

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

ANDE Rapid DNA		
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ANDE® Rapid DNA

1 General

- 1.1 A-chips allow for 5 buccal samples to run simultaneously. See [Section 4](#) for Chip Setup Procedure.
- 1.2 See ANDE® Rapid DNA - Sample Collection, Preparation, and Examination manual for sample preparation instructions.
- 1.3 For Safety Precautions, General Information and troubleshooting user login errors, see [QC370-ANDE® 6C Rapid DNA Maintenance and Troubleshooting manual](#).
- 1.4 Appendix [A](#) and [B](#) contain information found in the ANDE® 6C Rapid DNA Analysis™ System Product User Manual.

2 Power-up ANDE®

- 2.1 Be sure to login to the instrument BEFORE you unpack the chip. Instrument login issues should be resolved before the run commences.
- 2.2 If the ANDE® instrument is OFF, proceed as follows:
 - 2.2.1 Located on the back of the instrument, switch the power toggle to the “ON” position.
 - 2.2.2 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).
 - 2.2.3 If the POST fails, contact QA.
- 2.3 Once the warm-up process is complete (or if the instrument has already been ON), the log-in window will be displayed.
- 2.4 Log in with username and password.
- 2.5 See [QC370- ANDE® 6C Rapid DNA Maintenance and Troubleshooting](#) for user login errors.

3 Unpacking the Chips

- 3.1 Make sure to select the A-Chip box before opening. Be sure the steps below are followed to prevent damage to the chip and leakage of chemicals.

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- 3.2 Record the chip lot number in the LIMS test batch.
- 3.3 Ensure the shipping box is in the upright position. Upon opening the box, cold packs should be surrounding the Styrofoam packaging containing the chip. They can be discarded in regular trash.
- 3.4 Lift Styrofoam container out from the cardboard box using the tape handles attached and set the Styrofoam package onto a flat surface.
- 3.5 Remove tape holding Styrofoam package together and then remove the top piece of Styrofoam packaging to expose the wrapped chip. Remove the wrapped chip from the bottom Styrofoam packaging container by grasping the block (DO NOT HANDLE the chip by the opposite end; only by the block) and lifting the chip from the Styrofoam base.
- 3.6 Set the wrapped chip on a clean, flat surface and unfold the foil pouch.

NOTE: Chips must be used within 30 minutes of opening foil packaging.

- 3.7 Carefully cut open the foil pouch along the seams near the bottom and side seams opposite the block (taking care not to touch the chip) and take the chip out from the packaging by grasping the block portion. Avoid tilting the block as it is removed from the packaging.
- 3.8 Immediately place the chip on the sample loading fixture with block end over the cutout (to protect the detection window).

4 Chip Setup Procedure

- 4.1 Print LIMS output labels and affix to the side of the corresponding sample desiccator.
- 4.2 On the instrument click “Perform Run” on the operator menu.
- 4.3 Fill out the performed by tab in LIMS. This will add the date and time to the batch in LIMS.
- 4.4 Using the date and time listed in the Performed by tab, update the test batch description with the following format: RapidMM22YY_time.
- 4.5 **Chip Setup WITNESS:** Have a witness observe and verify that the following steps are performed:
- 4.6 Read the input and output sample ID for verification.
- 4.7 Scan the output LIMS label on the instrument and ensure that the output sample OCME ID number appears on the instrument screen.
 - 4.7.1 Hold the purple top of the swab (with embedded RFID tag) directly against the RFID scanner and listen for a low beep to confirm RFID recognition.

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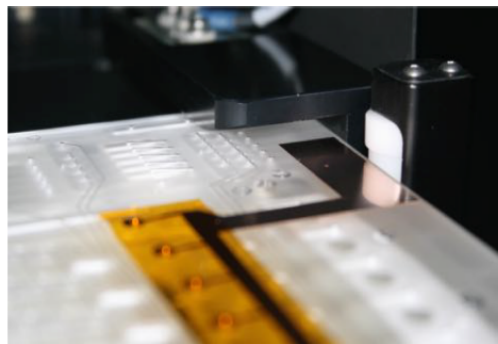
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- 4.8 Remove the plastic seal from the first swab chamber of the chip and remove the swab from the desiccator and insert the swab in its designated chamber on the chip and push down on the cap until you can hear a click.
- 4.9 Click “Next” on the touchscreen and repeat steps 4.5 – 4.8 for the remaining samples.
- 4.10 After four samples are loaded, the ANDE® instrument will prompt you to select which chip type you are running.
 - 4.10.1 Select the “A-chip” as the chip type for buccal swab samples. The instrument will prompt you to load a fifth sample. Confirm your choice by clicking “Yes” when prompted and repeat steps 4.5 - 4.8.

5 Instrument Loading Procedure

- 5.1 Once all the samples are loaded onto the chip, insert the chip into the ANDE® instrument as follows:
 - 5.1.1 Use one hand to hold the block; use the other hand to hold the right corner 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 the chip into the instrument.



- 5.1.2 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.

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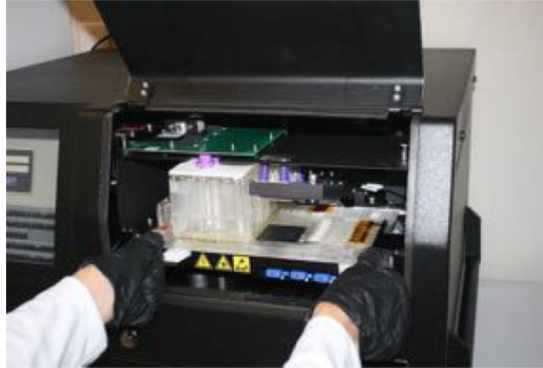
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- 5.1.3 Close instrument door by pressing firmly on the middle of the door. Hold the door down until it latches.
- 5.1.4 Once the chip access door is closed, the instrument will automatically begin sample processing. There is no “start” button or any further action that the user must take to initiate processing. The touchscreen will display the time remaining as the chip processing progresses.
- 5.2 Chip processing time is approximately 94 minutes for A-chips.

6 Run Completion and Chip Disposal

- 6.1 Remove the chip immediately when the touchscreen indicates run completion.
- 6.2 Close the instrument door, press firmly in the middle, and hold until it latches.
- 6.3 For chip disposal; seal the black gasket on the chip with the labels provided to prevent liquid from leaking out of the chip.
- 6.4 Return the chip to the foil pouch and seal with tape. Place used chip in red biohazard bag. Used chips should not be discarded in the regular trash. Discard all remaining packaging.

7 Results Summary

- 7.1 At the end of the run, a results summary will appear on the touchscreen.
 - 7.1.1 Refer to [Appendix A](#): Instrument Results Summary Index for more information on any symbols displayed.
- 7.2 Press “DONE”.
- 7.3 Once the “DONE” button is pressed, the touchscreen will return to the login screen.

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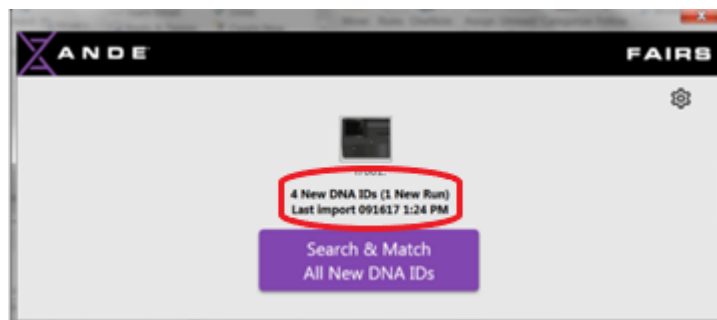
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8 Data Export from the ANDE® Instrument

- 8.1 Insert a USB flash drive in the side of the instrument.
- 8.2 In the operator menu, select “Manage Data”.
- 8.3 Select “Export Run Data”.
- 8.4 Select the date range for the desired run data.
- 8.5 Select the run file to be exported by touching the box to the left of the file.
- 8.6 Press the “Export Data to USB” button.
- 8.7 Once the data has been transferred to the USB, press “OK” and then “Done”.
- 8.8 Remove the USB from the instrument.
- 8.9 On the instrument main menu, press “Logout”.

9 Data Decryption & Exporting Results

- 9.1 Log on to the instrument laptop computer.
- 9.2 Open the FAIRS software. The FAIRS software **must** be opened before inserting the flash drive.
- 9.3 Insert the USB flash drive into the computer. **DO NOT INSERT USB BEFORE OPENING SOFTWARE.**
- 9.4 FAIRS software window should read “Importing...” at the top.
- 9.5 Verify that recent data has been imported by the “Last import” at the top of the window showing today’s date and time.



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

- 9.6 Close FAIRS and open the “FAIRS DATA” folder on the laptop desktop.
- 9.7 Select “i7065” folder
- 9.8 Select “Run data” folder (“RFID mismatches” folder will show up if the scanned RFID does not match).
- 9.9 Navigate to the most recent run. Runs are labelled based on the date and time the run was started on the instrument [i7065_YEARMONTHDATE4digittime.
- 9.10 Copy and paste the decrypted data onto the USB flash drive.
- 9.11 Remove the USB flash drive and log off instrument laptop.
- 9.12 Transfer the decrypted data on the USB to the FBIO network folder and name the data folder the same as the test batch.
- 9.13 Once done, delete decrypted data off the USB.

10 Printing Electropherograms

- 10.1 Navigate to the FBIO network run folder containing the decrypted data.
- 10.2 Create a folder in the STR_DATA→Casework→ANDE→YEAR→MONTH and then unzip the decrypted data into that folder.
- 10.3 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.
- 10.4 Instances where a ladder fails are indicated in the header of the ladder’s .PNG file.

10.5 Convert all files in the run folder with .PNG extension to .PDF based on the settings below:

- Paper size: Letter (8.50” x 11.00)
- Photo size: 8 x 10 in
- Page margins: Normal
- Fit: Shrink to fit
- Orientation: Portrait

10.5.1 Do not create PDFs for failed samples.

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

11 LIMS Data Import

- 11.1 Navigate to the network run folder containing the decrypted data.
- 11.2 Navigate to the “Instrument to LIMS” folder on the LIMS file share and in the Rapid folder create a folder with the same name as the LIMS test batch (e.g., Rapid121520_0818). Copy and paste the PDFs previously created and the excel file into the newly created folder on the LIMS file share.
- 11.3 Open the “LIMS import table macro” Excel and the alleleTable Excel and follow the macro instructions.
- 11.4 Navigate to the data entry table for output samples in the Rapid DNA test batch.
- 11.4.1 Click “Import Instrument Data”
- 11.5 Import the Excel file for the project made from the macro.
- 11.6 Attach the PDFs for the samples.

12 LIMS Post-Processing

- 12.1 In the **QCBatchParams**, indicate “Pass” or “Fail” for the ladder result. Fill in N/A for the thermomixer.

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13 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
Crystals observed inside instrument	Wipe crystals away with ethanol

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14 Appendix A: Instrument Results Summary Index

- 14.1 For passing samples the number of loci where data was obtained for the sample is indicated in parentheses. For male samples, the maximum number of loci is 27. For female samples, the maximum number of loci is 24,

	A green checkmark indicated that the sample successfully passed the Expert System rules and generated a sufficient number of CODIS loci. The maximum number of loci is 27 for males and 24 for females.
	A yellow checkmark indicates that the sample successfully passed the EXPERT system rules but should be reviewed by a forensic analyst.
	A red X indicates that the sample did not meet the minimum number of configured CODIS loci.

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15 Appendix B: ANDE® Expert System Data Evaluation (from ANDE® ADVANCED SCIENTIFIC TOPICS)

- 15.1 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.
- 15.2 If the sample had too little DNA, there may be no peaks observed.
- 15.3 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.
- 15.4 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.
- 15.5 For hemizygous loci (e.g., Y chromosome), the peak must be ≥ 200 RFU.
- 15.6 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.
- 15.7 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.
- 15.8 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.
- 15.9 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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