PREPARING SAMPLES FOR ANALYSIS ON THE GENETIC ANALYZER

Once the instrument has been prepared for a run, the amplified samples must be prepared for analysis by combining amplified DNA with Hi-Di formamide and the size standard. Remember the option to preheat the instrument to 60°C to save time.

- Sample set-up must be done in a Nucleic Acid Workstation located in the PCR Laboratory.

The pictures below shows the many components of the 96 well reaction plate and how they assemble as you progress.

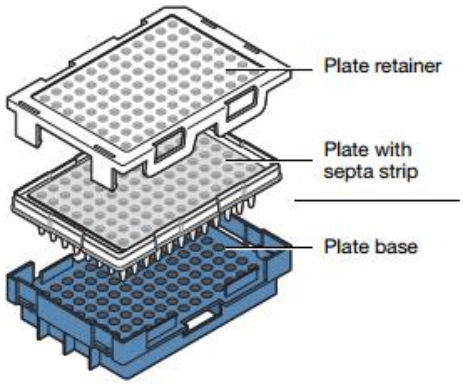

Plate Assembly for the 3500xL

Sample Set Up

Clean the interior surface of the Nucleic Acid Workstation using 10% bleach and kimwipes before beginning.

1. Remove the appropriate number of tubes of Hi-Di formamide and allow them to thaw. Note each kit requires a slightly different volume of Hi-Di formamide. The kit-specific CE set-up worksheet must be utilized for this step.

2. Briefly vortex the ILS. Note that each kit requires a different ILS. The kit-specific CE-set-up worksheet must be utilized. Spin the tubes in a microcentrifuge to remove any liquid from the caps.

3. Prepare a master mix, in a 1.7mL-microcentrifuge tube, based on the number of samples including ladder and controls, plus 2 extra samples to account for pipetting error.
   Controls: Extraction positive, if applicable
   Extraction negative
   Kit amplification positive
   Kit amplification negative
   Male specificity negative control, if applicable
   CE run positive (ladder)
   CE run negative

Note: One ladder should be injected per run.

Note: Warning Chemical Hazard. Formamide is a teratogen and is harmful by inhalation, skin contact, and ingestion. Use in a well-ventilated area. Use chemical-resistant gloves and open only when working in the Nucleic Acid Workstation.

4. Mix thoroughly with a brief vortex and spin the tube in a micro centrifuge to remove any liquid from the cap.

5. Set a 96 well reaction plate into a plate base. For each kit, follow the CE run set-up worksheet specific for that kit to dispense master mix into each well corresponding to a sample, control, or ladder.

6. Remove samples from the thermal cycler and set on the plate base. Place the samples inside the Nucleic Acid Workstation.

Note: Any condensation that may have developed in the top of the tubes must be spun down. Make sure all liquid is out of the caps before opening the tubes.

7. If applicable, remove the rubber lid and retainer clip covering the post-PCR tray and decontaminate. Cover the tray with a kimwipe to avoid contamination.

8. Dispense the amplified product into the corresponding well containing the formamide and size standard master mix according to the kit-specific CE run Set-up worksheet. If applicable, use the multichannel pipettor.

Note: Always change pipette tips immediately after dispelling the PCR product.

9. Cap or seal the tube(s) from which PCR product was just removed.

10. Continue pipetting amplified product into the corresponding wells and seal amplified product.

Note: There are no identifying marks on the wells! It is extremely important to note your position at all times with the sample tubes/wells, sample trays and appropriate worksheets. For samples needing to be re-setup from a different amplification tray, a witness must observe sample additions when manually added and initial the appropriate space on the worksheet.

11. After all the samples have been added, briefly vortex and centrifuge the ladder corresponding to the kit used to amplify the samples.

12. Pipette appropriate ladder corresponding to the kit used to amplify the samples into the designated wells.

Note: The run negative control contains formamide and size standard only.

13. Cover the 96 well reaction plate with 96 well plate septa.

14. Briefly vortex the reaction plate and place in the centrifuge. Set a tray across from it for balance. Make sure all bubbles are removed from the bottom of the tubes after the spin.

15. According to the kit procedure the plate may need to be denatured at 95ºC for 3 minutes. If not, proceed to step 17.

16. According to the kit procedure and after denaturization, the tray may need to be “quick chilled” in the metal ice block for at least 3 minutes. While the samples are being denatured, store the sealed PCR product in a labeled tray at -20°C.

17. Snap the white plastic plate retainer on the 96 well reaction plate. Make sure the plate retainer holes are aligned with the holes in the septa and that it is securely attached.

18. When finished, clean the interior surface of the workstation using 10% bleach and kimwipes and expose to UV light for 30 minutes.