

Tutorial and Instruction for Using FlowGate Infrastructure (<http://flowgate.jcvi.org>) for Computational Analysis of Flow Cytometry Data

Version of 28 September 2018

1. User Account Registration

The current version of FlowGate is invitation-only. When the user clicks the “Sign Up Here” on the landing page The following page will send user requests or comments to flowgate_helpdesk@jcvi.org (all the developers at JCVI can access this email).

Registration

FlowGate is being alpha-tested. Before its formal release, the account registration is invitation-only. Please send us your comments. We sincerely appreciate your support.

Your Name

Your Email

Comments for us

Comments for us

Submit your comments

2. Login to FlowGate

Currently the alpha-testing users should use the provided account to login at <http://flowgate.jcvi.org>. After FlowGate is officially released, the user can register for their own accounts (and “forgot your password” will also work to allow the user to reset the password).

Dataset from ImmPort SDY 180 (PI Palucka) at www.immport.org. Systems scale interactive exploration reveals quantitative and qualitative differences in response to 2009-2010 Fluze influenza vaccine and pneumococcal vaccine.

FlowGate

Sign In

Username

Password

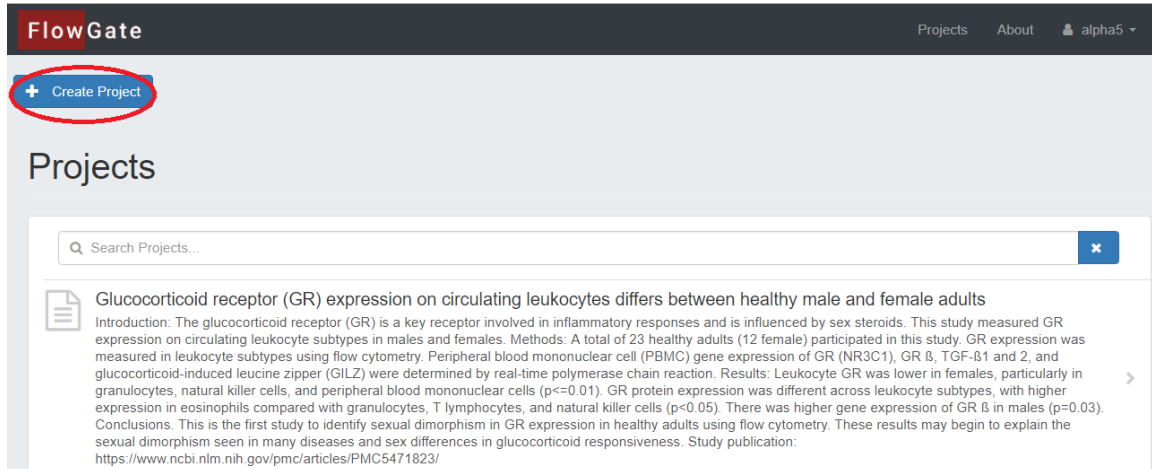
Login

[Sign Up Here](#) or [Forgot your password?](#)

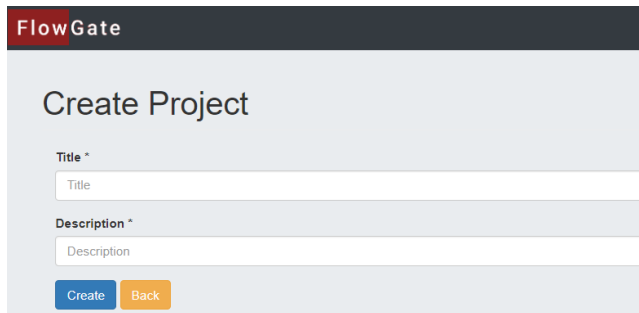
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3. Create a Project

On the first page after login, you will see a button “+ Create Project” for creating a project. A project is the data container that corresponds to your biomedical study with one or multiple pre-defined translational or clinical goal(s). One project may consist of multiple flow cytometry experiments, e.g., you may need to use multiple reagent panels to complete your study.



Click the button to see the page for creating a project, which requires only a title and description.



A project, after creation, cannot be changed or deleted as in the current version of FlowGate.

4. Create an Experiment

After you create a project, you will be able to see a button under the project description “+ Create Experiment”.



Click the button, and you will see the page for entering title, description, and hypothesis of a flow cytometry experiment. The “*” by the label means the field is required. After filling in the info, click “Create” to create the experiment.



The 'Create Experiment' form is a light gray box with three input fields. The first field is labeled 'Title *' with a red asterisk. The second field is labeled 'Description *' with a red asterisk. The third field is labeled 'Experiment Hypothesis' with a red asterisk. Below the fields are two buttons: 'Create' (blue) and 'Back' (orange).

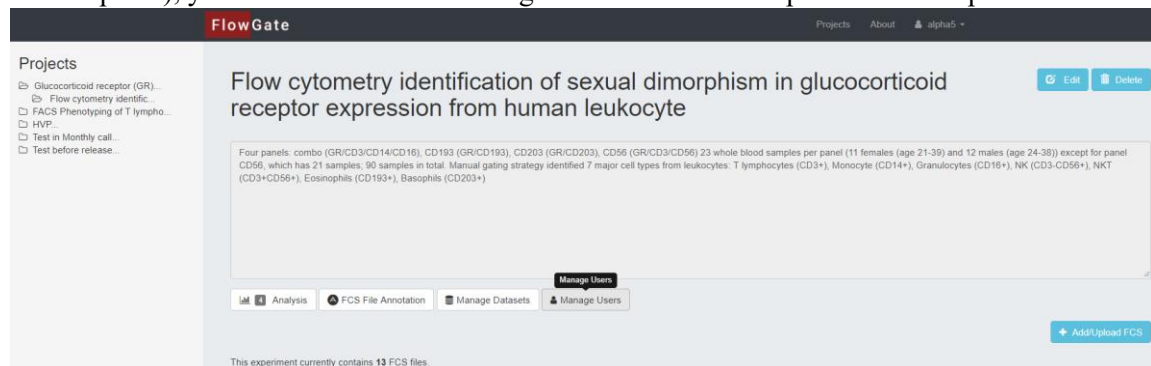
Usually one flow cytometry experiment involves only one reagent panel. Different staining panels imply different FCM experiments, although this is not strictly followed by all FlowGate users.

The title and description of an experiment can be edited. An experiment can also be deleted by the project owner by clicking the buttons to the right of the experiment title:

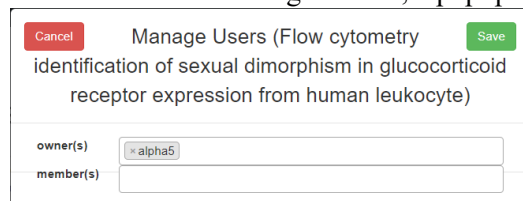


5. Manage Users of an Experiment

Click the experiment title under the project (or click the experiment name in the navigation tree in the left panel), you will see a button “Manage Users” under the experiment description.



Click the button “Manage Users”, a popup window will show



The 'Manage Users' popup window has a title bar with 'Cancel' and 'Save' buttons. The title is 'Manage Users (Flow cytometry identification of sexual dimorphism in glucocorticoid receptor expression from human leukocyte)'. It contains two input fields: 'owner(s)' with 'alpha5' entered and a small 'x' icon to its left, and 'member(s)' which is empty.

If you are the owner of the project (a project owner automatically owns all the experiments under the project), you will be able to share this experiment with other users of FlowGate by filling in their user names into the “member(s)” and click “Save”. The owner can also delete a member by clicking the “x” before the user name and then “Save”.

6. Upload FCS Files to an Experiment

Under the experiment description, you will see a button “+ Add/Upload FCS”

FlowGate

Projects About alpha5

Flow cytometry identification of sexual dimorphism in glucocorticoid receptor expression from human leukocyte

Edit Delete

Four panels: combo (GR/CD3/CD14/CD16), CD193 (GR/CD193), CD203 (GR/CD203), CD56 (GR/CD3/CD56) 23 whole blood samples per panel (11 females (age 21-39) and 12 males (age 24-38)) except for panel CD56, which has 21 samples; 90 samples in total. Manual gating strategy identified 7 major cell types from leukocytes: T lymphocytes (CD3+), Monocyte (CD14+), Granulocytes (CD16+), NK (CD3-CD56+), NKT (CD3+CD56+), Eosinophils (CD193+), Basophils (CD203+)

Analysis FCS File Annotation Manage Datasets Manage Users

+ Add/Upload FCS

This experiment currently contains 13 FCS files.

Click the button to see the upload file page:

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Projects About alpha5

Flow cytometry identification of sexual dimorphism in glucocorticoid receptor expression from human leukocyte

Upload FCS Files

Choose Files No file chosen

You can upload single or multiple .fcs file(s).

Or you can drag and drop your files in the box below to upload (Max size for each file is 1GB)

Drop files here

1016_gcr_CD3apc_cd16viob_cd14perc_2014-09-29.002.fcs (14.67 MB)	Uploaded Successfully	100%
1034_gcr_CD3apc_cd16viob_cd14perc_2015-06-09.001.fcs (14.69 MB)	Uploaded Successfully	100%
1033_gcr_CD3apc_cd16viob_cd14perc_2015-04-16.001.fcs (14.87 MB)	Uploaded Successfully	100%
1021_gcr_CD3apc_cd16viob_cd14perc_2014-11-21.001.fcs (15.11 MB)	Cancel	83%
1016_gcr_CD3apc_cd16viob_cd14perc_2014-09-29.001.fcs (15.25 MB)	Cancel	
1035_gcr_CD3apc_cd16viob_cd14perc_2015-06-09.001.fcs (14.37 MB)	Cancel	

Upload Back

You can click the button “Choose Files” to select FCS files from your local hard drive; or you can simply drag and drop FCS files into the area “Drop files here”. The files will be transferred sequentially with progress bars shown on the page. Please note that only FCS files can be uploaded, and the maximum size of each FCS file is 1GB, which should be more than enough in most cases.

After the files are uploaded, a total number of the files in the current experiment will be displayed under the experiment description.

7. Annotate FCS Files with Experiment Metadata

After some FCS files have been uploaded to your experiment, you can click the button “FCS File Annotation” under the experiment description to annotate each FCS file with experiment metadata.

FCS File Annotation

FlowGate

ProjectsAboutalpha5

[Test before release](#) > [HVP_August_subset](#) > Annotate Uploaded FCS Files with Experiment Metadata

Annotate Uploaded FCS Files with Experiment Metadata

The annotation is based on individual FCS files by user providing attribute categories, attribute names, and attribute values. The procedure consists of two steps:

Step 1 Upload a CSV (comma-delimited) or a TSV (tab-delimited) file that contains the metadata. Each row is one FCS file and each column is one attribute. The first column is the name of the FCS file. The name of the first column must be "FCS File Name", and the names of the attributes in the rest of the header. If the metadata table is small, you can also skip the Step 1 and manually create the metadata table in **Step 2**.

Download Template

File Type: ☒ CSV (,) ☐ TSV (Tab)

No file Selected

Select Annotation File

Upload

Step 2 Create a new or edit an existing metadata table (e.g., from user-uploaded metadata table)

By clicking "+" on the tab, a new attribute category will be created. The user can specify the category of an attribute when creating an attribute

By clicking "+" in the annotation table, a new attribute will be created. The user can specify its name, category, and possible values

After providing the possible values of an attribute, the user does not need to type in its value in the table, but just select the value from the drop down list and check/uncheck the corresponding checkbox for the FCS file.

Basicscategory1+

Save and Return

	Subject Id	Sex	Sample Type	Visit Number	Visit	Biosample
	Action	Action	Action	Action	Action	Action
FCS File Name	Candidate Values UBC_GR02	Candidate Values F	Blood	Candidate Values Visit 3	Candidate Values Day 0	Candidate Values UBC_SP_GR0
HVP_Tube_001.fcs	<input checked="" type="checkbox"/> UBC_GR02	<input checked="" type="checkbox"/> F	<input checked="" type="checkbox"/> Blood	<input checked="" type="checkbox"/> Visit 3	<input checked="" type="checkbox"/> Day 0	<input checked="" type="checkbox"/> UBC_SP_GR02_BL
HVP_Tube_002.fcs	<input checked="" type="checkbox"/> UBC_GR02	<input checked="" type="checkbox"/> F	<input checked="" type="checkbox"/> Blood	<input type="checkbox"/> Visit 4	<input type="checkbox"/> Day 1 - post dose 1	<input type="checkbox"/> UBC_SP_GR02_BL
HVP_Tube_003.fcs	<input checked="" type="checkbox"/> UBC_GR02	<input checked="" type="checkbox"/> F	<input checked="" type="checkbox"/> Blood	<input type="checkbox"/> Visit 5	<input type="checkbox"/> Day 3 - post dose 1	<input type="checkbox"/> UBC_SP_GR02_BL
HVP_Tube_004.fcs	<input checked="" type="checkbox"/> UBC_GR02	<input checked="" type="checkbox"/> F	<input checked="" type="checkbox"/> Blood	<input type="checkbox"/> Visit 6	<input type="checkbox"/> Day 7 - post dose 1	<input type="checkbox"/> UBC_SP_GR02_BL
HVP_Tube_005.fcs	<input checked="" type="checkbox"/> UBC_GR02	<input checked="" type="checkbox"/> F	<input checked="" type="checkbox"/> Blood	<input type="checkbox"/> Visit 7	<input type="checkbox"/> Day 14 - post dose 1	<input type="checkbox"/> UBC_SP_GR02_BL
HVP_Tube_011.fcs	<input type="checkbox"/> UBC_GR05	<input checked="" type="checkbox"/> F	<input checked="" type="checkbox"/> Blood	<input checked="" type="checkbox"/> Visit 3	<input checked="" type="checkbox"/> Day 0	<input type="checkbox"/> UBC_SP_GR05 BL

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A CSV template will be automatically generated based on the FCS files you have uploaded,

which can be downloaded by clicking the button

Download Template

You can open the CSV template in your Excel to input metadata of the FCS files for easy editing.

The first step (Step 1) of the annotation is to upload the CSV (TSV) file that you finish using Excel or other editors.

File Type: ☒ CSV (,) ☐ TSV (Tab)

No file Selected

Select Annotation File

Upload

If the metadata file is only a small table or there is only a small number of FCS files, you can also choose to skip the Step 1 and use the online editing function in the Step 2 to annotate your FCS files.

In Step 2, you can edit the metadata table you uploaded online. By clicking "+" on the tab, a new attribute category will be created. For example, you can create a category "Subject Demographics". A standardized representation of the metadata categories will ultimately help automate the downstream statistical comparison and visualization of the data analysis results. The user can specify the category of an attribute when creating an attribute. By clicking "+" in the annotation table, a new attribute will be created. The user can specify its name, category, and possible values. After providing the possible values of an attribute, the user does not need to type in its value in the table, but just select the value from the drop-down list and check/uncheck the corresponding checkbox for the FCS file.

After editing/changing the annotation, click "Save and Return"; otherwise, the change will be lost.

When an attribute is created, the user can specify which category the attribute belongs to, its possible values, as well as whether these values should be displayed in the dataset creation page as a filter.

Attribute Category * Basics

Attribute Name * Visit

For filtering out fcs files or creating cohorts for statistical comparison ☒

Day 0	Disp. Order *	1	Delete
Day 7	Disp. Order *	1	Delete

Add possible values for this attribute

Submit Cancel

In the metadata table on the annotation page, the action drop-down list supports hiding the column from display or from the dataset creation page. The user can also delete the attribute.

8. Create a Dataset for Analysis

By clicking the “Manage Datasets” button under the experiment, you will see the list of the current datasets (the list is blank if no dataset is created yet).

FlowGate Projects About alpha5

Flow cytometry identification of sexual dimorphism in glucocorticoid receptor expression from human leukocyte Edit Delete

Four panels: combo (GR/CD3/CD14/CD16), CD193 (GR/CD193), CD203 (GR/CD203), CD56 (GR/CD3/CD56) 23 whole blood samples per panel (11 females (age 21-39) and 12 males (age 24-38)) except for panel CD56, which has 21 samples; 90 samples in total. Manual gating strategy identified 7 major cell types from leukocytes: T lymphocytes (CD3+), Monocyte (CD14+), Granulocytes (CD16+), NK (CD3-CD56+), NKT (CD3+CD56+), Eosinophils (CD193+), Basophils (CD203+)

Manage Datasets new

Analysis FCS File Annotation Manage Datasets Manage Users

+ Add/Upload FCS

This experiment currently contains 19 FCS files.

Each experiment can contain multiple datasets. Each FCS file can be included into multiple datasets. A dataset is defined as a set of FCS files to be analyzed together in a single batch using a computational pipeline at FlowGate. **Therefor ALL the FCS Files in a dataset must be from the same reagent panel.**

Glucocorticoid receptor (GR) expression on circula... > Flow cytometry identification of sexual dimorphism... > List of Datasets for Analysis

List of Datasets for Analysis

[+ Create New Dataset](#) [Back](#)

Name	Description	Files
6FCSfiles_UCI_GR_ComboPanel		1029_gcr_CD3apc_cd16viob_cd14perc_2015-03-03.001.fcs 1030_gcr_CD3apc_cd16viob_cd14perc_2015-03-17.001.fcs 1019_gcr_CD3apc_cd16viob_cd14perc_2014-10-20.001.fcs 1018_gcr_CD3apc_cd16viob_cd14perc_2014-10-20.001.fcs 1020_gcr_CD3apc_cd16viob_cd14perc_2014-11-21.001.fcs 1022_gcr_CD3apc_cd16viob_cd14perc_2014-11-21.001.fcs
TestDataset3		1019_gcr_CD3apc_cd16viob_cd14perc_2014-10-20.001.fcs 1019_gcr_CD3apc_cd16viob_cd14perc_2014-10-20.001.fcs
Test dataset in Monthly call		1018_gcr_CD3apc_cd16viob_cd14perc_2014-10-20.001.fcs 1019_gcr_CD3apc_cd16viob_cd14perc_2014-10-20.001.fcs 1018_gcr_CD3apc_cd16viob_cd14perc_2014-10-20.001.fcs

Click the button “+ Create New Dataset” to create a new dataset based on uploaded FCS files and their metadata in this experiment:

Test before release > HVP_August_subset > List of Datasets for Analysis > Create Dataset

Create Dataset

Name *

Description

Filter ^

Subject Id	Sex	Visit Number	Visit
<input type="checkbox"/> UBC_GR02	<input type="checkbox"/> F	<input type="checkbox"/> Visit 3	<input type="checkbox"/> Day 0
<input type="checkbox"/> UBC_GR05	<input type="checkbox"/> M	<input type="checkbox"/> Visit 4	<input type="checkbox"/> Day 1 - post dose 1
<input type="checkbox"/> UBC_GR11		<input type="checkbox"/> Visit 5	<input type="checkbox"/> Day 3 - post dose 1
<input type="checkbox"/> UBC_GR18		<input type="checkbox"/> Visit 6	<input type="checkbox"/> Day 7 - post dose 1
		<input type="checkbox"/> Visit 7	<input type="checkbox"/> Day 14 - post dose 1

☐ Select All

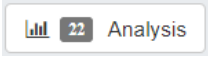
Files

<input type="checkbox"/> HVP_Tube_001.fcs	<input type="checkbox"/> HVP_Tube_013.fcs	<input type="checkbox"/> HVP_Tube_025.fcs
<input type="checkbox"/> HVP_Tube_002.fcs	<input type="checkbox"/> HVP_Tube_014.fcs	<input type="checkbox"/> HVP_Tube_041.fcs
<input type="checkbox"/> HVP_Tube_003.fcs	<input type="checkbox"/> HVP_Tube_015.fcs	<input type="checkbox"/> HVP_Tube_042.fcs
<input type="checkbox"/> HVP_Tube_004.fcs	<input type="checkbox"/> HVP_Tube_021.fcs	<input type="checkbox"/> HVP_Tube_043.fcs
<input type="checkbox"/> HVP_Tube_005.fcs	<input type="checkbox"/> HVP_Tube_022.fcs	<input type="checkbox"/> HVP_Tube_044.fcs
<input type="checkbox"/> HVP_Tube_011.fcs	<input type="checkbox"/> HVP_Tube_023.fcs	<input type="checkbox"/> HVP_Tube_045.fcs
<input type="checkbox"/> HVP_Tube_012.fcs	<input type="checkbox"/> HVP_Tube_024.fcs	

[Create](#) [Create and Analyze](#) [Back](#)

Click the “Create” button to return to the management of dataset page to see the new dataset; or click “Create and Analyze” to enter the analysis task creation and submission page with the created dataset.

9. Create and Submit an Analysis Task

Click the “Analysis” button under the experiment description  to see the list of the data analysis tasks. Each dataset can be used in multiple data analysis tasks.

HVP > August_mDCs > List of Analysis Task

List of Analysis Task

Show 10 entries Search:

Task Name	Task Status	Submitted on	Creator
alpha test 15	● results ready	28/08/2018 04:50:04	alpha5
alpha test 14	● results ready	28/08/2018 04:27:28	alpha5
alpha test 13	● results ready	28/08/2018 03:29:23	alpha5
alpha test 12.9	● results ready	28/08/2018 03:15:17	alpha5
alpha test 12.8	● results ready	27/08/2018 09:13:40	alpha5
alpha test 12.7	● results ready	27/08/2018 04:32:59	alpha5
alpha test 12.6	● results ready	27/08/2018 04:11:39	alpha5
alpha12.5	● results ready	27/08/2018 03:55:31	alpha5
Analysis12	● results ready	23/08/2018 02:12:22	alpha5
Analysis11	● results ready	23/08/2018 02:08:48	alpha5

Showing 1 to 10 of 21 entries Previous **1** 2 3 Next

[+ New Analysis](#)

The table records the task submission time. It also shows the status of each task (green: ready; red: error; yellow: pending). It will automatically paginate based on the user-specified number of entries shown on each page.

Click the button “+ New Analysis” at the bottom of the list to create a new analysis task:

Create New Analysis

Basic **Advanced**

Analysis Module*

Analysis Name*

Analysis Description

Input.Dir [dataset]

header_replace.txt [dir/file] No file chosen

header.list [dir/file] No file chosen

config.file [dir/file] No file chosen

rev.config.file [dir/file] No file chosen

Input.comp [dir/file] No file chosen

The user will need to choose an “Analysis Module” before seeing the input parameters needed in this module. If a generic DAFi pipeline is selected (e.g., DAFi.Complete.Pipeline), all the parameters need to be specified or uploaded by the user.

Create New Analysis

Basic Advanced

Analysis Module*

DAFi.for.UCI_GR

Analysis Name*

Analysis Description

Input.Dir [dataset]

group1

Submit

Cancel

If a customized module is selected (e.g., DAFi.for.UCI_GR), the user usually only needs to select a dataset from the drop-down list, which will display all the existing datasets in the current experiment.

Click the “Submit” button to submit the analysis task, which will be performed on the backend of FlowGate using the Comet Cluster at the San Diego Supercomputer Center. The status of the submitted tasks can be viewed in the List of Analysis Task page, which automatically refreshes. In this version of FlowGate, the data analysis results will be available in HTML format as a Jupyter Notebook Report.

10. View a Jupyter Notebook Report

When the status of an analysis task becomes green, click its title to see the result summary:

HVP > August_mDCs > List of Analysis Task > Analysis Results

Analysis Results

Experiment:	August_mDCs
Analysis Name:	alpha12.5
Analysis Description:	retest of 12 with updated description.txt
Analysis Status:	results ready
Job Number:	1485
Sent by:	alpha5
Date Created:	08/27/2018 03:55:31
Date Completed:	08/27/2018 09:00:47
Job Complete Status:	● The job finished without an error
Available Reports:	Reports/AutoReport.html Display Download
Job Results Archive:	launchJob.sh Download Job.18844152.tar.gz Download

Edit

Delete

Click the “Display” to open the Report in a new Tab in your browser.

11. Contact Us

The user can contact us from the web page “Contact Us” or send an email to flowgate_helpdesk@jcvl.org