1. Retrieve amplified samples from the thermal cycler or refrigerator.
2. Prepare thermal cyclers for snap dechill step. Set one thermal cycler to 95C (heat program) and one thermal cycler to 4C (chill program).
3. Spin down samples at 1000 RPM for one minute.
4. Retrieve the following reagents from the associated refrigerator and/or freezer.

PowerPlex Fusion® WEN ILS 500
PowerPlex Fusion® Allelic Ladder
HiDi Formamide*

***NOTE: HiDi Formamide must not be re-frozen.**

5. Record lot numbers of reagents on sheet.
6. Mastermix preparation:
   a. Prepare one mastermix for all samples, negative and positive controls, and allelic ladders as specified below:

(9.5 µL of HiDi + 0.5 µL of WEN ILS500 per sample)

# Samples + 2	**HiDi Form**	**ILS500**
16	171 uL	9 uL
32	323 uL	17 uL
48	475 uL	25 uL
64	627 uL	33 uL
80	779 uL	41 uL
96	931 uL	49 uL

7. Obtain a reaction plate and label the side with the run name.

8. Aliquot **10µL** of **mastermix** to each well.

    a. If an injection has less than 16 samples, add at least 10 uL of either dH_2O, formamide, HiDi, buffer or mastermix to all unused wells within that injection.

9. **Witness step.** Have another analyst witness the sample set-up.

10. Aliquot **1µL** of allelic ladder, positive control, negative control, and sample into their appropriate well.

    i. Sample order is as follows: A1, B1, C1… A2, B2, C2, etc.

11. 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.

12. Spin the plate in the centrifuge at 1000 RPM for one minute.

13. Denature plate for **3 minutes** with the thermal cycler set to 95C.

14. Chill plate for **3 minutes** with the thermal cycler set to 4C**.**

15. While plates are denature/chilling set up 3130 for run.

    a. Turn on oven and set for 60˚

        i. **Manual Control → Send Defined Command For:** click on **Oven.**

        ii. **Command Name** click on **Turn On/Off oven → Send Command**

iii. **Command Name** click on **Set Oven Temperature→60→Send Command**

16. Check if instrument needs a POP4 change. POP4 must be changed after seven days. POP4 change is needed if there is not enough to run the plate. A full piston chamber is ~200µL. A 6 injection plate requires ~450µL. **DO NOT OPEN INSTRUMENT DOORS IF AUTOSAMPLER IS IN MOTION.**

i. Remove a new bottle of POP4 from the refrigerator.

ii. Select **Wizards→Water Wash Wizard** and follow the wizard.

iii. When the **Fill Array** step has completed, remove the anode buffer jar, empty, and fill with 1X Buffer (~15 mL).

iv. Close instrument doors and wait for the steady green light.

v. Click **Finish**

17. Change buffer on instrument. (Buffer only needs to be changed once daily). **DO NOT OPEN INSTRUMENT DOORS IF AUTOSAMPLER IS IN MOTION.**

i. Remove reservoirs and anode buffer jar from instrument.

ii. Rinse all containers using distilled water and wipe dry with a lint free wipe.

iii. Make 1X buffer (45 ml Ultra Pure water, 5 ml 10X buffer) in a 50 mL conical tube

iv. Fill the buffer jar and anode buffer jar with 1X Buffer to their respective lines.

v. Fill the waste and water reservoirs with Ultra Pure water to their respective lines.

18. Import text file

a. **Plate Manager→ Import→ D:\AppliedBiosystems\Plate Records→ Plate Record for Current Run**

19. Set G5 spectral to most recent PPFusion spectral.

Spectral viewer → Dye Set drop down select G5 → List of Calibrations for Dye Set: G5 select PPFusionG5 spectral with the most recent date → click Set

Note: This spectral must be changed to Yfiler of Minifiler G5 spectral before running a Yfiler or Minifiler plate on this instrument. Due to the need to change spectrals between Fusion and Yfiler/Minifiler, Yfiler or Minifiler samples CANNOT be run on the same plate, or as the second plate on an instrument running a Fusion plate.

20. Spin down plate at 1000RPM for 1 minute.

21. Linking plate to instrument

i. **Run Scheduler →Plate View**

ii. Push the tray button on the bottom left of the machine and wait for the autosampler to move forward and stop at the forward position.

iii. Open the doors and place the tray onto the autosampler in the correct tray position, A or B. There is only one orientation for the plate. (The notched end faces away from the user.

iv. Ensure the plate assembly fits flat in the autosampler. The **Plate View** window will change from gray to yellow.

v. Type the exact plate name in the Plate ID window and click **"Search."** Or, click the "**Find All**" button and select the desired plate record.

vi. Click on plate record and then corresponding plate position in the **Plate View** screen. This will cause the yellow screen to turn green.

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

i. **Run Scheduler → Run View**

ii. Click on the run file to see the Plate Map or grid diagram.

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

i. If bubbles are present proceed to **Bubble Removal Wizard**. (Wizards→Bubble Removal Wizard)

24. Start run by pressing green **Run** button (play button).

25. Monitor instrument until event log shows the instrument as begun Pre-Run.

	U
Oven Temp	60°C
Pre-Run Voltage	15.0 kV
Pre-Run Time	180 sec
Injection Voltage	3 kV
Injection Time	5 sec
Run Voltage	13 kV
Run Time	2000 sec

26. Record lot numbers of buffer and POP4 on sheet.

27. Enter a LIMS log for current run.