Genexus™ Integrated Sequencer

Catalog Number A45727

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Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

The Ion Torrent™ Genexus™ Integrated Sequencer integrates library preparation, templating, sequencing, and data analysis into a single-instrument automated run. For more information on creating assays, adding or importing samples and library batches, creating plans for sample and library runs, starting a sequencing run, and data analysis, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910). This quick reference assumes familiarity with Genexus™ Software and the Genexus™ Integrated Sequencer, and is intended for more experienced users.

Create an assay

For information on how to create and manage assays in Genexus™ Software, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910). If you are using a system-installed assay without change, proceed to "Add samples and create a run plan in Genexus™ Software".

Add samples and create a run plan in Genexus™ Software

You can create run plans for two types of runs: sample runs that start from nucleic acid samples, and library runs that start from manually prepared libraries. Before creating a run plan in Genexus™ Software for either a sample run or a library run, you must first enter samples in the software to assign sample names and provide other information. Alternatively, you can import sample information from a data file.

Create a new sample

1. In the menu bar, click **Samples** ▸ **Manage Samples**.
2. In the **Manage Samples** screen, click + **Create Sample**.
3. In the **Create Sample** dialog box, complete the required fields.

   Attributes identified with a red asterisk (*) in the **Create Sample** dialog box are required when adding a new sample. If attribute information is not available when adding a new sample, substitute mock information to complete the required fields.
4. Click **Save**.

   The new sample is listed in the **Manage Samples** screen and will be available to use in your run.

Import samples

Sample data files can be used to capture, manage, and edit sample data. You can import sample data files in the following formats: TXT, XLS, XLSX, or CSV. For a list of the sample attributes that are included in the import file, and for information on downloading a Microsoft™ Excel™ example file to create an import file, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910), or *Genexus™ Software 6.2 Help*.

1. In the menu bar, click **Samples** ▸ **Manage Samples**, then click **Import Samples**.
2. In the **Import Samples** dialog box, click **Select samples file**.
3. Navigate to the file, then click **Open**.
4. Click **Upload**.

   A progress bar followed by an import report appears. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used).

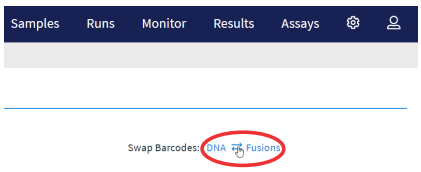
Successfully imported samples are listed in the **Samples / Manage Samples** screen.

Prepare a library batch

A library batch is a group of prepared libraries that are sequenced in the same library run. If you are planning a run starting from libraries that you have already prepared manually, you must first create a library batch in Genexus™ Software from samples that you have added. If you are planning a run starting from nucleic acid samples, skip this step and proceed to "Plan a sample run" on page 2.

1. In the menu bar, click **Samples** ▸ **Manage Samples**.
2. In the **Manage Samples** screen, in the **Filter Samples by...** dropdown menu, apply the **To Be Prepared** filter to limit the displayed samples to those samples that have not been placed in a library batch.
3. Select samples in the list by clicking the checkbox to the left of each sample, then click + **Prepare Library Batch**.
4. In the **Create Library batch** screen, in **Select Assay**, select the assay that you want to run.
5. In the expanded screen, in **Library Batch ID**, enter a unique identifier for the library batch.
6. Select the barcodes from the kit boxes into the appropriate fields.
7. Select the **Include NTC** checkbox to add no template control sample processing and reporting to the library batch.
8. Type a unique library name for each DNA and/or RNA library in the appropriate field.
9. Select the barcode ID of the adapter used to prepare each library. If appropriate, swap the default barcodes in the dialog box between DNA, RNA, and Fusions by clicking the **Swap Barcodes** swap image.

   For example, click the **DNA** and **Fusions** swap button.



IMPORTANT! Ensure that the actual barcodes that you used to create the libraries match the barcodes that you enter in the **Create Library batch** screen.

10. Enter the **Input Quantity** for each library.
11. Click **Submit** to save and submit your selections.

    The **Manage Libraries** screen opens, listing the library batch that you created. Libraries that are prepared in the same batch have the same **Library Batch ID**.

Plan a sample run

1. In the menu bar, click **Runs** ▸ **Plan Sample Run**.

   Note: You can also click + **Plan Sample Run** in the **Runs / Manage Runs** screen.
2. In the **Setup** step, enter or make the following selections.
   a. In the **Plan** section, enter a unique name.
   b. *(Optional)* In the **Reporting (Optional)** section, select one or more options if needed. You can select both options, or leave both options deselected.
      - **Generate Report**
      - **Upload BAM files to Ion Reporter™ Software**
   c. Click **Next**.
3. In the **Assays** step, select the assay or assays that you want to use in the run.

   Use the **Filter Assays By** list and the **Assay Name** search box to search, sort, and filter the list of assays.
4. In the **Include NTC** column for each assay that you select, click the **Include NTC** checkbox to include a no template control for the assay.
5. After you select an assay (or assays) and make the appropriate Ion Reporter™ Software selections (if applicable), click **Next**.
6. In the **Samples** step, select the samples from the list that you want to run with the assay, then click **Assign**.
7. If you selected more than one assay, repeat step 6 for each additional assay.
8. If needed, edit samples in one of the following ways, then click **Next**.
   - Click **View & Remove**, make your selections, then click **Update**.
   - Click **Remove All**, make your selections, then click **Assign**.
9. In the **Sample Plate** step, review sample positions in the sample plate. Drag and drop samples and no template controls to edit the location of samples and controls, if desired.
10. Modify the concentration of samples, if needed.
11. If sample plate information is correct, click **Next**.
12. In the **Review** step, review the run plan summary, then click **Save & Print** to print the run setup guide, if desired. Click **Save** to save the run without printing.

    After saving, the run appears in the **Manage Runs** screen in the run list with the name you specified.

After selecting the run and loading the sequencer, the run is started on the sequencer screen.

Plan a library run

1. In the menu bar, click **Runs** ▸ **Plan Library Run**.

   Note: You can also click + **Plan Library Run** in the **Runs / Manage Runs** screen.
2. In the **Setup** step, enter a name for the run, then configure the reporting options.
   a. In **Run Name**, enter a unique name.
   b. *(Optional)* In the **Reporting (Optional)** section, select one or more options if needed. You can select both options, or leave both options deselected.
      - **Generate Report**
      - **Upload Samples to Ion Reporter™ Software**
   c. Click **Next**.

3. In the **Assays** step, select the assay or assays that you want to use in the run, then click **Next**.

   Use the **Filter Assays By** list and the **Assay Name** search box to search, sort, and filter the list of assays.

4. In the **Library Batches** step, select the library batch or batches that you want to use in the run.

   Note: Only one library batch can be selected per assay. However, you can plan a multi-assay library run if you select multiple, different assays in the **Assays** step.

5. After you select a library batch (or batches), click **Next**.

6. In the **Review** step, review the run plan summary, then click **Save and Print** to print the run setup guide, if desired. Click **Save** to save the run without printing.

   After saving, the run appears in the run list on the **Manage Runs** screen with the name you specified.

The run is started on the sequencer screen after selecting a run and loading the sequencer.

Dilute the samples and load the sample plate

Before starting a run on the instrument, you must quantify and dilute the samples or sample libraries, then load the sample plate.

Dilute or concentrate the samples (if needed) and load the sample plate—sample run

Isolate DNA and RNA samples using one of the procedures and kits that are recommended in the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).

Samples with concentrations up to 1,024X of the target concentration for an assay (displayed as default values in the **Sample Plate** step screen in run planning) are in range for automated dilution and require no manual dilution. Enter the concentrations during sample run planning at the **Sample Plate** step (see step 9 on page 2).

1. For samples with concentrations that are out of range for automated dilution, manually dilute the sample with nuclease-free water, or concentrate the sample to a concentration ≤1,024X of the target concentration. For samples that are in range, go to step 2.

If the sample concentration is…	Then…
<0.11 ng/µL	Concentrate the sample to greater than or equal to the target concentration.
≥0.11 ng/µL, but less than the target concentration	Run is allowed but sample concentration may not be optimal for library preparation. Concentrate the sample to greater than or equal to the target concentration.
≤1,024X of the target concentration	No manual dilution is necessary. The sequencer dilutes the sample to the target concentration automatically during the run.
>1,024X of the target concentration	Manually dilute to the target concentration based on assay type, or to a concentration in range for automated dilution by the sequencer.

   Note:
   - If you enter a concentration <0.11 ng/µL or >10,000 ng/µL target concentration, a warning that the concentration is out of range appears, and you are not allowed to proceed to the next step.
   - If the concentration is ≤10,000 ng/µL, but >1,024X of the target concentration, you can proceed, but because the instrument cannot dilute samples more than 1,024-fold, the diluted sample concentration will be greater than the target concentration.

2. Add samples to the sample plate at the volume and positions that are specified in the run setup guide.

   The sample volume is not adjustable and depends on sample type, the number of primer pools in the assay, and library chemistry. The following table also provides loading volume.

Sample type	Number of primer pools	Volume
Ion AmpliSeq™ chemistry		
DNA	1	15 µL
DNA	2	25 µL
RNA	1	15 µL
RNA	2	25 µL
Ion AmpliSeq™ HD chemistry		
DNA	1	20 µL
RNA	1	20 µL
TNA	1	20 µL

3. Seal the plate with a sheet of Adhesive PCR Plate Foils (Thermo Fisher Scientific Cat. No. AB0626).

4. Keep the plate on ice until ready to load it in the sequencer.

Dilute and pool libraries, and load the sample plate—library run

1. Dilute each manually prepared and quantified sample library to 200 pM with nuclease-free water.

   Note: Each library must be barcoded with a unique barcode or barcode pair. Use this concentration as a starting point, then titrate up or down based on sequencing results, if needed.

2. Add equal volumes of each library to a new 1.5-mL low DNA retention tube so that the total volume is greater than the volume specified in the run setup guide provided by the software.

   Note: For information on combining DNA and RNA libraries recovered from sample runs using assays that include DNA and fusions, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).

3. Mix well by pipetting up and down five times, then transfer the specified volume of each library batch to the sample plate position specified in the run setup guide.

4. Seal the plate with a sheet of Adhesive PCR Plate Foils (Thermo Fisher Scientific Cat. No. AB0626).

5. Keep the plate on ice until you are ready to load it in the sequencer.

Load the sequencer and start a run

After you have planned a run in Genexus™ Software, use the run setup guide provided by the software to load samples in the sample plate, and to determine which consumables to load in the sequencer. Follow the step-by-step instructions in the sequencer touchscreen during run setup. The vision system of the sequencer tracks the addition of consumables in real-time and alerts you if a component is loaded in an incorrect position, or if an incorrect quantity is loaded.

Before you begin

1. Remove the library and templating strips from their boxes in the refrigerator or freezer, and ready them for loading in the sequencer.
   - Genexus™ Strip 1 and Genexus™ Strip 3-GX5™: equilibrate to room temperature for 30 minutes.
   - Genexus™ Strip 2-AS or Genexus™ Strip 2-HD, depending on your assay, and Genexus™ Strip 4: thaw on ice for at least 30 minutes. Keep the strips on ice until you load them in the sequencer.

   IMPORTANT! Confirm that the strip contents are completely thawed before installing in the sequencer.

2. Remove primer pool tubes in tube carriers that are needed for the run from the freezer, then thaw for at least 30 minutes on ice. After thawing, gently tap the primer pool tube or tubes on a bench surface to ensure that contents are collected at the bottom of the tubes. Keep the tubes and carriers on ice until you load them in the sequencer.

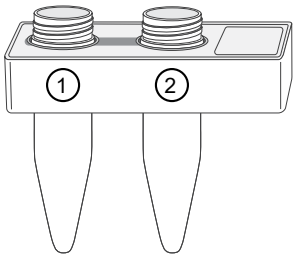
3. If you are installing a new Genexus™ Cartridge, thaw the cartridge at room temperature for 30 minutes before installing in the sequencer.

4. Genexus™ Strip 1 and Genexus™ Strip 3-GX5™ contain magnetic beads in one or two positions, yellow or brown in color, that sometimes get trapped in the upper "keyhole" of the tube. Dislodge these beads from the keyhole before installing the strip in the sequencer. Use the following procedure for each strip.
   a. Invert the strip 3–4 times to dislodge beads that are trapped in the keyholes.
   b. To remove any remaining beads and liquid from the keyholes, grasp the strip at one end with the strip seal facing up, then swing the strip with a rapid and downward centrifugal arm motion, ending with a sharp wrist-flick.
   c. Grasp the strip at the other end, then repeat the centrifugal motion.
   d. Check tube positions for any remaining beads that are trapped in keyholes, then repeat the centrifugal motion, if needed. It is acceptable if a few beads remain in the keyhole or on the tube wall, but most should be either in suspension or in a pellet at the bottom of the tube.

5. Inspect all the strips for large bubbles lodged under the surface of liquid in the tubes. Gently tap the strips on a benchtop to dislodge any bubbles without splashing the contents onto the upper tube walls. If tapping fails to dislodge a bubble, use the swing technique described in substep 4b until large bubbles are dislodged.

Fill Genexus™ Primer Pool Tubes *(custom assays only)*

If you are using a custom assay, Genexus™ Primer Pool Tubes must be manually filled with the custom Ion AmpliSeq™ or Ion AmpliSeq™ HD panels at the appropriate volume and in the correct primer pool tube positions. For Ion AmpliSeq™ library panels, use one carrier per DNA or RNA assay primer pool. The two positions in the primer pool tube carrier are designated as shown in the following figure:



① Position 1 tube: Contains Ion AmpliSeq™ DNA, Ion AmpliSeq™ RNA, or Ion AmpliSeq™ HD FWD primer pool

② Position 2 tube: Contains Ion AmpliSeq™ HD REV primer pool

1. Add primer pool at the indicated volume, appropriate to your assay type, to the Genexus™ Primer Pool Tubes using the following tables as a guide. Fill the number of tubes specified by the run plan summary.

Ion AmpliSeq™ DNA assays

Number of primer pairs per pool	Concentration	Volume in position 1	Volume in position 2
12–96	2X (400 nM)	140 µL	—
97–3,072	2X (100 nM)	140 µL	—
>3,072	2X ([3,072 / Number of primer pairs per pool] × 100 nM)[1]	140 µL	—

[1] For example, if a panel pool has 3,500 primer pairs, the 2X concentration is (3,072 / 3,500) × 100 nM = 87.8 nM.

Ion AmpliSeq™ RNA assays

Number of primer pairs per pool	Concentration	Volume in position 1	Volume in position 2
12-1,228	5X (250 nM)	75 µL	—
>1,228	5X ([1,228 / Number of primer pairs per pool] × 250 nM)[1]	75 µL	—

[1] For example, if a panel pool has 1,500 primer pairs, the 5X concentration is (1,228 / 1,500) × 250 nM = 205 nM.

Ion AmpliSeq™ HD assays

Primer pool type	Concentration	Volume in position 1	Volume in position 2
Ion AmpliSeq™ HD FWD	10X	50 µL	—
Ion AmpliSeq™ HD REV	10X	—	50 µL

IMPORTANT!

- If you are using Ion AmpliSeq™ library chemistry, leave the tube in position 2 empty and uncapped, but do not remove the tube from the carrier before loading in the sequencer. Do not add a second Ion AmpliSeq™ primer pool to the position 2 tube.
- If you are using Ion AmpliSeq™ HD library chemistry, add the FWD and REV primer pools to the appropriate tubes in the same carrier.
- Ensure that no bubbles are introduced at the bottom of the tube when adding the primer pool.

2. If you do not install the primer pool tube carriers in the sequencer immediately, cap the tubes that contain primer pools, then store the tube carriers on ice. Remember to uncap all tubes before installing.

Load the sequencer and start a run

1. Tap **Run** on the sequencer home screen to start the loading procedure.

2. In the **Run Selection** screen, select the run that you want to use from the list.

   Note: If you select a run that requires more lanes than are available on a currently installed chip, a dialog appears giving you the option to install a new chip, or cancel. If you proceed with a new chip, a postChipClean is performed, then the sequencer prompts you to perform the Clear Deck, UV Clean, Load Deck, Clear Sequencing Reagents, and Load Sequencing Reagents steps.

3. In the **Review Run** screen, confirm the run and assay selections, then tap **Next**.

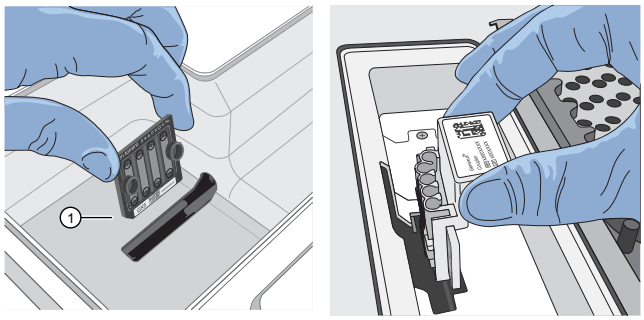
   The deck door opens automatically.

4. In the **Load Deck** screen, the sequencer instructs you step by step to load each required consumable in a highlighted position on the deck. The sequencer detects the loading of each consumable in real time and advances to the next component automatically.

IMPORTANT!

- Ensure that you remove the primer pool tube cap or caps before installing the tube carrier on the deck.
- Ensure that you load the correct type of barcode plate and library strip 2 for the type of run you are setting up. The sequencer displays a warning if you have installed consumables that are incompatible with the run you have selected, for example, a Genexus™ Barcodes AS plate or Genexus™ Strip 2-AS in an HD run.

5. If prompted, insert a new GX5™ Chip and Genexus™ Coupler. Insert the chip into the chip install slot with the chip notch oriented down and toward the front of the instrument.



① Notched corner of chip

IMPORTANT! Insert the Genexus™ Coupler so that it is level to ensure it will properly align with the GX5™ Chip. A coupler that is installed at an angle or is not level will not align properly to the chip and can result in a failed run.

6. When the deck consumables have been loaded, lock the library and templating strips in place by sliding the latches toward the rear of the deck.

   If a chip is detected and the strip latches are closed, the **Close Deck Door** screen appears.

7. Close the deck door, then tap **Next**.
   - If you installed a new chip in the sequencer, the sequencer prompts you to open the sequencing reagents bay doors to empty the waste and remove used sequencing reagents bay consumables. Proceed to step 8.
   - If you are using a chip that was previously installed and has sufficient lane capacity for the run, the sequencer prompts you to start the run.

IMPORTANT! The cartridge and bottles in the sequencing reagents bay must be replaced every time that a new chip is installed, regardless of how many lanes were used in the previous chip.

8. Follow on-screen instructions to empty the waste in the Waste Carboy, remove waste pipette tips, remove the used Genexus™ Bottle 1, Genexus™ Bottle 2, Genexus™ Bottle 3, and Genexus™ Cartridge, then tap **Next**.

IMPORTANT!

- Ensure that you empty and replace the Waste Carboy and the waste pipette tip bin.
- After replacing the emptied Waste Carboy, ensure that you reinsert the waste tube into the carboy.
- Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of consumables and liquid waste.

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9. Install a new Genexus™ Bottle 1, Genexus™ Bottle 2 (two required), Genexus™ Bottle 3, and Genexus™ Cartridge.

   After reagents have been installed, the **Close Sequencing Reagent Bay Door** screen appears.

10. Close the sequencing reagents bay doors.

    After the doors are closed, the sequencer automatically starts the run.

IMPORTANT! Do not tap **Start Run** in the **Close Sequencing Reagent Bay Door** screen. Tapping **Start Run** can cancel the run.

At the beginning of the run, the instrument verifies the chip, checks for leaks, then calculates run time.

A sequencing run encompasses the following stages:

- Starting
- Initializing
- Library Prep
- Templating
- Pre-sequencing
- Sequencing
- Cleaning

At each stage, the instrument shows the time remaining on the touchscreen.

When the run finishes, the sequencer displays the **Run Complete** screen.

Clear the instrument deck and perform a UV Clean

After a run completes, remove used consumables from the deck and perform a **UV Clean** to ready the instrument for the next run.

1. In the **Run Complete** screen, tap **Next** to start removal of used consumables.

   The deck door opens.

2. In the **Clear Deck** screen, the sequencer provides step-by-step instructions by highlighting the components to be removed. Unlock the library and templating strips by sliding the latches toward the front of the deck, then remove the used strips. Remove the remaining deck components specified by the sequencer.

3. Inspect the Genexus™ Filter in the liquid waste disposal port and verify that no standing liquid is present. If standing liquid is present, manually remove the liquid with a pipette, then pull out the filter. Test the filter with water to determine if a clog is present.

   - If the Genexus™ Filter is clogged, replace it with a new filter. For more information, see "*Replace the Genexus™ Filter*" in the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910).
   - If the Genexus™ Filter does not appear to be clogged, a line clog downstream of the filter is implicated. Contact Technical Support and report a possible deck liquid waste line clog.

4. When finished, close the deck door, then tap **Next**.

   A two-minute **UV Clean** starts.

5. After UV cleaning, if all the chip lanes were used, the sequencing reagents bay doors unlock. Open the doors, remove used components from the bay and empty the Waste Carboy, then tap **Next**.

IMPORTANT! Do **not** discard or remove the conical bottles, unless alerted by the sequencer to replace the bottles after a conical bottle flow rate test. For more information, see the *Genexus™ Integrated Sequencer User Guide*.

IMPORTANT! Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of Genexus™ Integrated Sequencer consumables and liquid waste.

CAUTION! The Genexus™ Bottle 1 (small waste bottle) contains small amounts of formamide. Dispose of this waste appropriately.

6. After removal of used components, close the sequencing reagents bay doors, then tap **Next**.

   The sequencer returns to the home screen.

Review data and results

You can review run results and data analysis and perform data management tasks in the **Results** menu. For more information, see the *Genexus™ Integrated Sequencer User Guide* (Pub. No. MAN0017910), or the *Genexus™ Software 6.2 User Guide* (Pub. No. MAN0018955).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Block 33 |
Marsiling Industrial Estate Road 3 |
#07-06, Singapore 739256

Product:
Genexus™ Integrated Sequencer

Manufacturer:
Life Technologies Corporation |
200 Oyster Point Blvd |
South San Francisco, CA 94080 | USA

Product:
Genexus™ Software

Manufacturer:
Life Technologies Corporation |
7335 Executive Way |
Frederick, MD 21704 | USA

Products:
GX5™ Chip and Genexus™ Coupler
Genexus™ Library Strips 1 and 2-AS
Genexus™ Library Strips 1 and 2-HD
Genexus™ Templating Strips 3-GX5™ and 4
Genexus™ Barcodes 1–96 AS
Genexus™ Barcodes 1–32 HD
Genexus™ Primer Pool Tubes
Genexus™ Pipette Tips
Genexus™ Sequencing Kit
Genexus™ Controls
Genexus™ Conical Bottles
Genexus™ Filter
Genexus™ GX5™ Starter Pack-AS
Genexus™ GX5™ Starter Pack-HD
Oncomine™ GX assays

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0017912

Revision	Date	Description
C.0	29 October 2020	• Changed the recommended concentration for manually prepared libraries from 125 pM to 200 pM. See "Dilute and pool libraries, and load the sample plate—library run" on page 3. • Updated "Load the sequencer and start a run" on page 5 to align with changes in Rev. C.0 of the *Genexus™ Integrated Sequencer User Guide*
B.0	30 June 2020	Updated for Genexus™ Software 6.2
A.0	11 November 2019	New Genexus™ Integrated Sequencer quick reference