

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

ANDE RAPID DNA		
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ANDE Rapid DNA

1 General

- 1.1 Rapid DNA is the fully automated (hands-free) process of developing a CODIS acceptable STR profile from a casework reference sample. The “swab in – profile out” process consists of automated extraction, amplification, separation, detection, and allele calling without human intervention.
- 1.2 A Rapid DNA cartridge (also known as a chip) is a preassembled set of reagents and other analytical components designed for use in a Rapid DNA instrument for the automated extraction, amplification, and separation of DNA samples.
- 1.3 A-chips allow for 5 samples to run simultaneously.

2 Buccal Sample Collection

- 2.1 Swabs used for the ANDE instrument are contained within desiccators whose caps have both a visible 2-D barcode, and an embedded RFID number to allow for sample tracking.
- 2.2 Ask the sample donor to rinse his/her mouth with water before collecting the sample.
- 2.3 To collect a buccal sample, swab each cheek vertically six times (the motion up-down is considered one time).
 - 2.3.1 The swab should be pressed against the inside of the cheek with enough pressure to make the cheek protrude slightly outward.
 - 2.3.2 Do not rotate the swab when swabbing. Simply move the swab from one cheek to the other for collection.
 - 2.3.3 Insert swab back into the desiccator after collection.
- 2.4 Refer to [Case Acceptance and Evidence Sign-in manual](#) for sample intake and scheduling.
- 2.5 Refer to the [Evidence Examination manual](#) for special instructions on examining buccal swabs for Rapid DNA.

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3 Power-up ANDE

NOTES:

- Be sure to log-in to the instrument BEFORE you unpack the chip. Instrument log-in issues should be resolved before the run commences.
- For issues with a locked user account, consult the [Rapid DNA QC manual](#).

3.1 If the ANDE instrument is OFF, proceed as follows:

3.1.1 In the back of the instrument, switch the power toggle to the “ON” position.

3.2 Wait for an 18-minute warm up cycle (includes a series of power on self-tests [POST] and calibrations to check the functionality of the critical modules of each subsystem).

3.3 Once the warm-up process is complete (or if the instrument has already been ON), the log-in window will be displayed.

3.3.1 Log in with username and password.

4 Unpacking the Chips

NOTE: Be sure the steps below are followed correctly to prevent damage to the chip and leakage of chemicals.

4.1 Record the chip lot number in the LIMS test batch.

4.2 Ensure the shipping box is in the upright position.

4.3 Carefully cut open the packaging tape of the shipping box and open the box.

4.3.1 Discard the clear packing gels in regular trash.

4.3.2 Pull the tape away from the white Styrofoam packaging container and use as a handle to lift the Styrofoam package from the box as shown:



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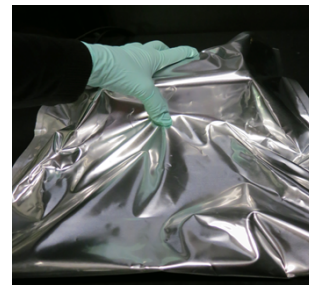
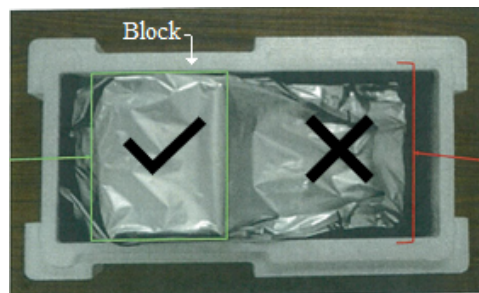
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4.4 Set the Styrofoam package onto a flat surface and follow these steps:

4.4.1 Remove tape holding the Styrofoam package together and then remove the top piece of Styrofoam packaging to expose the wrapped chip.



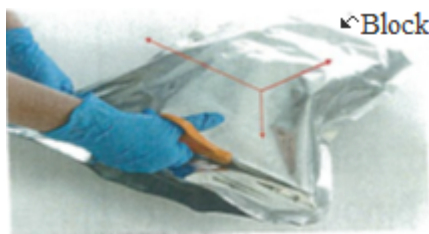
4.4.2 Remove the wrapped chip from the bottom Styrofoam packaging container by grasping the block (Do NOT handle chip by the opposite end; only by the block) and lifting the chip from the Styrofoam base.



4.4.3 Set the chip on a clean, flat surface and unfold the foil pouch.

NOTE: Use chips within 30 minutes of opening foil packaging.

4.4.4 Cut the foil pouch near the bottom and side seams opposite the block as shown:



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4.4.5 While holding the chip by the block, remove outer foil:



4.4.6 After unfurling the sides of the inner foil layer, repeat steps 4.4.4 and 4.4.5 to remove the inner layer. Use caution when opening the inner foil layer so the scissors do not damage the chip.

4.4.7 Open inner foil to expose the chip as shown below:



4.4.8 While holding the block, pull away the foil and gently lift the chip by the block to remove the foil packaging.



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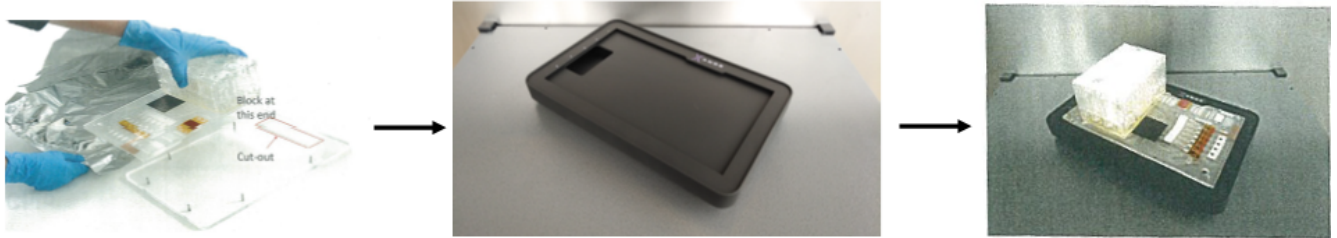
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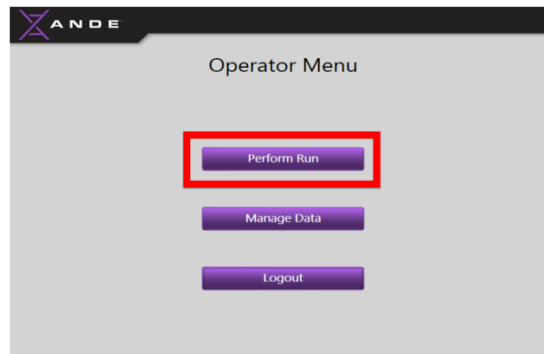
- 4.4.9 Immediately place the chip on the sample loading fixture with block end over the cutout (to protect the detection window).



5 Chip Set-Up Procedure

- 5.1 Print LIMS output labels and affix to the side of the corresponding sample desiccator.
- 5.2 Click “Perform Run” on the operator menu as shown below.

NOTE: Appearance of the menu may vary depending on the privileges given to SuperAdmin, Admin, and Operator.



NOTES:

- If buccal samples were collected on non-ANDE swabs, refer to Section **Error! Reference source not found.**: Non-ANDE Swabs.
 - Users experiencing problems with the handheld barcode scanner should refer to Section 15: Troubleshooting for alternative sample scanning options.
- 5.3 When prompted to scan the first sample, the operator should follow the steps below:
- 5.3.1 Read the input and output sample OCME ID numbers to the witness.
- 5.3.2 Use the handheld scanner to scan the output LIMS label.
- 5.3.3 Verify that the output sample OCME ID number appears on the instrument touchscreen.

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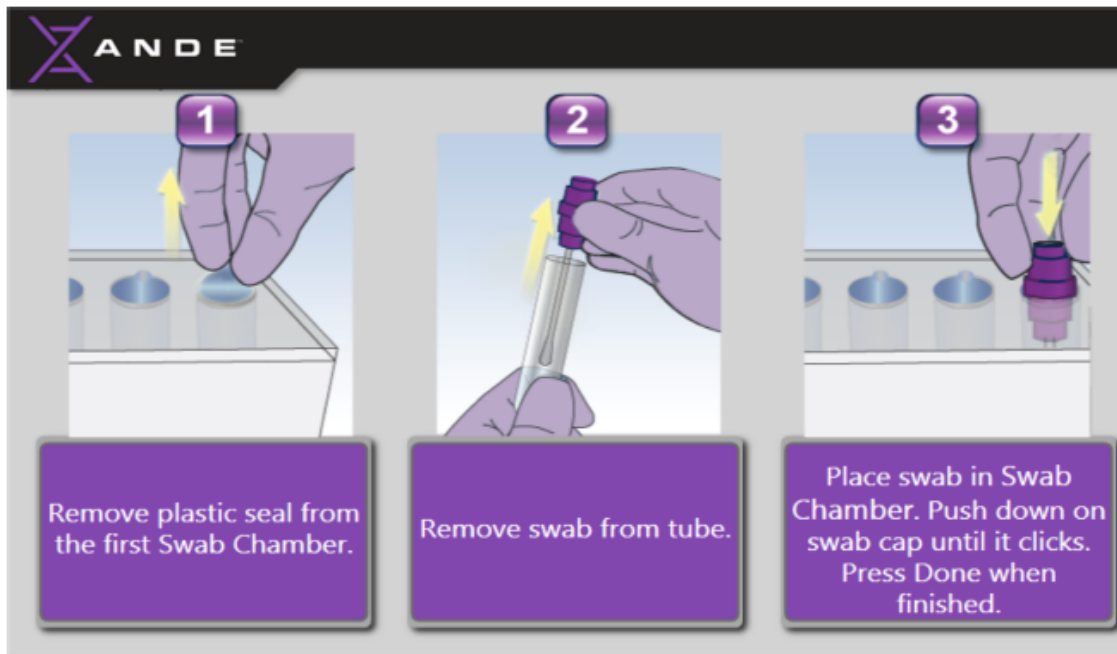
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- 5.3.4 Hold the purple top of the swab (with embedded RFID tag) directly against the RFID scanner as shown below:



- 5.3.5 Listen for a low beep to confirm RFID recognition.
- 5.3.6 Remove plastic seal from the first swab chamber (step 1 below).
- 5.3.7 Separate the swab from its desiccator cartridge (step 2 below).
- 5.3.8 With witness observing, insert the swab in its designated chamber on the chip and push down on the cap until it clicks (step 3 below).



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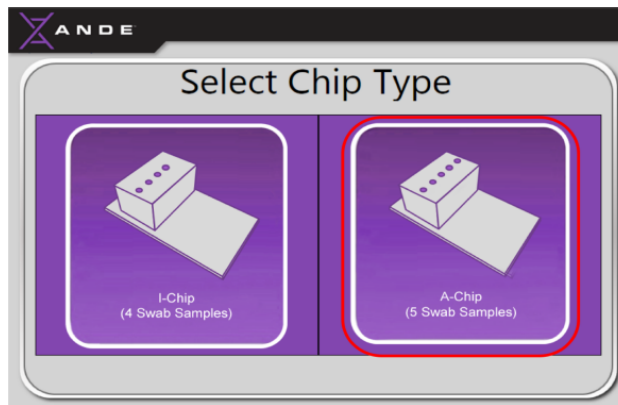
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- 5.4 Click “Next” on the touchscreen and repeat steps above for the remaining samples.
- 5.5 Discard all empty desiccator cartridges as swabs are loaded.
- 5.6 Select the “A-chip” as the chip type when prompted as shown below.

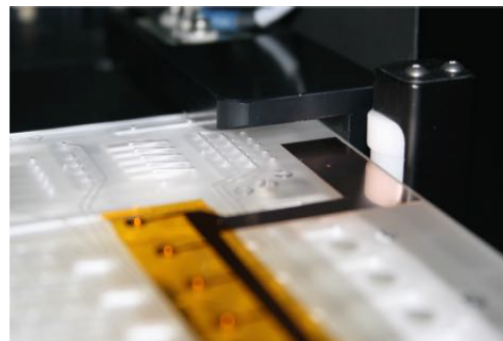
NOTE: the “Select Chip Type” prompt will appear after loading the 4th swab into the chip and selecting “Next” in the run wizard. Confirm settings as shown below if loading a 5th swab into the chip.



- 5.7 Confirm your choice by clicking “Yes” when prompted.
- 5.8 The witness procedure must be recorded in the LIMS test batch.

6 Instrument Loading Procedure

- 6.1 Insert chip into the ANDE as follows:
 - 6.1.1 Use your left hand to hold the block; use your right hand to hold the right side of the base. Align the base of the chip with the white wheels which will roll the chip inside the instrument as you gently push.



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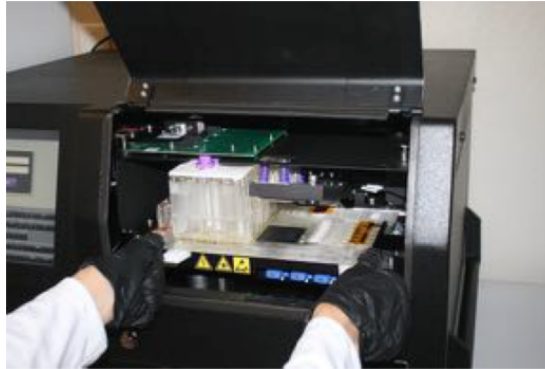
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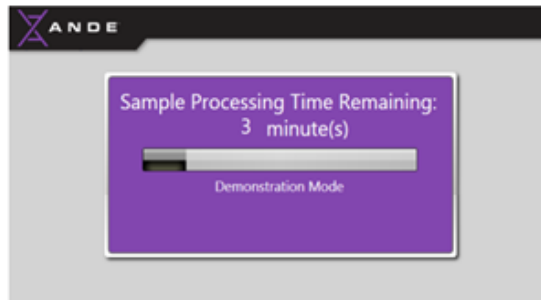
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- 6.1.2 Using both thumbs, slowly insert the chip all the way into the instrument until it is seated securely. You will know it is seated when the touchscreen prompts you to close the door.



- 6.2 Close instrument door, press firmly in the middle, and hold until it latches. Once the chip access door is closed, the instrument will automatically begin sample processing. There is no “start” button or any further action that the user must take to initiate processing.
- 6.3 The touchscreen displays the time remaining as the chip processing progresses.



- 6.4 Chip processing time is approximately 94 minutes for A-chips.

7 Run Completion and Chip Disposal

- 7.1 Remove the chip immediately when the touchscreen indicates run completion.
- 7.2 Close the instrument door, press firmly in the middle, and hold until it latches.
- 7.3 Chip disposal:

7.3.1 Seal the black gasket on the chip with the labels provided to prevent liquid from leaking out of the chip.

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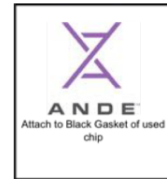
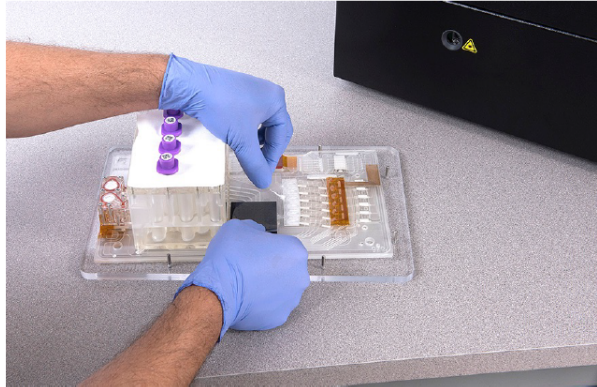
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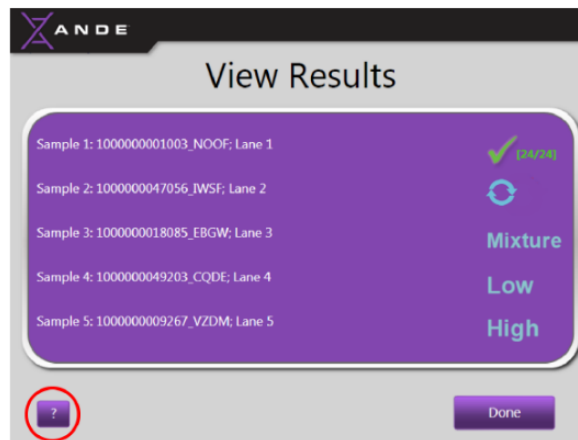
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- 7.3.2 Return the chip to its Styrofoam and shipping box.
- 7.3.3 Store the chip at room temperature until QA disposal. Used chips should not be discarded in regular trash.

8 Results Summary

- 8.1 At the end of the run, a results summary will appear on the touchscreen as shown below:



- 8.1.1 Refer to Appendix A: Results Summary for more information on interpreting any symbols displayed.
- 8.2 Press “Done”.

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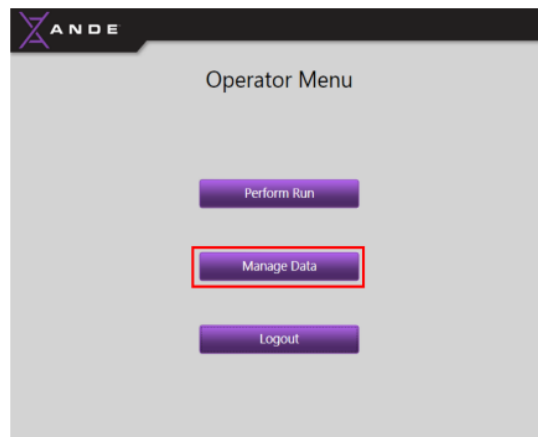
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9 Data Export

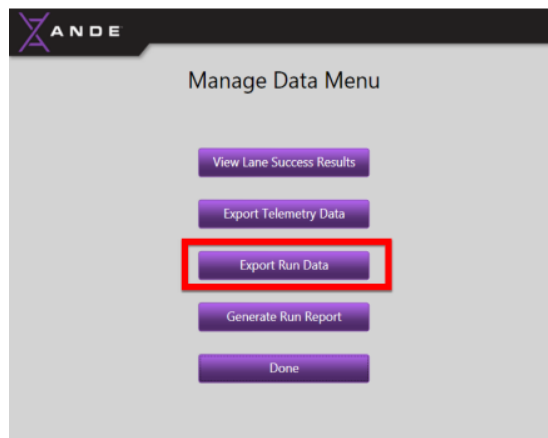
9.1 Insert a USB flash drive in the back of the instrument.



9.2 In the operator menu, select “Manage Data”.



9.3 Press “Export Run Data”.



9.4 Select the date range for the desired run data.

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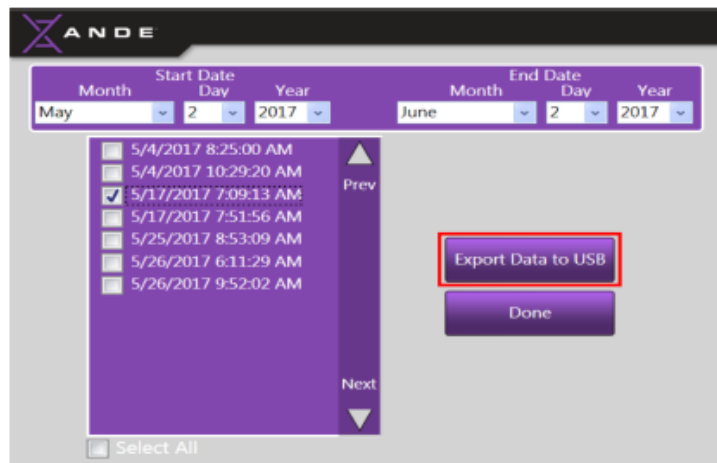
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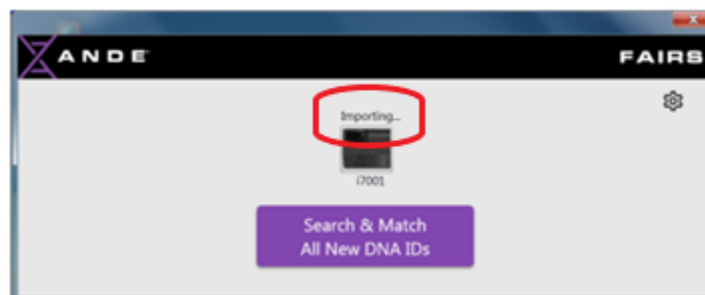
- 9.5 Select the run file to be exported by touching the box to the left of the file.
- 9.6 Press the “Export Data to USB” button.



- 9.7 Once the data has been transferred to the USB, press “OK” and then “Done”.
- 9.8 Remove the USB from the instrument.
- 9.9 On the instrument main menu, press “Logout”.

10 Data Decryption

- 10.1 Log on to the instrument laptop computer.
- 10.2 Open the FAIRS software.
- 10.3 Insert the USB flash drive into the computer.
- 10.4 FAIRS software window should read “Importing...” at the top.



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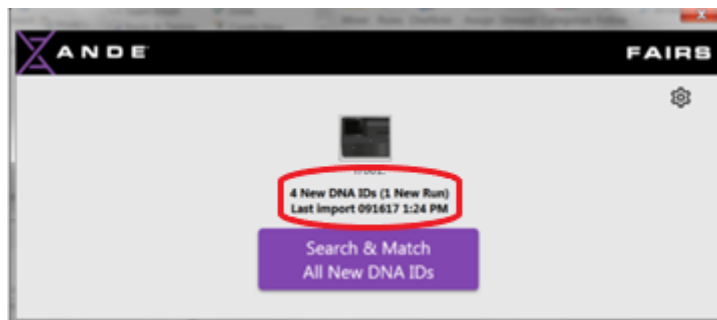
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- 10.5 Verify that recent data has been imported by the “Last import” at the top of the window showing today’s date and time.



NOTE: Data import into FAIRS automatically decrypts the data and saves it in the FAIRS DATA folder.

- 10.6 Close FAIRS and open the “FAIRS DATA” folder on the laptop desktop.
- 10.7 Navigate to most recent run.
- 10.8 Locate zipped folder containing decrypted data from most recent run.
- 10.9 Copy and paste decrypted data on to USB flash drive.
- 10.10 Remove USB flash drive and log off instrument laptop.

11 Network Data Import

- 11.1 Log on to a networked computer and insert USB flash drive.
- 11.2 Save the recently decrypted data to the ANDE DATA folder on the network.
- 11.3 Once done, delete decrypted data off USB.

12 Data Evaluation

- 12.1 Navigate to the network run folder containing the decrypted data.
- 12.2 There should be an Excel file which contains the Allele Table for all samples. For the allelic ladder, there should be a .PNG and an .FSA file. For each sample, there should be a .PNG, .FSA, and .XML file. The .XML file may not be present if the sample failed.
- 12.2.1 Instances where a ladder fails are indicated in the header of the ladder’s .PNG file.

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12.3 One by one, convert all files in the run folder with .PNG extension to .PDF using the “PDF Creator” command based on the settings below:

- Paper size: Letter
- Photo size: 8 x 10 in
- Page margins: Normal
- Fit: Shrink to fit

12.3.1 Do not create PDFs for failed samples.

12.3.2 Save the PDFs to the same run folder. Leave the file name the same as it was in .PNG format.

12.4 Open the allele table (Excel file) to view a tabular summary of the profiles generated. Any missing alleles should be investigated by opening the corresponding electropherogram (.PNG file). See Appendix B: Data Evaluation for more detailed information about peak requirements.

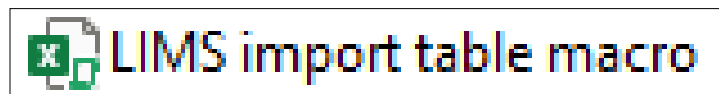
13 LIMS Data Import

13.1 Navigate to the network run folder containing the decrypted data.

13.2 Navigate to the “Instrument to LIMS” folder on the LIMS file share and create a folder with the same name as the LIMS test batch (e.g., Rapid121520_0818). Copy and paste the PDFs previously created into the newly created folder on the LIMS file share.

13.3 In the run folder, open the Excel file containing the Allele Table.

13.4 Open the “LIMS import table macro” Excel file in the ANDE network folder:



13.5 Click “Enable Content” and follow instructions for copying data from the Allele Table into the macro.

13.6 The LIMS import table should be saved in the LIMS import file format (i.e., Excel). Give the import file the same name as the LIMS test batch (e.g., Rapid121520_0818). This file must then be copied and pasted to the previously created run folder (already containing the run PDFs) in the “Instrument to LIMS” folder on the LIMS file share.

13.7 Navigate to the data entry table for output samples in the Rapid DNA test batch.

13.8 Click “Import Data” and import the previously saved LIMS import file.

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- 13.9 While still in the data entry table, choose “Select All”. Then select “Import Instrument Data”. In the window that appears, check the box for “electropherograms”. Click “Browse” and navigate to the previously saved PDFs on the LIMS file share.
- 13.10 “Select All” in the data entry table, release, and save.

14 LIMS Post-Processing

- 14.1 The user who imports the data to the LIMS test batch should click “Performed By” for the Rapid Run Complete task.
- 14.2 In the QC batch parameters, choose the appropriate ladder status from the options shown below, save, and release.
- Chip ladder passes
 - Pre-installed ladder passes
 - Fail

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15 Troubleshooting

Handheld barcode scanner not working	Users have the option of manually typing sample names on the touchscreen in instances when the handheld scanner is malfunctioning. Use the instrument touchscreen or keyboard to type the output OCME ID corresponding to each sample. If the instrument's on-board barcode scanner is enabled, this scanner may be used to scan both the output LIMS labels <u>and</u> RFID tags. The scanner will first emit a rapid red pulse, indicating that the user should hold the RFID tag directly against the scanner. After the beep is emitted to indicate RFID acceptance, the user should hold the LIMS <u>output</u> label about 2 to 3 inches away from the on-board scanner until the touchscreen indicates barcode recognition. Contact an instrument SuperAdmin to inspect scanner settings after the run has completed.
Swab loaded on chip prior to label scanning	If the swab is loaded on the chip before any sample scanning is done, the LIMS label affixed to the outside of the desiccator cartridge can still be scanned as usual. However, the chip should NOT be tilted sideways to allow scanning of the RFID tag. In this instance, allow the RFID scanning to time out. Proceed with running the chip and look for your sample files in an RFID mismatch folder. Samples will need to be re-collected and tested again.
Leaking fluid observed inside instrument	Notify QA and place a service call

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16 Appendix A: Results Summary

16.1 A green ✓ checkmark indicates that the sample passed.

16.2 A yellow ⚠ checkmark may appear due to artifacts such as pull-up. The data is considered to pass the Expert System rules but requires further review to confirm. For passing samples, the number of loci where data was obtained for the sample is indicated in parentheses.


16.3 For male samples, the maximum number of loci is 27.

16.4 For female samples, the maximum number of loci is 24.

16.5 A “Mixture” result indicates DNA detected from two or more individuals.

16.6 A “High” result indicates too much DNA.

16.7 A “Low” result indicates too little DNA.

16.8 A  symbol indicates inconclusive results; sample re-collection advised.

16.9 A red ✗ indicates no alleles detected.

17 Appendix B: Data Evaluation

17.1 If a sample had a failed ILS, peaks may be visible on the electropherogram but they will not be called and will not appear in the Allele Table.

17.2 If the sample had too little DNA, there may be no peaks observed.

17.2.1 For heterozygous loci, both peaks must be ≥ 250 RFU and the lower of the two peaks must have a height \geq the locus-specific peak height ratio (PHR). The peaks may be flagged for imbalance if the lower peak is $>15\%$ but $<$ the locus-specific PHR of the higher peak.

17.2.2 For homozygous loci, the main peak must be ≥ 300 RFU. If there is a minor peak in stutter position, it must be $\leq 15\%$ of the main allele height.

17.2.3 For hemizygous loci (e.g., Y chromosome), the peak must be ≥ 200 RFU.

17.2.4 Other reasons for peaks to be visible in the electropherogram but not reported in the Allele Table are when overall low signal is detected, when triallelic patterns are detected, or when a mixture is detected.

17.3 Peaks that were detected but not reported in the Allele Table may be displayed on the electropherogram with the numerical allele value enclosed in a colored box.

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17.4 An allele enclosed by a red box indicates that certain requirements were not satisfied. Alleles in a red box will not be displayed in the Allele Table.

17.5 An allele enclosed by a yellow box indicates that the peak satisfies some requirements but is off-ladder. Alleles in a yellow box will not be displayed in the Allele Table.

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