Ion AmpliSeq™ HD Library Kit

Catalog Numbers A37694, A37695

Pub. No. MAN0017774 Rev. C.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *Ion AmpliSeq™ HD Library Kit User Guide* (Pub. No. MAN0017392). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This quick reference is intended as a benchtop reference for experienced users of Ion AmpliSeq™ HD Library Kit (Cat. No. A37694). For detailed instructions, see the *Ion AmpliSeq™ HD Library Kit User Guide* (Pub. No. MAN0017392).

Prepare Ion AmpliSeq™ HD DNA libraries

This protocol is designed for use with 1-pool or 2-pool DNA primer panels. Modifications to the standard protocol can be made to accommodate 3-pool DNA primer panels. For more information, see *Ion AmpliSeq™ HD Library Kit User Guide* (MAN0017392).

Remove deaminated bases from FFPE DNA

IMPORTANT! If using DNA isolated from sources other than FFPE tissue, proceed directly to "Set up DNA target amplification reactions" on page 1.

1. For each FFPE DNA sample, add the following components to a single well of a 96-well PCR plate.

Component	Volume
20 ng FFPE DNA	≤7.3 µL
UDG, heat-labile	1 µL
Low TE	to 8.3 µL

2. Mix the reaction by pipetting at least half the total volume up and down at least 5 times, then seal the plate with MicroAmp™ Clear Adhesive Film. Centrifuge briefly to collect the contents.

3. Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate into the thermal cycler, then run the following program.

Temperature	Time
37°C	2 minutes
50°C	10 minutes
4°C	Hold (≤1 hour)

4. Remove the plate from the thermal cycler, then centrifuge briefly to collect the contents.

STOPPING POINT Reactions can be stored at –20°C long term.

5. Carefully remove the plate seal, then proceed immediately to "Set up DNA target amplification reactions", adding the target amplification reaction components to the well containing 8.3 µL of UDG treated FFPE DNA.

Set up DNA target amplification reactions

Set up DNA target amplification reactions using one of the following procedures for either 1- or 2-pool primer panels. To set up DNA target amplification reactions for 3-pool primer panels, see *Ion AmpliSeq™ HD Library Kit User Guide* (MAN0017392).

Prepare DNA target amplification reactions — 1-pool primer panel

IMPORTANT!

- Keep all reagents on ice or in a pre-chilled 4°C cold block during reaction setup.
- 4X Ion AmpliSeq™ HD Amplification Mix is viscous. Pipet slowly and mix thoroughly.
- Do not combine FWD and REV primer subpools for storage. Primer subpools must remain separate and combined only during the target amplification reaction setup.

1. Place a 96-well plate on ice or in a pre-chilled 4°C cold block.

2. Add the following components to a single well of a 96-well PCR plate. Prepare a master mix without sample DNA for multiple reactions.

Component	Volume
20 ng cfNA, cfDNA, or FFPE DNA	≤12.5 µL
4X Ion AmpliSeq™ HD Amplification Mix (purple cap)	7.5 µL
10X Pool 1 FWD (red cap)	3 µL
10X Pool 1 REV (yellow cap)	3 µL
CRC (yellow cap)	4 µL
Nuclease-free Water	to 30 µL

3. Mix by pipetting at least half the total volume up and down at least 5 times, then seal the plate with a MicroAmp™ Clear Adhesive Film. Centrifuge briefly to collect the contents.

Proceed to "Amplify the targets" on page 2.

Prepare DNA target amplification reactions — 2-pool primer panel

IMPORTANT!

- Keep all reagents on ice or in a pre-chilled 4°C cold block during reaction setup.
- 4X Ion AmpliSeq™ HD Amplification Mix is viscous. Pipet slowly and mix thoroughly.
- Do not combine FWD and REV primer subpools together for storage. Primer subpools must remain separate and combined only during the target amplification reaction setup.

1. Place a 96-well plate on ice or in a pre-chilled 4°C cold block.

2. Add the following components to two wells of a 96-well PCR plate. For multiple reactions, prepare a master mix without the sample DNA.

Component	Well 1, Pool 1 (volume)	Well 2, Pool 2 (volume)
20 ng cfNA, cfDNA, or FFPE DNA	≤6.3 µL	≤6.3 µL
4X Ion AmpliSeq™ HD Amplification Mix (purple cap)	3.7 µL	3.7 µL
10X Primer Pool 1 FWD (red cap)	1.5 µL	–
10X Primer Pool 1 REV (yellow cap)	1.5 µL	–
10X Primer Pool 2 FWD (blue cap)	–	1.5 µL
10X Primer Pool 2 REV (green cap)	–	1.5 µL
CRC (yellow cap)	2 µL	2 µL
Nuclease-free Water	to 15 µL	to 15 µL

3. Mix thoroughly by pipetting at least half the total volume up and down at least 5 times, then seal the plate with MicroAmp™ Clear Adhesive Film. Centrifuge briefly to collect the contents.

Amplify the targets

IMPORTANT! If you are using challenging primer panels or panels that have ≥500 primer pairs, amplify the targets using cycling option A in Table 1.

1. Place a MicroAmp™ Optical Film Compression Pad on the plate, then load the plate into the thermal cycler.

2. Run one of the following cycling programs, depending on the type of panel that is used.

   - *(Recommended)* Use the following cycling program for all primer panel types, including challenging panels and panels with ≥500 primer pairs.

     Table 1 Cycling option A

Stage	Step	Temperature	Time
3 cycles	Denature	99°C	30 seconds
	Anneal	64°C	2 minutes
		60°C	12 minutes
		66°C	2 minutes
	Extend	72°C	2 minutes
Hold	Final extension	72°C	2 minutes
Hold	—	4°C	≤1 hour

   - Use the following alternative cycling program if excessive amounts of primer dimer is produced with cycling option A.

     Table 2 Cycling option B

Stage	Step	Temperature	Time
3 cycles	Denature	99°C	30 seconds
	Anneal	64°C	2 minutes
		60°C	6 minutes
	Extend	72°C	30 seconds
Hold	Final extension	72°C	2 minutes
Hold	—	4°C	≤1 hour

Combine target amplification reactions (for 2-pool DNA primer panel libraries)

Note: Combining target amplification reactions is only required for DNA libraries with 2 or more primer pools.

For instructions on combining target amplification reactions for 3-pool DNA primer panel libraries, see the *Ion AmpliSeq™ HD Library Kit User Guide* (MAN0017392).

1. Remove the plate from the thermal cycler, then centrifuge briefly to collect the contents.

2. Carefully remove the plate seal.

3. For each sample, combine both 15-µL target amplification reactions into a single well.

Proceed to step 2 of "Partially digest amplicons" on page 3.

Partially digest amplicons

IMPORTANT!

- Ion AmpliSeq™ HD SUPA Reagent is viscous. Pipet slowly and mix thoroughly. Perform this step on ice or a cold block, then quickly proceed to incubation.
- Do not substitute any assay components with reagents from other kits.

1. Tap the plate gently on a hard flat surface, or centrifuge briefly to collect the contents at the bottom of the wells, then remove the plate seal.

2. **Add 5 µL of Ion AmpliSeq™ HD SUPA Reagent** (green cap) to each well.

   IMPORTANT! FuPa Reagent is NOT a substitute for the Ion AmpliSeq™ HD SUPA Reagent.

3. Mix by pipetting at least half the total volume up and down at least 5 times, then seal the plate with a MicroAmp™ Clear Adhesive Film. Centrifuge briefly to collect the contents.

4. Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate into the thermal cycler, then run the following program.

Temperature	Time
30°C	15 minutes
50°C	15 minutes
55°C	15 minutes
25°C	10 minutes
98°C	2 minutes
4°C	Hold (≤1 hour)

Amplify the library with barcoded primers

Ion AmpliSeq™ HD Dual Barcode Kit 1–24 primers are provided ready-to-use with different barcode primers per well. These primers are required for library preparation when sequencing one or multiple libraries per sequencing chip. If you are sequencing multiple libraries on a single chip, make sure to use different primers with each library.

1. Remove the plate from the thermal cycler, then centrifuge briefly to collect the contents.

2. Carefully remove the adhesive film from the plate.

3. **Add 4 µL of the selected barcode primers from the Ion AmpliSeq™ HD Dual Barcode Kit 1–24** to each well.

4. Mix by pipetting at least half the total volume up and down at least 5 times, then seal the plate with MicroAmp™ Clear Adhesive Film. Centrifuge briefly to collect the contents.

5. Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate into the thermal cycler, then run the following program.

Stage	Step	Temperature	Time
Hold	Activate the enzyme	99°C	15 seconds
5 cycles	Denature	99°C	15 seconds
	Anneal	62°C	20 seconds
	Extend	72°C	20 seconds
12–15 cycles[1], see Table 3	Denature	99°C	15 seconds
	Extend	70°C	40 seconds
Hold	Final extension	72°C	5 minutes
Hold	—	4°C	Indefinite

[1] For FFPE DNA, using 17 cycles can improve library yields.

Table 3 Recommended number of amplification cycles

Primer pairs per pool	Number of cycles
12–500	15
501–1,000	14
1,001–2,000	13
2,001–5,000	12

STOPPING POINT Library amplification products can be stored at 4°C overnight on the thermal cycler. For longer periods, store at –20°C.

Purify the library

1. Bring the Agencourt™ AMPure™ XP Reagent to room temperature, then vortex thoroughly to disperse the beads before use.
2. Briefly centrifuge the plate to collect the contents in the bottom of the wells.
3. Carefully remove the plate seal, transfer each library into a separate new well, then add 10 µL of Low TE to each library. Mix by pipetting at least half the total volume up and down 5 times.
4. Add 39 µL (1X sample volume before Low TE addition) of the Agencourt™ AMPure™ XP Reagent to each library, then pipet up and down 5 times to thoroughly mix the bead suspension with the DNA.
5. Incubate the mixture for 5 minutes at room temperature.
6. Place the plate in a magnetic rack such as the DynaMag™–96 Side Magnet, then incubate for 2 minutes or until the solution clears.
7. Carefully remove, then discard the supernatant without disturbing the pellet.
8. Add 150 µL of freshly prepared 70% ethanol, then move the plate side-to-side in the two positions of the magnet 5 times to wash the beads.
9. Carefully remove, then discard the supernatant without disturbing the pellet.
10. Repeat step 8–step 9 one more time.
11. Ensure that all ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 5 minutes.
12. Remove the plate from the magnet, add 50 µL of Low TE buffer to each well, then resuspend the Agencourt™ AMPure™ XP beads by pipetting up and down.
13. Incubate the plate at room temperature for 5 minutes.
14. Place the plate back in a magnetic rack such as the DynaMag™–96 Side Magnet, then incubate for 2 minutes or until the solution clears.
15. Transfer 50 µL of the purified library into a new well.
16. Add 50 µL (1X sample volume) of Agencourt™ AMPure™ XP Reagent to each library, then pipet up and down 5 times to thoroughly mix the bead suspension with the DNA.
17. Incubate the mixture for 5 minutes at room temperature.
18. Place the plate in a magnetic rack such as the DynaMag™–96 Side Magnet, then incubate for 2 minutes or until the solution clears.
19. Carefully remove, then discard the supernatant without disturbing the pellet.
20. Add 150 µL of freshly prepared 70% ethanol, then move the plate side-to-side in the two positions of the magnet 5 times to wash the beads.
21. Carefully remove, then discard the supernatant without disturbing the pellet.
22. Repeat step 20–step 21 one more time.
23. Ensure that all ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 5 minutes.
24. Remove the plate from the magnet, add 50 µL of Low TE buffer to each well, then resuspend the Agencourt™ AMPure™ XP beads by pipetting up and down.
25. Incubate the plate at room temperature for 5 minutes.
26. Place the plate back in a magnetic rack such as the DynaMag™–96 Side Magnet, then incubate for 2 minutes or until the solution clears.
27. Transfer the cleared library solution into a new tube or well in a plate.

Prepare Ion AmpliSeq™ HD RNA libraries

This protocol is designed for use with 1-pool RNA primer panels and panels that contain both DNA and RNA assays in a single primer pool.

Reverse transcribe RNA

IMPORTANT! Warm the 5X VILO™ Reaction Mix to room temperature for at least 20 minutes, then vortex to mix before pipetting. If there is any visible precipitate, mix further by vortexing until the 5X VILO™ Reaction Mix is completely resuspended.

1. If the RNA was prepared from FFPE tissue and not previously heat-treated, heat at 80°C for 10 minutes, then cool to room temperature.

2. For each sample, add the following components to a single well of a 96-well PCR plate. For multiple reactions, prepare a master mix without sample RNA.

Component	Volume
5X VILO™ Reaction Mix	2.4 µL
10X SuperScript™ Enzyme Mix	1.2 µL
≥20 ng cfNA or FFPE RNA	≤ 8.4 µL
Nuclease-free Water	to 12 µL

3. Mix by pipetting at least half the total volume up and down at least 5 times, then seal the plate with MicroAmp™ Clear Adhesive Film. Centrifuge briefly to collect the contents.

4. Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate in the thermal cycler, then run the following program.

Temperature	Time
42°C	30 minutes
85°C	5 minutes
4°C	Hold

STOPPING POINT Samples can be stored at 4°C for up to 16 hours in the thermal cycler. For longer term, store at –20°C.

5. Gently tap the plate on the bench to ensure that reactions are at the bottom of the wells.

Prepare cDNA target amplification reactions

IMPORTANT!

- Keep all reagents on ice or in a pre-chilled 4°C cold block during reaction setup.
- 4X Ion AmpliSeq™ HD Amplification Mix is viscous. Pipet slowly and mix thoroughly.
- Do not combine FWD and REV primer subpools for storage. Primer subpools must remain separate until combined during the target amplification reaction setup.
- **DO NOT** use CRC in RNA library preparation. The reagent is not compatible with the VILO™ Reaction Mix.

1. Place a 96-well plate on ice or in a pre-chilled 4°C cold block.

2. Add the following components to a single well of a 96-well PCR plate. Prepare a master mix without sample cDNA for multiple reactions.

Component	Volume
cDNA from 20 ng cfNA or FFPE RNA	12 µL[1]
4X Ion AmpliSeq™ HD Amplification Mix (purple cap)	7.5 µL
10X Pool 1 FWD (red cap)	3 µL
10X Pool 1 REV (yellow cap)	3 µL
Nuclease-free Water	to 30 µL

[1] cDNA volume in each target amplification reaction is the entire volume of the reverse transcription reaction from "Reverse transcribe RNA" on page 5.

3. Mix by pipetting at least half the total volume up and down at least 5 times, then seal the plate with a MicroAmp™ Clear Adhesive Film. Centrifuge briefly to collect the contents.

Amplify the targets

IMPORTANT! If you are using challenging primer panels or panels that have ≥500 primer pairs, amplify the targets using cycling option A in Table 4.

1. Place a MicroAmp™ Optical Film Compression Pad on the plate, then load the plate into the thermal cycler.

2. Run one of the following cycling programs, depending on the type of panel that is used.

   - *(Recommended)* Use the following cycling program for all primer panel types, including challenging panels and panels with ≥500 primer pairs.

     Table 4 Cycling option A

Stage	Step	Temperature	Time
3 cycles	Denature	99°C	30 seconds
	Anneal	64°C	2 minutes
		60°C	12 minutes
		66°C	2 minutes
	Extend	72°C	2 minutes
Hold	Final extension	72°C	2 minutes
Hold	—	4°C	≤1 hour

   - Use the following alternative cycling program if excessive amounts of primer dimer is produced with cycling option A.

     Table 5 Cycling option B

Stage	Step	Temperature	Time
3 cycles	Denature	99°C	30 seconds
	Anneal	64°C	2 minutes
		60°C	6 minutes
	Extend	72°C	30 seconds
Hold	Final extension	72°C	2 minutes
Hold	—	4°C	≤1 hour

Partially digest amplicons

IMPORTANT!
- Ion AmpliSeq™ HD SUPA Reagent is viscous. Pipet slowly and mix thoroughly. Perform this step on ice or a cold block, then quickly proceed to incubation.
- Do not substitute any assay components with reagents from other kits.

1. Tap the plate gently on a hard flat surface, or centrifuge briefly to collect the contents at the bottom of the wells, then remove the plate seal.

2. **Add 5 µL of Ion AmpliSeq™ HD SUPA Reagent** (green cap) to each well.

   IMPORTANT! FuPa Reagent is NOT a substitute for the Ion AmpliSeq™ HD SUPA Reagent.

3. Mix by pipetting at least half the total volume up and down at least 5 times, then seal the plate with a MicroAmp™ Clear Adhesive Film. Centrifuge briefly to collect the contents.

4. Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate into the thermal cycler, then run the following program.

Temperature	Time
30°C	15 minutes
50°C	15 minutes
55°C	15 minutes
25°C	10 minutes
98°C	2 minutes
4°C	Hold (≤1 hour)

Amplify the library with barcoded primers

Ion AmpliSeq™ HD Dual Barcode Kit 1–24 primers are provided ready-to-use with different barcode primers per well. These primers are required for library preparation when sequencing one or multiple libraries per sequencing chip. If you are sequencing multiple libraries on a single chip, make sure to use different primers with each library.

1. Remove the plate from the thermal cycler, then centrifuge briefly to collect the contents.

2. Carefully remove the adhesive film from the plate.

3. **Add 4 µL of the selected barcode primers from the Ion AmpliSeq™ HD Dual Barcode Kit 1–24** to each well.

4. Mix by pipetting at least half the total volume up and down at least 5 times, then seal the plate with MicroAmp™ Clear Adhesive Film. Centrifuge briefly to collect the contents.

5. Place a MicroAmp™ Optical Film Compression Pad on the plate, load the plate into the thermal cycler, then run the following program.

Stage	Step	Temperature	Time
Hold	Activate the enzyme	99°C	15 seconds
5 cycles	Denature	99°C	15 seconds
	Anneal	62°C	20 seconds
	Extend	72°C	20 seconds
12–15 cycles, see Table 6 (17 cycles for FFPE RNA and cfNA)	Denature	99°C	15 seconds
	Extend	70°C	40 seconds
Hold	Final extension	72°C	5 minutes
Hold	—	4°C	Indefinite

Table 6 Recommended number of amplification cycles

Primer pairs per pool	Number of cycles
12–500	15
501–1,000	14
1,001–2,000	13
2,001–5,000	12

STOPPING POINT Library amplification products can be stored at 4°C overnight on the thermal cycler. For longer periods, store at –20°C.

Purify the library

1. Bring the Agencourt™ AMPure™ XP Reagent to room temperature, then vortex thoroughly to disperse the beads before use.
2. Briefly centrifuge the plate to collect the contents in the bottom of the wells.
3. Carefully remove the plate seal, transfer each library into a separate new well, then add 10 µL of Low TE to each library. Mix by pipetting at least half the total volume up and down 5 times.
4. Add 39 µL (1X sample volume before Low TE addition) of the Agencourt™ AMPure™ XP Reagent to each library, then pipet up and down 5 times to thoroughly mix the bead suspension with the cDNA.
5. Incubate the mixture for 5 minutes at room temperature.
6. Place the plate in a magnetic rack such as the DynaMag™–96 Side Magnet, then incubate for 2 minutes or until the solution clears.
7. Carefully remove, then discard the supernatant without disturbing the pellet.
8. Add 150 µL of freshly prepared 70% ethanol, then move the plate side-to-side in the two positions of the magnet 5 times to wash the beads.
9. Carefully remove, then discard the supernatant without disturbing the pellet.
10. Repeat step 8–step 9 one more time.
11. Ensure that all ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 5 minutes.
12. Remove the plate from the magnet, add 50 µL of Low TE buffer to each well, then resuspend the Agencourt™ AMPure™ XP beads by pipetting up and down.
13. Incubate the plate at room temperature for 5 minutes.
14. Place the plate back in a magnetic rack such as the DynaMag™–96 Side Magnet, then incubate for 2 minutes or until the solution clears.
15. Transfer 50 µL of the purified library into a new well.
16. Add 50 µL (1X sample volume) of Agencourt™ AMPure™ XP Reagent to each library, then pipet up and down 5 times to thoroughly mix the bead suspension with the DNA.
17. Incubate the mixture for 5 minutes at room temperature.
18. Place the plate in a magnetic rack such as the DynaMag™–96 Side Magnet, then incubate for 2 minutes or until the solution clears.
19. Carefully remove, then discard the supernatant without disturbing the pellet.
20. Add 150 µL of freshly prepared 70% ethanol, then move the plate side-to-side in the two positions of the magnet 5 times to wash the beads.
21. Carefully remove, then discard the supernatant without disturbing the pellet.
22. Repeat step 20–step 21 one more time.
23. Ensure that all ethanol droplets are removed from the wells. Keeping the plate in the magnet, air-dry the beads at room temperature for 5 minutes.
24. Remove the plate from the magnet, add 50 µL of Low TE buffer to each well, then resuspend the Agencourt™ AMPure™ XP beads by pipetting up and down.
25. Incubate the plate at room temperature for 5 minutes.
26. Place the plate back in a magnetic rack such as the DynaMag™–96 Side Magnet, then incubate for 2 minutes or until the solution clears.
27. Transfer the cleared library solution into a new tube or well in a plate.

Quantify the library with the Agilent™ 2100 Bioanalyzer™ instrument

We recommend that you determine library concentration using the Agilent™ 2100 Bioanalyzer™ instrument, as this method also allows you to assess the library profile. After quantification, determine the dilution factor that results in a concentration of ~100 pM, which is appropriate for template preparation using an Ion template kit.

Quantify the library and calculate the dilution factor

Analyze 1 µL of the library on the Agilent™ 2100 Bioanalyzer™ instrument with the Agilent™ High Sensitivity DNA Kit (Cat. No. 5067-4626).

1. Determine the molar concentration of the library using the Bioanalyzer™ software. Briefly:
    a. Select the **Data** icon in the Contexts panel, then view the electropherogram of the sample to be quantified.
    b. Select the **Region Table** tab below, then create a region spanning the desired amplicon peaks. Correct the baseline if needed.
    c. The molarity is automatically calculated and displayed in the table in pmol/L (pM).
2. Based on the calculated library concentration, determine the dilution that results in a concentration of ~100 pM.
3. Dilute library to ~100 pM, then proceed to "Combining libraries, templating, sequencing, and data analysis" on page 8 or store libraries as described in "Store libraries" on page 8.

Store libraries

You can store libraries at 4–8°C for up to 1 month. For longer lengths of time, store at –30°C to –10°C.

Combining libraries, templating, sequencing, and data analysis

For instruction on combining libraries, and guidelines for templating, sequencing, and data analysis see the *Ion AmpliSeq™ HD Library Kit User Guide* (Pub. No. MAN0017392).

Limited product warranty

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Manufacturer:
Life Technologies Corporation |
5781 Van Allen Way |
Carlsbad, CA 92008

Products:
Ion AmpliSeq™ HD Library Kit,
Ion AmpliSeq™ HD Dual Barcode Kit 1–24

Manufacturer:
Life Technologies Corporation |
6055 Sunol Blvd |
Pleasanton, CA 94566 USA

Products:
Ion AmpliSeq™ HD Made-to-Order Panels

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Revision history: Pub. No. MAN0017774

Revision	Date	Description
C.0	08 December 2020	• Updated the DNA target amplification reaction setup to include the CRC reagent as a standard component in "Prepare DNA target amplification reactions — 1-pool primer panel" on page 1, and "Prepare DNA target amplification reactions — 2-pool primer panel" on page 2. • Removed the low concentration input reverse transcription reaction option in "Reverse transcribe RNA" on page 5. • Added the required additional round of library purification steps in "Purify the library" on page 4 and "Purify the library" on page 7. • Updated method recommendation in "Quantify the library with the Agilent™ 2100 Bioanalyzer™ instrument" on page 8.
B.0	21 June 2019	• Updated procedures to include information for improved library amplification multiplexy (12–5,000 primer pairs per pool). • Added a note to keep all reagents on ice or in a pre-chilled cold block during target amplification reaction setup (page 1, page 2, and page 5). • Updated "Prepare Ion AmpliSeq™ HD DNA libraries" on page 1 to include the procedure for using the optional CRC reagent. • Added reference to the protocol for preparing 3-pool DNA primer panel libraries in "Set up DNA target amplification reactions" on page 1. • Added a second cycling option in "Amplify the targets" on page 2 and 5. • Added reference to the protocol for combining target amplification reactions for 3-pool DNA primer panel libraries in "Combine target amplification reactions (for 2-pool DNA primer panel libraries)" on page 3. • Updated "Reverse transcribe RNA" on page 5 and "Prepare cDNA target amplification reactions" on page 5 to include reaction set up for low concentration input. • Added cycling recommendation for different amounts of primer pairs per panel in "Amplify the library with barcoded primers" on page 3 and 6.
A.0	11 June 2018	New Quick Reference Guide for the Ion AmpliSeq™ HD Library Kit.