Twist TrueAmp Polymerase Mix Datasheet

INTRODUCTION

Polymerase amplification is a critical step in next-generation sequencing workflows and can influence library yield, molecular complexity, coverage uniformity, error profile, and downstream variant-calling accuracy. While PCR is often required to generate sufficient library material for sequencing or target enrichment, amplification can also introduce bias when fragments are not amplified uniformly. This is especially relevant for libraries containing GC-rich, AT-rich, damaged, low-input, or otherwise difficult templates.

Amplification performance can also be affected by variables introduced during sample preparation and library construction. DNA input amount, primer concentration, thermal cycling conditions, buffer composition, purification carryover, and sample-derived inhibitors can all affect PCR efficiency. When amplification is sensitive to these variables, users may need to perform workflow-specific optimization.

The Twist TrueAmp Polymerase Mix was engineered to provide robust, high-fidelity amplification for NGS library preparation and target enrichment workflows. This datasheet summarizes the performance of TrueAmp Polymerase Mix across a series of amplification conditions relevant to NGS workflows, including input mass, primer concentration, thermal cycling variation, inhibitor tolerance, purification carryover, GC/AT sequence context, polymerase fidelity, and compatibility across Twist NGS workflows.

RESULTS

UNIFORMITY ACROSS GC-RICH AND AT-RICH GENOMES

Library yield alone does not fully describe amplification performance. A polymerase may generate sufficient material for sequencing while still introducing bias if some fragments amplify more efficiently than others. This is especially important for templates with high GC or AT content, where PCR-induced bias can result in uneven coverage, regional dropout, or reduced sensitivity in downstream analysis.

The Twist TrueAmp Polymerase Mix was evaluated using a mixed-genome library containing organisms with different base compositions. The resulting libraries were compared to a PCR-free condition. This design allows amplification uniformity to be assessed based on how closely each amplified library preserves the representation observed in the PCR-free condition.

A value closer to 0 indicates greater similarity to the PCR-free reference and therefore lower amplification-induced bias. In this experiment, the Twist TrueAmp Polymerase Mix had the most uniform read distribution across all genomes tested when compared with other amplification master mixes (**Figure 1**). When the Twist TrueAmp Polymerase Mix data are aligned to specific bacterial genomes of varying GC content, normalized coverage is highly uniform for all cases (**Figure 2**). These data support the use of the Twist TrueAmp Polymerase Mix in applications where preservation of library representation across difficult sequence contexts is important. When using the Twist TrueAmp Polymerase Mix, amplification to generate sufficient library mass for downstream steps does not require the sacrificing of coverage uniformity.

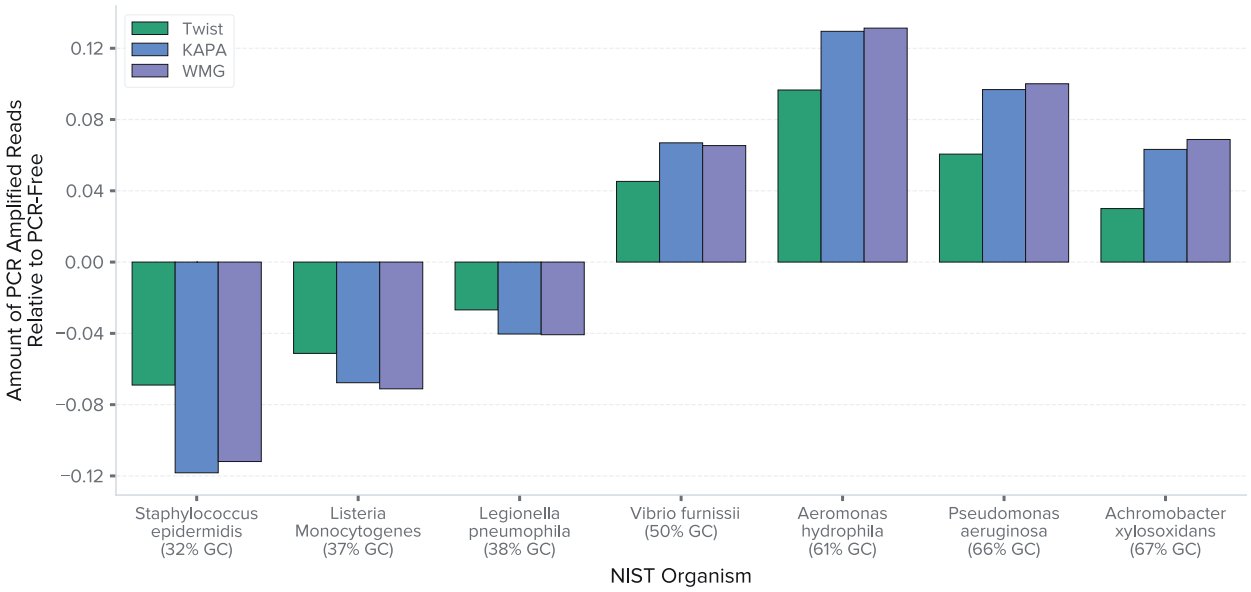
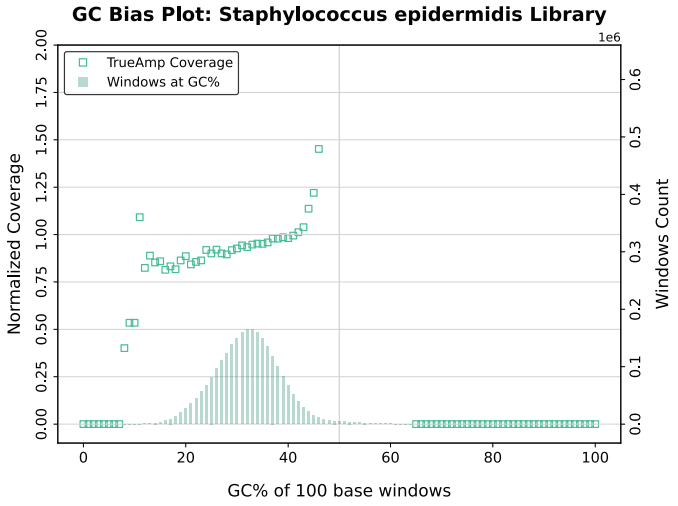
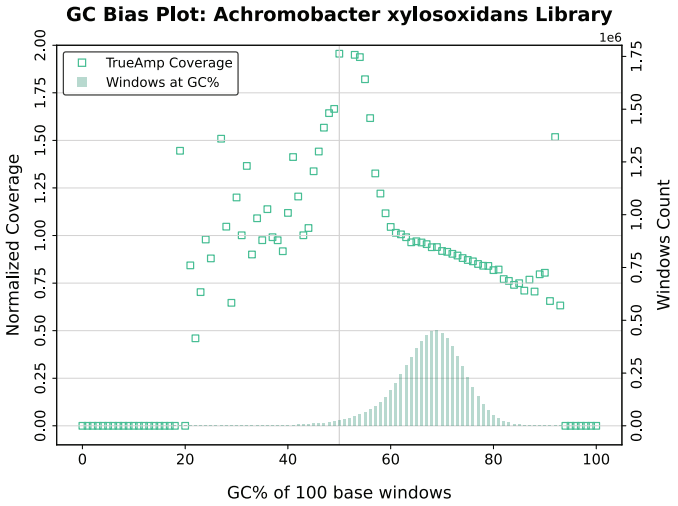


Figure 1. Read Distribution of GC Diverse Genomes. Data showing Twist TrueAmp Polymerase Mix's metagenomic uniformity compared to other amplification master mixes. Libraries were generated from a blend of seven genomes with Twist TrueAmp Library Preparation Kit. After ligation, the library was split across three polymerase mixes and amplified with 6 PCR cycles. The libraries were then sequenced, downsampled, and compared to the PCR-free condition. Twist TrueAmp Polymerase Mix has the least GC bias and the most uniform read distribution (closest to PCR-free at 0) for all genomes included in the blend.





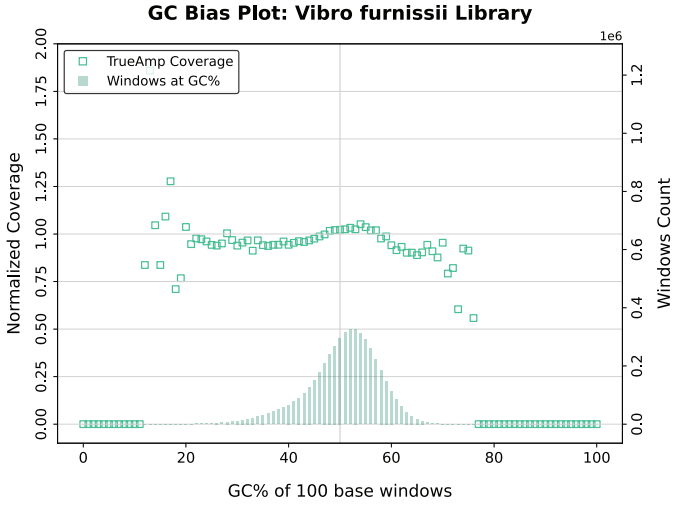


Figure 2. GC Bias Plots. Twist TrueAmp Polymerase Mix sequencing data is aligned to three individual bacterial genomes of low (S. epidermidis), moderate (V. furnissii), and high (A. xylosoxidans) GC percentage. The histograms above and to the right show the GC% window distribution across each bacterial genome and the square points show the normalized coverage of each window. A normalized coverage value of 1.0 is perfect uniformity.

AMPLIFICATION ACROSS DNA INPUT MASS

Library input into PCR is not always fixed or predictable. In NGS workflows, the amount of amplifiable material can vary based on sample availability, DNA quality, library conversion efficiency, purification recovery, or upstream target enrichment performance. If PCR performance is impaired at low input, users may fail to generate enough material for sequencing. If high numbers of cycles are required, there may be an increase in amplification bias, a reduction in library complexity, or the generation of artifacts.

For these reasons, a robust amplification mix should support a broad input range while allowing the number of PCR cycles to be adjusted based on the amount of starting library. The Twist TrueAmp Polymerase Mix's performance was assessed based on DNA template inputs from 100 fg to 100 ng using input-appropriate PCR cycle numbers (**Figure 3**). The results show that Twist TrueAmp Polymerase Mix can amplify a wide range of DNA template inputs.

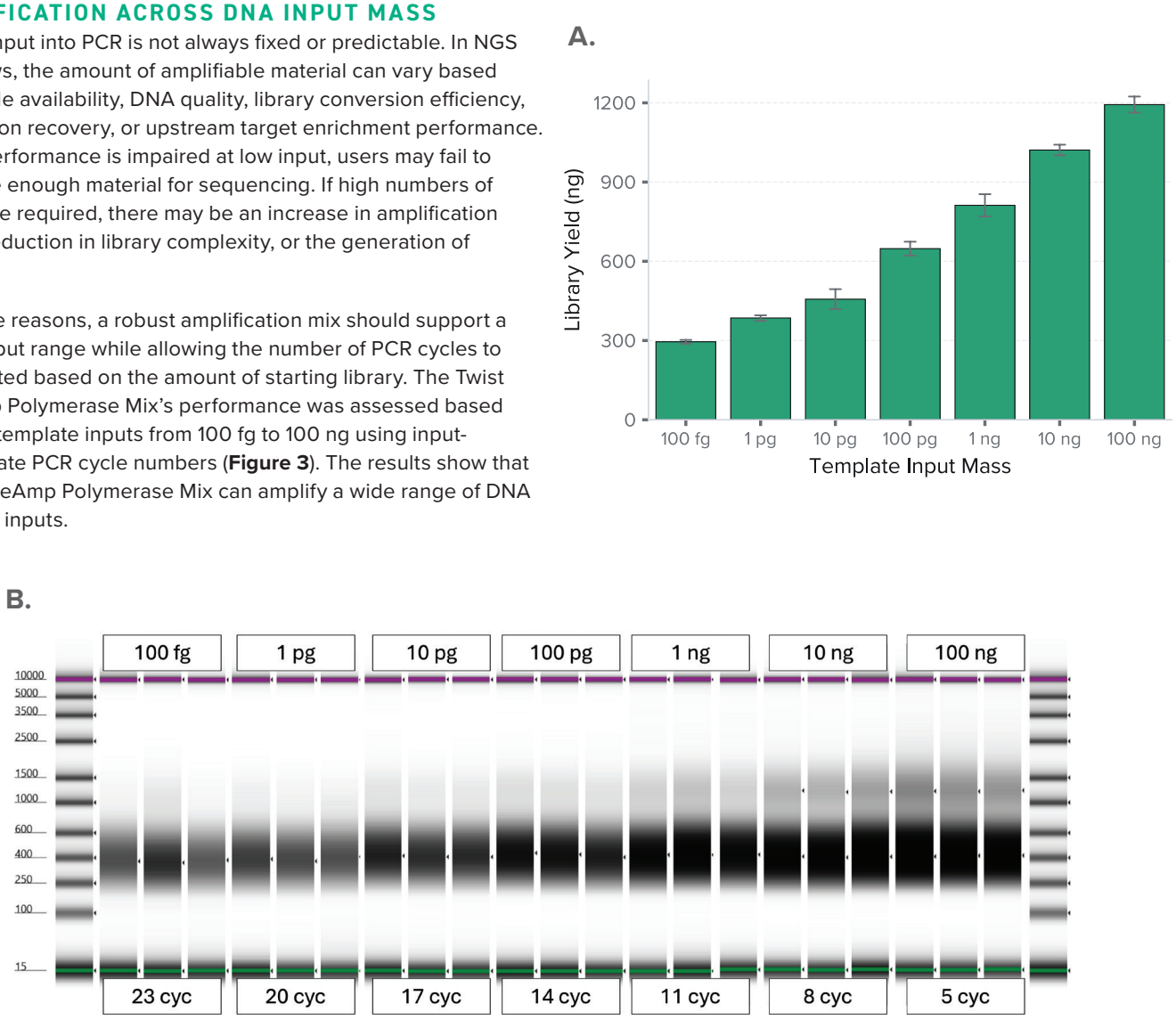


Figure 3. Library Yield by Varying DNA Mass Input. (A) Triplicate data showing Twist TrueAmp Polymerase Mix's capacity to amplify a wide range of DNA template input from 100 fg to 100 ng with the appropriate number of PCR cycles. The 100 fg, 1 pg, 10 pg, 100 pg, 1 ng, 10 ng, and 100 ng inputs were amplified using 23, 20, 17, 14, 11, 8, and 5 PCR cycles, respectively. **(B)** Electrophoresis gel banding corresponds to the conditions outlined above.

POLYMERASE FIDELITY

Fidelity is a key metric of polymerase activity that is tied to false positive signal during variant detection. The Twist TrueAmp Polymerase Mix fidelity was evaluated using PacBio HiFi sequencing. Single-molecule sequencing provides a sensitive method for observing the products of DNA synthesis and detecting low-frequency polymerase-introduced errors. This approach enables comparison of polymerase error profiles against commercial amplification mixes.

These results demonstrate that the Twist TrueAmp Polymerase Mix maintains high-fidelity amplification (**Figures 4 and 5**) for NGS applications in need of low error contribution from PCR.

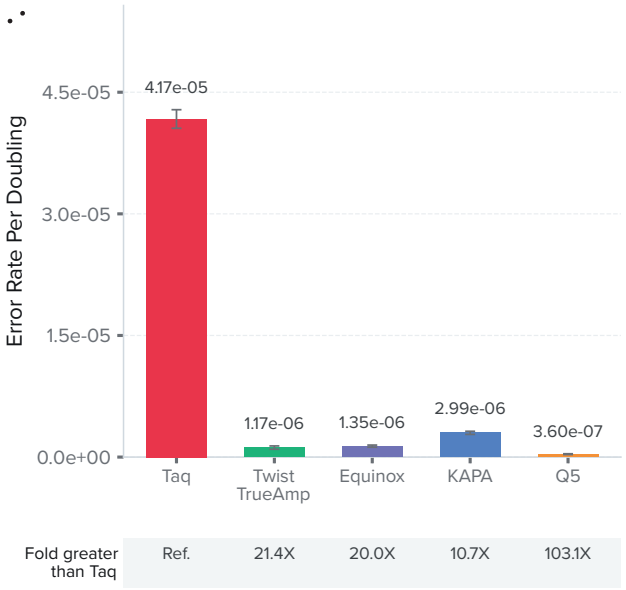


Figure 4. Error Rate of Various Polymerase Mixes. Triplicate data of PacBio CCS Sequencing showing error rate of several polymerase mixes available on the market. Amplification was performed on 1 ng of plasmid at 15 cycles PCR with various polymerases; unamplified plasmid was used as control. Libraries were constructed from 2-kb clonal gene amplicons using the PacBio SMRTBell Library Prep 3.0 kit. Libraries were sequenced on a PacBio Vega instrument downsampled to 37,500x coverage.

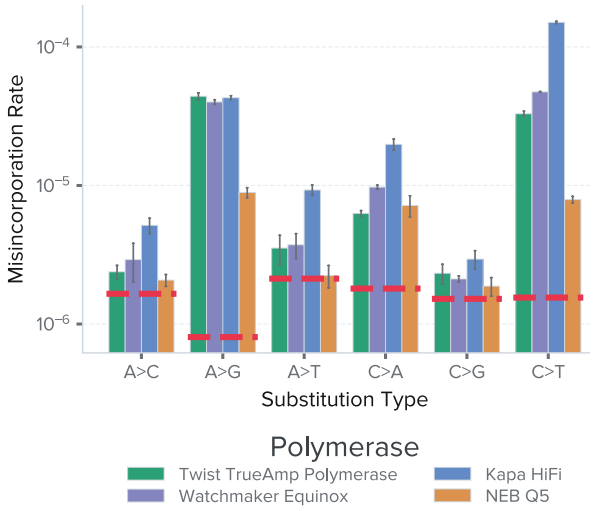


Figure 5. Base Misincorporation Rates of Various Polymerase Mixes. Base misincorporation derived from triplicate data of amplicon sequencing using PacBio CCS Sequencing across several commercially available polymerase mixes. Amplification was performed on 1 ng of plasmid at 15 cycles PCR; unamplified plasmid (PCR Free) was used as control. Libraries were constructed from 2-kb clonal gene amplicons using the PacBio SMRTBell Library Prep 3.0 kit. Libraries were sequenced on a PacBio Vega instrument downsampled to 37,500x coverage. The red dotted line represents misincorporation rates with the PCR-free control.

SET-UP ROBUSTNESS FOR AUTOMATION

Hot-Start

The Twist TrueAmp Polymerase Mix features a highly effective hot-start formulation designed to deliver optimal PCR amplification. By inhibiting enzyme activity at ambient temperatures, this formulation is particularly advantageous for extended reaction setups and automated workflows, where premature enzymatic activity can degrade single-stranded primers and templates, leading to non-specific amplification.

Twist TrueAmp Polymerase Mix's hot-start mechanism leverages a DNA aptamer that binds to the polymerase at room temperature, temporarily neutralizing its activity. Upon reaching high temperatures during the initial PCR denaturation step, the aptamer denatures, restoring full enzymatic function. The DNA aptamer is chemically inert, does not degrade over time, and will not act as a primer during amplification. In this way, the aptamer hot-start mechanism is reversible and the aptamer can refold to inhibit enzymatic activity at low temperatures even after initial denaturation.

In order to assess the functional aptamer performance, the Twist TrueAmp Polymerase Mix was incubated with primers and template for 2 hours at 25°C. After 2 hours, PCR thermal cycling begins and the PCR reaction is subsequently purified with a 1x SPRI ratio. The no pre-incubation control is included as a baseline yield measurement. Both conditions yield similar library mass and library profile remains intact (**Figure 6**).

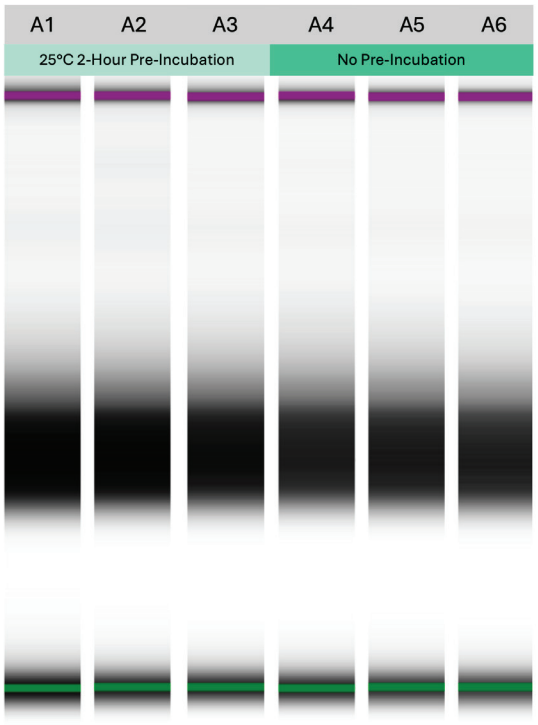


Figure 6. Library Profile by Incubation Method. Twist TrueAmp Polymerase Mix is prepared in a master mix format with template and primers. One master mix is prepared for both conditions. The reactions are either incubated at 25°C for 2 hours or undergo PCR thermal cycling immediately with no incubation. All reactions undergo a 1x SPRI and are visualized on the TapeStationDNA D5000 assay.

Thermalcycling robustness

PCR performance can vary between labs even when users follow the same protocol due to variation in set-up speed, equipment, and technique. Differences in thermal cycler model, block calibration, ramp behavior, maintenance history, and plate-to-block contact can cause small but meaningful changes in effective annealing temperature. This variability can lead to inconsistent library yield, especially when amplification conditions have a narrow operating window.

The Twist TrueAmp Polymerase Mix was evaluated across various annealing temperature conditions and was shown to consistently generate high library yields (**Figure 7**). A polymerase mix with broader thermal tolerance can reduce the risk that small site-to-site or instrument-to-instrument differences become a source of PCR failure or inconsistent library yield.

Primer concentration was also evaluated as an additional measure of PCR robustness. While most users will follow the recommended primer input, primer concentration can vary because of pipetting error, reaction-volume changes, indexing-primer format, custom workflow adaptation, or intentional optimization. Successful amplification across a wide range of primer concentrations suggests that the Twist TrueAmp Polymerase Mix has a forgiving amplification window and is less sensitive to minor setup variation (**Figure 8**).

Together, these data support the use of TrueAmp Polymerase Mix in environments where PCR needs to be robust across different workflows, users, sites, instruments, and minor setup differences. This is particularly relevant for NGS kits that are implemented across many laboratories, transferred into automated workflows, or used in environments where thermal cycler performance and setup consistency may vary.

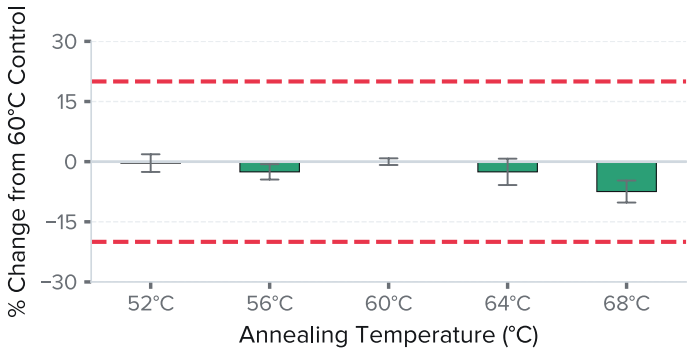


Figure 7. Library Yield by Temperature During Annealing. Triplicate data showing 10 ng of template library amplified with the Twist TrueAmp Polymerase Mix and 6 PCR cycles. This figure displays tolerance and robustness to variances in thermal cycler temperatures for the annealing step. Red dotted lines indicate a 20% variation acceptability threshold.

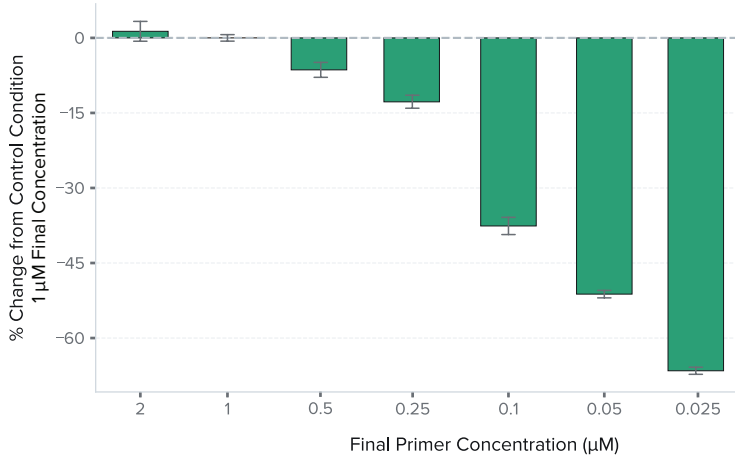


Figure 8. Library Yield by Primer Concentration. Triplicate data showing 10 ng template library amplified using the Twist TrueAmp Polymerase Mix and 6 PCR cycles, with P5/P7 primer titrations in a 50 µl reaction. Even with very low concentrations of amplification primers, quantifiable libraries were successfully amplified.

INHIBITOR AND SAMPLE BUFFER TOLERANCE

NGS workflows can be disrupted by inhibitors introduced from sample extraction, storage buffers, purification steps, or upstream enzymatic reactions. These substances can reduce PCR efficiency, lower final library yield, or cause amplification failure. In many cases, users may not know the exact composition of carryover material from upstream sample processing, making broad inhibitor tolerance an important attribute for a robust amplification mix.

The Twist TrueAmp Polymerase Mix was evaluated against multiple common inhibitors and buffer conditions, including ethanol, EDTA, buffers across a range of pH values, heparin, and SDS. Each of these stressors represents a different mechanism of potential PCR inhibition. For example, ethanol can be carried over from SPRI cleanup, EDTA from input buffers can chelate magnesium and reduce polymerase activity, and variable pH can affect reaction efficiency and enzyme performance. Additionally, heparin, a common blood sample contaminant, can inhibit amplification, and SDS, a protein denaturant, can disrupt polymerase function.

Twist TrueAmp Polymerase Mix performs well under less-than-ideal sample and workflow conditions, where inhibitor concentrations can be high (**Figure 9**). This is particularly relevant for workflows involving clinical research samples, DNA stored in buffers, automated purification, or high-throughput processing, where small amounts of carryover reagents may be difficult to eliminate completely.

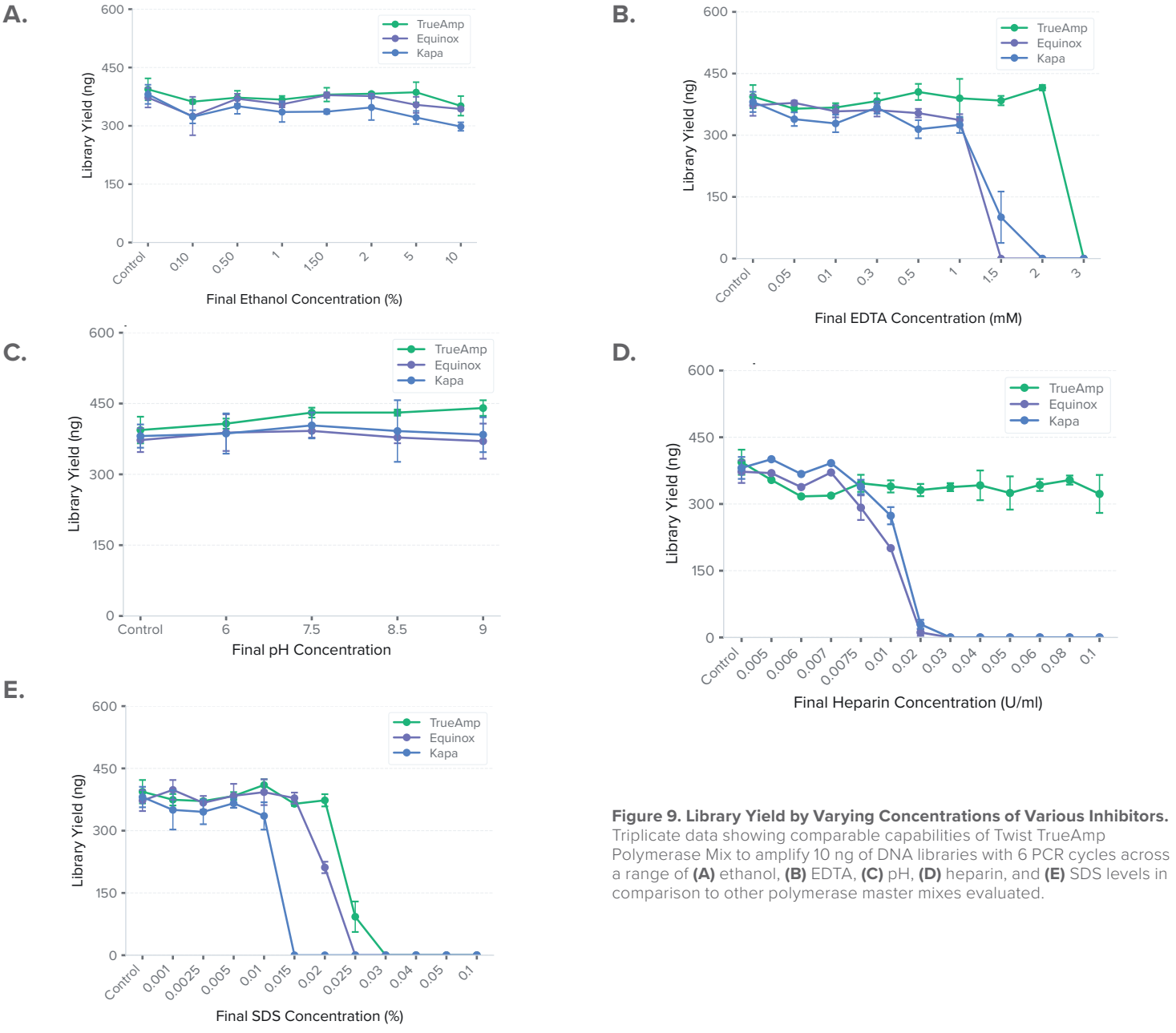


Figure 9. Library Yield by Varying Concentrations of Various Inhibitors. Triplicate data showing comparable capabilities of Twist TrueAmp Polymerase Mix to amplify 10 ng of DNA libraries with 6 PCR cycles across a range of **(A)** ethanol, **(B)** EDTA, **(C)** pH, **(D)** heparin, and **(E)** SDS levels in comparison to other polymerase master mixes evaluated.

PURIFICATION BEAD CARRYOVER TOLERANCE

Magnetic bead purification is commonly used throughout NGS library preparation and target enrichment workflows. While bead-based cleanup enables efficient purification and size selection, bead carryover into downstream enzymatic reactions can occur, especially in automated or high-throughput workflows. Bead carryover may reduce amplification efficiency, affect liquid handling, or create variability between samples.

The Twist TrueAmp Polymerase Mix was tested in the presence of substantial DNA purification bead carryover to evaluate whether amplification could still generate capture- and sequencer-ready libraries.

Twist TrueAmp Polymerase Mix successfully amplifies libraries even with 134.4 µl of Twist DNA Purification Beads carryover in a Twist FlexPrep workflow (**Figure 10**). Additionally, the Twist TrueAmp Polymerase Mix was tested with various types of purification beads commonly used in NGS workflows and showed high resilience in all cases (**Figure 11**). These results support the use of the Twist TrueAmp Polymerase Mix in workflows where robustness to purification variability is important.

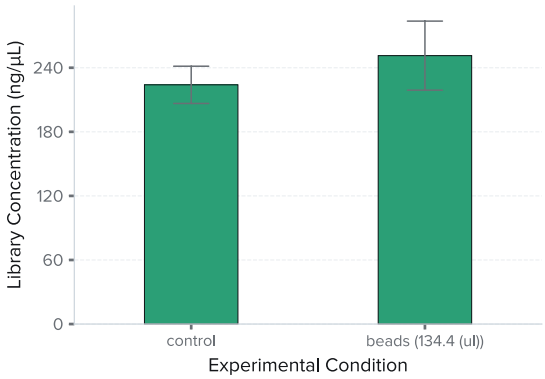


Figure 10. Library Yield by Volume of DNA Purification Beads. Triplicate data showing the capability of the Twist TrueAmp Polymerase Mix to successfully amplify capture- and sequencer-ready DNA libraries, in the presence of a large volume of DNA Purification Beads (134.4 µl). Libraries were generated using the Twist FlexPrep UHT Library Preparation workflow, with 50 ng input and 6 cycles of PCR with P5/P7 primers in a 50 µl PCR reaction, after pooling of 12 samples from a single row of a 96-well plate. Amplification was evaluated in both P5/P7 amplification and UDI indexing amplification contexts.

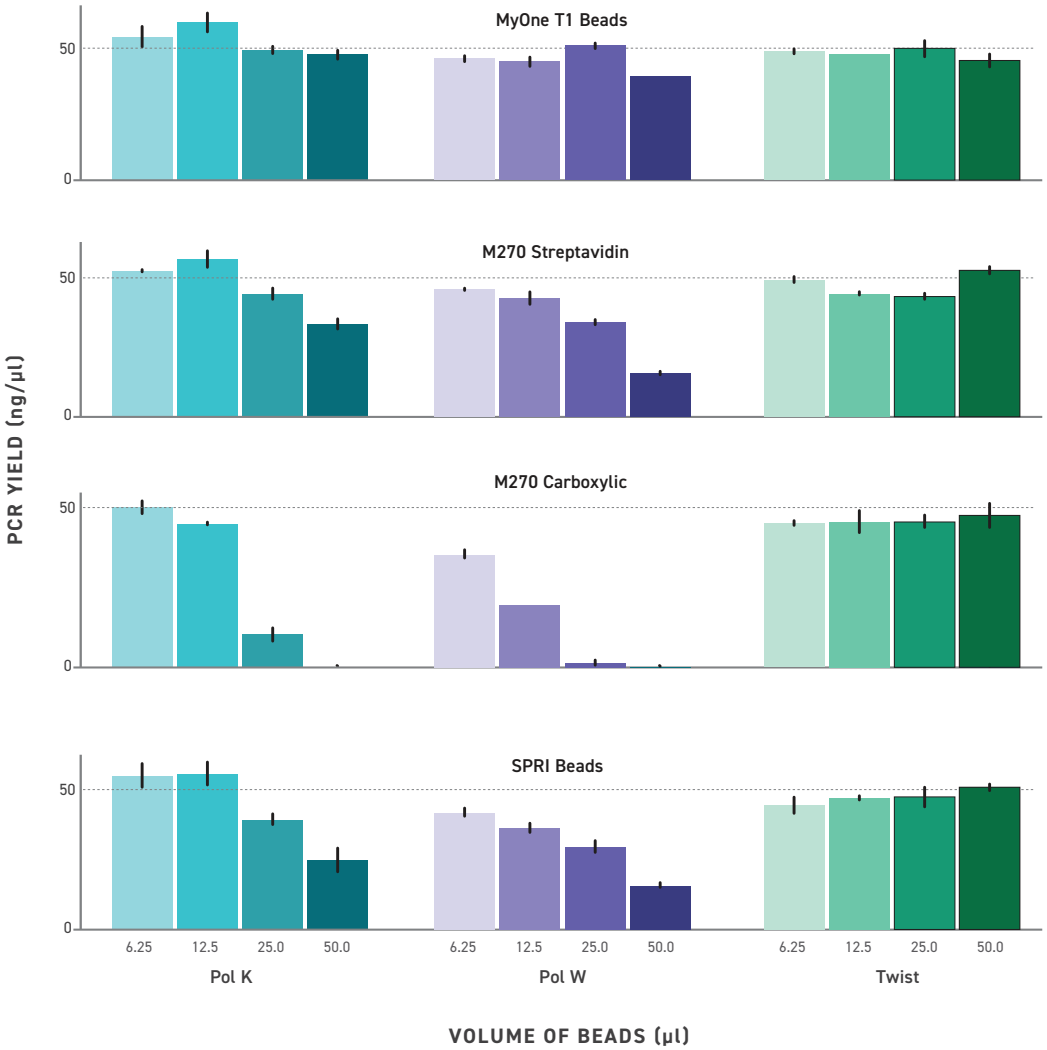


Figure 11. PCR Yield of Various Polymerase Mixes by Varying Volumes of Various DNA Purification Beads. PCR reactions were spiked with 6.25 µl, 12.5 µl, 25 µl, and 50 µl of MyOne T1 Beads, M270 Beads (Invitrogen), and Twist DNA Purification Beads. Reactions were purified after bead removal and quantified on Qubit dsDNA Broad Range Assay.

CONCLUSIONS

The Twist TrueAmp Polymerase Mix was engineered to support robust, high-fidelity amplification for NGS library preparation and target enrichment workflows. Across the conditions evaluated, the Twist TrueAmp Polymerase Mix demonstrated robust amplification from broad ranges of DNA input amounts, tolerance to primer and thermal cycling variation, resistance to common inhibitors and sample buffer conditions, and successful amplification in the presence of substantial bead carryover.

NGS performance is not determined by library yield alone; preservation of library complexity and uniformity across difficult regions is also critical for downstream sequencing quality. Twist TrueAmp Polymerase Mix can improve uniformity and fidelity across challenging GC-rich and AT-rich sequence contexts, supporting more representative amplification of sequencing libraries.

During development, the Twist TrueAmp Polymerase Mix was validated across many Twist library preparation and target enrichment workflows. Together, these data support the use of the Twist TrueAmp Polymerase Mix as a robust amplification solution for modern NGS workflows where yield, consistency, inhibitor tolerance, uniformity, and fidelity are important considerations.