Magelia®, a versatile multi-OMICs platform for full automation and miniaturization of complex molecular biology applications: high quality PCR-free WGS library prep on low input samples

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Introduction

- Multi-OMICs are providing an integrated perspective to power discovery across multiple levels of biology, accelerating research and medicine. However, upstream sample preparation applications are lagging due to low input biological samples (low quantity and/or low quality) and the requirement for highly trained staff. The need to treat low-input samples, or to partition them for multiple analyses, paired with an innovative automation approach is thus clear.
- The Magelia® platform, Inorevia's multi-OMICs solution, combines patented technologies to unlock automation of multiple challenging molecular biology protocols while showcasing outstanding performance for low-input sample preparation.
- Preparation of sequencing-ready libraries without PCR amplification presents significant advantages in data quality for Human WGS, such as marked reduction in base specific biases due to DNA polymerases, reduced duplication rates, reduced false positives, greater sensitivity for indel and copy number variants calling, better evenness of coverage across the whole genome than with-PCR approaches.
- New England Biolabs' NEBNext® Ultra™ II FS DNA PCR-free Library Prep Kit for Illumina® enables full library preparation featuring stable enzymatic fragmentation with a single protocol, while removing PCR bias, regardless of DNA input amount or GC content.
- Here, we showcase high-quality, high-yield fully automated library prep in Magelia®, using standard DNA samples at and below the kit's low limit (50 ng).

Key Benefits of Magelia® Technology

- **High efficiency on low quantities**
- **Full automation of complex workflows**
- **Time savings on hands-on & turn around time**
- **Automated reagent aliquoting**
- **Versatility: compact benchtop multi-OMICs platform**
- **Unparalleled precision: no cross-contamination or evaporation**

Methods

Standard gDNA samples NA12878 (Coriell Institute) and Human gDNA (G1521, Promega®) were treated for WGS using 50 and 20 ng as input, respectively. Samples were treated in parallel manually and in Magelia® using NEB's NEBNext® Ultra™ II FS DNA PCR-free Library Prep Kit for Illumina® following the manufacturer's instructions (Figure 1). Samples were treated in 8 replicates per condition.

Library concentrations were measured using NEB's Luna® Universal qPCR Master Mix and Thermo Fisher Scientific's Qubit™ with the dsDNA HS kit. Library profiles were verified using Agilent's 2100 Bioanalyzer and their High Sensitivity DNA kit.

NA12878 were prepared using the kit's low limit as input (50 ng). Libraries were then sent for shallow sequencing to NEB Sequencing Core. Four manual and four Magelia® treated samples were sequenced in a NextSeq 500 Mid output v2.5 (150 cycles) cartridge, targeting 1 million pairs per sample (pooled with additional samples from other projects). NA12878 libraries were then sequenced using the Novaseq targeting 30x coverage.

To challenge the kit's low limit, we next tested library prep using 20 ng of Human standard gDNA (G1521, Promega®). **Importantly, this is 2.5x less than the kit's low limit of 50 ng genomic DNA.**

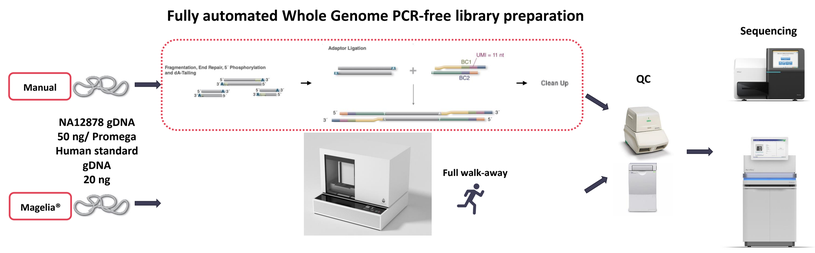

FIGURE1: PCR-FREE LIBRARY PREP WORKFLOW FOR VALIDATION OF LOW INPUT GDNA STANDARDS IN MAGELIA®

Samples were Qced as previously described, then shallow sequenced using a Miseq 2x150 Standard V2 cartridge, targeting 2.5 million pairs.

FASTQ files were evaluated using the FastQC tool for quality control inspection. The reads were then trimmed and mapped to the Homo sapiens (human) genome assembly GRCh37 (hg19) version.

Magelia® shows reproducible PCR-free library prep at the kit's low limit, while reducing incubation times

Figure 2 shows NA12878 library profiles for an 8-sample run in Magelia® and one manually treated sample as an example. Although the nature of the library biases its size distribution in the Bioanalyzer (300 bp targeted), all samples show a tight distribution. This was further confirmed through sequencing (Figure 3B).

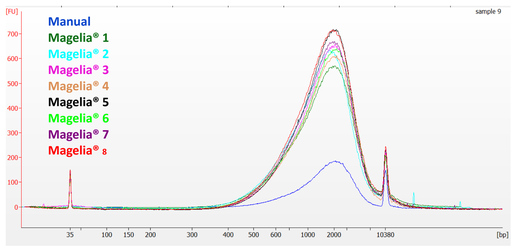

FIGURE 2: LIBRARY PROFILES FOR N12878 SAMPLES USING 50 NG AS INPUT

Differences in fluorescence between Magelia® and manually treated samples are due to elution in 3x less volume for Magelia® treated samples. Comparable and sufficient output concentrations of sequenceable material were secured for all treated samples.

Due to reaction miniaturization, enzymatic fragmentation was shortened 4x in the Magelia® platform while displaying good reproducibility.

At the QC level, these data show consistent and fully automated PCR-free library prep, securing sufficient material for sequencing from sample treatment in Magelia®.

Magelia® treated samples show quality data and stable size distribution at the kit's low limit

Figure 3A shows GC% for Magelia® and manually treated samples. GC% were comparable, with Magelia® treated samples showing less variability in AT rich regions than in the manual treatment.

Figure 3B shows average peak insert size for all shallow sequenced samples. Magelia® treated samples display less variability in terms of peak insert size.

Deep sequencing of these libraries confirmed high quality results for both Magelia® and manually treated samples, with percentages of unmapped reads, duplicates, chimeras and adapter dimers below 1.5 %. Improved average insert size was confirmed for Magelia® treated samples, as previsouly mentionned.

At the shallow and deep sequencing levels, these data show reproducible and fully automated PCR-free library prep, securing high quality material for sequencing in Magelia®.

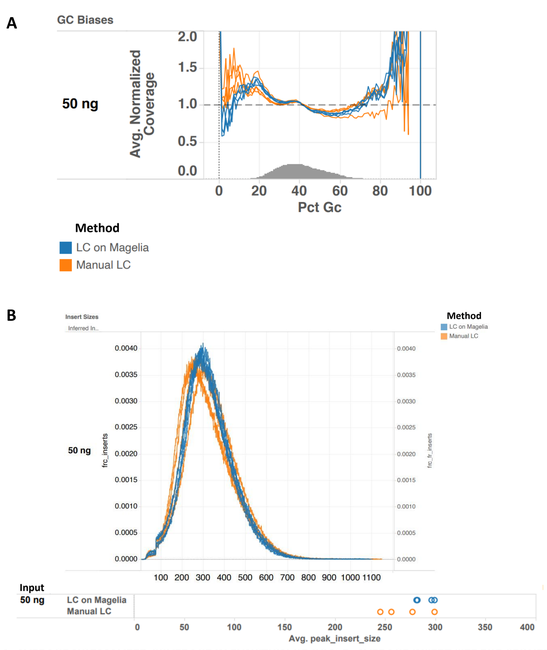

FIGURE 3: A. AVERAGE NORMALIZED COVERAGE IN FUNCTION OF GC % B. AVERAGE INSERT SIZE FOR SEQUENCED LIBRARIES

Magelia® secures more quality material for sequencing using less than the kit's low limit of input

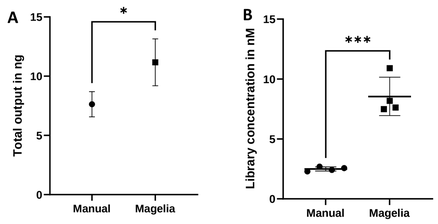

FIGURE 4: A. TOTAL OUPUT IN NG FOR MANUALLY AND MAGELIA® TREATED SAMPLES B. LIBRARY CONCENTRATION IN NM FOR MANUALLY AND MAGELIA® TREATED SAMPLES

While challenging the kit's low limit, we tested library prep using 20 ng of Human standard gDNA.

As shown on Figure 4, significantly more sequencing ready material was secured for Magelia® treated samples, irrespective of elution volume.

This translates into sufficient material for three independent sequencing runs per Magelia® treated sample versus one for manually treated samples (depending on the sequencing application).

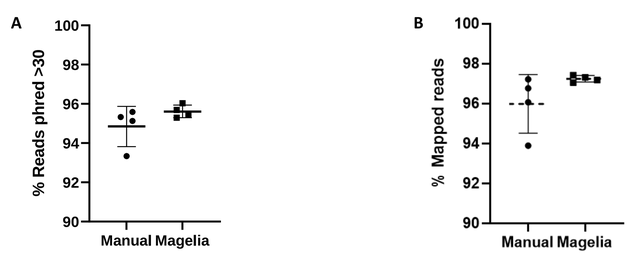

FIGURE 5: A. PERCENTAGE READS WITH PHRED SCORE >30 B. % PERCENTAGE MAPPED READS

Sequencing shows high quality data while starting with quantities below the kit's low limit input

Shallow sequencing of manually and Magelia® treated samples showed that high quality reads were secured for all samples, with an average 95.98% and 97.24% of reads with PHRED score >30, respectively. In terms of percentages of mapped reads, 94,85% and 95,61% for manually and Magelia® treated samples, respectively.

Although comparable quality was secured for all treated samples, a clear, manually treated outlier was present, and generally these samples behaved more variably.

Furthermore, a similar trend was observed for the percentage of true pairs (Figure 6A).

Finally, as shown on Figure 6B, mappings on the mitochondrial chromosome showed less duplicates for Magelia® treated samples, except for manually treated replicate 1.

Shallow sequencing showed that high quality sequencing data can be secured from less than half of the low limit of gDNA required by the manual of the protocol, while securing 3x more sequencing material in Magelia® treated samples.

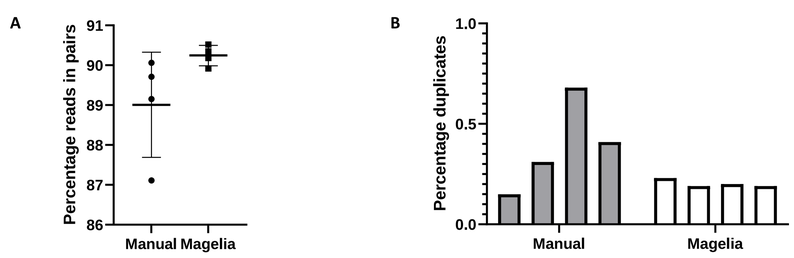

FIGURE 6: A. PERCENTAGE TRUE PAIRS FOR MANUALLY AND MAGELIA® TREATED SAMPLES B. PERCENTAGE DUPLICATES FOR MANUALLY AND MAGELIA® TREATED SAMPLES

Conclusion & Perspectives

Inorevia's Magelia® disruptive technology paired with NEB's NEBNext® Ultra™ II FS DNA PCR-free Library Prep Kit for Illumina®embodies an automated, robust and reproducible solution for full WGS library prep, securing more quality sequencing material while extending the low limits of the kit. The kit's robustness is complemented by the platform's capacity to treat low-input samples

We are continuously developing a variety of miniaturized applications optimized for low-input samples:

- RNA-seq library prep with rRNA depletion/mRNA capture, challenging the limits of material required and securing more material with the same amount of starting RNA, while reducing amplification cycle number.
- WGS PCR+ and PCR-free library prep with mechanical fragmentation, challenging the low limit of material required and reducing adapter dimer ratios.
- ChIP-seq reducing the number of cells required by the kit and yielding high quality data with low duplication levels. And many more to come!