Tagify™ UMI Reagents
Early Access Version
(Part No. 301200)

User Guide

v20230531

Introduction

seqWell's Tagify™ UMI 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/R1 priming sequences that also contain an 8-base barcode and a 10-base unique molecular identifier (UMI) region. These reagents may be incorporated as part of targeted sequencing assays, UDiTaS[1] or RGen-Seq[2] applications, CRISPR QC, and Cell and Gene Engineering QC. Eight different barcoded UMI reagents are included in the product. 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 in sequencing activities may require a license from a third party.

Table 1. Components of the Tagify UMI Reagent Set, Early Access

Reference	Component	P/N	Description	Storage	Qty
301200	Tagify UMI N501 Reagent	UMI501	0.5 ml tube, green cap, 10 µl	-20°C	1
	Tagify UMI N502 Reagent	UMI502	0.5 ml tube, green cap, 10 µl	-20°C	1
	Tagify UMI N503 Reagent	UMI503	0.5 ml tube, green cap, 10 µl	-20°C	1
	Tagify UMI N504 Reagent	UMI504	0.5 ml tube, green cap, 10 µl	-20°C	1
	Tagify UMI N505 Reagent	UMI505	0.5 ml tube, green cap, 10 µl	-20°C	1
	Tagify UMI N506 Reagent	UMI506	0.5 ml tube, green cap, 10 µl	-20°C	1
	Tagify UMI N507 Reagent	UMI507	0.5 ml tube, green cap, 10 µl	-20°C	1
	Tagify UMI N508 Reagent	UMI508	0.5 ml tube, green cap, 10 µl	-20°C	1

Note: seqWell's 3X Coding Buffer (101000), X Solution (101001) and MAGWise Paramagnetic Beads (101002 or 101003) are required to successfully use the Tagify UMI Reagents. These products are sold separately.

User-Supplied Reagents, Equipment, & Consumables

Reagents

- Genomic DNA
- 80% Ethanol (freshly prepared)
- Tris-HCl, pH 8.0
- 3X Coding Buffer (seqWell 101000)
- X Solution (seqWell 101001)
- MAGwise paramagnetic beads (seqWell 101002 or 101003)

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), or
- 0.2 ml PCR 8-tube strips and caps
- Magnetic stand for paramagnetic bead separation
- Vortex mixer
- Agilent TapeStation® or similar instrument for analysis of NGS libraries

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 UMI Reagents. Individual use, including DNA input amounts, volumes required, and incubation times/temperatures may require optimization depending on application.

1. Tagging Reaction

a. Add reagents, in order, to a PCR tube in the volumes and concentrations specified in the table below:

Table 2. Tagging Reaction Components

Reagent	Volume
Genomic DNA (50ng total, average 6.25ng/µl)	8.0 µl
10mM Tris-HCL, pH8.0	3.3 µl
Tagify UMI Reagent	2.0 µl
3X Coding Buffer	6.7 µl

Notes:

- Add 3X Coding buffer last to prevent premature DNA condensation.
- Reaction components can be scaled proportionally to accommodate more DNA input.
- Volume of Tris-HCL may be adjusted to accommodate larger or smaller volumes of genomic DNA.

b. Incubate samples for 15 min at 55°C. Hold at 20°C until proceeding to next step.

2. Stop Reaction

a. Add 10 µl X Solution to each sample.

b. Incubate samples for 10min at 68°C. Hold at 20°C until proceeding to next step.

3. Tagged DNA Purification

Note: DO NOT allow bead pellets to air dry.

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 or storage, pulse spin the tubes.

c. Add 30 µl (1X equivalent) of **MAGwise Paramagnetic Beads** to each sample using a pipette. Flush tips to ensure a complete dispense. Mix thoroughly.

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

e. Place the sample tubes on a magnet and let the beads settle completely, about 2 minutes. A bead pellet should form on the inner walls of each tube 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 sample tube 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 supernatant, without disturbing the bead pellet. **DO NOT** air dry bead pellet or DNA recovery may be compromised.
   iii. Repeat the previous step for a total of 2 washes with 80% ethanol. Remove and discard supernatant.
   iv. Cap tube, remove from magnet, pulse spin and return to magnet, letting beads settle (<30 seconds). Remove any residual ethanol at the bottom of the tube. Proceed immediately to the next steps through Tris addition.

h. Add 30 µl of 10 mM Tris to each sample. Remove the tubes from the magnetic stand and pipette the solution along the inner wall of the tubes multiple times to thoroughly resuspend the bead pellet.

i. Incubate at room temperature for at least 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).

k. When the supernatant has completely cleared, carefully transfer 28 µl of DNA eluate from each sample to a fresh tube. The transferred supernatant contains the purified, tagged DNA product.

SAFE STOPPING POINT

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

4. DNA QC and Quantification

For each set of samples processed, check tagged DNA quality and quantity of the samples on a High Sensitivity D5000 Agilent DNA ScreenTape or similar assay and PicoGreen DNA Assay, respectively.

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

b. Evaluate DNA from samples on a High Sensitivity D5000 Agilent DNA ScreenTape or similar assay following the manufacturer's instructions. Example traces are provided below:

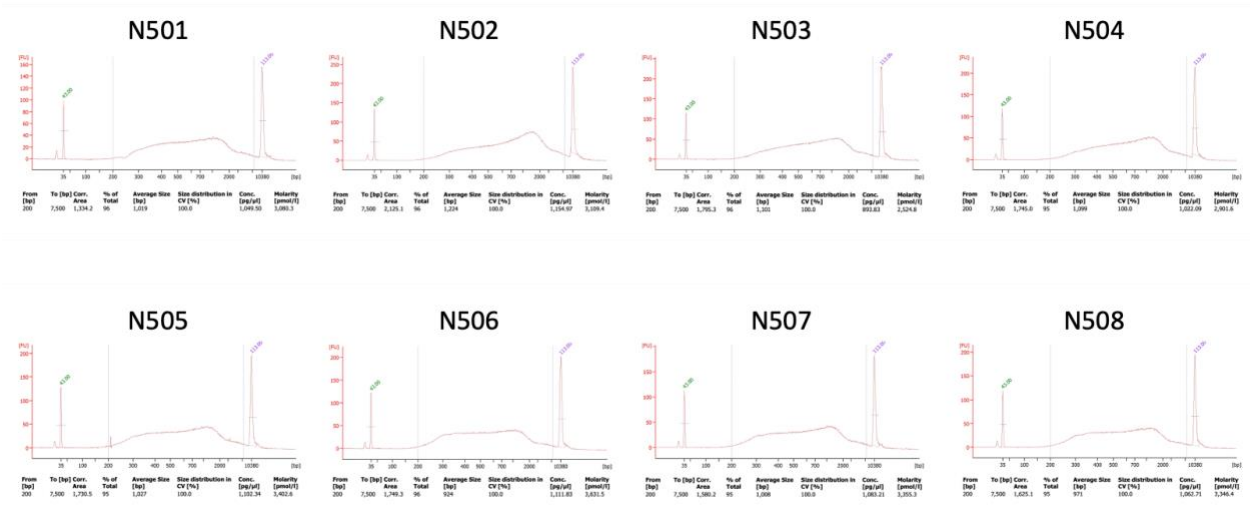

Figure 1. Example tagged DNA traces from an Agilent BioAnalyzer.

SAFE STOPPING POINT

Proceed immediately with Library Preparation or store purified DNA at -20°C.

Utilization of Tagged DNA

The final product of the Tagify UMI Reagent protocol is fragmented DNA containing one of the following sequences at the ends:

Tagify Reagent	Sequence
N501	AATGATACGGCGACCACCGAGATCTACACTAGATCGCNNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
N502	AATGATACGGCGACCACCGAGATCTACACCTCTCTATNNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
N503	AATGATACGGCGACCACCGAGATCTACACTATCCTCTNNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
N504	AATGATACGGCGACCACCGAGATCTACACAGAGTAGANNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
N505	AATGATACGGCGACCACCGAGATCTACACGTAAGGAGNNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
N506	AATGATACGGCGACCACCGAGATCTACACACTGCATANNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
N507	AATGATACGGCGACCACCGAGATCTACACAAGGAGTANNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
N508	AATGATACGGCGACCACCGAGATCTACACCTAAGCCTNNNNNNNNNNTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG

Blue text = 8bp barcode

Green text = 10bp UMI sequence

Appendix A: Incorporation of the Tagify 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 UMI reagents into the relevant areas of the protocol.

- Section 3:
  - Skip this step. Use of the Tagify UMI reagents no longer requires oligo annealing.
- Section 4:
  - Skip this step. Use of the Tagify UMI reagents no longer requires transposome assembly.
- Section 5:
  - Replace this section with the protocol described on pages 4 – 5 of this user guide.
- 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
20230531	May 31, 2023	NA	First Version

Technical Assistance

For technical assistance, contact seqWell Technical Support.
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