

A Column-Free, SPRI-Based Cleanup Workflow for High-Molecular-Weight DNA Extraction from Tissue Samples

Compatible with Oxford Nanopore sequencing, demonstrated on high-lipid brain tumor tissue.

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Summary

High-molecular-weight (HMW) genomic DNA is a critical input for Oxford Nanopore Technology (ONT) sequencing, enabling the resolution of structural variants, copy-number alterations and methylation-based tumor classification. Conventional column-based extraction workflows typically involve 10–15 pipetting steps and can deliver fragmented DNA that limits the performance advantages of long-read platforms, particularly from lipid-rich matrices such as brain tissue.

This application note describes a column-free cleanup workflow based on ClassiX™ CleanUp (RUO), a magnetic bead-based SPRI purification reagent. The workflow consolidates the cleanup into approximately five pipetting steps and preserves HMW DNA integrity. Feasibility was evaluated on brain tumor biopsies, a deliberately challenging sample type and benchmarked against a column-based reference protocol.

The method yielded an N₅₀ read length of ~14 kb, 99.7% nanopore retention across sequential runs, and ~17,000 passed reads per MinION flow cell. These results demonstrate technical feasibility on high-lipid tissue and support the use of ClassiX™ CleanUp for research applications where HMW DNA recovery and reduced hands-on variability are priorities. The workflow is highly compatible with fast lysis workflows even with complicated sample types (such as brain, blood, etc.).



ClassiX™ CleanUp (RUO)
(Cat. No. CXCU-10mL)

APPLICATION NOTE

Introduction

Long-read sequencing platforms such as Oxford Nanopore Technology (ONT) offer superior resolution for applications including tumor genotyping, structural variant detection, copy-number analysis and methylation profiling. The performance of these platforms depends directly on the quality of input DNA: fragment integrity, purity, and the effective removal of short degradation products all influence library behaviour and pore occupancy.

Solid Phase Reversible Immobilization (SPRI) chemistry, first described by DeAngelis et al. (1995), offers a tuneable mechanism for size-selective DNA purification. The concentration of polyethylene glycol (PEG) and salt controls the size threshold at which DNA binds to paramagnetic beads, allowing selective retention of HMW fragments while short contaminants, primers and residual RNA are removed (Lis & Schleif, 1975; Hawkins et al., 1994).

Compared with column-based cleanup, a magnetic-bead SPRI workflow offers two advantages familiar to users working with long-read sequencing: the elimination of spin columns, which can shear HMW fragments and a lower pipetting step count, which reduces hands-on variability across operators and runs.

Brain tumor tissue was selected as the validation matrix precisely because it represents one of the more challenging sample types for nucleic acid extraction, rich in lipids, cellularly heterogeneous and high in RNA. A workflow that performs reliably on this matrix is likely to transfer to less demanding sample types.

Experimental Methods

Tumor tissue biopsies (~12 mg) were processed using the optimized workflow summarized below. The full workflow comprises approximately five hands-on pipetting operations across lysis, binding, washing and elution.

Step	Time	Description
1. Tissue lysis	~6 min	12 mg tissue in 200 µL Buffer TLS (Xpedite Diagnostics, TLS-25mL) with Proteinase K (1 mg/mL; Xpedite Diagnostics, PRK-1mL), ± RNase A (0.1 mg/mL; QIAGEN, Cat. No. 19101); SwiftX Grinding Tubes with 6 × 2 mm metal beads (Xpedite Diagnostics, SXG-SMB); 2 min on TissueLyser (QIAGEN, Cat. No. 85300) at 45 Hz, followed by 4 min at 65 °C.
2. SPRI binding & size selection	3–6 min	Add 0.35× ClassiX™ CleanUp (Xpedite Diagnostics, CXCU-10mL) binding mix to 200 µL input; 2–5 min incubation at room temperature; 1 min magnetic separation. Selectively captures HMW DNA (>1.5–2 kb); smaller fragments are removed in the supernatant.
3. Washes	~3 min	Two washes with 1 mL 70% ethanol; 30 s drying on the magnetic rack (Xpedite Diagnostics, MAG-12).
4. Elution	~1.5 min	Elute with 100 µL 10 mM Tris, pH 7.6 (molecular biology grade) at 50 °C for 1 min; 0.5 min magnetic separation on MAG-12 rack.

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Note: magnetic separation was performed using the Xpedite Diagnostics magnetic rack (Cat. No. MAG-12, MAG-12combo). Two preparation conditions were compared in parallel with and without RNase A during lysis, to evaluate the impact of RNA contamination on downstream sequencing performance.

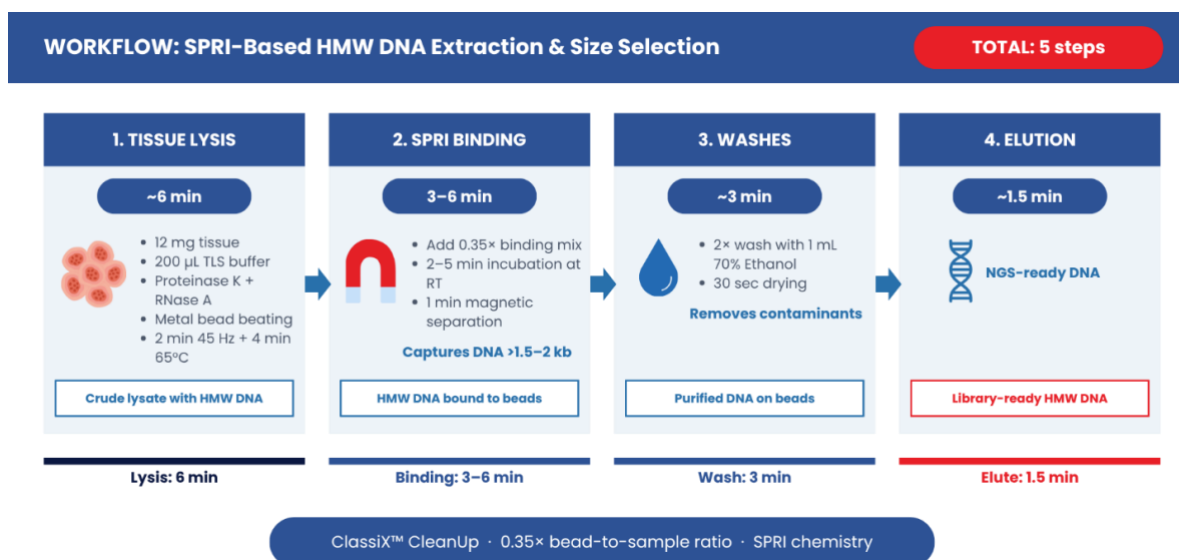


Figure 1 Optimized SPRI-based workflow for HMW DNA extraction from tumor tissue using ClassiX™ CleanUp.

Extracted DNA was quantified by NanoDrop spectrophotometry and Qubit fluorometry. PCR-free library preparation and sequencing was performed on an Oxford Nanopore MinION flow cell with the Rapid Sequencing Kit V14 (SQK-RAD114); quality metrics recorded included active pores, pore occupancy, reads called (passed/failed), bases called, mean read length and N₅₀ read length.

Results

Validation on brain tumor tissue using Oxford Nanopore sequencing demonstrated that the ClassiX™ CleanUp workflow preserves high-molecular-weight DNA integrity and delivers sequencing performance consistent with the requirements of ONT long-read applications. RNase A treatment during lysis was identified as a critical optimization step for this RNA-rich tissue type.

Table 1 Sequencing performance metrics.

Parameter	With RNase A
Active pores (pore scan)	907
Pore occupancy	57.5%
Reads called (passed / failed)	17.27 k / 4.38 k (79.7%)
Bases called (passed / failed)	43.51 Mb / 3.71 Mb (92.1%)
Mean read length	5.23 kb
N ₅₀ read length (vs. reference method)	~14 kb (vs. ~8.5 kb)

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Gel electrophoresis confirmed the presence of HMW DNA bands well above 5,000 bp in both conditions. RNase A treatment yielded a cleaner absorbance profile and a higher DNA concentration by Qubit (43.60 ng/ μ L with RNase vs. 38.6 ng/ μ L without).

The SPRI-based workflow achieved approximately 2 \times longer N₅₀ read length than the column-based reference method (~14 kb vs. ~8.5 kb), with minimal nanopore damage (only 3 pores lost across sequential runs). RNase A treatment improved passed-read yield by over 100% (17.27 k vs. 8.57 k reads) and pore occupancy by approximately 5 percentage points, confirming its importance for more RNA-rich tissue matrices.

Note on throughput: The column-based reference method generated a higher total read count over the same timeframe, consistent with its shorter fragment profile. At ~17,000 passed reads per MinION flow cell, users whose applications require higher total read counts would need to scale to multiple flow cells or adjust the bead-to-sample ratio to trade fragment length for read count.

Discussion

This study demonstrates that a column-free, SPRI-based cleanup workflow can deliver HMW DNA of sufficient quality for Oxford Nanopore sequencing from a lipid-rich tissue matrix. The workflow was found highly compatible with fast lysis workflows even with complicated sample types (such as brain, blood, etc.). The two operational advantages most likely to matter to users working with long-read platforms are the elimination of spin columns and the reduction to approximately five hands-on pipetting steps, compared with 10–15 in comparable column-based workflows. Both reduce a recognized source of user-to-user variability.

The 0.35 \times bead-to-sample ratio was selected to retain fragments in the target range for ONT library preparation while removing shorter contaminant DNA. This behaviour is consistent with canonical SPRI size selection: higher PEG concentrations (higher ratios) capture smaller fragments, while lower ratios selectively retain HMW DNA (Lis & Schleif, 1975; Schmitz & Riesner, 2006). ClassiX™ CleanUp is validated for bead-to-sample ratios from 0.5 \times (HMW enrichment) to 1.8 \times (standard cleanup), allowing researchers to tune fragment retention to their specific workflow requirements.

RNase A inclusion during lysis emerged as a critical optimization. Brain tissue is particularly RNA-rich, and residual RNA competes for pore loading during nanopore sequencing. The doubling of passed-read yield and measurable improvement in pore occupancy with RNase A treatment provide a clear recommendation for laboratories working with similar tissue types.

Importantly, the cleanup process was gentle on the sequencing hardware: only 3 nanopores were lost out of 907 across two sequential runs, indicating that ClassiX™ CleanUp does not introduce contaminants that damage flow cells. Moreover, the method is compatible with the Rapid Sequencing kit (PCR-free library preparation, no transposase inhibition in library preparation step).

APPLICATION NOTE

The results presented here establish technical feasibility on a challenging high-lipid tissue type. The summary statistics support the use of the workflow for research applications where HMW DNA recovery and reduced operator variability are the primary goals.

The strong performance of ClassiX™ CleanUp (RUO) on brain tissue, one of the more demanding matrices for nucleic acid extraction due to its high lipid content, cellular heterogeneity, and RNA abundance, suggests broad applicability to other complex tissue types, including FFPE samples, other solid tumor biopsies (pancreatic, hepatic, fibrotic tissues), NGS library cleanup workflows, and environmental monitoring applications where nucleic acid recovery from complex matrices benefits from tunable SPRI binding conditions.

Literature

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ClassiX™ CleanUp is for Research Use Only (RUO). Not for use in diagnostic procedures.

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