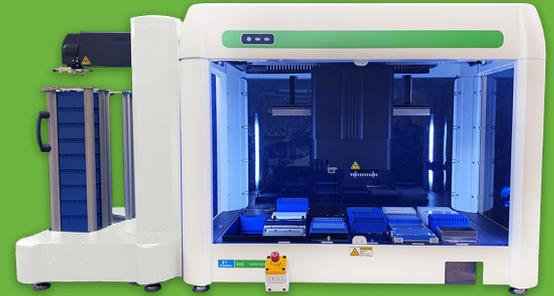


Optimizing On-deck Thermal Cycling

Sciclone® G3 NGSx iQ™ Workstation



Summary

With high capacity tip storage, on-deck thermal accessories, and highly reproducible liquid transfer technology, the Sciclone® G3 NGSx iQ™ workstation offers hands free, high throughput automation of Next Generation Sequencing (NGS) library prep workflows.

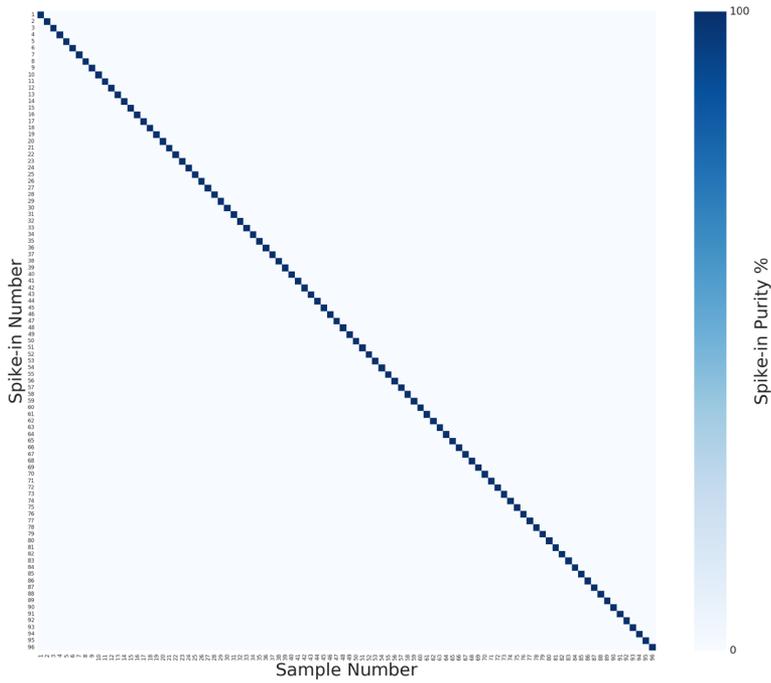
When processing NGS samples on a liquid handler with an on deck thermal cycler (ODTC) preventing evaporation and sample contamination are important considerations. PerkinElmer's disposable PCR plate lid is an automation-friendly solution suited for high throughput NGS sample processing designed to address these issues.

Preventing Cross Contamination

To analyze for cross contamination, 96 libraries were produced using the NEXTFLEX® Pre-plated automated Rapid XP DNA-seq kit on a Sciclone® NGSx iQ™ workstation. Each library was made from 140 ng of Human NA12878 genomic DNA. 10 µl of a 25 pg/µl unique identifiable spike-in control (available from PerkinElmer) were added to each genomic DNA sample prior to library preparation. A single PerkinElmer disposable PCR plate lid was used for the Fragmentation/End Repair/A-tailing reaction step and the PCR step (3 cycles) which were performed on the ODTC. After sequencing each library was analyzed informatically to identify any instances of cross contamination.

Results

Each barcoded library should have just one spike-in amplicon control associated with it. If any other spike-ins were detected not associated with that barcode, cross-contamination occurred. As can be seen in Figure 1, each library was determined to be 100% pure. No cross-contamination between libraries or sample wells occurred.

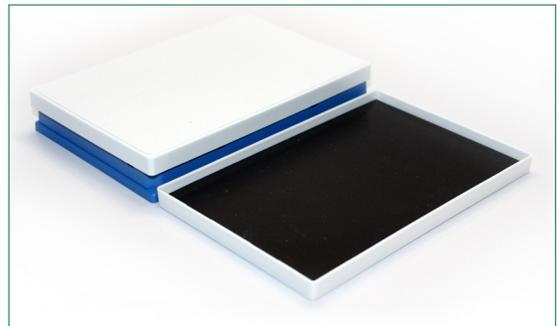


The pattern of unique identifiable spike-in controls indicates no cross-contamination between libraries or wells occurred during library prep.

Reducing Evaporation

PerkinElmer's disposable PCR plate lid was designed to decrease sample evaporation which is essential to obtain consistent results. To analyze for evaporation 50 µl water was pipetted into each well of a 96-well PCR plate and 15 cycles of a standard PCR profile run. The mass of the plate and sample was measured before and after the thermal cycling step and it was determined there was a 2% loss of volume which is consistent with other lids and seals tested.

When using PerkinElmer's disposable PCR plate lid, we found no cross contamination across sample wells and minimal evaporation that is consistent with other commonly used lids and seals. PerkinElmer's disposable PCR plate lid is an automation friendly solution to use when constructing NGS libraries on the Sciclone® G3 NGSx iQ™ workstation.



PerkinElmer Disposable PCR Plate Lid