

# Normalized Library Preparation and Simplified Hybridization for Targeted Sequencing Applications

sptlabtech

T W I S T  
BIOSCIENCE

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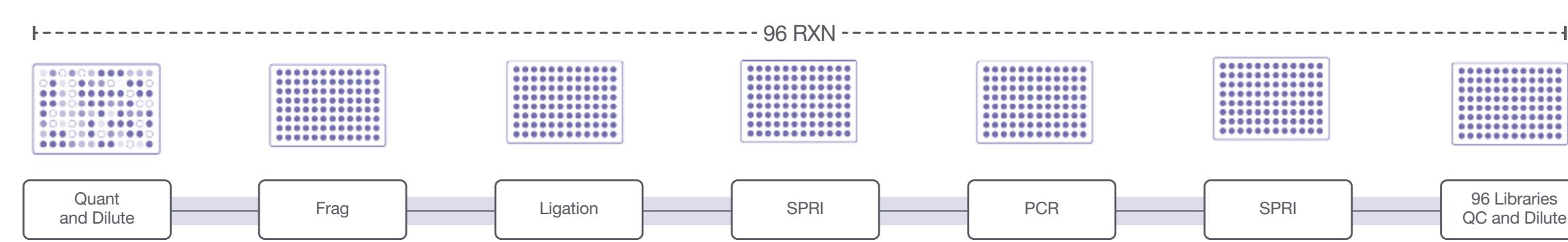
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## Abstract

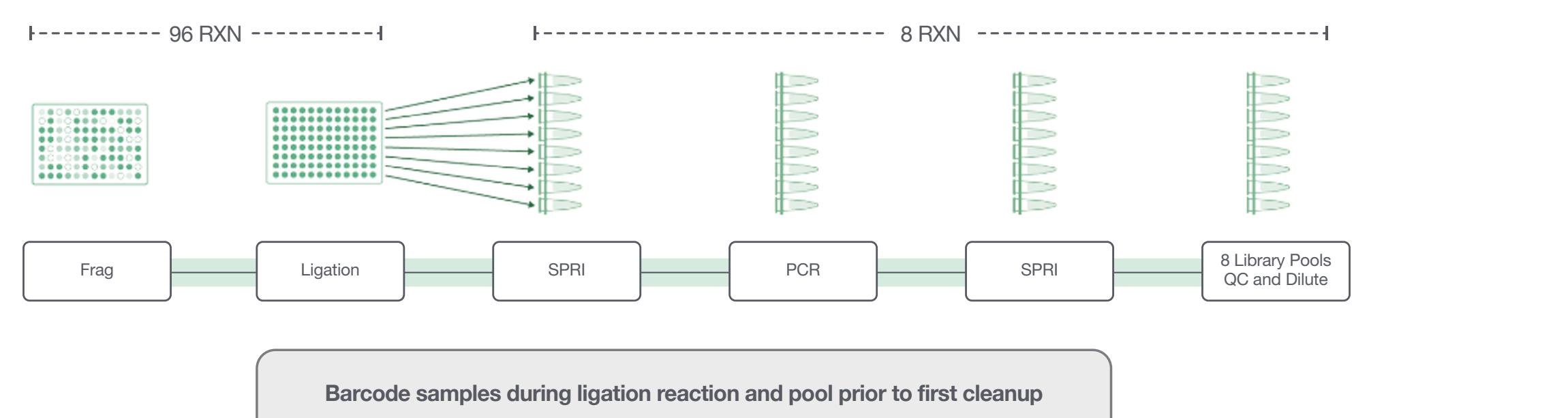
- Next-generation sequencing (NGS) platforms continue to advance by increasing throughput while reducing sequencing costs, shifting the primary bottleneck in large-scale genomics studies from sequencing to library preparation. For population-scale genomics and targeted sequencing applications, library preparation remains labor-intensive, time-consuming, and costly when performed manually.
- The **Twist FlexPrep™ UHT Library Preparation and Hybridization Kit** addresses these challenges through two core innovations. First, Normalization by Ligation™ (NBL) enables consistent conversion of genomic DNA (gDNA) into sequencing-ready libraries independent of input mass, eliminating the need for upfront quantification and normalization. Second, early incorporation of inline barcodes allows samples to be pooled immediately after ligation, reducing downstream reaction volumes and enabling up to a 12-fold reduction in post-ligation processing steps. In combination, these features support high-throughput 96-pool target capture, substantially decreasing reagent usage and hands-on time while maximizing sequencer utilization.
- Here, we demonstrate automated implementation of the Twist FlexPrep UHT Library Preparation and Hybridization workflow on **SPT Labtech's firefly® liquid handling platform**, using 384 samples, Bovine gDNA (Sigma Aldrich 36231-M) – 4 inputs concentrations: 30, 60, 90, 120 ng in quadrants 1, 2, 3 & 4 respectively to enable scalable, walk-away library preparation with high reproducibility, demonstrating data quality comparable to established genomic analysis methods.
- By combining the Twist FlexPrep UHT chemistry with SPT Labtech's firefly and Element Biosciences Trinity™ chemistry to enable on-sequence hybridization and target enrichment using Twist Biosciences Bovine panel, this workflow provides a robust, flexible, and cost-effective solution for high-throughput genomics studies, enabling efficient interrogation of known and novel genomic regions across large sample cohorts.
- Following automated library preparation and QC, pooled libraries were processed using **Element Biosciences Trinity™** chemistry, which integrates target hybridization directly onto the sequencing cartridge. This on-sequence hybridization approach eliminates traditional bead-based capture, wash steps, and post-hybridization amplification, reducing workflow complexity, hands-on time, and turnaround. When combined with Twist Biosciences target enrichment panels, the SPT Labtech–Twist–Element workflow enables streamlined, high-quality targeted sequencing with uniform coverage across large sample cohorts.

## FlexPrep Workflow

Typical enzymatic fragmentation library prep for 96 samples



FlexPrep UHT library prep for 96 samples

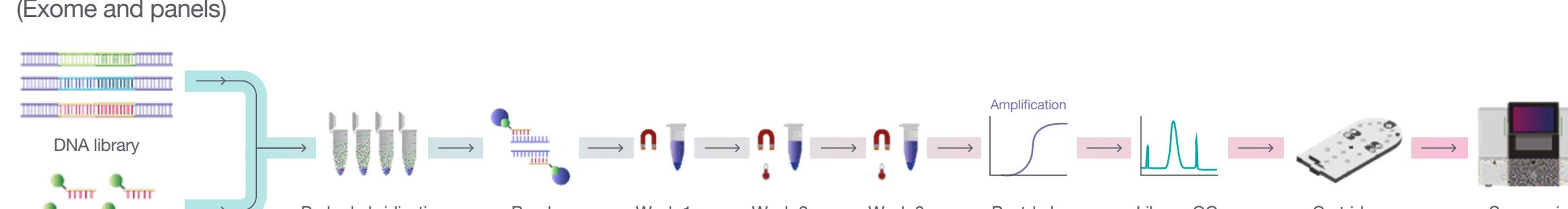


**Figure 3. Comparison of traditional enzymatic library preparation and Twist FlexPrep™ UHT workflows.**

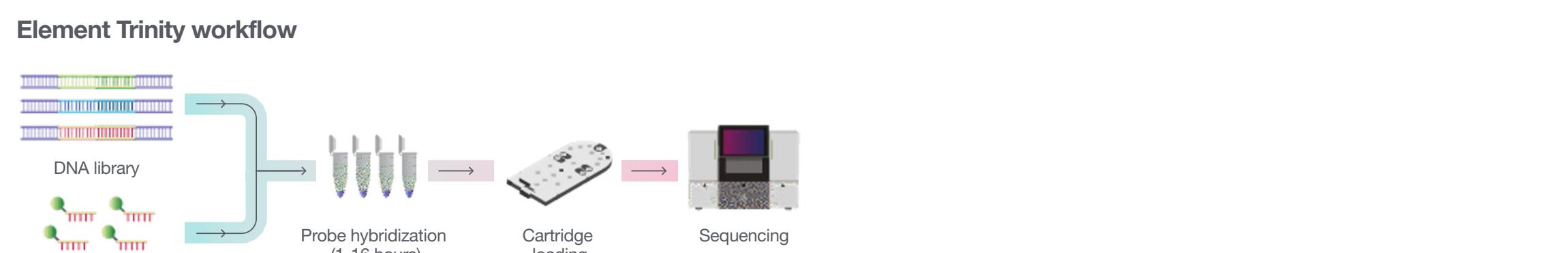
Schematic comparison of a conventional enzymatic fragmentation-based library preparation workflow and the Twist FlexPrep™ UHT library preparation workflow for 96 samples. In a traditional workflow, each step—from DNA quantification and dilution through fragmentation, ligation, cleanup, PCR, and final QC—is performed at full reaction scale, resulting in 96 reactions throughout the process. In contrast, the FlexPrep™ UHT workflow incorporates inline barcoding during the ligation step, enabling samples to be pooled prior to the first SPRI cleanup. Early pooling reduces downstream processing to eight pooled reactions, substantially decreasing reagent consumption, hands-on time, and cost while maintaining library quality and consistency. This streamlined workflow supports scalable, high-throughput library preparation suitable for automated implementation.

## Trinity™ chemistry / hyb overview

Traditional hybrid selection process (Exome and panels)



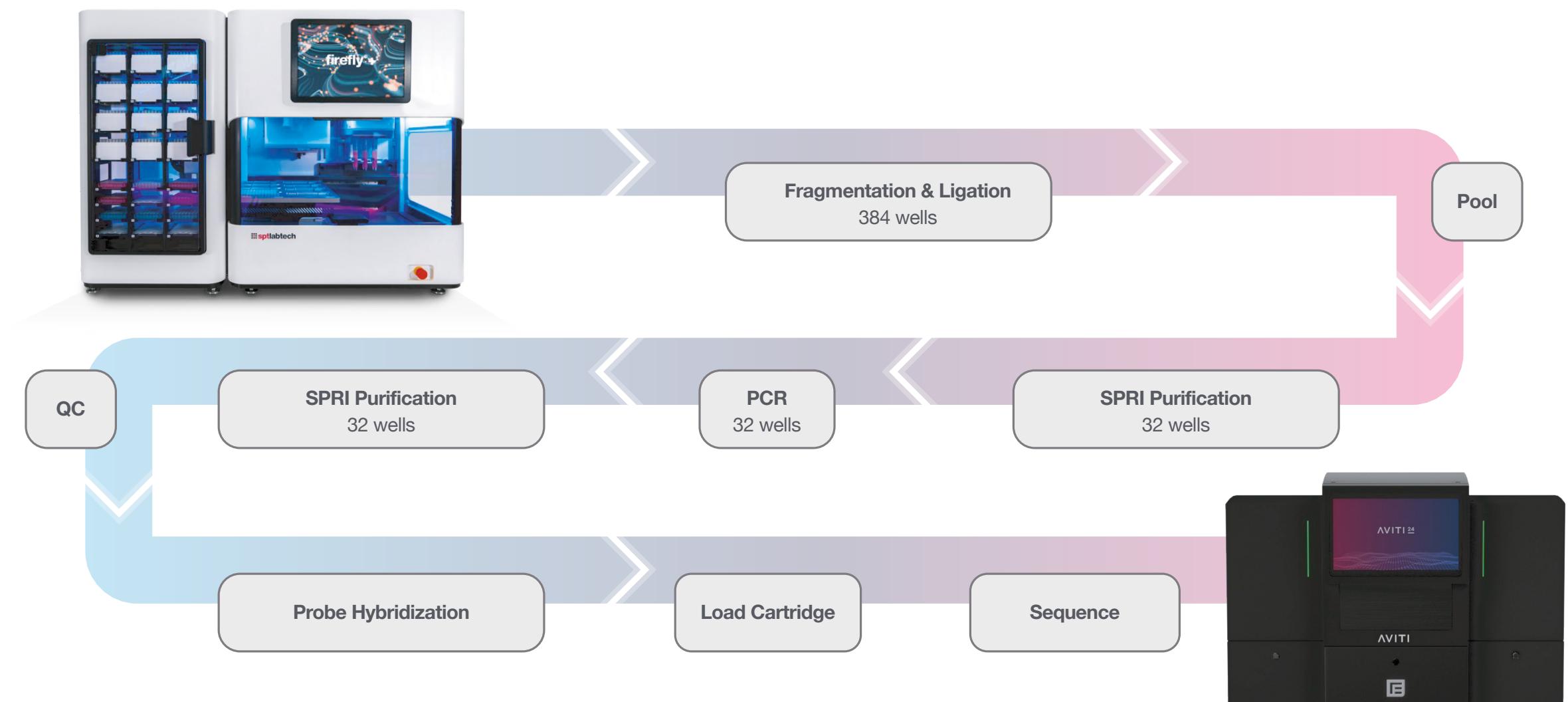
Element Trinity workflow



**Figure 4. Comparison of traditional hybrid selection and Element Trinity™ on-sequence hybridization workflows.**

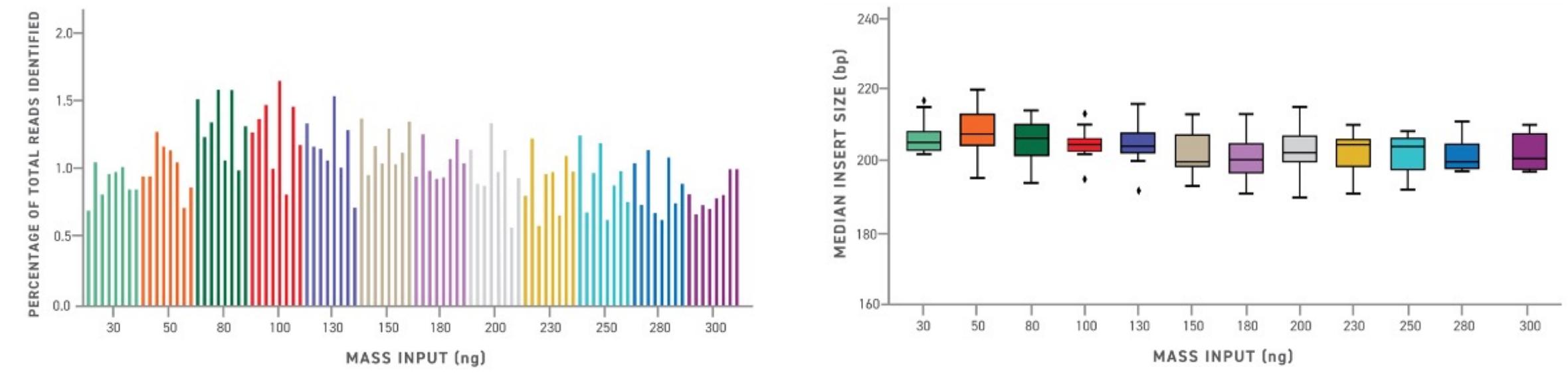
Schematic comparison of a conventional hybrid selection workflow for exome and targeted panel sequencing versus the Element Trinity™ on-sequence hybridization workflow from Element Biosciences. Traditional hybrid selection requires extended probe hybridization followed by bead binding, multiple wash steps, post-hybridization amplification, library QC, and pooling prior to cartridge loading and sequencing. In contrast, the Trinity workflow streamlines target enrichment by integrating hybridization directly onto the sequencing cartridge, eliminating bead-based capture, wash steps, and post-hybridization amplification. This simplified workflow reduces hands-on time, shortens turnaround, and minimizes complexity while maintaining robust target enrichment and sequencing performance.

## Twist Bioscience's FlexPrep UHT Library Preparation on firefly



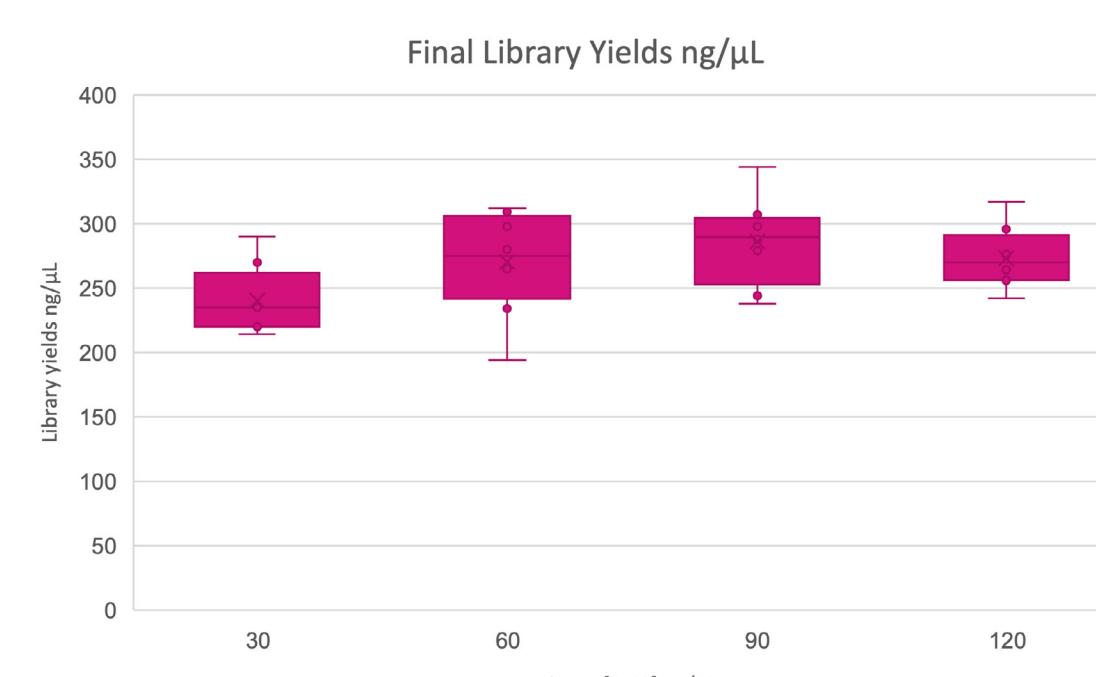
**Figure 1. Integrated firefly and Element Trinity™ workflow for automated library preparation, target enrichment, and sequencing.** Overview of the end-to-end workflow combining automated library preparation on SPT Labtech's firefly platform (blue) with on-sequence hybridization and sequencing using Element Biosciences Trinity™ (purple). Genomic DNA samples are processed on firefly through fragmentation and ligation at full 384-well scale, followed by early pooling enabled by inline barcoding. Post-ligation cleanup, PCR amplification, and SPRI purification are performed on pooled libraries at reduced reaction scale (32 wells), substantially lowering reagent consumption and hands-on time while maintaining library quality. After QC, libraries transition to the Trinity workflow, where probe hybridization occurs directly on the sequencing cartridge, followed by cartridge loading and sequencing.

## FlexPrep Normalization

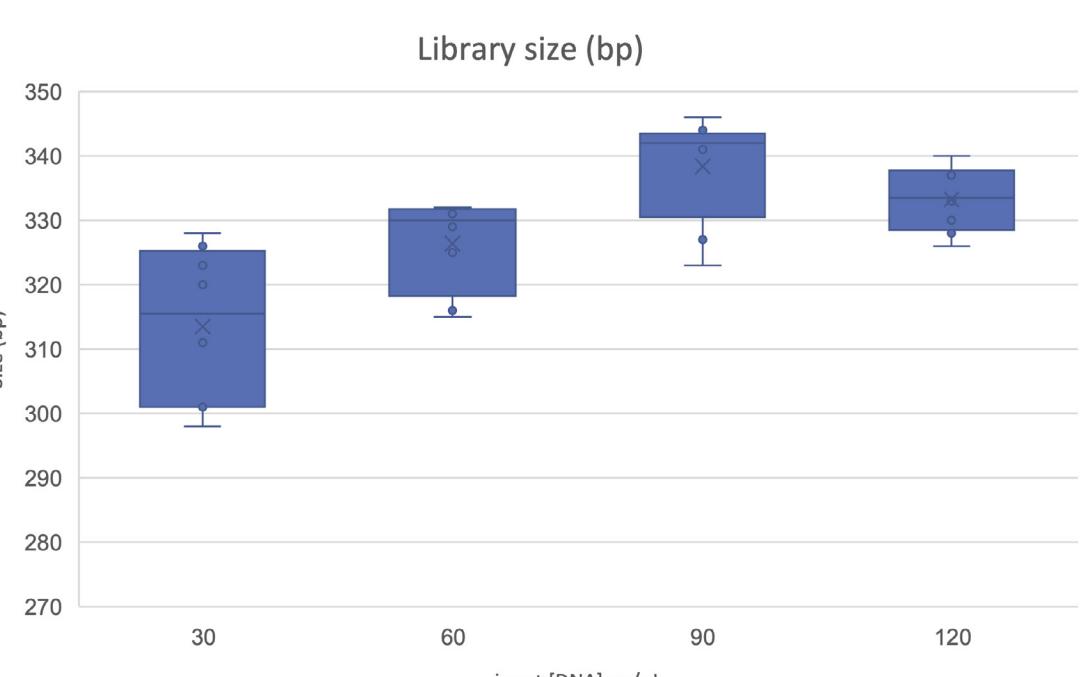


**Figure 2. FlexPrep™ Normalization by Ligation enables consistent library output across a wide DNA input range.** Evaluation of library normalization performance using the Twist Bioscience FlexPrep™ UHT Library Preparation chemistry across genomic DNA input masses ranging from 30 to 300 ng. Panel A (top) shows uniform NGS read depth across variable DNA inputs, eliminating tedious upfront quantitation. Panel B (bottom) shows consistent median insert sizes across the same input range, highlighting the ability of Normalization by Ligation™ (NBL) chemistry to normalize library yield while maintaining desired insert size while addressing performance issues seen with transposase-based approaches.

## Results – Final Library QC



**Figure 5. Final library yields across a range of gDNA input concentrations.** Final library yields (ng/µL) generated using the Twist FlexPrep™ UHT Library Preparation and Hybridization Kit automated on SPT Labtech's firefly liquid handling platform. Libraries were prepared from bovine genomic DNA across four input concentrations (30, 60, 90, and 120 ng), distributed across quadrants to assess robustness to variable input mass. Consistent library yields were observed across all input levels, demonstrating effective normalization enabled by Normalization by Ligation™ (NBL) chemistry. These results highlight the ability of the firefly-automated Twist FlexPrep workflow to deliver reproducible library preparation while eliminating the need for upfront DNA quantification and normalization.



**Figure 6. Library fragment size consistency across gDNA input concentrations.** Average library fragment sizes (bp) obtained from libraries prepared using the firefly-automated Twist FlexPrep™ UHT workflow across four genomic DNA input concentrations (30, 60, 90, and 120 ng). Library size distributions remained consistent across all inputs, indicating robust and reproducible fragmentation and library construction independent of starting material quantity. This uniformity supports scalable, high-throughput library preparation suitable for downstream hybridization, target enrichment, and sequencing applications.

## Results – Hyb metrics



**Figure 7. Hybridization and sequencing performance metrics following Trinity™ on-sequence hybridization.** Target enrichment and sequencing performance metrics for libraries prepared using the Twist FlexPrep™ UHT Library Preparation workflow and sequenced with Element Biosciences Trinity™ on-sequence hybridization chemistry. Metrics shown include percent off-target reads (PCT\_OFF\_BAIT), hybrid-selection library size (HS\_LIBRARY\_SIZE), percent duplicate reads (PCT\_EXC\_DUPE), mean target coverage (MEAN\_TARGET\_COVERAGE), percent of targets with zero coverage (ZERO\_CVG\_TARGETS\_PCT), and fold 80 base penalty (FOLD\_80\_BASE\_PENALTY). Results are shown across multiple firefly-automated library preparation pools (FP1–FP32). Consistent performance across all metrics demonstrates robust hybridization efficiency, uniform target coverage, and reproducible sequencing quality enabled by the integrated firefly–Twist–Element workflow.