Twist TrueAmp Polymerase Mix

Many PCR enzymes are developed to amplify single targets with accuracy and specificity for applications such as qPCR or cloning. Few are developed to meet the challenges of next-generation sequencing (NGS) and target enrichment, which require high yields alongside uniform and sensitive amplification of a wide range of fragment sizes and GC content. As a result, NGS amplification is generally biased towards short and GC- and structure-neutral molecules.

The Twist TrueAmp Polymerase Mix (2X) was engineered for best-in-class GC uniformity and unbiased amplification of complex NGS libraries with improved fidelity and yield. It features an engineered proof-reading hot-start DNA polymerase with a DOE-optimized buffer, dNTPs, and proprietary uniformity enhancers. Additionally, unlike many commercially available polymerases, where the hot-start mechanism only inhibits polymerization activity, the Twist TrueAmp Polymerase Mix features a novel aptamer-based hot-start mechanism that reversibly binds and inhibits the polymerase enzyme, which allows for long incubations during high-throughput automation.

HIGHLIGHTS

Save on sequencing economics with high uniformity

- Ability to amplify libraries with a wide GC range (5% to 95%) with minimal bias
- Maintain coverage visibility in challenging regions

Deliver on sensitivity with consistent high yields from complex libraries

- Robust amplification with DNA input as low as 100 fg
- Formulation optimized for robustness via design of experiment (DOE)

Gain higher confidence in error and artifact calls

- Significant improvement in fidelity via 20% fewer C->T misincorporations from deaminated cytosines
- High processivity reduces polymerase slippage in >8 homopolymer stretch regions

Increase throughput with automation

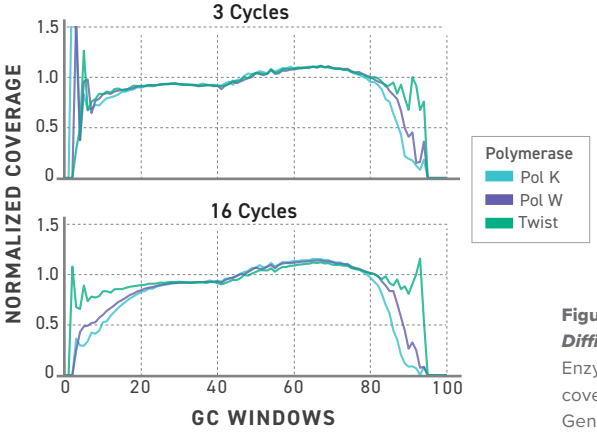
- Hot-start functionality blocks activity at room temperature
- Aptamer-based mechanism for reversible inhibition after PCR

Streamline workflow

- Single-tube ready-to-use 2X mastermix for increased robustness
- Validated with Twist library preparation and Twist target enrichment reagents
- Drop-in improvement to existing Twist amplification solutions

Low bias amplification

The Twist TrueAmp Polymerase Mix includes an engineered polymerase tolerant to destabilizing uniformity enhancers, providing improved coverage of high- and low-GC regions that drop out in other polymerase solutions (**Figure 1**). This improves sequencing economics and allows users to gain insights into targets within challenging regions that would otherwise be missed.



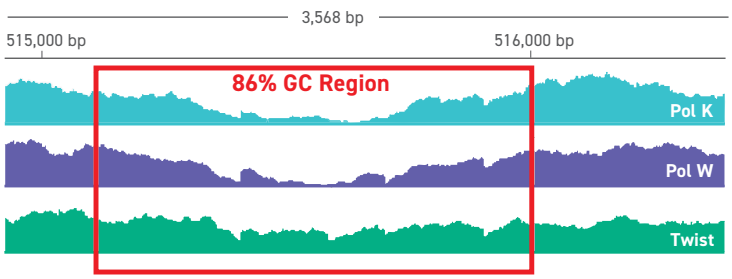


Figure 1. Coverage Data From Illumina WGS Sequencing of a 50:50 Microbial Genomes Mix: *C. Difficile* (AT-rich) and *B. Pertussis* (GC-rich). Libraries were prepared with Twist Library Preparation Enzymatic Fragmentation Kit 3.0 and amplified with different polymerases and cycles. (A) Normalized coverage against GC window plots comparing polymerases at 3 vs 16 cycles of PCR. (B) Integrated Genome Viewer view of a challenging 86% GC region in *B. Pertussis* after 3 PCR cycles.

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Efficient and robust amplification

Twist TrueAmp Polymerase Mix's improved efficiency lowers the number of PCR cycles required for yield targets, which minimizes artifacts and bias introduced with high-cycle PCRs. The mix is also robust in demanding workflows where low-yield libraries are amplified, such as target enrichment.

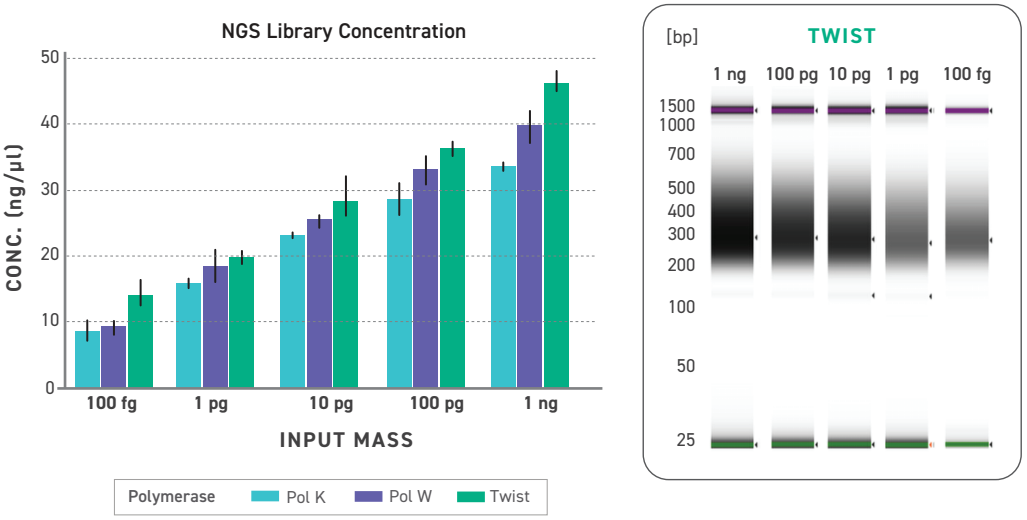

Figure 2. Yield Data From Serially Diluted (100 fg to 1 ng) gDNA Libraries After Enzymatic Shearing, Repair, and Ligation With Twist Library Preparation Enzymatic Fragmentation Kit 3.0, Split for Amplification With Different Polymerases and PCR Cycles. (A) NGS library concentration after 11, 14, 17, 20, and 23 PCR cycles for 1 ng, 100 pg, 10 pg, 1 pg, and 100 fg, respectively, with N = 3 replicates. NGS libraries were SPRI-purified and eluted in the same volume for quantification with Qubit dsDNA Broad Range Assay. (B) NGS library visualization on Tapestation D1000 for quality control on specificity and purity of amplified libraries.

High fidelity and reduced slippage

The Twist TrueAmp Polymerase Mix contains an engineered proofreading and high-processivity polymerase that significantly reduces C->T misincorporations, which are typically a product of cytosine deamination events. Its high processivity also helps reduce slippage events when amplifying homopolymer tracks. This enables higher confidence in applications where complex variants are of interest.

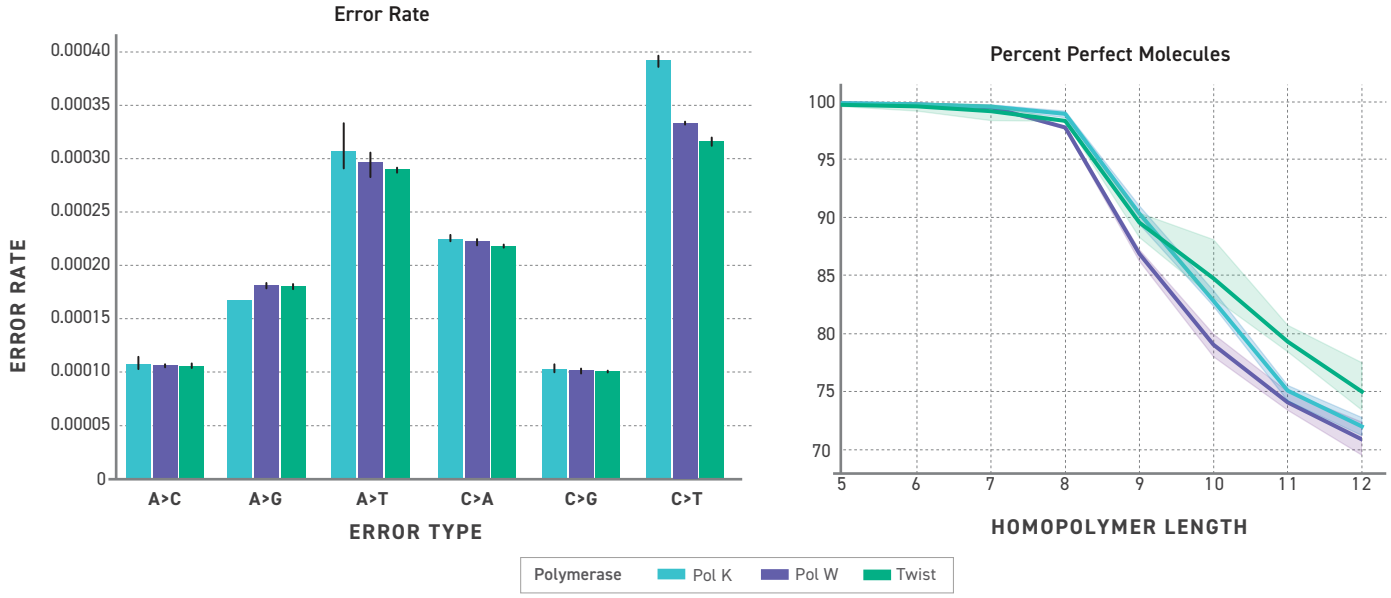

Figure 3. Fidelity Data via Substitutions and Slippage Deletions With Different Polymerases. (A) Substitution rates of gDNA libraries that were sequenced after >7 million base incorporation events. (B) Clonal plasmids with different lengths of homopolymer stretches were ordered from Twist Bioscience as templates for library preparation. The same ligated molecules were split to be amplified with different polymerases and sequenced on an Illumina NextSeq 550. Anchor sequences flanking the homopolymers were used to filter reads and exact counts of perfect homopolymers were calculated.

Bead tolerance

Magnetic beads are used in many library preparation and target enrichment workflows for purification and size selection purposes, but beads may impact PCR yield through inhibition of polymerase activity. The Twist TrueAmp Polymerase Mix has high bead tolerance and maintains higher sequencing read counts than other commonly used polymerases when tested at various amounts of magnetic beads. This bead tolerance allows for increased robustness in workflows and improves data quality.

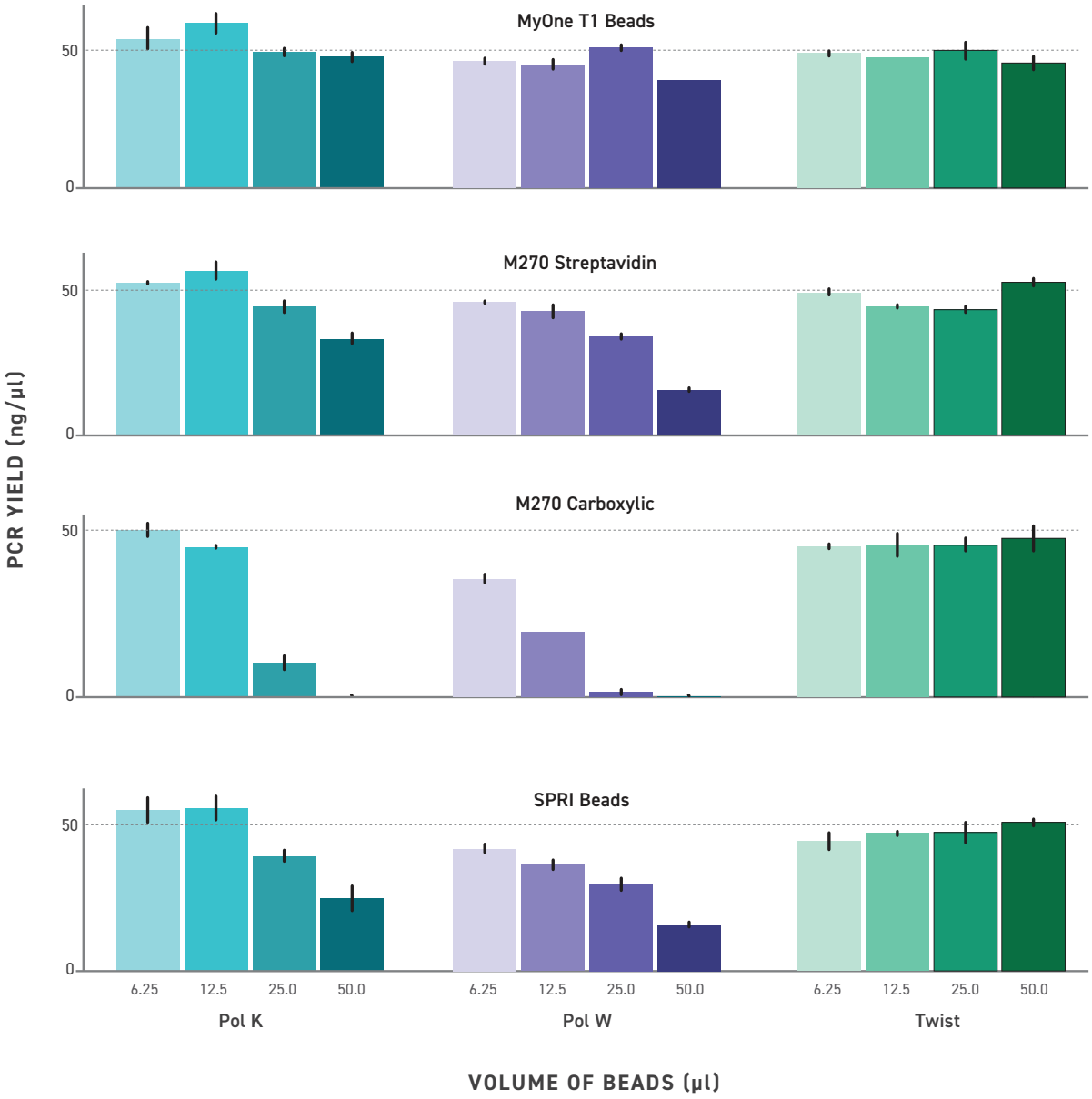

Figure 4. Tolerance to Paramagnetic Beads During PCR. PCR reactions were spiked with 6.25 µl, 12.5 µl, 25 µl, and 50 µl of MyOne T1 Beads, M270 Beads (Invitrogen), and Twist DNA Purification Beads. Reactions were purified post bead-removal and quantified on Qubit dsDNA Broad Range Assay.

LEARN MORE

twistbioscience.com/ngs
sales@twistbioscience.com

ORDERING INFORMATION

116471: Twist TrueAmp Polymerase Mix, 16 reactions
116472: Twist TrueAmp Polymerase Mix, 96 reactions