# Ion Total RNA-Seq Kit v2 for Whole Transcriptome Libraries

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This quick reference covers library preparation for up to 200-base-read sequencing on the Ion PGM™, Ion Proton™, Ion S5™, and Ion S5™ XL Systems.

**Note:** For safety and biohazard guidelines, refer to the "Safety" section in the *Ion Total RNA-Seq Kit v2 User Guide* (Pub. No. MAN0010654). For every chemical, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

## Fragment the whole transcriptome RNA

See the *Ion Total RNA-Seq Kit v2 User Guide* (Pub. No. MAN0010654) for detailed guidelines for preparing RNA.

### Fragment the RNA using RNase III

1. On ice, assemble a reaction for each RNA sample in a 0.2-mL PCR tube:

| Order | Component (add in order shown) | Volume per reaction |
|---|---|---|
| 1 | RNA sample and Nuclease-free Water:<br>• Poly(A) RNA: 1–500 ng<br>• rRNA-depleted total RNA: 10–500 ng<br>• WT Control RNA: 500 ng | 8–10 µL |
| 2 | 10X RNase III Reaction Buffer | 1 µL |
| 3 | RNase III | 1 µl |
| — | **Total Volume** | **10–12 µL** |

**IMPORTANT!** To reduce fragmentation variability, accurately pipet 1 µL of 10X RNase III Reaction Buffer and 1 µL of RNase III to each sample. Do not make a master mix that contains only 10X RNase III Reaction Buffer and RNase III.

2. Flick the tube or pipet up and down five times to mix, then centrifuge briefly to collect the liquid in the bottom of the tube.

3. Incubate the reaction in a thermal cycler at 37°C according to library and input quantity:

| RNA type | Amount | Reaction time |
|---|---|---|
| Poly(A) RNA | 1 to <100 ng | 3 min |
| | 100–500 ng | 10 min |
| rRNA-depleted RNA | 10 to <100 ng | 3 min |
| | 100–500 ng | 10 min |
| Total RNA | 500 ng | 10 min |

4. *Immediately* after the incubation, add 20 µL of Nuclease-free Water, then place the fragmented RNA on ice.

**IMPORTANT!** Proceed immediately to the next section, "Purify the fragmented RNA," or leave the fragmented RNA on ice for less than 1 hour.

### Purify the fragmented RNA

#### Before you begin

- Add 44 mL of 100% ethanol to the bottle of Wash Solution Concentrate and mix well. Mark the label on the bottle to indicate that you added ethanol. Store the solution at room temperature (15°C to 30°C).
- If you see a white precipitate in the Binding Solution Concentrate, warm the solution at 37°C, then shake the solution to dissolve any precipitate before use.
- Incubate the Nuclease-free Water at 37°C for ≥5 minutes.

#### Purify the fragmented RNA

1. Prepare beads for each sample:
   a. Gently vortex the Nucleic Acid Binding Beads tube to completely resuspend the magnetic beads.
   b. Add 5 µL beads to wells on the Processing Plate.
   c. Add 90 µL Binding Solution Concentrate to each well, then mix the Concentrate and beads by pipetting up and down ten times.
2. Bind the fragment RNA products to the beads:
   a. Transfer each 30-µL fragment RNA reaction to a bead-containing well on the Processing Plate.

b. Set a P200 pipettor at 150 µL. Attach a new 200-µL tip to the pipettor, then pre-wet the tip with 100% ethanol by pipetting the ethanol up and down three times.

c. Without changing tips, add 150 µL of 100% ethanol to each well.

**IMPORTANT!** While dispensing the ethanol, do not force out the last drops. Remove the last drop by touching the drop to the well wall. Change the tip and repeat steps 2b and 2c for the remaining wells only if the tip touches the wall. Accurate pipetting of 100% ethanol is critical for best results.

d. Set a single or multi-channel P200 pipettor at 150 µL. Attach new 200-µL tips to the pipettor, then mix the suspension in each well thoroughly by pipetting the wells up and down ten times.

**Note:** The color of the mixture should be homogeneous after mixing.

e. Incubate the samples for 5 minutes at room temperature off of the magnetic stand.

3. Remove the supernatant from the beads:

a. Place the Processing Plate on a magnetic stand for 5–6 minutes to separate the beads from the solution. Wait for the solution to clear before proceeding to the next step.

b. Leave the Processing Plate on the magnetic stand, then aspirate and discard the supernatant from the plate.

**IMPORTANT!** Do not disturb the magnetic beads. If any beads are aspirated, leave 2–3 µL of supernatant behind.

4. Wash the beads with Wash Solution Concentrate with ethanol:

a. Leave the Processing Plate on the magnetic stand.

b. Add 150 µL of Wash Solution Concentrate with ethanol to each sample.

c. Incubate the samples at room temperature for 30 seconds.

5. Remove the supernatant from the beads:

a. Aspirate and discard the supernatant from the plate.

b. Use a P10 or P20 pipettor to remove any residual liquid.

**IMPORTANT!** Do not disturb the separated magnetic beads. Remove all of the Wash Solution Concentrate from each well.

c. Air-dry the beads at room temperature to remove all traces of ethanol by leaving the Processing Plate on the magnetic stand for 1–2 minutes.

**IMPORTANT!** Do not overdry the beads (overdried beads appear cracked). Overdrying significantly decreases elution efficiency.

6. Elute the fragmented RNA from the beads:

a. Remove the Processing Plate from the magnetic stand.

b. Add 12 µL of pre-warmed (37°C) Nuclease-free Water to each sample, then mix the Nuclease-free Water and beads by pipetting up and down ten times.

c. Incubate at room temperature for 1 minute.

d. Place the Processing Plate on the magnetic stand for 1 minute to separate the beads from the solution. Wait for the solution to clear before proceeding to the next step.

e. For each sample, collect the eluant.

## Assess the yield and size distribution of fragmented RNA

**Note:** We do not recommend evaluating the yield and size for poly(A) fragmented RNA samples from <5-ng poly(A) RNA due to low input amount.

1. Quantify the yield of the fragmented RNA using the Qubit™ RNA Assay Kit with the Qubit™ Fluorometer.

   See the *Qubit™ RNA Assay Kit Protocol* (Pub. No. MAN0002327) or the *Qubit™ 3.0 Fluorometer User Guide* (Pub. No. MAN0010866) for instructions.

2. Evaluate the size distribution of the fragmented RNA:

a. If needed, dilute 1 µL of the sample to 50–5000 pg/µL with Nuclease-free Water.

b. Run the diluted sample on an Agilent™ 2100 Bioanalyzer™ instrument with the RNA 6000 Pico Kit. Follow the manufacturer's instructions for performing the assay.

c. Using the 2100 expert software, review the size distribution. The fragmentation procedure produces a distribution of RNA fragment sizes from 35 nt to several hundred or a few thousand nt, depending on your sample type. The average size is 100–200 nt.

**Note:** For instructions on how to review the size distribution, see the *Agilent™ 2100 Bioanalyzer™ Expert User's Guide* (Pub. No. G2946-90004).

If the profile for the fragmented RNA does not meet the typical results, see the "Troubleshooting" section in the *Ion Total RNA-Seq Kit v2.0 User Guide* (Pub. No. MAN0010656) for guidance.

3. Proceed according to the amount of fragmented RNA you have in 3 µL:

| Amount of fragmented RNA in 3 µL | Action |
|---|---|
| • ≥50 ng of poly(A) RNA<br>• ≥100 ng of rRNA-depleted total RNA<br>• ≥100 ng of WT Control RNA | Proceed to "Construct the whole transcriptome library."<br>Store the remaining RNA at –86°C to –68°C. |

| Amount of fragmented RNA in 3 µL | Action |
|---|---|
| • <50 ng of poly(A) RNA<br>• <100 ng rRNA-depleted total RNA | 1. Dry all of the RNA completely in a centrifugal vacuum concentrator at low or medium heat (≤40°C); this takes 10–20 minutes.<br>2. Resuspend in 3 µL of Nuclease-free Water, then proceed to "Construct the whole transcriptome library." |

## Construct the whole transcriptome library

IMPORTANT! The Ion Adaptor Mix v2, Ion RT Primer v2, and Ion PCR primers are unique to the Ion Total-RNA Seq Kit v2. Do *not* use the reagents from the Ion Total-RNA Seq Kit (first version) to prepare libraries with this user guide.

### Hybridize and ligate the RNA

1. On ice, prepare the hybridization master mix:

| Component | Volume per reaction[1] |
|---|---|
| Ion Adaptor Mix v2 | 2 µL |
| Hybridization solution | 3 µL |
| **Total Volume** | **5 µL** |

[1] Include 5–10% excess volume to compensate for pipetting error when preparing the master mix.

2. Add 5 µL of hybridization master mix to 3 µL of fragmented RNA sample:
   - Fragmented poly(A) RNA: up to 50 ng
   - Fragmented rRNA-depleted total RNA: up to 100 ng

   **Note:** If <50 ng of fragmented poly(A) RNA or <100 ng rRNA-depleted total RNA is recovered after fragmentation, we recommend using all fragmented RNA for ligation.

3. Slowly pipet the solution up and down ten times to mix, then centrifuge briefly.

4. Run the hybridization reaction in a thermal cycler:

| Temperature | Time |
|---|---|
| 65°C | 10 min |
| 30°C | 5 min |

5. On ice, add the RNA ligation reagents to the 8-µL hybridization reactions:

| Component | Volume per reaction[1] |
|---|---|
| Hybridization reaction | 8 µL |
| 2X Ligation Buffer | 10 µL |
| Ligation Enzyme Mix | 2 µL |
| **Total volume** | **20 µL** |

[1] Include 5–10% excess volume to compensate for pipetting error when preparing the master mix.

IMPORTANT! If the 2X Ligation Buffer contains a white precipitate, warm the tube at 37°C for 2–5 minutes or until the precipitate is dissolved. 2X Ligation Buffer is very viscous; pipet slowly to dispense it accurately.

6. Flick the tube or slowly pipet the solution up and down five times to mix well, then centrifuge briefly to collect the liquid in the bottom of the tube.

7. Incubate the 20-µL ligation reactions in a thermal cycler at 30°C according to input type and amount:

| RNA type | Amount into fragmentation | Reaction time |
|---|---|---|
| Poly(A) RNA | 1–5 ng | 1 hour |
| | >5 ng | 30 min |
| rRNA-depleted RNA | 10–100 ng | 1 hour |
| | >100 ng | 30 min |

IMPORTANT! Set the temperature of the thermal cycler lid to match the block temperature; turn OFF the heated lid; or leave the thermal cycler open during the incubation.

### Perform reverse transcription (RT)

1. On ice, prepare the RT master mix:

| Component | Volume per reaction[1] |
|---|---|
| Nuclease-free Water | 2 µL |
| 10X RT Buffer | 4 µL |
| 2.5 mM dNTP Mix | 2 µL |
| Ion RT Primer v2 | 8 µL |
| **Total Volume** | **16 µL** |

[1] Include 5–10% excess volume to compensate for pipetting error when preparing the master mix.

2. Incubate the RT master mix with the ligated RNA sample:
   a. Add 16 µL of the RT master mix to each 20-µL ligation reaction.
   b. Gently vortex the reaction to mix thoroughly, then centrifuge the reaction briefly to collect the liquid in the bottom of the tube.

c. Incubate in a thermal cycler with a heated lid at 70°C for 10 minutes, then snap-cool on ice.

3. Perform the reverse transcription reaction:
   a. Add 4 µL of 10X SuperScript™ III Enzyme Mix to each ligated RNA sample.
   b. Gently vortex to mix thoroughly, then centrifuge briefly.
   c. Incubate in a thermal cycler with a heated lid at 42°C for 30 minutes.

STOPPING POINT The cDNA can be stored at –30°C to –10°C for 2 weeks, stored at –86°C to –68°C for long-term storage, or used immediately.

## Purify the cDNA

### Before you begin

- If you have not done so already, add 44 mL of 100% ethanol to the Wash Solution Concentrate and mix well. Mark the label on the bottle to indicate that you added ethanol. Store the solution at room temperature (15°C to 30°C).
- If you see a white precipitate in the Binding Solution Concentrate, warm the solution at 37°C, then shake the solution to dissolve any precipitate before use.
- Incubate the Nuclease-free Water at 37°C for ≥5 minutes.

**IMPORTANT!** Accurate pipetting of bead cleanup reagents is critical for best results.

### Purify the cDNA

1. Prepare beads for each sample:
   a. Gently vortex the Nucleic Acid Binding Beads tube to completely resuspend the magnetic beads.
   b. Add 5 µL beads to wells on the Processing Plate.
   c. Add 120 µL Binding Solution Concentrate to each well, then mix the Binding Solution Concentrate and beads by pipetting up and down ten times.

2. Bind the cDNA to the beads:
   a. Add 60 µL of Nuclease-free Water to each of the 40-µL RT reaction.
   b. Transfer each 100-µL RT reaction to a bead-containing well on the Processing Plate.
   c. Set a P200 pipettor at 125 µL. Attach a new 200-µL tip to the pipettor, then pre-wet the tip with 100% ethanol by pipetting the ethanol up and down three times.
   d. Without changing tips, add 125 µL of 100% ethanol to each well.

   **IMPORTANT!** While dispensing the ethanol, do not force out the last drops. Remove the last drop by touching the drop to the well wall. Change the tip and repeat steps 2c–2d for the remaining wells only if the tip touches the wall. Accurate pipetting of 100% ethanol is critical for best results.

   e. Set a single or multi-channel pipettor at 150 µL. Attach new 200-µL tips to the pipettor, then mix the suspension in each well thoroughly by pipetting the wells up and down ten times.

      **Note:** The color of the mixture should be homogeneous after mixing.

   f. Incubate the samples for 5 minutes at room temperature off of the magnetic stand.

3. Remove the supernatant from the beads:
   a. Place the Processing Plate on the magnetic stand for 5–6 minutes to separate the beads from the solution. Wait for the solution to clear before proceeding to the next step.
   b. Leave the Processing Plate on the magnetic stand, then aspirate and discard the supernatant from the plate.

      **IMPORTANT!** Do not disturb the magnetic beads. If any beads are aspirated, leave 2–3 µL of supernatant behind.

4. Wash the beads with Wash Solution Concentrate with ethanol:
   a. Leave the Processing Plate on the magnetic stand.
   b. Add 150 µL of Wash Solution Concentrate with ethanol to each sample.
   c. Incubate the samples at room temperature for 30 seconds.

5. Remove the supernatant from the beads:
   a. Aspirate and discard the supernatant from the plate.
   b. Use a P10 or P20 pipettor to remove residual ethanol.

      **IMPORTANT!** Do not disturb the separated magnetic beads. Remove all of the Wash Solution Concentrate from each well.

   c. Air-dry the beads at room temperature to remove all traces of ethanol by leaving the Processing Plate on the magnetic stand for 1–2 minutes.

**IMPORTANT!** Do not overdry the beads (overdried beads appear cracked). Overdrying significantly decreases elution efficiency.

6. Elute the cDNA from the beads:
   a. Remove the Processing Plate from the magnetic stand.
   b. Add 12 µL of pre-warmed (37°C) Nuclease-free Water to each sample, then mix the Nuclease-free Water and beads by pipetting up and down ten times.
   c. Incubate at room temperature for 1 minute.
   d. Place the Processing Plate on the magnetic stand for 1 minute to separate the beads from the solution. Wait for the solution to clear before proceeding to the next step.
   e. For each sample, collect the eluant.

## Amplify the cDNA

1. For each cDNA sample, set up the PCR reaction, according to the non-barcoded or barcoded library tables.

   - For non-barcoded libraries:
     a. Prepare the Non-barcoded Library PCR Mix according to the following table.

| Contents | Volume per reaction[1] |
|---|---|
| Platinum™ PCR SuperMix High Fidelity[2] | 45 µL |
| Ion 5' PCR Primer v2 | 1 µL |
| Ion 3' PCR Primer v2 | 1 µL |
| **Total Volume** | **47 µL** |

[1] Include 5–10% excess volume to compensate for pipetting error when preparing master mix.
[2] Platinum™ PCR SuperMix High Fidelity contains a proofreading enzyme for high-fidelity amplification.

     b. Transfer 6 µL of cDNA to a new PCR tube.
     c. Transfer 47 µL of the Non-barcoded Library PCR Mix to each 6 µL of cDNA sample.
     d. Proceed to step 2.
   - For barcoded libraries
     a. Prepare the Barcoded Library PCR Mix according to the following table.

| Contents | Volume per reaction[1] |
|---|---|
| Platinum™ PCR SuperMix High Fidelity[2] | 45 µL |
| Ion Xpress™ RNA 3' Barcode Primer | 1 µL |
| **Total Volume** | **46 µL** |

[1] Include 5–10% excess volume to compensate for pipetting error when preparing master mix.
[2] Platinum™ PCR SuperMix High Fidelity contains a proofreading enzyme for high-fidelity amplification.

     b. Transfer 6 µL of cDNA sample to a new PCR tube.
     c. Transfer 46 µL of the Barcoded Library PCR Mix to each 6 µL of cDNA sample.
     d. Add 1 µL of the selected Ion Xpress™ RNA-Seq Barcode BC primer (choose from BC01–BC16) to each PCR tube.

2. Flick the tube or slowly pipet the solution up and down five times to mix well, then centrifuge briefly to collect the liquid in the bottom of the tube.

3. Run the reactions in a thermal cycler:

| Stage | Temp | Time |
|---|---|---|
| Hold | 94°C | 2 min |
| Cycle (2 cycles) | 94°C | 30 sec |
| | 50°C | 30 sec |
| | 68°C | 30 sec |
| Cycle<br>• 16 cycles for 1–5 ng poly(A) RNA or 10–100 ng rRNA-depleted RNA<br>• 14 cycles for >5 ng poly(A) RNA or >100 ng rRNA-depleted RNA | 94°C | 30 sec |
| | 62°C | 30 sec |
| | 68°C | 30 sec |
| Hold | 68°C | 5 min |

## Purify the amplified cDNA

### Before you begin

- If you have not done so already, add 44 mL of 100% ethanol to the Wash Solution Concentrate and mix well. Mark the label on the bottle to indicate that you added ethanol. Store the solution at room temperature (15°C to 30°C).
- If you see a white precipitate in the Binding Solution Concentrate, warm the solution at 37°C, then shake the solution to dissolve any precipitate before use.
- Incubate the Nuclease-free Water at 37°C for ≥5 minutes.

### Purify the amplified cDNA

1. Prepare beads for each sample:
   a. Gently vortex the Nucleic Acid Binding Beads tube to resuspend the magnetic beads completely.
   b. Add 5 µL of bead suspension to wells on the Processing Plate.
   c. Add 180 µL Binding Solution Concentrate to each well, then mix the Concentrate and beads by pipetting up and down ten times.

2. Bind the amplified cDNA to the beads:
   a. Transfer 53 µL of each amplified cDNA sample to a bead-containing well on the Processing Plate.
   b. Set a P200 pipettor at 130 µL. Attach a new 200-µL tip to the pipettor, then pre-wet the tip with 100% ethanol by pipetting the ethanol up and down three times.
   c. Without changing tips, add 130 µL of 100% ethanol to each well.

   **IMPORTANT!** While dispensing the ethanol, do not force out the last drops. Remove the last drop by touching the drop to the well wall. Change the tip and repeat steps 2b and 2c for the remaining wells only if the tip touches the wall. Accurate pipetting of 100% ethanol is critical for best results.

   d. Set a single or multi-channel P200 pipettor at 150 µL. Attach new 200-µL tips to the pipettor, then mix the suspension in each well thoroughly by pipetting the wells up and down ten times.
   e. Incubate the samples for 5 minutes at room temperature.

3. Remove the supernatant from the beads:
   a. Place the Processing Plate on a magnetic stand for 5–6 minutes to separate the beads from the solution. Wait for the solution to clear before proceeding to the next step.
   b. Leave the Processing Plate on the magnetic stand, then aspirate and discard the supernatant from the plate.

**IMPORTANT!** Do not disturb the magnetic beads. If any beads are aspirated, leave 2–3 µL of supernatant behind.

4. Leave the Processing Plate on the magnetic stand.
   a. Leave the Processing Plate on the magnetic stand.
   b. Add 150 µL of Wash Solution Concentrate with ethanol to each sample.
   c. Incubate the samples at room temperature for 30 seconds.

5. Remove the supernatant from the beads:
   a. Aspirate, then discard the supernatant from the plate.
   b. Use a P10 or P20 pipettor to remove remaining ethanol.

**IMPORTANT!** Do not disturb the separated magnetic beads. Remove all the Wash Solution Concentrate from each well.

   c. To remove all traces of ethanol, air-dry the beads at room temperature by leaving the Processing Plate on the magnetic stand for 1–2 minutes.

**IMPORTANT!** Do not overdry the beads (overdried beads appear cracked). Overdrying significantly decreases elution efficiency.

6. Elute the cDNA from the beads:
   a. Remove the Processing Plate from the magnetic stand.
   b. Add 15 µL of pre-warmed (37°C) Nuclease-free Water to each sample, then mix the Nuclease-free Water and beads by pipetting up and down ten times.
   c. Incubate at room temperature for 1 minute.
   d. Place the Processing Plate on a magnetic stand for 1 minute to separate the beads from the solution. Wait for the solution to clear before proceeding to the next step.
   e. For each sample, collect the eluant.

## Assess the yield and size distribution of the amplified DNA

1. Measure the concentration of the purified DNA with a NanoDrop™ Spectrophotometer or the dsDNA HS Assay Kit with the Qubit™ Fluorometer.

2. Analyze 1 µL of the library using the appropriate chip on the Agilent™ 2100 Bioanalyzer™ instrument. If the library concentration is:
   - 1–50 ng/µL: Use the Agilent™ DNA 1000 Kit.
   - 5–1000 pg/µL: Use the Agilent™ High Sensitivity DNA Kit.

3. Using the 2100 expert software, perform a smear analysis to:
   a. Quantify the percentage of DNA that is ≤160 bp: Use size range 50–160 bp.
   b. Determine the molar concentration (nM) of the cDNA libraries: Use size range 50–1000 bp.

**Note:** For instructions on how to perform the smear analysis, see "Perform a smear analysis" in the *Ion Total RNA-Seq Kit v2 User Guide* (Pub. No. MAN0010654).

4. Use molar concentration of the cDNA libraries from 3b of "Pool barcoded whole transcriptome libraries" on page 6 and "Determine the library dilution required for template preparation" on page 7.

| If the percent of DNA in 50–160 bp the range is | Action |
|---|---|
| <50% | Proceed to the next section, "Pool barcoded whole transcriptome libraries," or "Determine the library dilution required for template preparation." |
| 50–60% | Perform another round of purification on the amplified DNA using components from the Magnetic Bead Cleanup Module:<br>1. Bring the sample volume to 53 µL with Nuclease-free Water.<br>2. Follow the steps in "Purify the amplified cDNA" on page 5.<br>*or*<br>Proceed to the next section, "Pool barcoded whole transcriptome libraries," or "Determine the library dilution required for template preparation," but expect to see a slightly higher percentage of filtered reads in your sequencing data when compared to libraries with less than 50% of DNA in the range. |
| >60% | We recommend that you perform another round of purification on the amplified DNA using components from the Magnetic Bead Cleanup Module:<br>1. Bring the sample volume to 53 µL with Nuclease-free Water.<br>2. Follow the steps in "Purify the amplified cDNA" on page 5. |

## Pool barcoded whole transcriptome libraries

**Note:** If you are not pooling libraries, skip this section and proceed to "Determine the library dilution required for template preparation".

1. Determine the molar concentration (nM) of each barcoded cDNA library with the Agilent™ DNA 1000 Kit or the Agilent™ High Sensitivity DNA Kit.

   **Note:** 50–1000 bp size range is typically used to determine library concentration. If needed, adjust the range to include all the library peaks.

2. Dilute each barcoded cDNA library to the same molar concentration (nM).

   For example, if you have 3 different barcoded libraries that are 45, 55, 65 nM, select a concentration that is equal or lower than the lowest concentration of the three libraries, such as 30 nM. Dilute all or part of each library to 30 nM.

3. Mix an equal volume of each diluted library to prepare a pool of the barcoded libraries.

   The final molar concentration of the pooled library is the same for each diluted library. For example, if you dilute each library to 30 nM, the concentration of the pooled library is 30 nM.

   Use the final molar concentration to determine the Template Dilution Factor. Alternatively, you can determine the molar concentration of the pooled libraries with the Agilent™ DNA 1000 Kit or the Agilent™ High Sensitivity DNA Kit (see "Assess the yield and size distribution of fragmented RNA" on page 2).

### Determine the library dilution required for template preparation

With less than 50% of the amplified DNA in the 50–160 bp range, you can proceed to the template preparation procedure to prepare templated beads for sequencing on the Ion PGM™, Ion Proton™, Ion S5™, or Ion S5™ XL Systems.

Determine the dilution factor that gives a concentration of ~100 pM. This concentration is suitable for downstream template preparation. Use the following formula:

Dilution factor = (Library or pooled library concentration in pM)/100 pM

**Example:**

The library or pooled library concentration is 15,000 pM.

Dilution factor = 15,000 pM/100 pM = 150

Thus, 1 µL of library or pooled library mixed with 149 µL of Low TE (1:150 dilution) yields approximately 100 pM. Use this as the starting library dilution for template preparation.

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