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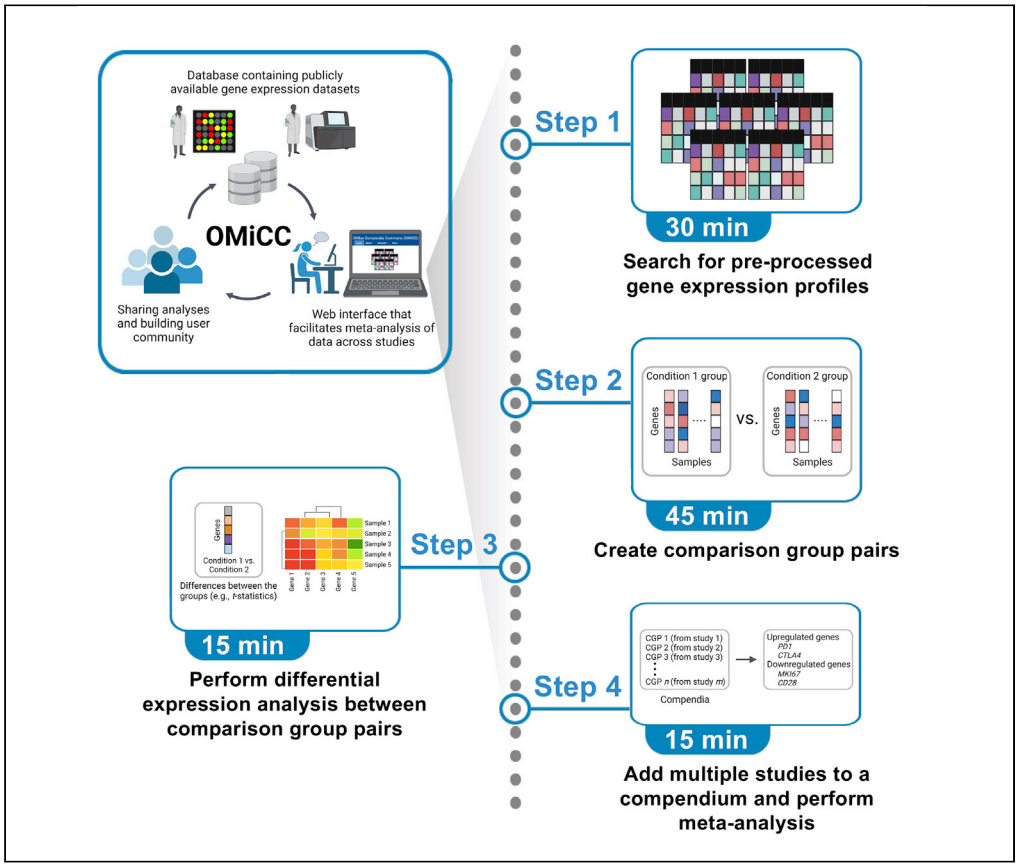
OMiCC: An expanded and enhanced platform for meta-analysis of public gene expression data



OMiCC (OMics Compendia Commons) is a biologist-friendly web platform that facilitates data reuse and integration. Users can search over 40,000 publicly available gene expression studies, annotate and curate samples, and perform meta-analysis. Since the initial publication, we have incorporated RNA-seq datasets, compendia sharing, RESTful API support, and an additional meta-analysis method based on random effects. Here, we provide a step-by-step guide for using OMiCC.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

OMiCC (OMics Compendia Commons) is a free web-based tool for gene expression data reuse

Search publicly available studies to perform sample group comparisons to explore a disease

In meta-analysis, multiple studies are combined to identify coherent signals

OMiCC supports crowd-sharing and users can share their own analyses with the community

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SUMMARY

OMiCC (OMics Compendia Commons) is a biologist-friendly web platform that facilitates data reuse and integration. Users can search over 40,000 publicly available gene expression studies, annotate and curate samples, and perform meta-analysis. Since the initial publication, we have incorporated RNA-seq datasets, compendia sharing, RESTful API support, and an additional meta-analysis method based on random effects. Here, we provide a step-by-step guide for using OMiCC.

For complete details on the use and execution of this protocol, please refer to Shah et al. (2016).

BEFORE YOU BEGIN

Overview

Advances in microarray and RNA sequencing technologies have led to a rapid increase in the amount of gene expression data deposited in public data repositories such as the Gene Expression Omnibus (GEO) (Barrett et al., 2013) and Array Express (Rustici et al., 2013). Integration and meta-analysis of data across studies is a powerful tool to incorporate heterogeneity into analysis and increase the statistical power for generating and testing hypotheses (Andres-Terre et al., 2015; Chaussabel and Baldwin, 2014; Chen et al., 2014; Dudley et al., 2011; Granlund et al., 2013; Khatri et al., 2013; Segal et al., 2005; Sirota et al., 2011; Sweeney et al., 2015; Teslovich et al., 2010). The ever-growing volume of publicly available data enables such meta-analysis, but much of the data remains under-utilized (Haynes et al., 2017; Rung and Brazma, 2013), partly because integrating data across multiple studies is not trivial and requires the user to search databases for relevant studies, manually annotate samples, transform data into different formats, normalize the data, and finally perform meta-analysis (Ramasamy et al., 2008; Rung and Brazma, 2013). Significant statistical and computational experience is required for many of these steps, which may dissuade biologists with less computational training to perform such analyses.

To bridge the gap between the volumes of publicly available data and the integration of this data to generate new biological insights, we developed a biologist-friendly web platform for data reuse and

meta-analysis. OMiCC (OMics Compendia Commons) is a freely available tool that enables biologists with little computational training to search existing data sets easily, annotate and curate data, and perform differential expression analysis and meta-analysis (Shah et al., 2016). Users can add multiple publicly available datasets to a compendium, a collection of studies focused on the biological question of interest, and on which differential analysis and meta-analysis can be performed. To date, OMiCC contains pre-processed datasets from more than 40,000 studies from GEO and the Sequencing Read Archive (SRA) (Leinonen et al., 2011). OMiCC is continually supported and updated with new studies deposited in GEO and SRA. Since the original publication (Shah et al., 2016), we have added multiple new features, including incorporating RNA-seq data, sharing of compendia, RESTful API support, and an additional meta-analysis method. In this paper, we provide users with a clear, step-by-step workflow for performing a complete analysis in OMiCC, from study selection to meta-analysis, as well as walk users through the new features of OMiCC.

The metadata available in large public repositories is often not standardized, and annotating samples (i.e., assigning labels such as disease state, gender, age) and collating datasets (i.e., creating control and treatment groups) is a time-consuming step (Ramasamy et al., 2008; Rung and Brazma, 2013). To streamline this process and enable the generation of gene expression signatures based on two-group comparisons, OMiCC supports creating sample groups and comparison group pairs (CGPs) using a simple point-and-click interface. A CGP contains two sample groups, for example, a control group and a treatment group from the same experiment/study, on which differential gene expression analysis can be performed. CGPs can be added to a compendium, which represents a group of CGPs relevant to a biological question. Users can add annotations to sample groups specifying perturbation, disease, sample, or source type. Users can then perform two types of analysis, differential expression analysis within a study and meta-analysis across studies, using an easy-to-use web interface. Users are able to retrieve the analyzed data directly from OMiCC.

Moreover, an important aspect of OMiCC is its crowdsourcing feature. In OMiCC, annotations and curated data sets created by users are stored and can be made available to other OMiCC users, who can integrate and use these data sets for their own analyses. One of the new features allows sharing an entire compendium containing data, structured and reusable annotations, and analyses integrated across multiple datasets. Users are also able to provide professional information on their profile, such as a link to a professional or LinkedIn page; this allows others to determine if a user shares biological interests or level of expertise. Thus, OMiCC provides the broad biomedical research community with the capacity to participate in community-wide collaborations. In 2016, we conducted a crowdsourcing "jamboree" exercise within the National Institutes of Health, where groups were tasked with using OMiCC to assess transcriptomic signatures of several autoimmune diseases (Lau et al., 2016; Sparks et al., 2016). We reported encouraging findings, providing evidence that OMiCC can facilitate and accelerate the pace by which publicly available data can be used to generate new biological insights.

While we provide evidence that biologists with little computational experience can use OMiCC to perform meta-analysis, wider adoption requires biologists to invest the time to familiarize themselves with the platform and its features. As the OMiCC user community grows, more users will create and share their datasets. This protocol provides an important resource towards achieving wider adoption by providing a clear guide and walking users through a complete workflow.

Comparison with other methods

While there are other useful resources that can be applied for data reanalysis and meta-analysis, it takes considerable programming to connect these tools in a complete workflow (Ramasamy et al., 2008). Microarray Retriever searches and retrieves data from GEO and ArrayExpress (Ivliev et al., 2008). ProfileChaser (Engreitz et al., 2011) and ExpressionBlast (Zinman et al., 2013) are web tools that take a gene expression profile as input and query GEO studies by similarity to the provided data. NetworkAnalyst (Xia et al., 2015) takes a list of genes or proteins or gene expression data as

input and performs meta-analysis and network visualization. GenePattern (Kuehn et al., 2008) provides a web interface for a broad array of computational tools for analyzing gene expression data. These tools focus on one or a subset of the steps in the complete workflow, while OMiCC provides a simple user-interface for the entire workflow from searching for samples to performing meta-analysis.

A similar tool, Crowd Extracted Expression of Differential Signatures (CREEDS), is a web resource that contains disease signatures that were annotated and analyzed through a crowdsourcing exercise (Wang et al., 2016). Through an online course on Coursera, participants developed over 2,000 single-gene perturbation signatures, over 800 disease signatures, and over 900 drug perturbation signatures. This further demonstrates the value of crowdsourcing in meta-analyzing existing data sets. While CREEDS contains thousands of previously curated signatures, OMiCC allows users to develop their own signatures by creating their own CGPs and compendia. Therefore, OMiCC facilitates the creation of CREEDS-like signatures without any programming. Furthermore, communities can be built using the crowdsourcing features in OMiCC, as evidenced by our jamboree event (Lau et al., 2016).

Terminology used in OMiCC

1. Sample group: A collection of samples from a single study. Two sample groups are required to create a comparison group pair. For example, one sample group can be made up of samples before an influenza challenge, while the second sample group can be made up of samples collected 12 h after an influenza challenge. Sample groups must be annotated using at least two MeSH (Medical Subject Headings) terms. MeSH is a controlled vocabulary used to index and search biomedical databases such as MEDLINE/PubMed (https://www.nlm.nih.gov/mesh/meshhome.html).
2. Comparison group pair (CGP): Composed of two sample groups from the same study that a user wants to compare. To compare the changes in gene expression after an influenza challenge, a user can add the sample groups described above to a CGP. Multiple CGPs can be made from a single study. For example, if one wants to compare gene expression between males and females, a user can create a CGP from the study mentioned above with a healthy male sample group and a healthy female sample group.
3. Compendium: A collection of CGPs. Users can add CGPs from multiple studies to a compendium.
4. Differential expression profile (DEPs): Gene expression differences of all genes between the two sample groups in a CGP.
5. Meta-analysis: Method for extracting statistically coherent signals from multiple CGPs, even when CGPs are from different studies or generated using different technology platforms. OMiCC provides two methods for meta-analysis, RankProd (Hong et al., 2006) and MetaIntegrator (Haynes et al., 2017).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
OMiCC (OMics Compendia Commons)	(Shah et al., 2016)	OMiCC URL: https://omicc.niaid.nih.gov/
recount2	(Collado-Torres et al., 2017)	https://jhubiostatistics.shinyapps.io/recount/
RankProd	(Hong et al., 2006)	https://bioconductor.org/packages/RankProd/
MetaIntegrator	(Haynes et al., 2017)	https://CRAN.R-project.org/package=MetaIntegrator
Deposited data		
Gene Expression Omnibus (GEO)	(Barrett et al., 2013)	http://www.ncbi.nlm.nih.gov/geo/
Sequencing Read Archive (SRA)	(Leinonen et al., 2011)	https://www.ncbi.nlm.nih.gov/sra

MATERIALS AND EQUIPMENT

To use OMiCC, all required is a computer with an internet connection. We recommend Chrome or Safari internet browsers (tested on Chrome version 98.0.4758.80 and Safari version 14.1.2).

STEP-BY-STEP METHOD DETAILS

Herein we provide users with a clear, step-by-step workflow for performing a complete analysis in OMiCC, from study selection to meta-analysis, as well as walk users through the new features of OMiCC.

Register

Timing: 5 min

This step describes how to register for an account on OMiCC.

1. To utilize the full functionality of the website, register for a free account with OMiCC.
   a. Go to https://omicc.niaid.nih.gov/ and click the "Register" button.
2. Enter an account name, password, name, and email.
   a. Users enter information about their area of expertise and organization, and can link to a professional home page, PubMed search term, LinkedIn ID, or ResearchGate ID.
3. An email is sent to the address provided to complete the registration process.

Search for studies and existing CGPs or compendia using the "search" tab

Timing: 15 min

This step describes how to search for gene expression studies on OMiCC, and how to search for existing CGPs and compendia created by other users.

Under the "Search" tab/menu at the top:

4. Select "On Study" to search for GEO or SRA studies using keywords extracted from the study and filtering by subject or technology platform (Figure 1).
   a. The search can be restricted to human or mouse only, or to specific platforms.
   b. Select "Studies with public Comparison Group Pairs" to search studies with at least one publicly available CGP.
   c. After applying a filter, click "Search".
   d. To get a list of all studies in the OMiCC database, click "Search" without any keywords or click "Browse All".
   e. Expand the study by clicking on the "+" icon to view study details.
   f. Click the icon under the "Study" column to be linked to the study on the GEO website to view experimental details, study design and contributors, and any available citations.
   g. Save studies by clicking on "Save to my study list" to return to these studies later.
5. Select "On Sample Groups" to search for sample groups by using keywords extracted from sample group annotations or metadata associated with the sample group, such as the user ID of the owner.
   a. Select the "Public Groups" option to show only sample groups that an owner makes public.
   b. Click "Search" after applying a filter.
6. Select "On Comparison Group Pairs" to search for comparison group pairs (CGPs) by using keywords extracted from the CGP title, the user ID of the owner, or the MeSH terms that are used to annotate the sample groups.
   a. Select "Public CGPs" to show only CGPs that the owner makes public.
   b. Click "Search" after applying a filter.

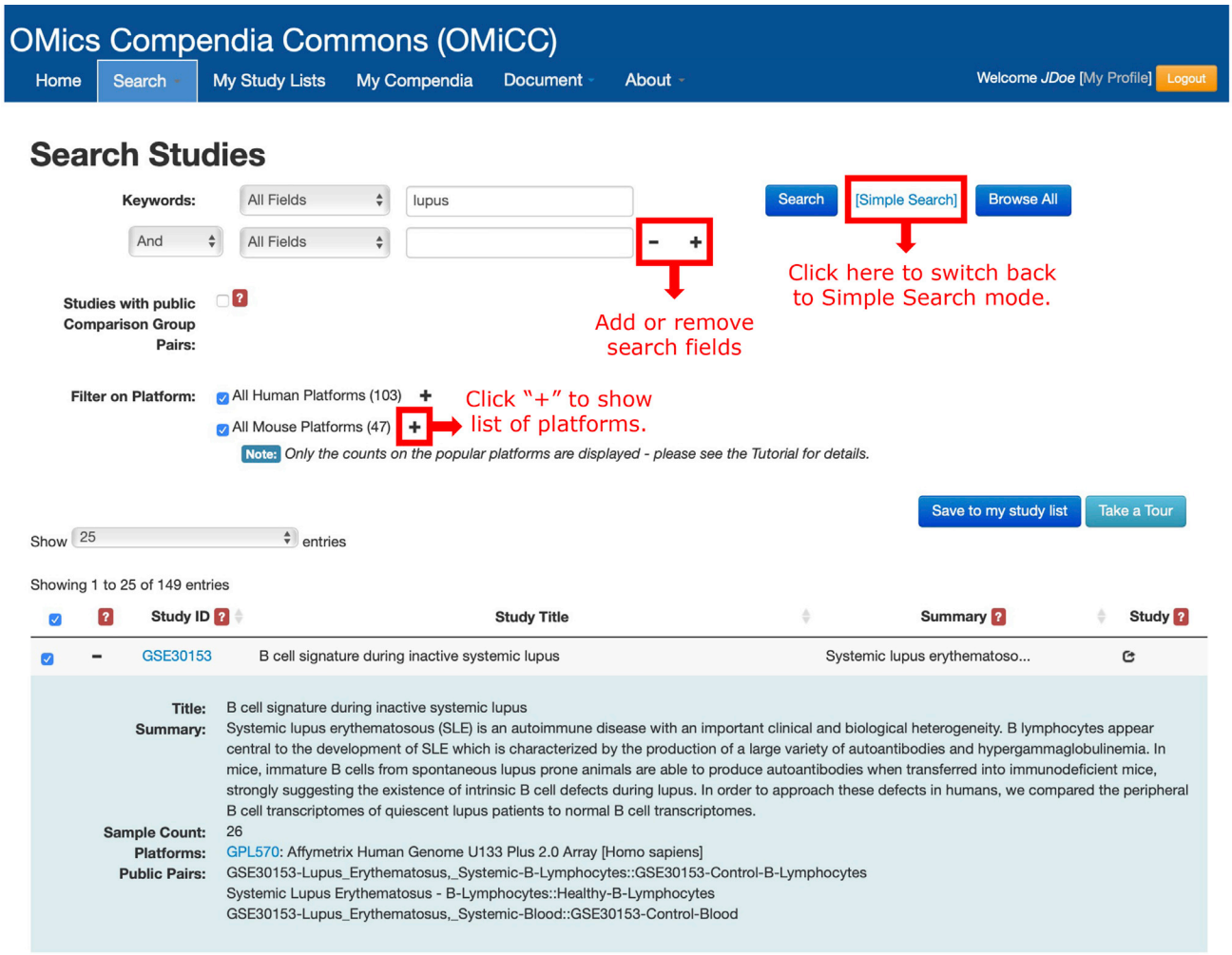


Figure 1. Search for studies

Users can search for studies using keywords on all fields or restricted data fields. The search results are presented in a list format, while detailed information can be displayed without leaving the search result list.

c. Select CGPs and click the "Add to Compendium" button to add them to an existing compendium.

7. Select "On Compendia" to search for compendia using keywords extracted from the compendia name, description, or user ID of the owner.
   a. Select "Public Compendia Only" to search on compendia that the owner makes public.
   b. Click "Search" after applying a filter.
   c. To add CGPs to your own compendium, click the "+" button in front of the compendium name, then check the specific CGPs to add.
   d. To add all the CGPs in a compendium, select the associated checkbox to the left of the compendium name and click the green "Add to the Compendium" button.

Create sample groups

Timing: 30 min

This step describes how to create sample groups from publicly available gene expression data.

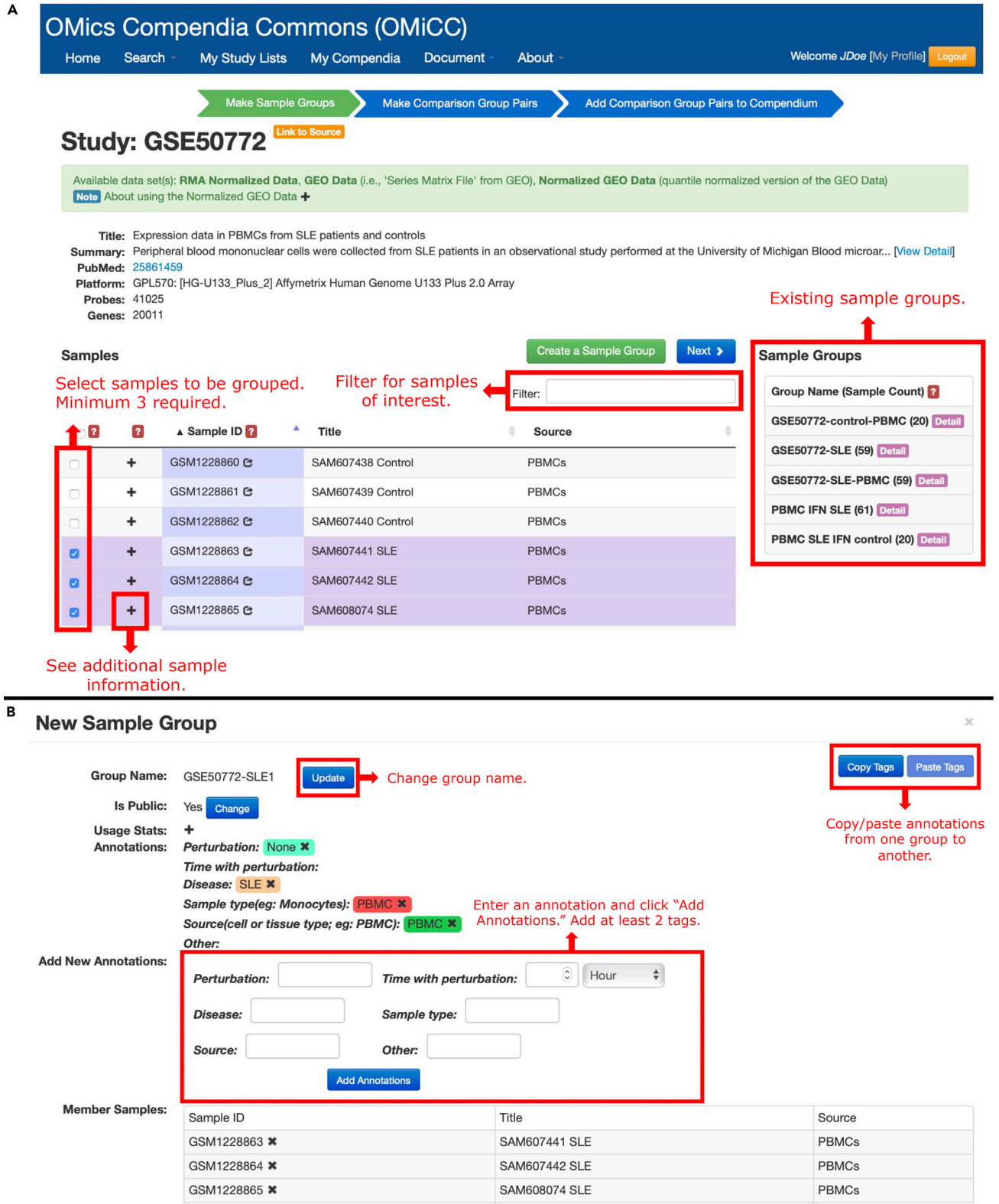


Figure 2. Create sample groups

(A) Operations on a specific study can be followed with the green and blue arrows at the top of the page. To view sample metadata, users can click the "+" and select samples to add to a Sample Group. Filter for samples of interest by inputting key words. Select the samples to include (a minimum of

Figure 2. *Continued*
3 samples) in a sample group by checking the boxes. Once created, the sample group name appears in the sample group list alongside previously created sample groups created by other OMiCC users. (B) Upon clicking the "Create a Sample Group" button, a pop-up panel appears where the user can add annotations (a minimum of 2 annotation tags) to the selected samples. To copy the annotation tags to another group, click the "Copy Tags" button, then the "Paste Tags" button in the other group. You can change the group name by clicking the "Update" button. After saving the Sample Group, the user can proceed to the "Make Comparison Group Pairs" step by clicking the "Next" button.

8. From the study search results, click on the Study ID to access the study page.
9. To create your own sample group, select samples to form a group (i.e., cases or controls).
   a. Use the filter to identify samples of interest.
   b. For example, if you are interested in samples from systemic lupus erythematosus (SLE) patients, enter "SLE" into the filter to display only those samples.
   c. At least 3 samples are required for each sample group (Figure 2A).
10. After selecting the samples, click the "Create a Sample Group" button. A pop-up window appears for the new sample group (Figure 2B).
11. Enter at least two annotations for the sample group.
   a. We provide a controlled vocabulary (MeSH) to annotate sample groups.
   b. MeSH annotation suggestions appear as you type into the annotation box.
   c. While any text is allowed, we encourage users to use the MeSH terms.
   d. Click the "Add Annotations" button after entering in the annotations.
12. The sample group name generates automatically based on the annotations.
   a. To change the sample group name, click the "Update" button, type in the desired group name, then click "Save".
13. To remove unwanted annotations, click the "X" next to the annotation term.
14. To copy the annotation tags to another group, click the "Copy Tags" button, then the "Paste Tags" button in the other group.
15. To make the sample group public, click the "Change" button next to the "Is Public" field.
   a. Once a sample group is saved and public, the sample group cannot be edited.
   b. If the sample group is not being used by any other OMiCC user, the owner of the group can change the status back to private and edit it.
16. Click "Save" to create the sample group.
   a. If at least 3 samples are not added to the sample group, the "Save" button is opaque and cannot be clicked.
17. If other users create sample groups using the study, the sample groups show up in bold in a pane on the right side of the browser.
   a. Click the purple "Detail" button to view annotations and sample details.
18. At least two sample groups are needed to create a CGP. Once at least two sample groups are listed under the "Sample Group" panel on the right side, click the "Next" button.

Create comparison group pair (CGP)

Timing: 15 min

This step describes how to create comparison group pairs (CGPs) using the previously created sample groups.

19. Choose two sample groups to add to the CGP.
   a. Select a sample group from the list of existing sample groups, then click on the "Add to Comparison Group Pair" button.
   b. Assign the selected sample group as "Condition 1" or "Condition 2 (reference)" (Figure 3A).
20. After both conditions are assigned, a pop-up window appears (Figure 3B).
   a. By convention, OMiCC treats the "Condition 2" sample group as the reference group for downstream differential expression analysis. A positive change in gene expression means

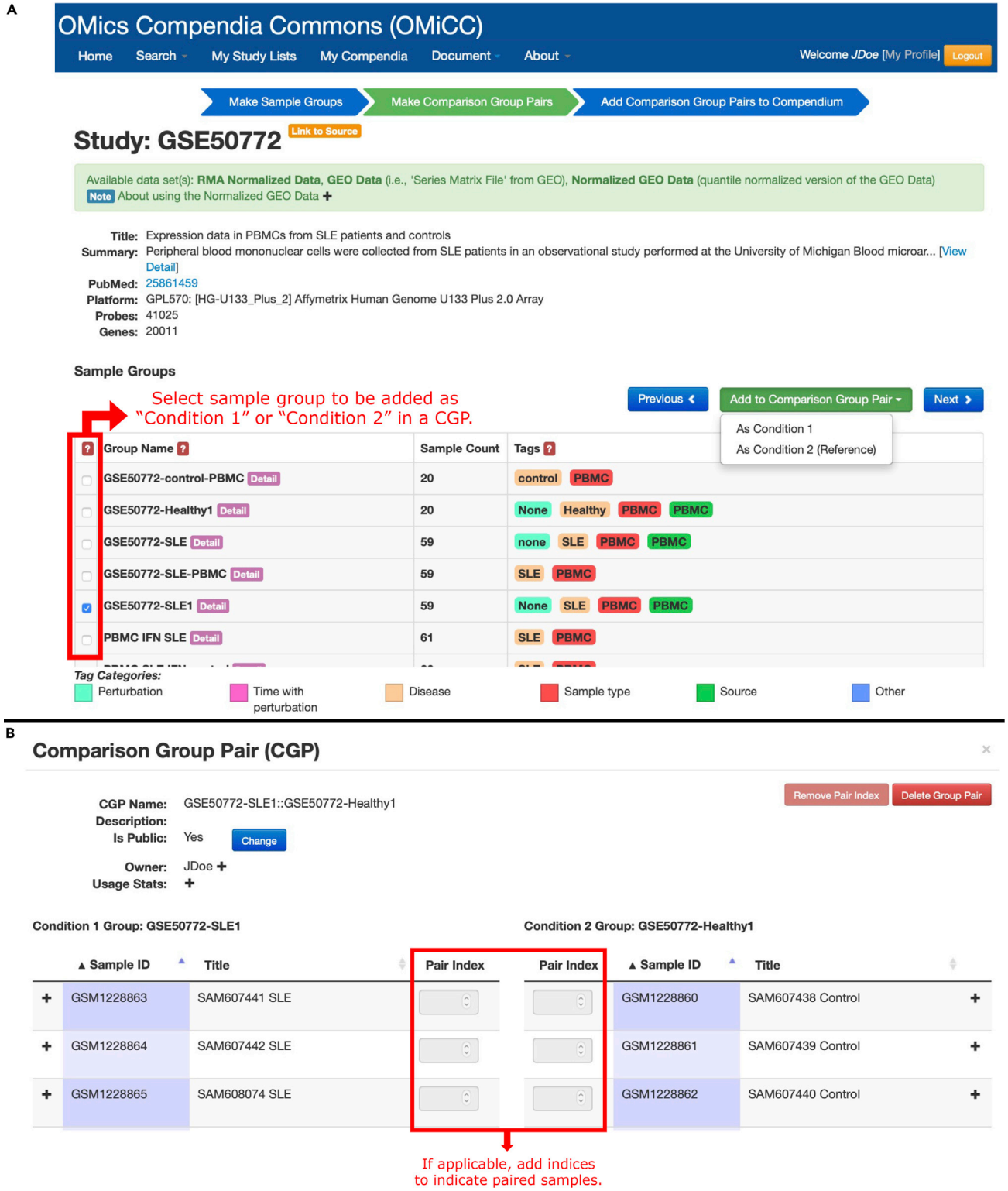


Figure 3. Create a comparison group pair (CGP)

(A) Users can select a sample group and add it either "As Condition 1" or "As Condition 2 (Reference)".

(B) Upon adding sample groups to both Condition 1 and Condition 2, a pop-up panel appears where the user can modify the CGP. If samples are paired, the user can add indices to indicate which samples are paired, which is used later in the analysis.

that the transcript level is higher in Condition 1 than Condition 2 and vice versa for down-regulated transcripts.

21. Optionally, samples in the two groups of a CGP can be paired (Figure 3B).
    a. For example, samples obtained before and after a perturbation from the same subject should be paired.
    b. If the samples are paired, paired analyses are performed downstream, for example, a paired t-test.
    c. If the samples to be paired are in the same order, click on "Set Default Sample Pair Index" to automatically create the pair index.
    d. The pair indices can also be manually changed.
    e. Pairing can only be done when the CGP is private. If the CGP is public, it must be made private for pair indices to be changed.
    f. Click "Save Sample Pair Index" to save the pair index for the samples.
22. To make the CGP public, click the "Change" button next to the "Is Public" field.
23. Click "Close" to save the CGP.
24. If other users create CGPs using the study, they appear in bold under "Comparison Group Pairs" at the bottom of the page.
    a. Click the green "CGP Detail" button to view sample details (Figure 4A).
25. Click "Next" on the study page to add CGP(s) to a compendium.

Create compendium

Timing: 5 min

This step describes how to create a compendium using the previously created CGPs.

26. Select the CGP(s) to add to a compendium (Figure 4A).
27. Choose an existing compendium or click the "Create New Compendium" button.
28. Click the green "Add to Compendium" button.
29. After adding CGPs to a compendium, you can go back to the search study page, create more sample groups in the current study, or go to the compendium to perform analyses. The toolbar on the top of the page can also be used to navigate the website.

Compute differential expression profiles (DEPs)

Timing: 15 min

This step describes how to compute differential expression profiles (DEPs) between the sample groups in a CGP.

30. Click on "My Compendia" on the toolbar to see a list of all compendia.
    a. Click on the desired compendium.
31. On the "Compendium" page, the "CGPs" tab lists the CGPs that are in the compendium.
    a. This tab includes the number of samples in each condition of the CGP, the number of features, the number of genes, the study of origin, platform, and whether it is public.
    b. Click the "Make Public" button to make CGPs public.
32. To export the raw expression data, select the desired CGPs and click the "Export Raw Data On" button and choose whether to export the data in probe or gene space.
    a. When the selected CGPs originate from different platforms or from RNA-seq studies, users can only export data in gene space.
33. To compute DEPs with default settings, click the "Compute DEPs with Default Settings" button.
    a. The default settings use limma with BH multiple-testing correction, using normalized GEO data.

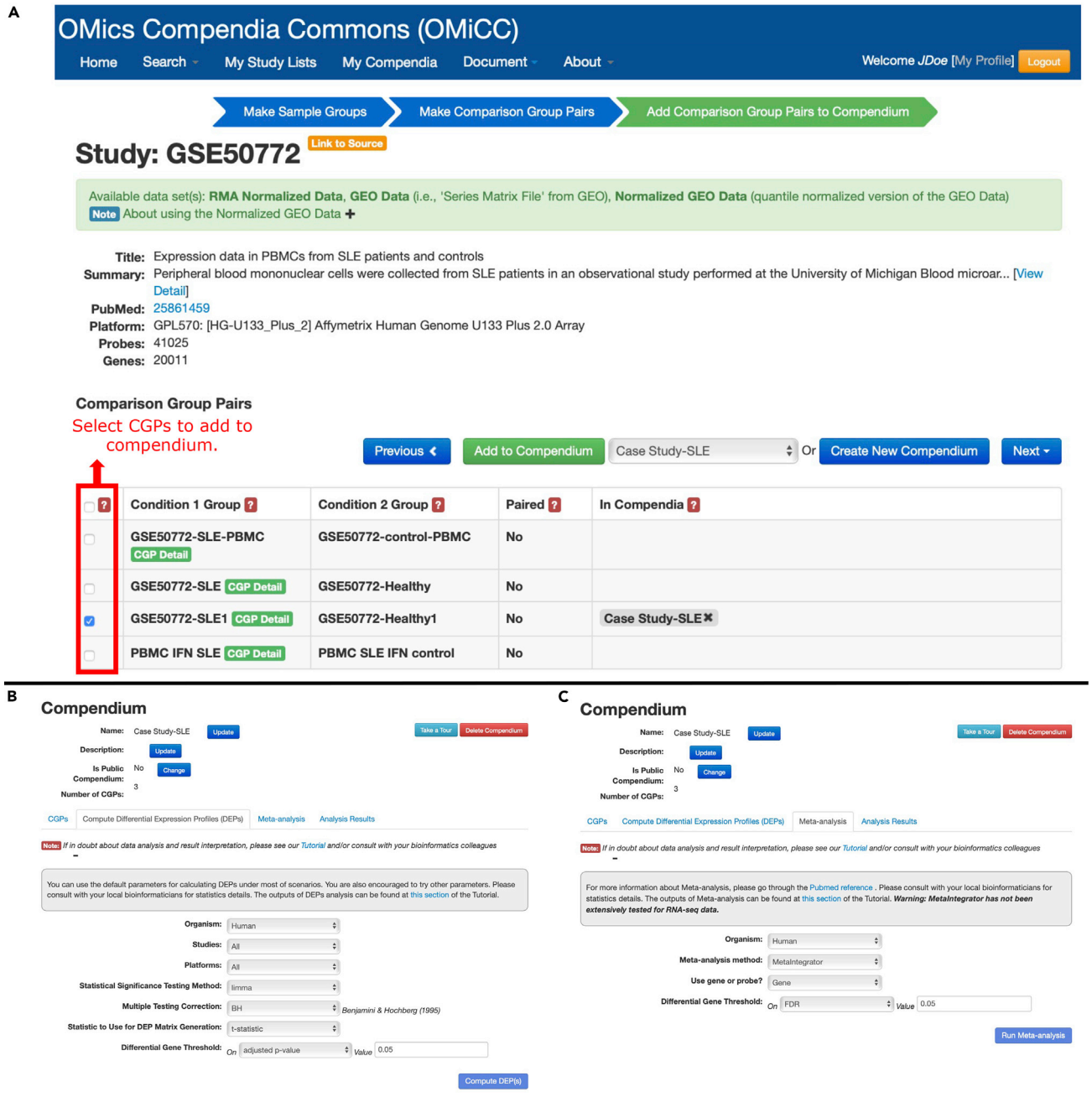


Figure 4. Create a compendium and run analysis

(A) Users can add CGPs to an existing compendium or create a new compendium.

(B) Users can compute Differential Expression Profiles (DEPs). Users can choose which studies or platforms to include, as well as modify the statistical significance testing method, multiple testing correction, statistic to use for DEP matrix generation, and differential gene thresholds.

(C) Users can perform meta-analysis. Users can choose the meta-analysis method to use, as well as the differential gene thresholds.

b. See "quantification and statistical analysis" section below for more information on normalization methods) and using t-statistics to generate a gene-by-CGP matrix (i.e., the DEP matrix).

c. CGPs with studies using platforms with missing probe-to-gene mapping information or where normalized GEO data is unavailable cannot be analyzed using this "one-click" approach.

34. To change the default settings used to compute DEPs, click on the "Compute Differential Expression Profiles (DEPs)" tab (Figure 4B).
35. Filter by organism, studies, or platform using the drop-down menus.
36. Choose the statistical parameters for performing differential analysis. Select from the following (Table 1):
37. Click on the check box next to the CGP to include that CGP in the DEP analysis.
38. If the CGP is generated from a study using a microarray platform, choose the data source (GEO, RMA normalized, or normalized GEO) and whether to perform analysis in gene or probe space.
39. If the CGP is generated from RNA-seq data, the data is normalized internally, and analyses are done in gene space.
40. Click the "Compute DEP(s)" button.

Perform meta-analysis

Timing: 15 min

This step describes how to perform meta-analysis of the studies in a compendium. Users can choose between two methods – RankProd and MetaIntegrator.

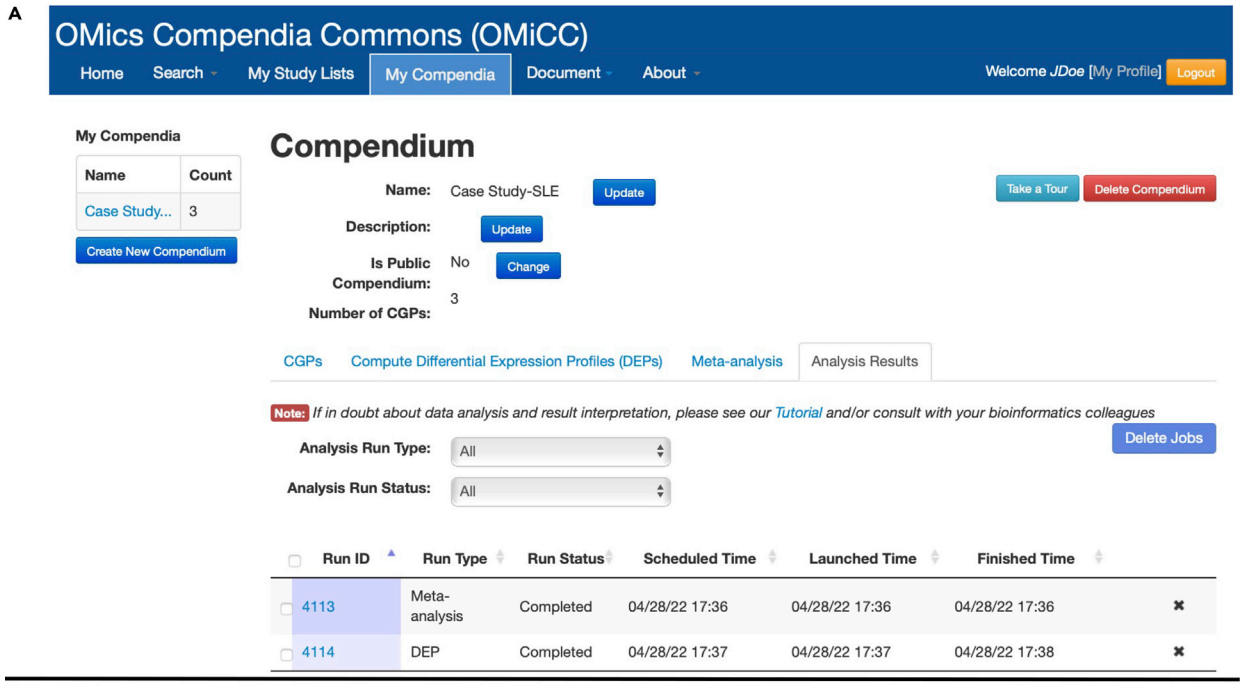
41. On the "Compendium" page, click on the "Meta-analysis" tab (Figure 4C).
42. Select which meta-analysis method to use, RankProd (rank product method) or MetaIntegrator (random effect model).
43. If the CGPs in the compendium are generated from studies using a microarray platform, select to perform the analysis in gene or probe space.
    a. If working with RNA-seq data, analysis is performed in gene space.
44. If using RankProd, select p-value or adjusted p-value and a threshold for differential expression analysis. Default is an adjusted p-value threshold of 0.05.
    a. Selecting the adjusted p-value uses the percentage of false positive predictions (pfp) value. RankProd calculates the pfp value as the estimated percentage of false predictions.
45. If using MetaIntegrator, select FDR or effect size and a threshold for differential expression analysis. Default is an FDR threshold of 0.05.
46. Click on the check box next to the CGP to select CGPs to include in the analysis.
47. If the CGP is generated using a study using a microarray platform, select the data source (GEO, RMA normalized, or normalized GEO).
48. Select the reference condition for each CGP so the comparison is biologically consistent across CGPs.
    a. For example, one CGP has patients with a disease status labeled as "Condition 1" and healthy subjects as "Condition 2". In a second CGP, healthy subjects are "Condition 1" and disease subjects are "Condition 2".
    b. The reference conditions need to be made uniform to either the healthy or disease groups.

⚠ CRITICAL: The reference conditions need to be standardized correctly for the results to have biological significance.

49. Click the blue "Run Meta-analysis" button.

Table 1. Statistical parameters for performing differential analysis

Parameter	Options
Statistical Significance Testing Method	limma, Mann-Whitney test, student's t-test
Multiple Testing Correction	Benjamini & Hochberg, Benjamini & Yekutieli, Holm, Hochberg, Hommel, Bonferroni
Statistic to Use for DEP Matrix Generation	log fold-change, average expression, t-statistic, b-statistic, p-value, adjusted p-value, -log(p-value), -log(adjusted p-value)
Differential Gene Threshold	Adjusted p-value, p-value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Name
Count
Case Study...
3
Create New Compendium
Compendium
Name:
Case Study-SLE
Update
Description:
Update
Is Public Compendium:
No
Change
Number of CGPs:
3
Take a Tour
Delete Compendium
CGPs
Compute Differential Expression Profiles (DEPs)
Meta-analysis
Analysis Results
Note: If in doubt about data analysis and result interpretation, please see our Tutorial and/or consult with your bioinformatics colleagues
Analysis Run Type:
All
Analysis Run Status:
All
Delete Jobs
Run ID
Run Type
Run Status
Scheduled Time
Launched Time
Finished Time
4113
Meta-analysis
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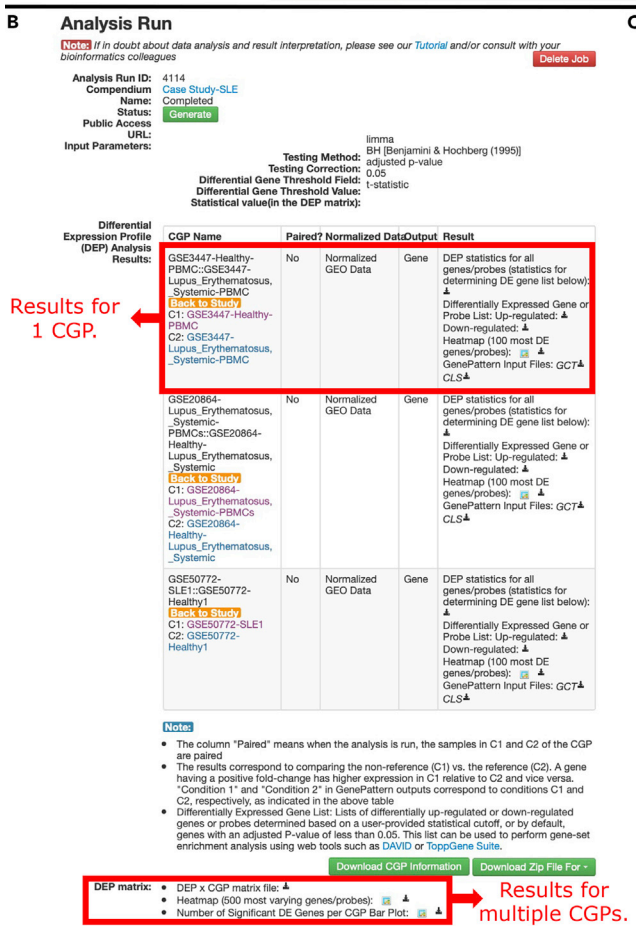
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Analysis Run
Note: If in doubt about data analysis and result interpretation, please see our Tutorial and/or consult with your bioinformatics colleagues
Delete Job
Analysis Run ID: 4114
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limma
Testing Method: BH [Benjamini & Hochberg (1995)]
Testing Correction: adjusted p-value
Differential Gene Threshold Field: 0.05
Differential Gene Threshold Value: t-statistic
Differential Expression Profile (DEP) Analysis Results:
CGP Name
Paired?
Normalized Data
Output
Result
GSE3447-Healthy-PBMC::GSE3447-Lupus_Erythematosus,_Systemic-PBMC
Back to Study
C1: GSE3447-Healthy-PBMC
C2: GSE3447-Lupus_Erythematosus,_Systemic-PBMC
No
Normalized GEO Data
Gene
DEP statistics for all genes/probes (statistics for determining DE gene list below):
Differentially Expressed Gene or Probe List: Up-regulated: Down-regulated:
Heatmap (100 most DE genes/probes):
GenePattern Input Files: GCT CLS
Results for 1 CGP.
GSE20864-Lupus_Erythematosus,_Systemic-PBMCs::GSE20864-Healthy-Lupus_Erythematosus,_Systemic
Back to Study
C1: GSE20864-Lupus_Erythematosus,_Systemic-PBMCs
C2: GSE20864-Healthy-Lupus_Erythematosus,_Systemic
No
Normalized GEO Data
Gene
DEP statistics for all genes/probes (statistics for determining DE gene list below):
Differentially Expressed Gene or Probe List: Up-regulated: Down-regulated:
Heatmap (100 most DE genes/probes):
GenePattern Input Files: GCT CLS
GSE50772-SLE1::GSE50772-Healthy1
Back to Study
C1: GSE50772-SLE1
C2: GSE50772-Healthy1
No
Normalized GEO Data
Gene
DEP statistics for all genes/probes (statistics for determining DE gene list below):
Differentially Expressed Gene or Probe List: Up-regulated: Down-regulated:
Heatmap (100 most DE genes/probes):
GenePattern Input Files: GCT CLS
Note:
The column "Paired" means when the analysis is run, the samples in C1 and C2 of the CGP are paired
The results correspond to comparing the non-reference (C1) vs. the reference (C2). A gene having a positive fold-change has higher expression in C1 relative to C2 and vice versa. "Condition 1" and "Condition 2" in GenePattern outputs correspond to conditions C1 and C2, respectively, as indicated in the above table
Differentially Expressed Gene List: Lists of differentially up-regulated or down-regulated genes or probes determined based on a user-provided statistical cutoff, or by default, genes with an adjusted P-value of less than 0.05. This list can be used to perform gene-set enrichment analysis using web tools such as DAVID or ToppGene Suite.
Download CGP Information
Download Zip File For
DEP matrix:
DEP x CGP matrix file:
Heatmap (500 most varying genes/probes):
Number of Significant DE Genes per CGP Bar Plot:
Results for multiple CGPs.

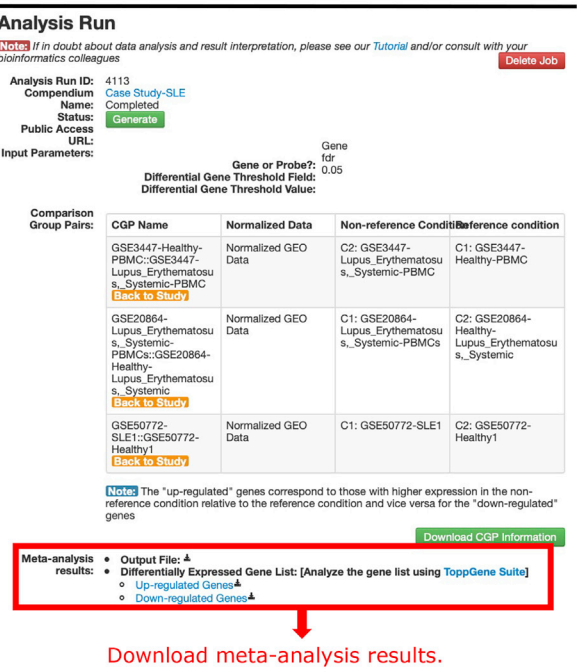
C
Analysis Run
Note: If in doubt about data analysis and result interpretation, please see our Tutorial and/or consult with your bioinformatics colleagues
Delete Job
Analysis Run ID: 4113
Compendium Name: Case Study-SLE
Status: Completed
Generate
Public Access URL:
Input Parameters:
Gene
Gene or Probe?: fdr
Differential Gene Threshold Field: 0.05
Differential Gene Threshold Value:
Comparison Group Pairs:
CGP Name
Normalized Data
Non-reference Condition
Reference condition
GSE3447-Healthy-PBMC::GSE3447-Lupus_Erythematosus,_Systemic-PBMC
Back to Study
Normalized GEO Data
C2: GSE3447-Lupus_Erythematosus,_Systemic-PBMC
C1: GSE3447-Healthy-PBMC
GSE20864-Lupus_Erythematosus,_Systemic-PBMCs::GSE20864-Healthy-Lupus_Erythematosus,_Systemic
Back to Study
Normalized GEO Data
C1: GSE20864-Lupus_Erythematosus,_Systemic-PBMCs
C2: GSE20864-Healthy-Lupus_Erythematosus,_Systemic
GSE50772-SLE1::GSE50772-Healthy1
Back to Study
Normalized GEO Data
C1: GSE50772-SLE1
C2: GSE50772-Healthy1
Note: The "up-regulated" genes correspond to those with higher expression in the non-reference condition relative to the reference condition and vice versa for the "down-regulated" genes
Download CGP Information
Meta-analysis results:
Output File:
Differentially Expressed Gene List: [Analyze the gene list using ToppGene Suite]
Up-regulated Genes
Down-regulated Genes
Download meta-analysis results.

Figure 5. Access analysis results

(A) Users can view results of any analyses under the "Analysis Results" tab in a compendium.

(B) For DEP analysis, users can download results for each individual CGP by clicking on the black download icons corresponding to each type of result. If multiple CGPs were included, additional results are available.

(C) For meta-analysis, users can download the output file of the chosen meta-analysis method, as well as lists of differentially up-regulated or down-regulated genes or probes.

Access and interpret analysis results

Timing: 10 min

This step describes how to view and download the results from both differential expression analysis and meta-analysis. The outputs of both analyses are described here.

50. On the "Compendium" page, click on the "Analysis Results" tab to see all analyses performed for a compendium (Figure 5A).
51. Filter analyses by run type (Derive DEPs or Meta-analysis) or run status (queued, running, completed, failed).
52. Click on a run ID to see the detailed results for those analyses.
53. Click the green "Generate" button in the "Public Access URL" field to generate a URL to share the analysis results.
54. For DEP analysis, download results for each CGP or the DEP matrix (Figure 5B).
    a. Click the black download icon next to each output type to download the results for each CGP.
    b. Click the heatmap icon to view a heatmap of differentially expressed genes in a pop-up window.
    c. These are the outputs (Table 2):
    d. Click the green "Download CGP Information" button to download an Excel file with information about each CGP used in the analysis (e.g., the number of subjects, platform, samples included).
    e. Click the green "Download Zip File For" button and select to download data for all differential expression profiles, all differentially expressed genes or probes, all heatmaps, or all GenePattern input files.
    f. Additional outputs are available if two or more CGPs are included. Click the black download icon to download files. Click on the heatmap icons to view the graphs in a pop-up window.
    g. These are the outputs (Table 3):
55. For meta-analysis, click on the black download icons to download the output of the chosen method (Figure 5C).
    a. The output file has these fields if the user selects the RankProd method (Table 4):

Table 2. Output of DEP analysis

Output name	Description
Differential Expression Profile (DEPs)	A file containing analysis result statistics of each gene or probe from condition 1 vs. condition 2 comparison. The statistics returned depend on the statistical method used. For example, if using the limma method, results include log(fold change), average expression, t-statistic, p-value, adjusted p-value, and b-statistic.
Differentially Expressed Gene or Probe List	A list of differentially expressed genes or probes determined based on the defined statistical cut-off. This list can be used to perform gene-set enrichment analysis.
Heatmap (100 most DE genes/probes)	A heatmap of the top 100 differentially expressed genes or probes showing all samples in the CGP. The values shown in the heatmap reflect the data source the user selected. If the "one-button" approach was used, by default the "Normalized GEO Data" is shown.
GenePattern Input Files	Two files (GCT and CLS formatted) are provided containing gene expression values and sample grouping information for down-stream analysis outside of OMiCC. This format is supported by tools such as Gene Pattern, Gene Set Enrichment Analysis, and Integrative Genomics Viewer.

Table 3. Additional outputs for two or more CGPs

Output name	Description
DEP × CGP matrix file	A merged matrix of DEPs (using the user-selected statistics) with genes or probes as rows and CGPs as columns.
Heatmap (500 most varying genes/probes)	A clustered heatmap of the 500 most varying genes or probes across CGPs. The value displayed is the user-selected statistic for the DEP × CGP matrix.
Number of Significant DE Genes per CGP Bar Plot	A bar plot showing the number of statistically significant differentially expressed genes for each CGP.

b. The output file has these fields if the user selects the MetaIntegrator method (please see MetaIntegrator paper (Haynes et al., 2017) for more details) (Table 5):
c. Click on the black download icons to download lists of differentially up-regulated or down-regulated genes or probes.
d. Click the green "Download CGP Information" button to download an Excel file with information about each CGP in the analysis (e.g., the number of subjects, platform, samples included).

56. Open any of the downloaded tables in Excel or a similar program to inspect the results of your analysis further.

RESTful API

Timing: 10 min

This step describes how to access OMiCC data using an API. A RESTful API (application programming interface) defines a set of functions through which users can interface directly with the OMiCC software from their computer to receive information from OMiCC.

57. In the toolbar, click "Document" then "RESTful APIs" for a list of API functions to access OMiCC's internal data, such as GEO studies, samples, platforms, public sample groups and CGPs.
58. Using the APIs requires users to acquire an access token from OMiCC. Use the following URL to get the token: https://omicc.niaid.nih.gov/api/login with your username and password in the request. For details, please refer to the RESTful API documents.

EXPECTED OUTCOMES

The results from any analysis run can be accessed through the "Analysis Results" tab of a compendium. The results of DEP analysis include the differential expression profiles of each CGP, a list of differentially expressed genes, and a heatmap of the 100 most differentially expressed genes or probes. GCT and CLS files are provided that can be used in downstream tools, such as GenePattern (Reich et al., 2006), Gene Set Enrichment Analysis (GSEA), and the Integrative Genomics Viewer (IGV). In addition to the CGP-specific results, DEP analysis returns a matrix of DEP × CGP, a heatmap of the 500 most varying genes or probes across CGPs, and a bar plot showing the number of DE genes per CGP. The results of meta-analysis include the output file specific to the meta-analysis method chosen (described above in the step-by-step method details), as well as lists of differentially expressed genes.

Table 4. Outputs using the RankProd statistical method

Parameter	Description
pfp	Estimated percentage of false positive (pfp) up to the position of each gene per direction of change (i.e., up-regulated and down-regulated genes). Pfp is similar to the false discovery rate (FDR).
Pval	Estimated p-value for being up-regulated or down-regulated for each gene
Fc.avg	Log fold change of average expression in condition 1 over average expression in condition 2
Fc (per CGP)	Log fold change for each CGP (labeled by the name of the CGP)

Table 5. Outputs using the MetaIntegrator statistical method

Parameter	Description
effectSize	Summary effect size computed using a random effect model
effectSizeStandardError	Standard error for the summary effect size
effectSizePval	Given summary effect size and standard error, p-value calculated based on a standard normal distribution
effectSizeFDR	Benjamini-Hochberg FDR correction for multiple hypothesis testing
tauSquared	Inter-dataset variation as estimated by the DerSimonian-Laird method
numStudies	Number of studies in which the gene was present
cochranesQ	Cochrane's Q value for evaluating heterogeneity of effect size estimates between studies
heterogeneityPval	p-value of Cochrane's Q calculated against a chi-squared distribution
fisherStatUp	Log sum of p-values that each up-regulated gene using Fisher's method
fisherPvalUp	p-value under a chi-squared distribution
fisherFDRUp	Benjamini-Hochberg FDR correction for multiple hypothesis testing
fisherStatDown	Log sum of p-values that each gene is down-regulated using Fisher's method
fisherPvalDown	p-value under a chi-squared distribution
fisherFDRDown	Benjamini-Hochberg FDR correction for multiple hypothesis testing

An example case study on performing a meta-analysis to investigate differential gene expression between patients with systemic lupus erythematosus, also called lupus, and healthy individuals without lupus is available to users (Methods videos S1, S2, S3, and S4).

This protocol walks users through data reuse and meta-analysis on publicly available gene expression data using the OMiCC web interface. We envision that as the user base of OMiCC grows, the number of annotated studies and publicly available CGPs and compendia will grow as well, facilitating more efficient data integration. OMiCC thus enables "virtual" collaborations in the broader biomedical research community and promotes the generation of new biological insights.

QUANTIFICATION AND STATISTICAL ANALYSIS

Both microarray and RNA-seq data are available in OMiCC. For microarray data, more than 40,000 human and mouse data sets and associated metadata were retrieved from GEO and incorporated into the OMiCC database. Three microarray data types are available in OMiCC depending on data availability in GEO: GEO series matrix data set; quantile normalized version of the series matrix data set; and for Affymetrix platforms, RMA normalized data derived from the raw CEL files. By default, OMiCC uses the quantile-normalized GEO series matrix file, but the user has the option of selecting other data types. Quality control is performed on all GEO studies and samples flagged as outliers by our pipeline are removed. A major update since our initial publication is the addition of RNA-seq data to the OMiCC database from the recount2 platform (Collado-Torres et al., 2017), a resource of RNA-seq data downloaded from SRA and uniformly processed using a Rail-RNA pipeline (Nellore et al., 2017) (Nellore et al., 2017). Gene count data from recount2 is transformed from coverage to read counts, then incorporated into the OMiCC database. If the user chooses RNA-seq data to use in their analysis, TMM (trimmed mean of M-values) normalization (Robinson and Oshlack, 2010), which adjusts for differences in total RNA production, and logCPM (counts per million) are performed on the data.

There are many tools in R, Python, and other environments that can perform statistical analysis, but they often require programming on the user's part (Tseng et al., 2012). Assembling the data into data structures can be a demanding task for biologists with little computational training. OMiCC assembles these data structures for the users through an easy-to-use interface and can also perform two types of analysis, differential expression analysis and meta-analysis. Differential expression analysis is performed on each individual CGP according to the user-specified parameters. Using drop-down menus, users can choose the statistical significance testing method, multiple testing correction method, and differential gene threshold. The three significance testing methods that can be

used in OMiCC are limma (Ritchie et al., 2015), an empirical Bayesian approach; the Mann-Whitney U test, also known as the Wilcoxon rank-sum test; and the Student's t-test.

There are many methods to perform meta-analysis on gene expression data, including combining ranks, combining effect sizes, combing p-values, and directly merging raw data using advanced normalization techniques (Tseng et al., 2012). While each has its own advantages, we use two methods to incorporate into OMiCC. RankProd is an R package that extends the rank product method and detects differential expression using a non-parametric test (Hong et al., 2006). An advantage of non-parametric methods is that they do not assume normality. While microarray data is continuous and can be assumed as normal distribution after normalization, RNA-seq data is a count distribution and thus cannot be assumed as normal distribution (Tseng et al., 2012). A disadvantage of RankProd is that it does not output a combined effect size across studies. Therefore, another new feature we incorporated is the meta-analysis package MetaIntegrator, which uses a random-effects model with the assumption that the results from each study in the analysis are drawn from a single distribution and that inter-study differences are a random effect (Haynes et al., 2017). It performs a DerSimonian and Laird random-effects meta-analysis and a Fisher's sum-of-logs test between cases and controls for each study and requires that a gene is significant using both methods. MetaIntegrator returns q-values, which evaluates whether each gene is differentially expressed between cases and controls in the included studies. An advantage of MetaIntegrator is that it returns a combined effect-size. Users should be aware that MetaIntegrator was developed for microarray studies and has not been extensively tested for RNA-seq data. While OMiCC does allow users to choose RNA-seq studies to use with MetaIntegrator and performs a TMM and CPM normalization on RNA-seq data, users should use cautious when interpreting these results. The results from either meta-analysis method can be downloaded directly from the OMiCC web interface.

LIMITATIONS

While OMiCC was developed to assist biologists without computational training to analyze publicly available data, it is not meant to replace collaborations with bioinformaticians or computational biologists. It can be dangerous to cherry-pick results and statistical expertise is often necessary to interpret meta-analysis results. If in doubt, we recommend users consult with a bioinformatics expert to determine which multiple-testing correction method and statistical test for differential expression analysis are appropriate for the studies of interest.

Selecting studies to include in the analysis is an important step. Certain microarray platforms are only used in a small number of studies or cover a small number of genes and may not be easily comparable with other platforms. To assist the user in choosing studies, OMiCC highlights the most popular microarray platforms. OMiCC also contains both microarray and RNA-seq data. While OMiCC allows the comparison of data across the two technologies, users should be aware that data generated from different technologies may not be directly comparable. The meta-analysis methods RankProd and MetaIntegrator were developed for microarray studies and have not been extensively tested for RNA-seq data. While OMiCC does allow users to choose RNA-seq studies to use with these methods and performs a TMM and CPM normalization on RNA-seq data, users should use caution when interpreting these results. Users can use both RankProd and MetaIntegrator methods and look for results consistent with both approaches. Users should also be aware that different library preparation protocols may heavily influence RNA-seq results (Alberti et al., 2014; Kumar et al., 2017; Sun et al., 2013).

Metadata is often not standardized and sometimes not reported in public databases, so it may be helpful to refer to the original publication for information on experimental design and batching. Meta-analysis methods are meant to mitigate the effect of biological and technical heterogeneity across studies, and therefore results that show a coherent signal across multiple studies and platforms are more robust.

TROUBLESHOOTING

Problem 1

The sample group cannot be edited (step 15).

Potential solution

If a sample group is public and being used by another OMiCC user, the group cannot be edited. Unfortunately, you cannot edit a sample group if it is being used by another user.

Problem 2

Some CGPs cannot be included in generation of a DEP or meta-analysis (steps 37 and 46).

Potential solution

OMiCC uses the gene symbol as a common link across studies. If the probe-to-gene map is not available, there is no way to generate a cross-study analysis. Unfortunately, there is no way to use this CGP in a cross-study analysis.

Problem 3

Error when opening text file in Excel indicating text file is SYLK file (step 56).

Potential solution

This error occurs because the text file begins with the term "ID". You can ignore this error. Click OK and proceed to inspect the file.

Problem 4

When creating a sample group, the "Save" button is opaque and cannot be clicked (step 16).

Potential solution

This error occurs because there is an insufficient number of samples in the sample group. OMiCC requires at least three samples per sample group. The only solution is to add more samples to the sample group.

Problem 5

When searching for studies, the number of displayed studies does not change when trying to limit the results to human or mouse platforms (step 4).

Potential solution

Click "Search" again after checking or unchecking boxes to identify specific platforms of interest on which to search.

Problem 6

There is no button visible to add a compendia to your personal compendia collection after searching on compendia (step 7).

Potential solution

Ensure that you are logged into OMiCC. One can search studies, sample groups, CGPs and compendia when not logged in to OMiCC, but cannot create sample groups, CGPs, or copy compendia without being logged in.

Problem 7

In the meta-analysis results, the fold-change of a gene is very different across CGPs (step 55).

Potential solution

In the CGPs, confirm that "Condition 1" and "Condition 2" are assigned consistently across studies (step 19). When performing meta-analysis, confirm that the correct group is assigned under "Reference Condition" (step 48). Users can also try to adjust the differential gene threshold or meta-analysis method. When in doubt, consult with your local bioinformatician.

Problem 8

In the meta-analysis results, the downloaded files of up-regulated or down-regulated genes are empty (step 55c). For the differential expression analysis, the summary of the analysis run states that there are no genes or probes that passed the threshold for differential expression (step 52).

Potential solution

For the meta-analysis, these files will be empty when none of the tested genes pass the significance threshold. Download and open the meta-analysis results (steps 55 and 56) to evaluate the full results of the meta-analysis. For the differential expression analysis, download and open the "DEP statistics for all genes/probes" file (steps 54 and 56) to evaluate the full results.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, John S. Tsang (john.tsang@nih.gov).

Materials availability

This study did not generate new unique materials or reagents.

Data and code availability

No new datasets were generated or analyzed during this study.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2022.101474.

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AUTHOR CONTRIBUTIONS

Conceptualization, J.S.T.; Methodology, C.C.L, Y.G., W.W.L., and J.S.T.; Software, Y.G., W.W.L., and C.C.L.; Formal Analysis, Y.G., W.W.L., and C.C.L.; Investigation, C.C.L., W.W.L., K.L.V., and R.S.; Writing – Original Draft, C.C.L., W.W.L., K.L.V., and R.S.; Writing – Review & Editing, C.C.L., W.W.L., K.L.V., R.S., and J.S.T.; Visualization, C.C.L., K.L.V., and R.S.; Supervision, R.S. and J.S.T.; Funding Acquisition, J.S.T.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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