Introduction

The National Institute of Standards and Technology (NIST) facilitated the development of this Wildlife Forensic Biology 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 Wildlife Forensic Biology Subcommittee).

This Wildlife Forensic Biology Process Map (Current Practices) captures details about the various procedures, methods and decision points most frequently encountered in the wildlife forensic biology process from a national perspective and ***is intended to reflect current practices****. The discipline requires examiners to make many decisions that can impact the quality and accuracy of results. The Wildlife Forensic Biology Process Map can benefit the discipline by providing a behind-the-scenes perspective into the various components and decision points in the examination 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 an examiner 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 wildlife forensic biology activities* ***currently performed*** *by examiners. For this reason, it is important to state that the OSAC Wildlife Forensic Biology Subcommittee does not 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, judgments 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 examiner, or agency. Processes and decisions may also be dictated by agency policy or law.

Process Map Applications:

The Wildlife Forensic Biology 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 Wildlife Forensic Biology Process Map:

The scope of the Wildlife Forensic Biology Process Map is limited to core processes within the discipline of wildlife forensic biology such as the analysis and comparison of wildlife evidence including species ID, sex determination, matching, geographic origin, and familial relationship. Several topics are omitted from this map including morphology. These and other topics may subsequently be addressed by the process mapping team, an individual entity or a standardization committee.

OSAC
Organization of Scientific Area Committees for Forensic Science

Suggested Citation: Taylor M, Waltke H, (2025), OSAC Wildlife Forensics Biology Subcommittee, Wildlife Forensics Biology Process Map (Current Practice). U.S. Department of Commerce, National Institute of Standards and Technology. 2025. Published May 2025

Request Received

1000 - Administrative Assessment

- Case intake assessment:
  - Resource availability and lab capacity
  - Suitability
  - Safety
  - Scope
  - Communication with requestor
  - Chain of custody
- Evidence submission
- Documentation of evidence submitted (photo or notes)
- Assess evidence items (including packaging, labels, etc.)
- Issue resolution

2000 - Technical Assessment

- Analyst review of request
- Review and assessment of evidence items
- Suitability assessment (e.g., for amount of material, contamination, etc.)
- Issue resolution and troubleshooting
- Initial test plan
- Item Characterization

3000 - 5000 Pre-Analysis

- Screening including presumptive tests, (e.g., blood etc.), and morphology
- Evaluate for contamination/ commingling
- Morphology: Triage and Pre-Processing
  - Morphology visualization e.g.,
    - Hair
    - Feathers
    - Ivory
    - Leather
    - Whole/partial
- Sample evaluation for biological material e.g. number of sampling sites
- Determine subsample techniques (blood, bone, hair, tissue, saliva, buccal swabs, feces, objects)
- Subsampling
- Extraction
- Clean up
- Quantitation

6000-9000 - Analysis: Amplification, Genetic Visualization, Interpretation, and Comparison (Choose applicable method(s))

- Taxonomic/species ID
- Sex determination
- Matching/minimum number of individuals
- Familial determination
- Population assignment/Geographic origin

- Quantitative Polymerase Chain Reaction (qPCR)
- Sequencing
- Fragment Analysis, e.g., Short Tandem Repeats (STR), etc.

10,000 - Reporting

- Case file management (download photos, catalog etc.)
- Drafting report
- Review
- Determination for further analysis
- Report submission

11,000 - Other - Evidence types and processes not covered in this map*

- Morphological Identification
- Next Generation Sequencing (NGS)
- Restriction Fragment Length Polymorphism (RFLP)
- Random Amplified Polymorphic DNA (RAPD)

*The processes, methods and techniques that are indicated in the map are limited to those conducted on-scene; also not included is the collection of evidence requiring certain types of specialized expertise.

Legend

- Process start and end
- **Input Box** Outlines the inputs at the beginning of each section
- **Output Box** Outlines the outputs at the end of each section
- Process (action)
- Decision
- Subprocess
- Linkage TO/FROM another page
- Linkage TO/FROM within the same page
- *Documentation*
- Underlined Word Word that will be defined in the glossary
- Discontinuation of Assessment or Examination

OSAC Organization of Scientific Area Committees for Forensic Science

This process map provides a visual description and attempts to represent all reasonable variations of casework currently performed by wildlife biologists. OSAC does not necessarily support or endorse (as best practices) all of the different steps and paths depicted in this process map.

1000 - Administrative Assessment (1 of 2): Case Intake and Evidence Submission

Case Intake

1002
Request received via evidence receipt, request from another unit within the lab, or phone call (or other communication method)

1004
Is the request only for an investigative lead?
- Yes → GO TO 2260
- No

1006
Is this a direct submission (e.g., is the evidence already waiting at the lab)?
- Yes → 1008 Review request and any associated paperwork → 1010
- No → 1016

1010
Assess the ability to process, e.g.:
- Resources available?
- Eligible for testing (e.g., eligible purposes and/or requesting organization)?
- Technically feasible?
- Analysis requested appropriate?
- Packaging and sealing appropriate?
- Appropriate PPE (or vaccinated personnel) available?

1012
Is there a need to communicate with agent/submitter (e.g., to answer questions or address concerns)?
- Yes → 1014 Communicate with submitter regarding questions or issues (e.g., scope of request, safety, testing required, packaging issues etc.)
- No

1016
Assess request via phone call (or other communication):
- May be detailed (lots of background, specific tests) or very general, e.g.:
  - Assess the scope of request (e.g., questions customer seeks to answer, evidence type, tests, appropriate shipping methods)
  - Suggest what type of tests are/may be required
  - Coach/suggest to customer as to what part(s) of evidence to sample, package, ship
  - Evaluate potential safety issues

1018
Assess the ability to process, e.g.:
- Resources available?
- Eligible for testing (e.g., eligible purposes and/or requesting organization)?
- Technically feasible?
- Analysis requested appropriate?
- Appropriate PPE (or vaccinated personnel) available?

1020
Accept all items (e.g. fulfill the entire request)?
- Yes
- No

1022
Fulfill a partial request (e.g. accept some of the items)?
- Yes → 1040
- No

1040
Retain (or accept) remaining item(s) that will not move forward for further processing?
- Yes → 1046 Retain item(s) in secure location; retain packaging as needed; keep any packaging organized according to evidence item(s)
- No

1042
Return any items to submitter (as applicable)?
- Yes → 1048 Arrange return to submitter PAP
- No → 1044 Refer or outsource any remaining portion(s) of the case as applicable (See referral/outsource path)

Referral/outsource path

1024
Refer requestor to another facility?
- Yes → 1028 Refer requestor to designated facility PAP
- No

1026
Send to another laboratory section or facility/contract lab for processing?
- Yes → 1030 Arrange to send to appropriate section of lab for processing
- No

1032
Log into case management system?
- Yes → 1036
- No → 1034 STOP/Proceed PAP

1036
Log item into case management system and perform any additional tasks as needed PAP e.g.:
- View submittal paperwork and any chain of custody submitted
- Create unique case identifier
- Create unique item designation for evidence as needed
- Generate and provide receipt to submitting agency
- Initiate/update laboratory chain of custody

Evidence Submission

1050
Collect the evidence myself?
- Yes → 1052 Arrange to pick up/collect evidence due to factors such as warrant issue or bulk seizure:
  Assess tasks, e.g.:
  - Travel to the scene
  - Perform sample selection
  - Perform subsampling as needed
  - Perform evidence custodian tasks
- No

1054
Has evidence already been received?
- Yes
- No → 1056 Await evidence receipt (if needed)

1058
Evaluate submission materials and initiate evidence receiving PAP
- Ensure the submitter is authorized to request the services
- Log item into case management system
  - Create unique case identifier
  - Create unique item designation for evidence if required
- View submittal paperwork and any chain of custody submitted
- Initiate/update laboratory chain of custody
- Ensure appropriate documentation is present for evidence processing PAP (e.g., appropriate paperwork is enclosed and filled out for permissions, necessary forms completed, etc.)
- Generate and provide receipt to submitting agency
- Ensure payment is complete (if necessary)

1060
Document and/or photograph including condition of outer packaging/container etc. upon receipt → GO TO 1102

This process map provides a visual description and attempts to represent all reasonable variations of casework currently performed by wildlife biologists. OSAC does not necessarily support or endorse (as best practices) all of the different steps and paths depicted in this process map.

1100 - Administrative Assessment (2 of 2): Open Container and Assess Evidence

FROM 1060

1102 Assign case now?

Yes → 1104 Assign case to analyst or self-assign PAP

No

Open and Document Evidence

1106 Open any *outermost* package(s) as needed and inventory evidence; document opening of the outer container.

1108 Document evidence as received and compare to documentation PAP:

- Note whether items were appropriately packaged
- Document and begin internal CoC including item number(s) if necessary
- Compare documentation and evidence item(s) to Chain of Custody (CoC) and/or other documentation to verify contents appear to be what is described on paperwork
- Take descriptor photos
- Assign unique laboratory item identifier(s) as needed

1110 Are there any discrepancies or other issues to be addressed (e.g., items in the container not as described, documentation inaccurate and incomplete, or has any evidence been removed, spilled out or contaminants introduced etc.)?

Yes → 1112

No

Assessment and Issue Resolution

1112 Address issues that may be in need of resolution (choose all that apply PAP):

- Improper seal(s) (e.g., PAP-seal unmarked, hole in packaging, missing tape, leakages, tears, rips, etc.)
- Missing, incorrect or illegible label(s)
- Commingled item(s)
- Incorrect description(s)
- Missing item(s)
- Chain of custody issues
- Mislabeled packaging
- Inappropriately packaged item(s) PAP
- Contaminated item(s)
- Evidence unsuitable (e.g. moldy etc)
- Missing or inappropriate markings (e.g., biohazard)

1114 Document/photograph and proceed PAP

1116 Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action(s):

- Fix on own (including opening individual items and performing any repackaging if needed)
- Reach out to evidence or property management unit
- Contact submitter to advise on how to proceed (send back, guidance on how to fix issue etc)
- Communicate with submitter and advise of potential STOP for certain item(s)

1118 Identify and evaluate the type of test or process(es) being requested based on submittal paperwork or instructions as needed, e.g.:

- Whether there are multiple processes requested
- Order of processing (e.g., morphology first etc.)

1120 Assess item(s) or sample(s) for safety issues as needed based on submission materials e.g.:

- Diseases
- Airborne or bloodbourne pathogens
- Poisons
- Sharp objects, bones, or other protrusions etc.

1122 Output

- Case assigned (possibly)
- Payment received as needed
- Evidence custodial review and assessment of:
  - Paperwork
  - Packaging
  - Evidence items requested indicated in submission
  - Safety considerations
  - Request for services

1124 Has case been assigned yet?

Yes → 1130 Assess request and associated item(s) or sample(s) as needed for potential safety issues, e.g.:

- Diseases
- Airborne or bloodbourne pathogens
- Poisons
- Sharp objects, bones, or other protrusions etc.

GO TO 2002

No → 1128 Assign case to analyst or self-assign PAP

2000 - Technical Assessment (1 of 3): Analyst Review, Assessment, and Suitability Assessment for Proceeding

FROM 1130

2002
Input:

- Analyst is reviewing the case
- Evidence custodial review and assessment of:
  - Paperwork
  - Packaging
  - Evidence items requested indicated in submission
  - Safety considerations
  - Request for services

Analyst Review and Assessment of Case

2004
Is more information needed prior to proceeding?
Yes

2006
Gather additional information as necessary, e.g.:

- Perform literature review
- Assess database or standard(s) availability
- Assess availability of validated method(s)
- Consider other limiting case factors PAP
- Request ingredient list or original manufacturer packaging

2008
Evaluate the type of test or process(es) being requested, e.g.:

- Multiple processes or personnel needed for testing
- Destructive vs. non-destructive processing
- Order of processing (e.g., morphology, genetics, etc.)

No

Communication with Requestor

2010
Are there issues with the request?
Yes

2012
Is the issue due to the type of material being requested for processing?
Yes

2020
Communicate with requestor regarding the type of material requested for analysis?
Yes

2022
Communicate with requestor regarding any limitations or potential inability to process the material.

No

2014
Is the issue due to time, or available resources or expertise?
Yes

2016
Communicate with requestor to determine if an alternative or a portion of the request can be fulfilled.

2018
Can lab fulfill an alternative or portion of the request?
Yes

GO TO 2102

No

GO TO 2102

2024
Proceed with material?
Yes

2026
Proceed noting limitations

GO TO 2102

No

2028
Choose all that apply (PAP)

- Document for future capabilities
- Take a sample
- Update CoC

GO TO 2050

Issue Resolution

2030
Can the issue be addressed?
Yes

2032
Resolve issue(s) PAP

2034
Document any issues observed and any resolution

GO TO 2102

No

2036
Send back to requestor?
Yes

2038
Return evidence to requestor and proceed PAP

No

2040
Continue with request, accept evidence, noting limitations?
Yes

GO TO 2102

2042
STOP/Proceed PAP

No

Outsourcing and Referral

2050
Facilitate sending to another facility?
Yes

2052
Refer requestor and facilitate (via phone call or other means) sending to designated facility PAP

2054
Has a report been generated?
Yes

2056
Forward report to submitting agency PAP

No

2058
Refer requestor to another facility?
Yes

2060
Refer requestor to designated facility PAP

No

2062
Send to another laboratory section or facility/contract lab for processing?
Yes

2064
Send to appropriate section of lab for processing

No

2068
STOP/Proceed PAP

This process map provides a visual description and attempts to represent all reasonable variations of casework currently performed by wildlife biologists. OSAC does not necessarily support or endorse (as best practices) all of the different steps and paths depicted in this process map.

2100 - Technical Assessment (2 of 3)

FROM 2010/2018/2026/ 2034/2040

Open and Document Individual Evidence Item(s)

2102
Open any individual evidence item package(s) as needed and inspect individual evidence items; document opening and whether seal(s) were cut

2104
Document evidence as received and compare to documentation PAP:

- Note whether items were appropriately packaged
- Document and begin internal CoC including item number(s) if necessary
- Compare documentation and evidence item(s) to Chain of Custody (CoC) and/or other documentation to verify contents appear to be what is described on paperwork
- Take descriptor photos
- Assign unique laboratory item identifier(s) as needed

2106
Are there any discrepancies or other issues to be addressed (e.g., items not as described, documentation inaccurate and incomplete, or has any evidence been removed, spilled, or contaminants introduced etc.)?

Yes

No

GO TO 2114

Item Assessment and Issue Resolution

2108
Address issues that may be in need of resolution (choose all that apply PAP):

- Improper seal(s) (e.g., PAP- seal unmarked, hole in packaging, missing tape, leakages, tears, rips, etc.)
- Missing, incorrect or illegible label(s)
- Commingled item(s)
- Incorrect description(s)
- Missing item(s)
- Chain of custody issues
- Mislabeled packaging
- Inappropriately packaged item(s) PAP
- Contaminated item(s)
- Evidence unsuitable (e.g. moldy etc)
- Missing or inappropriate markings (e.g., biohazard)
- No biological material present

2110
Document/photograph and proceed PAP

2112
Use knowledge, training, experience, preference, and/or agency policy to choose the appropriate action(s):

- Fix on own
- Reach out to evidence or property management unit
- Contact submitter to advise on how to proceed (send back, guidance on how to fix issue etc)
- Reach out to another colleague/expert for advice
- Refer to another unit for evaluation
- STOP/Discontinue processing certain item(s)

2114
Proceed with all item(s)?

Yes

No

2116
Communicate with submitter?

Yes

2118
Communicate with submitter as needed regarding item(s) that will not be processed and proceed PAP (e.g., return evidence to requestor, initiate evidence disposition PAP, etc.)

No

2120
Proceed with any item(s)?

Yes

2122
Assess evidence for processing:

- Sufficient quality?
- Sufficient quantity?
- Exemplar(s) collected properly?

GO TO 2202

No

2124
Is there more evidence to assess?

Yes

RETURN TO 1004

No

2126
STOP/Proceed PAP

2200 - Technical Assessment (3 of 3): Develop Initial Test Plan, and Evaluate for any All-consuming or Destructive Processes

FROM 2122

2202 Are there any issues?
- Yes → 2204 Document/photograph and proceed PAP noting any limitations
- No

Assess For Item Consumption

2206 Consume the entire item?
- Yes → 2208 Seek permission to consume entire item?
  - Yes → 2230 Seek permission from requestor
  - No
- No

2232 Permission Obtained?
- Yes
- No → 2234 Process anyway with less material (noting limitations)?
  - Yes → GO TO 2210
  - No → 2236 Consider morphology?
    - Yes → 2238 Send to appropriate section of lab for processing PAP
    - No → 2240 STOP/Proceed PAP → GO TO 10,000 Reporting

Assess for Destructive Process

2210 Use destructive process on item(s)?
- Yes → 2212 Did requestor specify no destruction of item(s)?
  - Yes → GO TO 2220
  - No → 2214 Seek permission to use destructive process?
    - Yes → 2216 Seek permission → 2218 Permission obtained?
      - Yes
      - No
    - No
- No

2220 Process anyway using alternate technique (noting limitations)?
- Yes
- No → 2221 Submit to another section prior to continuation?
  - Yes → 2224 Send to appropriate section of lab for processing PAP
  - No → 2226 STOP/Proceed PAP → GO TO 10,000 Reporting

2250
Develop initial test plan as per request, SOP, and evidence type taking into account factors such as:

- Sample selection
  - Choose representative samples from evidence as needed
  - Target unique individuals
  - Perform subsampling (if needed)- (e.g., how many units to collect from sample, how much volume of blood or tissue to collect, type of tissue etc.)
- Order of item processing (ex. choose destructive vs. non-destructive if multiple processes are requested)
- PPE and other safety considerations
- Test methods (including presumptive testing) and supplies needed
- Turnaround time
- Batching
- Sample prep
- Extraction methods
- PCR primers
  - Sequence
  - qPCR
  - STRs
- Morphology (if needed)

2260 Choose applicable sample type(s):

*Tissue includes items such as:
- Meat/Flesh/Salted Leather/Unprocessed Leather/Dyed or Processed Leather
- Hair follicle
- Dried Blood (e.g., flakes or on other material)
- Eggs
- Claw

- GO TO 3002 -Tissue*
- GO TO 3702 - Blood (liquid)
- GO TO 3802 - Bones, claws, teeth, and keratin based
- GO TO 3902 - Non-Tissue/ Inorganic
- GO TO 4102 - Shed Hair (multiple strands)
- GO TO 4202 - Scat
- GO TO 4252 - Vomit
- GO TO 4282 - Urine
- GO TO 4302 - Saliva
- GO TO 4402 - Other/ Unfamiliar

3000 - Tissue: Swabs and Assessment of Sample Type

FROM 2260/4120/4130

3002
Input: Tissue, e.g.:
- Meat (e.g., filets or other frozen muscle tissue)
- Fat
- Hair follicle
- Blood (dried; swab; if large enough amount e.g., larger than pin head, 10-20 microliters; visible dried blood flake)
- Saliva off clothing or swab
- Scat (if it is on a swab)
- Swab of unknown origin
- Eggs (incl. caviar)
- Claws (with pulp/tissue attached)
- Salted or unprocessed leather
- Leather/skin/hide
- Antler velvet

3004
Is it a swab (e.g., blood, scat, saliva)?
- Yes → Process Swab(s)
- No → Assess Tissue Sample Type(s)

Assess Tissue Sample Type(s)

3006
Evaluate for sources of possible contamination, e.g.,:
- Commingled DNA
  - Ground tuna - may be different types
- Multiple ingredients (whether there appears to be more than one type of tissue in a container)
- Dirt, soil, or other non-biological contaminants
- Different tissue types from the same animal commingled
  - Ground meats
- Fur (hairs or clumps w tissue attached)

3008
Is additional information or assistance needed?
- Yes → 3010
- No → 3012

3010
Obtain additional information or assistance:
- Ask colleague or someone with experience with this type of material for advice/assistance
- Use trial and error to ascertain information needed for further processing
- Obtain photo and/or list of ingredients

3012
Choose applicable sample type(s):
- GO TO 3102 - Meat/Flesh/Salted Leather/ Unprocessed Leather/Dyed or Processed Leather
- GO TO 3202 - Hair Follicle
- GO TO 3302 - Dried Blood (flakes or on other material)
- GO TO 3402 - Eggs
- GO TO 3502 - Claw

Process Swab(s)

3020
Does blood appear to be present?
- Yes → 3022
- No → 3028

3022
Perform presumptive testing for blood?
- Yes → 3024
- No → 3028

3024
Perform presumptive testing

3026
Document method and results (including the presence or absence of blood)

3028
Is it a fully saturated swab?
- Yes → 3034
- No → 3030

3030
Take subsample cutting?
- Yes → 3034
- No → 3032

3032
Continue processing; put whole swab in tube

3034
Subsampling
- Cut in order to create subsample based on:
  - Permission to consume sample
  - Knowledge and experience
  - Protocol
  - Other factors as needed (e.g., DNA richness of tissue, expectation of inhibitors, whether blood vs. saliva, etc.)
- Preserve the remaining sample PAP

GO TO 3610

3100 - Tissue Prep -Screening - Meat/Salted Leather/Unprocessed Leather/Dyed or Processed Leather

FROM 3012

Sample Assessment and Prep

3102
Is there evidence of a suspected mixture?
- Yes → 3104
- No

3104
Document
- Any contamination that may have taken place
- Any commingling

3106
Separate items, e.g.,:
- Divide into pieces/filets/subitems (e.g., "fish brick"; to delineate meat/flesh types and ascertain how many pieces there are)
- Rinse material if needed
- Target certain items or areas:
  - Small pieces to ensure the pieces are from one individual
  - Areas with higher anticipated DNA yield (e.g., ability to amplify)
  - Areas that are not highly pigmented as inhibitors may be present
- Separate materials (e.g., by color, texture, smell, etc. based on animal type such as game animal vs. pork, hide from other parts) using knowledge, training, and experience
- Review ingredients list as needed (if applicable)

3108
Are there commingled items?
- Yes → 3110
- No

3110
Group samples by type (e.g., color, texture, smell, etc.) and take a percentage of the similar items in each grouping for a representative sample

3112
Determine which samples to proceed with based on investigative questions, quantity, minimum anatomical features, etc.

3114
Rinse material as needed

3116
Sample the area on item that is most DNA rich with least amount of inhibitors

3118
Is further sample preparation needed?
- Yes → 3120
- No → GO TO 3122

3120
Prepare any sample(s) as needed, e.g.:
- Use freezer mill
- Soak in water or buffer
- Mince with razor
- Separate fat from meat, e.g., using color, consistency, and other characteristics

Subitem Designation and Selection

3130
Assign unique id for subitems?
- Yes → 3132
- No

3132
Assign unique id for each subitem as needed (e.g. by type of animal or tissue type)

3134
Repackage remaining material (e.g. not being used for further analysis) as needed or PAP

3136
Is there more than one subitem to be analyzed (see Analysis Considerations)?
- Yes → 3138
- No

3138
Select one subitem for processing

Analysis Considerations
- Instructions from submitter
- Which item(s) are unknown(s)
- What is being asked by submitter (e.g., pathology, suspected poisoning etc.)
- Whether any item(s) will go to another section

3122
Are there more subitems in need of prep?
- Yes → 3138
- No → GO TO 3602

3200 - Tissue Prep -Screening - Hair Follicle

FROM 3126

Assessment and Visual inspection

3202 Is flesh attached? — Yes → GO TO 3602

No →

3204 Does there appear to be blood present? — Yes → 3206 Separate out hairs from blood → 3208 Disposition hairs PAP → GO TO 3610

No →

3210 Received single hairs? — Yes → 3212 Conduct microscopic review? — Yes → 3214 Review under microscope for follicle, characteristics concerning taxonomic ID, color, and/or texture

No → 3212 — No →

3216 Is the hair commingled with other material? — Yes → 3218 Separate out hairs from other material and rinse as needed

No →

3220 Separate out individual hairs and rinse if needed → 3222 Conduct microscopic review? — Yes → 3224 Review each hair as needed under microscope for follicle, and group hairs by taxonomic characteristics, color, and/or texture

3222 — No →

Evaluate for Species of Interest and Presence or Absence of Follicle, etc.

3230 Based on the visual inspection, is the hair from the species of interest? — Yes → 3234 Based on visual inspection, continue with hairs from species of interest and note other species present as needed for reporting purposes PAP → 3236 Is a follicle present? — Yes → 3238 Separate the follicle from the rest of the hair? — Yes → 3240 Cut/separate follicle(s) as needed, and repackage hair PAP → GO TO 3602

3238 — No →

3236 — No →

3230 — No →

3232 Report on species present? — Yes → GO TO 10,000 Reporting

3232 — No →

3242 Continue? — Yes → 3244 Document lack of follicle(s) → GO TO 3602

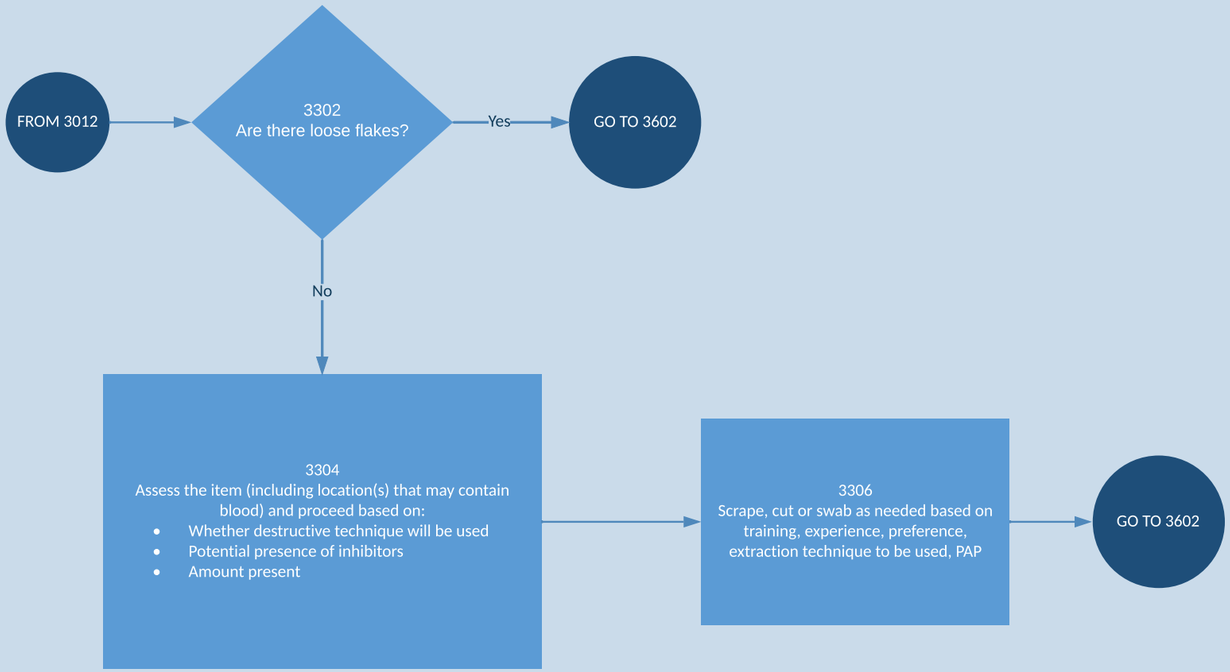
3242 — No →

3246 Is there more evidence to assess? — Yes → RETURN TO 1004

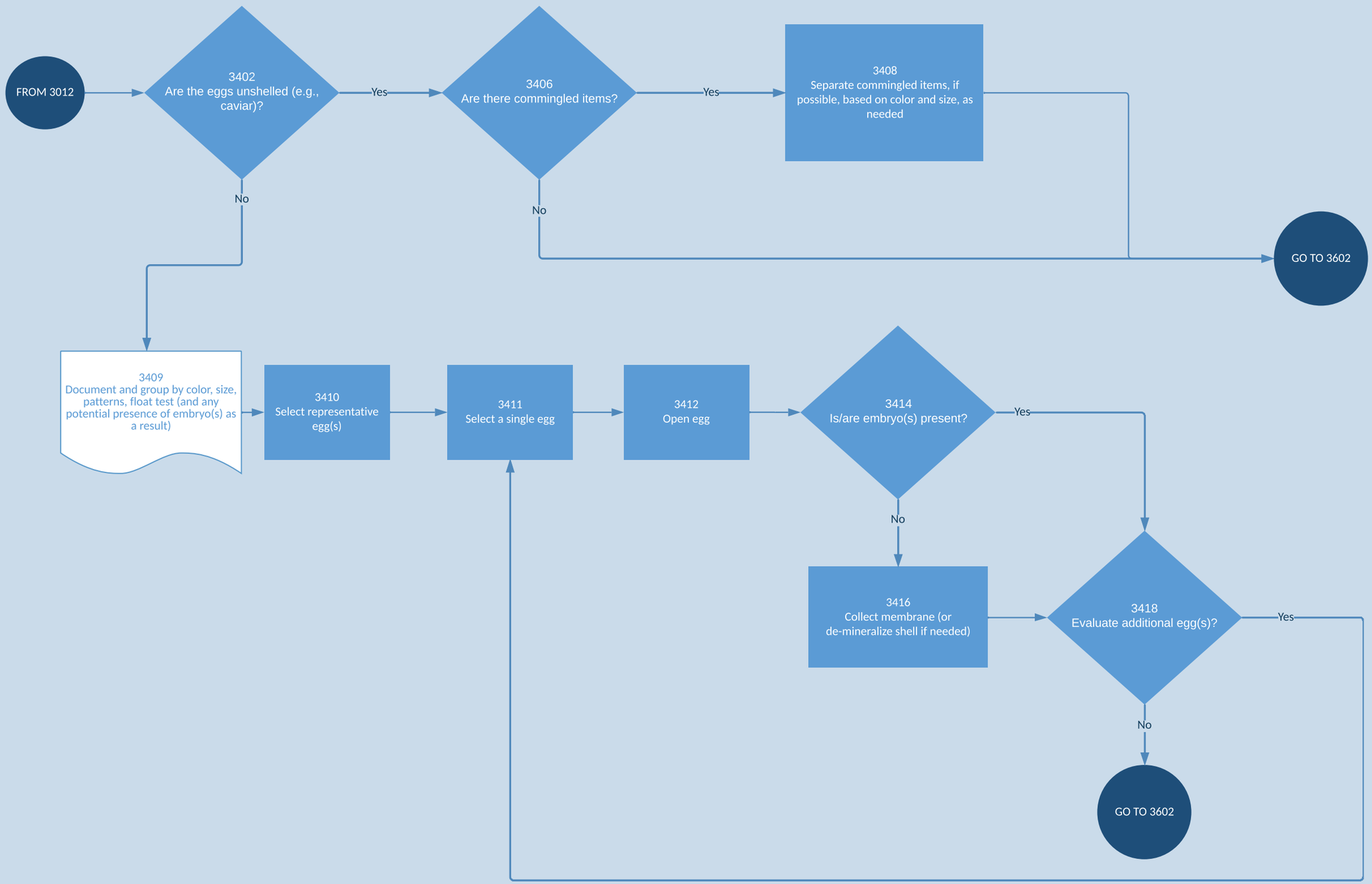
3246 — No →

3248 STOP/Proceed PAP → GO TO 10,000 Reporting

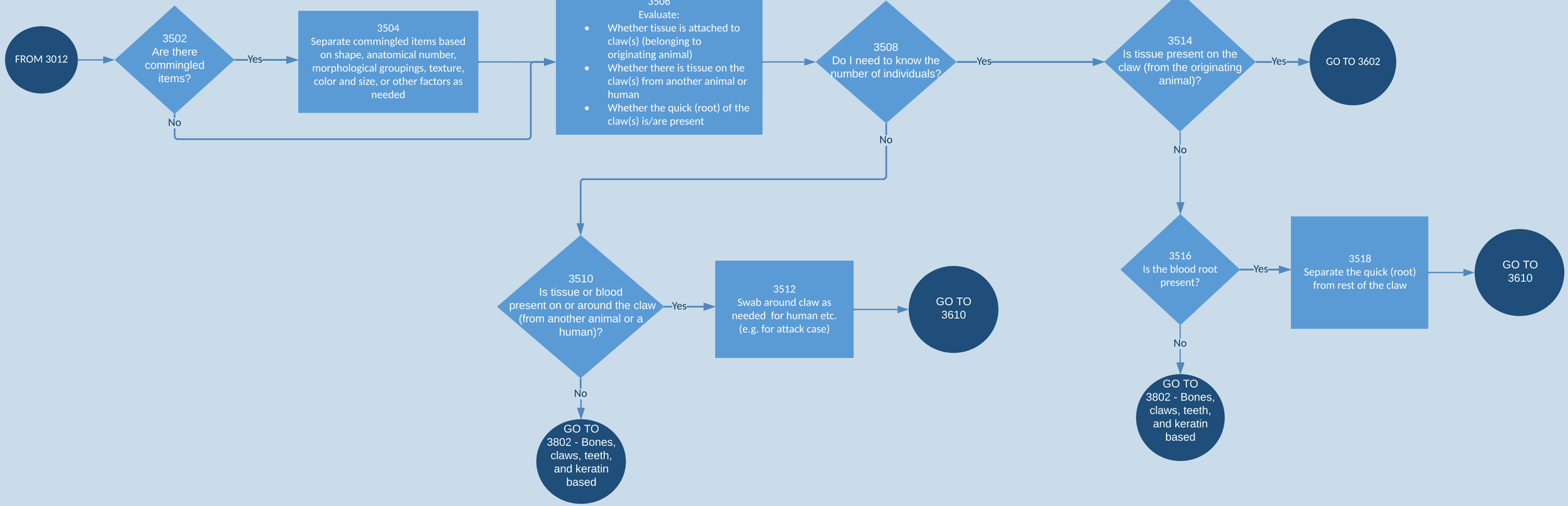
3300 - Tissue Prep - Screening - Dried Blood



3400 - Tissue Prep - Screening - Eggs



3500 - Tissue Prep - Screening - Claw



This process map provides a visual description and attempts to represent all reasonable variations of casework currently performed by wildlife biologists. OSAC does not necessarily support or endorse (as best practices) all of the different steps and paths depicted in this process map.

3600 - Tissue: Subsampling and Lysis

FROM 3122/3202/3238/3240/3244/3302/3306/3406/3408/3418/3514

3602
Is subsampling needed?

Yes

3606
Use knowledge, training, experience, and/or SOP in addition to other factors to determine appropriate subsampling method (e.g., cut, grind, swab, etc.), size, and/or quantity.

Consider:
- Tissue type
- Context (e.g., increase number of samples if material is homogeneous however due to context, may represent multiple individuals)
- Number of items submitted for analysis
- Anticipated DNA yield
- DNA quality

3608
Document
- Subsampling
  - Number or amount taken from item(s)

No

3604
Based on knowledge, training, experience and/or SOP, ascertain then use appropriate technique (e.g., cutting, scraping, grinding, pulling, etc.) to collect all or a portion of the item as needed for further analysis.

Consider:
- Tissue type
- Anticipated DNA yield
- DNA quality

3610
Choose appropriate lysis buffer and/or additives (i.e., if inhibitors may be present) as needed based on knowledge, experience, workflow preference, method and kit type anticipated for extraction, and/or SOP

3612
Incubate or otherwise leave to digest with heat or agitation

3614
Assess the solution for further analysis (e.g., tissue dissolved or otherwise looks sufficiently ready for analysis; clear, no longer chunky or congealed)

3616
Does the tissue appear ready for further analysis (e.g. dissolved)?

Yes

3624
Document as needed
- Appearance of solution if unusual
- Irregularities

3626
Output: Tube with liquid containing broken down tissue in lysis buffer (possibly with additives that were used to break down tissue)

GO TO 5002

No

3618
Obtain additional information (e.g., literature search, consult colleague, etc.)

Yes

3620
Obtain additional information as needed from applicable source(s)

No

3622
Try another lysis buffer/method?

Yes

RETURN TO 3610

No

3700 - Sample Prep: Liquid Blood (1 of 2)

FROM 2260

3702
Input: Blood (liquid)

3704
Is blood in a vial (e.g., medical vial, lab provided collection vial)?
- Yes → 3714
- No → 3706

3714
Evaluate vial/tube for additives (e.g. anticoagulants; based on color cap on tube in which blood arrived)

3716
Document any additive(s) that may cause potential limitations or other issues with analysis (e.g. heparin or other additive(s)

3718
Is the blood in the appropriate vial for processing?
- Yes → 3720
- No → 3724

3720
Does there appear to be EDTA present?
- Yes → 3722
- No → GO TO 3740

3722
Identify the appropriate (i.e. specialized) extraction technique necessary (e.g., isolate the white blood cells away from the red blood cells)

GO TO 3740

FROM 3906

3706
Is the blood diluted in any way (e.g. mixed with snow, etc.)
- Yes → 3708
- No → GO TO 3740

3708
Concentrate samples into multiple tubes (e.g., if there is a lot of liquid; too much for one tube)?
- Yes → 3712
- No → 3710

3712
Concentrate into multiple tubes as needed using the appropriate method (e.g., filter vs drying down, across multiple tubes if needed)

3710
Concentrate as needed using the appropriate method (e.g., filter vs drying down, across multiple tubes if needed)

GO TO 3740

3724
Communicate with submitter regarding the possible collection of new sample

3726
Am I able to obtain a new/clean/appropriate sample?
- Yes → 3728
- No → GO TO 3740

3728
Send original vial back to submitter?
- Yes → 3730
- No → 3732

3730
Return to submitter

3732
New appropriate item/sample received?
- Yes → GO TO 3740
- No → GO TO 3740

3734
Continue with existing sample?
- Yes → 3740
- No → 3736

3736
Is there more evidence to assess?
- Yes → RETURN TO 1004
- No → 3738

RETURN TO 1004

3738
STOP/Proceed PAP

GO TO 10,000 Reporting

3740
Use knowledge, training, experience, and/or SOP in addition to other factors to determine appropriate amount needed for analysis including any appropriate subsampling as needed

Consider:
- Potential additives (e.g., red top vs. purple top vial)
- Context (e.g., increase number of samples if material is homogeneous however due to context, may represent multiple individuals)
- Number of items submitted for analysis
- Anticipated DNA yield (e.g. mammal vs. non-mammalian)
- DNA quality

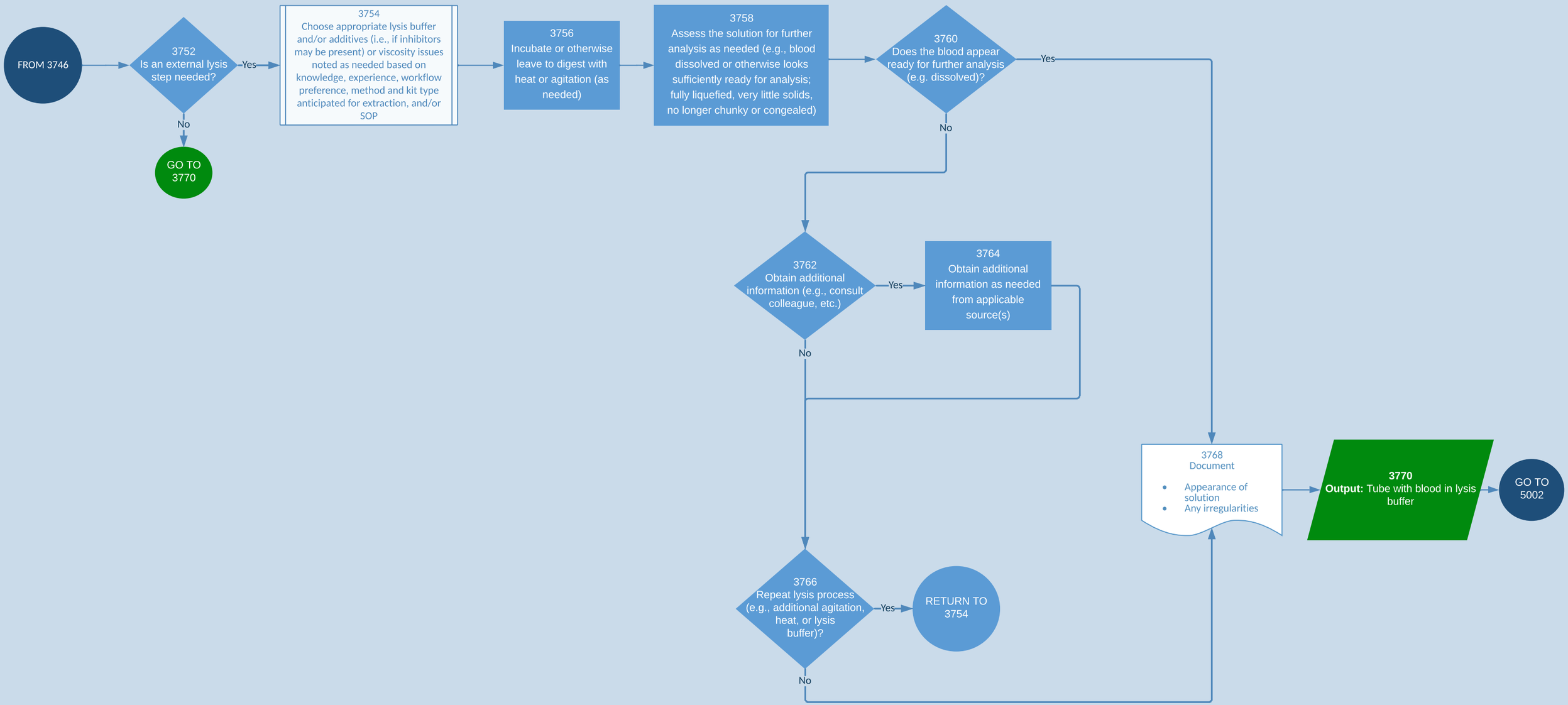
3742
Consume all sample?
- Yes → 3744
- No → 3746

3744
Prepare to consume all sample PAP (including any and notifications)

3746
Document
- Whether all sample was consumed
- Viscosity
- Texture
- Color
- Unusual characteristics (odor etc.)
- Subsampling
  - Number or amount taken from item(s)

GO TO 3752

3750 - Sample Prep: Liquid Blood (2 of 2)



3800 - Sample Prep: Bones/Teeth/Keratin (1 of 2)

FROM 2260

3802
Input:
- Bones
- Teeth
- Antlers
- Keratin based tissue (e.g., hooves, fish slime, claws with no blood attached, horns, tortoiseshell, ivory, etc.).

3804
Is there any grouping or organization that is needed?
- Yes
- No

3806
Separate and/or group items as needed, if possible, based on request (including any specific instructions from submitter), color, pattern, item type, anatomical features, and size, as needed (e.g., antlers, teeth, bone fragments, etc.)

3808
Evaluate any treatment and/or other factors that may affect processing such as:
- Environmental conditions (collection, storage, exposure to elements)
- Stage of growth (e.g., antlers)
- Color
- Porosity**
- Area(s) of blood flow
- Whether boiled* or heated*
- Bleaching

3810
Photograph and/or document?
- Yes
- No

3812
Document and/or photograph as appropriate

3814
Choose item(s) for further analysis (choose all that apply):
- Best sample based on:
  - Request (including specific instructions from submitter)
  - Case information
  - DNA availability and quantity:
    - Morphology, e.g., whether there is:
      - Pulp cavity from teeth
      - Marrow from bone
      - An area of bone with blood supply; i.e., underneath horns etc.
    - Other factors
      - Taphonomic condition (e.g., decay, exposure, treatment, etc.)
      - Size of bone
      - Color of bone
      - How claws have been cut
      - Whether/how much can be destructively sampled
- Representation
  - Random or representative sample as appropriate
  - Specific anatomical elements/features

3816
Assign unique identifier(s)?
- Yes
- No

3820
Assign unique identifiers as appropriate

3822
Wash or otherwise prep before attempting to access DNA?
- Yes
- No

3824
Wash and/or prep the evidence using the appropriate method(s) to assist in removing inhibitors and/or potential contaminants such as exogenous DNA, human DNA, or bacteria (choose all that apply):
- Bleaching
- Sanding
- UV radiation
- Washing
- Cut/drill/saw/smash
- Liquid nitrogen assisted smash

3826
Is there tooth root/pulp or bone marrow from a long bone present?
- Yes
- No

3828
Target the specific region(s) containing the pulp, root, marrow etc. and perform necessary activities to access the area using appropriate method (e.g., pulverize, scrape, drill; saw; dremel; shave; freezer mill; hammer, liquid nitrogen facilitated smashing, etc.)

See Factors for Subsampling

3830
Target the region(s) on the item with highest expected DNA yield as needed in order to subsample based on knowledge, experience, tools available, preference, and/or SOP (e.g., pulverize, scrape, drill; saw; dremel; shave; freezer mill; hammer, liquid nitrogen facilitated smashing, etc.)

See *Factors for Subsampling*

GO TO 3852

Factors for Subsampling

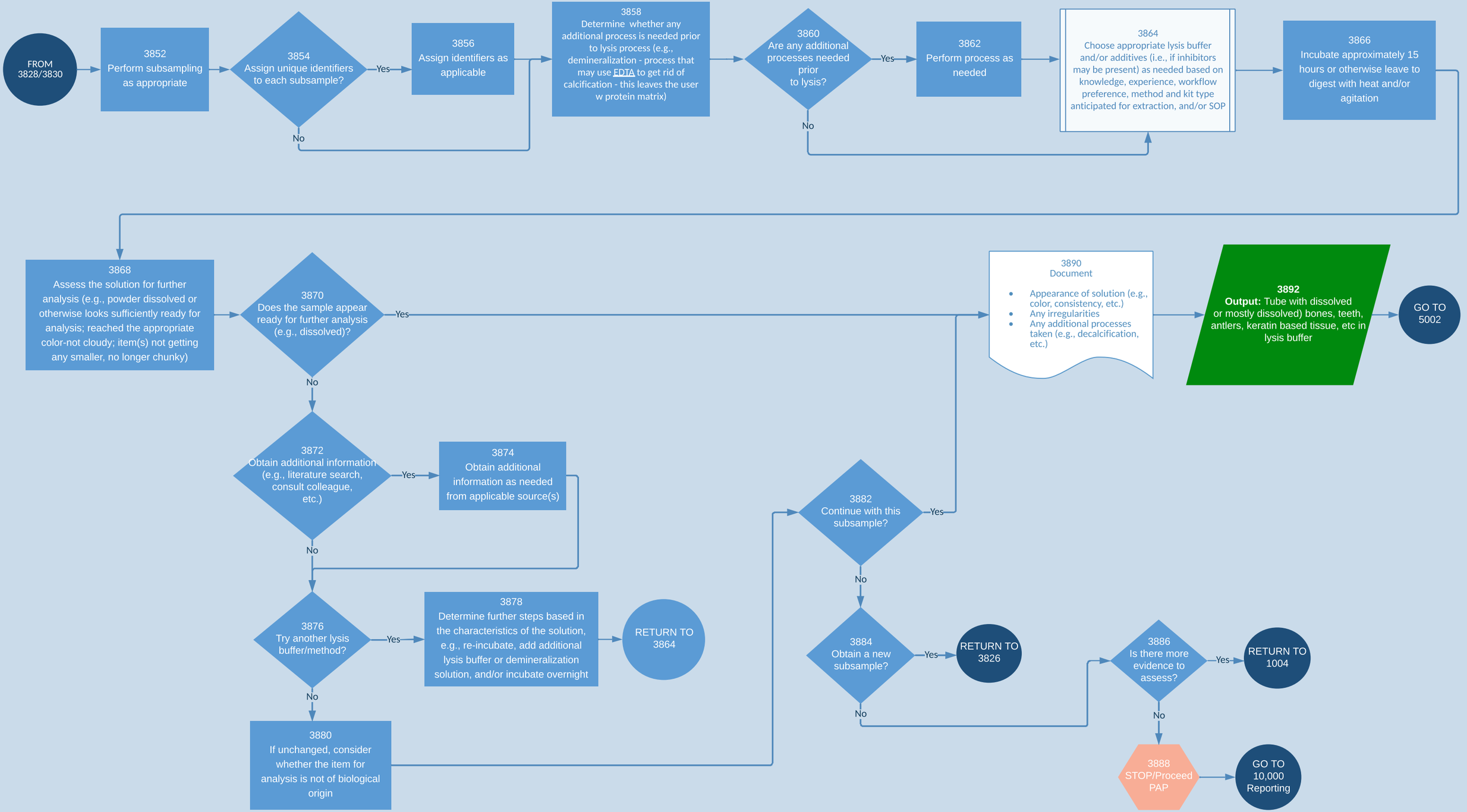
Use knowledge, training, and experience in addition to other factors to determine appropriate subsampling method, size, number of subsamples and/or quantity.

Consider:
- Amount
- Anticipated DNA yield
- DNA quality
- **Porosity
- Any treatment the item may have received (e.g., *boiling or heating etc.)

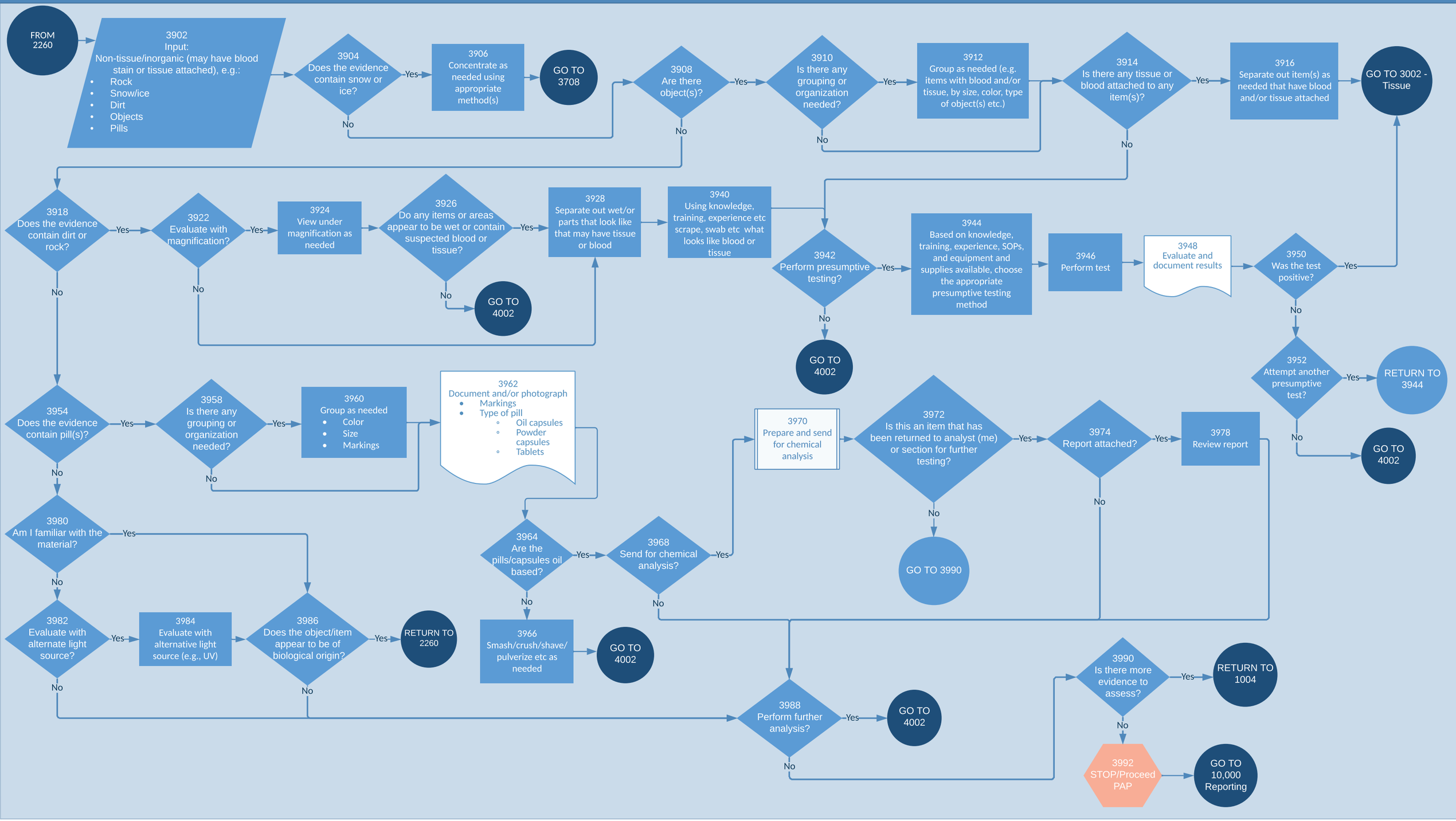
*boiled or heated may affect the position of sampling or area of sampling on the item

**porosity may affect the amount of sample needed for processing

3850 - Sample Prep: Bones/Teeth/Keratin (2 of 2)

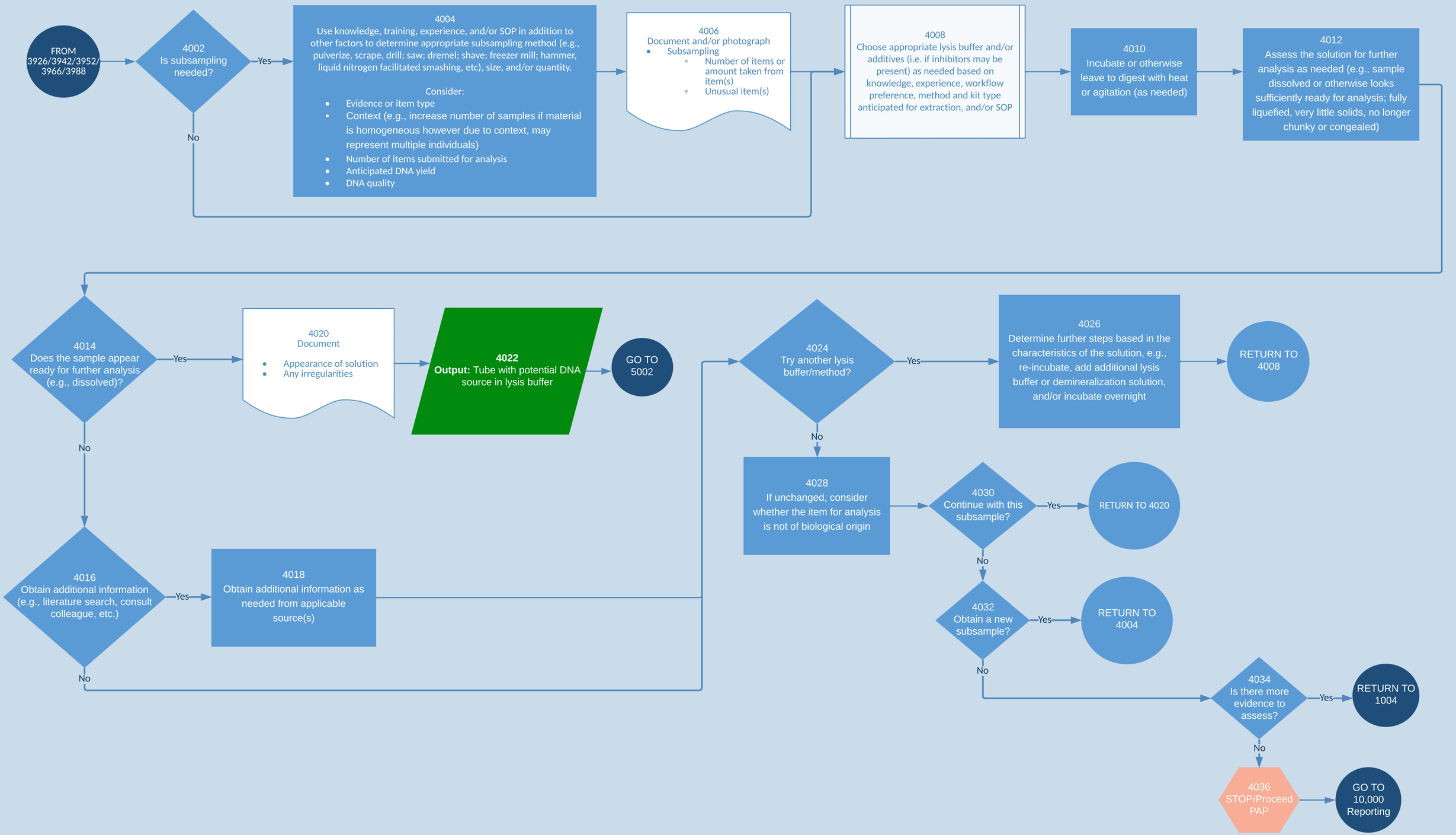


3900 - Sample Prep: Non-Tissue/Inorganic (1 of 2)

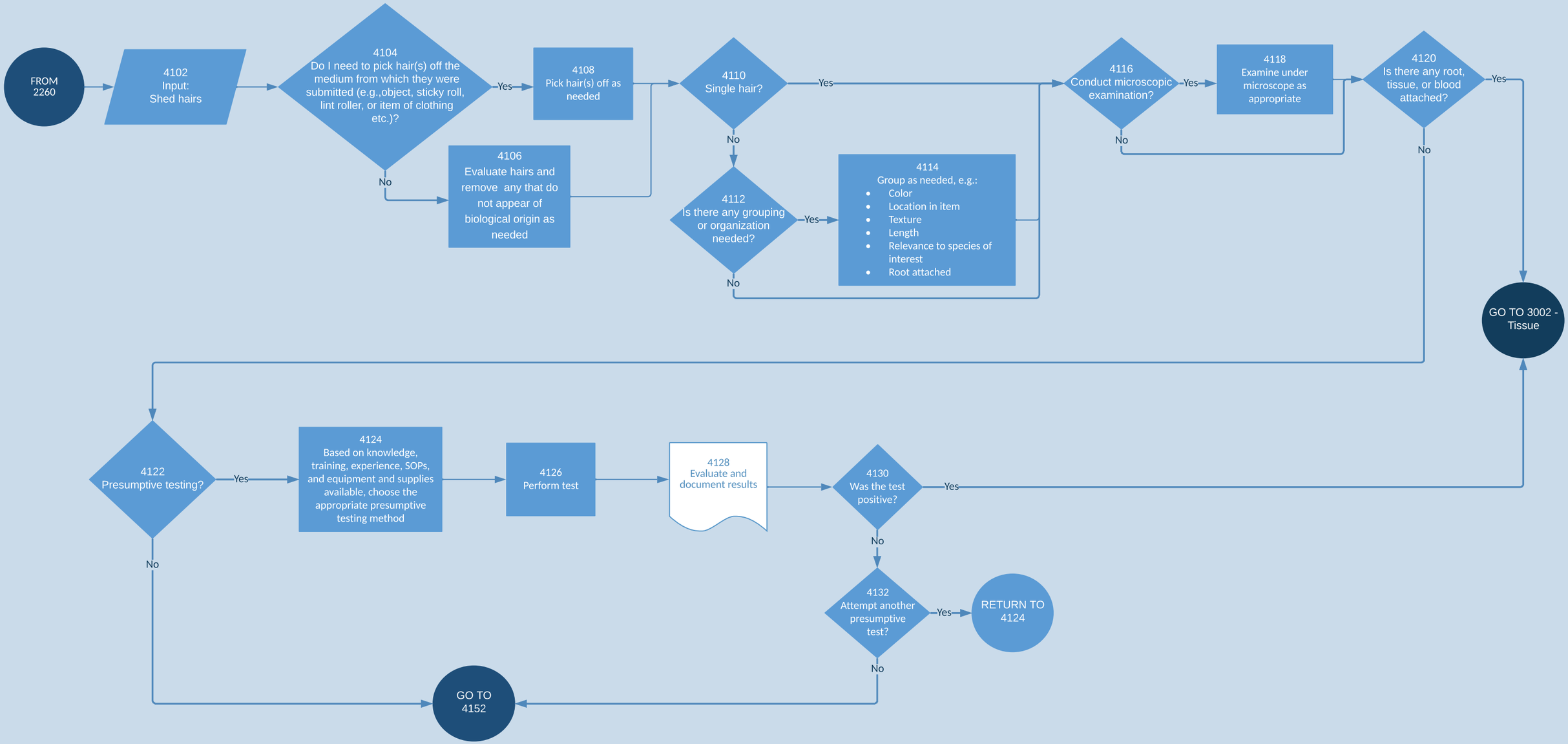


This process map provides a visual description and attempts to represent all reasonable variations of casework currently performed by wildlife biologists. OSAC does not necessarily support or endorse (as best practices) all of the different steps and paths depicted in this process map.

4000 - Sample Prep: Non-Tissue/Inorganic (2 of 2)

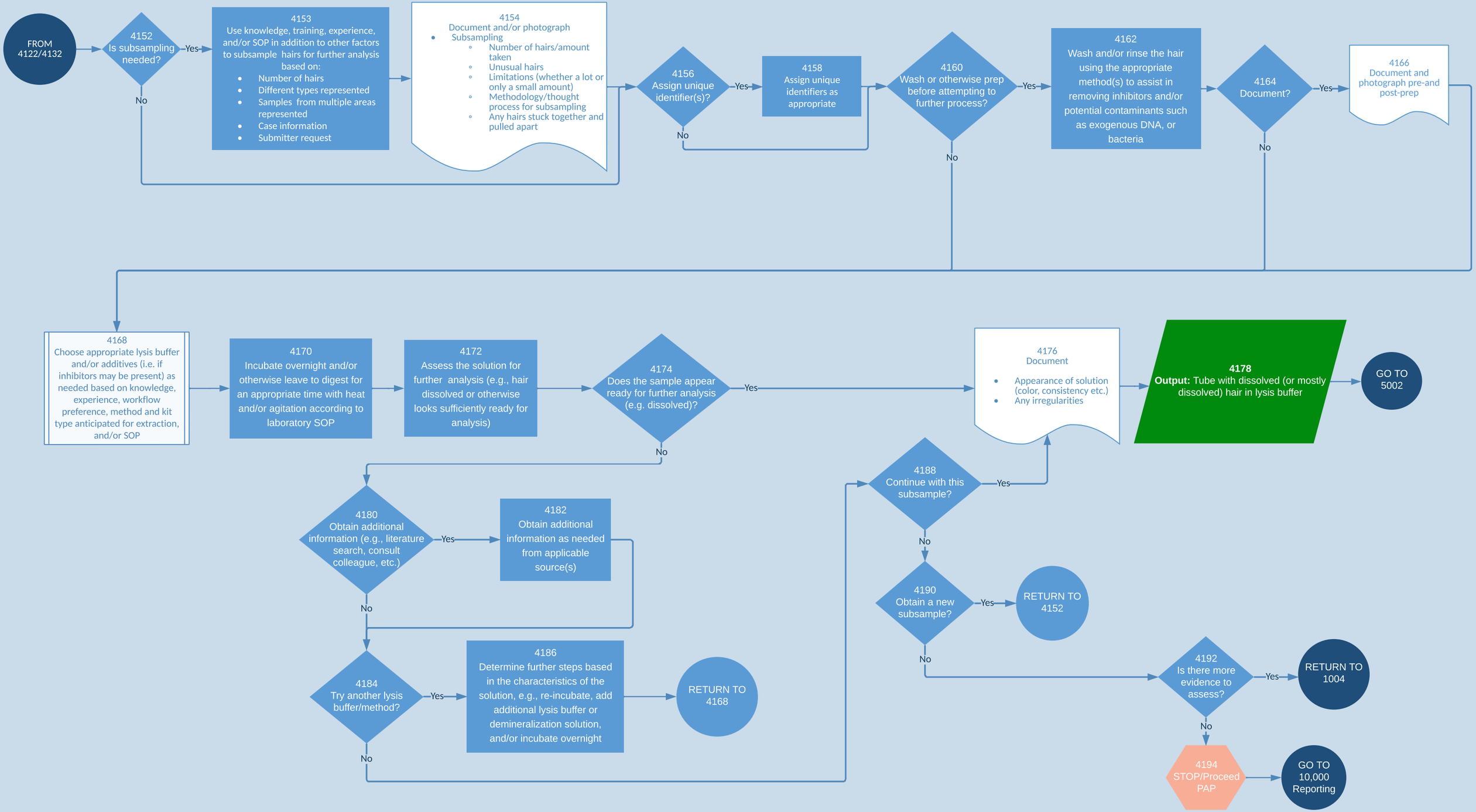


4100 - Sample Prep: Shed Hair (1 of 2)

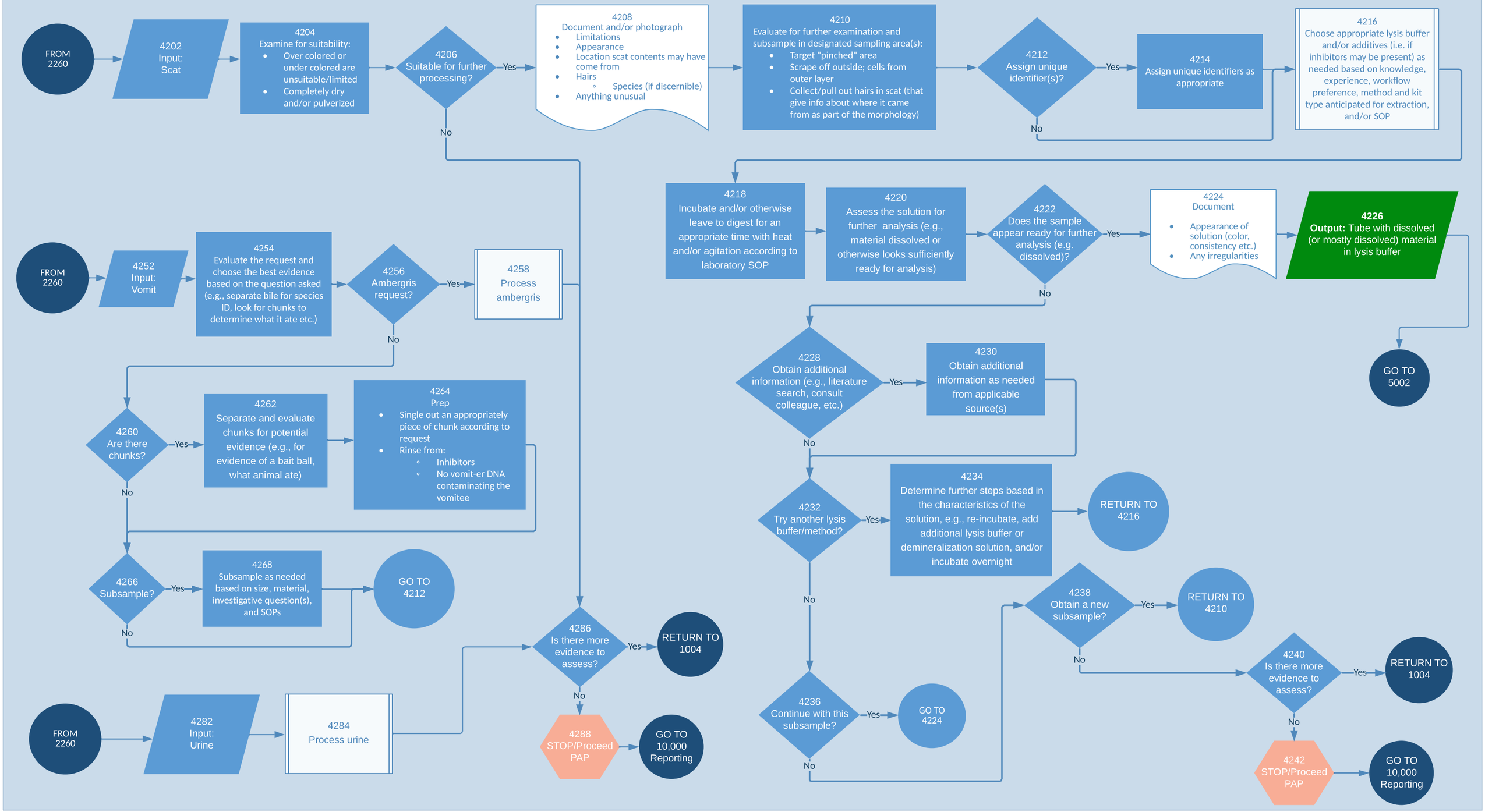


This process map provides a visual description and attempts to represent all reasonable variations of casework currently performed by wildlife biologists. OSAC does not necessarily support or endorse (as best practices) all of the different steps and paths depicted in this process map.

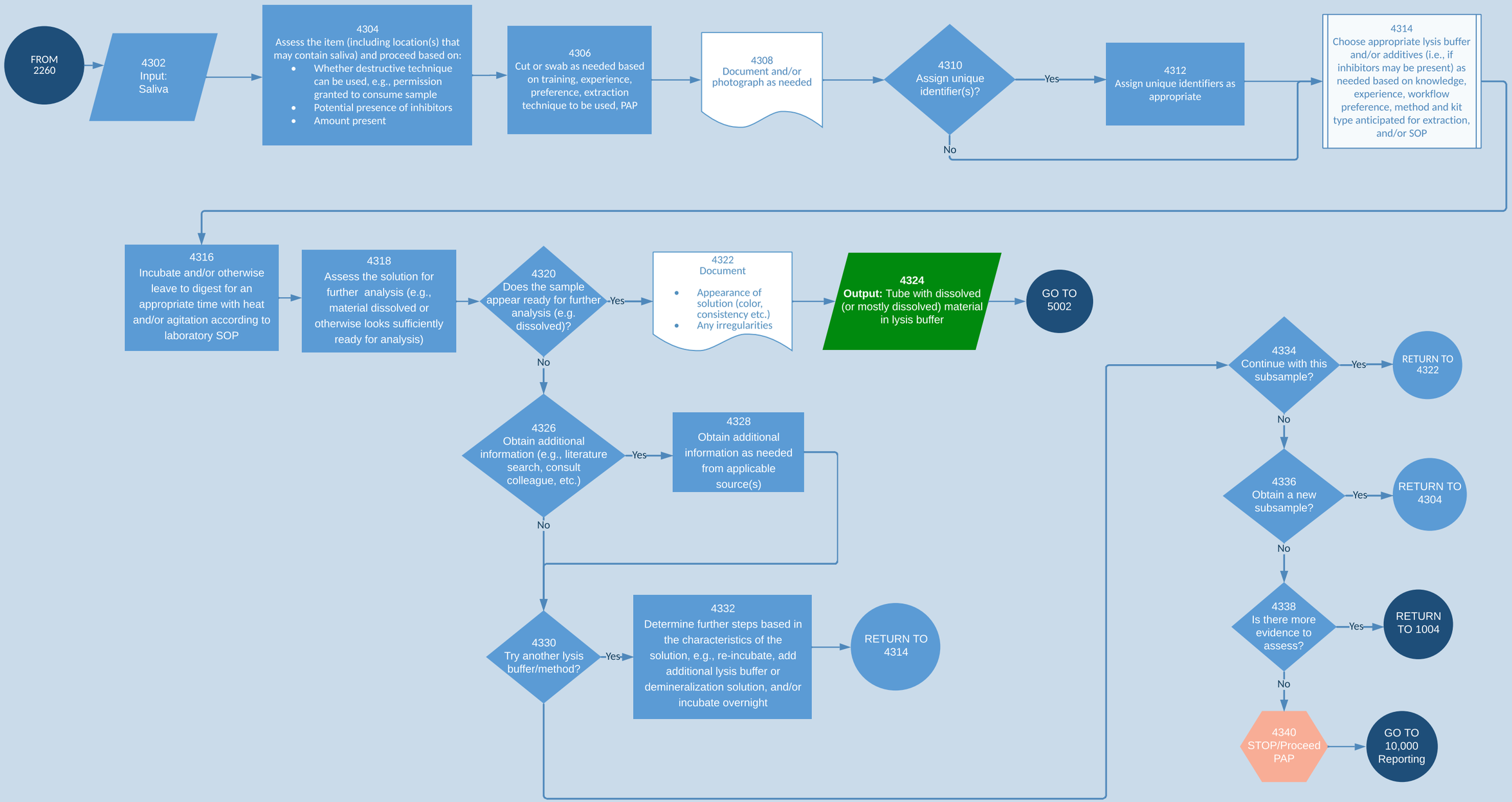
4150 - Sample Prep: Shed Hair (2 of 2)



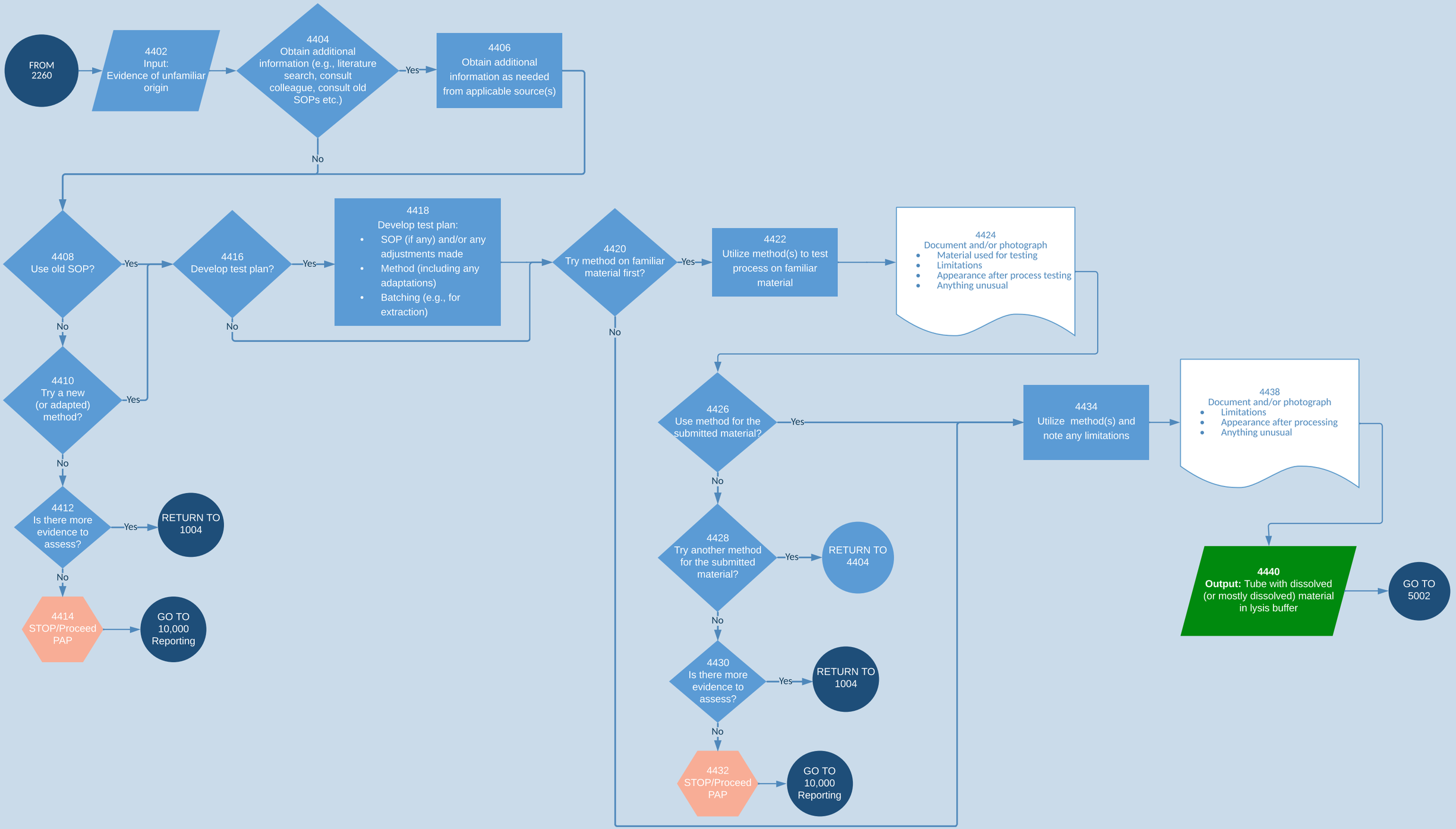
4200 - Sample Prep: Scat, Vomit, Urine



4300 - Sample Prep: Saliva



4400 - Sample Prep: Other/Unfamiliar



5000 - Extraction and Cleanup

FROM 3626/3770/3892/4022/4178/4226/4324/4440

5002
Input: Tube with liquid containing presumed biological material in lysis buffer (possibly w additives that were used to break down tissue)

Prep and Perform Extraction

5004
Assess sample(s) for analysis including available methods for extraction, potential batching, etc.

5006
Use case information, screening results, sample type, and agency policy to identify any samples that may be batched (if any)

5008
Determine appropriate extraction method based on knowledge, training, experience, skill level, sample/material type, potential yield, time constraints, available technology, preference, amount (or how many) of sample available, SOP, testing plan (if applicable), etc.

5010
Take aliquot(s) from tube(s)?
- Yes → 5012
- No → 5014

5012
Determine appropriate volume(s) based on knowledge, training, experience, kit, instrument, extraction method, SOP, etc.

5014
Document and/or photograph?
- Yes → 5016
- No → 5018

5016
Document and/or photograph as appropriate

5018
Any additional PPE needed?
- Yes → 5020
- No → 5022

5020
Determine and acquire appropriate PPE based on knowledge, training, experience, kit, extraction method, SOP, etc.

5022
Are there any additional prep steps needed?
- Yes → 5024
- No → 5026

5024
Perform any sample preparation as needed

5026
Perform extraction method

5028
Document results?
- Yes → 5030
- No → 5032

5030
Document and/or photograph results as appropriate

Evaluate Results and Perform any Troubleshooting

5032
Evaluate and assess solution for suitability based on knowledge, training, experience, SOP, etc. (e.g., for clarity, color, volume, remnants, sample based expectations, instrument data)

5034
Is the solution suitable for further analysis (e.g., is it clear or otherwise suitable)?
- Yes → 5046
- No → 5036

5036
Attempt troubleshooting?
- Yes → 5038
- No → 5046

5038
Based on knowledge, training, experience, SOP, and/or available technology choose appropriate troubleshooting method, e.g.,:
- Add more buffer
- Spin down again
- Scrape and spin down again (low volume)
- Silica cleanup
- Cleanup kit
  - What is available
  - Specific for extraction method
- Extract again with the same or a different kit

5040
Perform troubleshooting steps

5042
Is the solution suitable for further analysis?
- Yes → 5046
- No → 5044

5044
Attempt additional troubleshooting?
- Yes → RETURN TO 5038
- No → 5046

5046
Document and/or photograph as appropriate, e.g.:
- Limitations
- Unusual occurrences
- Inconsistencies
- Any troubleshooting performed (if applicable)

5048
Output: Extracted potential DNA in a tube with buffer and/or other substances (i.e. inhibitors); any potential sample limitations

5050
Quant?
- Yes → GO TO 5102
- No → GO TO 5202

5100 - Quantitation (Quant)

FROM 5050

5102
Input: Extracted presumed DNA in a tube with buffer; any sample limitations

Perform Quant and Evaluate Results

5104
Determine appropriate method for analysis based on knowledge, training, experience, preference, kit, instrument, method, and/or SOP, e.g.:
- Rt-PCR
- Fluorescence
- Spectrophotometry
- Gel or column electrophoresis

5106
Determine appropriate volume(s) to subsample (e.g., 1-2 microliters) based on knowledge, training, experience, kit, instrument, method, SOP etc.

5108
Perform steps for analysis as needed

5110
Evaluate and assess results based on knowledge, training, experience, SOP, etc. (e.g., presence of a value from zero and up; make sure it has numeric value)

5112
Is the sample suitable for further analysis (e.g., is it clear or otherwise suitable)?
- Yes
- No

5114
Attempt troubleshooting?
- Yes
- No

Troubleshooting

5116
Based on knowledge, training, experience, SOP, and/or available technology choose appropriate troubleshooting method. e.g.,:
- Re-dilute
- Concentrate down
- Silica cleanup
- Cleanup kit
  - What is available
  - Specific for extraction method

5118
Perform troubleshooting steps

5120
Is the solution suitable for further analysis?
- Yes
- No

5122
Attempt additional troubleshooting?
- Yes → RETURN TO 5116
- No

5124
Re-quant?
- Yes → RETURN TO 5104
- No

5126
Re-extract?
- Yes → RETURN TO 5002
- No

5128
Document and/or photograph as appropriate, e.g.:
- Limitations
- Unusual occurrences
- Inconsistencies or aberrations
- Purity
- Degree of degradation (if any)
- RNA ratio
- Inhibitors present
- Any troubleshooting steps taken (including cleanup)

5130
Output: Quantity and quality of DNA

GO TO 5202

5200 - Select Method(s) for Analysis

FROM 5050/5130

5202
Input:
- Presumed DNA in tube
- Notations regarding process
- Noted limitations
- Quantity and quality of DNA
- Request type

5204
Using knowledge, training, experience, preference, kit, instrument, method, taxonomy, and/or SOP, determine appropriate method(s) for analysis based on request type:
- Species ID
- Sex ID
- Genotyping activities
  - Matching/Individualization
    - Minimum number of individuals
  - Familial determination
  - Population assignment, e.g.:
    - Pack
    - Farm vs wild
    - Geographic region
    - Temporal distribution

5206
Choose appropriate method(s) for analysis based on request type

GO TO 6002 - Species ID

GO TO 6802 - Sex ID

GO TO 7002 - Matching/ Individualization

GO TO 8002 - Familial Determination

GO TO Population Assignment

6000 - Species ID: Choose Method and qPCR

6000 - Choose Method

FROM 5206/6802/7004/7006/8004/8006

6002
Based on the request type (e.g., looking for a targeted species vs. open ended), preference, and test availability, will I utilize qPCR?

Yes

No

6004
Will sequencing be performed?

Yes → GO TO 6102

No

6006
Perform STR? is there access to a suitable database (e.g., likely to have representative allele frequencies, etc.)

Yes → GO TO 6402

No

6008
STOP/Proceed PAP → GO TO 10,000 Reporting

Perform Testing

6010
Conduct sample prep:
- Determine appropriate aliquot volume(s) to subsample based on test plan, knowledge, training, experience, kit, instrument, method, concentration (if applicable), sample number, SOP, etc.
- Determine appropriate positive control(s) to run with the negative control(s) based on knowledge, training, preference, protocol, etc.

6012
Perform qPCR

6014
Evaluate qPCR results based on experience, training, SOP, assay/panel protocols, e.g.:
- Melting curve
  - Shape
    - Target curve is as expected
    - Later than expected curve
    - No curve/fail
  - Data values
- Control/blank data
  - Any amplification in blanks
  - Failure of positive
  - Location of positive (e.g., whether it is in the right place)

6016
Document as needed (e.g., via notes, software generated imaging, etc.)

Evaluate Results

6018
Did the results meet expectations?

Yes

6022
Document and/or photograph as appropriate, e.g.:
- Limitations
- Unusual occurrences
- Inconsistencies or aberrations

6024
Was the test positive for a/the species of interest?

Yes

6025
Conduct an additional assay for confirmation (e.g., with increased sensitivity)?

Yes → RETURN TO 6002

No

6032
Species of interest identified

No

6026
Conduct an additional assay for confirmation (e.g., with increased sensitivity)?

Yes → RETURN TO 6002

No

6028
Non- Reactive/Unable to identify species

6030
Document as needed (e.g., via notes, software generated imaging, etc.)

No

6020
Choose a different method for species ID?

Yes → RETURN TO 6002

No

6034
Document as needed (e.g., via notes, software generated imaging, etc.)

6036
Are there any other investigative questions to be answered?

Yes → RETURN TO 5206

No

6038
Is there more evidence to assess?

Yes → RETURN TO 1004

No

6040
STOP/Proceed PAP → GO TO 10,000 Reporting

6100 - Species ID: Sequencing (1 of 4)

FROM 6004

6102
Select primer set(s) to use based on expectations (e.g., case, morphological and/or, investigator information; knowledge, etc.)

6104
Is quant data needed to determine template volume or concentration?
- Yes → 6106
- No → 6110

6106
Is quant data available?
- Yes → 6110
- No → 6108

6108
Run quant

6110
Conduct sample prep:
- Determine appropriate aliquot volume(s) to subsample based on test plan, knowledge, training, experience, kit, instrument, method, concentration (if applicable), sample number, SOP, etc.
- Determine appropriate positive and negative control(s) to run based on knowledge, training, preference, protocol, etc.

6112
Run PCR

6114
Check for PCR products?
- Yes → 6116
- No → GO TO 6150

6116
Load and run PCR products on medium in order to visualize

6118
Evaluate PCR results:
- PCR Product detected
  - Product on gel or other medium
- Control/blank data
  - Any amplification in blanks
  - Failure of positive

6120
Document image of gel showing absence or presence of PCR product

6122
Was PCR product detected?
- Yes → GO TO 6150
- No → 6124

6124
Go to sequencing anyway?
- Yes → GO TO 6150
- No → 6126

6126
Troubleshoot?
- Yes → 6140
- No → 6128

6128
Non- Reactive/Unable to identify species

6130
Document as needed (e.g., via notes, software generated imaging, etc.)

6132
Perform any additional species ID testing methods?
- Yes → RETURN TO 6002
- No → 6134

6134
Are there any other investigative questions to be answered?
- Yes → RETURN TO 5206
- No → 6136

6136
Is there more evidence to assess?
- Yes → RETURN TO 1004
- No → 6138

6138
STOP/Proceed PAP → GO TO 10,000 Reporting

6140
Re-extract?
- Yes → 6144
- No → 6142

6142
Select and perform appropriate troubleshooting steps for individual samples as needed:
- Re-amplify using-
  - Same settings (e.g., material and equipment parameters and volume etc.)
  - Different settings (e.g., cleanup, change thermocycler settings, change mastermix, volume, modify DNA concentrations etc.)

→ RETURN TO 5110

6144
Create new subsample?
- Yes → 6146
- No → GO TO 5002

6146
Choose and return to the appropriate map page for applicable sample type to perform subsampling and other steps as necessary:

- GO TO 3604 - Tissue
- GO TO 4210 - Scat
- GO TO 3740 - Blood (liquid)
- GO TO 4268 - Vomit
- GO TO 3826 - Bones, claws, teeth, and keratin based
- GO TO 4284 - Urine
- GO TO 4004 - Non-Tissue/ Inorganic
- GO TO 4304 - Saliva
- GO TO 4152 - Shed Hair
- GO TO 4404 - Other/ Unfamiliar

6150 - Species ID: Sequencing (2 of 4)

FROM 6114/6122/6124

6152 Perform PCR cleanup

6154 Set up sequencing reaction

6156 Load and run sample(s) for sequencing on capillary or gel based electrophoresis medium in order to visualize

6158 Evaluate sequencing results:
- Whether there is data present, including distinguishable bases
- Control/blank data
  - Any interpretable sequence in blanks
  - Failure of positive

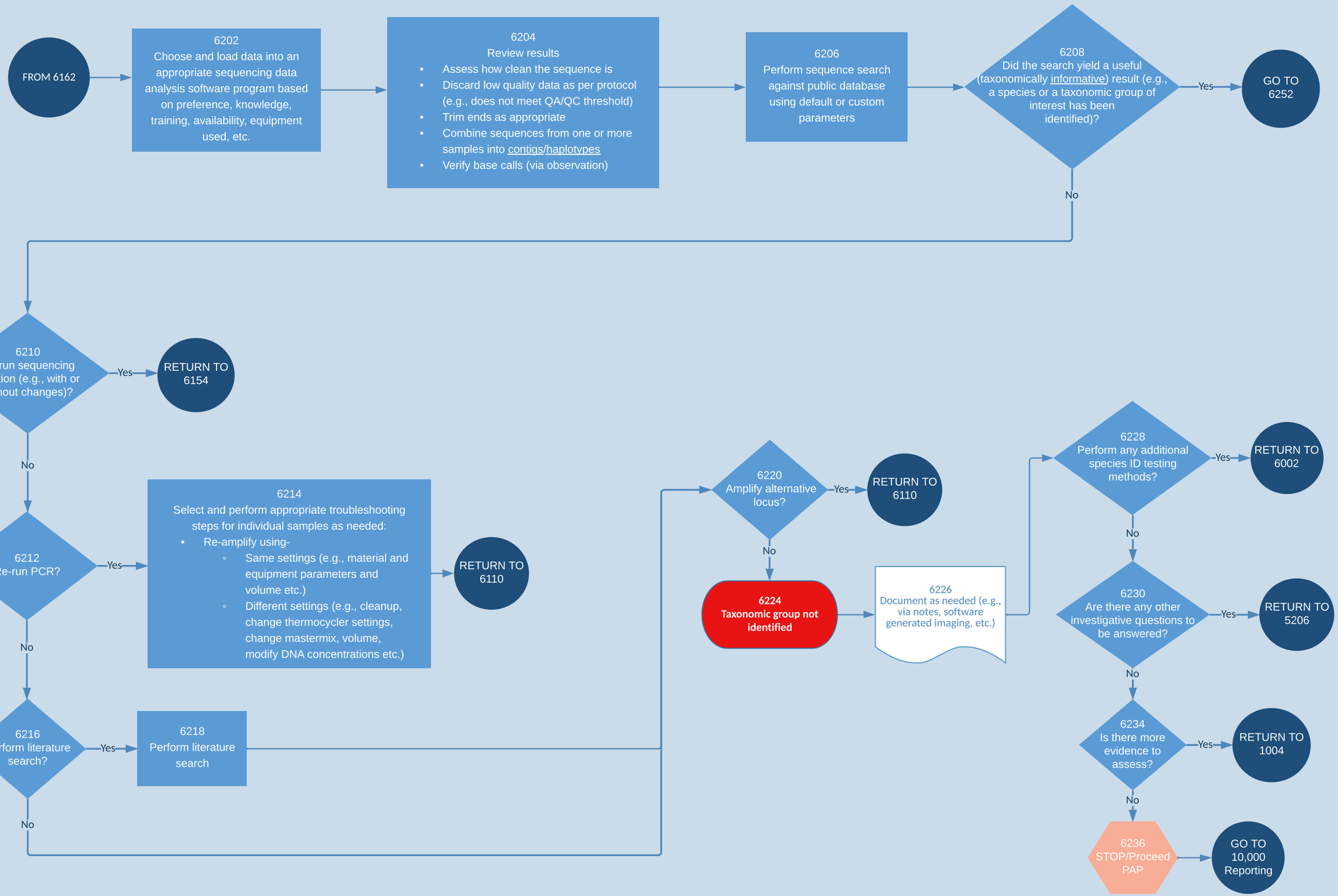
6160 Document as appropriate (e.g., via notes, software generated images, etc.)

6162 Is the sample data suitable to continue (e.g., controls performed as expected, sequence data appears adequate)?
- Yes → GO TO 6202
- No → 6164 Re-run sequencing reaction?
  - Yes → RETURN TO 6154
  - No → 6166 Troubleshoot?
    - Yes → 6180 Re-inject existing sequencing product on genetic analyzer?
      - Yes → 6182 Select and perform appropriate troubleshooting steps for individual samples as needed:
        - **Re-inject** existing sequencing product on genetic analyzer using-
          - Same settings (e.g., existing equipment parameters)
          - Different settings (e.g., run time, injection time, etc.)

        → RETURN TO 6158
      - No → 6184 Re-run PCR?
        - Yes → 6186 Select and perform appropriate troubleshooting steps for individual samples as needed:
          - Re-amplify using-
            - Same settings (e.g., material and equipment parameters and volume etc.)
            - Different settings (e.g., cleanup, change thermocycler settings, change mastermix, volume, modify DNA concentrations etc.)

          → RETURN TO 6110
        - No → 6188 Create new subsample and re-extract → 6190 Choose and return to the appropriate map page for applicable sample type to perform subsampling and other steps as necessary:
          - GO TO 3604 - Tissue
          - GO TO 4210 - Scat
          - GO TO 3740 - Blood (liquid)
          - GO TO 4268 - Vomit
          - GO TO 3826 - Bones, claws, teeth, and keratin based
          - GO TO 4284 - Urine
          - GO TO 4004 - Non-Tissue/ Inorganic
          - GO TO 4304 - Saliva
          - GO TO 4152 - Shed Hair
          - GO TO 4404 - Other/ Unfamiliar
    - No → 6168 **Non-reactive/No results were obtained** → 6170 Document as needed (e.g., via notes, software generated imaging, etc.) → 6172 Perform any additional species ID testing methods?
      - Yes → RETURN TO 6002
      - No → 6174 Are there any other investigative questions to be answered?
        - Yes → RETURN TO 5206
        - No → 6176 Is there more evidence to assess?
          - Yes → RETURN TO 1004
          - No → 6178 STOP/Proceed PAP → GO TO 10,000 Reporting

6200 - Species ID: Sequencing (3 of 4)



6250 - Species ID: Sequencing (4 of 4)

FROM 6208

6252 Accept results from search as final?
- Yes → **6254 Species or taxonomic group has been identified**
- No → 6280

6256 Document as needed (e.g., via notes, software generated imaging, etc.)

6258 Perform any additional species ID testing methods?
- Yes → RETURN TO 6002
- No → 6260

6260 Are there any other investigative questions to be answered?
- Yes → 6262
- No → GO TO "Is there more evidence to assess?'

6262 Was matching requested?
- Yes → 6264
- No → RETURN TO 5206

6264 Is there information regarding a known (e.g., targeted) species?
- Yes → 6266
- No → 6274

6266 Did any samples originate from the targeted species?
- Yes → 6268
- No → 6274

6268 Did all the samples provided originate from the same species?
- Yes → GO TO 7000 Matching
- No → 6270

6270 Group samples by species, e.g., those from targeted species vs. samples that are not of the targeted species

6272 Document species present in the samples including those that may or may not proceed to matching

6274 Proceed to matching, e.g., for a potential intercomparision of evidentiary samples?
- Yes → 6266
- No → 6276

6276 Is there more evidence to assess?
- Yes → RETURN TO 1004
- No → 6278

6278 STOP/Proceed PAP → GO TO 10,000 Reporting

6280 Is an in-house database available for further analysis?
- Yes → 6282
- No → 6302

6282 Align evidence samples to the database → 6284

6284 Is the alignment suitable?
- Yes → 6292
- No → 6286

6286 Change alignment parameters?
- Yes → 6288 Change alignment parameters → 6284
- No → 6290 Manually edit → 6284

6292 Assess genetic distance?
- Yes → 6294 Assess genetic distance → 6296
- No → 6296

6296 Build phylograms (tree building)?
- Yes → 6298 Build phylograms (tree building) → 6300
- No → 6300

6300 Consider factors for determining possible species identification based on knowledge, experience, and expertise, e.g.:
- Genetic distances (e.g., inter- and intra-species distance)
- Uniqueness of species of interest (ex. only one species in a genus)
- Position on phylogram
- Evolutionary history of the species of interest
- Any known geographic origin
- Completeness of database

6302 Based on the analysis, and appropriate parameters (e.g., inter- and intra- species distances, uniqueness, etc.), has the species been identified?
- Yes → **6304 Species or taxonomic group has been identified** → 6306
- No → 6308

6306 Document as needed (e.g., via notes, software generated imaging, etc.) → GO TO 6258

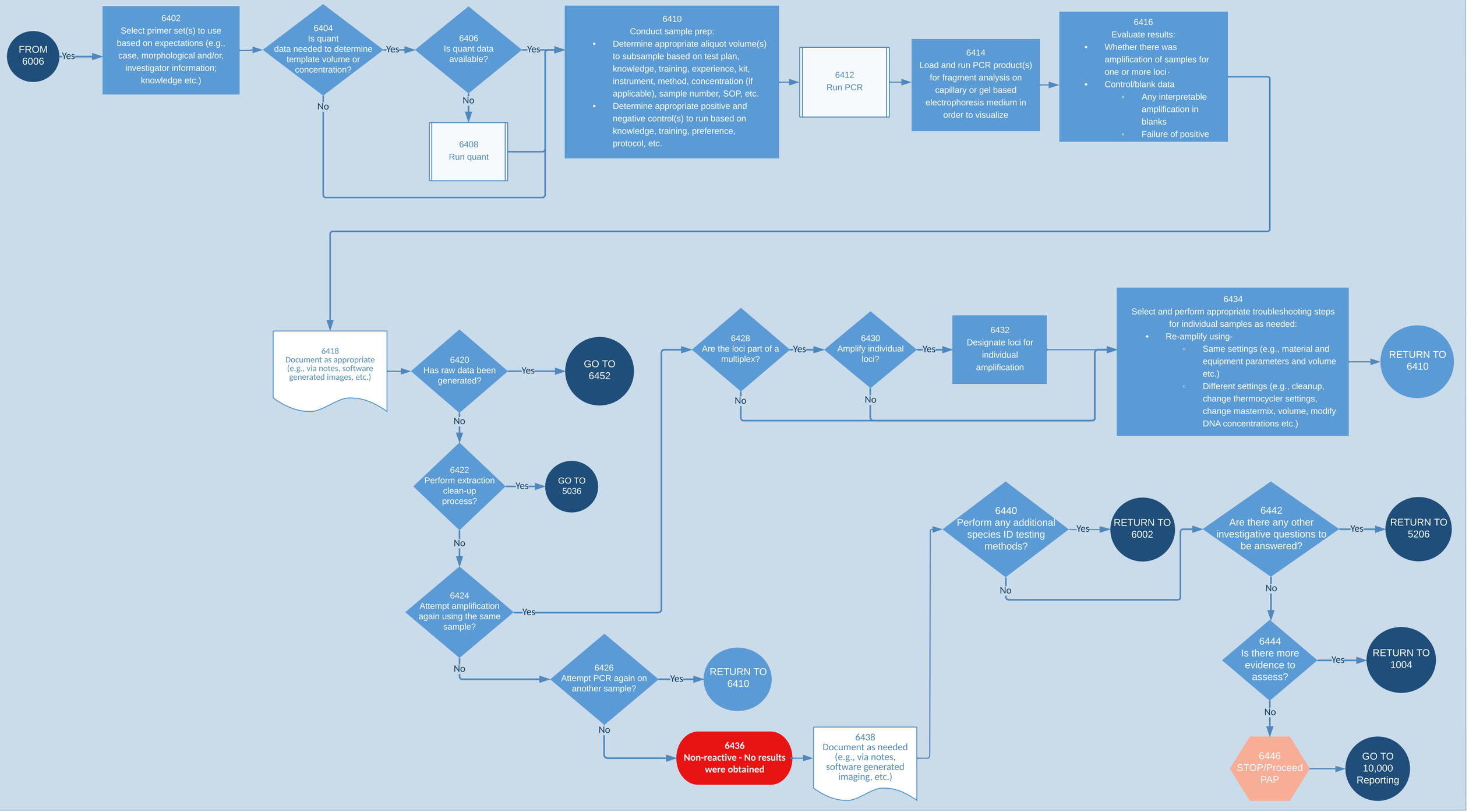
6308 Based on case info, is testing for population assignment necessary to determine species?
- Yes → GO TO 9008 Population Assignment
- No → 6310

6310 ID at a higher taxonomic level now?
- Yes → **6312 Report at a higher taxonomic level** → 6306
- No → 6314

6314 Is another database available (e.g., via colleague, etc.)?
- Yes → GO TO 6282
- No → **6316 Taxonomic group not identified** → 6318

6318 Document as needed (e.g., via notes, software generated imaging, etc.) → GO TO 6258

6400 - Species ID: STR (1 of 4)



6450 - Species ID: STR (2 of 4)

FROM 6420

6452
Choose and load data into an appropriate data analysis software program for QA/QC based on preference, knowledge, training, availability, equipment used, etc.

6454
Review results
- Assess the quality of data
- Discard low quality data as per protocol (e.g., does not meet QA/QC threshold)
- Verify size calls of fragment(s) (via observation)
- Evaluate completeness of profile

6456
Run data in allele call/software tool

6458
Review and edit resulting allele calls

6460
Based on results, does the sample appear to be free of contamination, including no evidence of a mixture, etc.?
- Yes → 6462
- No → 6464

6462
Are results otherwise suitable to proceed?
- Yes → GO TO 6510
- No → 6464

6464
Are there multiple runs from the sample (from my single lab item)?
- Yes → 6466
- No → 6470

6466
Combine to create a consensus profile (e.g., will creating a consensus profile create a more complete genotype)?
- Yes → 6468
- No → 6470

6468
Review generated report from allele call software and create consensus profile

6470
Does the consensus profile meet analysis criteria based on validation?
- Yes → **6510 Output:** Output(s) may include:
  - Suitable genotype for species ID
  - **Selected genetic database**
  → GO TO 6552
- No → 6472

6472
Troubleshoot?
- Yes → 6474
- No → 6478

6474
Re-load existing PCR product on genetic analyzer?
- Yes → 6476
- No → 6490

6476
Choose appropriate troubleshooting steps for individual samples as needed:
- Re-load existing PCR product on genetic analyzer using-
  - Same settings (e.g., material and equipment parameters and volume etc.)
  - Different settings (e.g., run time, injection time, etc.)

→ RETURN TO 6414

6478
No interpretable genotype was obtained - Taxonomic group not identified

6480
Document as needed (e.g., via notes, software generated imaging, etc.)

6482
Perform any additional species ID testing methods?
- Yes → RETURN TO 6002
- No → 6484

6484
Are there any other investigative questions to be answered?
- Yes → RETURN TO 5206
- No → 6486

6486
Is there more evidence to assess?
- Yes → RETURN TO 1004
- No → 6488

6488
STOP/Proceed PAP
→ GO TO 10,000 Reporting

6490
Re-run PCR?
- Yes → 6502
- No → 6492

6502
Are the loci part of a multiplex?
- Yes → 6504
- No → 6508

6504
Amplify individual loci?
- Yes → 6506
- No → 6508

6506
Designate loci for individual amplification

6508
Select and perform appropriate troubleshooting steps for individual samples as needed:
- Re-amplify using-
  - Same settings (e.g., material and equipment parameters and volume etc.)
  - Different settings (e.g., cleanup, change thermocycler settings, change mastermix, volume, modify DNA concentrations etc.)

→ GO TO 6410

6492
Perform extraction clean-up process?
- Yes → GO TO 5036
- No → 6494

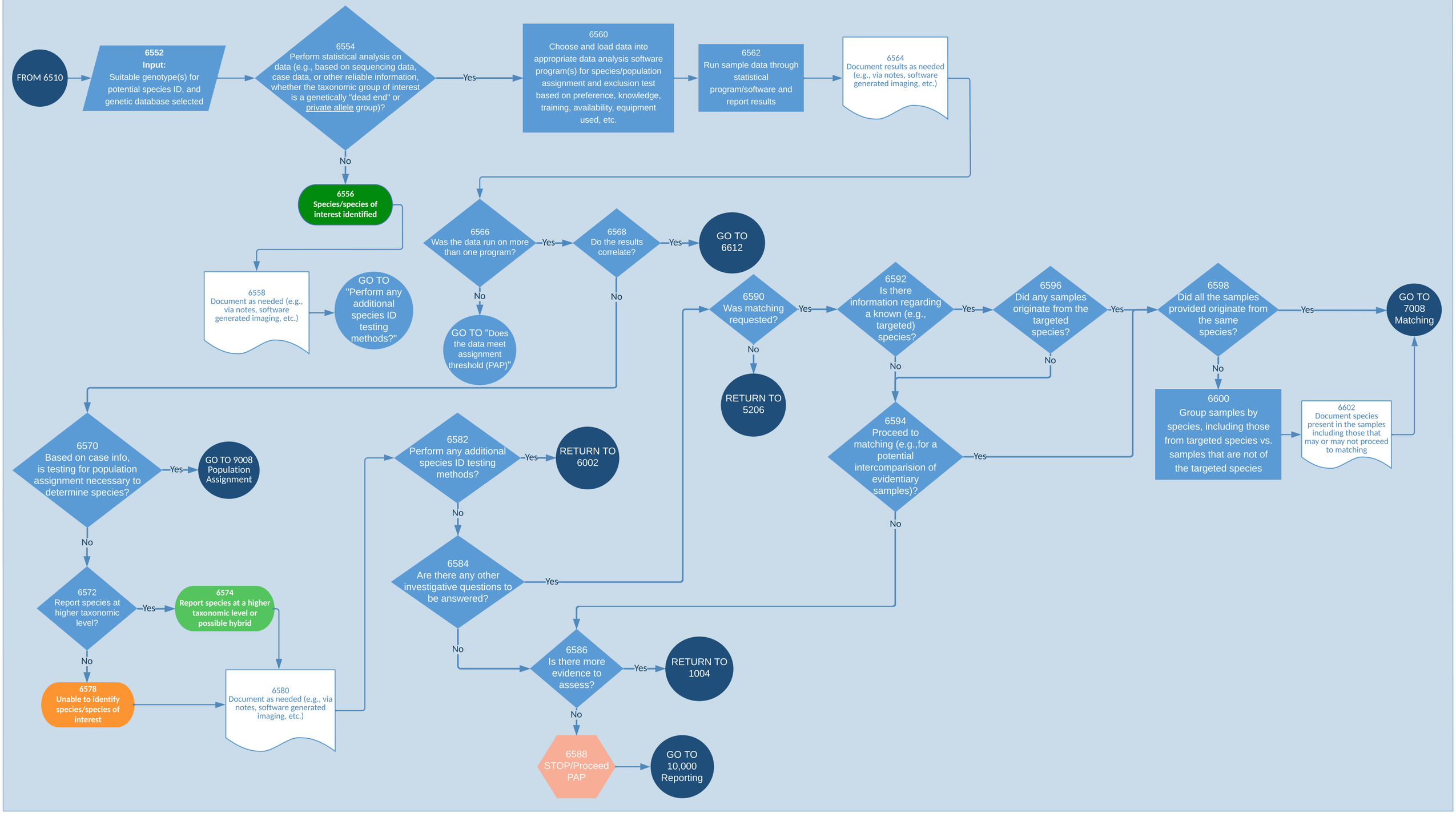
6494
Perform PCR cleanup?
- Yes → 6496 Perform PCR cleanup → GO TO 6410
- No → 6498

6498
Create new subsample and re-extract

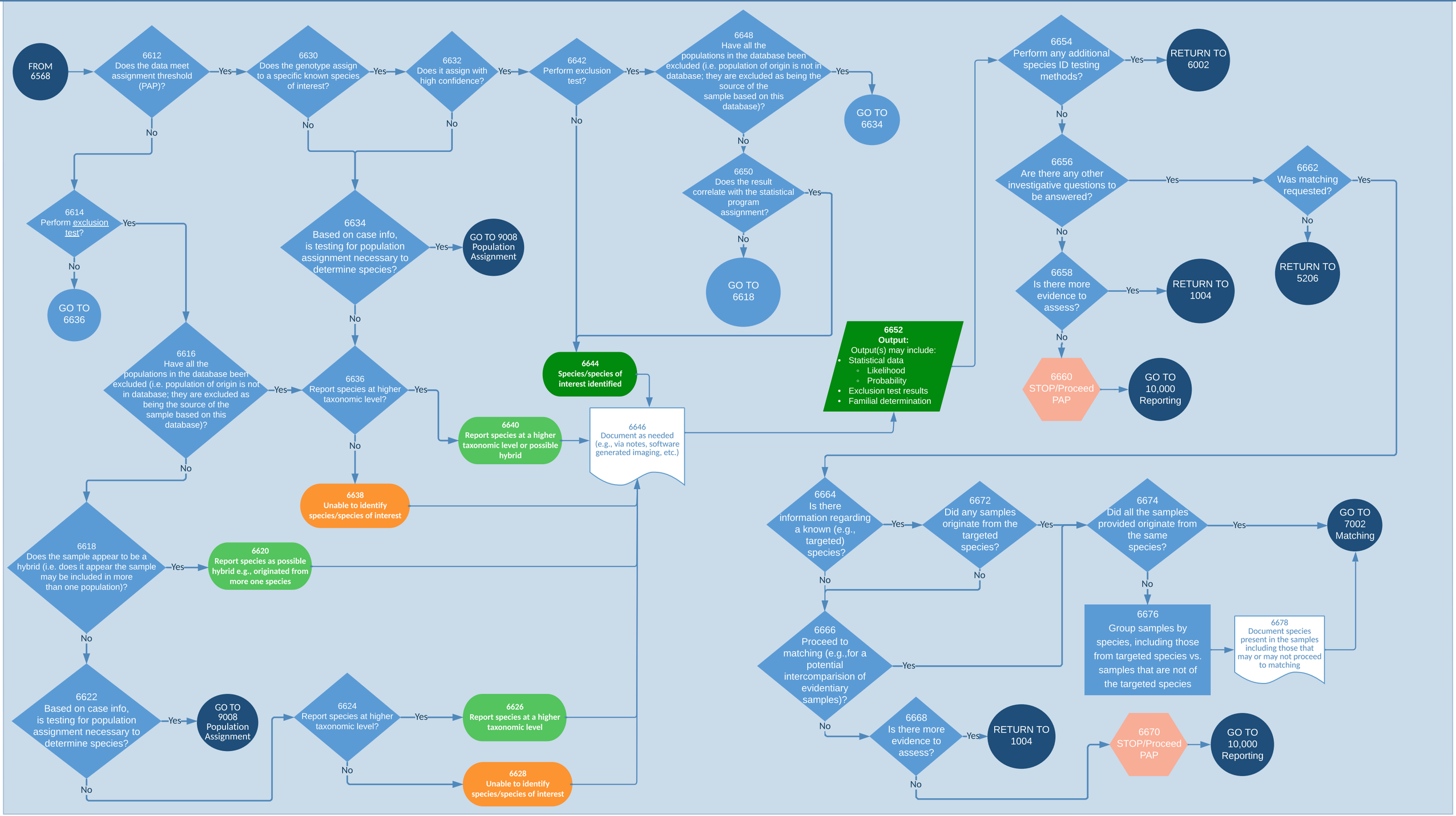
6500
Choose and return to the appropriate map page for applicable sample type to perform subsampling and other steps as necessary:

- GO TO 3604 - Tissue
- GO TO 4210 - Scat
- GO TO 3740 - Blood (liquid)
- GO TO 4268 - Vomit
- GO TO 3826 - Bones, claws, teeth, and keratin based
- GO TO 4284 - Urine
- GO TO 4004 - Non-Tissue/ Inorganic
- GO TO 4304 - Saliva
- GO TO 4152 - Shed Hair
- GO TO 4404 - Other/ Unfamiliar

6550 - Species ID: STR (3 of 4)



6610 - Species ID: STR (4 of 4)



6800 - Sex ID

FROM 5206

6802
Already know the species?

Yes

No

GO TO 6002

6804
Select marker(s) (e.g., y-STR or other type of marker) to use based on species and expectations

6806
Conduct sample prep:
- Determine appropriate aliquot volume(s) to subsample based on test plan, knowledge, training, experience, kit, instrument, method, concentration (if applicable), sample number, SOP, etc.
- Determine appropriate positive control(s) to run with the negative control(s) based on knowledge, training, preference, protocol, etc.

6808
Run PCR

6810
Load and run PCR products on capillary or gel based electrophoresis medium in order to visualize

6812
Evaluate PCR results via:
- PCR product(s) detected
  - Product on gel or other medium
  - Size and number of band(s)/peak(s)
- Control/blank data
  - Any amplification in blanks
  - Failure of positive
  - Location of positive (e.g., whether it is in the right place within range of peaks)

6814
Document as appropriate (e.g., via notes, software generated images, etc.)

6816
Did the results meet expectations?

Yes

No

6818
Troubleshoot?

Yes

6822
Choose appropriate troubleshooting steps:
- Re-run (i.e. new aliquot using same settings - material and equipment parameters and volume etc)
- Change settings/change mastermix
- Modify DNA concentrations

RETURN TO 6806

No

6820
STOP/Report PAP

6824
Document results via notes, software generated imaging, etc., e.g.:
- Whether controls worked
- Sex ID

6826
Can the sex be identified (e.g., via sex markers)?

Yes

6830
Sex identified

No

6828
Unable to identify sex

6832
Document as needed (e.g., via notes, software generated imaging, etc.)

6834
Are there any other investigative questions to be answered?

Yes

RETURN TO 5206

No

6836
Is there more evidence to assess?

Yes

RETURN TO 1004

No

6838
STOP/Proceed PAP

GO TO 10,000 Reporting

7000 - Matching/Individualization: Initial Assessment

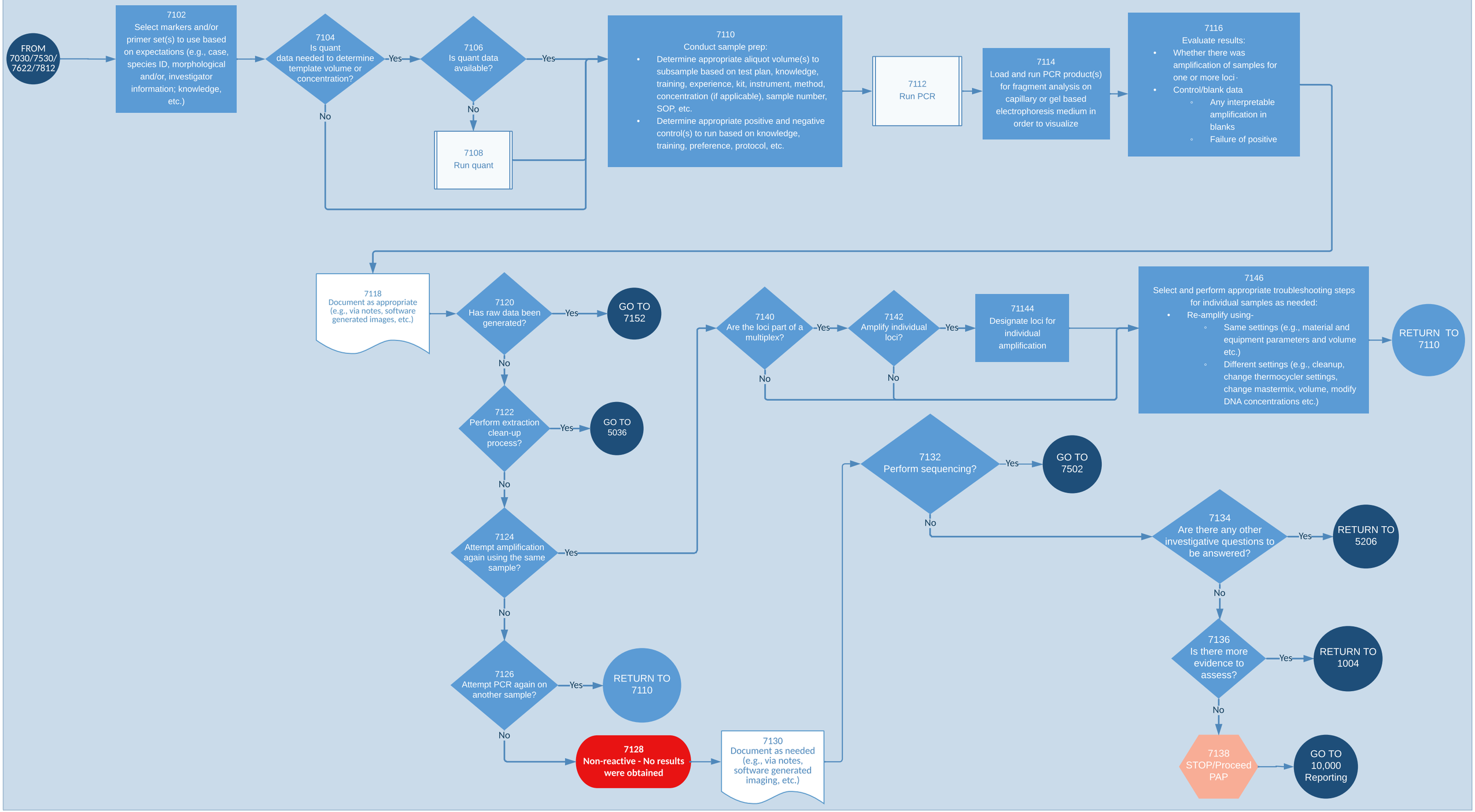
FROM TO 5206

7002 Is/are the species of all the sample(s) submitted for analysis known?
- Yes → FROM 6674/6678
- No → 7004 Is case information available indicating the species?
  - Yes → 7006 Perform species ID anyway?
    - Yes → GO TO 6002
    - No → 7008 Based on available information, is there access to a suitable database (e.g., likely to have representative allele frequencies, etc.)?
      - Yes → 7014 Is there a known sample for comparison?
        - Yes → 7016 Do I already have STR or sequencing data, e.g., from species ID, population, etc.?
          - Yes → 7018 Use the same data generated?
            - Yes → 7020 Use STR data?
              - Yes → GO TO 7302
              - No → GO TO 7802
            - No → Choose Method
          - No → Choose Method
        - No → 7022 Is a known sample necessary for comparison (e.g., as per request, case info, evidence samples submitted, etc.)?
          - Yes → 7024 Communicate with requestor regarding obtaining known
            - → 7026 Wait for known (e.g., Is a known sample necessary for comparison)?
              - Yes → 7028 Has known been obtained?
                - Yes → Choose Method
                - No → 7032 Continue waiting for known?
                  - Yes → RETURN TO 7028
                  - No → 7034 Have multiple unknowns been submitted?
              - No → 7034 Have multiple unknowns been submitted?
          - No → 7034 Have multiple unknowns been submitted?
        - 7034 Have multiple unknowns been submitted?
          - Yes → 7036 Intercompare the submitted unknowns?
            - Yes → GO TO 7030
            - No → 7038 Process for future comparison (or to otherwise obtain sample data for investigative purposes)?
              - Yes → GO TO 7030
              - No → 7040 STOP/Hold for future analysis
                - → 7042 Has any previous testing been run on the sample? (i.e. do I have anything to report?)
                  - Yes → **7048 Some results obtained; Unable to further test for matching or minimum number of individuals** → 7050 Document any testing information as appropriate (e.g., via notes, etc.)
                  - No → **7044 Unable to further test for matching or minimum number of individuals** → 7046 Document as appropriate (e.g., via notes, etc.)
                - 7050 / 7046 → 7052 Are there any other investigative questions to be answered?
                  - Yes → RETURN TO 5206
                  - No → 7054 Is there more evidence to assess?
                    - Yes → RETURN TO 1004
                    - No → 7056 STOP/Proceed PAP → GO TO 10,000 Reporting
    - No → 7010 Proceed with further laboratory analysis anyway (PAP) (e.g., case will proceed without statistical support, or a database may not be necessary based on case data)?
      - Yes → 7008
      - No → 7012 STOP/Hold for future analysis → GO TO "Are there any other investigative questions to be answered?"
- No → GO TO 6002

Choose Method
- 7030 Perform STR?
  - Yes → GO TO 7102
  - No → GO TO 7502

This process map provides a visual description and attempts to represent all reasonable variations of casework currently performed by wildlife biologists. OSAC does not necessarily support or endorse (as best practices) all of the different steps and paths depicted in this process map.

7100 - Matching/Individualization: Choose Method/STR (1 of 4)



This process map provides a visual description and attempts to represent all reasonable variations of casework currently performed by wildlife biologists. OSAC does not necessarily support or endorse (as best practices) all of the different steps and paths depicted in this process map.

7150 - Matching/Individualization: STR (2 of 4)

FROM Matching STR P1

7150
Choose and load data into an appropriate data analysis software program for QA/QC based on preference, knowledge, training, availability, equipment used, etc.

7152
Review results
- Assess the quality of data
- Discard low quality data as per protocol (e.g., does not meet QA/QC threshold)
- Verify size calls of fragment(s) (via observation)
- Evaluate completeness of profile

7154
Run data in allele call/software tool

7156
Review and edit resulting allele calls

7158
Based on results, does the sample appear to be free of contamination?
- Yes → 7160
- No → 7168

7160
Does there appear to be a mixture?
- Yes → 7162
- No → 7164

7162
Treat as a single source?
- Yes → 7164
- No → GO TO 7302

7164
Are results otherwise suitable to proceed (e.g., appropriate number of diagnostic loci,)?
- Yes → 7166
- No → 7168

7166
Evaluate another item?
- Yes → GO TO 7102
- No → GO TO 7206

7168
Troubleshoot?
- Yes → 7174
- No → 7170

7170
Non-reactive/Inconclusive/No interpretable genotype was obtained /Unable to proceed with matching/Unable to detect minimum number of individuals

7172
Document as needed (e.g., via notes, software generated imaging, etc.)

RETURN TO 7132

7174
Re-load existing PCR product on genetic analyzer?
- Yes → 7176
- No → 7178

7176
Choose appropriate troubleshooting steps for individual samples as needed:
- Re-load existing PCR product on genetic analyzer using-
  - Same settings (e.g., material and equipment parameters and volume etc.)
  - Different settings (e.g., run time, injection time, etc.)

RETURN TO 7114

7178
Re-run PCR?
- Yes → 7180
- No → 7188

7180
Are the loci part of a multiplex?
- Yes → 7182
- No → 7186

7182
Amplify individual loci?
- Yes → 7184
- No → 7186

7184
Designate loci for individual amplification

7186
Select and perform appropriate troubleshooting steps for individual samples as needed:
- Re-amplify using-
  - Same settings (e.g., material and equipment parameters and volume etc.)
  - Different settings (e.g., cleanup, change thermocycler settings, change mastermix, volume, modify DNA concentrations etc.)

RETURN TO 7110

7188
Perform extraction clean-up process?
- Yes → GO TO 5036
- No → 7190

7190
Perform PCR cleanup?
- Yes → 7192
- No → 7194

7192
Perform PCR cleanup

7194
Create new subsample and re-extract

7196
Are there multiple runs from the sample (from my single lab item)?
- Yes → 7198
- No → GO TO 7168

7198
Combine to create a consensus profile (e.g., will creating a consensus profile create a more complete genotype)?
- Yes → 7200
- No → GO TO 7168

7200
Review generated report from allele call software and create consensus profile

7202
Does the consensus profile meet analysis criteria based on validation?
- Yes → 7204
- No → GO TO 7168

7204
Based on species and relevant validation data, are results suitable to proceed, e.g., specific loci and marker(s) (including number, database marker threshold, etc.)?
- Yes → 7206
- No → 7208

7206
Output:
- Output(s) may include:
- Suitable genotype for potential assignment
- Selected genetic database

GO TO 7402

7208
Unable to proceed/inconclusive based on marker threshold established during validation

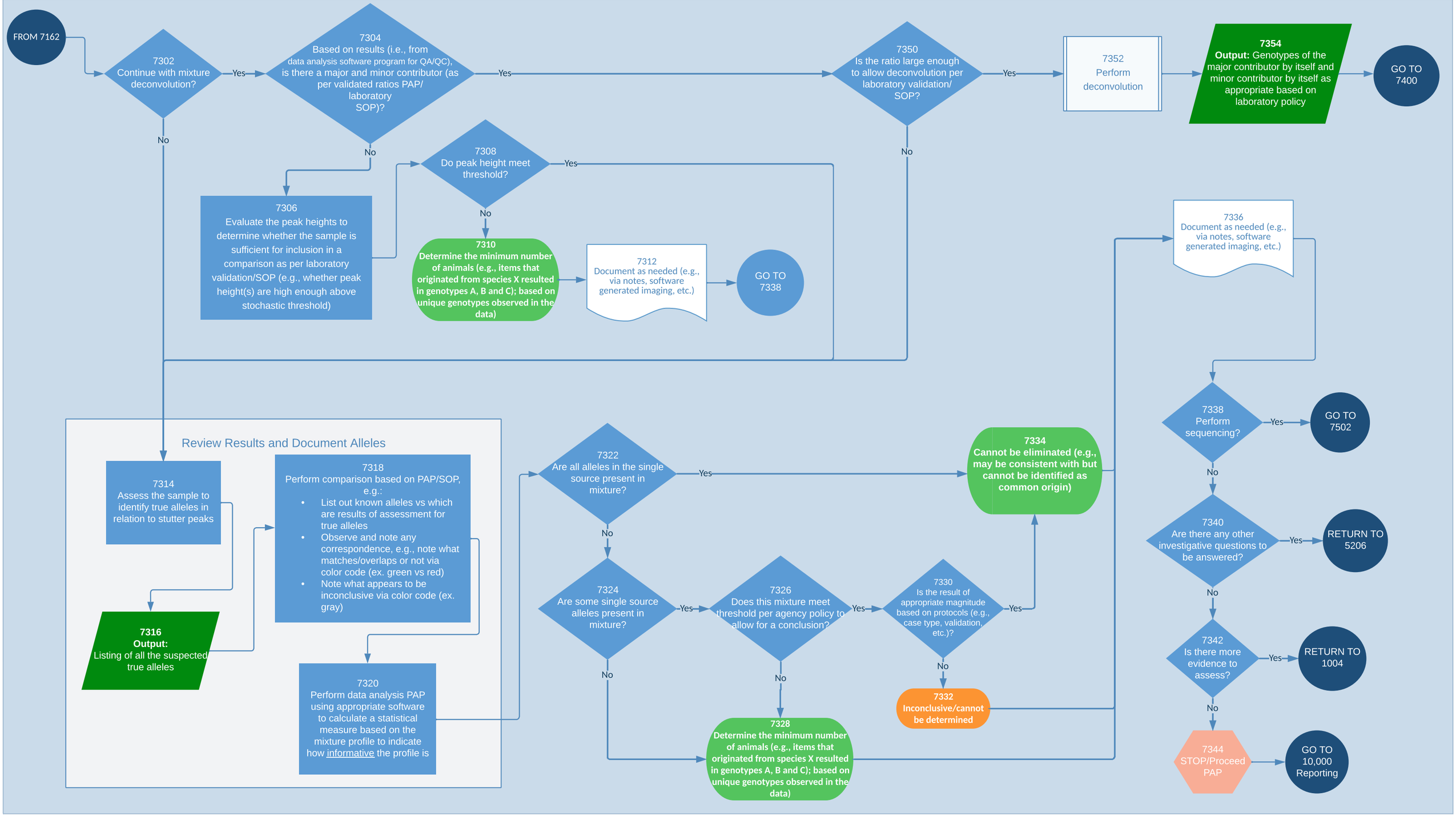
7210
Document as needed (e.g., via notes, software generated imaging, etc.)

RETURN TO 7132

Choose and return to the appropriate map page for applicable sample type to perform subsampling and other steps as necessary:

- GO TO 3604 - Tissue
- GO TO 4210 - Scat
- GO TO 3740 - Blood (liquid)
- GO TO 4268 - Vomit
- GO TO 3826 - Bones, claws, teeth, and keratin based
- GO TO 4284 - Urine
- GO TO 4004 - Non-Tissue/ Inorganic
- GO TO 4304 - Saliva
- GO TO 4152 - Shed Hair
- GO TO 4404 - Other/ Unfamiliar

7300 - Matching/Individualization: STR Mixtures (3 of 4)



7400 - Matching/Individualization: STR (4 of 4)

FROM Matching STR P2

7402
Input:
Suitable genotype(s) for comparison and genetic database selected

7404
Choose genotypes to compare

7406
Perform analysis, e.g.:
- Compare each sample's alleles to one another or to a known
- Determine number of unique genotypes

7408
Are there any samples with matching genotypes?

Yes

7410
Do all the results from genotype determination meet agency requirements to perform statistical analysis? (e.g., there are sufficient identical loci to perform statistical analysis, and/or a known sample is available)

Yes

7420
Input data into appropriate statistical analysis software program(s) e.g., based on species, region, knowledge, availability, equipment used, etc.

7422
Document statistical results as needed (e.g., via spreadsheet, allele cards, notes, software generated imaging, etc.)

7424
Does the likelihood ratio (LR) or random match probability (RMP) fall within the confidence interval?

Yes

7428
Is the LR or RMP result acceptable based on case type, knowledge of population, validation, protocols, etc.)?

Yes

7436
Document genotypic results as needed (e.g., via notes, software generated imaging, etc.)

7438
Identical genotypes indicating items originate from the same individual (include statistical support)

7440
Document as needed (e.g., via notes, software generated imaging, etc.)

No

7426
Double check the numbers/allele calls entered into program/software and data calls

No

7434
Seek assistance

No

7412
Do any of the results meet agency requirements?

Yes

No

No

7414
Document genotypic results as needed (e.g., via notes, software generated imaging, etc.)

7416
Determine the minimum number of animals (e.g., items that originated from species X resulted in genotypes A, B and C); based on unique genotypes observed in the data)

7418
Document as needed (e.g., via notes, software generated imaging, etc.)

7430
Seek additional assistance (e.g., colleague, literature, etc.)?

Yes

No

7432
Inconclusive/cannot be determined

7442
Output:
Statistical data, e.g.:
- Likelihood
- Probability

7444
Are there additional samples with matching genotypes to analyze?

Yes

RETURN TO 7408

No

7446
Perform sequencing?

Yes

GO TO 7502

No

7448
Are there any other investigative questions to be answered?

Yes

RETURN TO 5206

No

7450
Is there more evidence to assess?

Yes

RETURN TO 1004

No

7452
STOP/Proceed PAP

GO TO 10,000 Reporting

7500- Matching/Individualization: Sequencing (1 of 4)

FROM 7030/7132/ 7338/7446

7502 Is quant data needed to determine template volume or concentration?
- Yes → 7504 Is quant data available?
  - Yes → 7508
  - No → 7506 Run quant → 7508
- No → 7508

7508 Conduct sample prep:
- Determine appropriate aliquot volume(s) to subsample based on test plan, knowledge, training, experience, kit, instrument, method, concentration (if applicable), SOP, etc.
- Determine appropriate panel (or species) specific positive control(s) to run with the negative control(s) based on knowledge, training, preference, protocol, etc.

→ 7510 Run PCR

→ 7512 Check for PCR products?
- Yes → 7514 Load and run PCR products on medium in order to visualize
- No → GO TO 7602

7514 → 7516

7516 Evaluate PCR results:
- PCR Product detected
  - Product on gel or other medium
- Control/blank data
  - Any amplification in blanks
  - Failure of positive

→ 7518 Document image of gel showing absence or presence of PCR product

→ 7520 Was PCR product detected?
- Yes → GO TO 7602
- No → 7522 Go to sequencing anyway?
  - Yes → GO TO 7602
  - No

7524 Troubleshoot?
- Yes → 7540 Re-extract?
  - Yes → 7544 Create new subsample?
    - Yes → 7546 Choose and return to the appropriate map page for applicable sample type to perform subsampling and other steps as necessary:
      - GO TO 3604 - Tissue
      - GO TO 4210 - Scat
      - GO TO 3740 - Blood (liquid)
      - GO TO 4268 - Vomit
      - GO TO 3826 - Bones, claws, teeth, and keratin based
      - GO TO 4284 - Urine
      - GO TO 4004 - Non-Tissue/ Inorganic
      - GO TO 4304 - Saliva
      - GO TO 4152 - Shed Hair
      - GO TO 4404 - Other/ Unfamiliar
    - No → RETURN TO 5002
  - No → 7542 Select and perform appropriate troubleshooting steps for individual samples as needed:
    - Re-amplify using-
      - Same settings (e.g., material and equipment parameters and volume etc.)
      - Different settings (e.g., cleanup, change thermocycler settings, change mastermix, volume, modify DNA concentrations etc.)

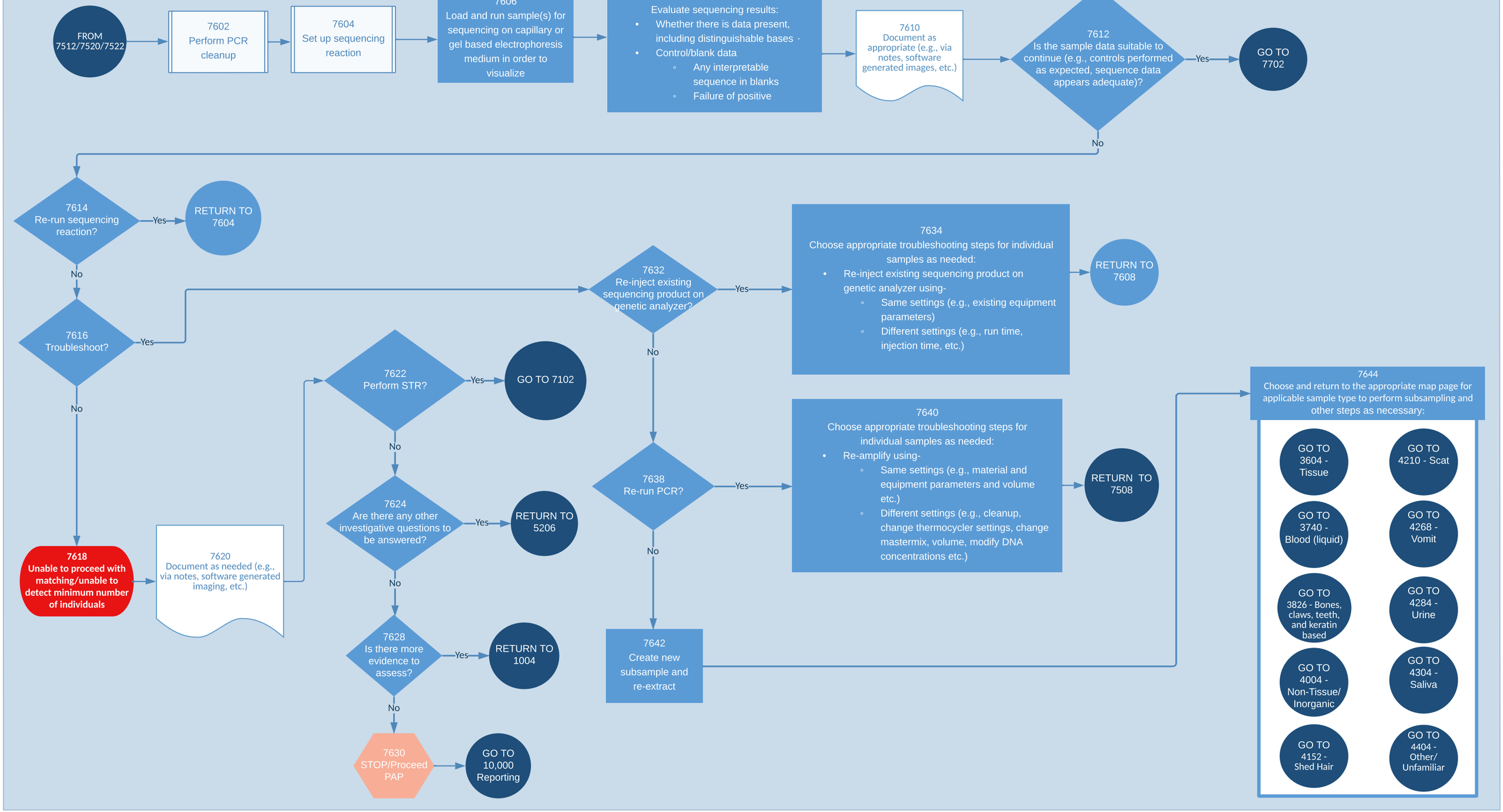
    → RETURN TO 7508
- No → **7526 No interpretable genotype was obtained /Unable to proceed with matching/unable to detect minimum number of individuals**

→ 7528 Document as needed (e.g., via notes, software generated imaging, etc.)

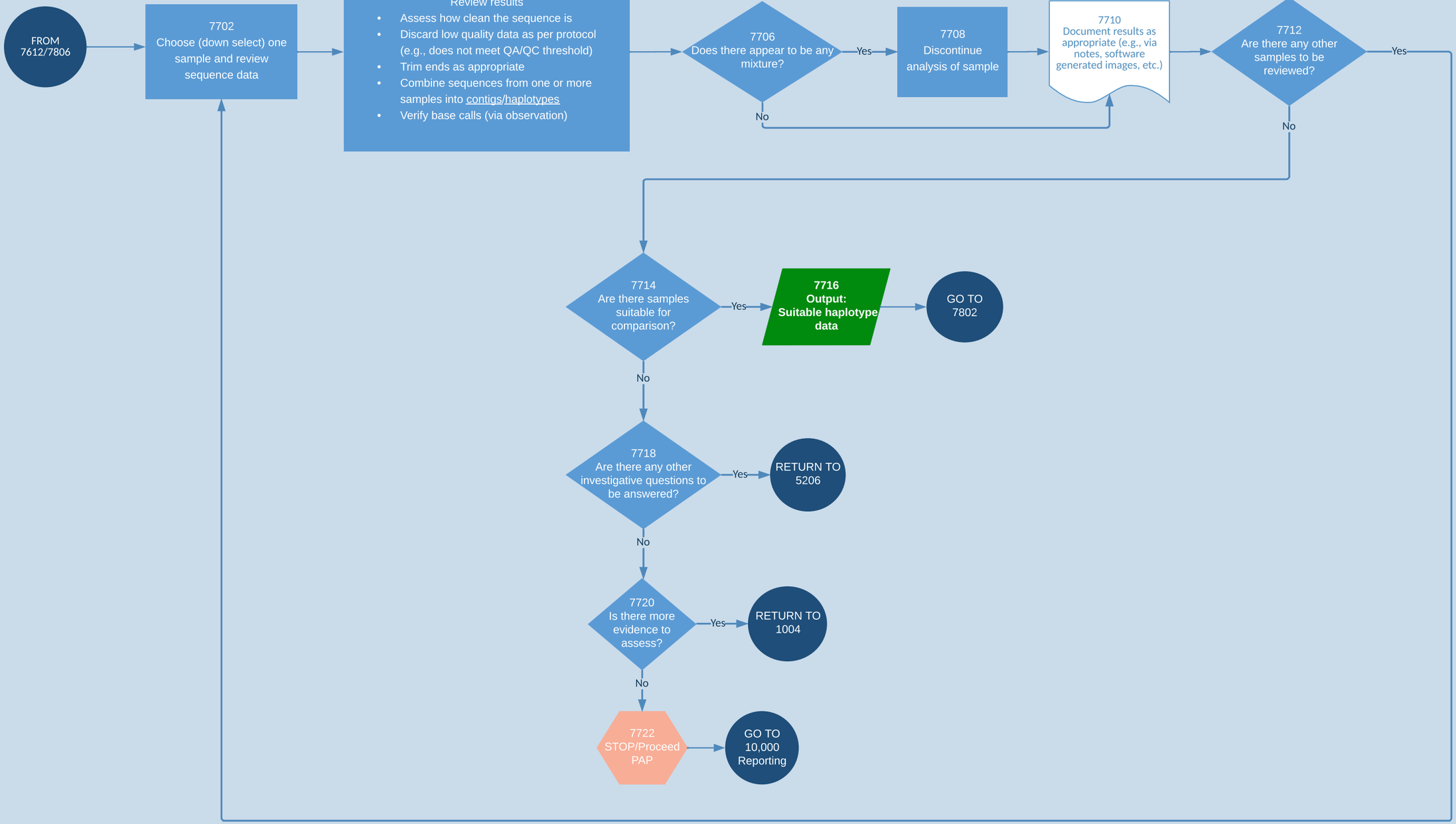
→ 7530 Perform STR?
- Yes → GO TO 7102
- No → 7532 Are there any other investigative questions to be answered?
  - Yes → RETURN TO 5206
  - No → 7534 Is there more evidence to assess?
    - Yes → RETURN TO 1004
    - No → 7536 STOP/Proceed PAP → GO TO 10,000 Reporting

This process map provides a visual description and attempts to represent all reasonable variations of casework currently performed by wildlife biologists. OSAC does not necessarily support or endorse (as best practices) all of the different steps and paths depicted in this process map.

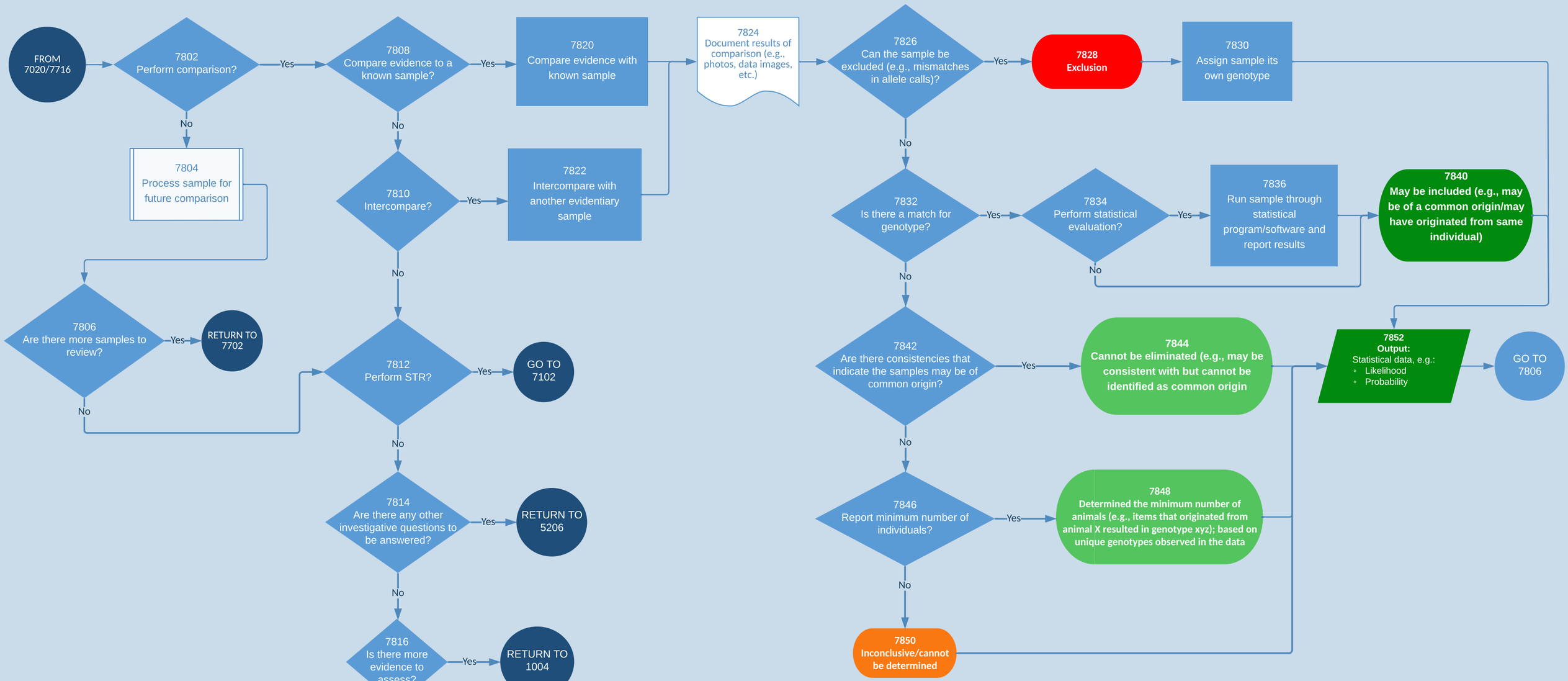
7600 - Matching/Individualization: Sequencing (2 of 4)



7700 - Matching/Individualization: Sequencing (3 of 4)



7800 - Matching/Individualization: Sequencing (4 of 4)



8000 - Familial Determination: Initial Assessment

FROM TO 5206

8002 Is/are the species of all the sample(s) submitted for analysis known?
- Yes
- No

8004 Is case information available indicating the species?
- Yes
- No

8006 Perform species ID anyway?
- Yes → GO TO 6002
- No

GO TO 6002

8010 Is there access to a validated genetic database that may potentially contain information concerning the species (e.g., based on information such as geographic range,subspecies, information from validation study(ies), etc.)?
- Yes

8012 Do I have confidence in {the information in} the database?
- Yes
- No

8014 Are there validated markers available for parental exclusion (i.e., exclude based on paternity)?
- Yes
- No

8016 Create a new database?
- Yes → 8030 Create own database
- No → 8018 STOP/Hold for future analysis

*Population types may include:
- Temporal distribution
- Geographic region
- Farm vs. Wild

8032 Do I already have STR or sequencing data, e.g., from species ID, population, etc.?
- Yes
- No

8034 Use the same data generated?
- Yes
- No

8036 Use STR data?
- Yes → GO TO 8202
- No

Choose Method

8038 Perform STR?
- Yes → GO TO 8102
- No

8040 Attempt to exclude a potential mother from offspring based on maternal data via sequencing?
- Yes → 8046 Perform sequencing (i.e., in order to attempt to exclude) and report results PAP
- No

8042 Attempt to exclude parentage of male offspring based on paternal data via sequencing?
- Yes → 8046
- No

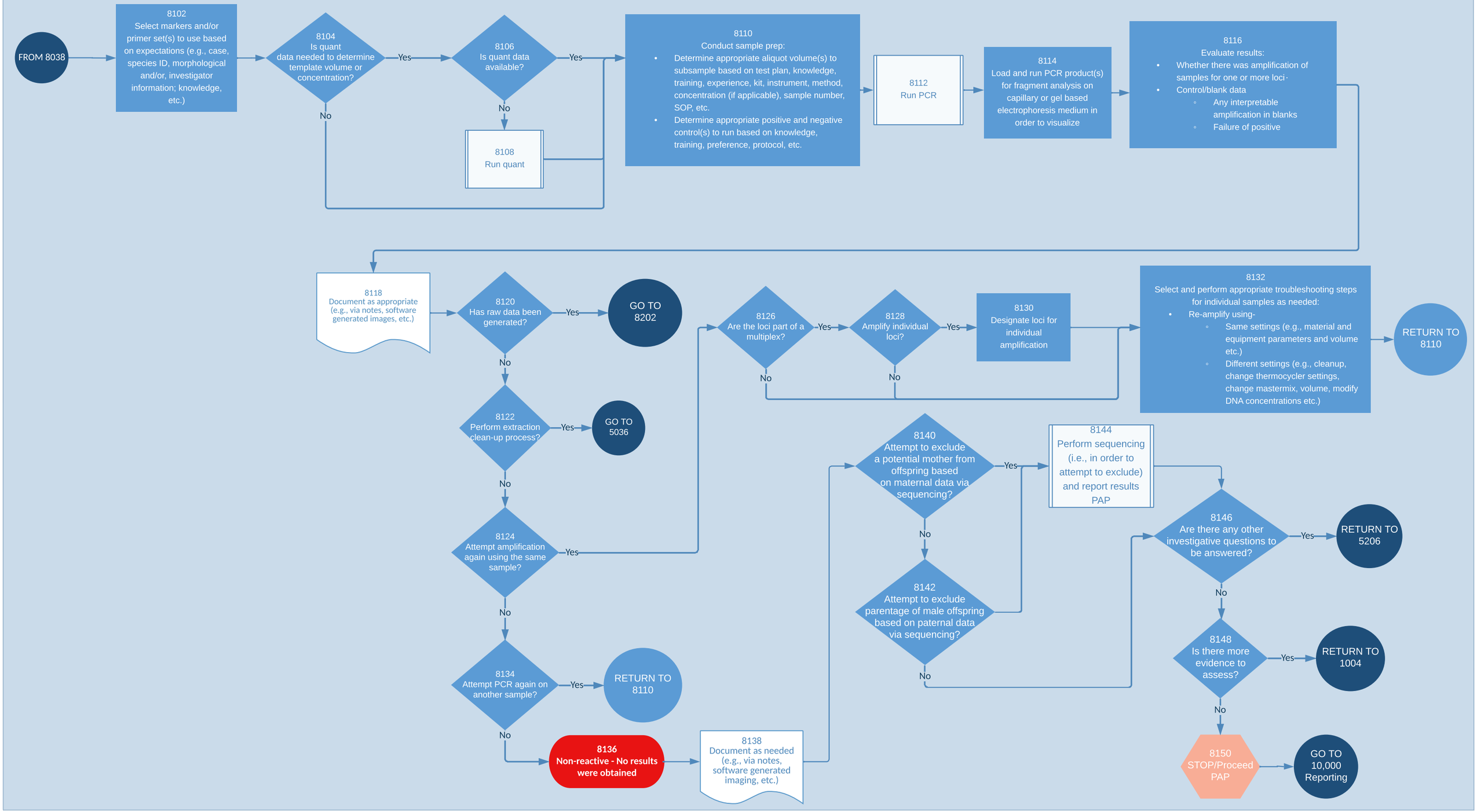
8020 Has any previous testing been run on the sample? (i.e. do I have anything to report?)
- Yes → **8026 Some results obtained; Unable to further test for relatedness** → 8028 Document any testing information as appropriate (e.g., via notes, etc.)
- No → **8022 Unable to test for relatedness** → 8024 Document as appropriate (e.g., via notes, etc.)

8048 Are there any other investigative questions to be answered?
- Yes → RETURN TO 5206
- No

8050 Is there more evidence to assess?
- Yes → RETURN TO 1004
- No

8052 STOP/Proceed PAP → GO TO 10,000 Reporting

8100 - Familial Determination: STR (1 of 3)



8200 - Familial Determination: STR (2 of 3)

FROM 8036/8120

8202
Choose and load data into an appropriate data analysis software program for QA/QC based on preference, knowledge, training, availability, equipment used, etc.

8204
Review results
- Assess the quality of data
- Discard low quality data as per protocol (e.g., does not meet QA/QC threshold)
- Verify size calls of fragment(s) (via observation)
- Evaluate completeness of profile

8206
Run data in allele call/software tool

8208
Review and edit resulting allele calls

8210
Based on results, does the sample appear to be free of contamination, including no evidence of a mixture, etc.?
- Yes → 8212
- No → 8214

8212
Are results otherwise suitable to proceed?
- Yes → GO TO Output
- No → 8214

8214
Are there multiple runs from the sample (from my single lab item)?
- Yes → 8216
- No → 8218

8216
Combine to create a consensus profile (e.g., will creating a consensus profile create a more complete genotype)?
- Yes → 8218
- No → 8218

8218
Review generated report from allele call software and create consensus profile

8220
Does the consensus profile meet analysis criteria based on validation?
- Yes → 8254
- No → GO TO 8222

GO TO 8222

8222
Troubleshoot?
- Yes → 8228
- No → 8224

8224
Non-reactive/No interpretable genotype was obtained /Unable to establish familial relatedness

8226
Document as needed (e.g., via notes, software generated imaging, etc.)

GO TO 8354

8228
Re-load existing PCR product on genetic analyzer?
- Yes → 8230
- No → 8232

8230
Choose appropriate troubleshooting steps for individual samples as needed:
- Re-load existing PCR product on genetic analyzer using-
  - Same settings (e.g., material and equipment parameters and volume etc.)
  - Different settings (e.g., run time, injection time, etc.)

RETURN TO 8114

8232
Re-run PCR?
- Yes → 8234
- No → 8244

8234
Are the loci part of a multiplex?
- Yes → 8236
- No → 8240

8236
Amplify individual loci?
- Yes → 8238
- No → 8240

8238
Designate loci for individual amplification

8240
Select and perform appropriate troubleshooting steps for individual samples as needed:
- Re-amplify using-
  - Same settings (e.g., material and equipment parameters and volume etc.)
  - Different settings (e.g., cleanup, change thermocycler settings, change mastermix, volume, modify DNA concentrations etc.)

8244
Perform extraction clean-up process?
- Yes → GO TO 5036
- No → 8246

GO TO 5036

8246
Perform PCR cleanup?
- Yes → 8248
- No → 8250

8248
Perform PCR cleanup

RETURN TO 8110

8250
Create new subsample and re-extract

8254
Based on species and relevant validation data, are results suitable to proceed, e.g., specific loci and marker(s) (including number, database marker threshold, etc.)?
- Yes → 8264
- No → 8256

8256
Evaluate another sample item (e.g., attempt to combine partial profiles from separate samples)?
- Yes → 8258
- No → 8260

8258
Perform analysis including but not limited to:
- Obtaining multiple consensus profiles from different samples
- Performing matching as needed (e.g., evaluate the samples to assess whether any match)
- Creating new consensus profile based on any potential matching samples

GO TO 8220

8260
Familial relationship could not be determined

8262
Document as needed (e.g., via notes, software generated imaging, etc.)

GO TO 8354

8264
Output:
Output(s) may include:
- Suitable genotype for familial determination
- Selected genetic database

GO TO 8302

8252
Choose and return to the appropriate map page for applicable sample type to perform subsampling and other steps as necessary:

- GO TO 3604 - Tissue
- GO TO 4210 - Scat
- GO TO 3740 - Blood (liquid)
- GO TO 4268 - Vomit
- GO TO 3826 - Bones, claws, teeth, and keratin based
- GO TO 4284 - Urine
- GO TO 4004 - Non-Tissue/ Inorganic
- GO TO 4304 - Saliva
- GO TO 4152 - Shed Hair
- GO TO 4404 - Other/ Unfamiliar

8300 - Familial Determination: STR (3 of 3)

FROM 8264

8302
Input:
Inputs may include:
- Suitable genotype(s) for potential assignment
- Genetic database selected
- Validated allelic panels (e.g., for exclusion)
- Case information
- Life history of the species (e.g., when they reach reproductive age, etc.)

8304
Perform exclusion* only?

Yes

No

*Parental exclusions only

8340
Use case information to identify the presumptive parent or offspring

8342
Compare alleles

8344
Is there a mismatch (e.g., does the potential offspring exhibit a combination of alleles that are not possible given the laws of Mendelian inheritance)

Yes

8448
Exclusion of parentage

No

8346
Cannot be excluded as having a potential parental relationship

8350
Document as needed (e.g., via notes, software generated imaging, etc.)

8352
Output:
Output(s) may include:
- Statistical data
  - Likelihood
  - Probability
- Exclusion test results
- Familial determination*

***Potential Outcomes of Familial Determination:**
- Parent
- Sibling
- Half-sibling
- Inconclusive (e.g., parent or sibling are equally likely; unrelated or related equally likely; not enough statistical power to determine relatedness; appear related but life history doesn't correlate or otherwise make sense)
- Unrelated

8306
Run sample data through statistical software (either to test for specific hypotheses or run sample data open ended) and document results as needed (e.g., via spreadsheet, allele cards, notes, software generated imaging, etc.)

8308
Output:
- Likelihood (goodness of fit) or
- Probability
  - Ex., Parentage or sibling depending on the sample and data

8310
Choose most consistent hypothesis(es) for familial determination based on confidence interval/score, case info, etc. such as parent, sibling, half-sibling, or unrelated

8312
Based on the available data, did the software results support a specific familial relationship?

Yes

No

8314
Run through software to test more specific hypothesis(es)?

Yes

8316
Run sample data through statistical software to test for specific relationship hypotheses and document results as needed (e.g., via spreadsheet, allele cards, notes, software generated imaging, etc.)

8318
Assess data (e.g., p-value, etc.)

No

8320
Can a determination be made (i.e., is the p-value significant)?

Yes

No

8324
Does there appear to be a familial relationship between the individuals?

Yes

No

8326
Unrelated

8322
May be related but cannot determine specific relationship

8328
Report the most likely relationship based on the data (including case information, software data, etc.)

8330
Familial relationship has been determined (i.e., parent-offspring, siblings, half-sibling)

8332
Document as needed (e.g., via notes, software generated imaging, etc.)

GO TO 8352

FROM 8226/8262

8354
Attempt to exclude a potential mother from offspring based on maternal data via sequencing?

Yes

No

8356
Attempt to exclude parentage of male offspring based on paternal data via sequencing?

Yes

No

8358
Perform sequencing (i.e., in order to attempt to exclude) and report results PAP

8360
Are there any other investigative questions to be answered?

Yes

RETURN TO 5206

No

8362
Is there more evidence to assess?

Yes

RETURN TO 1004

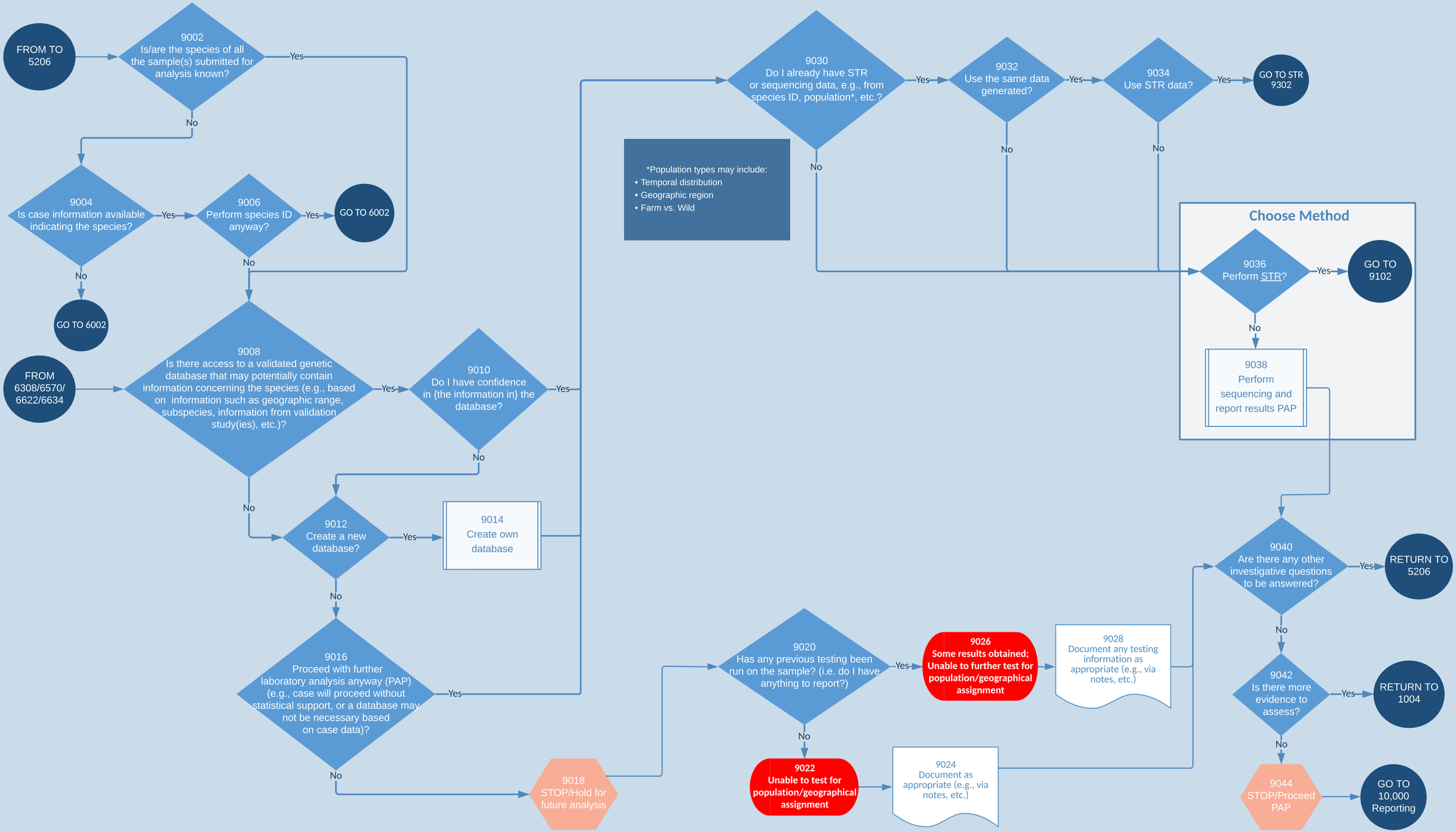
No

8364
STOP/Proceed PAP

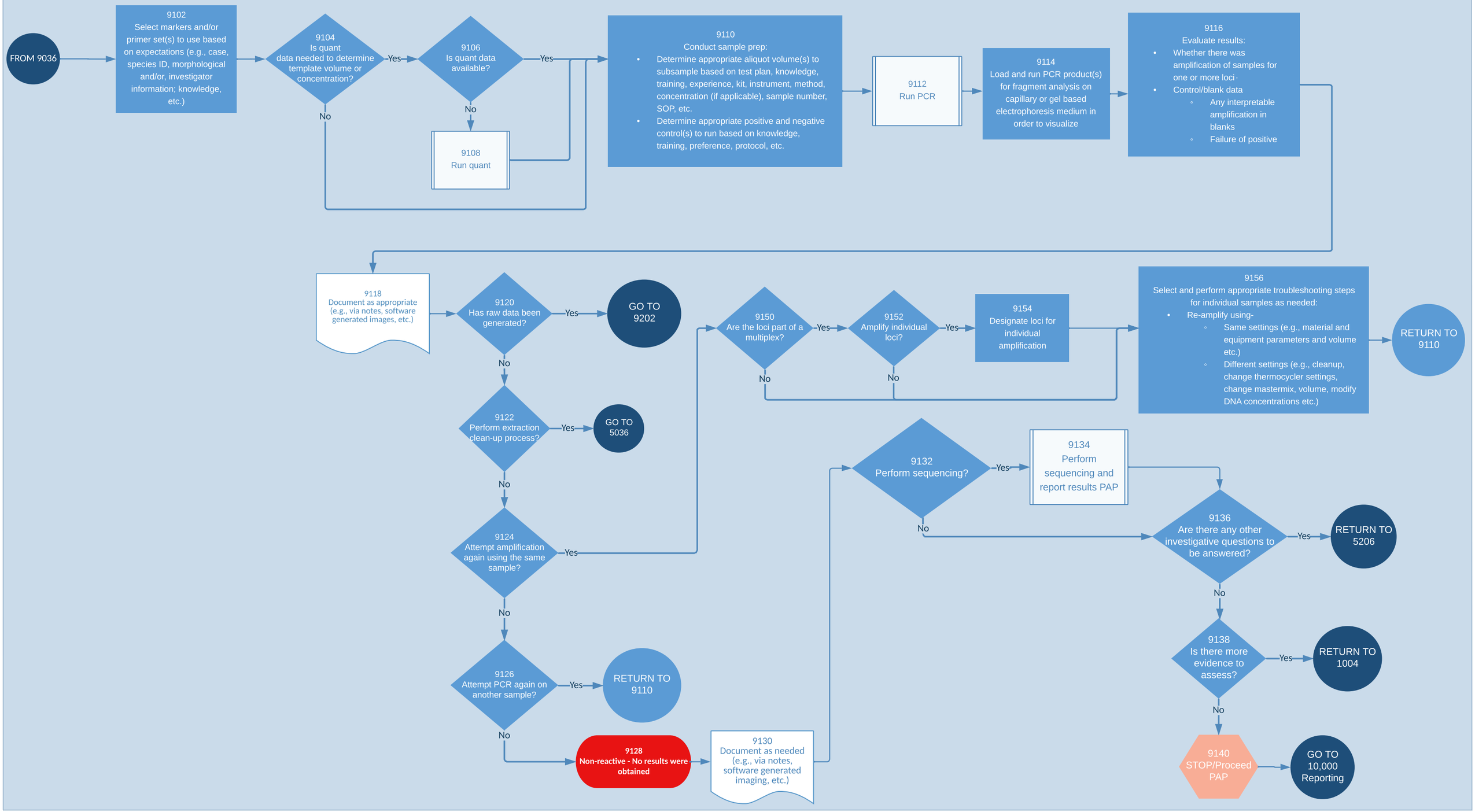
GO TO 10,000 Reporting

This process map provides a visual description and attempts to represent all reasonable variations of casework currently performed by wildlife biologists. OSAC does not necessarily support or endorse (as best practices) all of the different steps and paths depicted in this process map.

9000 - Population Assignment: Initial Assessment



9100 - Population Assignment: STR (1 of 3)



This process map provides a visual description and attempts to represent all reasonable variations of casework currently performed by wildlife biologists. OSAC does not necessarily support or endorse (as best practices) all of the different steps and paths depicted in this process map.

9200 - Population Assignment: STR (2 of 3)

FROM 9034/9120

9202
Choose and load data into an appropriate data analysis software program for QA/QC based on preference, knowledge, training, availability, equipment used, etc.

9204
Review results
- Assess the quality of data
- Discard low quality data as per protocol (e.g., does not meet QA/QC threshold)
- Verify size calls of fragment(s) (via observation)
- Evaluate completeness of profile

9206
Run data in allele call/software tool

9208
Review and edit resulting allele calls

9210
Based on results, does the sample appear to be free of contamination, including no evidence of a mixture, etc.?
- Yes → 9212
- No → 9224

9212
Are results otherwise suitable to proceed?
- Yes → GO TO 9280
- No → 9214

9214
Are there multiple runs from the sample (from my single lab item)?
- Yes → 9216
- No → 9218

9216
Combine to create a consensus profile (e.g., will creating a consensus profile create a more complete genotype)?
- Yes → 9218
- No → 9218

9218
Review generated report from allele call software and create consensus profile

9220
Does the consensus profile meet analysis criteria based on validation?
- Yes → 9270
- No → 9224

9224
Troubleshoot?
- Yes → 9226
- No → 9230

9226
Re-load existing PCR product on genetic analyzer?
- Yes → 9228
- No → 9242

9228
Choose appropriate troubleshooting steps for individual samples as needed:
- Re-load existing PCR product on genetic analyzer using-
  - Same settings (e.g., material and equipment parameters and volume etc.)
  - Different settings (e.g., run time, injection time, etc.)

RETURN TO 9114

9230
No interpretable genotype was obtained - unable to determine population assignment

9232
Document as needed (e.g., via notes, software generated imaging, etc.)

9234
Perform sequencing?
- Yes → 9135
- No → 9236

9135
Perform sequencing and report results PAP

9236
Are there any other investigative questions to be answered?
- Yes → RETURN TO 5206
- No → 9238

9238
Is there more evidence to assess?
- Yes → RETURN TO 1004
- No → 9240

9240
STOP/Proceed PAP

GO TO 10,000 Reporting

9242
Re-run PCR?
- Yes → 9244
- No → 9252

9244
Are the loci part of a multiplex?
- Yes → 9246
- No → 9250

9246
Amplify individual loci?
- Yes → 9248
- No → 9250

9248
Designate loci for individual amplification

9250
Select and perform appropriate troubleshooting steps for individual samples as needed:
- Re-amplify using-
  - Same settings (e.g., material and equipment parameters and volume etc.)
  - Different settings (e.g., cleanup, change thermocycler settings, change mastermix, volume, modify DNA concentrations etc.)

GO TO 9110

9252
Perform extraction clean-up process?
- Yes → GO TO 5036
- No → 9254

9254
Perform PCR cleanup?
- Yes → 9256
- No → 9258

9256
Perform PCR cleanup

9258
Create new subsample and re-extract

9270
Based on species and relevant validation data, are results suitable to proceed, e.g., specific loci and marker(s) (including number, database marker threshold, etc.)?
- Yes → **9280 Output:** Suitable genotype for potential assignment, and selected genetic database → GO TO 9302
- No → 9272

9272
Evaluate another sample item (e.g., attempt to combine partial profiles from separate samples)?
- Yes → 9274
- No → **9276 Unable to proceed/inconclusive based on marker threshold established during validation** → 9278

9274
Perform analysis including but not limited to:
- Obtaining multiple consensus profiles from different samples
- Performing matching as needed (e.g., evaluate the samples to assess whether any match)
- Creating new consensus profile based on any potential matching samples

GO TO 9204

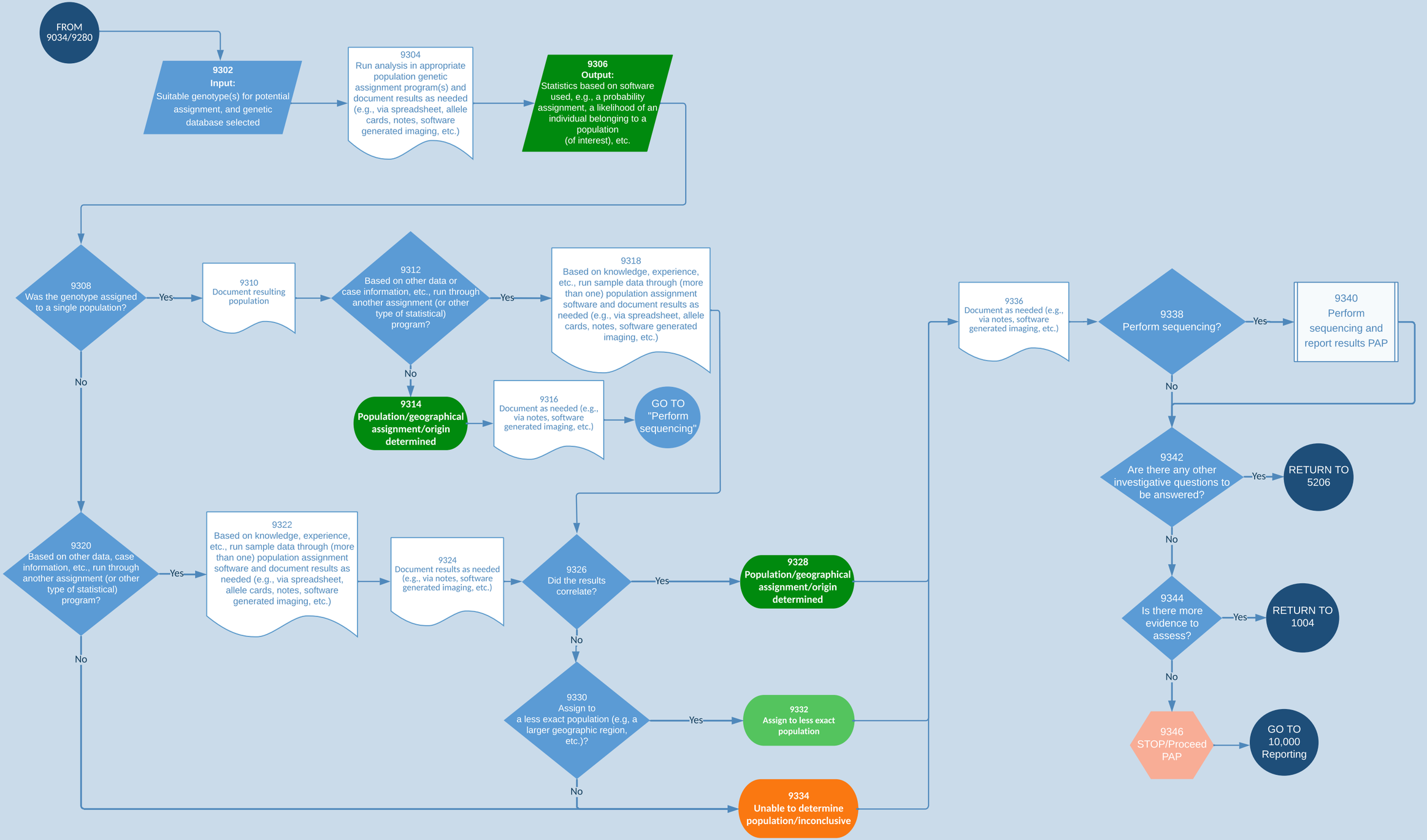
9278
Document as needed (e.g., via notes, software generated imaging, etc.)

GO TO 9234

9260
Choose and return to the appropriate map page for applicable sample type to perform subsampling and other steps as necessary:

GO TO 3604 - Tissue
GO TO 4210 - Scat
GO TO 3740 - Blood (liquid)
GO TO 4268 - Vomit
GO TO 3826 - Bones, claws, teeth, and keratin based
GO TO 4284 - Urine
GO TO 4004 - Non-Tissue/ Inorganic
GO TO 4304 - Saliva
GO TO 4152 - Shed Hair
GO TO 4404 - Other/ Unfamiliar

9300 - Population Assignment: STR (3 of 3)



10,000 - Reporting

FROM Various Areas in the Map

10002
Input:
- Case information, e.g.:
  - Request
  - Item(s) submitted
  - Submitter name and agency
  - CoC info (e.g., date received and by whom)
- Examinations conducted, e.g.:
  - Method(s) used
  - Type(s) of analyses
- Results (e.g., data, notes, photographs, etc.)
- Conclusions

10004
Perform any necessary case file management activities PAP (e.g., combine notes, data, etc. into a case file or pdf; combine communications into a file, etc.)

10006
Formal report needed?
Yes
No

10008
Issue informal report via email or other communication?
Yes
No

10010
Relay findings as appropriate

10012
Document reason(s) as needed PAP (e.g., via notes, in case file, etc.)

Stop/End Process

Perform Report Drafting Activities

10020
Issue a separate report for morphology (e.g., no genetic results, no DNA results obtained or warranted, etc.)?
Yes
No

10022
Draft morphology report including information such as:
- Evidence examined
- Observations
- Any examination(s) performed
- Results

10024
Issue report on genetics?
Yes
No

GO TO 10028

10026
Draft genetics report

10028
Draft final report which may be accomplished via software, notes or archiving tools etc.:

Report may include (but not be limited to) any or all of the following:

- Chain of custody
  - Who received from/when/how
  - Responsible officer/agency
- Who performed analyses
- Case information
  - Request information
  - Purported species information
- Examinations conducted
  - Date(s) examination(s) conducted
  - Methods used
    - Sequencing, STR, etc.
  - Types of analyses
    - Based on question(s) being asked, e.g., matching, species ID, sex ID, etc.
  - Other information, e.g.:
    - Known samples
    - Version and name of database(s) used
    - Loci used
- Results
  - Data, e.g.:
    - Statistical analysis
    - Sequence results
    - Sex ID results
    - STR analysis results
    - Morphological results
- Conclusions
  - Interpretation of results and/or expert opinion of results
- Evidence disposition
- Signature(s) of analysts
- Role(s) of personnel/analysts

GO TO 10030

Conduct Report Review(s)

10030
Perform tech review?
Yes
No

10032
Conduct technical review (including for notes, allele calls, any ID results, etc.)

10034
Are there any issues in need of correction?
Yes
No

10036
Additional analysis or re-analysis needed?
Yes
No

10038
Determine necessary re-analysis steps and conduct any additional re-analysis as needed

10040
Take appropriate corrective action(s) and edit report as necessary

RETURN TO 10030

10050
Conduct administrative review

10052
Are there any issues in need of correction?
Yes
No

10054
Edit report as necessary

10056
Perform additional admin review?
Yes
No

10060
Enter report into case information management system

10062
Issue report to appropriate personnel PAP

Stop/End Process

This process map provides a visual description and attempts to represent all reasonable variations of casework currently performed by wildlife biologists. OSAC does not necessarily support or endorse (as best practices) all of the different steps and paths depicted in this process map.

Glossary of Terms and Definitions*

Contigs: Contiguous sequence generated by overlapping series of sequence reads

Genetic distance: Genetic distance is a genetic divergence measurement between either species or populations within a species; A genetic distance is not a physical distance but an implied probability of a crossover occurring along the distance between loci on a chromosome

Haplotype: Combination of alleles at multiple loci on one chromosome that tend to be inherited together

Hide: Coming from larger animal with thicker skin; including with fur trade/trapping – may or may not be processed

Informative: Pertaining to information (statistically, taxonomically, etc.) that may help the analyst to decide whether or not to continue moving forward in the process with sample, evidence, etc.

Laboratory item number: see "subsample"

Leather: Tanned, and can be hide or a skin and is always processed

Mendelian Inheritance: refers to an inheritance pattern that follows the laws of segregation and independent assortment in which a gene inherited from either parent segregates into gametes at an equal frequency.

Mixture: Containing material from two or more DNA profiles.

Phylogram/phylogenetic tree: A diagrammatic representation of the evolutionary relationship among various taxa; mathematical objects which summarize the most recent common ancestor relationships between a given set of organisms

Private Allele: Allele unique to only one species

Skin: Generally coming from a smaller animal or reptile; may or may not be processed; not all hair on skin is classified as fur

Submitter item number: see "subsample"

Subsample: Breakdown:
Terms:
Evidence/case --- "submitter item number" may differ than what submitter designates as an item
- Item - "laboratory item number 1"
- "Item ..1a, 1b, 1c", or "sample 1a, 1b, 1c etc."
- Cutting
- Section
- Swab
- "piece from item 1a"" = Subsample
- All these wind up in different tubes
- Subitem (official vs. unofficial)/portion of the item/or also subsample
- Sample (verb)> Subsample
- Sample(n) = subsample

Additional **Sampling/Subsampling** terms:
- Swab
- Cutting
- **Sample**
- **Subsample**
- Item
- Portion
- Section
- Evidence

Taxonomic group: Grouping based on similarities; based on the description, identification, nomenclature, and classification of organisms

Abbreviations:

EDTA: Ethylenediamine tetra-acetic acid
PAP: Per Agency Policy
PCR: Polymerase Chain Reaction
qPCR: Quantitative Polymerase Chain Reaction
RFLP: Restriction Fragment Length Polymorphism
y-STR: Y-Chromosome Short Tandem Repeats