APPLICATION NOTE

Miniaturized automated library preparation using the NEXTFLEX Rapid XP v3 DNA-Seq kit.

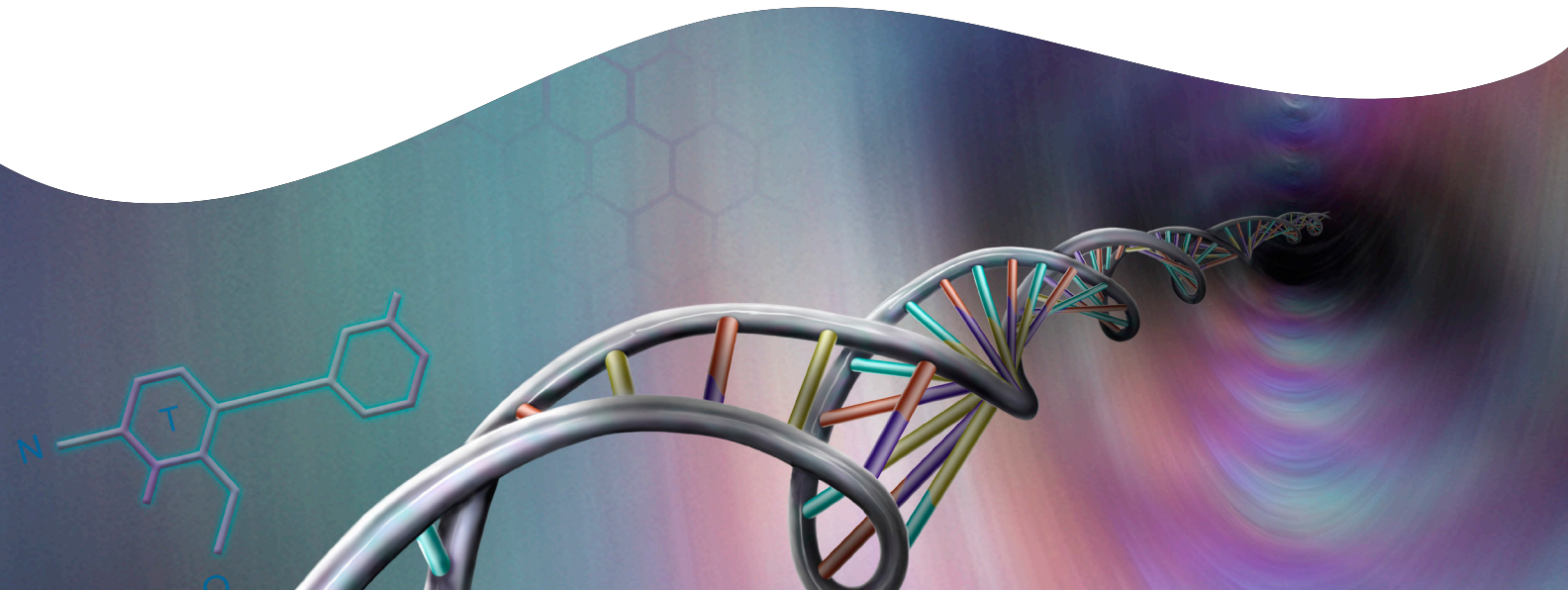
Introduction

Whole-genome sequencing (WGS) applications increasingly require library preparation workflows that can support high sample throughput while maintaining consistent library quality, automation compatibility, and efficient reagent use. As WGS adoption expands across population-scale, agricultural, translational, and routine genomic applications, laboratories are under increasing pressure to reduce total workflow cost without compromising data quality or operational robustness. This is particularly important for laboratories processing large sample cohorts, where workflow scalability and cost efficiency must be balanced against the need for reproducible sequencing-ready libraries.

The NEXTFLEX™ Rapid XP v3 DNA-Seq is a Revvity solution designed to support high-throughput WGS library preparation. To evaluate its suitability for miniaturized automated implementation, the workflow was tested at one-quarter reaction volume in a 96-well plate format using the FlexDrop™ Plus Non-Contact Dispenser (Revvity) and G.PURE™ GEN 2 NGS Clean-Up Device (Dispendix).

The study assessed whether the miniaturized workflow could generate libraries of suitable concentration and size distribution while maintaining clean negative controls and avoiding detectable adapter or primer-dimer contamination.

This application note summarizes the miniaturized library preparation workflow and pre-sequencing quality-control results, sequencing performance, coverage uniformity, and genotype concordance using a well-characterized Mimix™ Reference Standard.



Materials and methods

Experimental design

The NEXTFLEX Rapid XP v3 DNA-Seq workflow was evaluated in a 96-well target plate format using a checkerboard sample layout. 86 input wells contained 25 ng of Mimix Male gDNA Reference Standard (Revvity), while 10 random wells contained 0 ng input and served as no-template controls (Figure 1).

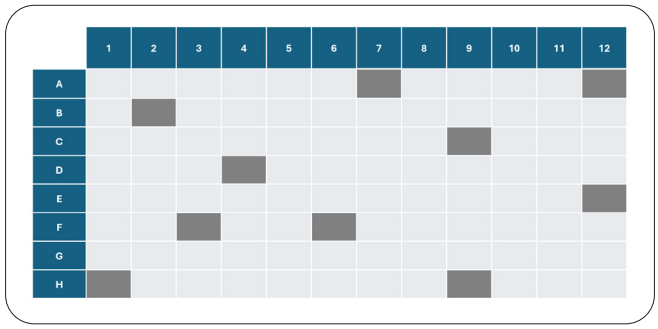


Figure 1: Checkerboard for 96-well format at one-quarter reaction volume. The 10 wells corresponding to no-template controls are presented in dark shading.

Miniaturized workflow

The standard NEXTFLEX Rapid XP v3 DNA-Seq workflow was miniaturized to one-quarter reaction volume as indicated (Figure 2). Full-volume reactions use a total reaction scale of 20 µL, whereas the one-quarter volume protocol scales reactions down to 5 µL, reducing library-prep reagent consumption while maintaining the same overall workflow.

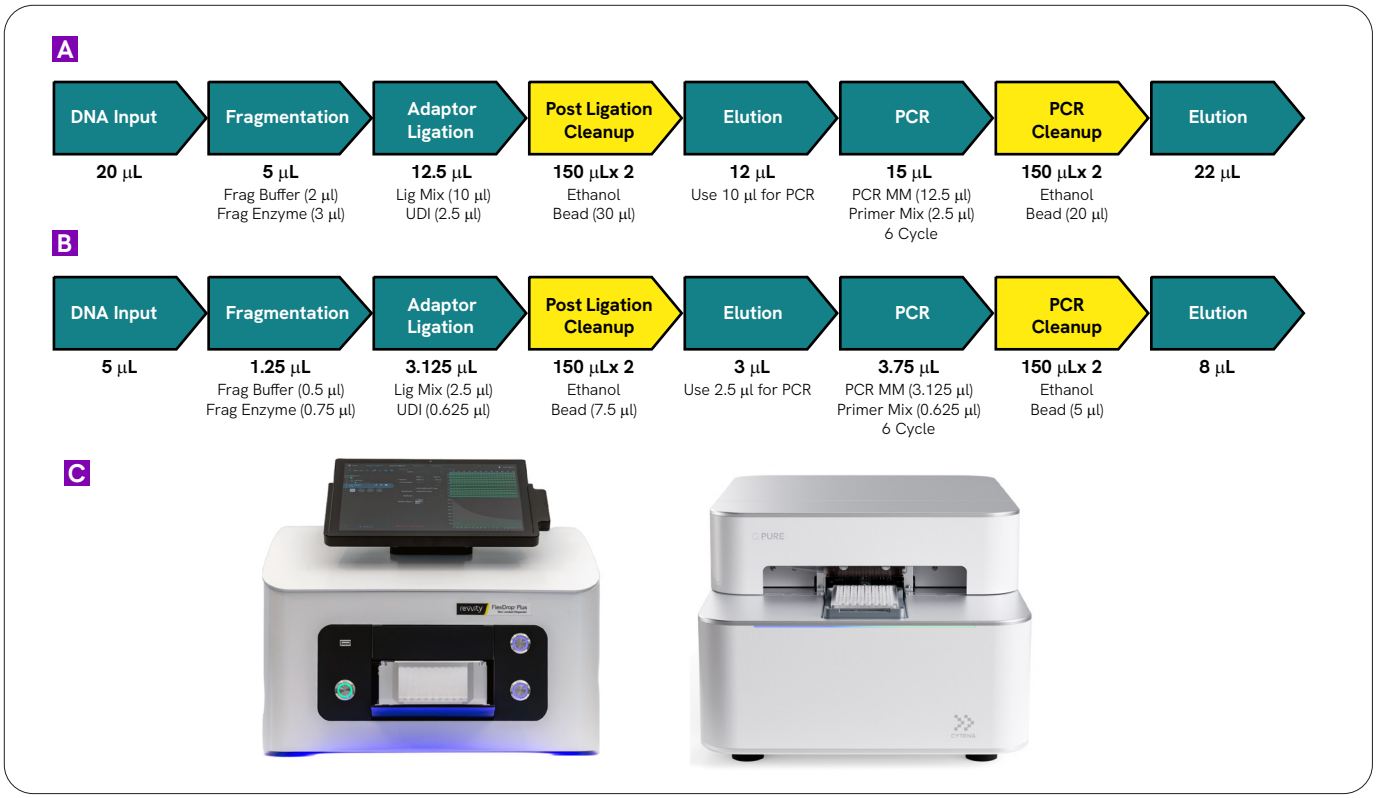


Figure 2: Comparison of the standard and miniaturized NEXTFLEX Rapid XP v3 DNA-Seq workflows. A. Standard NEXTFLEX Rapid XP v3 DNA-Seq kit workflows. B. Miniaturized workflow. The FlexDrop Plus system was used for reagent dispensing steps, shown in blue, while the Dispendix G.PURE™ device was used for cleanup-related steps, shown in yellow. Liquid-class settings and offset were adjusted for the different reagent classes to support accurate dispensing at reduced reaction volumes. C. The FlexDrop Plus dispenser, left, and the Dispendix G.PURE™ device, right, used in the automated miniaturized workflow.

Library QC, sequencing and data analysis

Final libraries were quantified using the Thermo Scientific® Qubit® assay and run on the Agilent TapeStation® HS D1000 to review size distribution. Eight libraries corresponding to a representative column of the plate (column 5) were pooled and run on an S2 flow cell on the Illumina® NovaSeq® 6000 platform at 2x150 bp read length. Analysis was performed using a custom script, using the VCF file provided for the Mimix Reference Standard and GRCh38 build as reference.

Results

Miniaturized workflow generated consistent libraries across the 96-well plate

Qubit quantification showed that input wells produced libraries with average library preparation of 61 ng/µL (Figure 3A). This indicates that the miniaturized workflow generated sufficient material for downstream sequencing. By contrast, the negative control wells produced Qubit values of zero, indicating no measurable DNA carryover by this assay under the tested conditions.

TapeStation analysis was performed for all libraries, including the negative controls. All 86 input wells showed clean profiles with appropriate size distribution for WGS (Figure 3B). No library peak was detected in the no-template controls by TapeStation analysis.

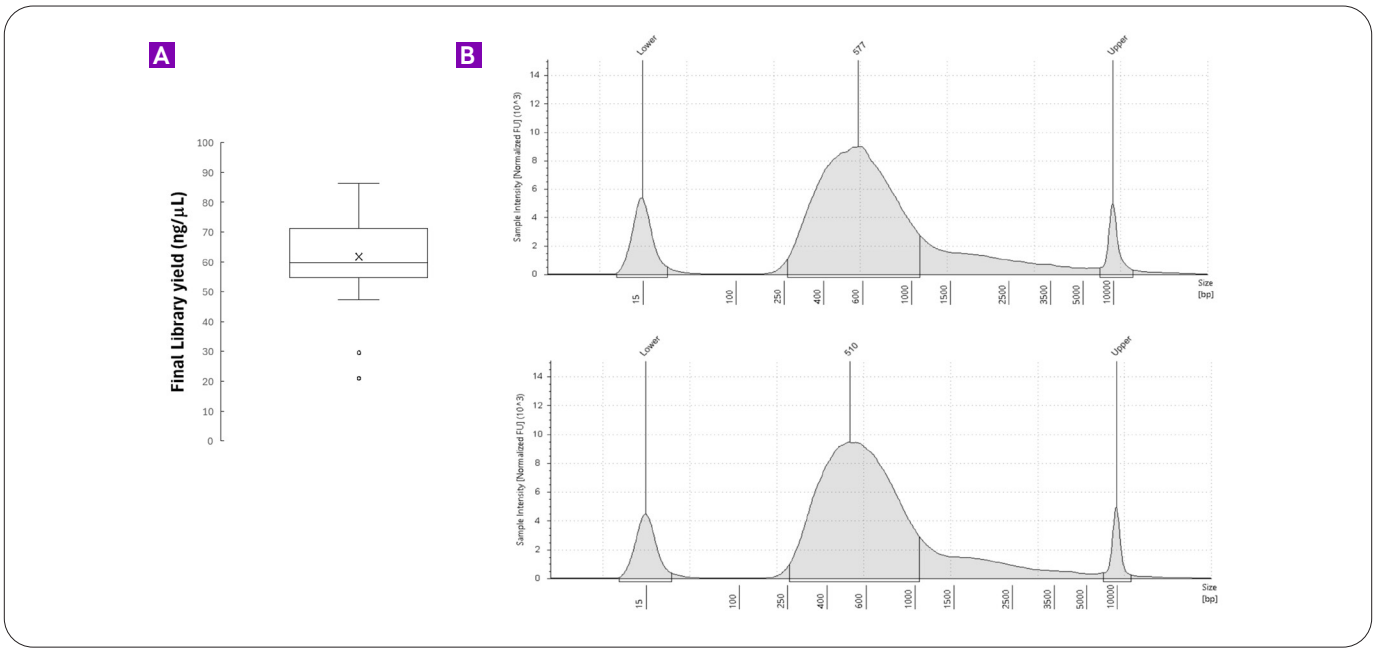


Figure 3: A. Yields obtained with NEXTFLEX Rapid XP v3 DNA-Seq kit across the 86 input samples. B. Representative TapeStation profiles of the libraries obtained. This distribution is consistent with an insert size of ~400 bp. No detectable adapter dimer was observed.

Coverage uniformity

Sequencing generated an average of 399 million read pairs per sample. On average, 98.3 % of reads aligned to the human genome, resulting in a mean genome coverage of 35X.

Coverage uniformity is an important quality metric for reliable and efficient detection of genetic variation across the genome. In WGS studies, uneven coverage can lead to reduced sensitivity in poorly covered regions, inefficient use of sequencing output, and potential bias in variant calling. To evaluate the uniformity of coverage distribution, Guanine-Cytosine (GC) bias was assessed across the eight replicates (Figure 4).

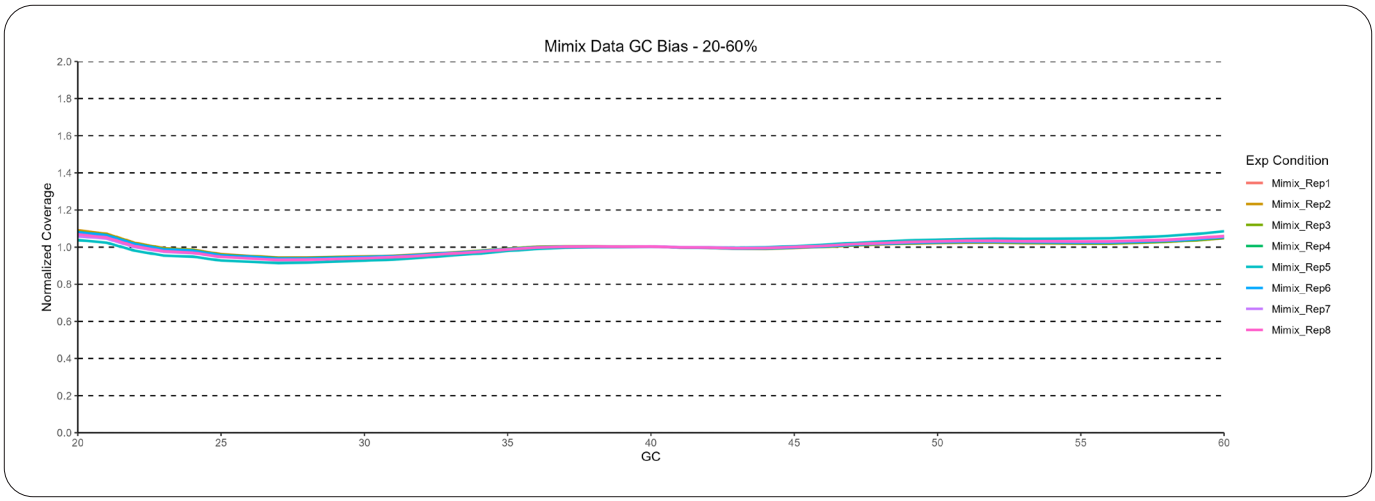


Figure 4: Normalized coverage across varying % GC content for the 8 replicates analyzed. Normalized coverage remained close to 1.0 across the evaluated GC-content range for all eight sequenced libraries, indicating limited GC-associated coverage bias.

Concordance between expected genotype from Mimix Reference Standard and observed genotype

The Mimix Male Genomic DNA Reference Standard was selected as input material because it contains a well-characterized set of 2,663 variants with population allele frequency below 10 %, distributed across multiple genomic regions.

This makes it a suitable reference material to assess whether miniaturization of the WGS workflow affects variant detection sensitivity, particularly for low-frequency variants that may be more challenging to recover consistently.

Among these variants, 269 are classified as missense variants. These are especially relevant in genome-wide screening studies because they result in amino acid substitutions and may therefore directly affect protein sequence and potentially protein function.

For this reason, the concordance analysis focused on this subset of missense variants, comparing the expected genotypes defined for the Mimix Reference Standard with the genotypes observed after sequencing and variant calling.

This approach provides a practical measure of whether the miniaturized workflow preserves accurate variant detection performance under reduced reaction-volume conditions.

Concordance between expected genotype from Mimix Reference Standard and observed genotype was 100% for the 269 variants studied. Table 1 lists some of those variants.

For each variant, the table reports the affected gene, public variant identifiers, the expected alternate allele fraction in the reference material, the reference and alternate alleles, and the mean allele-specific coverage observed across the eight sequenced libraries.

Table 1: Representative subset of 15 Mimix missense variants analyzed. Expected Alt fraction (%) represents the expected sample-level allele fraction for the variant, not the population allele frequency. Ref is the reference allele at that genomic position. Alt is the alternate allele expected in the Mimix reference material. All listed variants were concordant with the expected genotype. A full list of the 269 variants can be provided upon request.

Gene	dbSNP ID	ClinVar	Expected Alt fraction (%)	Ref	Alt	Mean Ref coverage	Mean Alt coverage
HPS1	rs58548334	175335	69.20 %	A	G	13.25	32.9
RBM20	rs942077	53184	68.00 %	G	C	14	30.0
BAG3	rs3858340	53943	48.80 %	C	T	15	29.0
ECHS1	rs1049951	370659	100.00 %	G	A	0	17.3
ECHS1	rs10466126	1245298	100.00 %	A	G	0	16.4
DCLRE1C	rs12768894	253714	61.80 %	T	C	16	13.8
CDH23	rs779974496	1481561	45.50 %	C	T	17	17.4
CDH23	rs1449510921	1319554	48.80 %	G	A	16	18.5
CDH23	rs1227065	55102	100.00 %	A	G	0	36.0
CDH23	rs1227051	55120	100.00 %	G	A	0	38.4
CDH23	rs41281330	55122	45.80 %	G	A	18	18.5
CDH23	rs11592462	55162	40.00 %	C	G	18	18.6
BMPR1A	rs11528010	50221	72.20 %	C	A	13	14.9
DBT	rs12021720	134332	100.00 %	T	C	0	25.1
ATM	rs1801516	133907	31.80 %	G	A	12	15.9

Conclusion

This evaluation demonstrates that the NEXTFLEX Rapid XP v3 DNA-Seq kit can be successfully miniaturized to one-quarter reaction volume and implemented in an automated 96-well format using the FlexDrop Plus dispenser and the Dispendix G.PURE™ system..

Across 86 input wells, the workflow generated consistent library yields and clean size profiles. Sequencing of eight representative libraries further demonstrated high alignment rates, low adapter-dimer contribution, uniform coverage, and concordance between expected and observed genotypes, supporting accurate variant detection under reduced reaction-volume conditions.