

FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

Yfiler™ – Capillary Electrophoresis		
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Yfiler™ – Capillary Electrophoresis

Refer to the “Identifiler Analysis on the ABI 3130xl Genetic Analyzer” procedures for instructions on how to:

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

A. Preparation of 3130xl 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.

B. Mastermix and Sample Addition for Yfiler™

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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 + 0.3 μL 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 3130x/ 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
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.

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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 3130xl.

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

D. 3130xl Settings

3130xl 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 160 μA
- Laser current: 5.0A \pm 1.0

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

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Table 2

	M	MR
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

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