xGen Broad-Range RNA Library Prep for flexible transcriptomics

Abstract

Transcriptome profiling applications require enrichment strategies that maximize recovery of biologically relevant RNA while aligning with sample quality and study objectives. Two commonly used approaches are poly(A) enrichment and RNA exome capture, each offering distinct advantages for specific transcriptomic applications.

The xGen Broad-Range RNA Library Preparation Kit supports both workflows through stranded RNA library preparation. Poly(A) enrichment selectively isolates polyadenylated messenger RNA prior to library construction, providing a streamlined approach for gene expression studies using high-quality RNA. RNA exome capture enriches coding transcript sequences through hybridization capture following library preparation, making it well suited for challenging sample types, including formalin-fixed paraffin-embedded (FFPE) tissue.

This application note highlights the capabilities of both workflows using representative data generated with the xGen Broad-Range RNA Library Preparation Kit. Together, these complementary solutions enable researchers to tailor transcriptome profiling strategies based on sample quality, project requirements, and sequencing objectives while leveraging a common library preparation workflow.

Introduction

RNA sequencing has become a foundational tool for studying gene expression, transcript structure, and biological pathways across a wide range of research applications. Because ribosomal RNA comprises the majority of total cellular RNA, transcriptome profiling workflows typically employ enrichment strategies to increase the proportion of informative sequencing reads and improve data quality. The xGen Broad-Range RNA Library Preparation Kit can be incorporated into both poly(A) enrichment and RNA exome capture workflows, enabling researchers to select an enrichment strategy based on sample quality, sequencing objectives, and study requirements.

Figure 1 illustrates the two enrichment approaches evaluated in this application note. Although both workflows generate stranded RNA-seq libraries using a common library preparation foundation, they differ in how transcript enrichment is achieved. Poly(A) enrichment selectively isolates polyadenylated messenger RNA prior to library preparation, while RNA exome capture enriches coding transcript sequences through hybridization capture following library construction. While RNA exome capture is frequently associated with FFPE and degraded samples, the workflow is also compatible with high-quality RNA, enabling researchers to select enrichment strategies based on experimental goals, transcript coverage requirements, and sample quality. Together, these complementary workflows provide flexibility for both routine gene expression studies and analysis of challenging RNA samples.

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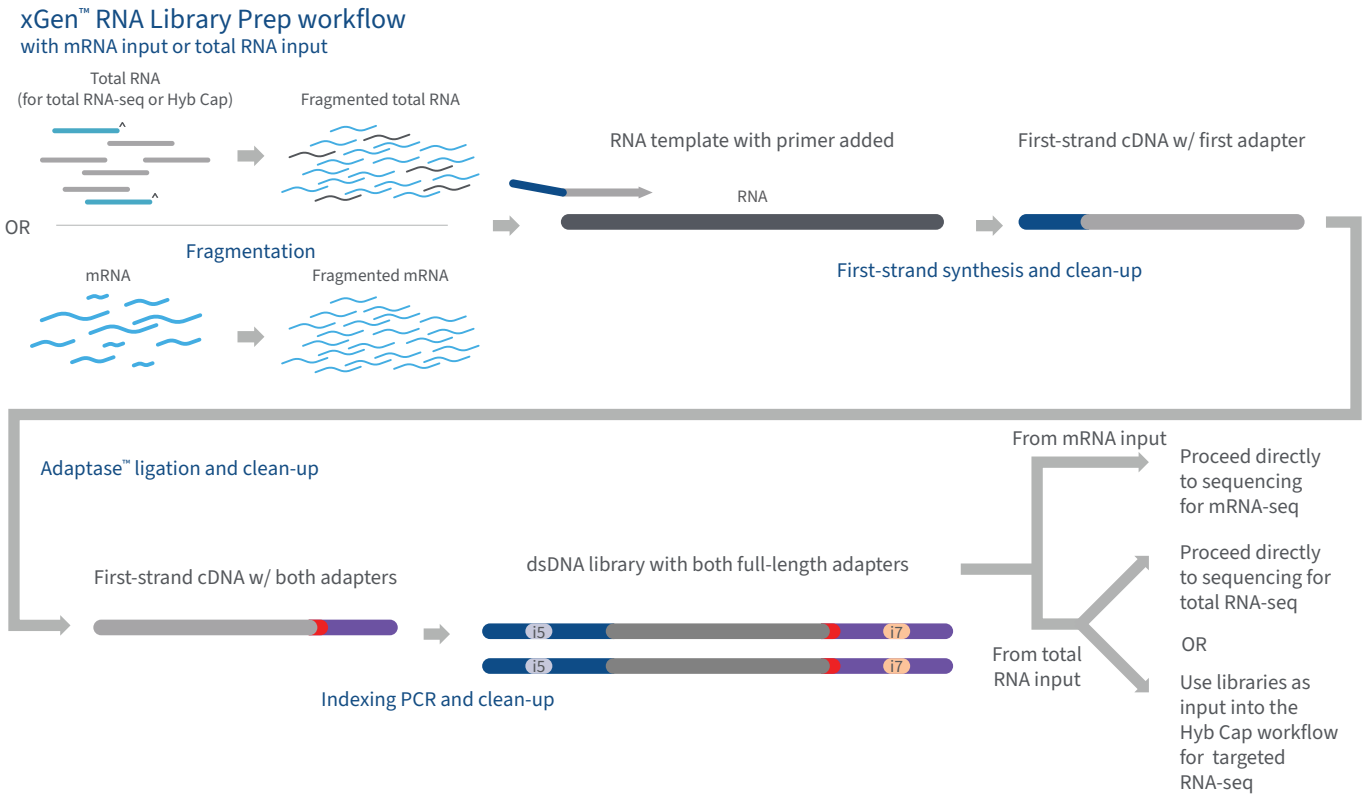


Figure 1. Transcriptome enrichment workflows supported by the xGen Broad-Range RNA Library Preparation Kit. Poly(A) enrichment selectively isolates polyadenylated messenger RNA prior to library preparation, providing an efficient workflow for gene expression studies using high-quality RNA samples. RNA exome capture enriches coding transcript sequences following library preparation through hybridization-based target capture, supporting transcriptome profiling of degraded RNA, FFPE tissue, and other challenging sample types. Both workflows leverage the xGen Broad-Range RNA Library Preparation Kit as a common stranded library preparation foundation.

Choosing an appropriate transcriptome enrichment strategy depends on several experimental factors, including RNA quality, study design, and biological objectives. Poly(A) enrichment is commonly used for high-quality RNA samples when efficient sequencing of protein-coding transcripts is the primary goal. However, degraded RNA samples, including many FFPE specimens, often contain fragmented transcripts that no longer retain intact polyadenylated tails. Because poly(A) enrichment relies on capture of these polyadenylated RNA molecules, transcript recovery can be reduced as RNA degradation increases. Additionally, poly(A) enrichment may not capture certain non-polyadenylated RNA species. In contrast, RNA exome capture enriches coding transcript sequences after library preparation through hybridization-based target capture, making it less dependent on transcript integrity and well suited for FFPE tissue, archival specimens, oncology samples, and other challenging RNA inputs. Key considerations for selecting between these workflows are summarized in Table 1.

Consideration	Poly(A) Enrichment	RNA Exome Capture
Primary target	Polyadenylated mRNA	Coding transcript sequences
Enrichment step	Before library preparation	After library preparation
Sequencing focus	Polyadenylated transcripts	Captured exonic targets
Compatibility with high-quality RNA	Well suited	Well suited
Compatibility with degraded RNA	Limited	Well suited
Best applications	Differential expression, bulk RNA-seq, discovery studies, gene expression profiling	FFPE profiling, translational research, oncology, retrospective cohorts
Recommended sample types	Fresh-frozen tissue, cell lines, high-quality RNA	FFPE tissue, archival specimens, challenging RNA samples

Table 1: Comparison of transcriptome workflows

Results and discussion

The following sections present representative data from independent studies evaluating RNA exome capture and poly(A) enrichment workflows using the xGen Broad-Range RNA Library Preparation Kit.

Part I: RNA Exome Capture

RNA exome capture enriches coding transcript sequences through hybridization-based target capture following RNA-seq library preparation. By targeting exonic regions of the transcriptome, xGen Exome Panels enable efficient sequencing of protein-coding transcripts while reducing sequencing dedicated to off-target regions. Because enrichment occurs after library construction, RNA exome workflows are particularly well suited for transcriptome profiling of challenging samples, including FFPE tissue, archival specimens, and other partially degraded RNA sources. Common applications include translational research, oncology studies, biomarker discovery, and retrospective cohort analysis.

RNA Exome Performance Across Diverse Sample Types

Input RNA quality can substantially impact RNA-seq data quality and influence the choice of enrichment strategy. To evaluate workflow performance across a range of sample qualities, RNA exome capture was assessed using both Universal Human Reference (UHR) RNA and breast cancer FFPE samples.

RNA quality is commonly assessed using RNA Integrity Number (RIN) and DV200 values. High-quality RNA samples with RIN ≥7 are generally well suited for poly(A) enrichment workflows. In contrast, degraded samples, including many FFPE specimens, often benefit from hybridization capture approaches that are less dependent on intact polyadenylated transcripts. For these challenging samples, DV200, which measures the percentage of RNA fragments longer than 200 nucleotides, can provide additional insight into the extent of RNA degradation and help guide library preparation conditions. To assess workflow performance, sequencing quality metrics including gene detection, mapping rate, exonic read fraction, and residual rRNA content were evaluated across all sample types.

RNA exome capture demonstrated consistent performance across both intact reference RNA and degraded FFPE samples (Table 2). Despite FFPE samples exhibiting RIN near 2, more than 17,000 genes were detected in all samples, indicating robust transcriptome representation across a wide range of RNA qualities. Mapping rates ranged from 87.9% to 89.6% and exonic read fractions ranged from 88.1% to 91.2%, confirming efficient enrichment of coding transcript sequences. Residual rRNA content remained below 1.5% across all samples, minimizing sequencing waste and maximizing reads allocated to biologically relevant transcripts. Collectively, these results demonstrate that RNA exome capture supports high-quality transcriptome profiling across diverse sample types, including highly degraded FFPE specimens.

Sample	RIN	STAR mapping rate	Genes detected	Exonic rate (%)	rRNA rate (%)
UHR RNA	8.9	89.6	17,758	91.2	1.3
FFPE Sample 1	2.1	88.8	17,009	89.2	0.9
FFPE Sample 2	2.0	87.9	17,024	88.1	1.0

Table 2: Summary of RNA exome sequencing performance metrics for representative intact and FFPE RNA samples. Libraries were prepared from 100 ng UHR RNA and breast cancer FFPE RNA using the xGen Broad-Range RNA Library Preparation Kit and enriched with the xGen Exome Hybridization Panel. Libraries were sequenced on a MiniSeq 2 × 75 bp and downsampled to 3 million reads before analysis with STAR [1], Picard [2], and RNA-SeQC [3]. Mapping rates approaching 90%, exonic read fractions near 90%, residual rRNA content below 1.5%, and detection of more than 17,000 genes demonstrate consistent performance across varying RNA qualities.

Multiplexed RNA Exome Capture Supports Cost-Effective Transcriptome Profiling

RNA exome capture workflows support multiplexing of multiple libraries within a single hybridization capture reaction, helping improve throughput and reduce per-sample costs. To evaluate the impact of multiplexing on transcriptome profiling performance, libraries prepared from the same Universal Human Reference (UHR) RNA and FFPE RNA samples above, and 4 additional low-quality FFPE RNA samples ranging in RIN from 2-2.3, were pooled and captured at varying multiplex levels.

RNA quality is often a major challenge in transcriptome profiling studies, particularly when working with FFPE or archival specimens. Despite substantial differences in RNA quality, RNA exome capture generated highly reproducible expression profiles across both UHR RNA and FFPE samples. Expression measurements remained highly concordant across the evaluated multiplexing conditions for both UHR and FFPE RNA, with R^2 values greater than 0.99 (**Figure 2**). Together, these results demonstrate that RNA exome capture provides a robust and reliable workflow for transcriptome profiling, delivering high-quality and reproducible expression data from both intact and degraded RNA inputs while supporting efficient sample multiplexing of up to 6 samples per hybridization capture reaction.

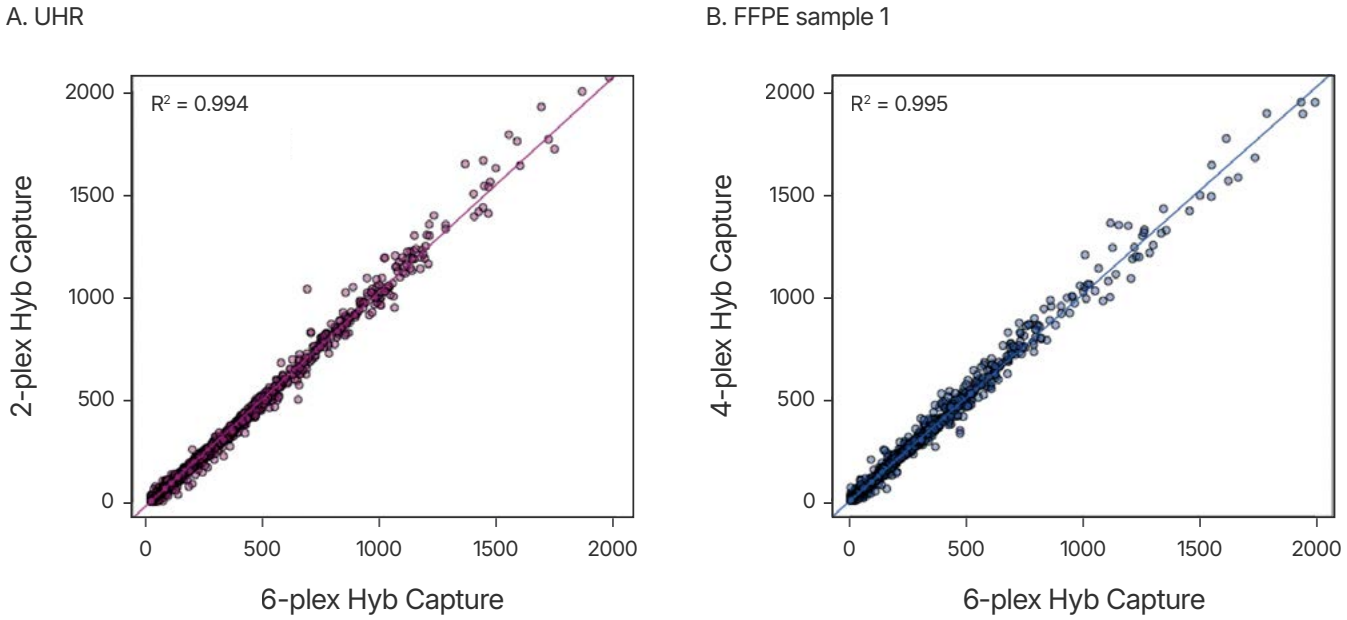


Figure 2. Gene expression concordance across multiplexed exome-captured RNA libraries. Libraries prepared from UHR RNA (A) and FFPE RNA (B) using the xGen Broad-Range RNA Library Preparation Kit were enriched with the xGen Exome Hybridization Panel. Expression measurements were compared between 2-plex and 6-plex hybridization captures for UHR RNA and between 4-plex and 6-plex captures for FFPE RNA. R^2 values greater than 0.99 demonstrate high concordance across the evaluated multiplexing conditions.

Part II: Poly(A) Enrichment

Poly(A) enrichment is a widely adopted RNA-seq approach for gene expression studies because it selectively enriches polyadenylated messenger RNA while reducing sequencing reads derived from ribosomal RNA and other non-coding RNA species. By focusing sequencing capacity on mature messenger RNA transcripts, poly(A) enrichment provides an efficient workflow for differential expression analysis, transcript quantification, and other standard bulk RNA-seq applications when working with high-quality RNA samples.

To evaluate enrichment performance, libraries were prepared from 100 ng and 1,000 ng RNA inputs using the xGen mRNA Enrichment Module and a leading commercially available poly(A) enrichment workflow (Supplier N). Evaluating both low- and high-input amounts helps determine whether enrichment performance remains consistent across sample inputs commonly encountered in gene expression studies, providing confidence in workflow robustness and experimental flexibility. Sequencing performance was assessed using mapping rate, residual rRNA content, strandedness, and transcriptome recovery metrics (Table 3).

The xGen mRNA Enrichment Module delivered high-quality transcriptome profiling across both tested input amounts, achieving mapping rates greater than 95% and residual rRNA content below 1%. Approximately 16,000 genes and more than 75,000 transcripts were detected across all tested conditions, indicating broad transcriptome representation across a 10-fold RNA input range. Performance across the reported metrics was comparable to a leading commercially available poly(A) enrichment workflow, demonstrating consistent transcriptome recovery across both low- and high-input conditions.

PolyA Bead Vendor	Input (ng)	Mapping Rate	rRNA Rate	Genes Detected	Transcripts Detected
Supplier N	1000	95.5%	0.9%	16,139	77,804
Supplier N	100	95.7%	0.7%	16,061	76,309
IDT	1000	95.1%	0.8%	15,956	75,822
IDT	100	95.8%	0.7%	15,899	75,154

Table 3: Sequencing and transcriptome profiling metrics for poly(A)-enriched RNA-seq libraries. Libraries were prepared from 100 ng and 1,000 ng UHR RNA using the xGen mRNA Enrichment Module (IDT) or a leading commercially available poly(A) enrichment workflow (Supplier N). Libraries were sequenced on a NextSeq 2000 using 2 × 151 bp reads and downsampled to 6.98 million reads. Comparable mapping rates, residual rRNA content, and transcriptome recovery were observed across the evaluated enrichment workflows and RNA input amounts.

Workflow Components for Transcriptome Profiling

Once an enrichment strategy has been selected, the reagents required to implement each workflow differ. Both workflows use the xGen Broad-Range RNA Library Preparation Kit as a common library preparation foundation, with workflow-specific reagents for poly(A) enrichment or RNA exome capture. **Figure 3** outlines the reagents, part numbers, and reaction sizes needed to generate 96 RNA-seq libraries using either workflow.

Workflow	Poly(A) Enrichment	Reaction Size	Part Number(s)	RNA Exome Capture	Reaction Size***	Part Number(s)
Library Prep Kit	xGen Broad-Range RNA Library Prep Kit	96	10009813	xGen Broad-Range RNA Library Prep Kit	96	10009813
Indexing Primers	xGen UDI Primer Plate 1	96	10005975	xGen UDI Primer Plate 1	96	10005975
Enrichment Kit	xGen mRNA Enrichment Module*	96	10017546	xGen Exome V2 + Spike-in Hyb Cap Panels**	16	10005152 & 10030779
Hybridization & Wash Reagents	Not Required	—	—	xGen Hybridization & Wash Kit V3/V2	16	10028311/ inquire
Universal Blockers	Not Required	—	—	xGen Universal Blockers, TS	16	1075474
Post-Hyb Cap Amplification	Not Required	—	—	xGen Library Amplification Primer Mix	16	1077675
Relative Cost	$	—	—	$$	—	—

*The xGen mRNA Enrichment Module must be purchased with the xGen Broad-Range RNA Library Preparation Kit and is not sold separately.

**xGen Exome Panels or custom exome panels can be selected based on the desired target content; the xGen Exome v2 Research Panel + Spike-In Panel are recommended.

***RNA exome reaction sizes are based on 6-plex hybridization capture reactions. Other sizes and configurations are available

Table 4. Reagents and product configurations required to generate 96 RNA-seq libraries using poly(A) enrichment or RNA exome capture workflows. Both workflows use the xGen Broad-Range RNA Library Preparation Kit as a common library preparation foundation. The figure summarizes the workflow-specific reagents, part numbers, and reaction sizes needed to generate 96 RNA-seq libraries. For RNA exome capture, reagent quantities are based on 6-plex hybridization capture reactions.

Conclusions

Transcriptome profiling workflows must balance transcript coverage, sequencing efficiency, and compatibility with diverse sample types. The xGen Broad-Range RNA Library Preparation Kit provides a common stranded library preparation foundation for both poly(A) enrichment and RNA exome capture workflows. RNA exome capture delivered consistent transcriptome profiling across intact reference RNA and degraded FFPE samples, including highly concordant expression measurements across multiplexed capture conditions. The xGen mRNA Enrichment Module provided high-quality transcriptome recovery with performance comparable to a leading commercially available poly(A) enrichment workflow. Together, these complementary approaches extend the utility of a common library preparation workflow across a range of RNA sample types and transcriptomics applications, giving researchers flexibility to select an enrichment strategy that aligns with their samples and experimental objectives.

References

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3. DeLuca DS, Levin JZ, Sivachenko A, et al. RNA-SeQC: RNA-seq metrics for quality control and process optimization. Bioinformatics. 2012;28(11):1530-1532.

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