Tagify™ i5 UMI Adapter-loaded Transposase 24/96 Reagents

(Tagify™ i5 UMI 24 Reagent Set, Cat No. 301230)
(Tagify™ i5 UMI 96 Reagent Set, Cat No. 301210)

User Guide

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Introduction

seqWell's Tagify™ i5 UMI Adapter-loaded Transposase Reagents are designed to catalyze the reaction to fragment and tag DNA with an oligonucleotide payload via Tn5 transposase. Specifically, these reagents deliver oligos that consist of full-length, Illumina-compatible P5/i5/UMI/R1 priming sequences that also contain a 10-base barcode and a 10-base unique molecular identifier (UMI) region. These reagents may be incorporated as part of targeted sequencing assays, such as UDiTaS[1] or RGen-Seq[2] applications, CRISPR QC, and Cell and Gene Engineering QC. The product is provided as either 24 or 96 different barcoded UMI reagents. This user guide describes the general use of the reagents and is not intended to serve as a full protocol for a specific library preparation method. The individual user is advised to review their application[3] and modify as required.

1. UDiTaS Method: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5861650/
2. RGEN-Seq Method: https://pubmed.ncbi.nlm.nih.gov/34880355/
3. Commercial use of these reagents may require a license from a third party.

Table 1. Components of the Tagify i5 UMI 24 Reagent Set, Cat No. 301230

Reference	Component	Description	Storage	Qty
301231	**Tagify i5 UMI 24 Reagent Plate**	**Fully skirted 96-well PCR plate**	**-20°C**	**1**
101284	**3X Coding Buffer**	**2 ml tube, white cap**	**Ambient**	**1**
101285	**X Solution**	**2 ml tube, black cap**	**Ambient**	**2**
101003	**MAGWise Paramagnetic Beads**	**Bottle containing 5ml**	**4°C**	**1**

Table 2. Components of the Tagify i5 UMI 96 Reagent Set, Cat No. 301210

Reference	Component	Description	Storage	Qty
301211	**Tagify i5 UMI 96 Reagent Plate**	**Fully skirted 96-well PCR plate**	**-20°C**	**1**
101284	**3X Coding Buffer**	**2 ml tube, white cap**	**Ambient**	**2**
101285	**X Solution**	**2 ml tube, black cap**	**Ambient**	**3**
101003	**MAGWise Paramagnetic Beads**	**Bottle containing 5ml**	**4°C**	**1**

User-Supplied Reagents, Equipment, & Consumables

Reagents

- Genomic DNA
- 80% Ethanol (freshly prepared)
- Tris-HCl, pH 8.0

Equipment & Consumables

- Single-channel pipettors (1-20 µl, 20-200 µl, 100-1,000 µl)
- Multi-channel pipettors (1-10 µl, 10-200 µl)
- Pipette tips (low-retention barrier tips)
- Eppendorf Tubes® (1.5 ml & 2.0 ml, DNA LoBind® Tubes)
- 0.2 ml PCR 8-tube strips and caps
- Magnetic stand or magnetic plate for paramagnetic bead separation
- 96-well thermal cycler (compatible with fully-skirted PCR plates and 8-tube PCR strips)
- Benchtop centrifuge to pulse-spin tubes and 8-tube PCR strips
- Plate centrifuge
- PCR plate seals (must be evaporation-resistant)
- Vortex mixer
- Agilent Bioanalyzer or Agilent TapeStation using Genomic DNA ScreenTape

Thermal Cycler Programs (all with lid-heating on)

- TAG: 55°C for 15 minutes; 25°C hold.
- STOP: 68°C for 10 min; 25°C hold.

Before Starting Procedure:

Pulse-spin kit components. Liquids can condense or shift locations inside containers during shipment or storage. Before dispensing from reagent tubes, pulse-spin in a suitable centrifuge to gather the reagents at the bottom of the tube. If the kit components freeze during shipment or storage, thaw, mix and pulse-spin before use.

Equilibrate MAGWise Paramagnetic Beads to room temperature. Warm MAGWise beads to room temperature for 30 minutes before use. Vortex to thoroughly resuspend beads prior to use. To transfer volumes accurately, pipette slowly and do not pre-wet pipette tips.

Prepare 80% ethanol fresh daily. Volume required will depend on the number of samples processed.

Prepare 10 mM Tris-HCl, pH 8.0. Prepare 10 mM Tris-HCl, pH 8.0 from a concentrated stock solution diluted with ultrapure water (molecular-biology grade). Do not use EDTA-containing solutions (e.g., TE).

Procedure: Tagging Protocol

This protocol describes a general use of the Tagify i5 UMI Reagents. As described, the protocol is designed to fragment 50 ng of genomic DNA to an average size of 0.8 – 2 kb (as measured by Agilent Bioanalzyer). Simultaneous to fragmentation, the DNA is tagged with an 82 base sequence that contains an Illumina-compatible P5/i5/UMI/R1 sequence composed of a 10 base i5 barcode and a 10 base UMI sequence (see Appendix A for more information). Individual use, including DNA input amounts, volumes required, and incubation times/temperatures may require optimization depending on application.

The Tagify i5 UMI 24/96 Reagents are supplied in a format that allows for use of anywhere from 1-96 reactions at a time. Each well of the plate is designed for single use, but remaining reagents can be preserved for future use by sealing wells required at the time of your experiment.

1. Tagging Reaction

If preparing libraries from fewer than 96 samples, please refer to subsection 1g below prior to starting:

a. Pulse-spin the Tagify i5 UMI Reagent plate in a centrifuge. Remove heat seal carefully to avoid splashing/contamination of reagents.
b. Add 5.0 µl of 10mM Tris-HCl, pH 8.0 to each well being used. Mix thoroughly and slowly by pipetting up and down (10 times at 5 µl) to avoid introducing bubbles.
c. After mixing, transfer 5.3 µl of the buffer/Tagify i5 UMI Reagent mixture to a new 8-tube PCR strip(s) or plate labeled REACTION TUBE or PLATE.
d. To each well being used in the REACTION TUBE or PLATE, add the following, **in order**, mix after each addition by pipette up and down slowly:

Table 3. Reagents to be added to the buffer/Tagify i5 UMI Reagent mixture

Reagent	Volume
Genomic DNA (50 ng)	8.0 µl
3X Coding Buffer	6.7 µl
Total Volume	20.0 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.

e. Close the REACTION TUBE or heat seal REACTION PLATE. Gently vortex the tubes or plate to mix and pulse spin.

f. Transfer the REACTION TUBE or PLATE to a thermal cycler and run the TAG program below, with lid-heating on:

55°C for 15 minutes

20°C hold

Special instructions for preparing libraries in batches of fewer than 96 samples:

g. Using a scalpel or razor blade, only open and peel the heat seal from the wells of the Tagify i5 UMI Reagent Plate corresponding to the total number of samples that will be processed.

h. Follow the instructions for reaction setup in the Tagify i5 UMI Reagent Plate above (steps 1a – 1c). After mixing the Tagify i5 UMI Reagents and the Tris-HCL buffer together in the plate, transfer all the contents to a clean 8-tube PCR strip(s) or a clean PCR plate labeled REACTION TUBE or PLATE.

i. After verifying that the seals on the unused portion of the Tagify i5 UMI Reagent Plate are still intact, cover the used wells to prevent contamination of the unused reagents when the plates are handled again. Then return the sealed/resealed plates to the freezer for later use.

j. Continue following the instructions for steps 1d – 1f to the REACTION TUBE or PLATE.

2. Stop Reaction

a. Pulse-spin the REACTION TUBE or PLATE in a centrifuge and carefully open the tube or remove the seal.

b. Add 10 µl X Solution to each well being used in the REACTION TUBE or PLATE. Mix thoroughly and slowly by pipetting up and down (10 times at 10 µl), being careful not to introduce excessive bubbles.

***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 or PLATE, then transfer to a thermal cycler and run the STOP program, below, with lid heating on:

68°C for 10 minutes

20°C hold

3. Tagged DNA Purification

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

b. Remove the samples from the thermocycler, pulse spin the REACTION TUBE or PLATE.

c. Add 30 µl (1X equivalent) of **MAGWise Paramagnetic Beads** to each sample. Fully expel tips to ensure a complete dispense and mix thoroughly.

d. Incubate on-bench for ≥5 minutes to allow DNA to bind.

e. Place the REACTION TUBE or PLATE on a magnet 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.

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

g. Wash beads with 80% ethanol.
   i. With the REACTION TUBE or PLATE on the magnetic stand or plate, add 200 µl of freshly prepared 80% ethanol to each well without disturbing the beads.
   ii. After ≥30 seconds, remove and discard the supernatant, without disturbing the bead pellet. **DO NOT** air dry bead pellets or DNA recovery may be compromised.
   iii. Repeat the previous steps (Steps 3g.i and 3g.ii) for a total of 2 washes with 80% ethanol. Remove and discard the supernatant.
   iv. Cap the REACTION TUBE or PLATE and remove from magnet; pulse spin and return to magnet 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.**

h. Add 20 µl* of 10 mM Tris to each sample. Remove the REACTION TUBE or PLATE from the magnet and pipette the solution along the inner wall of the tubes or wells multiple times to thoroughly resuspend the bead pellet.

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

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

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

k. When the supernatant has completely cleared, carefully transfer 18 µl* 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.

DNA QC and 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 and its quantity on PicoGreen™ DNA Assay or similar assay.

a. Quantify tagged DNA from samples using a PicoGreen DNA Assay (recommended) or similar assay.

b. Evaluate tagged DNA fragment sizes on a Agilent DNA Bioanalyzer Chip (recommended) or Agilent TapeStation using the genomic DNA ScreenTape following the manufacturer's instructions. Example traces are provided below:

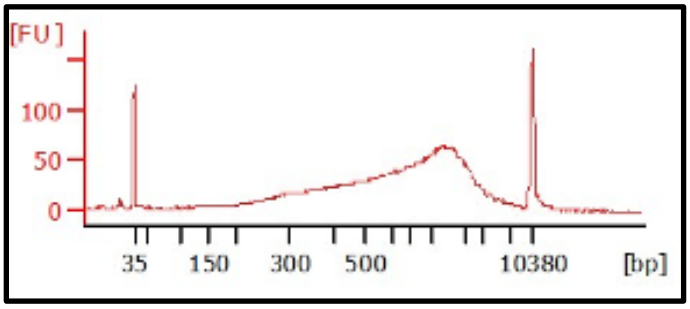

Figure 1. Example tagged DNA trace from an Agilent Bioanalyzer High Sensitivity DNA Kit.

For the example above, the region analysis used is 200 - 7500 bp with the average size being 1116 bp.

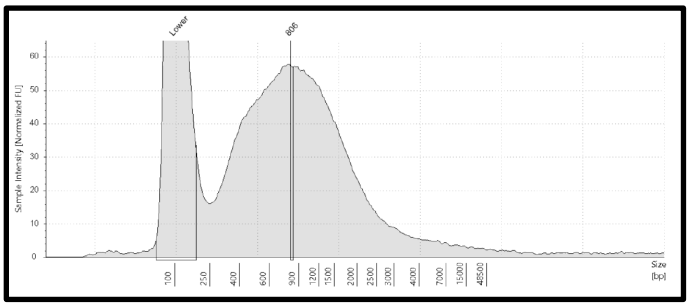

Figure 2. Example tagged DNA trace from an Agilent TapeStation using genomic DNA ScreenTape.

For the example above, the region analysis used is 200 - 7500 bp with the average size being 1124 bp.

***NOTE*:** Due to the nature of the interim tagged product that is not a complete NGS library, sizing data and traces obtained from other methods, such as Agilent TapeStation HS D5000 ScreenTape or Femto Pulse instruments, will vary. To note, the sizing range for Agilent Bioanalyzer and TapeStation HS D5000 ScreenTape may be the reason for the size discrepancy in the two assays. The sizing range for the Bioanalyzer HS DNA kit is 50-7000 bp while the Tapestation HS D5000 ScreenTape is 100-5000 bp. The tagged DNA has fragments between 5000-7000 bp which are not

taken into account on the Tapestation D5000 ScreenTape, thus using the genomic DNA ScreenTape will be the comparable assay to the recommended Bioanalyzer assay.

Please contact support@seqwell.com for more information or for sizing optimization recommendations.

SAFE STOPPING POINT

Proceed immediately with downstream application protocols or store purified tagmented DNA at -20°C .

Appendix A: Tagify i5 UMI Sequences

For each Tagify i5 UMI sequence, the structure of the inserted sequence is as follows:

5'-AATGATACGGCGACCACCGAGATCTACACTXXXXXXXXXXNNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3', where the blue X's indicate the 10bp barcode and the green N's indicate the UMI sequence.

For the 96 different sequences, the i5 barcode is listed below:

Plate Layout

i5 indices for Tagify UMI Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	GTAACACAGA	CAAGAGCGTG	ATCTCCACGG	TTACTGCTGC	CCGAGGTTAG	CTGATTAGGA	TGGAGCGATG	TCATACGTTC	TTGTCCTTCC	TCTCAACAAG	CGTGATACTT	CACACAGTCG
B	TAAGTTGTGG	AATACTCGGC	CTCGCTGCTT	TTGTTCTGCG	CCGTAATCGA	CAATGCGGAG	TTCGGTGCAT	TTGTGTCCGA	GGAGTTCTAT	TCGCATGACC	TACAATCCGG	TACCATCTAG
C	CACCTACCTC	GTATCTTAGG	CTCAGTAGAC	TCGCGGACAT	CCATTCACTT	ATTCCGCTTA	CCTCTGAACA	CTTATCCAGG	ATCTTAACCG	TACGAAGCAA	TAACCATTCG	TTGTAGCATC
D	ATATCAGTCC	TGGTCTGAGG	CTTGGTCCTT	ATCGAATTGC	TCCGACTACC	CTGACAACGG	TCCACCATAG	CGGTAACTCC	GGAGCTTGTT	CCAACGCTTA	TGCTACTGTT	TTCTTCCGTA
E	GGTTAAGTAG	CAAGGACATT	CGTCCAAGAG	TGTAGGATAG	TAGAACCTGA	GCATGTTAAG	CAAGCATGAA	TAGGACCATG	GTTACAGGTT	CAGTTAGCTC	CGTCAATTCG	TCTAGCGTTG
F	TGGTGGATAT	CGGTTGAGAA	GTATGGAACA	ATGTTGCTGA	ATATGGTGCA	TCGAGAAGCG	TCAGTTGGTT	CACAATTACG	CAACCAACAT	GTATGCTAAC	TTCACGTTGC	TCTAAGTCTG
G	TAGCTGGCAC	TACCTCGCTA	AATCGGTACG	TGAGGATGAA	TTGAGTATGG	TGCAGAGTGA	CCATTATACC	AACGCGAATA	ATGTTCCGTT	GTTGAGGCAC	GGCTTGAATT	CTCATATCCA
H	TTACCGTTGG	TAAGCTCGTT	CTAATGTCGA	TGTCTTCCAA	ATTCTGGACA	TACACAACCG	AAGTGCAAGG	TCACCAGTAT	CGAGGAACAG	AACAGAACGG	GTTCCTTCTT	TGGAGGTATA

For ease of use, please refer to the Tagify i5 UMI 96 Index List that can be found online at www.seqwell.com/resources

Appendix B: Incorporation of the Tagify i5 UMI Reagents into the UDiTaS™ method

The UDiTaS™ method, as published by Giannoukos, et al (see https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5861650/), is a well-known protocol for detecting indels and genome rearrangements that may occur during genome editing experiments. Below is a guide for substituting the Tagify i5 UMI reagents into the relevant areas of the protocol.

- Section 3:
    - Skip this step. Use of the Tagify i5 UMI reagents no longer requires oligo annealing.
- Section 4:
    - Skip this step. Use of the Tagify i5 UMI reagents no longer requires transposome assembly.
- Section 5:
    - Replace this section with the protocol described on pages 5 – 7 of this user guide.
    - *NOTE*: Tagged product elution volume for the UDiTaS™ method is 11 µl.
- Sections 6 – 13:
    - Proceed with the remainder of the protocol as specified in the UDiTaS™ method.

Revision History

Version	Release Date	Prior Version	Description of changes
20231204	20231204	NA	First Version
20230521	20230521	20231204	Early Access
20240618	20240624	20230521	Version 1.0

Technical Assistance

For technical assistance, contact seqWell Technical Support.
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