February 2020

Supplementary Protocol

QIAseq® miRNA Library Ion Chef™ and Ion S5™ setup

This protocol describes Ion Chef and Ion S5 setup using the QIAseq miRNA Library Kit (cat. no. 331502 or 331505).

IMPORTANT: Please consult the "Safety Information" and "Important Notes" in the *QIAseq miRNA Library Kit Handbook for Thermo Fisher Scientific NGS Systems*, **www.qiagen.com/HB-2573**, before beginning this procedure.

Important points before starting

- Following recommendations for library dilution concentrations and library loading concentrations are based on a Qubit™ Fluorimeter:
  - Ion Chef: 25 pM
  - Ion OneTouch™: 4 pM
- Perform 100 bp read length or 250 flows for optimal results. Our data analysis tool requires complete read length from UMIs to miRNAs.
- Set up the sequencing run without a reference genome to get unaligned .bam files for our data analysis. In addition, due to the special read structure of QIAseq miRNA libraries, reads start with 12 bp UMI.
- Do not run the plugin or Ion reporter. Due to the special structure of the library, it poses a problem for correct read processing.

Procedure

Create a planned run

1. Log into the Torrent server via the Torrent Browser.
2. Click the **Plan** tab. Look at the templates, select the application that you want to run (**RNA Seq**), and then choose one of the following 2 options:
   - Option 1: **Plan New Run** (on the right side of the screen) to plan a new run using the generic template for the selected application (**Figure 1A**)
   - Option 2: **Plan Run** in the dropdown menu under the **Settings** tab to the right of the existing template you selected from the template list (**Figure 1B**)

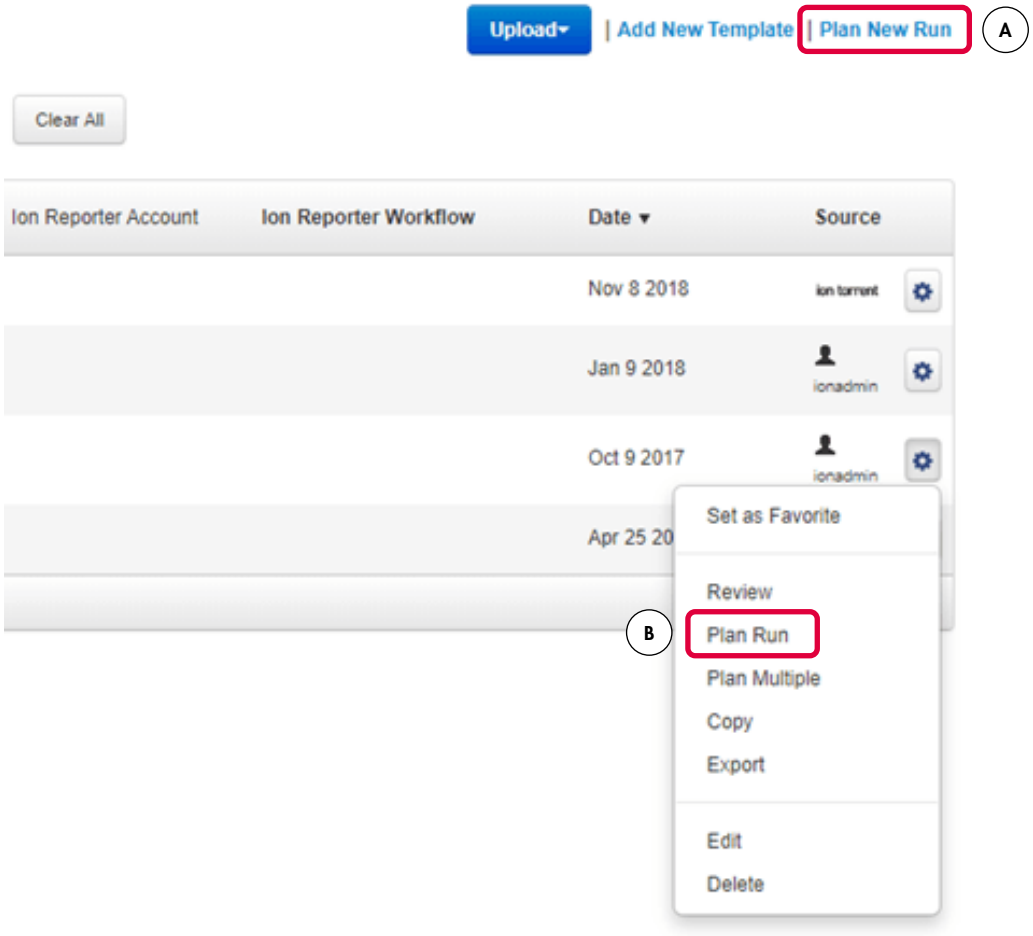

Figure 1. Start creating a planned run.

3. Using the **Planned Run** wizard: In the **Create Plan** tab, make the appropriate selections as below:
   - Template Name: **Ion RNA - miRNA**
   - Run Plan Name (required): [Enter a planned run name]
   - Analysis Parameters: **Default (Recommended)**
   - Number of barcodes: [Enter the number of samples]

   Important: Don't select a reference genome.

   Click **Next**.
4. In the **Ion Reporter** tab, under **Ion Reporter Account**, select **None**. And then, click **Next**.
5. In the **Research Application** tab: Under **Research Application**, select **RNA**. Under **Target Technique**, select **RNA Sequencing**. Click **Next**.
6. In the **Kits** tab, select the following (Figure 2, next page):
   - Instrument: **Ion GeneStudio™ S5 System**
   - Chip Type: [Select the appropriate chip type from the drop-down list]
   - Barcode Set: **IonXpress**
   - Template Kit: **IonChef**
   - Flows: **250**
   - Sequencing Kit: **Ion S5 Sequencing Kit**
   - Advanced Settings: **Use Recommended Defaults**

   Click **Next**.
7. In the **Plugins** tab, select **FileExporter**. Click **Next**.
8. In the **Projects** tab, select or create the appropriate project. Click **Next**.
9. Under the **Plan** tab:
   - Run Plan Name (required): [Enter a planned run name]
   - Analysis Parameters: **Default (Recommended)**
   - Sample Tube Label: [Enter or scan the barcodes of the Ion Chef Library Sample Tubes]
   - Chip Barcode: [Scan the barcodes of the chip]
10. When you have completed your selections, click **Plan Run** at the bottom right of the screen to save the run. The run is listed on the **Planned Runs** page under the name that you specified and is automatically used by the Ion Chef System when the associated sample is loaded.

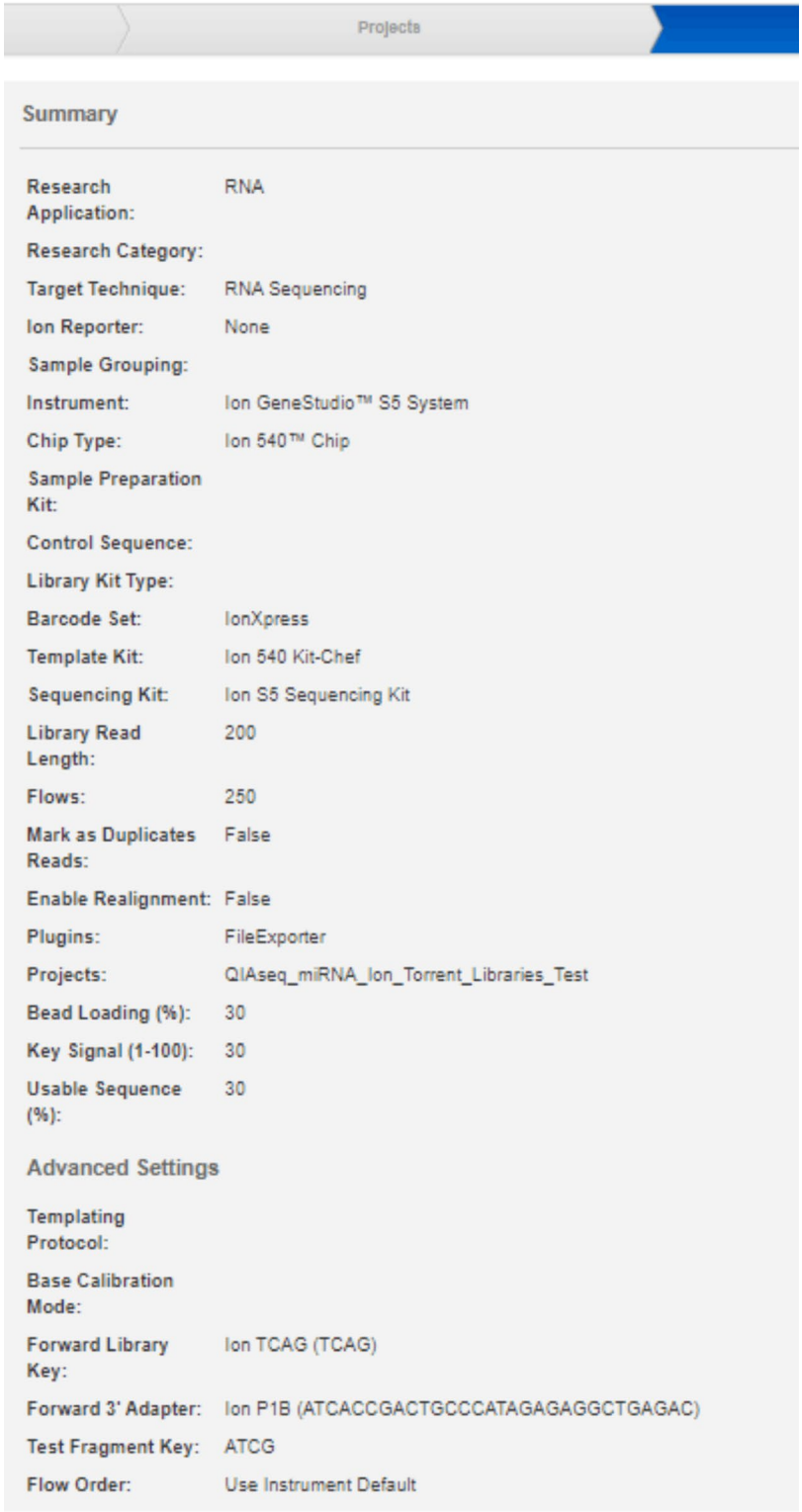

Figure 2. Overview of all settings.

11. Run the Ion Chef system according to manufacturer's instructions.
12. When the run is complete, unload the Ion Chef Instrument and sequence the chips immediately on Ion S5 according to manufacturer's instructions.
13. If the template is prepared by Ion OneTouch instead of Ion Chef: In the **Kits** tab, for **Template Kit**, select **OneTouch** (Figure 3). Other sequencing parameters set up on Ion S5 should be the same as above described with the Ion Chef.

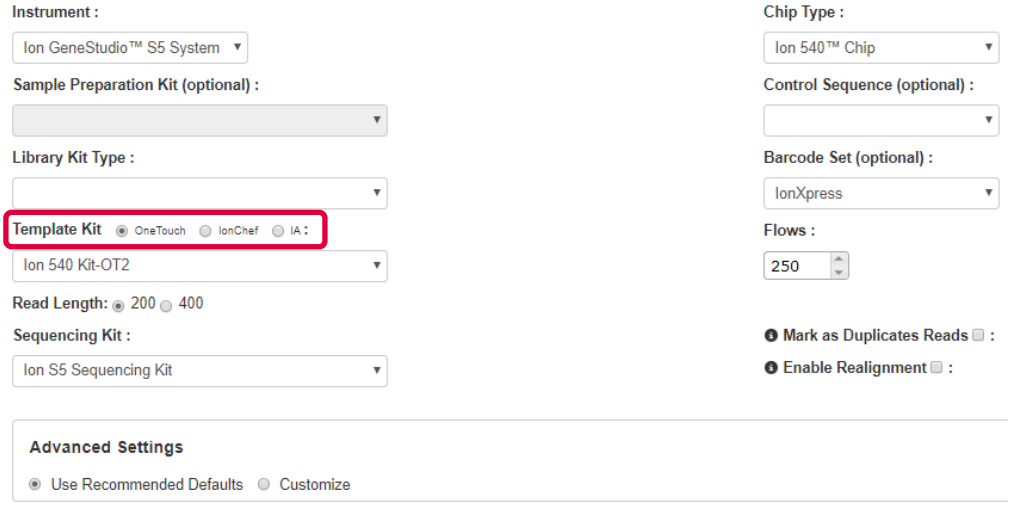

Figure 3. Settings for template prepared by Ion OneTouch.

Document revision history

Date	Changes
02/2020	Initial release

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.