Tagify™ TnX™ Universal Adapter Control Assay

Introduction

The Tagify TnX Universal Adapter reagent is designed to catalyze the reaction to fragment and tag DNA with an oligonucleotide payload via TnX transposase (Figure 1).

1 unit of loaded transposase has been optimized to fragment 100 ng of ***E. coli*** genomic DNA (gDNA) to an average size of 650 – 850 bps as measured by Agilent TapeStation HSD5000 (Figure 2), while simultaneously tagging the resulting fragments with Illumina-compatible P5 & P7 priming sequences.

Tagged DNA can then be seamlessly incorporated as part of a variety of assays and applications for NGS sequencing. This Technical Note outlines the control experiment from the Certificate of Assurance (CoA) to be used as a baseline assay for verifying the activity of the reagent upon receipt, and as a guideline for use of this reagent in their assay-specific protocols.

Adapter sequence (P5/R1):

*5′-**TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG***

Adapter sequence (P7/R2):

*5′-**GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG***

Figure 1. Molecular diagram of Tagify TnX Universal Adapter.

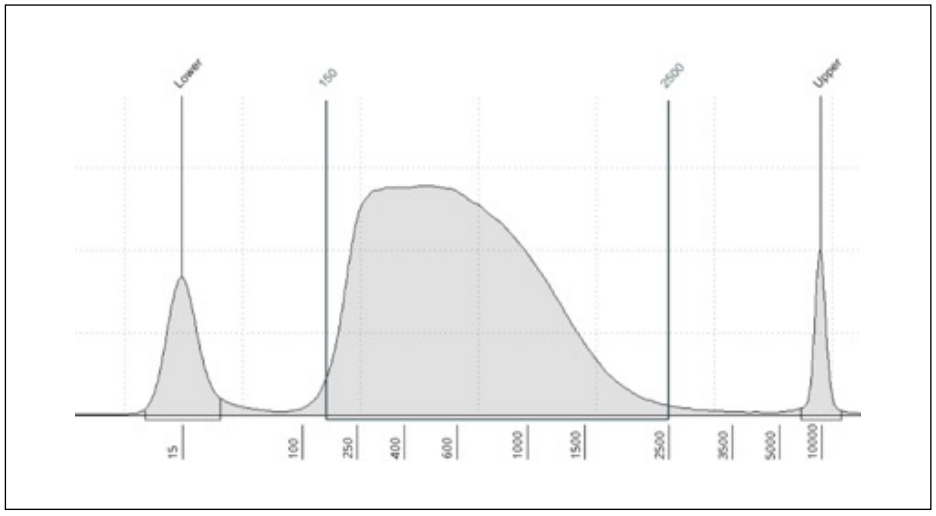


Figure 2. The fragment analysis trace generated by an Agilent TapeStation HSD5000 shows a trace of tagged DNA by using 1 unit of Tagify TnX Universal Adapter from 100 ng of ***E. coli*** genomic DNA. The region analysis used is 150 – 2500 bp with the average size being 687 bp.

TnX Transposase

Tagmentation-based chemistries primarily use Tn5, or a hyperactive form of Tn5, to fragment and tag DNA for a variety of NGS assays. Tn5 tagmentation is helpful in its ability to skip the need for a mechanical / enzymatic fragmentation step, an end-repair step, and a ligation step. However, Tn5 also has a well-characterized insertion bias that may make it unsuitable for some NGS assays (Figure 3).

The TnX transposase was specifically designed for NGS assays, over many rounds of directed evolution. It was derived from a synthetic transposase and contains the same payload format as Tn5, making it a suitable drop in for Tn5-based assays. The benefits of TnX over

traditional Tn5 are a significantly reduced insertion bias (Figure 3), along with higher activity and improved robustness. For many assays, Tn5 will be able to be directly replaced with TnX, loaded with a similar sequence payload, and may only require a titration assay to determine the comparable activity for a given application.

The following pages outline seqWell's control activity assay, and the materials required to run the assay.

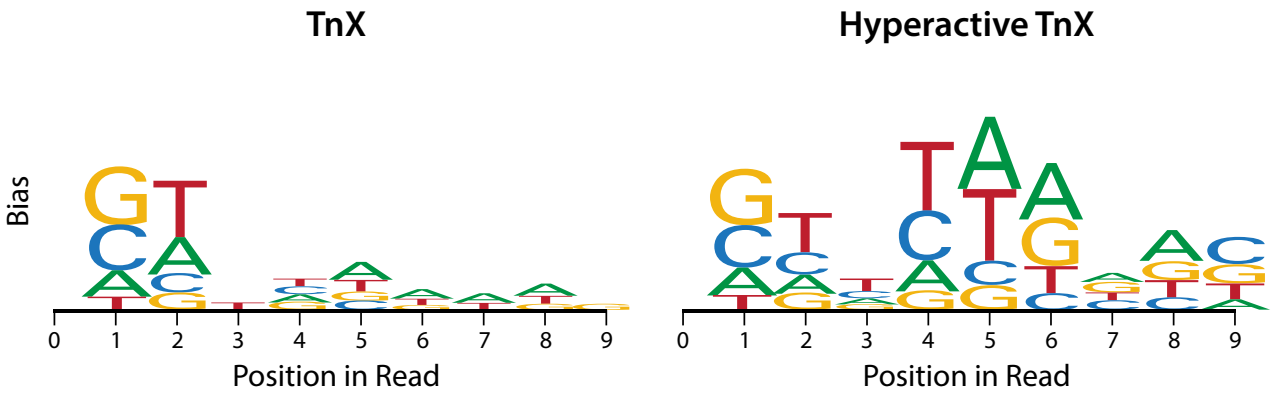


Figure 3. Insertion sequence bias of TnX (left) and Tn5 (right) for the first 9 bases.

Table 1. Reagent Components

Reference	Component	Description	Storage
301506	Tagify TnX Universal Adapter	10 units in a 2 mL tube	-20°C
301507	Tagify TnX Universal Adapter	40 units in a 2 mL tube	-20°C
301511	Tagify TnX Universal Adapter	Bulk format	-20°C

Table 2. Available Ancillary Reagents

Reference	Component	Description	Storage	*Control Assay: 1 unit
300145	Modified Tn5 Storage Buffer	For diluting transposase reagent	Ambient	N/A
101284	3X Coding Buffer	Tagmentation reaction buffer	Ambient	16.7 µL
101285	X Solution	Buffer for denaturation and removal of transposase	Ambient	25 µL
101003	MAGWise Paramagnetic Beads	Purification beads	4°C	90 µL

*The volume of each ancillary reagent required to replicate the control assay with 1 unit of reagent

Thermal Cycler Programs (all with lid heating on to 105°C)

- **TAG**: 55°C for 5 minutes; 25°C hold.
- **STOP**: 68°C for 10 minutes; 25°C hold.

Control Assay

The following control assay outlines the use of 1 unit of the Tagify TnX Universal Adapter reagent to tagment 100 ng of high-quality gDNA to 650 – 850 bps. DNA input amounts, volumes required, and incubation times/temperatures may require optimization depending on application.

1. Tagging Reaction

a. Pulse spin the Tagify TnX Universal Adapter reagent in a centrifuge.

b. To a new tube labeled REACTION TUBE, set up the tagging reaction by adding the following, in order, mixing after each addition by pipette up and down (≥10x at the transfer volume) slowly.

Reagent	Example
Tagify TnX Universal Adapter	1 unit
100 ng Genomic DNA in 10 mM Tris HCl, pH 8.0	28.3 µL
3X Coding Buffer	16.7 µL
Total Volume	50 µL

NOTE: The 3X Coding Buffer is viscous; pipette carefully to avoid introducing excessive bubbles. Add 3X Coding Buffer last to prevent premature DNA condensation.

c. Close the REACTION TUBE, gently vortex the tube, pulse spin, and transfer the REACTION TUBE to a thermal cycler and run the TAG program below, with lid heating on:

55°C for 5 minutes

25°C hold

d. Once the program is complete, proceed directly to the next step.

2. Stop Reaction

a. Pulse spin the REACTION TUBE in a centrifuge and carefully open the tube.

b. To the TUBE, add the following volume of X Solution to each well in use. Mix thoroughly and slowly by pipetting up and down (10 times at X Solution volume), being careful not to introduce excessive bubbles.

Reagent	Example
X Solution	25 µL
Total Volume	75 µL

NOTE: This solution contains SDS and vigorous mixing will cause it to foam. Pipetting slowly and under the surface of the solution will give the best results.

c. Securely reseal and pulse spin the REACTION TUBE, then transfer to a thermal cycler and run the STOP program, below, with lid heating on:

68°C for 10 minutes

25°C hold

d. Once the program is complete, proceed directly to the next step.

3. Tagged DNA Purification

a. Vortex room temperature MAGWise Paramagnetic Beads (MAGWise) to ensure that the beads are fully resuspended.

b. Remove the sample(s) from the thermocycler, pulse spin the REACTION TUBE, and carefully open the tube.

c. Add 90 µL of MAGWise Paramagnetic Beads, for a 1.2X bead ratio*, to each sample and mix thoroughly by pipetting up and down 10 times. Incubate on-bench for ≥5 minutes to allow DNA to bind.

NOTE: *MAGWise Beads volume ratio can be changed based on application to target different size selections.

d. Place the REACTION TUBE on a magnetic stand and let the beads pellet completely (≥2 minutes). A bead pellet should form on the inner walls of each tube or well and the supernatant should be visibly clear.

e. Remove and discard supernatant with a pipette. Be careful not to disturb the pellet.

f. Wash beads with 80% ethanol.

i. With the REACTION TUBE still on the magnetic stand, add 200 µl of freshly prepared 80% ethanol to each well without disturbing the beads.

ii. After ≥30 seconds, remove and discard the supernatant being careful not to dislodge the bead pellet.

DO NOT air dry bead pellets or DNA recovery may be compromised.

iii. Repeat the previous steps (Steps 3f.i and 3f.ii) for a total of 2 washes with 80% ethanol. Remove and discard the supernatant.

iv. Cap the REACTION TUBE and remove from magnetic stand; pulse spin and return to magnetic stand to let the beads pellet again (<30 seconds). **DO NOT** air dry bead pellets. Remove any residual ethanol at the bottom of the tube.

Proceed immediately to the next steps through Tris addition.

g. Add 20µl* of 10 mM Tris-HCl, pH 8.0 to each sample. Remove the REACTION TUBE from the magnetic stand and pipette the solution along the inner wall of the tube or multiple times to thoroughly resuspend the bead pellet.

NOTE: *Product elution volume is dependent on application and desired product concentration.

h. Incubate at room temperature for ≥5 minutes to elute the purified DNA off the beads.

i. Return the tube to the magnetic stand and allow beads to pellet on the inner walls of the wells (~2 minutes). DO **DO NOT** air-dry bead pellets or DNA recovery may be compromised.

j. When the supernatant has completely cleared, carefully transfer 18µl* of DNA eluate from each sample to a fresh tube. The transferred supernatant contains the purified, tagged DNA product.

NOTE: *Product elution volume is dependent on application and desired product concentration.

SAFE STOPPING POINT

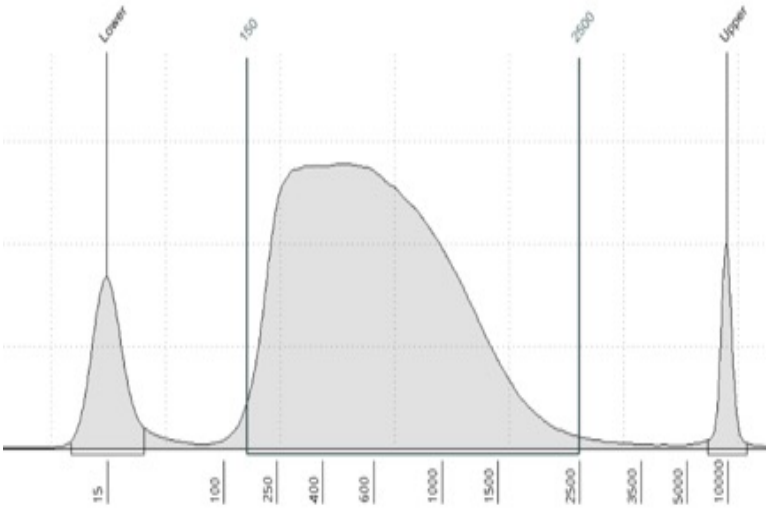
Proceed immediately with Tagged Product QC or store purified Tagged Product at -20°C.

Tagged DNA QC & Quantification

For each set of samples processed, check tagged DNA quality on an Agilent DNA Bioanalyzer Chip or Agilent TapeStation using the genomic DNA ScreenTape.

Evaluate tagged DNA fragment sizes on an Agilent TapeStation HSD5000, following the manufacturer's instructions.

For 1 unit of Tagify TnX Universal Adapter with 100 ng high-quality gDNA the average fragment size on the Agilent TapeStation HSD5000 should be between 650 - 850 bps (using a region analysis of 150-2500 bp).



www.seqwell.com