**Nextera XT DNA Library Prep Kit Protocol: Part 1/4 - Tagment Genomic DNA and Amplify Libraries**

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Part 1 of 4: Tagment Genomic DNA and Amplify Libraries

Links:

* [Part 1: Tagment and Amplify](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part1)
* [Part 2: Clean Up Libraries](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part2)
* [Part 3: Normalize Libraries](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part3)
* [Part 4: Pool Libraries](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part4)

With this protocol, your robot can perform the Nextera XT DNA Library Prep Kit protocol describe by the [Illumina Reference Guide](https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/samplepreps_nextera/nextera-xt/nextera-xt-library-prep-reference-guide-15031942-03.pdf).

This is part 1 of the protocol, which includes the steps (1) Tagment Genomic DNA and (2) Amplify Libraries. The Tagmentation step uses Nextera transposase to fragment DNA into sizes suitable for sequencing, and then tags the DNA with adapter sequences. The library amplification step increases the yield of the tagmented DNA using PCR. PCR adds the Index 1 (i7), Index 2 (i5), and full adapter sequences to the tagmented DNA from the previous step. This protocol assumes you are taking your plate off the OT-2 and thermocycling on a stand-alone PCR machine according to the [Illumina Reference Guide](https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/samplepreps_nextera/nextera-xt/nextera-xt-library-prep-reference-guide-15031942-03.pdf).

After the two steps carried out in this protocol, you can safely stop work and return to it at a later point. If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days.

**Process**

1. Input your number of samples.
2. Download your protocol.
3. Upload your protocol into the [OT App](https://opentrons.com/ot-app).
4. Set up your deck according to the deck map below.
5. Calibrate your labware, tiprack and pipette using the OT App. For calibration tips, check out our [support article](https://support.opentrons.com/ot-2/getting-started-software-setup/deck-calibration).
6. Hit "Run".

**Additional Notes**

* 2-mL Tuberack Reagent Setup:
  + Amplicon Tagment Mix: **A1**
  + Tagment DNA Buffer: **B1**
  + Neutralize Tagment Buffer: **C1**
  + Nextera PCR Master Mix: **D1**
  + Index 1 (i7) adapters: **A2-D3**
  + Index 2 (i5) adapters: **A5-D6**
* If number of samples is less than 25, arrange 6 tubes of Index 1 (A2-B3) and 4 tubes of Index 2 (A5-D5) in the tuberack. Otherwise, fill Index 1 and Index 2 according to the tuberack setup as instructed above.
* Review the reference guide before proceeding to confirm kit contents and make sure you have the required equipment and consumables.

**Nextera XT DNA Library Prep Kit Protocol: Part 2/4 - Clean Up Libraries**

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Part 2 of 4: Clean Up Libraries

Links:

* [Part 1: Tagment and Amplify](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part1)
* [Part 2: Clean Up Libraries](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part2)
* [Part 3: Normalize Libraries](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part3)
* [Part 4: Pool Libraries](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part4)

With this protocol, your robot can perform the Nextera XT DNA Library Prep Kit protocol describe by the [Illumina Reference Guide](https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/samplepreps_nextera/nextera-xt/nextera-xt-library-prep-reference-guide-15031942-03.pdf).

This is Part 2 of the protocol, which consists of just step (3) of the overall process: clean up libraries. This step uses AMPure XP beads to purify the library DNA and remove short library fragments after the previous step, library amplification.

After this step, it is safe to stop the workflow and return to it at a later point. If you are stopping, seal the plate and store at -15°C to -25°C for up to seven days.

### Process

1. Input your number of samples (make sure it is consistent with what you entered in Part 1).
2. Input your desired volume of the PCR product.
3. Input your desired bead ratio (use 1.8 if the input size is in the range 300-500 bp, use 0.6 if greater 500 bp).
4. Input your desired dry time.
5. Download your protocol.
6. Load your protocol into the [OT App](https://opentrons.com/ot-app).
7. Set up your deck according to the deck map.
8. Calibrate your labware, tiprack, and pipette using the OT App. For calibration tips, check out our [support article](https://support.opentrons.com/ot-2/getting-started-software-setup/deck-calibration).
9. Hit "Run".

### Additional Notes

* Trough Setup:
  + Resuspension Buffer: **A1**
  + AMPure XP Beads: **A2**
  + 80% Ethanol: **A3**
* Review the reference guide before proceeding to confirm kit contents and make sure you have the required equipment and consumables.

# Nextera XT DNA Library Prep Kit Protocol: Part 3/4 - Normalize Libraries

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Part 3 of 4: Normalize Libraries

Links:

* [Part 1: Tagment and Amplify](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part1)
* [Part 2: Clean Up Libraries](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part2)
* [Part 3: Normalize Libraries](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part3)
* [Part 4: Pool Libraries](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part4)

With this protocol, your robot can perform the Nextera XT DNA Library Prep Kit protocol describe by the [Illumina Reference Guide](https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/samplepreps_nextera/nextera-xt/nextera-xt-library-prep-reference-guide-15031942-03.pdf).

This is part 3 of the protocol, which is step (4) of the overall workflow: normalize libraries. This step normalizes the concentration of each library to ensure correct library representation in the donwstream pooled libraries.

It is safe to stop after this step and re-start work at a later point. If you are stopping, seal the plate and store at -25°C to -15°C for up to seven days.

### Process

1. Input your number of samples (make sure it is consistent with part 1, and 2).
2. Download your protocol.
3. Upload your protocol into the [OT App](https://opentrons.com/ot-app).
4. Set up your deck according to the deck map.
5. Calibrate your labware, tiprack and pipette using the OT App. For calibration tips, check out our [support article](https://support.opentrons.com/ot-2/getting-started-software-setup/deck-calibration).
6. Hit "Run".

### Additional Notes

* Tuberack Setup:
  + Library Normalization Additives 1: **A1**
  + Library Normalization Beads 1: **B1**
  + Library Normalization Wash 1: **C1**
  + Library Normalization Storage Buffer 1: **D1**
  + 0.1 N NaOH: **A2**
* Review the reference guide before proceeding to confirm kit contents and make sure you have the required equipment and consumables.

# Nextera XT DNA Library Prep Kit Protocol: Part 4/4 - Pool Libraries

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Part 4 of 4: Pool Libraries

Links:

* [Part 1: Tagment and Amplify](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part1)
* [Part 2: Clean Up Libraries](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part2)
* [Part 3: Normalize Libraries](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part3)
* [Part 4: Pool Libraries](https://protocol-delivery.protocols.opentrons.com/protocol/872-cosmosid-ngs-library-prep-part4)

With this protocol, your robot can perform the Nextera XT DNA Library Prep Kit protocol describe by the [Illumina Reference Guide](https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/samplepreps_nextera/nextera-xt/nextera-xt-library-prep-reference-guide-15031942-03.pdf).

This is part 4 of the protocol, which is step (5) of the overall workflow: pool libraries. This step combines equal volumes of normalized libraries into a single tube so they can all be loaded onto the sequencer at once. After pooling, follow the instructions for your sequencer to dilute and heat-denature the library pool before loading libraries for the sequencing run.

Store unused pooled libraries in the PAL tube or SGP plate at -25°C to -15°C for up to 7 days.

### Process

1. Input your number of samples (make sure it is consistent with part 1, 2, and 3).
2. Input the number of pooled libraries you would like to generate.
3. Input the volume of each library to be transferred to the tube.
4. Download your protocol.
5. Load your protocol into the [OT App](https://opentrons.com/ot-app).
6. Set up your deck according to the deck map.
7. Calibrate your labware, tiprack and pipette using the OT App. For calibration tips, check out our [support article](https://support.opentrons.com/ot-2/getting-started-software-setup/deck-calibration).
8. Hit "Run".

### Additional Notes

* Review the reference guide before proceeding to confirm kit contents and make sure you have the required equipment and consumables.