

# FORENSIC BIOLOGY PROTOCOLS FOR FORENSIC STR ANALYSIS

Yfiler™ – Capillary Electrophoresis		
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## Yfiler™ – Capillary Electrophoresis

### 1 Additional Resources:

1.1 Refer to the “[PowerPlex Fusion – Capillary Electrophoresis](#)” procedures for instructions on how to:

1.1.1 set up the 3130x/ instrument

Note: The spectral must be changed to Yfiler G5 spectral before running a Yfiler plate on the 3130xl instrument. Due to the need to change spectrals between Fusion and Yfiler/Minifiler, Yfiler samples CANNOT be run on the same plate, or as the second plate, on an instrument running a Fusion plate. See Section [5](#) below.

1.1.2 create, import, and link the plate record

1.1.3 troubleshoot

### 2 Preparation of 3130x/ Batch

2.1 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

2.2 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.

2.3 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. **The entire amp tube label must be read for each sample.**

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### 3 Mastermix and Sample Addition for Yfiler™

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

**Table 2**

# Samples + 2	HiDi Form (8.7 $\mu$ L per sample)	GS 500 LIZ Std (0.3 $\mu$ L 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.**

- 3.2 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.
- 3.3 Aliquot 9  $\mu$ L of mastermix to each well.
- 3.4 Aliquot samples as follows:
- 3.4.1 For samples being run at normal parameters: Aliquot the following:
- Allelic Ladder: 1  $\mu$ L
  - Positive/Negative Controls: 1  $\mu$ L
  - Samples: 1  $\mu$ L
- 3.4.2 For samples being run at high parameters: Aliquot the following:
- Allelic Ladder: 1  $\mu$ L
  - Positive/Negative Control: 1  $\mu$ L
  - Samples: 1  $\mu$ L

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- 3.5 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.
- 3.6 If an injection has less than 16 samples, add at least 9  $\mu\text{L}$  of either  $\text{dH}_2\text{O}$ , formamide, HiDi, buffer or mastermix to all unused wells within that injection.
- 3.7 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.
- 3.8 Continue to Denature/Chill

### 4 Denature/Chill:

- 4.1 Spin plate in centrifuge at 1000 RPM for one minute
- 4.2 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.)
- 4.3 Select the “dechillYF” program for Yfiler (95°C for 3 minutes followed by 4°C for 3 minutes).
- 4.4 Make sure the volume is set to 10  $\mu\text{L}$ .
- 4.4.1 NOTE: If samples from another system are on the plate with the Yfiler samples, use the Dechill procedure and volume for the system with the largest sample volume.
- 4.5 Press **Run** on the Thermal Cycler.
- 4.6 Update usage log.
- 4.7 While the denature/chill is occurring, you can turn on the oven on the ABI 3130xl.

### 5 Changing the Spectral

- 5.1 Set G5 Spectral to most recent YFiler spectral
  - 5.1.1 Go to Spectral Viewer → at Dye Set drop down select G5 → click on drop down and choose the latest passing “YFMFG5” spectral
  - 5.1.2 Click on the ‘Set’ button if necessary
  - 5.1.3 Active Calibration should be set to “YFMFG5” Spectral

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### 6 3130xl Settings

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

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 160µA  
   Laser current: 5.0A ± 1.0

**Table 3**

	<b>M</b>	<b>MR</b>
<b>Oven Temp</b>	60°C	60°C
<b>Pre-Run Voltage</b>	15.0 kV	15.0 kV
<b>Pre-Run Time</b>	180 sec	180 sec
<b>Injection Voltage</b>	3 kV	5 kV
<b>Injection Time</b>	10 sec	20 sec
<b>Run Voltage</b>	15 kV	15 kV
<b>Run Time</b>	1500 sec	1500 sec