Targeted DNA Sequencing Library Preparation with the Juno LP—192.24 IFC

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

1 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 24 Assay Pools with Overage (µL)
TSP Assay Loading Reagent (Fluidigm PN 101-0409)	2.5	75
PCR Water (Fluidigm PN 100-5941)	37.5	1,125
Total	**40.0**	**1,200**

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

Prepare the 10X Assay Pools

1 Obtain the Targeted DNA Seq Library Assays.

2 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.

3 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 Pool (µL)
Assay pre-mix (See Prepare the Assay Pre-Mix.)	40.0
Targeted DNA Seq Library Assays or PCR water*	10.0
Total	**50.0**

*For unused assay inlets, replace the Targeted DNA Seq Library Assays with 10.0 µL of PCR Water.

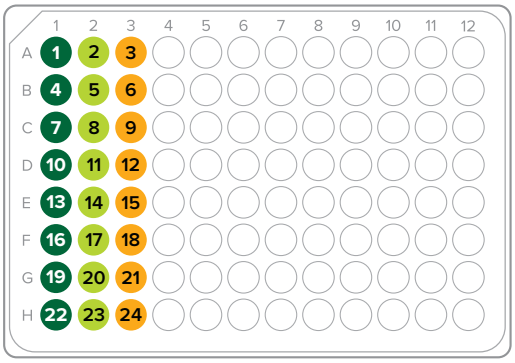


Figure 1. Layout of 10X Assay Pools

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

Prepare the Sample Pre-Mix

1 In a DNA-free hood, combine in a new 1.5 mL microcentrifuge tube:

Component if using kit PN 101-5668 or 101-5669*	Volume per Reaction (µL)	Volume per IFC (µL)
PCR Water	0.30	72
4X TSP Master Mix (Fluidigm PN 101-5786)	1.25	300
TSP Sample Loading Reagent (Fluidigm PN 101-0407)	0.25	60
TSP DNA Polymerase (Fluidigm PN 101-0995)	0.20	48
Total	**2.00**	**480**

*If you are using reagent kit PN 101-0406 or 101-2773, see the appendix on page 5 for the sample pre-mix volumes.

2 Vortex the sample pre-mix for 10–20 seconds, and then briefly centrifuge it to bring down all components.

Prepare Sample Mixes

1 Obtain two 96-well plates to prepare 192 individual sample mix solutions.

2 In a DNA sample hood, pipet into each 96-well plate:

Component if using kit PN 101-5668 or 101-5669*	Volume per Reaction (µL)
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**

*If you are using reagent kit PN 101-0406 or 101-2773, see the appendix on page 5 for the sample mix volumes.

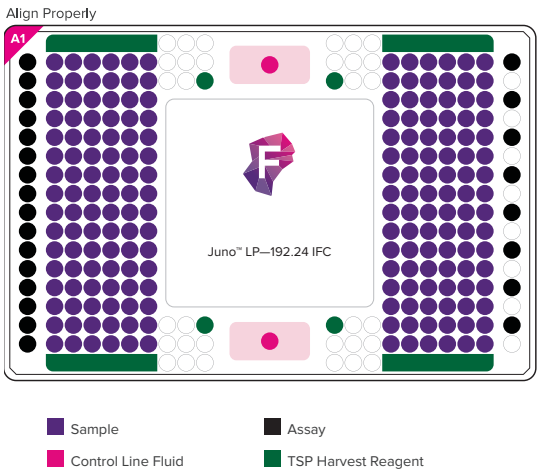
3 Seal each plate with clear adhesive film.

Load and Run the Juno LP—192.24 IFC

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 Review the map for the Juno LP—192.24 IFC:



2 Pull the protective tape down and away from the bottom of the IFC. Do not turn the IFC upside down.

3 Print the life-size map of the Juno LP—192.24 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.)

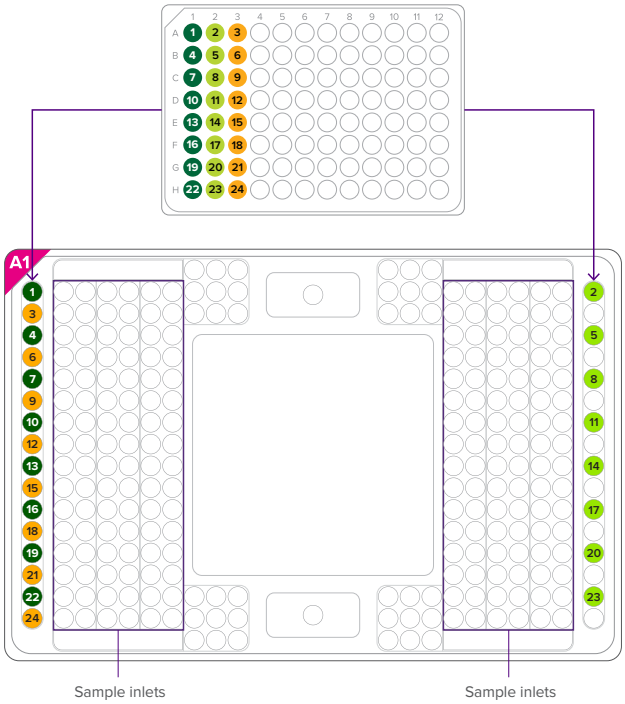
4 Load an entire syringe of Juno Control Line Fluid 192.24 into one accumulator and a second syringe into the other accumulator (pink circles on the map). Use only Juno Control Line Fluid 192.24.

5 Pipet 27 µL of TSP Harvest Reagent (Fluidigm PN 101-0743) into each of the P1, P2, P3, and P4 Inlets (green circles on the map in step 1).

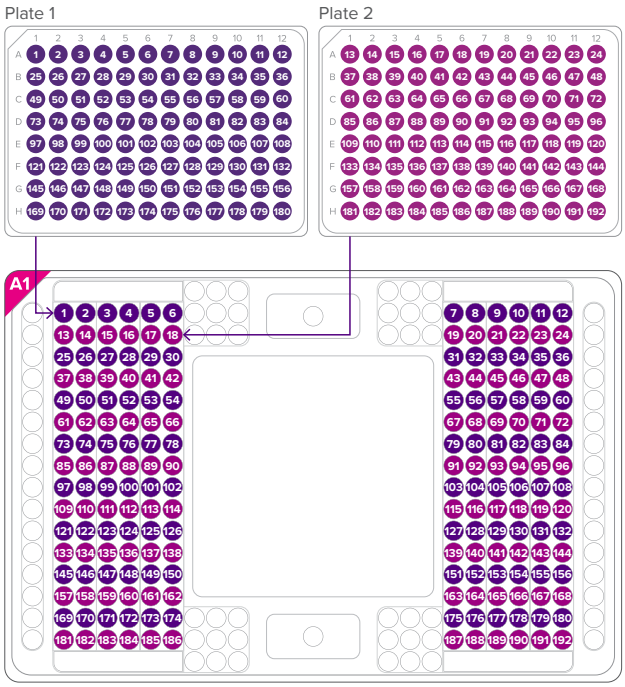
6 Pipet 220 µL of TSP Harvest Reagent into each of the H1, H2, H3, and H4 harvest reservoirs (green rectangles on the map).

7 Immediately before transferring into the IFC, vortex the 10X 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.

8 Pipet 3.5 µL of each 10X assay pool into an assay inlet (also see black circles on the map):



9 Pipet 3.5 µL of each sample mix into a sample inlet (also see dark purple circles on the map). The two different colors used here illustrate the pipetting scheme:



10 Cover all sample inlets with the Juno LP—192.24 Barrier Tape with the Barrier Tape Applicator. Be sure to gently pull one of the four tabs of the barrier tape up to remove the protective sheet and expose the adhesive surface. (For detailed instructions, see the Targeted DNA Sequencing Library Preparation with Juno Getting Started Guide.)

11 Ensure that the Interface Plate TX is installed in Juno. Tap **OPEN**.

12 Place the IFC on the tray, and then tap **One Step LP—192.24**. Select when the script should finish (if necessary, overnight harvest delay), and then tap **RUN**.

13 After the IFC is finished, tap **EJECT**.

IMPORTANT Eject the IFC ≤60 minutes after the run.

14 After ejecting the IFC, immediately proceed to the next section.

Pool the Harvested Samples from the Calculated Number of Samples

1 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 <192, create multiple pools.

2 Carefully remove the Juno LP—192.24 Barrier Tape from the IFC by putting the IFC on a flat surface, holding the IFC with one hand, and slowly peeling off the barrier tape with the other hand

3 Place the life-size map of the Juno LP—192.24 IFC under the IFC and align the notched A1 corner at the upper left of the IFC with the pink corner of the map. (For a life-size map, see the Targeted DNA Sequencing Library Preparation with Juno Getting Started Guide.)

4 In a post-PCR room, set an 8-channel pipette to 6.0 µL to transfer the entire harvest volumes from the Juno LP—192.24 IFC.

IMPORTANT Be sure to transfer the entire volume from each sample inlet for best barcode uniformity of mapped reads.

5 Based on the desired pool size, combine entire harvest volumes from the appropriate number of samples directly into an 8-well strip.

6 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 SPRI)

1 Remove the Agencourt® AMPure® XP magnetic beads from 4 °C, and then leave the beads at room temperature for 30 minutes before use.

2 Vortex the Agencourt AMPure XP magnetic beads vigorously to ensure that they are fully suspended.

3 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.

4 Vortex the Agencourt AMPure XP magnetic beads at high speed for 1 minute.

5 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.

6 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.

7 Vortex the suspension at high speed for 20 seconds.

8 Incubate the suspension at room temperature for 10 minutes.

9 Briefly centrifuge the tube, and then place the tube on a magnetic stand for 1–2 minutes.

10 Without disturbing the beads and keeping the tube on the magnetic stand, pipet the entire **supernatant** to a new tube.

11 Use a P10 pipette to transfer any residual volume to ensure that all supernatant has been transferred.

12 Pipet 75 µL of AMPure XP magnetic beads into the supernatant. Vortex the suspension at high speed for 20 seconds.

13 Incubate the suspension at room temperature for 10 minutes.

14 Briefly centrifuge the tube, and then place the tube on a magnetic stand for 1–2 minutes.

15 Without disturbing the beads and 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 Without disturbing the beads and 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: 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.

22 To the dried beads, pipet 30 µL of DNA Dilution Reagent (Fluidigm 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 °C for up to one week or at –20 °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 Without disturbing the beads and 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 Without disturbing the beads and 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: 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.

13 To the dried beads, pipet 30 µL of DNA Dilution Reagent. Vortex for 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 °C for up to 1 week or at –20 °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 °C or perform QC.

2 Perform QC by estimating the purified library concentration using a fluorometer and, if necessary, analyzing the purified library by Agilent® Bioanalyzer®.

Add the Sequencing Adapter to the Purified Library

1 In a post-PCR room, combine in a new PCR tube:

Component if using kit PN 101-5668 or 101-5669*	Volume per Reaction (µL)
4X TSP Master Mix (Fluidigm PN 101-5786)	7.5
TSP Adapter Mix (Fluidigm PN 101-0408)†	6.0
Purified library (before sequencing adapter is added)	4.5
PCR Water	12.0
Total	**30.0**

* **If you are using reagent kit PN 101-0406 or 101-2773,** see the appendix on page 5 for the sequencing adapter volumes.

† 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 °C	15 min
PCR	10	95 °C	15 sec
		60 °C	90 sec
		68 °C	90 sec
Final extension	1	68 °C	3 min
Hold	1	4 °C	∞

Clean Up the PCR Product (0.8X SPRI)

1. In a post-PCR room, in a new 1.5 mL microcentrifuge tube, pipet 25 µL of PCR product into 25 µL 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 µL of room temperature AMPure XP magnetic beads into the same tube with the 50 µL 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. Without disturbing the beads and keeping the tube on the magnetic stand, remove and discard the supernatant.
8. Use a P10 pipette to remove any residual supernatant from tube.
9. Keeping the tube on the magnetic stand, pipet 190 µL of 80% ethanol to wash the beads.
10. Incubate the tube at room temperature for 30–60 seconds.
11. Without disturbing the beads and 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: 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 µL 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 new, 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. Estimate the concentration of the sequencing library (after sequencing adapter is added) by using a fluorometer.
19. Compare the purified library (before sequencing adapter is added) to the sequencing library (after sequencing adapter is added) using the Agilent Bioanalyzer to ensure that the library with the sequencing adapter passes QC requirements.
20. Sequence the sequencing library (after sequencing adapter is added) on an Illumina® sequencer. Perform data analysis.

Appendix: Reagent Mixes for Kits PN 101-0406 and 101-2773

Sample Pre-Mix

Component	Volume per Reaction (µL)	Volume per IFC (µL)
TSP Master Mix (Fluidigm PN 101-0994)	2.50	600
TSP Sample Loading Reagent (Fluidigm PN 101-0407)	0.25	60
TSP DNA Polymerase (Fluidigm PN 101-0995)	0.20	48
PCR Water	0.05	12
Total	**3.00**	**720**

Sample Mix

Component	Volume per Reaction (µL)
Sample pre-mix	3.0
Genomic DNA sample, 100–200 ng/µL	1.0
Barcode primer (Fluidigm PN 101-0744)	1.0
Total	**5.0**

Sequencing Adapter

Component	Volume per Reaction (µL)
TSP Master Mix (Fluidigm PN 101-0994)	15.0
TSP Adapter Mix (Fluidigm PN 101-0408)*	6.0
Purified library (before sequencing adapter is added)	4.5
PCR Water	4.5
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).

For technical support visit fluidigm.com/support.

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