# Yfiler™ – Capillary Electrophoresis

Refer to the "Identifiler Analysis on the ABI 3130*xl* Genetic Analyzer" procedures 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 |
|---|---|---|---|
| Yfiler™ | Normal | M | 3 kV for 10 sec |
| | High | MR | 5 kV for 20 sec |

1. 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…G1, H1, A2, B2, C2…G2, H2, A3, B3, 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.

2. Have another analyst **witness** the tube setup by comparing the tube labels and positions indicated on the Load Plate Screen in the LIMS system with the tube labels and positions of the tubes themselves.

ARCHIVED


© NYC OFFICE OF CHIEF MEDICAL EXAMINER

## B. Mastermix and Sample Addition for Yfiler™

1. Prepare one mastermix for all samples, negative and positive controls, allelic ladders as specified in the table below (mastermix calculation, add 8.7 µL HiDi + 0.3µL GS 500 LIZ standard per sample).

| **# Samples + 2** | **HiDi Form** (8.7 µL per sample) | **GS 500 LIZ Std** (0.3 µL per sample) |
|---|---|---|
| 16 | 156.6 | 5.4 |
| 32 | 295.8 | 10.2 |
| 48 | 435.0 | 15.0 |
| 64 | 574.2 | 19.8 |
| 80 | 713.4 | 24.6 |
| 96 | 852.6 | 29.4 |
| 112 | 991.8 | 34.2 |
| 128 | 1131.0 | 39.0 |

**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.

3. For samples being run at normal parameters: Aliquot the following:

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

4. For samples being run at high parameters: Aliquot the following:

| | |
|---|---|
| Allelic Ladder: | 1 µL |
| Positive/Negative Control: | 1 µL |
| Samples: | 1 µL |

ARCHIVED


© NYC OFFICE OF CHIEF MEDICAL EXAMINER

5. 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.

6. If an injection has less than 16 samples, add at least 9 µL of either $dH_2O$, formamide, HiDi, buffer or mastermix to all unused wells within that injection.

**C. Denature/Chill - For Yfiler™ 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:

   a. 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.)
   b. Select the "dechillYF" program for Yfiler (95°C for 3 minutes followed by 4°C for 3 minutes). Make sure the volume is set to 10 µL.
   c. Press **Run** on the Thermal Cycler.
   d. Update usage log.
   e. While the denature/chill is occurring, you can turn on the oven on the ABI 3130*xl*.

NOTE: If Identifiler and Yfiler samples are on the same plate, the Dechill procedure for Identifiler should be used.

**D. 3130*xl* Settings**

| | |
|---|---|
| 3130*xl* visible settings: | EP voltage 15kV<br>EP current (no set value)<br>Laser Power Prerun 15 mW<br>Laser Power During run 15mW<br>Laser Current (no set value)<br>Oven temperature 60°C |
| Expected values are: | EP current constant around 120 to 160µA<br>Laser current: 5.0A ± 1.0 |

ARCHIVED


© NYC OFFICE OF CHIEF MEDICAL EXAMINER

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

**Table 2**

| | **Y10** | **YR20** |
|---|---|---|
| **Oven Temp** | 60°C | 60°C |
| **Pre-Run Voltage** | 15.0 kV | 15.0 kV |
| **Pre-Run Time** | 180 sec | 180 sec |
| **Injection Voltage** | 3 kV | 5 kV |
| **Injection Time** | 10 sec | 20 sec |
| **Run Voltage** | 15 kV | 15 kV |
| **Run Time** | 1500 sec | 1500 sec |

ARCHIVED

Controlled versions of Department of Forensic Biology Manuals only exist in the Forensic Biology Qualtrax software. All printed versions are non-controlled copies.
© NYC OFFICE OF CHIEF MEDICAL EXAMINER