Technical Note

Tagify™ i5 UMI Transposase Reagent and Input DNA Titration for the Modification of Fragment Size to Facilitate the Development of Multiplexed Genome-editing NGS Applications

Introduction

The Tagify i5 UMI Adapter-loaded Transposase Reagent is designed to catalyze the reaction that simultaneously fragments and tags DNA with an oligonucleotide payload via Tn5 transposase (Figure 1). Each loaded transposase has been optimized to fragment 50 ng of genomic DNA (gDNA) to an average size of 0.8 – 2kb as measured by Agilent Bioanalyzer (Figure 2), while simultaneously tagging the resulting fragments with an 82 base sequence that contains an Illumina-compatible P5+i5+UMI (unique molecular identifier)+R1 sequence. Tagged DNA can then be seamlessly integrated into downstream applications*, such as UDiTaS, RGEN-seq, and TTIS-seq.

The Tagify i5 UMI Transposase Reagent User Guide describes the general use of these reagents. It is not intended to serve as a full protocol for library preparation as part of a specific application. This Technical Note outlines the expected fragment sizes of tagged DNA in relation to DNA input and Tagify i5 UMI reagent volume that can be used as a guideline to generate the fragment size of interest to easily integrate Tagify i5 UMI reagents within their assay-specific protocols.

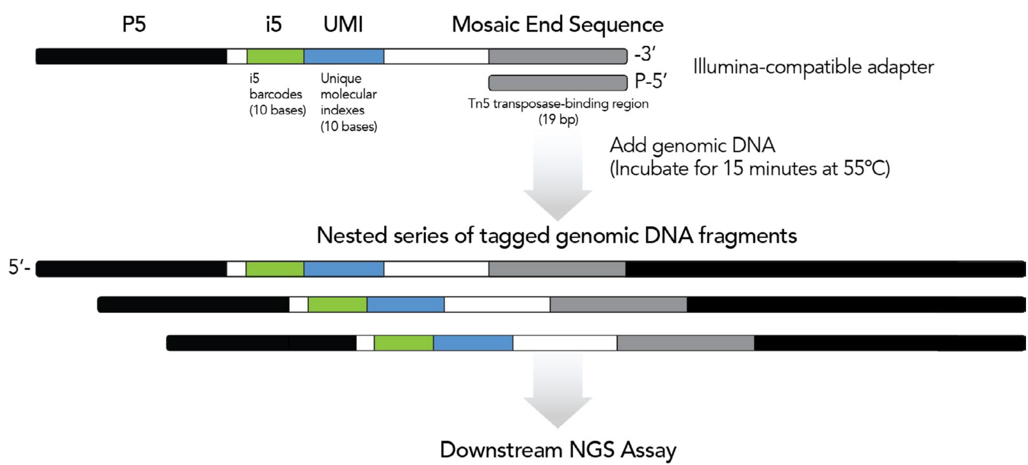


Figure 1. Molecular diagram of Tagify i5 UMI reagent workflow. Tagmenting DNA using Tagify i5 UMI loaded transposases requires a single 15-minute incubation at 55°C followed by reaction clean-up. Tagged DNA can then seamlessly integrate into downstream NGS assay.

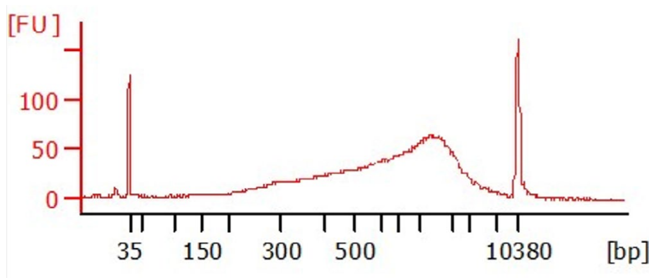


Figure 2. The electropherogram generated by an Agilent Bioanalyzer High Sensitivity DNA kit shows a trace of tagged DNA using 2 μl of Tagify i5 UMI reagent from 50 ng of human genomic DNA. The region analysis used is 200 - 7500 bp with the average size being 1116 bp.

Standard Method

The data presented within this Technical Note were generated following the protocols detailed within the product User Guide. Specifically, 96 individual libraries were made using 50 ng of human gDNA (NIST HG001, Coriell Institute for Medical Research), Tagify i5 UMI Adapter-loaded Transposase Reagents, and its validated i7 index. The 96 libraries were quantified and sized using Agilent TapeStation 2200 HSD5000 and subsequently sequenced on MiSeq v3 150 cycles. Please note that libraries created using Tagify i5 UMI Loaded-Transposase Reagents may be sequenced using other NGS platforms.

Table 1 shows the sequencing performance summary for the 96 libraries made using the Tagify i5 UMI Adapter-loaded Transposase Reagents. Several key performance metrics to assess how well the UMIs capture the diversity of tagged DNA molecules are defined below:

- Observed Unique UMI: Number of different UMI sequences observed
- Inferred Unique UMI: Number of different inferred UMI sequences derived
- Inferred UMI Entropy: Entropy of the inferred UMI sequences, indicating the effective number of bases in the inferred UMIs.
- UMI Percent Duplicate: The fraction of mapped sequence that is marked as duplicate.

The high number of average inferred UMI entropy and low number of average UMI percent duplication, 8.53 and 0.11%, respectively, (Table 1) for 96 libraries within these studies reflects the high diversity of molecular tags in the sequencing experiment, suggesting that there are many different original DNA molecules being captured.

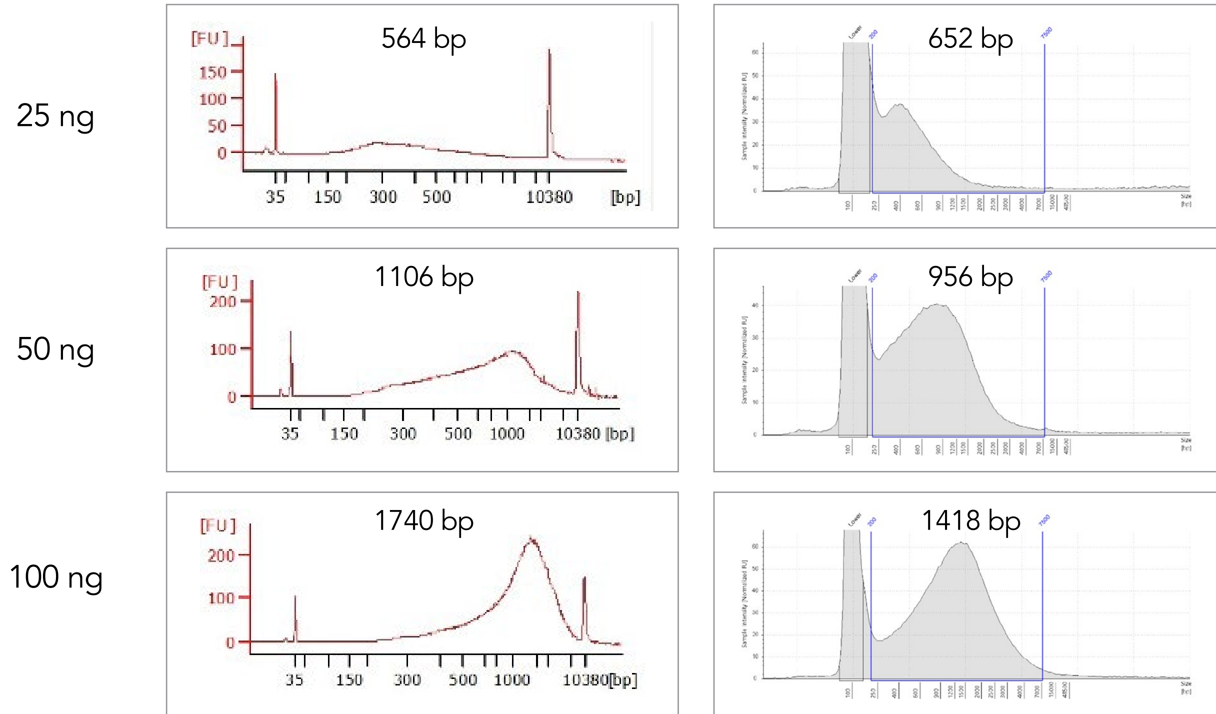
Modifying fragment size via titration of total mass of input DNA

In some cases, it may be desirable to modify the tagged DNA fragment size by adjusting the starting total mass DNA input. This Technical Note demonstrate a titration of total mass input using 25, 50, and 100 ng of human gDNA following standard protocols (Figure 3). For each set of samples processed, tagged DNA fragment sizes were evaluated on an Agilent DNA Bioanalyzer Chip and an Agilent Tapestation using the genomic DNA ScreenTape following the manufacturer's instructions. Each DNA input point was run in duplicate. The libraries were then sequenced on MiSeq v3 150 cycle kit.

Average PF Cluster	Average Median Insert Size	Average Observed Unique UMI	Average Inferred Unique UMI	Average Inferred UMI Entropy	Average UMI Percent Duplication
223,539	218 nt	148,379	148,375	8.53	0.11%

Table 1. Performance summary of 96 libraries generated using Tagify i5 UMI Reagents on MiSeq v3 150 cycle.

3A.



3B.

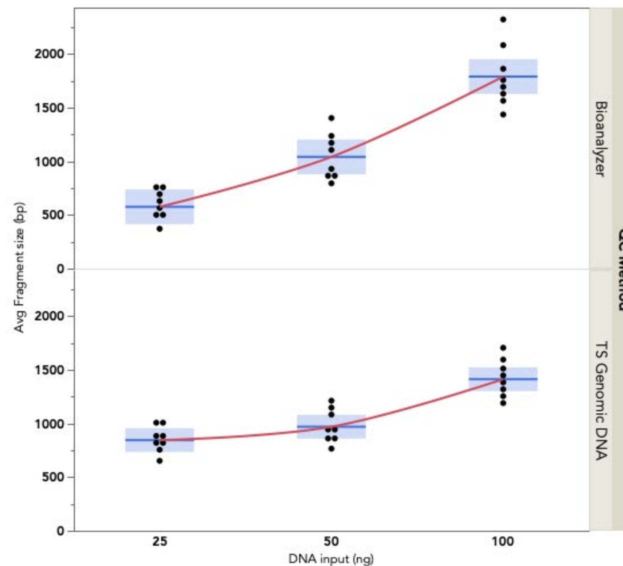


Figure 3. (A) The electropherograms generated by an Agilent Bioanalyzer High Sensitivity DNA kit (left) and an Agilent Tapestation using genomic DNA ScreenTape (right) show traces of tagged DNA using 2 µl of Tagify i5 UMI reagent from 25, 50, and 100 ng of human gDNA. The region analysis used was 200 – 7,500 bp. **(B)** A box-and-whisker plot shows tagged DNA fragment size increases as total mass DNA input increases for both QC methods: Agilent Bioanalyzer (top) and Agilent Tapestation (bottom).

Table 2 shows the range of median fragment size from 187 nucleotides to 277 nucleotides as total mass DNA input increased from 25 to 100 ng. Another factor that is affected by adjusting the total mass DNA input is the percent of UMI duplicates – it decreases as the amount of DNA input increases (ANOVA: Single Factor test, p-value <0.05). The average inferred UMI entropy remained consistent regardless of the total mass DNA input used in the reaction.

Modifying fragment size via titration of Tagify i5 UMI Transposase Reagents

In some cases, it may be desirable to adjust the tagged DNA fragment size by adjusting the volume of the Tagify i5 UMI Transposase Reagent used in the reaction. In Figure 4, a titration of the Tagify reagent was conducted using 1, 2, and 3 µl of Tagify i5 UMI reagent and 50 ng of human gDNA following

DNA Input Used	Number of samples	Average PF Cluster	Average Median Insert Size	Average Observed Unique UMI	Average Inferred Unique UMI	Average Inferred UMI Entropy	Average UMI Percent Duplication
25 ng	2	2,371,775	187 nt	582,722	582,710	9.40	0.27%
50 ng	4	2,516,447	236 nt	585,878	585,871	9.39	0.14%
100 ng	2	1,958,955	277 nt	552,001	551,997	9.37	0.11%

Table 2. Performance summary of 8 libraries made using Tagify i5 UMI Reagents with varying concentrations of input gDNA.

4A.

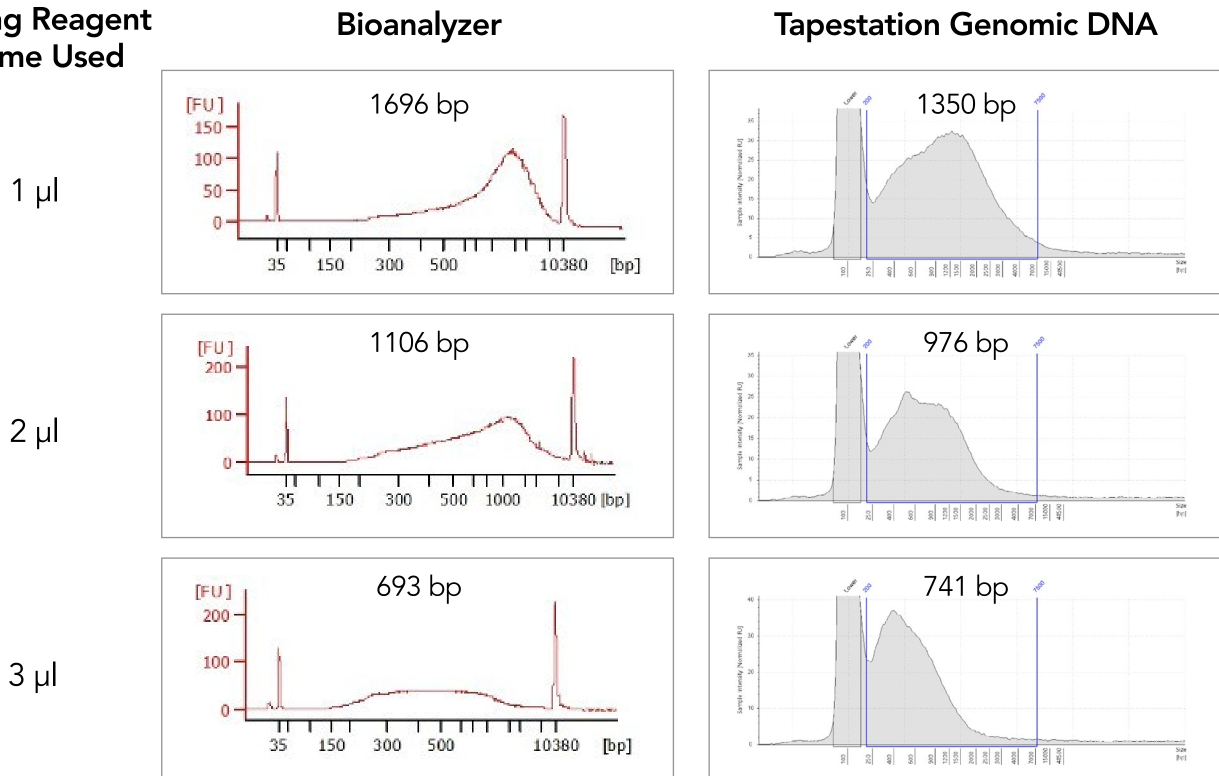


Figure 4A. The electropherograms generated by an Agilent Bioanalyzer High Sensitivity DNA kit (left) and an Agilent Tapestation using gDNA ScreenTape (right) show traces of tagged DNA using 1, 2, and 3 µl of Tagify i5 UMI reagent from 50 ng of human gDNA. The region analysis used was 200 – 7,500 bp.

4B.

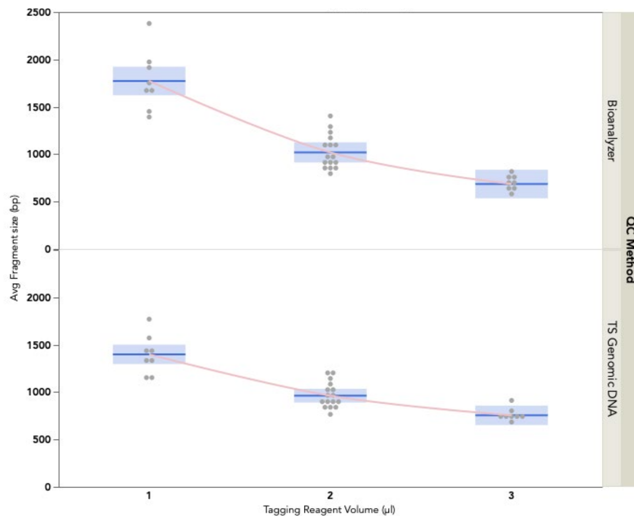


Figure 4B. A box-and-whisker plot shows tagged DNA fragment size decreases as tagging reagent used increases in the reaction for both QC methods: Agilent Bioanalyzer (top) and Agilent Tapestation (bottom).

standard protocols. For each set of samples processed, tagged DNA fragment sizes were evaluated on an Agilent DNA Bioanalyzer Chip and an Agilent Tapestation using the genomic DNA ScreenTape following the manufacturer's instructions. Each Tagify reagent volume point was run in duplicate. The libraries were then sequenced on MiSeq v3 150 cycle kit (Table 3).

The increase in the volume of Tagify i5 UMI reagent used during tagmentation (the simultaneous fragmenting and tagging of DNA) resulted in a decreased median insert size from 281 nucleotides to 196 nucleotides (Table 3). The average inferred UMI entropy and the average UMI percent duplication remained consistent regardless of the volume change.

Conclusions

Tagify i5 UMI Adapter-loaded Transposase Reagents are ready-to-use reagents designed to help researchers quickly fragment and add a UMI tag gDNA via loaded Tn5 transposase in a simple, streamlined workflow. By providing a consistent source of reliable, fully QC'd reagents, researchers can seamlessly integrate UMI-tagged DNA fragment libraries into downstream applications. The individual use of these reagents within a range of applications is possible, however, the desired size of the fragmented and tagged DNA may require adjustments. This Technical Note demonstrates the flexibility of adjusting UMI-tagged DNA fragment size by modifying total mass DNA input or the volume of tagging reagent used in the reaction. Such protocol optimization is encouraged to maximize the utility of these reagents within specific assays.

Tagging Reagent Used	Number of samples	Average PF Cluster	Average Median Insert Size	Average Observed Unique UMI	Average Inferred Unique UMI	Average Inferred UMI Entropy	Average UMI Percent Duplication
1 µl	2	1,972,802	281 nt	548,108	557,384	9.36	0.12%
2 µl	4	2,516,447	236 nt	585,878	584,871	9.39	0.14%
3 µl	2	1,972,157	196 nt	550,033	545,689	9.36	0.13%

Table 3. Performance summary of 8 libraries using varying volumes of Tagify i5 UMI Reagents on MiSeq v3 150 cycle.

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