Precision ID SNP Panels with the HID Ion S5™/HID Ion GeneStudio™ S5 System: Library Preparation on the HID Ion Chef™ System

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Pub. No. MAN0017769 Rev. B.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the following product documentation: *Precision ID SNP Panels with the HID Ion S5™/HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0017767). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Extract, then quantify input DNA

1. Extract gDNA using one of the recommended genomic DNA extraction kits listed in the *Precision ID SNP Panels with the HID Ion S5™/HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0017767).

2. Quantify gDNA using one of the recommended DNA quantification kits listed in the *Precision ID SNP Panels with the HID Ion S5™/HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0017767).

   Note: Use 1 ng gDNA in target amplification reactions.

Dilute the gDNA samples

Dilute samples to 20 pg/µL with nuclease-free water. Prepare 15 µL of each diluted sample (1 ng total) to prepare up to 8 libraries per Ion Chef™ run.

Thaw the reagents, then prepare the instrument

1. Before the run, thaw one Precision ID DL8 Reagents cartridge at room temperature for 20 minutes.

2. Thaw Precision ID primer pools.

3. If not performed after a previous run, unload, then clean the Ion Chef™ Instrument.

4. Verify that the Ion Chef™ Instrument has a connection to the Torrent Server. On the Ion Chef™ home touchscreen, tap **Settings**, then **Torrent Server** to view the connection status of your instrument.

Create a sample set

1. Sign into the Torrent Suite™ Software.

2. In the **Plan** tab, click **Samples**, then click **Add or Update Sample Set/Samples**.

3. Click **Enter New Sample**.

   a. In the **Add Sample** dialog box, enter the following information for each sample in the library preparation.

      - **Sample Name**—enter a sample name.
      - **PCR Plate Position**—select a plate position of A to H.
      - **Barcode Kit**—enter ***IonCode Barcodes 1–32***.
      - **Barcode**—enter the barcode for the appropriate IonCode Plate and plate position. For example, ***IonCode_0101 for Red plate, well A***.

   b. Click **Done**.

      Your new samples and sample attributes appear in the **Enter Samples** list.

   c. Repeat for each sample in the library preparation.

4. Click **Create Sample Set** to add the samples to a sample set.
   a. Enter the following information for the Sample Set.
      - **Sample Set Name**—enter a name for the sample set.
      - **Group Type**—select **DNA and Fusions**.
      - **Library Prep Type**—enter **AmpliSeq on Chef**.
      - **Library Prep Kit**—**Precision ID Chef DLB**.
   b. Click **Save Sample Set**.

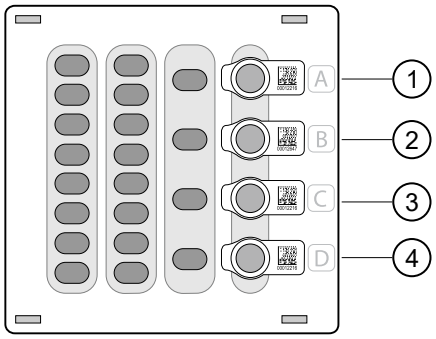
Add Precision ID primer pool to Positions A and B of the Reagents cartridge

1. Uncap all 4 tubes in positions A, B, C, and D in the Precision ID DL8 Reagents cartridge. Save the caps.

2. Add primer pool to the Precision ID DL8 Reagents cartridge using the following guidelines.

Panel	Action
Precision ID Ancestry Panel	Pipet 150 µL of the primer pool into the Position A tube and 150 µL into the Position B tube.
Precision ID Identity Panel	
Custom Ion AmpliSeq™ SNP Panel (2X)[1]	

[1] If the pool is at 5X concentration, first dilute to 2X. For dilution information, see the AmpliSeq guide. (Jessica to provide Pub. No.)



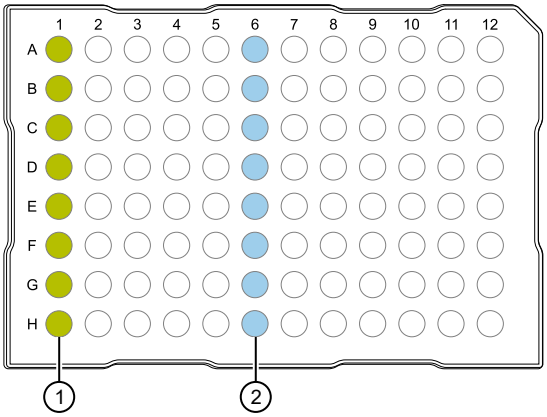
① Position A (150 µL of primer pool)
② Position B (150 µL of primer pool)
③ Position C (Empty tube)
④ Position D (Output tube)

Note: If input DNA is <1 ng, then the library concentration is <100 pM and library quantification with qPCR is required.

Add the DNA to the Precision ID DL8 IonCode™ Barcode Adapters

1. Remove the plate seal from a Precision ID DL8 IonCode™ Barcode Adapters Plate.

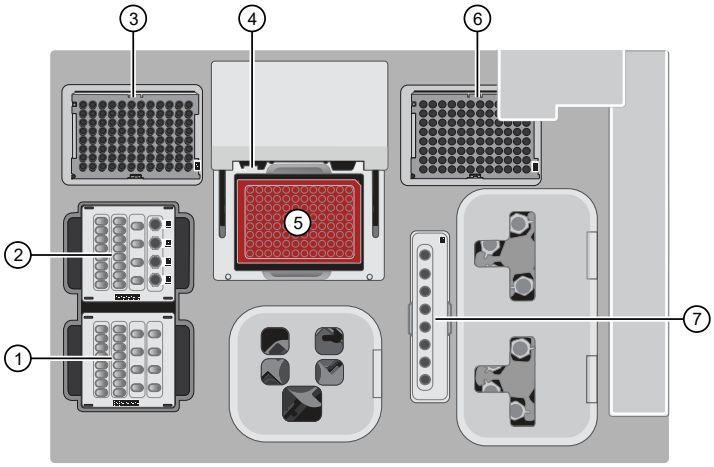
2. Pipet 15 µL of each DNA sample (67 pg/µL, 1 ng total) into wells A1 to H1 of the plate as shown in the following figure.



① Column 1 wells containing 15 µL of each diluted DNA sample (67 pg/µL).
② Column 6 wells containing 8 dried-down IonCode™ barcodes. Lowest number is in A6 and highest is in H6. All appear light blue in the actual plates.

Load the Ion Chef™ Instrument for library preparation

Follow the procedure below to load the Ion Chef™ Instrument. A completely loaded instrument is shown in the following figure.



① Precision ID DL8 Solutions cartridge
② Precision ID DL8 Reagents cartridge
③ Ion AmpliSeq™ Tip Cartridge L8
④ Framed PCR Foil Seal
⑤ Precision ID DL8 IonCode™ Barcode Adapters 96 Well Plate and PCR Plate Frame
⑥ Empty Tip Cartridge L8
⑦ Enrichment Cartridge

1. Open the instrument door:
   a. On the instrument touchscreen, tap ⏏ **(Open Door)**, then wait for the latch to open.
   b. Lift the instrument door to the top of the travel until the latch mechanism engages.

2. Load the Precision ID DL8 Solutions cartridge into the Solutions station.
   a. Gently tap the cartridge on the bench to force the reagents to the bottoms of the tubes.
   b. Load the cartridge into the Solutions station so that it snaps into place, and is level on the deck.

3. Gently tap the Precision ID DL8 Reagents cartridge on the bench to force the reagents to the bottom of the tubes, then load the cartridge into the Reagents station so that it snaps into place.

4. Load a new Ion AmpliSeq™ Tip Cartridge L8 into the New Pipette Tip station (left side of deck).
   a. Unwrap the Ion AmpliSeq™ Tip Cartridge L8, then remove the cover to expose the pipette tips.
   b. Pull the tip station catch backwards to open the locking bracket. Load the Ion AmpliSeq™ Tip Cartridge L8, then push the locking bracket closed.

5. Load an empty tip cartridge from a previous run into the Used Tip station.

6. Load the Precision ID DL8 IonCode™ Barcode Adapters 96 Well Plate containing gDNA onto the thermal cycler block and press down to seat it.

7. With the white dot on the PCR Plate Frame facing upward, load the PCR Plate Frame into the thermal cycler sample block pressing down firmly on each corner, then insert a new Frame Seal v2 underneath the automated heated cover. Ensure that the PCR Plate Frame is pressed completely down onto the thermal cycler block and that the PCR Plate Frame sits lower than the PCR Plate.

8. Close the instrument door by first lifting it up slightly to disengage the locking mechanism, then pushing down on the door so that the lower locks engage.

Start the run for the Precision ID Ancestry Panel or Precision ID Identity Panel

Perform the following steps to start an Ion AmpliSeq™ run on the Ion Chef™ Instrument.

1. On the Ion Chef™ home touchscreen, tap **Set up run**.

2. Tap **Step by step**, then tap **AmpliSeq** on the **Run Options** screen.

   Note: To bypass the step by step deck loading guide, tap **Quick start**.

3. Ensure that you have loaded the Ion Chef™ deck with Precision ID DL8 Kit consumables by advancing through the step by step deck loading steps on the instrument touchscreen.

4. Tap **Start check** on the **Close Door** screen. The Ion Chef™ Instrument performs a Deck Scan.

5. After Deck Scan completes (~3 minutes), tap **Next**.

6. In the **Data Destination** screen perform the following actions, then tap **Next**.
   - Verify the server information.
   - Choose the sample set created on page 1.

7. Enter the appropriate number of primer pools, target amplification cycles, and an anneal/extension time for your run, then tap **Next**.

Amount of input gDNA added	# of primer pools	Cycle number	Anneal & extension time
1 ng (~300 copies)	1	22 cycles	4
<1 ng (<~300 copies)	1	22 cycles + 1 to 5 cycles	4

8. Review panel and amplification entries for the run in the **AmpliSeq Confirmation Window**, then tap **Start Run**.

9. After approximately 7 hours, return to the Ion Chef™ Instrument. On the **Run Complete** screen, tap **Next** to go to the unloading and cleaning steps.

IMPORTANT! The Ion Chef™ Instrument holds the barcoded libraries in the tube in Position D of the Reagents cartridge. Remove, then cap the tube as soon as possible after run completion. Do not leave the tube in the instrument longer than 24 hours after the start of the run. After 24 hours from the start of the run, the instrument chiller stops actively cooling, and the sample is held at 27°C.

Unload the Ion Chef™ Instrument

1. Open the instrument door:
   a. In the instrument touchscreen, tap ⏏ **(Open Door)**, then wait for the latch to open.
   b. Lift the instrument door to the top of the travel until the latch mechanism engages.

2. Remove the Precision ID DL8 Reagents cartridge. Remove and cap the combined library tube from Position D, then discard the cartridge.

3. Remove, then discard the Precision ID DL8 Solutions cartridge.

4. Remove, then discard the Precision ID DL8 IonCode™ Barcode Adapters 96 Well Plate with the PCR Plate Frame and Frame Seal v2 from the PCR sample block in unison.

IMPORTANT! Do not attempt to separate the PCR Plate Frame from the PCR Plate and Frame Seal v2, as this may cause PCR product to splash and contaminate the instrument deck.

5. Remove, then discard the box of used pipette tips from the waste tip position.

IMPORTANT! Do not reuse the waste pipette tip rack. Always move the empty Tip Cartridge L8 from the new tip position to the waste tip position.

6. Move the empty Tip Cartridge L8 from the New Pipette Tip station to the Used Pipette Tip station.

IMPORTANT! Do not discard the empty Tip Cartridge L8.

7. Remove, then discard the Enrichment Cartridge.

Quantify the combined library by qPCR

After unloading the Ion Chef™ Instrument, determine the concentration of the combined library pools by qPCR with the Ion Library TaqMan™ Quantitation Kit (Cat. No. 4468802).

Dilute the combined library for quantification

1. If the combined library tube has been stored at 4°C, vortex the tube, then centrifuge briefly to collect droplets.
2. Prepare a 1:100 dilution by combining 2 µL with 198 µL of nuclease-free water.
3. After removing the aliquot, store the tube at 4°C.

Quantify the combined library by qPCR

Use the Ion Library TaqMan™ Quantitation Kit to analyze the combined sample library, standard, and negative control in duplicate 20-µL reactions.

1. Prepare three 10-fold serial dilutions of the *E. coli* DH10B Control Library (~68 pM; provided in the kit) at the concentrations listed in the following table. Label them as standards, then use these concentrations in the qPCR experiment setup.

Standard	Control Library volume	Nuclease-free water volume	Concentration
1	5 µL (undiluted)	45 µL	6.8 pM
2	5 µL Std 1	45 µL	0.68 pM
3	5 µL Std 2	45 µL	0.068 pM

2. Prepare sufficient reaction mixture for replicate reactions for each sample, negative control, and control library dilution. Add an extra reaction to compensate for pipetting error. For each reaction, combine 10 µL of Ion Library qPCR Master Mix and 1 µL of Ion Library TaqMan™ Quantitation Assay, 20X in a tube, then mix thoroughly.

Component	Volume (1 reaction)
Ion Library TaqMan™ qPCR Mix	10 µL
Ion Library TaqMan™ Quantitation Assay, 20X	1 µL

3. Aliquot 11 µL into each reaction well (two wells per reaction) of a PCR plate.
4. Add 9 µL of the diluted (1:100) sample library, control library dilution, or negative control to reaction wells, for a total reaction volume per well of 20 µL.
5. Set up the real-time PCR instrument.
   a. Enter the concentrations of the control library standards.
   b. Select ROX™ Reference Dye as the passive reference dye.
   c. Enter a reaction volume of 20 µL.
   d. Select FAM™ dye/MGB as the TaqMan™ probe reporter/quencher.
   e. Enter the following run parameters, depending on your system.

Real-time PCR System	Stage	Temperature	Time
7500 Real-Time PCR Instrument with SDS Software v1.2.3	Hold	50°C	2 minutes
	Hold	95°C	20 seconds
	40 Cycles	95°C	3 seconds
		60°C	32 seconds
7500 Real-Time PCR Instrument with HID Real-Time PCR Analysis Software v1.1 or later	Hold	50°C	2 minutes
	Hold	95°C	20 seconds
	40 Cycles	95°C	3 seconds
		60°C	30 seconds
QuantStudio™ 5 Real-Time PCR System	Hold	50°C	2 minutes
	Hold	95°C	20 seconds
	40 Cycles	95°C	3 seconds
		60°C	30 seconds

6. Run the reactions, then collect the real-time data.

Proceed to “Dilute, pool, and store the combined libraries”.

Dilute, pool, and store the combined libraries

Dilute the combined libraries

IMPORTANT! Dilute the combined libraries to the optimal input concentration before proceeding to template preparation. The quality of sequencing data relies on achieving the correct concentration of starting library.

Dilute libraries as described in the following table. Then use polyclonality and low-quality filter results from a sequencing run performed with ISPs templated at the starting concentration to titrate up or down to achieve optimal concentrations, if needed.

Dilute to	Minimum volume	Templating size in Planned Run setup
30 pM	25 µL	200 bp

(Optional) Combine library pools

After diluting library pools prepared on the Ion Chef™ Instrument to the target concentration (pM), you can "super-pool" libraries by combining equal volumes of the library pools, provided the pools were prepared with different Precision ID DL8 IonCode™ Barcode Adapters. Use the combined library pools in template preparation reactions on the Ion Chef™ Instrument.

Use the following recommendations for the number of Ion Chef™-prepared sample libraries loaded per chip. The recommendations are based on at least 100X coverage of 97% of markers. You may need to adjust the number of samples per chip based on your individual coverage requirements, sample quality, and throughput.

Panel	Samples per Ion S5™ Chip		
	Ion 510™ Chip	Ion 520™ Chip	Ion 530™ Chip
Precision ID Ancestry Panel	30	32[1]	32[1]
Precision ID Identity Panel	32[1]	32[1]	32[1]
Custom Ion AmpliSeq™ SNP Panel	The number of samples will vary depending on your panel. We recommend that you perform a baseline run to establish panel performance, then adjust the number of samples accordingly to meet the desired coverage.		

[1] The number of Precision ID DL8 IonCode™ Barcode Adapters is currently limited to 32.

Store the libraries

Store both diluted and undiluted libraries at 2°C to 8°C for up to 1 month. For long-term storage, store libraries at –30°C to –10°C.

Clean the Ion Chef™ Instrument

IMPORTANT! Clean the Ion Chef™ Instrument after every run. To prevent contamination, do not operate the instrument unless it has been recently cleaned.

1. Close the instrument door by first lifting it slightly to disengage the locking mechanism, then pushing down on the door until the locks engage.
2. On the Ion Chef™ Instrument touchscreen that appears after run completion, tap **Next**.
3. Ensure that you have removed all consumables from the Ion Chef™ Instrument, then tap **Next**.
4. With the door closed, tap **Start**.

   The instrument performs a Deck Scan before starting the cleaning routine. The Ion Chef™ Instrument stops ventilation and illuminates the ultraviolet (UV) light in the instrument.

CAUTION! The Ion Chef™ Instrument emits UV light at 254 nm. Wear appropriate eye wear, protective clothing, and gloves when working near the instrument. Do not look directly at the UV light while it is illuminated during the cleaning routine.

Limited product warranty

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Life Technologies Corporation | 5781 Van Allen Way | Carlsbad, California 92008 USA

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0017769 B.0

Revision	Date	Description
B.0	5 October 2023	• Added instructions for creating a sample set. See “Create a sample set” on page 1. • Updated instructions to include loading the PCR Plate Frame. See “Load the Ion Chef™ Instrument for library preparation” on page 2. • Updated instructions to include removing the PCR Plate Frame. See “Unload the Ion Chef™ Instrument” on page 3.
A.0	9 October 2018	New Quick Reference.