### Abstract

Preparation of large quantities of limiting, difficult samples such as ChIP DNA can be a time-consuming, challenging process, with the end result of sequencing data that does not support the desired goals of the project. Here, we present enabling the generation of high-quality data from these limiting, difficult samples by harnessing the power of Accel-NGS® 2S library preparation kits with high-throughput automation on the Perkin Elmer Sciclone® G3 NGSX Workstation to prepare large quantities of samples in a timely, robust manner. Highly efficient library preparations that maximize complexity and uniform representation of the genome make the most efficient use of next generation sequencing (NGS) reads and enable comprehensive analysis of DNA samples of limited quality or quantity. End repair of both the 3' and 5' DNA termini utilized in the Accel-NGS 2S Plus and PCR-Free DNA Library Kits allows greater efficiency of adapter ligation. Additionally, sequential adapter attachment requires no adapter ligation for lower input quantities. Together, these deliver a more complex library requiring less sequencing, reducing the overall sequencing cost for a given sample and enabling production of high-quality libraries from low yield ChIP. As little as 100 pg of ChIP starting material can be used to generate confident peak calls that are equivalent to ng input quantities, which decreases the number of cells required without loss of data quality. The minimal sequence-dependent bias of Accel-NGS 2S adapter attachment results in libraries that faithfully represent the ChIP sample. Data quality was determined by analysis of data generated from sequencing on an Illumina® MiSeq®. Optimized Accel-NGS 2S kit configuration combined with the flexible workflow options and optimized pipetting of the Sciclone G3 NGSX Workstation minimize consumable use, sample loss, and increase library efficiency with a walk-away solution capable of generating up to 96 libraries in 4 hours. The technology provides the opportunity to use one kit and one automated program to generate libraries from multiple sample types, regardless of their quality and, in a reproducible and accurate manner.

### Data Fidelity of ER ChIP-Seq at Low Inputs

- A majority of 1000 pg ChIP library peaks overlapped with 30 pg ChIP library peaks.
- Even when starting with undetectable amounts of ChIP DNA, most of the peaks overlapped with 1000 pg ChIP library peaks.

### Molecular Identifiers (MIDs) Retain More ChIP-Seq Data

- Standard de-duplication methods cannot differentiate PCR duplicates from strand and fragmentation duplicates.
- Strands and fragmentation duplicates align to the same genomic location, but are in fact unique library molecules.
- Single read sequencing (e.g., ChIP-Seq) is especially sensitive to standard de-duplication methods.
- MIDs prevent strand and fragmentation duplicates from being removed during de-duplication, which preserves library complexity.

### Conclusions and Acknowledgements

- Accel-NGS 2S Plus Library Kits efficiently construct libraries, even when starting from undetectable amounts of ChIP DNA.
- When used together with the Sciclone G3 NGSx Workstation, Accel-NGS 2S Plus Kits can perform 96 library prep in 4 hours.
- Using 2S MID Indexing Kits with Accel-NGS 2S Plus will further increase the usable amount of sequencing data.
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### Attend our Exhibitor Event to Learn More

Advancing Epigenetics NGS Sequencing and Analysis to the Single-Cell Level
Thursday October 20 at 1:00 to 2:30 PM
Room 8/15, Convention Centre East Building

Dr. Joseph Ecker, Sak Institute for Biological Studies

"Single Cell Methylation Distiguishes Brain Cell Types"

Dr. Ecker will present results of a large scale study in progress using the Single Cell Methyl workflow.

Dr. Adam Blatter, Active Motif

"New Tools for Studying the Epigenomes of Clinical Samples"

Dr. Blatter will present a novel workflow for low input ChIP-Seq using molecular identifiers (MIDs).