Introduction

The National Institute of Standards and Technology (NIST) facilitated the development of this Human Forensic DNA Analysis Process Map through a collaboration between the NIST Forensic Science Research Program and the NIST administered Organization of Scientific Area Committees (OSAC) for Forensic Sciences (specifically OSAC's Human Forensic Biology Subcommittee) with contributions from the Scientific Working Group on DNA Analysis Methods (SWGDAM).

This Human Forensic DNA Analysis Process Map (Current Practices) captures details about the various procedures, methods and decision points most frequently encountered in the discipline of human forensic biology/DNA analysis from a national perspective and ***is intended to reflect current practices****. The discipline requires analysts to make many decisions that can impact the quality and accuracy of results. The Human Forensic DNA Analysis Process Map can benefit the discipline by providing a behind-the-scenes perspective into the various components and decision points in the human forensic biology/DNA analysis process.*

Process mapping is the visual representation of critical steps and decision points of a process. Components of the process are deconstructed, placed into specific shapes within a flowchart and connected by one-way arrows to indicate directionality regarding decisions as well as progression throughout the overall process. The shape of each box assists the reader by representing a specific type of activity.

This process map captures the ***diverse*** *practices of multiple laboratories, with the goal of allowing a human forensic biology/DNA analyst to find their process represented in the map. To ensure this, the mapping team avoided creating a map of what* ***should*** *be done (e.g., best practices) and instead attempted to represent all reasonable variations of casework* ***currently performed*** *by human forensic biology/DNA analysts. For this reason, it is important to state that neither the OSAC Human Forensic Biology Subcommittee nor SWGDAM necessarily support or endorse (as best practices) all of the different steps and paths depicted in this process map.*

This map is not intended to be a step-by-step instruction manual outlining minutia, nor is it intended to be so broad that it lacks utility. Rather, judgements were made by the process mapping group as to which steps should be combined and which steps should be divided further. Certain processes represented in the map have a required sequence while other components may vary by analyst or agency. Processes and decisions may also be dictated by agency policy or law.

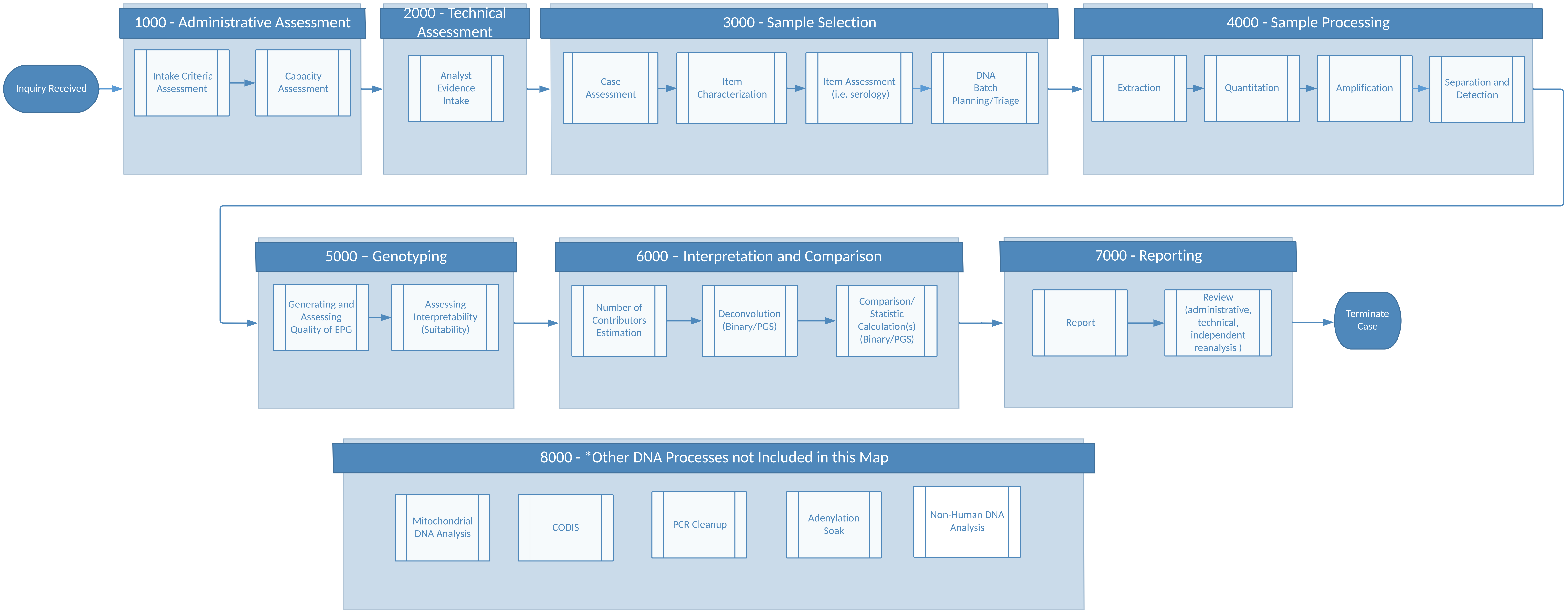
Process Map Applications:

The Human Forensic DNA Analysis Process Map is intended to be used to help improve efficiencies while reducing errors, highlight gaps where further research or standardization would be beneficial, and assist with training new examiners. It may also be used to develop specific laboratory policies and identify best practices.

Scope of the Human Forensic DNA Analysis Process Map:

The scope of Human Forensic DNA Analysis Process Map is limited to core processes within the discipline of human forensic biology/DNA analysis. Several topics are omitted from this map including mitochondrial DNA, CODIS, PCR cleanup, adenylation soak, and non-human DNA analysis. These topics may subsequently be addressed by the process mapping team, an individual laboratory or a standardization committee.

OSAC
Organization of Scientific Area
Committees for Forensic Science



Inquiry Received

1000 - Administrative Assessment
- Intake Criteria Assessment
- Capacity Assessment

2000 - Technical Assessment
- Analyst Evidence Intake

3000 - Sample Selection
- Case Assessment
- Item Characterization
- Item Assessment (i.e. serology)
- DNA Batch Planning/Triage

4000 - Sample Processing
- Extraction
- Quantitation
- Amplification
- Separation and Detection

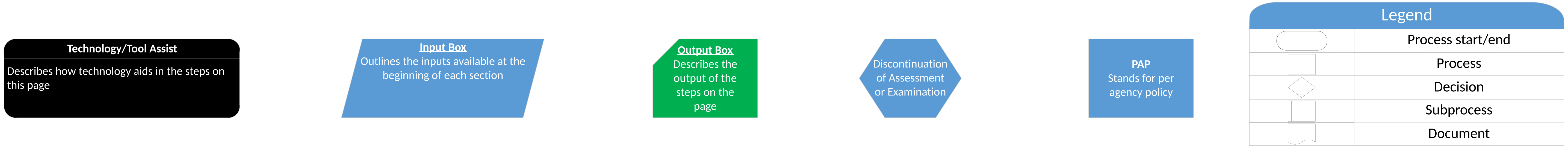
5000 – Genotyping
- Generating and Assessing Quality of EPG
- Assessing Interpretability (Suitability)

6000 – Interpretation and Comparison
- Number of Contributors Estimation
- Deconvolution (Binary/PGS)
- Comparison/ Statistic Calculation(s) (Binary/PGS)

7000 - Reporting
- Report
- Review (administrative, technical, independent reanalysis)

Terminate Case

8000 - *Other DNA Processes not Included in this Map
- Mitochondrial DNA Analysis
- CODIS
- PCR Cleanup
- Adenylation Soak
- Non-Human DNA Analysis



Technology/Tool Assist
Describes how technology aids in the steps on this page

Input Box
Outlines the inputs available at the beginning of each section

Output Box
Describes the output of the steps on the page

Discontinuation of Assessment or Examination

PAP
Stands for per agency policy

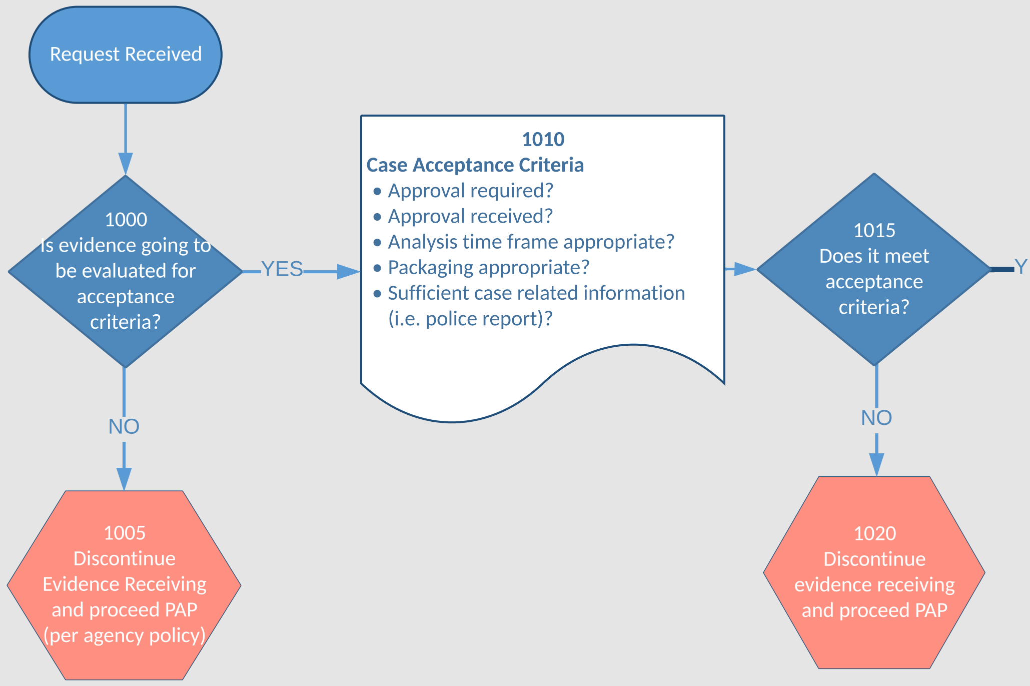
Legend	
▭	Process start/end
□	Process
◇	Decision
▯	Subprocess
▯	Document

This Process Map provides a visual description of the various steps of the casework process performed by DNA analysts and is an attempt to represent all reasonable variations in current practice. OSAC and SWGDAM do not explicitly support or endorse (as best practices) all of the different steps and/or paths as depicted on this current-practices process map.

There was a statement by the group that certain assumptions need to be made.

These are:
All reagents that are in use have been appropriately QC'ed PAP and in accordance with appropriate standards.
All instruments in use have been properly maintained PAP and in accordance with appropriate standards.
The movement through this map reflects a single item and appropriate separation of evidence from exemplars (separated by time and space) is followed.

1000 - Intake Criteria Assessment



Request Received

1000
Is evidence going to be evaluated for acceptance criteria?

YES

NO

1005
Discontinue Evidence Receiving and proceed PAP (per agency policy)

1010
Case Acceptance Criteria

- Approval required?
- Approval received?
- Analysis time frame appropriate?
- Packaging appropriate?
- Sufficient case related information (i.e. police report)?

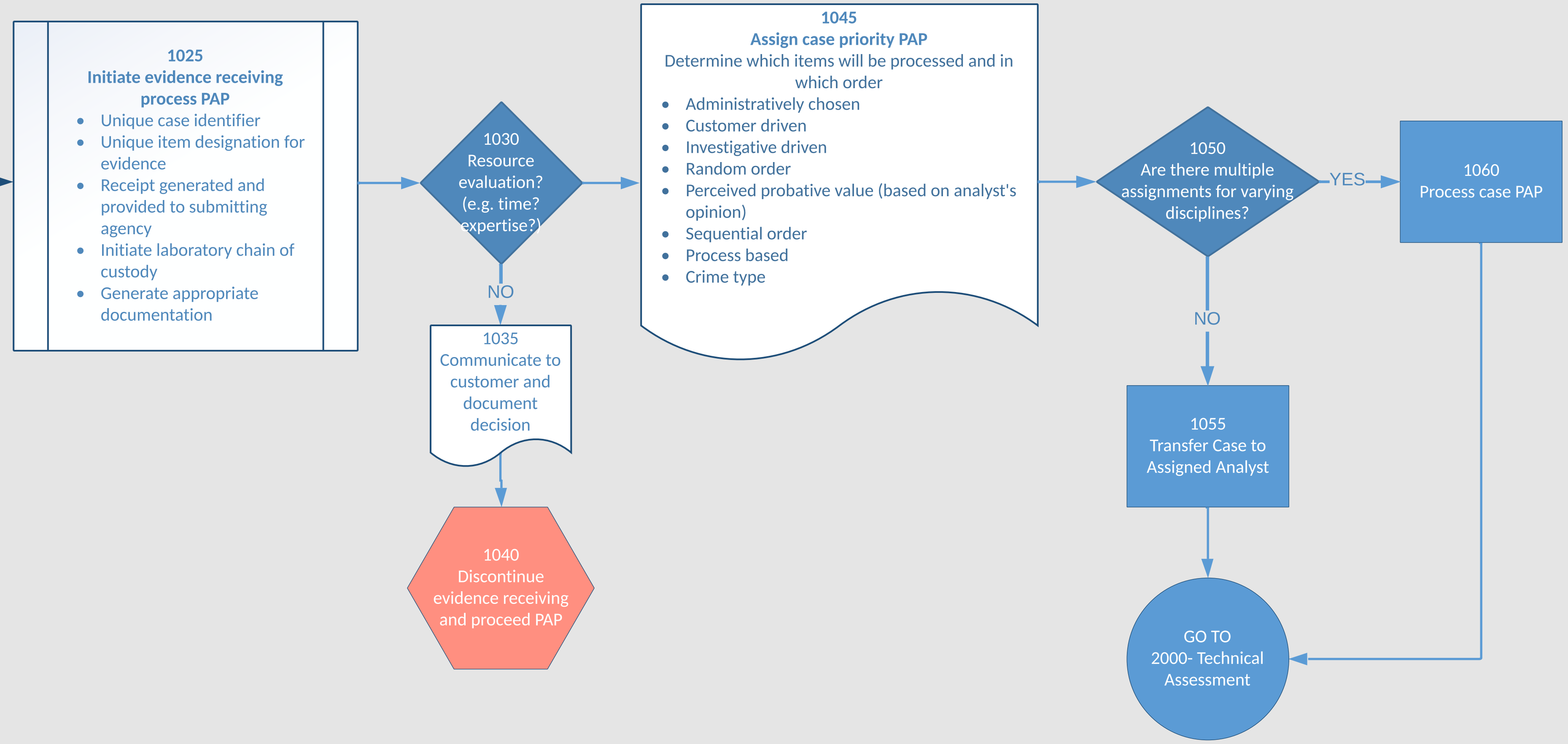
1015
Does it meet acceptance criteria?

YES

NO

1020
Discontinue evidence receiving and proceed PAP

1025 - Capacity Assessment



1025
Initiate evidence receiving process PAP

- Unique case identifier
- Unique item designation for evidence
- Receipt generated and provided to submitting agency
- Initiate laboratory chain of custody
- Generate appropriate documentation

1030
Resource evaluation? (e.g. time? expertise?)

NO

1035
Communicate to customer and document decision

1040
Discontinue evidence receiving and proceed PAP

1045
Assign case priority PAP
Determine which items will be processed and in which order

- Administratively chosen
- Customer driven
- Investigative driven
- Random order
- Perceived probative value (based on analyst's opinion)
- Sequential order
- Process based
- Crime type

1050
Are there multiple assignments for varying disciplines?

YES

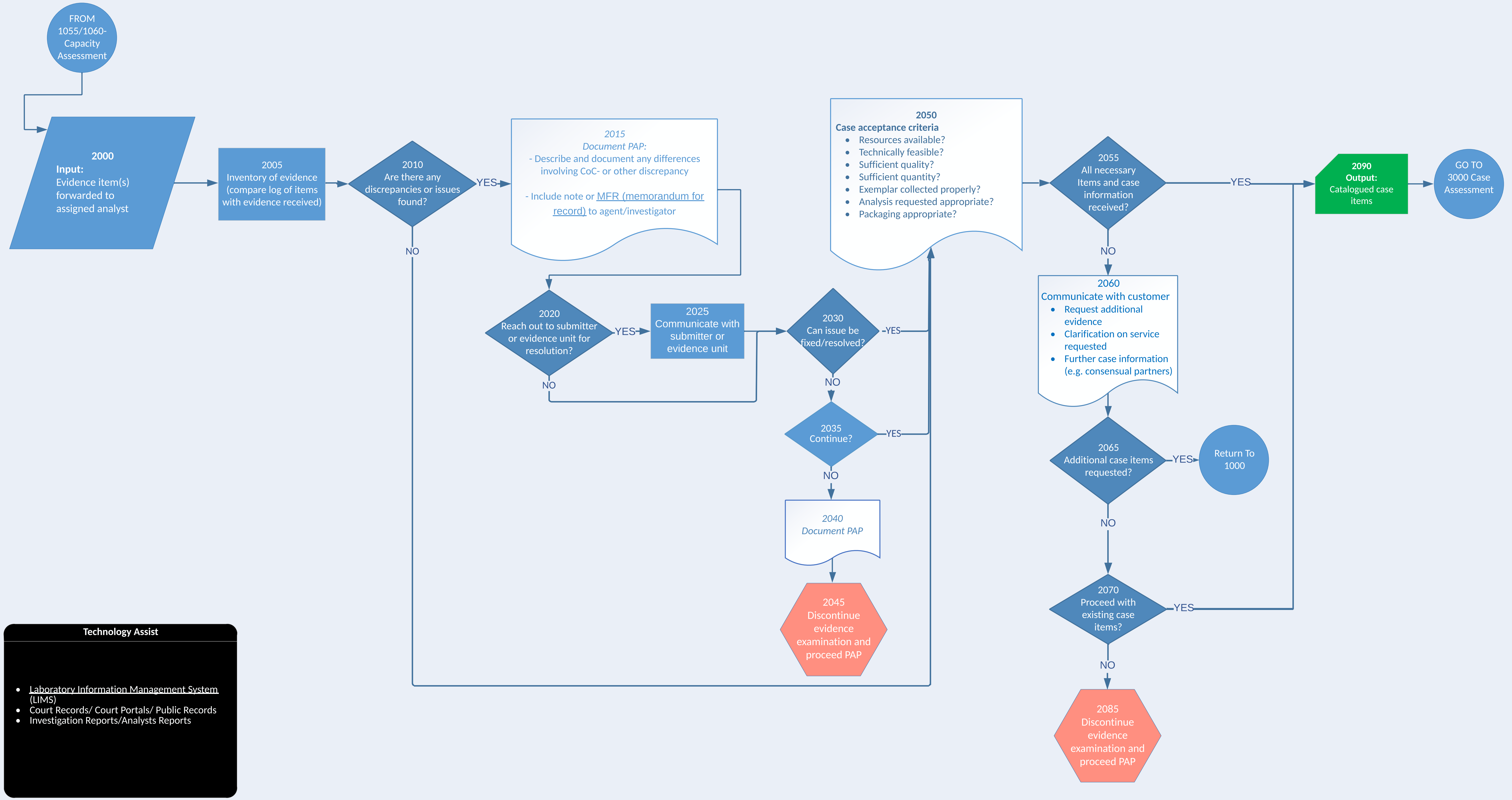
NO

1055
Transfer Case to Assigned Analyst

1060
Process case PAP

GO TO
2000- Technical Assessment

2000 - Technical Assessment



FROM 1055/1060-Capacity Assessment

2000
Input:
Evidence item(s) forwarded to assigned analyst

2005
Inventory of evidence (compare log of items with evidence received)

2010
Are there any discrepancies or issues found?

2015
Document PAP:
- Describe and document any differences involving CoC- or other discrepancy
- Include note or MFR (memorandum for record) to agent/investigator

2020
Reach out to submitter or evidence unit for resolution?

2025
Communicate with submitter or evidence unit

2030
Can issue be fixed/resolved?

2035
Continue?

2040
Document PAP

2045
Discontinue evidence examination and proceed PAP

2050
Case acceptance criteria
- Resources available?
- Technically feasible?
- Sufficient quality?
- Sufficient quantity?
- Exemplar collected properly?
- Analysis requested appropriate?
- Packaging appropriate?

2055
All necessary Items and case information received?

2060
Communicate with customer
- Request additional evidence
- Clarification on service requested
- Further case information (e.g. consensual partners)

2065
Additional case items requested?

Return To 1000

2070
Proceed with existing case items?

2085
Discontinue evidence examination and proceed PAP

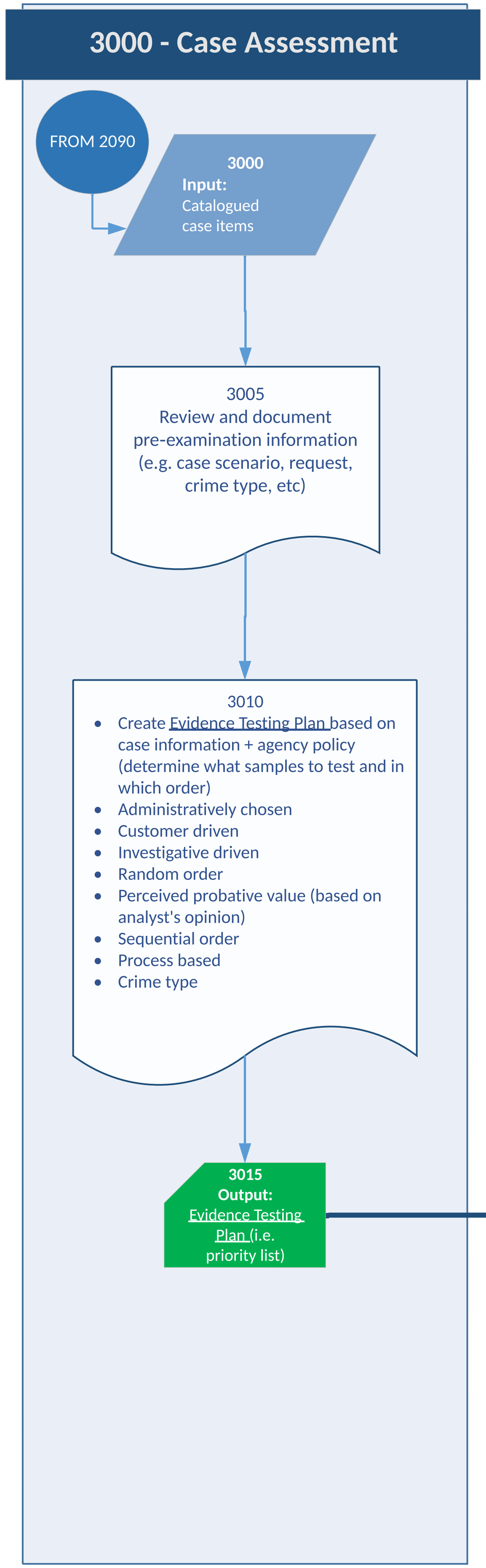
2090
Output:
Catalogued case items

GO TO 3000 Case Assessment

Technology Assist

- Laboratory Information Management System (LIMS)
- Court Records/ Court Portals/ Public Records
- Investigation Reports/Analysts Reports

3000 - Case Assessment



FROM 2090

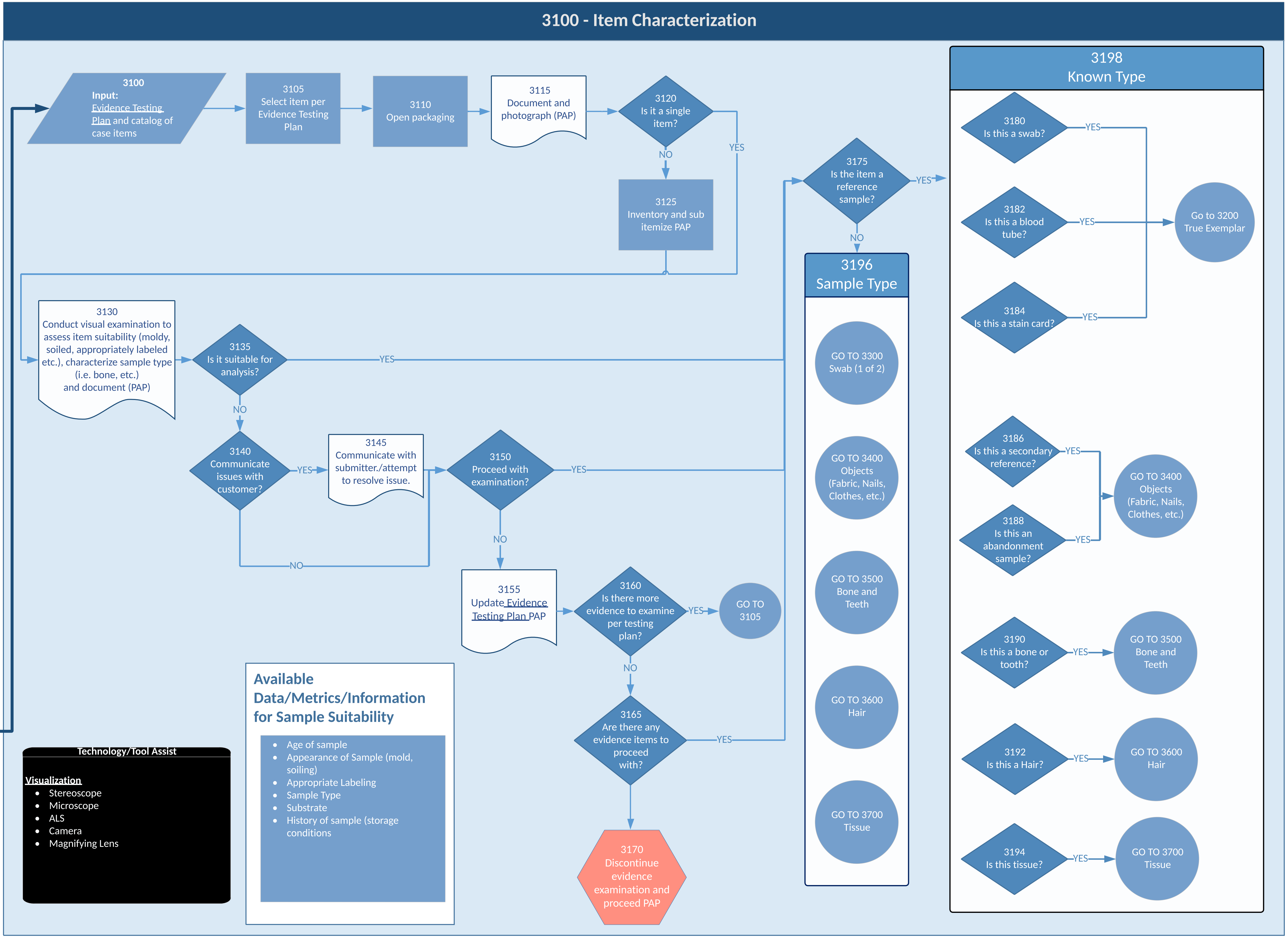
3000
Input:
Catalogued case items

3005
Review and document pre-examination information (e.g. case scenario, request, crime type, etc)

3010
- Create Evidence Testing Plan based on case information + agency policy (determine what samples to test and in which order)
- Administratively chosen
- Customer driven
- Investigative driven
- Random order
- Perceived probative value (based on analyst's opinion)
- Sequential order
- Process based
- Crime type

3015
Output:
Evidence Testing Plan (i.e. priority list)

3100 - Item Characterization



3100
Input:
Evidence Testing Plan and catalog of case items

3105
Select item per Evidence Testing Plan

3110
Open packaging

3115
Document and photograph (PAP)

3120
Is it a single item?

3125
Inventory and sub itemize PAP

3130
Conduct visual examination to assess item suitability (moldy, soiled, appropriately labeled etc.), characterize sample type (i.e. bone, etc.) and document (PAP)

3135
Is it suitable for analysis?

3140
Communicate issues with customer?

3145
Communicate with submitter./attempt to resolve issue.

3150
Proceed with examination?

3155
Update Evidence Testing Plan PAP

3160
Is there more evidence to examine per testing plan?

GO TO 3105

3165
Are there any evidence items to proceed with?

3170
Discontinue evidence examination and proceed PAP

3175
Is the item a reference sample?

3196 Sample Type

GO TO 3300 Swab (1 of 2)

GO TO 3400 Objects (Fabric, Nails, Clothes, etc.)

GO TO 3500 Bone and Teeth

GO TO 3600 Hair

GO TO 3700 Tissue

3198 Known Type

3180
Is this a swab?

3182
Is this a blood tube?

3184
Is this a stain card?

Go to 3200 True Exemplar

3186
Is this a secondary reference?

3188
Is this an abandonment sample?

GO TO 3400 Objects (Fabric, Nails, Clothes, etc.)

3190
Is this a bone or tooth?

GO TO 3500 Bone and Teeth

3192
Is this a Hair?

GO TO 3600 Hair

3194
Is this tissue?

GO TO 3700 Tissue

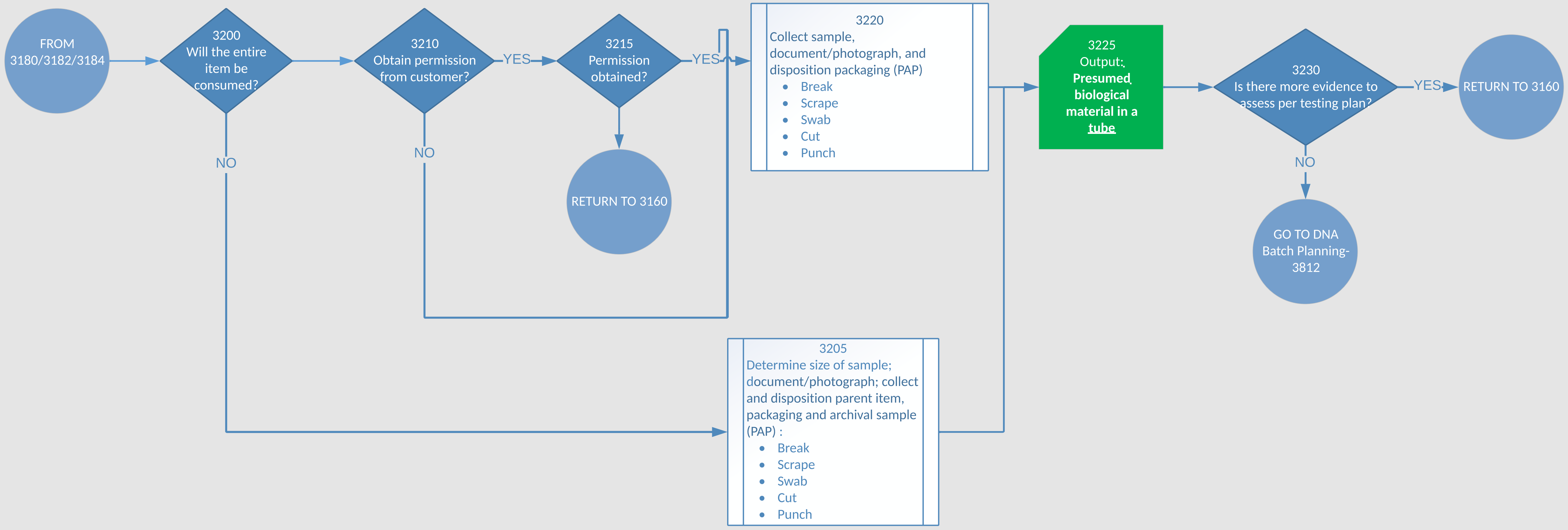
Technology/Tool Assist

Visualization
- Stereoscope
- Microscope
- ALS
- Camera
- Magnifying Lens

Available Data/Metrics/Information for Sample Suitability

- Age of sample
- Appearance of Sample (mold, soiling)
- Appropriate Labeling
- Sample Type
- Substrate
- History of sample (storage conditions

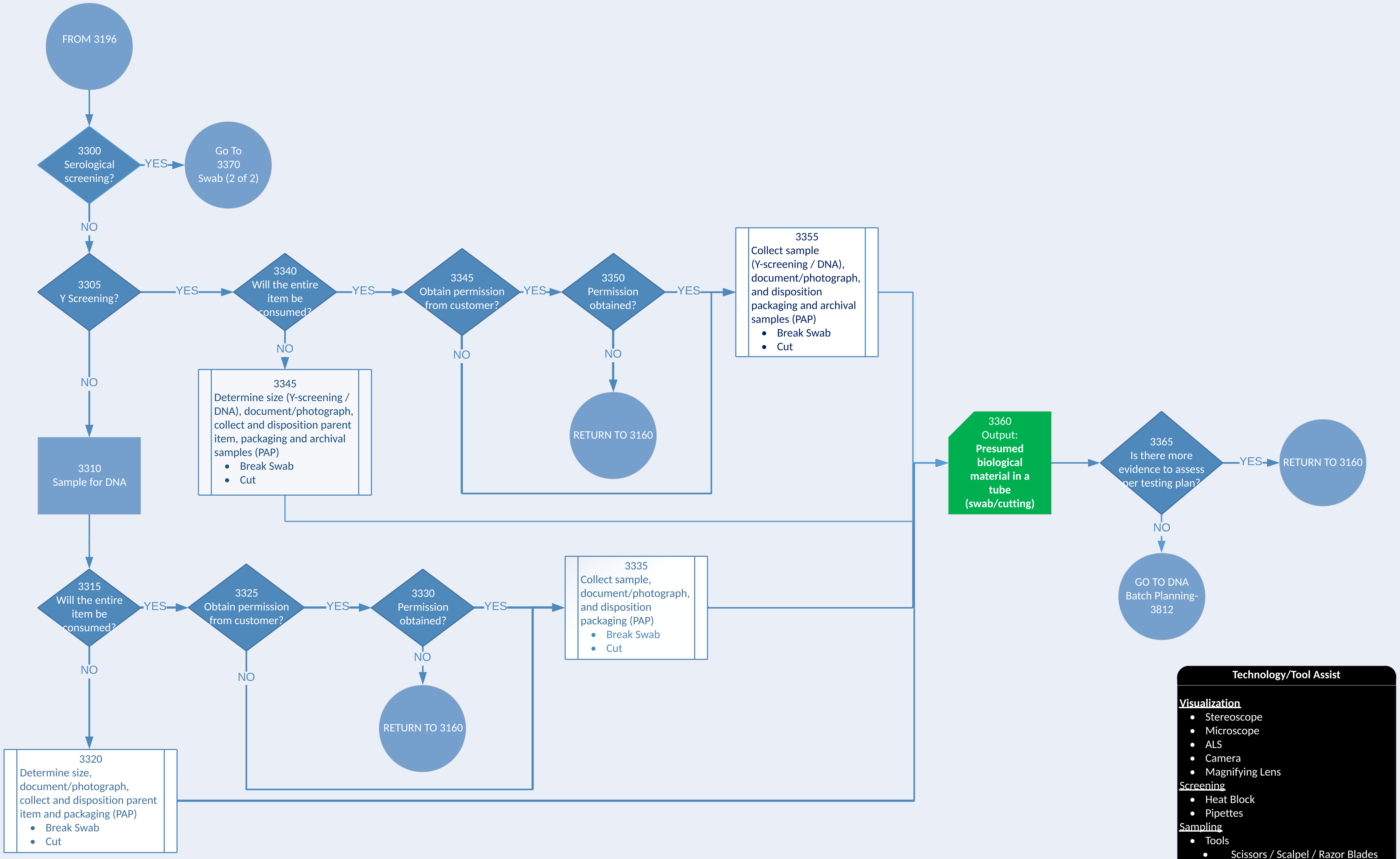
3200 - True Exemplar



Technology/Tool Assist

- Punch Robot
- Pipette
- Scissors / Scalpel / Razor Blades
- Tweezer / Forceps

3300 - SWAB (1 of 2)



FROM 3196

3300 Serological screening?

YES → Go To 3370 Swab (2 of 2)

NO → 3305 Y Screening?

YES → 3340 Will the entire item be consumed?

NO → 3345 Determine size (Y-screening / DNA), document/photograph, collect and disposition parent item, packaging and archival samples (PAP)
- Break Swab
- Cut

YES → 3345 Obtain permission from customer?

NO / YES → 3350 Permission obtained?

NO → RETURN TO 3160

YES → 3355 Collect sample (Y-screening / DNA), document/photograph, and disposition packaging and archival samples (PAP)
- Break Swab
- Cut

3305 NO → 3310 Sample for DNA

3315 Will the entire item be consumed?

NO → 3320 Determine size, document/photograph, collect and disposition parent item and packaging (PAP)
- Break Swab
- Cut

YES → 3325 Obtain permission from customer?

NO / YES → 3330 Permission obtained?

NO → RETURN TO 3160

YES → 3335 Collect sample, document/photograph, and disposition packaging (PAP)
- Break Swab
- Cut

3360 Output: **Presumed biological material in a tube (swab/cutting)**

3365 Is there more evidence to assess per testing plan?

YES → RETURN TO 3160

NO → GO TO DNA Batch Planning- 3812

Technology/Tool Assist

Visualization
- Stereoscope
- Microscope
- ALS
- Camera
- Magnifying Lens

Screening
- Heat Block
- Pipettes

Sampling
- Tools
  - Scissors / Scalpel / Razor Blades
  - Tweezer / Forceps
  - Vacuum
  - Measuring Tape
- Swabs
- Tape

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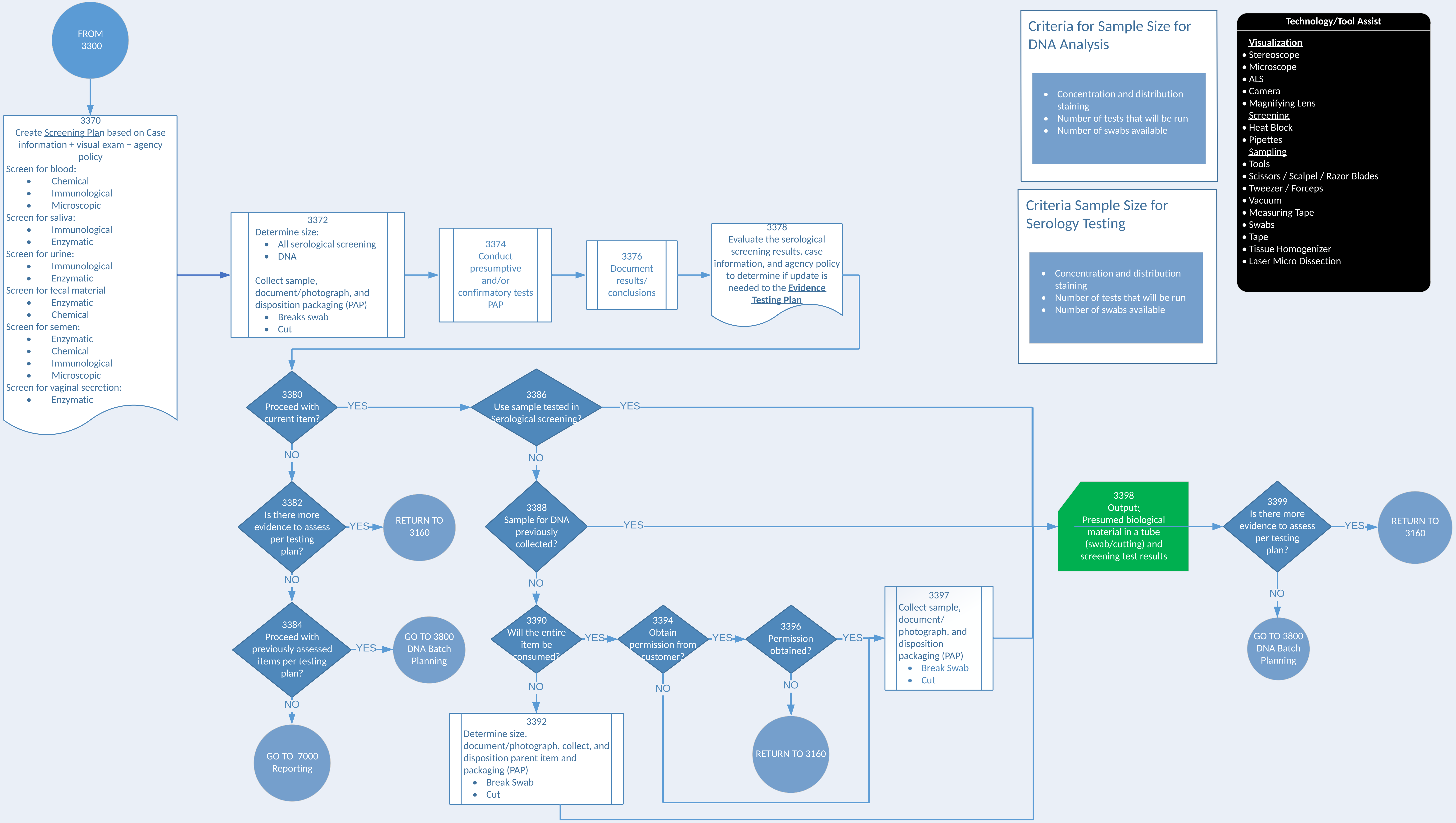
3300 - SWAB (2 of 2)



FROM 3300

3370
Create Screening Plan based on Case information + visual exam + agency policy
Screen for blood:
- Chemical
- Immunological
- Microscopic

Screen for saliva:
- Immunological
- Enzymatic

Screen for urine:
- Immunological
- Enzymatic

Screen for fecal material
- Enzymatic
- Chemical

Screen for semen:
- Enzymatic
- Chemical
- Immunological
- Microscopic

Screen for vaginal secretion:
- Enzymatic

3372
Determine size:
- All serological screening
- DNA

Collect sample, document/photograph, and disposition packaging (PAP)
- Breaks swab
- Cut

3374
Conduct presumptive and/or confirmatory tests PAP

3376
Document results/ conclusions

3378
Evaluate the serological screening results, case information, and agency policy to determine if update is needed to the **Evidence Testing Plan**

3380
Proceed with current item?
YES
NO

3386
Use sample tested in Serological screening?
YES
NO

3382
Is there more evidence to assess per testing plan?
YES
NO

RETURN TO 3160

3388
Sample for DNA previously collected?
YES
NO

3384
Proceed with previously assessed items per testing plan?
YES
NO

GO TO 3800 DNA Batch Planning

GO TO 7000 Reporting

3390
Will the entire item be consumed?
YES
NO

3392
Determine size, document/photograph, collect, and disposition parent item and packaging (PAP)
- Break Swab
- Cut

3394
Obtain permission from customer?
YES
NO

3396
Permission obtained?
YES
NO

RETURN TO 3160

3397
Collect sample, document/ photograph, and disposition packaging (PAP)
- Break Swab
- Cut

3398
Output;
Presumed biological material in a tube (swab/cutting) and screening test results

3399
Is there more evidence to assess per testing plan?
YES
NO

RETURN TO 3160

GO TO 3800 DNA Batch Planning

Criteria for Sample Size for DNA Analysis

- Concentration and distribution staining
- Number of tests that will be run
- Number of swabs available

Criteria Sample Size for Serology Testing

- Concentration and distribution staining
- Number of tests that will be run
- Number of swabs available

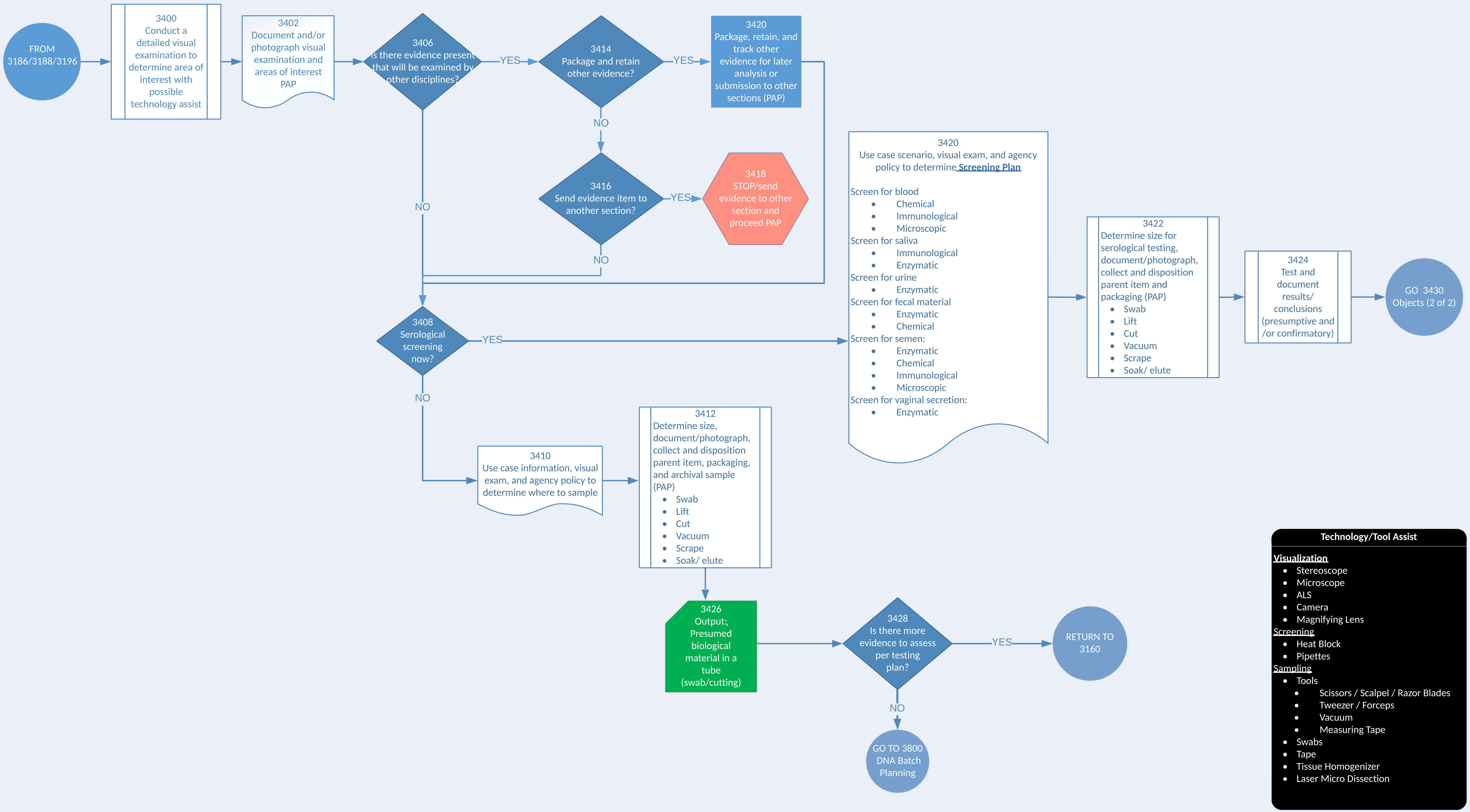
Technology/Tool Assist

Visualization
- Stereoscope
- Microscope
- ALS
- Camera
- Magnifying Lens

Screening
- Heat Block
- Pipettes

Sampling
- Tools
- Scissors / Scalpel / Razor Blades
- Tweezer / Forceps
- Vacuum
- Measuring Tape
- Swabs
- Tape
- Tissue Homogenizer
- Laser Micro Dissection

3400 - OBJECTS (Fabric, Nails, Clothes, etc.) (1 of 2)



FROM 3186/3188/3196

3400
Conduct a detailed visual examination to determine area of interest with possible technology assist

3402
Document and/or photograph visual examination and areas of interest PAP

3406
Is there evidence present that will be examined by other disciplines?

YES

3414
Package and retain other evidence?

YES

3420
Package, retain, and track other evidence for later analysis or submission to other sections (PAP)

NO

3416
Send evidence item to another section?

YES

3418
STOP/send evidence to other section and proceed PAP

NO

NO

3408
Serological screening now?

YES

3420
Use case scenario, visual exam, and agency policy to determine **Screening Plan**

Screen for blood
- Chemical
- Immunological
- Microscopic

Screen for saliva
- Immunological
- Enzymatic

Screen for urine
- Enzymatic

Screen for fecal material
- Enzymatic
- Chemical

Screen for semen:
- Enzymatic
- Chemical
- Immunological
- Microscopic

Screen for vaginal secretion:
- Enzymatic

3422
Determine size for serological testing, document/photograph, collect and disposition parent item and packaging (PAP)
- Swab
- Lift
- Cut
- Vacuum
- Scrape
- Soak/ elute

3424
Test and document results/ conclusions (presumptive and /or confirmatory)

GO 3430 Objects (2 of 2)

NO

3410
Use case information, visual exam, and agency policy to determine where to sample

3412
Determine size, document/photograph, collect and disposition parent item, packaging, and archival sample (PAP)
- Swab
- Lift
- Cut
- Vacuum
- Scrape
- Soak/ elute

3426
Output:
Presumed biological material in a tube (swab/cutting)

3428
Is there more evidence to assess per testing plan?

YES

RETURN TO 3160

NO

GO TO 3800 DNA Batch Planning

Technology/Tool Assist

Visualization
- Stereoscope
- Microscope
- ALS
- Camera
- Magnifying Lens

Screening
- Heat Block
- Pipettes

Sampling
- Tools
  - Scissors / Scalpel / Razor Blades
  - Tweezer / Forceps
  - Vacuum
  - Measuring Tape
- Swabs
- Tape
- Tissue Homogenizer
- Laser Micro Dissection

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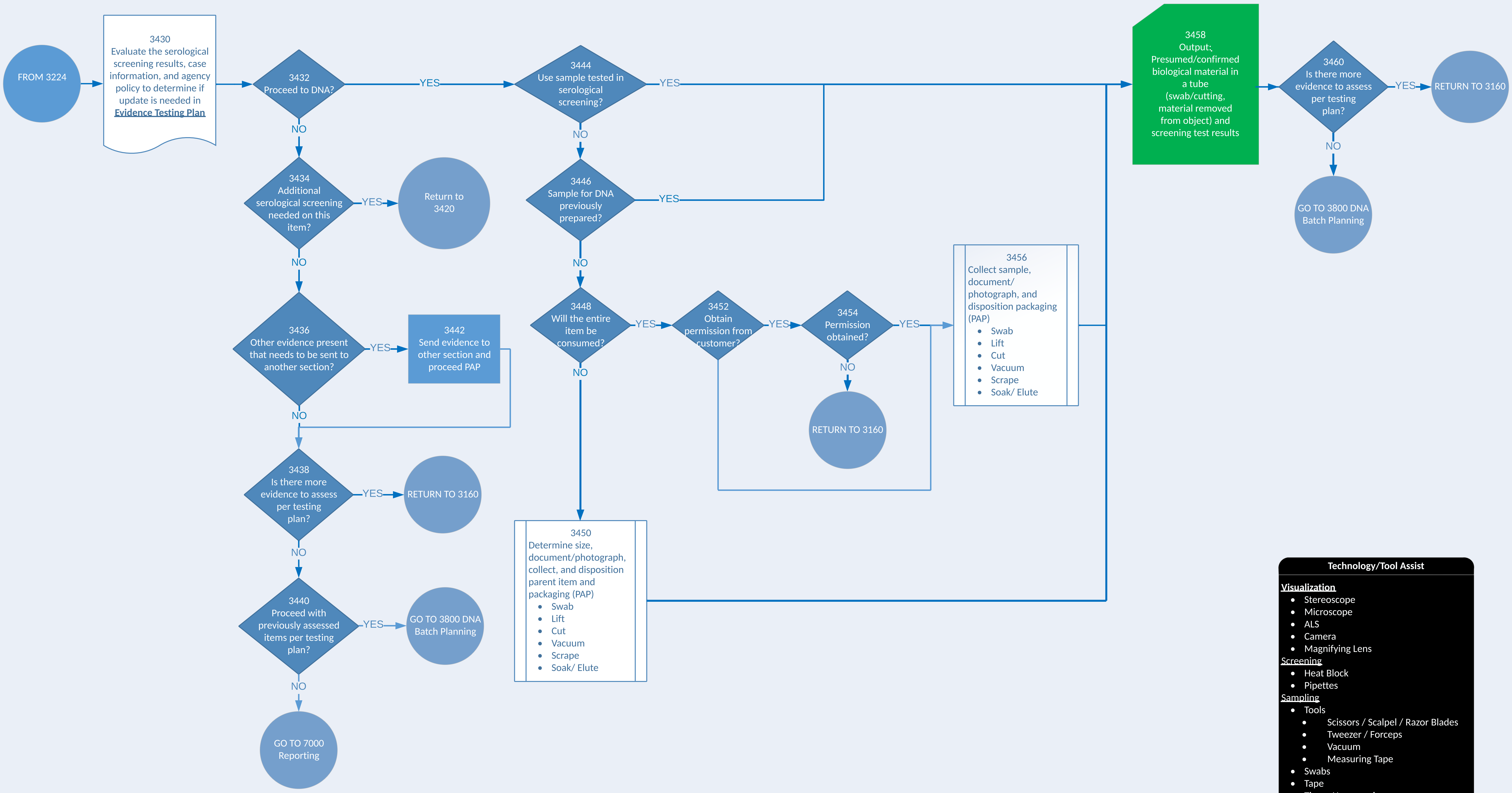
3400 - OBJECTS (Fabric, Nails, Clothes, etc.) (2 of 2)



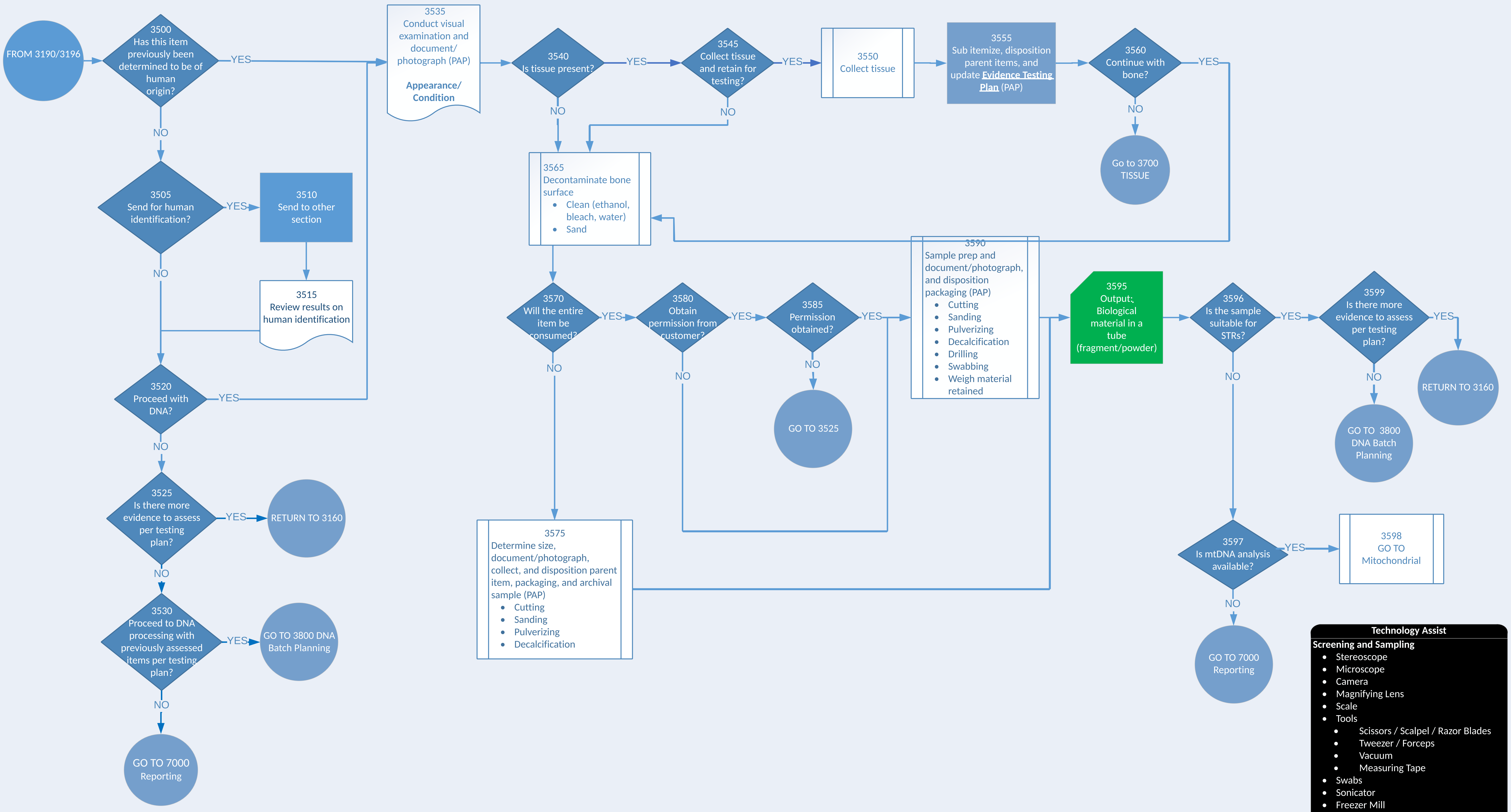
Technology/Tool Assist

Visualization
- Stereoscope
- Microscope
- ALS
- Camera
- Magnifying Lens

Screening
- Heat Block
- Pipettes

Sampling
- Tools
  - Scissors / Scalpel / Razor Blades
  - Tweezer / Forceps
  - Vacuum
  - Measuring Tape
- Swabs
- Tape
- Tissue Homogenizer
- Laser Micro Dissection

3500 - BONE AND TEETH



FROM 3190/3196

3500
Has this item previously been determined to be of human origin?
YES / NO

3505
Send for human identification?
YES / NO

3510
Send to other section

3515
Review results on human identification

3520
Proceed with DNA?
YES / NO

3525
Is there more evidence to assess per testing plan?
YES / NO

RETURN TO 3160

3530
Proceed to DNA processing with previously assessed items per testing plan?
YES / NO

GO TO 3800 DNA Batch Planning

GO TO 7000 Reporting

3535
Conduct visual examination and document/ photograph (PAP)

Appearance/ Condition

3540
Is tissue present?
YES / NO

3545
Collect tissue and retain for testing?
YES / NO

3550
Collect tissue

3555
Sub itemize, disposition parent items, and update **Evidence Testing Plan** (PAP)

3560
Continue with bone?
YES / NO

Go to 3700 TISSUE

3565
Decontaminate bone surface
- Clean (ethanol, bleach, water)
- Sand

3570
Will the entire item be consumed?
YES / NO

3575
Determine size, document/photograph, collect, and disposition parent item, packaging, and archival sample (PAP)
- Cutting
- Sanding
- Pulverizing
- Decalcification

3580
Obtain permission from customer?
YES / NO

3585
Permission obtained?
YES / NO

GO TO 3525

3590
Sample prep and document/photograph, and disposition packaging (PAP)
- Cutting
- Sanding
- Pulverizing
- Decalcification
- Drilling
- Swabbing
- Weigh material retained

3595
Output:
Biological material in a tube (fragment/powder)

3596
Is the sample suitable for STRs?
YES / NO

3599
Is there more evidence to assess per testing plan?
YES / NO

RETURN TO 3160

GO TO 3800 DNA Batch Planning

3597
Is mtDNA analysis available?
YES / NO

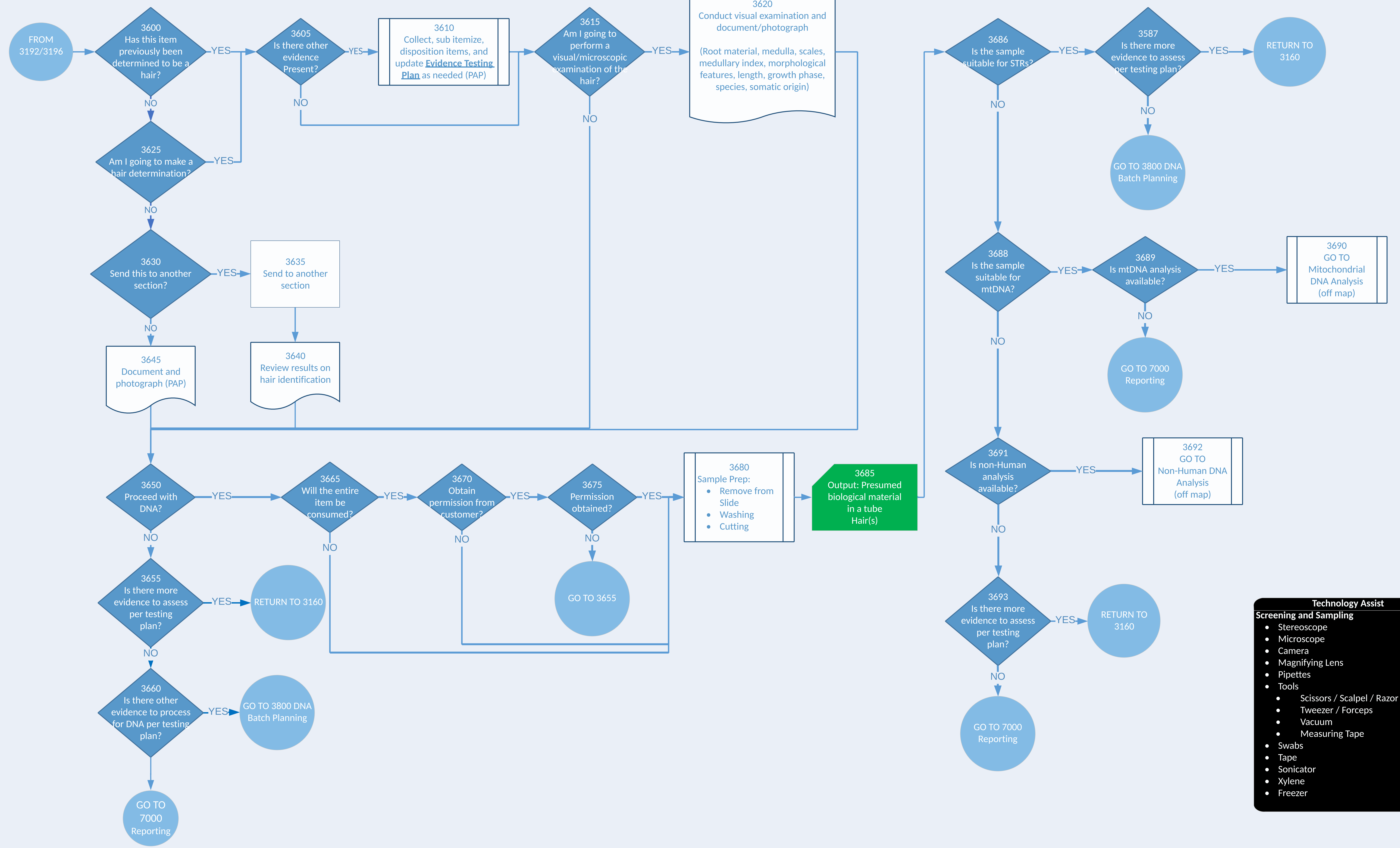
3598
GO TO Mitochondrial

GO TO 7000 Reporting

Technology Assist

Screening and Sampling
- Stereoscope
- Microscope
- Camera
- Magnifying Lens
- Scale
- Tools
  - Scissors / Scalpel / Razor Blades
  - Tweezer / Forceps
  - Vacuum
  - Measuring Tape
- Swabs
- Sonicator
- Freezer Mill
- Bone Saw
- Drill/Dremel
- Rotator (incubator)

3600 - HAIR (Unknown, Known, Mounted)



FROM 3192/3196

3600 Has this item previously been determined to be a hair? YES → 3605; NO → 3625

3605 Is there other evidence Present? YES → 3610; NO → 3615

3610 Collect, sub itemize, disposition items, and update **Evidence Testing Plan** as needed (PAP)

3615 Am I going to perform a visual/microscopic xamination of th hair? YES → 3620; NO → 3650

3620 Conduct visual examination and document/photograph

(Root material, medulla, scales, medullary index, morphological features, length, growth phase, species, somatic origin)

3625 Am I going to make a hair determination? YES → 3605; NO → 3630

3630 Send this to another section? YES → 3635; NO → 3645

3635 Send to another section

3640 Review results on hair identification

3645 Document and photograph (PAP)

3650 Proceed with DNA? YES → 3665; NO → 3655

3655 Is there more evidence to assess per testing plan? YES → RETURN TO 3160; NO → 3660

3660 Is there other evidence to process for DNA per testing plan? YES → GO TO 3800 DNA Batch Planning; NO → GO TO 7000 Reporting

3665 Will the entire item be consumed? YES → 3670; NO → 3680

3670 Obtain permission from customer? YES → 3675; NO → 3680

3675 Permission obtained? YES → 3680; NO → GO TO 3655

3680 Sample Prep:

- Remove from Slide
- Washing
- Cutting

3685 Output: Presumed biological material in a tube Hair(s)

3686 Is the sample uitable for STRs? YES → 3587; NO → 3688

3587 Is there more evidence to assess er testing plan? YES → RETURN TO 3160; NO → GO TO 3800 DNA Batch Planning

3688 Is the sample suitable for mtDNA? YES → 3689; NO → 3691

3689 Is mtDNA analysis available? YES → 3690; NO → GO TO 7000 Reporting

3690 GO TO Mitochondrial DNA Analysis (off map)

3691 Is non-Human analysis available? YES → 3692; NO → 3693

3692 GO TO Non-Human DNA Analysis (off map)

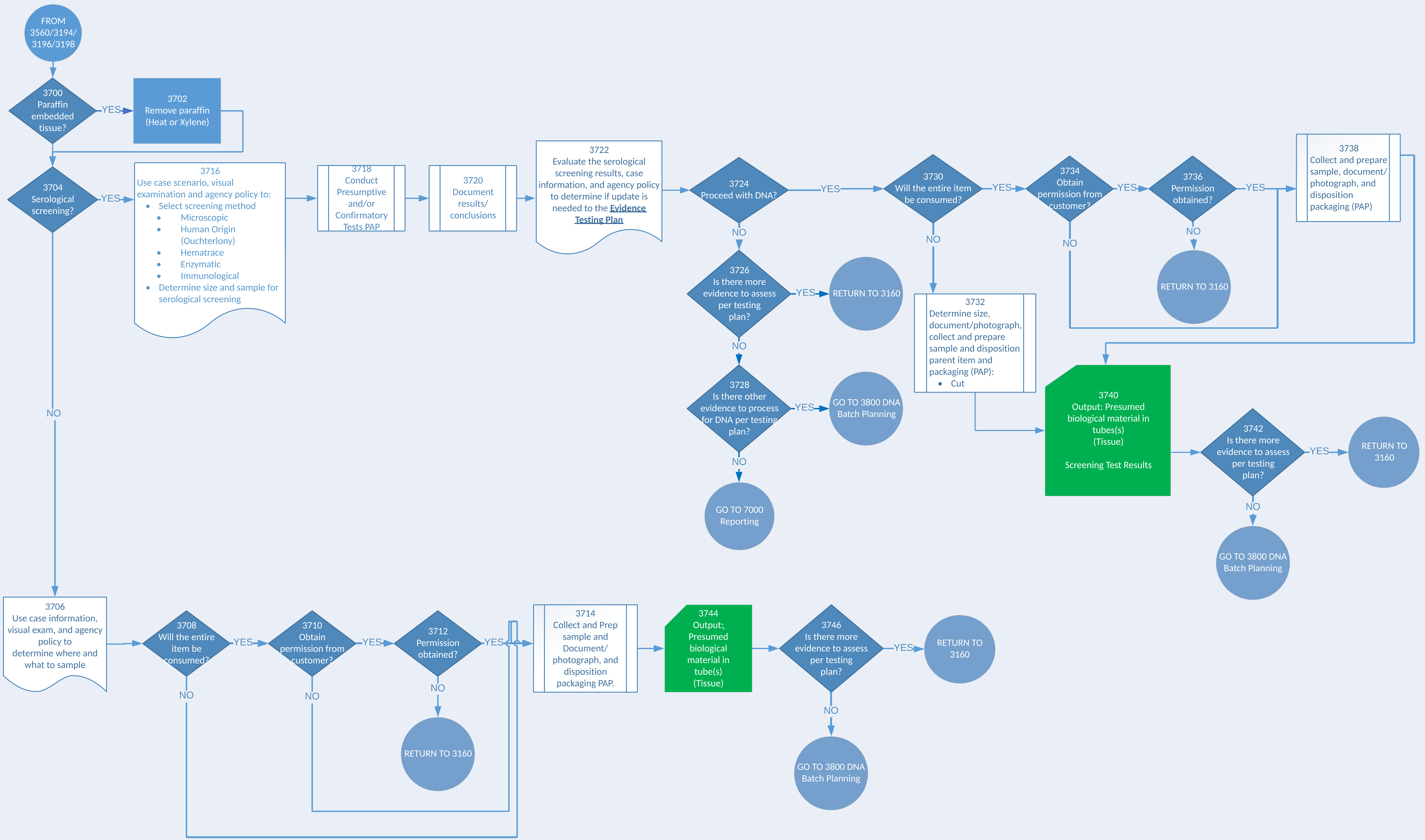
3693 Is there more evidence to assess per testing plan? YES → RETURN TO 3160; NO → GO TO 7000 Reporting

Technology Assist

Screening and Sampling

- Stereoscope
- Microscope
- Camera
- Magnifying Lens
- Pipettes
- Tools
  - Scissors / Scalpel / Razor Blades
  - Tweezer / Forceps
  - Vacuum
  - Measuring Tape
- Swabs
- Tape
- Sonicator
- Xylene
- Freezer

3700 - TISSUE



FROM 3560/3194/ 3196/3198

3700 Paraffin embedded tissue?

YES

3702 Remove paraffin (Heat or Xylene)

3704 Serological screening?

YES

3716 Use case scenario, visual examination and agency policy to:
- Select screening method
  - Microscopic
  - Human Origin (Ouchterlony)
  - Hematrace
  - Enzymatic
  - Immunological
- Determine size and sample for serological screening

3718 Conduct Presumptive and/or Confirmatory Tests PAP

3720 Document results/ conclusions

3722 Evaluate the serological screening results, case information, and agency policy to determine if update is needed to the **Evidence Testing Plan**

3724 Proceed with DNA?

YES

NO

3726 Is there more evidence to assess per testing plan?

YES

RETURN TO 3160

NO

3728 Is there other evidence to process for DNA per testing plan?

YES

GO TO 3800 DNA Batch Planning

NO

GO TO 7000 Reporting

3730 Will the entire item be consumed?

YES

NO

3732 Determine size, document/photograph, collect and prepare sample and disposition parent item and packaging (PAP):
- Cut

3734 Obtain permission from customer?

YES

NO

3736 Permission obtained?

YES

NO

RETURN TO 3160

3738 Collect and prepare sample, document/ photograph, and disposition packaging (PAP)

3740 Output: Presumed biological material in tubes(s) (Tissue)

Screening Test Results

3742 Is there more evidence to assess per testing plan?

YES

RETURN TO 3160

NO

GO TO 3800 DNA Batch Planning

NO

3706 Use case information, visual exam, and agency policy to determine where and what to sample

3708 Will the entire item be consumed?

YES

NO

3710 Obtain permission from customer?

YES

NO

3712 Permission obtained?

YES

NO

RETURN TO 3160

3714 Collect and Prep sample and Document/ photograph, and disposition packaging PAP.

3744 Output:, Presumed biological material in tube(s) (Tissue)

3746 Is there more evidence to assess per testing plan?

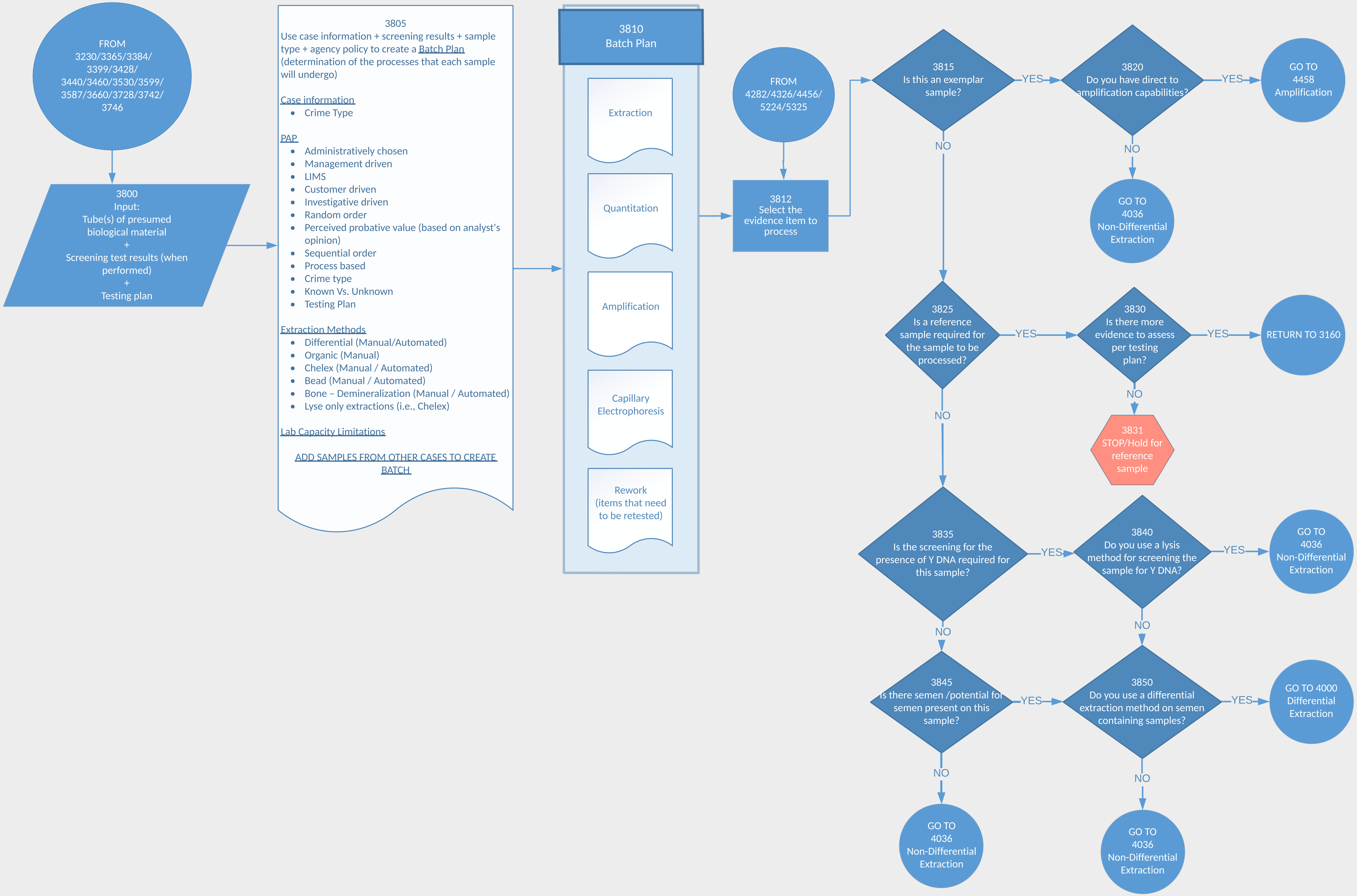
YES

RETURN TO 3160

NO

GO TO 3800 DNA Batch Planning

3800 - DNA Batch Planning



FROM
3230/3365/3384/
3399/3428/
3440/3460/3530/3599/
3587/3660/3728/3742/
3746

3800
Input:
Tube(s) of presumed biological material
+
Screening test results (when performed)
+
Testing plan

3805
Use case information + screening results + sample type + agency policy to create a Batch Plan (determination of the processes that each sample will undergo)

Case information
- Crime Type

PAP
- Administratively chosen
- Management driven
- LIMS
- Customer driven
- Investigative driven
- Random order
- Perceived probative value (based on analyst's opinion)
- Sequential order
- Process based
- Crime type
- Known Vs. Unknown
- Testing Plan

Extraction Methods
- Differential (Manual/Automated)
- Organic (Manual)
- Chelex (Manual / Automated)
- Bead (Manual / Automated)
- Bone – Demineralization (Manual / Automated)
- Lyse only extractions (i.e., Chelex)

Lab Capacity Limitations

ADD SAMPLES FROM OTHER CASES TO CREATE BATCH

3810
Batch Plan

Extraction

Quantitation

Amplification

Capillary Electrophoresis

Rework
(items that need to be retested)

FROM
4282/4326/4456/
5224/5325

3812
Select the evidence item to process

3815
Is this an exemplar sample?
YES → 3820; NO → 3825

3820
Do you have direct to amplification capabilities?
YES → GO TO 4458 Amplification
NO → GO TO 4036 Non-Differential Extraction

3825
Is a reference sample required for the sample to be processed?
YES → 3830; NO → 3835

3830
Is there more evidence to assess per testing plan?
YES → RETURN TO 3160
NO → 3831 STOP/Hold for reference sample

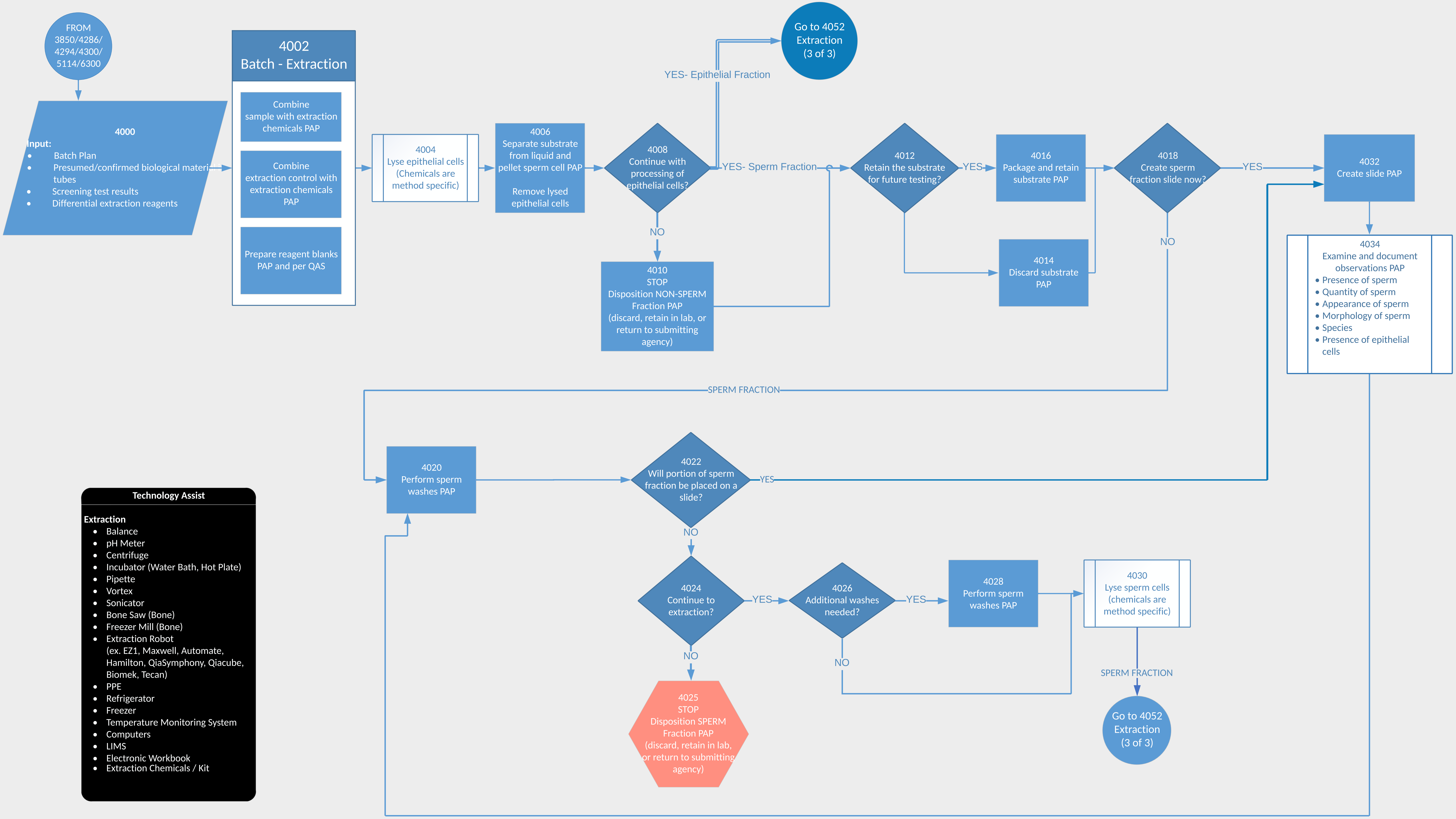
3835
Is the screening for the presence of Y DNA required for this sample?
YES → 3840; NO → 3845

3840
Do you use a lysis method for screening the sample for Y DNA?
YES → GO TO 4036 Non-Differential Extraction
NO → 3850

3845
Is there semen /potential for semen present on this sample?
YES → 3850
NO → GO TO 4036 Non-Differential Extraction

3850
Do you use a differential extraction method on semen containing samples?
YES → GO TO 4000 Differential Extraction
NO → GO TO 4036 Non-Differential Extraction

4000 - EXTRACTION (1 of 3): Differential Extraction



FROM 3850/4286/ 4294/4300/ 5114/6300

4000
Input:
- Batch Plan
- Presumed/confirmed biological materi tubes
- Screening test results
- Differential extraction reagents

4002
Batch - Extraction
- Combine sample with extraction chemicals PAP
- Combine extraction control with extraction chemicals PAP
- Prepare reagent blanks PAP and per QAS

4004
Lyse epithelial cells (Chemicals are method specific)

4006
Separate substrate from liquid and pellet sperm cell PAP
Remove lysed epithelial cells

4008
Continue with processing of epithelial cells?

Go to 4052 Extraction (3 of 3)

4010
STOP
Disposition NON-SPERM Fraction PAP
(discard, retain in lab, or return to submitting agency)

4012
Retain the substrate for future testing?

4016
Package and retain substrate PAP

4014
Discard substrate PAP

4018
Create sperm fraction slide now?

4032
Create slide PAP

4034
Examine and document observations PAP
- Presence of sperm
- Quantity of sperm
- Appearance of sperm
- Morphology of sperm
- Species
- Presence of epithelial cells

4020
Perform sperm washes PAP

4022
Will portion of sperm fraction be placed on a slide?

4024
Continue to extraction?

4025
STOP
Disposition SPERM Fraction PAP
(discard, retain in lab, or return to submitting agency)

4026
Additional washes needed?

4028
Perform sperm washes PAP

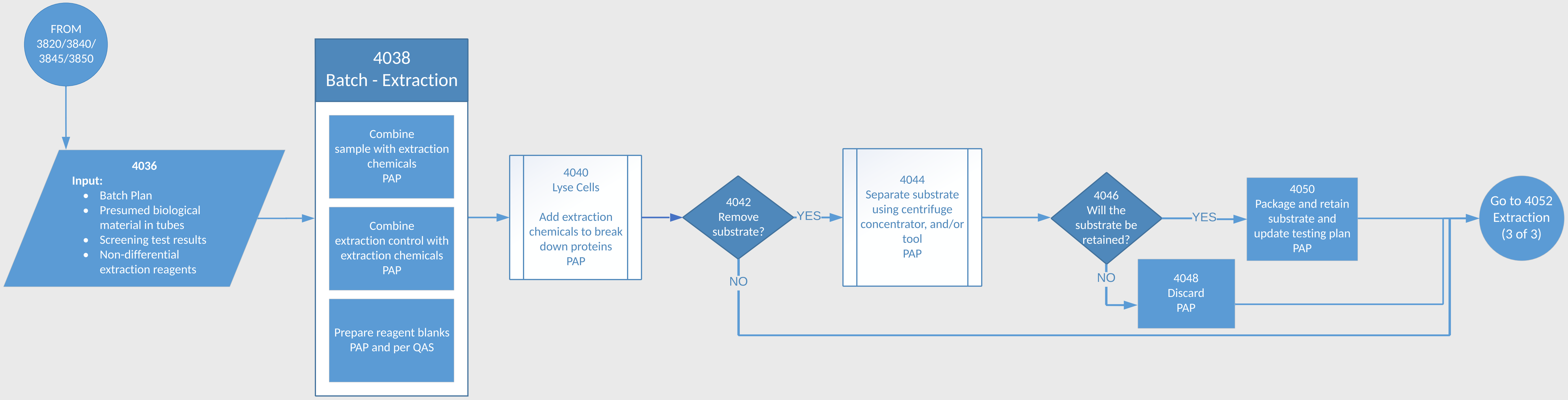
4030
Lyse sperm cells (chemicals are method specific)

Go to 4052 Extraction (3 of 3)

Technology Assist

Extraction
- Balance
- pH Meter
- Centrifuge
- Incubator (Water Bath, Hot Plate)
- Pipette
- Vortex
- Sonicator
- Bone Saw (Bone)
- Freezer Mill (Bone)
- Extraction Robot
  (ex. EZ1, Maxwell, Automate, Hamilton, QiaSymphony, Qiacube, Biomek, Tecan)
- PPE
- Refrigerator
- Freezer
- Temperature Monitoring System
- Computers
- LIMS
- Electronic Workbook
- Extraction Chemicals / Kit

4000 - EXTRACTION (2 of 3): Non-Differential Extraction


FROM 3820/3840/ 3845/3850

4036
Input:
- Batch Plan
- Presumed biological material in tubes
- Screening test results
- Non-differential extraction reagents

4038
Batch - Extraction

Combine sample with extraction chemicals
PAP

Combine extraction control with extraction chemicals
PAP

Prepare reagent blanks PAP and per QAS

4040
Lyse Cells
Add extraction chemicals to break down proteins
PAP

4042
Remove substrate?

YES

NO

4044
Separate substrate using centrifuge concentrator, and/or tool
PAP

4046
Will the substrate be retained?

YES

NO

4050
Package and retain substrate and update testing plan
PAP

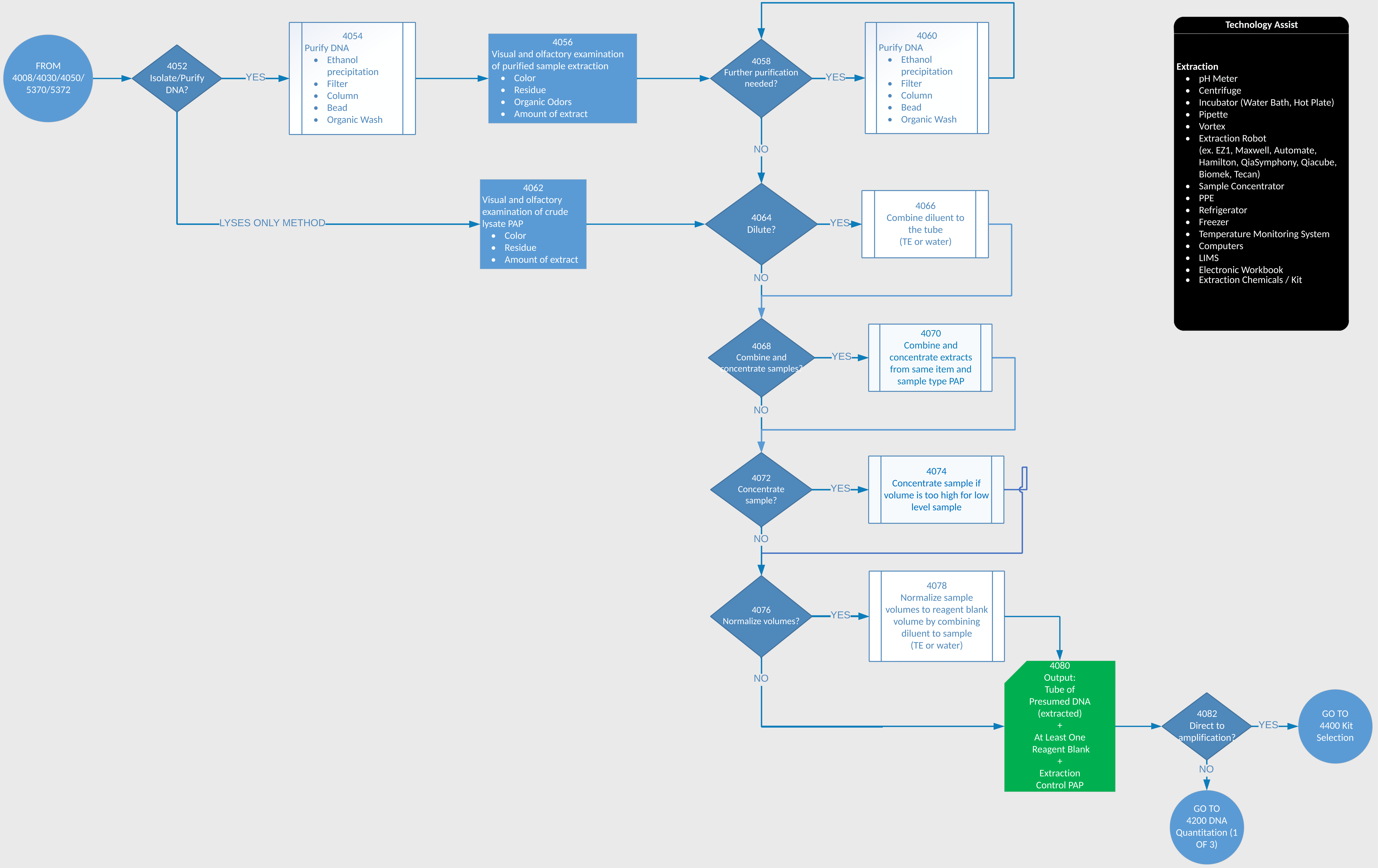
4048
Discard
PAP

Go to 4052 Extraction (3 of 3)

Technology Assist

Extraction
- Balance
- pH Meter
- Centrifuge
- Incubator (Water Bath, Hot Plate)
- Pipette
- Vortex
- Sonicator
- Bone Saw (Bone)
- Freezer Mill (Bone)
- Extraction Robot (ex. EZ1, Maxwell, Automate, Hamilton, QiaSymphony, Qiacube, Biomek, Tecan)
- PPE
- Refrigerator
- Freezer
- Temperature Monitoring System
- Computers
- LIMS
- Electronic Workbook
- Extraction Chemicals / Kit

4000 - EXTRACTION (Isolate and Purify) (3 of 3)



FROM 4008/4030/4050/5370/5372

4052 Isolate/Purify DNA?

YES

4054 Purify DNA
- Ethanol precipitation
- Filter
- Column
- Bead
- Organic Wash

4056 Visual and olfactory examination of purified sample extraction
- Color
- Residue
- Organic Odors
- Amount of extract

4058 Further purification needed?

YES

4060 Purify DNA
- Ethanol precipitation
- Filter
- Column
- Bead
- Organic Wash

NO

LYSES ONLY METHOD

4062 Visual and olfactory examination of crude lysate PAP
- Color
- Residue
- Amount of extract

4064 Dilute?

YES

4066 Combine diluent to the tube (TE or water)

NO

4068 Combine and concentrate samples?

YES

4070 Combine and concentrate extracts from same item and sample type PAP

NO

4072 Concentrate sample?

YES

4074 Concentrate sample if volume is too high for low level sample

NO

4076 Normalize volumes?

YES

4078 Normalize sample volumes to reagent blank volume by combining diluent to sample (TE or water)

NO

4080 Output: Tube of Presumed DNA (extracted) + At Least One Reagent Blank + Extraction Control PAP

4082 Direct to amplification?

YES

GO TO 4400 Kit Selection

NO

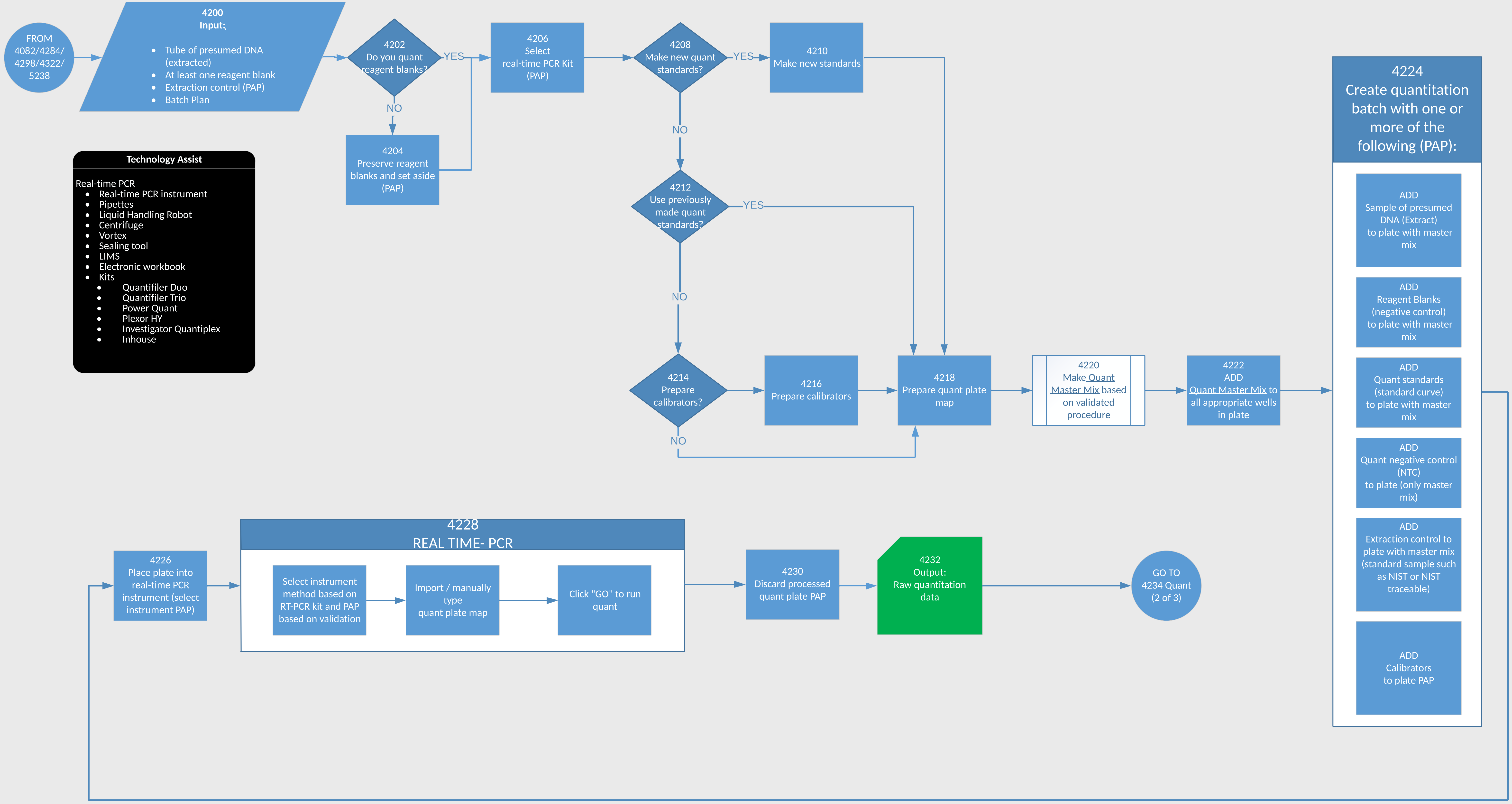
GO TO 4200 DNA Quantitation (1 OF 3)

Technology Assist

Extraction
- pH Meter
- Centrifuge
- Incubator (Water Bath, Hot Plate)
- Pipette
- Vortex
- Extraction Robot (ex. EZ1, Maxwell, Automate, Hamilton, QiaSymphony, Qiacube, Biomek, Tecan)
- Sample Concentrator
- PPE
- Refrigerator
- Freezer
- Temperature Monitoring System
- Computers
- LIMS
- Electronic Workbook
- Extraction Chemicals / Kit

4200 - QUANTITATION (1 of 3)

Return to Overview



FROM 4082/4284/4298/4322/5238

4200
Input:

- Tube of presumed DNA (extracted)
- At least one reagent blank
- Extraction control (PAP)
- Batch Plan

Technology Assist

Real-time PCR
- Real-time PCR instrument
- Pipettes
- Liquid Handling Robot
- Centrifuge
- Vortex
- Sealing tool
- LIMS
- Electronic workbook
- Kits
    - Quantifiler Duo
    - Quantifiler Trio
    - Power Quant
    - Plexor HY
    - Investigator Quantiplex
    - Inhouse

4202 Do you quant reagent blanks? YES → 4206; NO → 4204

4204 Preserve reagent blanks and set aside (PAP)

4206 Select real-time PCR Kit (PAP)

4208 Make new quant standards? YES → 4210; NO → 4212

4210 Make new standards

4212 Use previously made quant standards? YES → 4218; NO → 4214

4214 Prepare calibrators? → 4216; NO → 4218

4216 Prepare calibrators

4218 Prepare quant plate map

4220 Make Quant Master Mix based on validated procedure

4222 ADD Quant Master Mix to all appropriate wells in plate

4224 Create quantitation batch with one or more of the following (PAP):

- ADD Sample of presumed DNA (Extract) to plate with master mix
- ADD Reagent Blanks (negative control) to plate with master mix
- ADD Quant standards (standard curve) to plate with master mix
- ADD Quant negative control (NTC) to plate (only master mix)
- ADD Extraction control to plate with master mix (standard sample such as NIST or NIST traceable)
- ADD Calibrators to plate PAP

4226 Place plate into real-time PCR instrument (select instrument PAP)

4228 REAL TIME- PCR

Select instrument method based on RT-PCR kit and PAP based on validation → Import / manually type quant plate map → Click "GO" to run quant

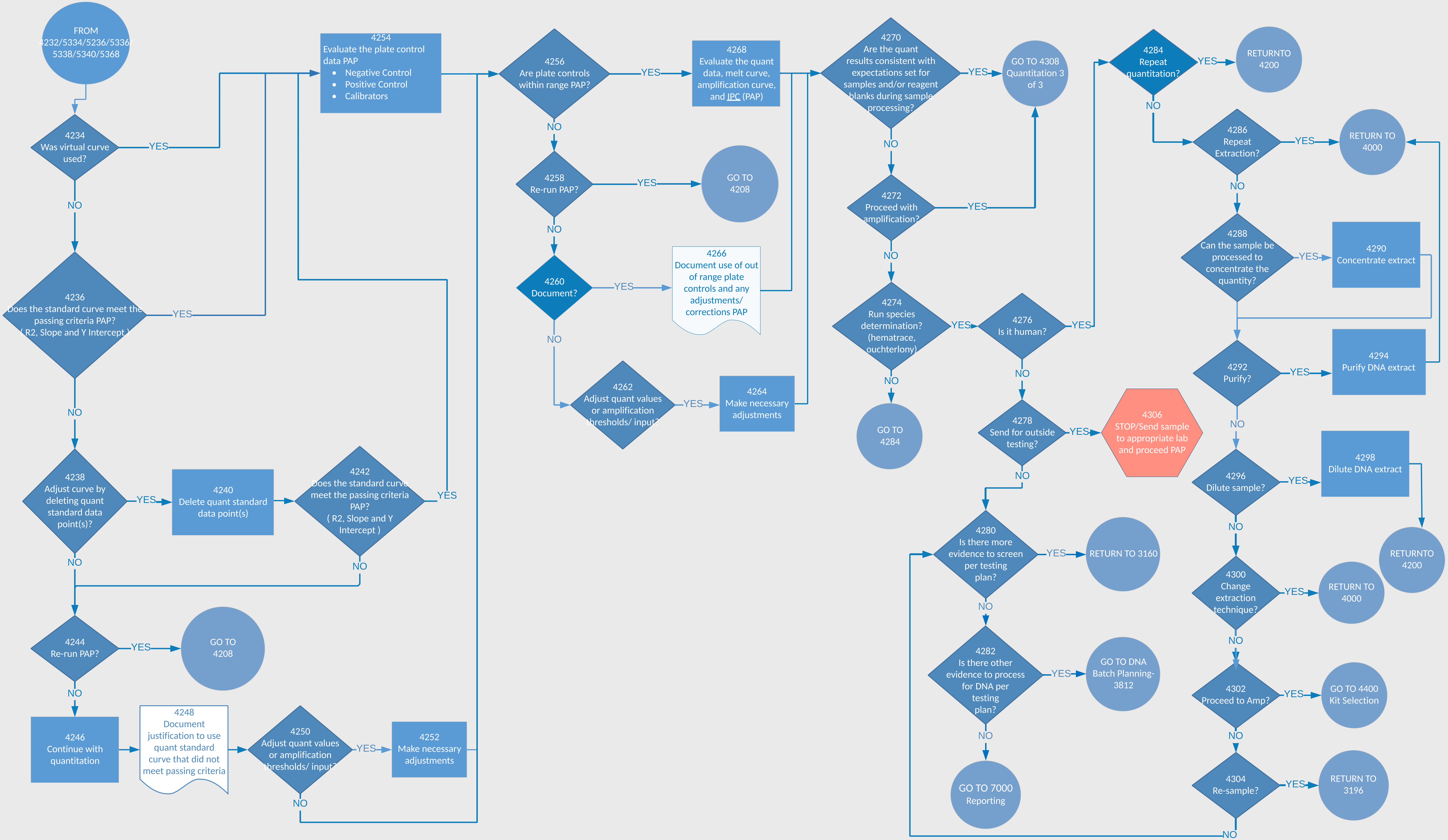
4230 Discard processed quant plate PAP

4232 Output: Raw quantitation data

GO TO 4234 Quant (2 of 3)

This Process Map provides a visual description of the various steps of the casework process performed by DNA analysts and is an attempt to represent all reasonable variations in current practice. OSAC and SWGDAM do not explicitly support or endorse (as best practices) all of the different steps and/or paths as depicted on this current-practices process map.

4200 - QUANTITATION (2 of 3)



FROM 4232/5334/5236/5336/ 5338/5340/5368

4234 Was virtual curve used?

4236 Does the standard curve meet the passing criteria PAP? (R2, Slope and Y Intercept)

4238 Adjust curve by deleting quant standard data point(s)?

4240 Delete quant standard data point(s)

4242 Does the standard curve meet the passing criteria PAP? (R2, Slope and Y Intercept)

4244 Re-run PAP?

GO TO 4208

4246 Continue with quantitation

4248 Document justification to use quant standard curve that did not meet passing criteria

4250 Adjust quant values or amplification thresholds/ input?

4252 Make necessary adjustments

4254 Evaluate the plate control data PAP
- Negative Control
- Positive Control
- Calibrators

4256 Are plate controls within range PAP?

4258 Re-run PAP?

GO TO 4208

4260 Document?

4266 Document use of out of range plate controls and any adjustments/ corrections PAP

4262 Adjust quant values or amplification thresholds/ input?

4264 Make necessary adjustments

4268 Evaluate the quant data, melt curve, amplification curve, and IPC (PAP)

4270 Are the quant results consistent with expectations set for samples and/or reagent blanks during sample processing?

GO TO 4308 Quantitation 3 of 3

4272 Proceed with amplification?

4274 Run species determination? (hematrace, ouchterlony)

GO TO 4284

4276 Is it human?

4278 Send for outside testing?

4306 STOP/Send sample to appropriate lab and proceed PAP

4280 Is there more evidence to screen per testing plan?

RETURN TO 3160

4282 Is there other evidence to process for DNA per testing plan?

GO TO DNA Batch Planning-3812

GO TO 7000 Reporting

4284 Repeat quantitation?

RETURNTO 4200

4286 Repeat Extraction?

RETURN TO 4000

4288 Can the sample be processed to concentrate the quantity?

4290 Concentrate extract

4292 Purify?

4294 Purify DNA extract

4296 Dilute sample?

4298 Dilute DNA extract

RETURNTO 4200

4300 Change extraction technique?

RETURN TO 4000

4302 Proceed to Amp?

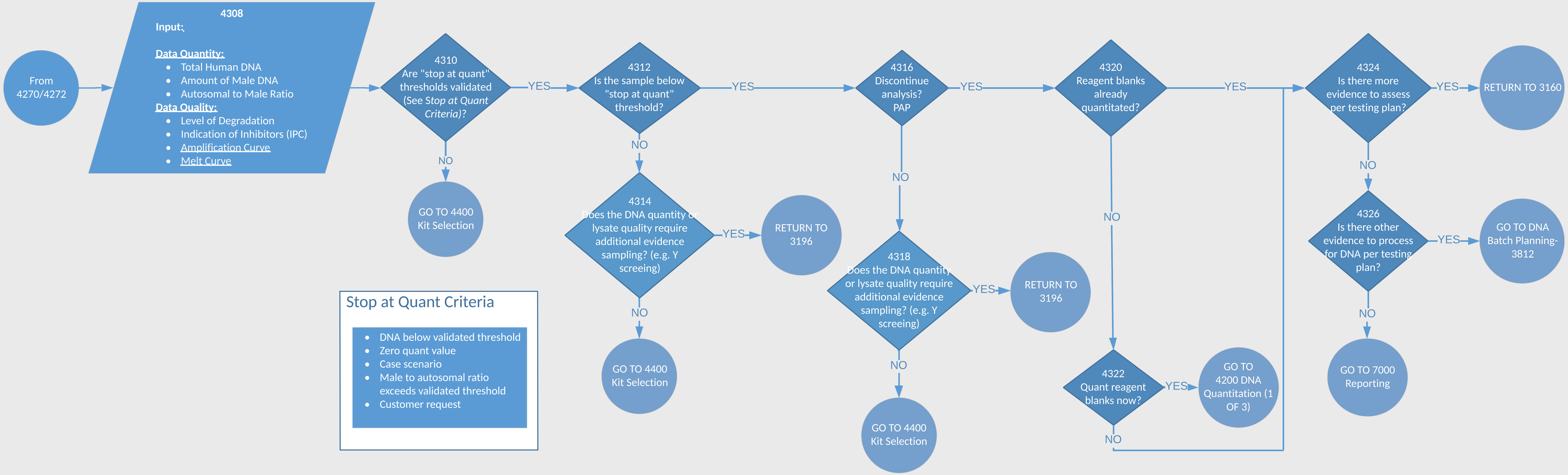
GO TO 4400 Kit Selection

4304 Re-sample?

RETURN TO 3196

This Process Map provides a visual description of the various steps of the casework process performed by DNA analysts and is an attempt to represent all reasonable variations in current practice. OSAC and SWGDAM do not explicitly support or endorse (as best practices) all of the different steps and/or paths as depicted on this current-practices process map.

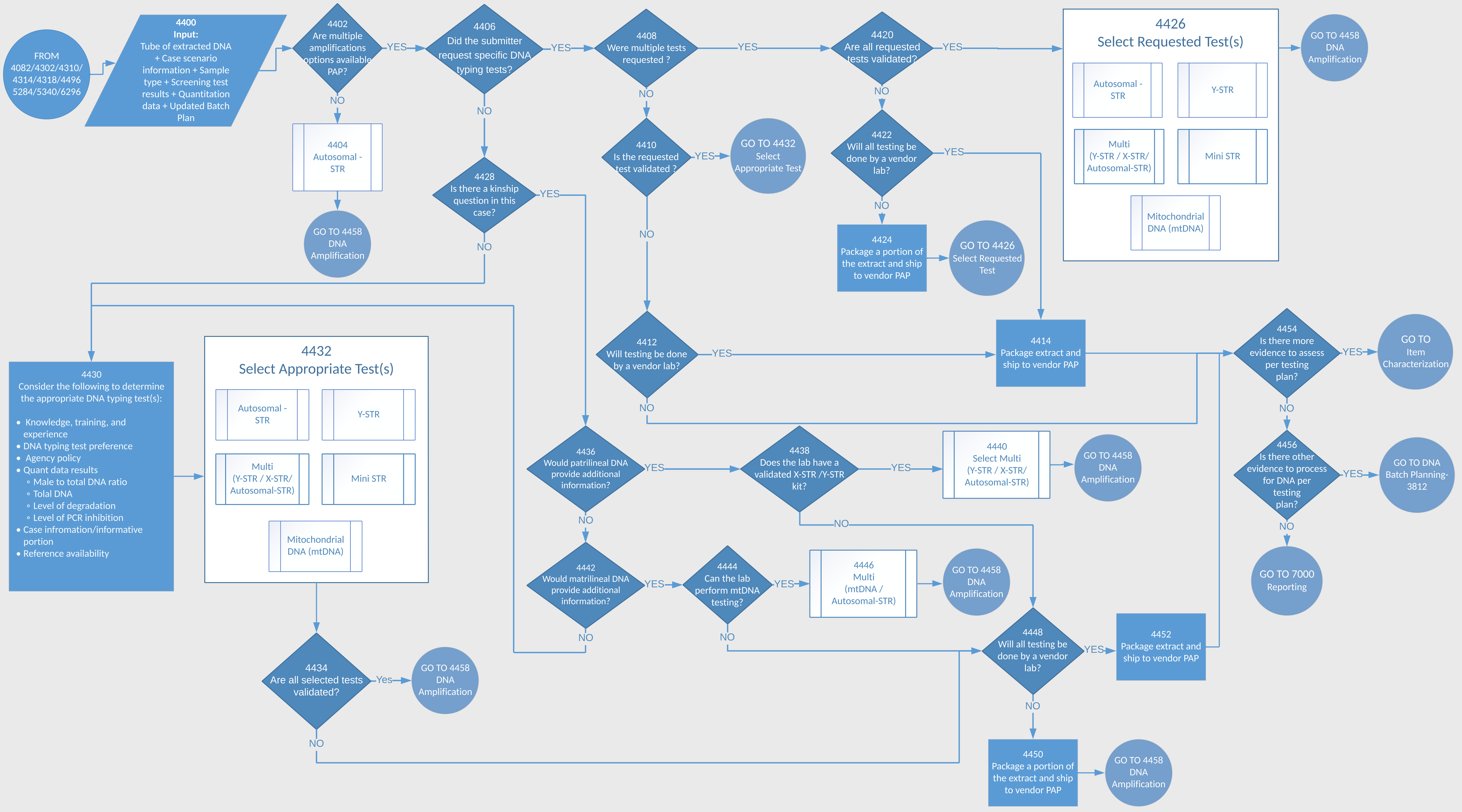
QUANTITATION (3 OF 3)



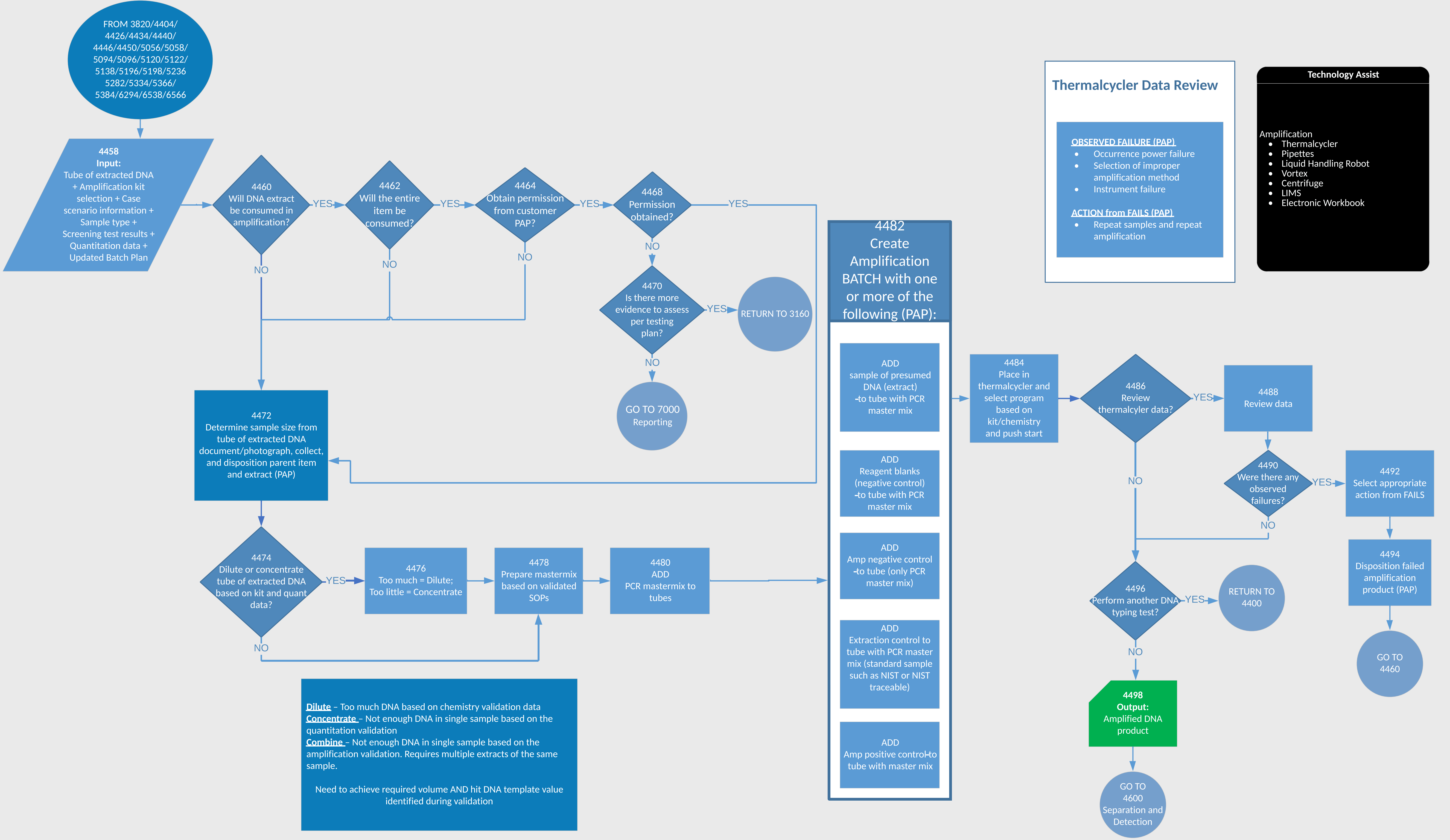
Stop at Quant Criteria

- DNA below validated threshold
- Zero quant value
- Case scenario
- Male to autosomal ratio exceeds validated threshold
- Customer request

4400 - KIT SELECTION



4400 - AMPLIFICATION



FROM 3820/4404/ 4426/4434/4440/ 4446/4450/5056/5058/ 5094/5096/5120/5122/ 5138/5196/5198/5236 5282/5334/5366/ 5384/6294/6538/6566

4458
Input:
Tube of extracted DNA + Amplification kit selection + Case scenario information + Sample type + Screening test results + Quantitation data + Updated Batch Plan

4460 Will DNA extract be consumed in amplification? YES / NO

4462 Will the entire item be consumed? YES / NO

4464 Obtain permission from customer PAP? YES / NO

4468 Permission obtained? YES / NO

4470 Is there more evidence to assess per testing plan? YES / NO

RETURN TO 3160

GO TO 7000 Reporting

4472 Determine sample size from tube of extracted DNA document/photograph, collect, and disposition parent item and extract (PAP)

4474 Dilute or concentrate tube of extracted DNA based on kit and quant data? YES / NO

4476 Too much = Dilute; Too little = Concentrate

4478 Prepare mastermix based on validated SOPs

4480 ADD PCR mastermix to tubes

Dilute – Too much DNA based on chemistry validation data
Concentrate – Not enough DNA in single sample based on the quantitation validation
Combine – Not enough DNA in single sample based on the amplification validation. Requires multiple extracts of the same sample.

Need to achieve required volume AND hit DNA template value identified during validation

4482 Create Amplification BATCH with one or more of the following (PAP):

- ADD sample of presumed DNA (extract) to tube with PCR master mix
- ADD Reagent blanks (negative control) to tube with PCR master mix
- ADD Amp negative control to tube (only PCR master mix)
- ADD Extraction control to tube with PCR master mix (standard sample such as NIST or NIST traceable)
- ADD Amp positive control to tube with master mix

4484 Place in thermalcycler and select program based on kit/chemistry and push start

4486 Review thermalcycler data? YES / NO

4488 Review data

4490 Were there any observed failures? YES / NO

4492 Select appropriate action from FAILS

4494 Disposition failed amplification product (PAP)

GO TO 4460

4496 Perform another DNA typing test? YES / NO

RETURN TO 4400

4498
Output:
Amplified DNA product

GO TO 4600 Separation and Detection

Thermalcycler Data Review

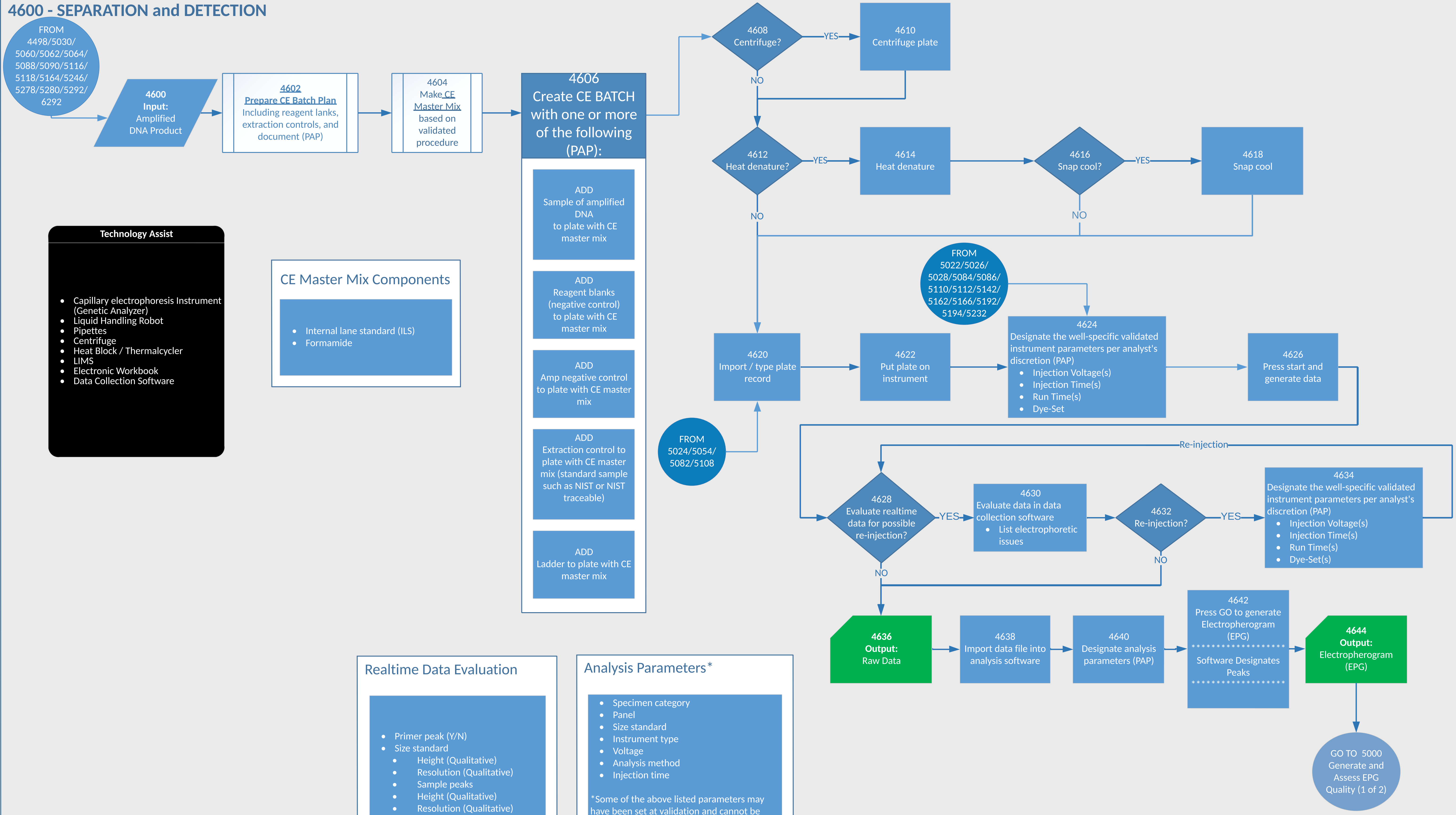
OBSERVED FAILURE (PAP)
- Occurrence power failure
- Selection of improper amplification method
- Instrument failure

ACTION from FAILS (PAP)
- Repeat samples and repeat amplification

Technology Assist

Amplification
- Thermalcycler
- Pipettes
- Liquid Handling Robot
- Vortex
- Centrifuge
- LIMS
- Electronic Workbook

4600 - SEPARATION and DETECTION



FROM 4498/5030/5060/5062/5064/5088/5090/5116/5118/5164/5246/5278/5280/5292/6292

4600
Input:
Amplified DNA Product

4602
Prepare CE Batch Plan
Including reagent lanks, extraction controls, and document (PAP)

4604
Make CE Master Mix based on validated procedure

4606
Create CE BATCH with one or more of the following (PAP):

- ADD Sample of amplified DNA to plate with CE master mix
- ADD Reagent blanks (negative control) to plate with CE master mix
- ADD Amp negative control to plate with CE master mix
- ADD Extraction control to plate with CE master mix (standard sample such as NIST or NIST traceable)
- ADD Ladder to plate with CE master mix

4608
Centrifuge? YES → 4610 Centrifuge plate; NO

4612
Heat denature? YES → 4614 Heat denature; NO

4616
Snap cool? YES → 4618 Snap cool; NO

FROM 5024/5054/5082/5108

4620
Import / type plate record

4622
Put plate on instrument

FROM 5022/5026/5028/5084/5086/5110/5112/5142/5162/5166/5192/5194/5232

4624
Designate the well-specific validated instrument parameters per analyst's discretion (PAP)
- Injection Voltage(s)
- Injection Time(s)
- Run Time(s)
- Dye-Set

4626
Press start and generate data

4628
Evaluate realtime data for possible re-injection? YES; NO

4630
Evaluate data in data collection software
- List electrophoretic issues

4632
Re-injection? YES; NO

4634
Designate the well-specific validated instrument parameters per analyst's discretion (PAP)
- Injection Voltage(s)
- Injection Time(s)
- Run Time(s)
- Dye-Set(s)

Re-injection

4636
Output:
Raw Data

4638
Import data file into analysis software

4640
Designate analysis parameters (PAP)

4642
Press GO to generate Electropherogram (EPG)
****
Software Designates Peaks
****

4644
Output:
Electropherogram (EPG)

GO TO 5000
Generate and Assess EPG Quality (1 of 2)

Technology Assist

- Capillary electrophoresis Instrument (Genetic Analyzer)
- Liquid Handling Robot
- Pipettes
- Centrifuge
- Heat Block / Thermalcycler
- LIMS
- Electronic Workbook
- Data Collection Software

CE Master Mix Components

- Internal lane standard (ILS)
- Formamide

Realtime Data Evaluation

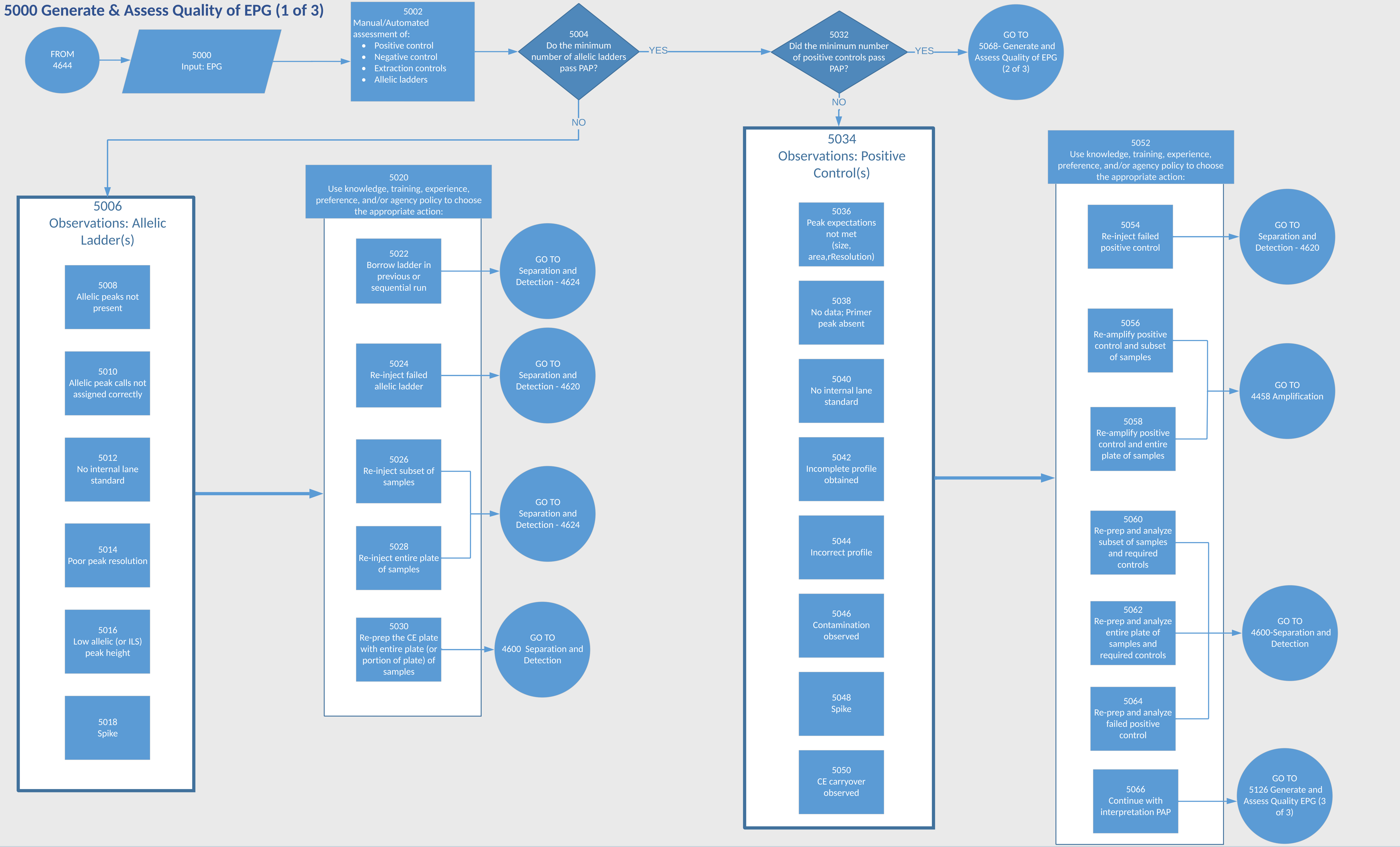
- Primer peak (Y/N)
- Size standard
  - Height (Qualitative)
  - Resolution (Qualitative)
  - Sample peaks
  - Height (Qualitative)
  - Resolution (Qualitative)

Analysis Parameters*

- Specimen category
- Panel
- Size standard
- Instrument type
- Voltage
- Analysis method
- Injection time

*Some of the above listed parameters may have been set at validation and cannot be varied PAP

5000 Generate & Assess Quality of EPG (1 of 3)



FROM 4644

5000
Input: EPG

5002
Manual/Automated assessment of:
- Positive control
- Negative control
- Extraction controls
- Allelic ladders

5004
Do the minimum number of allelic ladders pass PAP?

YES →

5032
Did the minimum number of positive controls pass PAP?

YES → GO TO 5068- Generate and Assess Quality of EPG (2 of 3)

5004 NO →

5006
Observations: Allelic Ladder(s)

5008
Allelic peaks not present

5010
Allelic peak calls not assigned correctly

5012
No internal lane standard

5014
Poor peak resolution

5016
Low allelic (or ILS) peak height

5018
Spike

5020
Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action:

5022
Borrow ladder in previous or sequential run → GO TO Separation and Detection - 4624

5024
Re-inject failed allelic ladder → GO TO Separation and Detection - 4620

5026
Re-inject subset of samples

5028
Re-inject entire plate of samples

5026, 5028 → GO TO Separation and Detection - 4624

5030
Re-prep the CE plate with entire plate (or portion of plate) of samples → GO TO 4600 Separation and Detection

5032 NO →

5034
Observations: Positive Control(s)

5036
Peak expectations not met (size, area,rResolution)

5038
No data; Primer peak absent

5040
No internal lane standard

5042
Incomplete profile obtained

5044
Incorrect profile

5046
Contamination observed

5048
Spike

5050
CE carryover observed

5052
Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action:

5054
Re-inject failed positive control → GO TO Separation and Detection - 4620

5056
Re-amplify positive control and subset of samples

5058
Re-amplify positive control and entire plate of samples

5056, 5058 → GO TO 4458 Amplification

5060
Re-prep and analyze subset of samples and required controls

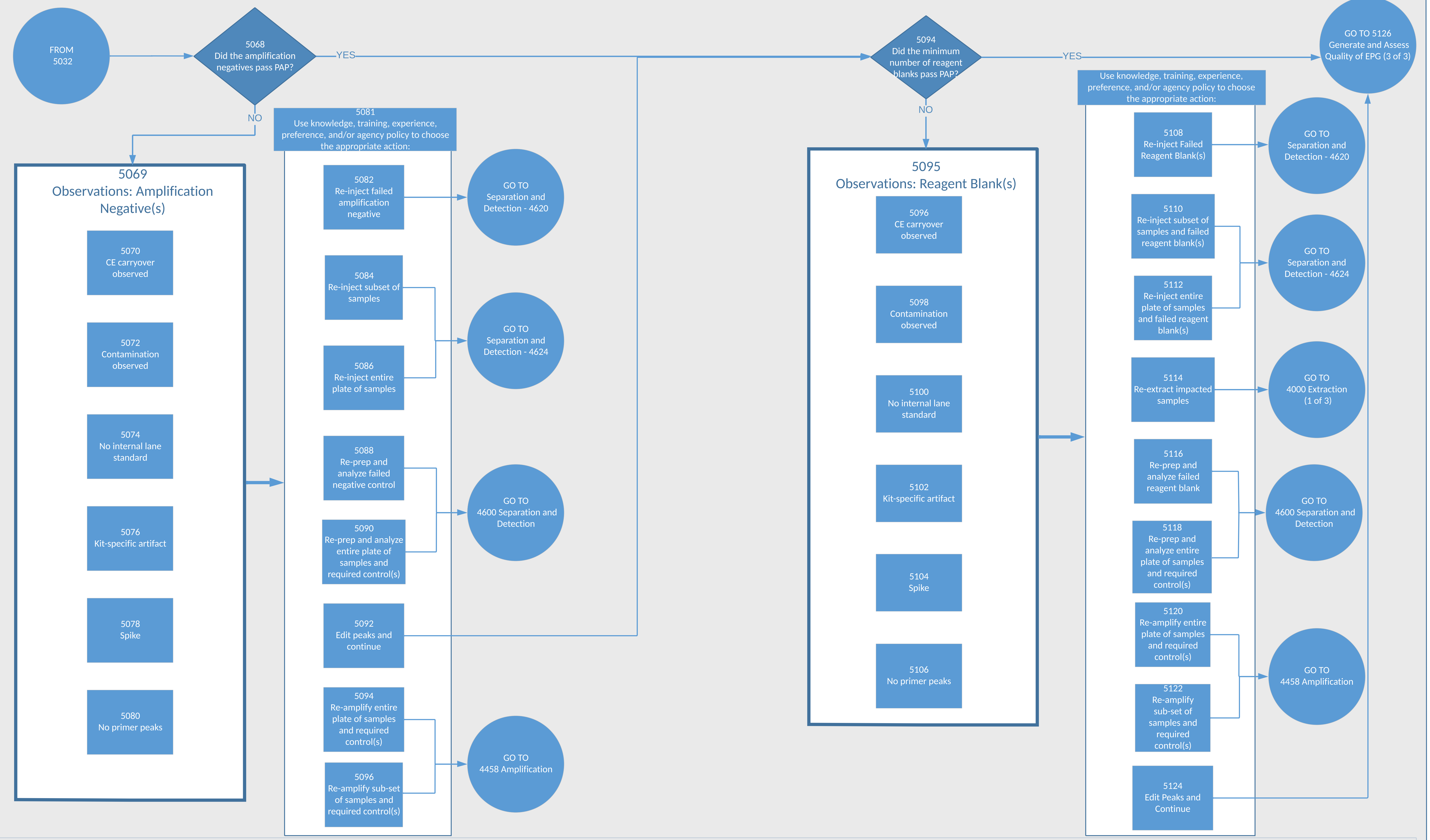
5062
Re-prep and analyze entire plate of samples and required controls

5064
Re-prep and analyze failed positive control

5060, 5062, 5064 → GO TO 4600-Separation and Detection

5066
Continue with interpretation PAP → GO TO 5126 Generate and Assess Quality EPG (3 of 3)

5000 - Generate & Assess Quality of EPG (2 of 3)



FROM 5032

5068 Did the amplification negatives pass PAP?

YES

NO

5069 Observations: Amplification Negative(s)

5070 CE carryover observed

5072 Contamination observed

5074 No internal lane standard

5076 Kit-specific artifact

5078 Spike

5080 No primer peaks

5081 Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action:

5082 Re-inject failed amplification negative

GO TO Separation and Detection - 4620

5084 Re-inject subset of samples

5086 Re-inject entire plate of samples

GO TO Separation and Detection - 4624

5088 Re-prep and analyze failed negative control

5090 Re-prep and analyze entire plate of samples and required control(s)

GO TO 4600 Separation and Detection

5092 Edit peaks and continue

5094 Re-amplify entire plate of samples and required control(s)

5096 Re-amplify sub-set of samples and required control(s)

GO TO 4458 Amplification

5094 Did the minimum number of reagent blanks pass PAP?

YES

NO

5095 Observations: Reagent Blank(s)

5096 CE carryover observed

5098 Contamination observed

5100 No internal lane standard

5102 Kit-specific artifact

5104 Spike

5106 No primer peaks

Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action:

5108 Re-inject Failed Reagent Blank(s)

GO TO Separation and Detection - 4620

5110 Re-inject subset of samples and failed reagent blank(s)

5112 Re-inject entire plate of samples and failed reagent blank(s)

GO TO Separation and Detection - 4624

5114 Re-extract impacted samples

GO TO 4000 Extraction (1 of 3)

5116 Re-prep and analyze failed reagent blank

5118 Re-prep and analyze entire plate of samples and required control(s)

GO TO 4600 Separation and Detection

5120 Re-amplify entire plate of samples and required control(s)

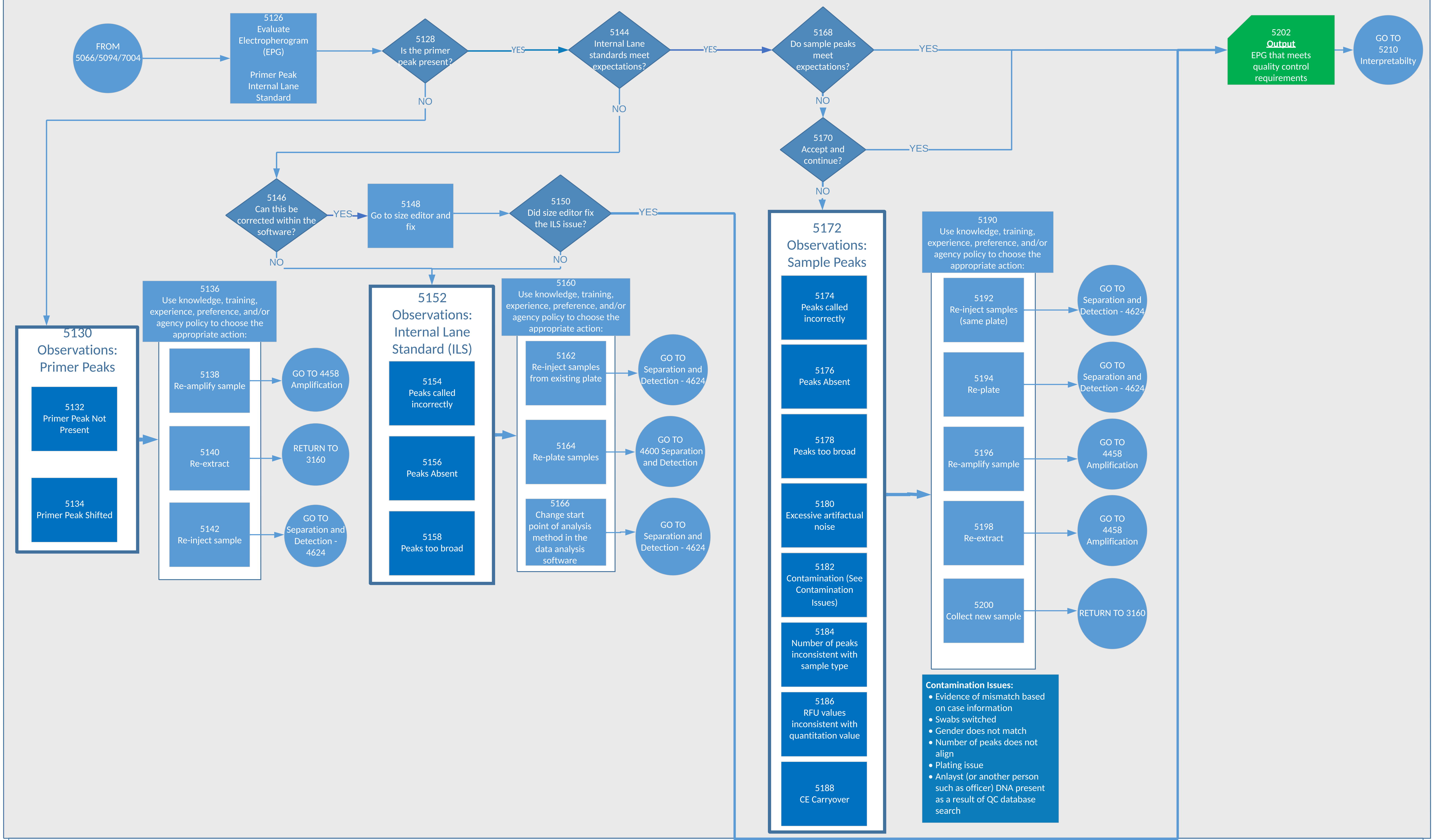
5122 Re-amplify sub-set of samples and required control(s)

GO TO 4458 Amplification

5124 Edit Peaks and Continue

GO TO 5126 Generate and Assess Quality of EPG (3 of 3)

5000 - Generate & Assess Quality of EPG (3 of 3)



FROM 5066/5094/7004

5126
Evaluate Electropherogram (EPG)
Primer Peak Internal Lane Standard

5128
Is the primer peak present?

5144
Internal Lane standards meet expectations?

5168
Do sample peaks meet expectations?

5170
Accept and continue?

5202
Output
EPG that meets quality control requirements

GO TO 5210 Interpretabilty

5146
Can this be corrected within the software?

5148
Go to size editor and fix

5150
Did size editor fix the ILS issue?

5130
Observations: Primer Peaks

5132
Primer Peak Not Present

5134
Primer Peak Shifted

5136
Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action:

5138
Re-amplify sample
GO TO 4458 Amplification

5140
Re-extract
RETURN TO 3160

5142
Re-inject sample
GO TO Separation and Detection - 4624

5152
Observations: Internal Lane Standard (ILS)

5154
Peaks called incorrectly

5156
Peaks Absent

5158
Peaks too broad

5160
Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action:

5162
Re-inject samples from existing plate
GO TO Separation and Detection - 4624

5164
Re-plate samples
GO TO 4600 Separation and Detection

5166
Change start point of analysis method in the data analysis software
GO TO Separation and Detection - 4624

5172
Observations: Sample Peaks

5174
Peaks called incorrectly

5176
Peaks Absent

5178
Peaks too broad

5180
Excessive artifactual noise

5182
Contamination (See Contamination Issues)

5184
Number of peaks inconsistent with sample type

5186
RFU values inconsistent with quantitation value

5188
CE Carryover

5190
Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action:

5192
Re-inject samples (same plate)
GO TO Separation and Detection - 4624

5194
Re-plate
GO TO Separation and Detection - 4624

5196
Re-amplify sample
GO TO 4458 Amplification

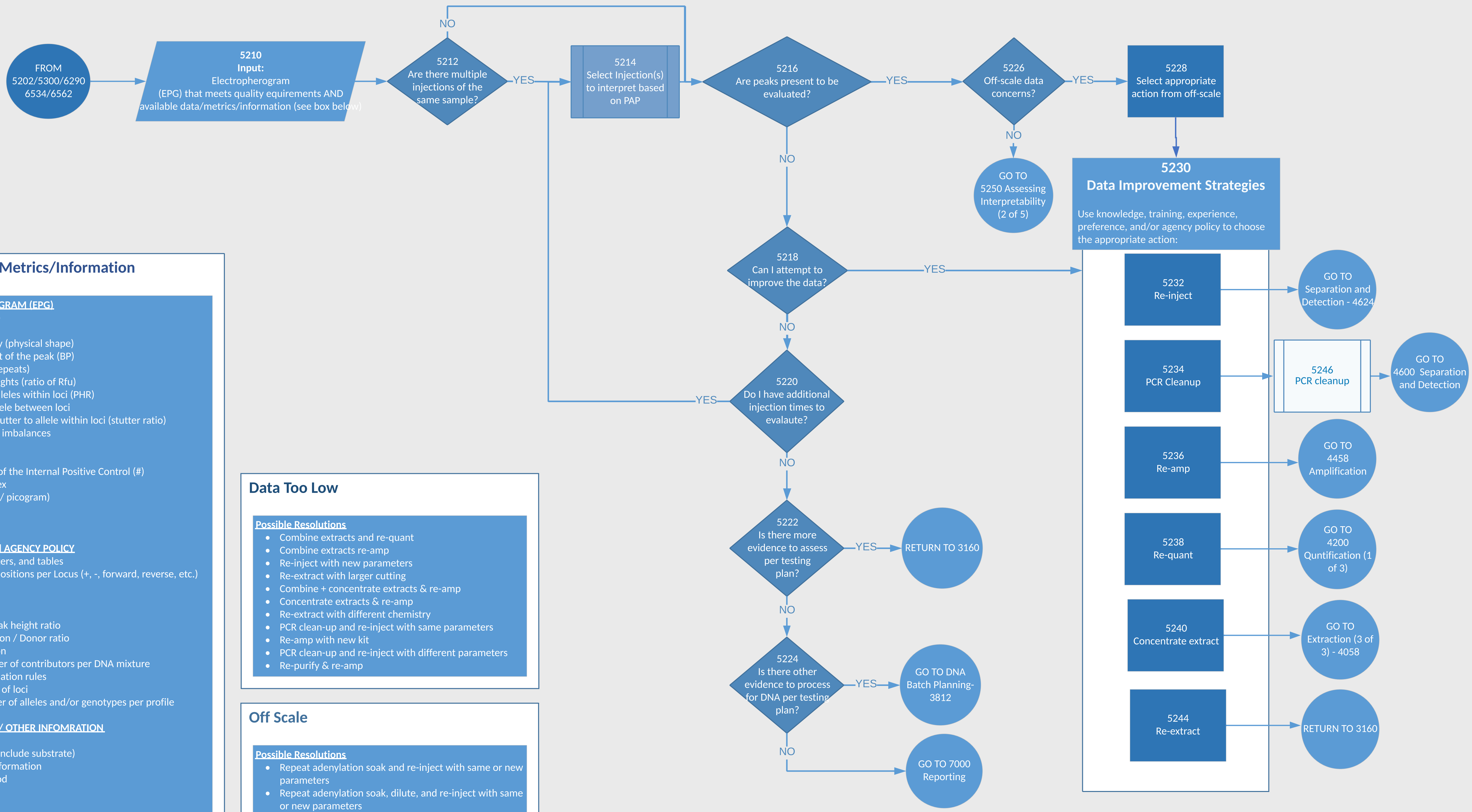
5198
Re-extract
GO TO 4458 Amplification

5200
Collect new sample
RETURN TO 3160

Contamination Issues:
- Evidence of mismatch based on case information
- Swabs switched
- Gender does not match
- Number of peaks does not align
- Plating issue
- Anlayst (or another person such as officer) DNA present as a result of QC database search

5000 - Assessing Interpretability (Suitability) (1 of 5): Data Reliability and Allele Designation

Return to Overview



FROM 5202/5300/6290 6534/6562

5210 Input: Electropherogram (EPG) that meets quality equirements AND available data/metrics/information (see box below)

5212 Are there multiple injections of the same sample? — YES → 5214; NO → 5216

5214 Select Injection(s) to interpret based on PAP

5216 Are peaks present to be evaluated? — YES → 5226; NO → 5218

5226 Off-scale data concerns? — YES → 5228; NO → GO TO 5250 Assessing Interpretability (2 of 5)

5228 Select appropriate action from off-scale

5218 Can I attempt to improve the data? — YES → 5230; NO → 5220

5220 Do I have additional injection times to evalaute? — YES → 5214; NO → 5222

5222 Is there more evidence to assess per testing plan? — YES → RETURN TO 3160; NO → 5224

5224 Is there other evidence to process for DNA per testing plan? — YES → GO TO DNA Batch Planning-3812; NO → GO TO 7000 Reporting

5230 Data Improvement Strategies

Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action:

- 5232 Re-inject → GO TO Separation and Detection - 4624
- 5234 PCR Cleanup → 5246 PCR cleanup → GO TO 4600 Separation and Detection
- 5236 Re-amp → GO TO 4458 Amplification
- 5238 Re-quant → GO TO 4200 Quntification (1 of 3)
- 5240 Concentrate extract → GO TO Extraction (3 of 3) - 4058
- 5244 Re-extract → RETURN TO 3160

Available Data/Metrics/Information

Data ELECTROPHEROGRAM (EPG)
- Peak height (Rfu)
- Peak count (#)
- Peak morphology (physical shape)
- Molecular weight of the peak (BP)
- Allele size (# of repeats)
- Relative peak heights (ratio of Rfu)
    - Alleles to alleles within loci (PHR)
    - Allele to allele between loci
    - Potential stutter to allele within loci (stutter ratio)
    - Other peak imbalances

QUANT DATA
- Cycle Threshold of the Internal Positive Control (#)
- Degradation Index
- Yield (nanogram / picogram)
- Male to total
- Curves

VALIDATION DATA and AGENCY POLICY
- Stutter ratios, filters, and tables
- Defined stutter positions per Locus (+, -, forward, reverse, etc.)
- Threshold
    - Stochastic
    - Analytical
- Heterozygote peak height ratio
- Mixture proportion / Donor ratio
- Sex determination
- Maximum number of contributors per DNA mixture
- Minimum information rules
    - Percentage of loci
- Minimum number of alleles and/or genotypes per profile

EVIDENCE / PROCESS / OTHER INFORMATION
- Sample size
- Sample type (to include substrate)
- Case scenario/Information
- Extraction method
- Serology results
- Color of extract
- Published data

Data Too Low

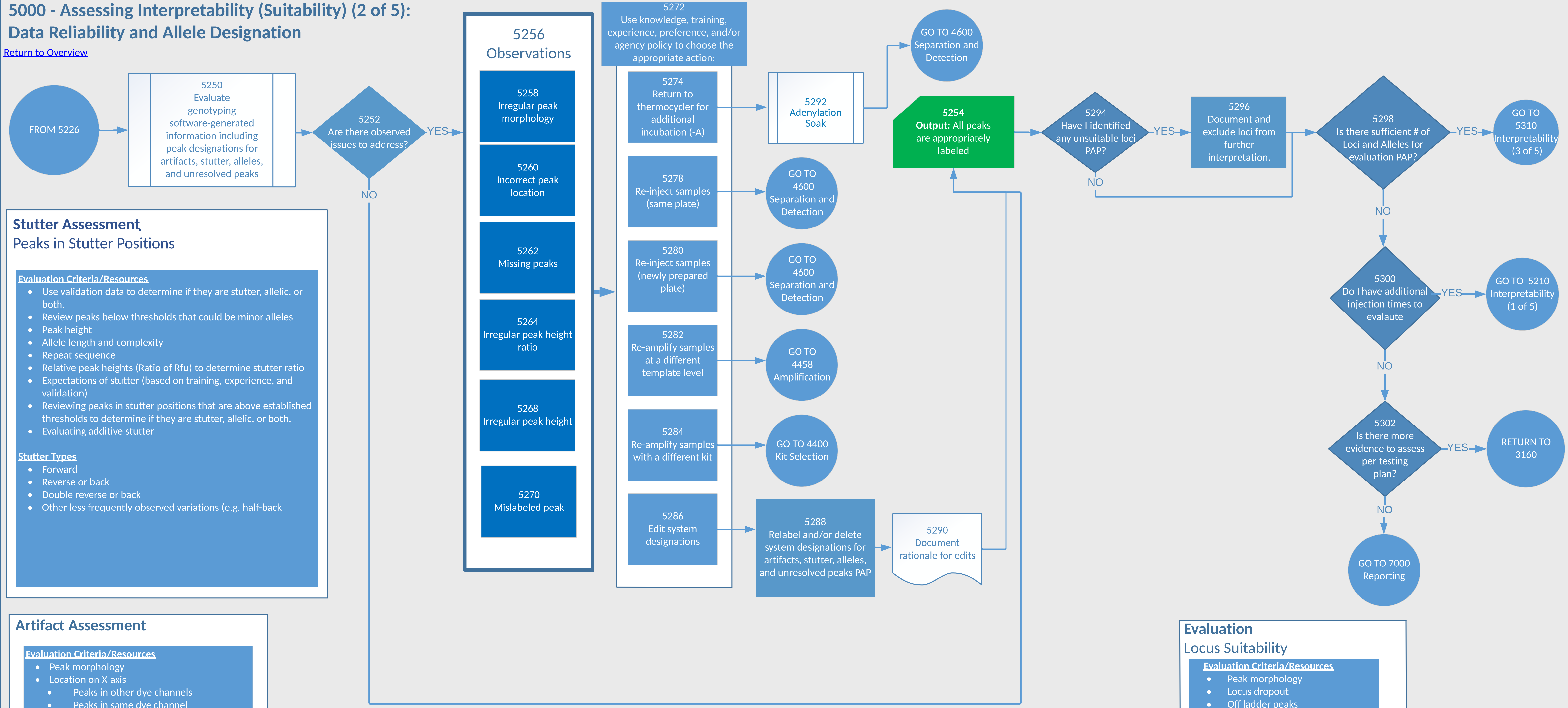
Possible Resolutions
- Combine extracts and re-quant
- Combine extracts re-amp
- Re-inject with new parameters
- Re-extract with larger cutting
- Combine + concentrate extracts & re-amp
- Concentrate extracts & re-amp
- Re-extract with different chemistry
- PCR clean-up and re-inject with same parameters
- Re-amp with new kit
- PCR clean-up and re-inject with different parameters
- Re-purify & re-amp

Off Scale

Possible Resolutions
- Repeat adenylation soak and re-inject with same or new parameters
- Repeat adenylation soak, dilute, and re-inject with same or new parameters
- Re-inject with new parameters
- Dilute PCR product and re-inject with same parameters
- Re-amp extract
- Dilute extract and re-amp
-

5000 - Assessing Interpretability (Suitability) (2 of 5): Data Reliability and Allele Designation

Return to Overview



FROM 5226

5250
Evaluate genotyping software-generated information including peak designations for artifacts, stutter, alleles, and unresolved peaks

5252
Are there observed issues to address?

5256
Observations

5258
Irregular peak morphology

5260
Incorrect peak location

5262
Missing peaks

5264
Irregular peak height ratio

5268
Irregular peak height

5270
Mislabeled peak

5272
Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action:

5274
Return to thermocycler for additional incubation (-A)

5292
Adenylation Soak

GO TO 4600 Separation and Detection

5278
Re-inject samples (same plate)

GO TO 4600 Separation and Detection

5280
Re-inject samples (newly prepared plate)

GO TO 4600 Separation and Detection

5282
Re-amplify samples at a different template level

GO TO 4458 Amplification

5284
Re-amplify samples with a different kit

GO TO 4400 Kit Selection

5286
Edit system designations

5288
Relabel and/or delete system designations for artifacts, stutter, alleles, and unresolved peaks PAP

5290
Document rationale for edits

5254
Output: All peaks are appropriately labeled

5294
Have I identified any unsuitable loci PAP?

5296
Document and exclude loci from further interpretation.

5298
Is there sufficient # of Loci and Alleles for evaluation PAP?

GO TO 5310 Interpretability (3 of 5)

5300
Do I have additional injection times to evalaute

GO TO 5210 Interpretability (1 of 5)

5302
Is there more evidence to assess per testing plan?

RETURN TO 3160

GO TO 7000 Reporting

Stutter Assessment

Peaks in Stutter Positions

Evaluation Criteria/Resources
- Use validation data to determine if they are stutter, allelic, or both.
- Review peaks below thresholds that could be minor alleles
- Peak height
- Allele length and complexity
- Repeat sequence
- Relative peak heights (Ratio of Rfu) to determine stutter ratio
- Expectations of stutter (based on training, experience, and validation)
- Reviewing peaks in stutter positions that are above established thresholds to determine if they are stutter, allelic, or both.
- Evaluating additive stutter

Stutter Types
- Forward
- Reverse or back
- Double reverse or back
- Other less frequently observed variations (e.g. half-back

Artifact Assessment

Evaluation Criteria/Resources
- Peak morphology
- Location on X-axis
  - Peaks in other dye channels
  - Peaks in same dye channel
- Frequency of observed artifacts public datasets (star base), manufacturer technical notes

Artifact Types
- Stutter (e.g. -1 repeat, +1 repeat)
- Shoulder (e.g. -A)
- Dye blobs
- Spike
- Pull up
- Pull down (Raised baseline)
- Non-human peaks
- Non specific amplification peak

Unresolved/Indistinguishable Peak Determination

Evaluation Criteria/Resources
- Peak morphology
- Peak label

Evaluation

Locus Suitability

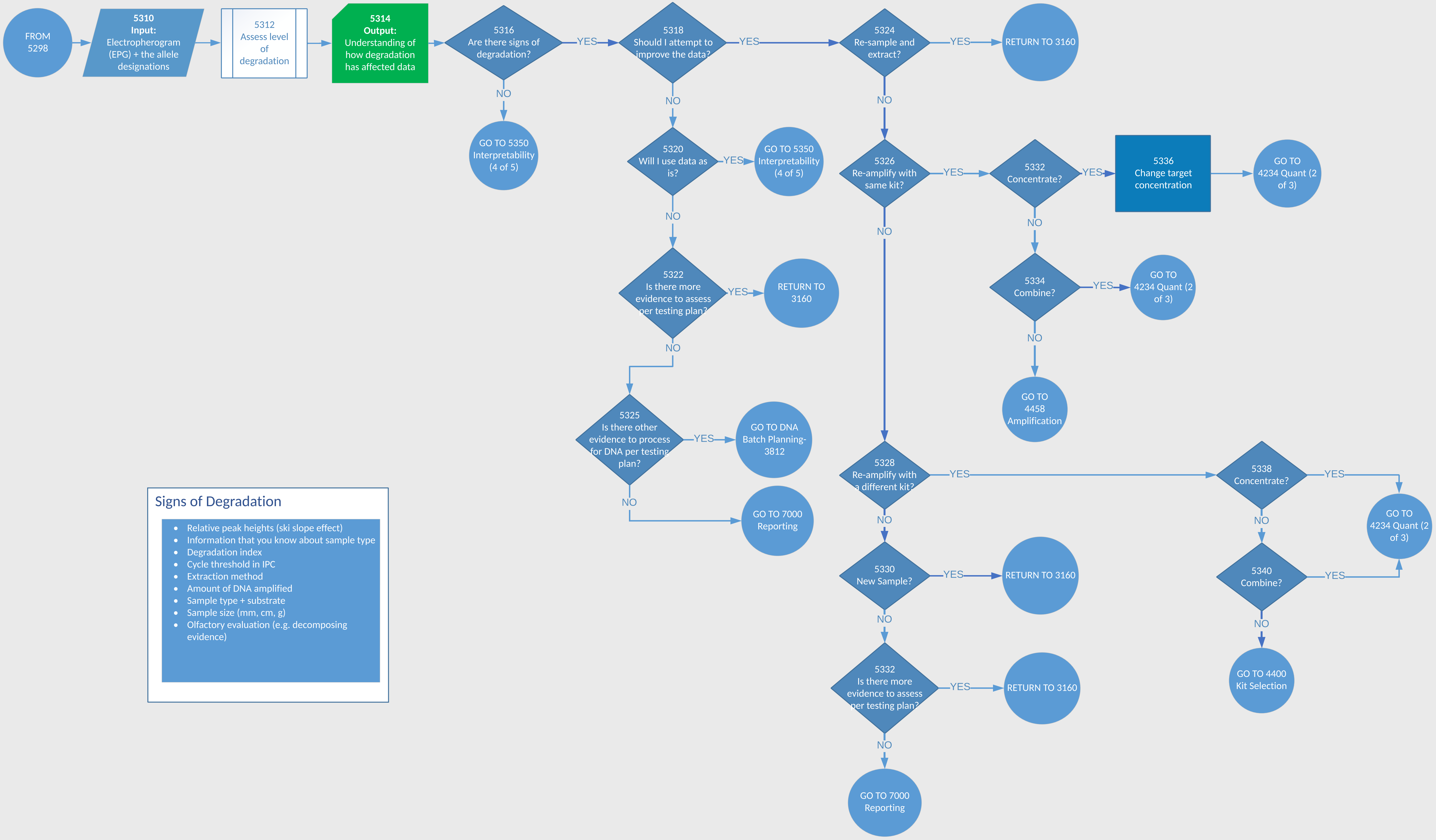
Evaluation Criteria/Resources
- Peak morphology
- Locus dropout
- Off ladder peaks

Expectations

Quality and Quantity

Inputs for Expectations
- Case scenario
- Sample type
- Data gathered
  - Amplification
  - Quantitation
  - Screening test results

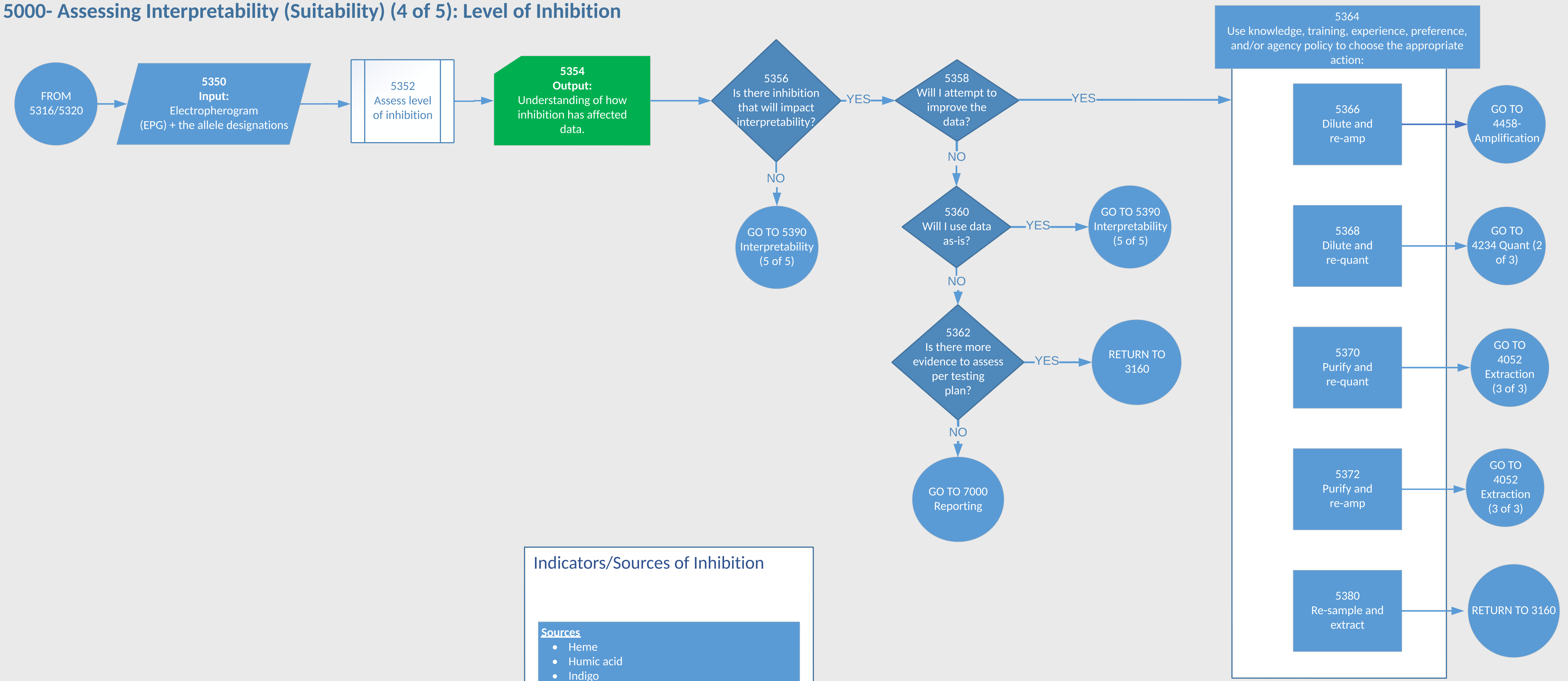
5000 - Assessing Interpretability (Suitability) (3 of 5): Level of Degradation



Signs of Degradation

- Relative peak heights (ski slope effect)
- Information that you know about sample type
- Degradation index
- Cycle threshold in IPC
- Extraction method
- Amount of DNA amplified
- Sample type + substrate
- Sample size (mm, cm, g)
- Olfactory evaluation (e.g. decomposing evidence)

5000- Assessing Interpretability (Suitability) (4 of 5): Level of Inhibition



FROM 5316/5320

5350
Input:
Electropherogram (EPG) + the allele designations

5352
Assess level of inhibition

5354
Output:
Understanding of how inhibition has affected data.

5356
Is there inhibition that will impact interpretability?

NO → GO TO 5390 Interpretability (5 of 5)

YES →

5358
Will I attempt to improve the data?

YES →

5364
Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action:

5366
Dilute and re-amp → GO TO 4458- Amplification

5368
Dilute and re-quant → GO TO 4234 Quant (2 of 3)

5370
Purify and re-quant → GO TO 4052 Extraction (3 of 3)

5372
Purify and re-amp → GO TO 4052 Extraction (3 of 3)

5380
Re-sample and extract → RETURN TO 3160

NO →

5360
Will I use data as-is?

YES → GO TO 5390 Interpretability (5 of 5)

NO →

5362
Is there more evidence to assess per testing plan?

YES → RETURN TO 3160

NO → GO TO 7000 Reporting

Indicators/Sources of Inhibition

Sources

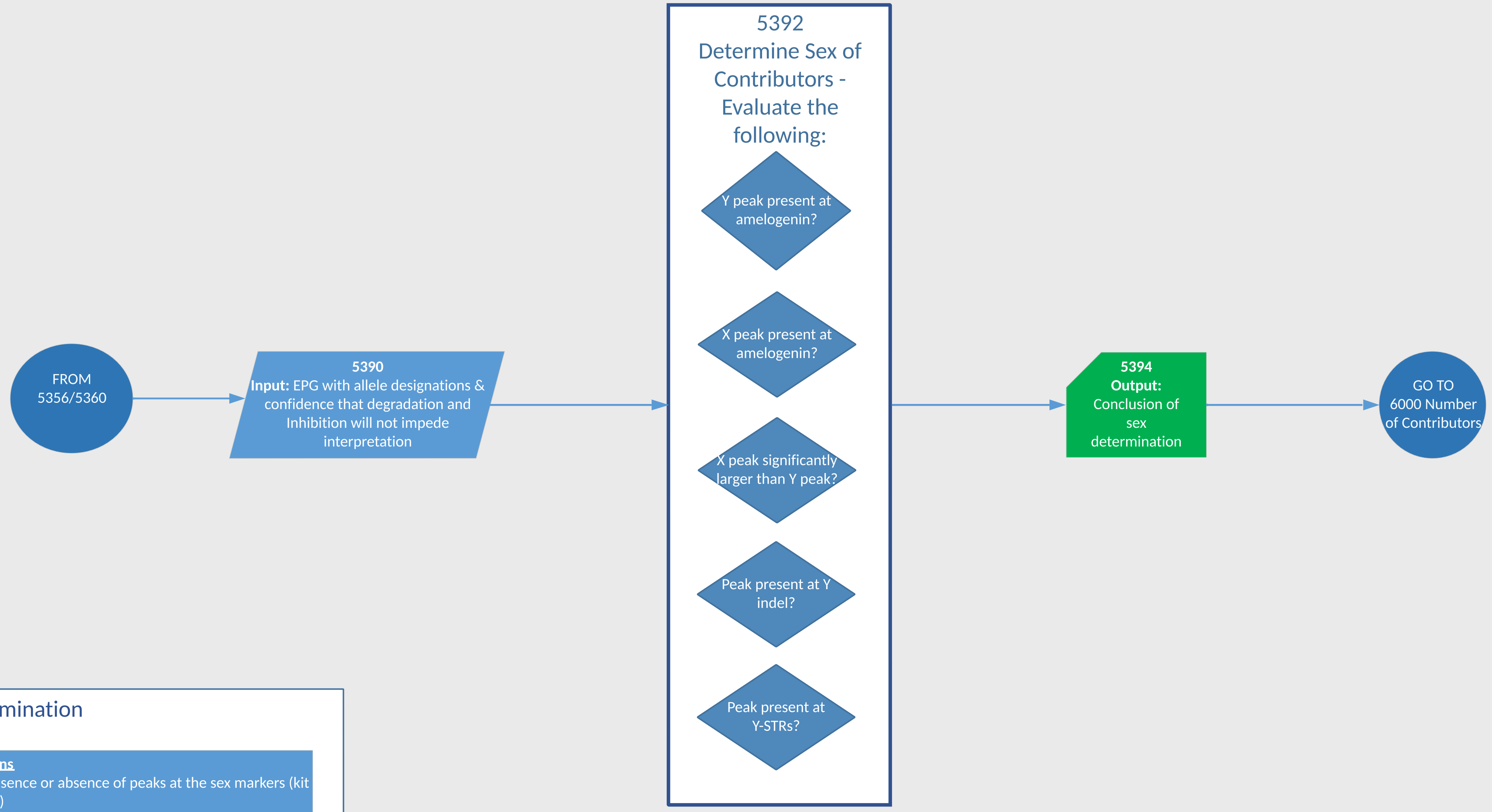
- Heme
- Humic acid
- Indigo
- Phenol
- Metal salts (e.g. ca, mg)
- Tannic acid
- Melanin
- Magnetic latent print techniques

Indicators

- Locus drop out, especially when not based on DNA length
- qPCR data (e.g.Cycle threshold in IPC)
- Information that you know about sample type + substrate
- Extraction method
- Olfactory evaluation
- Color of extract
- Amount of DNA amplified
- Sample size (mm, cm, g)

Assessing Interpretability (Suitability) (5 of 5): Sex of Contributors

Return to Overview



FROM 5356/5360

5390
Input: EPG with allele designations & confidence that degradation and Inhibition will not impede interpretation

5392
Determine Sex of Contributors - Evaluate the following:

Y peak present at amelogenin?

X peak present at amelogenin?

X peak significantly larger than Y peak?

Peak present at Y indel?

Peak present at Y-STRs?

5394
Output:
Conclusion of sex determination

GO TO 6000 Number of Contributors

Sex Determination

Considerations

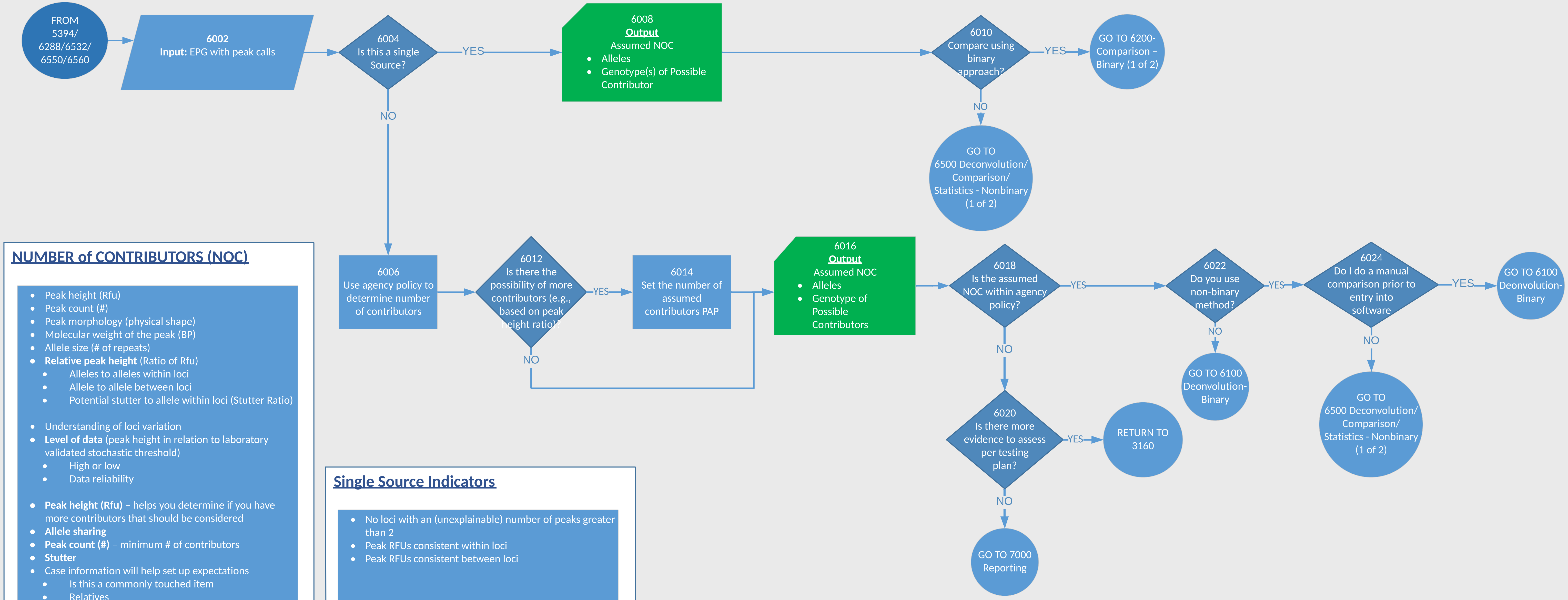
- The presence or absence of peaks at the sex markers (kit specific)
    - Amel
    - Y-indel
    - Y-STRs
- Peak height ratio of Amel will indicate number and sex of contributors
- Male to total DNA ratio from quant data
- Consider degradation or inhibition as an explication for missing sex markers
- Consider degradation or inhibition for explanation of unexpected results
- Expectations set based on sample type, case information, and SOP
- Consider number of contributors and expected/assumed contributors
- Determine if any imbalance is related to the amount of DNA, mutations, or mixture.

Conclusions

- Male DNA
- Male DNA that limits ability to determine if female is present
- Male/Female DNA mixture
- Female DNA
- No Conclusion

6000 - Number of Contributors

Return to Overview



FROM 5394/ 6288/6532/ 6550/6560

6002
Input: EPG with peak calls

6004
Is this a single Source?

YES

6008
Output
Assumed NOC
- Alleles
- Genotype(s) of Possible Contributor

6010
Compare using binary approach?

YES

GO TO 6200- Comparison – Binary (1 of 2)

NO

GO TO 6500 Deconvolution/ Comparison/ Statistics - Nonbinary (1 of 2)

NO

6006
Use agency policy to determine number of contributors

6012
Is there the possibility of more contributors (e.g., based on peak height ratio)?

YES

NO

6014
Set the number of assumed contributors PAP

6016
Output
Assumed NOC
- Alleles
- Genotype of Possible Contributors

6018
Is the assumed NOC within agency policy?

YES

6022
Do you use non-binary method?

YES

6024
Do I do a manual comparison prior to entry into software

YES

GO TO 6100 Deonvolution-Binary

NO

GO TO 6500 Deconvolution/ Comparison/ Statistics - Nonbinary (1 of 2)

NO

GO TO 6100 Deonvolution-Binary

NO

6020
Is there more evidence to assess per testing plan?

YES

RETURN TO 3160

NO

GO TO 7000 Reporting

NUMBER of CONTRIBUTORS (NOC)

- Peak height (Rfu)
- Peak count (#)
- Peak morphology (physical shape)
- Molecular weight of the peak (BP)
- Allele size (# of repeats)
- **Relative peak height** (Ratio of Rfu)
    - Alleles to alleles within loci
    - Allele to allele between loci
    - Potential stutter to allele within loci (Stutter Ratio)

- Understanding of loci variation
- **Level of data** (peak height in relation to laboratory validated stochastic threshold)
    - High or low
    - Data reliability

- **Peak height (Rfu)** – helps you determine if you have more contributors that should be considered
- **Allele sharing**
- **Peak count (#)** – minimum # of contributors
- **Stutter**
- Case information will help set up expectations
    - Is this a commonly touched item
    - Relatives
    - Sexual assault
        - # of reported assailants
        - # of consensual partners
        - relatives
- Expected/Assumed contributor profiles (quick comparison to see if this helps determine # of contributors
- Consider carryover as an explanation for what you see
- Assumed # of contributors (Prob Gen)
- Minimum # of contributors (CPI/CPE)
- Look at peaks below detection level to determine if there are indication of additional contributors

Administrative Information
- Sample type
- Case type
- Potential Relatives in the mixture

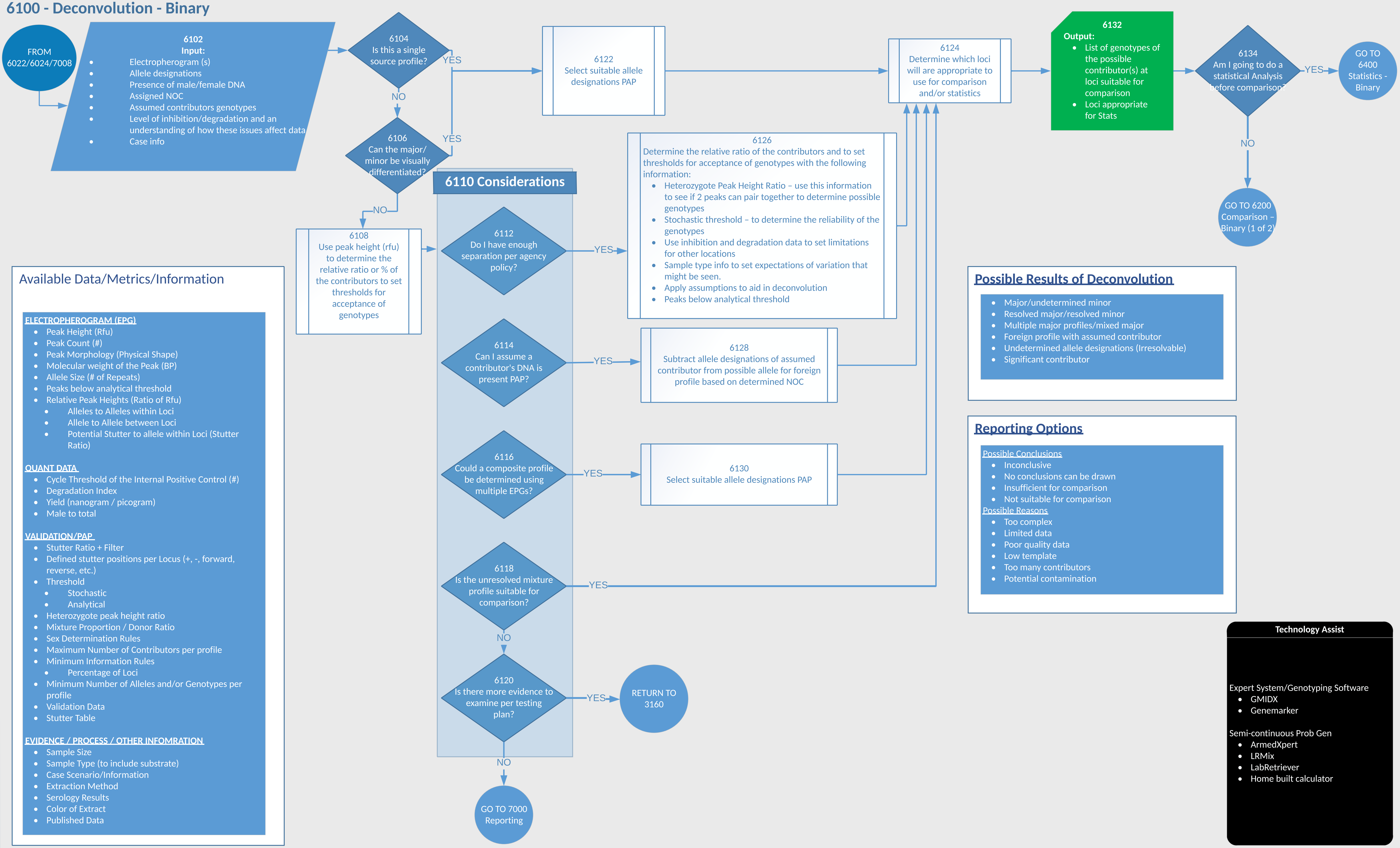
Single Source Indicators

- No loci with an (unexplainable) number of peaks greater than 2
- Peak RFUs consistent within loci
- Peak RFUs consistent between loci

Methods for Determining NOC

- Total number of peaks divided by 2
- Accounting for/Subtracting out assumed contributor
- Use information from both fractions of a differentially extracted sample
- Peak height ratios
- Peak heights
- Use a software tool to assist (e.g., PACE and NOCIt)

6100 - Deconvolution - Binary



FROM 6022/6024/7008

6102
Input:
- Electropherogram (s)
- Allele designations
- Presence of male/female DNA
- Assigned NOC
- Assumed contributors genotypes
- Level of inhibition/degradation and an understanding of how these issues affect data
- Case info

6104
Is this a single source profile?

6106
Can the major/ minor be visually differentiated?

6108
Use peak height (rfu) to determine the relative ratio or % of the contributors to set thresholds for acceptance of genotypes

6122
Select suitable allele designations PAP

6124
Determine which loci will are appropriate to use for comparison and/or statistics

6132
Output:
- List of genotypes of the possible contributor(s) at loci suitable for comparison
- Loci appropriate for Stats

6134
Am I going to do a statistical Analysis before comparison?

GO TO 6400 Statistics - Binary

GO TO 6200 Comparison – Binary (1 of 2)

6110 Considerations

6112
Do I have enough separation per agency policy?

6114
Can I assume a contributor's DNA is present PAP?

6116
Could a composite profile be determined using multiple EPGs?

6118
Is the unresolved mixture profile suitable for comparison?

6120
Is there more evidence to examine per testing plan?

RETURN TO 3160

GO TO 7000 Reporting

6126
Determine the relative ratio of the contributors and to set thresholds for acceptance of genotypes with the following information:
- Heterozygote Peak Height Ratio – use this information to see if 2 peaks can pair together to determine possible genotypes
- Stochastic threshold – to determine the reliability of the genotypes
- Use inhibition and degradation data to set limitations for other locations
- Sample type info to set expectations of variation that might be seen.
- Apply assumptions to aid in deconvolution
- Peaks below analytical threshold

6128
Subtract allele designations of assumed contributor from possible allele for foreign profile based on determined NOC

6130
Select suitable allele designations PAP

Available Data/Metrics/Information

ELECTROPHEROGRAM (EPG)
- Peak Height (Rfu)
- Peak Count (#)
- Peak Morphology (Physical Shape)
- Molecular weight of the Peak (BP)
- Allele Size (# of Repeats)
- Peaks below analytical threshold
- Relative Peak Heights (Ratio of Rfu)
    - Alleles to Alleles within Loci
    - Allele to Allele between Loci
    - Potential Stutter to allele within Loci (Stutter Ratio)

QUANT DATA
- Cycle Threshold of the Internal Positive Control (#)
- Degradation Index
- Yield (nanogram / picogram)
- Male to total

VALIDATION/PAP
- Stutter Ratio + Filter
- Defined stutter positions per Locus (+, -, forward, reverse, etc.)
- Threshold
    - Stochastic
    - Analytical
- Heterozygote peak height ratio
- Mixture Proportion / Donor Ratio
- Sex Determination Rules
- Maximum Number of Contributors per profile
- Minimum Information Rules
    - Percentage of Loci
- Minimum Number of Alleles and/or Genotypes per profile
- Validation Data
- Stutter Table

EVIDENCE / PROCESS / OTHER INFOMRATION
- Sample Size
- Sample Type (to include substrate)
- Case Scenario/Information
- Extraction Method
- Serology Results
- Color of Extract
- Published Data

Possible Results of Deconvolution

- Major/undetermined minor
- Resolved major/resolved minor
- Multiple major profiles/mixed major
- Foreign profile with assumed contributor
- Undetermined allele designations (Irresolvable)
- Significant contributor

Reporting Options

Possible Conclusions
- Inconclusive
- No conclusions can be drawn
- Insufficient for comparison
- Not suitable for comparison

Possible Reasons
- Too complex
- Limited data
- Poor quality data
- Low template
- Too many contributors
- Potential contamination

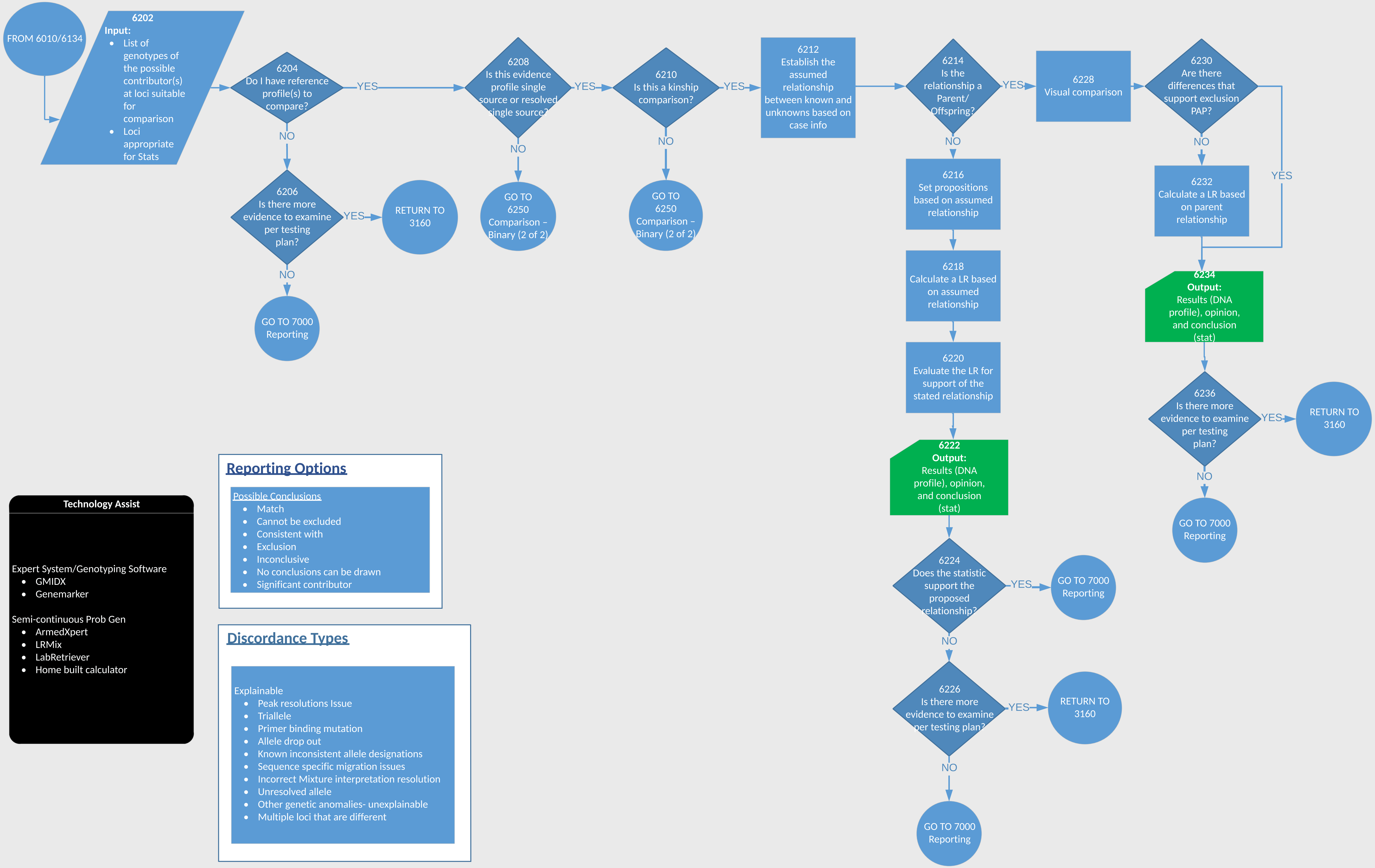
Technology Assist

Expert System/Genotyping Software
- GMIDX
- Genemarker

Semi-continuous Prob Gen
- ArmedXpert
- LRMix
- LabRetriever
- Home built calculator

6200 - Comparison – Binary (1 of 2)

Return to Overview



FROM 6010/6134

6202
Input:
- List of genotypes of the possible contributor(s) at loci suitable for comparison
- Loci appropriate for Stats

6204
Do I have reference profile(s) to compare?
YES → 6208; NO → 6206

6206
Is there more evidence to examine per testing plan?
YES → RETURN TO 3160; NO → GO TO 7000 Reporting

6208
Is this evidence profile single source or resolved single source?
YES → 6210; NO → GO TO 6250 Comparison – Binary (2 of 2)

6210
Is this a kinship comparison?
YES → 6212; NO → GO TO 6250 Comparison – Binary (2 of 2)

6212
Establish the assumed relationship between known and unknowns based on case info

6214
Is the relationship a Parent/ Offspring?
YES → 6228; NO → 6216

6216
Set propositions based on assumed relationship

6218
Calculate a LR based on assumed relationship

6220
Evaluate the LR for support of the stated relationship

6222
Output:
Results (DNA profile), opinion, and conclusion (stat)

6224
Does the statistic support the proposed relationship?
YES → GO TO 7000 Reporting; NO → 6226

6226
Is there more evidence to examine per testing plan?
YES → RETURN TO 3160; NO → GO TO 7000 Reporting

6228
Visual comparison

6230
Are there differences that support exclusion PAP?
NO → 6232; YES → 6234

6232
Calculate a LR based on parent relationship

6234
Output:
Results (DNA profile), opinion, and conclusion (stat)

6236
Is there more evidence to examine per testing plan?
YES → RETURN TO 3160; NO → GO TO 7000 Reporting

Technology Assist

Expert System/Genotyping Software
- GMIDX
- Genemarker

Semi-continuous Prob Gen
- ArmedXpert
- LRMix
- LabRetriever
- Home built calculator

Reporting Options

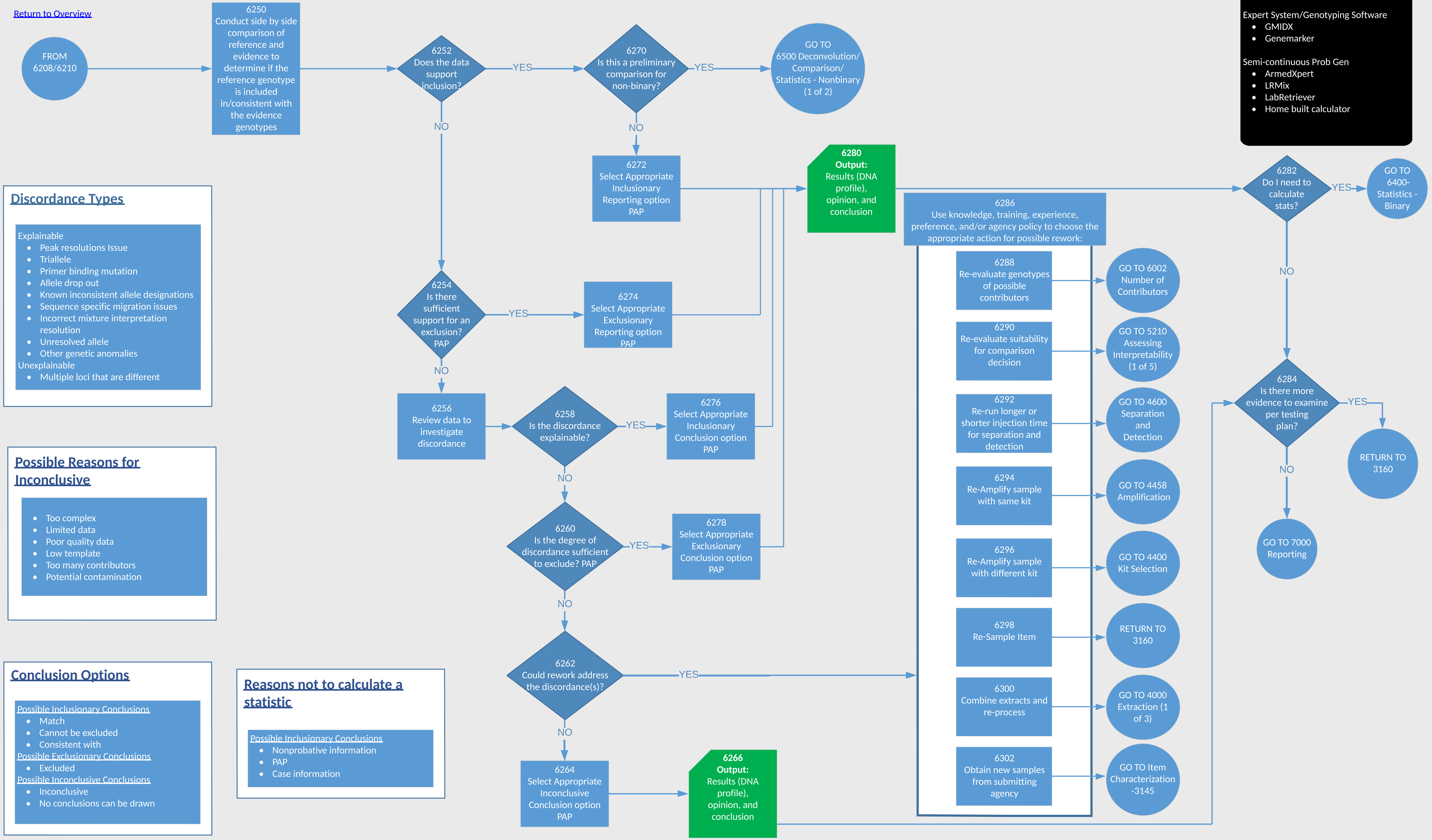
Possible Conclusions
- Match
- Cannot be excluded
- Consistent with
- Exclusion
- Inconclusive
- No conclusions can be drawn
- Significant contributor

Discordance Types

Explainable
- Peak resolutions Issue
- Triallele
- Primer binding mutation
- Allele drop out
- Known inconsistent allele designations
- Sequence specific migration issues
- Incorrect Mixture interpretation resolution
- Unresolved allele
- Other genetic anomalies- unexplainable
- Multiple loci that are different

6200 - Comparison – Binary (2 of 2)

Return to Overview



FROM 6208/6210

6250 Conduct side by side comparison of reference and evidence to determine if the reference genotype is included in/consistent with the evidence genotypes

6252 Does the data support inclusion?
- YES → 6270
- NO → 6254

6270 Is this a preliminary comparison for non-binary?
- YES → GO TO 6500 Deconvolution/ Comparison/ Statistics - Nonbinary (1 of 2)
- NO → 6272

6272 Select Appropriate Inclusionary Reporting option PAP

6254 Is there sufficient support for an exclusion? PAP
- YES → 6274
- NO → 6256

6274 Select Appropriate Exclusionary Reporting option PAP

6256 Review data to investigate discordance

6258 Is the discordance explainable?
- YES → 6276
- NO → 6260

6276 Select Appropriate Inclusionary Conclusion option PAP

6260 Is the degree of discordance sufficient to exclude? PAP
- YES → 6278
- NO → 6262

6278 Select Appropriate Exclusionary Conclusion option PAP

6262 Could rework address the discordance(s)?
- YES → 6286
- NO → 6264

6264 Select Appropriate Inconclusive Conclusion option PAP

6266 Output: Results (DNA profile), opinion, and conclusion

6280 Output: Results (DNA profile), opinion, and conclusion

6282 Do I need to calculate stats?
- YES → GO TO 6400-Statistics - Binary
- NO → 6284

6284 Is there more evidence to examine per testing plan?
- YES → RETURN TO 3160
- NO → GO TO 7000 Reporting

6286 Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action for possible rework:
- 6288 Re-evaluate genotypes of possible contributors → GO TO 6002 Number of Contributors
- 6290 Re-evaluate suitability for comparison decision → GO TO 5210 Assessing Interpretability (1 of 5)
- 6292 Re-run longer or shorter injection time for separation and detection → GO TO 4600 Separation and Detection
- 6294 Re-Amplify sample with same kit → GO TO 4458 Amplification
- 6296 Re-Amplify sample with different kit → GO TO 4400 Kit Selection
- 6298 Re-Sample Item → RETURN TO 3160
- 6300 Combine extracts and re-process → GO TO 4000 Extraction (1 of 3)
- 6302 Obtain new samples from submitting agency → GO TO Item Characterization -3145

Technology Assist

Expert System/Genotyping Software
- GMIDX
- Genemarker

Semi-continuous Prob Gen
- ArmedXpert
- LRMix
- LabRetriever
- Home built calculator

Discordance Types

Explainable
- Peak resolutions Issue
- Triallele
- Primer binding mutation
- Allele drop out
- Known inconsistent allele designations
- Sequence specific migration issues
- Incorrect mixture interpretation resolution
- Unresolved allele
- Other genetic anomalies

Unexplainable
- Multiple loci that are different

Possible Reasons for Inconclusive

- Too complex
- Limited data
- Poor quality data
- Low template
- Too many contributors
- Potential contamination

Conclusion Options

Possible Inclusionary Conclusions
- Match
- Cannot be excluded
- Consistent with

Possible Exclusionary Conclusions
- Excluded

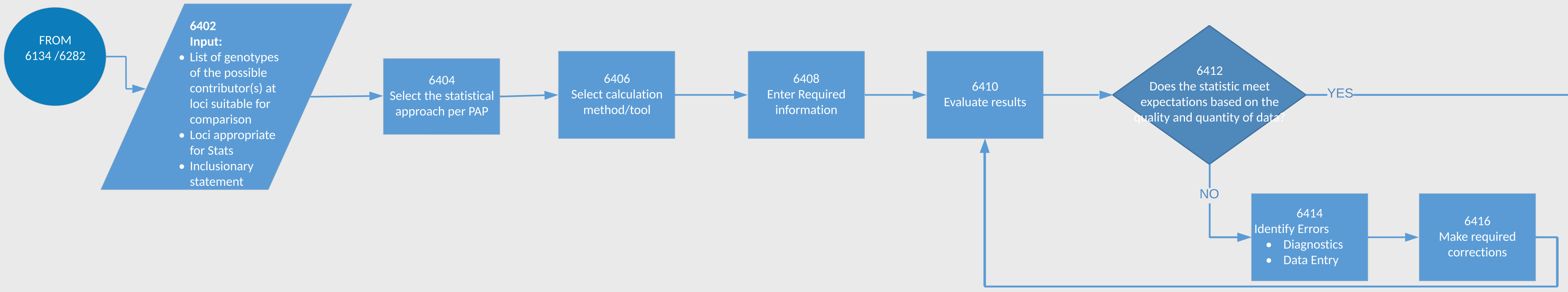
Possible Inconclusive Conclusions
- Inconclusive
- No conclusions can be drawn

Reasons not to calculate a statistic

Possible Inclusionary Conclusions
- Nonprobative information
- PAP
- Case information

6400 - Statistics - Binary

Return to Overview



FROM 6134 /6282

6402
Input:
- List of genotypes of the possible contributor(s) at loci suitable for comparison
- Loci appropriate for Stats
- Inclusionary statement

6404
Select the statistical approach per PAP

6406
Select calculation method/tool

6408
Enter Required information

6410
Evaluate results

6412
Does the statistic meet expectations based on the quality and quantity of data?

6414
Identify Errors
- Diagnostics
- Data Entry

6416
Make required corrections

6418
Output:
Quantitative evaluation of inclusionary statements

GO TO 7000 Reporting

Technology Assist

Expert System/Genotyping Software
- GMIDX
- Genemarker
-

Semi-continuous Prob Gen
- ArmedXpert
- LRMix
- LabRetriever
- Home built calculator

Statistical Software
- Home built calculator
- POPStats

Statistical Method Options

Combined Probability of Inclusion (CPI)/ Combined Probability of Exclusion (CPE)
- Sample type: Mixtures
- Information provided: The combined probability that a randomly selected unrelated individual is not excluded or is included from being a source of DNA evidence

Random Match Probability (RMP)
- Sample type: Single source or resolved single source
- Information provided: The probability of observing the evidence profile at random among unrelated individuals in population

Modified Random Match Probability (mRMP)
- Sample type: Mixtures
- Information provided: The probability of observing the evidence profile at random among unrelated individuals in population)

Likelihood Ratio (LR)
- Sample type: Mixtures, single source, resolved single source
- Information provided: The ratio of probability of the evidence given two competing hypotheses)

Y frequency estimate
- Sample type: Single source or resolved single source, mixtures
- Information provided: Frequency estimate in the population

Y match probability
- Sample type: Single source or resolved single source, mixtures
- Information provided: The probability that that Y haplotype would be observed in the population given that has been observed previously

Statistical Method Inputs

Combined Probability of Inclusion (CPI)/ Combined Probability of Exclusion (CPE)
- Allele calls
- Frequencies from database PAP

Random Match Probability (RMP)
- Allele calls
- Frequencies from database PAP

Modified Random Match Probability (mRMP)
- Allele calls
- Frequencies from database PAP

Likelihood Ratio (LR)
- Allele calls
- Frequencies from database PAP
- Propositions

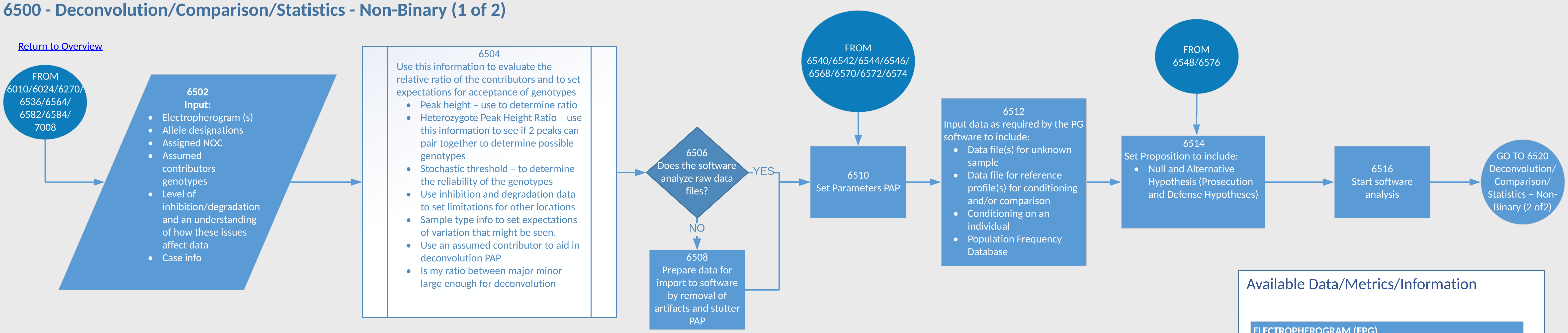
Y frequency estimate
- Allele calls
- Frequencies from haplotype database PAP

Y match probability
- Sample type: Single source or resolved Allele calls
- Frequencies from haplotype database PAP

This Process Map provides a visual description of the various steps of the casework process performed by DNA analysts and is an attempt to represent all reasonable variations in current practice. OSAC and SWGDAM do not explicitly support or endorse (as best practices) all of the different steps and/or paths as depicted on this current-practices process map.

6500 - Deconvolution/Comparison/Statistics - Non-Binary (1 of 2)

Return to Overview



FROM 6010/6024/6270/ 6536/6564/ 6582/6584/ 7008

6502
Input:
- Electropherogram (s)
- Allele designations
- Assigned NOC
- Assumed contributors genotypes
- Level of inhibition/degradation and an understanding of how these issues affect data
- Case info

6504
Use this information to evaluate the relative ratio of the contributors and to set expectations for acceptance of genotypes
- Peak height – use to determine ratio
- Heterozygote Peak Height Ratio – use this information to see if 2 peaks can pair together to determine possible genotypes
- Stochastic threshold – to determine the reliability of the genotypes
- Use inhibition and degradation data to set limitations for other locations
- Sample type info to set expectations of variation that might be seen.
- Use an assumed contributor to aid in deconvolution PAP
- Is my ratio between major minor large enough for deconvolution

6506
Does the software analyze raw data files?

6508
Prepare data for import to software by removal of artifacts and stutter PAP

FROM 6540/6542/6544/6546/ 6568/6570/6572/6574

6510
Set Parameters PAP

6512
Input data as required by the PG software to include:
- Data file(s) for unknown sample
- Data file for reference profile(s) for conditioning and/or comparison
- Conditioning on an individual
- Population Frequency Database

FROM 6548/6576

6514
Set Proposition to include:
- Null and Alternative Hypothesis (Prosecution and Defense Hypotheses)

6516
Start software analysis

GO TO 6520 Deconvolution/ Comparison/ Statistics – Non-Binary (2 of2)

Data Import

Prob Gen (Continous)
- Allele (STRmix)
- Height (STRmix)
- Base pair size (STRmix)
- Raw data file (TrueAllele)

Semi-Continuous
- Allele
- Height

Technology Assist

Expert System
- GMIDX
- Genemarker

Probabilistic Genotyping
- Fully continuous Prob Gen
    - STRmix
    - TrueAllele
    - DNA-View
    - GenoProof Mixture
- Semi-continuous Prob Gen
    - ArmedXpert
    - LRMix
    - LabRetriever
    - Home built calculator

Parameters

- Number of Contributors (NOC)
- # Burn-in accepts
- # MCMC accepts
- Drop in settings
- Degradation settings
- Variance settings
- Threshold(s)
- MCMC settings
- Loci to be ignored due to tri-alleles, unresolved peaks, or artifacts
- Use of replicates

Basis for Setting the Proposition(s)

- Case Information
- Customer driven
- Investigative driven
- Administratively chosen
- Management driven
- Perceived probative value (based on analyst's opinion)
- Process based
- Crime type

Available Data/Metrics/Information

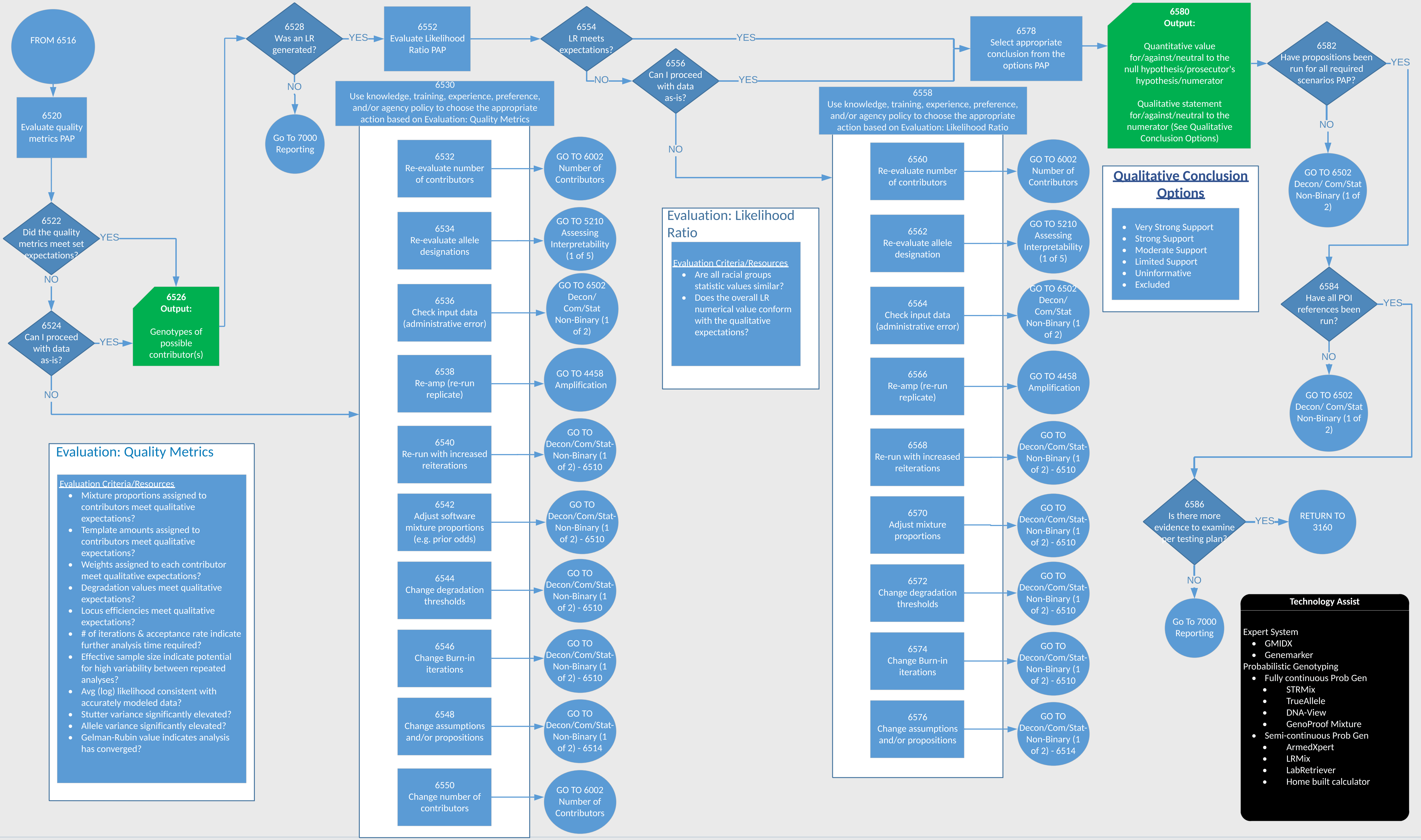
ELECTROPHEROGRAM (EPG)
- Peak height (Rfu)
- Peak count (#)
- Peak morphology (Physical Shape)
- Molecular weight of the Peak (BP)
- Allele size (# of Repeats)
- Relative peak heights (Ratio of Rfu)
    - Alleles to allele within a locus
    - Allele to allele between loci
    - Potential stutter to allele within loci (Stutter Ratio)

QUANT DATA
- Cycle threshold of the internal positive control (#)
- Degradation Index
- Yield (nanogram / picogram)
- Male to total

VALIDATION/PAP
- Stutter ratio + filter
- Defined stutter positions per locus (+, -, forward, reverse, etc.)
- THRESHOLD
    - Stochastic
    - Analytical
- Heterozygote peak height ratio
- Mixture proportion / Donor ratio
- Sex determination rules
- Maximum number of contributors per profile
- Minimum information rules
    - Percentage of loci
- Minimum number of alleles and/or genotypes per profile
- Validation data
- Stutter table

EVIDENCE / PROCESS / OTHER INFOMRATION
- Sample size
- Sample type (to include substrate)
- Case scenario/Information
- Discussion of imbalances
- Extraction method
- Serology results
- Color of extract
- Published data

6500 - Deconvolution/Comparison/Statistics - Non-Binary (2 of 2)



FROM 6516

6520
Evaluate quality metrics PAP

6522
Did the quality metrics meet set expectations?

6524
Can I proceed with data as-is?

6526
Output:

Genotypes of possible contributor(s)

6528
Was an LR generated?

Go To 7000 Reporting

6552
Evaluate Likelihood Ratio PAP

6554
LR meets expectations?

6556
Can I proceed with data as-is?

6530
Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action based on Evaluation: Quality Metrics

6532
Re-evaluate number of contributors

GO TO 6002 Number of Contributors

6534
Re-evaluate allele designations

GO TO 5210 Assessing Interpretability (1 of 5)

6536
Check input data (administrative error)

GO TO 6502 Decon/ Com/Stat Non-Binary (1 of 2)

6538
Re-amp (re-run replicate)

GO TO 4458 Amplification

6540
Re-run with increased reiterations

GO TO Decon/Com/Stat-Non-Binary (1 of 2) - 6510

6542
Adjust software mixture proportions (e.g. prior odds)

GO TO Decon/Com/Stat-Non-Binary (1 of 2) - 6510

6544
Change degradation thresholds

GO TO Decon/Com/Stat-Non-Binary (1 of 2) - 6510

6546
Change Burn-in iterations

GO TO Decon/Com/Stat-Non-Binary (1 of 2) - 6510

6548
Change assumptions and/or propositions

GO TO Decon/Com/Stat-Non-Binary (1 of 2) - 6514

6550
Change number of contributors

GO TO 6002 Number of Contributors

Evaluation: Quality Metrics

Evaluation Criteria/Resources

- Mixture proportions assigned to contributors meet qualitative expectations?
- Template amounts assigned to contributors meet qualitative expectations?
- Weights assigned to each contributor meet qualitative expectations?
- Degradation values meet qualitative expectations?
- Locus efficiencies meet qualitative expectations?
- # of iterations & acceptance rate indicate further analysis time required?
- Effective sample size indicate potential for high variability between repeated analyses?
- Avg (log) likelihood consistent with accurately modeled data?
- Stutter variance significantly elevated?
- Allele variance significantly elevated?
- Gelman-Rubin value indicates analysis has converged?

Evaluation: Likelihood Ratio

Evaluation Criteria/Resources

- Are all racial groups statistic values similar?
- Does the overall LR numerical value conform with the qualitative expectations?

6558
Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action based on Evaluation: Likelihood Ratio

6560
Re-evaluate number of contributors

GO TO 6002 Number of Contributors

6562
Re-evaluate allele designation

GO TO 5210 Assessing Interpretability (1 of 5)

6564
Check input data (administrative error)

GO TO 6502 Decon/ Com/Stat Non-Binary (1 of 2)

6566
Re-amp (re-run replicate)

GO TO 4458 Amplification

6568
Re-run with increased reiterations

GO TO Decon/Com/Stat-Non-Binary (1 of 2) - 6510

6570
Adjust mixture proportions

GO TO Decon/Com/Stat-Non-Binary (1 of 2) - 6510

6572
Change degradation thresholds

GO TO Decon/Com/Stat-Non-Binary (1 of 2) - 6510

6574
Change Burn-in iterations

GO TO Decon/Com/Stat-Non-Binary (1 of 2) - 6510

6576
Change assumptions and/or propositions

GO TO Decon/Com/Stat-Non-Binary (1 of 2) - 6514

6578
Select appropriate conclusion from the options PAP

6580
Output:

Quantitative value for/against/neutral to the null hypothesis/prosecutor's hypothesis/numerator

Qualitative statement for/against/neutral to the numerator (See Qualitative Conclusion Options)

Qualitative Conclusion Options

- Very Strong Support
- Strong Support
- Moderate Support
- Limited Support
- Uninformative
- Excluded

6582
Have propositions been run for all required scenarios PAP?

GO TO 6502 Decon/ Com/Stat Non-Binary (1 of 2)

6584
Have all POI references been run?

GO TO 6502 Decon/ Com/Stat Non-Binary (1 of 2)

6586
Is there more evidence to examine per testing plan?

RETURN TO 3160

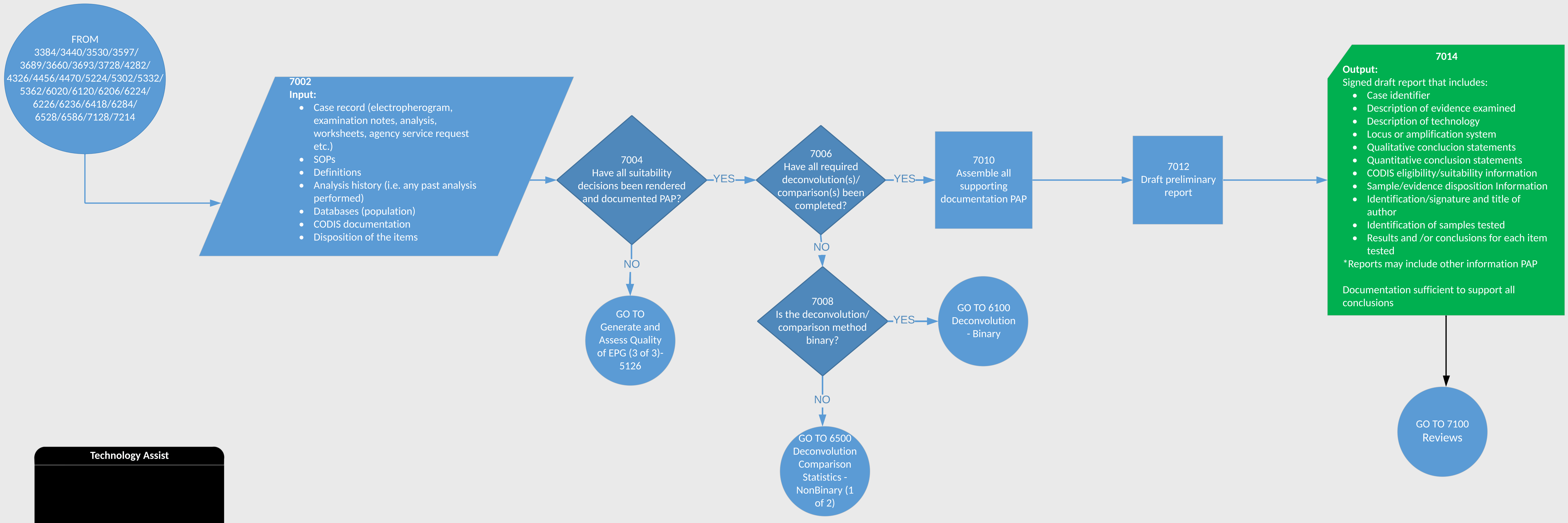
Go To 7000 Reporting

Technology Assist

Expert System
- GMIDX
- Genemarker

Probabilistic Genotyping
- Fully continuous Prob Gen
  - STRMix
  - TrueAllele
  - DNA-View
  - GenoProof Mixture
- Semi-continuous Prob Gen
  - ArmedXpert
  - LRMix
  - LabRetriever
  - Home built calculator

7000 - Reporting



FROM
3384/3440/3530/3597/
3689/3660/3693/3728/4282/
4326/4456/4470/5224/5302/5332/
5362/6020/6120/6206/6224/
6226/6236/6418/6284/
6528/6586/7128/7214

7002
Input:
- Case record (electropherogram, examination notes, analysis, worksheets, agency service request etc.)
- SOPs
- Definitions
- Analysis history (i.e. any past analysis performed)
- Databases (population)
- CODIS documentation
- Disposition of the items

7004
Have all suitability decisions been rendered and documented PAP?

GO TO
Generate and Assess Quality of EPG (3 of 3)-5126

7006
Have all required deconvolution(s)/ comparison(s) been completed?

7008
Is the deconvolution/ comparison method binary?

GO TO 6100
Deconvolution - Binary

GO TO 6500
Deconvolution Comparison Statistics - NonBinary (1 of 2)

7010
Assemble all supporting documentation PAP

7012
Draft preliminary report

7014
Output:
Signed draft report that includes:
- Case identifier
- Description of evidence examined
- Description of technology
- Locus or amplification system
- Qualitative conclusion statements
- Quantitative conclusion statements
- CODIS eligibility/suitability information
- Sample/evidence disposition Information
- Identification/signature and title of author
- Identification of samples tested
- Results and /or conclusions for each item tested

*Reports may include other information PAP

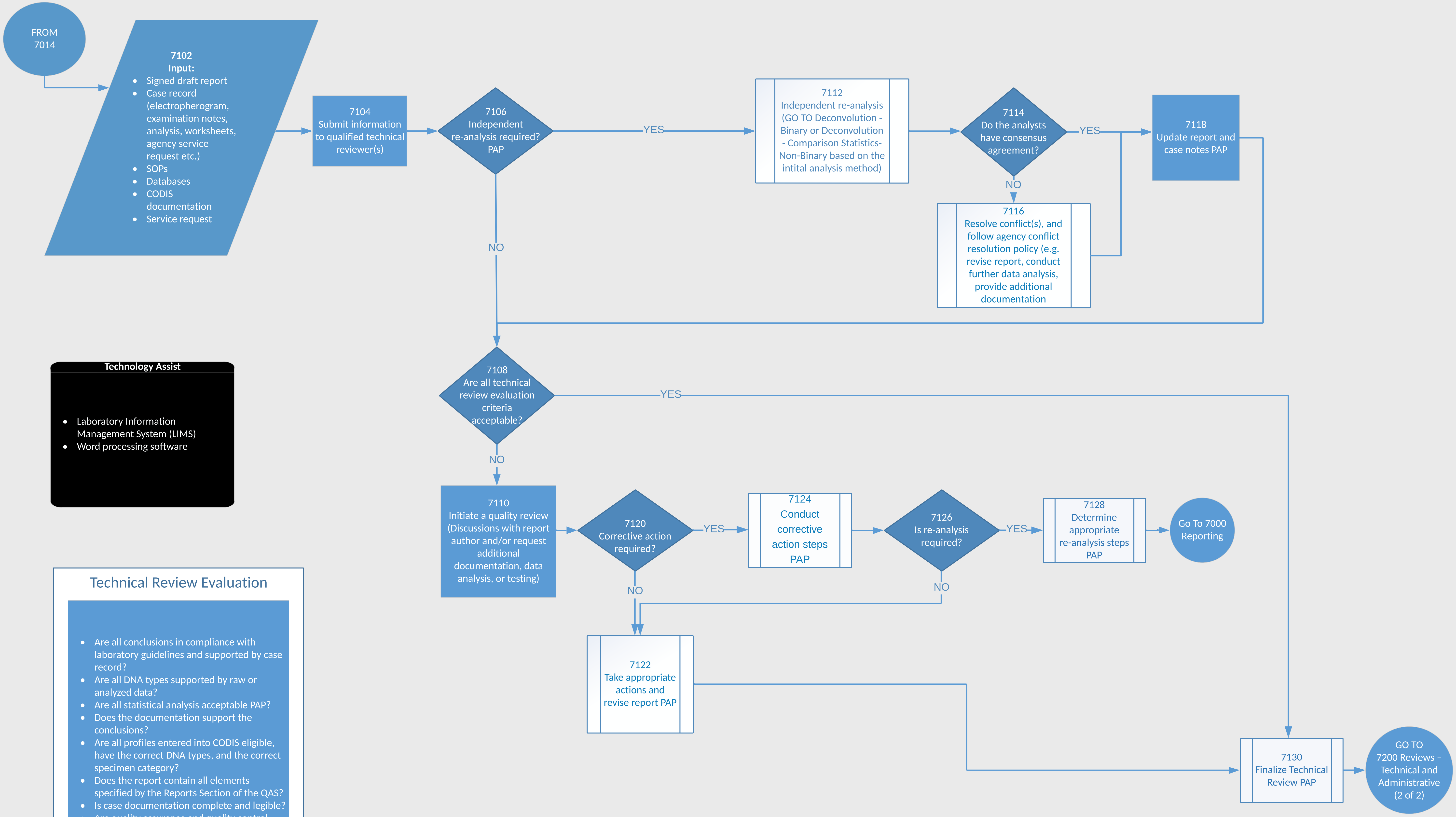
Documentation sufficient to support all conclusions

GO TO 7100
Reviews

Technology Assist

- Laboratory Information Management System (LIMS)
- Word processing software

7100 - Reviews- Technical and Administrative



FROM 7014

7102
Input:
- Signed draft report
- Case record (electropherogram, examination notes, analysis, worksheets, agency service request etc.)
- SOPs
- Databases
- CODIS documentation
- Service request

7104
Submit information to qualified technical reviewer(s)

7106
Independent re-analysis required? PAP

YES

7112
Independent re-analysis (GO TO Deconvolution - Binary or Deconvolution - Comparison Statistics- Non-Binary based on the intital analysis method)

7114
Do the analysts have consensus agreement?

YES

7118
Update report and case notes PAP

NO

7116
Resolve conflict(s), and follow agency conflict resolution policy (e.g. revise report, conduct further data analysis, provide additional documentation

NO

7108
Are all technical review evaluation criteria acceptable?

YES

NO

7110
Initiate a quality review (Discussions with report author and/or request additional documentation, data analysis, or testing)

7120
Corrective action required?

YES

7124
Conduct corrective action steps PAP

7126
Is re-analysis required?

YES

7128
Determine appropriate re-analysis steps PAP

Go To 7000 Reporting

NO

NO

7122
Take appropriate actions and revise report PAP

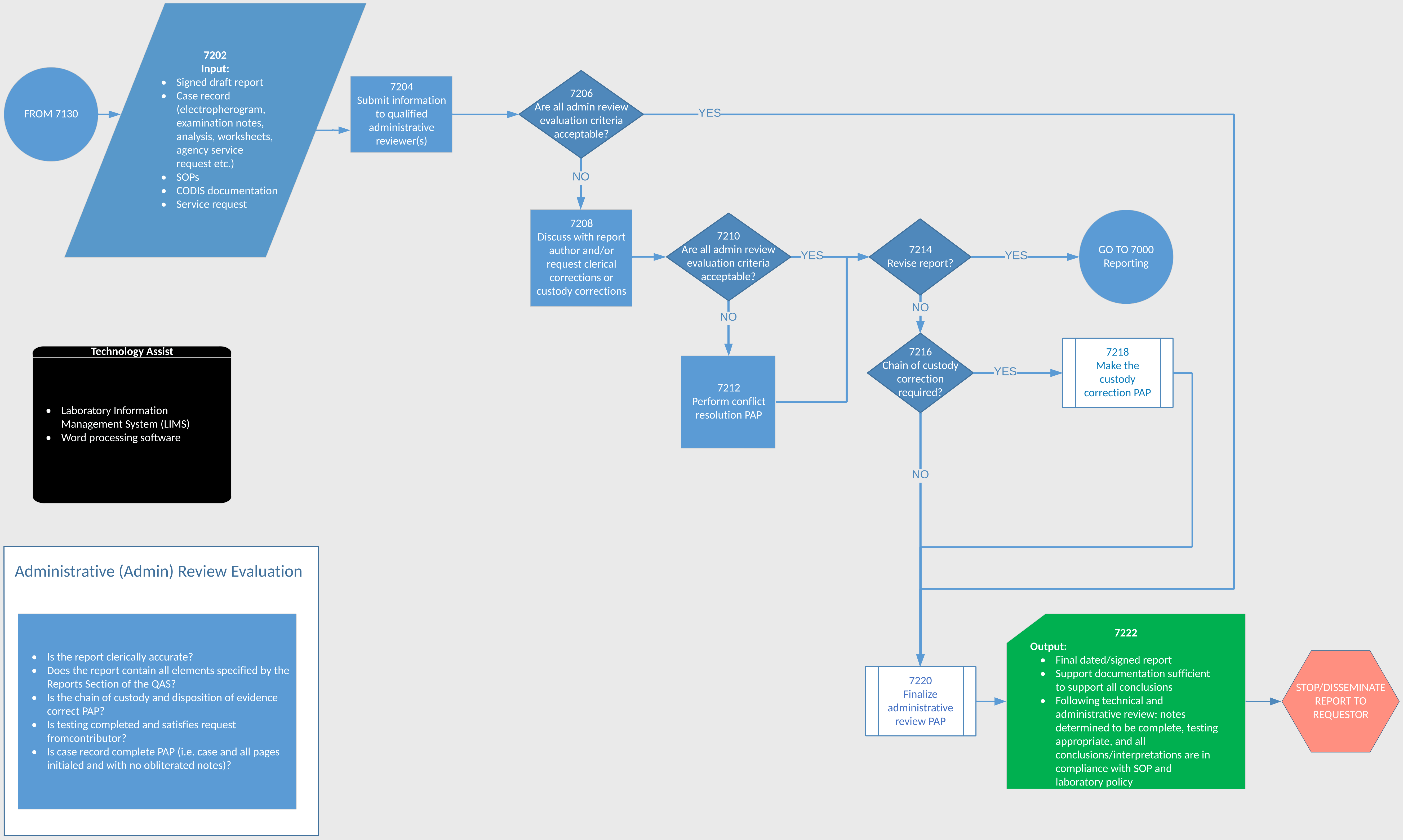
7130
Finalize Technical Review PAP

GO TO 7200 Reviews – Technical and Administrative (2 of 2)

Technology Assist
- Laboratory Information Management System (LIMS)
- Word processing software

Technical Review Evaluation
- Are all conclusions in compliance with laboratory guidelines and supported by case record?
- Are all DNA types supported by raw or analyzed data?
- Are all statistical analysis acceptable PAP?
- Does the documentation support the conclusions?
- Are all profiles entered into CODIS eligible, have the correct DNA types, and the correct specimen category?
- Does the report contain all elements specified by the Reports Section of the QAS?
- Is case documentation complete and legible?
- Are quality assurance and quality control (QA/QC) requirements satisfied?

7200 - Reviews – Technical and Administrative (2 of 2)



FROM 7130

7202
Input:
- Signed draft report
- Case record (electropherogram, examination notes, analysis, worksheets, agency service request etc.)
- SOPs
- CODIS documentation
- Service request

7204
Submit information to qualified administrative reviewer(s)

7206
Are all admin review evaluation criteria acceptable?

7208
Discuss with report author and/or request clerical corrections or custody corrections

7210
Are all admin review evaluation criteria acceptable?

7212
Perform conflict resolution PAP

7214
Revise report?

GO TO 7000 Reporting

7216
Chain of custody correction required?

7218
Make the custody correction PAP

7220
Finalize administrative review PAP

7222
Output:
- Final dated/signed report
- Support documentation sufficient to support all conclusions
- Following technical and administrative review: notes determined to be complete, testing appropriate, and all conclusions/interpretations are in compliance with SOP and laboratory policy

STOP/DISSEMINATE REPORT TO REQUESTOR

Technology Assist

- Laboratory Information Management System (LIMS)
- Word processing software

Administrative (Admin) Review Evaluation

- Is the report clerically accurate?
- Does the report contain all elements specified by the Reports Section of the QAS?
- Is the chain of custody and disposition of evidence correct PAP?
- Is testing completed and satisfies request fromcontributor?
- Is case record complete PAP (i.e. case and all pages initialed and with no obliterated notes)?