HANDOUT - STUDENT STEP-BY-STEP PROTOCOL

Part 1: Using NCBI BLAST (15 minutes)

Step 1: Open NCBI BLAST

1. Go to https://blast.ncbi.nlm.nih.gov/
2. Click on "Nucleotide BLAST" (the blue button)
3. Wait for the page to load completely

Step 2: Prepare Your Query Sequence

4. Copy the DNA sequence for Evidence A from your case file
5. Paste it into the "Enter Query Sequence" box
6. Make sure the entire sequence is pasted (no missing nucleotides)

Step 3: Select the Database

7. Look for the "Database" dropdown menu
8. Select "Nucleotide collection (nr/nt)"
9. This searches all available DNA sequences in the database

Step 4: Check Algorithm Settings

10. Click "Algorithm parameters" to expand more options
11. Make sure "Expect threshold" is set to 10 (default)
12. Leave other settings at their default values

Step 5: Run the BLAST Search

13. Click the blue "BLAST" button at the bottom
14. Wait 30-60 seconds for results (this may take longer if the server is busy)
15. Do not close the page or start a new search while waiting

Step 6: Analyze the Results

16. When results appear, look at the "Descriptions" table at the top:

- Record the top species match (the first one in the list)
- Record the Percent Identity (% ID column) - should be >97%
- Record the E-value - should be very small (close to 0.0)
- Record the Query Coverage (% Cov column) - how much of your sequence matched

Step 7: Verify the Match Quality

17. Click on the top result to see the full alignment details
18. Look at how well your sequence aligns with the database sequence
19. Check if there are many gaps or mismatches (there should be very few)
20. If the alignment looks good, record this as your species identification

Step 8: Repeat for Evidence B and C

21. Clear the search box and paste Evidence B sequence
22. Repeat steps 2-7 for Evidence B
23. Repeat steps 2-7 for Evidence C
24. Record all results in Part 1 of your worksheet

Understanding BLAST Results - Quick Reference:

Term	What It Means
Percent Identity (%)	How similar your sequence is to the match. >97% = excellent match
E-value	Statistical significance. Smaller numbers = more confident match. 0.0 is the best
Query Coverage (%)	How much of your sequence matched the database sequence. Should be 90%+

Part 2: Confirming with BOLD Systems (10 minutes)

BOLD Systems is a specialized database for DNA barcodes. Use it to verify your NCBI BLAST results.

Step 1: Access BOLD Identification Engine

25. Go to http://www.boldsystems.org/
26. Click "Identification" in the top menu
27. Select "Animal ID (COI)" option

Step 2: Submit Evidence A Sequence

28. Copy the DNA sequence for Evidence A
29. Paste it into the search box
30. Click "Submit"
31. Wait for the identification results to appear

Step 3: Review BOLD Results

32. Note the species match in the results
33. Find the BIN (Barcode Index Number) - a unique identifier for this species group
34. Check if the BOLD result matches your NCBI BLAST result

Step 4: Explore Additional Information

35. Click on the species name to see full taxonomic information
36. Look at the taxonomy (family, genus, species)
37. View specimen data and photos if available
38. Record this information in Part 2 of your worksheet

Step 5: Repeat for Evidence B and C

39. Submit Evidence B sequence to BOLD
40. Record the species and BIN number
41. Repeat for Evidence C

- 💡 Pro Tip: If BOLD and NCBI BLAST give different results, use BOLD - it specializes in barcoding!

Part 3: Conservation Status Research (15 minutes)

Now that you know which species were identified, research their conservation status.

For Each Identified Species, Find:

- Conservation Status: Is it Endangered? Threatened? Vulnerable?
- CITES Protection: Is it listed in CITES appendices?
- Legal Trade Status: Is it legal to trade this species?
- Key conservation information: Why is it important to protect?

Websites for Research:

- CITES Species Database: https://cites.org/ (search for your species)
- IUCN Red List: https://www.iucnredlist.org/ (conservation status)
- Google Search: "[Species Name] conservation status" for quick information

What You're Looking For:

Endangered: Species facing very high risk of extinction

Vulnerable: Species at high risk of extinction

CITES Appendix I: Highest protection level - trade is banned except in very special cases

CITES Appendix II: Trade allowed but controlled

Record all conservation information in Part 3 of your worksheet.

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Part 4: Suspect Analysis and Case Conclusion (10 minutes)

Connect the DNA evidence to each suspect and make your final recommendation.

Step 1: Connect Evidence to Suspects

42. Evidence A (tissue from vehicle trunk) → Who was driving the vehicle?
43. Evidence B (blood on jacket) → Whose jacket had the blood stain?
44. Evidence C (hair in warehouse) → Whose warehouse was searched?

Step 2: Analyze Each Suspect's Statement

- Suspect 1 (Alex Chen): Claims tissue is from chicken for pet snake
- Suspect 2 (Jordan Martinez): Claims blood is from hiking injury
- Suspect 3 (Morgan Li): Denies any biological materials in warehouse

Step 3: Evaluate the Evidence Against Each Statement

45. Does the DNA evidence SUPPORT their statement?
46. Or does it CONTRADICT their statement?
47. Fill in Part 4: Suspect Analysis - connect evidence to suspects and evaluate statements

Part 5: Case Conclusion (10 minutes)

Step 4: Make Your Recommendation

48. Based on the DNA evidence and conservation status:
49. Which suspect(s) committed wildlife trafficking?
50. What evidence most strongly supports your conclusion?
51. Are there any suspects whose statements are contradicted by the DNA?

Step 5: Complete Your Worksheet

52. Fill in Part 5: Case Conclusion - make your final recommendation with reasoning
53. Be sure to explain your reasoning using evidence

Critical Thinking Questions to Consider:

- Does the DNA evidence support or contradict each suspect's story?
- Which suspect has the weakest alibi?
- Could multiple suspects be working together in a trafficking ring?
- What additional evidence would strengthen the case?
- Why is wildlife trafficking a serious crime?