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;

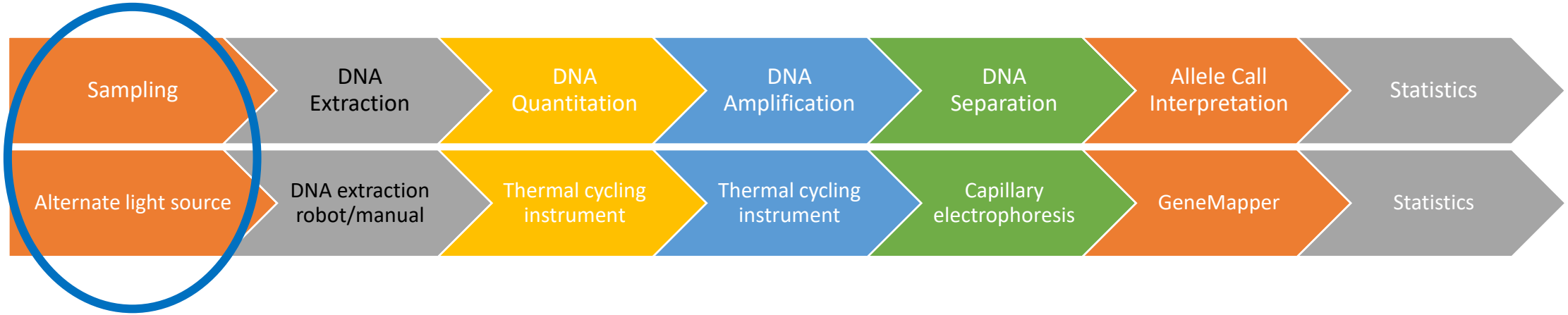
2. DNA separation and detection instruments and parameters;

3. software parameters associated with instruments;

4. maintenance and calibration;

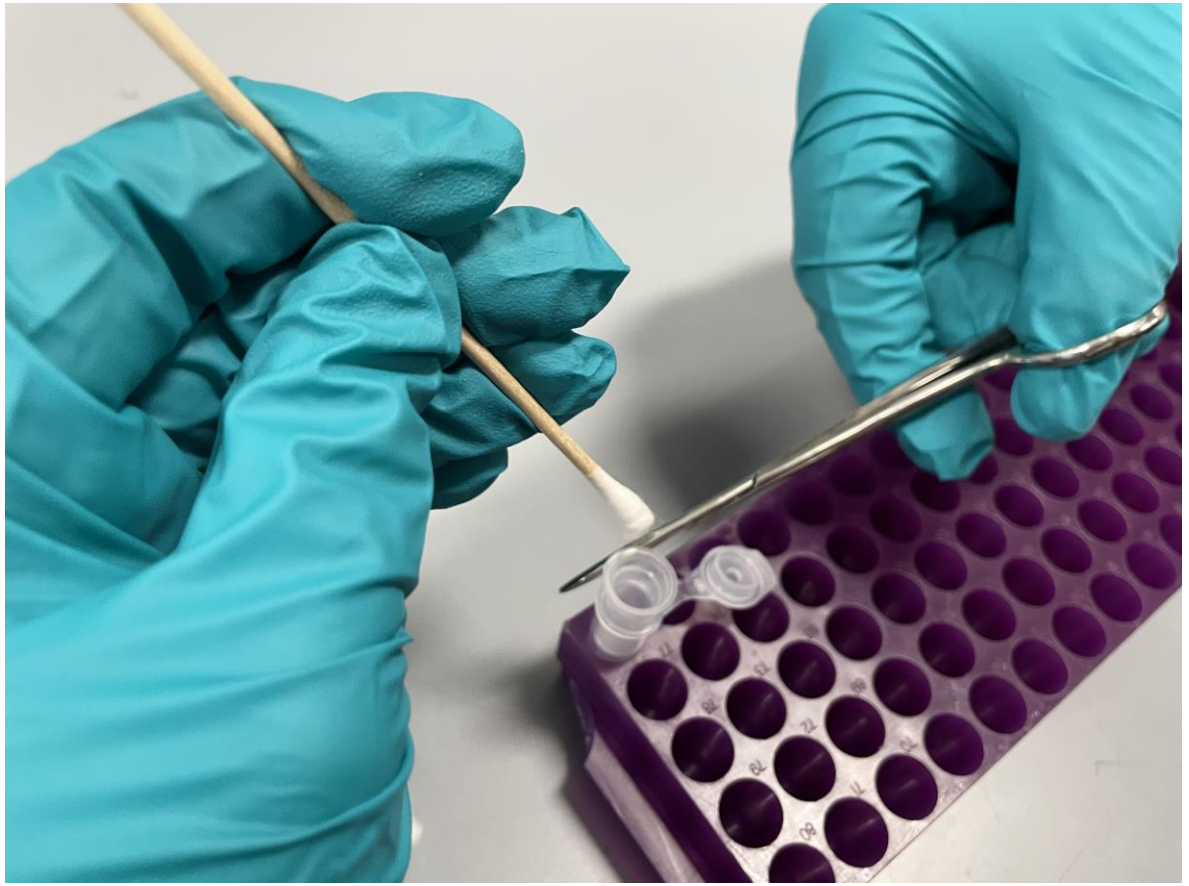
5. storage of STR typing kit and DNA separation reagents.

DNA Typing Process & Instrumentation

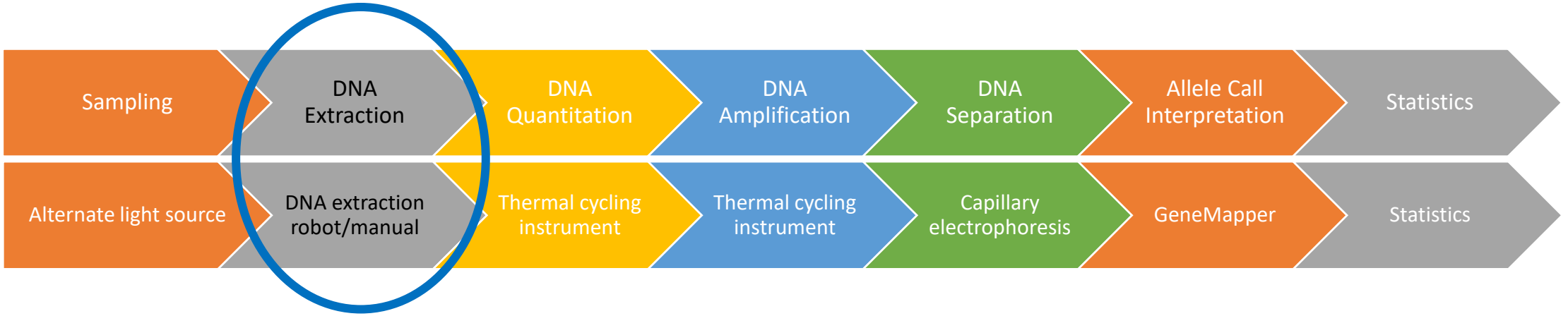


Prior to DNA Extraction: Sampling

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

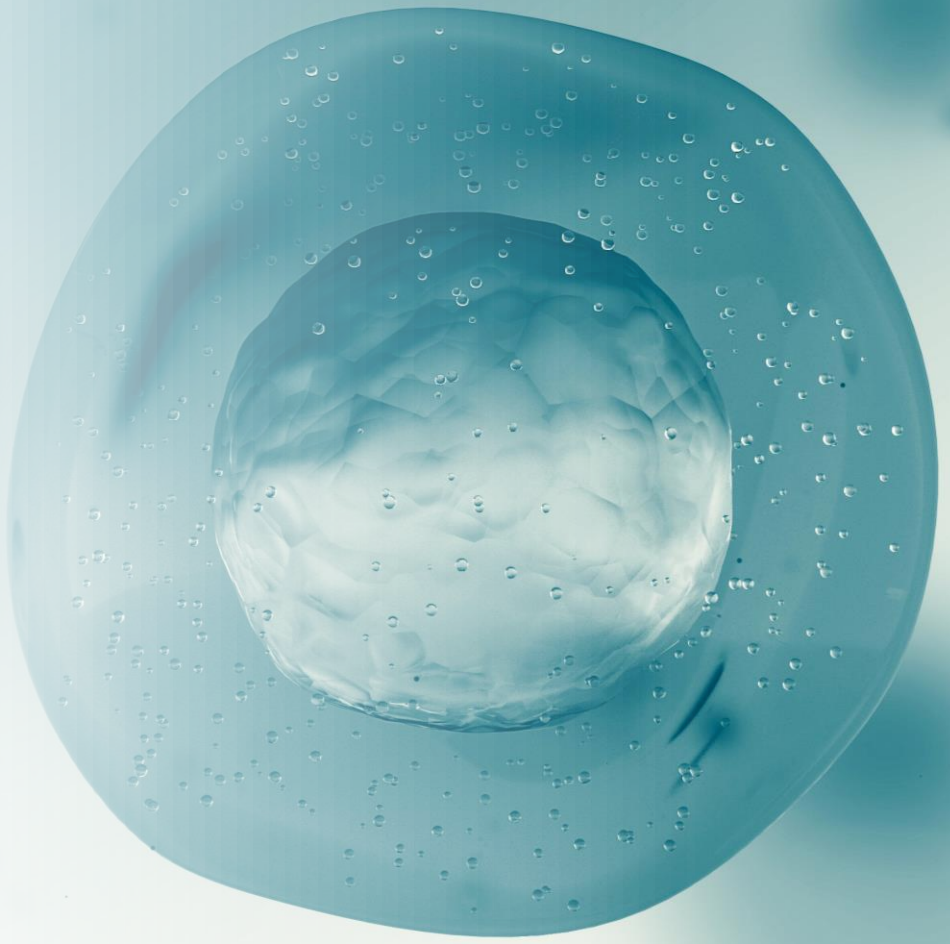


DNA Typing Process & Instrumentation



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

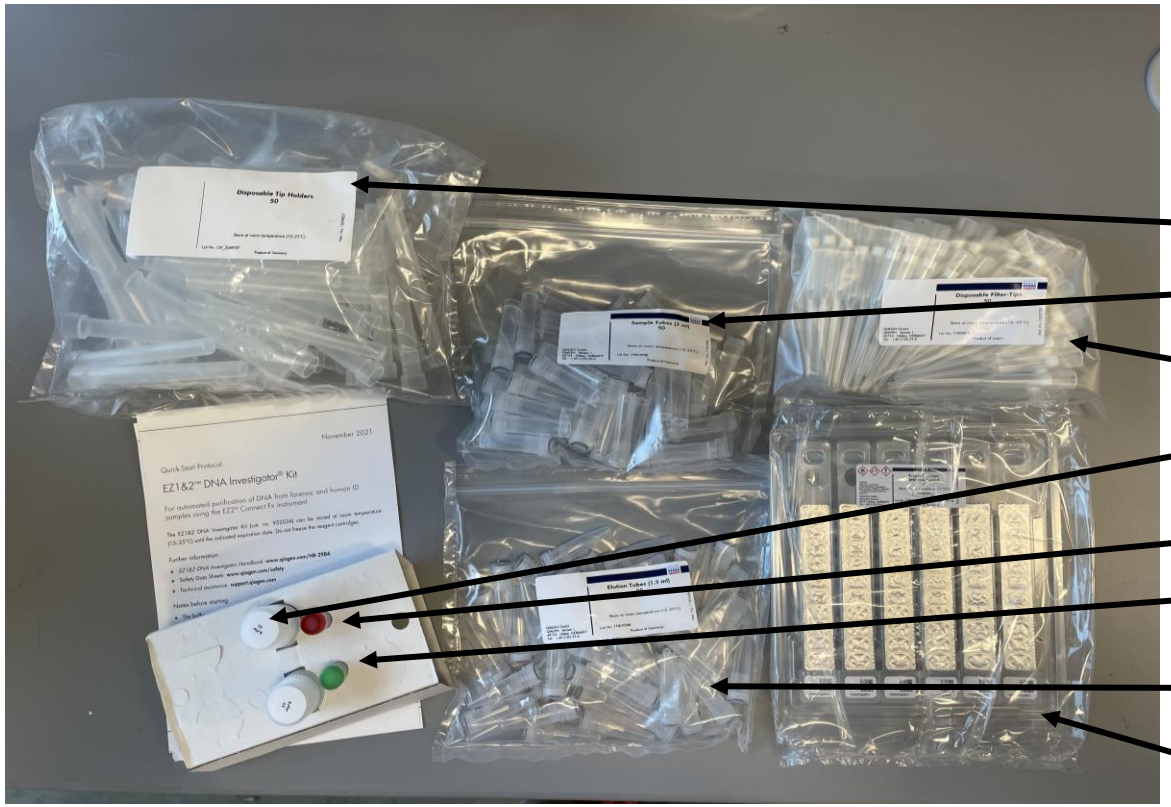
DNA Extraction Instruments

- 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

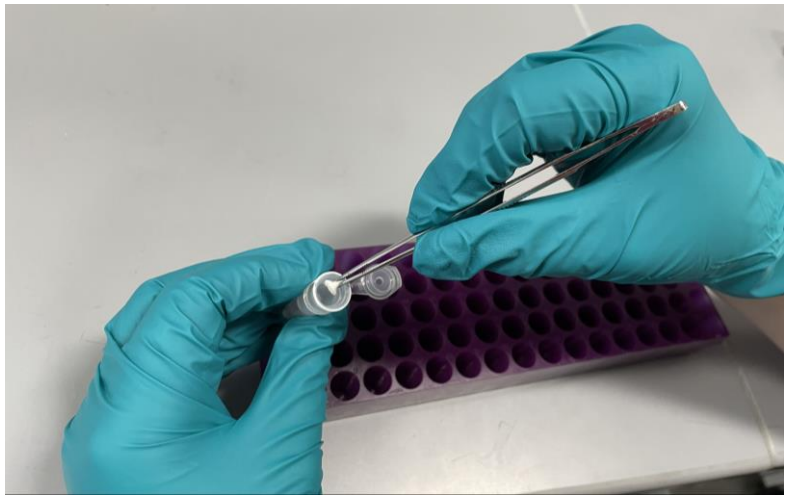
Elution tubes

Reagent cartridges

DNA Extraction: Pre-Extraction Steps

- Incubation
  - Thermomixer

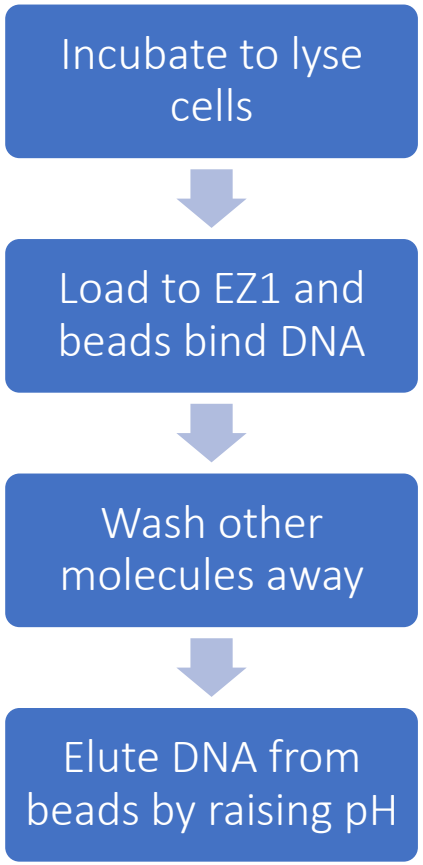
- Substrate removal
  - Spin basket and tweezers



- 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

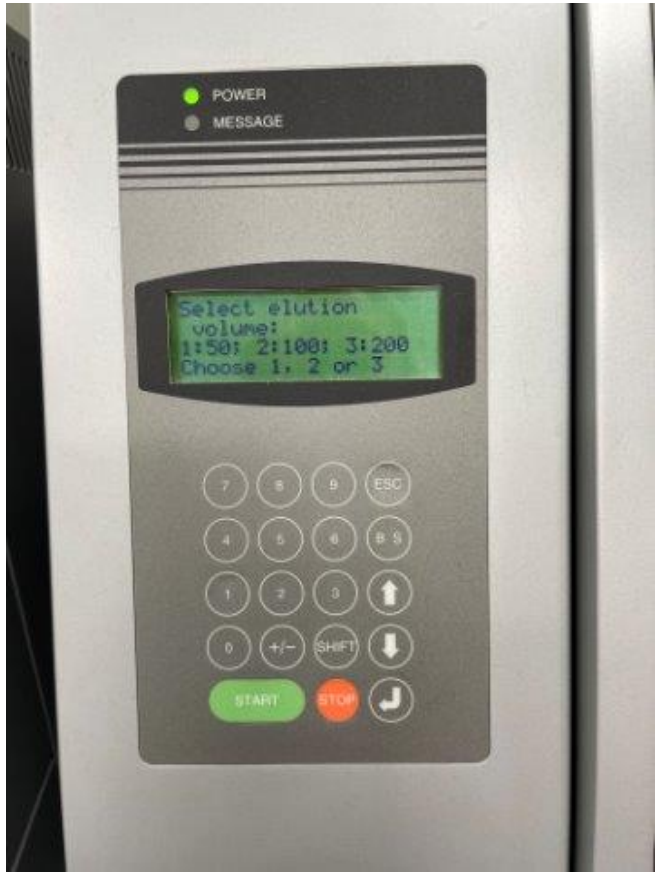
- Apply validated or manufacturer's protocol
  - Trace- for low quantity samples
  - Trace Tip Dance (TD)- protocol for when sample cutting remains in tube
  - Normalization – eliminates the need to quantify post-extraction
  - Large Volume- protocol for larger input buffer and reagent volumes

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 µL) for more concentrated DNA
- Can use to concentrate DNA from a larger volume to a smaller volume

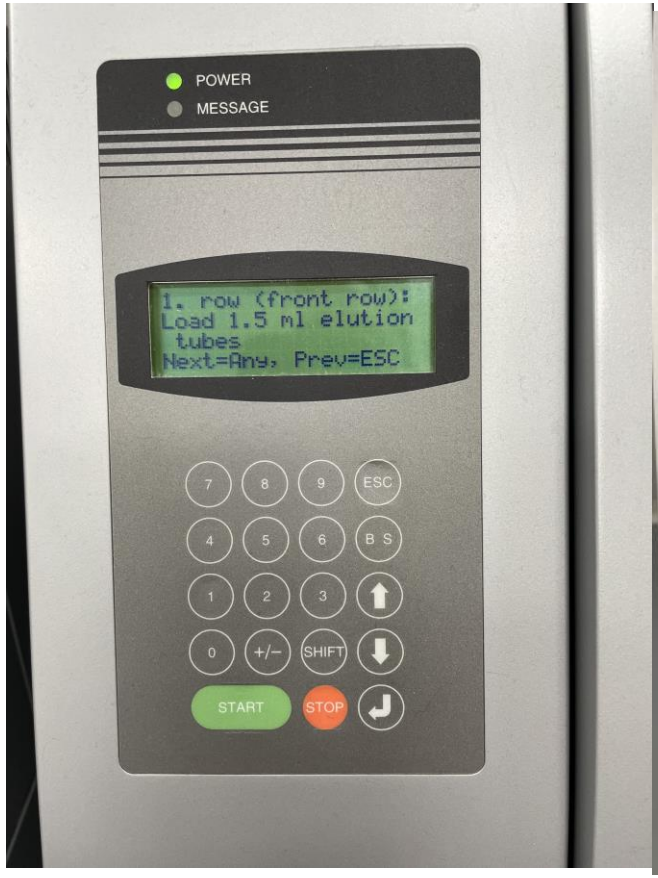


DNA Extraction Instrument Run Parameters: EZ1

- 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

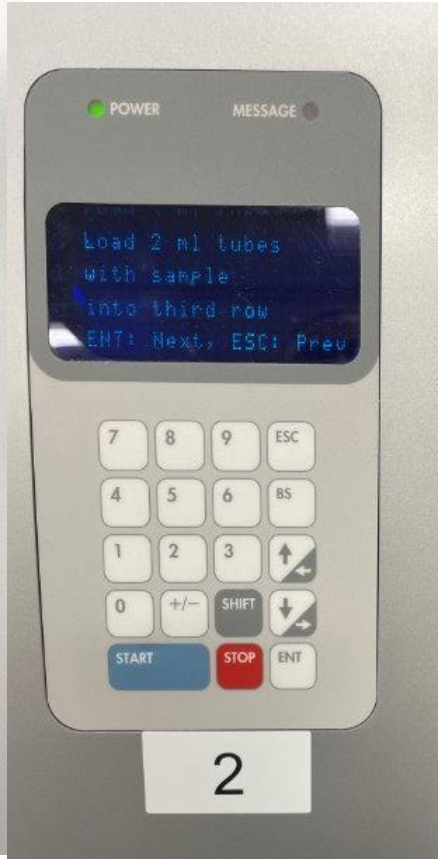


DNA Extraction Instrument Run Parameters: EZ1

- 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



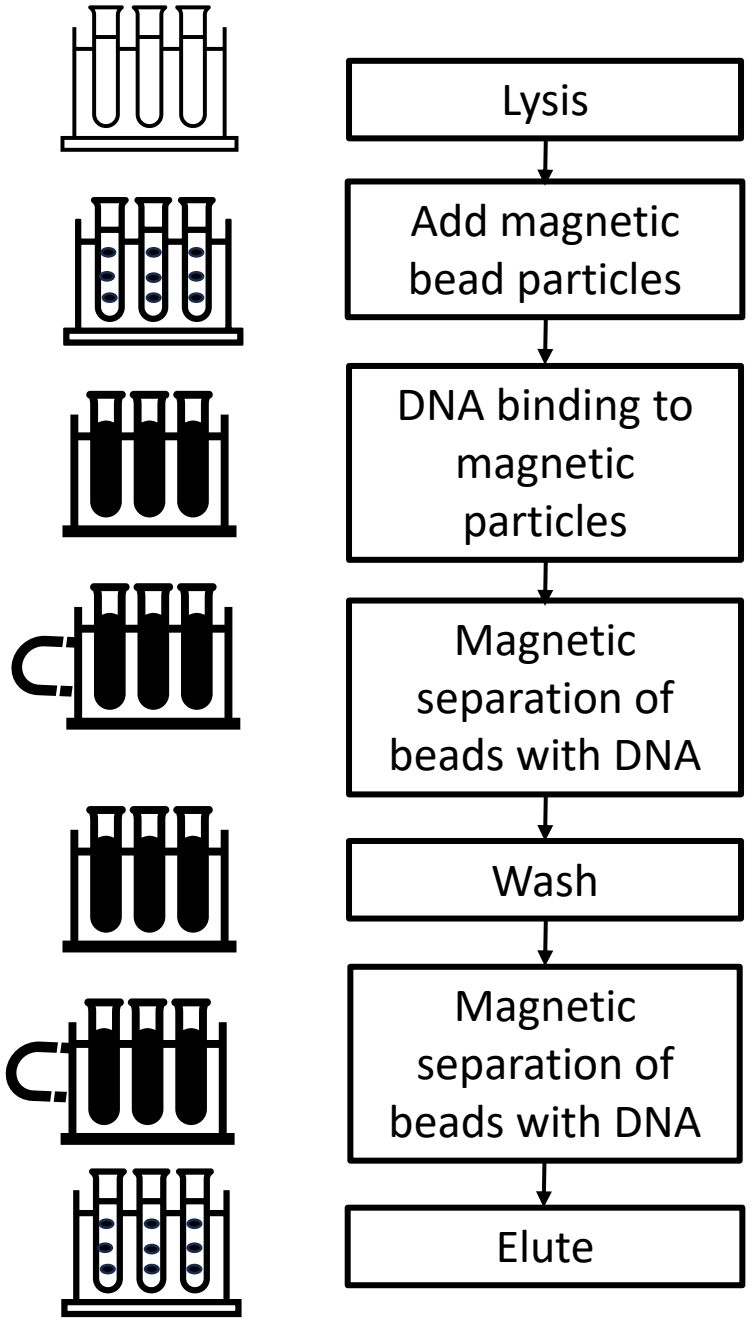
DNA Extraction Instrument Run Parameters: EZ1

- 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

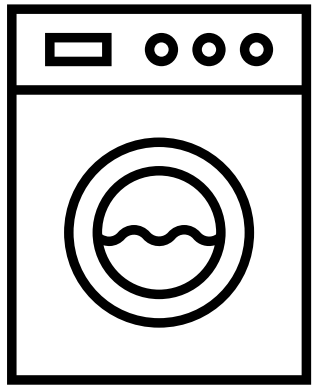
DNA Extraction Instrument EZ1: Binding

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?

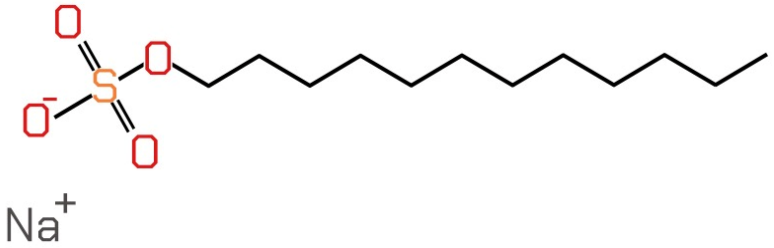


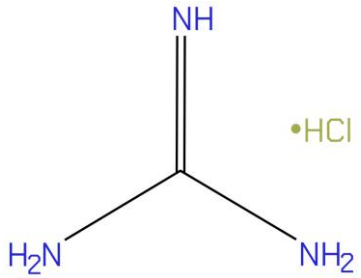
DNA Extraction: Lysis Agents

- 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





$Na^+ \ ^-O\text{-}H$

Figure credit: Nihal Chana

DNA Extraction: Lysis Agents

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

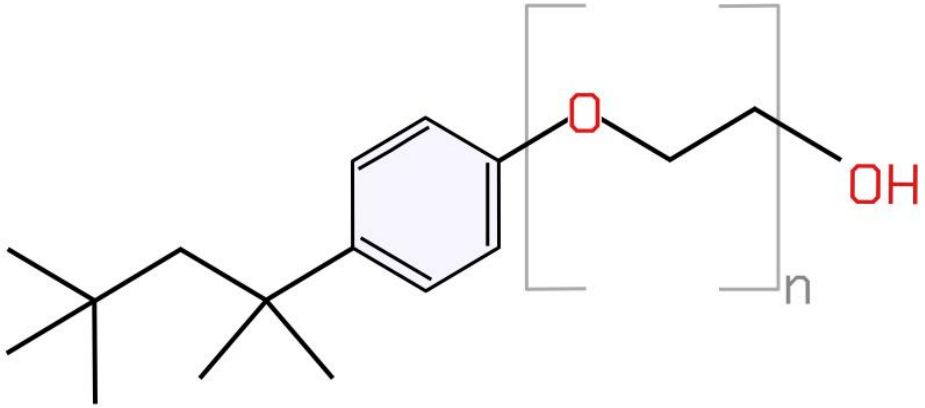


Figure credit: Nihal Chana

DNA Extraction: Lysis Agents

- Chemical agents
  - Tween 20 – non-ionic detergent for emulsifying and solubilizing protein

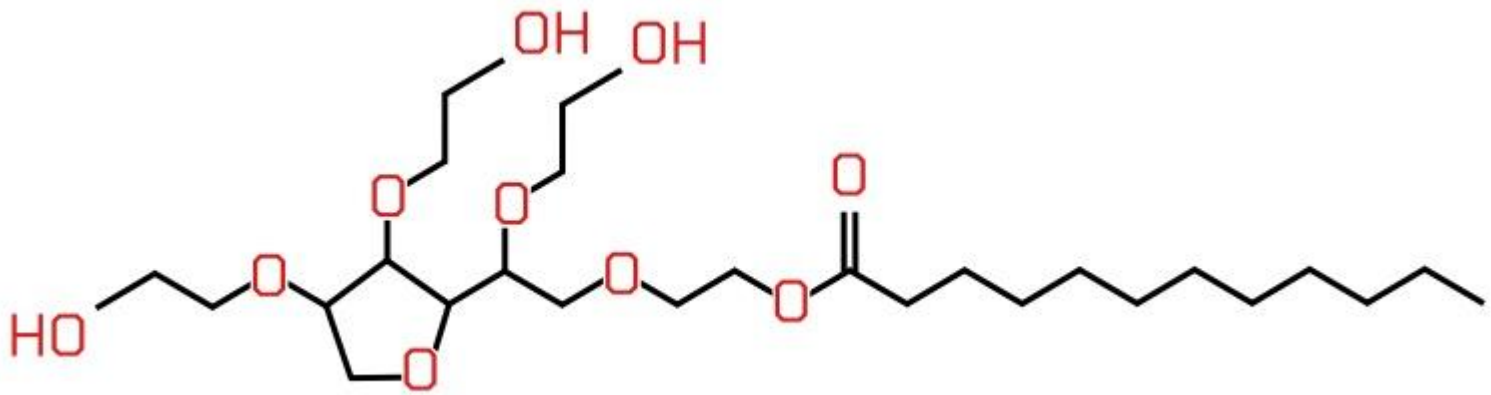
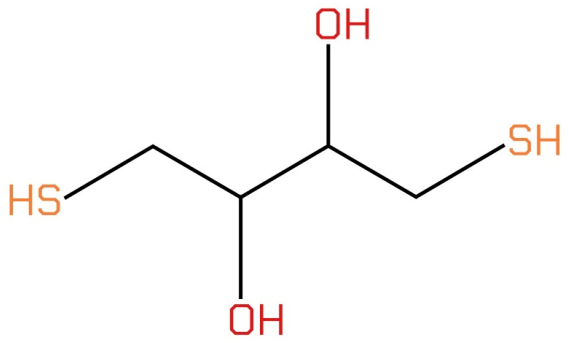


Figure credit: Nihal Chana

DNA Extraction: Lysis Agents

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



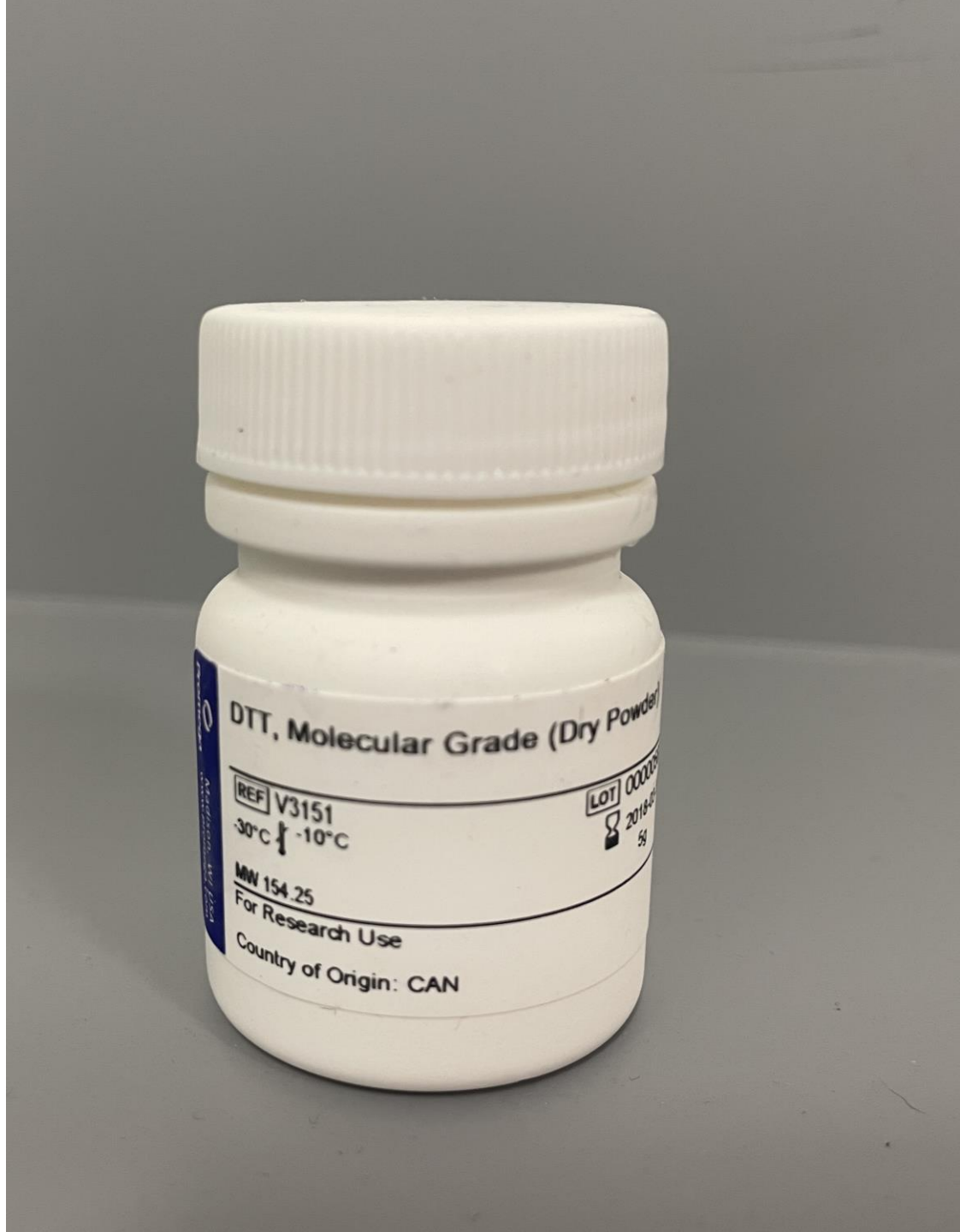
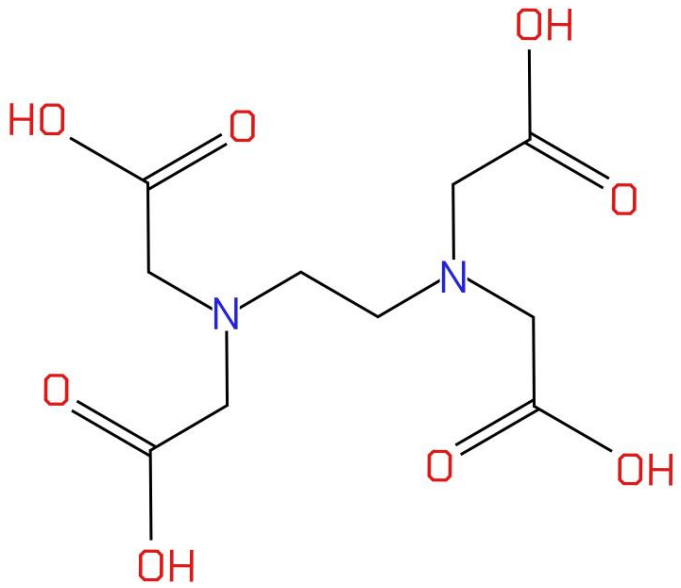


Figure credit: Nihal Chana

DNA Extraction: Lysis Agents

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



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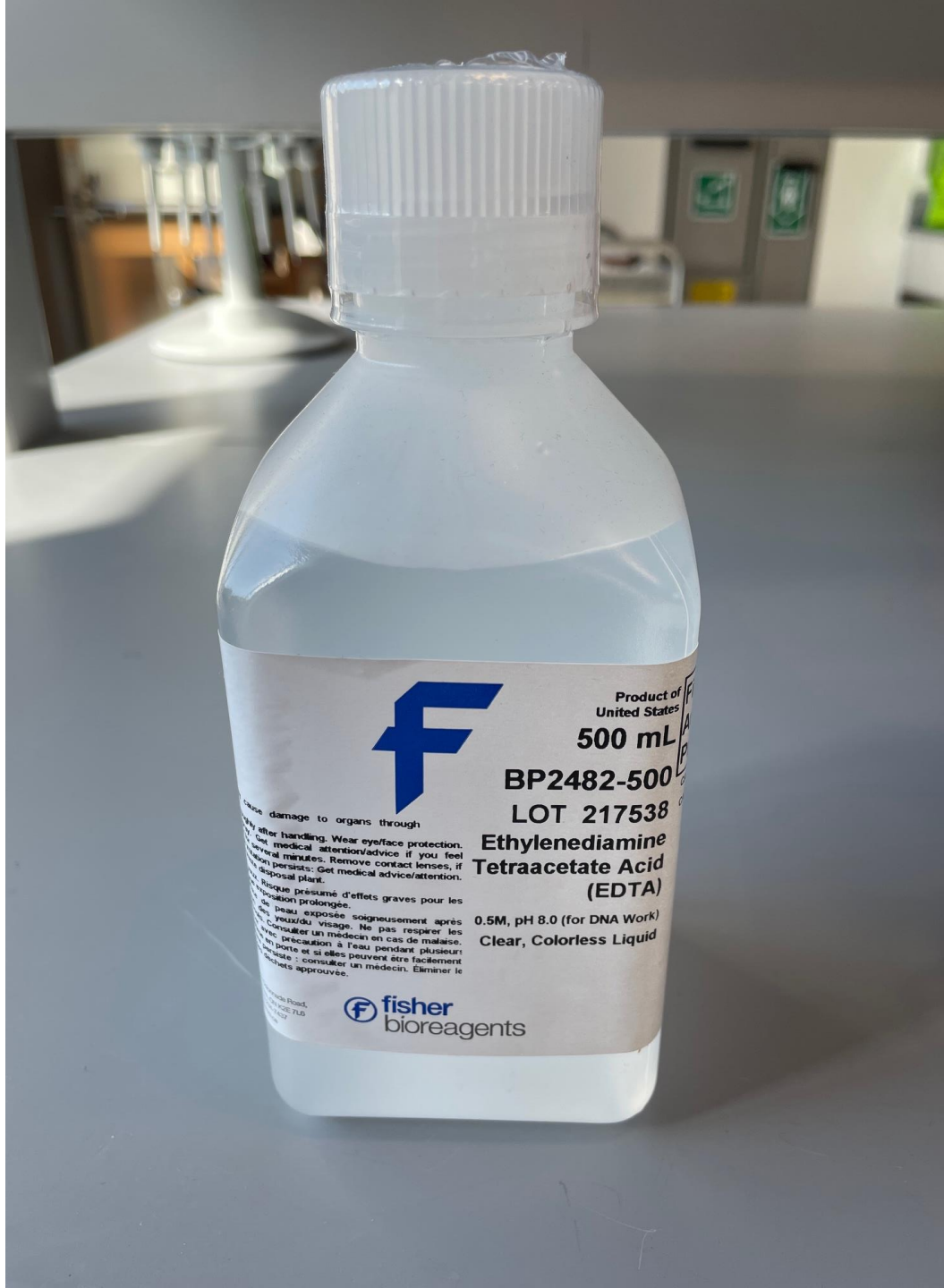


DNA Extraction: Lysis Agents

- Ethanol (CH_3-OH)
  - Precipitates DNA

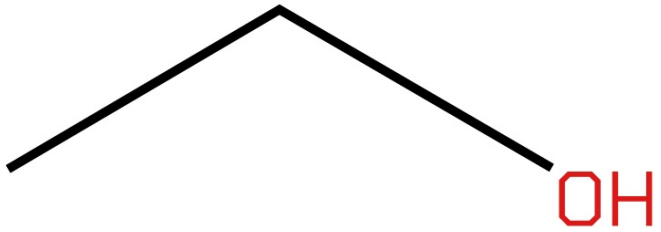
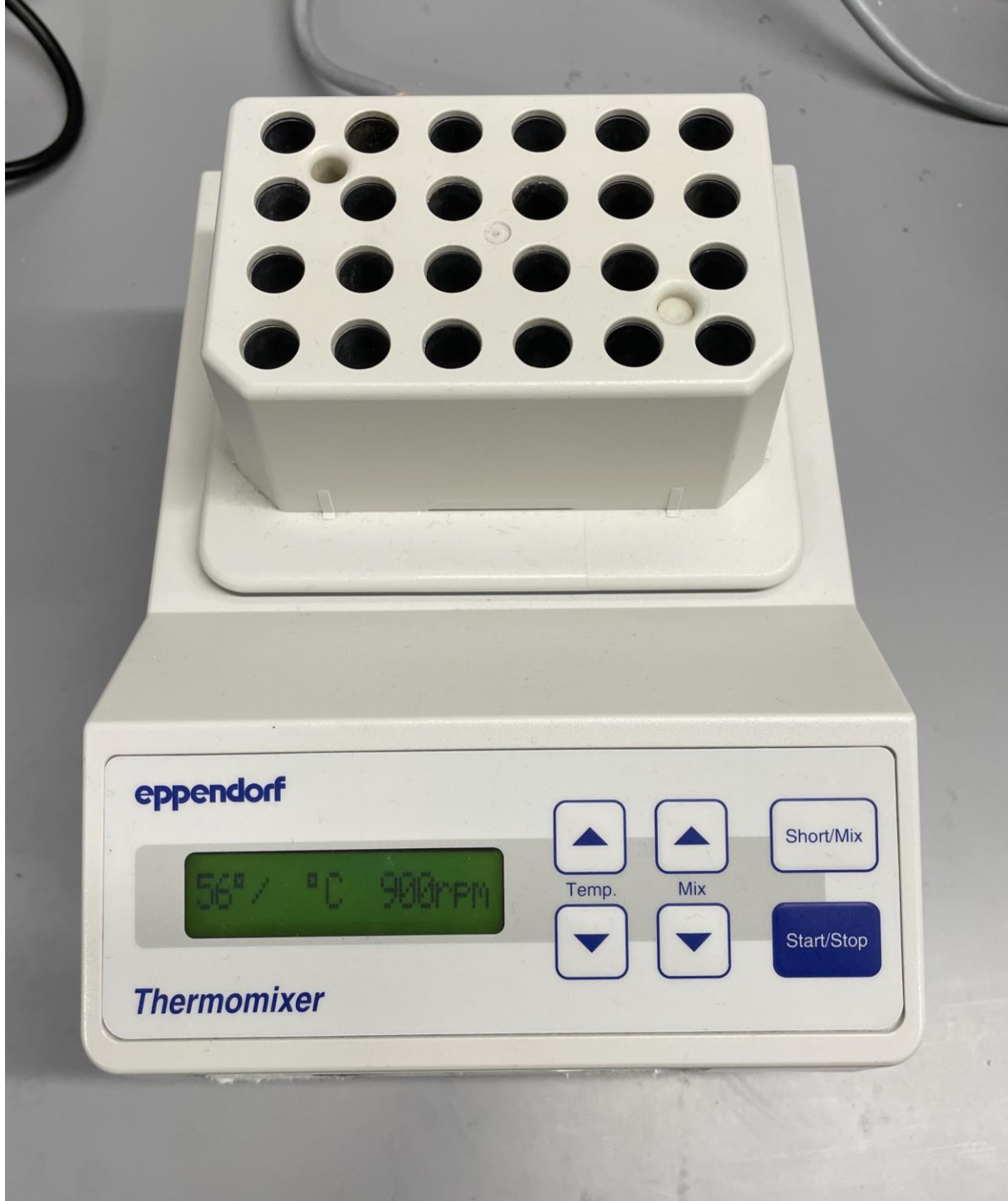


Figure credit: Nihal Chana

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 µL
140-190 µL G2, 10 µL ProK

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

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

Soil -– Trace/Tip Dance
Up to 0.5 g
100 µL G2

Blood/saliva stain - Trace
0.5 cm^2
290 µL G2, 10 µL ProK

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

Nail clipping - Trace
1 clipping
160 µL G2, 20 µL ProK, 20 µL DTT

Semen - Large Volume
>0.5 cm^2
455 µL G2, 25 µL ProK, 20 µL DTT

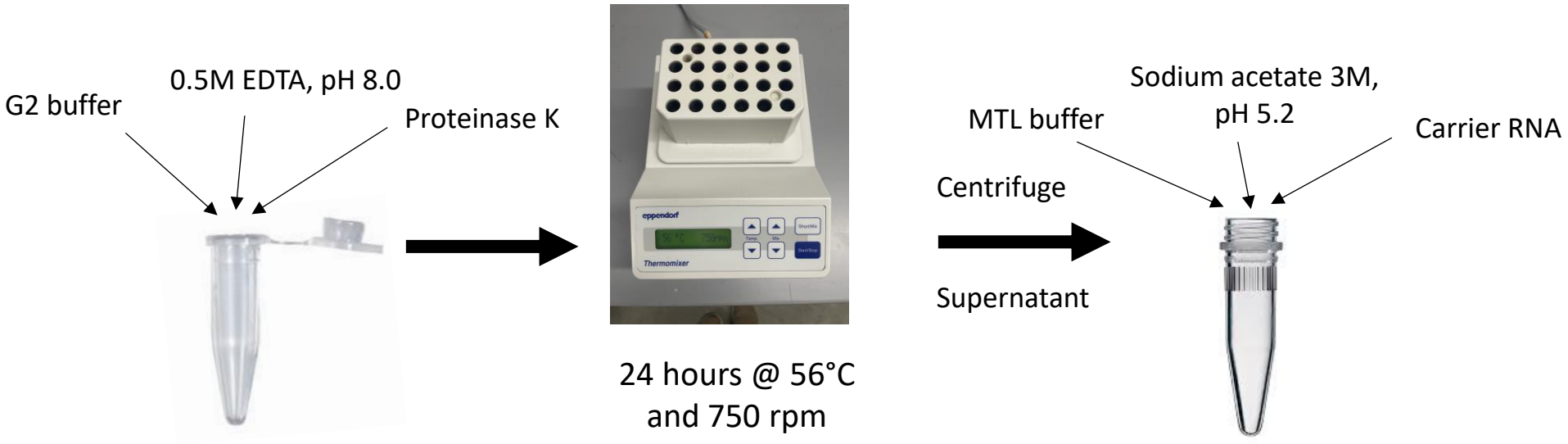
Tissues - Trace
Up to 10 mg
190 µL G2, 10 µL ProK

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

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

Cigarette butts – Trace/Tip Dance
1 cm^2
190 µL G2, 10 µL ProK

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



< 200 mg bone powder

225 µL G2, 25 µL ProK, 250 µL of 0.5 M EDTA pH 8.0
400 µL MTL buffer, 50 µL 3 M sodium acetate pH of 5.2, 1 µL 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 cm^2 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.

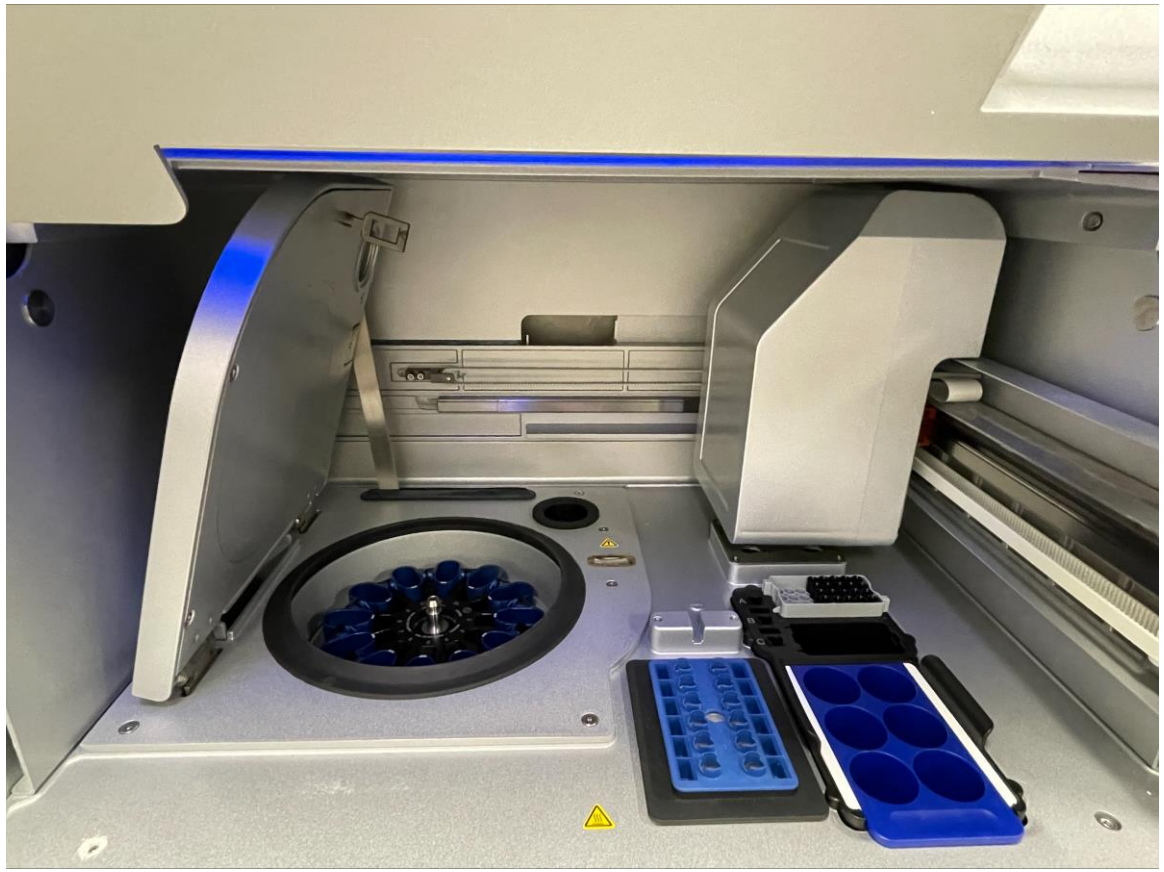
- Remove the **supernatant** to a new **non-sperm fraction** tube.

- 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

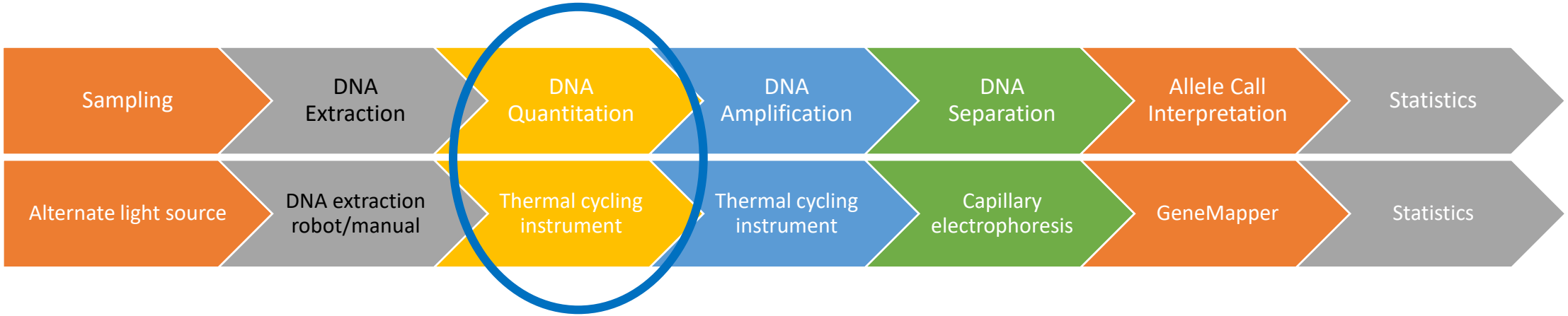
DNA Extraction: Sonication

- Digestion buffer
- Proteinase K
- Sonicator

Post-DNA Extraction- Concentration

- Ethanol precipitation
- Microcon
- EZ1 reextraction

DNA Typing Process & Instrumentation

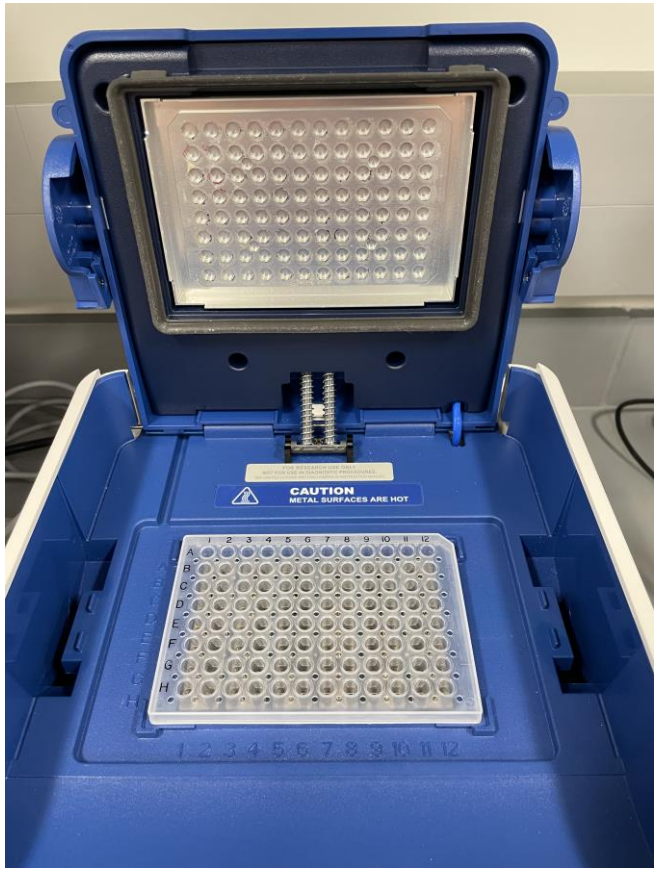


Thermal Cycling Instruments

- 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 snuggly 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

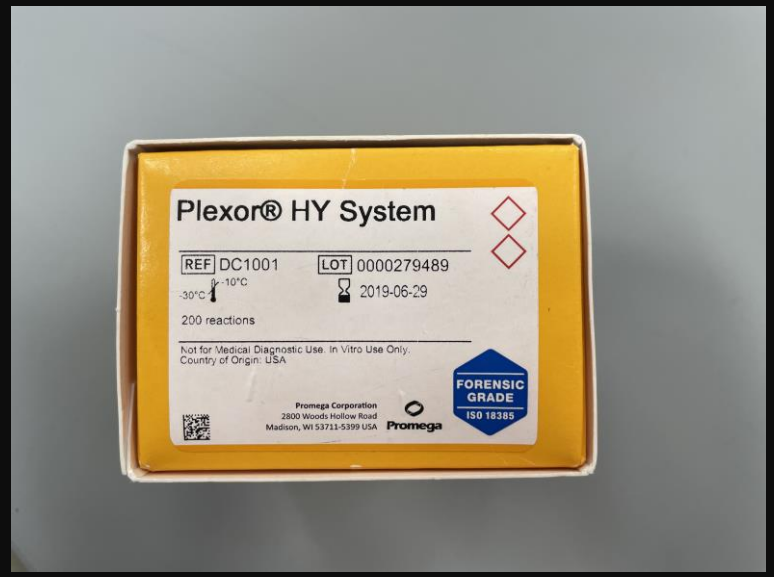


Thermal Cycling Parameters

- 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)

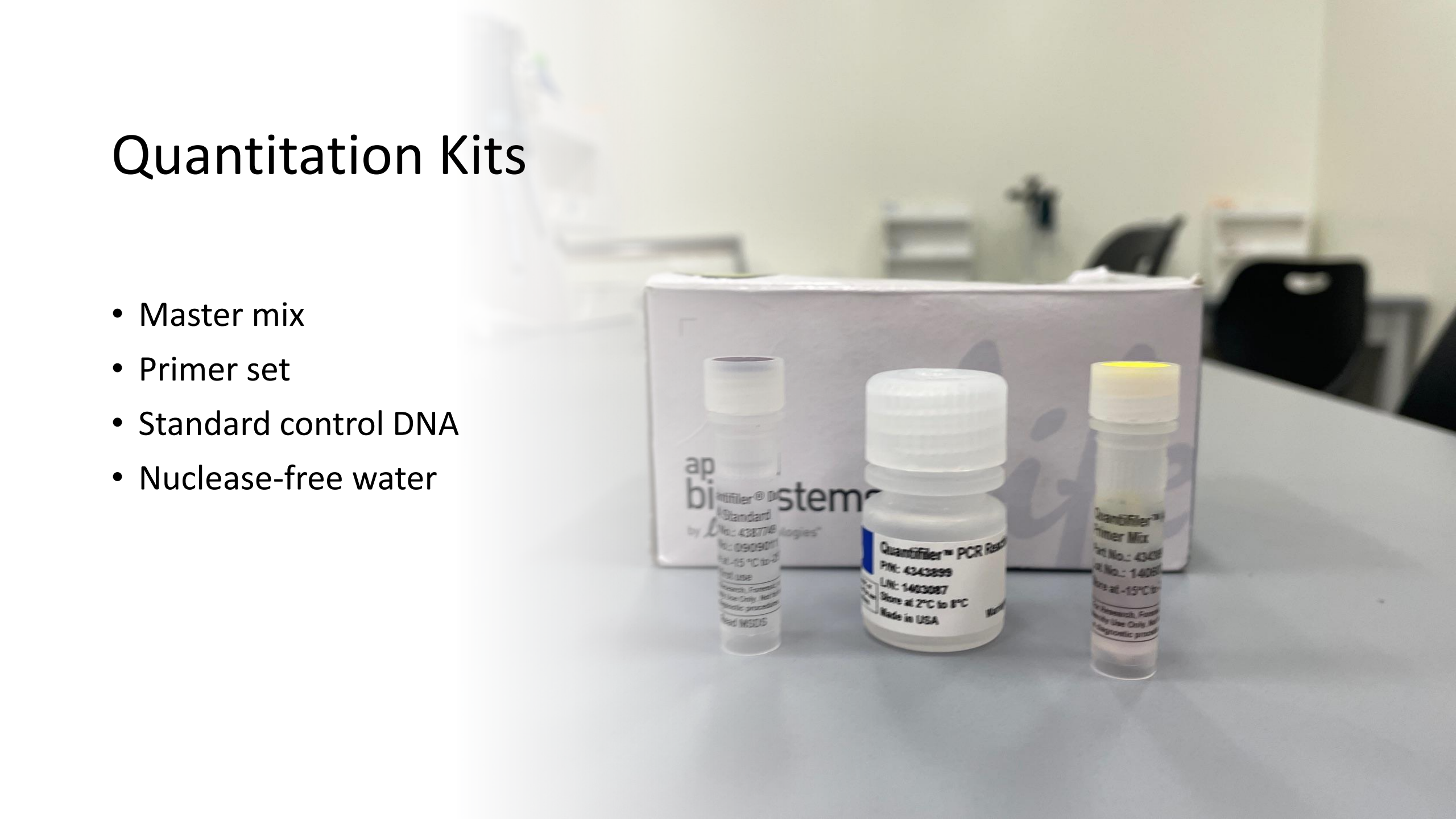
PCR DNA Quantitation Kits for Forensic Applications

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



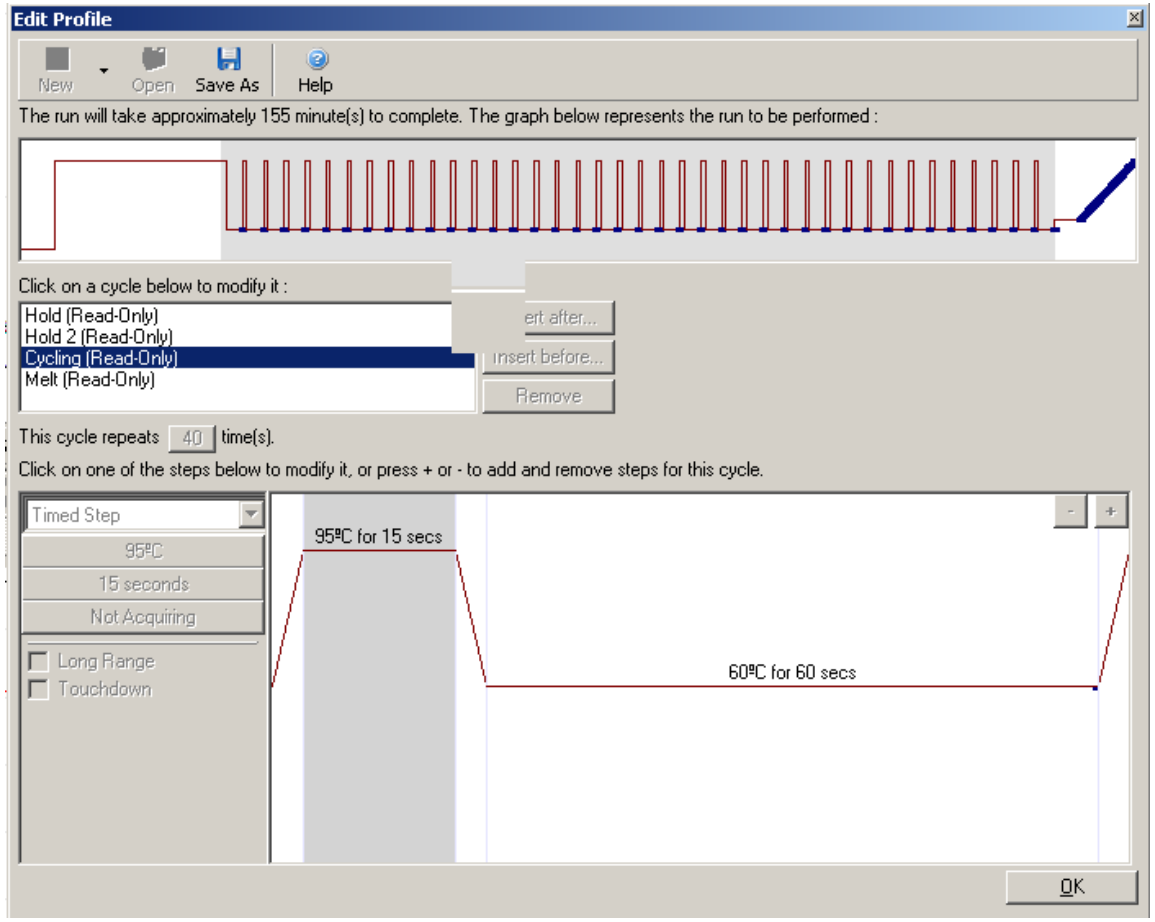
Quantitation Kits

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



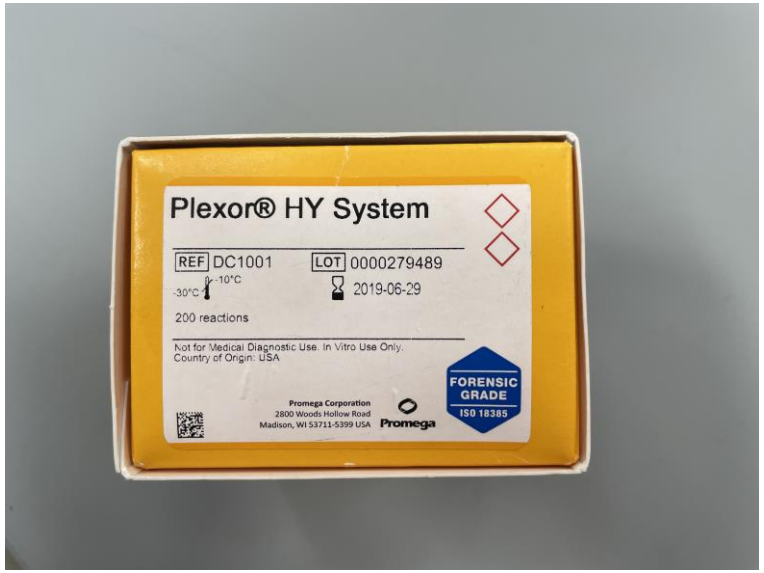
Thermal Cycler Run Parameters: Quantifiler™

- 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



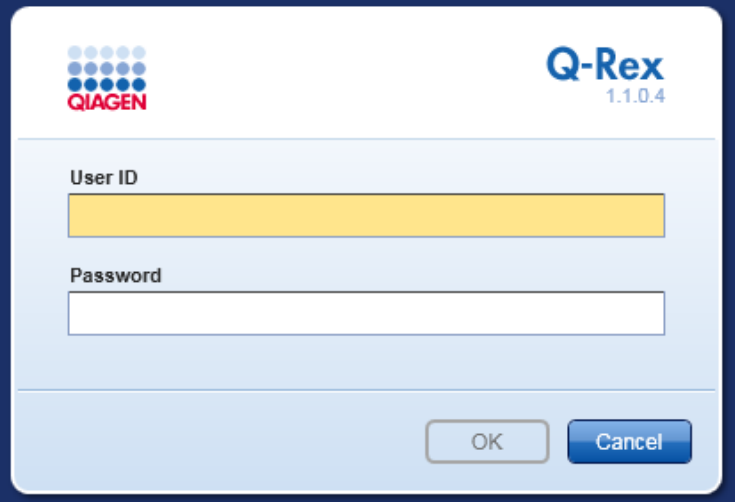
Thermal Cycler Run Parameters: Plexor® HY System

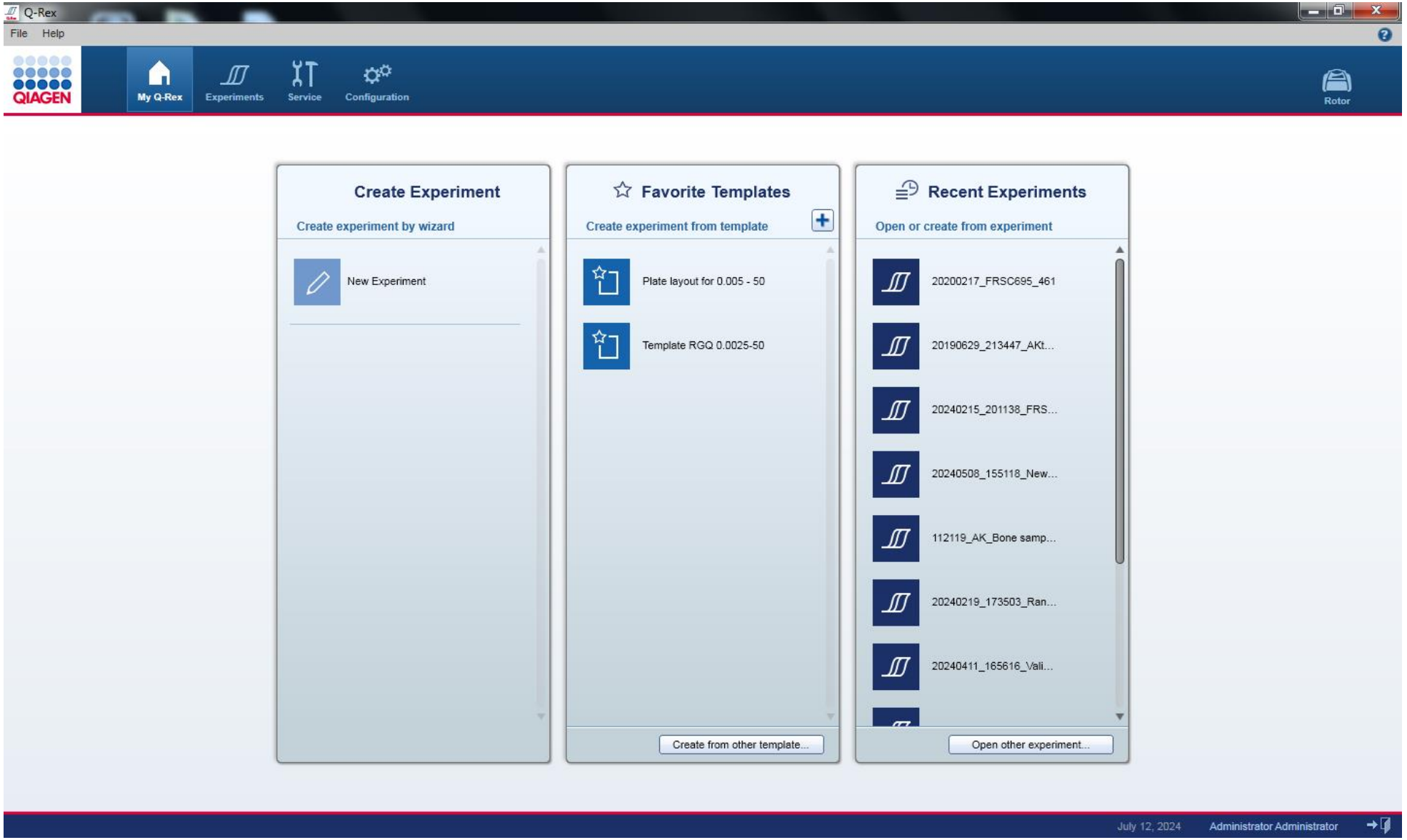
- Standards: 0.0032-50 ng/mL
- 10 µL PCR master mix, 1 µL primer/IPC mix, 7 µL amplification grade water, 2 µL of sample, standard, or control
- Denature
  - 95 °C for 2 min
- Two-step denature-anneal/extend cycle (38x):
  - 95 °C for 5 s
  - 60 °C for 35 s



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

- Standards: 0.0025-50 ng/mL
- 9 µL PCR master mix, 9 µL primer mix, 2 µL 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)




Q-Rex
File
Help
QIAGEN
My Q-Rex
Experiments
Service
Configuration
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, 2024
Administrator Administrator

Q-Rex

File Help

My Q-Rex | Experiments | Service | Configuration | Rotor

*New Experiment

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

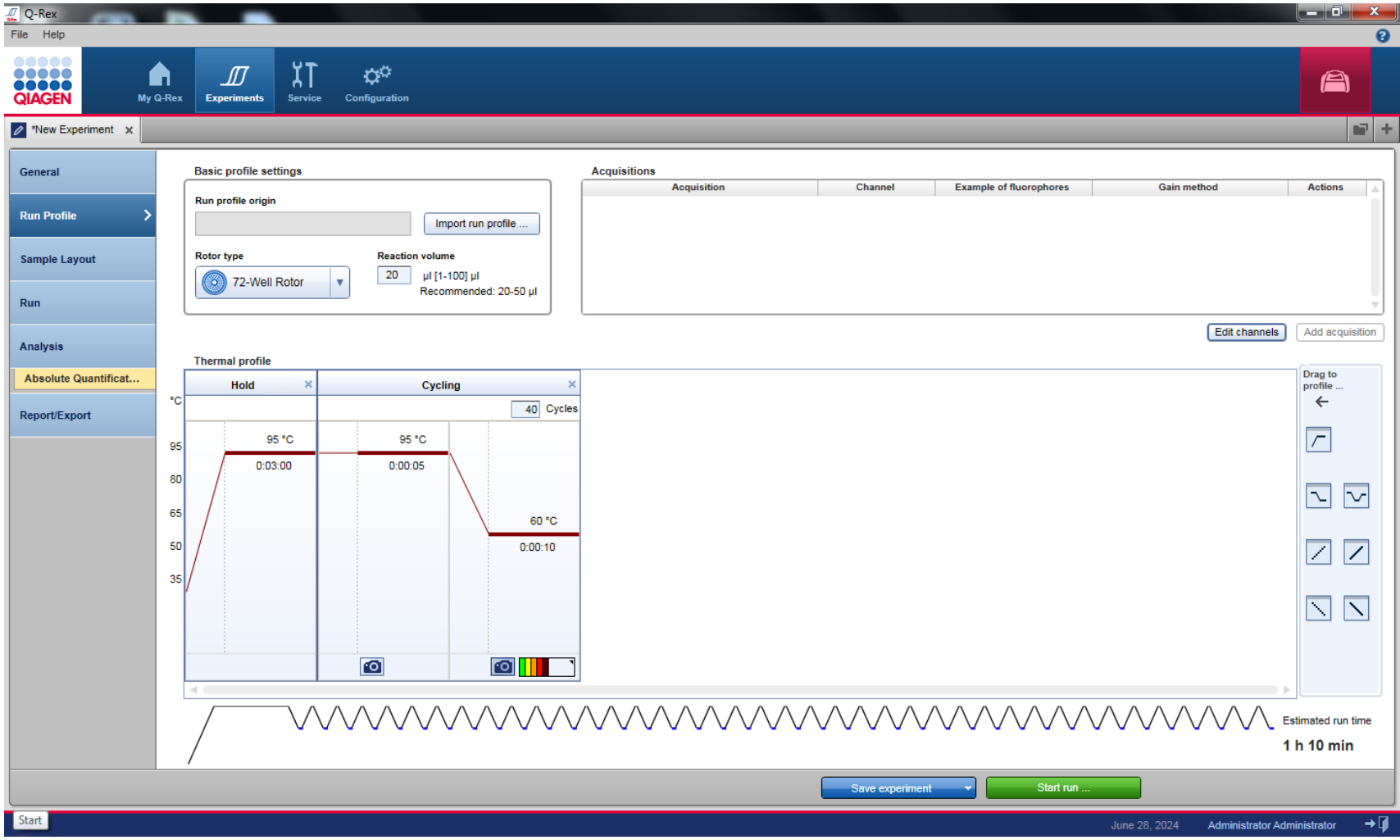
Kit name	Catalog / Material number	Lot number	Kit expiry date	
Investigator Quantiplex Pro RGQ Kit	-		9/11/2023	✕

Add QIAGEN kit ...

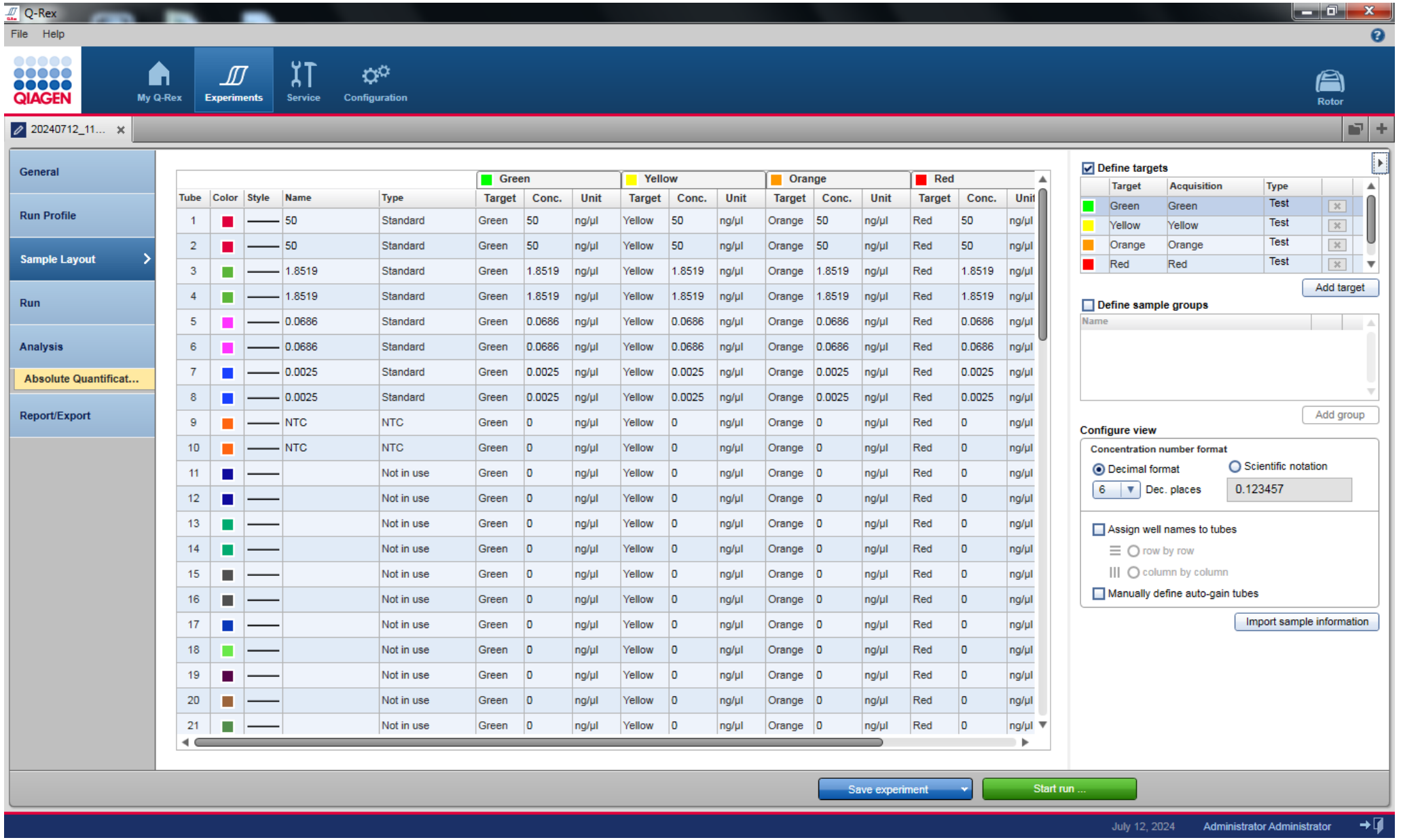
Save experiment | Start run

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

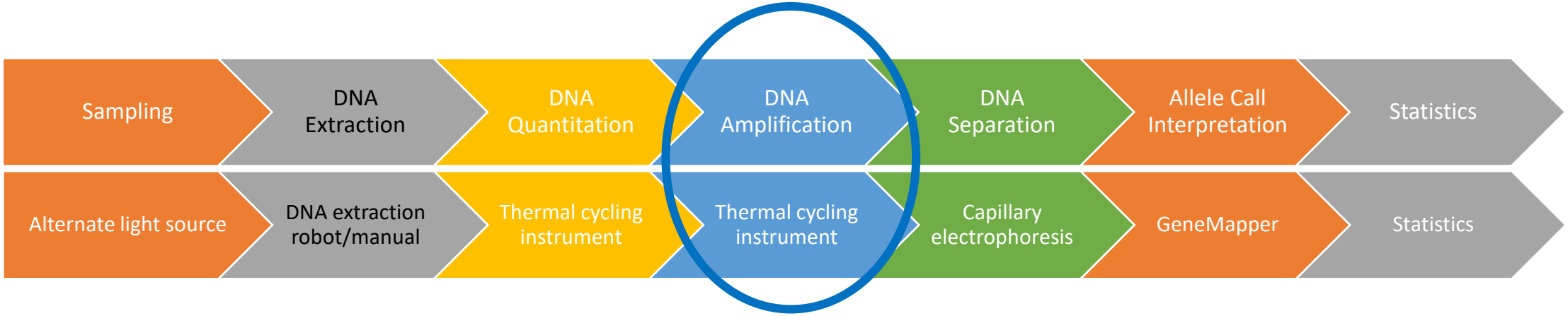


Thermal Cycle Parameters: Quantiplex Pro RGQ in Q-Rex Standard Input



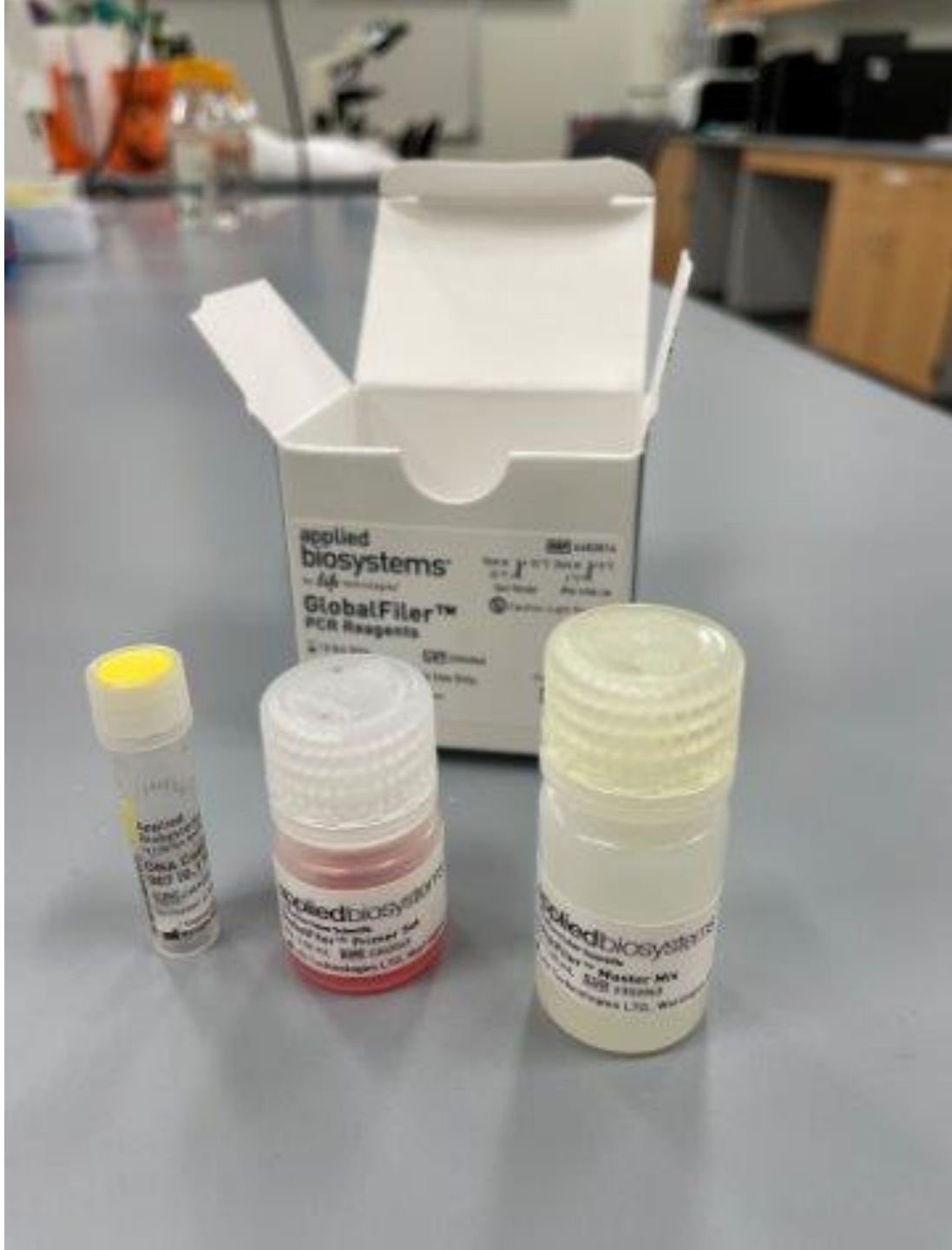
					Green			Yellow			Orange			Red		
Tube	Color	Style	Name	Type	Target	Conc.	Unit	Target	Conc.	Unit	Target	Conc.	Unit	Target	Conc.	Unit
1			50	Standard	Green	50	ng/µl	Yellow	50	ng/µl	Orange	50	ng/µl	Red	50	ng/µl
2			50	Standard	Green	50	ng/µl	Yellow	50	ng/µl	Orange	50	ng/µl	Red	50	ng/µl
3			1.8519	Standard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

DNA Typing Process & Instrumentation



STR Amplification Kits

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

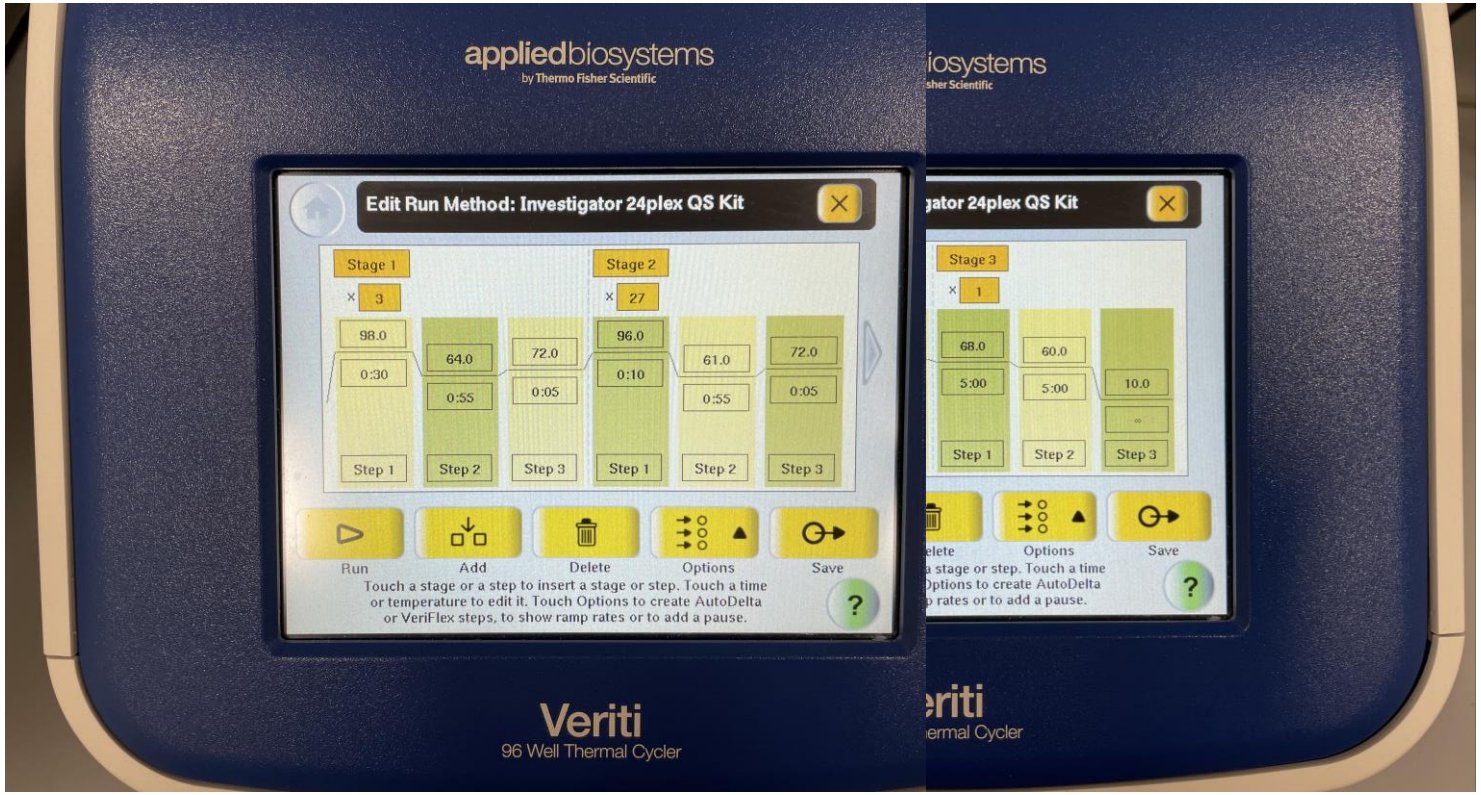


Thermal Cycle Parameters: GlobalFiler™

- 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

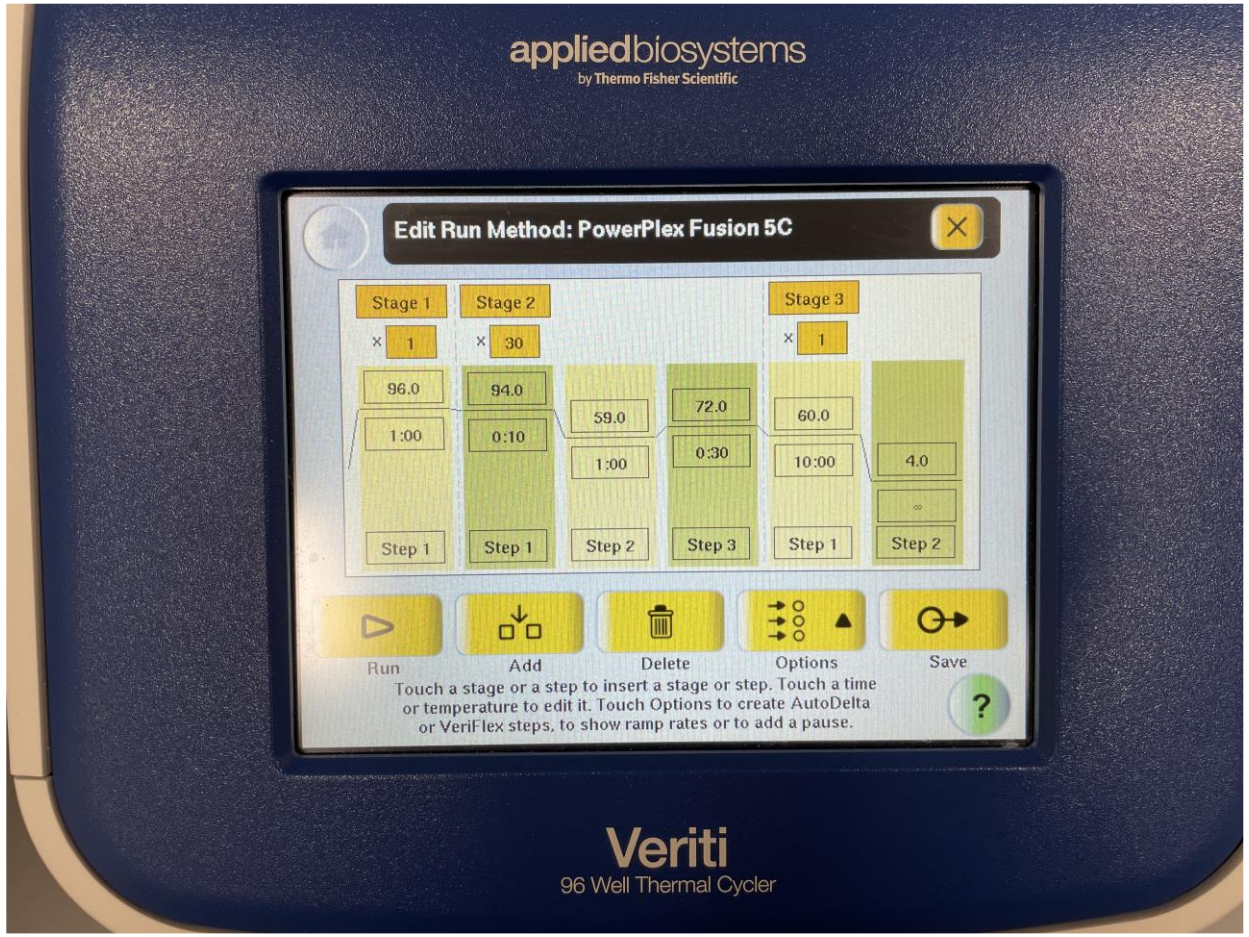
Thermal Cycle Parameters: Investigator 24plex QS

- 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

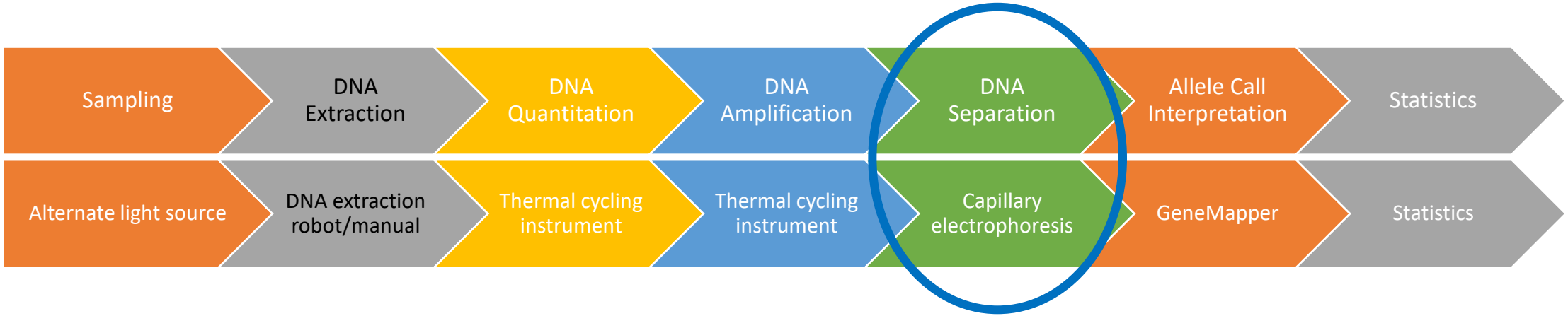
Thermal Cycle Parameters: PowerPlex 35GY

- 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

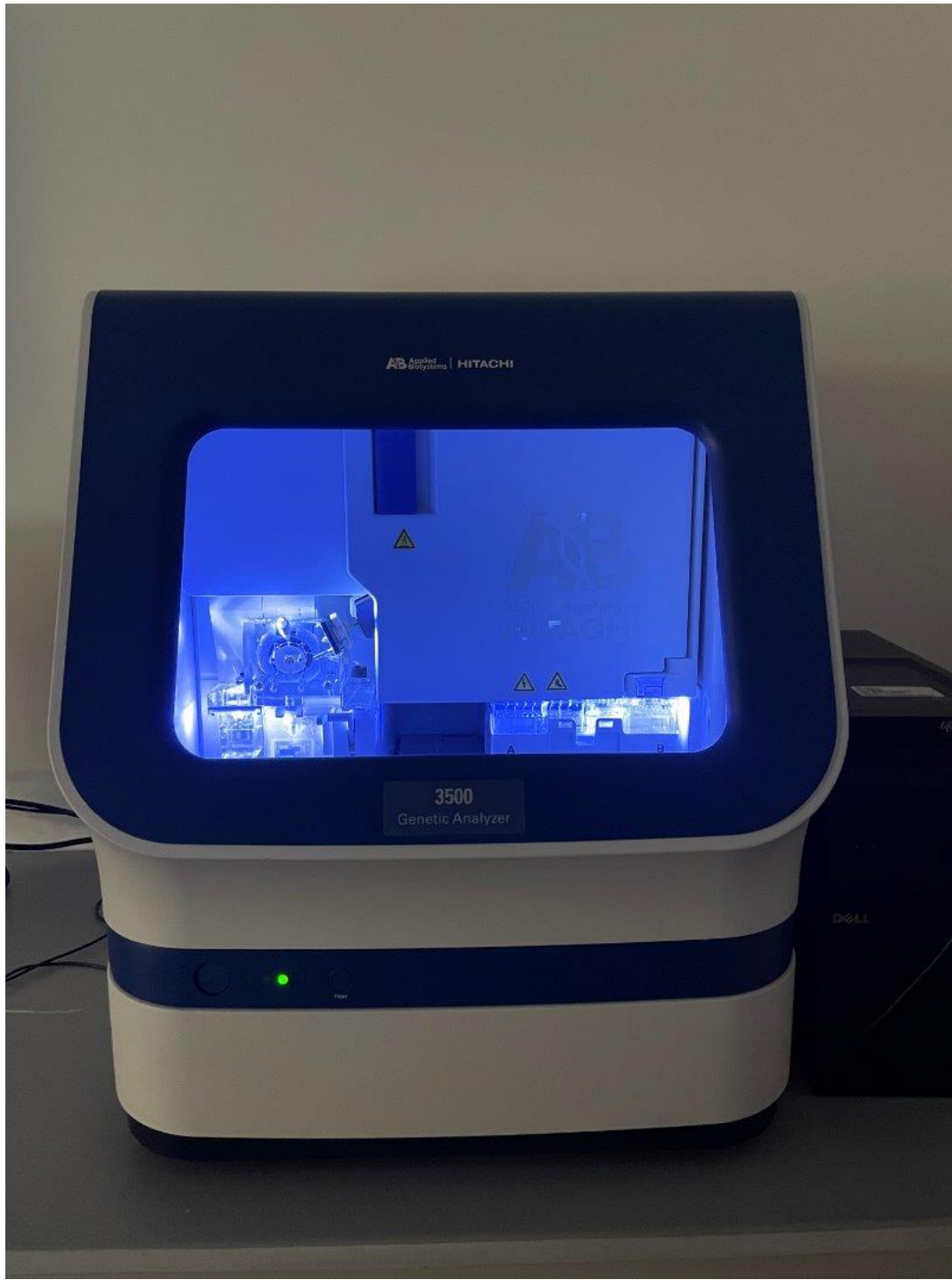
- Routine, according to requirements for accreditation and lab SOP
- Examples include temperature verification and run cycle performance

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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3500
3500 Results


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by life technologies
3500 Series
Data Collection Software 3
Version 3.1
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Not for use in diagnostic procedures.
Launching 3500 Series Data Collection Software 3...
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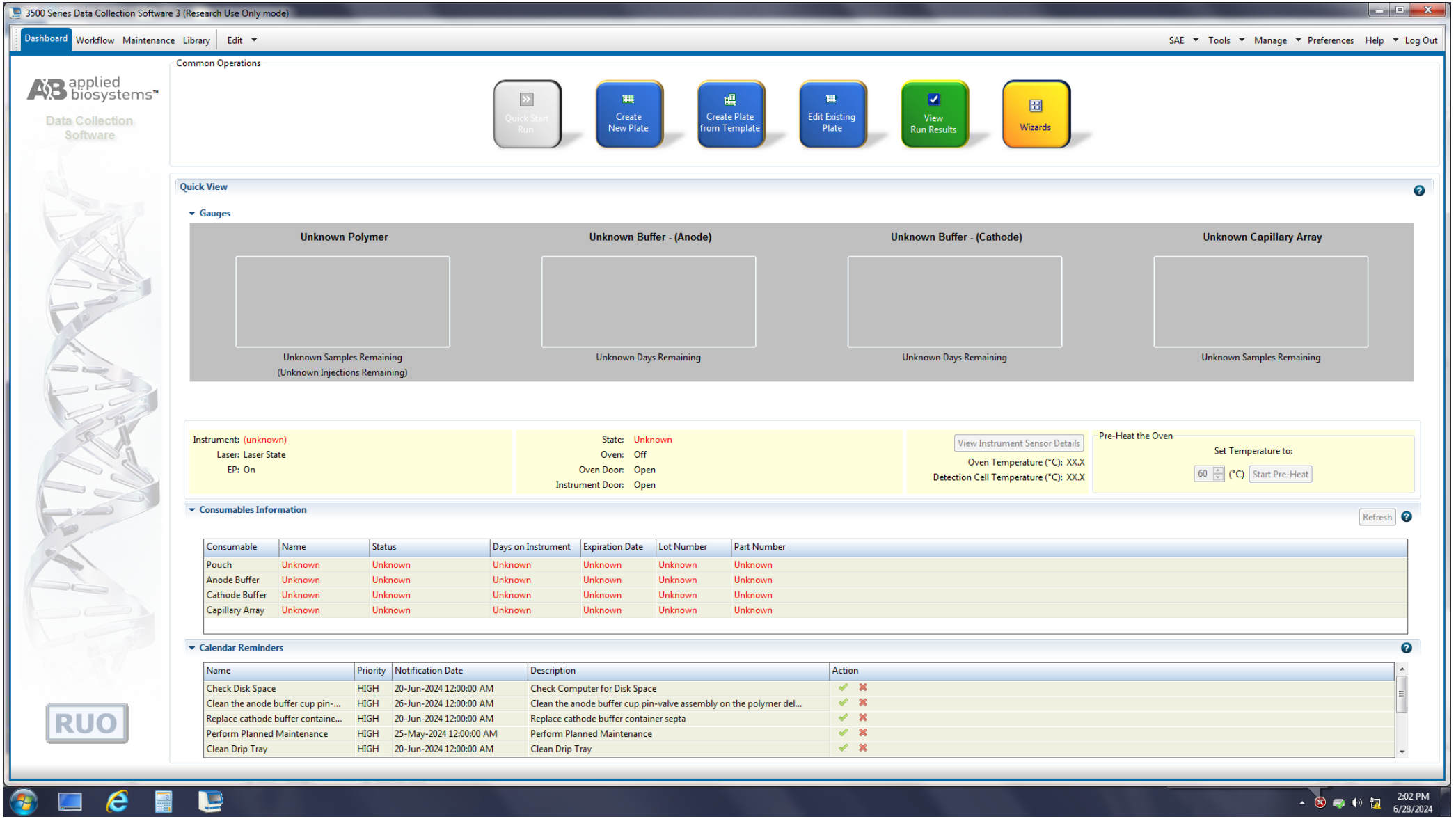
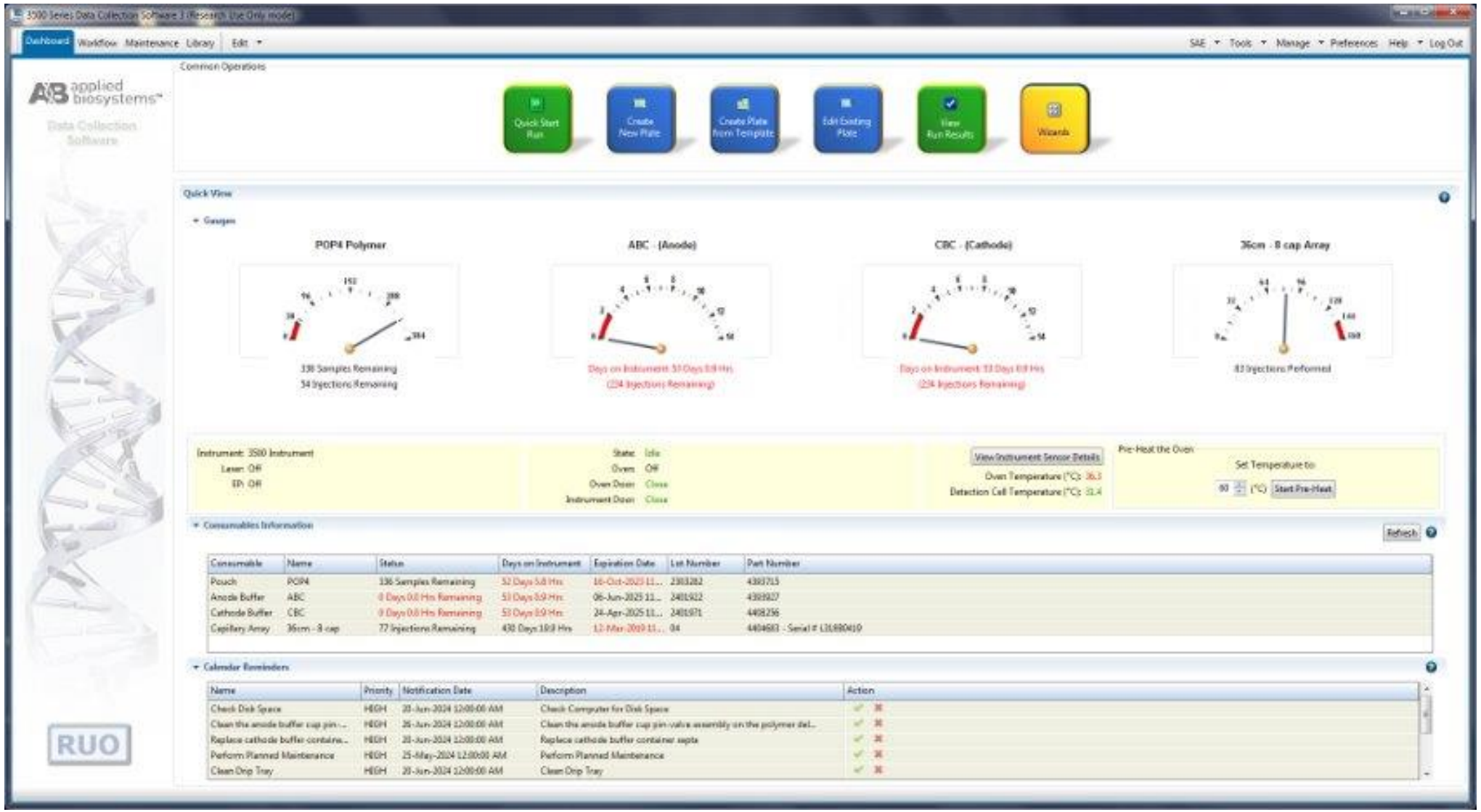
AB 3500 Dashboard: Instrument Not Connected

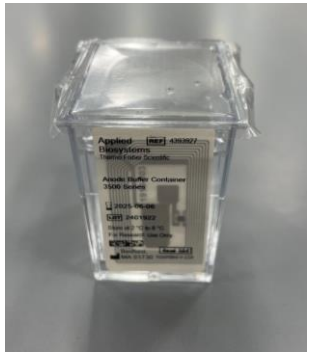


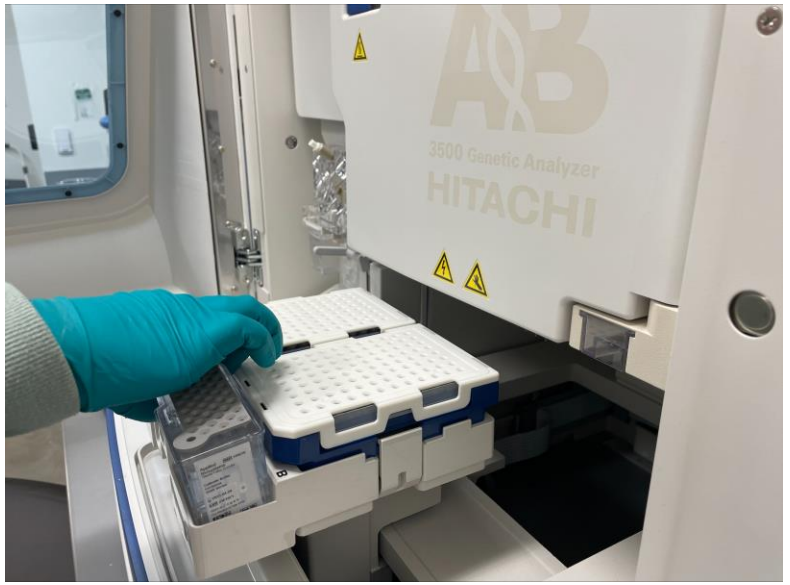
AB 3500 Dashboard: Instrument Connected



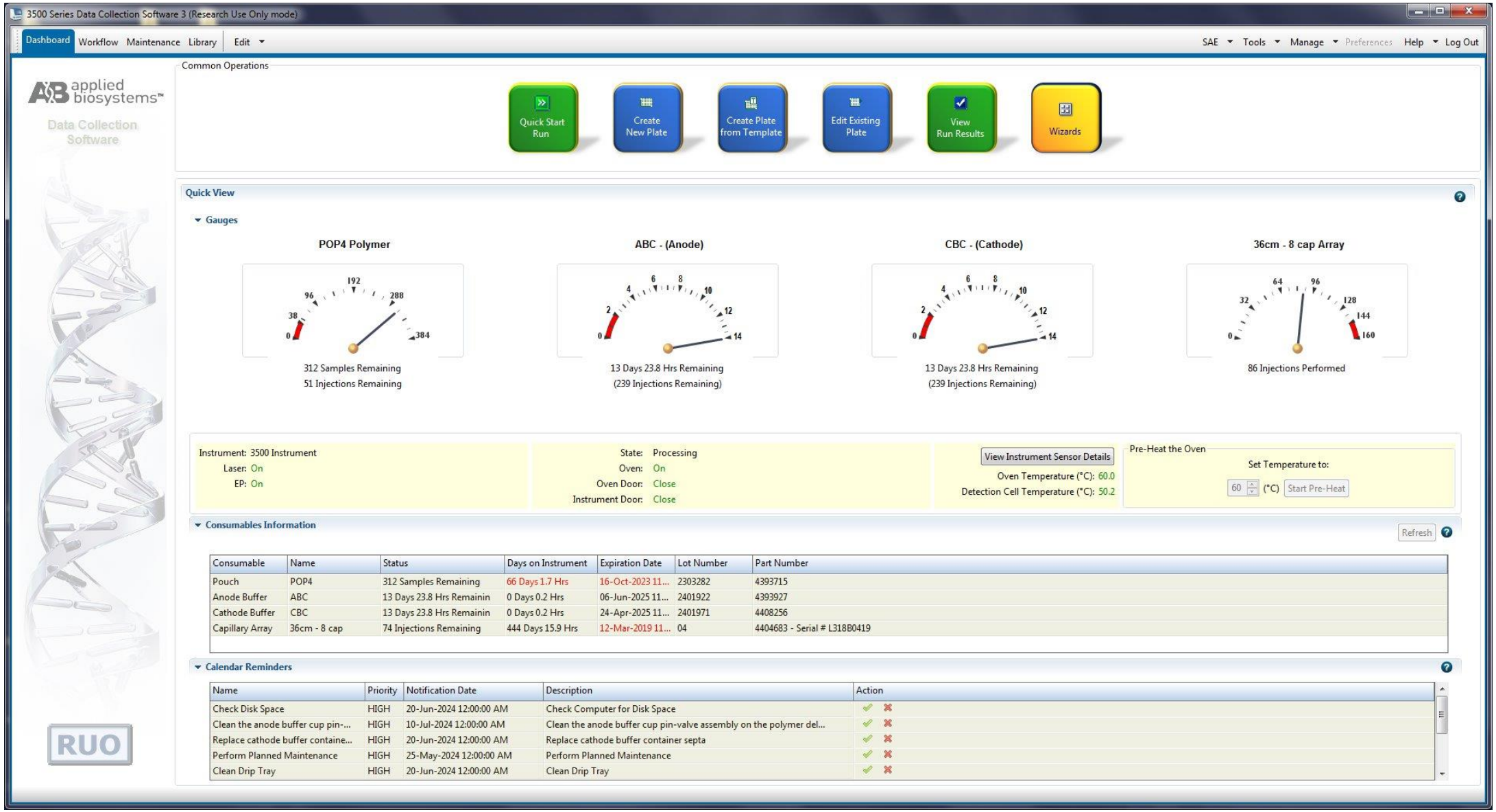
AB 3500 Dashboard: Replacing Anode and Cathode Buffer

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

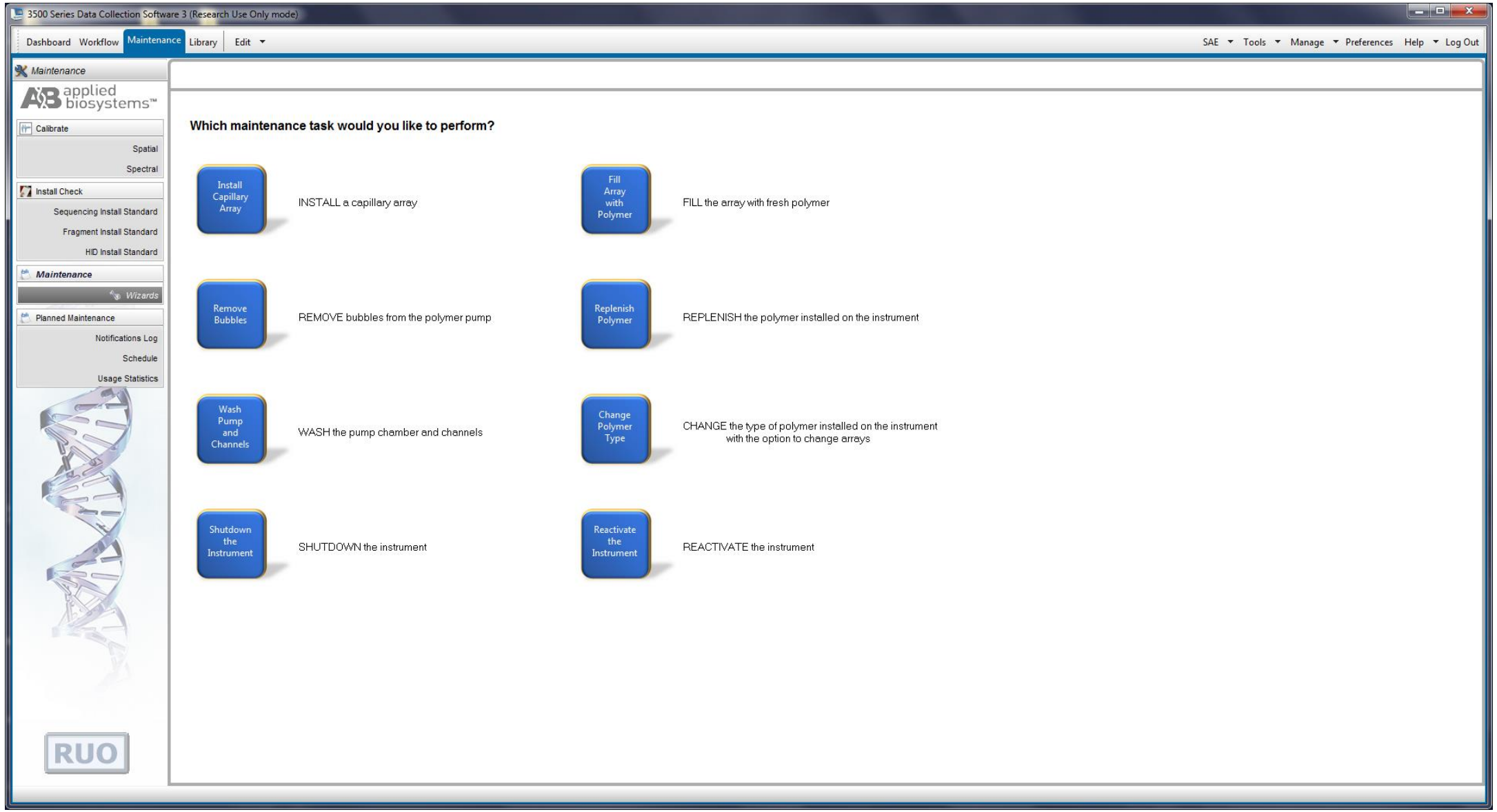




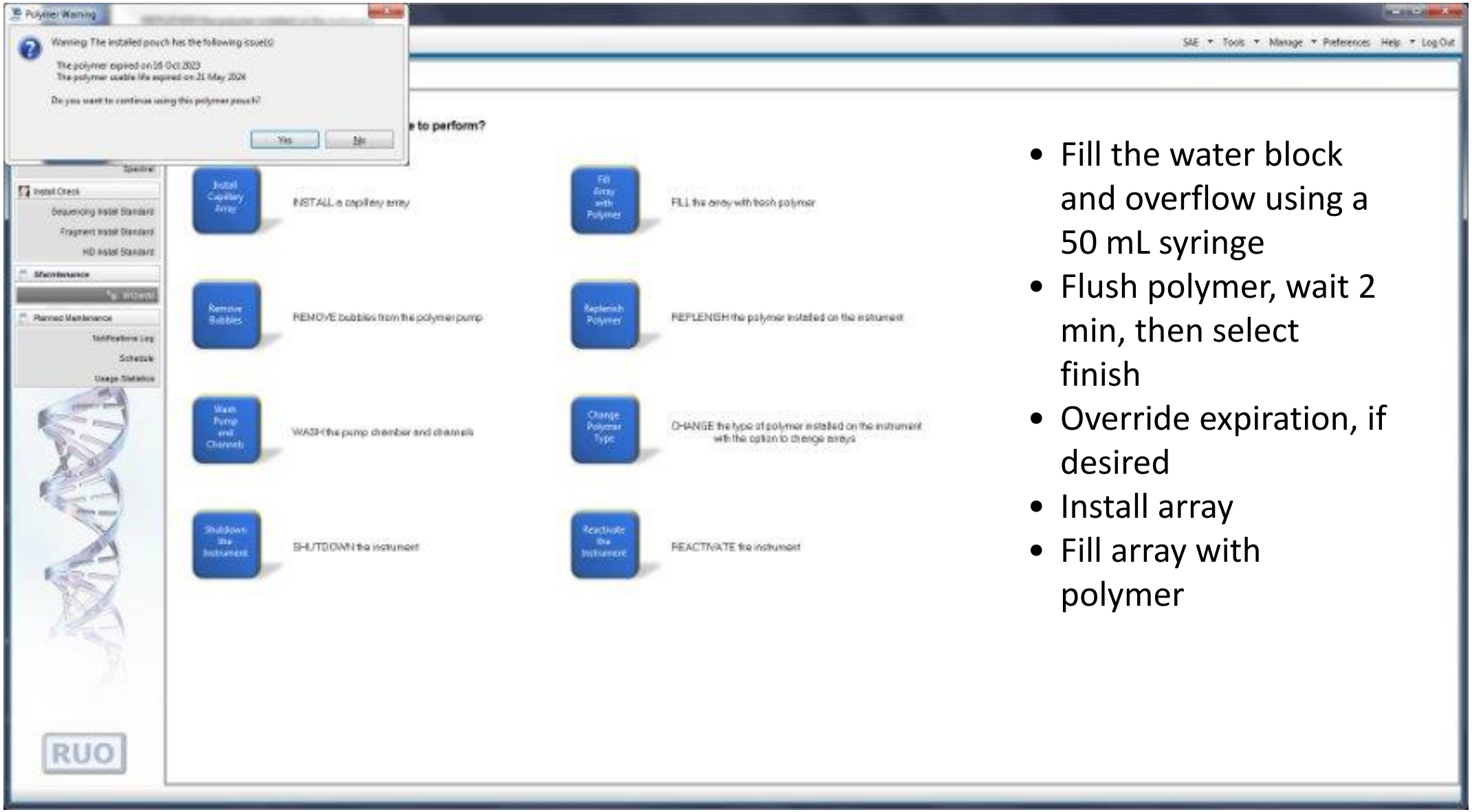
AB 3500 Dashboard: Buffers Replaced



AB 3500 Maintenance Wizard

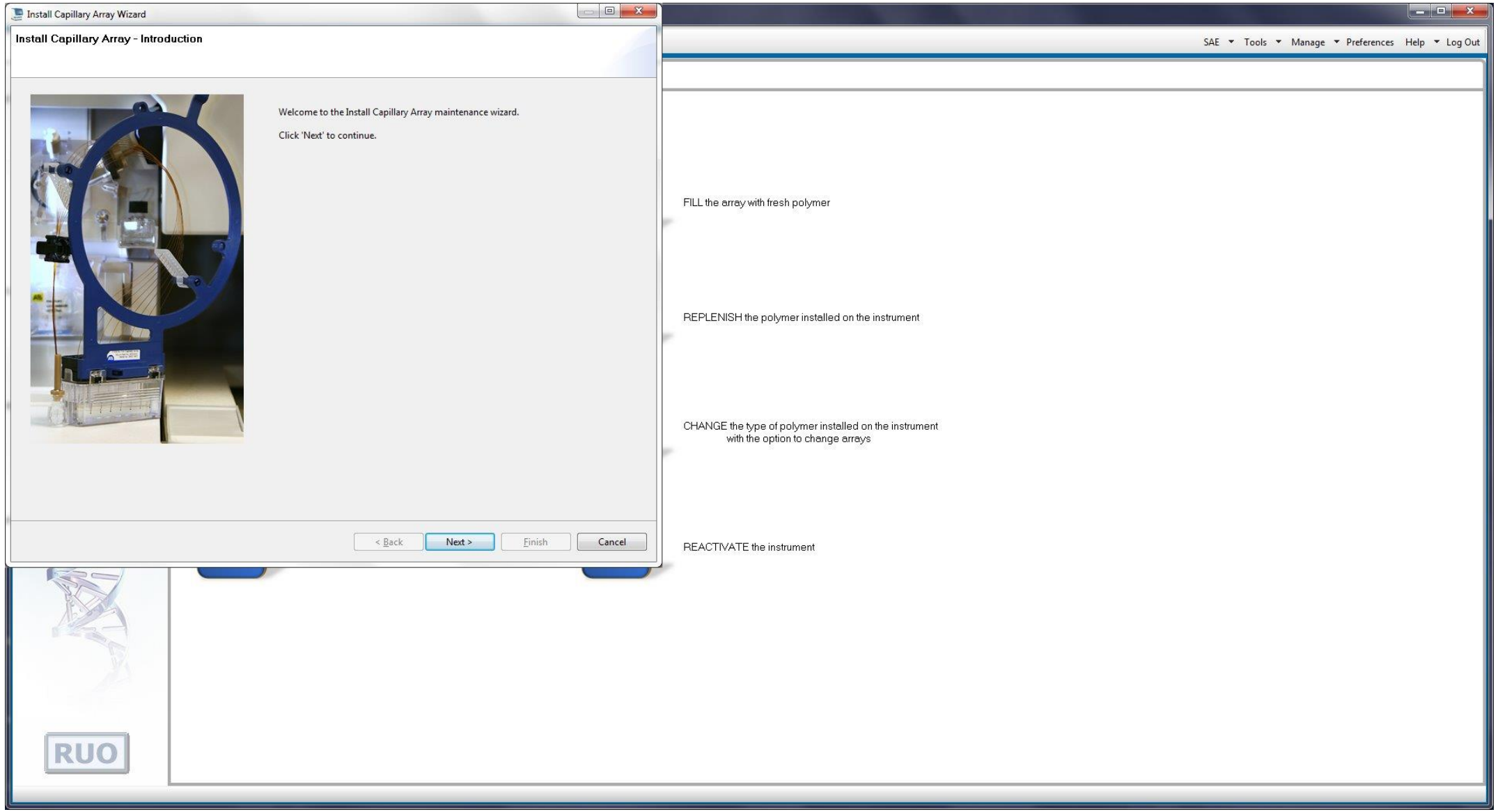


AB 3500 Expiration Warning

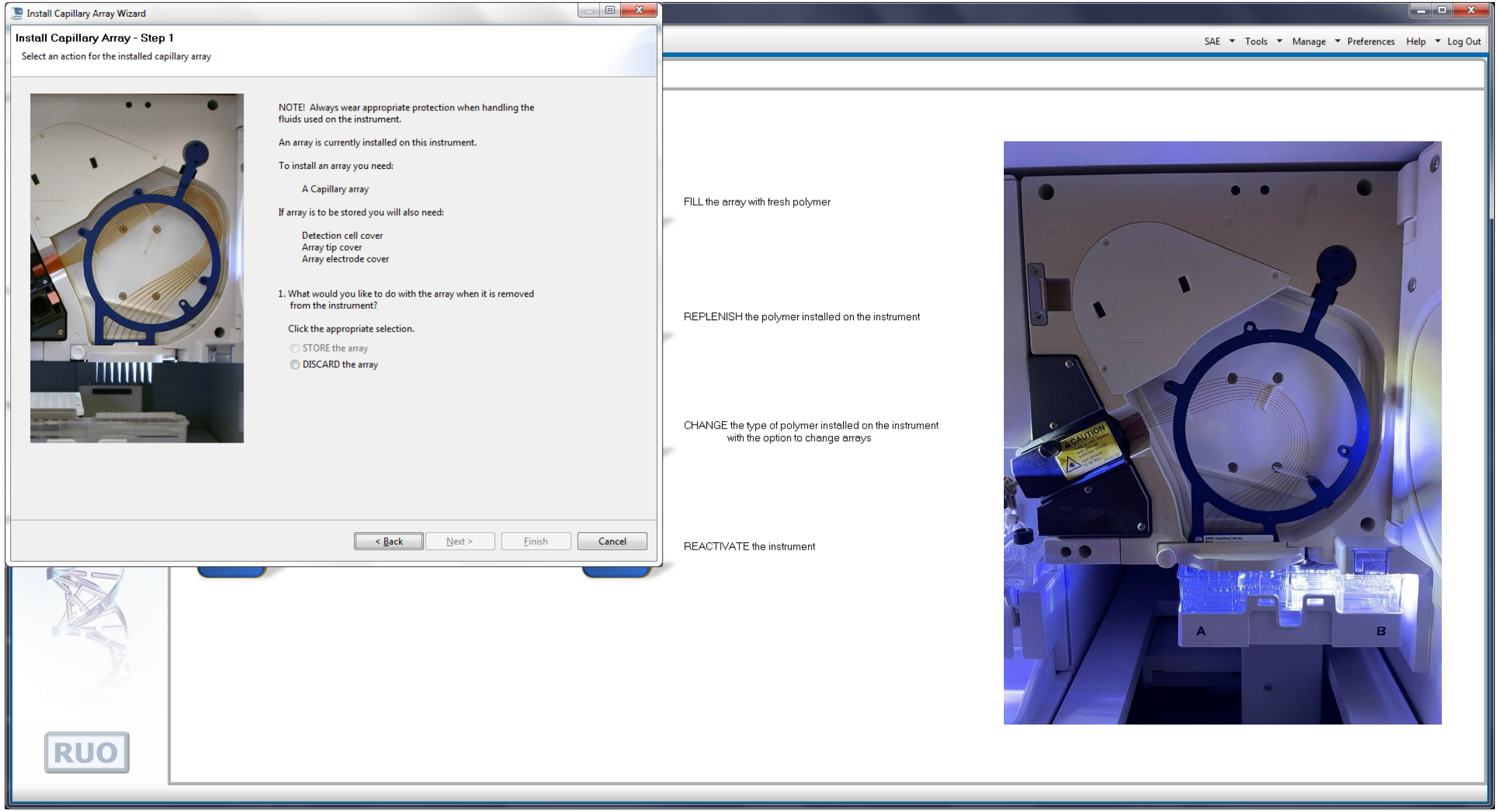


- Fill the water block and overflow using a 50 mL syringe
- Flush polymer, wait 2 min, then select finish
- Override expiration, if desired
- Install array
- Fill array with polymer

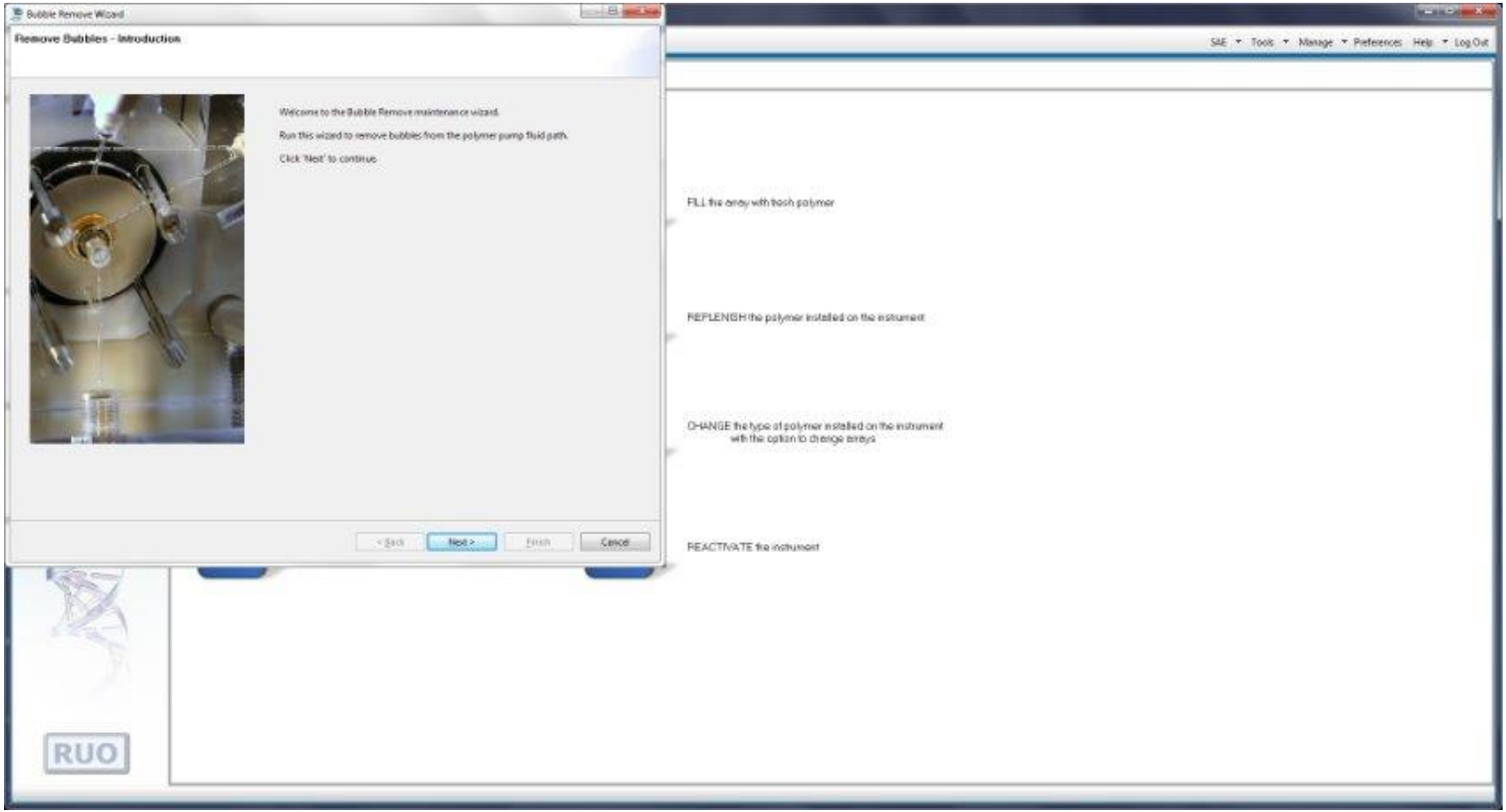
AB 3500 Install Array



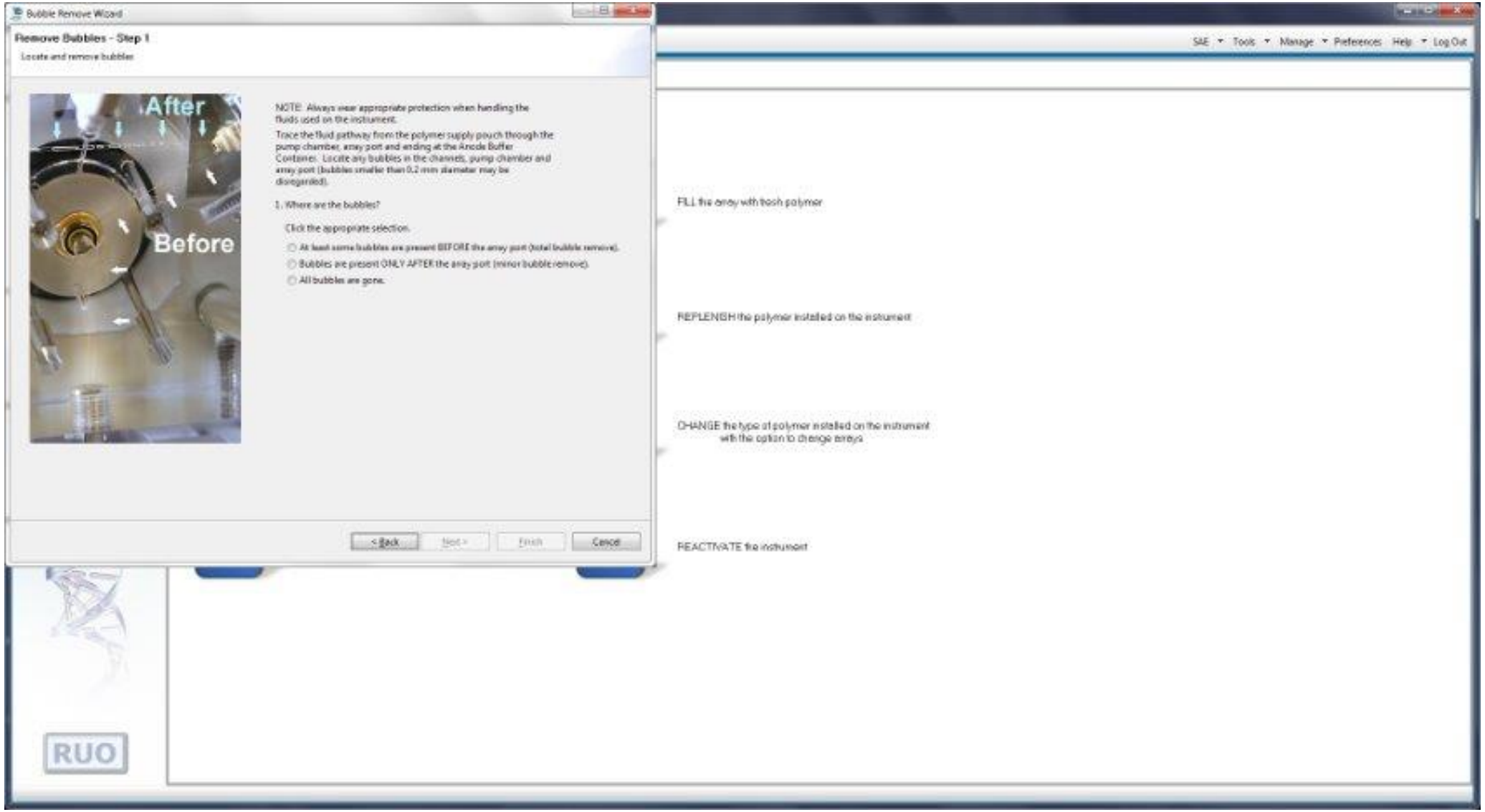
AB 3500 Install Array



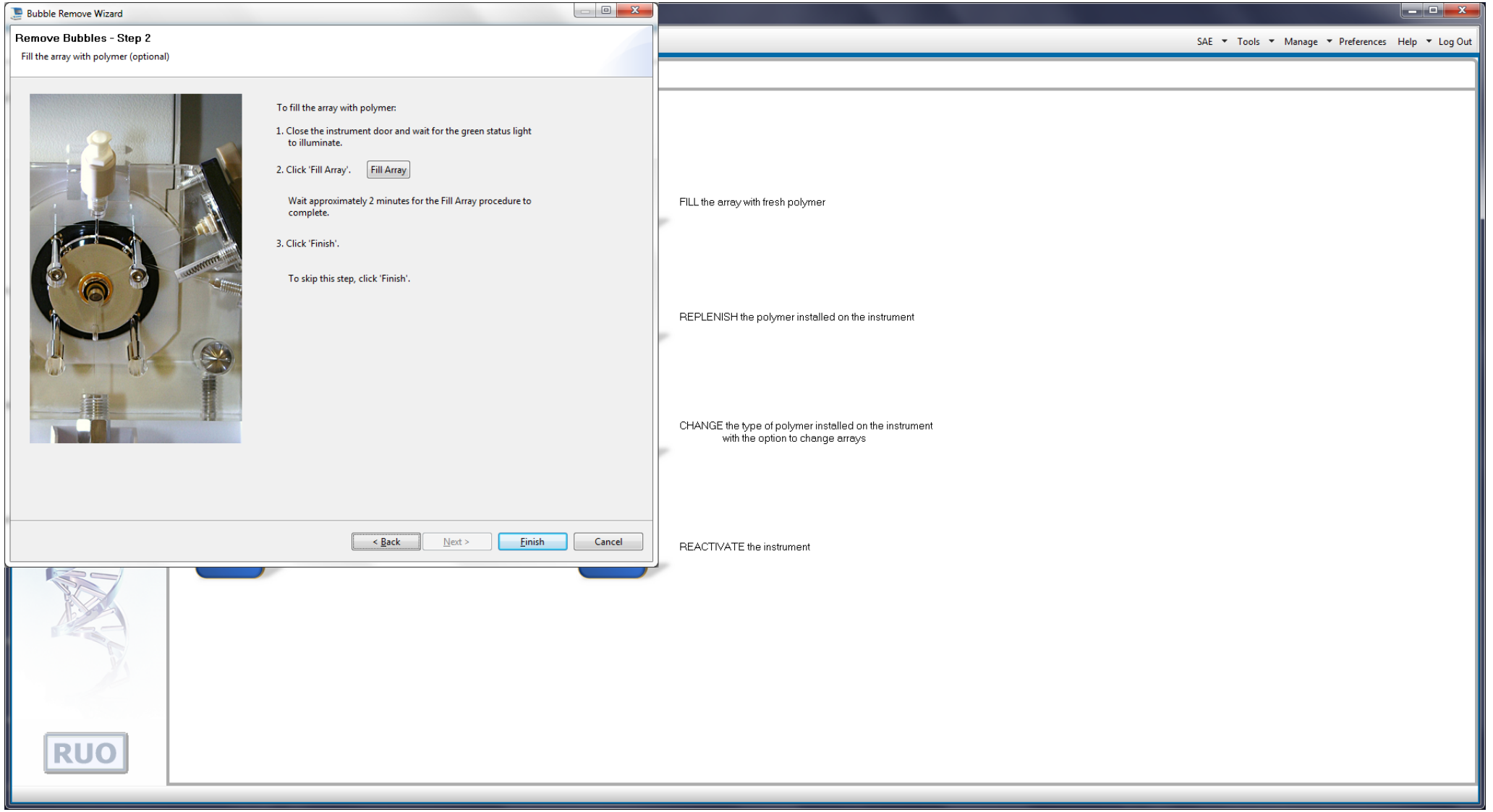
AB 3500 Fill Array & Remove Bubbles



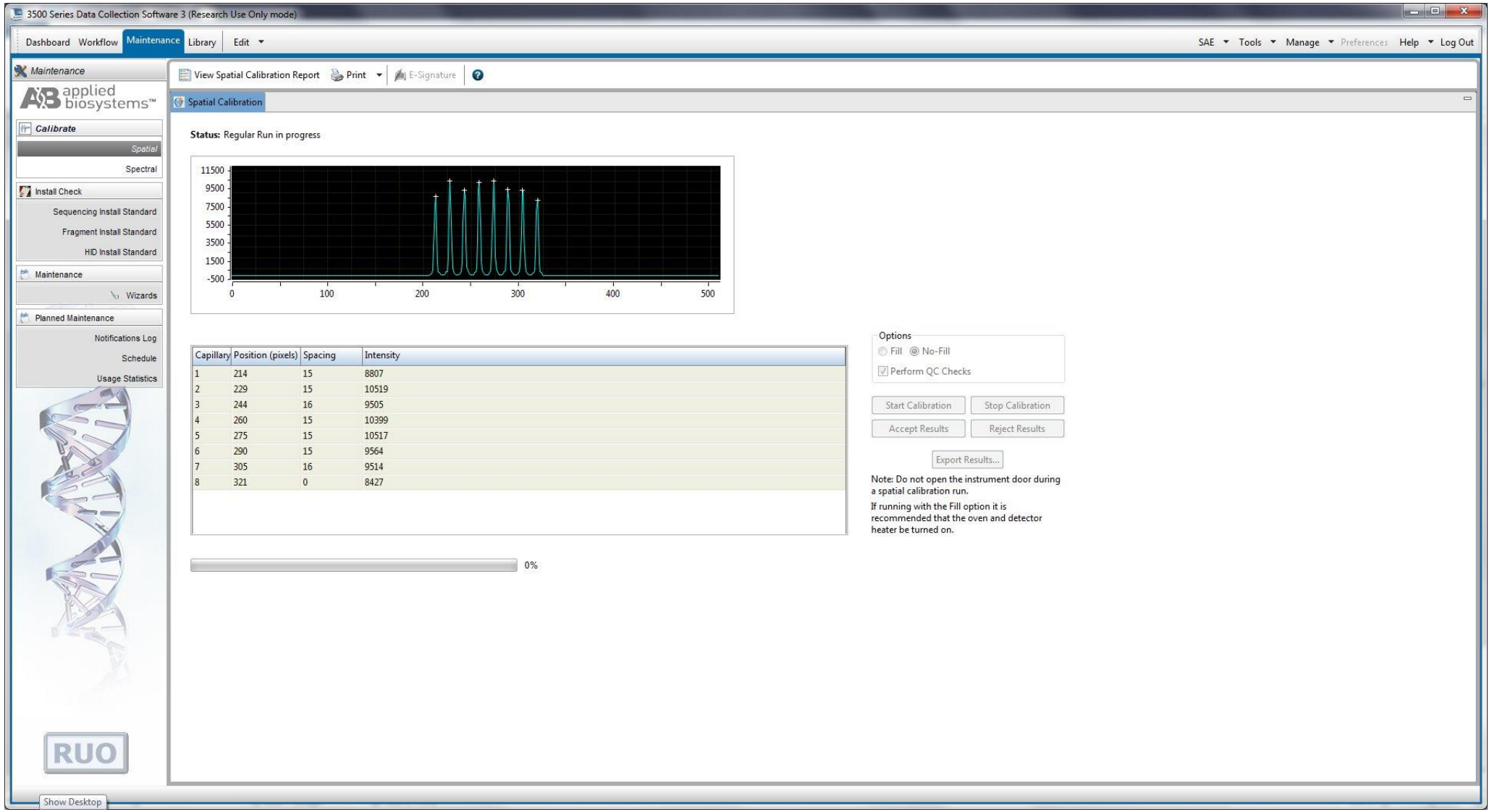
AB 3500 Fill Array & Remove Bubbles



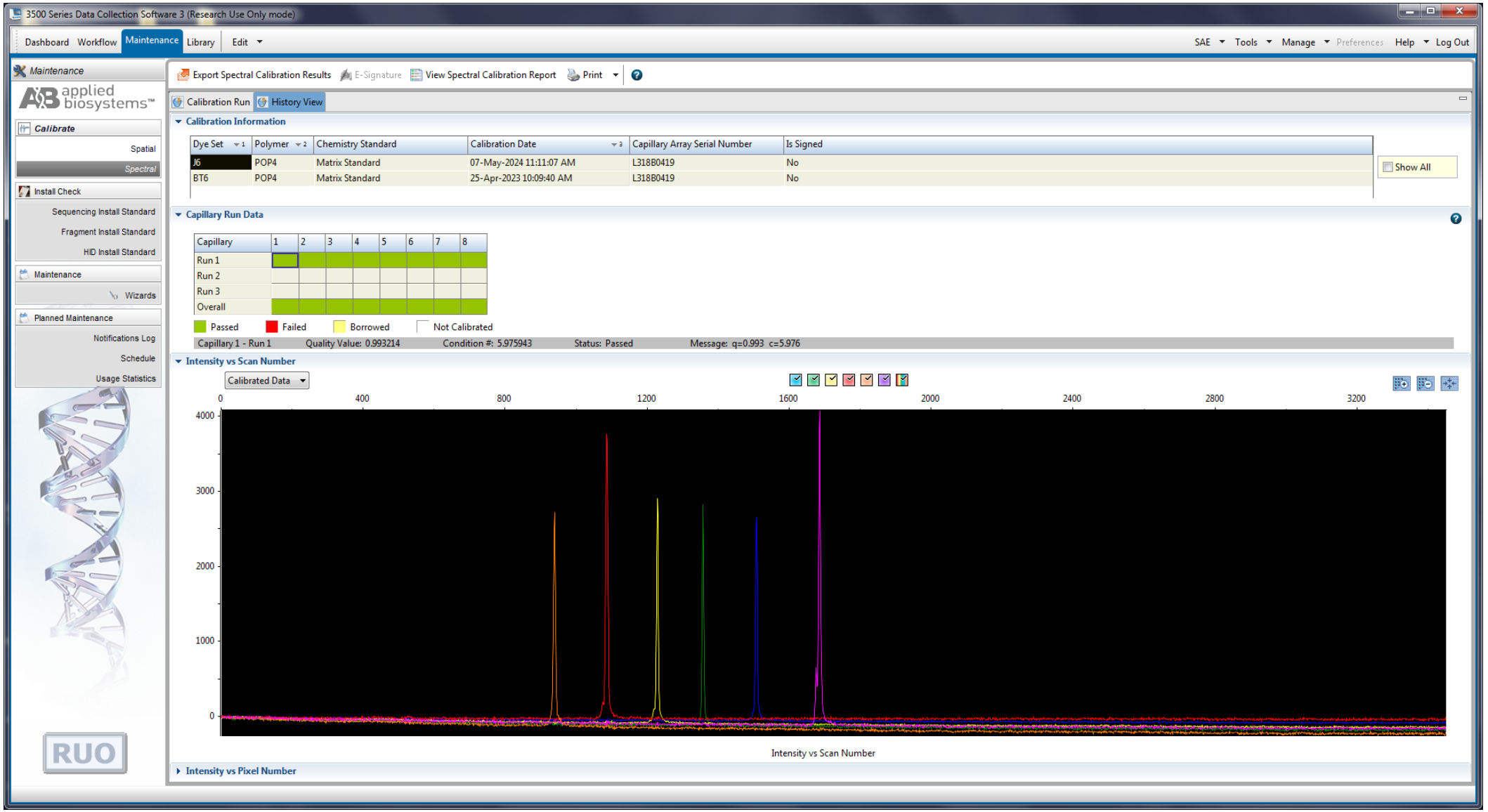
AB 3500 Fill Array & Remove Bubbles



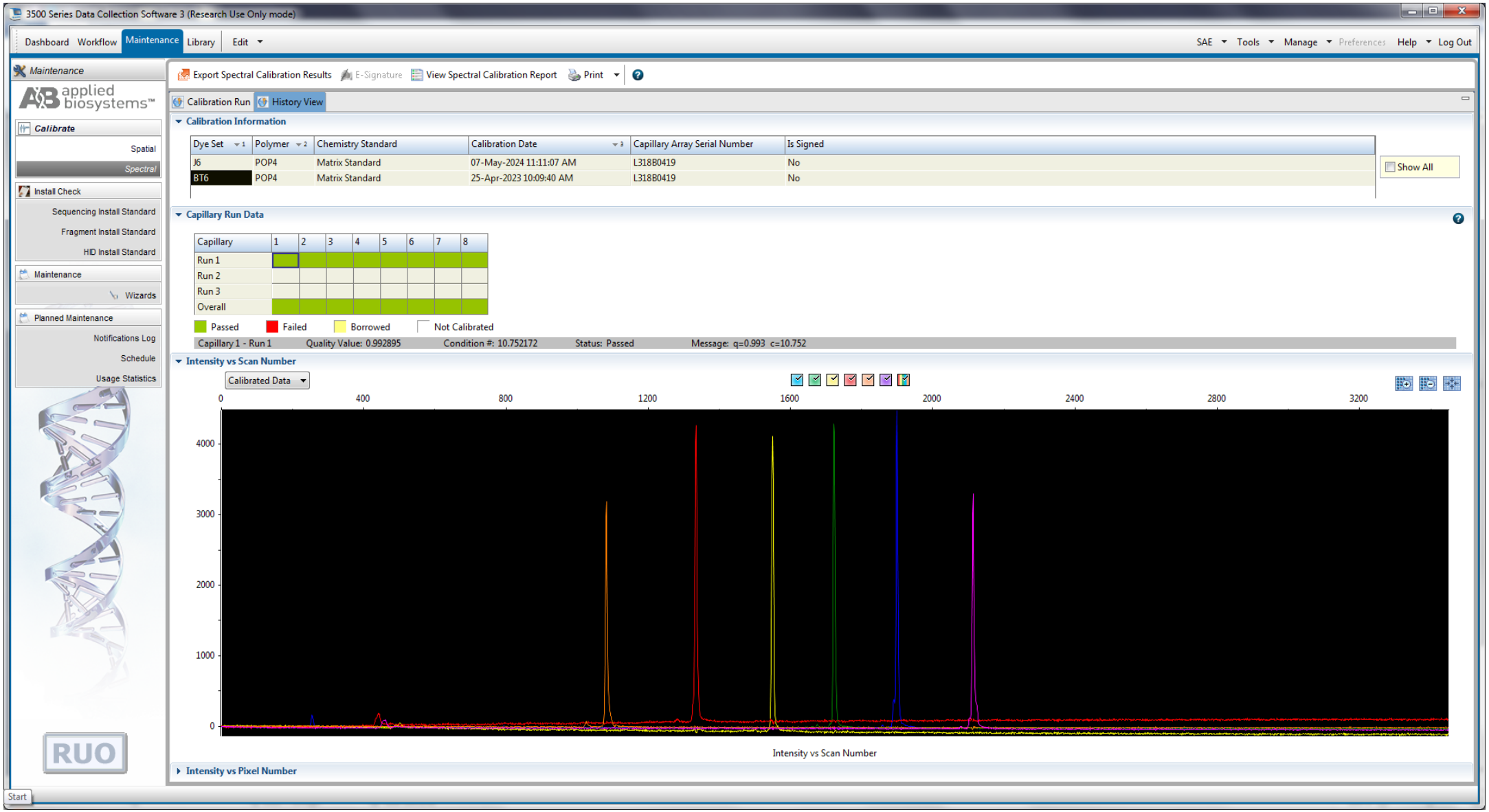
AB 3500 Spatial Calibration



AB 3500 Spectral Calibration: J6

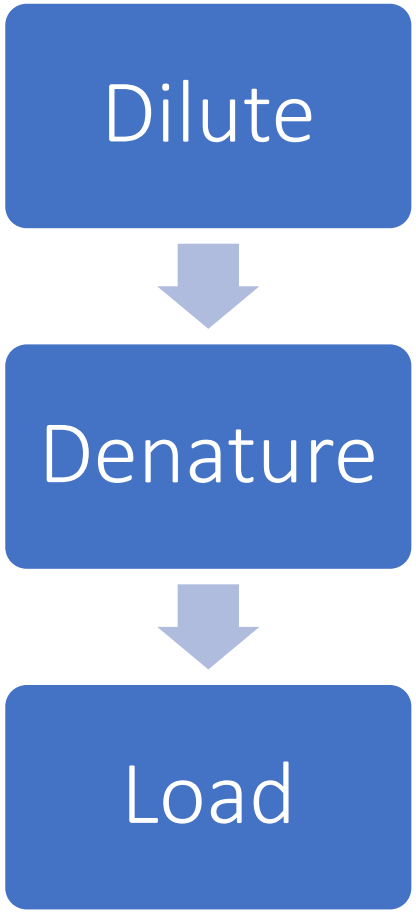


AB 3500 Spectral Calibration: BTO

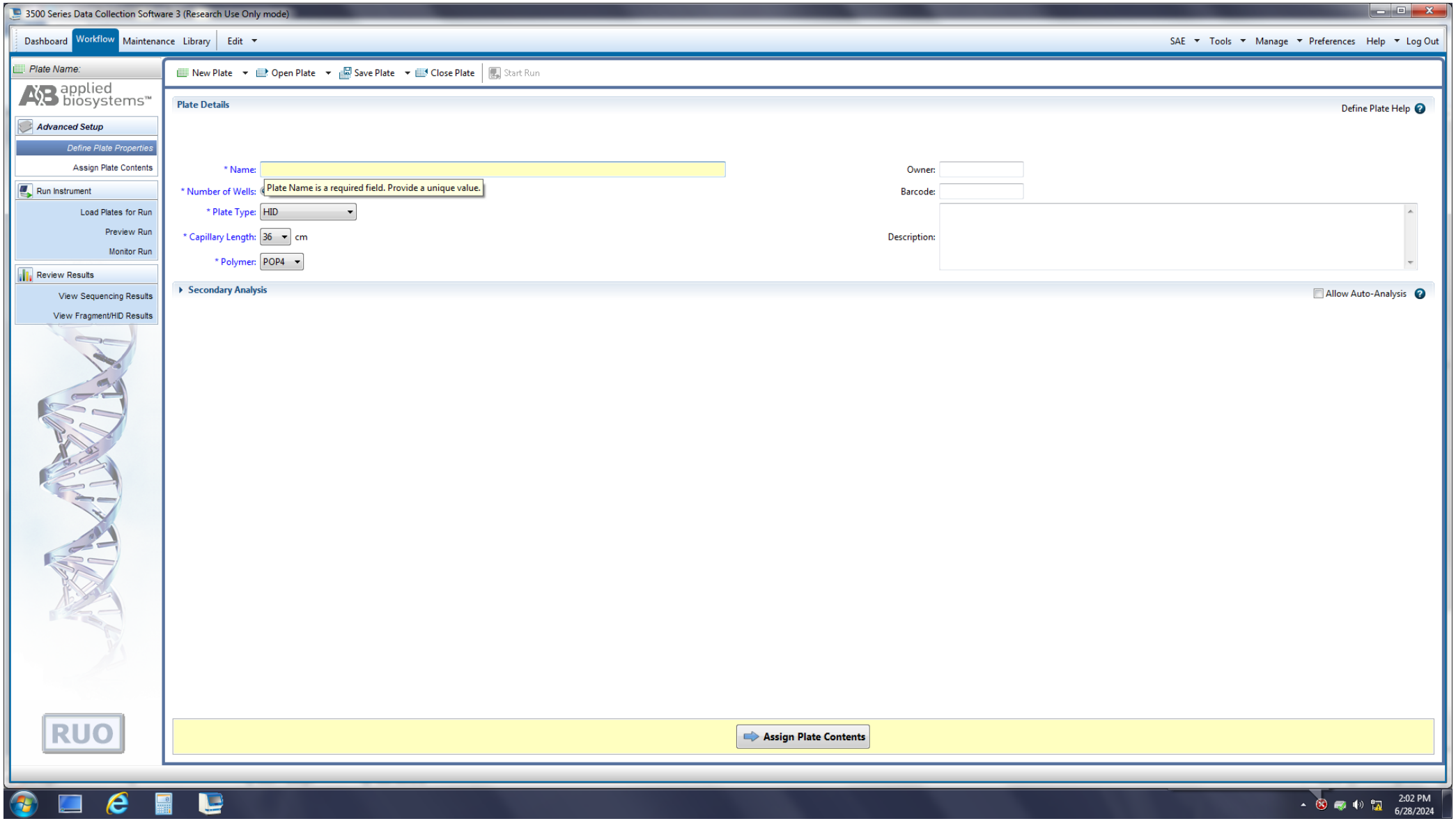


Prepare Samples to Run

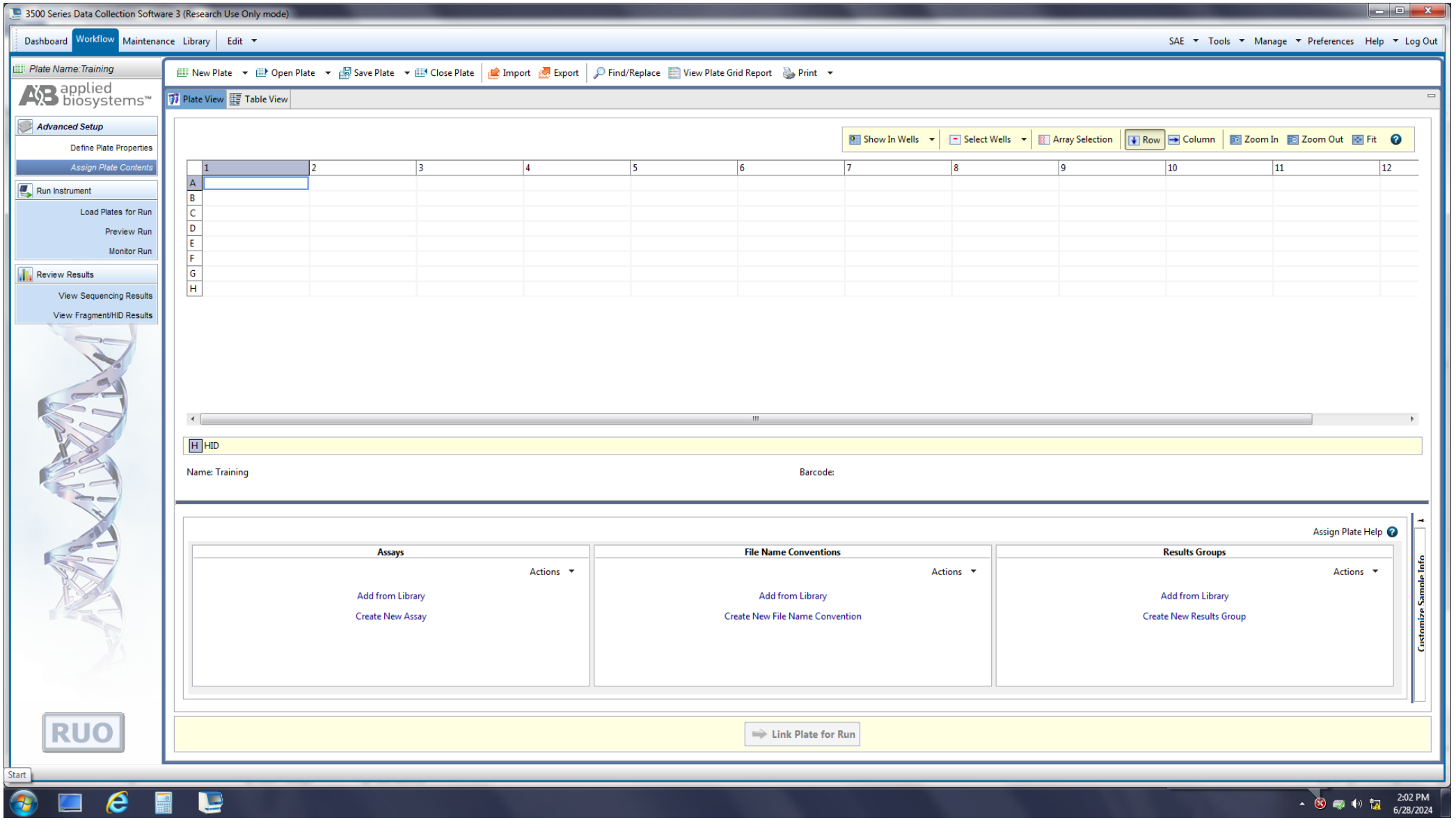
- To a 96-well skirted plate, combine
  - 0.5 µL size standard (vortexed well)
  - 8.5-12 µL Hi-Di formamide
  - Create master mix for total number of samples
- Add 1 µL of amplified sample or allele ladder (before and after samples)
- Add 10-12 µL per well to plate, pipetting down the side to avoid 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



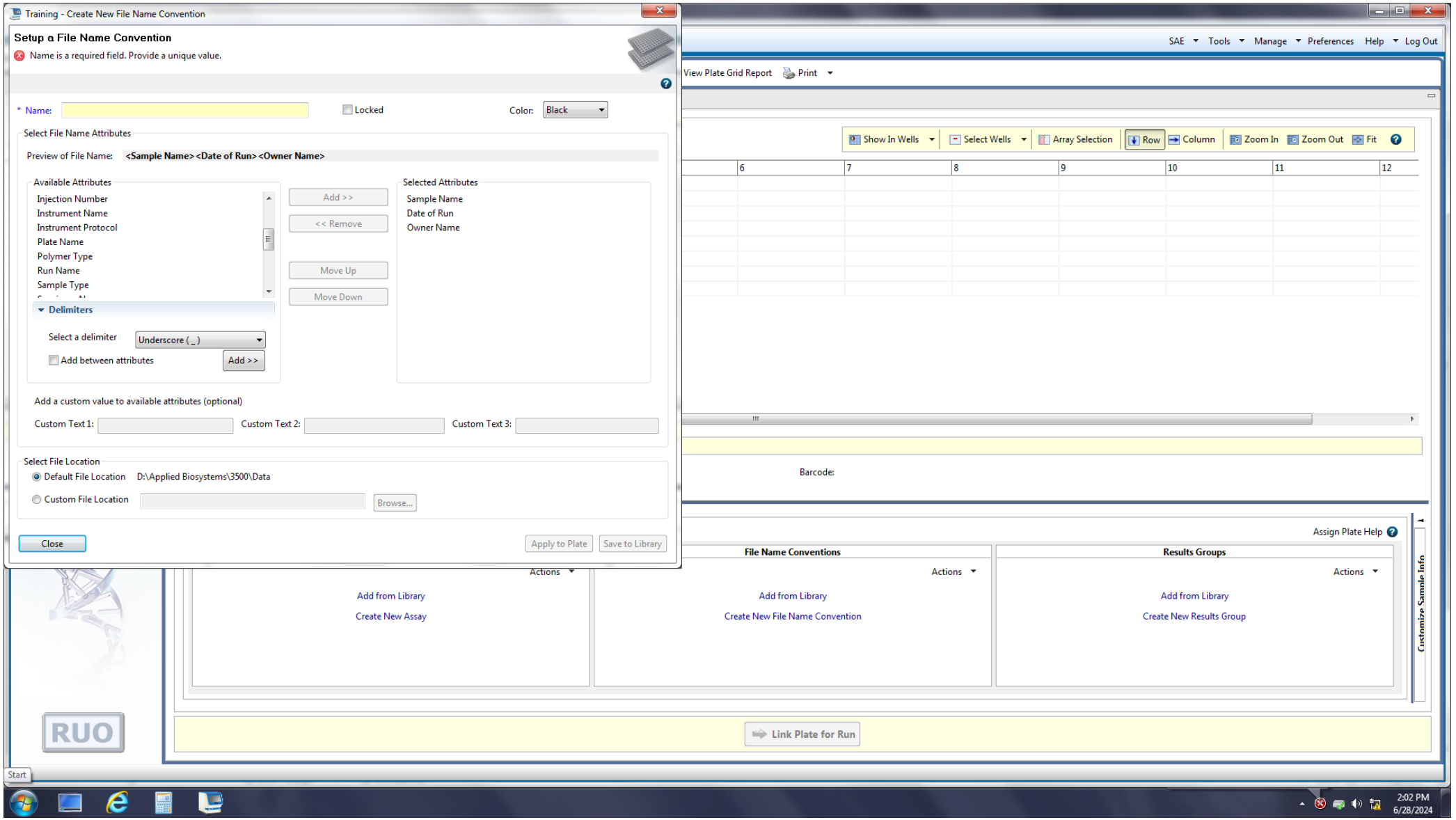
AB 3500 Setup New Plate

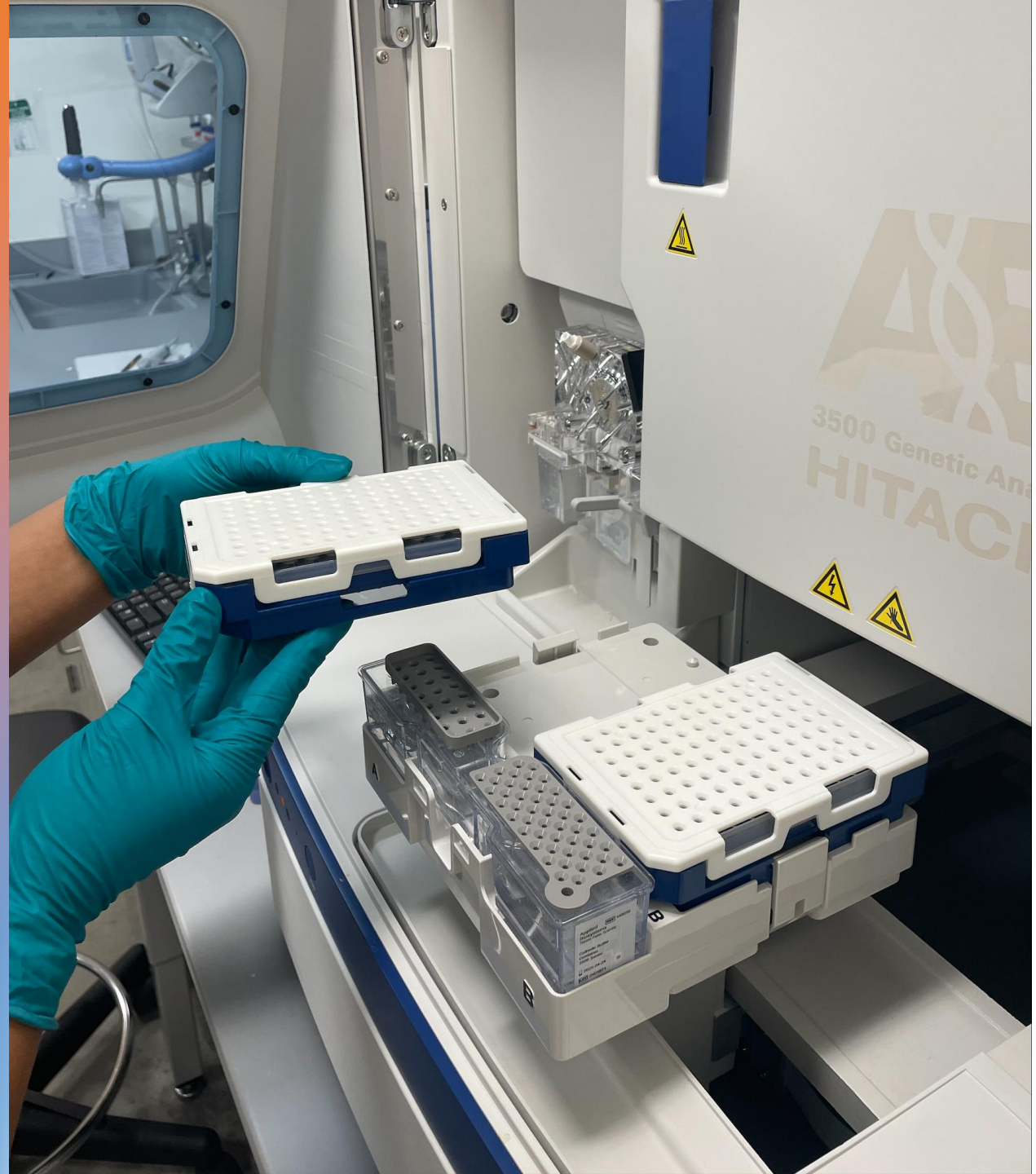


AB 3500 Assign Plate Contents



AB 3500 Assign Plate File Name Convention



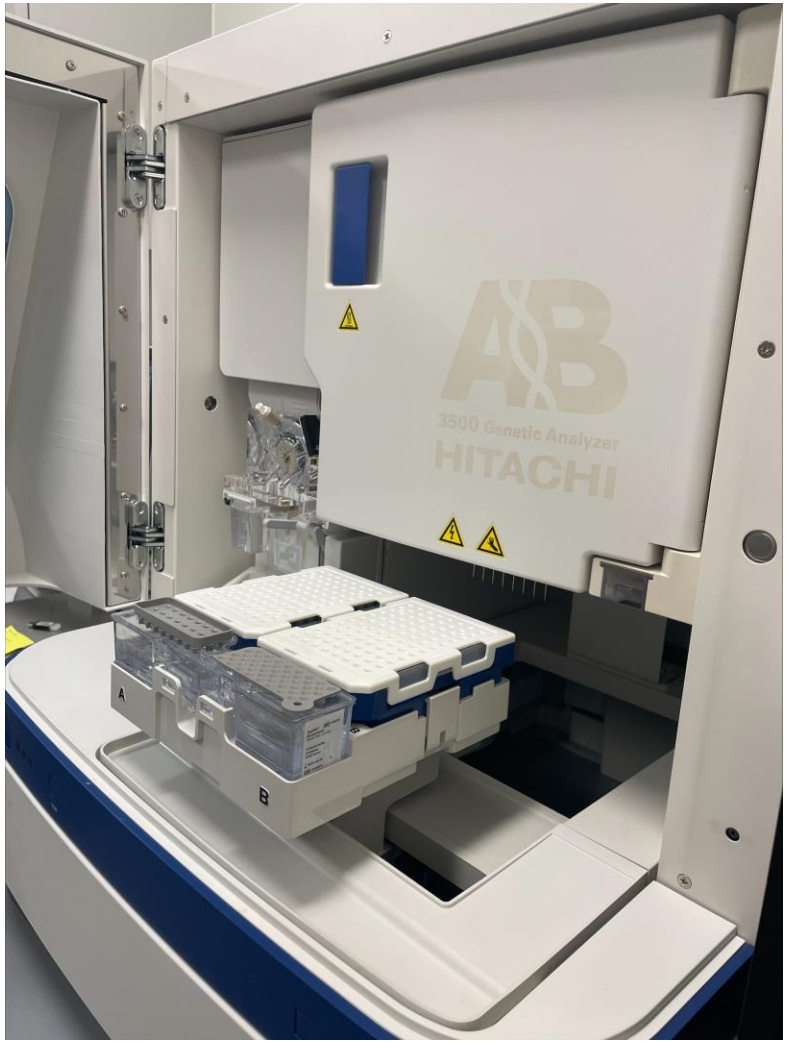


AB 3500 Load Plate to Run

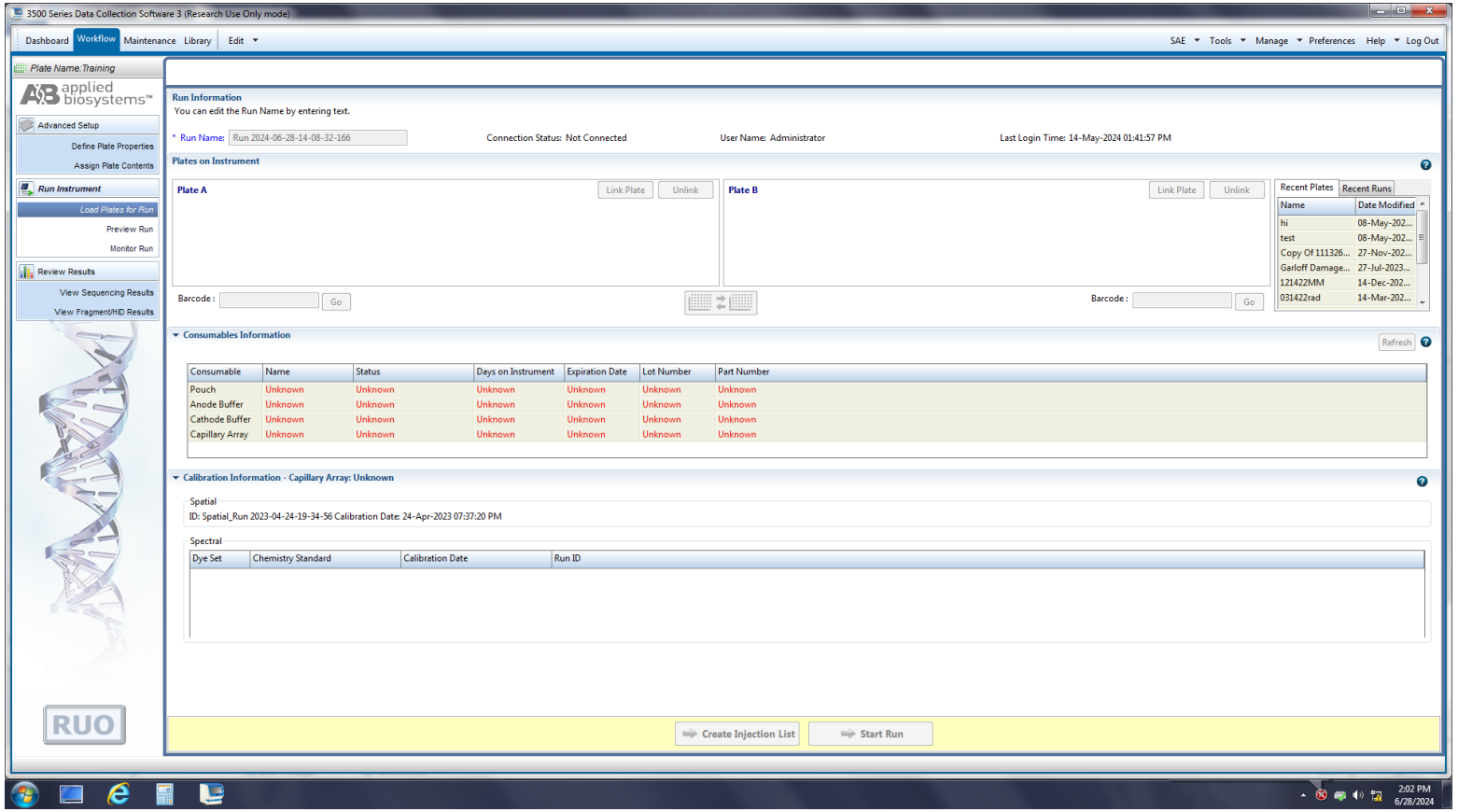
- Select the tray button on the front of the instrument to home the tray
- Insert the plate in a white and blue cartridge
- To run in A, put your plate in the A spot
- To load the plate, pull side tab to put in and close

AB 3500 Ready to Run Plate

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

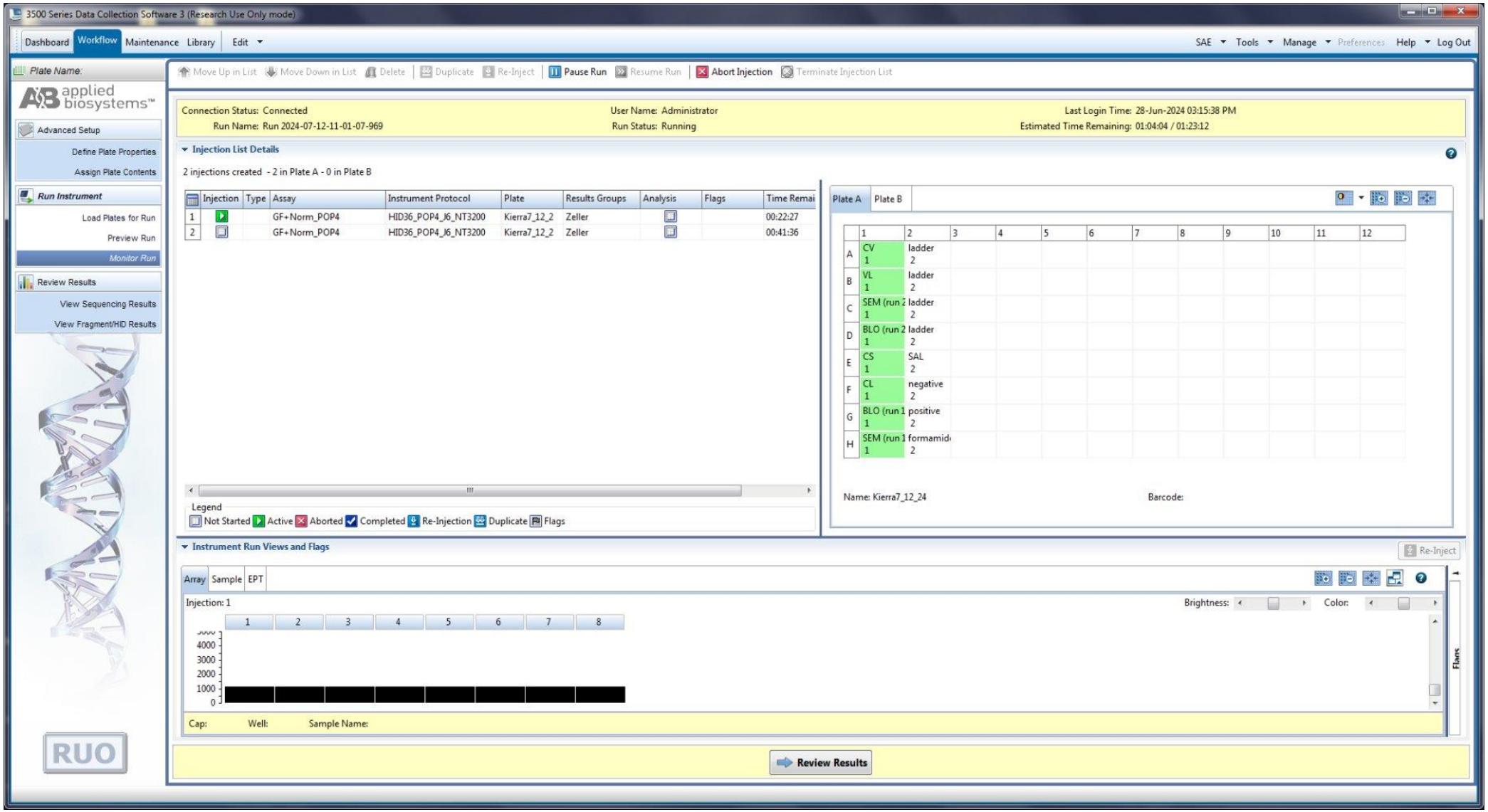


AB 3500 Start Run

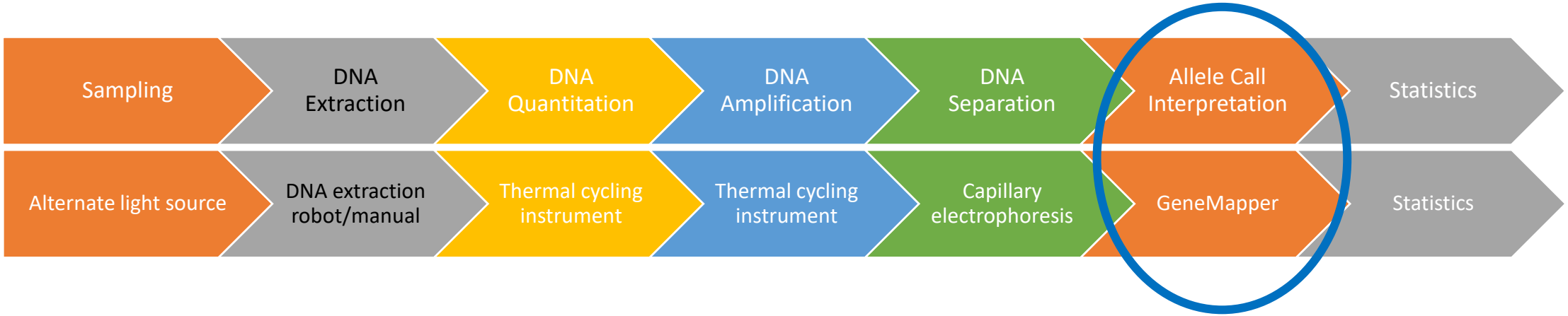


- In tray view, select sample type: sample, positive or negative control, or allele ladder
- In plate view, highlight all samples to run
- Link plate to run, default is plate A
- Save changes-Yes
- Start run

AB 3500 Running a Plate

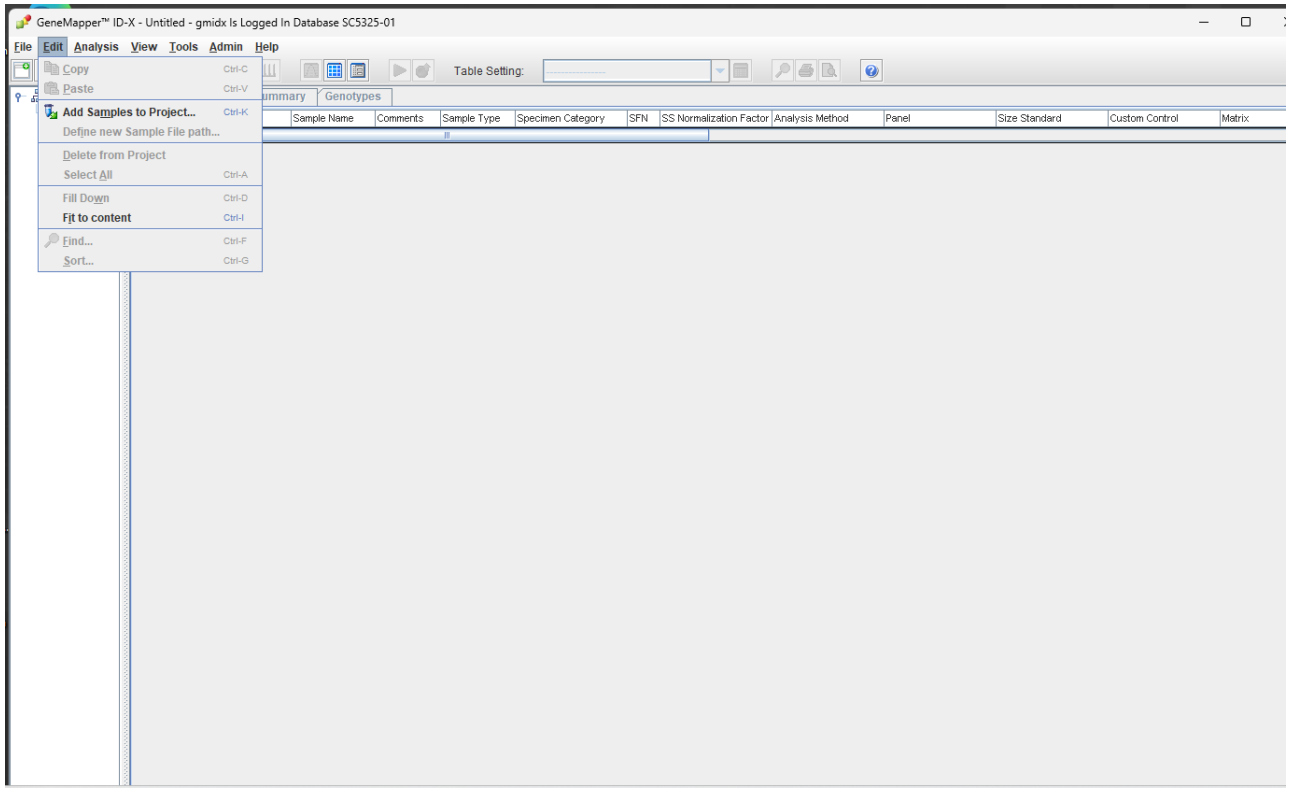


DNA Typing Process & Instrumentation



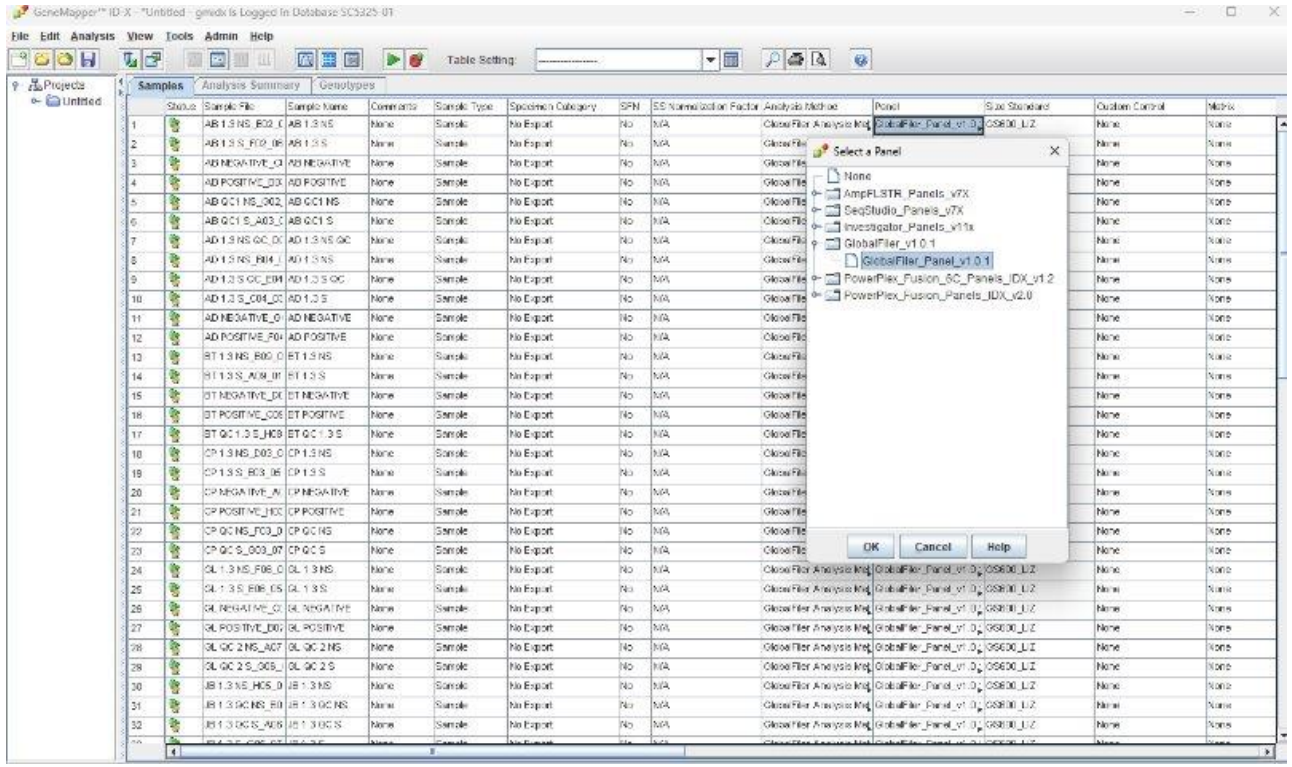
Software: GeneMapper

- Analysis of raw .hid files



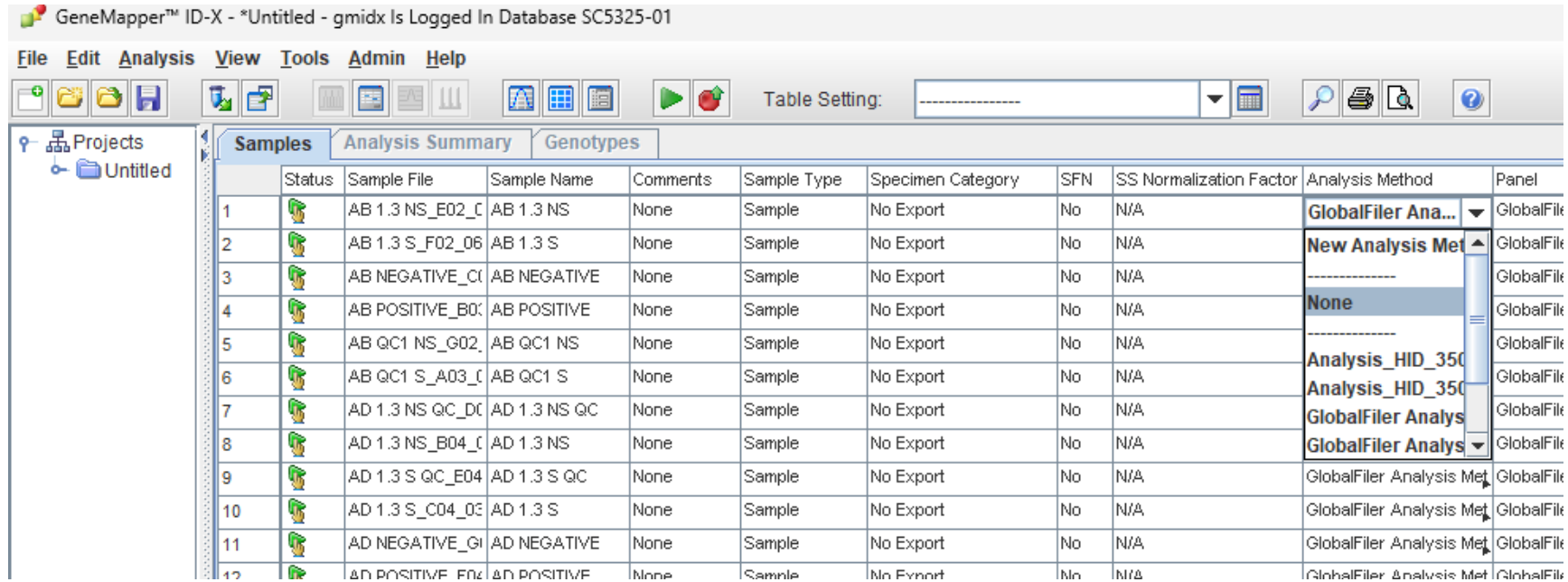
Software: GeneMapper

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



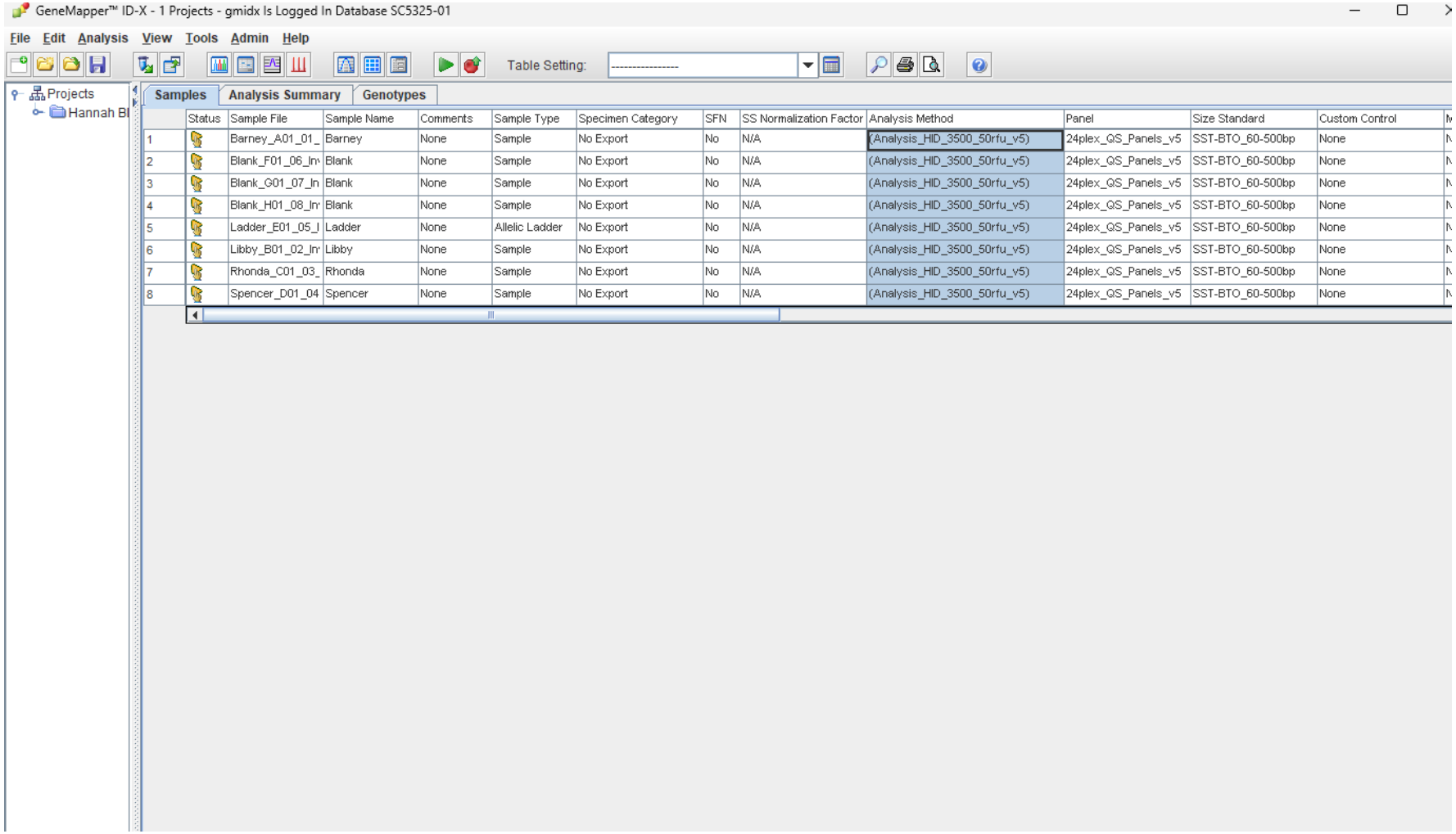
Software: GeneMapper

- Select Analysis Method
- Thresholds

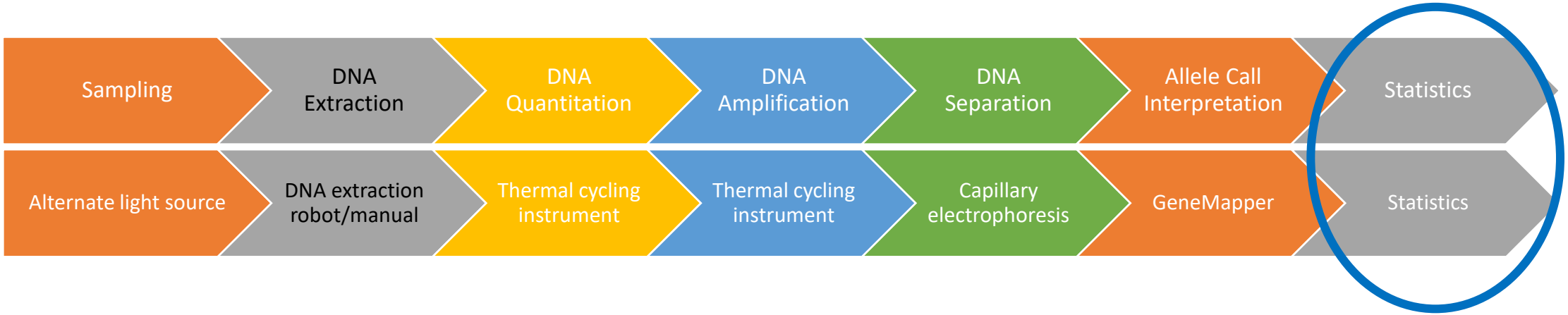


Software Parameters

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

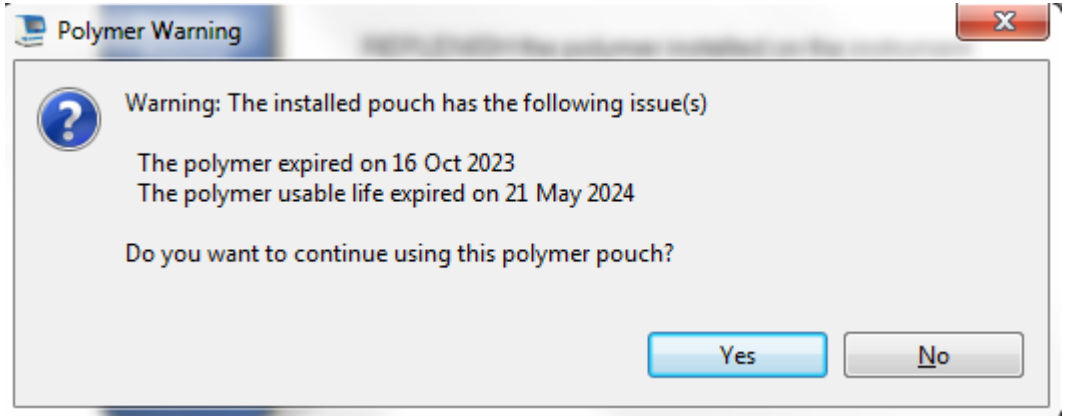


DNA Typing Process & Instrumentation



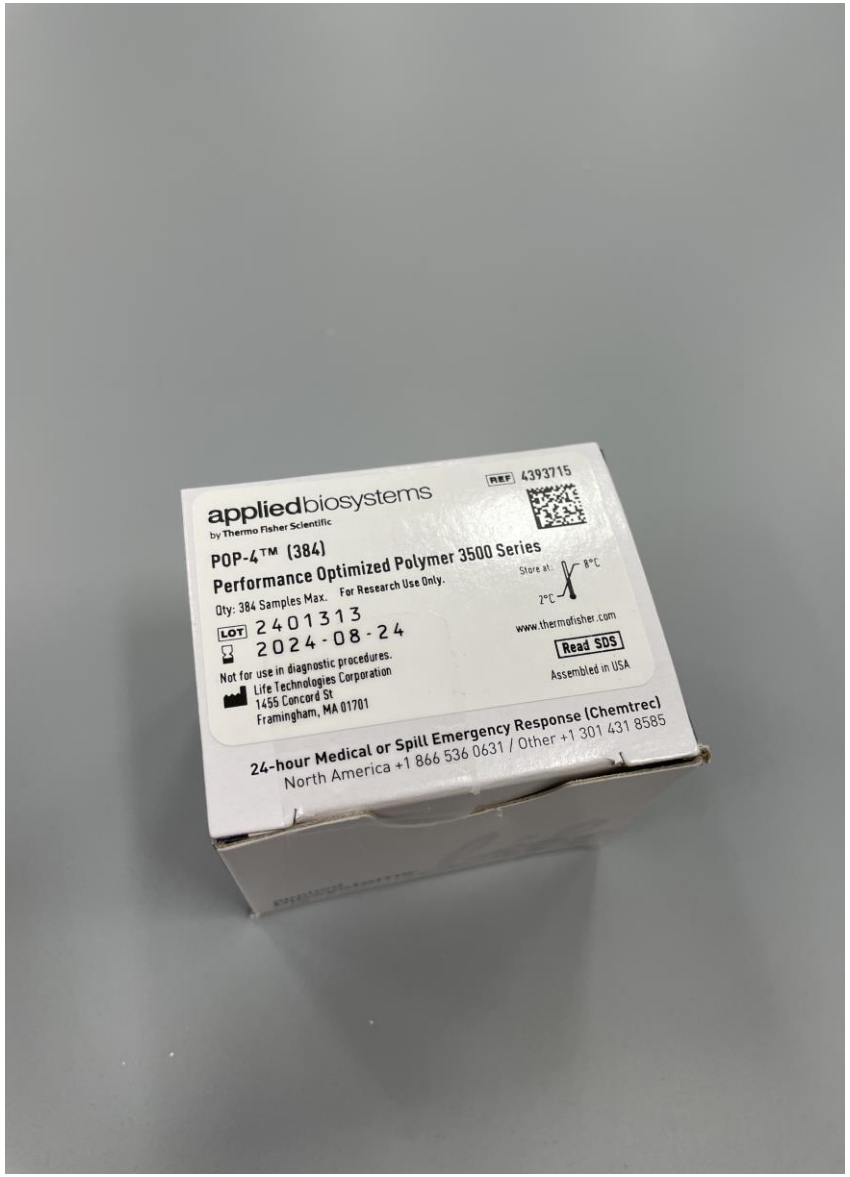
Maintenance

- 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



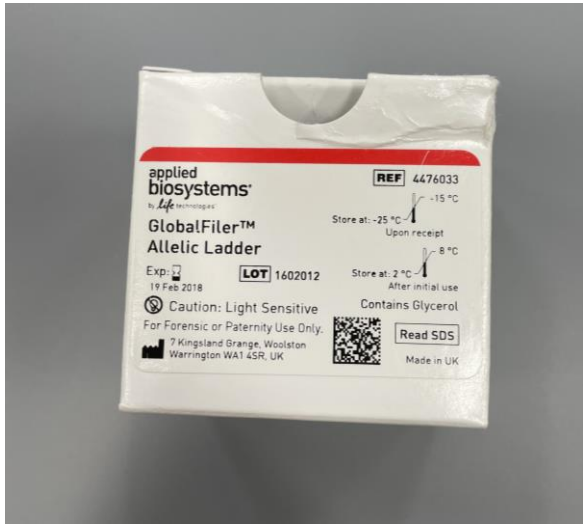
Storage of DNA Separation Reagents

- 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 fingermark 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.