

## FAQs - Tagify™ i5 UMI Adapter-loaded Transposase

1. What is included in the Tagify i5 UMI reagent set?
  - A 96-well plate of Tn5s (each loaded with a UMI, p5, and i5), 3X Coding Buffer, Stop Solution, and MAGWise beads.
2. How long are the UMIs and the i5s included?
  - The UMI and i5 are 10bp each.
3. What downstream applications can be used after the Tagify i5 UMI reagent?
  - The Tagify i5 UMI reagent is designed for use in a variety of downstream applications that require the addition of a UMI. These reagents may be incorporated as part of targeted sequencing assays, such as UDiTaS<sup>1</sup> or RGen-Seq<sup>2</sup> applications, CRISPR QC, and Cell and Gene Engineering QC. However, commercial use of these reagents in sequencing activities may require a license from a third party.
4. Does the Tagify i5 UMI reagent generate a fully sequencable molecule?
  - The Tagify i5 UMI reagent generates a one-sided tagged fragment and will still need an additional barcode for sequencing.
5. How large of fragments does the Tagify i5 UMI reagent generate?
  - The Tagify i5 UMI reagent should generate 800 - 2000 bp fragments when following the protocol using 50 ng of high quality gDNA input.
6. How many times can each well of the reagent plate be used?
  - Each well in the plate contains enough reagent for 1 reaction.
7. Can less than 96 reactions be used at once?
  - The reagents have been tested for multiple freeze-thaw cycles without issue. To use less than 96 reactions of Tagify i5 UMI reagent, it is recommended to use a scalpel or razor blade to 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. Please refer to the user guide for more detailed instructions.

8. What assay should be used for QCing the tagged fragments?
- seqWell uses the Agilent High Sensitivity DNA Bioanalyzer Chip to verify fragment sizing. 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.
9. What i7 should I use?
- Depending on the application, users may wish to create a unique dual index (UDI) strategy for demultiplexing final sequencing data. In this case, seqWell has validated a matching set of i7 index sequences that can be used in combination with the i5 indexes included in the Tagify i5 UMI 96 Reagent set. The i7 index sequences are listed below.

**Plate Layout**

		i7 indices to match to Tagify UMI 96 i5 indices											
		1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>		GTCAAGTCCA	CAACTAAGTC	ATAACCTGAC	CAGGTACTTC	AACCGAGCCA	CAACGTCATT	ATTGGTCAGA	ATGTTGCGGA	CACCAATAAC	TGTCCTCTTT	CGAAGGACTG	TAGTTATCGC
<b>B</b>		TATCTCTTCC	GTAAGGATT	TGCGGTTCCA	CAGGAATATG	TGGATTCAAG	AAGAACGATG	AAGACCTGTT	AATGCTAACC	GCGTCCACAA	CATGAGTAAC	TCTACCGTCA	TGCAGGTGAT
<b>C</b>		CCGCGAAGAA	CATCGGAGGA	AATACTTGCC	AACGCACAAT	CAACAGATAC	CTGCAATTAC	CGCTAATGAA	GTAACACGTA	ATGTGCGCTT	CTGCGCGAAT	GGTAATATCG	TGTGAAGCTA
<b>D</b>		CCTACTCGGA	ATTCTGATGG	AATCGCGGAA	ATTGAGAAGG	AAGGTAAGTC	CGCCGATGAT	CAGAGTGCAT	GATATACGGA	TATCTAGTGC	CTGTAGTATG	TGTGCGAGTT	CAACTCCTGA
<b>E</b>		TTCGTATCAC	TATCGTTACC	CAGAACGCGA	ATTGCACCTT	TGACAATACG	CGGACAAGAC	GSTAAGTGA	ATCCGAGAGG	GCCTACAATG	GAGCCGTACA	TTATCGCTGA	TGTAGCAACG
<b>F</b>		TCCTCCATCC	TGGTCTGTGA	TGAACCAAGG	AAGTGGATAC	GATTGTGCAT	CGTACTCCTC	GTATTTCAGTG	CAATTCACAC	AAGCTCAGTT	CATTCTTAGG	GGTTGAGTTC	CGGTAACGCA
<b>G</b>		ATAACATCGC	TGCCAACATG	TGTTAGTCAG	GATAAGATGC	GCACACCATT	TGCATGAGTT	GCGCTACGTT	TTGTCAGTTC	TACGCTTAGA	TGACCGACAA	ATTGGACGCC	GTAGCAGCAG
<b>H</b>		GATATGCGTT	TCATTACACG	CAGTAGGTAA	TACCTCGACA	TCGTTATTCC	AATCTGGAGC	TTGTCATAGC	ATACGCCATT	ATCCACTAGG	TGTGTAACCG	TATGTGTGTG	CTACAGCCGA

Please contact [support@seqwell.com](mailto:support@seqwell.com) for index list.

- UDiTaS Method: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5861650/>
- RGEN-Seq Method: <https://pubmed.ncbi.nlm.nih.gov/34880355/>