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 3130xl 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 3130*xl* 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 Spin down samples at 1000 RPM for one minute.

2.3 Prepare dilutions of amplified samples, if necessary. 0.1X TE^{-4} should be used to make the dilutions. Pipette mix prior to aliquoting for dilution. Ensure that TE lot number is recorded.

2.3.1 When manually recording lot numbers, include the entire series of letters and numbers (i.e., 0.1XTE1612155668) in the Notes section of the test batch.

2.4 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.

2.5 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.**

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 µL HiDi + 0.3µL GS 500 LIZ standard per sample).

Table 2

# 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.

3.2 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.

3.3 Aliquot 9 µL 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 µL
Positive/Negative Controls:	1 µL
Samples:	1 µL

3.4.2 For samples being run at high parameters: Aliquot the following:

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

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 µL of either dH_2O, 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 µL.

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 While the denature/chill is occurring, set up the ABI 3130*xl*.

4.6.1 Turn on oven and set for 60°C

4.6.2 Import the Instrument Plate Record

4.6.3 Set G5 spectral to most recent YFMFG5 spectral

4.7 Following the denature/chill, spin down plate at 1000RPM for 1 minute.

4.8 Place plate into plate assembly and ensure white top is secured to the black base. The notch in the plate will align to the same corner as the notch in the plate assembly.

4.9 Link the plate to the instrument.

4.10 Check Run View to ensure correct number of samples and injections are being run.

4.11 Ensure instrument is ready to go by checking the reservoirs are filled properly, the plate is linked correctly, and no bubbles are present in the polymer block.

4.12 Start run by pressing green **Run** button (play button). When the Processing Plate dialog box opens (You are about to start processing plates…), click OK.

4.13 Enter a LIMS usage log for current run, recording lot numbers of buffer and POP4 in the usage log if they were changed for run.

4.14 Amplification tubes should be stored at 4°C-8°C, filed by instrument and run name.

4.14.1 Amplification tubes should be stored with their most recent 3130 run. For example, a sample initially run on Newton is rerun on Meyer. That tube should now be stored with the samples on the Meyer run. Do not return the tube to its initial run storage box.

4.14.2 If a positive control is being pulled for use from a previous run, the tube should be returned to its original run box after use to remain associated with its original amp set.

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

6 3130*xl* Settings

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

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

Table 3

	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