

Targeted DNA Sequencing Library Preparation with the LP-48.48 IFC on Juno

For safety information and detailed procedures, see the Targeted DNA Sequencing Library Preparation with Juno Getting Started Guide (PN 101-0414) and the Juno System User Guide (PN 100-7070).

IMPORTANT Be certain that all reagents are thawed completely to room temperature and mixed thoroughly prior to use.

Determine the Number of Samples to be Sequenced in the Same Run

Determine the number of samples to pool based on the sequencing platform and desired read depth.

Prepare the Assay Pre-Mix

- In a DNA-free hood, combine the following components in a new 1.5 mL microcentrifuge tube:

Component		Volume per Assay Pool (μL)	Volume for 48 Assay Pools with Overage (μL)
TSP Assay Loading Reagent (PN 101-0409)	●	2.5	150
PCR Water (PN 100-5941)	○	27.5	1,650
Total		30.0	1,800

- Vortex the assay pre-mix for ≥20 seconds, and then briefly centrifuge it to bring down all components.

Prepare the 20X Assay Pools

- Obtain the Targeted DNA Seq Library Assays.
- Immediately before use, ensure that the stock assay plate is securely sealed, and then vortex for 10–20 seconds to mix. Centrifuge the assay plate at 3,000 x g for 5 minutes.
- In a DNA-free hood, combine the following components in wells of a new PCR plate or in 8-well strips according to the layout shown (see Figure 1):

Component	Volume per Assay Solution (μL)
Assay pre-mix (See Prepare the Assay Pre-Mix .)	30.0
Targeted DNA Seq Library Assays or PCR Water*	20.0
Total	50.0

*For unused assay inlets, replace the Targeted DNA Seq Library Assays with 20.0 μL of PCR Water.

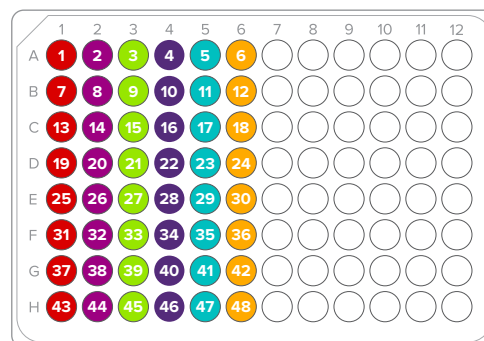


Figure 1. Layout of 20X assay pools and sample mixes

- Seal the assay plate with clear adhesive film or cap the 8-well strips.

Prepare the Sample Pre-Mix

- In a DNA-free hood, combine the following components in a new 1.5 mL microcentrifuge tube:

IMPORTANT Components must be combined in the order below. You add 4X TSP Master Mix to the PCR Water to dilute it before adding the remaining reagents.

Component		Volume per IFC (μL)
PCR Water (PN 100-5941)	○	33.6
4X TSP Master Mix (PN 101-3050)	●	90.0
TSP Sample Loading Reagent (PN 101-3049)	●	18.0
TSP DNA Polymerase (PN 101-0995)	●	14.4
Total		156.0

- Vortex the sample pre-mix for 10–20 seconds, and then briefly centrifuge it to bring down all components.
- Pipet 16 μL of the sample pre-mix into each well of a new 8-well strip.

Prepare Sample Mixes

- 1 Obtain one 96-well plate to prepare 48 individual sample mix solutions.
- 2 In a DNA sample hood, pipet into each well of the 96-well plate (follow the layout shown in Figure 1):

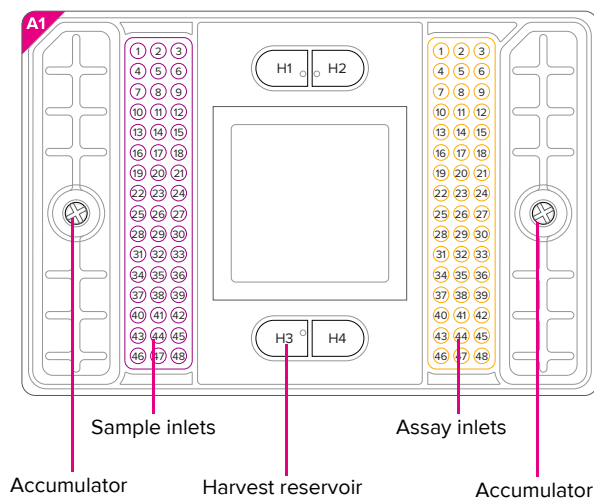
Component	Volume per Reaction (µL)
Sample pre-mix (See Prepare the Sample Pre-Mix.)	2.0
Genomic DNA sample, 50–100 ng/µL	2.0
Barcode primer (Fluidigm PN 101-0744)	1.0
Total	5.0

- 3 Seal each plate with clear adhesive film.

Prime the LP–48.48 IFC on Juno

IMPORTANT

- Control line fluid on the integrated fluidic circuit (IFC) or in the inlets makes the IFC unusable. Use a new IFC.
 - Vortex thoroughly and centrifuge all assay and sample mixes before pipetting into IFC inlets. Failure to do so may result in decreased data quality.
 - Do not go past the first stop on the pipette.
 - Before loading, ensure that there are no bubbles in the inlets.
- 1 Ensure that the Interface Plate MX is installed in the instrument. [See the Juno System User Guide (PN 100-7070).]
 - 2 Review the LP–48.48 IFC:



- 3 Pull the protective tape down and away from the bottom of the IFC. Do not invert the IFC.
- 4 Print the life-size map of the LP–48.48 IFC, place it under the IFC, and align the notched A1 corner at the upper left of the IFC with the pink corner of the IFC map. (For a life-size map, see the Targeted DNA Sequencing Library Preparation with Juno Getting Started Guide.)

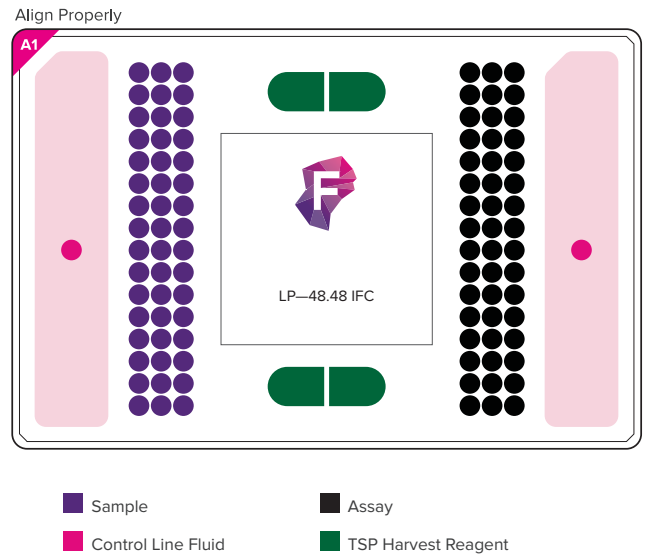


Figure 2. Map of the LP–48.48 IFC

- 5 Load an entire syringe of LP–48.48 Control Line Fluid into one accumulator and a second syringe into the other accumulator (dark pink circles in Figure 2). Use only LP–48.48 Control Line Fluid.
- 6 Pipet 650 µL of TSP Harvest Reagent (PN 101-0743) into each of the H1, H2, H3, and H4 harvest reservoirs (green hemispheres in Figure 2).
- 7 Place the IFC on the tray of the Juno™ instrument, and then tap **LOAD**. [For detailed instructions on Juno operation, see the Juno System User Guide (PN 100-7070).]
- 8 On the Juno Scripts screen, tap **Prime LP–48.48**. Priming the IFC takes ~5 minutes.
- 9 After the script is finished, tap **EJECT** to eject the IFC.

Load and Run the LP–48.48 IFC on Juno

- 1 Place the life-size map of the Juno LP–48.48 IFC under the IFC and align the notched A1 corner at the upper left of the IFC with the pink corner of the IFC map. (For a life-size map, see the Targeted DNA Sequencing Library Preparation with Juno Getting Started Guide.)
- 2 Immediately before transferring into the IFC, vortex the 20X assays and sample mixes for 10–20 seconds to mix, and then centrifuge both plates at 3,000 x g for 5 minutes. If necessary, remove any large bubbles from the wells and centrifuge again at 3,000 x g for 5 minutes.
- 3 Pipet 4.0 µL of each 20X assay pool into an assay inlet. [See Figure 2 (black circles) and the IFC pipetting scheme in Figure 3.]
- 4 Pipet 4.0 µL of each sample mix into a sample inlet. [See Figure 2 (dark purple circles) and the IFC pipetting scheme in Figure 3.]

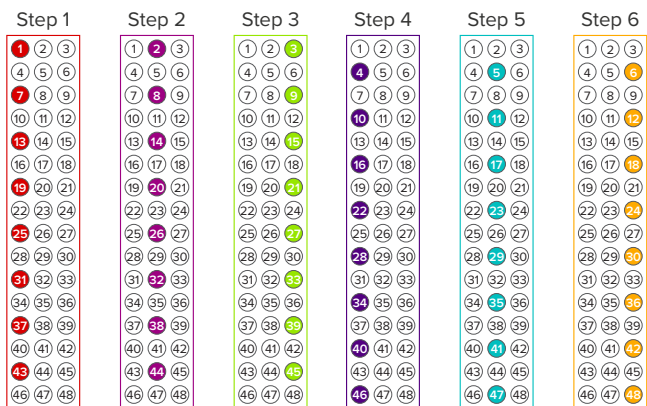
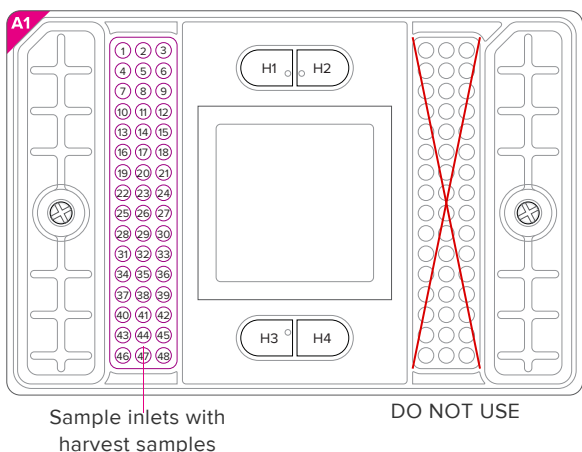


Figure 3. IFC pipetting scheme for samples and assay pools

- Cover sample and assay inlets with LP—48.48 Barrier Tape. (See the Targeted DNA Sequencing Library Preparation with Juno Getting Started Guide.)
- On Juno, tap **OPEN** and place the IFC on the tray, tap **LOAD**, and then tap **One Step LP—48.48**. Select when the script should finish (if necessary, adjust the harvest time), and then tap **RUN**.
- After the IFC is finished, tap **EJECT**.
IMPORTANT Eject the IFC ≤60 minutes after the run.
- After ejecting the IFC, immediately proceed to the next section.

Pool the Harvested Samples from the Calculated Number of Samples

- Confirm the number of samples to be sequenced together. (See the Targeted DNA Sequencing Library Preparation with Juno Getting Started Guide.) If the calculated number of samples is <48, create multiple pools.
- Review the map for harvesting samples:



- Carefully remove the LP—48.48 Barrier Tape from the sample chamber of the IFC by putting the IFC on a flat surface, holding the IFC with one hand, and slowly pulling the tab of the barrier tape until the tape is peeled away from the chamber

- Place the life-size map of the LP—48.48 IFC under the IFC and align the notched A1 corner at the upper left of the IFC with the pink corner of the IFC map. (For a life-size map, see the Targeted DNA Sequencing Library Preparation with Juno Getting Started Guide.)
- Set an 8-channel pipette to 12.0 μL to transfer the entire harvest volumes from the LP—48.48 IFC.
IMPORTANT Be sure to transfer the entire volume from each sample inlet for best barcode uniformity of mapped reads.
- Based on the desired pool size, combine entire harvest volumes from the appropriate number of samples directly into an 8-well strip.
- Combine volumes from each 8-well strip into a single new 1.5 mL microcentrifuge tube per sample pool.

STOPPING POINT Store the 1.5 mL tube of pooled samples at 4 °C for up to one week or at –20 °C for longer storage.

First Cleanup (0.4X/0.9X Double-Sided Solid-Phase Reversible Immobilization)

- Warm Agencourt® AMPure® XP magnetic beads to room temperature.
- Vortex the Agencourt AMPure XP magnetic beads vigorously to ensure that they are fully suspended.
- Prepare 5 mL of fresh 80% ethanol per library: Pipet 1 mL of DNase-free water into a graduated tube, and then add absolute ethanol to 5 mL. Cap the tube and mix.
- Vortex the Agencourt AMPure XP magnetic beads at high speed for 1 minute.
- In a new 1.5 mL microcentrifuge tube, pipet 150 μL of pooled samples. If the volume of pooled samples is <150 μL , add DNA Dilution Reagent or PCR Water to bring the volume to 150 μL .
- Pipet 60 μL of AMPure XP magnetic beads into the same tube with the 150 μL of pooled samples. Expel any beads left in the pipette tip by pipetting the suspension up and down 10 times.
- Vortex the suspension at high speed for 20 seconds.
- Incubate the suspension at room temperature for 10 minutes.
- Briefly centrifuge the tube, and then place the tube on a magnetic stand for 1–2 minutes.
- Keeping the tube on the magnetic stand, pipet the entire **supernatant** to a new tube.
- Use a P10 pipette to transfer any residual volume to ensure that all supernatant has been transferred.
- Pipet 75 μL of AMPure XP magnetic beads into the supernatant. Vortex the suspension at high speed for 20 seconds.
- Incubate the suspension at room temperature for 10 minutes.
- Briefly centrifuge the tube, and then place the tube on a magnetic stand for 1–2 minutes.
- Keeping the tube on the magnetic stand, remove and discard the supernatant.

- 16 Use a P10 pipette to remove any residual supernatant from the tube.
- 17 Keeping the tube on the magnetic stand, pipet 400 μL of 80% ethanol to wash the beads.
- 18 Incubate the tube at room temperature for 30–60 seconds.
- 19 Keeping the tube on the magnetic stand, remove and discard the ethanol.
- 20 Repeat steps 17–19 two more times. Remove all ethanol.
- 21 Remove the tube from the magnetic stand and dry the beads at 37 $^{\circ}\text{C}$ for 1 minute, or at room temperature for 10–15 minutes.

IMPORTANT Ensure that both the beads and tube are dry before proceeding, but be careful not to over dry the beads. If over dried, beads look cracked.
- 22 To the dried beads, pipet 30 μL of DNA Dilution Reagent (PN 100-9167). Vortex for 20 seconds.
- 23 Incubate the suspension at room temperature for 2 minutes.
- 24 Briefly centrifuge the tube, and then place the tube on a magnetic stand for 1–2 minutes.
- 25 Keeping the tube on the magnetic stand, pipet 30 μL of the eluate to a new tube.

STOPPING POINT Store at 4 $^{\circ}\text{C}$ for up to one week or at –20 $^{\circ}\text{C}$ for longer storage.

Second Cleanup (0.8X SPRI)

- 1 Vortex the AMPure XP magnetic beads at high speed for 20 seconds.
- 2 Pipet 24 μL of Agencourt AMPure XP magnetic beads into the same tube with the 30 μL of eluate from the first cleanup. Expel any beads left in the pipette tip by pipetting the suspension up and down 10 times.
- 3 Vortex the suspension at high speed for 20 seconds.
- 4 Incubate the suspension at room temperature for 10 minutes.
- 5 Briefly centrifuge the tube, and then place the tube on a magnetic stand for 1–2 minutes.
- 6 Keeping the tube on the magnetic stand, remove and discard the supernatant.
- 7 Use a P10 pipette to remove any residual supernatant from the tube.
- 8 Keeping the tube on the magnetic stand, pipet 190 μL of 80% ethanol to wash the beads.
- 9 Incubate the tube at room temperature for 30–60 seconds.
- 10 Keeping the tube on the magnetic stand, remove and discard the ethanol.
- 11 Repeat steps 8–10 two more times. Remove all ethanol.
- 12 Remove the tube from the magnetic stand and dry the beads at 37 $^{\circ}\text{C}$ for 1 minute, or at room temperature for 10–15 minutes.

IMPORTANT Ensure that both the beads and tube are dry before proceeding, but be careful not to over dry the beads. If over dried, beads look cracked.

- 13 To the dried beads, pipet 30 μL of DNA Dilution Reagent. Vortex 20 seconds.
- 14 Incubate the suspension at room temperature for 2 minutes.
- 15 Briefly centrifuge the tube, and then place the tube on a magnetic stand for 1–2 minutes.
- 16 Keeping the tube on the magnetic stand, pipet 30 μL of the eluate to a new tube.




STOPPING POINT Store the tube at 4 $^{\circ}\text{C}$ for up to 1 week –20 $^{\circ}\text{C}$ for longer storage.

Third Cleanup (0.8X SPRI) and Quality Control

- 1 Repeat steps 1–16 in **Second Cleanup (0.8X SPRI)** with 30 μL eluate from the second cleanup. This is the purified library (before sequencing adapter is added). Store the purified library from the third cleanup at 4 $^{\circ}\text{C}$ or perform QC.
- 2 Perform QC by estimating the purified library concentration by fluorometer and, if necessary, analyzing the purified library by Agilent® Bioanalyzer®.

Add the Sequencing Adapter to the Purified Library

- 1 Combine in a new PCR tube:

Component		Vol. per Reaction (μL)
4X TSP Master Mix (PN 101-3050)		7.5
TSP Adapter Mix * (PN 101-0408)		6.0
Purified library (before sequencing adapter is added)		4.5
PCR Water (PN 100-5941)		12.0
Total		30.0

*For dual barcoding, replace the TSP Adapter Mix with a dual index adapter mix from the Targeted DNA Seq Library Adapter Set (PN 101-2412).

- 2 Perform PCR using a stand-alone thermal cycler:

Description	Cycles	Temp.	Time
Hot start	1	95 $^{\circ}\text{C}$	15 min
PCR	10	95 $^{\circ}\text{C}$	15 sec
		60 $^{\circ}\text{C}$	90 sec
		68 $^{\circ}\text{C}$	90 sec
Final extension	1	68 $^{\circ}\text{C}$	3 min
Hold	1	4 $^{\circ}\text{C}$	∞

Clean Up the PCR Product (0.8X SPRI)

- 1 In a new 1.5 mL microcentrifuge tube, pipet 25 μ L of PCR product into 25 μ L of DNase-free water. Mix, and then briefly centrifuge the tube.
- 2 Ensure that the Agencourt AMPure XP magnetic beads are at room temperature, and then vortex the beads at high speed for 20 seconds.
- 3 Pipet 40 μ L of room temperature AMPure XP magnetic beads into the same tube with the 50 μ L of diluted PCR product. (See step 1.) Expel any beads left in the pipette tip by pipetting the suspension up and down 10 times.
- 4 Vortex the suspension at high speed for 20 seconds.
- 5 Incubate the suspension at room temperature for 10 minutes.
- 6 Briefly centrifuge the tube, and then place the tube on a magnetic stand for 1–2 minutes.
- 7 Keeping the tube on the magnetic stand, remove and discard the supernatant.
- 8 Use a P10 pipette to remove any residual supernatant from the tube.
- 9 Keeping the tube on the magnetic stand, pipet 190 μ L of 80% ethanol to wash the beads.
- 10 Incubate the tube at room temperature for 30–60 seconds.
- 11 Keeping the tube on the magnetic stand, remove and discard the ethanol.
- 12 Repeat steps 9–11 two more times. Completely remove and discard all of the 80% ethanol.
- 13 Remove the tube from the magnetic stand and dry the beads at 37 °C for 1 minute, or at room temperature for 10–15 minutes.
IMPORTANT Ensure that both the beads and tube are dry before proceeding, but be careful not to over dry the beads. If over dried, beads look cracked.
- 14 To the dried beads, pipet 45 μ L of DNA Dilution Reagent. Vortex for 20 seconds.
- 15 Incubate the suspension at room temperature for 2 minutes.
- 16 Briefly centrifuge the tube, and then place the tube on a magnetic stand for 1–2 minutes.
- 17 Keeping the tube on the magnetic stand, pipet the entire eluate to a labeled tube. The eluate is the sequencing library.
STOPPING POINT Store the sequencing library at 4 °C for up to one week or at –20 °C for longer storage.

- 18 Compare the purified library (before sequencing adapter is added) and the sequencing library (after sequencing adapter is added) using the Agilent Bioanalyzer to ensure that the library with the sequencing adapter passes QC requirements.
- 19 Sequence the sequencing library (after sequencing adapter is added) on an Illumina® sequencer. Perform data analysis.

For technical support visit fluidigm.com/support.

North America +1 650 266 6100 | Toll-free: 866 358 4354 in the US | techsupport@fluidigm.com
China (excluding Hong Kong) +86 21 3255 8368 | techsupportchina@fluidigm.com
All other Asian countries +1 650 266 6100 | techsupportasia@fluidigm.com

Europe +33 1 60 92 42 40 | techsupporteurope@fluidigm.com

Japan +81 3 3662 2150 | techsupportjapan@fluidigm.com

Central and South America +1 650 266 6100 | techsupportlatam@fluidigm.com

For Research Use Only. Not for use in diagnostic procedures.

Information in this publication is subject to change without notice. Use standard laboratory safety protocols. **Safety data sheet information:** fluidigm.com/sds. **Patent and license information:** fluidigm.com/legalnotices. **EU's WEEE directive information:** fluidigm.com/compliance. Fluidigm, the Fluidigm logo, and Juno are trademarks or registered trademarks of Fluidigm Corporation in the United States and/or other countries. All other trademarks are the sole property of their respective owners. © 2016 Fluidigm Corporation. All rights reserved. 07/2016