Tagify™ i5 UMI Adapter-loaded Transposase

Catalog numbers:

301230: Tagify i5 UMI 24 (1x24x3µL) Reagent Set

Custom dispense available upon request

User Guide

v20250606

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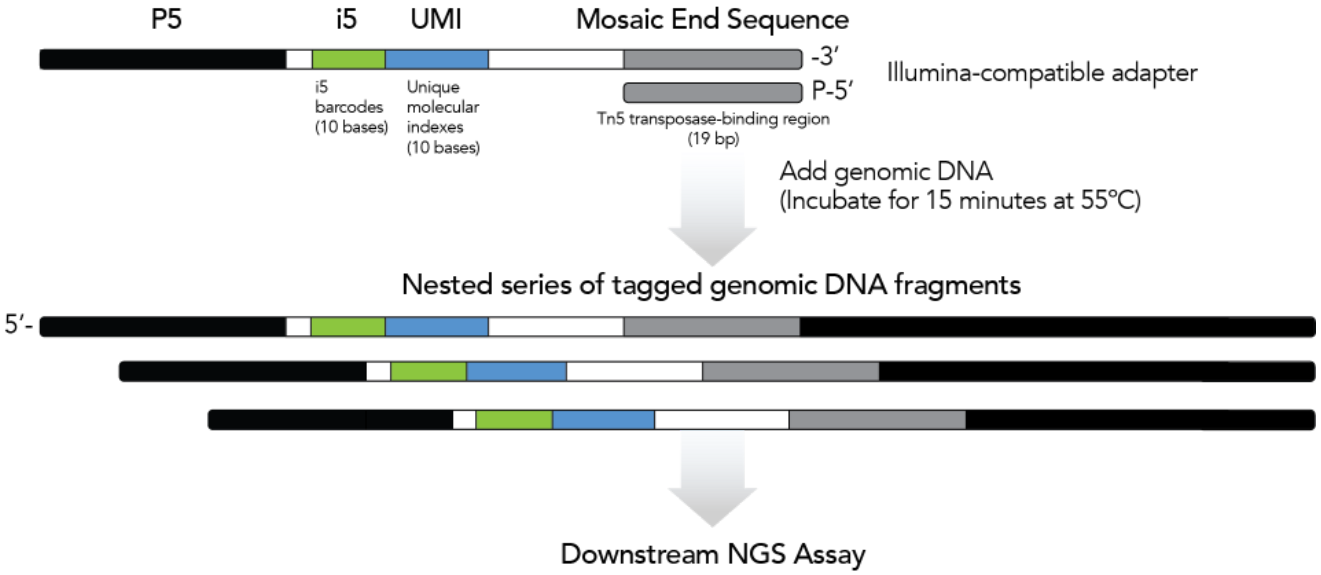
Introduction

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 (See Appendix A for more barcoding information). 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. 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.

The Tagify i5 UMI Adapter-loaded Transposase reagents can be used to prepare gDNA for a variety of targeted sequencing assays. An example would be tagging 50 ng of human gDNA to average fragment sizes of 0.8 – 2 kb (measured by Agilent Bioanalyzer). Then, a downstream targeted sequencing assay that utilizes two rounds of nested PCR can be employed. Standardly, up to 24 unique i5 UMI barcodes are supplied, pre-loaded, in the kit at a 3µL dispense, and can be combined with a variety of user supplied i7 barcodes for additional multiplexing. If additional unique i5 indexes or a different dispense volume is needed, please inquire to sales@seqwell.com.

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.

Tagify i5 UMI Adapter-loaded Transposase Molecular Diagram



Tagify i5 UMI Adapter-loaded Transposase Components

Table 1. Tagify i5 UMI 24 Reagent Set Components

Tagify i5 UMI 24 Reagent Set (1x24x3µL)

Catalog No.: 301230

Item	Component	REF	Description	Storage	Qty
1	Tagify i5 UMI 24 (1x24x3µL) Reagent Plate	301231	Fully skirted 96-well PCR plate, 24 reactions of 3µL each	-20° C	1
2	3X Coding Buffer	101284	2 ml tube, white cap	Ambient	1
3	X Solution	101285	2 ml tube, black cap	Ambient	2
4	MAGwise Paramagnetic Beads	101003	Bottle containing 5 ml of MAGwise beads	4° C	1

Note: Please reach out if a custom volume dispense of the Reagent Plate is required.

User-Supplied Reagents, Equipment, Consumables, and Thermal Cycler Programs

Required Reagents

- Genomic DNA
- 100% Ethanol (molecular biology grade)
- 10 mM Tris-HCl, pH 8.0
- Ultrapure Water (PCR grade)
- Qubit™ 1x dsDNA High Sensitivity (HS) Assay Kit (ThermoFisher P/N: Q33230), Quant-iT™ PicoGreen™ dsDNA Assay Kits (ThermoFisher P/N: P7589), or other validated dsDNA quantification assay
- Agilent High Sensitivity DNA Kit for the Bioanalyzer (Agilent P/N: 5067-4626) or Genomic DNA ScreenTape Assay for the TapeStation (Agilent P/N: 5067-5365)

Equipment & Consumables

- Single-channel pipettors (1-20 µl, 20-200 µl, 100-1,000 µl)
- Multichannel pipettors (1-10 µl, 10-200 µl)
- Pipette tips (low-retention barrier tips)
- Eppendorf Tubes® (1.5 ml and 2 ml, DNA LoBind® Tubes)
- PCR plate seals (must be evaporation-resistant)
- 96-well thermal cycler (compatible with fully skirted PCR Plates and 8-tube PCR strips)
- Magnetic stand for 8-tube PCR strip or 96-well plate
- 0.2 ml 8-tube PCR strips and caps/seals
- Benchtop centrifuge to pulse-spin tubes and 8-tube PCR strips
- Plate centrifuge
- Vortex mixer
- Agilent TapeStation or Agilent Bioanalyzer using Genomic DNA ScreenTape

Thermal Cycler Programs (all with lid-heating on at 105°C):

- **TAG:** 55°C for 15 minutes
  25°C hold

- **STOP:** 68°C for 10 minutes
  25°C hold

Before starting the procedure:

Review FAQs. FAQs can be found here: https://seqwell.com/resource-category/faqs/. Please review these prior to your first run.

Measure and adjust input DNA concentration. Assay the DNA concentration of each sample using Qubit, PicoGreen, or other validated dsDNA assay. Adjust the input DNA concentration for each application using 10 mM Tris-HCl, pH 8.0, if necessary. DO NOT use EDTA-containing solutions (e.g., TE buffer) to dissolve or dilute input DNA because EDTA can suppress enzymatic activity. Please refer to your downstream targeted assay for determining the appropriate DNA input.

Program thermal cycler. For convenience, set up all thermal cycler programs described in the protocol before starting.

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

Equilibrate MAGwise Paramagnetic Beads to room temperature. MAGwise beads can be stored for up to 2 weeks at room temperature or for longer periods at 2 - 8°C. If stored cold, warm at 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.

Check the X Solution for precipitate before use. If a precipitate is visible, incubate at 37°C for 5 minutes (or longer). Mix gently by inversion until the precipitate dissolves (**DO NOT** vortex).

> ***Note:*** X Solution contains SDS and will precipitate if stored below room temperature. Overly vigorous mixing will cause foaming.

Please note that the 3X Coding Buffer is viscous. Store 3X Coding Buffer at room temperature. To transfer volumes accurately, pipette slowly and do not pre-wet pipette. While adding 3X Coding Buffer to reactions, mix completely by slowly pipetting up and down several times with the same pipette tip(s) used for addition. Always change pipette tips before adding 3X Coding Buffer to different reactions.

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 (both molecular-biology grade). Do not use EDTA-containing solutions (e.g., TE).

Protocol

This protocol describes a general use of the Tagify i5 UMI Reagents. Individual use, including DNA input amounts, volumes required, and incubation times/temperatures may require optimization depending on application.

The Tagify i5 UMI Adapter-loaded Transposase reagents are supplied in a format that allows for use of anywhere from 1-total reactions in the kit. Each of the wells of the plate are designed for single use, but unused reagents can be preserved for future use by only unsealing wells required at the time of your experiment. See step 1e for more details.

This application is best suited for **high quality DNA (DIN 8.0 ≥ recommended)** eluted in 10mM Tris HCl, pH 8.0.

The Tagify i5 UMI reagent was optimized for fragmenting 50 ng of human gDNA with 2µL of stock Tagify i5 UMI tagging reagent to an average size of 0.8–2kb as measured by Agilent Bioanalyzer. This is referred to as the Standard (1X) reaction throughout this user guide. This is suited for the standard 3µL dispense of the reagent per well, however custom volumes can be dispensed upon request.

To achieve different target sizes, we recommend titration experiments to determine the optimal reaction setup. You can change the volume of the tagging reagent being used or the amount of DNA input. For more information please refer to our *Tagify i5 UMI Transposase Reagent and Input DNA Titration* technical note found here: https://seqwell.com/resource-product/tagify-i5-umi-reagents/.

1. Tagging Reaction

If preparing fewer samples than the total number of reactions in the kit, please refer to subsection 1e 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.

Note: Each well is a single-use well containing transposomes loaded with i5/UMI barcodes.

b. To a new 8-tube PCR strip(s) or 96-well plate labeled **Reaction Tube(s) or Plate**, set up the tagging reaction for each sample by adding the following, *in order*, mixing after each addition by pipette up and down (≥10x at the transfer volume) slowly. All reactions below will yield average fragment size of 0.8-2kb. For other target sizes see *Tagify i5 UMI Transposase Reagent and Input DNA Titration* technical note.

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

Reagent/Reaction Size	0.5X	Standard (1X)	1.5X	2X
Dilution of Tagify i5 UMI Reagent Plate	Dilute plate with 5µL Tris	Dilute plate with 5µL Tris	*No dilution needed**	*No dilution needed**
Tagify i5 UMI Reagent for Prep	2.7 µl	5.3 µl	3 µl	4 µl**
Genomic DNA (ng)	25 ng	50 ng	75 ng	100 ng
Genomic DNA volume in 10mM Tris HCl, pH 8.0	4 µl	8 µl	20 µl	22.7 µl
3X Coding Buffer	3.3 µl	6.7 µl	10 µl	13.3 µl
Total Volume	**10 µl**	**20 µl**	**30 µl**	**40 µl**
Stock Tagify i5 UMI Reagent Used	1µL	2µL	3µL	4µL**

*If the amount of stock Tagify i5 UMI Reagent needed is the same as the volume dispensed in the plate, you can add the other reagents directly to your plate.

**Please reach out for this volume dispense of reagent.

c. Close the **Reaction Tube(s)** or heat seal **Reaction Plate**, gently vortex the tubes or plate to mix, pulse-spin, and transfer the **Reaction Tube(s) or Plate** to a thermal cycler and run the TAG program below, with lid-heating on at 105°C:

55°C for 15 minutes

25°C hold

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

Special instructions for preparing reactions in batches of fewer than total kit:

e. Pulse-spin the **Tagify i5 UMI Reagent plate** in a centrifuge. Using a scalpel or razor blade, cut the seal after the wells that will be processed. Carefully 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.

f. Follow the instructions for reaction setup in the Tagify i5 UMI Reagent plate above (step 1b). Only using the uncovered wells of reagent that will be in use immediately.

g. 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.

h. Continue following the instructions for steps 1c – 1d to the **Reaction Tube(s) or Plate.**

2. Stop Reaction

a. Pulse-spin the **Reaction Tube(s) or Plate** in a centrifuge and carefully open the tube(s) or remove the seal.

b. To the **Reaction Tube(s) or Plate** 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.

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.

Reagent/Reaction Size	0.5X	Standard (1X)	1.5X	2X
X Solution	5µL	10µL	15µL	20µL
Total Volume	**15µL**	**30µL**	**45µL**	**60µL**

c. Securely reseal and pulse-spin the **Reaction Tube(s) or Plate**, then transfer to a thermal cycler and run the STOP program, below, with lid heating on at 105°C:

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 (or vigorously pipet) *room temperature* **MAGwise** to ensure that the beads are fully resuspended before use.

b. Remove the samples from the thermocycler, pulse-spin the **Reaction Tube(s) or Plate**, and carefully open the tube(s) or remove the seal.

c. Add 1X equivalent volume* of **MAGwise** to each sample and mix thoroughly by pipetting up and down ≥10 times.

Note: *MAGwise Beads volume ratio can be changed based on application to target different size-selections.

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

e. Place the **Reaction Tube(s) or Plate** 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.

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

g. Wash beads with 80% ethanol.
   i. With the **Reaction Tube(s) or Plate** still in 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 to not dislodge the bead pellet.
   iii. Repeat the previous 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(s) or Plate** and remove from the magnetic stand; pulse-spin and return to magnetic stand to let the beads pellet again (<30 seconds). Remove any residual ethanol at the bottom of the tube. **Do not air dry the bead pellets**. **Proceed immediately to the next step**

h. Add 20 µl* of 10 mM Tris-HCl, pH 8 to each sample. Remove the **Reaction Tube(s) or Plate** from the magnetic stand 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 from the beads.

j. Return tube to magnetic stand and allow the bead pellet to reform on the inner wall of the tube (~2 minutes). **Do not air-dry bead pellets** or DNA recovery may be compromised.

k. When the supernatant has cleared completely, carefully transfer 18 µl* of DNA eluate from each sample to a fresh 1.5 ml LoBind tube. The transferred eluate contains the purified, tagged DNA.

SAFE STOPPING POINT

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

Tagged DNA Quantification and QC

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 PicoGreen DNA Assay (recommended), or similar assay.

b. Evaluate tagged DNA fragment sizes on an Agilent DNA Bioanalyzer Chip (recommended) or Agilent TapeStation using the genomic DNA ScreenTape following the manufacturer's instructions. Example traces from the Standard (1X) protocol are provided below.

Note: seqWell defines successful use of the Tagify i5 UMI reagent as tagging 50 ng of genomic DNA input to 800-2000 bp fragments, measured by the Agilent High Sensitivity DNA Bioanalyzer Chip, when following the Standard (1X) protocol. Other QC assays and instruments are not supported for this reagent due to significant size discrepancies found when using assays, such as the Agilent TapeStation HS D5000 Screen Tape.

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

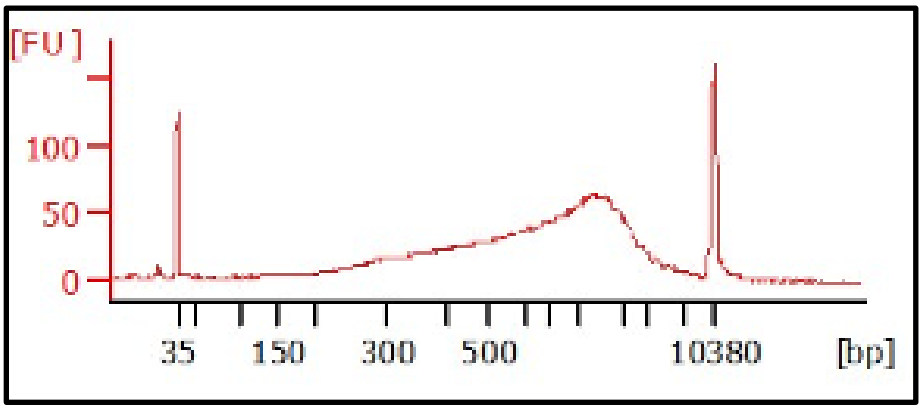


Figure 1. Example tagged DNA trace from an Agilent Bioanalyzer High Sensitivity DNA Kit. For the example above, the region analysis used is 200 - 7500 bp with the average size being 1116 bp.

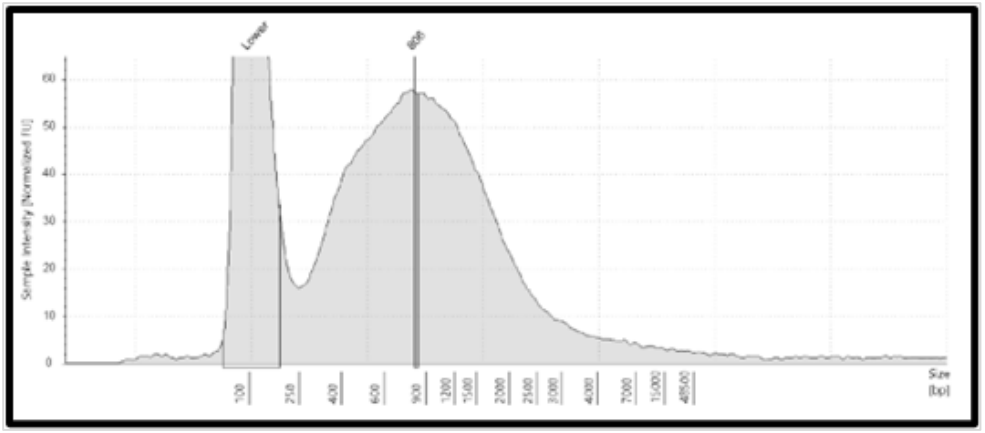


Figure 2. Example tagged DNA trace from an Agilent Bioanalyzer High Sensitivity DNA Kit. For the example above the region analysis used is 200 - 7500 bp with the average size being 1124 bp.

SAFE STOPPING POINT

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

Appendix A: Tagify i5 UMI Index Information and Demultiplexing Guidance

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 10-bp index and the green N's indicate the 10-bp UMI sequence.

There are 24 i5/UMI reagents available. The most up to date list of i5 indexes can be found in spreadsheet format at http://seqwell.com/resource-category/index-list/ and are also displayed below:

Well ID	i5 Index Forward Orientation	i5 Index Reverse Orientation
A01	GTAACACAGA	TCTGTGTTAC
B01	TAAGTTGTGG	CCACAACTTA
C01	CACCTACCTC	GAGGTAGGTG
D01	ATATCAGTCC	GGACTGATAT
E01	GGTTAAGTAG	CTACTTAACC
F01	TGGTGGATAT	ATATCCACCA
G01	TAGCTGGCAC	GTGCCAGCTA
H01	TTACCGTTGG	CCAACGGTAA
A02	CAAGAGCGTG	CACGCTCTTG
B02	AATACTCGGC	GCCGAGTATT
C02	GTATCTTAGG	CCTAAGATAC
D02	TGGTCTGAGG	CCTCAGACCA
E02	CAAGGACATT	AATGTCCTTG
F02	CGGTTGAGAA	TTCTCAACCG
G02	TACCTCGCTA	TAGCGAGGTA
H02	TAAGCTCGTT	AACGAGCTTA
A03	ATCTCCACGG	CCGTGGAGAT
B03	CTCGCTGCTT	AAGCAGCGAG
C03	CTCAGTAGAC	GTCTACTGAG
D03	CTTGGTCCTT	AAGGACCAAG
E03	CGTCCAAGAG	CTCTTGGACG
F03	GTATGGAACA	TGTTCCATAC
G03	AATCGGTACG	CGTACCGATT
H03	CTAATGTCGA	TCGACATTAG

The index orientation used will depend on the sequencer. Please refer to your sequencer's manual for determining the appropriate index direction. These 24 different i5s can be combined with a variety of i7s to enable additional multiplexing.

Optimal Demultiplexing Guidance:
In accordance with Illumina's guidelines, it is recommended to allow for 1 mismatch in the index reads during demultiplexing. This allows for the capture of many more reads than 0 mismatch at a reduced risk, due to sufficient Hamming distances between barcodes.

Refer to *Illumina's mismatch guidelines here:*
https://knowledge.illumina.com/software/general/software-general-reference_material-list/000007484

For the XLEAP chemistries:
For NovaSeq X, NextSeq 2000, and MiSeq i100 running XLEAP-SBS chemistry, an increased rate of 1 mismatch in the index reads has been observed. Hence, it is especially important to allow for 1 mismatch on these instruments to recover all expected data for each index.

Version	Release Date	Prior Version	Description of changes
V20231204	**March 12, 2023**	**N/A**	**First version**
V20230521	**May 5, 2023**	**V20231204**	**Early Access**
V20240618	**June 18, 2024**	**V20230521**	**Version 1.0**
V20250606	**June 3, 2025**	**V20240618**	• Updated the standard reagent product to Tagify UMI 24 (1x24x3uL) • Modified the reaction setups to accommodate 0.5, 1, 1.5, and 2X • Added: table of contents, FAQ link, molecular diagram, index list, and demultiplexing guidance.

Technical Assistance

Please review FAQs at https://seqwell.com/resource-category/faqs/.

For additional technical assistance with Tagify i5 UMI Adapter-loaded Transposase, contact seqWell Technical Support.

E-mail: support@seqwell.com

seqWell, Inc.
66 Cherry Hill Drive
Beverly, MA 01915
USA
+1-855-737-9355
https://seqwell.com/