Enabling High Throughput Next Generation Sequencing from Low Input ChIP

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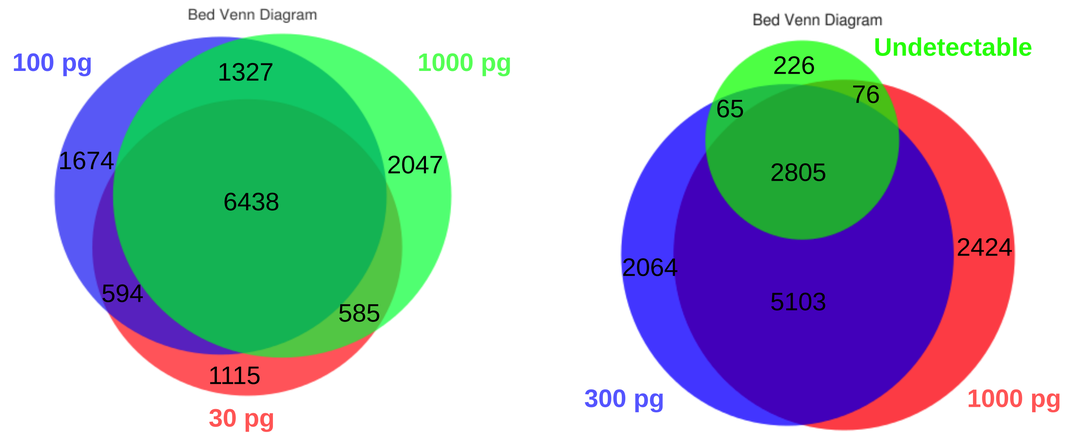
Abstract

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 titration 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 lab efficiencies 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 quantity, in a reproducible and accurate manner.

Data Fidelity of ER ChIP-Seq at Low Inputs

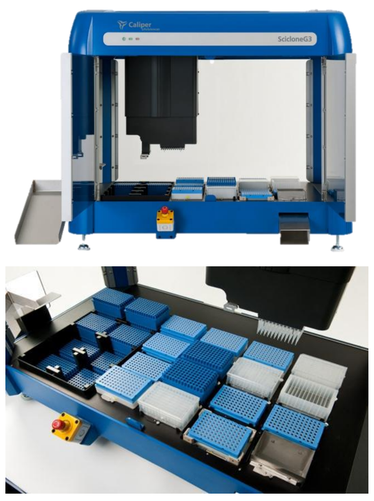


- A majority of 1000 pg ChIP library peaks overlapped with 30 pg ChIP library peaks.
- Even when starting with undetectable amounts of ChIP DNA, most of the peaks overlapped with 1000 pg ChIP library peaks.

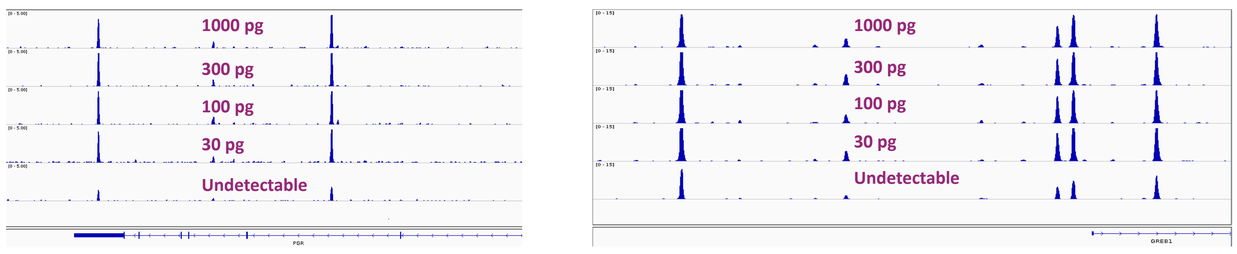
Sciclone G3 NGSx Workstation

Standardized deck layout

- Accel-NGS 2S on the Sciclone NGSx: 96 library preps in 4 hours
- Complete walk-away NGS library prep solution
- Partial tip loading capability
- 4 temp locations enable storage of master mixes and incubations
- Deck "cut out" for tip storage
- Enclosed deck
- Magnetic plates for SPRI™ cleanups
- Graphical User Interface (GUI) provides deck layout and master mix instructions

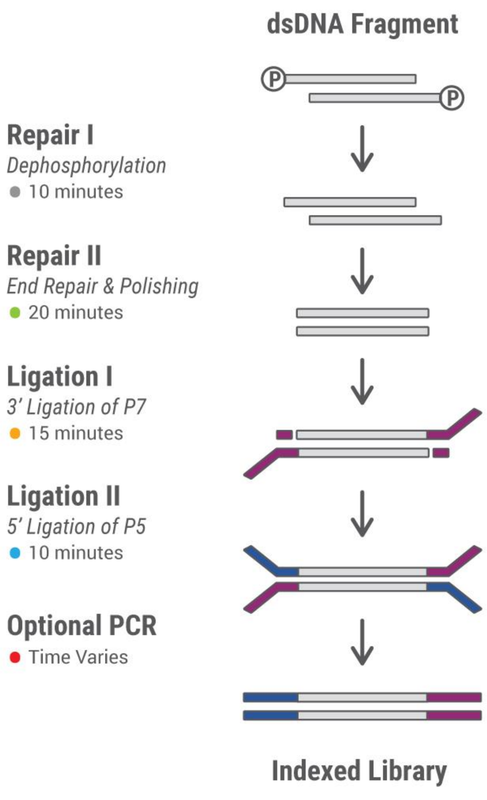


Estrogen Receptor ChIP-Seq Peaks



- Progesterone Receptor (PGR) and Growth Regulation By Estrogen In Breast Cancer 1 (GREB1) loci are known binding sites for the Estrogen Receptor transcription factor.
- Even when starting with undetectable amounts of ChIP DNA, similar peaks are observed at these loci.

Accel-NGS 2S Plus Workflow



Repair both 5' and 3' ends of damaged ChIP DNA fragments
- More efficient adapter attachment
- Increased library complexity

No adapter titration needed
- Automation friendly
- Reduce concerns of proper input quantification

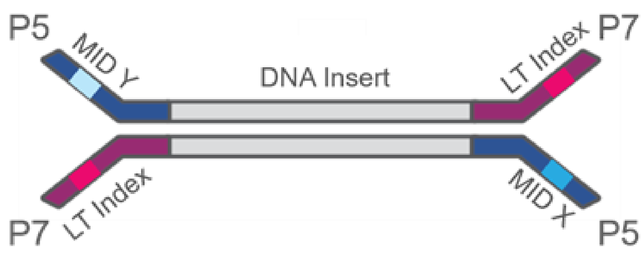
Sequential ligation
- Prevents adapter dimer formation
- Enables optimized ligation to each strand

Compatible with cfDNA and FFPE samples
- A single kit for all of your sample types
- PCR-free libraries from 10 ng of cfDNA

Balanced coverage of AT- and GC-rich regions
- Accurate representation of genome
- More complete genome coverage

Optional PCR
- PCR-free inputs down to 10 ng
- Inputs down to 10 pg with PCR

Molecular Identifiers (MIDs) Retain More ChIP-Seq Data



- Swift has introduced MIDs, paired with Accel-NGS 2S DNA Library Kits.
- MID technology is offered by Active Motif and Swift.
- MIDs enable identification of unique library molecules.

- Standard de-duplication methods cannot differentiate PCR duplicates from strand and fragmentation duplicates.
  - Strand 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.

Estrogen Receptor ChIP-Seq Sequencing Metrics

Sample	Quantity Used for ChIP	Quantity Used for Library Prep	Peaks	FRiP	Mappability	Redundancy	PCR Cycles	Library Concentration
MCF7 derivative	500 ng	undetectable*	3198	1.76%	86.03%	2.6%	15	21.00 nM
MCF7 derivative	10 µg	30 pg	8776	4.68%	86.14%	11.4%	15	4.71 nM
MCF7 derivative	10 µg	100 pg	10067	4.78%	88.40%	5.4%	12	1.61 nM
MCF7 derivative	10 µg	300 pg	10064	4.90%	88.95%	2.6%	12	4.35 nM
MCF7 derivative	10 µg	1000 pg	10440	4.85%	89.21%	0.0%	9	3.13 nM

Accel-NGS 2S Plus libraries were constructed from Estrogen Receptor (ER) ChIP DNA at a variety of quantities.
*Quantity was too low to quantify by Qubit®, and is considered <20 pg.

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 preps in 4 hours.
- Using 2S MID Indexing Kits with Accel-NGS 2S Plus will further increase the usable amount of sequencing data.
- Swift Biosciences would like to acknowledge the contribution of the following members from Dana Farber Cancer Institute: Paloma Cejas, Myles Brown, Rinath Jesselson, and Shirley Liu.

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, *Salk Institute for Biological Studies*
"Single Cell Methylomes Distinguish Brain Cell Types"
Dr. Ecker will present results of a large scale study in progress using the Single Cell Methyl workflow.

Dr. Adam Blattler, *Active Motif*
"New Tools for Studying the Epigenomes of Clinical Samples"
Dr. Blattler will present a novel workflow for low input ChIP-Seq using molecular identifiers (MIDs).

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