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Preparation and Sequencing of RNA Libraries with the Ion Personal Genome Machine® (PGM™) System

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Quantitate the library by Bioanalyzer™ Instrument analysis

Quantitate the library to determine its molar concentration. After determining the molar concentration of the library, calculate the Template Dilution Factor to dilute the library for preparation of template-positive Ion Sphere™ Particles (see "Determine the Template Dilution Factor of the RNA library" on page 4).

Note: For complete procedures including safety on RNA library preparation, refer to the *Ion Total RNA-Seq Kit v2 User Guide* (Pub. no. 4476286).

Required materials

Note: For all required materials, consumables, and equipment, refer to the appropriate user guide.

Required kits

Item†	Source/Cat. no.
Agilent® DNA 1000 Kit	Agilent 5067-1504
Agilent® High Sensitivity DNA Kit	Agilent 5067-4626

† For the Safety Data Sheet (SDS) of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

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Optional kits

Item†	Source/Cat. no.
Qubit® dsDNA HS Assay Kit	Life Technologies Q32851

† For the Safety Data Sheet (SDS) of any chemical not distributed by Life Technologies, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Required equipment

Item	Source/Cat. no.
Agilent® 2100 Bioanalzyer™ instrument	Agilent G2938A
NanoDrop® Spectrophotometer	Thermo Scientific

Optional equipment

Item	Source/Cat. no.
Qubit® 2.0 Fluorometer	Life Technologies Q32866

Required consumables

Item	Source
(*Optional*) Low TE Buffer	Major Laboratory Supplier (MLS)

Procedure

1. Quantitate the purified library using 1 µL of sample with a Qubit® 2.0 Fluorometer, NanoDrop® Spectrophotometer, or other UV spectrophotometer (OD 260 nm).
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. Determine the molar concentration of the library with the Agilent® 2100 Bioanalyzer™ Instrument Expert software: Perform a smear analysis by following the manufacturer's instructions. In the Bioanalyzer™ Instrument Expert software, select: **View ▸ Setpoints ▸ Smear Analysis**.

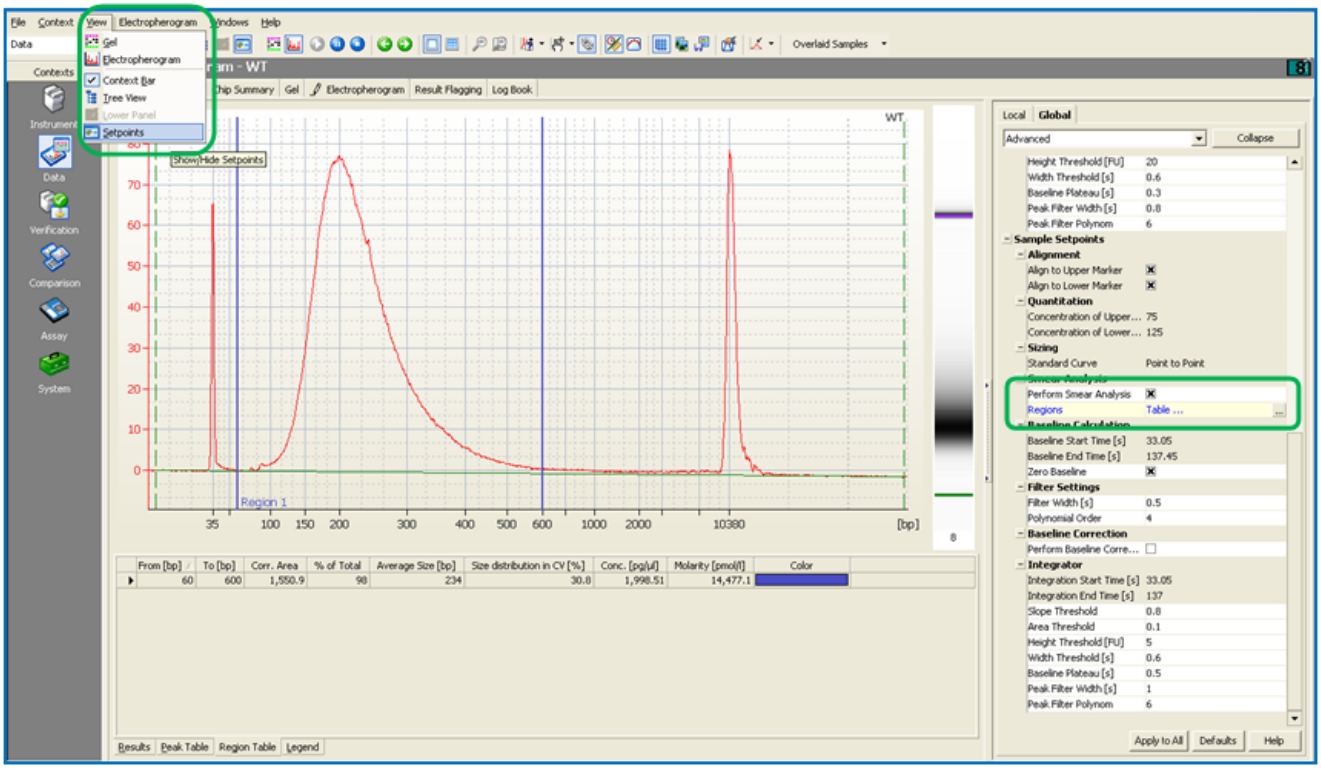
Example

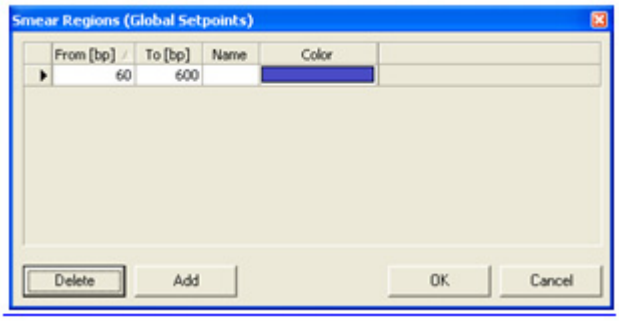
The following figure shows a 60–600 bp region set for smear analysis. The software automatically calculates the mass concentration, either in:

- ng/µL and displays nMol/L with the Agilent® DNA 1000 Kit

  or

- pg/µL and displays pmol/L with the Agilent® High Sensitivity DNA Kit:





Determine the Template Dilution Factor of the RNA library

The Template Dilution Factor is calculated from the molar concentration from Bioanalyzer™ Instrument analysis (see "Quantitate the library by Bioanalyzer™ Instrument analysis" on page 1). Calculate the Template Dilution Factor according to method. Next, use the Ion OneTouch™ 200 Template Kit v2 or the Ion PGM™ 200 Xpress™ Template Kit to prepare template-positive Ion Sphere™ Particles.

Note: For complete procedures on preparation of template-positive Ion Sphere™ Particles (ISPs) by the Ion OneTouch™ System, refer to the *Ion OneTouch™ 200 Template Kit v2 User Guide for use with the Ion OneTouch™ System* (Pub. no. 4478372).

Determine the Template Dilution Factor to prepare template-positive ISPs on the *Ion OneTouch™ System* for whole transcriptome and small RNA libraries

For the Template Preparation procedure using the Ion OneTouch™ 200 Template Kit v2, determine the Template Dilution Factor that gives 210 × 10^6 molecules of template per 20 µL for both whole transcriptome and small RNA libraries. Use a conversion factor of 8.3 nM = 5 × 10^9 molecules/µL, and the appropriate formula:

Template Dilution Factor =

(Library concentration in nM) × [(5 × 10^9 molecules/µL)/(8.3 nM)] × [20 µL/(210 × 10^6 molecules)]

If your library concentration is >20 nM, serially dilute 1:10 or 1:100 until your library concentration is ≤20 nM. (This step helps prevent a Template Dilution Factor of several thousands).

Example: If the library concentration is 10 nM:

Template Dilution Factor =

(10 nM) × [(5 × 10^9 molecules/µL)/(8.3 nM)] × [20 µL/(210 × 10^6 molecules)] = 574

Thus, 1 µL of library mixed with 573 µL of Nuclease-Free Water (1:574 dilution) yields approximately 210 × 10^6 molecules per 20 µL.

Determine the Template Dilution Factor to prepare template-positive ISPs with the *IKA® Ultra-Turrax® Tube Drive*

Note: For complete procedures on preparation of template-positive Ion Sphere™ Particles (ISPs) with the IKA® Ultra-Turrax® Tube Drive, refer to the *Ion PGM™ 200 Xpress™ Template Kit User Guide* (Pub. no. 4474291).

For the Template Preparation procedure using the Ion PGM™ 200 Xpress™ Kit, determine the Template Dilution Factor that gives 315 × 10^6 molecules of template per 18 µL for both whole transcriptome and small RNA libraries. Use a conversion factor of 8.3 nM = 5 × 10^9 molecules/µL, and the appropriate formula:

Template Dilution Factor =
(Library concentration in nM) × [(5 × 10^9 molecules/µL)/(8.3 nM)] × [18 µL/(315 × 10^6 molecules)]

If your library concentration is >20 nM, serially dilute 1:10 or 1:100 until your library concentration is less than or equal to 20 nM. (This step helps prevent a Template Dilution Factor of several thousands).

Example: The RNA library concentration is 400 nM

Dilute 1 µL of library in 99 µL of Nuclease-Free Water (1:100 dilution) to yield a final library concentration of 4 nM. Use this final library concentration to calculate the Template Dilution Factor:

Template Dilution Factor =
(4 nM) × [(5 × 10^9 molecules/µL)/(8.3 nM)] × [18 µL/(315 × 10^6 molecules)] = 138

Thus, 1 µL of the 4-nM library dilution mixed with 137 µL of Nuclease-Free Water (1:138 dilution) yields approximately 315 × 10^6 molecules per 18 µL. Use 18 µL of the final library dilution to prepare the aqueous master mix.

Set up the Ion PGM™ System for RNA libraries

1. Enter these settings on the Run Info screen of the Ion PGM™ Sequencer:

If the touchscreen prompt says...	Select...
Runtype	GENS
Library	Appropriate library reference
Barcode	• *Non-barcoded* libraries: **RNA_Barcode_None** • *Barcoded* libraries prepared with the Ion Xpress™ RNA-Seq Barcode 01–16 Kit: **IonXpressRNA**
Number of flows	• *Whole transcriptome libraries*: 520 flows (130 cycles) • *Small RNA libraries*: 160 flows (40 cycles) **Note:** To change cycling conditions for different read lengths, enter a different number of flows.

IMPORTANT! To insure proper trimming of the sequences prior to alignment with a reference, for all libraries prepared with the Ion Total RNA-seq Kit v2, you must select an option from the barcode pull-down menu. If the RNA-seq library does *not* have barcodes, select **RNA_Barcode_None** in the barcode dialog box. If the library was prepared with the Ion Xpress™ RNA-Seq Barcode 01–16 Kit, select **IonXpressRNA**.

2. Enter the remaining information as needed.
3. Click **Next**, then follow the remaining prompts to start the run.

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The information in this guide is subject to change without notice.

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