Precision ID GlobalFiler™ NGS STR Panel v2: Manual Library Preparation

Catalog Numbers A33114, A26435, A30941, A33586, 4468802

Pub. No. MAN0016130 **Rev.** B.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Precision ID GlobalFiler™ NGS STR Panel v2 with the HID Ion S5™/HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0016129). 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 GlobalFiler™ NGS STR Panel v2 with the HID Ion S5™/HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0016129).

2. Quantify gDNA using one of the recommended DNA quantification kits listed in the *Precision ID GlobalFiler™ NGS STR Panel v2 with the HID Ion S5™/HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0016129).

   Note: Use 1 ng gDNA in target amplification reactions.

Prepare the STR target amplification reaction

1. Add the following components to each well of a 96-well PCR plate.

   Note: For multiple reactions, prepare a master mix.

Component	Volume
5X Ion AmpliSeq™ HiFi Mix (red cap)	4 µL
Precision ID GlobalFiler™ NGS STR Panel v2	10 µL
gDNA, 1 ng[1]	X µL[2]
Nuclease-free Water	6 – X µL
Total	**20 µL**

[1] Less than 1 ng of gDNA can be used, but adjust the number of PCR cycles appropriately in "Amplify the targets".

[2] ≤6 µL

2. Seal the plate with a MicroAmp™ Clear Adhesive Film, vortex briefly, then centrifuge the plate to collect droplets.

Proceed to "Amplify the targets".

Amplify the targets

The cycle number for target amplification depends on the amount of input DNA. Cycle numbers can be increased if the quality or quantity of input DNA is uncertain.

IMPORTANT! When amplifying multiple samples in a single PCR plate, ensure that the input DNA across the samples is roughly equivalent, or the PCR cycle number is based on the sample with the *lowest* quantity. This ensures that the selected cycle number for target amplification is optimal for all the samples in the run. Expect higher stutter with higher cycle number.

Cycle number depending on input DNA

Amount of input gDNA	Number of cycles
1 ng (~300 copies)	23 cycles
0.5–1 ng	23 cycles
0.5 ng	24 cycles
0.250 ng	25 cycles
0.125 ng	26 cycles

To amplify target regions, run the following program:

Stage	Step	Temperature	Time
Hold	Activate the enzyme	99°C	2 minutes
Cycle number (see preceding table)	Denature	99°C	15 seconds
	Anneal and extend	60°C	4 minutes
Hold	—	10°C	Hold

STOPPING POINT The target amplification reactions can be held at 10°C overnight on the thermal cycler. For longer periods, store at –20°C for up to one month.

Partially digest amplicons

1. Remove the plate seal, then **add 2 µL of FuPa Reagent** (brown cap) to each amplified sample. The total volume is ~22 µL.
2. Seal the plate with a clear adhesive film, vortex thoroughly, then spin down to collect droplets.
3. Load in the thermal cycler, then setup and run the following thermal cycling conditions:

Temperature	Time
50°C	10 minutes
55°C	10 minutes
60°C	20 minutes
10°C	Hold (for up to 1 hour)

STOPPING POINT Store the plate at –20°C.

Ligate adapters to the amplicons, then purify

You *must* ligate a different barcode adapter to each library when:

- Sequencing multiple libraries on a single chip
- Sequencing multiple replicates of DNA libraries from the same sample on a single chip

IonCode™ barcode adapters are provided at the appropriate concentration, and include forward and reverse adapters in a single well. No further handling is necessary.

IMPORTANT! When handling barcode adapters, avoid cross-contamination.

Perform the ligation reaction

IMPORTANT! If there is visible precipitate in the Switch Solution, vortex or pipet up and down at room temperature to resuspend.

1. Carefully remove the plate seal, then add the following components to each well containing digested amplicons in the order listed.

   IMPORTANT! Add the DNA Ligase last. Do not combine DNA Ligase and adapters before adding to digested amplicons.

Order of addition	Component	Volume
1	Switch Solution (yellow cap)	4 µL
2	Precision ID IonCode™ Barcode Adapter	2 µL
3	DNA Ligase (blue cap)	2 µL
—	**Total volume**	**~30 µL**

2. Seal the plate with a new MicroAmp™ Adhesive Film, vortex thoroughly, then centrifuge to collect droplets.
3. Load the plate in the thermal cycler, then run the following thermal cycling conditions:

Panel	Temperature	Time
Precision ID GlobalFiler™ NGS STR Panel v2	22°C	30 minutes
	68°C	10 minutes
	10°C	Hold (for up to 24 hours)

Purify the libraries

1. Carefully remove the plate seal, then add 45 µL (1.5X sample volume) of Agencourt™ AMPure™ XP Reagent to each library.
2. Pipet up and down 5 times to mix the bead suspension with the DNA thoroughly, then incubate the mixture for 5 minutes at room temperature.

   Alternatively, use a plate mixer (such as the Eppendorf™ MixMate™ mixer with the 96 × 0.2-mL PCR tube holder) to mix the bead suspension. Seal the plate, mix for 5 minutes at 2,000 rpm at room temperature, then centrifuge the plate briefly to collect droplets.
3. Place the plate in a magnetic rack (such as the DynaMag™–96 Side Magnet; Cat. No. 12331D), then incubate for 2 minutes or until solution clears.
4. Carefully remove, then discard the supernatant without disturbing the pellet.
5. Add 150 µL of freshly prepared 70% ethanol, then move the plate side-to-side in the two positions of the magnet to wash the beads. Remove, then discard the supernatant without disturbing the pellet.
6. Repeat step 5 for a second wash.
7. Ensure that all ethanol droplets are removed from the wells. Keep the plate in the magnet, then air-dry the beads at room temperature for 5 minutes.

Elute the libraries

1. Remove the plate containing the library from the magnet, then add 50 µL of Low TE to the pellet to disperse the beads.
2. Seal the plate with a MicroAmp™ Clear Adhesive Film, then vortex thoroughly.
3. Incubate for 5 minutes at room temperature, then centrifuge to collect droplets.

   IMPORTANT! For maximum recovery, ensure that the suspension incubates for at least 5 minutes at room temperature.
4. Place the plate on the magnet for at least 2 minutes.

STOPPING POINT Samples can be stored with beads at 4°C for up to one month. For long-term storage at −20°C, place the plate in the magnet, then transfer the sample supernatants to a new plate. Do not store libraries at −20°C in the presence of beads.

Quantify the libraries by qPCR

After eluting each Precision ID library, determine concentration by qPCR with the Ion Library TaqMan® Quantitation Kit (Cat. No. 4468802).

Dilute the libraries for quantification

1. If samples have been stored at 4°C, vortex the plate, then centrifuge to collect droplets.
2. Place the plate in the magnetic rack for 2 minutes, or until the supernatant clears.
3. Prepare 1:100 dilutions by removing 2 µL of supernatant, then combine with 198 µL of Nuclease-free Water.
4. After removing the aliquots, store the plate at 4°C.

Quantify the libraries

Use the Ion Library TaqMan® Quantitation Kit to analyze each sample, 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, each 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 v1.2	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.

See "Dilute, pool, and store the libraries" on page 4 for library concentrations required for template preparation. Depending on your quantification results, proceed with one of the following options:

- If sufficient library was prepared, continue to "Dilute, pool, and store the libraries" on page 4.
- If insufficient library was prepared, continue to "(Optional) Amplify and purify the libraries" on page 4.
- Continue with less than optimal library concentration and proceed to "Dilute, pool, and store the libraries" on page 4. See "Troubleshooting" in the *Precision ID GlobalFiler™ NGS STR Panel v2 with the HID Ion S5™/HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0016129) for effects of using low library concentration.

(Optional) Amplify and purify the libraries

A library that yields less than the recommended concentration can be rescued by library amplification. See the *Precision ID GlobalFiler™ NGS STR Panel v2 with the HID Ion S5™/HID Ion GeneStudio™ S5 System Application Guide* (Pub. No. MAN0016129) for the full procedure.

Dilute, pool, and store the libraries

IMPORTANT! To ensure adequate coverage, we recommend that you pool no more than 32 barcoded libraries to run on a single Ion 530™ Chip.

Dilute the libraries

1. After the run is complete, calculate the average concentration of each undiluted library using the following equation:

   Avg concentration of undiluted library = (qPCR mean quantity) × (library dilution)

   For example:

   - qPCR mean quantity: 3 pM
   - Sample library dilution: 100

   The average concentration of the undiluted library:
   (3 pM) × (100) = 300 pM

2. Dilute libraries as described in the following table.

Panel	Dilute to	Minimum volume
Precision ID GlobalFiler™ NGS STR Panel v2	50 pM	25 µL

Note:

- To ensure accurate dilution of sample library, avoid pipetting volumes of 1 µL or less. For example, for a 1:30 dilution, dilute 2 µL of sample library with 58 µL of Low TE.
- If you are pooling sample libraries, the minimum volume of the pool must be 25 µL.

Pool the libraries

After diluting each sample library to 50 pM, pool equal volumes, then use the pooled library in a template preparation reaction on the Ion Chef™ Instrument.

Use the following recommendations for the number of manually-prepared sample libraries loaded per chip. The recommendations are based at least 100X coverage. 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 520™ Chip	Ion 530™ Chip
Precision ID GlobalFiler™ NGS STR Panel v2	16	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.

Customer and technical support

For support:

- **In North America**—Send an email to **HIDTechSupport@thermofisher.com**, or call **888-821-4443 option 1**.
- **Outside North America**—Contact your local support office.

For the latest services and support information for all locations, go to **thermofisher.com/support** to obtain the following information.

- Worldwide contact telephone numbers
- Product support
- Order and web support
- Safety Data Sheets (SDSs; also known as MSDSs)

Additional product documentation, including user guides and Certificates of Analysis, are available by contacting Customer Support.

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

Revision history: Pub. No. MAN0016130

Revision	Date	Description
B.0	15 November 2018	Updated to support use with Ion GeneStudio™ S5 Systems.
A.0	30 June 2017	New quick reference for manual Precision ID GlobalFiler™ NGS STR Panel v2 library preparation.