

Supplementary Protocol

QIAseq[®] Targeted RNA Panel Ion Chef^{f™} and Ion S5[™] set up

This protocol describes Ion Chef and Ion S5 setup using the QIAseq Targeted RNA Panels (cat. nos. 333002, 333005, 333022, 333025, 333027, 333217).

Important: Please consult the QIAseq Targeted RNA Panel Kit Handbook before beginning this procedure (<https://www.qiagen.com/HB-2067>).

Important points before starting

- The following recommendations for library dilution concentrations and library loading concentrations are based on QIAseq Library Quant System:
Ion Chef: 50 pM Ion
- Do 200 bp read length or 500 flows for optimal result.
- Set up sequencing without reference genome to get unaligned *.bam file for our data analysis.
- Do not run plugin or Ion Reporter[™] if possible. Due to the library special structure, it could pose potential problems for correct reads processing.

Procedure

Create a planned run

1. Log into the Ion Torrent[™] server via the Torrent Browser.
2. Click the **Plan** tab. Look at the templates, select the application that you want to run (RNAseq), 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).

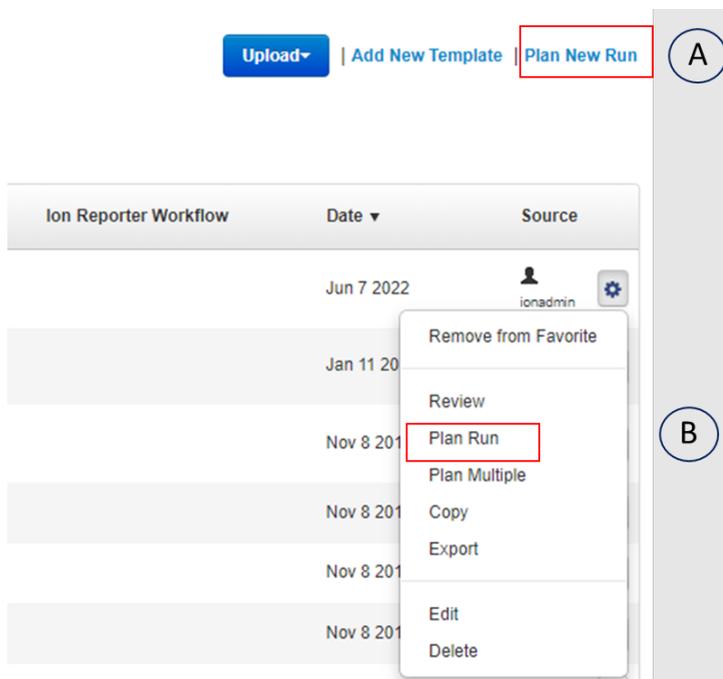


Figure 1. Start creating a planned run.

3. Run the Ion Chef system according to manufacturer's instruction.
4. When the run is complete, unload the Ion Chef instrument and sequence the chips immediately on S5 according to manufacturer's instruction.

Option 1

1. Click **Plan New Run**. Under the **Ion Reporter** tab, select **None**.
2. Under **Research application** tab, select **RNA** and **RNA Sequencing**.
3. Under **Kits** tab, make the following selections:
 - Instrument: Ion GeneStudio™ S5 System
 - Chip Type: Select the appropriate chip type from dropdown list, such as Ion 530™ Chip
 - Library Kit Type: none
 - Barcode Set: Ion Xpress™
 - Template Kit: IonChef (Ion 510 & Ion520 & Ion 530 Kit-Chef)
 - Sequencing Kit: Ion S5 Sequencing Kit
 - Flows: 500
 - For Advanced settings: Use Recommended Defaults

Kits Show Summary

Instrument :
 Ion GeneStudio™ S5 System

Sample Preparation Kit (optional) :
 [Dropdown]

Library Kit Type :
 [Dropdown]

Template Kit OneTouch IonChef IA.1
 Ion 510 & Ion 520 & Ion 530 Kit-Chef

Templating Size
Sequencing Kit :
 Ion S5 Sequencing Kit

Chip Type :
 Ion 530™ Chip

Control Sequence (optional) :
 [Dropdown]

Barcode Set (optional) :
 IonXpress

Flows :
 500

Mark as Duplicates Reads
 Enable Realignment

Advanced Settings

Use Recommended Defaults Customize

Warning! It's **not recommended** to change these settings, please consult your local field representative before modifying parameters below.

Templating Protocol :
 Chef Protocol - 200 bp

Forward Library Key :
 Ion TCAG

Test Fragment Key :
 ATCG

Base Calibration Mode :
 Default Calibration

Forward 3' Adapter :
 Ion P1B

Flow Order :
 Use Instrument Default

4. Under **Plugins** tab, select **FileExporter** and **sampleID**.

Create Plan Ion Reporter Research Application **Plugins** Projects Plan

Select plugins to execute, then click Next. Select All Clear Show Summary

ampliSeqRNA FieldSupport PGXAnalysis
 AssemblerSPAdes FileExporter RNASeqAnalysis
 coverageAnalysis FilterDuplicates RunTransfer
 DataExport ImmuneResponseRNA sampleID
 ERCC_Analysis molecularCoverageAnalysis variantCaller

← Previous Next →

5. Select or add appropriate project under **Project** tab.

Create Plan Ion Reporter Research Application Kits Plugins **Projects** Plan

Select the project(s) that will receive data from runs planned in this template, then hit next. Show Summary

AmpliSeq_QIASeq_Comparison
 Hilden
 QIAseq_DNA
 QIAseq_FX_All-in-One_DNA_Library_Hilden
 QIAseq_miRNA_Ion_Torrent_Libraries_Test
 QIAseq_Targeted_RNA_Panels

Search

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6. Under the **Plan** tab:

- Run Plan Name (required): Enter a **Planned Run** name
- Analysis Parameters: Default (Recommended)
- Number of barcodes: Enter sample number
- Sample Tube Label: Enter or scan the barcodes of the Ion Chef Library Sample Tubes
- Chip Barcode: Scan the barcodes of the chip

7. Enter a sample name for each barcode used (require at least one sample): manually input sample name and select **Barcode** in the dropdown list.

Create Plan | Ion Reporter | Research Application | Kits | Plugins | Projects | **Plan**

Show Summary

Run Plan Name (required):

Analysis Parameters: Default (Recommended) Custom Details -

Number of barcodes: + -

Sample Tube Label:

Chip Barcode:

Enter a sample name for each barcode used (require at least one sample) + - 🗑:

#	Barcode	Sample Name (required)	Sample ID	Sample Description	Reference	Target Regions	Hotspot Regions	Control Seq Type (optional)
1	IonXpressRNA_001 (CTAAGGTAAC)	Sample 1						

8. When you have completed your selections, click **Plan Run** at the bottom right of the Plan tab screen to save the run. The run is now 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.

Here is the overview of all the settings:

Summary

Research Application: RNA

Research Category:

Target Technique: RNA Sequencing

Ion Reporter: None

Sample Grouping: Other

Instrument: Ion GeneStudio™ S5 System

Chip Type: Ion 530™ Chip

Sample Preparation Kit:

Control Sequence:

Library Kit Type:

Barcode Set: IonXpress

Template Kit: Ion 510 & Ion 520 & Ion 530 Kit-Chef

Sequencing Kit: Ion S5 Sequencing Kit

Library Read Length: --

Flows: 500

Mark as Duplicates Reads: False

Enable Realignment: False

Plugins: FileExporter, sampleID

Projects: QIAseq_Targeted_RNA_Panels

Bead Loading (%): 30

Key Signal (1-100): 30

Usable Sequence (%): 30

Advanced Settings

Templating Protocol: Chef Protocol - 200 bp

Base Calibration Mode:

Forward Library Key: Ion TCAG (TCAG)

Forward 3' Adapter: Ion P1B (ATCACC GACTGCCCATAGAGAGGCTGAG AC)

Test Fragment Key: ATCG

Flow Order: Use Instrument Default

Option 2

- To create a template, click on **Add New Template** tab (see Figure 1), make the same selections shown in Option 1 for Ion Reporter, Research Application, Kits, Plugins, and Projects tabs, and then save the template
- 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)
- Under the **Create Plan** tab, make the following selections:
 - Run Plan Name (required): Enter a **Planned Run** name
 - Analysis Parameters: Default (Recommended)

- Number of barcodes: Enter sample number
 - Sample Tube Label: Enter or scan the barcodes of the Ion Chef Library Sample Tubes
 - Chip Barcode: Scan the barcodes of the chip
4. Enter a sample name for each barcode used (require at least one sample): manually input sample name and select **Barcode** via dropdown list
 5. When you have completed your selections, click **Plan Run** at the bottom right of the Plan tab screen to save the run. The run is now 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.

Document Revision History

Date	Description
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06/2023	Initial release
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