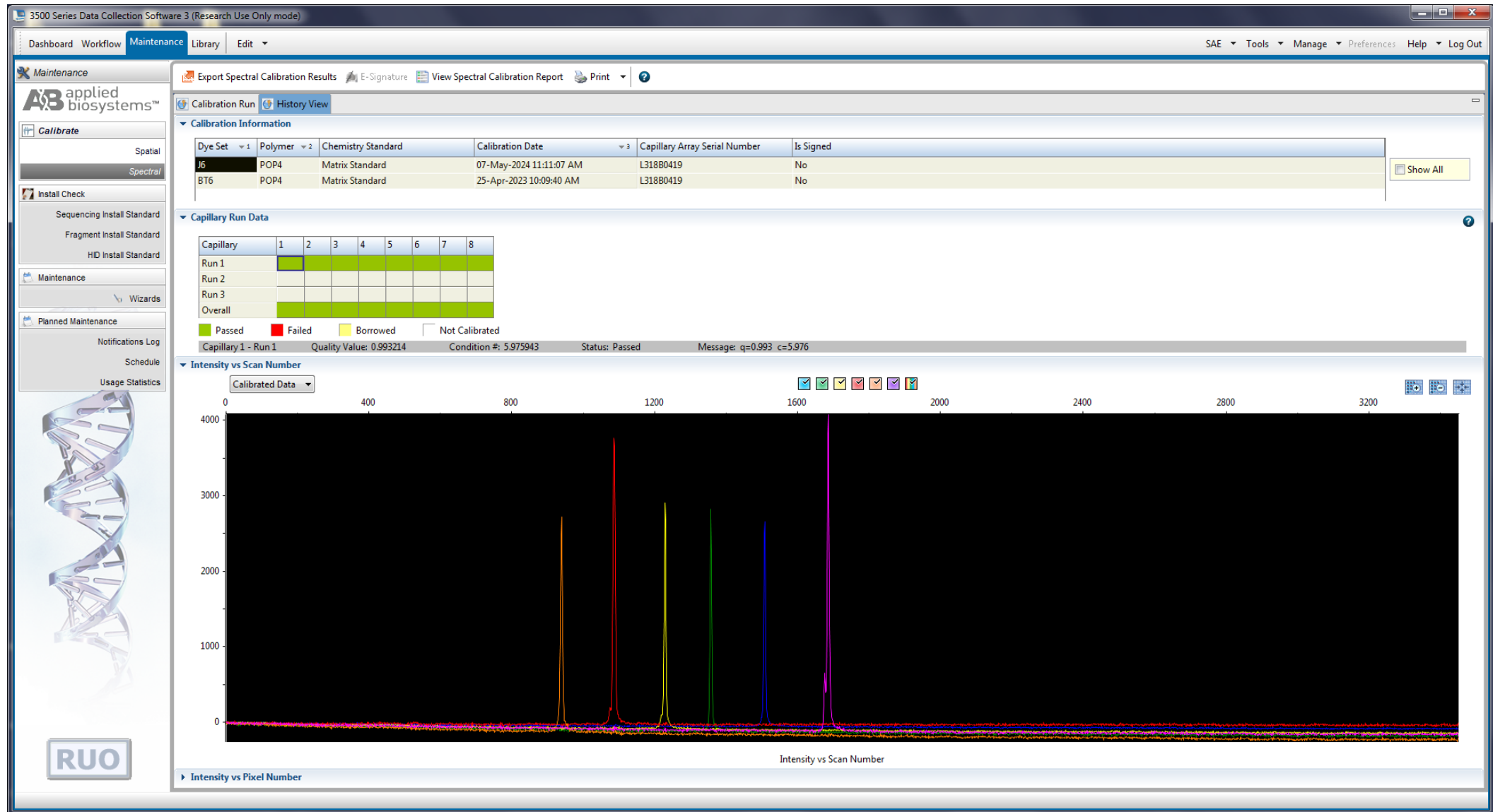
Export Results...

Note: Do not open the instrument door during a spatial calibration run.

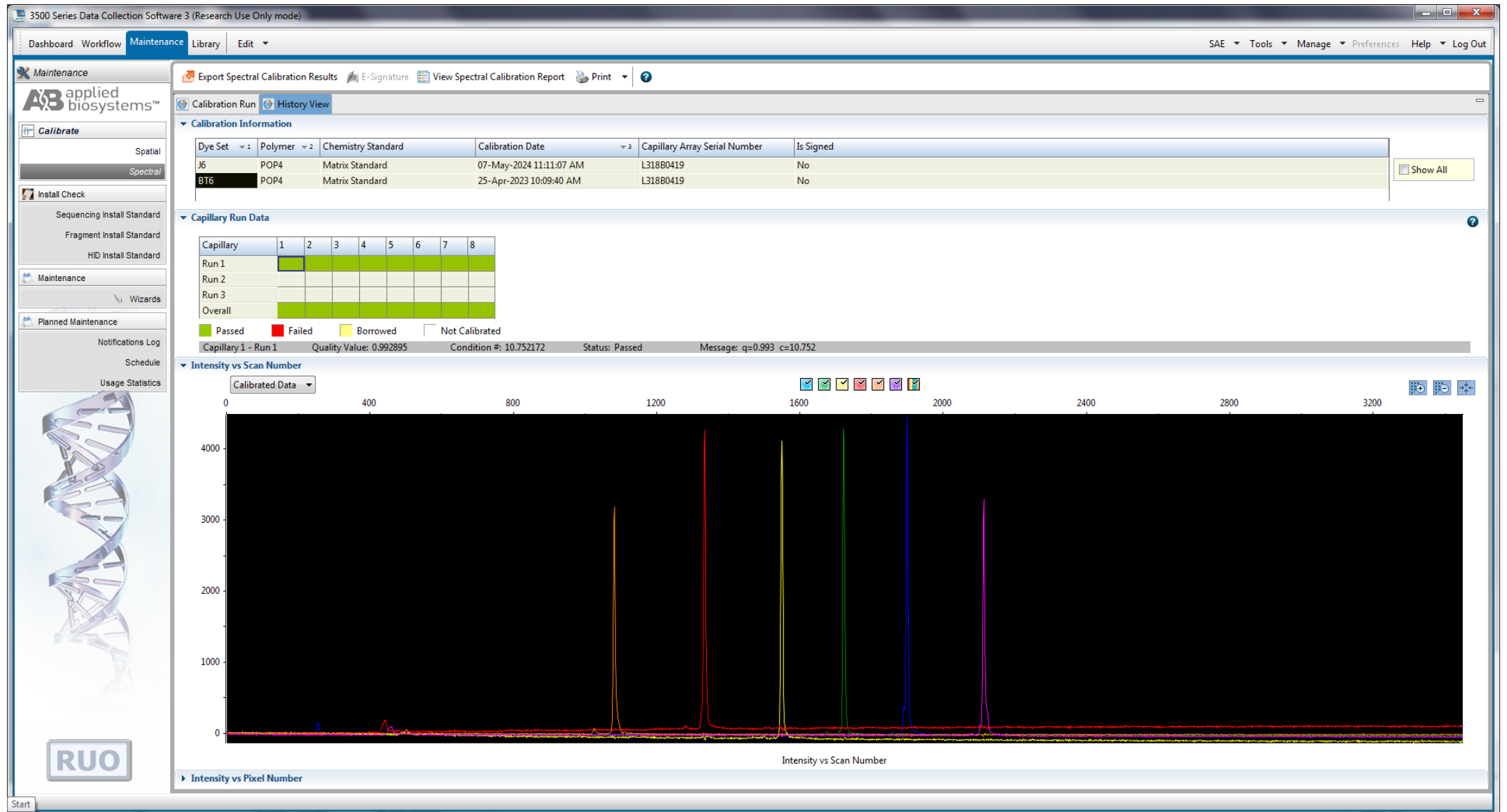
If running with the Fill option it is recommended that the oven and detector heater be turned on.

0%

AB 3500 Spectral Calibration: J6

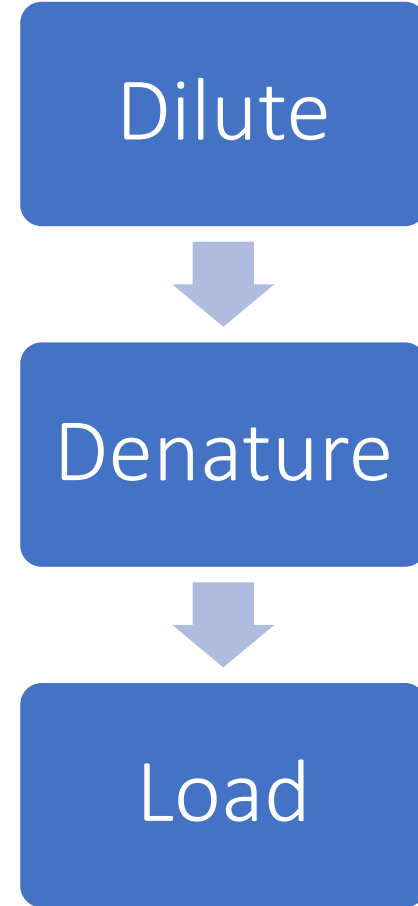


AB 3500 Spectral Calibration: BTO



Prepare Samples to Run

- To a 96-well skirted plate, combine
 - 0.5 μL size standard (vortexed well)
 - 8.5-12 μL Hi-Di formamide
 - Create master mix for total number of samples
- Add 1 μL of amplified sample or allele ladder (before and after samples)
- Add 10-12 μL per well to plate, pipetting down the side to avoid bubbles
- Lay septa on plate, press in
- Denature plate for 5 min at 95 °C, snap cool on cold block or ice
- Load all 8 wells in a column per injection (fill with formamide if an empty lane)
- Discard formamide



AB 3500 Setup New Plate

3500 Series Data Collection Software 3 (Research Use Only mode)

Dashboard Workflow Maintenance Library Edit SAE Tools Manage Preferences Help Log Out

Plate Name:

AB applied biosystems™

Advanced Setup

Define Plate Properties
Assign Plate Contents

Run Instrument
Load Plates for Run
Preview Run
Monitor Run

Review Results
View Sequencing Results
View Fragment/HID Results

RUO

New Plate Open Plate Save Plate Close Plate Start Run

Plate Details Define Plate Help

* Name: Plate Name is a required field. Provide a unique value.

Owner:

* Number of Wells:

Barcode:

* Plate Type:

Description:

* Capillary Length: cm

* Polymer:

▸ Secondary Analysis ☐ Allow Auto-Analysis

AB 3500 Assign Plate Contents

3500 Series Data Collection Software 3 (Research Use Only mode)

Dashboard Workflow Maintenance Library Edit SAE Tools Manage Preferences Help Log Out

Plate Name: Training

AB applied biosystems™

Advanced Setup

Define Plate Properties

Assign Plate Contents

Run Instrument

Load Plates for Run

Preview Run

Monitor Run

Review Results

View Sequencing Results

View Fragment/HID Results

RUO

New Plate Open Plate Save Plate Close Plate Import Export Find/Replace View Plate Grid Report Print

Plate View Table View

Show In Wells Select Wells Array Selection Row Column Zoom In Zoom Out Fit

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

HID

Name: Training Barcode:

Assays Actions

Add from Library

Create New Assay

File Name Conventions Actions

Add from Library

Create New File Name Convention

Results Groups Actions

Add from Library

Create New Results Group

Assign Plate Help

Customize Sample Info

Link Plate for Run

Start

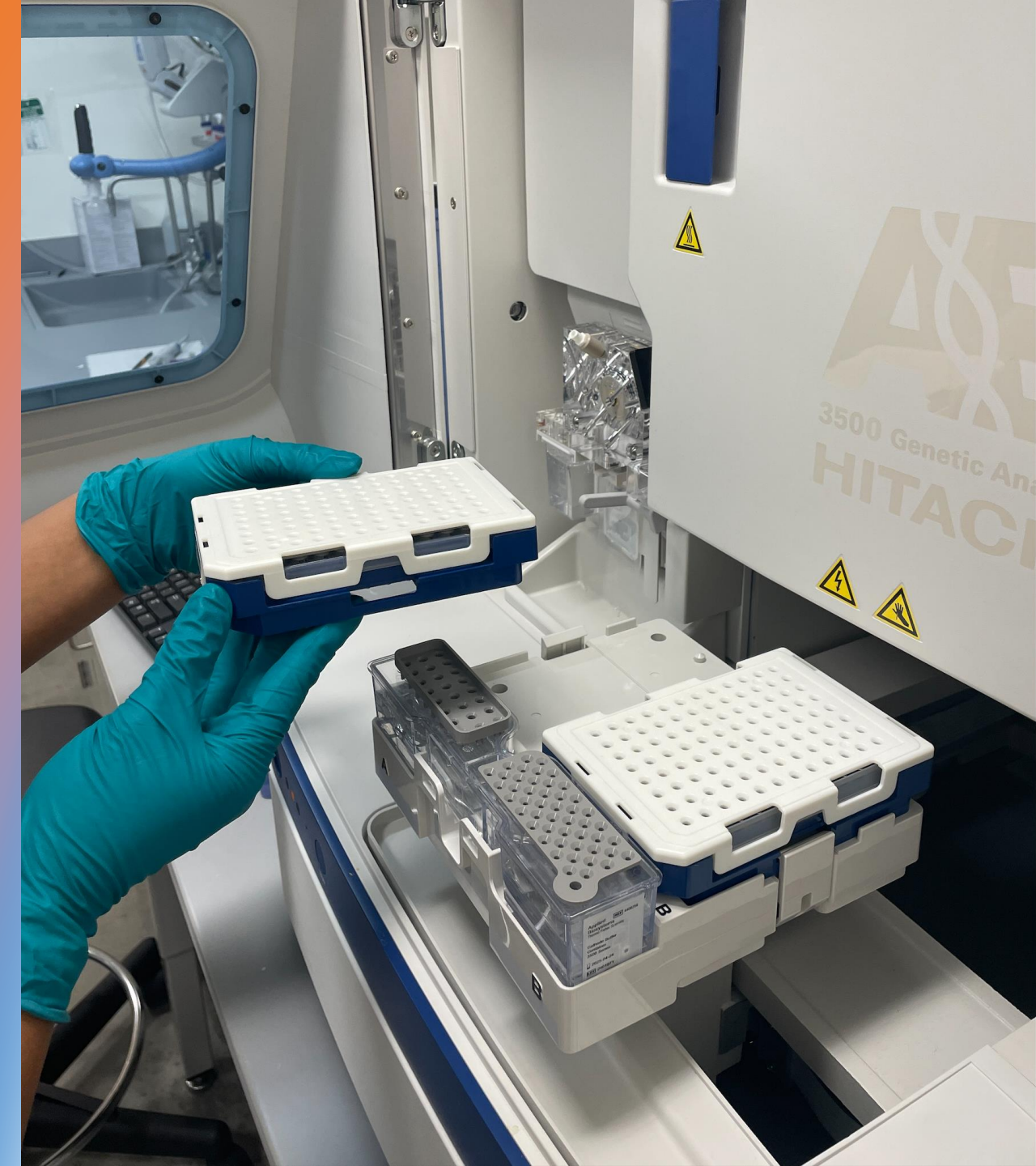
2:02 PM 6/28/2024

AB 3500 Assign Plate File Name Convention

The screenshot displays the AB 3500 software interface. A dialog box titled "Training - Create New File Name Convention" is open, showing the "Setup a File Name Convention" window. The dialog includes a "Name" field, a "Locked" checkbox, and a "Color" dropdown set to "Black". It also features a "Preview of File Name" section showing "<Sample Name> <Date of Run> <Owner Name>". The "Available Attributes" list includes Injection Number, Instrument Name, Instrument Protocol, Plate Name, Polymer Type, Run Name, and Sample Type. The "Delimiters" section shows a dropdown for "Underscore (_)" and a checkbox for "Add between attributes". The "Select File Location" section has radio buttons for "Default File Location" (selected) and "Custom File Location". The "Close" button is at the bottom left, and "Apply to Plate" and "Save to Library" buttons are at the bottom right.

The background application window shows the "View Plate Grid Report" interface. It includes a menu bar with "SAE", "Tools", "Manage", "Preferences", "Help", and "Log Out". Below the menu is a toolbar with "Show In Wells", "Select Wells", "Array Selection", "Row", "Column", "Zoom In", "Zoom Out", and "Fit". The main area displays a plate grid with columns 6 through 12. Below the grid is a "Barcode" section. At the bottom, there are three panels: "File Name Conventions", "Results Groups", and "Assign Plate Help". Each panel has an "Add from Library" button and a "Create New" button. A "Link Plate for Run" button is located at the bottom center.

The Windows taskbar at the bottom shows the Start button, several application icons, and the system clock indicating 2:02 PM on 6/28/2024.

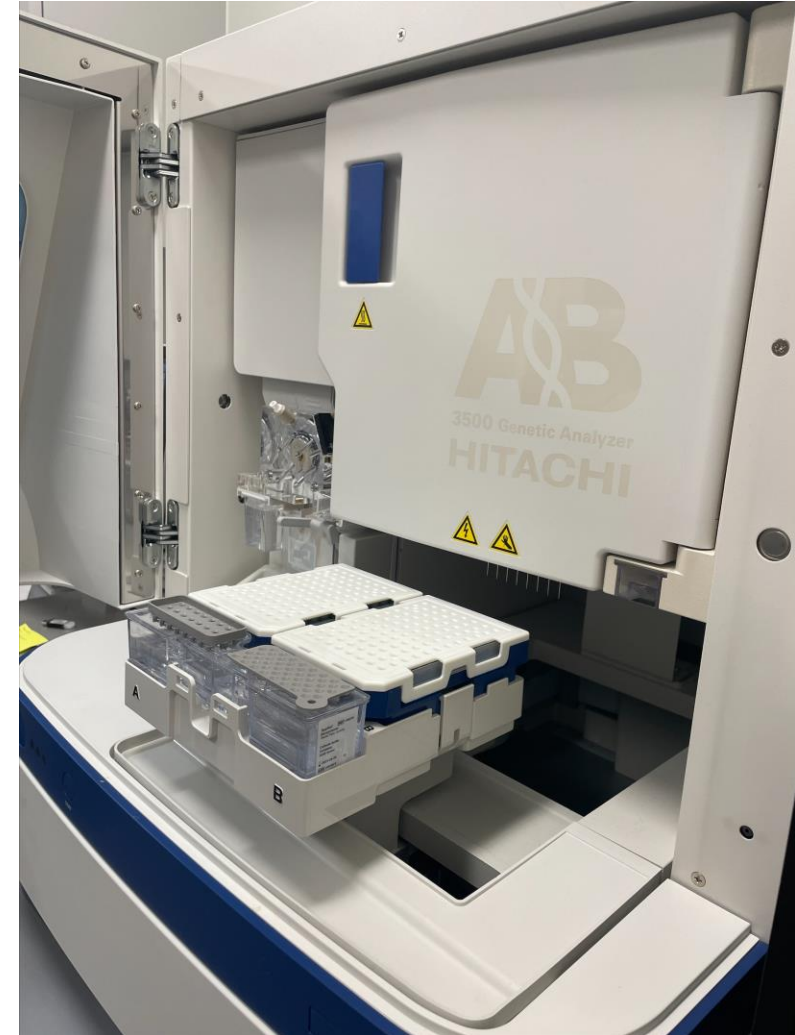


AB 3500 Load Plate to Run

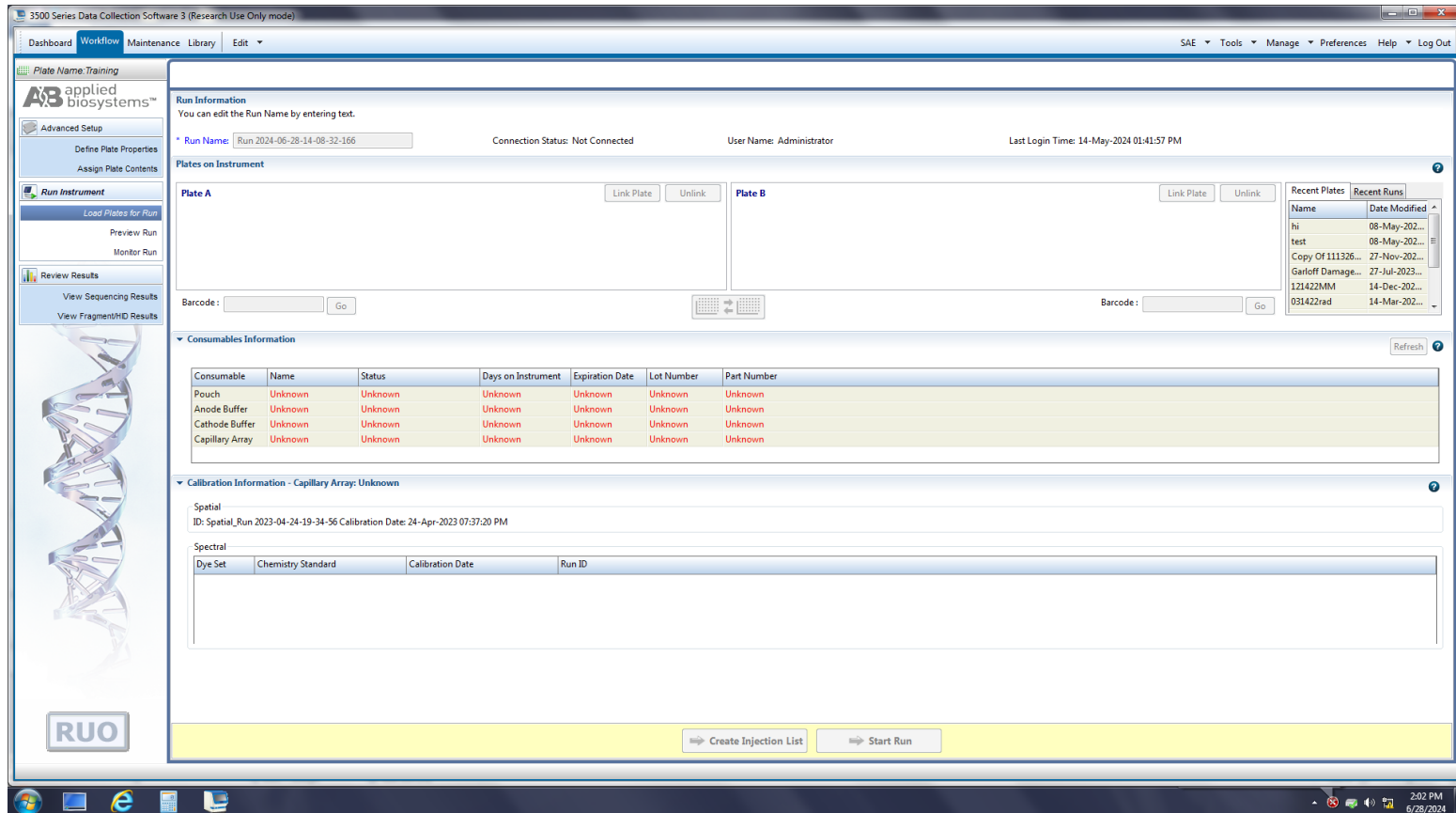
- Select the tray button on the front of the instrument to home the tray
- Insert the plate in a white and blue cartridge
- To run in A, put your plate in the A spot
- To load the plate, pull side tab to put in and close

AB 3500 Ready to Run Plate

- Close door and push button to home tray
- Start run program



AB 3500 Start Run



- In tray view, select sample type: sample, positive or negative control, or allele ladder
- In plate view, highlight all samples to run
- Link plate to run, default is plate A
- Save changes-Yes
- Start run

AB 3500 Running a Plate

3500 Series Data Collection Software 3 (Research Use Only mode)

Dashboard Workflow Maintenance Library Edit SAE Tools Manage Preferences Help Log Out

Plate Name: **applied biosystems™**

Advanced Setup

Define Plate Properties
Assign Plate Contents

Run Instrument

Load Plates for Run
Preview Run
Monitor Run

Review Results

View Sequencing Results
View Fragment/HD Results

Connection Status: Connected User Name: Administrator Last Login Time: 28-Jun-2024 03:15:38 PM
Run Name: Run 2024-07-12-11-01-07-969 Run Status: Running Estimated Time Remaining: 01:04:04 / 01:23:12

Injection List Details

2 injections created - 2 in Plate A - 0 in Plate B

Injection	Type	Assay	Instrument Protocol	Plate	Results Groups	Analysis	Flags	Time Remaining
1	Active	GF+Norm_POP4	HID36_POP4_J6_NT3200	Kierra7_12_2	Zeller			00:22:27
2	Not Started	GF+Norm_POP4	HID36_POP4_J6_NT3200	Kierra7_12_2	Zeller			00:41:36

Legend

Not Started Active Aborted Completed Re-Inject Duplicate Flags

Instrument Run Views and Flags

Array Sample EPT

Injection: 1

1 2 3 4 5 6 7 8

4000
3000
2000
1000
0

Cap: Well: Sample Name:

Brightness: Color:

Re-Inject

Review Results

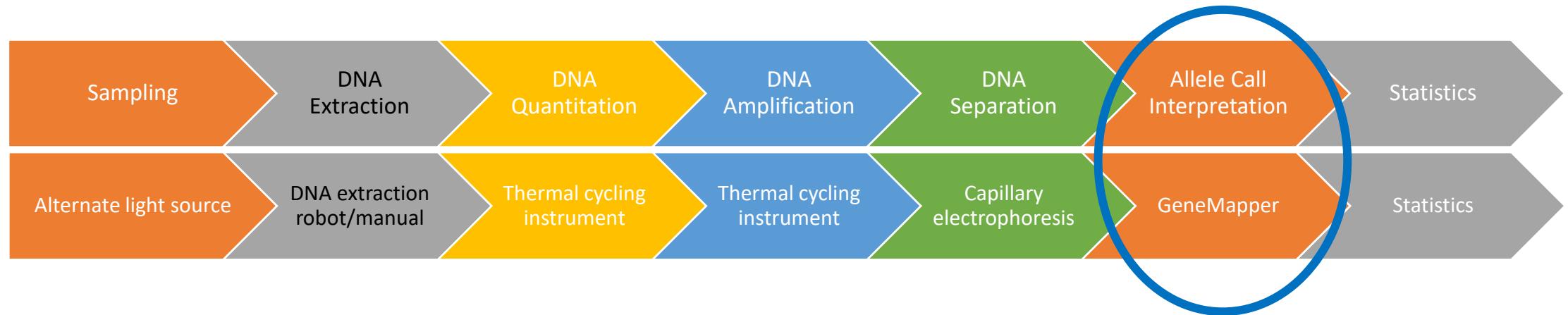
Plate A Plate B

	1	2	3	4	5	6	7	8	9	10	11	12
A	CV 1	ladder 2										
B	VL 1	ladder 2										
C	SEM (run 1) 1	ladder 2										
D	BLO (run 1) 1	ladder 2										
E	CS 1	SAL 2										
F	CL 1	negative 2										
G	BLO (run 1) 1	positive 2										
H	SEM (run 1) 1	formamide 2										

Name: Kierra7_12_24 Barcode:

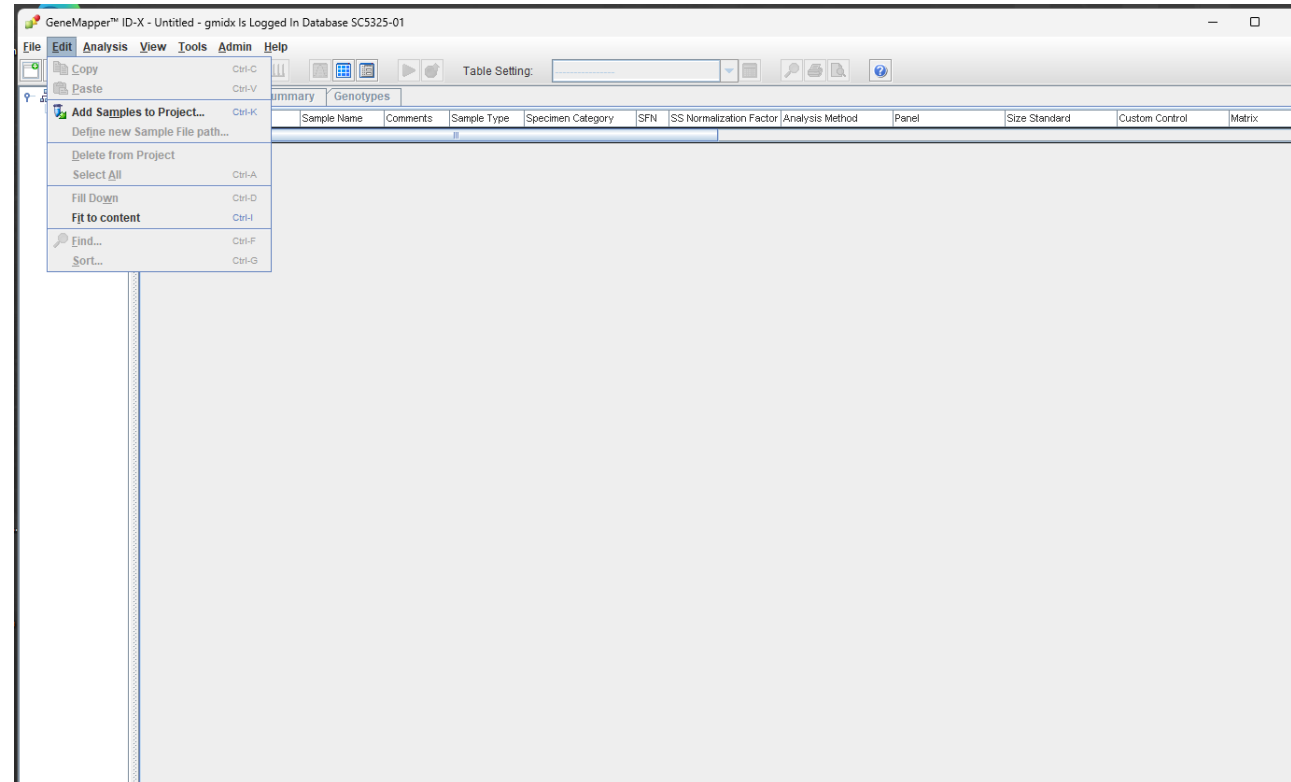
RUO

DNA Typing Process & Instrumentation

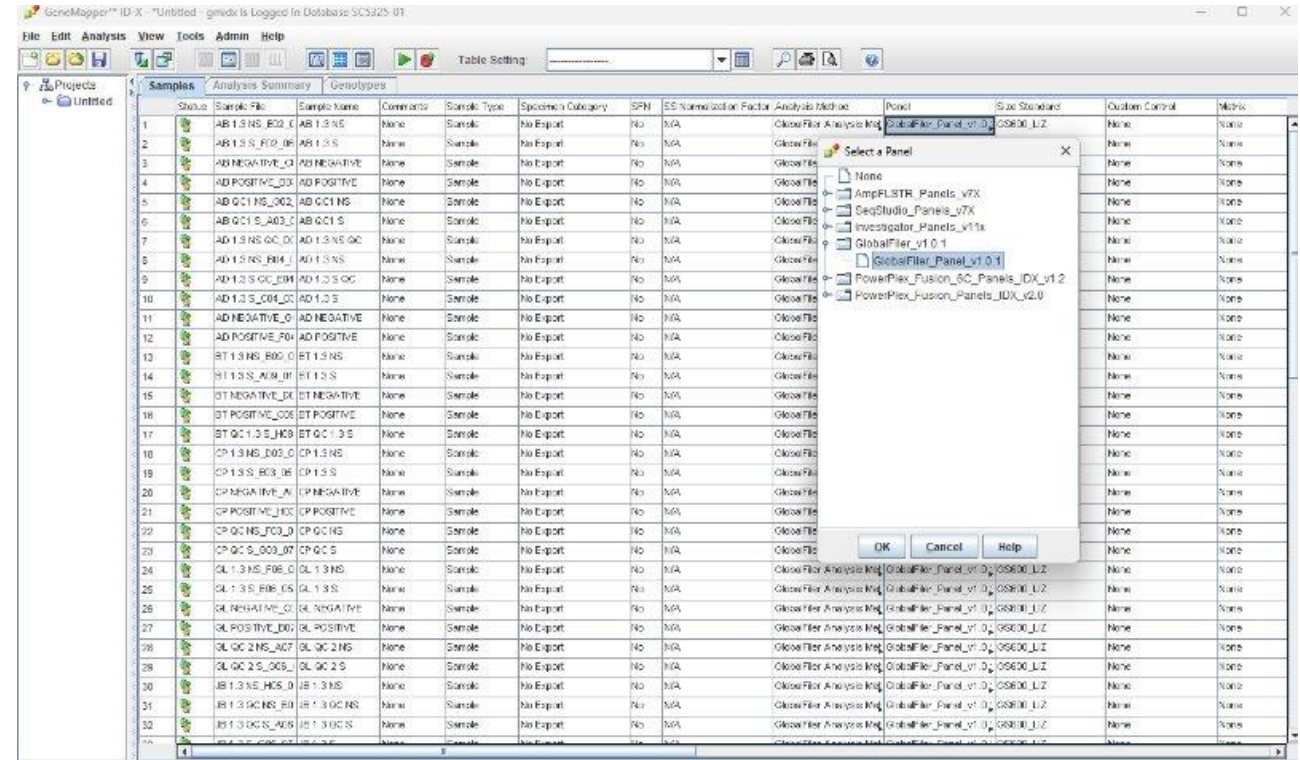


Software: GeneMapper

- Analysis of raw .hid files



- Analysis of .hid files
- Indicate STR kit and ISS used



Software: GeneMapper

- Select Analysis Method
- Thresholds

GeneMapper™ ID-X - *Untitled - gmidx Is Logged In Database SC5325-01

File Edit Analysis View Tools Admin Help

Table Setting: _____

Projects
Untitled

	Status	Sample File	Sample Name	Comments	Sample Type	Specimen Category	SFN	SS Normalization Factor	Analysis Method	Panel
1		AB 1.3 NS_E02_C	AB 1.3 NS	None	Sample	No Export	No	N/A	GlobalFiler Ana...	GlobalFiler
2		AB 1.3 S_F02_06	AB 1.3 S	None	Sample	No Export	No	N/A	New Analysis Met	GlobalFiler
3		AB NEGATIVE_C0	AB NEGATIVE	None	Sample	No Export	No	N/A	None	GlobalFiler
4		AB POSITIVE_B0	AB POSITIVE	None	Sample	No Export	No	N/A	None	GlobalFiler
5		AB QC1 NS_G02_	AB QC1 NS	None	Sample	No Export	No	N/A	Analysis_HID_350	GlobalFiler
6		AB QC1 S_A03_C	AB QC1 S	None	Sample	No Export	No	N/A	Analysis_HID_350	GlobalFiler
7		AD 1.3 NS QC_D0	AD 1.3 NS QC	None	Sample	No Export	No	N/A	GlobalFiler Analysis	GlobalFiler
8		AD 1.3 NS_B04_C	AD 1.3 NS	None	Sample	No Export	No	N/A	GlobalFiler Analysis	GlobalFiler
9		AD 1.3 S QC_E04	AD 1.3 S QC	None	Sample	No Export	No	N/A	GlobalFiler Analysis Met	GlobalFiler
10		AD 1.3 S_C04_03	AD 1.3 S	None	Sample	No Export	No	N/A	GlobalFiler Analysis Met	GlobalFiler
11		AD NEGATIVE_G0	AD NEGATIVE	None	Sample	No Export	No	N/A	GlobalFiler Analysis Met	GlobalFiler
12		AD POSITIVE_F0	AD POSITIVE	None	Sample	No Export	No	N/A	GlobalFiler Analysis Met	GlobalFiler

Software Parameters

- Set thresholds based on validation
 - Interpretation : 200 or 300 RFU
 - Analytical: 50 RFU

GeneMapper™ ID-X - 1 Projects - gmidx Is Logged In Database SC5325-01

File Edit Analysis View Tools Admin Help

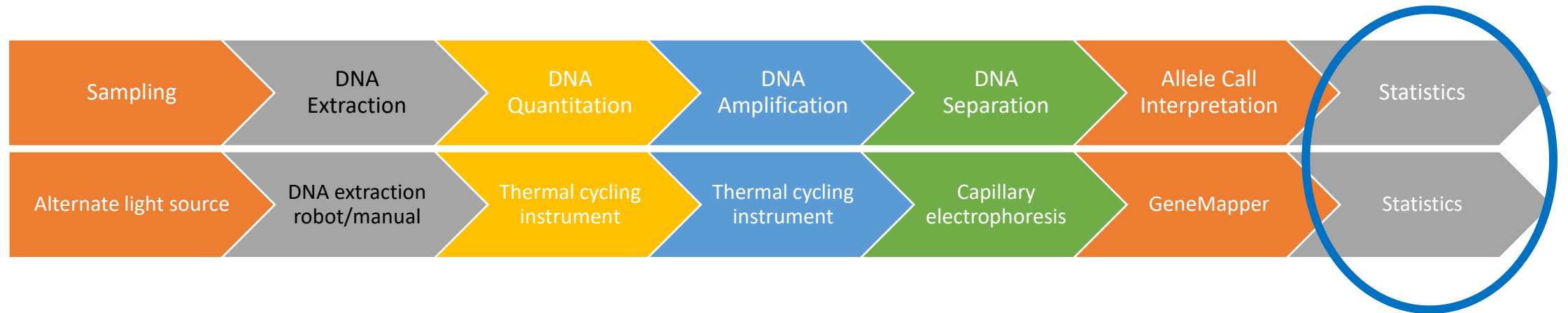
Table Setting: [v]

Projects
Hannah B

Samples Analysis Summary Genotypes

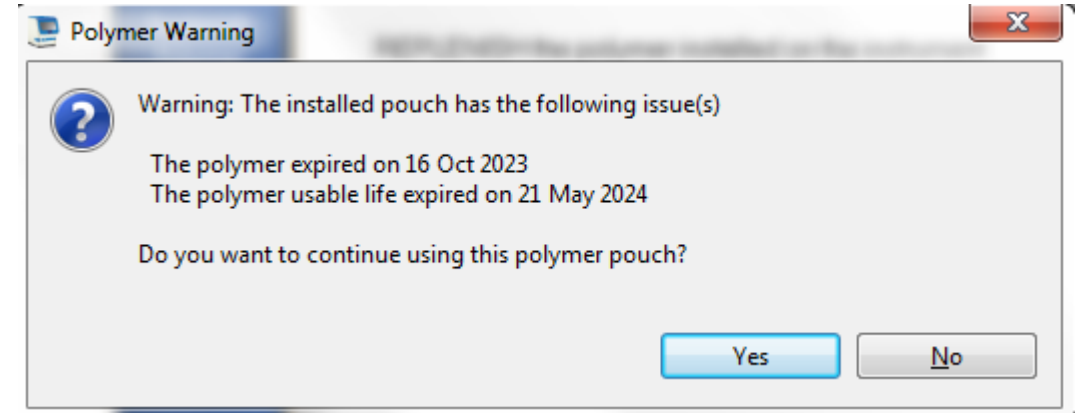
	Status	Sample File	Sample Name	Comments	Sample Type	Specimen Category	SFN	SS Normalization Factor	Analysis Method	Panel	Size Standard	Custom Control
1		Barney_A01_01_	Barney	None	Sample	No Export	No	N/A	(Analysis_HID_3500_50rfu_v5)	24plex_QS_Panels_v5	SST-BTO_60-500bp	None
2		Blank_F01_06_In	Blank	None	Sample	No Export	No	N/A	(Analysis_HID_3500_50rfu_v5)	24plex_QS_Panels_v5	SST-BTO_60-500bp	None
3		Blank_G01_07_In	Blank	None	Sample	No Export	No	N/A	(Analysis_HID_3500_50rfu_v5)	24plex_QS_Panels_v5	SST-BTO_60-500bp	None
4		Blank_H01_08_In	Blank	None	Sample	No Export	No	N/A	(Analysis_HID_3500_50rfu_v5)	24plex_QS_Panels_v5	SST-BTO_60-500bp	None
5		Ladder_E01_05_J	Ladder	None	Allelic Ladder	No Export	No	N/A	(Analysis_HID_3500_50rfu_v5)	24plex_QS_Panels_v5	SST-BTO_60-500bp	None
6		Libby_B01_02_In	Libby	None	Sample	No Export	No	N/A	(Analysis_HID_3500_50rfu_v5)	24plex_QS_Panels_v5	SST-BTO_60-500bp	None
7		Rhonda_C01_03_	Rhonda	None	Sample	No Export	No	N/A	(Analysis_HID_3500_50rfu_v5)	24plex_QS_Panels_v5	SST-BTO_60-500bp	None
8		Spencer_D01_04	Spencer	None	Sample	No Export	No	N/A	(Analysis_HID_3500_50rfu_v5)	24plex_QS_Panels_v5	SST-BTO_60-500bp	None

DNA Typing Process & Instrumentation



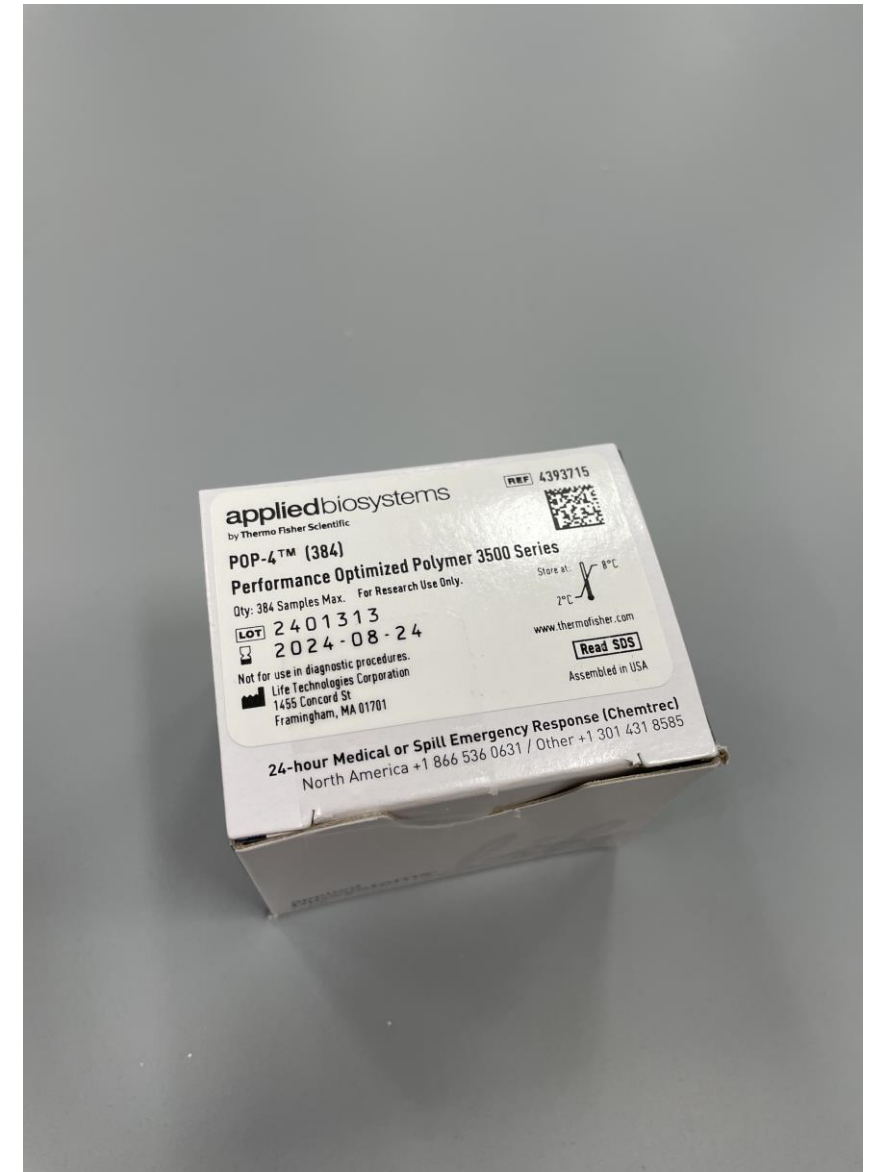
Maintenance

- Replacing rubber septa on plate and buffers when changed
- Replacing anode buffer - 14 days
- Replacing cathode buffer – 14 days
- Replacing the capillary array - 160 injections
- Refilling polymer - 384 injections
- Replacing fittings and o-rings – as needed



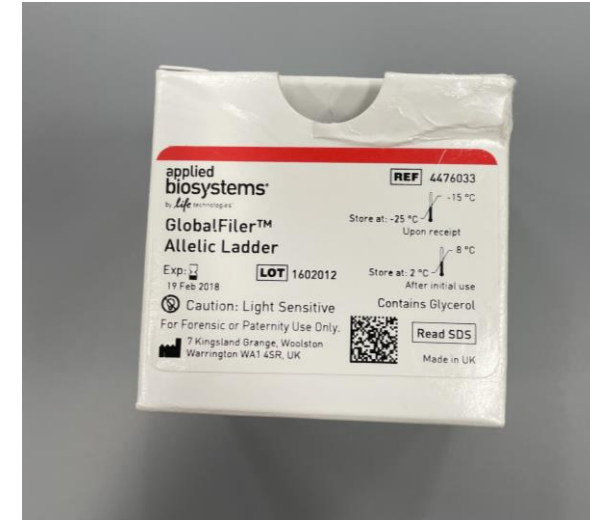
Storage of DNA Separation Reagents

- Refrigerator (2-8 °C)
 - Performance optimized polymer (POP-4™)
 - Anode buffer
 - Cathode buffer



Storage of DNA Separation Reagents

- Freezer (-15-25 °C)
 - Hi-Di formamide – highly deionized formamide prepared by formamide passing over ion exchange resin
 - Allele Ladder - specific to each kit with DNA fragments of the allele sizes



Study Questions

- What component of blood, semen and saliva contains the nuclear DNA?
- What agents are used for cell lysis and how does each work?
- What pH is used for DNA binding and elution in DNA extraction?
- What type of DNA is the final EZ1 eluted product?
- How is DNA separated in extraction instruments?
- What steps are performed by robotic DNA extraction instruments?
- What is three-step PCR and what happens at each step?
- What functions are performed by CE instruments?
- What calibration steps must be performed prior to running CE instruments?
- What maintenance steps must be performed prior to using the CE?

Suggested Readings

- Andersen J. and Bramble, S. The effects of fingerprint enhancement light sources on subsequent PCR-STR analysis of fresh bloodstains. *Journal of Forensic Sciences* 1997; 42(2): 303-306.
- Butler, J.M. *Advanced Topics in Forensic DNA Typing: Methodology*. Chapter 2: DNA Extraction Methods, 2011.
- Butler, J.M. *Advanced Topics in Forensic DNA Typing: Methodology* Ch. 6: Capillary Electrophoresis, 2011.
- Butler, J.M. *Advanced Topics in Forensic DNA Typing: Methodology*. Chapter 11: Low-Level DNA Testing: Issues, concerns, and solutions, 2011.
- Giusti, A.M., et al. Application of deoxyribonucleic acid (DNA polymorphisms to the analysis of DNA recovered from sperm. *J. For. Sci.*, 1986 31: 409-417.
- Hochmeister M.N., et al. PCR analysis from cigarette butts, postage stamps, envelope sealing flaps, and other saliva stained material. *Methods in Molecular Biology*, Vol 98: Forensic DNA Profiling Protocols. Edited by P.J. Lincoln and J. Thomson © Humana Press Inc., Totowa, NJ.
- Walsh P et al. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*: 10:506-513.