Frequently Asked Questions:

TrueAmp Library Preparation Kit

Core Workflow & Compatibility

What sample types and library preparation workflows are compatible with the TrueAmp Library Preparation Kit?

The TrueAmp Library Preparation Kit is designed for flexible DNA library construction across a wide range of sample types and input amounts. It supports an integrated workflow where fragmentation, end repair, and dA-tailing are performed in a single enzyme mix, simplifying the overall process.

The kit performs robustly across diverse DNA inputs, including FFPE samples or samples with varying GC content and is compatible with DNA in common storage buffers such as TE, Tris-HCl, or water.

It also enables consistent and reliable fragmentation using a single standardized protocol, while also allowing users to tune insert size by adjusting incubation time or temperature. The streamlined workflow can be completed in approximately 3 hours, with less than 15 minutes of hands-on time, and is designed to maximize library yield through high reaction efficiency and minimal sample loss.

What input amount is recommended for library preparation using the TrueAmp Library Preparation Kit?

The TrueAmp Library Preparation Kit supports a broad range of DNA input amounts, from as low as 100 pg up to 1 µg. This flexibility enables reliable library preparation across low-input and high-input sample types.

Can I use the TrueAmp Library Preparation Kit for bisulfite conversion or EM-seq workflows?

No, the TrueAmp Library Preparation Kit is not compatible with bisulfite conversion or EM-seq workflows. The enzymatic steps in the TrueAmp Library Preparation workflow include DNA repair, which can alter or remove native methylation signatures.

For methylation-sensitive applications such as bisulfite or EM-seq, we recommend using mechanical shearing (e.g., sonication) followed by the Twist cfDNA Library Preparation Kit, which is better suited for preserving methylation information.

Can I use the TrueAmp Library Preparation Kit for cfDNA Samples?

The TrueAmp Library Preparation Kit is not recommended for cfDNA workflows. Since cfDNA is already fragmented, the enzymatic fragmentation step is unnecessary. While it is possible to skip fragmentation and proceed with end repair and dA-tailing, the one-pot workflow may not deliver optimal conversion efficiency for these short fragments.

For best performance and highest library conversion from cfDNA, we recommend using the Twist cfDNA Library Preparation Kit, which is specifically optimized for cell-free DNA.

Can I use degraded or FFPE-derived DNA with this kit?

Yes, the TrueAmp Library Preparation Kit performs well with degraded and FFPE-derived DNA. The workflow includes DNA repair activity, which can help restore damaged bases commonly found in FFPE samples.

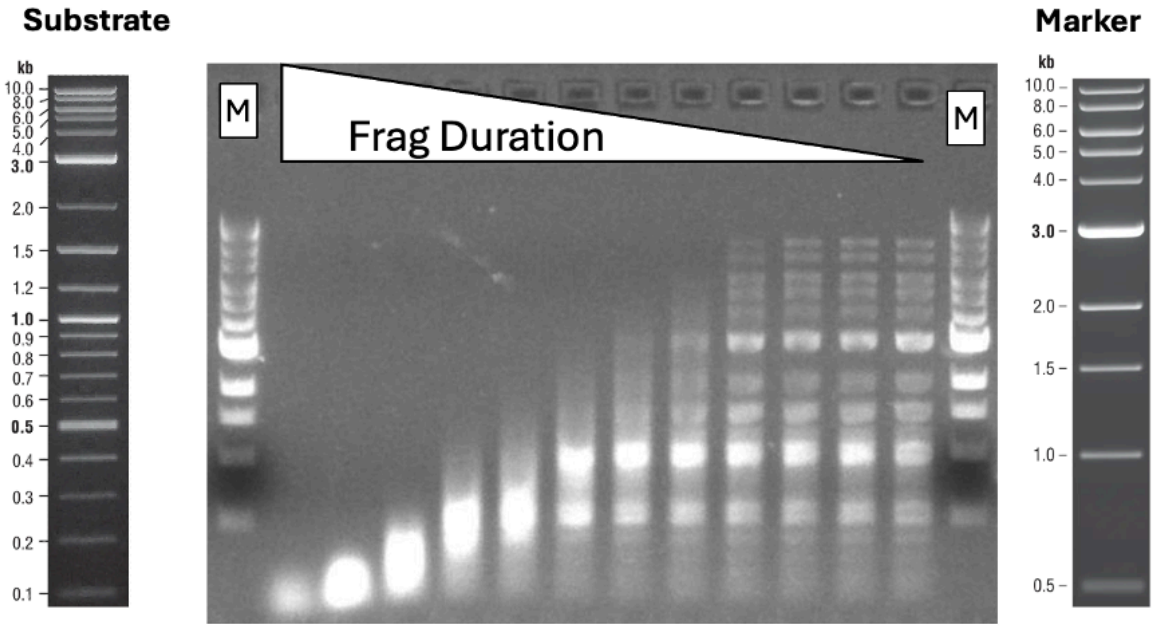
As a result, users often observe improved library yield and recovery compared to workflows without repair. Some optimization (e.g., fragmentation time, PCR cycles) may be required depending on sample quality.

Can I use plasmids, PCR products or mixed amplicons with this kit?

Yes. The TrueAmp fragmentation chemistry is broadly compatible with a range of double-stranded DNA inputs, including genomic DNA (gDNA), plasmids, and PCR-derived amplicons. Under consistent reaction conditions, high-quality DNA substrates, regardless of origin, typically yield comparable fragment size distributions, indicating minimal sequence or topology-dependent bias.

During development, fragmentation performance was evaluated using DNA ladders spanning a wide range of fragment sizes. This study demonstrates that the enzymatic fragmentation

preferentially processes larger DNA molecules while maintaining representation of shorter fragments, resulting in a controlled size distribution without significant loss of diversity across the input population.



Can I use this kit for targeted enrichment workflows?

Yes, the TrueAmp Library Preparation Kit is well suited for targeted enrichment workflows, including hybrid capture applications. It generates high library yields and promotes uniform molecule conversion, which supports efficient and consistent downstream target capture performance.

Can I use this with automation platforms (Hamilton, etc.)?

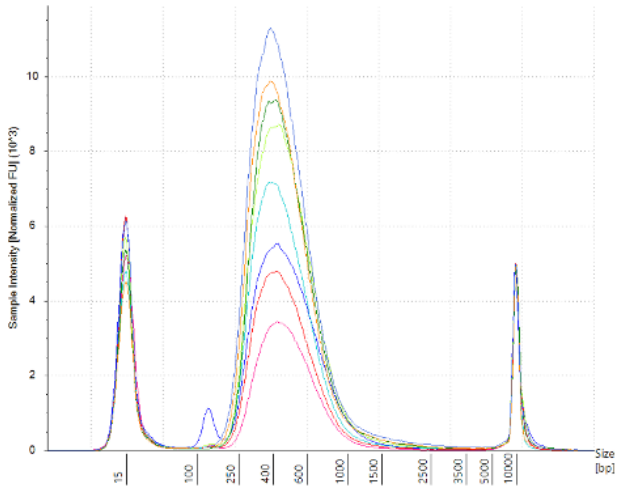
Yes, the TrueAmp Library Preparation Kit is compatible with liquid handling automation platforms such as Hamilton systems. The streamlined workflow, reduced number of steps, and minimal hands-on time make it well suited for automation. Minor optimization (e.g., mixing, bead handling, or liquid classes) may be required depending on the platform and configuration.

Reaction Setup

Do I need to adjust fragmentation conditions based on DNA input amount?

No. The enzymatic fragmentation chemistry is designed to produce consistent fragment sizes across the recommended input range, so fragmentation time and temperature do not need to be adjusted based on DNA mass.

Under the same conditions, samples with similar DNA quality ranging from 0.1 ng to 500 ng will generate comparable fragment size distributions. Demonstrated above, human genomic DNA inputs from 0.1 ng to 500 ng fragmented at 32°C for 22 minutes produced similar final library sizes (~450–480 bp), with PCR cycle number increasing as input mass decreased.



Mass Input (ng)	PCR Cycles	Average Size (bp)
500	3	452
100	5	456
50	6	463
25	7	469
10	8	469
5	9	460
1	10	479
0.1	14	480

How do I control fragment sizes with the TrueAmp Library Preparation Kit?

Fragment size can be controlled by adjusting fragmentation reaction temperature and incubation time. Increasing either parameter generally results in shorter DNA fragments, while reducing them produces longer fragments.

Because fragmentation performance can vary depending on thermocycler calibration, DNA input quality, and storage buffer composition, we recommend performing a small design of

experiments (DOE) with your specific samples to determine optimal conditions prior to scaling up.

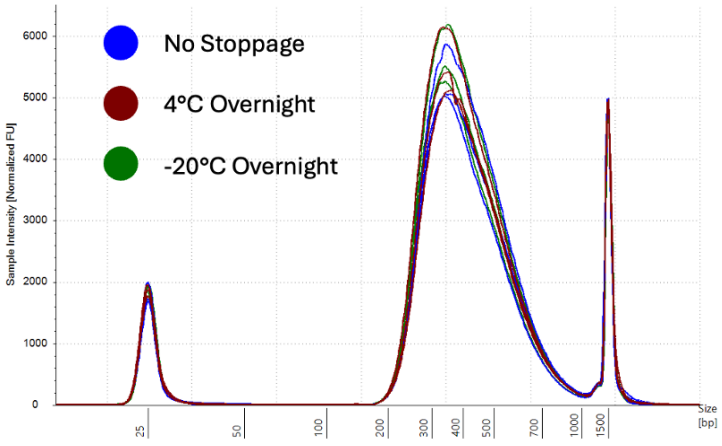
For additional guidance and example data, please refer to the Twist TrueAmp Library Preparation Kit technical note on insert size optimization.

How does input DNA quality impact final insert size?

Input DNA quality directly affects fragment size. High-quality, intact DNA produces predictable insert sizes, while degraded or pre-sheared DNA (e.g., FFPE) typically results in shorter fragments under the same conditions. In these cases, reducing fragmentation time or temperature and applying size selection may help achieve the desired insert size.

After fragmentation and end repair/dA-tailing, can samples be stored overnight before proceeding to ligation?

Yes, fragmented and end-prepped DNA can be safely stored at either 4 °C or -20 °C (e.g., overnight) prior to ligation without any appreciable impact on library preparation performance as compared to samples processed immediately. This has been validated across technical replicates (n=3), where samples were stored under these conditions before ligation and showed consistent results.



Is it necessary to vortex the 10X Twist Fragmentation Enzyme Mix before use?

Yes. Vortexing the 10X Twist Fragmentation Enzyme Mix prior to use is important to ensure the solution is fully homogeneous. Failure to properly mix the enzyme solution may lead to variability in fragmentation performance and inconsistent results.

What adapters and primers are compatible with the TrueAmp Library Preparation Kit?

The TrueAmp Library Preparation Kit is designed for flexibility and is compatible with a wide range of 3′ T-overhang adapters. Note that adapter quality impacts overall library preparation efficiency. For optimal performance, we recommend using validated Twist adapter systems:

- Twist Universal Adapter System (SKU: 101308)
  - Requires PCR amplification with indexed primers to complete library construction.
- Twist UMI Adapter System (SKU: 105041)
  - Requires PCR amplification with indexed primers.
  - Enables incorporation of Unique Molecular Identifiers (UMIs) for improved error correction and duplicate handling.
- Twist Full-Length UDI Adapters (SKU: 107376)
  - Do not require PCR for index incorporation, as full-length P5/P7 sequences and indices are already present.
  - Optional PCR may still be performed for library amplification if additional yield is needed.

All three systems are fully compatible with the TrueAmp workflow. Selection should be based on your application needs (e.g., UMI requirements, workflow, or PCR-free indexing preference).

How do I optimize the amount of adapter input for each of those adapters?

Adapter input should be adjusted based on DNA input amount and fragment size to maintain sufficient adapter-to-insert molar excess for efficient ligation while minimizing adapter-dimer formation. Twist Universal Adapters are supplied at 10 µM and recommended conditions in the

protocol are designed to maintain an adapter-to-insert molar ratio of approximately ≥150:1 for robust ligation efficiency across a broad range of inputs. Too little adapter may reduce library conversion and yield, while excessive adapter can increase adapter-dimer carryover, particularly in low-input samples.

If optimization is required, titrate adapter input while monitoring final library yield and adapter-dimer levels. The optimal condition is the lowest adapter amount that maintains strong library yield with minimal adapter dimer.

For ultra-low input samples (<1 ng), additional optimization may be required to balance ligation efficiency against adapter-dimer formation, as reduced insert mass increases the likelihood of adapter self-ligation products. Conversely, for higher DNA input amounts, increasing adapter concentration beyond the recommended range may further improve ligation efficiency in some workflows.

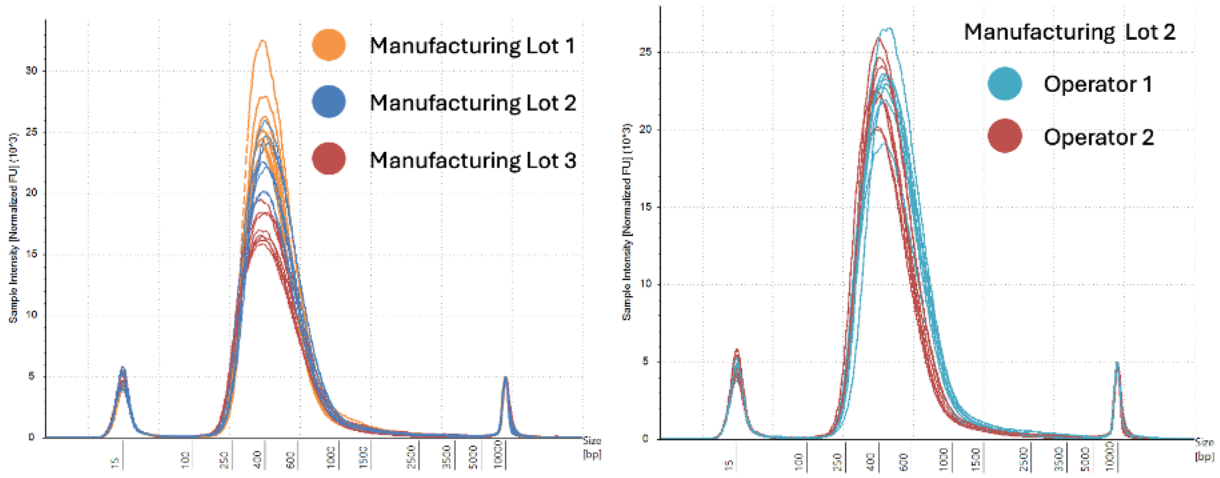
Are there inhibitors that can impact fragmentation efficiency?

Yes, contaminants such as salts, EDTA, ethanol, detergents, and extraction reagent carryover can impact enzymatic fragmentation. These may either inhibit or enhance enzyme activity, leading to inconsistent or shifted fragment sizes.

In some cases, fragmentation can be tuned by adjusting incubation time or temperature to compensate. However, if inhibitors significantly disrupt enzyme activity and no library is generated, we recommend performing an additional DNA cleanup before library preparation.

Handling & Storage

What is the variability in fragment length we can expect from manufacturing or operator variability?



Three independent manufacturing lots were evaluated to assess fragmentation variability, with an additional operator performing replicate testing on a separate day to characterize operator-to-operator effects.
Fragmentation with the TrueAmp Library Preparation Kit demonstrates high reproducibility within a given lot, with technical replicates (n=8) showing tight fragment size distributions and a standard deviation of ~7–10 bp (~2% CV). Across manufacturing lots, modest shifts in the mean fragment size are observed (~30–40 bp, or ~5–8% deviation), while maintaining consistent distribution shape and overall library performance. Operator-to-operator variability can introduce similar shifts in the average fragment size, as observed within the same lot; however, these differences should not meaningfully impact the distribution profile or downstream performance.

Are freeze–thaw cycles acceptable for the TrueAmp reagents?

Yes. The TrueAmp reagents have been tested through up to four freeze-thaw cycles with no observed impact on performance. These studies were conducted over multiple days to reflect

typical laboratory use. As a best practice, repeated freeze–thaw cycles should still be minimized when possible.

What is the shelf life of the TrueAmp Library Preparation Kit?

The TrueAmp Library Preparation Kit has a shelf life of 12 months when stored as recommended. This is supported by accelerated aging studies conducted over 13 months, which showed no measurable impact on performance or stability.

Do you offer customizations to the TrueAmp Library Preparation Kit?

Yes, we offer flexible customization options to support a wide range of specialized requirements or unique applications. For more information or to discuss your specific requirements, please contact our team.

Troubleshooting

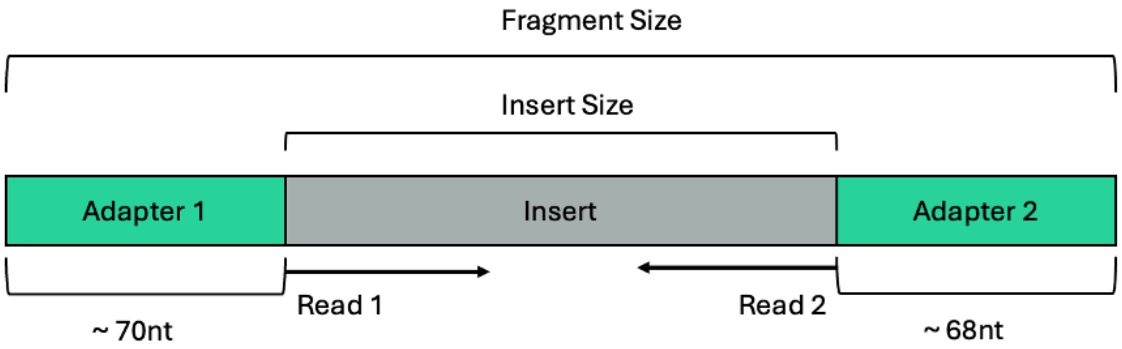
When should I not use the TrueAmp Library Preparation Kit?

The TrueAmp Library Preparation Kit is not recommended for workflows where DNA is already sheared or where native DNA modifications must be preserved. This includes applications such as cfDNA library preparation and methylation-sensitive workflows (e.g., bisulfite or EM-seq). For these use cases, alternative workflows or kits optimized for those applications are recommended.

Can you help explain Insert size vs Library/Fragment size?

The insert is the DNA of interest generated during fragmentation. The insert size refers to the length of this DNA region. The fragment (or sequencing fragment/library) includes the insert plus the sequencing adapters ligated to both ends. Therefore, fragment size = insert size + adapter sequences. Traditional adapter sequences are roughly 65-70nt long but dependent on the presence of UMIs or length of indexes. In paired-end sequencing, reads are generated from both ends of the insert. If the insert is longer than the combined read length, a portion in the

middle remains unsequenced. If the insert is shorter, reads may overlap or extend into adapter sequences.



Why is insert size important in library preparation?

Insert size impacts library yield, sequencing performance, and downstream applications. Smaller fragments typically amplify more efficiently and can result in higher yields, while larger fragments may reduce cluster generation efficiency on some sequencing platforms. Insert size also influences data quality metrics such as coverage uniformity, duplication rates, and alignment efficiency.

In targeted enrichment workflows, especially multiplexed captures where the same mass input (e.g., 500 ng) represents a different number of molecules depending on fragment size. Smaller libraries contain more molecules than larger ones at the same mass, which can affect capture efficiency and sample balance.

Additionally, larger insert sizes in target enrichment workflows can increase the proportion of near-bait reads, as longer fragments extend beyond the probe-binding region. Insert size should also be considered relative to sequencing read length. For paired-end sequencing (e.g., 2×75 bp or 2×150 bp), inserts that are too short may result in overlapping reads or sequencing into adapter regions, reducing usable data. Selecting an appropriate and consistent insert size for your application helps ensure optimal sequencing performance and reliable downstream analysis.

Why are my fragment sizes smaller than expected?

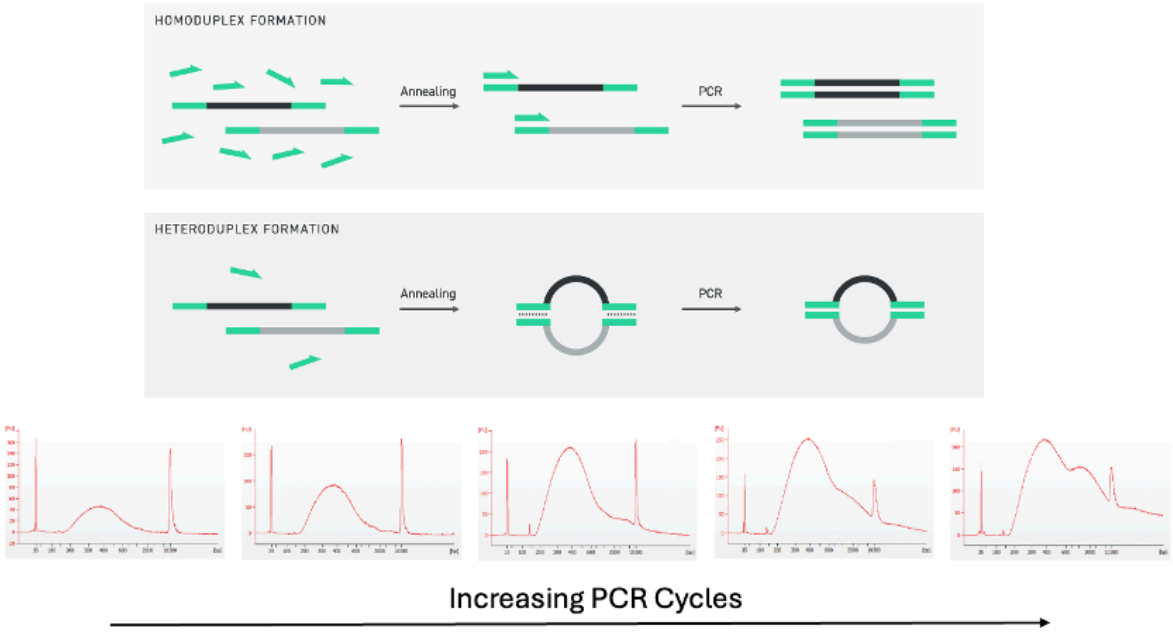
Shorter-than-expected fragments typically result from degraded or pre-sheared input DNA, excessive fragmentation time or temperature, or buffer components (e.g., salts) that increase enzymatic activity. We recommend reducing fragmentation time or temperature and confirming input DNA quality to help achieve the desired insert size. If needed, performing a cleanup step prior to library preparation may improve consistency.

Why do I see multiple peaks or unexpectedly large fragments in my library?

If your DNA has been sufficiently fragmented, the presence of multiple peaks, especially those appearing larger than the expected fragment size, is typically an artifact of library preparation rather than true size distribution:

1. PCR overamplification and heteroduplex formation.
During excessive PCR cycling, DNA strands from different templates can anneal to each other, forming heteroduplexes. These structures migrate more slowly during capillary electrophoresis (e.g., TapeStation or Bioanalyzer), which can create the appearance of larger fragment sizes or additional peaks.

For additional information, please refer to the technical note on heteroduplexes: https://www.twistbioscience.com/resources/white-paper/heteroduplexes-affect-library-size-determination-without-impacting-targeted

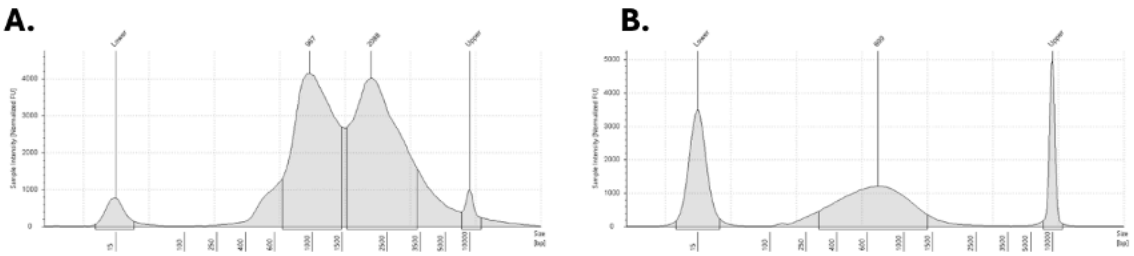


Overcycling can introduce heteroduplex artifacts in adapter-ligated libraries. Samples of the gDNA library were subjected to 2x increments in rounds of PCR amplification. **Top/Middle:** Schematic of homoduplex and heteroduplex formation during PCR amplification. With appropriate amplification, library molecules form homoduplex products. With excessive PCR cycling, related but non-identical library molecules can misanneal, forming heteroduplex species that migrate aberrantly during electrophoresis. **Bottom:** Bioanalyzer electropherograms representing increasing PCR cycles (left to right, 4 - 12 cycles). PCR libraries show progressively broad, irregular peaks and apparent high-molecular-weight species, consistent with heteroduplex formation from overamplification.

2. Adapter structure effects (full-length Y adapters)

Libraries constructed with full-length Y adapters (containing both P5 and P7 sequences) can exhibit altered migration behavior due to their partially single-stranded structure. This can cause fragments to appear larger than their true size during electrophoretic analysis.

These 2 effects can be validated by performing a short “reconditioning PCR”: Use a small number of cycles (e.g., 2–5 cycles) with the inclusion of a high concentration of primers This step promotes the formation of fully matched homoduplexes, which migrate more accurately during electrophoresis. After this treatment, the apparent fragment size distribution should shift closer to the expected profile.



Resolution of library fragment size following PCR amplification. 250ng of gDNA was fragmented at 25°C for 15 mins for a PCR-free protocol **(A)** TapeStation trace of a library immediately following ligation of Twist Full-Length UDI Adapters. The electropherogram shows multiple broad and irregular peaks, reflecting heterogeneous migration behavior caused by the Y-shaped adapter structure. These structural features can result in apparent high-molecular-weight species and poor size resolution. **(B)** TapeStation trace of the same library after 3 cycles of PCR amplification. PCR converts adapter-ligated molecules into fully double-stranded homoduplex products, resolving structural heterogeneity and yielding a defined fragment size distribution.

Why is my library yield lower than expected?

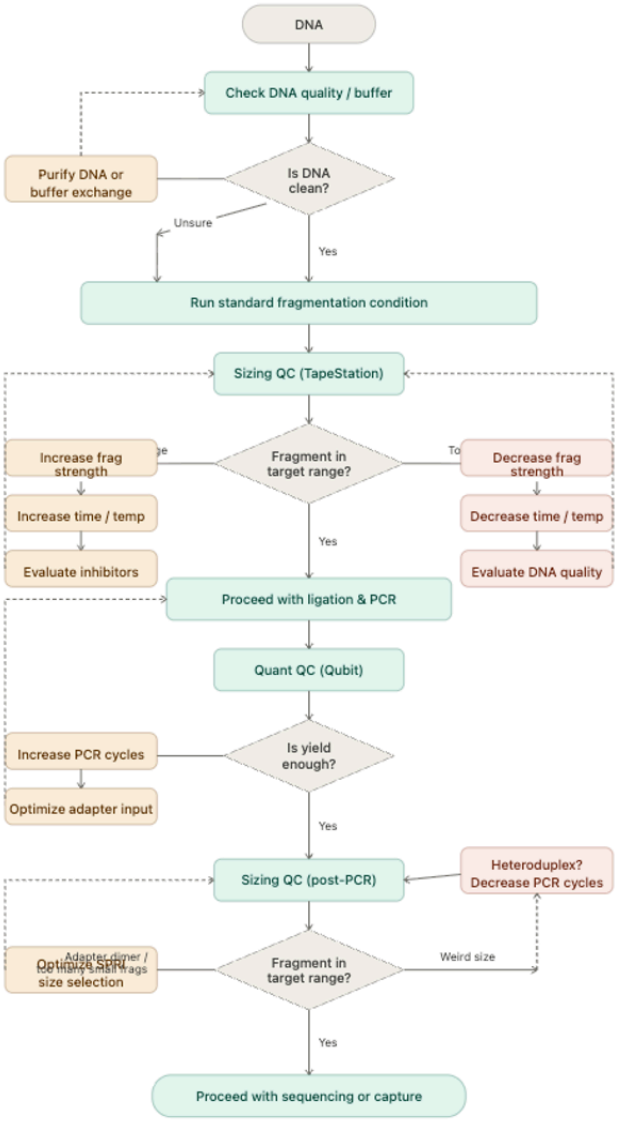
Low library yield can result from low input DNA, poor DNA quality, over- or under-fragmentation, or the presence of inhibitors that affect enzymatic steps. Additional factors such as improper size selection, incomplete ethanol removal, over-dried SPRI beads, or insufficient mixing during reactions may also reduce yield. Ensuring clean DNA input, optimizing fragmentation conditions, and following recommended SPRI handling and mixing practices should improve performance.

Why do I see adapter dimers or short fragments in my library?

Adapter dimers or short fragments are typically observed with low input DNA, excess adapter concentration, or insufficient size selection. Additional causes include improper adapter handling (e.g., degradation or incorrect dilution) or pre-mixing adapters with ligation reagents. Optimizing adapter concentration, ensuring proper adapter handling, and performing appropriate SPRI cleanup (e.g., adjusting bead ratios or repeating cleanup) can help reduce these artifacts.

What conditions should I start with to achieve ~200–250 bp insert sizes?

As a general starting point, the baseline fragmentation times and temperatures in the protocol are recommended to achieve insert sizes in the 200-250 bp range. Fragmentation behavior can vary depending on thermocycler calibration, DNA quality, and buffer composition. For best results, we recommend performing a small time-course optimization (DOE) using your specific samples and follow this decision tree for optimization of the target insert size.



How many PCR cycles should I use?

The number of PCR cycles depends on input DNA amount and desired library yield. Lower input samples typically require more cycles to generate sufficient material, while higher input samples require fewer cycles to minimize duplication and preserve library complexity. Optimization may be required to balance yield and sequencing performance.