# Automation of Sample Extraction and Library Preparation for Cell-Based Next Generation Sequencing

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## Overview

This work focuses on the development of a novel single touchpoint automated workflow that:

- Performs lysis of cell membranes and nuclei
- Extracts and purifies the freed gDNA from cells
- Performs library preparation of the extracted DNA
- Has total hands on time of 30 minutes
- Produces reproducible, sequence ready library with comparable QC metrics to manual workflows

## Introduction

As Next Generation Sequencing (NGS) has gained traction as a viable and efficient method of furthering clinical diagnostics and genomics research, it has become vital to prioritize accessibility and ease of use. This work focuses specifically on cell-based NGS, which involves extraction of DNA from specific tissues or cell cultures. Sequencing these cells has numerous applications including:

- Organoid research
- Tissue specific diagnostics
- Non-specific diagnostics
- Characterization of cell homogeneity

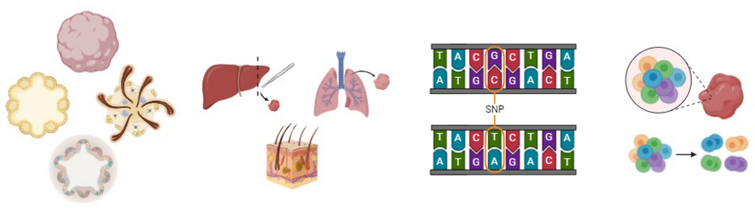



*Figure 1: Applications of cell-based sequencing[1]*

Although significant strides have been made in increasing accessibility of NGS through decreasing costs, required workload, and procedure time, the library preparation process remains a major bottleneck in the NGS workflow. The difficulties of NGS library prep, and specifically cell-based library prep include:

- High sensitivity to human error
- Significant hands-on time (8-9 hours)
- Sensitivity in handling cells during extraction
- Reproducibility of exact conditions

Therefore, there is a need for a stand-alone automated workflow that can mitigate these burdens and allow for low expense sequencing research and diagnostics.

## Methods

***Automated Device:*** The workflow was developed on a capillary based device (BioQule™, Revvity, Waltham, MA), with two axis of movement for liquid handling, thermal zone for thermocycling, and a magnetic plate for purification steps.

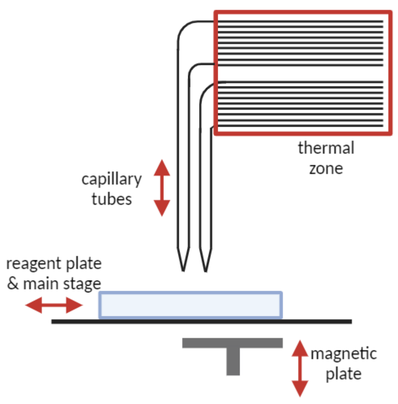



*Figure 2: Schematic of automated device components and axis of motion[1]*

***Automated Workflow:*** The automated workflow has two main processes; extraction and library preparation.

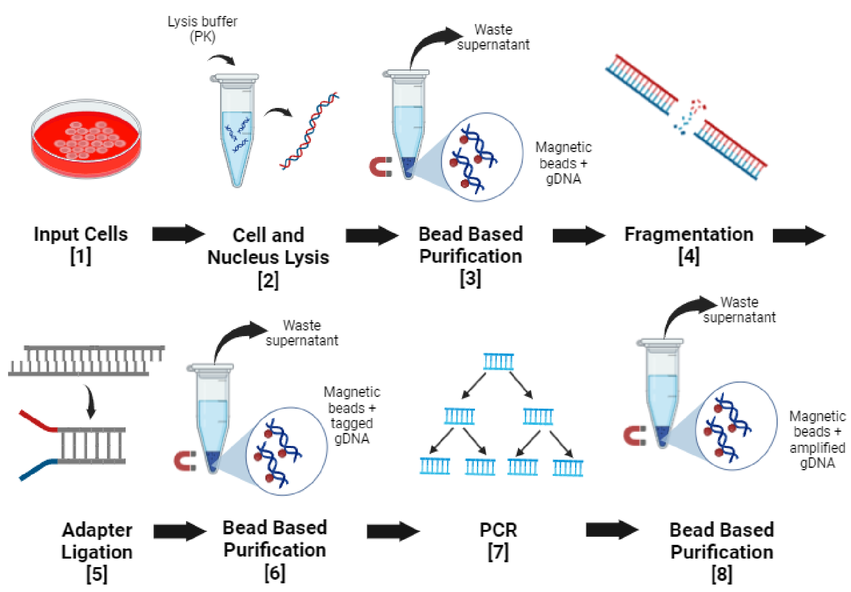



*Figure 3: Workflow for gDNA extraction and library preparation. All steps are automated on the device[1]*

***Extraction:*** Following optimization of lysis procedures, the following conditions/processes were used for extraction.

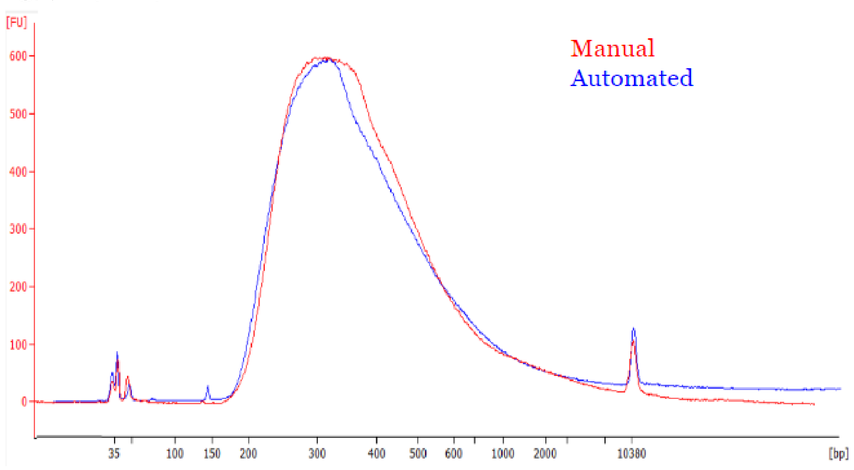
- Proteinase K (PK) was used as lysis buffer for both cell membrane and nucleus
- Magnet bead purification was conducted using AMPure®XP beads (Beckman Coulter®, Brea, CA)

***Library Preparation:*** Reagents from the Watchmaker DNA Library Preparation Kit[2] (Watchmaker Genomics®, Boulder, CO) were used, with additional wash steps, washing detergents, and modified reagent quantities to optimize library preparation on the automated system.

***Validation:*** AML12 cells (0.5 million cells) were used for workflow validation with the *Watchmaker DNA Library Preparation Kit* (Watchmaker Genomics®, Boulder, CO) and *IDT® for Illumina® DNA/RNA UD Indexes Set B, Tagmentation* (Illumina®, San Diego, CA) kits. Following library preparation, automated and manual samples were validated on the Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA) and Qubit Flurometer (ThermoFisher Scientific, Waltham, MA). Sequencing was performed on the Illumina® MiniSeq® sequencer (Illumina®, San Diego, CA) with paired-end 150 bp read lengths for automated and manual samples.

## Results

The full automated protocol yielded 13.82 ng/µL of dsDNA (n=7), comparable to manual library preparation of 14.22 ng/µL (n=4).



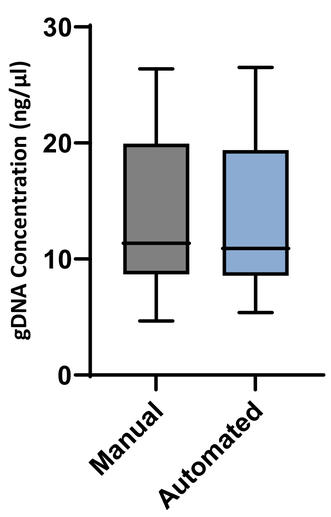




*Figure 4 (above): Comparison of dsDNA on Bioanalyzer (Agilent Technologies, Santa Clara, CA) for manual and automated procedures showing near identical distributions*

*Figure 5 (left): Comparison of dsDNA yield for automated and manual procedures on Qubit Flurometer (ThermoFisher Scientific, Waltham, MA)*

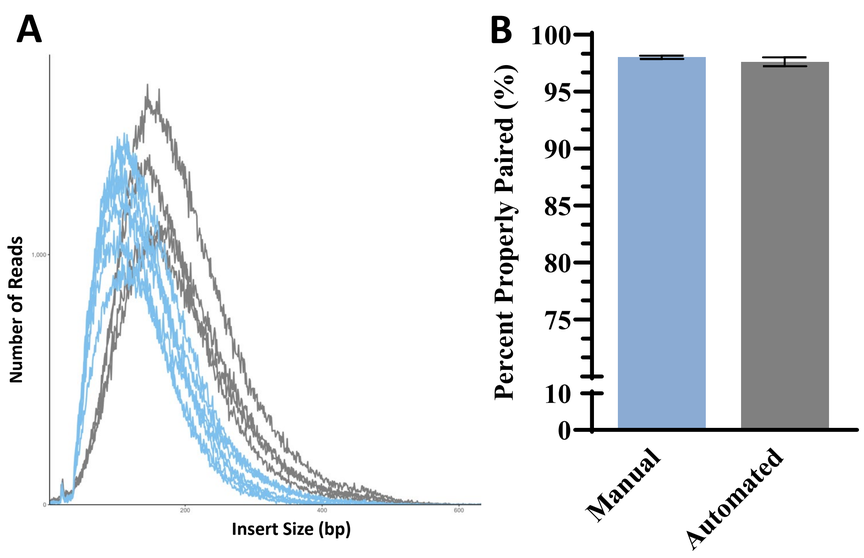



*Figure 6: (A) Comparison of distribution of insert sizes for manual and automated protocols. Blue represents automated and grey represents manual (B) Comparison of properly paired alignment percentages between manual and automated protocols. The mus musculus mm39 build was used as the reference genome*

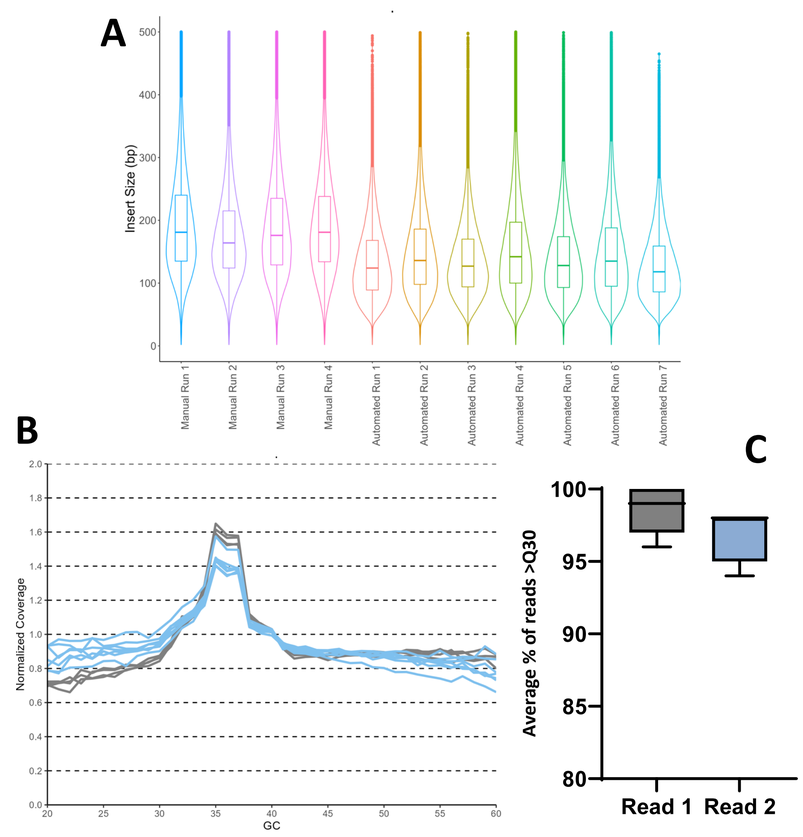



*Figure 7: (A) Violin plots of distributions of insert sizes for individual manual and automated runs. Workflow is consistent and reproducible across runs (B) Normalized GC bias coverage from 20-60%. Manual samples are shown in grey, and automated samples in blue (C) Analysis of average quality scores for paired end reads (forward and reverse) in the automated runs. Averages are above Illumina standards*

## Conclusions / Future Directions

- Single touch point workflow for preparation of sequencing ready samples directly from cell suspension
- Comparable concentrations and sequencing results to manual controls
- Potential to assist cell-based research and clinical diagnostics
- Validation to be conducted on an expanded range of cell lines
- Potential to expand workflow to include tissue dissociation, for entirely automated tissue to sequencing procedure

## Acknowledgements

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## References

[1] Figures generated with BioRender

[2] Watchmaker Genomics, Watchmaker DNA Library Preparation Kit. 2024.