

Optimized NEBNext UltraExpress® workflows enable high-performance RNA and DNA library construction from low inputs



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Introduction

The increasing demand for rapid, streamlined, and automation-friendly library preparation workflows has driven the development of three NEBNext UltraExpress kits: UltraExpress RNA, UltraExpress DNA, and UltraExpress FS DNA. These workflows were originally designed to provide fast, single-tube protocols with reduced incubation times and fewer cleanup steps, supporting efficient and scalable next-generation sequencing (NGS) library construction across standard input ranges. Recent workflow improvements have now enabled substantially lower input requirements, expanding their utility for a broader spectrum of research applications where sample quantity is limited.

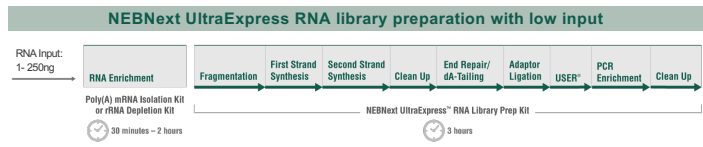
NEBNext UltraExpress RNA was initially recommended for 25–250 ng of total RNA with compatibility across multiple enrichment strategies. The workflow has now been optimized to generate high-quality RNAseq libraries from as little as **1 ng** of RNA, enabling efficient library preparation even when only minimal material is available.

NEBNext UltraExpress DNA (mechanically sheared) originally supported DNA inputs ranging from 10–200 ng. With updated workflow refinements, the kit now performs reliably with inputs down to **500 pg**, enabling robust library generation from extremely low quantities of DNA.

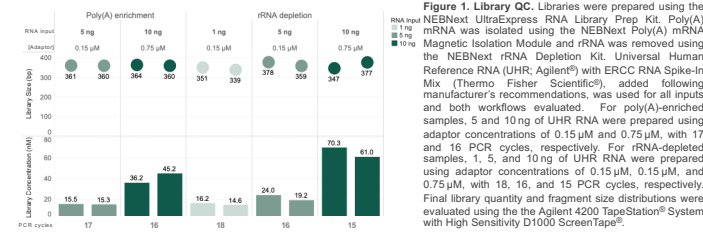
NEBNext UltraExpress FS DNA (enzymatically sheared) integrates fragmentation and library construction and was initially recommended for 10–200 ng of DNA. The workflow has now been extended to support inputs as low as **100 pg**, substantially reducing the amount of starting material required.

The expanded low-input performance of NEBNext UltraExpress RNA, DNA, and FS DNA workflows deliver a powerful solution for researchers working with limited sample material, all while maintaining the rapid processing times associated with UltraExpress. Across all three workflows, high library yields and strong sequencing metrics were maintained even at these reduced input levels. The streamlined formats remain compatible with high-throughput and automated processing, supporting efficient integration into larger-scale workflows.

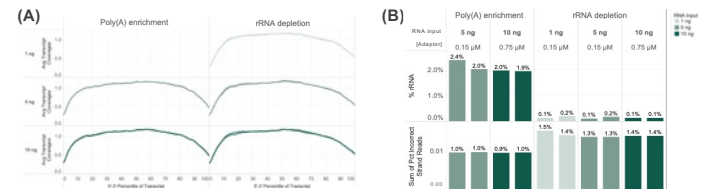
Results



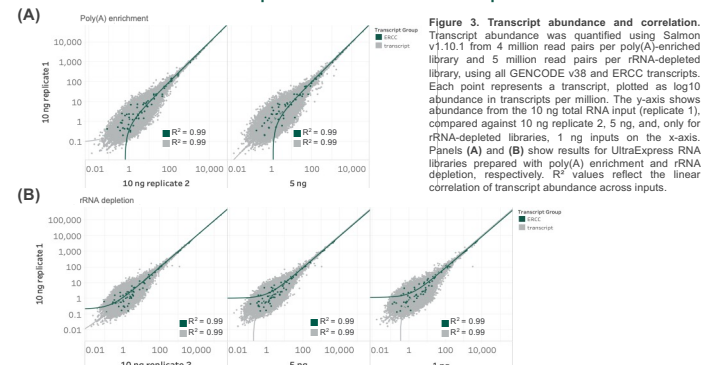
Library yields and sizes from low inputs



RNA sequencing metrics from low inputs

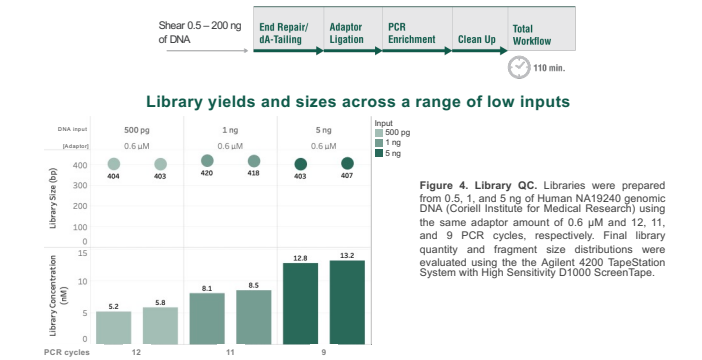


Transcript correlations across low inputs

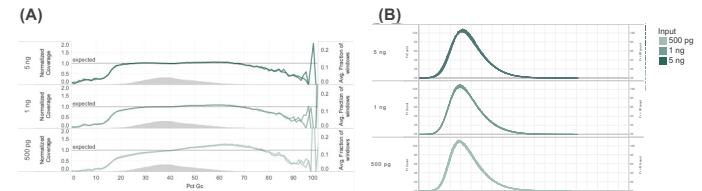


Results continued

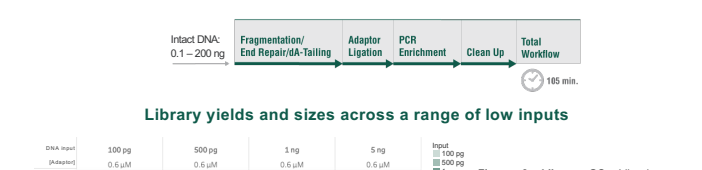
NEBNext UltraExpress DNA library preparation with low input



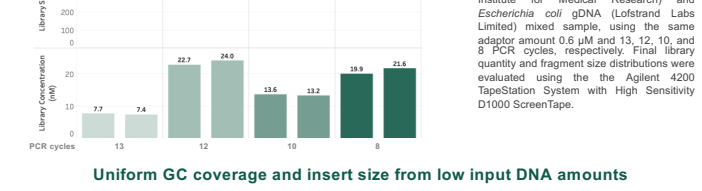
Uniform GC coverage and insert size from a range of low input DNA amounts



NEBNext UltraExpress FS DNA library preparation with low input



Uniform GC coverage and insert size from low input DNA amounts



Conclusions

- Shorter hands-on time** → NEBNext UltraExpress RNA:
 - Protocol now accommodates lower inputs down to 1 ng with depletion and 5 ng with poly(A) enrichment
 - Total RNA to library in 4–5 hours
- Fewer steps and consumables**
- Flexibility with sample types and protocols** → NEBNext UltraExpress DNA and FS:
 - Protocol now compatible with inputs down to 500 pg for UltraExpress DNA and 100 pg for UltraExpress FS
 - DNA sample to library in < 2 hours
- Automation friendly volumes**

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