



Intermountain Forensics

SOP #

AMP-203

Revision #

02

Forensic DNA Technical Leader Approval

Issue Date

03/01/2023

ABI 3500 Instrument Load

1. Purpose

This document describes the steps to set up a plate and loading it on the 3500 Genetic Analyzer instrument.

2. Summary

Directions for setting up and loading a plate on the 3500 genetic analyzer instrument.

3. Procedure

Documentation

1. Open a DOC-307 Case Chronicle document and save a copy to the Batches in Progress folder on the Shared Drive as the Batch ID with the following naming format: YYMMDDAnalyst Initials (Example: 230215SW)
 - a. If previous processing was performed for this batch, then open the previously saved document.
2. Click on the 3500 tab of the saved Case Chronicle.
3. Enter the following information into the header of the document:
 - a. Load Plate (which is the batch ID)
 - b. Performed by
 - c. Date of CE run set-up
 - d. Reagent Lot Numbers
4. Select the kit type from the drop-down list.
5. Add the number of Samples to the Sample field of the header.
 - a. This information is used by the DOC-307 Case Chronicle to calculate the volume of master mix components and should include the total number of samples, reagent blanks, positive control, and amplification negative control. An additional 15% is automatically included for overage to account for pipetting error.
6. Enter the sample identification numbers for all samples that will be included on the tray.
 - a. Each plate should have more than one Allelic Ladder. At least 1 ladder every 3 columns is recommended.
7. To create an import file:
 - a. Click on the Load Import Tab for the kit being utilized.
 - b. Click on File > Save As
 - c. Save the file as a .txt file with the batch ID as the file name.



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Plate Preparation

1. Prepare the reaction reagents by thawing completely, then vortex 3 to 5 seconds and centrifuge briefly before opening.
2. Create a master mix for each Sample/Reagent Blank and Allelic Ladder, including overage, and mix thoroughly by vortexing.
 - a. Refer to the header of the 3500 tab of DOC-307 Case Chronicle for exact volumes suitable for the number of samples.

Kit	ILS	ILS Volume	Hi-Di Formamide Volume
Globalfiler	GeneScan™ 600 LIZ™ Size Standard v2.0	0.4µL	9.6µL
Investigator 24Plex	DNA Size Standard 24plex (BTO)	0.5µL	12.0µL
YFiler Plus	GeneScan™600 LIZ™ Size Standard v2.0	0.4µL	9.6µL

3. Add the appropriate volume of the master mix for the amplification kit into each reaction well that will be used.

Kit	ILS
Globalfiler	10 µL
Investigator 24Plex	12 µL
YFiler Plus	10 µL

4. Pipette 1µL of PCR product or Allelic Ladder to the applicable wells.
5. Pipette 10µL (12µL for Investigator) Formamide to any empty wells that will be included in an injection.
6. Seal the load plate with an appropriately sized septa to cover all wells used.
7. Centrifuge the plate to remove any bubbles.

Running the Instrument

1. Place the load plate on the genetic analyzer:
 - a. Press the **Tray** button on the 3500 instrument to present the auto sampler.
 - b. Place the load plate on a plate base.
 - i. The flat corner should be oriented to the corresponding corner of the plate base.
 - c. Snap the plate retainer over the plate and plate base.
 - d. Open the instrument doors and place the plate assembly onto the auto sampler with the notched end of the plate base away from the user. Ensure that the plate assembly lies flat in the auto sampler.
 - e. Close the doors.



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2. In the Dashboard, click **Create Plate from Template** to display the Open Plate Template from Library Dialog Box.
3. Select the template and click **Open**.
4. In the **Define Plate Properties** screen, enter the plate name. Verify the number of wells, plate type, capillary length, and polymer are already defined in the template.
 - a. Number of Wells: 96
 - b. Plate Type: HID
 - c. Capillary Length: 36
 - d. Polymer: POP4
5. Select **Assign Plate Contents** from the bottom of the define plate properties screen.
6. In the tool bar click **Import** then select the plate import file.
7. From the Plate View select the wells containing, sample controls, or ladders.

Note: Do not include empty wells when assigning assays, results groups, or file name conventions.
8. Verify the assay, file name convention, and results group imported correctly.
 - a. Assay:
 - i. GF-EX+Norm_POP4 (Globalfiler or Yfiler)
 - ii. Investigator 24Plex
 - b. File Name Convention: IMF
 - c. Results Group: IMF
9. Save the plate document.
10. Select **Link Plate for Run** at the bottom of the screen.
11. Select **Load Plates**.
12. Link the plate name to the plate position.
13. Select create injection list.
14. From the injection list select the **Start Run** button to initiate the run.

Reinjections of Previously Analyzed Samples

1. Re-injections and re-preparations of load plates require a separate import file, which is created under a new plate name (Original plate name-2, -3, etc.).
2. The positive and negative controls, applicable sample(s), and at least one allelic ladder are required.
3. Follow the procedures above for plate preparation and instrument operation steps.



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A handwritten signature in black ink, appearing to read "Lisa E. Walker".

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4. *References*

GlobalFiler™ PCR Amplification Kit USER GUIDE
Yfiler™ Plus PCR Amplification Kit USER GUIDE
Investigator® 24plex QS Handbook
Applied Biosystems 3500/3500xL Genetic Analyzer User Guide

5. *Definitions*

N/A