DebarcodeR Example Protocol

Abbreviations

RMD	R Markdown file
FCS	Flow Cytometry Standard

Defined Terms

STAINED_CELLS FCS	**FCS** file containing the flow cytometry measurements from the **STAINED_CELLS** experimental sample for a set of 48 barcoded conditions.
FCB_CELLS FCS	**FCS** file containing the flow cytometry measurements from the **FCB_CELLS** experimental sample for a set of 48 barcoded conditions.
UTC_CELLS FCS	**FCS** file containing the flow cytometry measurements from the **UTC_CELLS** experimental sample for a set of 48 barcoded conditions.
PARENT DIRECTORY	Designated folder for storing debarcode R input files for a given experiment: **STAINED_CELLS FCS** file(s), **FCB_CELLS FCS** file(s), **UTC_CELLS FCS** file(s), **RMD,** and plate map.
PLATE DIRECTORY	Folder generated within **PARENT DIRECTORY** as part of the debarcodeR computational workflow that stores the debarcodeR output files for a given set of 48 barcoded conditions.

Before you Begin

Preparing to Run DebarcodeR

The debarcodeR protocol described below can be used to computationally deconvolute **FCS** files corresponding to a given set of 48 barcoded conditions. Typically, we run two barcoded 48-well experiments on a single 96-well plate. One experiment in columns 1-6 and another in columns 7-12. Prior

to running DebarcodeR for the first time, R, RStudio and rtools must be downloaded. The following guide walks through how to set up the R environment with R, rtools, and RStudio for both Windows and Mac Operating Systems: https://ohdsi.github.io/Hades/rSetup.html#Instructions_for_Windows. The following protocol presents an example debarcodeR workflow that interfaces with Cytobank, a cloud-based data analysis platform for working with single-cell data sets, however, is intended to be a broadly applicable reference irrespective of platform.

Debarcode Data using DebarcodeR

Prepare Folder with Requisite Files

Timing: 10 minutes

1. Create and name a new folder. This folder is referred to as **PARENT DIRECTORY** from here on.
2. Navigate to the following Github repository: https://github.com/cytolab/DebarcodeR/tree/master/Protocols
3. Download the debarcodeR **RMD,** "DebarcodeR_Template_RMD.rmd"**,** from the above Github link and transfer to **PARENT DIRECTORY.**
4. Prepare plate map:
    a) Download the plate map template Excel workbook, "DebarcodeR_Template_Platemap.xlsx", from the above Github link and transfer to **PARENT DIRECTORY**.
    b) Open the workbook.
    c) Do not change the columns labeled "plate.row" or "plate.col"
    d) In the "Doses" column, input the corresponding individual well label (i.e. Etoposide, Nocodazole, Staurosporine, 11, 12....).
    e) In the "Conditions" column, input the experimental group that the well belongs to (i.e. "Compound" or "Control").

f) Name each tab of the Excel workbook according to the name of each assay plate listed on the corresponding **FCS** files.

Note: Typically, we run two 48-well barcoded experiments on a single 96-well plate.

5. Download **FCS** files from Cytobank:
    a) Navigate to the Cytobank experiment containing the experimental files.
    b) Click *Actions* > *Export* > *Download files.*
    c) Click the checkbox for the **STAINED_CELLS FCS**, **FCB_CELLS FCS**, and **UTC_CELLS FCS** files corresponding the name of the plate being debarcoded.
    d) Click *Zip & Download Files***.**
    e) An email with the **FCS** files will be sent to the email associated with the Cytobank account. Download the emailed zip file and unzip into **PARENT DIRECTORY**.
    f) Additional guided instructions can be found here: https://support.cytobank.org/hc/en-us/articles/205047378-Download-FCS-files-and-other-files-from-the-Cytobank-platform.

Debarcoding plate in RStudio

Timing: 30 minutes (per 48-well barcoding set)

Note: Within the **RMD** each chunk is labeled with the corresponding step as presented in the following protocol.

1. Open the **RMD** from **PARENT DIRECTORY**.
2. Change the title, author, and date at the top of the **RMD.**
3. If this is the first time DebarcodeR is being run, run the {r download packages} chunk. Otherwise, skip this chunk and proceed to step 4.
4. Run the {r load packages} chunk.
5. Generate an API Token in Cytobank:
    a. Navigate to the ‘Account Settings’ page by clicking on the username drop-down menu in the top right of the navigation bar.

b. Click on Account Settings.

c. On the Account Settings page, find the API Token section and click *Generate API Token***.** A box will appear with the API Token which will last for 8 hours.

d. Copy the API Token.

e. Additional guided instructions can be found here: https://support.cytobank.org/hc/en-us/articles/115003798071-Generate-an-API-token-in-Cytobank.

6. Fill out the {r collect exp info} chunk:

   a. Replace the existing API Token in the **RMD** with the newly generated API Token.

   b. Fill out the experiment ID (eg. 41810) of the parent Cytobank experiment.

   c. Set the base directory to be the file path for **PARENT DIRECTORY**.

Note: the format of a file path differs based on operating system

   d. Run the {r collect exp info} chunk.

7. Fill out the {r input plate name, columns, platemap, select FCB/UTC/stain files} chunk:

   a. Input the name of the plate as is listed in the **FCS** files and the tab of the Excel spreadsheet containing the corresponding plate map.

   b. Input the columns of the plate half being debarcoded (“1-6” or “7-12”)

   c. Input the cell type used.

   d. Input the initials of the user.

   e. Input the name of the gate containing “Live cells for analysis”.

   f. Input name of compensation matrix.

   g. Input the file path for the excel workbook containing plate map.

   h. Input the exact names of **STAINED_CELLS FCS, FCB_CELLS FCS**, and **UTC_CELLS FCS** files including the “.fcs” extension.

   i. Run the {r input plate name, columns, platemap, select FCB/UTC/stain files}

      i. **Note:** the names of the **STAINED_CELLS FCS, FCB_CELLS FCS**, and **UTC_CELLS FCS** files are returned at the end of the chunk. Check that the file names correspond to the desired plate name and column.

8. Run the {r gate, scale and compensate} chunk. This chunk applies the “Live cells for analysis” gate and compensation to the **STAINED_CELLS FCS, FCB_CELLS FCS**, and **UTC_CELLS FCS** files.
9. Run the {r debarcoding fcb file} chunk.
    a. Check the newly created folder within **PARENT DIRECTORY** named according to the plate and columns being debarcoded. This folder is referred to as **PLATE DIRECTORY** from here on. Within this folder should be 4 plots that can be used to assess quality of debarcoding (**Figure 1**).

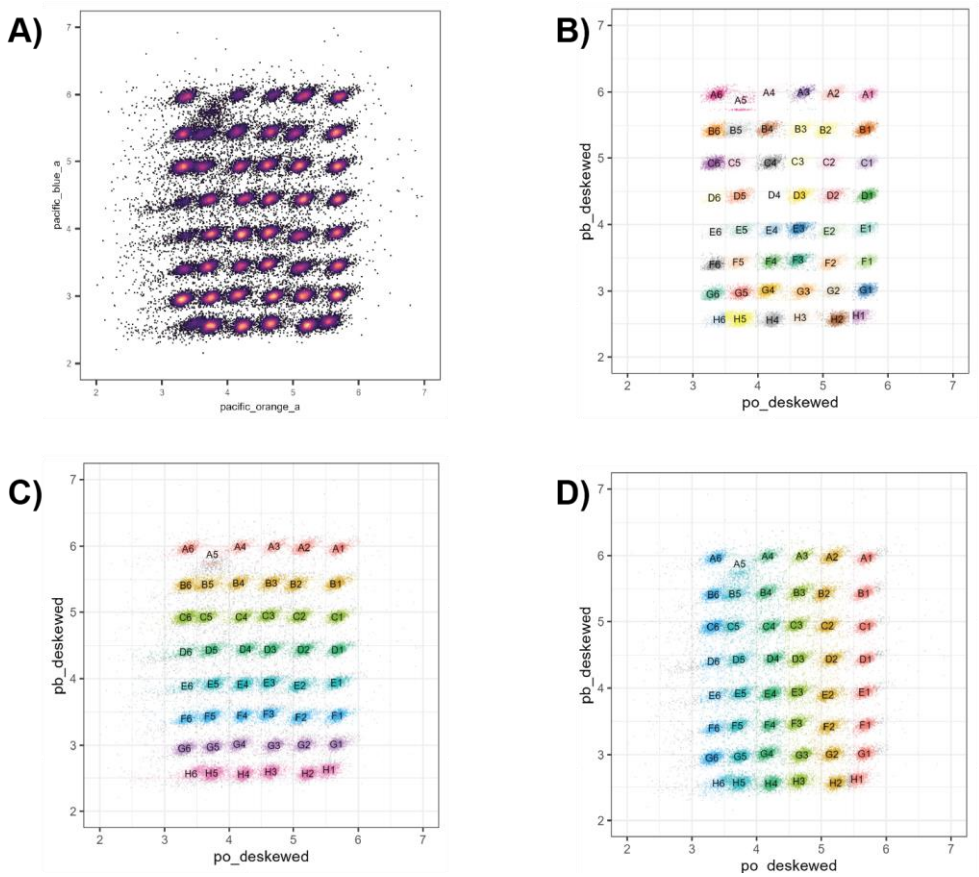

Figure 1 - Example quality control plots outputted in the process of running debarcodeR (Steps 9 and 11). A) “deskewed” plot depicts the 48 distinct populations resulting from the adjustment and prediction of dye uptake for each well. B) “well_all” plot demonstrates the 48 well assignments resulting from the clustering of deskewed data to estimate the probability cells came from a given barcoding level. C) “well_pblevel” plot shows estimated **PB** level assignments. D) “well_polevel” plot shows estimated **PO** level assignments.

10. Run the {r output debarcoded fcb} chunk. This chunk creates a new **FCS** file for each well from the **FCB_CELLS FCS** file and outputs these files into a new folder named “debarcoded” within **PLATE DIRECTORY**.

Note: within the “debarcoded” folder there should be 50 total files: 48 **FCB_CELLS FCS** files (one for each well) + 1 **FCB_CELLS FCS** file (for unassigned cells) + 1 annotation text file.

11. Run the {r debarcoding stained} chunk. The same plots generated in step 9 will be outputted to **PLATE DIRECTORY** for the **STAINED_CELLS FCS** data.
12. Run the {r output debarcoded stained files} chunk. As with step 10, this chunk creates a new **FCS** file for each well from the **STAINED_CELLS FCS** file and outputs these files the “debarcoded” folder within **PLATE DIRECTORY**.

Note: within the “debarcoded” folder there should now be 99 total files: 48 **FCB_CELLS FCS** files (one for each well) + 1 **FCB_CELLS FCS** file (for unassigned cells). 48 **STAINED_CELLS FCS** files (one for each well) + 1 **STAINED_CELLS FCS** file (for unassigned cells) + 1 annotation text file.

13. Run the {r clone exp} chunk. This chunk creates a clone of the original Cytobank experiment and uploads the **STAINED_CELLS FCS, FCB_CELLS FCS**, and **UTC_CELLS FCS** files in addition to the 98 newly debarcoded files (101 total **FCS** files) to the clone. Once the chunk finishes running, there will be a new experiment listed under “Clones” in the “Experiment summary” page of the original Cytobank experiment.
14. Click on the newly cloned experiment. Do not proceed to step 15 until all 101 **FCS** files are shown under “FCS files” on the “Experiment summary” page. This takes around a minute and requires refreshing the page a few times.
15. Run the {r upload sample tags based on uploaded platemap} chunk. This assigns sample tags to the newly created Cytobank experiment based on the plate map provided in the {r input plate name, columns, platemap, select FCB/UTC/stain files} chunk.
16. Run the {r create debarcodeR output plot} chunk. A plot will be generated and outputted to **PLATE_DIRECTORY.**