COFACTOR

THE LEADERS IN RNA

Cofactor Prism Software
Version 2.4

Introduction

Cofactor Genomics' ImmunoPrism™ Immune Profiling Kit enables researchers to profile the immune response at the site of a solid tumor. The protocol accepts total RNA extracted from solid tumor tissue and delivers a fully-analyzed immune report.

This document explains how to use Prism, a web portal that facilitates cloud-based analyses of samples prepared using the ImmunoPrism Kit. Prism allows users to upload FASTQ files of their samples, generate Individual Sample Reports and a Biomarker Discovery Report, and view the status of submitted projects and results from completed projects.

IMPORTANT: Analyses in Prism were specifically developed for libraries using the optimized reagents and protocol of the ImmunoPrism Kit. As a result, immune profiling results for samples prepared without the ImmunoPrism Kit in the Individual Sample and Biomarker Discovery Reports will be dramatically aberrant, and are not supported.

0. FASTQ Preparation

The ImmunoPrism Kit generates libraries to sequence for Illumina sequencing. Depending on the sequencer model and the IT infrastructure of users, sequenced reads via FASTQ may be generated in two methods.

Many users will have a locally run pipeline to demultiplex sequencing runs and generate a single FASTQ file per sample.

Other users may have sequencing runs upload in real-time to BaseSpace from select sequencing machines (e.g MiSeq) and demultiplex runs using BaseSpace tools. This setup and procedure is beyond this guide. Please refer to your machine manual to understand how to configure machines and how to setup runs to upload directly to BaseSpace. For additional assistance, we recommend the "New to BaseSpace" article for configuration and demultiplexing in BaseSpace: https://help.basespace.illumina.com/articles/new-to-basespace. When employing this method, each sample should have only 1 FASTQ file and files will need to be downloaded to a local computer to upload via Prism.

Other methods may exist for generating FASTQ files from a sequencer. Any method that creates an individual, compressed (via gzip) FASTQ file per sample can be used to run analysis on Prism.

FASTQ files must be compressed via gzip and follow standard industry file extensions (.fastq.gz or .fq.gz). Files that are non-compressed, are compressed via other algorithms, or follow other file extensions, are not accepted.

1. Creating and Logging into a Prism Account

The Prism web portal may be accessed through: https://prism.cofactorgenomics.com/.

The first step to run the immune profiling analysis is to create a Prism account. Visit the web portal at the above link and click "Login to Start".

From this login page, click "Create User". Follow the form and input the relevant information to create your account. Note the username and password; this will be used in later sessions to log into your account and access current and past analyses. Upon account creation, you will be directly logged in. From here, you can proceed to instructions for submitting a project for analysis (Step 2).

If you already have an account, log into the Prism web portal visit the above web page and click "Login to Start". Then input your account credentials and click "Login". From here, you can proceed to instructions for submitting a project for analysis (Step 2) or looking at previous results (Step 3).

2. Submitting a Project for Analysis

To submit a project on your Prism account, you will upload FASTQ files and input the pertinent meta data to facilitate analysis. Before you start the submission process it is useful to have FASTQ files organized and ready to upload via the browser.

After logging in, click "Submit New Project" button or "Submit New Project" in the top toolbar from any page in Prism. This action will navigate you to the submission portion of the user home page. The submission page has step by step instructions. You will get feedback in real-time on each step: a red cross means the step is incomplete, a green check means the step is complete, and an orange exclamation mark needs your attention. All steps must have a green check or an orange exclamation mark to submit the project.

Note: The orange exclamation mark may appear when submitted samples are in a single group. This is just to remind you that no Biomarker Discovery Report will be run.

Briefly, the steps to submission are as follows:

1. Upload compressed (via gzip) FASTQ files.
2. Drag "Available" files into Group 1, if only generating Individual Sample Reports, or into Group 1 and Group 2, if generating a Biomarker Discovery Report.
3. Name the group(s).
4. Specify the disease of samples.
5. Click "Analyze" to submit your project for analysis

Congratulations, you have successfully created a Prism analysis project!

You will receive an email notification when your project has completed analysis. A link in the email will take you directly to view the project analysis results. To view the status of this and other submitted projects, proceed to instructions for looking at project status and analysis results (Step 3).

Notes:

- Uploaded FASTQ files will be available for 24 hours. Therefore, these files can be used for several projects or groupwise comparisons. After 24 hours, the FASTQ files will need to be re-uploaded.
- FASTQ files must have a minimum of 10 million reads. If applicable, FASTQ files will be trimmed to 40 million reads per sample by the analysis pipeline.
- FASTQ files must be compressed by the gzip algorithm and use .fastq.gz or .fq.gz as the file extension.
- Some manipulations of gzip files render them corrupt for our file uploader. If you find that uploaded gzip files are being rejected, please uncompress and recompress the files and try uploading again. If you have any issue with the uploader, please do not hesitate to contact us (See Step 4).

3. View Project Status and Analysis Results

Prism enables a user to see the status of submitted projects and to view Individual Sample Reports and a Biomarker Discovery Report per project. While logged in, click "See Results" in the top toolbar or any page of Prism. Here you will see a table of projects you have created on Prism. The table has three columns for the status, name, and the date of submission.

The status of each project can be:

- "Running", where the project analysis is currently running;
- "Success", where the project analysis is complete and reports are available; and

If a project has finished analysis (indicated by a "Success" status), Individual Sample Reports and a Biomarker Discovery Report will be available. To access these reports, return to the table of projects and click on the name of the project. On this project page, you will see a table with a row for each sample in the project. Click the link in each row, under the "Report" column, to access the report of each sample. Immediately below the table, click the link for the Biomarker Discovery Report. If no links are in this page, it is because your project has not competed analysis.

Note: The Biomarker Discovery Report will only be available if the project includes a minimum of three samples per group, and the samples have been placed in two individual groups for comparison.

4. Getting Help

If you encounter any challenges while using Prism, or have questions about project status or reports, please contact support@cofactorgenomics.com or visit our support website: https://support.cofactorgenomics.com/.

5. Revision History

Version	Description	Date
0.7.1	Initial Release	09/2018
1.0.1	Full Product Release with updates	11/2018
1.0.2	New Upload Instructions	08/2019
2.4.0	Version Adjustment	08/2019
2.4.1	Gzip File Requirement	09/2019
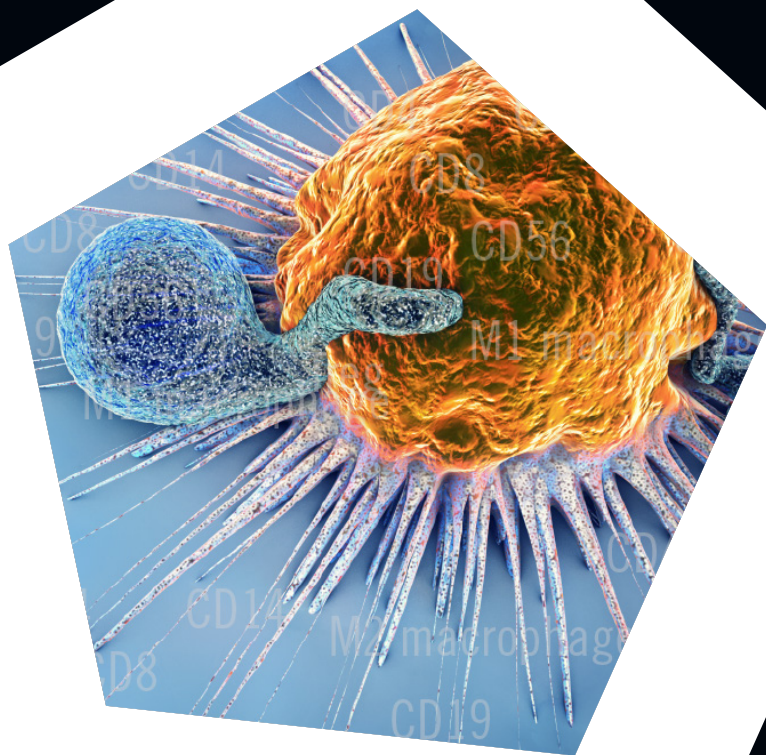
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