NovaSeq Xp Workflow Checklist

This document is not a replacement for the *NovaSeq 6000 System Guide (document # 1000000019358)*. Use the most recent versions of the system guide and NovaSeq Control Software.

Prepare Reagents

☐ 1 Thaw SBS and cluster (CPE) cartridges in room temperature water as follows.

Cartridge	Duration of Thaw
SP, S1, and S2 SBS cartridge	4 hours
SP, S1, and S2 cluster cartridge	Up to 2 hours
S4 SBS cartridge	4 hours
S4 cluster cartridge	Up to 4 hours

☐ 2 Thoroughly dry the cartridge bases.
☐ 3 Blot the foil seals dry if needed.
☐ 4 Invert each cartridge 10 times.
☐ 5 Gently tap the bottom of each cartridge on the bench.
☐ 6 **[Optional]** Store thawed reagents at 2°C to 8°C for up to 24 hours.

Prepare Instrument

☐ 1 Complete the post-run wash.
☐ 2 Empty used reagent bottles and return them to the buffer drawer.

Pool and Dilute Library

☐ 1 Bring a flow cell package to room temperature for at least 10 minutes.
☐ 2 Thaw DPX1, DPX2, and DPX3, and then place on ice.
☐ 3 Pool libraries to the desired plexity.

Mode	Total Volume of Pool Per Lane (µl)
SP/S1	18
S2	22
S4	30

☐ 4 Dilute library to final loading concentration as follows.

Library Type	Final Loading Concentration (pM)	Pooled Loading Concentration (nM)
DNA PCR-free library pool	115–235	0.575–1.175
DNA PCR-amplified library pool	200–400	1.0–2.0
Single Cell	175–275	.875–1.375

Denature Library

☐ 1 Prepare 0.2 N NaOH by diluting stock NaOH with laboratory-grade water.
☐ 2 **[Optional]** Spike-in 1% nondenatured PhiX as follows.
  ☐ a Dilute 10 nM PhiX to 0.25 nM using 10 mM Tris-HCl, pH 8.5.
  ☐ b For each lane, add the appropriate volume of PhiX to the tube of nondenatured library pool.

Mode	Nondenatured 0.25 nM PhiX (µl)	Nondenatured Library Pool (µl)
SP/S1	0.7	18
S2	0.8	22
S4	1.1	30

☐ 3 For each lane, add 0.2 N NaOH to the nondenatured library as follows.

Mode	0.2 N NaOH (µl)	Nondenatured Library Pool (µl)
SP/S1	4.0	18.0
S2	5.0	22.0
S4	7.0	30.0

☐ 4 Cap and then vortex briefly.
☐ 5 Incubate at room temperature for 8 minutes.
☐ 6 For each lane, add 400 mM Tris-HCl, pH 8.0 to neutralize as follows.

Mode	400 mM Tris-HCl, pH 8.0 (µl)	Resulting Volume
SP/S1	5.0	27.0 µl, or 27.7 µl with PhiX
S2	6.0	33.0 µl, or 33.8 µl with PhiX
S4	8.0	45.0 µl, or 46.1 µl with PhiX

☐ 7 Cap and then vortex briefly.
☐ 8 Place on ice until use.

SAFE STOPPING POINT

If you cannot immediately proceed, cap the tube and store at -25°C to -15°C for up to three weeks.

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Prepare ExAmp Master Mix

- ☐ 1 Place flow cell onto the flow cell dock and place the manifold over the flow cell.
- ☐ 2 Close the clamp.
- ☐ 3 Invert or vortex briefly to mix DPX1 and DPX2.
- ☐ 4 Briefly vortex DPX3 to mix.
- ☐ 5 Briefly centrifuge DPX1, DPX2, and DPX3.
- ☐ 6 For each flow cell, combine the following volumes in a suitable microcentrifuge tube in the order specified.

Addition Order	Reagent*	Volume for Two-Lane Flow Cell (SP/S1/S2) (µl)	Volume for Four-Lane Flow Cell (S4) (µl)
1	DPX1	126	315
2	DPX2	18	45
3	DPX3	66	165

*DPX reagent tube caps may be color coded (red, yellow, and blue for DPX1, DPX2, and DPX3, respectively). Make sure that color coding is preserved when replacing tube caps.

- ☐ 7 These volumes result in 210 µl ExAmp master mix for SP, S1, or S2 mode, or 525 µl Master Mix for S4 mode.
- ☐ 8 **Pipette and dispense slowly to avoid bubbles.**
- ☐ 9 Vortex for 20–30 seconds.
- ☐ 10 Centrifuge at up to 280 × g for up to 1 minute.
- ☐ 11 **For the best sequencing performance, immediately proceed to the next step. If necessary, ideal storage of the master mix is up to 1 hour on ice. Use within 30 minutes if storing at room temperature.**

Load Library Onto the Flow Cell

- ☐ 1 For each lane, add ExAmp Master Mix to each denatured library pool as follows.

Mode	Denatured Library Pool (µl)	ExAmp Master Mix (µl)	Resulting Volume (µl)
SP/S1	27	63	90
S2	33	77	110
S4	45	105	150

- ☐ 2 If using tube strips, pipette to mix until homogenous.
- ☐ 3 Centrifuge at up to 280 × g for up to 1 minute.
- ☐ 4 Add the appropriate volume of library/ExAmp mixture to each manifold well.

Mode	Library/ExAmp Mixture per Well (µl)
SP/S1	80
S2	95
S4	130

- ☐ 5 After adding the ExAmp/library mixture to all manifold wells, wait approximately 2 minutes for the mixture to reach the opposite end of each lane.
- ☐ 6 **Start the sequencing run within 30 minutes of loading libraries onto the flow cell.**

Load Flow Cell Onto the Instrument

- ☐ 1 From the Home screen, select **Sequence**, and then select a single or dual flow cell run:
  - **A+B**—Set up a dual flow cell run.
  - **A**—Set up a single flow cell run on side A.
  - **B**—Set up a single flow cell run on side B.
- ☐ 2 Remove flow cell from the flow cell dock:
  - ☐ a Open the clamp that secures the flow cell and manifold.
  - ☐ b Carefully remove and discard the manifold.
  - ☐ c Grasp the sides of the flow cell and remove it from the dock.
- ☐ 3 Load flow cell onto the instrument:
  - ☐ a Invert the flow cell around the long axis.
  - ☐ b Place the flow cell on the flow cell stage, and then select **Close Flow Cell Door**.

Load Cartridges Onto the Instrument

- ☐ 1 Place an empty library tube into position #8 of the cluster cartridge.
- ☐ 2 Remove the used SBS and cluster cartridges.
- ☐ 3 Load the SBS and cluster cartridges into the reagent chiller drawer.
- ☐ 4 Remove the used buffer cartridge.
- ☐ 5 Place a new buffer cartridge into the buffer drawer.

Start Sequencing

- ☐ 1 **[Optional]** Sign in to BaseSpace Sequence Hub.
- ☐ 2 Select **Run Setup**, and then **NovaSeq Xp**.
- ☐ 3 Enter the required parameters and settings.
- ☐ 4 Select **Start Run**.