Targeted DNA Sequencing Library Preparation with the LP—48.48 IFC on Access Array

For safety information and detailed procedures, see the IFC Controller AX User Guide (PN 68000157), the Targeted DNA Sequencing Library Preparation with Access Array™ Getting Started Guide (PN 101-2737), and the FC1 User Guide (PN 100-1279).

IMPORTANT This guide is for preparing targeted DNA sequencing libraries with Targeted DNA Seq Library Reagent kits only. To prepare sequencing libraries with Access Array reagents, see the Access Array System for Illumina Sequencing Systems User Guide (PN 100-3770).

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

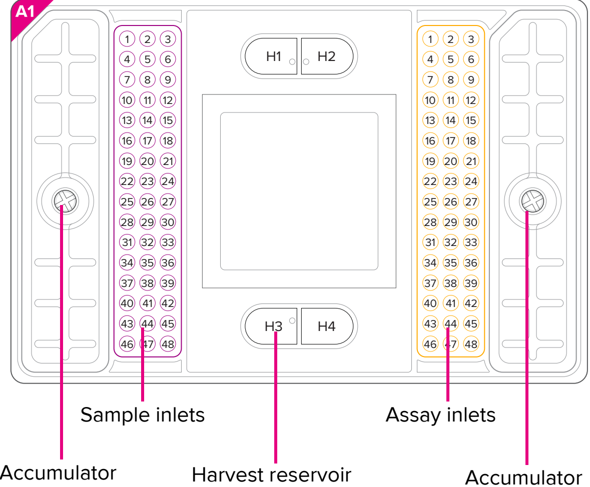
Determine the number of samples to pool based on the sequencing platform and desired read depth.

Prime the LP—48.48 IFC

IMPORTANT

- Use the LP—48.48 IFC (integrated fluidic circuit) within 24 hours after opening package.
- Load the IFC ≤60 minutes after priming.
- Be certain that all reagents are thawed completely to room temperature and mixed thoroughly prior to use.
- Due to different accumulator volumes, only use 48.48 syringes containing LP—48.48 Control Line Fluid.
- Control line fluid on IFC or in the inlets makes IFC unusable.

1 Review the LP—48.48 IFC:



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

3 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 Access Array Getting Started Guide.)

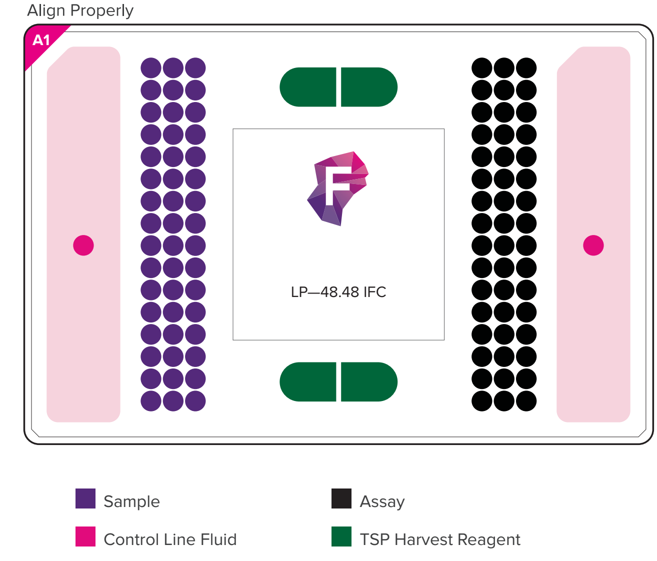


Figure 1. Map of the LP—48.48 IFC

4 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 1). Use only LP—48.48 Control Line Fluid.

5 Pipet 500 µL of TSP Harvest Reagent (PN 101-0743) into each of the H1, H2, H3, and H4 harvest reservoirs (green hemispheres in Figure 1 and see diagram in Prime the LP—48.48 IFC).

6 Place the IFC into the pre-PCR IFC Controller AX land run script **PRIME (155x)**.

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 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**

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

Prepare the 20X 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 2):

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.

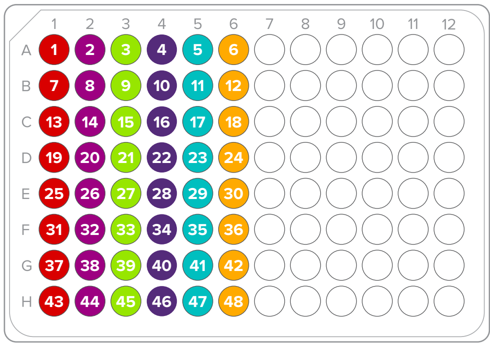


Figure 2. Layout of 20X assay pools and sample mixes

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 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**

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

Prepare Sample Mixes

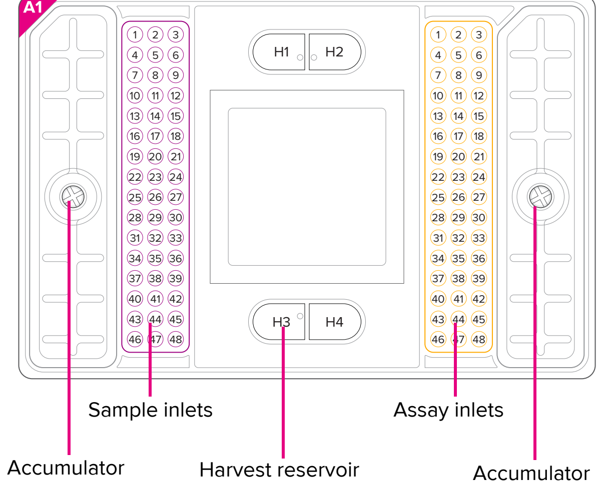
1. Obtain one 96-well plate to prepare 48 individual sample mix solutions.
2. In a DNA sample hood, pipet these components into each well of the new 96-well plate (follow the layout shown in Figure 2):

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 the plate with clear adhesive film.

Load the LP—48.48 IFC

1. Review the LP—48.48 IFC:



IMPORTANT
- 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.

2. 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 Access Array Getting Started Guide.)

3 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.

4 Pipet 4.0 µL of 20X assay pool into each of the assay inlets. [See Figure 1 (black circles) and the IFC pipetting scheme in Figure 3.]

5 Pipet 4.0 µL of sample mix into each of the sample inlets. [See Figure 1 (dark purple circles) and the IFC pipetting scheme in Figure 3.]

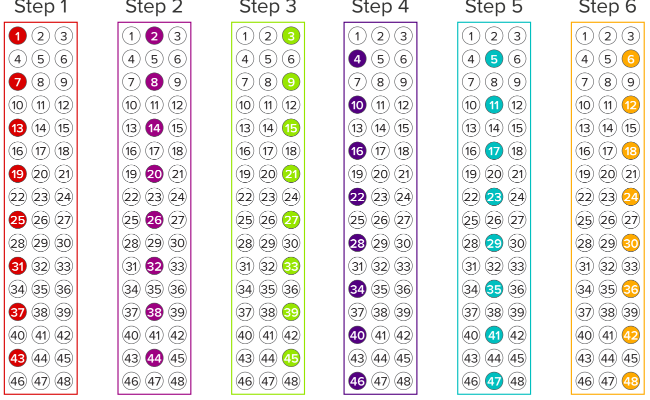


Figure 3. IFC pipetting scheme for samples and assay pools

6 Place the IFC into the pre-PCR IFC Controller AX and run script **LOAD MIX (155x)**.

Thermal-Cycle the LP—48.48 IFC

Place the IFC onto the Fluidigm FC1™ cycler and start PCR by selecting the **LP—48x48 PCR** protocol. [(See the FC1 Cycler Usage Quick Reference (PN 100-1250).]

Harvest the LP—48.48 IFC

1 After PCR has finished, move the LP—48.48 IFC into the post-PCR lab for harvesting.

2 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 Access Array Getting Started Guide.)

3 Remove remaining fluids from the H1–H4 harvest reservoirs [See Figure 1 (green hemispheres) and diagram in Prime the LP—48.48 IFC.]

4 Pipet 650 µL of fresh TSP Harvest Reagent into the H1–H4 harvest reservoirs. [See Figure 1 (green hemispheres) and diagram in Prime the LP—48.48 IFC.]

5 Pipet 2 µL of TSP Harvest Reagent into each of the sample inlets on the IFC:

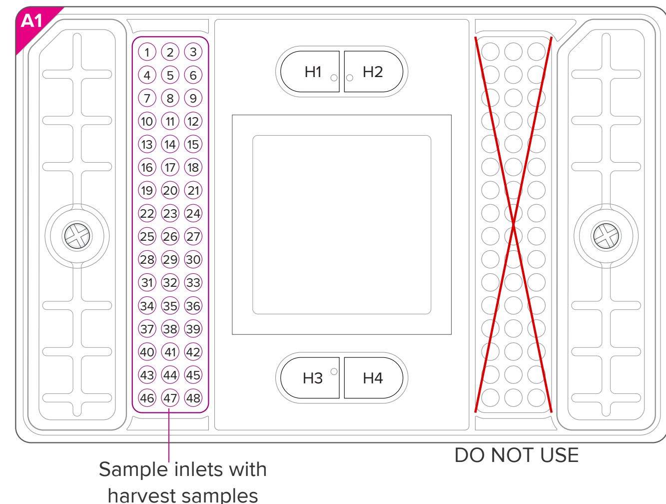


Figure 4. Map for harvesting samples from the LP—48.48 IFC

6 Place the IFC into the post-PCR IFC Controller AX and run script **HARVEST (155x)**.

7 When the script has finished, remove the IFC from the post-PCR IFC Controller AX.

8 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 Access Array Getting Started Guide.) If the calculated number of samples is <48, create multiple pools.

2 Review the map for harvesting samples. (See Figure 4.)

3 Set an 8-channel pipette to 14.0 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.

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

5 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)

1. Warm Agencourt® AMPure® XP magnetic beads to room temperature.
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. 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. 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 °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 °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. 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 °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 –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 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 standard 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 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. 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 µL 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 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 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.

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