Minifiler – Capillary Electrophoresis

Refer to the "Identifiler Analysis on the ABI 3130*xl* Genetic Analyzer" manual for instructions on how to:

1. set up the 3130*xl* instrument
2. create, import, and link the plate record
3. troubleshoot

A. Preparation of 3130*xl* batch

Ensure that the appropriate System is filled into the "Sys" column.

Table 1

Amplification System/Cycle)	Specification	Run Module Code	Parameters
MiniFiler™	Normal	F	3 kV for 10 sec

B. Master Mix and Sample Addition for MiniFiler™

1. Prepare one master mix for all samples, negative and positive controls, and allelic ladders as specified in the table below (master mix calculation: add 8.7 µL HiDi + 0.3 µL LIZ500 standard per sample).

# Samples + 2	HiDi Form (8.7 µL per sample)	LIZ500 Std (0.3 µL per sample)
16	157 µL	6 µL
32	296 µL	11 µL
48	436 µL	16 µL
64	575 µL	20 µL
80	714 µL	25 µL
96	853 µL	30 µL
112	992 µL	35 µL
128	1132 µL	40 µL

NOTE: HiDi Formamide cannot be re-frozen.

2. Obtain a reaction plate and label the side with the name used for the 3130*xl* Run ID and place the plate in an amplification tray or the plate base. Aliquot **9 µL of mastermix** to **each** well.

C. Adding Samples:

a. Arrange amplified samples in a 96-well rack according to how they will be loaded into the 96- well reaction plate. Sample order is as follows: A1, B1, C1, D1... G1, H1, A2, B2, C2...G2, H2, A3, B3, C3, etc. Thus the plate is loaded in a columnar manner where the first injection corresponds to wells A1-H2, the second A3-H4 and so on.

b. Have someone witness the tube setup by comparing the tube labels and positions indicated on the sample sheet with the tube labels and positions of the tubes themselves.

c. Aliquot the following:

Allelic Ladder:	1 µL
Positive/Negative Controls:	1 µL
Samples:	1 µL

d. When adding PCR product, make sure to pipette the solution directly into the formamide and gently flush the pipette tip up and down a few times to mix it.

e. If an injection has less than 16 samples, add 10µL of either dH_2O, HiDi formamide, or master mix to all unused wells within that injection.

D. Denature/Chill – For MiniFiler™ After Sample Addition:

1. Once all of the samples have been added to the plate, place a new 96-well Septa over the reaction plate and firmly press the septa into place.

2. Spin plate in centrifuge at 1000 RPM for one minute.

3. For Denature/Chill:

   i. Place the plate on a 9700 Thermal Cycler (Make sure to keep the Thermal Cycler lid off of the sample tray to prevent the septa from heating up).

ii. Select the "denature/chill" program. Make sure the volume is set to 10 µL. (or highest volume amount if multiple systems are being run on the same plate)

iii. Press **Run** on the Thermal Cycler. The program will heat denature samples at 95°C for 5 minutes followed by a quick chill at 4°C (this will run indefinitely, but the plate should be left on the block for at least **5 min**).

iv. Update usage log.

v. While the denature/chill is occurring, you can turn on the oven on the ABI 3130*xl*.

E. 3130*xl* Settings

3130*xl* visible settings:

EP voltage 15kV
EP current (no set value)
Laser Power Prerun 15 mW
Laser Power During run 15mW
Laser Current (no set value)
Oven temperature 60°C

Expected values are:

EP current constant around 120 to 160µA
Laser current: 5.0A ± 1.0

It is good practice to monitor the initial injections in order to detect problems.

Table 2

	F
Oven Temp	60°C
Pre-Run Voltage	15.0 kV
Pre-Run Time	180 sec
Injection Voltage	3 kV
Injection Time	10 sec
Run Voltage	15 kV
Run Time	1500 sec